UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

Relationship between Portuguese consumer preferences and textural properties, chemical composition and nutritional value of beef

Ana Cristina Saragoça Melgado Gonçalves Monteiro

Tese de Doutoramento em Ciências Veterinárias

Especialidade de Produção Animal

CONSTITUIÇÃO DO JURI

Presidente:
Reitor da Universidade Técnica de Lisboa

Vogais:
Doutor José António Mestre Prates
Doutor José Pedro Cardoso Lemos
Doutor José Manuel Bento Santos Silva
Doutor Miguel Nuno Geraldo Viegas Santos Elias
Doutora Teresa de Jesus da Silva Matos
Doutor Rui José Branquinho de Bessa

ORIENTADOR
Doutor José Pedro Cardoso Lemos

CO-ORIENTADOR
Doutor José António Mestre Prates

2012

Lisboa

UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

Relationship between Portuguese consumer preferences and textural properties, chemical composition and nutritional value of beef

Ana Cristina Saragoça Melgado Gonçalves Monteiro

Tese de Doutoramento em Ciências Veterinárias

Especialidade de Produção Animal

CONSTITUIÇÃO DO JURI

Presidente:
Reitor da Universidade Técnica de Lisboa

Vogais:
Doutor José António Mestre Prates
Doutor José Pedro Cardoso Lemos
Doutor José Manuel Bento Santos Silva
Doutor Miguel Nuno Geraldo Viegas Santos Elias
Doutora Teresa de Jesus da Silva Matos
Doutor Rui José Branquinho de Bessa

ORIENTADOR
Doutor José Pedro Cardoso Lemos

CO-ORIENTADOR
Doutor José António Mestre Prates

2012

Lisboa

À minha Bomboquita

ACKNOWLEDGEMENTS

To all those who contributed for the accomplishment of this PhD thesis, I would like to express my sincerely gratefulness, in particular to:

Faculdade de Medicina Veterinária from Universidade Técnica de Lisboa and Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), for all the institutional support granted.

To Professor José Pedro Cardoso Lemos, my supervisor and Professor José António Mestre Prates my co-supervisor, for the guidance, the knowledge transmitted and the critical review of this thesis.

To "Montado Alentejano e Tradição" for the collaboration in the first phase of the present study.

To Regional abattoir of Alto Alentejo, Sousel for allowing the sample collection and carcass measurements.

To Professor Rui Bessa for the knowledge transmitted, guidance and papers review. I am deeply grateful by the precious advices, the encouragement, but especially by the support and patience demonstrated in a very difficult moment of my professional and personal life.

To Professor António Barreto for the opportunity to work in the laboratory of the Departamento de Tecnologia. Also to Engª Maria José Fernandes for coordinating efforts in the use of equipment, material and space, but also for her sympathy and assistance.

To Professor Berta São Brás for allowing the use of the spectrophotometer from the laboratory of the Departmento de Farmacologia e Toxicologia. I am also grateful to Drª Belmira Carrapiço, Ana Lúcia and Adriana for their assistance and kindness.

To Dª Paula, always an example of availability and efficiency, I am thankful for her helpfulness and sympathy.

To Maria João Fradinho, with whom I share the cabinet and the tempestuous period of writing the PhD thesis, by the sympathy, friendship and encouragement.

To Cristina Alfaia, Sofia Costa and Marta Madeira for their support and friendship. Also to Paulo Costa for making possible the sensory analysis in the last trial. I want to express my thanks for their encouraging words.

To all my dear colleagues and friends, Benedita Pinheiro, Catarina Guerreiro, Helena Santos, Inês Viegas, Joana Brás, Márcia Correia, Patrícia Ponte, Paula Lopes, Susana Martins, Teresa Ribeiro, Vânia Ondina, Virgínia Pires and the only man among women, Fernando Dias, for all… the lunches in an atmosphere of pleasant gossip, the friendship, the continuous support, the encouragement. Their friendship has been of central importance during the last years in Faculdade de Medicina Veterinária. I am grateful that our relationship has become personal.

To my parents which have always been the pillars of my life, without them none of this would be possible. Also to my broters, sister and sister in law for their support, and to my nephews who fill my life with joy.

The last but not the least, I am deeply grateful to my dearest husband Rui and to my wonderful daughter Joana. She is my greatest love, making my heart almost burst with love and joy. I know that during the writing of this thesis I have not always been the nicest person, I am grateful for your patience, encouragement and love. It is amazing how a three years old child can be so understanding.

Thank you all!

This work was co-financed by Fundação para a Ciência e Tecnologia (Grant SFRH/BD/31091/2006) and by Ministério da Agricultura (AGRO 422/2004)

ABSTRACT

Relationship between Portuguese consumer preferences and textural properties, chemical composition and nutritional value of beef

Beef is a nutritionally rich product with a high economic value. However in the last decades beef has been target of negative publicity by the media, mainly due to the high content of cholesterol as well as saturated and *trans* fatty acids (FA). The increasing number of food scares across the Europe over the last years has increased even more consumers' concerns about beef quality and safety. Despite the health concerns beef sensory properties still remain the main purchasing and repeat purchasing criteria for the consumers. Tenderness has been considered the major palatability characteristic of beef and defines its commercial value. However, tenderness variability has been considered a major problem to the beef industry. In this context, the aim of this proposal was to ascertain the organoleptic properties and lipid nutritional value of beef and to relate them with the Portuguese consumer` preferences. The experimental work was divided in two trials. In the first trial, the meat quality and lipid nutritional value from "Vitela Tradicional do Montado"-PGI veal, Mertolenga-PDO veal and Mertolenga-PDO beef were studied. The results suggested that the three products are not very different, but the two veal types are more alike, which means that age had a more powerful effect than the crossbred or gender. Nevertheless, despite the similarity in FA profile and of the production systems of the animals sampled, it was possible to discriminate the meat types. Moreover, the PGI veal seems to have higher variability than the other two meat types studied. In the second trial, three beef types from the Portuguese market place, Carnalentejana-PDO, imported from Brazil and national undifferentiated beef, were studied and compared to characterise nutritional value and variability of beef lipids as well as the organoleptic properties and their variability. We also intended to relate the sensory attributes with instrumental measurements in order to realise if it is possible to predict sensory evaluation through instrumental measurements. Brazilian beef had the highest lipid nutritional value and lipid stability as this beef presented higher contents of α-tocopherol, β-carotene, CLA and PUFA. The data indicated that all beef types seem to have α-tocopherol values high enough to inhibit lipid oxidation and discoloration. Energy intake was calculated for the main FA and compared with the recommended daily intake. All beef types seem to have low cholesterol and SFA contents. Concerning the relation between the sensory attributes and the instrumental measurement, sensory tenderness was correlated with WBSF but not with TPA hardness. Nevertheless, TPA chewiness was well correlated with sensory tenderness indicating TPA usefulness to give addicional information of beef texture. Off-flavour was the

main determinant of overall acceptability and Brazilian beef presented the highest and lowest values, respectively. Brazilian beef also had the lowest juiciness score, indicating that this beef type despite having the best lipid nutritional value can be rejected due to it sensory attributes. All beef types were considered slightly to moderately tender regarding WBSF and sensory tenderness mean values. National undifferentiated beef seem to have higher variability in the colour measurements, whilst Carnalentejana-PDO seems to depicted higher tenderness variability. Despite having mean values in the aforementioned attributes that indicates a consumer high acceptability, the high variability in both cases can be detrimental in the moment of sale.

Key words: Beef; meat quality; tenderness; colour; lipid profile; nutritional value; texture profile analysis.

RESUMO

Relação entre as preferências do consumidor português e a textura, composição química e valor nutricional da carne de bovino

A carne bovina é um produto nutricionalmente rico e de elevado valor económico. No entanto, nos últimos anos tem sido alvo de publicidade negativa por parte da comunicação social devido ao seu conteúdo em colesterol e em ácidos gordos saturados e *trans*. O crescente número de surtos alimentares na Europa aumentou a preocupação dos consumidores quanto à segurança e qualidade alimentar. No entanto, apesar das referidas preocupações, os atributos sensoriais ainda permanecem a principal razão que leva os consumidores a comprar e a repetir a compra de carne de bovino. A tenrura tem sido considerada o atributo mais importante da carne bovina e define o seu valor comercial, mas a sua variabilidade tem representado um problema para a indústria. Neste contexto, o objectivo deste trabalho foi determinar as propriedades organolépticas e o valor nutricional da fracção lipídica da carne bovina e relacioná-las com as preferências dos consumidores. O trabalho experimental consistiu em dois ensaios. No primeiro ensaio foram estudados a qualidade organoléptica e o valor nutricional da fracção lipídica da carne de vitela "Vitela Tradicional do Montado"-IGP e Mertolenga-DOP e do novilho Mertolenga-DOP. Os resultados sugerem que as duas vitelas têm composições similares, o que pode significar que o efeito da idade foi maior do que o efeito da raça ou género. Apesar das semelhanças do sistema de produção e no perfil lipídico as três carnes foram bem discriminadas. No segundo ensaio foram estudados a qualidade organoléptica e o valor nutricional dos lípidos das carnes de bovino Carnalentejana-DOP, importada do Brasil e nacional indiferenciada. Também aqui se pretendeu prever a qualidade sensorial das carnes com base nas medições instrumentais. Todos os tipos de carne apresentaram baixo teor em colesterol, e teor em α-tocoferol suficiente para inibir a oxidação lipídica e a descoloração. A carne Brasileira apresentou o melhor valor nutricional do perfil lipídico e potencial anti-oxidante, visto ter apresentado conteúdos superiores em α-tocoferol, β-caroteno, CLA e ácidos gordos poliinsaturados. A ingestão de energia foi calculada para os ácidos gordos mais importantes e comparada com as recomendações de ingestão diárias. Todos os tipos de carne apresentaram baixo conteúdo em colesterol e ácidos gordos saturados. Os atributos sensoriais apresentaram correlações baixas com os parâmetros instrumentais, e apenas a força de corte se correlacionou com a tenrura sensorial. No entanto, a tenrura sensorial correlacionou-se com a mastigabilidade indicando que o TPA pode ser útil, dando informação adicional sobre a textura da carne. O *off-flavour* foi o principal determinante da

aceitabilidade da carne, apresentando a carne Brasileira os valores mais e menos elevados, respectivamente. A carne Brasileira foi também a menos suculenta, indicando que apesar de apresentar o melhor valor lipídico pode, no entanto, ser rejeitada pelas suas características sensoriais. Todas as carnes foram consideradas ligeira a moderadamente tenras, tendo em conta os seus valores de força de corte e de tenrura sensorial avaliada pelo painel de provadores. As carnes nacionais apresentaram heterogeneidade da sua composição química, a Carnalentejana-DOP na tenrura e a nacional indiferenciada na cor, o que é indesejável e pode condicionar a sua comercialização, pelo que este aspecto deve ser melhorado.

Palavras-chave: Qualidade da carne; carne bovina; tenrura; cor; perfil lipídico; valor nutricional; análise de textura.

CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND SYMBOLS

Aº	angstrom
ADP	adenosine diphosphate
AOAC	Association of Official Analytical Chemists
ATP	adenosine triphosphate
ATPase	adenosine trifosfatase
Ag^{+}-HPLC	silver-ion high performance liquid chromatography
BD	bolsa de doutoramento
BHT	butylated hydroxytoluene
BSE	bovine spongiform encephalopathy
C	carbon
c	*cis*
ºC	Celsius degree
Ca^{2+}	ion calcium
CHD	cardiovascular heart disease
CIISA	centro de investigação interdisciplinar em sanidade animal
CL	cooking losses
CLA	conjugated linoleic acid
cm	centimetres
cm^2	square centimetres
cm^3	cubic centimetres
CoA	coenzyme A
COMA	Committee on Medical Aspects of Food Policy
CV	coefficient of variation
CW	carcass weight
D	dimension
D^2	squared Mahalanobis distance
Dad	diode array detector
DFD	dark firm and dry
DHA	docosahexaenoic acid
DHLNL	dihydroxylysinonorleucine
DM	dry matter
DPA	docosapentaenoic acid
E	energy value
EC	economic community
EDTA	ethylenediaminetetraacetic acid
EEC	European economic community
EFSA	European food safety authority
e.g.	*exempli gratia*
EPA	ecosapentaenoic acid
et al.	and others
F	Fisher
FA	fatty acid
FAO	food and agriculture organization
FAME	fatty acids methyl esters
Fe^{2+}	ferrous ion
Fe^{3+}	ferric ion
FCT	Fundação para a Ciência e a Tecnologia
FID	flame ionization detector
g	gram
GC	gas chromatography

Gly	glycine
GPa	gigaPascal
GPP	gabinete de planeamento e política
h	hour
HCl	hydrochloride acid
HDL	high density lipoprotein
HHMD	histidinohydroxymerodesmosine
HI	Hanna Instruments
HLNL	hydroxylysinonorleucine
H_2O_2	hydrogen peroxide
HP	hydroxylysylpyridinoline
hPa	hectoPascal
HPLC	high performance liquid chromatography
IDRHa	Instituto do desenvolvimento rural e hidráulica
i.e.	*id est*
IMF	intramuscular fat
kcal	kilo calories
kDa	kilodalton
kg	kilograma
KOH	potassium hydroxide
l	litre
LC	long chain
L-Ca	L-carnitine
LDL	low density lipoprotein
LP	Lysylpyridinoline
M	Molar
m	meter
Max	Maximum
MCP	multicatalytic proteinase complex
MDA	maloaldeyde
MFI	myofibrillar fragmentation index
MHC	myosin heavy chain
mg	milligram
min	minute
Min	minimum
ml	millilitre
MLC	myosin light chain
mm	millimetre
mM	millimolar
MPa	megapascal
mRNA	mensenger ribonucleic acid
MUFA	monounsaturated fatty acid
n	number
N	Nitrogen
NaOH	Sodium hydroxide
nm	nanometer
NP	Norma Portuguesa
ns	no significant
NU	national undifferentiated
OA	overall acceptability
P	probability
PDO	protected designation of origin

PF-IY	peak force yield
PGI	protected geographical indication
pH	hidrogenionic potential
pI	isoelectric point
Pi	phosphate inorganic
ppm	parts per million
PSE	pale, soft and exsudative
PUFA	polyunsaturated fatty acids
r	correlation coefficient
r^2	determination coefficient
RCF	relative centrifugal force
RDI	recommended daily intake
rpm	revolutions per miute
s	second
SAS	statistical analysis system
SD	standard deviation
SDH	succinate dehydrogenase
SEM	standard error of the mean
SFA	saturated fatty acids
T	temperature
t	*trans*
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TCA	trichloroacetic acid
TFA	trans fatty acids
TI	thrombogenic index
ton	Tons
TPA	texture profile analysis
TSG	traditional speciality guaranteed
U	unit
UK	United Kingdom
USA	United States of America
UV	ultraviolet
V	volume
WBSF	Warner-Bratzler shear force
WHO	world health organization
WSC	water soluble carbohydrates
α	alpha
β	beta
γ	gamma
Δ	delta
µ	micro
ω	omega
µg	microgram
µl	micro litre
µm	micro meter
µmol	micromole
µM	micro molar
=	equal
>	mayor
<	minor
%	percent

1 – Introduction

The changes in the world meat markets over the past decade and the improvement in the educational and economical conditions of most consumers have increased the demands for high quality in meat (Andersen *et al.*, 2005). The food scares that happened in the last 10 to 15 years warn consumers about the food hazards to which they may be subjected. Alerts like those that forced Coca-Cola to withdraw the stock produced in Belgium from the European market, or the crisis caused by the presence of melamine in milk powder for babies in China, are two major examples of world food scares. Nevertheless, much of the food scares that have shaken food markets have been related to meat. Bovine spongiform encephalopathy (BSE) crisis in beef, aflatoxins in avian and dioxins in avian and swine meat have increased animal welfare concerns, the need for traceability and environmental legislation, which in turns have increased pressure at various points across the food chain (Scollan *et al.*, 2006). All these food safety problems have resulted in a decline of meat consumption in European countries over the last two decades, more pronounced in the affected specie of each food scare. Beef consumption was the most affected and was often replaced by consumption of other meats (Eurostat, 2009). In addition, the higher beef price compared with other meats and human health concerns (fat intake, saturated fatty acids and related diseases) (Gatellier *et al.*, 2005), have also contributed for beef consumption decrease.

In 2008, there was a significant increase in beef production in Portugal that reached 109.000 tonnes (+18.3% than in the previous year). This increase was outstanding in veal, the production of which increased 61.8%, but also in beef that increased 11.1%. This reflects a recovery in relation to 2007 (92.000 tonnes), which presented frankly weak production results. Moreover, there was a reversal of the picture seen since the BSE crisis, which in Portugal only effected beef production from 1998 on. Despite slow, the autochthonous beef breeds production has increased in the last years. However it still occupies a market niche that does not even reached the 3% of total national beef production (1997-2007) (GPP, 2007a). The cattle population in Portugal is concentrated mainly in three regions: Alentejo (41% of total), North (23%) and Azores (17%) (GPP, 2007b). These three regions account for 81% of the total cattle population in Portugal. Beef cattle production has a major importance in the regions of Alentejo and in the north, since the number of beef cattle in these regions is higher than the number of dairy cattle (GPP, 2007b). Moreover, due to the slopes in the north and the poor and thin soils in Alentejo, the cattle production in semi-extensive system, is often the only solution for most families. Since these families cannot compete with intensive

production systems rearing autochthonous breeds gives them the opportunity to improve their income.

With the aim of improving the profitability of farms that rear local cattle breeds, the policy has moved to guarantee meat quality using the European Union Regulation 2081/92 to establish regional and quality assurance labels. Protected designation of origin (PDO), Protected geographic indication (PGI) and Traditional specialties guaranteed (TSG) are quality labels normally taken under geographical groupings and with specific genotypes and production systems. Biological and/or organic meats are also quality labels protected by special legislation, similar to those previously referred to. These quality labels are a guarantee of safety and quality to the consumer. Portugal have several veal and beef certified products, which deserves a more deeply study towards an assessment of all aspects of product quality and in order to verify whether these are in line with consumer expectations. Despite certified beef consumption has increased in the last years (IDRHa, 2006), this is still a market niche, and commercial crossbred bovines produced under intensive regimens provide the main supply of beef (GPP, 2007b).

In response to food scares, consumers became more self-conscientious and start searching for meat with characteristics that differ from the most consumed meat. The promotion of meat products having certain characteristics that could be of considerable benefit to the rural economy, in particular to less-favoured or remote areas, more animal and environment friendly, and with a more "green" production system, conforms the increasing demand by consumers for quality guarantees (Guerreiro, 2001).The health concerns related with the close association between diet and health, particularly by its involvement in the incidence of cancer, atherosclerosis, obesity and type 2-diabetes helped to change consumer's beef perception. Beef consumption have been overshadowed due to the highlight given to several negative traits, like cholesterol, saturated fatty acid and trans fatty acid content as well as total omega 6 content comparatively to omega 3 content. Health guidelines point to the decrease in consumption of saturated fats and omega 6 fatty acids and to increase of omega 3 polyunsaturated fatty acids consumption, especially of long chain polyunsaturated fatty acids (World Health Organization, 2003). Knowledge of these relationships has augmented consumer interest in the nutritional quality of food in a way that this is becoming one of the greatest dimensions of product quality. But also, drive researchers to investigate about bioactive compounds such as conjugated linoleic acid (CLA), L-carnitine, carnosine, taurine,creatine, amongst others (Purchas *et al.*, 2006), with the aim of meeting functional foods, even though much remains to be done. Studies in biological effects of CLA swarmed in the last decade in the perspective of finding health positive effects of this group of isomers.

Despite all the discussion around these issues, it is clear that lean red meat remains an important food source for people, and beef fat content is generally low. The composition of beef is closer to an ideal protein for human consumption, providing all the amino acids that are essential for the human adult (Oddy *et al.*, 2001). Moreover beef is also a good source of iron, zinc and phosphorus, and vitamins A, B6, B12, D and E (Scollan *et al.*, 2006).

Quality is a subjective term, the meaning of which varies depending on whom use it. Moreover, meat is a very complex matrix and beef quality is multifaceted comprising sensory, nutritive, hygienic or toxicological and technological factors (Hofmann, 1994). Thus, despite all health, animal and environmental concerns, it is true that organoleptic traits of meat still rules consumers' purchase. In general it is assumed that colour, price and freshness are the primary attributes that consumers search in meat before purchasing, whilst taste and tenderness are experienced attributes, because they are only known after consumption (Sepúlveda *et al.*, 2008). These experienced attributes are responsible for consumers repeated purchase. Moreover, without a minimum of tenderness being attended consumer does not repeat the purchase. When a minimum of tenderness is guaranteed, juiciness and flavour are the beef attributes that assume the greatest importance for consumers. Nevertheless, these attributes are difficult to measure and often require the use of taste panels to assess the complex parameters involved in the eating experience (Gill *et al.*, 2010). It is important for research and industry purposes that any assessment of tenderness made in a laboratory to be highly correlated with sensory assessment of these criteria (Perry *et al.*, 2001). Attempts to predict beef tenderness have been endeavour for a long time, but the results have not been brilliant. The efforts to correlate instrumental methods with sensory tenderness obtained by a sensory panel have not always been successful. The same is observed with the correlation between sensory tenderness and instrumental methods with consumer's preferences. Sensory assessment of tenderness or toughness is based on different elements that occur during eating. No laboratory analyses approximate to all the actions of biting and chewing and merge these into a single measure of tenderness. Rather, these actions are simplistically mimicked by a series of objective tests. Moreover, the explanation for tenderness differences based on physic and chemical approaches are needed to understand what causes those differences. In the scope of all the issues above referred to were designed the trials that led to this thesis.

To achieve the objectives several experiments were conducted. Subsequent to the practical development of the study, the bibliographic review was developed. Each bibliographic review sub-chapter, tried to answer a question. Chapter 2.1 - What is the composition of muscle?; Chapter 2.2 - How does muscle turns into meat?; Chapter 2.3 – What changes occur in meat

during cooking?; Chapter 2.4 – How Meat Quality is defined? (What defines meat quality?); Chapter 2.5 – What does consumers look for in beef?

1.2 – Objectives

Development of studies concerning the relationship between the palatability attributes, textural and chemical characteristics and overall consumer acceptance can be useful to the beef industry. Moreover, the actual motivation of consumers to purchase healthy foods is a new challenge to the market place. For food products to have an optimum consumer appeal, they must not only provide a high degree of eating satisfaction, but must also be consistent in their palatability attributes. Among other quality queues of beef, palatability remains the primary determinant of consumers' acceptance, and the main attribute influencing consumers' acceptance is tenderness. In Portugal, as far as know it cannot be find in the literature studies concerning the real knowledge of consumers` needs and expectations when purchasing beef nor considering the impact of relative differences in palatability attributes in overall acceptance of beef by those consumers.

In this context, the overall aim of the present study was to ascertain the sensory properties of beef through chemical, instrumental and sensory analysis and to relate them with the Portuguese consumer` preferences.

At the light of this main purpose, the specific objectives can be defined as questions summarised as follows:

1. How do textural properties and chemical components of beef correlate with the consumers' choice?
2. Does the consumer choose the healthiest beef?
3. Is it possible to predict early on the beef chain the sensory quality based on its textural and chemical properties?

The experimental work was divided in two trials. The aim of the first trial was to assess the meat lipids and the physical, chemical and sensory characteristics of Portuguese "Vitela Tradicional do Montado"-PGI veal, Mertolenga-PDO veal and Mertolenga-PDO beef, like consumer purchase it in the marketplace. These three certified meat types has in common the Mertolenga breed: Vitela Tradicional do Montado-PGI (calves from crosses of Mertolenga with exotic breeds), Mertolenga-PDO veal (calves of purebred Mertolenga) and Mertolenga-PDO beef. Trial was drive in strict collaboration with "Montado Alentejano" (Évora, Portugal) Association, who was the entity responsible for the commercialization of the meat.

Samples were collected in the slaughter house "Matadouro Regional do Alto Alentejo", in Sousel (Portugal). This trial originated two papers entitled: "Intramuscular lipids of Mertolenga-PDO beef, Mertolenga-PDO veal and "Vitela Tradicional do Montado"- PGI veal" and "Eating quality of Vitela Tradicional do Montado- PGI, Mertolenga-PDO veal and beef".

In the second trial there were used three beef segments commercialised in Portuguese market, which are quality branded (certified), imported and national undifferentiated beef. For this purpose we chose in the first group Carnalentejana-PDO, as it is commercially the most important from all certified beef. In the imported beef group we choose Brazilian beef seeing that these beef has been gaining market share. The beef samples from the three beef types were collected in a Portuguese hypermarket. We intended to elucidate the actual quality of beef marketed in Portugal. The aim of the second trial was to relate the composition of beef in cholesterol, lipid-soluble antioxidant vitamins, fatty acids with the greatest impact on health, and L-carnitine from the market place with the daily intake recommendations by FAO/WHO (2009). Moreover, we also intended to explore the relationship between WBSF, TPA and sensory analysis of the three beef types, in order to realize which technique (WBSF or TPA) is the most useful to predict sensory characteristics of beef. Finally we also aimed to determine the physical and chemical characteristics, as well as the sensory attributes, of the three types of beef from Portuguese marketplace plus the variability of the referred characteristics and attributes, and to relate them with the previous consumers' choices.

The results of the second trial have been reported in three manuscripts, entitled: "Nutritional value and variability of lipids from the three main beef types marketed in Portugal", "Relationship between texture profile analysis and sensory analysis of the three main beef types marketed in Portugal" and "Characterization of beef quality from the Portuguese market".

2 – BIBLIOGRAPHIC REVIEW

This review will try to elucidate some of the main concepts concerning muscle physiology, muscle contraction process, *post-mortem*, *rigor mortis* and conditioning, meat quality attributes, meat nutritional value and the interrelation between these issues. Meat is a very complex matrix and the research concerning all the aspects previously refereed to, is not new. Nevertheless, much remains to be done. Despite some important discoveries in this field have been achieved a long time ago, many aspects have not been clarified yet. Researchers often disagree on the theories and mechanisms underlying meat science. Pollack (2003) once said," Muscle is considered one of the most important and best achievements of the Mother Nature, and she seems to closely guard her secrets!" That seems to be very true!

2.1 – Structure and composition of muscle

Mammals have three types of muscle tissue: skeletal, cardiac and smooth. This review will focus only on skeletal muscle tissue, which is formed by bundles of very long, cylindrical, multinucleated cells with transverse striates, with quick and powerful contraction subjected to voluntary control (Junqueira & Carneiro, 2004). Their function is to produce force and to allow motion. Muscles can cause either locomotion of the organism itself or movement of internal organs.

Skeletal muscle is a very heterogeneous tissue, with a complex architecture. Moreover it is composed by several tissues and cell types including muscle fibres (75 to 90% of total volume), along with connective tissue, intramuscular adipocytes and vascular and nervous tissues (Lefaucheur, 2010). Both the lipid and protein fraction markedly affect the organoleptic characteristics of meat (Wood, 1990). Muscle tissue has a variable composition where even the compounds present in small amounts can have a great importance in the sensory characteristics of meat (Touraille, 1991). However, the underlying muscle composition is 75% water, 20% protein, 3% fat and 2% soluble non-protein substances. The latter 2% are 3% of minerals and vitamins, 45% of non-protein nitrogen-containing substances, 34% of carbohydrates and 18% of inorganic compounds (Tornberg, 2005).

The proteins that constitute the skeletal muscle tissue can be of three types (Tornberg, 2005):

- Myofibrillar proteins (about 50-55% of total protein content);
- Sarcoplasmic proteins (aproximately 30-34% of total protein content), and;
- Connective tissue proteins (around 10-15% total protein content).

Myofibrillar proteins are the main proteins of the skeletal muscle tissue, and will be further discussed in a row. Sarcoplasmic proteins will only shortly be discussed, since these proteins have only a minor role in meat quality. Connective tissue proteins will be discussed in the section dedicated to the connective tissue.

2.1.1- Skeletal muscle fibre

Skeletal muscle fibre, also called myofibre, is composed of cylindrical, extremely elongated contractile cells, bound together by collagenous supporting tissue. Muscle fibres have a variable length and 10 to 100 µm in diameter (depending on animal species). They are multinucleated due to their formation by fusion of myoblasts during foetal development, representing 75–90% of the muscle volume (Lefaucheur, 2010).

Skeletal muscle can be composed of a large variety of functionally diverse fibre types. Myofibre is bounded to the sarcolemma, which is the cellular membrane (Lee, Joo & Ryu, 2010). The skeletal muscle fibre consists of a complex network of protein filaments, in which coexist more than 65 different proteins (Fraterman *et al.*, 2007).

These cylindrical structures are formed by a succession of sarcomeres, with about 2.3 µm in length. The sarcomere is the structural unit of muscle fibre and is situated between two Z lines. Towards the centre of the sarcomere lies the dark A band (anisotropic in polarized light), which contains the bipolar thick filaments comprised of myosin and associated proteins (Clark *et al.*, 2002) and flanked by two light I half-bands (isotropic in polarized light) (Figure 1). The structures responsible for the contraction of skeletal muscle, the myofibrils, are cylindrical structures with 1-2 µm in diameter, arranged parallel to the axis of the skeletal muscle fibre. The myofibrils structure consists of four systems of filaments: the thick filaments of myosin; the thin filaments of actin, troponin and tropomyosin; the elastic filament of titin; and the inextensible filaments of nebulin. There are also two transversal structures, the M line, which crosses the A band and the Z line, which crosses the I band (see Figure 1). Beneath the A band there is a lighter area containing the myosin filaments, which is called H band. In turn, beneath the H band there is a darker band, the M line. The M line is composed by the esqueletin filament (Junqueira & Carneiro, 2004). The C-terminal portion of titin also spans the A band and M line (Clark *et al.*, 2002). Beneath the Z line there is the desmin filament that composes the intermediate filaments, linking the myofibrils to each other (Junqueira & Carneiro, 2004). In addition to myosin, other proteins have been identified as components of the thick filaments and M line of the sarcomere (Clark *et al.*, 2002). The I band contains actin filaments only, whereas the A band contains the miosin filaments and the end

of the actin filaments. The thin filaments pointed ends inter-digitated with the thick filaments, within the band A. The globular heads of the myosin molecules, also known as cross-bridges, extend from the core of the thick filaments and cyclically interact with the thin filaments (Bottinelli & Reggiani, 2000). As can be seen in Figure 1-I, each myosin filament is surrounded by six filaments of actin in a hexagonal arrangement.

2.1.1.1 – Myofibrillar proteins

The myofibrillar proteins can be divided in three classes: 1 - the myofilamentous fibrous proteins, as myosin and actin, which builds the myofibrillar structure up; 2 - the regulatory proteins, as the tropomyosin-actin complex, α- and β-actinin, M-protein, C-protein; and 3 - the scaffold proteins, such as titin, nebulin, desmin, vimentin and synemin, that supports the whole myofibrillar structure (Tornberg, 2005). Together the myosin and the actin comprise 55% of the total proteins in the skeletal muscle (Junqueira & Carneiro, 2004).

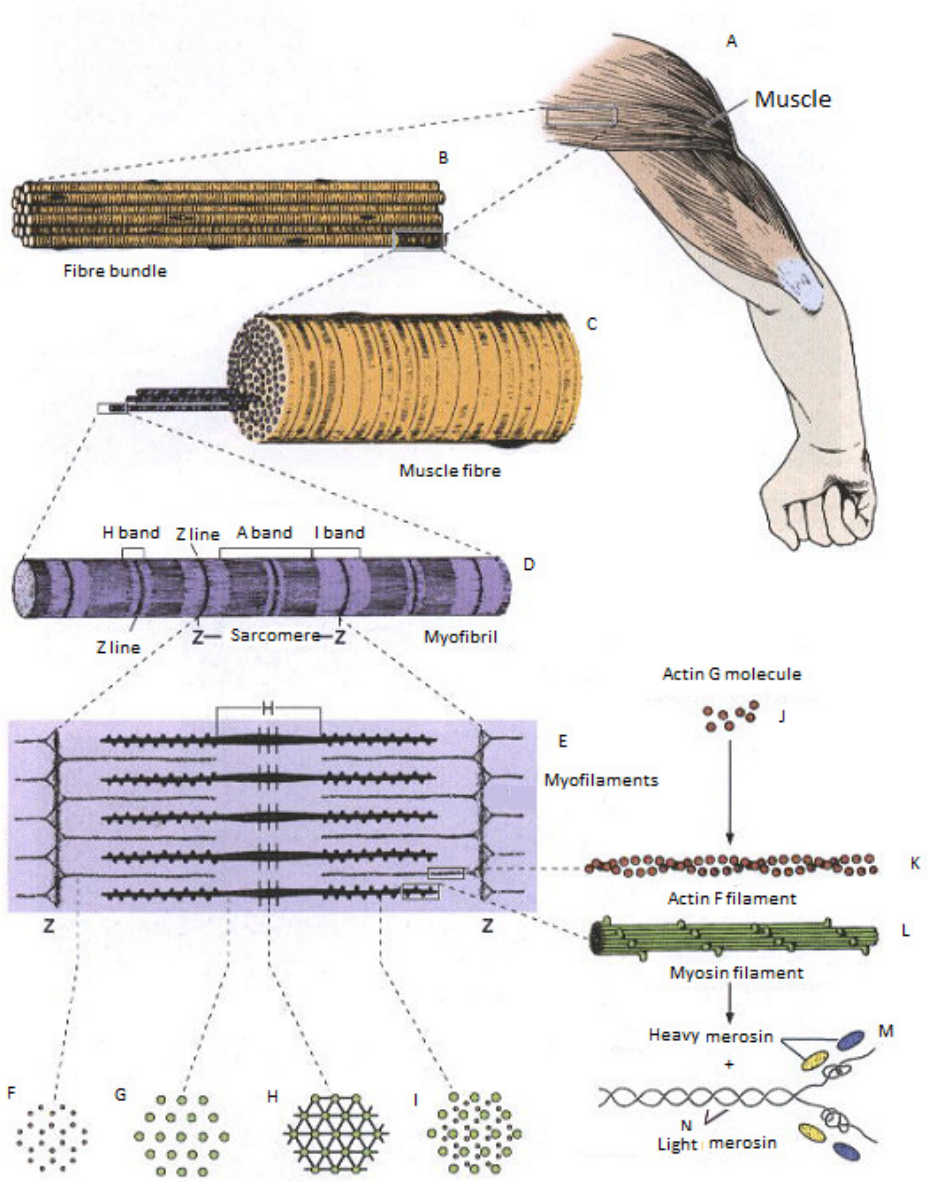
Myosin is the best known molecular motor protein, which is able to convert chemical energy into mechanical energy through structural change (Choi & Kim, 2009). There are several myosin classes (about 15), and striated muscle tissue myosin belongs to the class II (Clark *et al.*, 2002). The myosin molecule consists of a globular region with two heads attached to a very long stick, the tail. Each head is composed by a heavy chain (MHC) that contains the N-terminal region and two of the light chains (MLC) (Choi & Kim, 2009). The C-terminal tail have a coiled-coil morphology, holding the two heavy chains together (Clark *et al.*, 2002). The head are the ATPase activity sites and are responsible for binding the actin molecules (Junqueira & Carneiro, 2004).

The heavy merosin can be split in three fragments, the linear part of the tail adjoining the heads (merosin S2), and the two heads (merosin S1), which are the parts that hold all the functions attributed to the heavy merosin (Clark *et al.*, 2002).

The binding between myosin and actin molecules occurs through cross-links between the two proteins, during the process of muscle contraction, forming the actin-myosin complex, which will be discussed latter (Clark *et al.*, 2002).

The actin molecule is implicated in a series of functions in the striated muscle tissue, namely motility, cytokinesis and contraction. Actin is anchored in the Z-disc, span the I-band and extend towards the middle of the sarcomere (Clark *et al.*, 2002). The actin is a helical structure, called F actin, composed of two chains of globular G actin monomers, coiled in a double alpha helix. Each half-helical coil of the thin filament is comprised of 7 actin monomers. Single molecules of nebulin, polymers of tropomyosin and the troponin complex,

Figure 1. Organization of skeletal muscle.



Skeletal muscle (A) is mainly composed of bundles of muscle fibres (B), where each fibre (C) comprises numerous myofibrils (D). Each myofibril is composed of myofilaments (E) of actin (K) and myosin (L). The myosin molecule (M) is composed by two parts, the heavy merosin and the light merosin (N) (Adapted from Junqueira and Carneiro, 2004).

are present along the length of the actin filaments (Clark *et al.*, 2002). The tropomyosin and the troponin complex are also part of the actin filaments. Each actin G molecule displays regions for interaction with myosin, with a nucleotide (ATP or ADP) and a divalent cation (calcium or magnesium) (Bandman, 1994). Besides tropomyosin and troponins, tropomodulin and actinin γ are also associated with actin filaments.

Tropomyosin forms homodimers or heterodimers to compose two polypeptide chains arranged in coiled-coil morphology (Junqueira & Carneiro, 2004). The principal function of tropomyosin is to work together with the troponins in regulating the interaction between the thin and thick filaments. This complex regulatory mechanism involves a Ca^{2+} ion and troponin mediated conformational shift in the position of tropomyosin on the thin filaments (Clark *et al.*, 2002). Additionally to its critical role in the contraction mechanism, tropomyosin also has a function in stabilizing the thin filaments. Binding of tropomyosin to actin increases the stiffness of the thin filaments and inhibits their fragmentation. Tropomyosin also stabilizes thin filaments by slowing depolymerization and polymerization at their pointed ends (Broschat 1990).

The troponin complex is composed by three proteins, troponin C, I and T, which binds strongly to each tropomyosin molecule in the filament. The troponin C has high affinity to the calcium ions, being responsible to grant calcium ions sensibility to the actomyosin complex. The troponin I inhibits the interaction between myosin and actin in relaxed muscle through the inhibition of the ATPase activity of actomyosin. Finally, the troponin T is the linking molecule, joining tropomyosin and the others troponins (Junqueira & Carneiro, 2004). When the calcium concentration in the sarcolemma increases calcium ions binds to the N-terminal regulatory domain in troponin C. This alters the interaction with troponin I, which transmits the activation signal further along the thin regulatory complex, resulting in the alteration of the troponin-tropomiosin complex conformation, with the exposure of the myosin binding site in the actin molecule. Together tropomyosin and the troponins are the contraction regulating molecules of vertebrates' skeletal muscle (Clark *et al.*, 2002).

Titin filaments have been also called gap filaments or G filaments. After actin and myosin, titin is the most abundant muscle protein, representing about 9% of the total protein content of the sarcomere (Trinick, 1991). The titin filaments consists of a globular head and a very long thin tail that extends from the Z line to the M line of the sarcomere, alongside to the microfilaments of actin and myosin. The segment of titin molecule, that lays in band I, has elastic properties, contrary to what happens to the segment that is in band A, whose main characteristic is to be closely linked to the myosin filaments. This proximity of the two structures, resulting from the ability of the titin segment in band A to connect to several proteins that comprise the macrofilament of myosin (the myosin tail and protein C), suggests an important role of titin in the regulation of band A ultra-structure (Prates, 2000). Moreover, the elastic property of titin appears to be a molecular spring that governs some aspects of myofibrillar stiffness (Clark *et al.*, 2002).

Nebulin is the fourth filament system of the skeletal muscle tissue. The main characteristics of longitudinal nebulin filaments are that they are inextensible. The C-terminal end of nebulin is partially inserted into the Z-lines, whereas its N-terminal end extends to the pointed ends of the thin filaments (McElhinny *et al,* 2001). The structural proximity between nebulin and actin microfilaments, together with the capability to interact with F actin along its entire length, suggests a role of this giant protein on the regulation of the ultrastructure of band I (Prates, 2000).

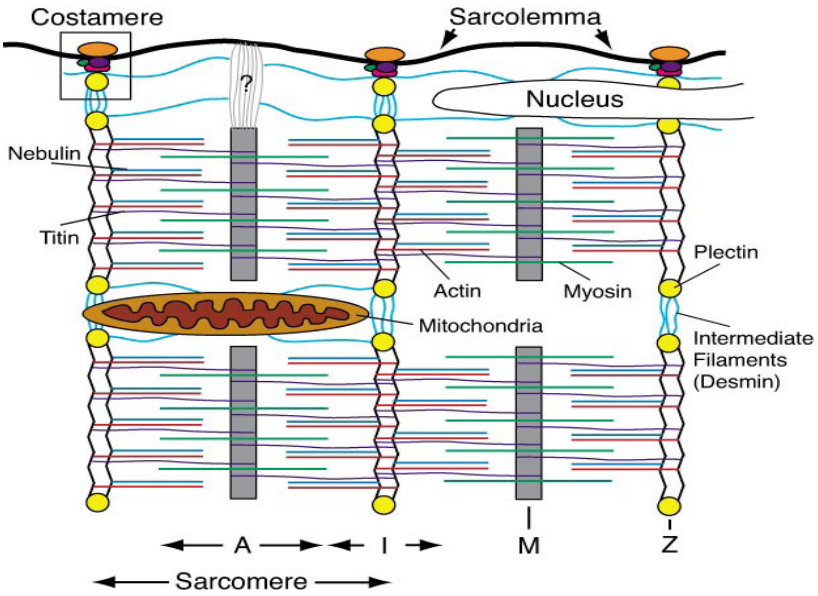
Some authors have found indications that this protein may have a direct role in contraction of skeletal muscle (Jin & Wang, 1991). Moreover, it appears that nebulin possess modules that inhibit actomyosin ATPase activity, as well as sliding velocities of actin filaments over myosin (Clark *et al.*, 2002). That way, nebulin may comprise another system that regulates the actin-myosin interaction, an idea supported by studies which demonstrated that nebulin may have multiple binding sites on the thin filament, analogous to tropomyosin (Lukoyanova *et al.* 2002).

The Z-discs represent the lateral boundaries of the sarcomere where the actin, titin, and nebulin filaments are anchored (Figure 2). Moreover, Z-discs are surrounded by transverse filaments of desmin, which are primarily responsible not only for the union of adjacent fibrils, but also by its binding to cellular organelles. Finally it is where the linking structures of the myofibrils, the costameres, bind to the sarcolemma (Taylor *et al.*, 1995). Z-discs also are implicated in mechanic sensation and signaling to the nucleus, which contribute to maintenance of muscle homeostasis (Clark *et al.*, 2002). Due to the referred anchoring property, Z-lines are the primary conduits of the force generated by contraction. The Z-lines of adjacent myofibrils are aligned, so that a way is provided to coordinate contractions between individual myofibrils, in the point where the Z-line is linked to the muscle membrane (Taylor *et al.*, 1995).

Striated muscle is surrounded by a basement membrane rich in extracellular matrix proteins (collagen, laminin). This matrix acts as an adherent substratum and provides structural stability to the cell (Clark *et al.*, 2002). The sarcolemma proteins form a complex network adjacent to the inner cytoplasmic membrane in close association with it (Taylor *et al.*, 1995).

The contractile apparatus attaches to the basement membrane at periodic membrane-associated plaques, the costameres. The costameres are linked to extracellular matrix proteins through integrin, which is an integral membrane protein. The cytoplasmic domain of integrin β unit is attached to tallinn, which in turn interacts with vinculin. The vinculin establishes connections with α-actinin and actin (Figure 2; Clark *et al.*, 2002).

Figure 2 - A schematic overview of cytoskeletal linkages in striated muscle (modified from Carlsson and Thornell, 2001).



Costameres co-ordinately transduce contractile force from the Z-line to the basement membrane, where the force is transmitted laterally to the muscle termini (Danowski *et al.* 1992). In addition, costameres are organizational points for the membrane cytoskeleton, which maintains the structural integrity of the membrane during contraction. These features are a result of at least three different cytoskeletal networks (integrins/focal adhesion complexes, the dystroglycan complex, and the spectrin-based cytoskeleton) acting in concert to promote the stable linkage between the myofibril and the muscle membrane (Clark *et al.*, 2002).

2.1.1.2 – Classification of myofibres

The diversity of skeletal muscle can be attributed to the heterogeneous characteristics of the individual muscle fibres and the mosaic composition of the numerous fibre types (Bottinelli & Reggiani, 2000). Fibre type composition can vary markedly in different species and muscle types, depending on function. There are several factors that contribute to the fibre type variation, such as gender, breed, hormones and physical activity (Choi & Kim, 2009). These fibre type variations differ according to their molecular, metabolic, structural and contractile properties. Myofibres can be characterized by their total number, cross-sectional area, length and contractile and metabolic properties. Skeletal muscle also exhibits remarkable adaptive

capabilities to a number of genetic, nutritional and environmental conditions (Lefaucheur, 2010), a good example is the adaptation to physical exercise.

Contractile characteristics of muscle are differentiated by muscle fibre types which have oxidative and /or glycolytic metabolism pattern in live skeletal muscle and carcass (Lee, Joo & Ryu, 2010). In general, muscle fibre is classified according to their contractile and metabolic properties (Lefaucheur, 2010).

2.1.2 - Sarcoplasmic proteins

The sarcoplasmic proteins are proteins of the sarcoplasma, soluble in water or at low ionic strength mediums, to which belong most of the enzymes of the glycolytic pathway, creatine kinase and myoglobin. These proteins constitute about 30 to 35% of the total muscle proteins. Circa 100 different proteins are known to be present in the sarcoplasmic fraction and they are globular proteins of relatively low molecular weight ranging from 17,000 (myoglobin) to 92,500 (Phosphorylase b) (Tornberg, 2005). These enzymes involved in the metabolism interact with sarcomeric components at the M-line region. For example, the enzyme creatine kinase is predominantly localized at cellular sites of energy production and consumption. The function of creatine kinase is to shift the N-phosphoryl group from phosphocreatine to ADP, and thus replenish ATP (Clark *et al.*, 2002). These proteins only have a secondary role in the mechanism of transformation of the muscle into meat and in meat quality. Their role will be discussed whenever the respective intervention in these mechanisms is relevant.

2.1.3 – Connective tissue

The connective tissue has an important role in tissue protection, since it involves the cells and tissues acting as an envelope (protection function); at gathering together several tissues and in the union of organelles with the tissues (mechanical function); and finally in cellular nutrition, since it is embedded in a viscous colloid solution consisting of polysaccharides (interstitial fluid) (nutritional function).

Connective tissue holds muscle fibres together, allowing the force of contraction generated by each fibre individually to act on the entire muscle, thus contributing to the contraction. The role of connective tissue has high functional importance because, in most cases, the fibres do not extend from one to the other end of the muscle (Junqueira & Carneiro, 2004). It is also through the connective tissue that the force of muscle contraction is transmitted to other structures like tendons, ligaments, fascia, periosteum, among others (Robelin, 1990).

Muscle connective tissues, endomysium, perimysium and epimysium, make up a network of collagen and elastin fibres embedded in a matrix of proteoglycans (Lepetit, 2008). The structure built up by the connective tissue starts with an external covering sheet of connective tissue, the epimysium, around the whole muscle. The perimysium is the connective tissue layer that separates each muscle into muscle fibre bundles or fascicles. There are large (primary) fascicles and smaller (secondary) fascicles, and therefore primary and secondary perimysial layers separating them. Collagen fibres in the perimysium are arranged in a crossed-ply arrangement of two sets of wavy collagen fibres. Finally, each muscle fibre is surrounded by another type of connective tissue, the endomysium, which is the thin connective tissue layer, over the plasmatic membrane. The vast majority of endomysium thickness is made up of a near-random feltwork of fine, wavy collagen fibres (Purslow, 2005). Epimysium fibres are usually of large diameter, but vary considerably with the type of muscle. The perimysium fibres are generally wider than those of the endomysium, with about 50-95 nm in diameter, whereas the endomysium fibres are around 40 nm being arranged in thin layers. The layers seem to be randomly oriented, even though there must be some degree of organization imposed by the stretching of the muscle when the muscle is under normal pressure (Bailey & Light, 1989). The endomysium is a non-fibrous layer surrounded by thin fibrils of reticulin.

The connective tissue is particularly abundant in cattle and has an important role in determining the organoleptic properties of meat, especially tenderness. The epimysium is easily separated from the flesh, not constituting a factor of texture variation, though. The perimysium and endomysium, do not separate easily from the flesh, and together form the intramuscular connective tissue. Seen at the microscopy the endomysium has a honeycomb structure, whilst the perimysium consists of thick sheets of collagen fibres (Nishimura *et al.*, 1998). Moreover, the perimysium exists in higher proportion in the muscle, representing circa 90% of the three layers of tissue, being a very important factor in meat quality (Bailey, 1985). Taken together, these morphological differences will influence meat quality, an issue that will be discussed later on.

There are three types of connective tissue fibres: collagen, elastin and reticulin. All of them are fibrous proteins. Intramuscular connective tissue is mainly comprised of collagen and elastin fibres, surrounded by a proteoglycan matrix. The total collagen content in beef muscles can vary from 1% to 15% of dry weight, whilst elastin, being a smaller component, varies from 0.6% to 3.7% of dry weight (Purslow, 2005). Collagen is a glycoprotein and it is the main structural component of the connective tissue (about 55-95% of the dry matter content). It is composed by tropocollagen monomers, which aggregate to form either extended fibres in

the epimysium and perimysium or mainly a structural matrix in the endomysium. The collagen content in the perimysium varies much more between muscles than in the endomysium (Purslow, 2005). Purslow (1999), in an extensive study of 14 beef muscles reported that perimysial collagen ranged from 0.45% to 4.76% (% of muscle dry weight), whereas endomysial collagen % varied from 0.47% to 1.20% (Purslow, 2005).

Elastin unlike collagen is highly elastic. The role of elastin in meat texture has been completely neglected over the years. It was assumed that this protein was present only in small quantities, less than 10% of the total collagen content, and was mainly associated with the blood vessels. However, in some muscles such as the semitendinosus, this protein represents around 40% of collagen. Elastin is not affected by heating, and if there is a significant amount present in muscle, it can increase the strength of shear and thus the toughness of the meat (Bailey, 1988).

Reticulin exists even in smaller quantities and is composed of tiny fibres that form a network around the cells. The reticulin fibres can be a precursor of collagen and elastin fibres.

2.1.3.1 – Collagen

Collagen exhibits a great diversity of structure and functions, which is manifested by: 1- the strong strings fibres present in tendons; 2 - the layers of intercrossed flexible fibres of the skin; 3 - the laminar layers of transparent thin fibres of the cornea; 4 - the amorphous membrane structure of capsules glomeruli; 5 - the lubricated cartilage of joints; 6 - the mineralized collagen of bones and teeth, and; 7 - the thin filaments that surround and support cells (Bailey, 1985).

Collagen molecule is approximately 300 nm long and 1.5 nm in diameter, made up of three polypeptide alpha chains, each possessing the conformation of a left-handed helix. These three left-handed helices are twisted together into a right-handed coiled coil, *i.e.* a triple helix, a cooperative quaternary structure stabilized by numerous hydrogen bonds.

Collagen is first synthesized and secreted as procollagen, a larger precursor protein (Koide & Nagata, 2005). Thus the structure of collagen results from the interaction of three tropocollagen chains, which have a very open and rigid helix, due to their high content of proline, an amino acid with a α-imino rigid structure without possibility of free rotation on the α-carbon atom (Bailey & Light, 1989). The three polypeptide chains are composed of long chains of a tripeptide whose general structure is Gly-XY, where Gly is glycine and X and Y are often proline and hydroxyproline, respectively, but can be any amino acid (with the exception of tryptophan, which has never been identified in collagen). Hydroxyproline is a

modified amino acid derived from proline by enzymatic post-translational hydroxylation (Koide & Nagata, 2005).

About 33% of the amino acids existing in tropocollagen are glycine, while proline and hydroxyproline together represent about 25%. The hydroxyproline exists in all types of collagen in a constant proportion of around 14%, this is the main reason why the determination of this amino acid is generally used to determine the content of collagen in meat and meat products. Other reason is the fact that this amino acid is only found in muscle tissue. For Bailey and Light (1989), this amino acid is the unique identifier of collagen.

Collagen exists in several different genetic forms, however only a few exist in the muscle. In muscle collagen types I and III are the far more abundant, but collagens type IV, V, VI, XII, XIV, XV and XIX are also present in minor quantities. Type I is present in the epimysium, types I and III are present in the perimysium, and types III, IV and V in the endomysium (Koide & Nagata, 2005; Tornberg, 2005).

Types IV collagen is the principal non-fibre-forming component of the basement membrane linking the fibrous (reticular) layer of the endomysium to the muscle cell membrane (sarcolemma). In mammalian species, type V is a minor component of intramuscular connective tissue (Purslow, 2005).

The different types of collagen can be grouped into three classes: fibrous collagen, which is the most common, non-fibrous collagen and collagen filamentous, which is present in much smaller amounts, even though it plays an extremely important role in some specific tissues (Junqueira & Carneiro, 2004).

The collagen molecule, despite having a simple structure, exhibits considerable biological diversity. This diversity is achieved by a series of translational modifications (Bailey & Light, 1989).

2.1.3.1.1 – Collagen chemical bonds

Collagen molecules initially associate via non-covalent interactions in the immature fibril, which is the reason why the initial fibril orientation is unstable. Collagen molecules can slide past one another and the immature fibre is therefore more subject to disruption by collagenolysis, variations in ionic strength and temperature. Tensile strength and functionality of the collagen fibril are due primarily to the formation of intermolecular crosslinks (McCormick, 1999). All the fibril-forming collagen types in higher vertebrates (types I, II, III, V and XI) are cross-linked through a mechanism based on the reactions of aldehydes, which are generated by lysyl oxidase from lysine (or hydroxylysine) side-chains (Eyre & Wu, 2005).

Crosslinking is initiated immediately upon fibril aggregation by the oxidative deamination of specific lysine or hydroxylysine residues by the enzyme lysyl oxidase, resulting in lysine- or hydroxylysine-derived aldehydes (allysine and hydroxyallysine, respectively). The head-to-tail lateral alignment of the collage molecules in quarter-stagger array allows allysine and hydroxyallysine to be enable to interact with other peptidyl aldehydes or unmodified lysine or hydroxylysine residues on adjacent alpha-chains (McCormick, 1999).

There are two kinds of bond in the collagen fibres, intramolecular bonds that exist within the collagen molecule, *i.e.* between procollagen chains, and intermolecular bonds that exist between different collagen molecules, preventing the latter slippage of adjacent molecules. The nature and quality of crosslinking determines the solubility of collagen, aspects that have an important influence on meat tenderness. Intramolecular bonds in collagen molecule can be of three distinct types:

1 - Disulphide bridges, which are confined to certain types of collagens, *e.g.* those with amino acids with sulfur atoms (cysteine and methionine) like type III and IV collagens, linking the alpha-chains of the procollagen molecules;

2 – Divalent cross-links or intermediate cross-links, which link two molecules of collagen fibrils in the same or different fibrils, and are formed from lysine and hydroxylysine aldehydes;

3 – Trivalent or mature cross-links, which are bonds formed from simple divalent reducible cross-links that join more than two collagen fibrils, being the most complex cross-links.

There are two types of reducible cross-links derived from lysine, intramolecular (within collagen molecule) and intermolecular (between collagen molecules). There is only one kind of intramolecular cross-link, the aldol, and two types of intermolecular cross-links, the aldimine and keto-imine, both Schiff bases (Bailey & Light, 1989).

It should be noted that the aldol links alone do not increase the stability and insolubility of collagen fibrils, since they are located within the molecule. It requires the presence of an intermolecular cross-linking to impart strength and stability to the collagen fibres and to the matrix (Bailey & Light, 1989).

In summary, the two major pathways that form crosslinks are the allysine pathway, based in the hydroxylation of the lysine aldehydes that produces aldimine reducible crosslinks, and the hydroxyallysine pathway that produces crosslinks arising from hydroxylysine aldehydes (McCormick, 1994; Eyre & Wu, 2005).

Amadori rearrangement of the initial aldimine crosslinks formed between lysine and hydroxylysine aldehydes can produce ketoamine derivatives (Eyre *et al.*, 1984). The reducible crosslinks vary in their stability, with ketoamine crosslinks being heat stable and aldimine

crosslinks heat labile (Allain *et al.*, 1978). The initial condensation products form reducible crosslinks because they contain Schiff base double bonds, which can be reduced. Hydroxylysinonorleucine (HLNL) is a reducible cross-link, whilst dihydroxylysinonorleucine (DHLNL) is the only stable divalent cross-link (Lepetit, 2007). DHLNL is the ketoamine crosslink precursor of the non-reducible hydroxylysylpyridinoline (HP) crosslink. In meat, divalent cross-links such as the HLNL, DHLNL and histidinohydroxymerodesmosine (HHMD) link two collagen molecules, whereas others, such as the pyridinoline crosslinks hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP), link three collagen molecules, being for that reason called trivalent cross-links (Eyre & Wu, 2005). Ehrlich Chromogen is also a trivalent crosslink (Kuypers *et al.*, 1994). The Ehrlich Chromogen is also a maturation product of the hydroxyallysine pathway, with the same ketoamine cross-link precursors as pyridinoline (Lepetit, 2007). LP residues are only present in negligible amounts in most tissues except bone (McCormick, 1999).

The proportion of both reducible intermolecular bonds is directly related to the extent of hydroxylation of the tissue or to the type of collagen. A given tissue may have different levels of hydroxylation, and as such, a different proportion of aldimine and keto-imine cross-links (Bailey & Light, 1989).

2.1.4 – Adipose tissue

Food fat plays an important role in the human diet. Fats are vital for a healthy body, provide it with energy and contribute to the absorption of fat-soluble vitamins. As a carrier of flavours and aromatic substances, fat also plays an important part in cooking. On the other hand, no other nutrient has to combat as many prejudices as fat. It is linked to obesity, type 2 diabetes, cancer, and coronary heart disease (Lichtenstein *et al.*, 1998). Adipose tissue has been considered to have low blood supply but in fact it is a highly vascular organ with a capillary bed equivalent to or exceeding that of muscle tissue. In the cells of white adipose tissue, fat appears as small droplets, which coalesce to form a large droplet. Those droplets fills and distend the cell, displacing the cytoplasm and the nucleus to one side and giving rise to the typical signet ring appearance of the adipose tissue cell (Leat, 1982). Depot of fat is derived mainly from dietary fat which is absorbed and transported to the fat depots as plasma chylomicrons and very low density lipoproteins, and by *de novo* synthesis mainly from carbohydrate and volatile fatty acids, which occurs almost exclusively in adipose tissue (Leat, 1982). The fatty acids formed by *de novo* synthesis are not stored as free fatty acids but as glycerol esters (Leat, 1982).

Adipose tissue can be localized under skin (subcutaneous fat), between the muscles (intermuscular fat) and within the muscle (intramuscular fat) (Schmid, 2011). Besides these three types of fat, that are related to muscle, other fat depots exist, like offal fat. Intramuscular fat and marbling are not synonymous. Marbling is the industry term used to describe the visual appearance of 'flecks' or spots of fat deposited within muscle (Oddy *et al.*, 2001; Warner *et al.*, 2010). Marbling is a visual score given to a piece of meat whereas intramuscular fat is the chemically measured fat content (includes membrane lipids) although the terms are often used interchangeably (Warner *et al.*, 2010). Intramuscular fat is the last adipose tissue to be deposited in finishing animals, although adipose tissue starts to accumulate in the early weaning periods (Harper & Pethick, 2004).

The accretion rate of intramuscular fat content depends not only on the variation during the development of number and intrinsic metabolic activity of adipocytes inside the muscle tissue, but also on the muscle growth rate and metabolic activity of other organs. For instance, animals having a high muscularity (*e.g.* double muscled cattle) or muscular tissue with a high glycolytic activity generally displays a reduced development of intramuscular fat (Hocquette *et al.*, 2010). This suggests that muscle cells and adipocytes interplay during growth. In addition, early events that influence adipogenesis inside the muscle (*i.e.* proliferation and differentiation of adipose cells; type of the connective structure embedding adipocytes) might be involved in inter individual differences in intramuscular fat content. Increasing muscularity will also dilute the final fat content of muscle (Hocquette *et al.*, 2010). Rate of fat deposition (including the intramuscular depot) is dependent also on nutrient intake. High intake relative to maintenance and to the capacity to deposit protein results in higher rates of fat deposition (Zembayashi 1994; Owens *et al.* 1995). Intramuscular fat in beef cattle predominantly consists of adipocytes (fat cells) in the connective tissue seams surrounding the muscle fibre bundles, with a small contribution as intracellular lipid droplets. Adipocytes arise from their precursors, pre-adipocytes, derived from stromovascular cells (Oddy *et al.*, 2001). Adipocytes are capable of synthesize and esterify fatty acids into triglycerides. Later they can hydrolyse triglycerides to provide fatty acids to other tissues (Robelin & Casteilla, 1990).

Adipose tissue is mainly constituted by lipids, but also by water and connective tissue. During animal growth the proportion of these three constituents changes, which has an important effect on meat quality. The proportion of lipids increases with age, as well as the adipocites size (Hocquette *et al.*, 2010).

Lipids can be subdivided into phospholipids, triacylglycerols or triglycerides, mono- and diacylglycerols, cholesterol, cholesterol esters and free fatty acids (Lefaucheur, 2010). The main constituents of muscle fat are phospholipids, triglycerides and cholesterol, but only the

two formers will be focused here. Cholesterol will be focused latter on nutritional value section.

The major lipid class in adipose tissue (> 90%) is triglycerides. Triglycerides are neutral lipids composed by fatty acids esters and glycerol, which are the main constituents of adipocytes (about 95% of tissue weight are triglycerides). For the synthesis of the glycerol radical it is necessary to have a glucose supply (Robelin & Casteilla, 1990).

Phospholipids are structural lipids, having a higher PUFA content than triglycerides, in order to perform its function as a constituent of cellular membranes (Wood *et al.*, 2008). Phospholipid amount is fairly constant or increases little, as the animal increases in fatness, and neutral lipid predominates in overall fatty acid composition. The contribution of phospholipids to total intramuscular fat varies between 0.7% and 0.9%, and is about 30% higher in oxidative than glycolytic muscles, likely in relation to the higher mitochondrial content of oxidative muscles (Alasnier, Rémignon, & Gandemer, 1996).

The level of triglycerides is high in slow-twitch type I fibres, moderate in oxido-glycolytic IIA fibres, and very low in glycolytic IIB fibres (Malenfant *et al.*, 2001). A small amount of triglycerides is localized as droplets mostly within type I myofibres, whereas an overwhelming amount of triglycerides is located in intramuscular adipocytes mostly grouped along the perimysium, or interspersed between myofibres (Essén-Gustavsson *et al.*, 1994; Gondret *et al.*, 1998). It is often stated that red oxidative muscles contain more total IMF than white glycolytic muscles. However, a careful analysis of available data does not confirm this statement, and shows that the amount and size of intramuscular adipocytes within a muscle would not be related to its fibre type composition (Lefaucheur, 2010).

Lipids are made of aliphatic chains, generally with more than eight carbon atoms. They are formed by structural units with a pronounced hydrophobicity, but are soluble in organic solvents, like ether and chloroform (Ratnayake & Galli, 2009). Nevertheless, some lipids are amphipathic molecules, since they contain both hydrophobic and hydrophilic moieties (glycerophospholipids, glyceroglycolipids, sphingophospholipids and sphingoglycolipids). As such, they are polar and different from neutral lipids (Belitz *et al.*, 2009). Lipids can therefore be classified according to their polarity. Fatty acids, mono, di and triacylglycerols, sterols, sterols esters, carotenoids, waxes and tocopherols are classified as neutral lipids. Lipids can also be classified according to the acyl residue characteristics in simple lipids (non saponifiable), like free fatty acids, isoprenoids lipids (sterols, carotenoids and monoterpenes) and tocopherols, and in acyl lipids (saponifiable), like mono, di and triacylglycerols, phospholipids, glycolipids, diol lipids, waxes and sterol esters (Belitz *et al.*, 2009).

2.1.4.1 – Fatty Acids

Fatty acids are mainly linked by ester or amide bonds and may be released by chemical or enzymatic hydrolysis. Fatty acids are organic compounds having one carboxyl group of hydrophilic nature (head), and a paraffin chain (tail), composed of carbon atoms linked by covalent hydrophobic bonds (Ratnayake & Galli, 2009). In animal tissues the fatty acids most common are those that contain 12 to 24 carbon atoms. However, shorter fatty acids appear in some animal products such as milk, which has fatty acids with 4 to 10 carbon atoms (Belitz *et al.*, 2009).

Acyl lipid hydrolysis releases aliphatic carboxylic acids which differ in chemical structure. They can be divided into groups by chain length, number, position and configuration of their double bonds, as well as by the occurrence of additional functional groups along the chains Belitz *et al.*, 2009).

In meat, saturated fatty acids encompass about 30-60% of total fatty acids (Schmid, 2011). Unbranched, straight-chain molecules with an even number of carbon atoms are dominant among the saturated fatty acids. The short-chain, low molecular weight fatty acids (<14:0) are only present in triglyceride of milk fat, coconuts oil and palmseed oil (Belitz *et al.*, 2009).

The saturated fatty acids in meat fat are myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0). Palmitic acid is generally the more abundant, comprising approximately one-fourth to one-third of all fatty acids. Stearic acid (18:0) comes next with a fatty acid share of approximately 10–20%, while the share of myristic acid is 3–6% (Diaz *et al.*, 2005; Valsta *et al.*, 2005).

Consumption of saturated fatty acids has been associated with increased plasma cholesterol and plasma low density lipoprotein (LDL), linked to a major risk of coronary heart disease (Grundy, 1987). High levels of palmitic acid are responsible for increasing total plasma and LDL cholesterol even though stearic acid is not hypercholesterolaemic (Williams, 2000).

In meat fat the monounsaturated fatty acids generally account for around 40–50% of the fat. Oleic acid (18:1 *c*9) not only comes top of the monounsaturated fatty acids, as in general is also the fatty acid most frequently found in meat fat. It generally accounts for 30–40% of fatty acids (Diaz *et al.*, 2005; Scheeder *et al.*, 2001), being generally more abundant in neutral lipids than in phospholipids. Oleic acid is formed from stearic acid by the enzyme stearoyl Co-A desaturase (Δ9 desaturase), a major lipogenic enzyme (Wood *et al.*, 2008). Δ9 desaturase is capable of desaturating a wide range of fatty acids, but has maximum activity against stearic acid (Leat, 1982).

As far as the effect on health is concerned, oleic fatty acid is considered to decrease LDL cholesterol levels (Diaz *et al.*, 2005), and therefore as beneficial effects on health.

The level of polyunsaturated fatty acids in beef is around 10–20% of total fat. Unsaturation decreases the melting point and the thermal stability of lipids. That is why fat with a high PUFA level is softer and more difficult to process, which can be a problem mainly in processed meat products. Moreover, the more polyunsaturated fatty acids present, the lower the oxidative stability of the fat, which can represent a nutritional value problem, and the lower the oxidative stability of the colour, which can decrease consumers' acceptability. The effect of fatty acids on shelf life is explained by the propensity of unsaturated fatty acids to oxidise, leading to the development of rancidity as display time increases (Wood *et al.*, 2003). The colour change is due to the oxidation of red oxymyoglobin into brown metmyoglobin, this reaction generally proceeding in parallel to that of rancidity. Several studies have shown that lipid oxidation products can promote pigment oxidation and vice versa, although the strength of the relationship between these two aspects of shelf life is sometimes low (Renerre, 2000). Moreover, rancidity also promotes the appearance of off-flavours, which is detrimental from consumer point of view.

The polyunsaturated fatty acids, which predominate in meat lipids, contain two or three allyl groups in their acyl residues. Fatty acids isolated double bonds (a methylene group inserted between the two cis-double bonds) are usually denoted as isolenic or non-conjugated fatty acids. The structural relationship that exists among the unsaturated, non-conjugated fatty acids, derived from a common biosynthetic pathway is distinctly revealed when the double bond position is determined by counting from the methyl end of the chain (the position designation using this method of counting requires the suffix "ω"). Acids with the same methyl ends are then combined into groups. Thus, three family groups exist in meat: ω3 (linolenic type), ω6 (linoleic type) and ω9 (Belitz *et al.*, 2009). In this section only polyunsaturated fatty acids will be considered, since monounsaturated fatty acid (containing only one double bond), which include ω9 family, has been already reviewed in the previous section.

Linoleic acid is derived entirely from the diet, since it cannot be synthesized by the mammals' body. This fatty acid and other members of the ω6 family are considered as essential fatty acids, and are required as building blocks for biologically-active membranes (Belitz *et al.*, 2009). In ruminants, this fatty acid is degraded into monounsaturated and saturated fatty acids in the rumen by microbial biohydrogenation and only a small proportion, around 10% of dietary 18:2 *n*-6, is available for incorporation into tissue lipids. In both sheep and cattle, this fatty acid is at higher levels in muscle than adipose tissue (Wood *et al.*, 2008).

Other important PUFA is α-linolenic acid (18:3 *n*-3), which is present in many concentrate feed ingredients but at lower levels than 18:2 *n*-6. This is a major dietary fatty acid for ruminants since it constitutes over 50% of total fatty acids in grass and grass products tissue (Wood *et al.*, 2008). Again, a high proportion is biohydrogenated to saturated fatty acids in the rumen. Doreau and Ferlay (1994) found that a variable proportion of dietary 18:3 *n*-3 (85–100%) and 18:2 *n*-6 (70–95%) is biohydrogenated, so less is available for incorporation into tissues. Hence a higher level of 18:2 *n*-6 than 18:3 *n*-3 in tissues is not only due to a higher affinity for incorporation into phospholipid molecules but also due to reduced biohydrogenation in the rumen (Wood *et al.*, 2008) of 18:2 *n*-6 fatty acid.

The proportion of 18:3 *n*-3 in ruminants is also higher in muscle than in adipose tissue. Muscle contains significant proportions of long chain (C20-22) PUFAs which are formed from 18:2 *n*-6 and 18:3 *n*-3 by the action of Δ5 and Δ6 desaturase and elongase enzymes (Wood *et al.*, 2008). The resulting products are arachidonic acid (20:4 *n*-6) and eicosapentaenoic acid (EPA, 20:5 *n*-3), respectively, which have various metabolic roles including eicosanoid production (Wood *et al.*, 2008).

In beef the concentration of *trans* fatty acids is generally between 2.8 and 9.5% total fatty acids. Meat and meat products therefore contain moderate amounts of *trans* fatty acids (TFA) (Schmid, 2011). The intramuscular lipids of bulls contain approximately 2.8–3.2% of total *trans* 18:1 isomers (Nuernberg *et al.*, 2005a), and the recommendations of TFA consumption are 1-2% of total energy consumption (Scollan *et al.*, 2006). *Trans* fatty acids are the result of the biohydrogenation of other fatty acids in the rumen. The double bonds in unsaturated fatty acids are usually of the *cis* type, *i.e.* the hydrogen atoms attached to the carbon atoms in the fatty acid chain, point in the same direction. In ruminants, as a result of biohydrogenation in the rumen, a significant proportion of double bonds are altered to the *trans* type, *i.e.* the hydrogen atoms point in different directions. These fatty acids have particularly low melting points as a result of this structure. Vaccenic acid (18:1 *t*11; TVA) is the most abundant *trans* 18:1 isomer in beef (Dannenberger *et al.*, 2004; Nuernberg *et al.*, 2005b), being a biohydrogenation product of 18:2 *n*-6 (Wood *et al.*, 2008). TVA is also a precursor for tissue synthesis of the conjugated linoleic acid isomer *c*9,*t*11. Despite the generality of *trans* fatty acids have a detrimental effect on health, there is evidence of a beneficial of TVA (Scollan *et al.*, 2006).

Conjugated linoleic acid (CLA) is a collective term of class of conjugated isomers of linoleic acid. All known isomers have double bonds with a single carbon bond in between (=conjugated double bonds) instead of the usual methylene-separation. These double bonds can either be *trans* (*t*) or *cis* (*c*) configured. CLA are defined as a combination of several

positional and geometric isomers with double bonds predominantly at 9 and 11, 10 and 12, or 11 and 13 carbon atoms with various combinations of *cis* and *trans* configuration at each double bond (Bessa *et al.*, 2000). The double bonds of CLA may be in the positions of 7,9; 8,10; 9,11; 10,12; or 11,13 and the 3-dimentional geometric combinations for cis and/or trans configurations (Belury, 2002).

CLA can be produced in the rumen but synthesis from TVA in tissues is quantitatively the most important contributor to tissue levels (Scollan *et al.*, 2006). TVA is converted into CLA (*c*9,*t*11 CLA isomer) in adipose tissue by the action of stearoyl Co-A desaturase, the same enzyme responsible for the production of 18:1 *c*9 from 18:0. Like 18:1 *c*9, both TVA and CLA are at higher proportions in neutral lipid than phospholipid and higher in adipose tissue than muscle. Dannenberger *et al.* (2004) reported 10 isomers of CLA in beef with *c*9,*t*11 CLA isomer representing approximately 70% of total CLA isomers. The major isomers in food are in the following rank order: *c*9*t*11 CLA (rumenic acid) > *t*7*c*9 CLA > *c/t* 11,13 CLA > *t*10*c*12 CLA isomer > other isomers (Belury, 2002). It is possible that a number of these CLA isomers have biological activity.

2.2 - Mechanisms underlying the transformation of muscle into meat

Adenosine triphosphate (ATP) is generated primarily through glycolytic pathway from glucose stored as glycogen during the *post-mortem* conversion of muscle to meat. By a natural process lactate, that is the final product of glycolysis in anaerobiosis, is accumulated in muscle due to stopped blood circulation. Lactate or acid lactic will decrease ultimate meat pH. If distribution of glycolytic fibres is predominant in individual muscles, rapid *post-mortem* glycolysis is induced, and accumulation of lactate results in a rapid muscle pH decline. Muscle with different fibre types composition have different patterns of *post-mortem* change during the conversion of muscle to meat, and may have an influence on ultimate meat quality (Ryu & Kim, 2005).

Low muscle pH, whilst temperature is still high will result in higher protein denaturation, paler meat colour, and poorer water holding capacity, *i.e.*, pale, soft and exudative (PSE) meat, which is more common in pork. Ryu *et al.* (2006) reported that the percentage of fibre type IIB was correlated with ultimate muscle pH. Likewise, muscles with higher percentage of type IIB fibres and lower percentage of type I fibres showed significantly higher glycogen and lactate contents at 45 min *post-mortem*, and also showed paler colour and higher drip loss in the early *post-mortem* period (Choi *et al.*, 2007). By the other hand a very slow pH decline, resulting in high ultimate pH, with high temperature will result in dark, firm and dry meat

(DFD), which by the way is frequent in beef. In both cases the meat quality is compromised, and should be avoided.

2.2.1 – Onset of *rigor mortis*

In resting muscle the ATP bounds to the ATPase in the myosin heads, which hydrolysis the ATP molecule in ADP and Pi (inorganic phosphate) releasing energy. Usually actin and myosin cannot link since troponin-tropomyosin complex blocks myosin head-binding sites on the outer domain of the thin filaments, the actin F (Choi & Kim, 2009).

Ion calcium binding to troponin C, pulls the tropomyosin molecule further inside the actin helix, tropomyosin shifts its position (likely more than once) to expose the weak myosin-binding sites on actin. Myosin binding activates the myosin ATPase and causes further translocation of tropomyosin on actin filaments, allowing strong, force generating interactions between myosin and actin (Clark *et al.*, 2002). The myosin and actin can disassociate when a new molecule of ATP is bound to the myosin head. Upon hydrolysis of each ATP molecule, the head domain that interacts with actin undergoes a large angular rotation, resulting in a displacement of 100 A°. After completion of this power stroke, ADP is dissociated and the actomyosin complex returns to the relaxed state. Each myosin head likely repeats this cycle several times in a single twitch (Clark *et al.*, 2002).

The energy required by contractile activity is provided by ATP hydrolysis to ADP and Pi. ADP is then re-synthesized to ATP from phosphocreatine through the creatine kinase reaction, from glycolytic processes in the sarcoplasma and from oxidative phosphorylation in the mitochondria. Glycolytic processes represent the initial stages of glycogen and glucose metabolism and lead to pyruvate or lactate production. Pyruvate, fatty acid, ketone bodies provide supply of acetyl-CoA which is the substrate for the mitochondrial oxidative processes (Bottinelli & Reggiani, 2000).

The early *post-mortem* development of rigor proceeds through gradual phases, first there is a delay period (pre-rigor phase) and after a rapid phase period. Immediately after exsanguination, during the delay period, very few rigor bonds are formed and the muscle is still very extensible. The pre-rigor phase starts few minutes *post-mortem* and finishes about 30 minutes *post-mortem* (Sentandreu *et al.*, 2002). During this period, the stop of the blood circulation interrupt the oxygen supply to the muscle, the level of ATP remaining constant, first because glycogen are still available to regenerate ATP from ADP. After glycogen exhaustion ATP is regenerated from ADP through creatine phosphate. However the creatine phosphate falls rapidly while there is a slow production of lactate (Tornberg, 1996; Huff-

Lonergan *et al.*, 2010; Lefaucheur, 2010). The rigor phase can be highly variable in length depending on muscle types, animal species and chilling conditions (Sentandreu *et al.*, 2002), which influences glycogen stores. Once the available reserves of creatine phosphate are depleted, the level of ATP begins to decline rapidly leading to the loss of the ability of myosin to disassociate from actin (Huff-Lonergan *et al.*, 2010), accompanied by a shortening of the muscle and the development of a force under the isometric conditions (Tornberg, 1996). Thus the development of rigor bonds proceeds at an even higher rate, leading to the potential shortening of the myofibril. Shortening is explained by the release of calcium ions into the myofibrillar space at ATP concentrations sufficiently high for contraction (Tornberg, 1996).

While there is ATP the concentration of calcium ions continues low, since it remains possible to pump them into the sarcoplasmic reticulum. Once glycogen stores are depleted also depletes ATP, which prevents the pump of calcium ions into the sarcoplasmic reticulum and hence ions diffuse into the sarcoplasm. The accumulation of these ions is also the result of the decreased ability of mitochondria to retain them (Taylor & Etherington, 1991), when temperature decreases below 20 ° C (Pearson & Young, 1989).

During the development of rigor is also observed the accumulation of lactate and thus the associated drop in pH (Lefaucheur, 2010). This occurs because during early *post-mortem* metabolism the muscle quickly becomes anaerobic forcing the anaerobic glycolytic pathway to be the primary generator of ATP (Ryu & Kim, 2005).

As muscle is converted to meat, many changes occur (Huff-Lonergan *et al.*, 2010), including:

1) a gradual depletion of available energy;

2) a shift from aerobic to anaerobic metabolism favouring the production of lactic acid resulting in the pH decrease to 5.4–5.8;

3) a rise in ionic strength, in part, because of the inability of the ATP dependent calcium, sodium, and potassium pumps to function;

4) an increasing inability of the cell to maintain reducing conditions.

All of these changes can have a profound effect on numerous proteins in the muscle cell, especially on one of the proteolytic enzyme systems that is thought to play a significant role in the tenderization that occurs during *post-mortem* ageing (Huff-Lonergan *et al.*, 2010). This issue will be discussed in a while.

During rigor development, not only longitudinal but also lateral contraction occurs (Tornberg, 1996). Offer *et al.* (1989a) have shown that a decrease of about 9% in the cross-sectional area of the myofibrils takes place during rigor. This decrease is suggested to be partly due to a fall in pH and partly due to attachment of myosin heads to the actin at rigor onset. The consequent of this shrinkage of the myofibrils is that the fibres shrink and the water that is left behind

accumulates, first along the perimysial network and later along the endomysial network, give rise to two types of extracellular compartments (Offer *et al.*, 1989b). All these alterations in muscle physiology and biochemistry affect meat quality (Tornberg, 2005).

2.2.1.1 - Factors that affect *rigor mortis*

Among some of the microenvironmental factors that can have a major influence on *post-mortem* changes, mainly on the calpain system, are temperature, pH, ionic strength and oxidative and nitrosylation status of the proteins in the cell.

It is widely recognised that high temperatures can produce contractures (heat-shortening) of a magnitude equivalent to cold contractures, but the effects on toughness are particularly different (Tornberg, 1996): whereas cold shortening increase the initial toughness and can, if sufficiently severe, be maintained even after ageing, conditions that produce significant heat rigor contractures have little effect on, or even reduce, initial (at *rigor mortis*) toughness and subsequent changes in tenderness are better described as a failure to tenderize rather than actual toughening (Simmons *et al.*, 2008). One possibility may relate to the extent of denaturation under conditions that produce heat-shortening. Denaturation of myosin is believed to be an important consequence that increases purge loss and colour (reflectance) as the myofibrillar lattice shrinks (Offer, 1991), and may well influence meat texture (Simmons *et al.*, 2008).

Rigor shortening is affected dramatically by the early *post-mortem* temperature of the muscle. A minimal degree of shortening occurs when pre-rigor muscle is exposed to temperatures in the range of 14–20 °C (Huff-Lonergan *et al.*, 2010). The degree of shortening in this temperature range is about 10%.The extent of shortening that occurs in the range of 0–10 °C can result in sarcomeres that are 50% of their normal length, and is named cold-shortening. On the other end of the temperature spectrum, significant shortening can also occur when the pre-rigor muscle is held at temperatures in the range of 20–40 °C. Shortening at these temperatures has been reported to be as much as 30% (Huff-Lonergan *et al.*, 2010), being named heat-shortening.

The susceptibility to cold shortening varies between species and between muscle types, red muscles being more susceptible than white ones (Hannula & Puolanne, 2004).

Muscle fibres also differ in their rate of ATP use. In addition, oxidative fibres are particularly susceptible to cold temperatures. One of the key events in cold shortening is the accelerated loss of calcium sequestering ability by the sarcoplasmic reticulum, especially in oxidative fibres. Therefore, another factor to consider is the fibre type composition of the muscles.

Rapid temperature decline could increase muscle shortening, especially if the muscles contain a high amount of oxidative fibres (Huff-Lonergan *et al.*, 2010). Several authors have reported that both cold and heat-shortening decreases tenderness and/or increases Warner-Bratzler shear force (WBSF; Koohmaraie, 1996; Savell *et al.*, 2005; Huff-Lonergan *et al.*, 2010).

Normal pH in skeletal muscle tissue is about 7.2 *in vivo*. However during *post-mortem* period, skeletal muscle pH decreases to around 5.4-5.7. In beef this pH is normally reached 24-48 hours *post-mortem*. The decrease in pH during the onset of *rigor mortis* has two components of high importance in the maturation and quality of meat, mainly in the holding capacity of water: the pH drop rate (glycolytic rate) and the final value of pH (glycolytic potential) (Touraille, 1991).

It has been observed in beef that a high or low pH at 3 h *post-mortem* may produce products with less acceptable tenderness than beef that has an intermediate pH 3 h *post-mortem*. Therefore, a moderately rate of pH decline may be quite beneficial to the development of tenderness, *i.e.* when a pH between 6.2 and 6.7 is reached 3 hours *post-mortem* (Klont *et al.*, 2000). One explanation for this phenomenon that has been proposed is that pH may exert an influence on the proteolytic enzymes that reside in the skeletal muscle cell (Simmons *et al.*, 2008). Muscle pH may be the cause of the differences observed in μ-calpain activity. It has been hypothesized that more rapid pH decline (higher glycolytic rate), accompanied with a higher Ca^{2+} concentration, resulted in increased μ-calpain autolysis, and earlier activation (Claeys *et al.* 2001) of this proteolytic protein and subsequently higher protein degradation and higher tenderness (Melody *et al.*, 2004) *early.post-mortem*. The glycolytic rate early *post-mortem* may play a pivotal role in regulating the rate of *post-mortem* tenderization (Melody *et al.*, 2004).

It is important to notice, that it is not the rapid pH fall *per se* that accelerates tenderisation but rather the interaction of the faster pH decline and temperature (Simmons *et al.*, 2008). Muscle pH and temperature interact continuously during rigor development, as they impact on both physical shortening, and proteolytic activity (Hwang & Thompson, 2001).

A low muscle pH while the muscle temperature is still high is a risk to meat quality (Hwang & Thompson, 2001). This condition is well recognised in pork as the pale, soft and exudative condition, which is caused by a spontaneously rapid pH decline in the early pre-rigor period. Despite this meat defect being generally detected in pork, a large pH decline caused by an excessive vigorous electrical stimulation can induce a similar condition in beef and lamb to produce an adverse effect on tenderness, water holding capacity, colour stability and overall eating quality (Geesink *et al.*, 2001). Most of the effects of excess stimulation can be attributed to denaturation of muscle structural proteins and enzymes. Moreover, a

combination of a very rapid pH decline with a slow chilling regime can cause an increase in toughness due to early exhaustion of μ-calpain at high temperatures and thus less ageing potential during chiller ageing (Hwang & Thompson, 2001).

2.2.2 - Post-*rigor mortis* or ageing

Most changes in the *post-rigor* meat texture result primarily from the weakening of the myofibrillar structure. Taylor *et al.* (1995) have shown that the greatest changes in tenderization occur within the first 3 or 4 days *post-mortem*, *i.e.*, involving the *rigor* period. Numerous structural changes occur, including:

1 – the loosing up of the intermediate filaments holding the myofibrils laterally in place;

2 – the degradation of titin and nebulin, which connect myosin filaments along their length and therefore cause weakening of the myofibrillar strength muscle

3 – Z-disk weakening, leading to myofibril fragmentation (Taylor *et al.*, 1995; Koohmaraie & Geesink, 2006).

The most consensual ultrastructure change in muscle *post-mortem* associated with tenderization, between the several work groups that has been studying this issue, is the disruption of the I band and the Z-disk (Taylor *et al.*, 1995; Sentandreu *et al.*, 2002; Koohmaraie & Geesink, 2006).

The proteins degraded during muscle fibre degradation are myofibrillar and cytoskeletal proteins, which include troponin-I, troponin-T, desmin, vinculin, meta-vinculin, dystrophin, nebulin and titin (Taylor *et al.*, 1995; Koohmaraie *et al.*, 2002). Three major cytoskeletal structures are degraded when meat is tender: Z- to Z-line attachments by intermediate filaments, Z- and M-line attachments to the sarcolemma by costameric proteins and the elastic filament of the protein titin (Taylor *et al.*, 1995). Z- to Z-line attachments are mostly composed by the desmin protein (Koohmaraie & Geesink, 2006). It should be noted that myosin and actin are little or not affected during tenderization process.

There is a consensus supporting the concept that the meat tenderization process is primarily enzymatic in nature, with physico-chemical conditions (pH, osmotic pressure) controlling the proteolytic action of endogenous peptidases (Sentandreu *et al.*, 2002). Despite the discussion generated for years concerning which proteolytic system is the main responsible for the tenderization process, several authors agreed that there are two conditions that are need to be fulfilled by the proteolytic systems considered. First, it must be able to mimic *post-mortem* pattern of ultrastructural changes. Second, it must have access to the substrate (Koohmaraie & Geesink, 2006).

The proteolytic systems that have been considered to have a role in tenderization process are calpains, cathepsins and proteosomes, also known as multicatalytic proteinase complex. More recently, Sentandreu and co-workers (2002) have also referred caspases and serine peptidases as proteolytic systems involved in the tenderization process. The three first proteolytic systems will be extensively exploited, since they are more often referred in the literature as having a potential effect in the tenderization.

2.2.2.1 - Muscle Calpains

Some authors reported that, the first evidence supporting the assumption that calpains have a primordial role in meat tenderization was the rapid loss of Z-disks in calpain-treated skeletal muscle myofibrils, a change often associated with meat tenderness (Taylor *et al.*, 1995; Koohmaraie *et al.*, 2002). Other authors even stressed forward that calpains are responsible for up to 95% of all proteolytically induced *post-mortem* tenderization (Delgado *et al.*, 2001).

Calpains are calcium-activated proteases with an optimum activity at neutral pH. In skeletal muscle, the calpain system consists of at least three proteases, μ-calpain or calpain 1 (requires to be active between 5 to 65 μM calcium concentration), m-calpain or calpain 2 (requires to be active between 0.3-1.0 mM calcium concentration) and calpain, p94 or calpain 3. The calpastatin, the specific inhibitor of μ- and m-calpain, is also present in this proteolytic system (Kohmaraie & Geesink, 2006; Huff-Lonergan *et al.*, 2010), having a crucial role. Despite the evidence that calpain 3 does autolyze in *post-mortem* muscle (Parr *et al.*, 1999), a major role of calpain 3 on *post-mortem* proteolysis and tenderization has been excluded. The main reason for such is that this enzyme is not inhibited by calpastatin (Sorimachi *et al.*, 1993), and it has been established that calpastatin activity has great influence on these events (Koohmaraie & Geesink, 2006). Therefore, seeing the lack of activity of this enzyme in post-mortem tenderization process, it will be left out of the discussion.

The μ- and m-calpains are heterodimers composed of two subunits, the 80 kDa catalytic subunit, that is responsible for the peptidase activity and is unique to each enzyme, and the 30 kDa regulatory subunit, which is common to all of them (Sentandreu *et al.*, 2002; Huff-Lonergan *et al.*, 2010). The activity of ubiquitous calpains is mainly controlled by calcium ions, phospholipids and calpastatin (Saido *et al.*, 1994).

Calpastatin, the specific inhibitor of the ubiquitous calpains, is a polymorphic protein comprising generally four similar domains each of them exhibiting calpain inhibitory activity and a N-terminal domain of variable size (Goll *et al.*, 1999). Calpastatin inhibitory process requires calcium concentrations that are reported to be close to or below those that are

required to activate calpain. Calpastatin is an unstructured protein but when it binds to calpains it adopts a structure which allows inhibition to take place. The binding of calcium to calpain causes changes in the calpain molecule enabling it to become active but also allowing calpastatin to interact with the enzyme (Kemp *et al.*, 2010). Calpastatin is also a substrate for the calpains and can be degraded in the presence of calcium. Degradation of calpastatin does not lead to complete loss of inhibitory activity, and even after extensive proteolysis some inhibitory activity remains. In ruminant species there is relationship between calpastatin activity in the muscle 24 h after slaughter and the degree of tenderisation achieved after conditioning, with differences in calpastatin accounting for 40% of the variation in tenderness (Shackelford *et al.*, 1994).

An important characteristic of μ- and m-calpain is that despite their need of calcium for being active, they undergo autolysis in the presence of calcium. Autolysis reduces the Ca^{2+} requirement for half maximal activity of μ- and m-calpain (Koohmaraie & Geesink, 2006).

Both μ-calpain and m-calpain have slower rates of activity against myofibrillar protein substrates at pH values and ionic strengths similar to those found in *post-mortem* muscle (Huff-Lonergan & Lonergan, 1999). Alterations in pH and/or ionic strengths may cause conformational changes that allow an increase in the hydrophobicity and aggregation of the enzyme (Huff-Lonergan *et al.*, 2010). Likewise, pH/ionic strength changes may alter the conformation of substrate proteins and render them less susceptible to cleavage by μ-calpain (Huff-Lonergan & Lonergan, 1999). Accelerated decline of early post-mortem pH appears to favour more accelerated autolysis and activation of μ-calpain as well as accelerated proteolysis of known calpain substrates (Melody *et al.*, 2004). More importantly, muscles that experience a slightly accelerated pH decline do also exhibit an accelerated rate of tenderization and, in some cases may have an advantage in water holding capacity (Melody *et al.*, 2004; Simmons *et al.*, 2008). It is possible that the decline in pH, as long as it is not too rapid may destabilize the structure of the μ-calpain molecule and allow for conformational changes that favour activation of the molecule to allow proteolysis of substrates and then subsequent autolysis (Huff-Lonergan *et al.*, 2010).

Because of the limited specificity of μ- and m-calpain, they do not degrade proteins to their constituent amino acids, nor do they degrade major myofibrillar proteins such as myosin or actin. A hypothesized role for calpains in muscle is the specific proteolysis of cytoskeletal proteins (titin and nebulin) and intermediate filaments (desmin) to initiate myofibrillar protein degradation (Huff-Lonergan *et al.*, 2010). In one proposed model (Neti *et al.*, 2009) calpains catalyse the release of myofilaments from the myofibril and made them available to the

proteasome and/ or lysosomes for complete degradation to amino acids (Huff-Lonergan *et al.*, 2010).

Discussion concerning if both enzymes μ- and m-calpain are responsible for tenderization or only one of them, lead to the evidence that μ-calpain is activated in early *post-mortem* (within 3 days of slaughter) during the period when *post-mortem* proteolysis of key myofibrillar proteins is known to take place (Taylor *et al.*, 1995). The m-isoform persists longer than the less stable μ-isoform in ageing muscle from all species studied, which suggests that μ-calpain, but not m-calpain, is responsible for *post-mortem* tenderization (Koohmaraie & Geesink, 2006), since after the inactivation of μ-calpain, the tenderization rate decreases. Additionally, the Ca^{2+} concentrations that exist in muscle *post-mortem* are less than that required of m-calpain for activation (Hwang & Thompson, 2001).

2.2.2.2 - Muscle cathepsins

Cathepsins are a group of peptidases located in the lysosomes and mostly active at acidic pH (Kemp *et al.*, 2010). This is indeed a complex group of enzymes that includes exo- and endo-peptidases belonging to cysteine (cathepsins B, H, L and X), aspartic (cathepsins D and E) and serine (cathepsin G) peptidase families. Of all these cathepsins, the ones expressed in muscle tissue include cathepsins B, D, H, L, S, F and K. However it seems that cathepsin D is not involved in the tenderizing process (Sentandreu *et al.*, 2002).

Cathepsins are synthesized as proenzymes in the lysosomes that are further transformed into the mature active enzymes by cleavage of the N-terminal pro-peptide. This process can be done either by autolytic cleavage or by the action of other peptidases (Turk *et al.*, 2000). Active cathepsins represent a high hydrolytic potential since their total concentration in cells can be higher than 1 mM (Lloyd & Mason, 1996). Their activity is controlled by several factors including pH, redox potential, extent of precursor activation and specific endogenous inhibitors. The low pH and the high temperature can originate the disruption of the lysosomal membrane and the failure of ionic pumps in lysosomal membranes during rigor development consecutively to the depletion of ATP stores (Taylor & Etherington, 1991).

Differences in sensitivity to pH can indicate different activities of these enzymes in the muscle *post-mortem*, *e.g.*, cathepsin L is very unstable at pH equal or higher than 7 but most of the other cathepsins are stable over a wide range of pH values. They are generally believed to work only in acidic environment like the lysosomal compartment (Turk *et al.*, 2000). By contrast, cathepsin S has been shown to be stable and active above pH 7.0, indicating a possible role outside lysosomes (Kirschke *et al.*, 1989).

Cystatins are the cathepsins inhibitors. *In vivo*, cystatins are located in the cytoplasm, separated from cathepsins, which remain inside lysosomes. In this situation, unwanted cathepsin activity outside lysosomes is prevented by cystatins. In *post-mortem* muscle, on the contrary, the physiological situation is different because lysosomal breakdown induces a release of cathepsins to the cystosol where cystatins are. It is the balance between cathepsins and cystatins that will drive the effective activity level of the cathepsins (Sentandreu *et al.*, 2002).

Ideas discarding the contribution of cathepsins in the development of meat tenderness are mainly based on two observations. First, cathepsin activities failed to explain differences in tenderness of meat samples, as the degradation patterns are different than those presented in muscle *post-mortem* (Koohmaraie & Geesink, 2006). Second, inhibition studies showed that some cathepsin inhibitors were not able to suppress *post-mortem* proteolysis (Sentandreu *et al.*, 2002). To support this, other authors also stated that there is little or no actin and myosin degradation during ageing, two proteins highly sensitive to the action of cathepsins (Koohmaraie *et al.*, 1991). Another major argument to support that cathepsins could not participate in *post-mortem* proteolysis has been the fact that they are contained into lysosomes, and it is doubtful that cathepsins are released from lisosomes in post-mortem muscle (Koohmaraie, 1996; Koohmaraie, & Geesink, 2006) in time, since the breakdown of the lysosomal membrane only occurs 10-14 days post-mortem, when tenderization seems to be almost completed.

Despite the low association found by several authors between cathepsins activities' and meat tenderness, there are some evidences that cathepsins B and L activities at 8 h *post-mortem* are positively correlate with tenderness in beef (O'Halloran *et al.*, 1997). Cathepsin L hydrolyses the largest number of myofibrillar proteins, including troponin T, I and C, nebulin, titin and tropomyosin, which are degraded during the *post-mortem* conditioning period as well as myosin and actin, in rabbit, beef and chicken myofibrils (Mikami *et al.*, 1987).

Moreover, incubation of myofibrils with a lysosomal extract results in some of the ultra-structural changes observed during meat ageing including the degradation of myofibrils near the N_2 lines and at the A–I junction area. Moreover, some fragments with molecular masses close to 155 and 90 kDa, probably originating from myosin heavy chain degradation, have been identified in stored meat (Yates *et al.*, 1983). Like myosin, actin seems to be also partially degraded but this degradation occurs probably after a minimum of 7–10 days *post-mortem* (Taylor *et al.*, 1995). Cathepsins degrade many other structural and contractile proteins, so it is possible that they show more affinity for these other proteins than for actin and myosin filaments in muscle.

Because all these structural changes identified in *post-mortem* muscle cannot be explained by the action of one proteolytic system, a synergistic action of calpains and cathepsins must be considered (Koohmaraie & Geensink, 2006). Therefore, while calpains may be responsible of the changes in the early *post-mortem* period (24 h), a relevant contribution of cathepsin could be expected thereafter (Zeece *et al.*, 1992).

2.2.2.3 - Proteasome complex

Proteasome complex formely known as multicatalytic proteinase complex (MCP) is a non lysosomal protein complex with strong peptidasic activity *in vivo*. The 20S proteasome is a 700,000 dalton, cylinder shaped structure arranged as four axially stacked heptameric rings composed exclusively of either alpha-subunits (two outer rings) or beta-subunits (two inner rings), respectively (Sentandreu *et al.*, 2002). Within the cell, the 20S proteasome exists either in a free state or associated with large regulatory complexes. It can thus bind one or two 19S complexes responsible for the ATP-ubiquitin dependent hydrolytic activity of the resulting 26S complex (Robert *et al.*, 1999). The activity of the 20S proteasome is under the control of specific activators and inhibitors. The 20S proteasome has been shown to degrade myofibrils and to cause significant damage of the M and Z-lines as does calpains (Taylor *et al.*, 1995; Robert *et al.*, 1999). It has been suggested by some authors, based on the enlargement of the Z-line followed by the appearance of an amorphous protein material, that the 20S proteasome might be the main proteolytic system of concern in *post-mortem* tenderization of meat (Ouali, 1999). As neither calpains nor cathepsins are able to mimic these structural changes, one possible candidate could be the 20S proteasome (Sentandreu *et al.*, 2002). However this theory has been more recently denied, as proteosomes could not be involved in meat tenderization *post-mortem,* because they do not mimic the degradation pattern observed in *post-mortem* muscle (Taylor *et al.*, 1995; Kohmaraie & Geesink, 2006). Kohmaraie and coworkers (1992) stated that myofibrils were a very poor substrate for proteasome complex. Of all myofibrillar proteins, only troponin-C and MLC-2 and -3 were degraded by proteasome complex. Based on phase and electron microscopy observations, proteasome complex had no detectable effect on myofibrils.

Nevertheless, the specific structural changes observed in high pH meat are wholly comparable to those obtained upon treatment of glycerinated fibres with purified 20S proteasome (Sentandreu *et al.*, 2002). The proteasome might be therefore a good candidate for *post-mortem* tenderization of high pH meat (Ouali, 1999).

In summary, it can be concluded that meat tenderization process *post-mortem* remains a controversial issue. From the three kinds of proteins present in skeletal muscle tissue, sarcoplasmic proteins are not structural proteins and so, they do not directly affect meat tenderness. By the other hand connective tissue determines the background toughness. Leftover the myofibrillar proteins, whilst have been object of intense discussion. Goll and coworkers (1992) stated that because the calpain system is the only proteolytic system capable of making the very specific cleavages needed to release myofilaments, it is the best candidate to initiate the removal of myofilaments from the surface of the myofibrils (Koohmaraie & Geensink, 2006). In a later phase, the proteasome complex is a good candidate to degrade proteins into amino acids, once myofilaments are disassembled into proteins, since it cannot degrade the released myofilaments into proteins (Koohmaraie *et al,.* 2002). Also Robert *et al.* (1999) defended a synergistic action between different peptidases notably calpains and proteosomes. However, protasome complex needs ATP, and at this post-rigor mortis stage ATP has already been exhausted. It is noteworthy that the ageing process typically takes 10–20 days in beef (Smulders *et al.*, 1990).

2.3 – Rheological properties of meat

Meat is mainly constituted by water (around 75%) and proteins (around 20%), which are the main responsible by the structural changes undergone by the meat during preparation until being consumed. Meat is composed by globular proteins (sarcoplasmic proteins) and fibrous proteins (myofibrillar and connective tissue proteins). Proteins are macromolecules formed by amino acids residues that have the ability to lose their native form conformation (denaturation). Given the rigidity of the molecule most dehydrated proteins are presented in solid form. In contact with the solvent, *e.g.* water, proteins unfold and become more flexible. This protein-solvent interaction, which depends on the protein amino acids' and on the solvent, determines the solubility and rheological behaviour. The degree of swelling influences the flexibility of the molecule and its unfolding will increase the hydrodynamic volume, reducing the distance between molecules, and increasing the viscosity. The intrinsic viscosity can be ten times higher in case of fibrous proteins (like collagen). When the protein is denatured, the large increase seen in the intrinsic viscosity is not due to a large amount of solvent, but to the increased size of the protein molecule unfolded (Tornberg, 2005).
Factors such as pH, temperature, ionic strength, salts and denaturing agents have an important role in the change of protein conformation.

2.3.1 – Behaviour of myofibrilhar proteins

The denaturation of myofibrillar proteins in solution usually results in gel formation. Myosin has a special characteristic since that it forms gels at very low concentration (0.5% by weight; Hermansson & Langton, 1988). When purified myosin is heated, the firmness of the gel reaches its maximum at 45 ºC when pH is 5.5 or at 60 ºC when pH is 7 (Sharp & Offer, 1992). When actin is present in the solution, a firmer gel is obtained. Ionic strength (mostly sodium chloride) and pH are important factors since they determine if the myosin exists in monomeric form or as filaments. When pH is neutral and the ionic strengths is higher than 0.3, the myosin molecules are dispersed as monomers, forming a coarse network with large pores (see Figure 3-a). At lower ionic strength the myosin molecules are assembled in filaments, resembling the natural thick filaments in the muscle. If these are the conditions during heating, a firmer gel is formed especially if the filaments are very long. Such a gel consists of a finer and more uniform network, with smaller pores (Sharp & Offer, 1992). This gel is clear and has good water holding capacity (Figure 3-b). However, myosin, both in monomers or filaments, has the ability to aggregate and form a cloudy gel with low water holding capacity.

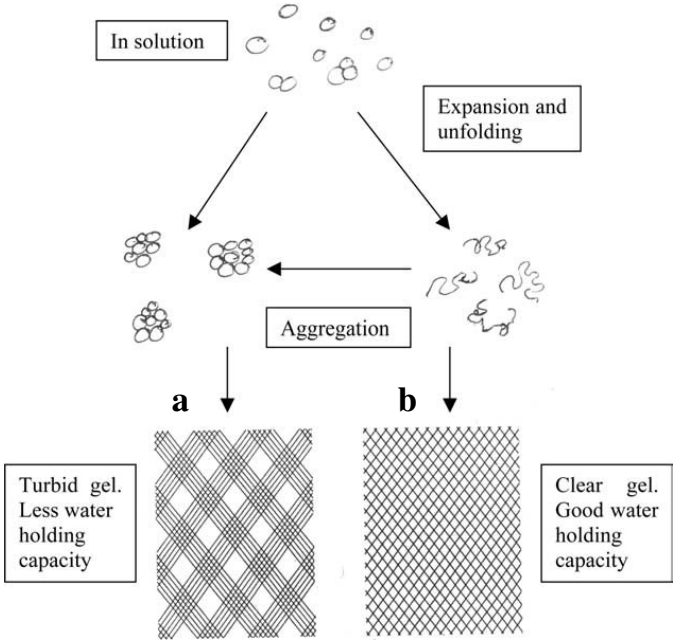
The gel formation of myosin occurs in two steps, in two separate temperature regions on heating. The first part of the reaction occurs between 30 and 50 ºC and the second step at temperatures above 50 ºC. The first step involves aggregation of the globular heads of myosin (Tornberg, 2005). With increasing heating on this temperature range, the two heads of some myosin molecules coalesce and some dimers are formed by aggregation through the heads. More and more myosin molecules aggregate through their heads to form a globular mass with the tails radiating outwards, no native myosin molecules left and the only monomers present had coalesced heads (Sharp & Offer, 1992).

Heating to 50 ºC resulted in further aggregation, being hard to distinguish between the individual tails, leading to the formation of large globular aggregates (Tornberg, 2005).

No tails were seen after these temperatures. The second stage involved structural changes in the helix structure of the myosin tail, leading to network formation, where hydrophobic groups interact with each other. The helix content of light meromyosin began to decrease at about 30 ºC and attained a minimum at 70 ºC. Simultaneously, heating to 65 ºC progressively increases the surface hydrophobicity, whereas at higher temperatures it decreases again. The decrease in hydrophobicity observed at the higher temperatures suggests that part of the hydrophobic residues take part in protein–protein interactions leading to a network formation of aggregates, *i.e.* to the formation of a gel (Tornberg, 2005). An interesting aspect of the

sarcoplasmic proteins is the tenderizing effect some of these enzymes can have, using low temperature and long time heating (heating rate of 0.1 ºC/min) on beef muscles.

Figure 3. Different type of gel formation on heating of globular proteins. Freely after Hermansson (1982).



2.3.2 – Behaviour of collagen

Collagen has a quasi-crystalline structure and a very elastic modulus in the raw state (Lepetit, 2007). However when heated to temperatures of 58-65 ºC there is a helix to coil transition of the collagen molecule. Collagen is denatured and contracts. In this state its behaviour is governed by the theory of rubber-like elasticity (Kopp & Bonnet, 1987). The denaturation of collagen is probably due to the breakage of hydrogen bonds loosing up the fibrillar structure, followed by the contraction of the collagen molecule (Tornberg, 2005). The thermal transition of collagen is progressive and during denaturation, crystalline and amorphous (rubber-like) regions coexist (Lepetit, 2007). When meat is heated above the temperature of collagen contraction, as collagen fibres and fibrils are initially wavy they can contract freely to a certain degree (Lepetit, Grajales, & Favier, 2000). Above that free contraction, the collagen fibres and fibrils start to apply pressure on muscle fibres and bundles, and can contract further. Therefore, the final contraction state of collagen fibres depends on the resistance of muscles fibres (Lepetit, 2007). When the heat treatment is further increased, the intermolecular cross-links are progressively destroyed and finally collagen goes into solution

(Lepetit, 2007), forming a gel, if heat-resistant intermolecular bonds do not stabilize it (Tornberg, 2005). Thus, if unrestrained, the collagen fibres shrink to one quarter of its resting length at heating temperatures between 60-70 ºC (Tornberg, 2005). Nevertheless, Horgan and coworkers (1991) have shown that when meat is heated to 80 ºC for 45 min, neither the amount of Pyridinoline nor the amount of Ehrlich Chromogen cross-links changes, since these two ketoamine cross-links are thermo-stables.

2.3.3 - Mechanical properties of collagen

Several authors have gather efforts in understanding the role of intramuscular connective tissue in coordinating force transmission in muscle. The two connective tissues (endomysium and perimysium) present in meat are composite networks of collagen and elastin fibres embedded in a matrix of proteoglycan. It has been shown that the easily-deformable tensile properties of the endomysial network allow it to follow necessary shape changes in contracting muscle fibres without restraining this process. Moreover the endomysium thickness is suitable for efficient load transfer over a wide range of physiological muscle fibre lengths (Purslow, 2005). It has been proposed that the division of a muscle into fascicles by the perimysium reflects the need to accommodate shape changes as the muscle contracts, which is achieved by allowing fascicles to slide past each other (Purslow, 2005).

Structural and mechanical studies of cooked beef have clearly demonstrated that separation of the perimysium from the endomysium of fibres on the surface of the fascicle is relatively easy in cooked meat, but the individual perimysial layers are very strong, dominating the fracture behaviour of meat. Thus perimysium is the major contributor to toughness (Purslow, 2005). Moreover, the perimysium has higher strength relative to the weaker endomysial– perimysial interface in cooked beef. Since the amount of perimysial collagen in muscle is much greater than the endomysial fraction (McCormick, 1999), it is not surprising that tenderness variations have been more linked to perimysial than to endomysial collagen.

At rest length, *i.e.* when the sarcomere length is about 2 µm, the average angle between each set of collagen fibres (perimysium) and the axis of muscle fibres is about 55º (Purslow, 1989) which is about the same for collagen fibrils (Purslow & Trotter, 1994). The degree of organization in endomysium is more random, though (Lepetit, 2008). In composite materials this angle of windings is known to give maximum resistance against internal pressure to a fibre reinforced cylinder. The waviness of collagen fibres or fibrils, which is defined as the ratio of their curved length to their straight length is highest at rest length (Lepetit, 2008). Its value is in the range 1.15–1.30 for perimysium fibres (Purslow, 1989) whereas it is about 1.1

for endomysium fibrils (Field & Faber, 1970). This waviness decreases and vanishes both for extreme shortening and stretching. The waviness of collagen fibres is a structural characteristic which plays an important role in collagen shrinkage that occur in meat during cooking (Lepetit, 2008). In the raw state, the elastic modulus of collagen fibres is about 0.5–1 GPa (Fung, 1981) whereas the elastic modulus of elastin is about 0.1– 0.41 MPa (Silver *et al.*, 1992). Collagen has a quasi-crystalline structure and a very high elastic modulus. But when heated to temperatures of 58–65 ºC, there is a transition from the native helical-ordered (crystalline) state to a randomly coiled (amorphous) structure of the collagen molecule. The collagen molecule is denatured and contracts. When this transition is complete the collagen behaves according to the theory of rubber-like elasticity (Kopp & Bonnet, 1987). However, the thermal transition is highly dependent on the ability of collagen to shrink. Maintaining constant stress or constant strain on collagen fibrils during heating delays the thermal transition of collagen and the consequence is that in these conditions crystalline regions in collagen fibres remain longer and collagen fibres are stiffer (Lepetit, 2008).

The tensile strength of collagen depends on the formation of covalent intermolecular cross-links between the individual protein subunits (Eyre & Wu, 2005).The stress developed by a rubber-like material is directly dependent on the number of cross-linked chains. This dependence is due to entropy. When the material is unstressed the chains have a random configuration and therefore maximum entropy. Stretching a rubber like material produces a reorientation of the chains, leading to a decrease in entropy. The stress necessary to stretch the material is directly linked to the decrease in entropy (Lepetit, 2008). Despite the random configuration, there is a statistical distribution of shapes for all the chains in a population of macromolecules, which are changing with time. This is why collagen fibres shrink when the hydrogen bonds in the collagen molecules are destroyed by heat above 60–65 ºC. The average thermal contraction of collagen fibres in a rest length aged meat is estimated at about 20–25% (Lepetit *et al.*, 2000). At this percentage of shortening, most of the collagen structure is already in an amorphous state (Wright & Wiederhorn, 1951), *i.e.*, denaturated. As the glass transition temperature of hydrated collagen is around -6 ºC (Brake & Fennema, 1999) then above this temperature amorphous collagen structure is rubber like (Lepetit *et al.*, 2008).

The breaks of the structure at high strain can occur anywhere in the chains or in the cross-links. The trivalent cross-links, which link three collagen molecules, such as HP, LP and Ehrlich Chromogen are the strongest ones (Eyre & Wu, 2005) (see section 2.1.3.1.2). The collagen elastic *moduli* depends on the number of divalent and trivalent cross-links, the functionally of the latter's being 1.5 times higher than the formers, though (Lepetit, 2008). Pyridinoline crosslinks alone, expressed as mole per volume of meat, predict relatively well

the variations of tenderness observed between muscles, gender and age as shown by Lepetit (2007). Moreover, the cooking loss increases with the amount of pyridinoline, which is in agreement with an increase in pressure developed by connective tissues (Listrat *et al.*, 2007).
Several studies have shown that small muscle fibre bundles give tender meat and also that the smaller muscle fibre diameter the tender the meat. However, Lepetit (2008) results revealed that it is not the inside of the muscle fibre that makes meat with small diameter fibres tenderer. Collagenous envelopes have an important role in the relationships between tenderness and the diameter of muscle fibres or muscle fibre bundles, which is explained by the link between pressure and radius in a cylinder. The pressure developed by collagen fibres during cooking is higher the smaller the muscle fibre diameter. Thus collagen fibrils around small fibres (and collagen fibres around small fibres bundles) are expected to shorten more, and consequently to have a lower final elastic modulus (Snowden *et al.*, 1977).

2.3.3.1 - Effect of pH

There are three main effects of pH on the mechanical properties of connective tissues after heating. The decrease of the pH of a piece of meat produces a swelling of both connective tissues and muscle fibres. The swelling of muscle fibres is limited by the surrounding endomysium (Offer *et al.*, 1989b). The swelling of collagen fibres by an acidic solution affects the volumic percentage of collagen in the fibres and hence their elastic modulus (Lepetit, 2008). The swelling of collagenous structures decreases as the amount of crosslink chains per volume increases.
As part of the theory of rubber like elasticity low pH is expected to produce a smaller decrease in strength of connective tissues in meat from old animals than from young ones, when all other variables are equal between them. This effect is not significant on tenderness of muscles with low amount of collagen (Gault, 1985).
Low pH decreases the temperature of thermal transition of collagen (Berge *et al.*, 2001). The rate at which the collagen is solubilised to gelatine is minimal at a pH between 5 and 6. A high level of collagen fibre hydration is known to decrease the temperature of thermal transition (Miles *et al.*, 2005). As the temperature of collagen thermal transition is decreased, the stress developed is lower (Lepetit, 2008). Usha and Ramasani (2000) found a decrease in the isometric tension developed by collagen fibres when pH is decreased. Consequently, the pressure developed by collagen fibres on muscle fibres and bundles during cooking in low pH meat is lower than in normal pH meat. This is one of the reasons why low pH meat retains more water on cooking than normal pH meat (Lepetit, 2008).

Offer and coworkers (1989b) suggested that one of the mechanisms of pH induced tenderisation of meat could be a breakage of covalent collagen cross-links and of some specific peptide bonds. In the same way, Usha and Ramasami (2000) suggested also that cleavage of acid labile cross-links can explain the weakening of tendon at low pH. Other authors showed that the transverse breaking stress of meat, which reflects connective tissue strength, shows a decrease with pH which cannot be explained by swelling alone. This suggests a real weakening of connective tissues at low pH (Lepetit, 2008).

2.3.3.2 – Effect of temperature

The heat treatment may have an effect on tenderness of meat by its intensity, the heating rate, the duration, or even by the interaction between these factors. As the temperature increases the proteins denature and the properties of denatured proteins contribute largely to determine the tenderness of meat (Bailey, 1988).

The effect of heat treatment on intramuscular connective tissue depends on its composition, more specifically the type of chemical bonds within and between collagen molecules in thermo-labile cross-links the degradation by the heat will be quicker and more extensive than in thermo-stable cross-links.

Earlier studies justified the increased in toughness between 40-60 ºC with the contraction of actin and myosin. McCormick (1999) stated that before 60 ºC is attained, both these proteins have undergone a transition from the gel state, initially posing little resistance to shear, to a hardened dehydrated form, although the water is still associated with the myofibre. The second increased in toughness was attributed to the contraction of collagen between 65-80ºC (McCormick, 1999). As isolated intramuscular connective tissue forcefully shrinks above 65 ºC when rapidly heated (Kopp & Bonnet, 1987), there is still a feeling that high-temperature shrinkage of collagen could cause the shrinkage of meat seen at 65–80 ºC, and that this shrinkage causes volume reduction in the muscle fibres, thus increasing their toughness (Lepetit *et al.*, 2000). However, it has been pointed out that the ratio of transverse to longitudinal shrinkage in meat on cooking, and especially how these vary with sarcomere length, is not simply explainable on the basis of collagen network shrinkage (Purslow, 2005). It is possible that other events, such as cytoskeletal protein denaturation, cause the shrinkage to toughen myofibrillar components at higher temperatures. Moreover, single muscle fibres lack perimysium, which accounts about 90% to the intramuscular connective tissue, so it is unlikely that contraction observed above 65 ºC is due to intramuscular connective tissue. It seems more likely that contraction seen at these temperatures is due to sarcoplasmic proteins

and actin contraction (Mutungi *et al.*, 1996). It looks like the relationship between toughness and temperature earlier used by some authors is now surpassed.

More recent studies, settled that a sharp increase in toughness occurs between 40 and 50 ºC, followed either a dip or a plateau in toughness, between 50 and 60 ºC, depending on measurement technique, attributed to a weakening of the collagenous connective tissue, due to denaturation, and then a second phase of increasing toughness above 65 ºC (Purslow, 2005).

A typical curve (Figure 4) from thermal transitions found in a muscle is composed of three major transition zones A, B and C. The first transition displays its maximum between 57 and 65 ºC, and has been attributed to the denaturation of myosin. The second transition, which occurs between 65 and 67 ºC, was assigned to collagen and to sarcoplasmic proteins. The third transition has been assigned to actin and is found between 80 and 83 ºC (Staburvisk & Martens, 1980).

For the second transition it has also been shown that actomyosin and myosin and its sub-units undergo transitions in the same temperature range (Wright & Wilding, 1984).

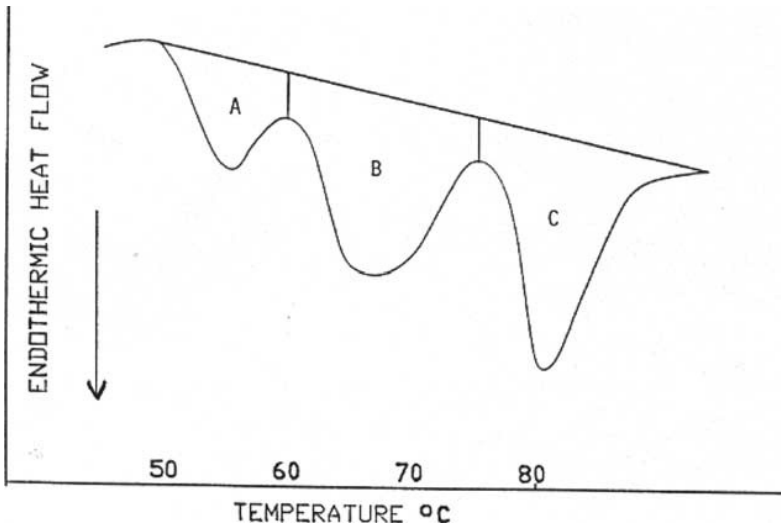
Most sarcoplasmic proteins aggregate between 40 and 60 ºC (Tornberg, 2005). Accordingly, Davey and Gilbert (1974) inferred that the sarcoplasmic proteins might have a role in the consistency of cooked meat in such a way that the heat-induced aggregated sarcoplasmic proteins can form a gel in between the structural meat elements and thereby link them together. On the other hand, the evidence that intramuscular connective tissue actually contributes to the first phase of toughening, at 40–60 ºC, goes back to studies in the 80s.

Bouton *et al.* (1981) concluded from the Warner-Bratzler PF-IY measurements that the connective tissue contribution to toughness is high at low cooking temperatures and decreases above 60 ºC. Lewis and Purslow (1991) shown that perimysial connective tissue strength increases in meat cooked up to 50 ºC and decreases above this temperature. Collagenase could remain active in the meat at cooking temperatures until 60 ºC, whereas at faster heating and reaching a higher end temperature of 70–80 ºC they were inactivated (Purslow, 2005).

Tornberg (2005) reinforced that there is shrinkage of the muscle fibres above 40 ºC and a cooperative contraction of connective tissues and muscle fibres at 60–70 ºC.

The resistance of heated muscle fibre is known to be affected by the state of ageing, sarcomere length and pH (Offer *et al.*, 1989b).

Figure 4. –Thermal transitions curve (freely after Tornberg, 2005).



2.3.4 - Changes on Water holding capacity

In all shrinkage and denaturation process over temperature of collagen and myofibrillar structures, there is another aspect that is interesting to notice, mainly due to its influence in final meat texture. This is water holding capacity, which is severely affected by cooking process. Intramuscular connective tissue shrinkage squeezes out fluid released from heated myofibrils thus affecting their properties (Lewis & Purslow, 1991). In whole meat, shrinkage and swelling of myofibrils are crucial factors affecting water losses. Water losses in raw meat can be obtained by evaporation from the surface or as exudates, when meat is cut. Exudates is not only composed by water, but a solution of sarcoplasmic proteins, that is drained from the cut surface of the meat by gravity, if the viscosity of the exudates is low enough and the capillarity forces do not retain it (Tornberg, 2005). It has been suggested by Offer and coworkers (1989a) that this drip loss arises predominantly from the longitudinal channels through the meat between the fibre bundles. Most of the water in the living muscle is held within the myofibrils (approximately 80%), in the spaces between the thick and thin filaments. Any large changes in the distribution of water within the meat structure, is originated from changes in this spacing. Lateral shrinkage of the filament lattice is brought about by a pH fall closer to the isoelectric point, rigor contraction and myosin denaturation (Offer *et al.*, 1989b).

Cooking induces structural changes, as already discussed, which decrease the water-holding capacity of the meat. As temperature increases to 50 ºC the amount of water around fibre bundles also increases, comparing to raw meat, which seems to be in accordance with the

transverse shrinkage of fibres and fibre bundles. Above 50 ºC this widened gap diminishes, again up to 70 ºC, probably mainly due to the shrinkage of the connective tissue (Tornberg, 2005).

At 60–70 ºC the connective tissue network and the muscle fibres cooperatively shrink longitudinally, the extent of shrinkage increasing with temperature. This shrinkage causes the great water loss that is obtained on cooking. It is then presumed that water is expelled by the pressure exerted by the shrinking connective tissue on the aqueous solution in the extracellular void (Tornberg, 2005). However, the increase of water in extracellular space when temperature rises from 70 to 90 ºC is not that easily understood it looks that a swelling of the perimysium occurs at these cooking temperatures (Tornberg, 2005).

2.4 – Meat Quality

Meat quality is a very difficult concept to define. The main cause is that meat quality can have different meanings depending to what is addressed. One of the primordial definitions of meat quality that appear in the literature, despite simplistic seems to remain updated, and was given by Hammond in 1955: "Quality can best be defined as that which public likes best and for which they are prepared to pay more than average price" (quoted by Steenkamp, 1997). Steenkamp (1997) stated that definition of quality is becoming increasingly complex as it encompasses the physical intrinsic qualities of the meat (colour, shape, appearance, tenderness, juiciness, flavour) and extrinsic qualities (brand, quality mark, origin, healthiness, production environment etc.). Therefore, concerning consumer point of view, meat quality has been defined by those attributes the consumer perceives as desirable which includes both visual and sensory attributes and credence attributes of safety, health and more intangible attributes such as 'clean' and 'green' or welfare status of the production system (Becker, 2000). Important visual attributes include lean colour and fat colour, amount and distribution of fat as well as the absence of excess water (purge) in the tray. Once cooked, consumer satisfaction is largely determined by how tender the meat is as well as its flavour and juiciness (Glitsch, 2000).

Tenderness is often referred by consumers as the most important palatability attribute however, when meat presents satisfactory tenderness value, flavour and juiciness are the following attributes. Importance of credence attributes of meat are increasingly and comprehend mainly safety and healthiness. Concerning healthiness, the importance of meat as a source of some nutrients (*e.g.* protein and some micronutrients, mainly vitamins A, B6, B12, D and E, as well as iron, zinc and selenium) is well recognized (Biesalski, 2005), but some

other potential beneficial components of meat, like L-carnitine, are much less known. In the last years consumers health concerns' compelled meat scientists to investigate meat nutritional value, and much investigation has been focused on the biological effects of conjugated linoleic acid (CLA). Moreover, the importance of nutritional index related to fatty acid composition, like P/S (polyunsaturated/saturated fatty acids) and n-6/n-3 (ω6 polyunsaturated fatty acids/ω3 polyunsaturated fatty acids), has been questioned. Animal products are a complex food with a highly structured nutritional composition and major source of cholesterol in the diet. It becomes edible and more digestible when it is subjected to cooking (Hur *et al.*, 2007). Nevertheless, beef cholesterol content in a balanced diet does not seem to be a health problem. All this issues will be further exploited and explained.

Because of the highly heterogeneous tissue and cellular composition of skeletal muscle, and the numerous factors affecting meat quality (pre-, peri-, and *post-mortem*), it remains difficult to identify what muscle biological traits are specifically involved in determination of meat quality (Lefaucheur, 2010).

2.4.1 - Organoleptic properties of meat

2.4.1.1 - Tenderness

Tenderness can be defined as the ability of meat to be cut or chew, or the ease with which the meat structure falls apart during the mastication process (Touraille, 1991).

Tenderness is one of the most determinants of meat quality and a common cause of unacceptability in meat products, when meat presents a low tenderness score. Several authors sustained that meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development, and *post-mortem* proteolysis of myofibrillar and myofibrillar associated proteins (Koohmaraie & Geesink, 2006). Nevertheless, the main determinant of ultimate tenderness has been attributed to the extent of proteolysis (Koohmaraie & Geesink, 2006; Kemp *et al.*, 2010). An increase in tenderness is observed as a result of enzymatic degradation of muscle tissue 24 hours *post-mortem*. The proteolysis contribution to meat tenderness is predominantly regulated by the protease levels in the muscle at slaughter, duration of post-rigor ageing and peptidase activity during ageing (Koohmaraie & Geesink, 2006). Temperature of storage can affect this enzymatic degradation, as well as other factors including: pH, muscle fibre type, amount and degree of cross-linking of connective tissue, and animal species (Smulders *et al.*, 1990). Intramuscular

fat contributes to meat tenderness directly and indirectly (Hocquette *et al.*, 2010) as does the rate and extent of *post-mortem* energy metabolism (Thompson *et al.*, 2006).

2.4.1.1.1 - Factors that affect meat tenderness

2.4.1.1.1.1 – Muscle fibres

The size and number of muscle fibres are factors that influence muscle mass and meat quality. It has been reported that the number and size of muscle fibres are negatively correlated (Lefaucheur, 2010). Myofibre hypertrophy (increase of cell volume) is a balance between protein synthesis and degradation. Thus, the relative changes in both processes strongly affect myofibre growth rate, as well as meat tenderization/tenderness through modulation of *post-mortem* proteolysis (Koohmaraie *et al.*, 2002).

During *post*-natal development, when the number of fibres is high fibres generally grow more slowly. Conversely when the number of fibres is low, fibres grow more rapidly (Choi & Kim, 2009). This means that muscle with high fibre number, probably will have fibres with low cross-sectional area. Generally, the decrease in fibre diameter during cooking is positively related with tenderness (Lepetit, 2007). However, large myofibre cross-sectional area might not be a cause of the decrease in meat tenderness (Lefaucheur, 2010). In cattle, the relationship between tenderness and cross-sectional area is controversial (Renand *et al.*, 2001). At a first glance, increasing total fibre number could be a good strategy to simultaneously increase lean meat content while preserving meat quality by reducing myofibre cross-sectional area. Some studies have shown that increasing the total fibre number *per se* would not be enough (Rehfekdt *et al.*, 2007). However, a high fibre number combined with a low percentage of type IIB white fibres would be a good strategy to improve both lean meat content and meat quality (Kim *et al.*, 2008; Ryu *et al.*, 2006).

The heterogeneity of meat fibre type of different skeletal muscles is known to influence meat tenderness. Muscles mainly composed by type II fast fibres are more susceptible to *post-mortem* proteolytic degradation than muscles mainly composed by type I slow fibres (Xiong *et al.*, 2007). Probably these muscles would present a better tenderness score. Therefore, increasing the proportion of fast-twitch glycolytic fibres could have beneficial effects on *post-mortem* ageing and tenderness in species exhibiting slow rate of *post-mortem* ageing, such as cattle (Lefaucheur, 2010).

Muscle types vary in their potential to cold shorten, red being more susceptible than white (Bendall, 1973). Because white muscle fibres tend to have higher amounts of glycogen, they

experience a more severe drop in pH earlier in the rigor process (Savell *et al.*, 2005), which means that probably muscles with higher amount of red fibres are more prone to be tougher.

The effect of sarcomere length on meat tenderness has originally thought to be mediated through the increase of the overlap of the myofilaments and consequently toughness comes from the myofibrillar structure (Lepetit *et al.*, 2000). The relationship of cold shortening and sarcomere length to toughness was first demonstrated by Herring *et al.* (1965). These authors have shown the direct relationship of sarcomere length to fibre diameter and toughness. Their theory is: the more contracted the sarcomere, the larger the fibre diameter becomes due to sliding of the filaments over one another. After cooking, meat with larger fibres is tougher (Savell *et al.*, 2005). The majority of the research suggests that sarcomere shortening is the causative factor of the decrease in tenderness of muscles from the time of slaughter to 24 h *post-mortem* (Savell *et al.*, 2005). Sarcomere length has been shown to contribute to decrease of proteolysis post-slaughter through its effect on limiting access of proteases to myofibrillar protein substrate in cold-shortened beef (Weaver *et al.*, 2008).

Moreover, some authors claimed that meat with sarcomeres shorter than 2 µm, not even after 14 days of conditioning has tenderized. Davis and coworkers (1979) comparing ‘‘less tender’’ with ‘‘more tender’’ steaks, found that ‘‘more tender’’ steaks had longer sarcomeres (based on mean values) than ‘‘less tender’’ steaks. Nevertheless, other studies have shown that sarcomere length does not affect tenderness (Culler *et al.*, 1978; Seideman & Koohmaraie, 1987; Shackelford *et al.*, 1994).

The rate of temperature and pH decline *post-mortem* is the main causative of sarcomere shortening. At intermediate rates of temperature decline, minimal shortening of sarcomeres occurs at rigor. If the temperature decline is too rapid and glycolysis is slow, cold shortening occurs resulting in a profound increase in toughness (Pike *et al.*, 1993). If the temperature fall is too slow and glycolysis is fast heat-toughening (also called *rigor*-toughening or *rigor* shortening) can occur with associated tougher meat, due to a failure of the tenderising processing during ageing (Pike *et al.*, 1993; Dransfield, 1994).

2.4.1.1.1.2 - Connective Tissue

Intramuscular connective tissue contribution to meat texture is certainly important, it is also rather immutable and forms a ‘‘background toughness’’ that we can do little about in practical terms (Sentandreu *et al.*, 2002). Given the large influence of intramuscular connective tissue on meat texture, even small manipulations of its expression and turnover may have considerable potential for reducing unwanted variations in meat tenderness (Purslow, 2005).

However, considering studies where it intends to handle the amount of perimysium to improve meat quality, this should be treated with caution, since that the variation in expression of perimysial tissue between muscles is tightly related to muscle activity, and so may not easily be manipulated without compromising muscle function in the live animal.

It has been discussed in the literature which is more important to meat tenderness, total collagen content or collagen composition. In recent studies in collagen composition, the nature of collagen cross-links is gathering greater relevance.

During *post-mortem* storage proteolysis of both collagenous and proteoglycans components of intramuscular connective tissue increases the amount of collagen that can be solubilised, reducing significantly the strength of perimysium in raw meat, after conditioning (Lewis & Purslow, 1991). However, this issue is not consensual since other authors referred that intramuscular connective tissue is macroscopically unaffected by *post-mortem* proteolysis (Harper, 1999). It is generally considered that connective tissue does not change or degrade post-slaughter although small changes during tenderstretch hanging may contribute to increase the tenderness (Harper, 1999).

In muscle the amount of perimysial collagen is much greater than the endomysial fraction (McCormick, 1999). For a given animal, the inter-muscle variations in the amount of perimysium have been shown to be better linked to the variations in meat tenderness than the amount of endomysium (Light *et al.*, 1985). Also, the thickness of perimysium was significantly correlated with the shear-force value (Liu *et al.*, 1996). Nevertheless, Purslow (2005) stated that precise correlations between textural measures such as Warner- Bratzler shear force (WBSF) and collagen content are poor. Moreover, correlations between WBSF and perimysial content or perimysial thickness are also low (Purslow, 2005). However in a study developed by De Smet and coworkers (1998) shear force measures in raw meat reflected background toughness and was directly related to collagen content (Ngapo *et al.*, 2002). It is important to notice that in muscles with low collagen content like *longissimus dorsi*, this tissue might provide a limited contribution to background toughness in comparison with myofibrillar toughness (Ngapo *et al.*, 2002).

McCormick (1994) observed that, during growth and ageing, little variation exists in the perimysial collagen content of skeletal muscles. In the age-related toughening of meat it has been suggested that it is the quality of collagen which is responsible for progressive toughening of meat as animals grow older (Bailey & Light, 1989).

Meat tenderness generally decreases with animal age and collagen-rich muscles show this effect more than those with low intramuscular connective tissue content (Bailey & Light, 1989). As an animal matures, covalent cross-links between collagen fibrils become heat-stable and these links significantly increase meat toughness (Bailey & Light, 1989; McCormick, 1994, 1999; Purslow, 2005). Succinctly, the cross-links determine the extent of tension generated during heating and the residual adhesion between muscle fibres, *i.e.* the thermal and mechanical stability of intramuscular connective tissue. Moreover, collagen in muscle is relatively slow to turnover. Rucklidge *et al.* (1992) reported a half-life of 45 days. This gives time for slow modifications *e.g.* the conversion of divalent to trivalent cross-links between collagen molecules (Purslow, 2005). It is therefore not the amount of collagen present, but rather the degree of structural linkage in collagen that determines its contribution to meat tenderness (Bailey & Light, 1989; McCormick, 1994, 1999; Purslow, 2005).

There is a general shift with chronological ageing to increased proportions of type I collagen (Kovanen & Suominen, 1989). Type III collagen fibrils are smaller in diameter than type I collagen fibrils suggesting they should pose less resistance to shear force (Bailey, 1988). However, type III collagen also possesses a few disulfide bonds (unlike the other fibrillar collagens) and in cooked meat is apparently less heat soluble than type I collagen (Burson & Hunt 1986). These factors may in part account for the association of collagen type III with negative textural changes in muscle (McCormick, 1994).

Lepetit *et al.* (2000) concluded from their study that it is unlikely that a universal relationship between collagen content and cooked meat toughness could be derived, even for a constant level of collagen cross-links, as the contribution of collagen depends on the ability of the collagen fibres to contract during cooking and this contraction is dictated by the muscle fibres it surrounds.

In an experiment where there are large variations in the number of crosslinks per mole of collagen and little variation in collagen amount, tenderness will be highly correlated with the number of cross-links per mole of collagen and less so with collagen amount and conversely. However, when comparing different samples where both the collagen amount and the number of collagen cross-links per mole of collagen differ, then low correlations are observed between tenderness and either the collagen amount or the number of collagen crosslinks per mole of collagen (Lepetit, 2008). McCormick (1999) concluded that mature cross-links and collagen concentration have an additive effect on the toughening of meat. Accordingly, Ngapo and coworkers (2002) have shown that cross-link concentrations (per gram wet meat) are correlated with collagen amount ($0.54 < r < 0.95$), which reinforces the additive effect of collagen content and number of collagen cross-links on meat tenderness. For rubber-like

materials there is a high correlation between the elastic modulus and the breaking strength. So, this is why variations of elastic modulus of connective tissues in meat can be used to explain variations in Warner Bratzler breaking strength (Lepetit, 2008).

2.4.1.1.1.3 - Adipose Tissue

Fat can play different roles in meat tenderness, depending on whether it is subcutaneous fat, inter or intramuscular fat. Fat thickness can play a significant role in the reduction of cold shortening during the chilling processes of beef (Dolezal *et al.*, 1982). Increased thicknesses of subcutaneous fat were found to improve tenderness by allowing the carcass to chill more slowly and to increase enzyme activity. The authors aforementioned postulated that increased fatness either decreased chilling rate because of a greater amount of insulation or because of increased total mass. Moreover, they established that carcasses with only 2.54 mm of external fat received the lowest sensory panel ratings for myofibrillar tenderness, and had the highest shear force values.

Another positive effect of carcass fat in meat quality is not directly linked to meat tenderness, but indirectly through better moisture content. Johnson *et al.* (1988, quoted by Savell, *et al.*, 2005) stated that lean tissue retained less water than adipose after 20 h *post-mortem* and that more moisture loss occurs from lean than from fat tissue (Savell *et al.*, 2005). Increased fatness may decrease shrinkage by serving as a barrier against moisture loss (preventing evaporation from the lean), or it may act to minimize the total moisture content in the carcass resulting in both cases in tenderness increase. In fact, several authors found positive correlations between subcutaneous fat and tenderness (May *et al.* 1992; Jones & Tatum, 1994).

On the other hand, it is generally accepted that intramuscular fat (IMF) positively influences flavour, juiciness, tenderness and/or firmness and the overall acceptability of meat in different species (Wood *et al.*, 2008), although research results are quite controversial. There is a general agreement that very low levels of IMF lead to dry and less-tasty meat (Hocquette *et al.*, 2010). Several authors have stipulated that the minimum amount of IMF to achieve acceptable consumer satisfaction is about 3% to 4% for beef (Savell & Cross, 1988). However, the minimum content of intramuscular fat content still remains a controversial issue and its optimal level is difficult to predict. Other authors referred a minimum level of 1% for obtaining an acceptable organoleptic quality (Møller & Inversen, 1993). Hocquette *et al.* (2010) suggested that marbling improves meat tenderness by reducing bulk density and decreasing strength of the connective tissue, due to the fact that adipose tissue deposited

between perimysium surrounding muscle bundles. Moreover, the lubrication effect may improve quality through increased juiciness (Fiems *et al.*, 2000), since fat stimulates saliva production by salivary glands. This increased juiciness also gives the sensation of a tender meat.

Jeremiah and coworkers (2003) mentioned that the contribution of intramuscular fat to the variation in sensory palatability may explain only 10-15% of the total variance in palatability (Hocquette *et al.*, 2010). Measures of sensory palatability incorporate attributes such as tenderness, juiciness and flavour. Although the relationship between intramuscular fat, objective and sensory measures of tenderness is poor (Monteiro *et al.* 2009), the relationship between intramuscular fat content and flavour is usually strong.

It is important to notice, that the increase in intramuscular fat results from the general carcass fat increase. Thus the effect of the increase of these two types of fat is interrelated.

2.4.1.1.2 - Methods to determine meat tenderness

Meat texture can be assessed by both sensory and instrumental methods. Some attempts to relate sensory tenderness with mechanical and structural changes in meat during cooking were made. Nevertheless, this goal has not been fully achieve, yet. To better understand the relationship between sensory and mechanical measurement of tenderness, it is necessary to review some of the physical properties of meat and of the mastication process.

Sensory measurements can be made by a sensory panel or a consumers' panel. The first one implies training of the panellists, whilst the latter does not. Both methods are time consuming and costly. However, whilst in sensory panel the time and money costs are mostly during panel training and afterwards they are much more diluted, in consumers' panel the cost are always high. Also sensory panel are limited to a group of 10 to 16 trained people, whereas consumers' sensory evaluation must be done with hundreds of consumers, being much more subjected to personal experience. The questions that can be performed to a sensory panel are more consistent and the answers are based in a structured scale. Consumers' questions are personal opinions about the product and should be simple and direct, like: "Do you like it'" or "Which one do you prefer?", which is limiting.

During the mastication of meat, deformation and fracture of the samples takes place. The mechanical forces acting on meat are shear, compression and tensile forces. As meat is a composite, it is important to study in which structural element failure takes place, as well as where does the cracks propagate in order to be able to understand its mechanical properties (Tornberg, 2005).

The instrumental measurement of texture is made by a dynamometer that provides mechanical energy at constant speed. The result is a force *versus* time curve where, according to the geometry used in the test, the texture variation of the material is registered.

The empirical method of the WBSF is the most widely used for the assessment of the texture of whole meat. However, this technique rely on measuring a single parameter, and none fully imitate the complexity of the chewing motion (Duizer *et al.*, 1996).The most commonly used configuration is the one in which the shearing plane is perpendicular to the muscle fibres. Tensile, shear and compression forces operate in this type of test (Tornberg, 2005). The WBSF technique is, however, the instrumental technique that usually yields the best correlation with sensory panel scores for meat toughness. More recently, texture profile analysis test (TPA) has started to be used for texture tests. However, despite being used with success in several food and non-food products, measuring several textural attributes at the same time and being as easy to implement as the WBSF technique, it is not used by meat researchers. TPA compresses a bite sized food, usually with 1 cm cube, in order to simulate the chewing action of the teeth. In this method frequently called "two bites test", the probe compresses twice the material with a lag time between the two actions. Compression is usually 80% of original length of the sample (Sahin & Sumnu, 2006). The main advantage of TPA is that with one measurement several variables can be assessed (Huidobro *et al.*, 2005). The variables measured with TPA test are:

Hardness – It gives the maximum force necessary to compress the sample. In mouth, hardness is the force required to bite completely through sample with the molars.

Fracturability – The force during the first compression at which the material fracture.

Cohesiveness – It gives the extent to which the sample could be deformed prior to rupture. In mouth, cohesiveness is felt like the amount of deformation undergone by the material before rupture when biting completely through the sample with molars.

Springiness – It represents the ability of the sample to recover its original form after the deforming force is removed. In mouth, springiness is the force with which the sample returns to its original shape or size after a partial compression, without failure, between the tongue and the palate.

Adhesiveness – It represents the force necessary to pull the plunger away from the beef. In mouth adhesiveness is the force required to remove product completely from palate, using tongue, after compression of the sample between tongue and palate.

Resilience – It represents the property of a material to absorb energy when is deformed elastically and then, upon unloading to have this energy recovered. In other words, it is the maximum energy per unit volume that can be elastically stored.

Chewiness – The energy necessary to chew a solid sample to a steady state of swallowing (hardness x cohesiveness x springiness).

Tornberg (2005) suggested that the reason for the toughness observed for the raw meat up to about 50 ºC is based on the fact that the applied stress during mastication is reduced by viscous flow in the fluid-filled channels in between fibres and fibre bundles. Between 50 and 65 ºC the formation of a gel of aggregated sarcoplasmic proteins glues the fibres and fibre bundles together. The viscous flow becomes then lower as the elasticity of the meat increases. Thus, there is a higher probability of the applied stress being transferred within the material to the crack, without any viscous dissipation of energy and therefore propagating it. Afterwards meat is more easily fractured in the mouth, mastication is facilitated and tenderness improved. However, above 65 ºC elasticity acts adversely and impairs the tenderness.

For a fully brittle fracture in a linearly elastic material (cooked meat above 65 ºC behaves rubber like, *i.e.* an elastic material; Lepetit *et al.*, 2000) higher elastic modulus gives rise to larger tensile stresses to extend a crack (Jowitt, 1979). Therefore, it seems that the contraction of the connective tissue gives rise to an increasing in the elasticity of the meat by forming a much denser material in the temperature region of 65-80 ºC and thereby a tougher meat (Lepetit *et al.*, 2000; Tornberg, 2005).

2.4.1.2 - Juiciness

Juiciness represents the character more or less dry of meat during the eating process. It can be divided in two components: first the juice that is released during the first mastication as a result of the breakage of the meat structure and second the increase production of saliva, due to the stimulation effect of intramuscular fat on the salivary glands.

Juiciness depends not only of each component of meat composition but also of the relation between them during mastication. Juiciness is dependent of water holding capacity of meat. The factors that affect one of them also affect the other (for more detailed explanation see 2.4.2.1 sub-section).

2.4.1.3 – Flavour

Flavour and aroma are sensory attributes that significantly influence consumer acceptability of beef (Shahidi, 1998). Although raw beef has little aroma and only a blood-like flavour, it contains all the compounds that will subsequently become flavours and flavour enhancers during the cooking process.

The flavour precursors include free amino acids, peptides, reducing sugars, nucleotides, lipids and vitamins. Interactions between these molecules and/or their degradation products via complex chemical reactions such as the Strecker degradation and the Maillard reaction produce a large number of intermediates and/or volatiles that create the flavour and aroma of beef (Melton, 1982; Mottram, 1998).

Some controversy remains about the relative contribution made by lipid components of meat as opposed to the other classes of molecules, in regard to the determination of flavour and aroma. Early research showed that the fat tissues in meat were the source of the characteristic species flavour (Mottram, 1998). Lipids take on their greatest significance in generation of unpleasant flavours in meat during storage (Skibsted *et al.* 1998). These flavours are generated by the hydrolysis of triacylglycerols and phospholipids, as well as the oxidation of fatty acids. Oxidative decomposition of unsaturated lipids is the main factor in off-flavour generation and deterioration of beef (Skibsted *et al.*, 1998). The effect of fatty acid on meat flavour is due to the production of volatile, odourous, lipid oxidation products during cooking. The unsaturated fatty acids from phospholipid are particularly important in flavour development (Mottram, 1998). Studies results showed that the samples with high *n*-3 polyunsaturated fatty acids (PUFA) concentrations produced higher concentrations of lipid degradation products, particularly saturated and unsaturated aldehydes, alcohols and ketones. Aldehydes were quantitatively the most important and since they have low odour thresholds they are thought to be the reason for the changes in flavour of the modified packaged beef (Elmore *et al*, 1999) and of grass fed animals (Larick & Turner, 1989; Priolo *et al.*, 2001). Some of these aldehydes are more likely to derive from 18:1 *c*9 and 18:2 *n*-6 than from 18:3 *n*-3. It is suggested that free radicals formed from the more unsaturated and easily oxidised *n*-3 PUFA initiated the oxidation of these more abundant fatty acids (Elmore *et al.*, 1999).

2.4.2 - Physic properties of meat

2.4.2.1 - Water Holding Capacity

Lean muscle contains approximately 75% of water. The water molecule is V-shaped. The electronegative oxygen atom pulls the electrons from the covalent bond between the oxygen atom and the hydrogen atoms. As a result hydrogen atom charges with a partial positive charge and the oxygen atom with a partial negative charge. Therefore, water molecules have a small size, a high dipole moment, an ability to form hydrogen bonds and a very high dielectric

constant (Puolanne & Halonen, 2010). These characteristics will influence water behaviour in the muscle fibre.

During meat processing one major problem is water loss, which is frequently expressed as drip loss, expressible water, cook loss, and cooling loss depending on the stage of processing in which it is measured. Water loss from raw meat can be obtained by evaporation from the surface, and as exudates, when a muscle is cut. The exudates is called drip loss, and is drained from the cut surface of the meat by gravity, if the viscosity of the exudates is low enough and the capillary forces do not retain it. Offer *et al.* (1989b) have confirmed that this drip loss arises predominantly from the longitudinal channels through the meat between the fibre bundles (Tornberg, 2005). The ability of meat to retain water is defined as water holding capacity (Grau & Hamm, 1956, quoted by Cheng & Su, 2008).

Loss of water has a substantial influence on product quality, as a higher loss of water gives an expectation of a less optimal quality, due to shrinkage (which if excessive can have an adverse effect on product appearance) of products. Moreover, greater water losses result in drier meat and consequently less juicy. It does not only affect the juiciness, one of the most important meat quality attributes for consumer, but also impacts negatively tenderness (Oddy *et al.*, 2001; Cheng & Su, 2008). It also reduces the weight of the product, which implies financial loss.

Since water is a dipolar molecule it is attracted to charged species (Puolanne *et al.*, 2010). Most of the water in the living muscle is held within the myofibrils (about 80%), in the spaces between the thick and thin filaments, and between the myofibrils and the sarcolemma (Huff-Lonergan & Lonergan, 2005). Water is in the muscle fibre as a lubricant, as well as a medium to transport metabolites in the fibre. Hydrophilic and hydrophobic areas in the proteins must be well organized to allow rapid translocations, as well as structural elements should provide optimal filament distances at each level of contraction (Puolanne & Halonen, 2010). Water holding is caused by the electrostatic repulsion between the myofibrillar proteins (myofilaments), which results in a swelling of the myofibrils, or in some cases (*e.g.* with salts or at very low or high pH) even a partial solubilisation of filaments (due to the repulsions between individual molecules). The various cross-bridges between the myofilaments (Z-lines, actomyosin cross-bridges and intermediate filaments) prohibit the unlimited swelling of the myofibrils.

The conversion of muscle to meat affects water holding capacity and consequently water losses. The first modification is a cellular swelling by the increased intracellular osmolarity *post-mortem*, where the membrane structures are rendered almost intact resulting in increased intracellular water volume. During the *rigor mortis* both lateral and longitudinal contraction

occurs, giving rise to the second water redistribution phase. As the lateral spacing of filaments declines, sarcoplasmic fluid is forced out from the myofibrillar structure into the extracellular space, giving rise to water compartments around the fibres and the fibre bundles (Oddy *et al.*, 2001; Tornberg, 2005). These compartments give rise to a more viscous behaviour in the raw state, as compared to the cooked.

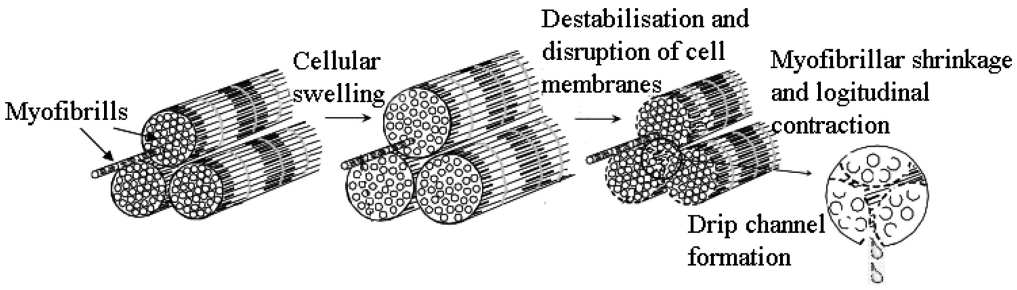
The rate of pH decline also influences the drip losses. The degradation of the muscle proteins in *post-mortem* muscle is associated with drip loss. The way it happens is strictly related with glycolytic rate. The most severe purge or drip loss is often found in PSE (Huff-Lonergan & Lonergan, 2005).

As the pH drops, near to the iso-electric point (myosin pI=5.4), the net charge of the proteins is zero. This means that all of the negatively and positively charged amino acid side chains are equal, which causes the maximal attraction between the two. This attraction holds the filaments closely together and does not allow any water to get in, greatly reducing the water-holding capacity (Smulders *et al.*, 1990).

Research has suggested that reduced degradation of proteins that tie the myofibril to the cell membrane (such as desmin) may allow shrinkage of the myofibril to result in shrinkage of the muscle cell. This shrinkage opens drip channels and results in increased drip loss (Morrison *et al.*, 1998; Kristensen & Purslow, 2001). Therefore, increased degradation of proteins (like desmin) could prevent myofibril shrinkage from being effectively transmitted to the entire cell and would allow more moisture to reside in the tissue.

The cooking procedure will induce mass loss of meat products, namely cooking loss, which is a combination of liquid and soluble matters lost from the meat during cooking. During cooking, compositions are lost from the meat in the form of vapor, which is almost entirely water, and drip, which is made up of liquefied fat and water (Cheng & Su, 2008). The water is probably lost due to heat-induced protein denaturation during cooking of the meat, which causes less water to be entrapped within the protein structures held by capillary forces.

Figure 5. Water loss mechanism in *post-mortem* muscle (adapted from Bertram *et al.*, 2002).



Several factors affect the cooking loss of meat products, including raw meat quality, pre-cooking treatment, cooking technique, heating rate, cooking and endpoint centre temperature. Cheng and Su (2008) reported that different cooking methodologies can introduce variation into cooking loss of meat products, and thus affect their eating quality. Cooking losses increases with temperature increasing, no matter de cooking technique. Shortened muscles show a higher cooking loss. The larger extracellular space, when shortened, gives more room for the connective tissue to contract without being constrained by the myofibrillar fraction (Tornberg, 1996). This in turn gives a higher number of fibres per unit cross-sectional area, hence a larger elastic modulus and consequently a higher WBSF (Tornberg, 2005).

The amount of mass loss depends also on the cooking rate, *i.e.* holding time at each temperature. Normally, since the cooking rate is related directly to the efficiency of heat transfer and temperature, a higher cooking temperature will lead to a higher heating rate and a shorter cooking time. A low media temperature (about 65 ºC) and slow heating rate could achieve a good texture with high binding ability (Cheng & Su, 2008). The greatest increments in cooking losses were in the temperature ranges 50–60ºC and 60–70ºC, which may be explained by the contracted protein system causing expulsion of water (Cheng & Su, 2008).

2.4.2.2 - Colour

Colour is the first meat attribute evaluated by consumers in the purchase moment. From the possible colours that beef can assume, bright red is generally the one preferred by consumer (Oddy *et al.*, 2001; Mancini & Hunt, 2005).

The characteristic colours of meat predominantly result from the interaction of oxygen with myoglobin in muscle. Meat colour is not merely a reflection of the myoglobin content of the different muscle fibre types within muscle, but is also a function of exposure to oxygen and light at point of sale (Kropf 1980), and the *in situ* biochemical changes that occur in *post-mortem* muscle.

The meat colour is mainly determined by the amount and chemical state of heme pigments, myoglobin and hemoglobin. Myoglobin is the main protein responsible for meat colour, although other heme proteins such as hemoglobin and cytochromes may also play a role in beef colour (Mancini & Hunt, 2005). Myoglobin concentration in muscle is dependent on dietary availability of iron and copper. In the living animal, only 10% of the total iron comes from myoglobin, but after the slaughter and bleeding of the animal, most of the hemoglobin is eliminated, so the iron proportion from myoglobin increases to 95% (Fenemma, 2007). However, the greatest influence on meat colour is the ultimate pH of meat (Shorthose &

Harris, 1991), which is mainly determined by glycogen content at slaughter. The low muscle glycogen and consequently high ultimate pH, results in *post-mortem* mitochondrial respiration extension, therefore prolonged deoxygenation of myoglobin, and hence dark meat. On contrary, high muscle glycogen at slaughter favours extended anaerobic glycolysis and increased lactate accumulation that generates a lower ultimate pH (in the range 5.4–5.5). Under these conditions, myoglobin is oxygenated and meat colour is light (Hunt, 2009).

Colour in meat is therefore related to ultimate pH, which in turn, is related to tenderness (Purchas 1990; Jeremiah *et al.* 1991). Hence, meat colour is not only of importance for visual appraisal by the consumer, but can also be used as a mean of grading meat to ascertain tenderness (Wulf & Wise 1999).

2.4.2.2.1 - Myoglobin chemistry

Myoglobin is a globular, water soluble protein containing eight α-helices linked by short non-helical sections. Myoglobin also contains a prosthetic group *i.e.*, the heme group. The heme ring, called porphyrin, has a centrally located iron atom that can form six bonds. Four of these bonds are with pyrrole nitrogens joined together by methane bridges. The 5th bond coordinates with the proximal histidine. A 6th site is available to reversibly bind a ligand. The ligand present and the valence of the iron dictate muscle colour (Mancini & Hunt, 2005). This ring structure form stable complexes with many metal ions, being the ones formed with the transition elements cobalt and iron, the biologically most important (Bandman, 1994).

Four major chemical forms of myoglobin are primarily responsible for meat colour (Mancini & Hunt, 2005). Deoxymyoglobin occurs when no ligand is present at the 6th coordination site and the heme iron is in ferrous (Fe^{2+}) form. This result in the purplish-red colour typically associated with vacuum packaged product and muscle immediately after cutting. However, when exposed to oxygen, oxygenation occurs with the development of a bright cherry-red colour. No change in iron's valence occurs during oxygenation although the sixth coordination site is now occupied by an oxygen molecule. In addition, the distal histidine interacts with bound oxygen, altering the structure and stability of myoglobin. As exposure to oxygen increases, the oxymyoglobin penetrates deeper beneath the meat's surface. Depth of oxygen penetration and thickness of the oxymyoglobin layer depend on the meat's temperature, oxygen partial pressure, pH, and competition for oxygen by other respiratory processes (Mancini & Hunt, 2005).

The oxidation of the central iron atom within the heme group is responsible for discoloration, a change from red oxymyoglobin to brownish metmyoglobin. In this case, ferrous heme iron

oxidizes to its ferric form (Fe^{3+}), oxygen is released and replaced by a water molecule (Faustman *et al.*, 2010). Metmyoglobin formation depends on numerous factors including oxygen partial pressure, temperature, pH, meat's reducing activity, and in some cases, microbial growth (Mancini & Hunt, 2005).

Reduction of metmyoglobin is crucial to meat colour life and greatly depends on muscle's oxygen scavenging enzymes, reducing enzyme systems, and the NADH pool, which is limited in *post-mortem* muscle (Faustman *et al.*, 2010). Both enzyme activity and the NADH pool are continually depleted as time *post-mortem* progresses. Thus, oxymyoglobin is not converted directly to deoxymyoglobin. Firstly, it proceeds through the ferric redox state at low-oxygen partial pressures, when occurs removal of oxygen atom via oxygen consumption, which likely results in oxidation of oxy- to metmyoglobin. From a practical standpoint, the next step is often troublesome because subsequent deoxymyoglobin formation will depend on the muscle's reducing capacity plus further reduction in oxygen tension (Mancini & Hunt, 2005).

2.4.2.2.2 - Instrumental Colour

Several options are available to measure colour, which encompasses different measure devices. Each instrument offers a variety of options concerning colour system (Hunter, CIE and tristimulus), illuminants (A, C, D65, and Ultralume), observation angle (2º and 10º) and aperture size (0.64-3.2 cm).

This review will only discuss the CIE, since on one hand it is commonly used by researchers to measure colour, and by the other hand, it was the one used in the trials that gave rise to this review. Briefly, in the CIE L* a* b* system, the L* component is called brightness (value), and a* and b* are called chromaticity coordinates. L* represents the difference between dark (0) and light (100). The coordinate a*(redness) measures the ratio between green (-60) and red (+60) and the coordinate b* measures the ratio between blue (-60) and yellow (+60). The chromaticity coordinates can also be used to calculate the hue angle (h*) and chroma (C*). The hue angle corresponds to what is commonly called colour, being function of the wavelength of reflected light, while chroma indicates how pure the colour is, *i.e.*, the degree of deviation for gray (Sahin & Sumnu, 2006). The chroma indicates the perceived intensity of a determined colour and depends mostly on the myofibrilar structure and on the ultimate meat pH (Renerre, 2000). The hue angle depends mainly on pigment content and chemistry (Renerre, 2000; Touraille, 1991).

The hue angle can be determined by $h^* = \tan^{-1} (b^*/a^*)$ and the chroma by $C^* = \sqrt{(a^{*2} + b^{*2})}$.

2.4.3 – Nutritional value of meat lipids

2.4.3.1 - Long chain fatty acids

The recent dietary guidelines are focused on the absolute amounts of specific PUFA intake and recommend mainly the increase in the consumption of long-chain PUFA (EPA and DHA). The *n*-3 LC-PUFA, particularly EPA and DHA, are widely recognized to have beneficial effects on human health. The beneficial effects include antiatherogenic, anti-thrombotic and anti-inflammatory effects and overall, increased intake leads to a reduced risk of coronary heart disease (Givens & Gibbs, 2006). They are critical for proper brain and visual development in the foetus, the maintenance of neural and visual tissues throughout life (Calder, 2004; Leaf *et al.*, 2003) and may have roles in reducing cancer and obesity/type-2 diabetes (WHO, 2003). Attention has also been focused on the extent to which consumption of the precursor of the *n*-3 series, α-linolenic acid can provide sufficient amounts of tissue EPA and DHA through the *n*-3PUFA elongation–desaturation pathway (Williams & Burdge, 2006). Recent reports as questioned the nutritional importance of increased α-linolenic acid concentration as α-linolenic acid is not as bioactive as are longer chain omega-3 fatty acids such as EPA and DHA (Harris, 2007), and its conversion to *n*-3 LC-PUFA is considered poor (Enser, 2001). Therefore, increasing the consumption of products rich in EPA and DHA became more important. These long chain fatty acids are only found in animal fat, fatty fish in particular being an excellent source (Schmid, 2011).

Conjugated linoleic acid (CLA) is a collective term for a group of conjugated isomers of linoleic acid. CLA is found predominantly in ruminant derived products (Griinari *et al.*, 2000). Biological effects have been widely investigated in CLA isomers, however, finding are only consistent for two of these isomers, *c9*,*t*11 CLA (rumenic acid) and *t10*,*c12* CLA. The anticarcinogenic and antiatherogenic effects of *c9*,*t11* CLA and the anti-obesity effects of *t10*,*c12* CLA have been well documented (Belury, 2002). In addition it is also believed that CLA modulates immunity and thrombosis as well as fatty acid biochemistry, lipid metabolism and gene expression in the liver, muscle and adipose tissue (Belury, 2002). Some authors have postulated that *t*10,*c*12 CLA is solely responsible for the reduction of body fat gain, whereas the *c*9,*t*11 CLA isomer enhances growth and feed efficiency in young rodents. In other cases the isomers act together to induce an effect (Pariza, 2004). Despite CLA have health benefits in the human diet meat from ruminants makes only a small contribution towards nutritionally significant levels, though.

2.4.3.1.1 – Nutritional indexes

It is important to refer also that the usefulness of the nutritional index like P/S (polyunsaturated/saturated fatty acids; (18:2 *n*-6 + 18:3 *n*-3) / (14:0 + 16:0 + 18:0)) and n-6/n-3 (ω6 polyunsaturated fatty acids/ω3 polyunsaturated fatty acids) has indicators of healthiness of the meat lipid profile has been demystified and considered misleading, as the fatty acids were grouped in a wrong way. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), as technical agencies of the United Nations, are charged with providing science-based guidance on food and nutrition to national governments and the international community. The aim is to define requirements, the nutritional requirement values and the corresponding nutrient-based recommendations (Burlingame *et al.*, 2009). The overall goal of these recommendations is to support the health and nutritional well-being of individuals and populations (Burlingame *et al.*, 2009). One of the recommendations is that the intake of SFA should not exceed 10% E to keep cholesterol levels in a normal range and to reduce the risk of CHD. However, individual SFA have different effects on plasma cholesterol levels. Lauric (12:0), myristic (14:0), and palmitic (16:0) FA increase low density lipoproteins (LDL) and high density lipoproteins (HDL) cholesterol (Katan *et al.*, 1994), which can be explained by the observed reduction in LDL receptor activity protein, and amount of mRNA (Fernandez & West, 2005). Nevertheless, stearic acid decrease LDL-cholesterol (Mensink, 2005). The different mechanism is attributed to the high conversion rate of 18:0 to 18:1 (Lichtenstein, 2006). Polyunsaturated fatty acids and MUFAs had similar effects on plasma LDL-cholesterol levels, whereas SFAs significantly raised LDL levels (Elmadfa & Kornsteiner, 2009). Considering, that stearic acid is considered a beneficial FA regarding its effect on plasma cholesterol in humans, or even to decrease LDL-cholesterol (Mensink, 2005), the P/S ratio might not be the most appropriate nutritional index to evaluate FA in ruminant meats.

Recently, the nutritional relevance of the *n-6/n-3* FA ratio, as a risk factor for some cancers and coronary heart disease of humans has been discredited (Griffin, 2008). First, there is evidence that increasing linoleic acid intake does not result in increased arachidonic acid in plasma or platelet lipids, and does not increase formation of pro-inflammatory mediators (Adam *et al.*, 2003). Furthermore, both *n*–6 and *n*–3 fatty acids have been shown to have anti-inflammatory properties that are protective of atherogenic changes in vascular endothelial cells (De Caterina *et al.*, 2000).

2.4.3.2 – Cholesterol

Cholesterol is a sterol that in higher animals is the precursor of several biological compounds like bile acids, vitamin D3 and steroid hormones. Cholesterol is also a key constituent of cell membranes, mediating their fluidity and permeability. It is mainly synthesized by the liver and other tissues, but a small part can also be absorbed from dietary sources. Dietary cholesterol enters the body by way of the chylomicron pathway and is removed from the plasma by the liver as a component of chylomicron remnants (Hur *et al.*, 2007). Cholesterol is a monounsaturated lipid with a double bond on carbon-5, and is susceptible to oxidation in the presence of oxygen, light, heat, radiation, free radicals, metal ions, and other factors (Hur *et al.*, 2007).

Cholesterol can be oxidised and the resulting oxidation products are also sterols with similar structure to cholesterol, containing an additional hydroxy, ketone or epoxide group on the sterol nucleus and/or a hydroxyl group on the side chain of their molecules, though. These cholesterol oxidation products exert a number of adverse effects in biological tissues and have been implicated in the initiation and progression of a number of diseases, particularly atherosclerosis (Kumar & Singhal, 1991).

The contribution of beef to dietary cholesterol and its effect on serum cholesterol is a controversial issue that has received much attention from both health professionals and consumers. This is due to the role of high serum cholesterol as one of the primary risk factors in the incidence of coronary heart disease. Previous studies concerning cholesterol content of beef provide no consensus as to the role of fat and lean in contributing to total cholesterol content. Cholesterol *per se* is not the agent because LDL, which contains cholesterol, is pro-atherogenic whereas HDL, which also contains cholesterol, is anti-atherogenic (Olson, 1998).

Chizzolini and co-workers (1999) presented a mean value for beef cholesterol of 60 mg/ 100 g muscle. However the mean value these authors presented for *longissimus dorsi* muscle (47 mg/ 100 g muscle) was slightly lower than that. Differences in fibre type might be the most likely reason for some of the differences observed in cholesterol content among different muscles of the same species, and from the same muscle of different species. Oxidative muscles are richer in phospholipids and the higher the phospholipids content the higher the cholesterol content (Chizzolini *et al.*, 1999). This relationship is related to the physical effect of cholesterol in ordering the phospholipids chains contributing to maintain the membrane fluidity (Alasnier *et al.*, 1996).

Cholesterol values in meat do not change appreciably on cooking, since negligible amounts are lost from membranes (Lewis *et al.*, 1993). The role of dietary cholesterol on serum

cholesterol concentrations, although disputed in some cases, appears to be important only for people genetically predisposed to hypercholesterolemia (Chizzolini *et al.*, 1999).

2.4.3.3 – Vitamin E

The antioxidant activity of vitamin E in meats has been widely investigated. D-α-, D-β-, D-λ- and D-δ-tocopherols, together with the corresponding tocotrienols, are the natural compounds with vitamin E activity (Kerry *et al.*, 2000). Although the major form of vitamin E in meat is α-tocopherol, minor amounts of other vitamin E homologues also exist. Tocopherol is a fat-soluble vitamin that acts as a potent antioxidant in biological systems, being capable of quenching free radicals and thus, protecting the highly oxidizable polyunsaturated fatty acids in cell membranes from peroxidation by reactive oxygen species, preventing flavour, colour and nutritional deterioration of meat during storage (Gray *et al.*, 1996; Morrissey *et al.*, 2000). α-Tocopherol is dependent upon micellar formation for transport across intestinal membranes (Hollander, 1981) where it is incorporated into lipoproteins and secreted into the intestinal lymph (Bjørneboe *et al.*, 1990) for distribution to other tissues. High concentrations of other components compete for these sites in the micelle and in the absorption and transport process (Yang *et al.*, 2002). Intestinal absorption of α-tocopherol is facilitated by high levels of dietary fat (Wolf, 1984). Meat of grazing cattle would be expected to have good colour stability and a reduced predisposition to oxidation or rancidity development, properties similar to those observed for vitamin E supplemented grain-fed cattle, since that grazing animals on good quality pasture resulted in elevated concentration of vitamin E in muscles (Arnold *et al.*, 1993).
It is interesting to note, that there is evidence that α-tocopherol is not degraded in the rumen (Leedle *et al.*, 1993).

2.4.3.4 – Pro-Vitamin A compounds

Carotenoids are a class of lipophilic pigments that are found in nature mainly in a wide variety of fruits and vegetables, but also in beef. β-Carotene is a pro-vitamin A, another important fat-soluble antioxidant that quenches sites localised within the hydrophobic region of biological membranes, contrasting with the scavenging activity of a-tocopherol close to the membrane surface (Fukuzawa *et al.*, 1998). β-Carotene is essentially the only carotenoid absorbed at the level of intestine and, therefore, the predominant carotenoid found in meat (Yang *et al.*, 2002). Intestinal absorption is facilitated by high levels of dietary fat. This

carotenoid has been suggested to have an important role in controlling oxidatively induced diseases, such as cancer and atherosclerosis (Decker *et al.*, 2000). Although β-carotene is less reactive than α-tocopherol, both antioxidants can exert a co-operative antioxidant activity at different positions within the membrane. However, high concentrations of vitamin E may interfere with the absorption of β-carotene and, as both β-carotene and α-tocopherol are bound to lipoproteins when transported in the blood, they may compete with each other for binding sites on the lipoprotein molecule (Yang *et al.*, 2002).

Descalzo *et al.* (2005) and Insani *et al.* (2008) realised that pasture-fed animals had significantly more β-carotene than grain-fed animals. It is also important concerning meat quality from consumer point of view that high concentrations of β-carotene can display an unpleasant yellow colour to the beef fat (Yang *et al.*, 2002).

Since colour is the first meat attribute evaluated by consumers, and that it is subject to *post-mortem* oxidation resulting in discoloration, the oxidative stability of muscle depends on the balance between anti-oxidants (α-tocopherol and β-carotene) and pro-oxidants including the concentration of PUFA and free iron in the muscle (Kanner, 1992) in order to control the oxidation rate of myoglobin.

2.4.3.5 – L-Carnitine

L-Carnitine (L-CA) is a small molecule derived from lysine. Trimethyllysine is formed from methylation of lysine, and subsequently converted to butyrobetaine in all tissues. The butyrobetaine is finally hydroxylated to carnitine in the liver and, in some animals, in the kidneys. It is released from these tissues and is then actively taken up by all other tissues (Bremer, 1983). Thus, in mammals L-CA is synthesized mainly in the liver and in some species in the testis and kidney (Demarquoy *et al.*, 2004). During the enzymatic pathway several cofactors are also needed such as vitamin C and iron, making this biosynthesis strongly influenced by the bioavailability of these elements. After its biosynthesis, carnitine is excreted out of the cell and transported by the blood stream to organs and tissues depending upon fatty acid oxidation for their energetic needs.

The primary function of L-CA is to allow the entry of esterified fatty acids into the mitochondrial matrix, where β-oxidation of fatty acids occurs (Demarquoy *et al.*, 2004). Thus, due to its important role in fatty acid transport towards mitochondrial membrane, L-CA is a key element in fat metabolism. Recent studies have shown that L-carnitine can act as antioxidant in meat, suppressing oxidative damage during ageing, inhibiting lipoperoxidation of linoleic acid and protecting against damage induced by H_2O_2 (Djenane *et al.*, 2004). Thus,

L-CA is involved in the peroxisomal oxidative metabolism and serves as a cofactor for various enzymatic reactions.

L-CA deficiency can cause some disorders like muscle atrophy, neurological disorders and heart diseases. These symptoms are linked to the main role of L-CA on fatty acid metabolism.

L-CA can be supplied to the body through endogenous biosynthesis (from lysine and methionine) and food intake (Vaz & Wanders, 2002). With a regular (omnivorous) diet, it seems that at least 80% of the L-CA found in the body comes from dietary intake. Most of the dietary L-CA is provided by meat, fish and dairy products (Demarquoy *et al.*, 2004), nevertheless salmon, which is one of fish with higher L-CA content, has about 10 times less L-CA them meat (Rigault *et al.* 2008). L-CA in tissues is present in free and esterified forms. Free L-CA occupies about 80% of total L-CA under normal circumstances (Demarquoy *et al.*, 2004). Rigault *et al.* (2008) realised that cooking do not significantly alter these values. In the quoted authors study, they obtained 335 µmol/ 100 g muscle of free L-CA percentage in *longissimus* muscle, which corresponded to 79% of the total L-CA. The recommended L-CA intake ranges from 2 to 12 µmol/kg/day (Galland *et al.*, 2001). Considering a 70 kg human being the daily average recommendation is of 490 µmol. To meet this recommendation, an average 100-150 g of meat should be daily eaten (Rigault *et al.* 2008).

2.5- Consumers' concerns and expectations

In the last decade, food scares have increased consumers' concern for food safety accompanied by significant reductions in the consumption of the affected products. Meat has been a prime target of these food scares, but within all meat types beef was by far the most affected (Eurostat, 2009). The perceived risk associated with beef also depends on specific incidents related to this product. However, consumers' concerns are not limited to the fear that bovine spongiform encephalopathy (BSE) might have significant impacts on human health, the hormone abuse in the meat sector, exemplified by the illegal use of the bovine growth hormone (Angulo & Gil, 2007) or the indiscriminate use of antibiotics are also motive for consumer concern. Verbecke (2001) reported that after the BSE crisis, consumers intended to decrease beef consumption in the future. Then in a follow-up study, after the dioxin scare, the consumer image of poultry and pork was negatively affected, whereas the safety perception of beef improved after it had been initially damaged by the BSE scare (Angulo & Gil, 2007). These situations lead to the replacement of some types of meat by others, with the consequently decrease consumption of the affected meat and increase of the others (Eurostat,

2009). As consequence, new trends occurred in the fresh beef market as reflected in the demand for higher quality and the drive to differentiate beef on the basis of product brandings, geographical origin, sensory or processing characteristics (Scollan *et al.*, 2006). Consumers' demand for beef is increasingly influenced by their concerns about the healthfulness, quality, nutritional content and safety of the foods they consume, and by the growing demand for intangible attributes such as animal welfare and environmental impacts of production and marketing. Consumers' quality judgments of food depend on the perceptions, needs and goals they have (Steenkamp, 1990). The concept of quality is essentially defined by the consumer and, therefore, is not easy to measure (Grunert *et al.*, 1996). Many aspects can be used by consumers to perform their food choices. Intrinsic (cut, colour and fat content) and extrinsic cues (price, origin, production and nutritional information) are used to form expectations about product quality attributes, the latter being classified in two categories: those experienced before or during consumption (experienced quality attributes; price and sensory attributes) and those not directly experienced, such as healthiness, naturalness and ethical aspects (Napolitano *et al.*, 2007). Even after the product consumption, these so called credence attributes, like animal welfare, environmental protection, food safety and origin (Becker, 2000), cannot be measured or in some way guaranteed. In this respect, quality labels have a positive effect on the quality of the meat perceived by the consumer. The creation of meat quality labels, normally under geographical groupings and with specific genotypes and production systems, conforms to the increasing demand by consumers for guarantees of quality (Guerreiro, 2001). Concerning food safety, certification strategies (traceability or quality labels) have been developed to improve consumer perception of food safety, which generates an increase of the product final price (Angulo & Gil, 2007).

Regarding animal welfare and environmental concerns, the promotion of products with certain characteristics of considerable benefit to the rural economy (particularly to less-favoured or remote areas), with a more conservative environment impact and being more animal friendly meets the European Union legislation, as part of the adjustment of the common agricultural policy. The great confidence consumers attribute to the quality labels is related to the growing concern for health, nutrition and food safety aspects, where quality labels are an indication that meat has undergone to a certain type of control (Verbecke & Ward, 2006) assuring food safety. The belief that organic farming is safer is related to the banning of fertilizers and pesticides in crop production, genetically modified organism food and the use in feeds of meals made with products of animal origin (Napolitano *et al.*, 2007). It seems interesting that quality label beef is perceived by consumers to be more expensive

compared to conventionally produced beef. However, the relationship between the higher price and the added quality value is not very clear. It is not surprising that meeting certification requirements usually implies higher production costs (Sepúlveda *et al.*, 2008), mainly because it is assumed that beef has a production system that undergoes strict controls which in turn imply higher costs. However, some consumers are willing to pay higher beef prices to reassure safety but others will trade off safety improvement against the price (Sepúlveda *et al.*, 2008). In addition, the main limit to purchasing certified meat remains the price. The higher price can be due to high production costs, which are affected by specification rules (*e.g.*, high space allowance and origin of feedstuffs) and by the small-scale production system of these products. One strategy to overcome this problem may be the introduction of increased willingness to pay by constant and reliable quality signaling systems capable to provide an ethical value to the product, which may become even higher if associated to traditional farming and typical meat productions (Napolitano *et al.*, 2007).

In some countries, studies revealed that amongst production credence quality attributes of beef, animal feeding and the region of production and/or of origin are the aspects most valued by consumers (Napolitano *et al.*, 2007; Schnettler *et al.*, 2009; Sepúlveda *et al.*, 2008). Our work team realised that in Portugal origin and brand are also most valued by consumers (Banovic *et al.*, 2010). Despite all the importance consumers assign to the extrinsic meat quality, as Wandel and Bugge (1996) point out, the expressed concerns of consumers in relation to environmental and animal welfare issues do not mean that behaviour has changed accordingly (Bernués *et al.*, 2003). Several authors stated that unless beef attained a minimum value of sensory quality, mainly in tenderness, consumers will not choose or accept it.

Delivering the quality attributes demanded by the consumer, together with impartial and reliable information (cues), are key actions that will enable many meat industries to stay in business or to expand (Bernués *et al.*, 2003). This is particularly important concerning certified beef, which is more expensive than commercial beef rear in an intensive manner. That is also because certified beef create more expectations to the consumers not only on beef quality but also on product homogeneity. Consumers expect that when repeat the purchase the quality remains the same. Logically, the relative importance of these attributes will differ between consumers with different social, cultural and economic characteristics (Verbeke & Viaene, 1999).

3 - Intramuscular lipids of Mertolenga-PDO beef, Mertolenga-PDO veal and "Vitela Tradicional do Montado"- PGI veal

A.C.G. Monteiro, M. A. Fontes, R.J.B. Bessa, J.A.M. Prates & J. P.C. Lemos

CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Avenida da Universidade Técnica, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal

Adapted from *Food Chemistry* (2012), 132, 1486-1494

Intramuscular lipids of Mertolenga-PDO beef, Mertolenga-PDO veal and "Vitela Tradicional do Montado"- PGI veal

Abstract

Quality of three branded meats (*n*=68), "Vitela Tradicional do Montado"-PGI veal, Mertolenga-PDO veal and Mertolenga-PDO beef were analyzed for cholesterol (HPLC-UV), α-tocopherol (HPLC-FD), fatty acid composition (GC-FID), including conjugated linoleic acid (CLA) isomeric profile (Ag^+-HPLC), and nutritional value of lipids. All the meats analyzed had similar contents (P>0.05) of cholesterol, α-tocopherol and free fat. In contrast, the percentage of 18:0 was lower for PGI veal, and that of 18:1 *c*9 was higher in PDO veal, whilst the percentage of 18:2 *n*-6 was higher in PDO beef, relative to other two meats. The content of total CLA and the percentage of its *t*11,*c*13 isomer were higher, and the *n-6/n-3* ratio was lower, in PDO veal, relative to the other two meats. The data suggested that PGI veal has higher variability for most fatty acids than the other two types of meat. Finally, a discriminant analysis was conducted and the three meat types were well discriminated using the meat fatty acid profile as variables.

Keywords: Beef, Cholesterol, CLA isomers, Fatty acids, Meat quality, α-Tocopherol.

3.1 Introduction

The Protected Designation of Origin (PDO) and Protected Geographic Indication (PGI) meat types are certified by European Union legislation and are supposed to present unique quality and organoleptic traits especially associated with the specific properties of their lipid fraction (Regulation n. 510/2006 of 20 March 2006, EC). This way the promotion of these products could be of considerable benefit to the rural economy, by improving the incomes of farmers and by retaining the rural population in less-favoured or remote areas, where desertification is a reality (Regulation n. 510/2006 of 20 March 2006, EC). In Portugal, the consumption of certified beef has increased in the last years (GPP, 2007a). The certified beef products in Portugal are all originated from animals raised in a traditional semi-extensive production system, according to the approved product specifications. One such case is Mertolenga-PDO veal and beef, which are obtained from the Mertolenga purebred calves and young bulls, respectively. Moreover, meat obtained from Mertolenga crossbreed calves, mainly with the Limousin and Charolais breeds can be certified as "Vitela Tradicional do Montado"-PGI veal. These products must comply with specifications: "Vitela Tradicional do Montado"-PGI veal are obtained from crossbred calves with age until 12 months and 180 kg of carcass weight. The Mertolenga-PDO veal is obtained from purebred animals with age until 15 months and with carcass weight lower than 220 kg, whilst the Mertolenga-PDO beef is obtained from purebred animals with 16-30 months of age and more than 220 kg of carcass weight. It is noteworthy that these three meat types are obtained from animals produced in similar conditions and commercialised by the same Group (as defined in Regulation no. 510/2006 of 20 March 2006, EC).

The certified beef has a higher price in the market mainly due to the higher production and certification costs. Moreover, the certification strategies (traceability or quality labels) have been developed to improve consumer perception of food safety, which generates an increase of the product final price (Angulo & Gil, 2007). The consumers are willing to pay more for these products because the production system of such meat is perceived as less harmful to the environment and more animal friendly and meat is expected to be healthier and more palatable (Sepúlveda *et al.*, 2008). As reviewed recently (Schmid, 2011), beef produce on extensive production systems has healthier lipid profile. The *n-6/n-3* PUFA ratio is relatively low in these meats as grass contains high levels of α-linolenic acid, in opposition to cereal-based diets, which are rich in linoleic acid, producing an undesirably high *n-6/n-3* PUFA ratio (Schmid, 2011).

The meat produced under extensive, and hence less controlled production system can be expected to be less homogenous regarding sensory and nutritional attributes which might occasionally frustrate the consumers' expectations. The specifications production rules defined for each PDO and PGI meat type, attempts to obtain distinctive and fairly homogenous products. Nevertheless, the studies on variability of quality attributes of PDO and PGI meat types have been mostly overlooked.

The Mertolenga-PDO beef fatty acid composition, including CLA isomers, has already been reported by our team (Alfaia *et al.*, 2006b; Costa *et al.*, 2008). However, no comparative studies concerning lipid composition and variability of all types of Mertolenga certified meat have been conducted. Thus, the aim of this study was to assess the meat lipids of "Vitela Tradicional do Montado"-PGI veal, Mertolenga-PDO veal and Mertolenga-PDO beef, like consumer purchase it in the marketplace.

3.2 Material and Methods

3.2.1 Meat samples

This study was performed on 23 Mertolenga crossbred calves "Vitela Tradicional do Montado" (PGI veal), 23 purebred Mertolenga calves (PDO veal) and 22 purebred Mertolenga young bulls (PDO beef). PGI calves presented mean values of age and carcass weight of 10 months and 164 kg, respectively. Mean age (11 months) and carcass weight (162 kg) values presented by PDO calves were similar to those presented by PGI veal. PDO young bulls presented higher age (18 months; $P < 0.001$) and carcass weight (251 kg; $P < 0.001$). The calves from PGI veal are crossbred females since males are slaughtered latter and commercialised as "Novilho Tradicional do Montado" beef. The Mertolenga-PDO veal and beef are obtained almost exclusively from males since females are kept for herd replacement and so their contribution to meat production is very small. All animals were raised under the PGI and the PDO Book Specifications, in different representative private farms, under a semi-extensive system based on natural pastures under holm and cork oak. Supplementation with cereals and dry forages (hay and straw) was provided during periods of feed scarcity. The calves remained with their mothers on natural pastures until weaning (6-9 months of age), after that they were confined and fed with concentrate and straw. The concentrate composition fed to calves and young bulls are depicted in Table 1. The animals were slaughtered between June and September 2005, in an officially approved slaughterhouse, according to standard methods using a captive bolt stunner, followed by sticking and

bleeding. The carcasses were electrically stimulated, to avoid cold shortening, and kept six days at 0-1 ºC.

Table 1 – Chemical composition (%) of concentrate feed according to PGI veal and PDO veal and beef specifications

Characteristics	Calves (age < 12 months)	Young bulls (age > 12 months)
Crude Protein	16.0-16.5	16.0-18.0
Crude Fat	2.6-6.0	2.5-5.0
Crude Fibre	6.0-9.7	6.0-9.8
Starch	Min 25.0	Min 20.0
WSC	Min 4.0	Min 4.5
Ash	Max 9.0	Max 9.0
Calcium	1.0-1.2	0.95-1.2
Phosphorus	0.55-0.62	0.50-0.62

WSC = Water soluble carbohydrates

The samples of *longissimus lumborum* muscle were collected on the slaughterhouse, in different days, in order to assure different herd origin and consequently a more representative sampling. The samples were trimmed from their visible fat and connective tissue, and then minced, vacuum packaged and frozen at -80 ºC until analyses were performed.

3.2.2 Analysis of cholesterol and α-tocopherol

The total cholesterol and lipid-soluble antioxidant vitamins were extracted from homogenised meat samples, after saponification with KOH solution freshly prepared, according to the procedure described by Prates *et al.* (2006). The cholesterol and *α*-tocopherol were separated by normal-phase HPLC (Zorbax Rx-Sil with the corresponding 12.5 mm analytical guard column, 250 mm × 4.6 mm internal diameter, 5 μm particle size; Agilent Technologies Inc., Palo Alto, CA, USA), using an HPLC system (Agilent 1100 series, Agilent Technologies Inc.) equipped with an UV-Vis photodiode array detection for cholesterol (202 nm) and with a fluorescent detection for *α*-tocopherol (excitation wavelength of 295 nm and emission wavelength of 325 nm) in series. The solvent (30 ml/l isopropanol in *n*-hexane) flow rate was 1 ml/min and the injection volumes were 100 μl for cholesterol and 20 μl for *α*-tocopherol.
The total content of cholesterol and *α*-tocopherol in muscle were determined in duplicate, based on the external standard technique, from a standard curve for peak areas *vs.* compounds concentration.

3.2.3 Lipid extraction and methylation

The free fat content was measured according to the AOAC official method 945.16 (2000) in fresh samples, and expressed as mg/g muscle. As fresh meat was used for the free fat determination, 35-40 g of sodium phosphate was added in order to dehydrate the meat.
For total fatty acid (FA) quantification meat samples were previously lyophilised (-60 ºC and 2.0 hPa) to constant weight using a lyophilisator Edwards Modulyo (Edwards High Vaccum International, UK). Briefly, FA were directly extracted and methylated from 250 mg of lyophilised meat samples by a one-step procedure (adapted from Christie *et al.*, 2001). The FA were converted to their methyl esters (FAME) by a combined transterification procedure with NaOH in anhydrous methanol (0.5 M) followed by HCl/methanol (1/1 v/v) at 50 °C, during 30 and 10 min, respectively (Raes *et al.*, 2001). The nonadecanoic acid (19:0) was added as internal standard. The same FAME solution was used for the analysis of both FA composition and CLA isomeric profile.

3.2.4 Analysis of fatty acid methyl esters

The gas chromatography analysis of FAME were performed using a gas chromatograph Varian 3800 (Varian Inc, Walnut Creek, CA, USA) fitted with a flame ionization detector (FID) and an OmegaWax 250 (Supelco, Bellefont, CA, USA) capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Briefly, the gas chromatograph oven temperature was programmed to start at 150 ºC and held at this temperature for 15 min, followed by an increase of 3 ºC/min to 220 ºC and, finally, held at 220 ºC for 20 min. The injector and detector temperatures were 250 ºC and 280 ºC, respectively. The helium was used as carrier gas and the split ratio was 1:20. The identification was accomplished by comparing the retention times of peaks from samples with those of FAME standard mixtures. The content of *trans*-octadecenoic FA was expressed as a single value (18:1 *t*) because of their incomplete chromatographic resolution. The sum of *trans* FA does not include the conjugated *trans* FA. The quantification of FAME was based on the internal standard technique, using 19:0 as internal standard and on the conversion of relative peak areas to weight percentage. The FA were expressed as the percentage of the sum of identified FA (g/100 g total FA).

3.2.5 Determination of CLA isomers

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm internal diameter, 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using a high performance liquid chromatography system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and diode array detector (DAD) adjusted to 233 nm. The mobile phase was 0.1% acetonitrile in *n*-hexane maintained at a flow rate of 1 ml/min, and injection volumes of 20 μl were used. The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial standards and with values published in the literature (Fritsche *et al.*, 2001). The identity of each isomer was controlled by UV spectra of CLA isomers from the DAD in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems (Agilent Technologies, 2001).

The total CLA contents were quantified by GC analysis of total FAME and the relative concentrations obtained by Ag^+-HPLC were used to calculate the unresolved peaks in the GC chromatogram. The calculation of the *c*9,*t*11, *t*7,*c*9 and *t*8,*c*10 CLA isomers were performed by comparing the HPLC areas of these isomer peaks with the peak area of the three co-eluted isomers from GC chromatogram. The quantification of the other CLA isomers was assessed from their Ag^+-HPLC areas relative to the area of the main isomer *c*9,*t*11. A detailed description of the quantification process of individual CLA isomers using these two complementary methods was described by Prates and Bessa (2009).

3.2.6 Statistical analysis

The effect of the beef type was studied by analysis of variance using the PROC MIXED procedure of Statistical Analysis Systems (SAS) software package, version 9.1 (SAS Institute Inc., Cary, NC, USA, 2004). The data were checked for normality and homocedasticy. For some variables significant differences of variances between types were found and thus were analysed by PROC MIXED model allowing for variance heterogeneity. For these variables, variances of 3 groups were pairwise compared in order to establish difference between groups.

The canonical discriminant analysis was applied to data in order to distinguish beef types. The variable selection for discriminant analysis was achieved using the significant variables after PROC MIXED analysis. The stepwise discriminant analysis (PROC STEPDISC, SAS, 2004) selected the variables with higher discriminant ability. Following this, the linear discriminant

functions were developed using the PROC DISCRIMINANT (SAS, 2004) to determine the coefficients of fatty acids that maximise the differences between the three meat types.

3.3 Results

3.3.1 Content of cholesterol and α-tocopherol in muscle

The total cholesterol (mg/g muscle), *α*-tocopherol (μg/g muscle), free fat (mg/g muscle) and total FA (mg/g muscle) in *longissimus lumborum* muscle of PGI veal, PDO veal and PDO beef are depicted in Table 2. No significant differences ($P > 0.05$) concerning total cholesterol and *α*-tocopherol content were observed. The total cholesterol and *α*-tocopherol mean values were 0.42 mg/g muscle and 2.46 μg/g muscle, respectively.

3.3.2 Muscle fatty acid content and composition

The total FA content did not differ ($P>0.05$) among meats and averaged 6.23 mg/g muscle (Table 2). The data on the FA composition (expressed as g/100 g total FA) and partial sums of FA of *longissimus lumborum* muscle of PGI veal, PDO veal and PDO beef are presented in Tables 2 and 3, respectively. Regardless of the meat type, total FA content consisted predominantly of SFA (averaging 40.0%) followed by monounsaturated FA (MUFA; ranging from 31.4 to 35.4%) and finally by PUFA (ranging from 13.1 to 16.7%). The small amounts of *trans* FA (TFA) were detected (averaging 3.6%).

The predominant FA in intramuscular fat were palmitic (16:0; averaging 21.6% total FA) and stearic (18:0; ranging 13.4-15.2% total FA) acids as SFA, oleic acid (18:1 *c*9; 28.1-31.5%) as MUFA and linoleic (18:2 *n*-6; ranging 8.1-11.5%) and arachidonic (20:4 *n-6*; 2.5-3.7%) acids as PUFA. PGI veal showed higher 17:0 ($P < 0.05$) and 20:3 *n*-6 ($P < 0.05$) than the other two meats, higher 12:0 ($P < 0.01$) and 20:4 *n*-6 ($P < 0.05$) than PDO veal, and higher 15:0 ($P < 0.05$), 17:1 *c*9 ($P < 0.05$), 20:5 *n-3* ($P < 0.05$) and 22:5 *n-3* ($P < 0.05$) than PDO beef. Conversely, the percentages of CLA ($P < 0.05$) and 20:0 ($P < 0.01$) were lower for PGI veal relative to PDO veal, and the percentage of 18:0 ($P < 0.001$) was lower for PGI veal relative to PDO beef. The 18:1 *c*9 ($P < 0.05$) was highest in PDO veal, despite not different from PDO veal, whilst the 18:2 *n*-6 ($P < 0.001$) was highest in PDO beef.

There were no differences in SFA among meat types. The percentages of MUFA and PUFA were the highest and the lowest in PDO veal, respectively (Table 3), despite not different from

PGI veal. This was mainly due to the contribution of 18:1 *c*9 and 18:2 *n*-6 to the MUFA and PUFA partial sums, respectively.

Table 2 - Total cholesterol, α-tocopherol, free fat content and fatty acid content and composition of the *longissimus lumborum* muscle from PGI veal, PDO veal and PDO beef.

	PGI veal		PDO veal		PDO beef		Signif
	Mean	SEM	Mean	SEM	Mean	SEM	
Cholesterol (mg/g muscle)	0.41	0.009	0.42	0.015	0.43	0.013	ns
α-Tocopherol (μg/g muscle)	2.62	0.207	2.17	0.159	2.58	0.276	ns
Free fat (mg/g muscle)	5.39	0.489	5.32	0.307	6.08	0.371	ns
Total FA (mg/g muscle)	5.82	0.610	6.49	0.600	6.38	0.547	ns
Fatty acid composition (g/100 g total FA)							
10:0	0.75	0.092	0.52	0.049	0.58	0.045	ns
12:0	0.09^{a}	0.007	0.06^{b}	0.004	0.07^{ab}	0.005	**
14:0	2.19	0.130	2.11	0.010	2.01	0.094	ns
14:1	0.42	0.024	0.41	0.040	0.36	0.032	ns
15:0	0.34^{a}	0.017	0.30^{ab}	0.013	0.28^{b}	0.014	*
16:0	21.75	0.521	22.09	0.446	20.98	0.387	ns
16:1 *c*9	2.56^{ab}	0.099	2.73^{a}	0.142	2.29^{b}	0.089	*
17:0	0.88^{a}	0.040	0.76^{b}	0.026	0.77^{b}	0.024	*
17:1	0.69^{a}	0.035	0.62^{a}	0.018	0.55^{b}	0.022	*
18:0	13.40^{b}	0.306	14.43^{ab}	0.338	15.24^{a}	0.327	***
18:1 *c*9	28.57^{ab}	1.284	31.49^{a}	0.840	28.05^{b}	0.746	*
18:1 *t*	3.26	0.447	3.01	0.378	3.89	0.304	ns
18:2 *n*-6	9.04^{b}	0.730	8.06^{b}	0.435	11.45^{a}	0.578	***
18:3 *n*-3	0.47	0.054	0.41	0.032	0.52	0.103	ns
CLA^{+}	0.35^{b}	0.025	0.46^{a}	0.030	0.39^{ab}	0.026	*
20:0	0.09^{b}	0.005	0.12^{a}	0.006	0.11^{ab}	0.004	**
20:1 *c*9	0.10	0.008	0.12	0.005	0.11	0.005	ns
20:2 *n*-6	0.11	0.017	0.08	0.004	0.10	0.006	ns
20:3 *n*-6	0.88^{a}	0.101	0.60^{b}	0.048	0.59^{b}	0.039	*
20:4 *n*-6	3.67^{a}	0.414	2.52^{b}	0.204	2.93^{ab}	0.190	*
20:5 *n*-3	0.48^{a}	0.089	0.33^{ab}	0.043	0.22^{b}	0.041	*
22:4 *n*-6	0.28	0.031	0.27	0.023	0.27	0.020	ns
22:5 *n*-3	0.83^{a}	0.107	0.70^{ab}	0.066	0.54^{b}	0.059	*
22:6 *n*-3	0.13	0.030	0.08	0.015	0.25	0.081	ns
Other FA	8.76	0.679	7.80	0.412	7.77	0.424	ns

Statistical probability of treatment: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean.

$^{+}$ The CLA content obtained by GC was corrected to total CLA content with the relative proportions of the individual isomers determined by HPLC, as described in the text (section 3.2.5).

The PDO beef had a higher (P<0.01) P/S ratio than PDO veal. The higher value of this FA ratio in PDO beef is mainly due to the greater percentage of 18:2 *n*-6 in this meat. The PDO beef had the highest *n-6/n-3* PUFA ratio (16.4; P<0.001), and the PGI and PDO veal depicted similar values (average of 8.7).

Table 3 - Partial sums of FA (g/100 g total FA) and nutritional ratios of fatty acids in *longissimus lumborum* muscle from PGI veal, PDO veal and PDO beef.

	PGI veal		PDO veal		PDO beef		*Significance*
	Mean	SEM	Mean	SEM	Mean	SEM	
SFA	39.48	0.746	40.39	0.380	40.03	0.624	ns
MUFA	32.34^{ab}	1.384	35.36^{a}	0.930	31.36^{b}	0.835	**
PUFA	15.92^{ab}	1.456	13.11^{b}	0.782	16.69^{a}	0.886	*
TFA	3.44	0.446	3.25	0.381	4.06	0.306	ns
n-6	13.99^{ab}	1.259	11.53^{b}	0.687	15.33^{a}	0.798	**
n-3	1.82	0.245	1.49	0.146	1.26	0.192	ns
Ratios							
P/S	0.27^{ab}	0.030	0.22^{b}	0.013	0.32^{a}	0.020	***
n-6/n-3	8.7^{b}	0.526	8.7^{b}	0.621	16.4^{a}	1.666	***

Statistical probability of treatment: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean.

P/S = (18 :2*n-6* + 18 :3*n-3*) / (14 :0 + 16 :0 + 18 :0)

The PDO veal had higher values of CLA content (24% higher) than PGI veal (Table 2). The data on the CLA isomeric profile (% total CLA) of PGI veal, PDO veal and PDO beef are depicted on Table 4. The total *trans/trans* and *cis/trans* (*cis, trans* and *trans, cis*) isomers were higher and lower in PDO veal, respectively, although not different from PGI veal.

The *cis/trans* isomers are the major CLA group with values from 79% to 85%. The major contributor to this percentage was the *c*9,*t*11 CLA isomer, with an average of 65%. The total *cis*/*cis* group, a minor CLA group, contributed with only 2% of total CLA content. The only *cis*,*cis* CLA isomer detected was the *c*9,*c*11. The PDO veal had the highest percentage of *t*10,*t*12 (3.7%) and the lowest relative proportion of *t*7,*c*9 (9.6%) CLA isomers than the other two meats studied, higher percentages of the *t*9,*t*11 (7.0%) and *t*11,*c*13 (2.7%) CLA isomers than PDO beef, as well as lower percentages of *c/t* 12,14 (0.8%) CLA isomer than PGI veal. The PDO beef presented higher value of *t*6,*t*8 CLA isomer (1.5%) than PGI veal.

Table 4 – The CLA isomers (% total CLA) of *longissimus lumborum* muscle from PGI veal, PDO veal and PDO beef.

	PGI veal		PDO veal		PDO beef		*Significance*
	Mean	SEM	Mean	SEM	Mean	SEM	
*t*12,*t*14	1.42	0.121	1.56	0.162	1.14	0.149	ns
*t*11,*t*13	2.25	0.305	2.45	0.178	1.91	0.303	ns
*t*10,*t*12	2.55^{b}	0.300	3.67^{a}	0.284	1.66^{c}	0.188	***
*t*9,*t*11	5.99^{ab}	0.505	7.02^{a}	0.587	4.67^{b}	0.475	**
*t*8,*t*10	1.38	0.236	1.69	0.341	1.25	0.211	ns
*t*7,*t*9	2.04	0.197	2.47	0.374	2.26	0.408	ns
*t*6,*t*8	1.00^{b}	0.086	1.32^{ab}	0.106	1.48^{a}	0.174	*
Total *trans/trans*	15.67^{ab}	1.106	18.88^{a}	1.398	13.57^{b}	0.945	**
*c/t*12,14	1.47^{a}	0.211	0.83^{b}	0.131	0.72^{b}	0.077	**
*t*11,*c*13	1.89^{ab}	0.411	2.73^{a}	0.510	1.21^{b}	0.199	*
*c*11,*t*13	1.78	0.297	1.08	0.159	1.10	0.130	ns
*c*9,*t*11	62.53	1.947	64.48	1.336	68.00	1.539	ns
*t*7,*c*9	15.35^{a}	1.455	9.59^{b}	0.861	13.47^{a}	1.252	**
Total *cis/trans*	82.21^{ab}	1.223	79.03^{b}	1.377	84.53^{a}	1.018	**
*c*9,*c*11	2.12	0.345	2.09	0.185	1.90	0.211	ns

Statistical probability of treatment: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean.

3.3.3 Discriminant analysis

The canonical discriminant analysis was applied to fatty acid composition including isomeric CLA distribution in order to discriminate the beef types used in this study. The results of canonical discriminant analysis, loadings of correlation matrix and discriminant functions are depicted in Table 5. The differences between meat types in the fatty acid profile can be evaluated by the values of squared Mahalanobis distance (D^2). The higher values of D^2 obtained correspond to larger differences between meat types. In line with this, the differences were smaller between PGI veal and PDO veal (6.5), than between PGI veal and PDO beef (21.6) or between PDO veal and PDO beef (21.1), as illustrated in Figure 6.

This means that the two veal types are more similar to each other than with the PDO beef. The application of canonical discriminant analysis to selected variables resulted in two discriminant functions, which maximise the ratio between class variance and minimises the ratio within class variance, discriminating the three meat types studied, although not completely.

Table 5 - Results of canonical discriminant analysis: loadings of correlation matrix between predictor variables (standardized canonical coefficients) and discriminant functions (roots 1 and 2), and some statistics for each function.

	Root 1	Root 2
Fatty acids		
18:2 *n*-6	-0.674	-0.159
20:3 *n*-6	6.188	-1.240
20:0	17.893	-13.702
12:0	-18.839	26.828
17:0	2.203	2.954
*t*9,*t*11 CLA	0.047	-0.033
16:1 *c*9	1.676	-0.911
*c*9,*t*11 CLA	-0.004	0.001
22:5 *n*-3	1.531	-0.695
18:0	-0.112	-0.009
20:4 *n*-6	-0.150	1.053
Statistics		
Cannonical R	0.905	0.731
Eigenvalue	4.555	1.148
Cummulative proportion	0.799	1.000
Probability	<0.0001	<0.0001

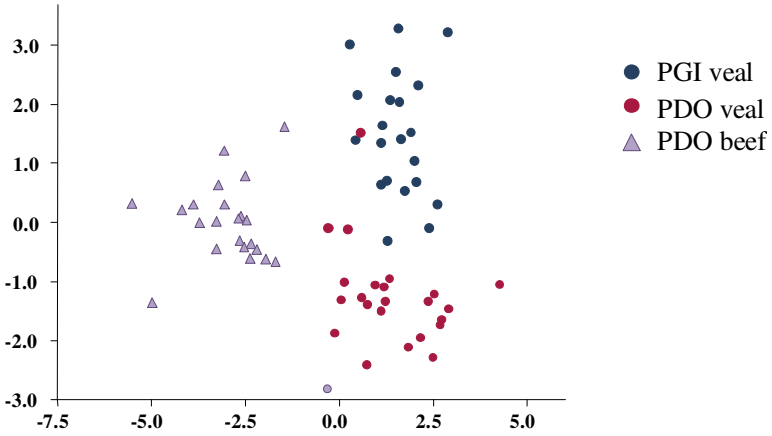
Table 6 – Classification matrix of cross-validation results for the three types of beef (PGI veal, PDO veal and PDO beef) using canonical discriminant analysis.

	Beef types		
	PGI veal	PDO veal	PDO beef
Classified as PGI veal	18	3	1
Classified as PDO veal	4	20	0
Classified as PDO beef	0	0	21
Total	22	23	22
% of correct classification	81.8	87.0	95.5

The discriminant functions obtained used 11 variables, from which 12:0, 20:0, 20:3 *n-6* and 17:0 had the highest discriminant power in both roots. The only CLA isomers that contributed to the discriminant functions were the *t*9,*t*11 and *c*9,*t*11. After cross-validation, results varied between 82 and 96% of correct classification, being the extreme values from PGI veal and PDO beef, respectively. The PDO veal presented a classification of 87% correct (Table 6).

As can be seen in the plot, both veal are located in the right side of the plot, whilst PDO beef is located in the left side (Figure 6). The second function (root 2) discriminates between both veal, having PGI veal positive loadings and PDO veal negative ones.

Figure 6 - Plot of the discriminant functions (root 1 *vs.* root 2) for classification of the three types of beef (PGI veal, PDO veal and PDO beef).



3.3.4 Variability of lipid traits

We checked if variances of studied variables differed between meat types (Table 7).

The cholesterol and most of MUFA and PUFA had significant differences in variances between meat types. In general, the PGI meat had higher variability in those variables where variance differences were detected. The exceptions were the cholesterol and 18:3 *n*-3. Regarding 18:3 *n*-3, the highest variance was observed for PDO beef and the lowest one for PDO veal. The variance differences were statistically significant in nine of the 13 CLA isomers detected in this study. From these nine isomers, PGI veal had higher variability in seven of them. However, the variance values of *t*10,*t*12 and in *t*11,*c*13 CLA isomers were not different from the values presented by PDO veal, whilst variance values of *t*11,*t*13 and *t*7,*c*9 CLA isomers were not different from the ones showed by PDO beef.

3.4 Discussion

3.4.1 Cholesterol and alpha-tocopherol content

The values reported here for total cholesterol (0.42 mg/g muscle) are similar to those presented by Alfaia *et al.* (2006b) (0.44 mg/g muscle) and Costa *et al.* (2008) (0.41-0.46 mg/g

muscle), in Mertolenga-PDO *longissimus dorsi* muscle. Other authors reported slightly higher cholesterol values in other breeds and/or muscles (*e.g.* 0.48-0.49 mg/g in Alfaia *et al.*, 2006a; 0.47-0.57 mg/g in Chizzolini *et al.*, 1999) than those presented in this work.

Table 7 – Variance of cholesterol and fatty acids including CLA isomers of *longissimus lumborum* muscle from PGI veal, PDO veal and PDO beef

	PGI veal	PDO veal	PDO beef	*Significance*
Cholesterol	0.002^{b}	0.005^{a}	0.004^{a,b}	*
10:0	0.19^{a}	0.05^{b}	0.04^{b}	**
16:1	0.21^{ab}	0.47^{a}	0.17^{b}	*
17:0	0.03^{a}	0.02^{ab}	0.01^{b}	*
17:1	0.03^{a}	0.01^{b}	0.01^{b}	*
18:1	36.27^{a}	16.26^{ab}	12.24^{b}	*
18:3 *n*-3	0.06^{b}	0.02^{c}	0.23^{a}	***
20:2 *n*-6	0.01^{a}	0.00^{b}	0.00^{b}	***
20:3 *n*-6	0.23^{a}	0.05^{b}	0.03^{b}	***
20:4 *n*-6	3.76^{a}	0.96^{b}	0.80^{b}	***
20:5 *n*-3	0.17^{a}	0.04^{b}	0.04^{b}	***
22:5 *n*-3	0.24^{a}	0.10^{b}	0.07^{b}	*
Other FA	8.76^{a}	7.80^{b}	7.77^{b}	*
*t*11,*t*13	2.33^{a}	0.73^{b}	2.03^{a}	*
*t*10,*t*12	2.25^{a}	1.85^{a}	0.78^{b}	*
*t*7,*t*9	0.97^{b}	3.21^{a}	3.67^{a}	**
*t*6,*t*8	0.19^{b}	0.26^{b}	0.67^{a}	**
c/t 12,14	1.11^{a}	0.40^{b}	0.13^{c}	***
*t*11,*c*13	4.22^{a}	5.97^{a}	0.87^{b}	***
*c*11,*t*13	2.20^{a}	0.58^{b}	0.37^{b}	***
*t*7,*c*9	52.92^{a}	17.06^{b}	34.46^{ab}	*
*c*9,*c*11	2.97^{a}	0.78^{b}	0.98^{b}	**

Statistical probability of treatment: *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05).

There were no difference (P>0.05) among α-tocopherol content in meats, which averaged 2.46 μg/g muscle (Table 2). These values are higher than those reported by Costa *et al.* (2008) in Mertolenga-PDO beef (1.4-1.9 μg/g). Our group reported α-tocopherol contents for other Portuguese PDO beef higher than the values presented here, namely 3.22 μg/g muscle for

Carnalentejana-PDO beef (Monteiro *et al.*, 2008) and 3.3-3.9 μg/g muscle for Barrosã-PDO veal (Prates *et al.*, 2006).

The present study confirmed the lack of an age (veal vs beef), sex (PGI veal *vs.* PDO veal) or genotype (purebred *vs.* crossbred) effect on the total cholesterol content in meat, previously reported by other authors (Rule *et al.*, 1997; Costa *et al.*, 2006), as well as on α-tocopherol content. The differences observed in the literature for α-tocopherol content might be due to dietary factors in particular pasture feeding and synthetic tocopherol acetate supplementation.

The low α-tocopherol content observed in our study indicates a low dietary intake. West and coworkers (1997) observed that the α-tocopherol contents in the *longissimus lumborum* muscle of pastured cattle varies between 3.7 and 7 μg/g, suggesting that there may be a large difference in the α-tocopherol levels found in pastures with different biomass compositions. The beef cattle producing PDO and PGI meat in this study were grazed on Alentejo, a South region of Portugal, where grass is scarce and of poor quality in the hottest months of the year (June to September).

The minimum α-tocopherol content needed to inhibit meat metamyoglobin formation, and to protect it from lipid oxidation was estimated to be 3.0 μg/g in minced muscle (Faustman *et al.*, 1989). The muscle *α*-tocopherol values of the three meats studied here were 18% lower than the recommended value, which implies that lipid stability of these meats might be affected. Therefore, the levels of *α* -tocopherol acetate inclusion in supplementary feed should be increased.

3.4.2 Fatty acid content and composition

The values obtained for concentration of total FA (averaging 6.2 mg/g muscle) in *longissimus lumborum* muscle are lower than the ones reported in other works with Mertolenga beef (averaging 17.7 mg/g muscle in Alfaia *et al.*, 2006b; averaging 10.1 mg/g muscle in Monteiro *et al.*, 2006; ranging from 14.3 to 18.2 mg/g muscle in Costa *et al.*, 2008). However, the animals used in this study where younger than the animals used in the trials of the aforementioned authors and it is well established that intramuscular fat is the last adipose tissue to be deposited (Vestegaard *et al.*, 2000). Thus, these animals probably have not deposited all the intramuscular tissue at the time they were slaughtered.

The differences in 18:1 *c*9 and 18:2 *n*-6 in beef has been related to feeding regimen and, hence, to differences in dietary intake (French *et al.*, 2000; Realini *et al.*, 2004) because pasture has lower contents of 18:1 *c*9 and 18:2 *n*-6 than cereals (Palmquist, 1988). However, Daniel and coworkers (2004) demonstrated that increased 18:1 *c*9 in sheep muscle in response

to concentrate rich diets is due to the up-regulation of stearoyl-CoA desaturase gene expression and not to 18:1 *c*9 dietary availability. The age and animal breed type differences on muscle 18:1 *c*9 concentration has also been reported (Smith *et al.*, 2009) due to differences in stearoyl-CoA desaturase expression. The increased concentration of 18:1 *c*9 on beef from concentrate-fed animals has also been explained by increased intramuscular fat content and, hence, due to a higher neutral lipid/ phospholipid ratio (Wood *et al.*, 2008).

In our study, PDO veal presented higher 18:1 *c*9 and lower 18:2 *n*-6 percentages than PDO beef. These differences could not be due to breed differences, since both animal types were Mertolenga purebred males. Rule *et al.* (1997) and Warren *et al.* (2008) explained the differences in FA composition based on an age effect. However, in contrast to our results, in those studies older animals had higher percentages of 18:1 *c*9 and lower relative proportions of 18:2 *n*-6. It is noteworthy that the age effect was accomplished with a fat content increased, since older animals were fatter, though the differences found could be due to differences in fat content rather than due to age differences. The FA content in *longissimus lumborum* muscle of PDO veal and beef was similar, which might explain the differences between our study and the studies of the quoted authors.

The main difference between the PDO veal and beef are the degree of sexual maturity. The PDO calves have an average age of 11 months and are just attaining puberty, whereas the PDO young bulls have an average age of 18 months. Although the effects of male sexual maturity in muscle FA deposition are not well exploited, some information is available for comparison of castrated and entire bovine males. Monteiro *et al.* (2006) found that muscle from Mertolenga steers had higher 18:1 *c*9 and lower 18:2 *n*-6 percentages than muscle from entire males, differences which remained even after correction for intramuscular fat. Thereby male sexual maturity might be a factor that affects the FA composition of beef. This is in agreement with the results obtained by Eichhorn and coworkers (1985), who reported a lower percentage of 18:1*c*9 and higher relative proportions of 18:2 *n*-6, 18:3 *n-3* and 20:4 *n*-6 in *longissimus dorsi* muscle of young bulls comparing with steers.

Although the PDO beef had a higher percentage of 18:2 *n-6* than the PDO veal, there were no differences between these two meats in 20:2 *n-6*, 20:3 *n-6*, 20:4 *n-6* and 22:4 *n-6*. On the contrary, the relative proportion of 18:2 *n*-6 was similar between the PDO veal and PGI veal but differences were observed in the *n*-6 series of long-chain (LC; > C18) PUFA, mainly due to the highest 20:4 *n-6* percentage in PGI veal.

Finally, the percentage of the precursor of the *n-3* series of LC-PUFA, the α-linolenic acid, is similar among meat types. Nevertheless, the PGI veal had the highest values for 20:5 *n-3* (EPA) and 22:5 *n-3* (DPA), although not different from PDO veal. In fact, Enser (2001)

reported that the conversion efficiency to EPA and DHA (22:6 *n-3*) is poor, thus being the direct intake of these LC-PUFA desirable.

Concerning CLA isomeric profile, Alfaia *et al.* (2006b) reported similar values of total CLA in *longissimus dorsi* muscle of Mertolenga-PDO beef (0.36 g/100 g lipids) to those presented here (0.40 g/100 g lipids). The *t*7,*c*9 isomer is mentioned frequently as the second most prevalent CLA isomer (Alfaia *et al.*, 2006a,b), which is in agreement with our results. Most research effort has been directed towards the effects of nutrition on CLA content and isomeric profile (Scollan *et al.*, 2006), and there are relatively few reports on the effect of breed, gender, age or slaughter weight (De La Torre *et al.*, 2006; Moreno *et al.*, 2008). The higher CLA content of PDO veal is not clear but might be associated with higher stearoyl-CoA desaturase activity (converting 18:1 *t*11 into *c*9t11 CLA isomer) as suggested by the simultaneous increase of its other products (*i.e.* 16:1 *c*9 and 18:1 *c*9). With the exception of the *c*9,*t*11 and *t*7,*c*9 isomers, the origin of all other CLA isomers is expected to be ruminal biohydrogenation of dietary unsaturated C18 FA (Collomb *et al.*, 2004). It is well established that pasture feeding compared with concentrate feeding, increases the proportion of *t*11,*c*13, *t*11,*t*13 and *t*12,*t*14 isomers, which are sensitive grass (linolenic acid) intake indicators, and decreases the percentage of the *t*7,*c*9 isomer in beef lipids (Dannenberger *et al.*, 2005). Despite the lack of differences between our meats in *t*11,*t*13 and *t*12,*t*14 CLA isomers, the PDO veal had a lower relative proportion of *t*7,*c*9 isomer than PGI veal and PDO beef and a higher percentage of *t*11,*c*13 than PDO beef. The differences in the percentages of these CLA isomers among meats may reflect a higher pasture intake by the calves from PDO veal relative to the other calves and young bulls.

3.4.3 Nutritional value of intramuscular fat

It is important to notice that the low intramuscular fat content of these meats makes them healthy, which is an advantage from the consumers' point of view. Moreover, if we consider a common serving beef of 100 g, any of the meats studied will only supply a modest cholesterol amount (42 mg), representing about 14% of the recommended daily cholesterol intake in adults (300 mg; British Department of Health, 1994).

In order to evaluate the nutritional value of intramuscular fat related to human health, PUFA/SFA (P/S) and *n-6/n-3* ratios of FA are described and presented on Table 3.

Ruminant meats have relatively low P/S ratios as a consequence of the extensive C18 PUFA biohydrogenation in the rumen, with the consequent stearic acid (18:0) accumulation. As stearic acid is considered a neutral (Scollan, 2003) or even a beneficial (Mensink, 2005) FA

regarding its effect on plasma cholesterol in humans, the P/S ratio might not be the most appropriate nutritional index to evaluate FA in ruminant meats (Santos-Silva *et al.*, 2002).

The *n-6/n-3* ratio values are higher than the described by Costa *et al.* (2008; averaging 10.4), and much higher than the ones presented by Monteiro *et al.* (2006; ranging from 2.8 to 4.2) for Mertolenga-PDO beef. Alfaia *et al.* (2006b) reported similar values (14.9) in early autumn Mertolenga-PDO beef, though. The nutritional relevance of the *n-6/n-3* FA ratio, as a risk factor for some cancers and coronary heart disease of humans has been recently discredited (Griffin, 2008). Recent dietary guidelines are more focused on the absolute amounts of specific PUFA intake and recommend mainly the increase in the consumption of LC-PUFA, mainly EPA and DHA. The sum of EPA and DHA ranged from 0.4 to 0.6 g/100 g total FA, which represents 2.6-3.6 mg/100 g of muscle of *n-3* LC-PUFA. These values correspond only to 1 to 2% of the minimum recommended daily intake (RDI) for humans' diet (250-2000 mg/day, Elmadfa & Kornsteiner, 2009). Considering a common serving beef of 100 g, the studied meats will account with 135 mg of palmitic the main hypercholesterolemic fatty acid, whilst it will only have 6.5 to 8.5 mg of *n-3* LC-PUFA (EPA, DPA and DHA) and 17.9 to 23.3 mg of *n-6* LC-PUFA (20:4 *n*-6 + 22:4 *n*-6). Total *n-3* and *n-6* LC-PUFA are far from the minimum RDI value.

3.4.4 Discriminatory ability and homocedasticy of intramuscular fatty acid pattern and isomeric profile

Consumers associate quality labelled products, like PDO and PGI beef, to healthy, more regular and above-average quality products. For that reason consumers are willing to pay more for a product with a guarantee of genuineness, added quality value and that promotes a higher development of the region of origin (Fontes *et al.*, 2006). Being so, it seems important to assess the homogeneity of these products regarding the lipid traits. It is also interesting to realise if we can distinguish them by its lipid profile. For such, we performed canonical discriminant analysis and tested homocedasticy.

Fatty acid profiles in meat and milk are good markers of production system (Bessa *et al.*, 2006). Several authors have been successful in using beef and veal fatty acid composition as discriminate tools to separate them into weaning classes (Moreno *et al.*, 2006), production systems (Dias *et al.*, 2008; Alfaia *et al.*, 2009) or breeds (Dias *et al.*, 2008). Our results clearly indicate that despite the minor differences obtained in meat fatty acid profile and the similarity of the production systems of the animals sampled, it was feasible to allocate meat samples into one of the three meat types with good accuracy. The higher value of D^2 obtained

between the two veal indicate that the lipid profiles from the two veal are more similar to each other than to PDO beef. This is likely due to the age differences between both veal and PDO beef and/or to the differences in the degree of sexual maturity.

Although the lack of homogeneity in beef quality has been identified as a major problem of the beef industry in several countries (Polkinghorne *et al.*, 2008), studies concerning variability of the quality traits have been directed mostly to meat eating quality, particularly tenderness. As certified PGI and PDO meats could be expected to have a distinctive lipid profile, the assessment of its variability deserve to be studied to ensure product homogeneity. Nevertheless, this approach has been mostly overlooked in the relevant literature. Moreover, healthiness is one of the credence attributes most highly valuated by consumer, and the interest in valuing meat with information about the nutritional value in labels has been increasing (Bernués *et al.*, 2003; Wezemael *et al.*, 2010).

PDO veal and PDO beef were more uniform meats regarding most fatty acids than PGI veal. As the production system is quite similar, the highest variability of PGI veal is probably due to the diversity in genetic backgrounds as the specifications of this PGI veal allow animals from different crosses.

The fat content of meat is a major determinant of beef sensory attributes (Wood *et al.*, 2008). The variance of muscle total fatty acid content did not differ between meat types and the coefficient of variation (CV) averaged 42.7%. This value is lower (16%) than the total fatty acids CV values reported PDO beef by Alfaia and co-workers (2006a; averaging 50.7%) in Carnalentejana-PDO, but higher than the one presented by Monteiro and co-workers (2006) in Mertolenga beef (averaging 15%). The free fat variance was higher in PGI veal (despite not different from PDO beef), which presented 44% of CV, against de 26% and 28% presented by PDO veal and beef, respectively. Costa *et al.* (2008) presented total intramuscular lipids CV averaging 35%, whereas Alfaia *et al.* (2006b) described a lower values of total intramuscular lipids CV (averaging 21%).

PGI veal had similar 18:1 *c*9 mean values for the two PDO meats studied, although PGI veal 18:1 *c*9 variance was 55% and 66% higher than the 18:1 *c*9 variance value presented by PDO veal (not statistically different) and PDO beef, respectively. As mentioned earlier bovine meats with high percentages of oleic acid, have generally, a higher score in taste panel evaluations (Zembayashi *et al.*, 1995), which means that the higher variability in this fatty acid presented by PGI veal can give rise to undesirable large sensory variation between meats. Moreover, the much higher variance value presented by PGI veal can be covering mean differences in 18:1*c*9 between meats.

Despite of the lack of mean differences between meat types in 18:3 *n-3*, PDO beef had the highest variance value (0.23), followed by PGI veal (0.06) and finally PDO veal (0.02). Fisher and co-workers (2000) found that the flavour intensity is correlated with 18:3 *n*-3 content, so the higher variability presented by PDO beef can affect that palatability of beef. Considering LC-PUFA (EPA and DPA), PGI veal had higher variability in these FA values than PDO beef, although not different from PDO veal. Once more, the higher variability presented by PGI veal can compromise the healthiness value of this meat.

Regarding CLA isomeric profile we can observe that meats had similar mean values for *t*7,*t*9, *c*11,*t*13 and *c9,c*11 isomers. However, when analysing variance of these CLA isomers, we realise that PGI veal had a higher variance than the two other meats in *c*11,*t*13 and *c9,c*11 isomers, as well as, a smaller variance in the *t*7,*t*9 isomer. Overall, the results suggest that the highest variability showed by PGI veal is probably due to the fact that in the specification of these certificated products are allowed animals from different crosses, *i.e.*, with different genetic backgrounds.

3.5 Conclusions

Data from cholesterol, α-tocopherol and FA content and composition, including CLA isomeric distribution, suggest only small differences among the lipid composition of Mertolenga PGI veal, PDO veal and PDO beef. This result indicates that within a similar production system the age/weight, gender and crossbreeding practices have minor effects on muscle FA composition. Nevertheless, it was possible to discriminate the type of meat using the FA profile, reinforcing the initial assumption that each PDO and PGI has unique attributes associated with the specific properties of their lipid fraction.

From a nutritional point of view it is difficult to decide which of the three types of meat is the healthiest, since Mertolenga-PDO veal has higher MUFA and total CLA contents than PDO beef and PGI veal, respectively, whereas PDO beef has higher total PUFA content and P/S ratio than PDO veal and, finally, PGI veal has a trend for a higher *n-3* LC-PUFA (EPA and DPA).

Finally, it is important to notice that PGI veal is the most heterogeneous of the three meat types studied, which is an issue that should deserve some attention by the Association of Producers in order to improve the homogeneity of these certified products.

Acknowledgments

The financial support (Grant AGRO/2004/422) and the individual fellowship from FCT to Ana Cristina Monteiro (SFRH/BD/2006/31091) are acknowledged.

4 - Eating quality of "Vitela Tradicional do Montado"- PGI veal, Mertolenga-PDO veal and beef

A.C.G. Monteiro, A. S. Barreto, M. Fraústo da Silva, M. A. Fontes & J.P.C. Lemos

CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa - Avenida da Universidade Técnica, Pólo Universitário Alto da Ajuda, 1300-477 Lisboa, Portugal

Eating quality of "Vitela Tradicional do Montado"- PGI veal, Mertolenga-PDO veal and beef

Abstract

Consumer demand for beef is highly influenced by health, animal and environmental concerns. The quality branded beef production systems seem to better address the consumer concerns'. The physical, chemical and sensory characteristics of "Vitela Tradicional do Montado"-PGI veal and Mertolenga-PDO veal and beef were compared in samples from *longissimus lumborum* muscle six days *post-mortem*. The T_3 (temperature 3 hours *post-mortem*) values reflected the age/carcass weight (CW) differences among groups, with PDO beef presenting the highest value. PDO beef had the lowest L* and the highest a*, b*, C*, pigment content, cooking losses and Warner-Bratzler shear force (WBSF) values. Age/CW and pigment content strongly influenced beef colour correlating negatively with L* and positively with a* and C*. Ultimate pH also influenced meat colour correlating negatively with b*, h* and C*. WBSF correlated positively with age/CW, T_3 and cooking losses, and negatively with myofibrillar fragmentation index, tenderness, juiciness and overall acceptability. Cooking losses and juiciness were the main contributors for tenderness differences. PGI and PDO veal had lighter colour and were tenderer than PDO beef. The three meat types were well discriminated using chemical, physical and sensory attributes by canonical discriminant analysis.

Keywords: Beef, Colour, Meat Quality, Mertolenga, Tenderness

4.1 Introduction

Consumer demand for beef is increasingly influenced by the concerns about beef quality, health issues, nutritional value and safety (Xue *et al.*, 2010), as well as environment and animal welfare requirements (Andersen *et al.*, 2005). Even though consumers may be influenced by information about the product nutritional value, food safety issues and the image associated with it, sensory properties such as appearance (colour and fatness), texture, juiciness and flavour still remain the main purchasing and repeat purchasing criteria (Haugen & Kvaal, 1998). However, the concept of quality is difficult to define, whilst in the northern European countries the quality is linked to health and hygiene aspects in the southern European countries quality refers more to palatability attributes, geographical and human environment, and it is mostly linked to specific methods of production and/or region (Hocquette & Gigli, 2004). The promotion of products having characteristics that could be of considerable benefit to the rural economy, in particular to less-favoured or remote areas, has been encouraged. The Protected Designation of Origin (PDO) and Protected Geographic Identification (PGI), are the quality labels (Regulation 510/2006 20th March 2006, EC), usually under geographical groupings and with specific genotypes and production systems, created to meet the increasing demand by consumers for quality guarantees, animal welfare and environment protection.

The edapho-climatic conditions in Portugal, mainly in the south region of the country, are characterized by several dry months. In the same region, the predominance of flat soils with low fertility and productivity and high hydraulic erosion limits the agricultural practice. The extensive and semi-extensive cattle production seems to be an adequate alternative to more intensive practices. In 2007 the Portuguese beef and veal production was 91742 tons, and PDO and PGI beef and veal production only represented 2.4% (2168 tons) of the total production (GPP, 2007a). Despite beef production being mainly based on intensive systems with crossbred cattle, the production of autochthonous beef breeds in Portugal (10 autochthonous beef breeds) have an important economic and social role in the populations/regions where they are performed, as it represents an income to the population, and thereby helping their fixation in the region. One such example is Mertolenga breed, which can be marketed as Mertolenga-PDO beef and Mertolenga-PDO veal when animals are purebred, or as "Vitela Tradicional do Montado"-PGI veal, when animals are crossbred, mainly with the Limousin and Charolais breeds. These three meat types are obtained from animals produced in similar conditions and commercialised by the same Group (as defined in Regulation 510/2006 20th March 2006, EC).

The Mertolenga purebred and crossbred animals are raised in a traditional semi-extensive production system in the Alentejo region of Portugal, characterized by natural pastures under holm and cork oak, which is usually referred to as "Montado". Supplementation with cereals and dry forages (hay and straw) can be provided during the periods of feed scarcity, as well as finishing with concentrate before slaughter. Raising cattle on pasture has been associated with toughening, decreased beef colour and flavour acceptability (Hedricks *et al.*, 1983), and the development of off-flavours during *post-mortem* ageing, even after a finishing period with cereals. Thus, attempting to fulfil the consumers' expectations for healthier food may have a negative impact on meat quality. Therefore, it seems important to characterize these meats. Despite being marketed for years the study of these meats chemical, physical and sensory characteristics has been overlooked.

The aim of this study was to determine the physical, chemical and sensory characteristics of Portuguese "Vitela Tradicional do Montado"-PGI veal and Mertolenga-PDO veal and beef, raised under the typical production systems. This study is part of a grant research program (AGRO/2004/422, Ministry of Agriculture, Portugal), in which we intended to relate beef physical and chemical composition with its sensory attributes and consumer preferences.

4.2 Materials and Methods

4.2.1 Animals

This study was performed on 68 animals of "Vitela Tradicional do Montado-PGI", Mertolenga-PDO calves and Mertolenga-PDO young bulls. According to the specification, "Vitela Tradicional do Montado"-PGI veal is obtained from animals with age until 12 months and 180 kg of carcass weight. The Mertolenga-PDO veal is obtained from animals with age until 15 months and with carcass weight lower than 220 kg, whilst Mertolenga-PDO beef is obtained from animals with age from 16 to 30 months and carcass weight from 220 kg.

The animals were distributed into the three meat groups as followed:

- 23 crossbred female "Vitela Tradicional do Montado-PGI" (PGI veal) calves averaging 10.1 ± 1.5 months of age and 164.2 ± 9.5 kg carcass weight;
- 23 purebred Mertolenga-PDO calves (PDO veal) averaging 11.1 ± 2.1 months of age and 162.1 ± 12.2 kg carcass weight and;
- 22 purebred Mertolenga PDO young bulls (PDO beef) averaging 18.0 ± 3.4 months of age and 251.1 ± 34.9 kg carcass weight.

The calves remained with their mothers on natural pastures until weaning (6-9 months of age), after that they were supplemented with concentrate and straw. The young bulls were finished on concentrate during 3-5 months. The concentrate composition fed to calves and young bulls are depicted in Table 8.

The calves from PGI veal are crossbred females, since males are slaughtered latter to be commercialised as "Novilho Tradicional do Montado" beef. Mertolenga-PDO beef is obtained almost exclusively from males, since females are kept for herd replacement, so their contribution to meat production is very small.

The animals were slaughtered and dressed in an officially approved slaughterhouse, according to standard methods, using a captive bolt stunner, followed by sticking and bleeding, from June to September. After slaughter, the carcasses were electrically stimulated to avoid cold shortening. Three hours *post-mortem*, when muscle temperature and pH were measured, the cooling chamber presented air velocity ranging between 0.1 and 0.6 m/s, temperature ranging between 3 and 6 ºC, and humidity ranging between 82 and 92%. The samples of *longissimus lumborum* muscle were collected in the slaughter house in several days, in order to assure different herd origins, and consequently, a more representative sampling.

4.2.2 Analysis of meat quality

The muscle pH and temperature were measured 3 hours *post-mortem* (pH_3 and T_3) at the 13th *thoracic vertebrae* level with a HI 99163 portable pH-meter (Hanna Instruments Inc., Rhode Island, USA). After 72-96 hours the carcasses were dressed and samples of *longissimus lumborum* were collected between the first and third *lumbar vertebrae* (around 0.7 – 1.0 kg). Those samples were *vaccum* packaged and stored at 0-1ºC until being processed. Six days after slaughter ultimate pH (pH_u), colour and dry matter (DM) were measured in the refrigerated samples. The pH_u was measured three times in each sample, and the value expressed was the mean of the three determinations.

Meat colour measurements were carried out with a Minolta CR 300 colorimeter (Konica Minolta Holdings Inc., Tokio, Japan) with a C iluminant and a 2º standard observer in the CIELAB space, after 1 hour of blooming to allow oxygenation. The colour coordinates were lightness (L*), redness (a*), yellowness (b*), chroma (C*) and hue (h*). The C* and h* were calculated from a*and b* values according to the device specifications. The dry matter content was determined in muscle samples by microwaves (Smart System 5, CEM Microwaves Technology Ltd., Buckingham, UK), following the device specifications. Afterwards, the samples were minced, *vacuum* packaged, frozen and stored at -18 ºC until the

laboratory analysis were performed. Two steaks were left intact for Warner-Bratzler Shear Force (WBSF) measurement and sensory analysis.

Table 8. Chemical composition (%) of concentrate feed according to PGI veal and PDO veal and beef specifications

Characteristics	Calves (age < 12 months)	Young bulls (age > 12 months)
Crude Protein	16.0-16.5	16.0-18.0
Crude Fat	2.6-6.0	2.5-5.0
Crude Fibre	6.0-9.7	6.0-9.8
Starch	Min 25.0	Min 20.0
WSC	Min 4.0	Min 4.5
Ash	Max 9.0	Max 9.0
Calcium	1.0-1.2	0.95-1.2
Phosphorus	0.55-0.62	0.50-0.62

WSC = Water soluble carbohydrates

Total pigment content was determined on two replicates, through the quantification of the cyanometmyoglobin and cyanomethemoglobin, by the method described by Wierbicki and coworkers (1955) and expressed as g/ 100 g DM.

The free fat content was measured according to the NP 1224 (2002) in fresh samples, and expressed as g/ 100 g DM. Briefly, 5 g of fresh minced muscle was weighted into cellulose extraction thimbles. As fresh meat was used 35-40 g of sodium phosphate was added in order to dehydrate the meat. The extraction was made with petroleum-ether for 6 hours. After evaporation of the petroleum-ether the Soxhlet extraction flasks (500 ml) were placed in the oven overnight at 103 ± 1 ºC to complete the evaporation process. Since hydrolysis of the samples was not performed the intramuscular fat content presented in this study corresponds to free fat content instead of total intramuscular fat content.

Total collagen concentration was determined through hidroxiproline quantification according to the Norma Portuguesa 1987 (2002) and adapted by Silva and coworkers (1999), and expressed as g/100 g DM. The collagen solubility was determined by the method described by Silva *et al.* (1999). The dilution steps were altered. Briefly, after hydrolysis for 16 hours at 105 ºC, the volume was adjusted with fresh distilled water (4 ºC) to 100 ml in soluble collagen and to 150 ml in insoluble collagen. The hydrolysates were filtered and aliquots of 25 ml (soluble collagen) and 10 ml (insoluble collagen) of the solutions were transferred to graduated tubes, where the volume was adjusted in both cases to 50 ml. The collagen

solubility was expressed as total collagen percentage. The absorbance was measured at 558 nm against a blank.

The myofibrillar fragmentation index (MFI) was determined as described by Silva *et al.* (1999). Briefly, 10 ml of a MFI solution was added to the 4 g of fresh minced muscle, the mixture was vortex-mixed for 10 s and centrifuged for 15 min at 4000 rpm. The supernatant was discarded and the procedure repeated. Afterwards, 15 ml of the MFI solution was added, vortex-mixed for 10 s and filtered with a strainer. This solution was used for the protein concentration measurement. The protein concentration of the suspension was determined by the Kjeldahl method (NP 1612, 2006). Samples protein concentration was uniformed for 0.5 g of protein/ml with MFI solution and then the absorbance was measured at 540 nm against a blank. The results were multiplied by a 200 factor.

Steaks with 2.5 cm thick were used for the WBSF determination, and were thawed at 0-4 ºC for 24 hours. The samples were weighted, grilled until it reachead 70 ºC of internal temperature, and weighted again for cooking losses determination. Grill cooking was conducted with a Modular 65/70 FTES electric griddle (Modular System Ltd., Italy) pre-heated at a temperature of 250 ºC. The temperature was controlled with a needle thermocouple probe, which was inserted horizontally at the midpoint of the steak's width. The steaks for WBSF were chilled until reached room temperature. Each sample provided a minimum of eight strips with a 1 cm^2 cross section. The cores were removed parallel to the muscle fibre orientation and were sheared perpendicular to the longitudinal orientation of the muscle fibres, using a TA-TX Plus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) equipped with a Warner-Bratzler shear blade. The beef sample resistance to shearing was recorded in a force-deformation plot. The maximum shear force in kg corresponded to the highest peak of the curve.

The steaks for sensory analysis were thawed at 0-4 ºC for 24 hours. The samples were wrapped in aluminium foil and cooked to an internal temperature of 70 ºC following the same procedure of WBSF measurements. After grilled samples were trimmed of the external connective tissue, and cut into 2 cm^2 cores. Each plate served had one piece of each meat type. Seven trained panellists assessed a profile composed by tenderness, juiciness, flavour and overall acceptability. The panellists were asked to score the samples on a ten-point structured line scale anchored at each end with the descriptors very tough/tender, very dry/juicy, very slight/strong and very much disliked/liked.

4.2.3 Statistical analysis

The statistical analysis was performed using the PROC MIXED procedure of Statistical analysis systems (SAS) software package, version 9.1 (SAS Institute Inc., Cary, NC, USA, 2004). Data was checked for normality and homocedasticy. For some variables significant differences of variances between the groups were found and thus were analysed by PROC MIXED model for variance heterogeneity. For these variables, variances of the three groups were pairwise compared in order to establish difference between the groups.

The relationship between the variables was determined using the Pearson's correlation coefficients (SAS, 2004).

The canonical discriminant analysis was applied to data in order to distinguish beef types. Variable selection for discriminant analysis was achieved using the significant variables after PROC MIXED analysis. The selection of the most significant variables was performed by forward 'stepwise' procedure (PROC STEPDISC) (SAS, 2004). Following this, the linear discriminant functions were developed using the PROC DISCRIMINANT (SAS, 2004) to determine the coefficients of the lipid composition variables that maximise the differences between the three meat groups. The statistical significance of each discriminant function was evaluated on the basis of the Wilks' Lambda factor after the function was removed. This parameter ranges from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power). Finally, to verify the power and the stability of the model, a 'leave-one-out' cross-validation discriminant analysis was performed. In this test known samples were used as 'unknowns' to validate the model built on the basis of a reduced set of cases. Statistical significance was set up at the $P<0.05$ level.

4.3 Results and Discussion

4.3.1 Individual variables

The concentrate composition fed to calves and young bulls are depicted in Table 8. Data on T_3, pH_3 and pH_u are presented on Table 9. As expected, PDO beef resulted from the oldest and heaviest animals at slaughter ($P<0.001$). Consequently, they had greater muscular mass, which leads to a slower temperature rate decline and hence to a higher T_3 ($P<0.001$). The higher value of T_3 observed in PDO veal relative to PGI veal was unexpected but can partly be explained by the significantly higher mean temperature of the chilling room (5.4 and 3.9 ºC, respectively) at three hours after slaughter.

There were no significant differences (P>0.05) between groups in the pH_3 of l*ongissimus lumborum* muscle. In some research, optimum tenderness was achieved by carcasses electrical stimulation, in order to produce a pH_3 of 6.0-6.1 (Chambaz *et al.*, 2003) which is similar to our results (Table 9). All meat types presented a pH_u within values considered normal ($5.4 \leq pH_u \leq 5.7$; Touraille, 1991) for beef and veal. The pH_u was significantly higher in PDO veal (P<0.001). The higher pH_u of PDO veal reflected the trend observed in pH_3 by this meat type. The ultimate pH is inversely related to quantity of glycogen present in the muscle at slaughter. Hence the higher pH_u of PDO veal could be due to stress before slaughter (Lawrie, 1998).

4.3.1.1 Meat Colour

The values of meat colour parameters are displayed in Table 9. The PDO beef had the lowest L* value (P<0.001) and the highest a* (P<0.001), b* (P<0.05; not different from PGI veal) and C* (P<0.001) values. On the contrary, the PGI veal presented the lowest a* and C* values. The PGI veal also presented the lowest and PDO beef the highest pigment content (P<0.001). The value presented by PDO beef in pigment content was 29% and 41% higher than the values presented by PDO veal and PGI veal, respectively. Moreover, PDO veal also presented pigment content 17% higher than PGI veal. The pigment content of muscle increases with age especially up to 24 months, and then, remains relatively stable (Renerre, 1986). This justifies the highest value of pigment content in PDO beef since these animals were older at slaughter. Accordingly, Gil *et al.* (2001) and Serra *et al.* (2008) also found higher pigment content in older animals when comparing several Spanish breeds.

It is also well known that males present higher pigment content than females. The higher pigment content of PDO veal group could be due to its male status, as it seems that at the same age the males has higher pigment content than the females (Lawrie, 1998), and/or to breed effect, given that the PDO calves were purebred and PGI calves were crossbred.

The colour is mainly influenced by the myoglobin nature and content, and by the composition and physical state of muscle (Renerre, 1986). The higher pigment content presented by PDO beef was reflected in a lower L* and higher a* and C* values, whilst the lower pigment content observed in PGI veal was also reflected in a higher L* and lower a* and C* values. The PDO veal presented intermediate values of pigment content, but also of a* and C* parameters of colour. The colour parameters suggest that PDO beef was darker and had a more saturated colour. Our results are in agreement with those from other authors suggesting that older animals have darker muscle colour (Warner, 1989; Lawrie, 1998).

Table 9 - Physical and chemical characteristics of *longissimus lumborum* muscle of "Vitela Tradicional do Montado"-PGI veal, Mertolenga-PDO veal and Mertolenga-PDO beef.

	PGI veal		PDO veal		PDO beef		*Significance*
	Mean	SEM	Mean	SEM	Mean	SEM	
T_3 (ºC)	16.0 [b]	0.339	18.3 [a]	0.494	19.9 [a]	0.931	***
pH_3	6.08	0.057	6.21	0.055	6.06	0.046	ns
pH_u	5.46 [b]	0.034	5.67 [a]	0.049	5.42 [b]	0.013	***
L*	35.73 [a]	0.499	34.87 [a]	0.594	31.52 [b]	0.339	***
a*	17.21 [c]	0.427	18.59 [b]	0.341	21.59 [a]	0.381	***
b*	2.88 [a b]	0.341	2.35 [b]	0.350	3.79 [a]	0.340	*
h*	9.19	1.042	6.92	1.086	9.74	0.758	ns
C*	17.51 [c]	0.452	18.81 [b]	0.352	21.95 [a]	0.420	***
Pigment (g/100 g DM)	1.05 [c]	0.043	1.26 [b]	0.088	1.79 [a]	0.073	***
Free Fat (g/ 100 g DM)	2.04	0.182	1.98	0.110	2.33	0.141	ns
Total Collagen (g /100 g DM)	2.22 [b]	0.053	3.00 [a]	0.109	2.82 [a]	0.099	***
Collagen Solubility (%)	19.51 [a]	0.775	15.31 [b]	0.732	17.08 [a b]	1.308	*
MFI	22.28	1.678	25.79	2.340	23.01	2.116	ns
Cooking Losses (%)	22.51 [b]	0.732	22.34 [b]	0.732	25.25 [a]	0.755	*
WBSF (kg)	5.16 [b]	0.377	5.56 [b]	0.521	7.43 [a]	0.455	**
Tenderness	6.05	0.321	5.08	0.391	4.94	0.355	ns
Juiciness	4.79	0.318	4.12	0.350	4.44	0.273	ns
Flavour	3.75	0.242	3.70	0.217	5.58	0.219	ns
Overall acceptability	5.31	0.290	4.48	0.419	4.59	0.333	ns

Statistical probability: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different subscripts are significantly different; SEM= standard error of the mean. T_3 = Muscle temperature measured 3 hours *post-mortem*; pH_3 = Muscle pH measured 3 hours *post-mortem*; pHu = Muscle pH measured 6 days *post-mortem*; DM = dry matter; MFI = myofibrillar fragmentation index; WBSF = Warner-Bratzler shear force.

It is assumed that the pH_u affect beef colour through its effect on myoglobin nature (Monin, 1991). However, this relationship was not clear in the present study since the PGI veal and PDO beef presented similar pH_u value, but different colour parameters. So, it seems that the pigment content had a higher influence on meat colour than pH_u.

In other study of our work team relating consumer visually appreciation of beef colour in samples where colour was also instrumentally measured, we realise that the three meat types presented colour parameters values which indicate that these meat types would have great acceptability by consumers (data not published).

4.3.1.2 Meat Tenderness

The data on meat texture characteristics are presented in Table 9. All meat types analysed had a similar (P>0.05) and low free fat content (1.98-2.33 g/100 g DM), which is an advantage from the consumers' point of view. These values are lower than those reported by Costa *et al.* (2008) (1.43-1.82 g/100 g fresh muscle) and Monteiro (2003) in Mertolenga-PDO beef (3.32 g/100 g DM) but the animals from these studies were older and heavier, which is known to increase intramuscular fat content (Vestergaard *et al.*, 2000). This tendency was seen in our study despite the lack of significant differences between PDO veal and beef. There were several factors that probably affected the intramuscular fat content of these animals. First, it is important to notice that our results report to the free fat content instead of the total intramuscular fat content, and it has been reported in the literature that animals with low fat content usually have greater proportion of phospholipids, which are not included in the free fat content. Second, these animals were slaughtered in a very dry spring/summer and considering they were raised on pasture we can postulate that these animals mobilized the body reserves attending to the food scarcity.

The PGI veal had lower total collagen content (P<0.001) than PDO veal and beef, and higher collagen solubility (P<0.05) than PDO veal. Monteiro (2003) reported collagen solubility values similar to the presented here (18.87%), but higher total collagen contents (4.14%) in Mertolenga beef. The lowest collagen content presented by PGI veal could be a gender effect since some authors reported that females have lower collagen content than males (Bailey & Light, 1989). Moreover, it could also be a breed effect, since PGI veal is from crossbred animals that are expected to have higher muscular development than purebred animals like PDO calves (PDO veal) and young bulls (PDO beef). The relatively low value of the collagen solubility (15.3%) observed in PDO veal (18% and 22% lower than the values presented by PDO beef and PGI veal, respectively), was unexpected attending to the age of the animals. The growth rate before slaughter affects collagen turnover. Bailey and Light (1989) defended that animals with a higher growth rate have younger collagen with less heat-stable cross-links, *i.e.* higher collagen solubility. The PGI veal is from crossbred animals that are expected to have a higher growth rate than the PDO purebred animals. This seems to be the obvious explanation for the higher collagen solubility presented by PGI veal. However, the lower collagen solubility value presented by PDO veal comparing to PDO beef remain unexplained.

The values of MFI were similar (P > 0.05) for all the analysed meat types, averaging 23.7. This mean value is somewhat lower than the values presented by Monteiro (2003) in the animals of the same breed (averaging 29.9%) and in Carnalentejana-PDO beef (averaging

27.4). These higher values could result from the longest ageing period applied, since that MFI is a measure of the *post-mortem* proteolysis. Various reports have shown that MFI increases with ageing, which seems to be related to the phenomenon of myofibrils breaking into shorter fragments at, or near, the Z-disk during *post-mortem* storage (Olson *et al.*, 1976).

The PDO beef had the highest value of cooking losses ($P<0.01$) and WBSF value ($P<0.01$). The cooking losses of PDO beef were 11% and 12% higher than the values presented by PGI veal and PDO veal, respectively. The values found in this study are slightly lower than those reported by Monteiro (2003) (30.33%) in Mertolenga-PDO beef. The value of PDO beef WBSF was 25% and 31% higher than the value of PDO veal and PGI veal, respectively. PDO beef also presented a trend for a lower sensory tenderness score ($P=0.06$).

The tenderness appears to be related to the rates of *post-mortem* degradation of the myofibrillar network, linked to the biochemical proteolysis and to the amount and nature of collagen present around and between the fibres (Bailey & Light, 1989). Moreover, Savell and Cross (1988) suggested that marbling also improves meat tenderness. The results of our study are not in accordance with the exposed. Considering our results we would expect that PDO veal also presented higher WBSF value than PGI veal. In fact, the PDO veal presented similar values of collagen content and solubility as well as of free fat content and MFI to PDO beef. In turn, PDO veal presented higher collagen content and lower collagen solubility than PGI veal. These results are in line with other studies reporting that the collagen content is neither related with WBSF nor with taste panel tenderness (Hunsley *et al.*, 1971). It looks like WBSF was more influenced by cooking losses than by collagen content or by *post-mortem* proteolysis. The values obtained for WBSF indicate that PGI veal and PDO veal are tenderer than PDO beef (Table 9). The WBSF value presented by the two veal (averaging 5.36 kg) is within the range tenderness values where beef is considered to have a large acceptability by the Portuguese consumers (Simões & Lemos, 2005).

The sensory tenderness value presented by PGI veal, despite not statistically different from the PDO meat groups, has tended to be higher (6.1 in a scale from 1 to 10) reinforcing the idea that this veal would probably have a great acceptability in the Portuguese market.

Regarding juiciness, flavour and overall acceptability, the panellists considered the three types of meat similar to each other. All meat types were scored as slightly dry and having slightly acceptability. PGI and PDO veal had a moderately slight beef flavour, whilst PDO beef had a slightly strong beef flavour.

4.3.2 Correlations between variables

The effect of age and CW will be discussed together, because it is difficult to distinguish the effect of each variable separately. The results from the correlation of each one of these two variables with the other variables studied were very similar. Nevertheless the correlation coefficients from the two variables are still presented (Table 10). These two variables were highly correlated with a correlation coefficient of 0.86 ($P<0.001$).

4.3.2.1 Meat Colour

The correlation coefficients among meat colour and age, CW, pH_3, pH_u and total pigment content of the PGI veal, PDO veal and PDO beef are presented in Table 10. There are several significant correlations suggesting that meat colour was affected by the variables analysed. The age/CW strongly influenced the beef colour, being negatively correlated with L* ($P<0.001$) and positively with a*, b* and C* ($P<0.001$). The muscle pigments had the same effect on the beef colour parameters ($P<0.001$), except for yellowness, as the pigment content was not correlated with b* parameter. Probably the effects of age/CW on the meat colour could be explained by the pigment content, as age and CW are strongly correlated with pigment content (correlation coefficients of 0.72 and 0.73, respectively; $P<0.001$). These results are in agreement with the results reported by Serra *et al.* (2004), who also reported that pigment content correlates negatively with L* and positively with a* parameters. Thus, beef from older and heavier animals with higher pigment content will be darker, with a more intense and saturated colour.

Our results also show an important effect of pH_u on beef colour as consequence of the strong correlation with b* and h* parameters ($P<0.001$), and a moderate negative correlation with a* ($P<0.05$) and C* ($P<0.01$) parameters. In line with our results, Abril *et al.* (2001) and Page and coworkers (2001) also found a negative correlation between pH and a* and b* values. The latter authors found a stronger correlation between these variables than between L* and pH.

It is well documented in literature that a high final pH is related to darker beef, but the results of Page *et al*. (2001) and ours suggest that pH affect the beef colour by altering h* through changes in b* values.

Table 10. Pearson's correlation coefficients between colour parameters and age, carcass weight (CW), pH_3, pH_u and total pigment content of *longissimus lumborum* muscle of PGI veal, PDO veal and PDO beef.

	Age	CW	pH_3	pH_u	Pigments
L*	-0.54***	-0.56***	0.08	-0.19	-0.71***
a*	0.66***	0.70***	-0.22	-0.29*	0.66***
b*	0.37***	0.39***	-0.18	-0.63***	0.17
h*	0.22	0.23	-0.15	-0.68***	-0.03
C*	0.66***	0.70***	-0.21	-0.31**	0.65***
Pigments	0.72***	0.73***	0.00	-0.01	-

Statistical probability of correlation coefficients: no superscript, P>0.05; *, P<0.05; ***, P<0.001. CW = Carcass weight; pH_3 = muscle pH measured at 3 hours *post-mortem*; pHu = Muscle pH measured 6 days *post-mortem*

4.3.2.2 Meat Tenderness

The correlation coefficients among meat chemical and physical characteristics and sensory attributes are shown in Table 11. The early pH and temperature have been suggested as factors affecting meat tenderness, as they influence the activity of endogenous enzymes (Dransfield, 1994). However, neither MFI nor WBSF were correlated with pH (P>0.05) or with T_3 (P>0.05).

The MFI and WBSF were inversely correlated (P<0.05), *i.e.*, a higher myofibrillar proteolysis resulted in a lower WBSF. Accordingly, Savell and Cross (1988) and Silva *et al.* (1999) also found an inverse correlation between MFI and WBSF. This result is not surprising as during *post-mortem* proteolysis the breakage of myofibrillar structure weakens the muscle fibre.

Hocquette *et al.* (2010) stated that marbling also affects tenderness, indirectly though. Accordingly, Savell and Cross (1988) suggested that marbling improves meat tenderness by reducing bulk density and decreasing strength of the connective tissue, since the intramuscular fat is deposited between the perimysium surrounding the muscle bundles. Moreover, the lubrication effect may improve quality by increasing juiciness, as fat stimulates saliva production by the salivary glands (Fiems *et al.*, 2000). Unexpectedly, our results are in contradiction with the results presented by those authors as the free fat content, although weakly, correlated positively with WBSF. This result is not easy to explain, so further investigation would be necessary to understand it. It should be notice though, that the free fat content in the three meat types is low.

The cooking losses correlated positively with T_3 (0.30; P<0.05). The high temperature and pH early *post-mortem* favours peptidasic activity, leading to structural changes in the muscle

during the ageing process. These structural changes, such as the destruction of all membranes, the transverse and longitudinal shrinkage of muscle fibres, the aggregation of sarcoplasmic proteins and shrinkage of the connective tissue, results in cooking losses (Honikel, 1998) and harder meat. Our results also showed a weak correlation (P=0.06) between T_3 and WBSF. The cooking losses correlated positively with WBSF (P<0.001) and inversely with sensory tenderness (P<0.01), which seems to be in agreement with the above explanation, and explained a WBSF and sensory tenderness of 14% and 23% ($r^2 \times 100$), respectively. Accordingly, Silva *et al.* (1999) Destefanis *et al.* (2000) and Serra *et al.* (2008) found cooking losses to be inversely correlated with tenderness, and with overall acceptability. The latter could be due to an indirect effect of a higher tenderness score, since this trait is highly correlated with overall acceptability.

4.3.2.3 Sensory Attributes

The meat tenderness and WBSF correlated inversely (P<0.01), which was expected and in agreement with the majority of the research published (*e.g.* Silva *et al.*, 1999; Destefanis *et al.*, 2000; Vestegaard *et al.*, 2000; Chambaz *et al.*, 2003). This is not surprising, as WBSF measures the hardness, which is the opposite of tenderness. The good correlation between WBSF and sensory tenderness turns instrumental WBSF technique a good predictor of sensory tenderness.

Collagen content and meat flavour (P<0.05) correlated inversely. Accordingly, Jeremiah *et al.* (1993) found an inverse correlation between total collagen content and flavour desirability.

The two major sensory attributes of meat, tenderness and juiciness, correlated positively (P<0.001). This relation has been reported previously by several authors (*e.g.* Silva *et al.*, 1999; Serra *et al.*, 2008), with correlation coefficients ranging from 0.58 to 0.88.

This relationship may be explained by the fact that, the tender the meat is, more quickly juices are released by chewing, which gives the sensation of a juicier meat (Savell & Cross, 1988). The sensory tenderness was also positively correlated with flavour (P<0.001) and with overall acceptability (P<0.001), which was expectable since consumers' acceptability is largely based on sensory tenderness (Destefanis *et al.*, 2000). The meat juiciness correlated positively with pH_u (P<0.05). With increasing pH more water becomes retained between the muscle fibre bundles, which will be released during the mastication process. The meat juiciness also correlated negatively with WBSF (P<0.001) and positively with flavour (P<0.001) and overall acceptability (P<0.001). Accordingly, Destefanis *et al.* (2000) also found similar correlations

Table 11. Correlation coefficients between physical and chemical characteristics of meat quality and sensory attributes in *longissimus lumborum* muscle of PGI veal, PDO veal and PDO beef.

	Age	CW	pH_3	T_3	pH_u	Free fat	CL	Collagen	Solubility	MFI	WBSF	Tenderness	Juiciness	Flavour
WBSF	0.41***	0.43***	-0.24	0.23	-0.19	0.31*	0.48***	0.12	0.07	-0.31*	-	-	-	-
Tenderness	-0.18	-0.21	0.07	-0.07	0.21	-0.19	-0.37**	0.04	0.03	-0.03	-0.38**	-	-	-
Juiciness	-0.00	-0.07	0.10	-0.05	0.29*	-0.16	-0.22	-0.15	-0.04	-0.15	-0.36**	0.72***	-	-
Flavour	-0.06	-0.16	-0.13	-0.07	-0.01	-0.10	-0.02	-0.30*	0.07	-0.03	-0.22	0.42***	0.44***	-
OA	-0.09	-0.16	0.06	-0.05	0.25	-0.20	-0.31*	-0.12	0.00	-0.09	-0.40**	0.90***	0.89***	0.50***

Statistical probability of correlation coefficients: no superscript, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001.

CW = Carcass weight; pH_3 = Muscle pH measured at 3 hours *post-mortem*; T_3 = Muscle temperature measured 3 hours *post-mortem;* pHu = Muscle pH measured 6 days *post-mortem*; CL = Cooking losses; Solubility = Collagen solubility; MFI = myofibrillar fragmentation index; WBSF = Warner-Bratzler shear force; OA = Overall acceptability.

between WBSF and overall acceptability. The flavour and overall acceptability (P<0.001) were also correlated. According to our results, Monsón *et al.* (2005) reported that tenderness (r=0.60), juiciness (r=0.59) and flavour (r=0.49) were the attributes that most influenced the acceptability of meat. The correlations observed between the sensory attributes could be due to the "halo effect", *i.e.* when an attribute is enhanced by other characteristics of the product (Gill *et al.*, 2010).

The age/carcass weight was correlated with the majority of the quality variables, excepting the sensory variables, indicating this could be the main effect between the meat types.

4.3.3 Discriminant analysis

The canonical discriminant analysis was applied to the meat quality variables in order to discriminate the beef types used in this study. The results of the canonical discriminant analysis, loadings of correlation matrix and discriminant functions are presented in Table 12. The differences between meat types in quality variables selected by the canonical discriminant analysis can be evaluated by the values of squared Mahalanobis distance (D^2).

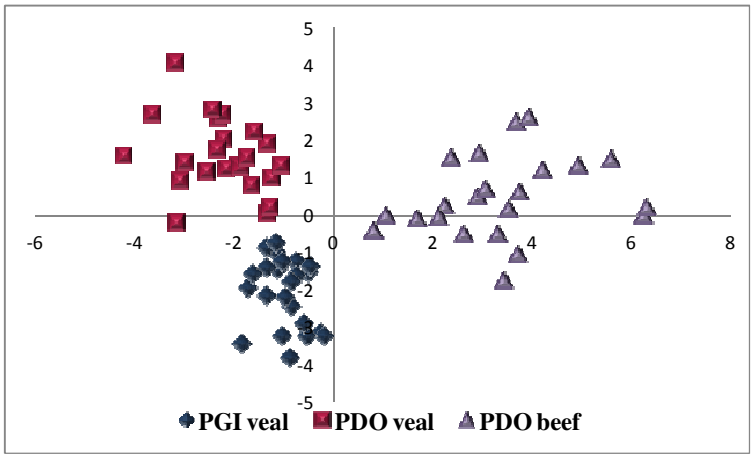
Table 12 - Results of canonical discriminant analysis: loadings of correlation matrix between predictor variables (standardized canonical coefficients) and discriminant functions (roots 1 and 2), and some statistics for each function.

	Root 1	Root 2
Variables		
pH_u	-3.3668	5.9908
a*	-3.3346	5.0445
L*	-0.5776	0.4065
C*	3.4423	-4.4586
GI	-1.8047	-0.8787
Total collagen	1.0654	1.6950
WBSF	0.2747	0.0553
Overall acceptability	0.0943	0.2516
Statistics		
Canonical R	0.874	0.825
Eigenvalue	3.968	2.275
Cumulative proportion	0.636	1.000
Probability	<0.0001	<0.0001

In line with this, the differences were smaller between VTM-PGI veal and Mertolenga-PDO veal (13.6), than between PGI veal and PDO beef (21.7) or between PDO veal and Mertolenga-PDO beef (18.0), as illustrated in Figure 7. This means that the two veal are more similar to each other than with the PDO beef, indicating that breed was not the discriminating factor between the meat types. Our work team compared the results of the discriminant analysis from the same three meat types, but having the lipid profile as discriminant variables, and realise that the distance between the veal types were likewise the smallest, and the distance between PGI veal and PDO beef was higher than the distance between the two PDO meat types (see Chapter 3, sub-section 3.3.3).

The application of canonical discriminant analysis to selected variables resulted in two discriminant functions, which maximise the ratio between class variance and minimises the ratio within class variance, discriminating the three meat types studied. The discriminant functions obtained used eight variables, from which pH_u, a* and C* parameters had the highest discriminant power in both roots.

Figure 7 - Plot of the discriminant functions (root 1 *vs.* root 2) for classification of the three types of beef (PGI veal, PDO veal and PDO beef).



After cross-validation, results varied between 90.0 and 100% of correct classification, being the lower from both PDO and the highest from PGI veal. As can be seen in the plot, both veal types are located in the left side of the plot, whilst the PDO beef is located in the right side (Figure 7). The second function (root 2) discriminates both veal, having the PGI veal positive loadings and PDO veal negative ones. The variables that discriminate the veal

types were pH_u, total collagen in both roots and T_3 in the first root and overall acceptability in the second one.

Our results clearly indicate that despite the small extent of the differences obtained in meat quality characteristics and the similarity of the production systems of the animals, it was feasible to allocated meat samples into one of the three meat types with good accuracy.

4.4 Conclusion

The PDO beef is darker and redder than the PGI and PDO veal. The darker colour presented by PDO beef does not seems to compromise PDO beef acceptability by the Portuguese consumer. However, WBSF values indicate that PDO beef is considered a hard meat probably due to an insufficient ageing period which compromises it acceptability by the Portuguese consumer. Thus, much has to be done in the Portuguese industry concerning the motivation of professionals to practice a longer ageing period in order to improve meat quality. On contrary, the PGI and PDO veal are medium tender and consider to have a great acceptability by the Portuguese consumer. Cooking losses had a moderate negative effect on sensory tenderness and affected slightly the WBSF value. The correlation between sensory tenderness and WBSF, despite moderate, can indicate that sensory tenderness can be predicted by instrumental means.

These results indicate that within a similar production system the veal from female crossbred calves and from male purebred calves were very similar, indicating that the gender and crossbreeding practices (purebred *vs.* crossbred) have minor effects on the muscle chemical and physical composition. In our study the age and/or carcass weight had shown a more significant effect on muscle characteristics. Nevertheless, it was possible to discriminate between PGI veal, PDO veal and PDO beef using the variables selected by canonical discriminant analysis.

Acknowledgements

The financial support from Ministry of Agriculture (AGRO/2004/422) and the individual grant from FCT to Ana C. G. Monteiro (SFRH/BD/31091/2006) are acknowledged.

5 - Nutritional value and variability of lipids from the three main beef types marketed in Portugal

A.C.G. Monteiro, M. A. Fontes, J.A.M. Prates, R.J.B. Bessa & J.P.C. Lemos

CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Avenida da Universidade Técnica, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal

Nutritional value and variability of lipids from the three main beef types marketed in Portugal

Abstract

The nutritional value of lipids from the three main beef types marketed in Portugal, Carnalentejana-PDO, imported from Brazil and national undifferentiated, were assessed to be characterised. The samples, from the Portuguese market place, were analysed for cholesterol, β-carotene, α- and γ-tocopherols, L-carnitine, fatty acid composition, including conjugated linoleic acid (CLA) isomeric profile, and nutritional value of lipids. All beef types had low cholesterol content and α-tocopherol level high enough to inhibit lipid oxidation and discoloration. The Brazilian beef had higher α-tocopherol, β-carotene and L-carnitine, as well as 18:0, 18:3 *n*-3, CLA, 20:5 *n*-3, 22:5 *n*-3, SFA and total *n*-3 fatty acids. In contrast, the Brazilian beef presented lower γ-tocopherol, TBARS, 18:2 *n*-6, PUFA, TFA and total *n*-6 fatty acids values. The energy value was calculated for the main fatty acids or sum of FA, considering a serving beef with 100 g and compared with the recommended daily intake. A 100 g serving beef provides from 1.79 to 2.07 g of fat from which, 457 mg are 16:0, 54.5-72.9 mg TFA, 70.6-167.8 mg 18:2 *n*-6, 5.0-21.9 mg 18:3 *n*-3, 32.8 mg *n-6* LC-PUFA (20:4*n*-6 + 22:4 *n*-6) and 7.8-21.5 mg *n-3* LC-PUFA (EPA and DPA). The detailed study of lipid composition variability suggests that the national undifferentiated beef was the most variable among the three beef types studied.

Keywords: Beef, Cholesterol, CLA isomers, Fatty Acids, Meat Quality, Vitamin E.

5.1 Introduction

The FAO/WHO established the criteria to describe the strength of evidence for developing dietary guidelines (Elmadfa & Kornsteiner, 2009). The recommendations take into account the fact that fat has the highest energy value of all macronutrients. The fat has a reducing satiating influence, which combine with the fact that induce palatability, results in an over consumption of fat (Blundell & MacDiarmid, 1997).

In the last decades, food scares have increased consumer' concerns for food safety accompanied by significant reductions in the consumption of the affected products. This has been particularly evident regarding beef. Consumer' demand for beef is increasingly influenced by healthfulness, quality, nutritional content and safety and more recently by the intangible attributes such as animal and environmental friendly production systems (Angulo & Gil, 2007). Thus, new trends occurred in the fresh beef market with the aim to differentiate beef on the basis of product brandings, geographical origin, sensory or processing characteristics (Scollan *et al.*, 2006).

Besides food scares, beef consumption has been also affected by the negative publicity on media, especially with regard to its composition in saturated and *trans* fatty acids (TFA), cholesterol, as well as the unbalanced relationship between the *n*-6 and *n*-3 fatty acids (FA). However, the nutritional relevance of indexes like P/S (polyunsaturated/saturated FA) and *n*-6/*n*-3 FA has been questioned. It is well established that ruminant edible products have relatively low P/S ratios as a consequence of the extensive C18 PUFA biohydrogenation in the rumen, with the consequent stearic acid (18:0) accumulation. Moreover, individual saturated fatty acids (SFA) have different effects on the plasma cholesterol levels. Whilst lauric (12:0), myristic (14:0), and palmitic (16:0) FA increase (Katan *et al.*, 1994), the 18:0 FA decrease the low density lipoproteins (LDL) cholesterol (Mensink, 2005). The specific biological effects observed for 18:0, relative to the other SFA, has been attributed to the high endogenous conversion rate of 18:0 to 18:1 conducted by the stearyl-CoA desaturase (Lichtenstein, 2006). Thus, the P/S ratio might not be the most appropriate nutritional index to evaluate FA in the ruminant meat as discussed by Santos-Silva *et al.* (2002). Recently, the nutritional relevance of the *n*-6/*n*-3 FA ratio, as a risk factor for some cancers and coronary heart disease of humans has been discredited (Griffin, 2008). Recent dietary guidelines from FAO/WHO are more focused on the absolute amounts of specific PUFA intake rather than on FA ratios, recommending the increase in the consumption of LC-PUFA, mainly EPA and DHA (Elmadfa & Kornsteiner, 2009).

Beef is also an important source of conjugated linoleic acid (CLA), vitamins and L-Carnitine. CLA is a collective term of class of conjugated dienoic isomers of linoleic acid. CLA is found predominantly in food products derived from ruminants (Griinari *et al.*, 2000). Important biological effects, including anticarcinogenic, antiatherosclerotic, antiadipogenic and antidiabetogenic activity, have been attribute to *c*9,*t*11 and *t*10,*c*12 CLA isomers (Belury, 2002).

While CLA effects on health have been issue of extensive discussion in the last decade, L-carnitine has been much less studied. L-carnitine is a small molecule derived from lysine, which can be supplied to the body through endogenous biosynthesis (from lysine and methionine) and food intake (Vaz & Wanders, 2002). The primary function of L-carnitine is to allow the entry of esterified fatty acids into the mitochondrial matrix, where β-oxidation of fatty acids occurs (Demarquoy *et al.*, 2004). Thus, due to its important role in fatty acid transport towards mitochondrial membrane, L-carnitine is a key element in fat metabolism. Recent studies have shown that L-carnitine can act as antioxidant in meat, suppressing oxidative damage during ageing, inhibiting lipoperoxidation of linoleic acid and protecting against damage induced by H_2O_2 (Djenane *et al.*, 2004). Thus L-carnitine content in muscle might contribute to its oxidative stability. Given the potential beneficial effects attributed to L-carnitine and the lack of studies concerning L-carnitine content in beef it seemed important to include this variable in the study.

Detailed information on lipid composition of beef (cholesterol, vitamins and fatty acids with the greatest impact on health) is necessary to estimate the contribution of beef to daily intake requirements. Therefore, the aim of this work was to stud lipid quality and variability from the main beef types available on the Portuguese market and their contribution to the recommended human dietary intake of lipids.

5.2 Material and Methods

5.2.1 Meat samples

This study was performed on 16 Carnalentejana-PDO, 15 Brazilian and 15 national undifferentiated beef samples. The samples of *longissimus lumborum* muscle were bought in a hypermarket, according to their availability. We intended to imitate the purchase performed by the regular consumer, in order to be as faithful as possible to what the Portuguese consumers meets in the market. The samples of *longissimus lumborum* muscle were collected in the hypermarket during five months and each sample belonged to a different animal. The

samples were representative of all the batches marketed in that commercial area during the referred period of time.

The Carnalentejana-PDO beef is obtained from Alentejana purebred young bulls produced in a traditional semi-extensive production system according to the product specifications. Protected Designation of Origin (PDO) beef is quality branded beef certified by the European legislation (Regulation n. 510/2006 of 20 March 2006, EC). These products follow strict rules detailed in the specification book for each product. These rules concern mainly the breed, origin and the production system. Among the several Portuguese PDO beef, Carnalentejana-PDO beef is commercially the most important.

Brazilian beef was the imported beef chosen, because this beef market share has been growing in the last years. In fact, the imported Brazilian beef increased from 2303.8 of fresh beef in 2005 (GPP, 2007b) to 2897.2 tonnes in 2007 (GPP, 2009).

Brazilian beef is obtained from crosses of local breeds, like Nelore (*Bos indicus*), with more exotic breeds (*Bos taurus*), and is usually produced in a traditional semi-extensive production system based on pastures followed by a finishing period with concentrates.

The national undifferentiated beef is originated from crossbred young bulls produced in a conventional intensive concentrate based system, being the most consumed type of beef in Portugal. This group consists of beef from different crossbred animals, mainly with Charolais and Limousin sires, of different ages and genders produced in Portugal.

The ageing period was significantly different between Brazilian beef and the two Portuguese beef groups (78 ± 9.0 days vs 12 ± 5.3 days in Carnalentejana-PDO and 13 ± 6.0 days in national undifferentiated beef).

Samples were trimmed from their visible fat and connective tissue and then minced, vacuum packaged and frozen at -80 ºC until analyses were performed.

5.2.2 Analysis of cholesterol and lipid soluble antioxidant vitamins

Total cholesterol and lipid-soluble antioxidant vitamins were extracted from homogenised meat samples, after saponification with KOH solution freshly prepared, according to the procedure described by Prates and coworkers (2006). The DL-α and D-γ-tocopherols standards were obtained from Calbiochem (Merck Biosciences, Darmstadt, Germany) and all-trans–carotene and cholesterol standards from Sigma Chemical Co. (St. Louis, MO, USA). Cholesterol, *α*- and γ-tocopherols were separated by normal-phase HPLC (Zorbax Rx-Sil with the corresponding 12.5 mm analytical guard column, 250 mm × 4.6 mm internal diameter, 5 μm particle size; Agilent Technologies Inc., Palo Alto, CA, USA), using an HPLC system

(Agilent 1100 series, Agilent Technologies Inc.) equipped with an UV-Vis photodiode array detection for cholesterol (202 nm) and β-Carotene (450 nm) and with a fluorescent detection for tocopherols (excitation and emission wavelengths of 295 and 325 nm, respectively) in series. The solvent (30 ml/l isopropanol in *n*-hexane) flow rate was 1 ml/min and the injection volumes were 100 µl for cholesterol and β-carotene, and 20 µl for α-and γ-tocopherols. Total contents of cholesterol, tocopherols and β-carotene in meat were determined in duplicate, from a standard curve of peak areas *versus* compounds concentration.

5.2.3 Analysis of tiobarbituric acid

The determination of oxidation was performed by thiobarbituric acid-reactive substances (TBARS) technique described by Botsoglou *et al.* (1994) and modified by Grau *et al.* (2000). Briefly, 2 g of fresh minced meat were weighted to which 1 ml of EDTA (0.3% in an aqueous solution), 5 ml of BHT (0.8% in hexane) and 8 ml of TCA (5% in an aqueous solution) were added. The mixture was homogenised at 19000 rpm for 30 seconds in a mixer, maintaining the tubes in ice, followed by centrifugation at 1900 g during 5 min. The upper layer of hexane was discarded and the remaining volume was filtered and adjusted to 10 ml with TCA solution. An aliquot of 2.5 ml was removed and 1.5 ml of the TBA solution was added. After that it was incubated during 30 min at 70ºC. After cooling the tubes in water, absorbance was measured at 532 nm against a blank sample (2.5 ml TCA + 1.5 ml TBA solutions). Beef samples had been frozen for two years before being analysed. All measurements were performed in duplicate and expressed as mg of malondialdehyde (MDA) per kg of meat.

5.2.4 Analysis of L-carnitine

Five grams of minced meat was used for free L-carnitine extraction and followed the enzymatic colorimetric procedure described by Shimada *et al.* (2004). The meat samples were homogenised at 8000 rpm for 30 seconds (two times 15 s) in a mixer, always maintaining the tubes in ice, after added 25 ml of percloric acid 0.3 M. The mixture was then centrifuged at 1140 g for 10 min, and filtered in glass wool. The previous steps were repeated after residue resuspension with 20 ml of percloric acid 0.3 M (homogenization, centrifugation and filtration). The final volume was adjusted to 50 ml with percloric acid 0.3 M. An aliquot of eight ml of the filtered pooled supernatant fraction was neutralized with KOH 1.2 M and centrifuged for 10 min at 8390 RCF. The mixture was ultra-filtered with a 0.45 µm Millipore filter and the volume adjusted to 10 ml. The absorbance reading was made after add 100 µl of

tissue extract and 100 µl of a buffer freshly prepared to each well of the microplate. The blank reading was made in a Microplate Reader (SpectraMax 340 Pc Molecular Devices), at a wavelength of 415 nm, after 10 min of incubation with shaking at 37 ºC. After that, 50 µl of 5.0 U carnitine acetyltransferase was added to each well in order to initiate the enzymatic reaction. The mixture was once more incubated for 10 min at 37 ºC, and the absorbance was again measured at 415 nm. The difference between final and initial absorbance readings was used to calculate the L-carnitine content. The L-carnitine content was determined in duplicate, from a standard curve of reading wavelength *vs.* compound concentration.

5.2.5 Lipid extraction and methylation

The beef samples for IMF and FA determination were lyophilised (-60 ºC and 2.0 hPa) to constant weight with a lyophilisator Edwards Modulyo (Edwards High Vacuum International, UK). The intramuscular fat content was measured according to the AOAC official method (945.16, 2000), and expressed as mg/ g muscle. Briefly, 1 g of lyophilised minced muscle was weighted, and after one hour of hydrolysis with HCl 4N, samples were filtrated with Whatman Nº 1 filters, which were afterwards dried at 103 ± 1 ºC for one hour. After have cooled at room temperature, the filters were inserted into cellulose extraction thimbles. The extraction was made with petroleum-ether for 6 hours. After evaporation of the petroleum-ether the Soxlet extraction flasks (500 ml) were placed in the oven overnight at 103 ºC to complete the evaporation process.

Briefly, FAME were directly extracted and methylated from 250 mg of lyophilised meat samples by a one-step procedure (adapted from Christie *et al.*, 2001). FA were converted to their methyl esters (FAME) by a combined transterification procedure with NaOH in anhydrous methanol (0.5 M) followed by HCl/methanol (1/1 v/v) at 50 °C, during 30 and 10 min, respectively (Raes *et al.*, 2001). The nonadecanoic acid (19:0) was added as internal standard. The same FAME solution was used for the analysis of both FA composition and CLA isomeric profile.

5.2.6 Analysis of fatty acid methyl esters

The gas chromatography analysis of FAME were performed using a gas chromatograph Varian 3800 (Varian Inc, Walnut Creek, USA) fitted with a flame ionization detector (FID) and an OmegaWax 250 (Supelco, Bellefont, CA, USA) capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Briefly, the gas chromatograph oven temperature

was programmed to start at 150 ºC and held at this temperature for 15 min, followed by an increase of 3 ºC/min to 220 ºC and, finally, held at 220 ºC for 20 min. The injector and detector temperatures were 250 ºC and 280 ºC, respectively. Helium was used as carrier gas and the split ratio was 1:20. Identification was accomplished by comparing the retention times of peaks from samples with those of FAME standard mixtures. The content of *trans*-octadecenoic FA was expressed as a single value (18:1*t*) because of their incomplete chromatographic resolution. The sum of TFA does not include the conjugated TFA. The quantification of FAME was based on the internal standard technique, using 19:0 as internal standard and on the conversion of relative peak areas to weight percentage. The FA were expressed as the percentage of the sum of identified FA (g/100 g total FA).

5.2.7 Determination of CLA isomers

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm internal diameter, 5 μm particle size, Chrompack, Bridgewater, USA), using a high performance liquid chromatography system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and diode array detector (DAD) adjusted to 233 nm. The mobile phase was 0.1% acetonitrile in *n*-hexane maintained at a flow rate of 1 ml/min, and injection volumes of 20 μl were used. The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial standards and with values published in the literature (Fritsche *et al.*, 2001). The identity of each isomer was controlled by UV spectra of CLA isomers from the DAD in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems (Agilent Technologies, 2001).

The total CLA content was quantified by GC analysis of total FAME and the relative concentrations obtained by Ag^+-HPLC were used to calculate the unresolved peaks in the GC chromatogram. The calculation of the *c*9,*t*11, *t*7,*c*9 and *t*8,*c*10 CLA isomers were performed by comparing the HPLC areas of these isomer peaks with the peak area of the three co-eluted isomers from GC chromatogram. The values for *c*9,*t*11 CLA content include those of *t*8,*c*10, CLA and the values for *t7*,*t*9 CLA include those of *t*8,*t*10 CLA because the complete separation of these CLA isomers was not possible by the GC under our experimental conditions (Fritsche *et al.*, 2001). Quantification of the other CLA isomers was assessed from their Ag^+-HPLC areas relative to the area of the main isomer *c*9,*t*11. A detailed description of the quantification process of individual CLA isomers using these two complementary methods was described by Prates and Bessa (2009).

5.2.8 Statistical analysis

The effect of the beef type was studied by analysis of variance using the PROC MIXED procedure of the Statistical Analysis Systems, version 9.1 (SAS Institute Inc., Cary, USA, 2004). Data were checked for normality and homocedasticy. The variables with significant differences of variances among groups were analysed by PROC MIXED model for variance heterogeneity. For these variables, the variances of the three groups were pairwise compared in order to detect significant differences of variances between groups.

Coefficients of variation (CV) were also determined by CV= ((SEM x $\sqrt{n}$)/ $\overline{X}$), where

SEM = standard error of the mean,

n = number of samples,

$\overline{X}$ = mean of the beef group.

The relationship between the variables was determined using the Pearson's correlation coefficients (SAS, 2004).

5.3 Results

5.3.1 Beef content of cholesterol, lipid soluble antioxidant vitamins, TBARS and carnitine

The content of intramuscular fat (mg/g muscle), total cholesterol (mg/g muscle), α- and γ-tocopherols (µg/g muscle), L-carnitine (µmol/g muscle) and TBARS (mg MDA/kg muscle) in *longissimus lumborum* muscle from Carnalentejana-PDO, Brazilian and national undifferentiated beef are presented in Table 13.

The content of intramuscular fat was similar ($P>0.05$) for the three meats analysed (averaging 19.5 mg/g muscle). No significant differences ($P>0.05$) concerning total cholesterol content were observed, which averaged 0.40 mg/ g muscle. The Brazilian beef had higher content of α-tocopherol (12.4 µg/g muscle; $p<0.001$) than the other two beef types, and greater content of L-carnitine (1.71 µmol/ g muscle; $p<0.01$) than the national undifferentiated beef. The Carnalentejana-PDO (1.66 µmol/g) and national undifferentiated (1.62 µmol/g) beef had similar values of L-carnitine.

The Brazilian beef presented lower content of γ-tocopherol (0.04 µg/g muscle; $p<0.001$) and TBARS (0.08 mg MDA/kg muscle; $p<0.001$) than the other two beef types. The β-carotene content of Carnalentejana-PDO and national undifferentiated beef were very low and

frequently under the detection limit of the method, ranging from not detected to 0.09 µg/g muscle in Carnalentejana-PDO beef and from not detected to 0.05 µg/g muscle in national undifferentiated beef. Conversely, all Brazilian beef samples presented detectable β-carotene value (averaging 0.21 µg/g muscle).

Table 13 – Intramuscular fat (mg/g muscle), cholesterol (mg/g muscle), α- and γ-tocopherol (µg/g muscle), TBARS (mg MDA/kg muscle) and L-carnitine (µmol/g muscle) of the *longissimus lumborum* muscle from Carnalentejana-PDO, Brazilian and national undifferentiated (NU) beef.

	Carnalentejana-PDO		Brazilian		NU		*Significance*
	Mean	SEM	Mean	SEM	Mean	SEM	
Intramuscular fat	17.9	1.23	20.7	1.55	19.9	2.05	ns
Cholesterol	0.40	0.006	0.40	0.005	0.40	0.009	ns
Alpha-tocopherol	3.22^{b}	0.446	12.44^{a}	0.974	3.71^{b}	0.461	***
Gama-tocopherol	0.13^{a}	0.010	0.08^{b}	0.017^{a}	0.16^{a}	0.020	**
TBARS	0.35^{a}	0.046	0.08^{b}	0.012	0.30^{a}	0.051	***
L-Carnitine	1.66^{b}	0.021	1.71^{a}	0.013	1.62^{b}	0.025	**

Statistical probability of treatment: ns, P>0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean.

5.3.2 Beef fatty acid content and composition

The intramuscular fat and the total fatty acid contents were similar among the three beef studied. In contrast, the three beef types differed in most of the FA (Table 14). The Carnalantejana-PDO and national undifferentiated beef presented a similar FA profile, although the 18:1 *t* was higher in Carnalantejana-PDO than in national undifferentiated beef. The Brazilian beef had higher proportions of most FA where significant differences were observed, except for the 18:2 *n*-6 (p<0.001) and 20:3 *n*-6 (P<0.05) in which the values were lower than in the other two beef types, and the 18:1 *t* (p<0.001) and 22:1 *c*9 (P<0.05) in which Brazilian beef presented a lower value than the Carnalentejana-PDO beef (Table 14).

Regardless the beef type, total FA content consisted predominantly of SFA (ranging from 41.2% to 45.7%) followed by monounsaturated FA (MUFA; averaging 37.3%) and finally by PUFA (ranging from 7.0 to 11.8%). Small amounts of *trans* FA (TFA) were detected (ranging from 2.7% to 4.1%).

The Brazilian beef had the highest SFA (p<0.01) and the lowest PUFA (p<0.01), whilst Carnalentejana-PDO beef had the highest total TFA (p<0.001).

Table 14 - Fatty acid content (mg/g muscle) and composition (g/100 g total FA) of the *longissimus lumborum* muscle from Carnalentejana-PDO, Brazilian and and national undifferentiated (NU) beef.

	Carnalentejana-PDO		Brazilian		NU		Significance
	Mean	SEM	Mean	SEM	Mean	SEM	
FA	12.7	1.176	14.8	1.475	14.3	1.348	ns
14:0	2.29^{b}	0.184	2.93^{a}	0.161	2.31^{b}	0.194	**
14:1	0.43^{b}	0.036	0.60^{a}	0.061	0.45^{b}	0.039	*
15:0	0.35^{b}	0.015	0.42^{a}	0.025	0.33^{b}	0.021	**
16:0	22.89	0.425	24.42	0.698	23.01	0.604	ns
16:1 *c*9	2.81	0.134	2.79	0.181	3.00	0.175	ns
16:1 *t*9	0.17^{b}	0.017	0.24^{a}	0.031	0.14^{b}	0.015	**
17:0	0.98^{a,b}	0.064	1.10^{a}	0.033	0.85^{b}	0.046	**
17:1 *c*8	0.73	0.067	0.75	0.040	0.70	0.048	ns
18:0	14.38^{b}	0.327	16.69^{a}	0.738	15.03^{b}	0.567	*
18:1 *c*9	32.06	1.002	32.53	0.896	33.68	0.964	ns
18:1 *t*	3.91^{a}	0.340	2.96^{b}	0.156	2.59^{b}	0.145	***
18:2 *n*-6	8.82^{a}	0.633	3.41^{b}	0.377	8.43^{a}	0.730	***
18:3 *n*-3	0.29^{b}	0.038	1.06^{a}	0.161	0.25^{b}	0.030	***
CLA	0.31^{b}	0.016	0.46^{a}	0.027	0.29^{b}	0.021	***
20:0	0.084^{b}	0.006	0.12^{a}	0.010	0.085^{b}	0.009	**
20:3*n*-6	0.42^{a}	0.040	0.29^{b}	0.031	0.40^{a}	0.033	*
20:4*n*-6	1.74	0.185	1.25	0.170	1.76	0.229	ns
20:5*n*-3	0.19^{a,b}	0.083	0.39^{a}	0.055	0.14^{b}	0.075	*
22:0	0.27^{a}	0.021	nd	nd.	0.19^{b}	0.035	*
22:4 *n*-6	0.16	0.018	nd	nd	0.14	0.025	ns
22:5 *n*-3	0.31^{b}	0.051	0.65^{a}	0.075	0.25^{b}	0.054	***
Others	6.38	0.374	6.69	0.472	6.15	0.333	ns
SFA	41.24^{b}	0.547	45.66^{a}	1.413	41.76^{b}	0.754	**
MUFA	36.56	1.124	37.01	1.116	38.19	1.053	ns
PUFA	11.80^{a}	0.920	7.04^{b}	0.829	11.15^{a}	1.005	**
TFA	4.07^{a}	0.344	3.21^{b}	0.143	2.74^{b}	0.147	***
n-6	11.14^{a}	0.845	4.95^{b}	0.563	10.67^{a}	0.977	***
n-3	0.66^{b}	0.118	2.10^{a}	0.277	0.48^{b}	0.108	***
n-6 LC-PUFA	1.89	0.198	1.25	0.170	1.90	0.237	0.058
n-3 LC-PUFA	0.50^{b}	0.083	1.04^{a}	0.127	0.39^{b}	0.098	***

Statistical probability of treatment: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean; nd, not detected.

The SFA percentage of Brazilian beef was 9.7% and 8.5% higher and the PUFA value 40.0% and 36.9% lower than the values for the same sum of FA presented by Carnalentejana-PDO and national undifferentiated beef, respectively. On the other hand, the TFA percentage of Carnalentejana-PDO beef was 21.1% and 32.7% higher than the values presented by Brazilian and national undifferentiated beef, respectively.

The sum of the *n*-6 PUFA ($p<0.001$) was 55.5% and 53.6% lower and the sum of the *n*-3 FA ($p<0.001$) 68.8% and 77.1% higher in Brazilian beef than in Carnalentejana-PDO and national undifferentiated beef, respectively. The Brazilian beef had also the highest long chain (LC) *n*-3-PUFA (20:5*n*-3 + 22:5 *n*-3; $p<0.001$) content.

Data on the CLA isomers (mg/100 g total FA) of Carnalentejana-PDO, Brazilian and national undifferentiated beef are presented in Table 15. The Brazilian beef presented 44 and 46% higher total CLA ($P<0.001$) content than Carnalentejana-PDO and national undifferentiated beef, respectively.

Table 15 - CLA isomers (mg/ 100 g total FA) of *longissimus lumborum* muscle from Carnalentejana-PDO, Brazilian and national undifferentiated (NU) beef.

	Carnalentejana-PDO		Brazilian		NU		Significance
	Mean	SEM	Mean	SEM	Mean	SEM	
Total CLA	294.8^{b}	18.23	516.4^{a}	28.11	282.3^{b}	21.82	***
*t*12,*t*14	1.9^{b}	0.47	4.4^{a}	0.71	0.9^{b}	0.25	***
*t*11,*t*13	4.2^{b}	0.60	12.0^{a}	1.35	3.7^{b}	0.71	***
*t*10,*t*12	5.7^{a}	0.31	2.5^{b}	0.25	4.7^{a}	0.49	***
*t*9,*t*11	17.1^{b}	1.29	29.4^{a}	2.36	18.0^{b}	1.48	***
*t*7,*t*9	5.9^{b}	0.72	10.4^{a}	1.62	5.1^{b}	1.06	*
*t*6,*t*8	6.4^{a}	0.68	3.7^{b}	0.52	5.7^{a}	0.65	**
c/t 12,14	1.4	0.43	1.7	0.52	1.2	0.59	ns
*t*11,*c*13	4.0^{b}	0.57	31.1^{a}	4.06	3.9^{b}	0.91	***
*c*11,*t*13	0.7	0.23	0.7	0.21	0.7	0.24	ns
*t*10,*c*12	9.6^{a}	0.73	3.8^{b}	0.53	9.0^{a}	1.28	***
*c*9,*t*11	207.4^{b}	16.4	398.9^{a}	21.2	196.4^{b}	16.4	***
*t*7,*c*9	30.6^{a}	2.69	17.7^{b}	0.68	32.8^{a}	3.24	***

Statistical probability of treatment: ns, $P>0.05$; *, $P<0.05$; **,$P<0.01$; ***, $P<0.001$; means in the same row with different superscripts are significantly different ($P<0.05$); SEM, standard error of the mean.

The *cis/trans* isomers are the major CLA group, wherein Brazilian beef presented the highest value (0.45 g/ 100 g FA) and the other two beef types presented similar value to each other (0.24 g/ 100 g FA). The proportion of total *cis/trans* isomers varied between 85.6% in

Carnalentejana-PDO and 87.9% in Brazilian beef. The national undifferentiated beef presented intermediate value (86%).

The major contributor to this percentage was the *c*9,*t*11 CLA isomer, which ranged between 68.8 and 77.2% (Carnalentejana-PDO and national undifferentiated beef presented the lowest and Brazilian beef the highest values).

Moreover, the Brazilian beef presented higher *t*12,*t*14 ($p<0.001$), *t*11,*t*13 ($p<0.001$), *t*9,*t*11 ($p<0.001$), *t*7,*t*9 ($p<0.05$), *t*11,*c*13 ($p<0.001$) and *c*9,*t*11 ($p<0.001$) CLA isomers content. The Brazilian beef also presented lower *t*10,*t*12 ($p<0.001$), *t*6,*t*8 ($p<0.01$), *t*10,*c*12 ($p<0.001$) and *t*7,*c*9 ($p<0.001$) CLA isomers content than the other two beef types, which presented similar values to each other in all the referred CLA isomers.

5.3.3 Homocedasticy of beef variables

The variables with variances significantly different among beef types are presented in Table 16. The Brazilian beef had significantly higher variance in α-tocopherol, 16:1 *t*9, 18:3 *n*-3, SFA and total *n*-3 and lower variance in TBARS and 18:2 *n*-6 than the other two beef types.

The higher variance presented in α-tocopherol and 18:3 *n*-3 is due to higher mean values observed in these two variables by this beef type as coefficient of variation (CV) are not increased compared with the other beef types. The Brazilian beef also presented higher variance and CV in 18:0 and lower variance and CV in 17:0 than Carnalentejana-PDO beef, although the mean values are similar.Otherwise, Carnalentejana-PDO beef presented higher variance and CV in 18:1 *t* and TFA content, than the other two beef types. Considering the isomers of CLA, the Brazilian beef presented higher variance for *t*11,*t*13 and *t*11,*c*13 than the other two beef types, higher variance for *t*12,*t*14 than national undifferentiated beef, as well as higher variance for *t*7,*t*9 than Carnalentejana-PDO beef. However, the CV values presented by the Brazilian beef in the majority of aforementioned CLA isomers were lower than the CV values presented by the other two beef types. In all other CLA isomers which variance was significantly different between beef types, the Brazilian beef presented lower variance than the two beef, which in turn presented similar values to each other.

Table 16 - Variance of alpha-tocopherol, TBARS and fatty acids of the *longissimus lumborum* muscle from Carnalentejana-PDO, Brazilian and national undifferentiated (NU) beef.

	Carnalentejana-PDO		Brazilian		NU		*Significance*
	Variance	CV	Variance	CV	Variance	CV	
Alpha-tocopherol	3.18[b]	55.40	12.33[a]	30.32	3.19[b]	48.13	*
TBARS	0.034[a]	52.57	0.002[b]	58.09	0.039[a]	65.84	***
16:1 *t*9	0.004[b]	40.00	0.014[a]	50.03	0.003[b]	41.50	*
17:0	0.065[a]	26.12	0.017[b]	11.62	0.032[a,b]	20.96	*
18:0	1.71[b]	9.10	8.17[a]	17.13	4.83[a,b]	14.61	*
18:1 *t*	1.85[a]	34.78	0.365[b]	20.41	0.317[b]	21.68	***
18:2 *n*-6	6.42[a]	28.71	2.13[b]	42.82	7.99[a]	33.54	*
18:3 *n*-3	0.024[b]	52.41	0.388[a]	58.83	0.014[b]	46.48	***
SFA	4.78[b]	5.31	29.96[a]	11.99	8.53[b]	6.99	**
TFA	1.89[a]	33.81	0.306[b]	17.25	0.325[b]	20.78	***
n-3	0.223[b]	71.52	1.15[a]	51.09	0.175[b]	87.14	***
*t*12,*t*14	3.59[a]	67.33	7.51[a]	55.79	0.91[b]	45,05	***
*t*11,*t*13	5.66[b]	38.28	27.20[a]	31.81	7.46[b]	46.14	**
*t*10,*t*12	1.50[a,b]	24.58	0.94[b]	23.54	3.60[a]	32.45	*
*t*7,*t*9	8.38[b]	69.07	39.18[a]	48.56	16.73[a,b]	46.11	*
*t*11,*c*13	5.23[b]	46.91	247.67[a]	43.45	12.41[b]	75.39	***
*t*10,*c*12	8.55[b]	41.39	4.27[b]	41.75	24.60[a]	51.32	**
*t*7,*c*9	115.86[a]	32.16	6.95[b]	15.22	157.42[a]	34.20	***

Statistical probability of treatment: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); CV, coefficient of variation.

5.4 Discussion

5.4.1 Beef contents of cholesterol, lipid soluble antioxidant vitamins, TBARS and L-carnitine

The values reported here for total cholesterol (0.40 mg/g muscle) were similar to those presented in the chapter 3 sub-section 3.3.1 (content of cholesterol and α-tocopherol in muscle; averaging 0.42 mg/g muscle) in Mertolenga-PDO *longissimus lumborum* muscle, and lower than those presented by Alfaia *et al.* (2006a) (averaging 0.49 mg/g muscle) in Carnalentejana-PDO *longissimus dorsi* muscle. The latter authors also presented a value for

total cholesterol in intensively raised cattle (0.37 mg/g muscle) that was slightly lower than the ones reported here.

The α- and γ-tocopherols were the only vitamin E homologues detected in the three meat types, being the latter available only in small amounts. The levels of α -tocopherol in Carnalentejana-PDO (3.2 µg/g muscle) and national undifferentiated beef (3.7 µg/g muscle) are in between the values reported in the literature for beef derived from grain-fed cattle (1.8–2.4 µg/g muscle) and from pasture-fed cattle (4.4–5.8 µg/g muscle) (Yang *et al.*, 2002). Nevertheless, the levels of α-tocopherol in Brazilian (12.4 µg/g muscle) meat are much higher than those reported in beef derived from pasture-fed cattle or grain-fed cattle receiving supra-nutritional doses of vitamin E (4.3–6.0 µg/g muscle) (Yang *et al.*, 2002). West and coworkers (1997) mentioned contents of α-tocopherol in the *longissimus lumborum* muscle of pastured cattle varying between 3.7 and 7 µg/g muscle, suggesting that there may be large differences in α -tocopherol levels of pastures with distinct biomass composition. In fact, it is well established that the content of α-tocopherol in meat depends on muscle characteristics, level of vitamin E in the diet and duration of feeding (Morrissey *et al.*, 2000). Röhrle and co-workers (2011) in a study where they intended to quantify the α-tocopherol content and to discriminate their provenance (concentrates *vs.* pasture) through stereoisometric separation realised that the Brazilian beef had greater concentration of α–tocopherol (8.13 µg/g muscle) comparing with beef samples from Europe and US. Moreover, those authors realised that stereoisomeric profile of Brazilian beef samples suggests consumption of a naturally rich source of feed combined with supplemental synthetic α–tocopherol from concentrates (Röhrle *et al.*, 2011).

All meat types have α -tocopherol concentrations that allow preserving its quality, since it has been reported that 3.0 µg/g of α-tocopherol is enough to retard metamyoglobin formation, and to protect it from lipid oxidation (Faustman *et al.*, 1989). The values of TBARS for all the three beef types were lower than 5 ppm, which is the concentration at which Brewer and Harbers (1991) realised that rancidity is detectable.

Mean ageing period was greater in Brazilian beef (78 days *vs.* 12 and 13 days in Carnalentejana-PDO and national undifferentiated beef). The need of a greater shelf life is related to the fact that Brazilian beef has a long journey to reach the European market. For oxidative stability of these beef samples do not be compromised they must be protected against fatty acid and myoglobin oxidation. It seems that these beef samples are subjected to some kind of treatment with antioxidant solutions, mainly vitamin E (α-tocopherol). The extremely high α-tocopherol value presented by Brazilian beef is accomplished by a very low

TBARS value (0.08 mg MDA/kg muscle; $p < 0.001$). The low value presented by Brazilian beef shows the efficacy of the great α-tocopherol content of these beef type.

It is important to notice that the TBARS values presented here were determined in the meat samples after 2 years of freezing. Nevertheless, the value obtained in Brazilian beef is similar to the values presented by other authors in fresh meat with days to few weeks of cooling (0.06 mg MDA/kg muscle Alfaia *et al.*, 2010; 0.05-1.0 MDA/kg muscle Djenane *et al.*, 2004; <0.1 mg MDA/kg muscle Luño *et al.*, 2000).

The values of γ-tocopherol obtained were very low and in almost half of the samples of Brazilian beef γ-tocopherol values were under the detection threshold. In Carnalentejana-PDO and national undifferentiated beef all samples presented detectable values (averaging 0.15 µg/g muscle). The values presented by these two beef types are similar to those presented by Prates and co-workers (averaging 0.14 µg/g muscle; 2006) in *longissimus lumborum* and *longissimus thoracis* of Barrosã-PDO veal. The value presented by Brazilian beef was 43% lower (0.08 µg/g muscle) than the mean value presented by the other two beef types. Pastures are rich in α-tocopherol but have low γ-tocopherol concentration while concentrates (depending on their ingredient composition) are usually richer in γ-tocopherol than pastures (Ponte *et al.*, 2008).

The β-Carotene was detected in all samples of Brazilian beef (mean value 0.21 µg/g muscle, ranging from 0.13 to 0.29 µg/g muscle). In contrast, very few samples of Carnalentejana-PDO and national undifferentiated beef presented detectable values of this soluble antioxidant vitamin. The values of β-carotene varied from not detected to 0.09 µg/g in Carnalentejana-PDO, and from not detected to 0.05 µg/g in national undifferentiated beef. The mean value of β-carotene presented by Brazilian beef was higher than the value presented in *longissimus dorsi* muscle from cattle grazed on a good green pasture (0.16 µg/g muscle) by other authors (Yang *et al.*, 2002). Thus, these results suggest that these bovines were grazed on good quality pastures, very rich in tocopherols and carotenoids.

Research on beef L-carnitine in the literature is scarce. Recently the interest in L-carnitine increased mainly due to its potential beneficial effect on health as a dietetic supplement. L-carnitine is an essential cofactor for the transport of fatty acids across the mitochondrial membranes. In the mitochondrial matrix β-oxidation of fatty acids occurs with energy production (Rigault *et al.*, 2008). Moreover, L-carnitine can act as antioxidant in meat which is an important effect in terms of health. The percentage of free L-carnitine in muscle is around 83-88% of total L-carnitine (Demarquoy *et al.*, 2004).

The available research data present in the literature concerning L-carnitine was obtained with different methodologies, which turns data comparison more difficult. The L-carnitine values

reported in the generality of the literature were higher than those presented here. Shimada *et al.* (2004) with the same extraction and quantification methodology, reported values of 3.47 µmol/g for Angus×Hereford beef (n=2) and 3.57 µmol/g of free carnitine for Japonese Black beef (n=3), which were higher than ours. However, the same authors also reported free L-carnitine values of 1.86 and 2.04 µmol/g for Holstein steers with 12 (n=4) and 24 (n=2) months of age, respectively, which were similar to those presented here (ranging from 1.62 to 1.71 µmol/g muscle). Those authors analysed very few samples of each animal species / breed which can compromise the validity of the results. Knüttel-Gustavsen and Harmeyer (2007) using a radioisotopic assay, obtained values of 2.76 and 4.03 µmol/g for total L-carnitine and 2.01 and 2.35 µmol/g for free L-carnitine in beef of the shoulder and beef steak, respectively. Despite of the different technique values for free L-carnitine in beef obtained by the quoted authors were not much different from ours.

Considering the putative antioxidant effect of L-carnitine, we tested the relationship between TBA and L-carnitine with a regression model including also α-tocopherol (as it is the main antioxidant compound analysed) and concluded that L-carnitine content have no significant relationship with TBA

5.4.2 Nutritional value of intramuscular fat

The FAO and WHO organizations jointly had prepared a document with the recommendations for daily intake of lipids, which has stipulated that total fat consumption should not exceed 30% of energy consumption, whilst cholesterol consumption should not exceed 300 mg *per* day (Elmadfa & Kornsteiner, 2009). Beef samples of Carnalentejana-PDO, Brazilian and national undifferentiated used in this study presented a low intramuscular fat content (averaging 19.5 mg/g muscle), which is an advantage from the consumers' health point of view. Considering a common serving beef with 100 g, any of the meats studied will only supply modest cholesterol content (40 mg), representing about 13% of the recommended daily cholesterol intake in adults.

The Carnalentejana-PDO, Brazilian and national undifferentiated beef presented a dry matter mean value of 26.8% (26.1%, 26.9% and 26.4%, respectively), which represents a water mean proportion of 73.2% (73.9%, 73.1% and 73.6%, respectively). The percentage of carbohydrates in beef is around 0.4% (Ferreira & Graça, 1985), the percentage of fat in the three beef types ranged between 1.8 and 2.1% and the protein ranged between 23.9 and 24.4%. Converting our values into energy density, a 100 g steak will have around 113.2 -

118.1 kcal (from which 16.0-18.9 kcal are from fat). Beef in a balanced diet can be beneficial for an adequate intake level of fat.

The intake of SFA should not exceed 10% of energy consumption to keep cholesterolaemic levels in a normal range and to reduce the risk of coronary heart disease (CHD), and TFA values should not exceed 1% of energy consumption (Elmadfa & Kornsteiner, 2009). The SFA and TFA content in a 100 g steak is 738 and 72.9 mg in Carnalentejana-PDO, 945 and 66.5 mg in Brazilian, and 831 and 54.5 mg in national undifferentiated beef, respectively. All beef types present a SFA energy value proportion lower than the recommended value (in a 100 g steak). The daily energy intake of an active man with 170 cm and of an active woman with 165 cm should not exceed 2600 and 2300 kcal, respectively. The SFA contribution in a 100 g serving beef represents 0.3% of total daily calories intake for a man or for a woman, being the Brazilian beef the greatest contributor (9 kcal *vs.* 7 kcal in the other two beef types). Concerning the proportion of the energy intake from SFA in a 100 g steak ranges between 5.9% in Carnalentejana-PDO and 7.2% in Brazilian beef, whilst the proportion of TFA is 0.7% in Carnalentejana-PDO, 0.6% in Brazilian, and 0.5% in national undifferentiated, respectively.

The FAO/WHO minimum recommended intake levels for essential fatty acids are 2.5% of energy consumption from 18:2 *n*-6 plus 0.5% of energy consumption from linolenic acid (18:3 *n*-3) to prevent deficiency symptoms. For chronic disease prevention, the effective intake levels lie between 6 and 11% of energy consumption from total PUFA (*n*–6 and *n*–3) (Elmadfa & Kornsteiner, 2009). In a beef with 100 g, the 18:2 *n*-6 values ranged from 70.6 mg in Brazilian beef to 167.8 mg in national undifferentiated beef, whereas the 18:3 *n*-3 values ranged from 5.0 mg in national undifferentiated beef to 21.9 mg in Brazilian beef.

The lower PUFA content presented by Brazilian beef was mainly due to the lower 18:2 *n*-6 ($p<0.001$) content which was reflected in the lower *n*-6 ($p<0.001$) content presented by this beef comparing to Carnalentejana-PDO and national undifferentiated beef. The sum of *n*-6 PUFA ($p<0.001$) was 55.6% and 53.6% lower in Brazilian beef than in Carnalentejana-PDO and national undifferentiated beef, respectively.

The nutritional importance of increased α-linolenic acid concentration is not clear since it is not recognized in α-linolenic acid the human health beneficial effects attributed to longer chain *n*-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Harris, 2007). The beneficial effects of EPA and DHA include antiatherogenic, anti-thrombotic and anti-inflammatory effects and overall increased intake leads to a reduced risk of CHD (Givens & Gibbs, 2006). Moreover, the conversion of α-linolenic acid to *n*-3 LC-PUFA is considered poor (Enser, 2001), thus the direct intake of *n*-3 LC-PUFA is

recommended. Accordingly, recent dietary guidelines are focused on the absolute amounts of specific PUFA intake and recommend mainly the increase in the consumption of long-chain PUFA (EPA and DHA). However, recommended intakes of *n*-3 PUFA (200 mg/day; COMA) are not being met by the diet in the majority of the population mainly because of low consumption of oil-rich fish. Animal derived foods are likely to have an important function in increasing intake of EPA and DHA LC-PUFA (Givens & Gibbs, 2006).

Brazilian beef presented the highest value of *n*-3 FA ($p<0.001$). The *n*-3 FA was 69.0% and 77.1% higher in Brazilian beef than in Carnalentejana-PDO and national undifferentiated beef, respectively. The sum of EPA and DPA ranged from 0.4 to 1.0 g/100 g total FA, whereas EPA ranged from 0.14 to 0.39 g/100 g total FA, which represents 7.8-21.5 mg/100 g of muscle and 2.8-8.1 mg/100 g of muscle, respectively. The recommendations concern only EPA and DHA, nevertheless in the present study only a few samples presented DHA value above the detection limit. Therefore the values presented for a 100g steak concern only to EPA values which corresponds only to 1.1 to 3.2% of the minimum recommended daily intake for humans' diet (250 mg/day, Elmadfa & Kornsteiner, 2009).

Interestingly, in the study of Howe and coworkers (2006), DPA contributed 29% of the total LC *n*-3 fatty acids, which is in line with the present results were DPA was 50% to 65% higher than EPA. These data do, however, highlight the need to better understand the physiological effects of DPA (Givens & Gibbs, 2006).

In summary, a common serving beef with 100 g, the studied meats will account with 457 mg of 16:0 the main hypercholesterolemic fatty acid, 54.5 to 72.9 mg of total TFA (national undifferentiated and Carnalentejana-PDO beef presenting the extreme values, respectively), 25.9 to 37.8 mg (averaging 32.8 mg) of *n-6* LC-PUFA (20:4*n*-6 + 22:4 *n*-6) and 7.8 to 21.5 mg of *n-3* LC-PUFA (EPA and DPA; national undifferentiated and Brazilian beef presenting the extreme values, respectively). The values presented in this study for 16:0 and 18:2 *n*-6 are lower than the normal values (890 mg and 200 mg) observed in lean beef according to EFSA (2010). However values presented for TFA (3-6% total fatty acids) and 18:3 *n*-3 (10 mg) are close to the values observed in lean beef (EFSA, 2010). Total *n-3* LC-PUFA and *n-6* LC-PUFA are far from the minimum daily recommended intake. Brazilian beef presented the biggest amount ($p<0.001$) of *n-3* LC-PUFA and national undifferentiated beef the lowest amount of the same LC-FA (despite not different from Brazilian beef).

5.4.3 Homocedasticy of beef variables

One major problem of the beef industry is to guarantee homogeneity of the product. Research concerning this issue was only developed regarding meat palatability, mainly tenderness. However, healthiness is one of the credence attributes most highly valuated by consumer, and the interest in valuing meat with information about the nutritional value in labels has been increasing (Bernués *et al.*, 2003; Wezemael *et al.*, 2010). As could be expected that certified PDO meats have a distinctive lipid profile (Regulation n. 510/2006 of 20 March 2006, EC), the assessment of its variability deserves to be studied to ensure product homogeneity. Nevertheless, this approach is not well established. Thus, it seems important to assess the homogeneity of these products regarding the lipid traits.

The fat content of meat is a major determinant of beef sensory attributes (Wood *et al.*, 2008). In this study, variance of meat total fatty acid content did not differ between meat types and coefficient the variation averaged 35.7%. This value is lower than the coefficient of variation (CV) of total fatty acids reported by Alfaia and co-workers (2006a; averaging 50.7%) in Carnalentejana-PDO, but higher than the one presented by Monteiro and co-workers (2006) in Mertolenga beef (averaging 15%). Intramuscular fat variance was also similar between meat types averaging 29.9% of CV.

The greater variability presented by Brazilian beef in SFA is mainly due to the greater variability presented in the 18:0. According to Realini and coworkers (2004), the pasture-fed animals have a higher concentration of 18:0 than animals fed concentrate. Moreover, Maggioni *et al.* (2010) realised that beef from purebred Nellore (*Bos indicus*) have a higher concentration of 18:0 than beef from animals with higher percentage of *Bos Taurus*. Moreover, Orellana and coworkers (2009) found higher 18:0 concentration in Bradford breed (*Bos indicus* x *Bos Taurus*) than in criollo Argentino (*Bos Taurus*). The greater variability in 18:0 concentration presented by Brazilian beef is probably due to different percentages of *Bos indicus* genotype from the animals that gave rise to those beef samples.

The greater mean associated with higher variance observed in 18:1 *t* from Carnalentejana-PDO was not in accordance with the mean and variance seen in *c*9,*t*11 CLA isomer. As *c*9,*t*11 CLA is highly correlated with 18:1 *trans-11* in ruminant meat (Palmquist *et al.*, 2004), these results suggests that in PDO animals there was an altered rumen metabolism with deposition of 18:1 *t10* rather than 18:1 *t*11 in PDO beef, characteristic of high concentrate diets with high PUFA levels (Bessa *et al.*, 2006). However, our chromatographic conditions did not allow us to resolve these two isomers.

The meat palatability is a strong determinant of whether consumer will repurchase a specific type of meat. Tenderness, juiciness and flavour are strongly associated with the perception of meat palatability. The fat content can influence all these attributes, the latter being also extremely influenced by fatty acid composition mainly by PUFAs. When the thermal oxidation of LC-PUFA occurs during cooking, oxidation contributes to the flavour and/or off-flavour formation (Stelzleni & Johnson, 2008). Several researchers have associated the 18:2 *n*-6 FA with flavour intensity, wherein grain fed animals produce beef with a more acceptable flavour (Melton, 1990; Elmore *et al.*, 2004). Fisher and co-workers (2000) found that flavour intensity is correlated with the 18:3 *n*-3 FA content. However, the 18:3 *n*-3 has a negative impact on flavour. Thus the higher variability presented by Brazilian beef in 18:3 *n*-3 can be reflected in high variability on the palatability and consequently on the acceptability of this beef type in the market place.

5.5 Conclusion

All beef types presented desirable low cholesterol content as well as α-tocopherol value high enough to inhibit lipid oxidation and discoloration. The beef types analysed present low content in γ-tocopherol but are a good source of L-carnitine. A serving beef with 100 g have cholesterol, SFA and TFA contents much lower than the minimum recommended values by FAO/WHO. The data indicate that Brazilian beef presents the greatest oxidative stability (low TBARS value) which seems to be mainly due to the greatest α-tocopherol content, but also to the greatest β-carotene content. Brazilian beef also seems to be the healthier beef analysed due to the greatest CLA and *n*-3 LC-PUFA content. Concerning lipid composition homogeneity the national undifferentiated beef was the most variable from the three beef types studied.

Acknowledgments

The financial support (Grants AGRO/2004/422 and PTDC/CVT/2006/66114) and the individual fellowship from FCT to Ana Cristina Monteiro (SFRH/BD/2006/31091) are acknowledged.

6 - Relationship between texture analysis and sensory attributes of the three main beef types marketed in Portugal

A.C.G. Monteiro, M. Aguiar Fontes, P.Costa, J.A.M. Prates & J.P.C. Lemos

CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa - Avenida da Universidade Técnica, Pólo Universitário Alto da Ajuda, 1300-477 Lisboa, Portugal

Relationship between texture analysis and sensory attributes of the three main beef types marketed in Portugal

Abstract

There are several methods to determine texture properties of beef, but studies relating them are scarce. Thus, the aim of this study was to assess the relationship between the sensory attributes determined by a trained sensory panel and instrumental measurements (texture profile analysis (TPA) and Warner-Bratzler shear force) in *longissimus lumborum* muscle of beef from the three main beef market segments in Portugal (branded-protected designation of origin, imported-Brazilian and national undifferentiated beef). Additionally we aimed to study beef homogeneity as it is a very important issue to the meat industry and to consumers. National undifferentiated beef had the highest hardness (similar to Brazilian beef), adhesiveness and juiciness, as well as a higher hardness coefficient of variation. Brazilian beef presented the highest off-flavour and the lowest overall acceptability. TPA hardness was not correlated with sensory tenderness. However, tenderness correlated negatively with chewiness and had a trend for a negative correlation with resilience. In line with this, chewiness correlated positively with WBSF, which correlated with tenderness. WBSF seems to be a better predictor of instrumental texture than TPA. However, TPA test give useful additional information about beef texture. All sensory attributes correlated with overall acceptability, being the higher value obtained with off-flavour. This suggests that off-flavour strongly influenced overall acceptability. Finally, national undifferentiated beef was the most heterogeneous from the beef types studied, which is undesirable from the consumer's point of view.

Keywords: Beef, Meat Quality, Sensory analysis, Tenderness, Texture profile analysis

6.1 Introduction

Meat quality, generally accepted as the ability to satisfy consumer expectations, is difficult to achieve because it depends on the consumer habits (Verbecke *et al.*, 2010). However, understanding the traits contributing to beef quality and how to increase them in order to satisfy consumer requirements, is a major current issue in meat science (Hocquette & Gigli, 2004). Moreover, variability in meat quality attributes are large and often an industry problem (Polkinghorne, Thompson, Watson, Gee & Porter, 2008). Among sensory attributes tenderness and juiciness are known to have a huge importance on consumer decision to purchase beef and repurchase it (Calkins & Hodgen, 2007). However, meat sensory attributes are difficult to measure and often require the use of taste panels to assess the complex parameters involved in the eating experience (Gill *et al.*, 2010). These measurements can be made by a trained sensory panel (TSP) or by a consumer panel. The first one implies the training of panellists, whereas the latter does not but it requires a larger number of tasters. In general TSP is limited to a group of 8 to 16 panellists, whereas consumer sensory evaluation should be carried out with hundreds of consumers. Despite both methods being costly and time consuming, TPS costs regards mostly to the panel training. Moreover, the TPS are trained to score specific attributes to the eating quality independently of the other sensory dimensions (Watson *et al.*, 2008). Thus, the information obtained using a TSP is more consistent and objective than that retained in a consumer's panel (Destefanis *et al.*, 2008).

To overcome some of those problems, more precisely the subjectivity of human assessments, several instrumental methods of predicting meat quality, in particular its texture, have been developed. The instrumental measurement of texture most commonly used are shear force and compression tests, which rely on measuring a single parameter, and thus do not fully imitate the complexity of the chewing motion, as humans measure and integrate sensory perceptions on a material that undergoes continuous transformation during chewing (Duizer, Gullett & Findlay, 1996). Some authors have pointed out that within instrumental tests the imitative, like texture profile analysis (TPA) test, are the best to mimic the consumption experience (Meullenet, Carpenter, Lyon & Lyon, 1997; Ross, 2009). The TPA was developed in the 60's and since then it has been used to measure several texture characteristics. In the TPA 'two bites test' the probe compresses twice the material with a lag time between the two actions, in order to simulate the chewing action of the teeth. The compression is usually 80% of original length of the sample (Sahin & Sumnu, 2006). The main advantage of TPA is that one test can assess several variables, including hardness, fracturability, cohesiveness and springiness, with the double compression cycle (Huidobro, Miguel, Blázquez & Onega, 2005). Despite the

advantages of this method and that it has been successfully used in several foods, TPA is not commonly used nor in the meat industry nor by meat researchers. Although previous reports indicate that TPA has similar capability to predict sensory tenderness of beef than WBSF (Warner-Bratzler shear force; Caine, Aalhus, Best, Dugan & Jeremiah, 2003), studies comparing these two methods are scarce. It is deeply important for research and industry purposes that any assessments of tenderness made in a laboratory to be highly correlated with sensory assessment. Moreover, the combination of sensory evaluation with instrumental analysis gives a great deal of information and a more complete picture of the product (Ross, 2009). Thus, the objective of this study was to explore the relationship between WBSF, TPA and trained sensory panel in order to realise wich technique is the most useful to predict sensory characteristics of beef. To reach this purpose the three main beef types market in Portugal (branded, imported and undifferentiated beef), were used and compared to understand if the beef type interferes with the results. A further objective was to determine the variability within and between each one of the retail beef samples of the three different origins from the Portuguese market place.

6.2 Materials and Methods

6.2.1 Animals and meat sample preparation

This study was performed on 16 Carnalentejana-protected designation of origin (PDO), 15 Brazilian and 15 national undifferentiated beef samples from *longissimus lumborum* muscle chosen in a hypermarket in order to reproduce consumer's purchase. The samples were collected during five months according to their availability and each one belonged to a different animal. We collected samples of all batches marketed in that commercial area during the referred period of time.

The Carnalentejana-PDO beef was obtained from Alentejana purebred young bulls produced in a traditional semi-extensive production system according to the Protected PDO specifications (Regulation n. 510/2006 of 20 March 2006, EC). The Carnalentejana-PDO production follows strict rules detailed in the specification book for Carnalentejana-PDO, which concerns mainly the breed, the geographic origin and the production system. Carnalentejana-PDO was chosen among the several Portuguese PDO beef as it is commercially the most important one.

The Brazilian beef was the imported beef chosen because its market share has been growing in the last years. In fact it is the fourth beef from the ranking of foreign trade beef and

increased from 2303.8 of fresh beef in 2005 (GPP, 2007b) to 2897.2 tonnes in 2007 (GPP, 2009). Brazilian beef is obtained from crosses of local breeds, like Nelore (*Bos indicus*), with European breeds (*Bos taurus*), and is usually produced in a traditional semi-extensive production system based on pastures followed by a finishing period with concentrates.

The national undifferentiated beef is the most consumed in Portugal, and is originated from crossbred (mainly with Charolais and Limousin sires) young bulls produced in a conventional intensive concentrate based system. This group consists of beef from crossbred animals of different ages and genders produced in Portugal.

It was observed that the ageing period was significantly different between Brazilian beef and the two Portuguese beef groups (78 ± 9.0 days vs 12 ± 5.3 days in Carnalentejana-PDO and 13 ± 6.0 days in national undifferentiated beef).

Three 25 mm thick steaks were cut from *longissimus lumborum* muscle, one for WBSF, one for TPA and one for sensory panel. Samples were trimmed from external connective tissue, vacuum packaged and frozen at -18 ºC until analyses were performed. Steaks were thawed at 4 ºC for 24 hours before analysis.

Grill cooking was conducted with a plate grill (Modular 65/70 FTES electric griddle Modular System Ltd., Italy) pre-heated at a temperature of 250 ºC. Grill setting were designed to achieve a final internal temperature of 71 ºC. Temperature was controlled with a needle thermocouple probe (Lufft C120, Munich, Germany), which was inserted horizontally at the midpoint of the steak's width.

6.2.2 Beef Warner-Bratzler shear force

After grilling steaks for WBSF were chilled until reached room temperature. Each sample provided a minimum of eight strips with a 1 cm^2 cross section. The cores were removed parallel to the muscle fibre orientation and were sheared perpendicular to the longitudinal orientation of the muscle fibres at a crosshead speed of 300 mm/min, using a TA-TX Plus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) equipped with a Warner-Bratzler shear blade (with a 30 kg load cell). The beef sample resistance to shearing was recorded in a force-deformation plot and the maximum shear force in kg corresponded to the highest peak of the curve. Data were collected with Texture Expert Exceed software (Stable Micro Systems Ltd., Surrey, UK).

6.2.3 Beef texture profile analysis

For TPA the cores were cut from the steaks perpendicular to the longitudinal orientation of the muscle fibres. Each sample was subjected to two cycles of compression with the same Texture Analyser, equipped with a cylindrical probe of 10 mm diameter moved at a constant speed of 5 mm/s. When the probe detected the sample, the thickness was automatically recorded by the software, and then the probe continued downward to 80% of the sample original thickness. Eight to twelve measurements were made in each sample. The results are presented in a force (kg) by time (s) curve plot. The TPA parameters studied were:

Hardness – peak force of the first compression cycle (kg). It gives the maximum force necessary to compress the sample. In mouth, hardness is the force required to bite completely through sample with the molars.

Fracturability – the force during the first compression at which the material fracture (kg).

Cohesiveness – ratio between the positive force area during the second compression to that during the first compression (Area 2/ Area1), and gives the extent to which the sample could be deformed prior to rupture. In mouth, cohesiveness is felt like the degree to which meat is compressed between the teeth before it breaks.

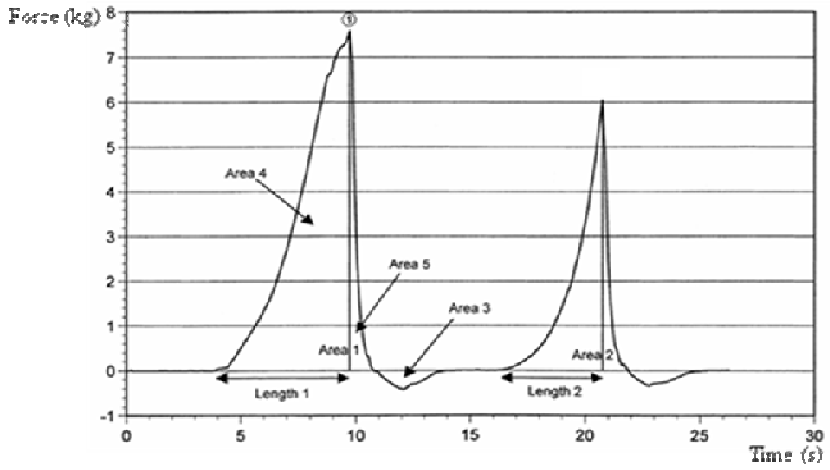
Springiness – ratio of the time duration of force input during the second compression to that during the first compression (Length 2/ Length 1). It represents the ability of the sample to recover its original form after the deforming force is removed. In mouth, springiness is the force with which the sample returns to its original shape or size after a partial compression, without failure, between the tongue and the palate.

Adhesiveness – negative area under the baseline between the compression cycles (Area 3). It represents the force necessary to pull the probe away from the beef. In mouth adhesiveness is the force required to remove the product completely from palate, using tongue, after compression of the sample between the tongue and palate.

Resilience – ratio of the negative force input to the positive force input during the first compression (Area 5/ Area 4). It represents the property of a material to absorb energy when is deformed elastically and then, upon unloading to have this energy recovered. In other words, it is the maximum energy per unit volume that can be elastically stored.

Chewiness – The energy necessary to chew a solid sample to a steady state of swallowing (hardness ×cohesiveness × springiness).

Figure 8 – Typical force by time graphic of the two compression cycles from texture profile analysis test. Parameters: hardness = peak force 1; adhesiveness = Area 3; cohesiveness = Area 2/Area 1; springiness = Length 2/Length 1; Resilience = Area 5/Area 4; chewiness = hardness x cohesiveness x springiness.



6.2.4 Beef sensory analysis

For sensory analysis the steaks were cut into 2 × 2 × 2 cm^3, discarding pieces with connective tissue or fat, and maintained at 60 ºC until tasting. The beef samples were randomly served to a 10 member sensory panel. Distilled water and unsalted crackers were provided to purge the palate between samples. The panellists scored the steaks on a 1 to 8 points scale for tenderness (defined as the opposite of the force required to bite the sample through the molars), juiciness (juice released from the sample after the first chews), beef flavour (the intensity with which the sample is recognized as beef), off-flavour (all flavours not considered as typical in beef, *i.e.*, found strange for a beef sample) and overall acceptability (1 = extremely tough, extremely dry, extremely weak, extremely weak, dislike extremely; 8 = extremely tender, extremely juicy, extremely strong, extremely strong, like extremely, respectively).

6.2.5 Statistical analysis

Statistical analysis was carried out by analysis of variance using the PROC MIXED procedure of Statistical Analysis Systems(SAS) software package, version 9.1 (SAS Institute Inc., Cary, USA, 2004). Data was checked for normality and homocedasticy. For some variables

significant differences of variances between groups were found and thus they were analysed by PROC MIXED model for variance heterogeneity. The model included the group Carnalentejana-PDO, Brazilian and national undifferentiated beef as fixed effects. The signifincant level was set at 5% ($P<0.05$).

Coefficients of variation (CV) were also determined through $CV = ((SEM \times \sqrt{n}) / \overline{X})$, where,

SEM = standard error of the mean,

n = number of samples,

$\overline{X}$ = mean of the beef group.

The relationship between the variables was determined using the Pearson's correlation coefficients (SAS, 2004).

6.3 Results

6.3.1 Beef texture and sensory parameters

Data on mean, SEM, minimum Min), maximum (Max) and CV of WBSF, TPA parameters and sensory attributes are depicted in Table 17. Beef samples had similar WBSF value ($P>0.05$), but national undifferentiated beef had the lowest CV, which was 37% and 23% lower than the one obtained in Carnalentejana-PDO and Brazilian beef, respectively.

The national undifferentiated beef presented higher TPA hardness ($P<0.01$) than Carnalentejana-PDO beef and a trend for a higher chewiness value ($P=0.08$). National undifferentiated beef also showed the highest coefficient of variation (CV) in both parameters (Table 17). The hardness and chewiness CVs of national undifferentiated beef were 50% and 25% higher than the value presented by Carnalentejana-PDO, as well as 24% and 52% higher than the value presented Brazilian beef, respectively.

All samples presented low adhesiveness, but the national undifferentiated beef presented the lowest value ($P<0.05$) in this parameter. Brazilian beef presented the highest adhesiveness CV, which was 36% and 53% higher than the one presented by Carnalentejana-PDO and national beef, respectively. Despite having similar means, Carnalentejana-PDO beef had cohesiveness and resilience CV values lower than Brazilian (32% and 54%, respectively) and national undifferentiated (26% and 59%, respectively) beef. Considering the variability in the TPA parameters assessed, adhesiveness had the highest CV value, which was followed by resilience, whilst springiness presented the lowest CV value in the three beef types.

Table 17 – Means, standard error of the means, minimum (Min), maximum (max) and coefficient of variations (CV) of *longissimus lumborum* texture parameters and sensory attributes from Carnalentejana-PDO, Brazilian and national undifferentiated (NU) beef

	Carnalentejana-PDO					Brazilian					NU					Significance
	Mean	SEM	Min	Max	CV	Mean	SEM	Min	Max	CV	Mean	SEM	Min	Max	CV	
Warner Bratzler shear force (kg)	5.48	0.387	3.23	8.29	28.26	5.28	0.319	3.01	7.72	23.39	5.42	0.251	3.43	6.96	17.93	ns
TPA parameters																
Hardness (kg)	4.68^{b}	0.156	3.82	5.95	13.33	5.30^{a,b}	0.280	3.17	7.16	20.44	6.08^{a}	0.422	2.77	8.05	26.88	**
Adhesiveness (kg x s)	-0.02^{b}	0.002	-0.04	-0.01	-35.82	-0.02^{b}	0.003	-0.05	0.00	-55.90	-0.03^{a}	0.002	-0.05	-0.02	-26.10	*
Cohesiveness	0.51	0.013	0.41	0.60	10.11	0.48	0.019	0.38	0.62	15.32	0.48	0.017	0.39	0.62	13.58	ns
Springiness	0.76	0.011	0.67	0.84	5.82	0.77	0.009	0.69	0.81	4.50	0.75	0.010	0.69	0.81	5.07	ns
Resilience	0.15	0.008	0.10	0.23	22.00	0.15	0.017	0.09	0.30	44.85	0.16	0.021	0.08	0.41	50.18	ns
Chewiness (kg)	1.85	0.092	1.47	2.79	19.88	1.94	0.063	1.54	2.48	12.57	2.18	0.149	1.23	3.04	26.40	ns
Sensory attributes																
Tenderness	5.43	0.223	3.90	6.50	15.91	5.31	0.238	3.40	7.20	17.36	5.60	0.186	3.70	7.00	12.86	ns
Juiciness	3.94^{a,b}	0.155	3.00	4.90	15.28	3.56^{b}	0.132	2.80	4.30	14.57	4.22^{a}	0.163	2.80	5.10	14.97	*
Flavour	4.46	0.157	3.50	5.30	13.17	4.11	0.179	2.90	5.00	16.87	4.37	0.143	3.50	6.10	12.68	ns
Off-flavour	1.46^{b}	0.213	1.00	4.10	56.50	3.68^{a}	0.336	1.70	4.10	35.36	1.80^{b}	0.320	1.00	3.80	68.85	***
Overall liking	5. 24^{a}	0.145	4.40	6.20	10.72	3.72^{b}	0.168	2.70	4.80	17.49	4.92^{a}	0.131	3.90	5.90	10.30	***

Statistical probability of treatment: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean; TPA, texture profile ananlysis.

Regarding trained sensory panel results tenderness and flavour were similar between the three beef types studied. The juiciness was higher in national undifferentiated beef, despite not different from Carnalentejana-PDO beef.Brazilian beef had the highest off-flavour ($P<0.001$) and the lowest overall acceptability values ($P<0.001$). All beef types presented similar CV values between the attributes studied, with exception of the off-flavour parameter, in which national undifferentiated beef presented the high CV value, followed by Carnalentejana-PDO and finally by Brazilian beef (ranging between 35 and 69%).

6.3.2 Correlation between texture and sensory parameters

The correlation coefficients (r) found among texture profile, WBSF and sensory attributes in *longissimus lumborum* muscle from Carnalentejana-PDO, Brazilian and national undifferentiated beef are shown in Table 18.

Moderately ($0.5>r>0.3$) negative correlations were observed between hardness, adhesiveness ($P<0.05$) and cohesiveness ($P<0.001$), as well as between WBSF and sensory tenderness ($P<0.001$) and between chewiness ($P<0.05$) and tenderness. Furthermore, moderately ($0.7>r>0.3$) positive correlations were observed between hardness and chewiness ($P<0.001$), between cohesiveness, springiness ($P<0.01$) and resilience ($P<0.001$), as well as between springiness, adhesiveness ($P<0.01$) and chewiness ($P<0.05$). Cohesiveness and chewiness were also inversely moderately correlated ($r=0.3$) with off-flavour, which apparently has no biological meaning.

WBSF presented a trend to correlate positively with adhesiveness ($P=0.06$), cohesiveness ($P=0.05$) and chewiness ($P=0.05$). Tenderness presented a trend to correlate negatively with resilience ($P=0.06$).

There were also moderately ($0.4>r>0.3$) significant positive correlations between sensory traits. Tenderness and juiciness were correlated ($P<0.01$) and all sensory traits ($P<0.01$) were correlated with palatability. Off-flavour correlated negatively with flavour ($P<0.01$) and with palatability ($P<0.001$).

The magnitude of the correlations between TPA parameters and sensory attributes were similar, ranging between 0.26 and 0.34 (absolute values).

Table 18 – Correlation coefficient between Warner-Bratzler shear force, texture profile analysis parameters and sensory attributes of *longissimus lumborum* from Carnalentejana-PDO, Brazilian and national undifferentiated beef

	Adhesiveness	Cohesiveness	Springiness	Resilience	Chewiness	WBSF	Tenderness	Juiciness	Flavour	Off-flavour	Palatability
Hardness	-0.31*	-0.54***	-0.21	-0.14	0.72***	0.05	-0.18	0.14	-0.12	-0.08	-0.20
Cohesiveness		IIIIIIIIII	0.40**	0.59***	0.14	0.29[c]	-0.17	-0.10	0.15	-0.34*	0.23
Springiness			IIIIIIIIII	0.04	0.29*	0.13	0. 05	0.01	0.09	0.11	-0.09
Resilience				IIIIIIIIII	0.21	0.16	-0.28	-0.18	0.14	-0.24	0.04
Chewiness					IIIIIIIIII	0.29[c]	-0.30*	0.12	-0.02	-0.30*	-0.09
WBSF						IIIIIIIIII	-0.52***	-0.15	0.11	-0.24	-0.06
Tenderness							IIIIIIIIII	0.48***	-0.05	0.15	0.46**
Juiciness								IIIIIIIIII	0.27	-0.24	0.42**
Flavour									IIIIIIIIII	-0.44**	0.45**
Off-flavour										IIIIIIIIII	-0.63***

Statistical probability of treatment: P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001

WBSF = Warner-Bratzler shear force; OA = overall acceptability

[c] Variables with a statistical probability of 0.05

6.4 Discussion

6.4.1 Beef texture and sensory parameters

The fracturability was excluded from the study as only a few samples presented fracturability. The higher value of TPA hardness presented by national undifferentiated beef was accomplished with a trend for a higher value in chewiness. Our results are similar to the results presented by Caine *et al.* (2003) but lower than those presented by Huidobro *et al.* (2005) in cooked meat after six days of ageing. The national undifferentiated beef also presented higher CV in both parameters (hardness and chewiness), indicating higher heterogeneity of this beef type.

The values obtained in this study for adhesiveness were lower than the ones obtained by other authors (Caine *et al.*, 2003). Adhesiveness is the force required to remove a product completely from palate, using tongue, after compression of the sample between tongue and palate. Therefore, low adhesiveness values in beef seem to be expected, seeing that beef is not supposed to be a very adhesive product. Caine *et al*. (2003), obtained similar values for cohesiveness, springiness and chewiness to the ones obtained in the present study. On contrary, Huidobro *et al.* (2005) obtained a higher springiness value in cooked meat. The asymmetric shape and similar initial slope of both of the compression curves indicate that steaks had a firm elastic (springiness) quality (Caine *et al.*, 2003). This finding is in agreement with the results obtained, seeing the intermediate values for cohesiveness and springiness and the low resilience. Moreover, the cohesiveness values are different from one which indicates the absence of sample recovery after the first compression. In the second compression cycle the sample lost some height so the area recorded was smaller than in the first cycle, as observed by other authors (Tabilo *et al.*, 1999).

Consistent beef tenderness is difficult to obtain and often an industry problem (Polkinghorne *et al.*, 2008). The CV is an indicator of the homogeneity between samples within each beef type, which is a desirable characteristic from the consumer point of view. The meat industry has to struggle against the heterogeneity of beef quality. The main reason is that consumers want to buy a similar product to the one previously bought, when the purchase is repeated. Furthermore, consumer expectations concerning quality branded products, like Carnalentejana-PDO beef are higher. Moreover, usually quality branded beef is more expensive, giving consumer an additional motive to be exigent. In most of the TPA parameters, Carnalentejana-PDO beef had the lowest CV values. The national undifferentiated beef had higher hardness and chewiness CV, indicating higher heterogeneity

of this beef type. The higher heterogeneity presented by national undifferentiated beef is because this beef is obtained from animals of different crosses, ages and genders, being the less homogeneous beef group represented in this study. On the other hand, the lower CV showed by Carnalentejana-PDO beef in these parameters is mainly because those animals are purebred and produced in a more restricted way, following the specifications book. Although many researchers use WBSF as an indicator of sensory tenderness in beef (Shackelford, Wheeler & Koohmaraie, 1995, 1997, 1999), in the present study the data variability was higher than in TPA hardness in Carnalentejana-PDO and Brazilian beef. In national undifferentiated beef the situation is reverse, *i.e.*, the greater data variability was observed in TPA hardness.

Considering the TPA parameters and the sensorial attributes assessed, unexpectedly the CV of most of the TPA parameters was higher than the CV of the sensory attributes, indicating that the instrumental measurements had more variability than the sensorial ones. However, the CV values of TPA parameters were similar or even slightly lower than those presented by other authors (Huidobro *et al.*, 2005). Within the sensory attributes the off-flavour CV was the exception, because had the greatest values of all measurements made. The higher off-flavour CV presented by Carnalentejana-PDO and national undifferentiated beef was due to the great number of the samples where the off-flavours were not detected and thus scored with the minimum value (1 in a 1 to 8 structured scale) whilst in a few number off-flavours were detectable and scored with medium values. Conversely, Brazilian beef presented detectable off-flavours in almost all samples tasted and though lower variability in this attribute. The Brazilian beef presented the highest value in off-flavour. The mean off-flavour value was 3.68 which means panellists classified the intensity of off-flavours presence as slightly weak. There are several hundreds of compounds in meat that contribute to flavour and aroma (Calkins & Hodgen, 2007).

The higher off-flavour value presented by Brazilian beef could be due to several reasons. First, Brazilian beef had higher ageing time (averaging 78 days *vs.* 12 and 13 days in Carnalentejana-PDO and national undifferentiated beef, respectively). It is well accepted that during ageing enzymatic reactions occur with production of volatile compounds, which alter the flavour of beef (Calkins & Hodgen, 2007).

Second, the typical production system of Brazilian beef is based on grazing. Other study developed by our work team, concerning determination of lipid profile clearly indicated that this meat was from animals produce on pasture. Grass fed cattle tend to have higher polyunsaturated fatty acid content, especially 18:3 *n*-3 and 22:5 *n*-3, which is in accordance with our results, it stand to reason that the thermal oxidation of these fatty acids may

contribute to increased level of off-flavours described as grassy and fishy (Vatansever *et al.*, 2000; Stelzleni & Johnson, 2008). This fatty acid profile can contribute to undesirable aroma and/or flavour formation (Mottram, 1987). It is also important to notice that the compounds existing in meat depend on feeding composition and on the amount of forage fed. Myer *et al.* (1992) reported an increase in off-flavours score when canola oil was fed to animals. Moreover, Melton (1990) suggested that several grasses in ruminant diets cause less desirable meat flavour. Hay was also found to produce less desirable meat flavour than corn silage.

Hexanal and 2,4-decadienal contribute positively to beef flavour, but may produce undesirable flavours at higher concentrations. Hexanal is the most prominent volatile compound in cooked meat with the amount being directly proportional to the thiobarbituric acid-reactive substances (TBARS), a measure of oxidation and inversely proportional to flavour acceptability (Calkins & Hodgen, 2007). This is not the case of our study, as Brazilian beef presented a very low value of malondialdehyde (MDA) measured by TBARS technique (see chapter 5, sub-section 5.3.1).

It seems more likely that the reason for such a high off-flavour value in Brazilian beef is the fact that panellists were trained with common beef and, according to Resconi and co-workers (2010), when panellists are accustomed to taste meat from animals reared mainly on concentrates might perceived the typical pastoral flavour as strange.

6.4.2 Correlation between texture and sensory parameters

From all the correlations detected the highest correlation coefficient was obtained between hardness and chewiness ($r = 0.72$), which is not surprising seeing that chewiness is product of hardness, cohesiveness and springiness. This suggests that resistance to the compression force was probably the main textural property determining tenderness characteristics.

Attributes for breakdown, bolus formation and final juiciness are required to discriminate texture, as well as the chewing measurement of resistance. Toughness in meat relates to work done during chewing, though this is determined more by the strain necessary to break connective tissue, than by the force or stress required for failure (Lillford, 2011). The mechanical properties of meat changes during cooking and are known to be affected by collagen. The helix to coil transition (denaturation) of the collagen molecules during heating changes the collagen structure, with the loss of the fibrillar structure due to the breakage of the hydrogen bonds (Lepetit, 2007). The higher the waviness of collagen fibres and fibrils is the greater the contraction amplitude will be. The contraction of collagen fibres and fibrils can be artificially divided into two phases. First a free contraction phase where collagen fibre can

contract without being restrained by the muscles fibres or muscle fibres bundles. Second a forced contraction phase, when collagen fibres and fibrils apply pressure on muscles fibres and bundles (Lepetit *et al.*, 2002). The pressure developed by connective tissues is opposed to the resistance of fibres and fibre bundles (resilience). The balance between the pressure and the resistance leads to a state of equilibrium and to a final value of collagen contraction state which is the determinant of the elastic modulus of collagen fibres and fibrils (Lepetit, 2008). The correlations between springiness and cohesiveness and between cohesiveness and resilience are in accordance with the above exposed.

A higher cohesiveness also contributes to a higher hardness as a result of a lower maturation or a higher contraction of the muscle fibres with the increased strain (Lepetit, 2007). All the above relationships could be reflected in a higher resilience (resistance to compression). Unexpectedly, TPA hardness and cohesiveness were inversely correlated, which is in contradiction with the above explanation. However, hardness measured through WBSF had a trend for a positive correlation with cohesiveness and chewiness. A more cohesive sample supports higher deformations before rupture, and consequently will be harder to chew, as cohesiveness is felt like the amount of beef deformation without rupture when biting completely through the sample with molars (Lawless & Heymann, 2010). Tenderness had a trend to correlate inversely with resilience. The resilience measures meat deformation, as it describes the ability of the meat to recover from deformation and offer resistance to a subsequent deformation (Veland & Torrissen, 1999). Thus, a more resilient beef will probably be less tender due to the higher resistance of the sample to compression. In addition, the harder the beef more chews are needed to reach a steady state of swallowing, which is in agreement with the negative correlation between sensory tenderness and chewiness.

Although WBSF is the most widespread and selected method used for meat tenderness evaluation (Huidobro *et al.*, 2005) because explains a substantial proportion of the TSP variation (Shackelford *et al.*, 1999), it has been considered as an imprecise predictor of beef tenderness determined by trained panellists (Shackelford *et al.*, 1995, 1997). Others suggested that WBSF explains a substantial proportion of the variation in sensory tenderness (Silva, Patarata & Martins, 1999; Chambaz, Scheeder, Kreuzer & Dufey, 2003). According to the latest authors, in our study WBSF was moderately negatively correlated with sensory tenderness, explaining 29% of the variation in sensory tenderness.

No significant correlations were found between TPA hardness and WBSF as well as between TPA hardness and sensorial tenderness ($P>0.05$). This result is in agreement with Duizer *et al.* (1996), which stated that TPA measurements only evaluate two compressions and maximum intensity perception seems to occur anywhere between the first and the fourth bite.

The aforementioned results indicate that TPA hardness is not a good predictor of sensory tenderness in beef. On contrary, WBSF was a better predictor of sensory tenderness considering the moderately high coefficient correlation between WBSF and sensory tenderness. It is also important to notice that evaluating the human response to the eating process relies on subjective human assessment, since there are no objective means of measuring the full range of interacting characteristics contributing to the eating quality (Warriss, 2000). This could be the main reason for the difficulties found by researchers in relating instrumental and sensory characteristics of beef.

The off-flavour was the sensory attribute that greatly differ between beef types, which suggests that off-flavour strongly influenced overall acceptability. Tenderness and juiciness were highly correlated conforming that these two attributes are strongly associated. This could be due to the fact that the tender the meat is more easily juices are released from the spaces between the muscles fibres creating a sensation of a juicer meat. Tenderness, juiciness and flavour were positively correlated with overall acceptability, but these results could be due to the "halo effect", *i.e.* when an attribute is enhanced by other characteristics of the product (Gill *et al.*, 2010).

6.5 Conclusion

national undifferentiated beef was the most variable of the three beef types studied, which can represent a market problem as this is undesirable from a consumer point of view.

WBSF was moderately correlated with tenderness, explaining 27% of the tenderness variation. Thus, according to this study WBSF is a better predictor of sensory tenderness than TPA hardness. TPA hardness was not correlated with the sensory perception of muscle texture. Nevertheless, TPA chewiness correlated with sensorial tenderness and tended to correlate with WBSF, suggesting that TPA can be a useful methodology in beef texture studies giving important additional information. Despite the correlation between sensory tenderness and TPA chewiness, the chewiness parameter only explained 9% of the tenderness variation.

Acknowledgments

Financial support (grant AGRO/2003/422) and individual fellowship to Ana Cristina Monteiro (SFRH/BD/2006/31091) and Paulo Costa (SFRH/BPD/46135/2008) are

acknowledged. We also like to acknowledge Paula Lopes and Cristina Alfaia for their colaboration on sample preparation for sensory analysis.

7 - Beef quality and variability in the Portuguese market

A.C.G. Monteiro, M. A. Fontes, & J. P.C. Lemos

CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Avenida da Universidade Técnica, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal

Beef quality and variability in Portuguese market

Abstract

Beef samples were collected in a hypermarket in order to study their composition and quality variability. The physical, chemical and sensory characteristics of *longissimus lumborum* of Carnalentejana-PDO, imported from Brazil (Brazilian) and national undifferentiated beef samples were assessed and compared. Brazilian beef presented much higher ageing period, MFI (despite not different from national undifferentiated beef) and off-flavour score, as well as lower juiciness (despite not different from Carnalentejana-PDO beef) and overall acceptability score. Carnalentejana-PDO beef presented lower pH than the other two beef types. Cooking losses were positively correlated with juiciness. WBSF was highly inversely correlated with tenderness, which in turns was highly correlated with juiciness. Flavour and off-flavour were also correlated. All sensory attributes were correlated with overall acceptability, being the highest coefficient of correlation obtained between off-flavour and overall acceptability. National undifferentiated beef presented higher variability in the colour traits, like a*, b* and pigment content, whilst Carnalentejana-PDO beef presented higher CV in MFI and WBSF. Carnalentejana-PDO beef presented greater beef samples percentage with extreme values in WBSF, *i.e.*, values lower than 4.5 and higher than 7.0. All beef types presented WBSF mean value lower than 5.4 and mean sensory tenderness score of 5.5 which means all beef types would be considered slightly (5) to moderately (6) tender. Tenderness variability of Carnalentejana-PDO beef and colour variability of national undifferentiated are detrimental for these beef types in the moment of sale from consumer point of view.

7.1 Introduction

The consumption of fresh beef has been negatively affected by several phenomena. At the level of the individual consumer, the increasing concerns for healthy and safe food, as well as environmental and ethical issues related with animal production are the main reasons for the consumption decrease (Verbecke & Vianne, 1999). Consumers have become increasingly anxious, mainly driven by safety crises like BSE, dioxin, aflotoxin and more recently *Escherichia coli*. Moreover, from a nutritional point of view, beef has suffered from a bad reputation mainly due to its saturated and *trans* fatty acids, as well as cholesterol content. However, recent studies have shown the opposite, *i.e.*, when meat is consumed moderately as part of a balanced diet, is an excellent source of vitamins, minerals, amino acids, has low fat and cholesterol content, and can be a useful source of long-chain fatty acids (Andersen *et al.*, 2005). Despite the health and safety concerns, sensory properties such as appearance (colour and fatness), texture, juiciness and flavour still remain the main purchasing and repeat purchasing criteria (Haugen & Kvaal, 1998). Moreover, it is important for industry that any assessment of tenderness made in a laboratory to be highly correlated with sensory assessment of the same attribute (Perry *et al.*, 2001).

The sensory assessment of meat quality is obtained by use of either trained taste panels or untrained consumer panels. Both of these can assess the separate components of tenderness, juiciness and flavour. Sensory components of toughness arise from interactions between structural characteristics of meat and the forces required to masticate it in the mouth (Oddy *et al.*, 2001). The consumer panels are essential to obtain feedback on consumer preferences, but are expensive and time consuming. When knowledge of preferences is not essential, the use of trained taste panels can offer an alternative which has been shown to be well correlated with scores given by consumer panels (Perry *et al.*, 2001). Nevertheless, trained taste panels are still expensive and time consuming.

Another problem that beef industry faces is the lack of beef consistency, which affects all the intervenient of the market place. Thus, it is important to be well succeeded in predicting sensory attributes through instrumental measurements. Moreover, consumers have difficulty in selecting beef because they are unsure of its quality, particularly tenderness (Huffman *et al.*, 1996). Thus attention must be paid to the eating and quality parameters variability.

The use of rustic or local breeds as an alternative beef production system has the advantage that these breeds are closely related to the environment and help to maintain the biodiversity in agricultural production, especially in depressed areas (Gil *et al.*, 2001). According to these needs and to consumerdemands for healthy and safe food, as well as environmental and

ethical concerns, the European agricultural policy started to promote label systems. The PDO (Protected designation of origin), PGI (Protected geographical indication) and TSG (traditional speciality guaranteed) labels aim to protect food names as a way to differentiate products and keep consumers confidence on food safety and quality. The PDO beef follows strict rules detailed in the specification book concerning mainly the breed, origin and the production system. In Portugal there are several PDO beef products and Carnalentejana-PDO is the economically most important one. The Carnalentejana-PDO beef is obtained from Alentejana purebred young bulls produced in a traditional semi-extensive production system according to the product specifications. Despite that producing branded beef has a huge importance to the rural economy and to retain population in less favoured areas, branded beef production is still a market niche. The other main beef types marketed in Portugal are imported beef and national undifferentiated beef.

The Brazilian beef was the imported beef chosen, because the market share has been growing in the last years. In fact it is the fourth origin from the ranking of foreign trade beef. It increased from 2303.8 tonnes of fresh beef in 2005 (GPP, 2007b) to 2897.2 tonnes in 2007 (GPP, 2009). Brazilian beef is usually obtained from crosses of local breeds, like Nelore (*Bos indicus*), with more exotic breeds (*Bos Taurus*), and is usually produced in a traditional semi-extensive production system based on pastures with a finishing period with concentrates.

The national undifferentiated beef is originated from crossbred young bulls produced in a conventional intensive concentrate based system, being the most consumed in Portugal. This group consists of beef from different crosses, ages and genders produced and slaughtered in Portugal.

The aim of this study was to determine the physical and chemical characteristics as well as sensory attributes of three types of beef from Portuguese market, Carnalentejana-PDO, imported from Brazil and national undifferentiated beef, plus the variability of the referred characteristics and attributes.

This study is part of a grant research program (AGRO/2004/422, Ministry of Agriculture, Portugal).

7.2 Materials and Methods

7.2.1 Sample preparation

This study was performed on 46 samples of *longissimus lumborum* muscle 16 from Carnalentejana-PDO, 15 samples from Brazilian and 15 samples from national

undifferentiated. Samples of *longissimus lumborum* muscle were chosen in a hypermarket in order to reproduce consumer's purchase as decribed in sub-section 6.2.1. The ageing period was significantly different between Brazilian beef and the two Portuguese beef groups (78 ± 9.0 days vs 12 ± 5.3 days in Carnalentejana-PDO and 13 ± 6.0 days in national undifferentiated beef).

7.2.2 Chemical and physical analysis

The ultimate pH (pH_u) of the samples chosen in the supermarket was measured with a HI 99163 portable pH-meter (Hanna Instruments Inc., Rhode Island, USA), being the value expressed the mean of three determinations.

Meat colour measurements were carried out after 1 hour of blooming to allow oxygenation, with a Minolta CR 300 colorimeter (Konica Minolta Holdings Inc., Tokio, Japan) with a C iluminant and a 2º standard observer in the CIELAB space (*Commission Internationale de l'Eclairage, 1976*). Colour coordinates were lightness (L*), redness (a*), yellowness (b*), chroma (C*) and hue (h*). C* and h* were calculated from a*and b* values according to the device specifications.

Dry matter content was determined in minced muscle samples by microwaves (Smart System 5, CEM Microwaves Technology Ltd., Buckingham, UK), following the device specifications.

Samples for laboratory analysis were minced, vacuum packaged, frozen and stored at -18 ºC until analysed. Two steaks were left intact for Warner-Bratzler shear force (WBSF) measurement and sensory analysis.

Total pigment content was determined on two replicates, through the quantification of the cyanometmyoglobin and cyanomethemoglobin (Wierbicki *et al.*, 1955). The intramuscular fat content was measured according to the AOAC official method (945.16, 2000) in lyophilised samples (-60 ºC and 2.0 hPa), as described in Chapter 5 (sub-section 5.2.5), and expressed as g/100 g dry matter.

Total collagen concentration was determined by hidroxiproline quantification according to the Norma Portuguesa 1987 (2002), adapted by Silva *et al.* (1999), and expressed as dry matter percentage. Collagen solubility was determined as described by Silva *et al.* (1999) (Chapter 4, sub-section 4.2.2). Collagen solubility was expressed as total collagen percentage. Absorbance was measured at 558 nm against a blank. The myofibrillar fragmentation index (MFI) was determined as described in Chapter 4 (sub-section4.2.2).

7.2.3 Warner Bratzler shear force and cooking losses

Steaks with 2.5 cm thick were used for WBSF determination, and were thawed at 4 ºC. Samples were weighted, grilled until it reached 71 ºC of internal temperature, and weighted again for cooking losses determination. Grill cooking was conducted with a Modular 65/70 FTES electric griddle (Modular System Ltd., Italy) pre-heated at a temperature of 250 ºC. The grill final internal temperature was 71 ºC. Temperature was controlled with a needle thermocouple probe (Lufft C120, Munich, Germany), which was inserted horizontally at the midpoint of the steaks' width.

After grilling, steaks for WBSF were chilled until reached room temperature. Each sample provided a minimum of eight strips with a 1 cm^2 cross section. The cores were removed parallel to the muscle fibre orientation and were sheared perpendicular to the longitudinal orientation of the muscle fibres, using a TA-TX Plus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) equipped with a Warner-Bratzler shear blade with v slot. The beef sample resistance to shearing was recorded in a force-deformation plot. The maximum shear force in kg corresponded to the highest peak of the curve.

7.2.4 Sensory analysis

Steaks for sensory analysis were thawed at 4 ºC for 24 hours. Cooking procedures were similar to WBSF determination. After cooking steaks were cut into 2 x 2 x 2 cm^3, discarding pieces with connective tissue or fat, and maintained at 60 ºC until tasting. Samples were randomly served to a 10 member sensory panel. The panellists score the steaks on a 1 to 8 points scale for tenderness, juiciness, beef flavour, off-flavour and overall acceptability, as described in chapter 6 (sub-section 6.2.4).

It was considered as off-flavour all flavours that panellists considered strange or unusual in beef. Thus, flavours that in other countries would be classified as normal flavours could in this study be classified as off-flavour as long as panellists find it strange.

7.2.5 Statistical analysis

The effect of the beef type was studied by analysis of variance using the PROC MIXED procedure of Statistical Analysis Systems (SAS) software package, version 9.1 (SAS Institute Inc., Cary, USA, 2004). Data were checked for normality and homocedasticy. For some

variables significant differences of variances between groups were found and thus were analysed by PROC MIXED model allowing for variance heterogeneity.

Canonical discriminant analysis was applied to data in order to distinguish Carnalentejana-PDO, Brazilian and national undifferentiated beef types. Variable selection for discriminant analysis was achieved using the significant variables after PROC MIXED analysis. The stepwise discriminant analysis (PROC STEPDISC, SAS, 2004) selected the variables with higher discriminant ability. Following this, the linear discriminant functions were developed using the PROC DISCRIMINANT (SAS, 2004) to determine the coefficients of physical and chemical characteristics as well as sensory attributes that maximise the differences between the three meat types.

The coefficients of variation (CV) were also determined as described previously in chapter 5, section 5.2.8.

7.3 Results

7.3.1 Means and coefficient of variation

Data on mean, standard error of the mean and coefficient of variation of dry matter, pH, colour parameters (L*, a*, b*, h* and C*), pigment content, intramuscular fat content, collagen content and solubility, myofibrillar fragmentation index (MFI), cooking losses and WBSF are presented in Table 19. Data on sensory attributes is presented in Table 17 (Chapter 6, section 6.3.1).

Brazilian beef presented higher ageing period ($P<0.001$) value than the other two beef types. Carnalentejana-PDO beef presented lower ultimate pH ($P<0.01$) than the other two beef types, and lower MFI ($P<0.05$) than Brazilian beef.

Brazilian beef also presented higher off-flavour value ($P<0.001$) and lower overall acceptability ($P<0.001$) than the other two beef types, and lower juiciness ($P<0.01$) than national undifferentiated beef.

Brazilian samples presented higher CV in overall acceptability and lower CV in ageing, b* and h* parameters of the colour, as well as in off-flavour. National undifferentiated beef presented higher CV in a*, b* and h* colour parameters, pigment and total collagen content. National undifferentiated beef presented higher a* variance ($P<0.05$) than the two other beef, higher b* variance ($P<0.01$) than Brazilian beef, and higher pigment variance ($P<0.01$) than Carnalentejana-PDO beef.

Table 19 – Physical and chemical characteristics of *longissimus lumborum* from Carnalentejana-PDO, Brazilian and national undifferentiated (NU) beef

Variables	Carnalentejana-PDO			Brazilian			NU			Signif
	Mean	SEM	CV	Mean	SEM	CV	Mean	SEM	CV	
DM (%)	26.05	0.216	3.32	26.91	0.307	4.41	26.38	0.332	4.88	ns
pH	5.63[b]	0.028	1.9	5.78[a]	0.037	2.4	5.78[a]	0.044	2.9	**
L*	33.69	0.618	7.1	33.45	0.709	7.9	33.28	0.859	9.7	ns
a*	21.01	0.356	6.6	20.80	0.371	6.7	19.90	0.642	12.1	ns
b*	3.83	0.386	39.0	4.44	0.279	23.5	3.39	0.576	63.5	ns
h*	9.86	1.079	42.4	12.33	0.854	25.9	9.01	1.536	63.8	ns
C*	21.41	0.386	7.0	21.30	0.372	6.5	20.69	0.476	8.6	ns
Pigment (% DM)	1.61	0.075	17.9	1.50	0.108	26.9	1.79	0.164	34.2	ns
Intramuscular fat (% DM)	6.82	0.429	25.2	7.66	0.555	28.1	7.46	0.703	36.5	ns
Total collagen (% DM)	2.38	0.092	15.1	2.34	0.099	15.8	2.45	0.119	24.3	ns
Collagen Solubility (%)	17.42	0.627	13.9	18.30	0.768	15.7	17.41	1.129	18.2	ns
MFI	51.56[b]	6. 503	50.5	76.81[a]	6.712	32.7	57.52[a,b]	4.372	28.4	*
Cooking Losses (%)	27.04	1.040	14.9	28.73	1.057	13.8	28.42	0.839	11.0	ns
WBSF (kg)	5.48	0.387	27.4	5.28	0.319	22.6	5.42	0.251	17.3	ns

Statistical probability of treatment: ns, P>0.05; *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean; CV, coefficient of variations; Signif, significance; DM,dry matter; MFI, myofibrillar fragmentation index; WBSF, Warner-Bratzler shear force

7.3.2 Correlations between variables

Correlation coefficients among beef chemical and physical parameters and sensory attributes are presented in Table 20.

Chemical and physical parameters were not well correlated with sensory attributes. There was a trend for pH (P=0.07) to correlate with off-flavours. Cooking losses (P<0.05) correlated inversely with juiciness, and WBSF (P<0.001) was moderately inversely correlated with tenderness. Considering sensory attributes, all were well correlated with overall acceptability, off-flavour (P<0.001) were highly correlated, though. Tenderness (P<0.001) and juiciness were highly positively correlated. Flavour (P<0.01) was also well correlated with off-flavours.

Table 20 – Correlation coefficients between chemical and physical characteristics and sensory attributes

	Tenderness	Juiciness	Flavour	Off-flavours	OA
pH	-0.01	-0.01	-0.17	0.27	-0.178
Intramuscular fat	-0.22	-0.01	-0.00	-0.12	0.07
Total collagen	0.08	0.04	0.12	-0.20	0.24
Collagen solubility	-0.02	-0.05	-0.16	0.18	-0.19
MFI	0.14	0.14	0.35*	0.24	-0.05
Cooking losses	-0.09	-0.31*	0.10	0.18	0.04
WBSF	-0.50***	-0.14	0.11	-0.23	-0.06
Tenderness	-	0.49***	0.09	0.13	048**
Juiciness		-	0.28	-0.26	0.43**
Flavour			-	-0.44**	0.47**
Off-flavours				-	-0.66***

7.3.3 Discriminant analysis

Canonical discriminant analysis was applied to data in order to discriminate the beef types used in this study. Results of canonical discriminant analysis, loadings of correlation matrix and discriminant functions are depicted in Table 21.

The application of canonical discriminant analysis to selected variables resulted in two discriminant functions, which maximise the ratio between class variance and minimises the ratio within class variance.The discriminant functions obtained used five variables, from which pH had the highest discriminant power in both roots. Overall acceptability followed by off-flavour had the highest discriminant power in the first root, whilst juiciness followed by overall acceptability had the highest discriminant power in the second root.

After cross-validation, results varied between 47 and 100% of correct classification, being the extreme values from national undifferentiated and Brazilian beef, respectively. Carnalentejana-PDO beef presented a classification of 64.3% correct (Table 22).

As can be seen in the plot (Figure 9), both Portuguese types of beef are located in the left side of the plot, whilst Brazilian beef is located in the right side. The second function (root 2) discriminates between both Portuguese beef types, having Carnalentejana-PDO beef negative loadings and national undifferentiated beef positive ones. Nevertheless, the two Portuguese beef type are not well separed and present a great overlapping. It is clear that national undifferentiated beef distribution in the plot is more spread than the other beef types, which is indicative of national undifferentiated beef higher variability.

Table 21 - Results of canonical discriminant analysis: loadings of correlation matrix between predictor variables (standardized canonical coefficients) and discriminant functions (roots 1 and 2), and some statistics for each function.

	Root 1	Root 2
Meat characteristics		
pH	1.234	5.066
IFM	0.031	-0.005
Juiciness	-0.452	1.241
Off-flavour	0.617	-0.312
Overal acceptability	-1.290	-0.614
Statistics		
Cannonical R	0.892	0.457
Eigenvalue	3.906	0.264
Cummulative proportion	0.937	1.000
Probability	<0.0001	<0.0576

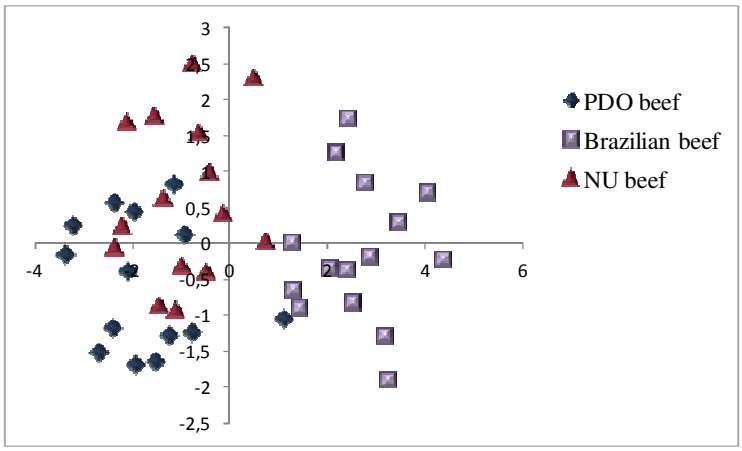
Table 22 – Classification matrix of cross-validation results for the three types of beef (Carnalentejana-PDO, Brazilian and national undifferentiated beef) using canonical discriminant analysis.

	Beef types		
	Carnalentejana-PDO	Brazilian	NU
Classified as PDO beef	9	1	4
Classified as Brazilian beef	0	15	0
Classified as NU beef	6	2	7
Total	15	18	11
% of correct classification	64.3	100.0	46.7

7.3.4 Distribution of relative frequencies

Beef tenderness has been considered for decades the most important palatability attribute. However, the lack of tenderness consistency in beef has been a major concern to the beef industry, mainly because it is the main reason for consumers to repeat or not repeat the beef purchase. The relative frequency distribution of the WBSF values for the three beef types studied can be seen in Figure 10.

Figure 9 - Plot of the discriminant functions (root 1 *vs.* root 2) for classification of the three types of beef, Carnalentejana-PDO, Brazilian and national undifferentiated beef.



In our study all beef types presented a similar shear force value, which averaged 5.39. However, despite the lack of mean differences the distribution of the beef samples was different from type to type. About 37.5% of Carnalentejana-PDO beef samples presented shear force lower than 4.5, whilst only 20% and 13% of Brazilian and national undifferentiated beef, respectively, presented value lower than 4.5 (Figure 10). Nevertheless, all three beef types presented around 60% of the samples with WBSF values lower than 5.5. These results indicate that Carnalentejana-PDO beef presented the highest percentage of samples with a tenderness value that makes it highly acceptable by the Portuguese consumers (Simões *et al.*, 2005). Carnalentejana-PDO beef was also the beef type with highest percentage of samples (25%) with WBSF higher than 7.

The relative frequency distribution for panel ratings tenderness for the three beef types studied is shown in Figure 11. The panel scores for tenderness ranged from 1 (extremely tough) to 8 (extremely tender). All three beef types were scored similarly, averaging 5.45 (between slightly tender and moderately tender). Aproximately, 87% of the national undifferentiated beef samples were scored by the panel between 5 and 7. Only Brazilian beef presented samples scored extremely tender. Carnalentejana-PDO beef presented samples with scores almost equally distributed (Figure 11), and with Brazilian beef presented the highest percentage of samples scored below 5 (33.3%).

Figure 10 – Relative frequency distribution of shear force values for Carnalentejana-PDO, Brazilian and national undifferentiated beef.

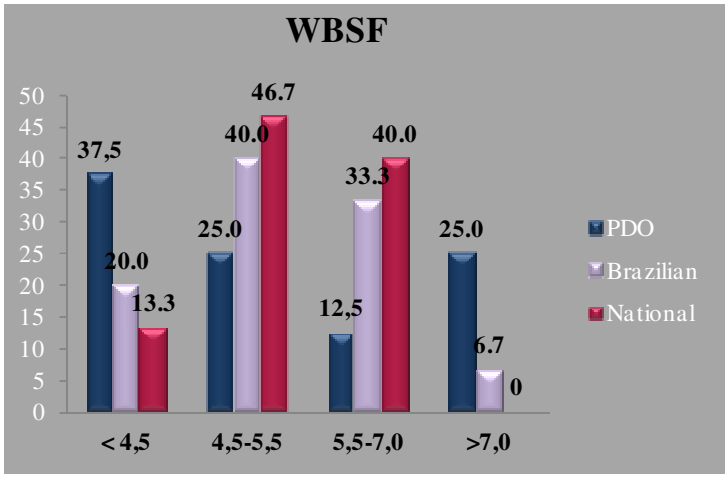
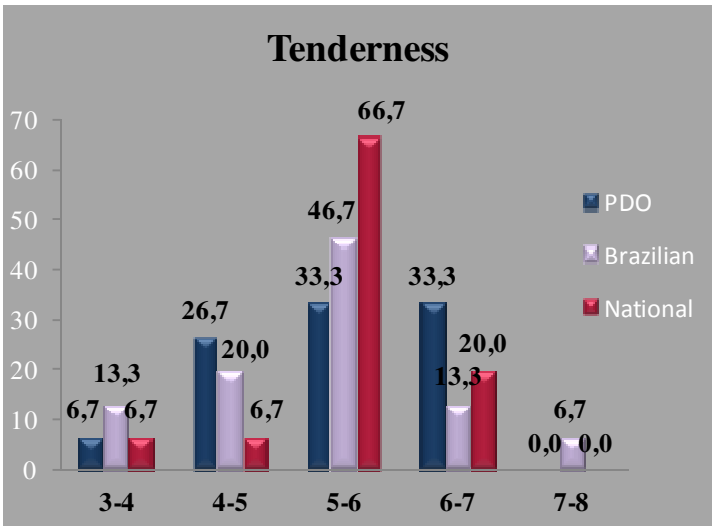


Figure 11 – Relative frequency distribution for sensory panel tenderness ratings of Carnalentejana-PDO, Brazilian and national undifferentiated beef.



7.4 Discussion

7.4.1 Means of beef quality traits

All beef types presented similar colour parameters values which were similar to those presented by Monteiro *et al.* (2005) in Carnalentejana-PDO beef. The same authors presented similar collagen solubility and cooking losses, but higher total collagen content and lower intramuscular fat and pigment content as well as lower WBSF value than the values presented in this study. Serra *et al.* (2004) in beef of a Spanish breed also observed similar L*, a* and

pigment content as well as a slightly lower cooking losses than the values presented in this study. The L* value as well as the pigment content were also similar to the values presented by Monteiro (2003) and in the chaper 4 of the present thesis in Mertolenga-PDO beef. In the study previously described in chapter 4 the Mertolenga-PDO beef also presented similar h* value and total collagen content (for more detailes see section 4.3.1).

All beef types presented a WBSF value lower than 5.5 which makes these beef types slightly tender. Other author presented similar WBSF values (Monteiro *et al.*, 2009), and considered beef as slightly tender. Also beef sensory tenderness was scored with an average value of 5.45, *i.e.*, between slightly and moderatadly tender. *Post-mortem* tenderization is largely due to the enzymatic activity of proteases, which breaks down the structural proteins with the consequent weakening of the myofibrilar matrix (Koohmaraie & Geesink, 2006). Several authors reported that ageing periods higher than 7 days improve beef tenderness, sensory and instrumentally measured (George *et al.*, 1999). However, the same authors referred that ageing periods longer than 11 days does not produce any additional improvement in tenderness. All beef types had an ageing period long enough to allow the beneficial effect of the enzymatic activity of proteases on myofibrillar structure. Moreover, despite the much longer ageing period Brazilian beef was suject to it did not bring any additional tenderness improvement. It could though, diminished the differences that may have existed due to *Bos indicus* nature of Brazilian beef. In cattle, it is well known that *Bos indicus* breeds are rated less tender and juicy than *Bos taurus* breeds (like Carnalentejana-PDO and national undifferentiated beef; Andersen *et al.*, 2005). This difference seems to be associated with differences in muscle protein turnover in the living cattle (Wheeler *et al.*, 1994). In agreement, the higher MFI presented by Brazilian beef did not reflected lower WBSF value or higher tenderness score. This higher MFI probably resulted from the longer ageing period (78 days).

The beef difference obtained in juiciness value, in which Brazilian beef presented the lowest score (despite not different from Carnalentejana-PDO beef), could also result from the longer ageing period as some authors referred that very long ageing period cause an excessive myofibrillar fragmentation, and consequently a decreased water holding capacity and therefore juiciness (Huff-Lonergan & Lonergan, 2005).

Myofibrillar toughness, connective tissue toughness and juiciness all contribute to sensory perception of cooked meat texture. For this reason, the objective measurements of shear force, compression and cooking losses should be useful in predicting sensory tenderness and juiciness assessment (Perry *et al.*, 2001). WBSF and sensory tenderness, as well as cooking losses and juiciness, were inversely correlated. The contribution for the tenderness/toughness

seemed to be linked to the myofibrillar component of beef, as the contribution of connective tissue content and solubility to meat tenderness/hardness was not evident. Purslow (2005) stated that precise correlations between textural measures such as WBSF and collagen content are poor. Other authors have referred that muscles with low collagen content like *longissimus lumborum* might provide a limited contribution to background toughness in comparison with myofibrillar toughness (Perry *et al*., 2001; Ngapo *et al.*, 2002). In the connective tissue structure modelling of Lepetit and coworkers (2000) it was concluded that it is unlikely that a universal relationship between collagen content and cooked meat toughness could be derived, even for a constant level of collagen cross-links, as the contribution of collagen depends on the ability of the collagen fibres to contract during cooking and this contraction is dictated by the muscle fibres it surrounds (Lepetit *et al.*, 2000). The lack of contribution from collagen content, collagen solubility and intramuscular fat to hardness measured by WBSF test or sensory tenderness has been reported by other author (Silva *et al.*, 1999; Monteiro *et al.*, 2005, 2009). The lack of correlation found may be due to the fact that soluble collagen contribution is more important in animals with great age differences, and the contribution of total collagen content is more important in muscles with different collagen contents (Dransfield, 1994).

Sensory perception of tenderness is multifaceted and is partly influenced by stimulation of the salivary glands as well as actual juiciness of beef *per se*. Although sensory tenderness and juiciness are treated as separated attributes of meat quality, they may have a degree of interdependence, because changes that occur in meat structure may affect both sensory attributes. It has also been suggested that there is a "halo effect" between sensory tenderness and juiciness, whereby a beef sample judged to be very tender would often also be judged as very juicy (Shorthose & Harris, 1991, Gill *et al.*, 2010). This is probably due to the fact that as tender the beef is more easily juices are released from the spaces between the muscles fibres creating a sensation of a juicer meat. Several other authors have found a positive correlation between these two sensory attributes (Silva *et al.*, 1999; Sierra *et al.*, 2010; Chapter 4, sub-section 4.3.2.3). Juiciness can be divided in two components: first the juice that is released during the first mastication as a result of the breakage of the meat structure and second the increase production of saliva, due to the effect of intramuscular fat on the salivary glands (Geay *et al.*, 2002). Factors that affect beef water holding capacity will also affect negatively cooking losses and juiciness (Perry *et al.*, 2001). The rigor process could result in mobilization of water out, not only out of the myofibril, but also out of the extra myofibril spaces as the overall volume of the cell is constricted (Oddy *et al.*, 2001; Huff-Lonergan & Lonergan, 2005). Therefore, a more shortened muscle shows higher cooking

losses. Moreover, during cooking liquefied fat and water are lost. The water is probably lost due to heat-induced protein denaturation during cooking of the meat, which causes less water to be entrapped within the protein structures held by capillary forces. The greatest increments in cooking losses were in the temperature ranges 50–60 ºC and 60–70 ºC, which may be explained by the contracted protein system causing expulsion of water (Cheng & Su, 2008). The greatest cooking losses will result in less juiciness with is in agreement with the negative correlation obtained between cooking losses and juiciness (r=-0.31), but also with the trend for a higher cooking losses value presented by Brazilian beef, which presented the highest MFI and the lowest juiciness.

The pH presented a trend to correlate positively with off-flavours. This is partly in accordance with the negative correlation obtained by Safari and co-workers (2001) between pH and flavour. In those authors work flavour, which is usually described as the typical flavour of beef, decreased as pH increased, the same way that in our study off-flavour, usually described as the presence of flavour not typical in beef, increased as pH increased. Moreover, the higher pH allows longer proteolitic activity, which can result in greater enzymatic reactions, more oxidation products and consequently the greater production of volatile compounds which will alter the normal flavour of beef (Calkins & Hodgen, 2007).

Off-flavour was highly correlated with overall acceptability, presenting Brazilian beef the highest and lowest values in these attributes, respectively. Several reasons can be point out for such a high off-flavour value presented by Brazilian beef. In first place, these beef presented the highest ageing period, and is well known that during ageing enzymatic reactions can produce volatile compounds, which alter the flavour of beef (Calkins & Hodgen, 2007). In addition, the typical production system of Brazilian beef is based on grazing. Several researches have reported that beef from pasture fed animals have more off-flavours than meat from concentrate fed animals. Moreover, flavours and/or off-flavours present in beef depend on the pasture composition (Calkins & Hodgen, 2007). The lower acceptance for beef obtained from grass fed animals, as Brazilian beef, can be attributed to the increased intensity of negative attributes as barny, bitter, grassy, gamey, and a decreased intensity of positive attributes such as juicy and umami (Maughan *et al.*, 2011). This would also explain the lower juiciness score of Brazilian beef. We know from a previous study that off-flavours present in Brazilian beef are not due to the presence of oxidation products as the malondyaldehyde value mesured by the TBARS technique presented by this beef type was low. Probably is just due to the different pasture composition and/or a result of panellists not being used to such flavours. Panellists that are accustomed consuming meat from animals reared mainly on concentrates might perceive the typical pastoral flavour as strange (Resconi *et al.*, 2010). Possibly, the

short period that animals were finished on concentrates (as it seems to be the case of Brazilian beef) was insufficient to reduce pastoral flavours (Resconi *et al.*, 2010). Priolo *et al.* (2001) suggested that at least three months of finishing with concentrates are necessary to reduce the pastoral flavour. The panel classification is relative to the eating habits of the panellists, which is a reflection of the Portuguese population. Consumers with different eating habits might rate differently beef off-flavours. These results indicate that off-flavour is the sensory attribute that defines the overall acceptability in this study explaining 43% (r^2 x 100) of overall acceptability variance.

The differences between beef types in the variables studied can be evaluated by the values of squared Mahalanobis distance (D^2). The higher values of D^2 obtained correspond to larger differences between beef types. In line with this, the differences were much smaller between Carnalentejana-PDO and national undifferentiated beef (2.1), than between Carnalentejana-PDO and Brazilian (19.3) or between Brazilian and national undifferentiated beef (13.5), as illustrated in Figure 9. This means that Carnalentejana-PDO and national undifferentiated beef presented more similar chemical, physical and sensory characteristics to each other than with the Brazilian beef. Moreover it can be seen that Brazilian beef is located in the right side and well separated from the other two beef types. This means that Brazilian beef and the two Portuguese beef types were well discriminated by the physical, chemical and sensory characteristics measured. However the same is not so clearly concerning the two Portuguese beef types, considering the great overlapping existing between them.

7.4.2 Variability of beef quality traits

The three beef types were analysed to determine the extent variation in their eating quality. The variability in ageing days (coefficient of variation) was much lower in Brazilian beef (15% *vs.* 46% in the other beef types), this is probably due to the fact that ageing period mean value from Brazilian beef was much higher.

The national undifferentiated beef presented higher variability in the generality of the colour parameters and in pigment content (CV). This was also seen in the higher variance value in a* parameter than the other two beef types, in the higher variance in b* parameter than Brazilian beef and in the higher variance in pigment content than Carnalentejana-PDO beef. The higher variability in a* parameter of colour presented by national undifferentiated beef could be a result of the higher variability presented by this beef type in total pigment content, as a* parameters reflects the pigment content of beef. Appearance, mainly colour, has been point out as the first criteria consumers use to judge meat. Moreover, consumers have colour

preferences well defined. The greater variability presented by national undifferentiated beef in colour can be detrimental in the purchasing moment. The higher variability in national undifferentiated beef samples could result from de fact that this group is more heterogeneous, *i.e.*, is constituted by samples with different backgrounds (animals from different crosses, genders and/or ages). Despite the highest variability presented by national undifferentiated beef in a* parameter, the value obtained was similar to the value obtained by Maher *at al.* (2004) for all the ageing periods studied by those authors. However, the b* parameter CV obtained by the quoted authors were much lower than the presented here.

The Carnalentejana-PDO beef presented higher variability in MFI and WBSF values (CV). Accordingly, Carnalentejana-PDO beef also presented higher data dispersion in WBSF. The higher variability in WBSF values could be due to differences in the weakening of the myofibrilar matrix by enzymatic activity of proteases, as indicated by the higher variability in the MFI. Various reports have shown that the MFI increases with ageing, and this increase with ageing seems to be related to the phenomenon of myofibrils breaking into shorter fragments at, or near, the Z-disk during *post-mortem* storage (Olson *et al.*, 1976). The ageing period of Carnalentejana-PDO beef ranged between 3 and 23 days long. According to George *et al.* (1999) beef samples with less than 7 days of ageing will probably have low myofibrillar fragmentation and consequently higher WBSF value. Moreover, a lower MFI could result from a higher glycolytic rate. This could not be measured as beef was bought in the supermarket. However, Carnalentejana-PDO beef had lower final pH which could result from a higher glycolytic rate, that would have a detrimental effect in proteolytic activity decreasing fragmentation of the myofibrillar structure (Silva *et al.*, 1999).

The WBSF CV presented by the beef types were similar to the value presented by George and coworkers (1999) in top sirloin, but a bit lower (excepting the value presented by Carnalentejana-PDO beef) than the value presented in strip loin samples. Also Maher *et al.* (2004) presented higher WBSF variability value in *longissimus dorsi* muscle. Intrinsic meat quality attributes such as texture and flavour are important factors that consumers consider when deciding to repurchase beef. This is particularly important for expensive products like branded quality beef as Carnalentejana-PDO beef. The great variability in tenderness presented by Carnalentejana-PDO beef represents a commercial problem.

The CV values observed in the sensory attributes studied were similar for all beef types, excepting the off-flavour attribute. The high off-flavour CV presented by Carnalentejana-PDO and national undifferentiated beef is mainly due to the fact that in the majority of the samples from these beef types off-flavours were under the detection limit of the panellists perception, and thus were scored with the minimum value (1 in a 1 to 8 scale), and only a few

samples presented medium off-flavour notes. In turns Brazilian beef presented off-flavour in all samples, and also presented higher consistency in the results. The CV values obtained in this study for the sensory attributes were similar to the ones presented by George *et al.* (1999) and Maher *et al.* (2004).

Variability differences between techniques to measure tenderness (instrumental and sensory) have been explained with differences in the muscle fibre orientation of samples. It is also important to note that the instrumental measurement of texture is made by a single compression (shear) step, while the sensory method of evaluation includes several steps outside and inside the mouth, from the first bite through mastication and swallowing (Szczesniak, 2002), and maximum intensity perception seems to occur anywhere between the first and the fourth bite (Duizer *et al.*, 1996).

7.5 Conclussion

Considering the physical and chemical characteristics measured the three beef types were considered similar. Nevertheless Carnalentejana-PDO beef was less consistent in tenderness and national undifferentiated beef in colour characteristics. The greatest differences between the three beef types were in sensory attributes with Brazilian beef presenting the worse score in juiciness, off-flavours and overall acceptability. Off-flavour strongly influenced the overall acceptability. Despite the similarities between the beef types, the differences in physical and chemical characteristics and sensory attributes were enough to discriminate them. Once more, Brazilian beef was the most distinguished over the beef types studied.

Acknowledgments

The financial support (Grant AGRO/2004/422) and the individual fellowship from FCT to Ana Cristina Monteiro (SFRH/BD/2006/31091) are acknowledged.

8. GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The work reported in this thesis was divided in two experimental trials. The results of the first trial were presented in chapter 3 and 4. The study was focused in meat quality (physical, chemical and sensory attributes), and in lipid nutritional value of three quality branded beef products that have in common their origin breed, *i.e.*, Mertolenga breed. The results revealed a general similarity between the two veal studied, "Vitela Tradicional do Montado"-PGI veal and Mertolenga-PDO veal, against Mertolenga-PDO beef. Table 23 provides an integrated review of the main results obtained in the first trial.

The three products were not very different in chemical, physical and sensory attributes, as well as in fatty acid profile, but the two veal types were more alike, which means that age had a more powerful effect than breed or gender. It is interesting to notice that despite the minor differences obtained in chemical, physical and sensory attributes, as well as in meat fatty acid profile and the similarity of the production systems of the animals sampled, it was feasible to allocated meat samples into one of the three meat types with good accuracy though. From a nutritional point of view, it is difficult to decide which of the three types of beef is the healthiest, since Mertolenga-PDO veal has higher MUFA and total CLA contents than PDO beef and PGI veal, respectively, whereas PDO beef has higher total PUFA content and P/S ratio than PDO veal and, finally, PGI veal has a trend for a higher *n-3* LC-PUFA (EPA and DPA).

Considering the organoleptic characteristics of beef, the PDO beef was darker, redder and harder than the two veal types. The WBSF values obtained for the three meat types allow to conclude that PDO beef is considered a hard meat, for which the higher cooking losses could have contributed, whilst PGI and PDO veal are medium tender meats. The high correlation between sensory tenderness and WBSF indicates that sensory tenderness can be predicted by instrumental means. Nevertheless, instrumental measurements seemed to be more sensitive to detect tenderness differences between groups than the sensory panel.

The overall results of the first trial suggested that the "Vitela Tradicional do Montado"-PGI showed the highest variability which was probably due to the fact that in the specification of this certificated products are allowed animals from different crosses, *i.e.*, with different genetic backgrounds. The greater heterogeneity of the PGI veal is an issue that should deserve some attention by the Association of Producers in order to improve the homogeneity of these certified products.

Table 23 – Selected fatty acids and nutritional ratios as well as chemical and physical characteristics of intramuscular fat of *longissimus lumborum* muscle from "Vitela Tradicional do Montado"-PGI veal, Mertolenga-PDO veal and Mertolenga-PDO beef.

	PGI veal		PDO veal		PDO beef		Significance
	Mean	SEM	Mean	SEM	Mean	SEM	
Fatty acid composition (g/100 g total FA)							
16:1*c*9	2.56^{ab}	0.099	2.73^{a}	0.142	2.29^{b}	0.089	*
18:0	13.40^{b}	0.306	14.43^{ab}	0.338	15.24^{a}	0.327	***
18:1 *c*9	28.57^{ab}	1.284	31.49^{a}	0.840	28.05^{b}	0.746	*
18:2 *n*-6	9.04^{b}	0.730	8.06^{b}	0.435	11.45^{a}	0.578	***
CLA	0.35^{b}	0.025	0.46^{a}	0.030	0.39^{ab}	0.026	*
20:0	0.09^{b}	0.005	0.12^{a}	0.006	0.11^{ab}	0.004	**
20:4 *n*-6	3.67^{a}	0.414	2.52^{b}	0.204	2.93^{ab}	0.190	*
20:5 *n*-3	0.48^{a}	0.089	0.33^{ab}	0.043	0.22^{b}	0.041	*
22:5 *n*-3	0.83^{a}	0.107	0.70^{ab}	0.066	0.54^{b}	0.059	*
FA sums and ratios							
MUFA	32.34^{a,b}	1.384	35.36^{a}	0.930	31.36^{b}	0.835	**
PUFA	15.92^{a,b}	1.456	13.11^{b}	0.782	16.69^{a}	0.886	*
n-6	13.99^{a,b}	1.259	11.53^{b}	0.687	15.33^{a}	0.798	**
P/S	0.27^{a,b}	0.030	0.22^{b}	0.013	0.32^{a}	0.020	***
n-6/*n*-3	8.7^{b}	0.526	8.7^{b}	0.621	16.4^{a}	1.666	***
T_3	16.0^{b}	0.716	18.3^{a}	0.716	19.9^{a}	0.716	***
pH_u	5.46^{b}	0.361	5.67^{a}	0.361	5.42^{b}	0.361	***
L*	35.73^{a}	0.500	34.87^{a}	0.500	31.52^{b}	0.500	***
a*	17.21^{c}	0.391	18.59^{b}	0.391	21.59^{a}	0.391	***
b*	2.88^{a,b}	0.349	2.35^{b}	0.349	3.79^{a}	0.349	*
Pigment (% DM)	1.05^{c}	0.007	1.26^{b}	0.007	1.78^{a}	0.007	***
Total collagen (% DM)	2.22^{b}	0.092	3.00^{a}	0.092	2.82^{a}	0.092	***
Collagen solubility (%)	19.51^{a}	0.978	15.31^{b}	0.978	17.08^{a,b}	0.978	*
Cooking losses (%)	22.51^{b}	0.751	22.34^{b}	0.751	25.25^{a}	0.751	*
WBSF (kg)	5.16^{b}	0.462	5.56^{b}	0.462	7.43^{a}	0.462	**

Statistical probability of treatment: *, P<0.05; **,P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of the mean; T_3, temperature 3 hours *post-mortem*; WBSF = Warner-Bratzler shear force; CLA= conjugated linoleic acid; MUFA= monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids; P/S=(18:2*n-6*+18:3*n-3*)/(14:0+16:0+18:0).

The results of the second trial were presented in chapters 5, 6 and 7. This second trial had as starting point the results of a previous study performed at a hypermarket. Consumers were asked to choose the preferred beef from three different types based on visual evaluation, simulating the purchasing moment, and to state the expected sensory quality of the chosen

one. This was followed by a blind test where consumers were asked to taste and choose the preferred beef (experienced quality) and to state the reason(s) of their choice (Banovic *et al.*, 2010).

Samples used in the present study were purchased at the same hypermarket and were from the same three beef types. The beef types were chosen considering their commercial importance on the Portuguese market place. It was chosen a certified beef with protected designation of origin, an imported beef and national undifferentiated beef. In the first case, the certified chosen beef was Carnalentejana-PDO beef, which is the Portuguese branded quality beef with the highest economic value. The imported beef chosen was Brazilian beef considering the great increase of the market share verified in the last years. The undifferentiated beef is the most consumed in Portugal, and it is mainly beef from crossebred animals raised in an intensive production system based on concentrates.

The main objective of this second trial was to understand consumers` needs and expectations when purchasing beef considering the impact of relative differences in palatability attributes in overall acceptance of beef by those consumers. For such, beef types were studied in order to characterise their chemical, physical and sensory characteristics as well as the nutritional value of their lipid profile.

A general overview of the nutrient composition from the three beef types analysed and the calories corresponding to each nutrient, as well as the total for a steak with 100 g (raw) is presented in Table 24. The percentage of carbohydrates in beef is tabled and average around 0.4% (Ferreira & Graça, 1985), as the percentage of fat in the three beef types ranged between 1.8 and 2.1% and considering the water content, the protein content was determined by difference and ranged between 23.9 and 24.4%.

Table 24 – Proportional composition and energy value (E) of a 100 g (raw) steak from Carnalentejana-PDO, Brazilian and national undifferentiated (NU) beef.

	Carnalentejana-PDO		Brazilian		NU	
Composition	Proportion	E (kcal)	Proportion	E (kcal)	Proportion	E (kcal)
Water	73.9%	0	73.1%	0	73.6%	0
Protein	23.9%	95.6	24.4%	97.6	24.0%	96.0
Carbohydrates	0.4%	1.6	0.4%	1.6	0.4%	1.6
Intramuscular fat	1.8%	16.0	2.1%	18.9	2.0%	18.0
Total	100.0%	113.2	100.0%	118.1	100.0%	115.6

As can be seen all beef types present a low energy value, mainly due to the low total fatty acid

content. The content and energy value of the fatty acids or groups of fatty acid for which there are recommended daily intake (RDI) values are discriminated in Table 25.

The three beef types presented low cholesterol content and alpha-tocopherol value high enough to inhibit lipid oxidation and discoloration. Beef is poor in γ-tocopherol and β-carotene, nevertheless, it is a good source of L-carnitine. In a serving beef with 100 g SFA and TFA content is much lower than the maximum RDI values by FAO/WHO. Despite *n*-6 and *n*-3 content being lower than the RDI value, beef can be give a good contribution to prevent deficiency symptoms of these LC-PUFA mainly in diets with low fish consumption. Contrary to what is transmitted to consumers, mainly through the media, beef has not high cholesterol or saturated fatty acid content, despite SFA being the greatest fatty acid group comprising between 41.2 and 45.7% of total FA. The low content in cholesterol and SFA was mainly due to the low intramuscular fat content. Thus, included in a balanced diet beef can contribute with high value protein, long chain fatty acids and intramuscular fat in a healthy way.

Data was calculated having into account the intramuscular fat content of each meat type, which was about 17.8 mg/g muscle in Carnalentejana-PDO, 19.7 mg/g muscle in national undifferentiated beef and 20.6 mg/g muscle in Brazilian beef.

Table 25- Recommended daily intake (RDI), composition and energy value (E) of the main fatty acids and fatty acid groups from *longissimus lumborum* muscle of Carnalentejana-PDO, Brazilian and national undifferentiated beef in a 100 g of raw steak.

Nutrients	RDI	Carnalentejana-PDO		Brazilian		National undifferentiated	
		content	E	content	E	content	E
Cholesterol	< 300 mg	40 mg	3.6	40 mg	3.6	40 mg	3.6
SFA	< 10% E	738 mg	6.6	945 mg	8.5	831 mg	7.5
TFA	< 1% E	72.9 mg	0.66	66.5 mg	0.60	54.5 mg	0.49
18:2 *n*-6	≥ 2.5% E	158 mg	1.42	71 mg	0.64	168 mg	1.51
18:3 *n*-3	≥ 0.5% E	5.19 mg	0.05	21.94 mg	0.17	4.98 mg	0.04
EPA	> 250 mg	3.40 mg	0.03	8.07 mg	0.07	2.79 mg	0.03
EPA+ DPA		8.95 mg	0.08	21.53 mg	0.19	7.76 mg	0.07

SFA = saturated fatty acids; TFA = *trans* fatty acids; EPA = ecosapentaenoic acid; DPA = docosapentaenoic acid; E = energy intake

The daily intake of 18:2 *n*-6, 18:3 *n*-3 and EPA provided by a 100 g steak are lower than the RDI. The recommendations of *n*-3 LC-PUFA concerns to EPA and DHA intake, however as in the present study DHA was below to the detection limit in the majority of the samples

analysed the values presented here only regards to EPA content. The EPA values correspond only to 1.1 to 3.2% of the minimum RDI for humans' diet (250 mg/day, Elmadfa & Kornsteiner, 2009). Therefore the values of EPA presented by the three beef types were much lower than the minimum value necessary to prevent deficiencies. There are not recommended values for the DPA intake. Interestingly, in the study of Howe *et al.* (2006), the DPA contributed with 29% to the total *n*-3 LC PUFA which is in line with the present results were DPA was 50% to 65% higher than EPA. These data do, however, highlight the need to better understand the physiological effects of DPA (Givens & Gibbs, 2006).

Considering the physical and chemical characteristics measured the three beef types were considered similar, nevertheless Carnalentejana-PDO beef was less consistent in tenderness and national undifferentiated beef in colour traits. Beef types presented greater differences considering sensory attributes, with Brazilian beef presenting the worse score in juiciness, off-flavours and overall acceptability.

Despite the similarities between the beef types, the differences in physical and chemical characteristics and sensory attributes were enough to discriminate them. Nevertheless, Brazilian beef was the most distinguished over the beef types studied, and the two Portuguese beef types presented some overlapping.

After the physical, chemical and sensory characterization of the three beef types has been achieved we intended to relate it with the results obtained in the market study. Thus, at the light of the main objective referred above, specific objectives were defined and questions formulated which we intended to answer.

Quite a few studies have indicated that consumers attribute great confidence to the quality labels (Verbecke & Ward, 2006) in respect to guarantee credence attributes, *i.e.*, attributes such as healthiness, animal welfare, environmental protection, food safety and origin (Becker, 2000). Moreover, consumers are willing to pay more for such beef (Sepúlveda *et al.*, 2008). In Portugal, studies relating consumer preferences with quality and nutritional value of meat as far as we know are inexistent. We intended to fill this gap with this study.

Several authors have reported that much is expected by consumers from quality branded beef. Consumers have more confidence in quality branded beef quality and safety but also believe that this type of products are more environmental and animal friendly.

In the shop, this believes are set in practice. Consumers assess quality of a product evaluating the available intrinsic and extrinsic cues which they believe reflect the product quality, this reflects the consumer expectations. After product purchasing and following its preparation, the product quality is experienced during its consumption, confirming or not those quality expectations (Banovíc *et al.*, 2010). In the aforementioned authors study, consumers

perceived beef colour and fat content differently. Carnalentejana-PDO beef was perceived has being dark coloured and having high intramuscular fat content. Brazilian beef was perceived has being medium red and having medium intramuscular fat content, whilst national undifferentiated beef was perceived has being light red and having low intramuscular fat content. Moreover, Carnalentejana-PDO beef was preferred over the other two beef in terms of extrinsic cues (origin, brand and label information), as well as regarded as more expensive beef. This is in agreement to other authors results in other countries (Verbecke & Ward, 2006; Sepulveda *et al.*, 2008). Between Brazilian and national undifferentiated beef, consumers perceived national undifferentiated beef as being more expensive, but preferred it in terms of origin and label information.

Consumers also perceived Carnalentejana-PDO beef as having higher expected quality than Brazilian and national undifferentiated beef in all quality traits considered, *i.e.*, in taste, tenderness, juiciness, nutrition, healthiness and safety.

After the blind test, consumers considered once more Carnalentejana-PDO beef as having better perceived quality, based on taste, tenderness and juiciness. The other two beef types, Brazilian and national undifferentiated beef, were considered similar in the aforementioned sensory attributes. In the remaining quality attributes, nutrition, healthiness and safety, they were considered similar. These attributes cannot be experienced, as they are credence attributes and consumers most often has difficulty to distinguish beef in terms of what they cannot measure. In terms of overall appreciation consumers chose Carnalentejana-PDO beef over Brazilian and national undifferentiated beef, with 53% of the respondents choosing this beef type (Banovíc *et al.*, 2010). The quoted authors also concluded from their study that the recognition of the brand name by consumers turns the labels information less important. Consumers' prior experience and confidence in credibility of Carnalentejana-PDO brand might have influence in both quality cues and quality aspects leading to increased expected quality of this beef.

This study in same way failed to answer the first question, *i.e.*, "How do textural properties and chemical components of beef correlate with the consumers' choice?". Consumers have chosen Carnalentejana-PDO beef as the preferred one however, these beef type presented WBSF and sensory tenderness value similar to the other two beef types. Looking more closely to those authors' results, we realised that despite the significant differences obtained between beef types in intrinsic experienced quality cues, that differences were very small. The cues of experienced quality were taste, tenderness and juiciness in which Carnalentejana-PDO beef presented 8%, 13% and 7% higher mean value than national undifferentiated beef, and 10%, 8% and 11% higher mean value than Brazilian beef. In the rest of the experienced

quality cues used (nutrition, healthiness and safety) there were no differences between beef types. In the present study, mean tenderness values measured either instrumentally (WBSF), or by the trained sensory panell were similar among the three types of beef. In addition Carnalentejana-PDO beef presented great variability in instrumental hardness, with great percentage of samples with values greater than 7 (hard beef) and lower than 4.5 (tender beef). We can only extrapolate that in the supermarket study the small differences in each of the sensory attributes obtained between the beef types all together were enough to make consumers to choose Carnalentejana-PDO.

Considering the instrumental measurement of colour, the colour parameters were similar between the beef types studied. However, as mentioned before, consumers considered Carnalentejana-PDO beef darker than the other two beef types, with national undifferentiated beef presenting the intermediate value. It is important to state that "couvettes" where beef are packaged had different colours which could compromise the evaluation. Juiciness and off-flavour were the only attributes, evaluated by the sensory panel, different between the beef types, presenting Brazilian beef the lowest and highest values, respectively. The differences in these two attributes were sufficient to influence the overall acceptability of beef, in which Brazilian beef scored lower.

Concerning the second question we proposed to answer, *i.e.*, "Does the consumer choose the healthiest beef?" we realised that they do not chose the healthier beef. From our study, Brazilian beef presented the healthier lipid profile over the other two beef. Brazilian beef presented a trend for a higher fatty acid content, and lower free fat content but higher intramuscular fat content. This suggests higher phospholipids content. This beef also presented higher vitamins content (α-tocopherol and β-carotene), L-carnitine, total CLA and *n*-3 LC-PUFA as well as lower *n*-6 LC-PUFA than the other two beef types, and lower TFA than Carnalentejana-PDO beef. The higher SFA content might seem at first glance as a detrimental aspect of this beef type, however, the higher SFA content is mainly due to the higher 18:0 FA content. This FA is considered as beneficial as it has been proved that it lowers low density lipoproteins cholesterol (Mensink, 2005).

The high α-tocopherol value presented by Brazilian beef was accomplished by a low value of TBARS, which could indicate that this beef had higher colour and FA stability. We realised that consumer preferences did not matched with the beef with better lipid nutritional value. Seeing that nutritional value is difficult to attain we used the acceptable macronutrient distribution ranges recommended by FAO/WHO (2009), and the information available concerning the effect of each major fatty acid on health, to evaluate lipid profile in beef.

In summary, all beef types presented low cholesterol contents and α-tocopherol values high enough to inhibit lipid oxidation and discoloration. Beef is a poor source of γ-tocopherol and beta-carotene, but a good source of L-carnitine. Moreover, beef has low intramuscular fat content providing thus low calories intake.

We concluded from our study that beef included in a balanced diet can contribute with high value protein, LC-PUFA and cholesterol in a healthy way.

A third question was proposed: "Is it possible to predict early on the beef chain the sensory quality based on its textural and chemical properties?".

Attempts to relate sensory attributes with instrumental measurements with the aim to predict consumers' choices concerning beef are not new. The options to measure beef quality include consumer or trained panels and objective measurements. Whilst objective measurements, such as shear force and compression tests, have the advantage of being relatively cheap, they are rather simplistic, one dimensional measures of a complex set of interactions which occur when cooked meat is chewed and masticated in the mouth (Watson *et al.*, 2008). Based on good results in other products, we thought that TPA could be an advantage over shear force, as several attributes can be measured at the same time, with a double compression cycle. Moreover, studies of TPA in beef are scarce.

The relationship between objective and sensory measurements was studied by several authors, and a great part of them concluded that shear force is an useful indicator of sensory tenderness (Silva *et al.*, 1999; Perry *et al.*, 2001; Monteiro *et al.*, 2009). However, other sensory attributes cannot be measured and the eating process involves more than tenderness. On the other hand, the expectation we had concerning TPA was not well succeeded, since TPA parameters were not well correlated with sensory attributes.

Several research studies tried to solve one of the major problems of the beef industry that affects all the market players, *i.e.*, beef consistency. We realised from our study that much has to be done. In general, national undifferentiated beef was the most variable from the three beef types studied. It is difficult without segmentation of this beef type by classes to have homogeneity, seeing that national undifferentiated beef includes beef from the most diverse origins, from different crosses, genders and ages. Carnalentejana-PDO beef presented the greatest variability in tenderness, as already referred, probably due to the greater ageing period variability. This can be solved using a range ageing period, starting with no less than 5 days of ageing.

Variability seems to be a problem in the Portuguese beef, as Carnalentejana-PDO beef presented the greatest variability in tenderness, national undifferentiated beef had the greatest variability in colour and in the first trial, "Vitela Tradicional do Montado"-PGI veal was the

most heterogeneous in the generality of chemical, physical and sensory attributes. Nevertheless, the higher variability presented by Carnalentejana-PDO beef in tenderness assumes more meanful proportions, as by one hand tenderness is the sensory characteristic most valuable by consumers, and by the other hand, this beef type is a quality branded beef which is most valued by consumers than common beef and have a higher market price.

From the present study and concerning the lack of information consumers have, it seems that nutritional labeling is a need to consumers. As can be seem in the literature beef has not a good equilibrium in fatty acid composition. Nevertheless, lean beef which is the common beef in the Portuguese market does not have the health problems related to fatty acid composition frequently attributed to beef. In addition, to promote efficiently beef, mainly quality branded beef it should be reinforce the concept that beef is a rich and healthy food. For such health beneficial nutrients in which beef is rich, like complex vitamin B and minerals should be study. It could also be helpful to differentiate beef types.

Work with the associations of beef breed (certified) producers must be done in order to improve tenderness, and significantly reduce variability. It seems that there is no connection between what consumers look for and the production sector. Thus, more information should be transmitted to producers in order to direct production to market needs.

REFERENCES

Abril, M., Campo, M.M., Önenç, A., Sañudo, C., Albertí, P., & Negueruela, A. I. (2001). Beef colour evolution as a function of ultimate pH. *Meat Science* 58, 69-78.

Adam, O., Wolfram, G. & Zollner, N. (2003). Influence of dietary linoleic acid intake with different fat intakes on arachidonic acid concentrations in plasma and platelet lipids and eicosanoid biosynthesis in female volunteers. *Annals of Nutrition and Metabolism,* 47, 31–36.

Agilent Technologies (2001). *Agilent chemstation for LC 3D systems - Understanding your spectra module user's guide* (p. 84). Palo Alto, CA, USA: Agilent Technologies Inc.

Alasnier, C., Rémignon, H. & Gandemer, G. (1996). Lipid characteristics associated with oxidative and glycolytic fibres in rabbit muscles. *Meat Science*, 43, 213-224.

Alfaia, C.M.M., Ribeiro, V.S.S., Lourenço, M.R.A., Quaresma, M.A.G., Martins, S.I.V., Portugal, A.P.V., Fontes, C.M.G.A., Bessa, R.J.B., Castro, M.L.F. & Prates, J.A.M. (2006a). FA composition, conjugated linoleic acid isomers and cholesterol in beef from crossbred bullocks intensively produced and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications. *Meat Science,* 72, 425-436.

Alfaia, C.M.M., Quaresma, M.A.G., Castro, M.L.F., Martins, S.I.V., Portugal, A.P.V., Fontes, C.M.G.A., Bessa, R.J.B. & Prates, J.A.M. (2006b). Fatty acid composition including isomeric profile of conjugated linoleic acid and, cholesterol in Mertolenga-PDO beef. *Journal of the Science of Food and Agriculture,* 86, 2196-2205.

Alfaia, C.M.M., Alves, S.P., Martins, S.I.V., Costa, A.S.H., Fontes, C.M.G.A., Lemos, J.P.C., Bessa, R.J.B., & Prates, J.A.M. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry,* 114, 939-946.

Alfaia, C.M.M., Alves, S.A., Lopes, A.F., Fernandes, M.J.E., Costa, A.S.H., Fontes, C. M.G.A., Castro, M.L.F., Bessa, R.J.B. & Prates, J.A.M. (2010). Effect of cooking method on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science,* 84*,* 769-777.

Allain, J.C., Le Lous, M., Bazin, S., Bailey, A.J. & Delaunay, A. (1978). Isometric tension developed during heating of collagenous tissues. *Biochimica et Biophysica Acta*, 533, 147-155.

Andersen, H.J., Oksbjerg, N. & Therkilden, M. (2005). Potential quality control tools in the production of fresh pork, beef and lamb demanded by the European society. *Livestock Production Science*, 94, 105-124.

Angulo, A.M. & Gil, J.M. (2007). Risk perception and consumer willingness to pay for certified beef in Spain. *Food Quality and Preference,* 18, 1106-1117.

AOAC 945.16 (2000). Oil in cereals adjuncts. Petroleum ether extraction method 945.16. In *Official methods of analysis* (17th ed., chapter 27 pp. 31). Gaithersburg, MA, USA: Association of Official Analytical Chemists International.

AOAC 990.26 (2000). Hydroxyproline in meat and meat products. Colorimetric method 990.26. In *Official methods of analysis* (17th ed., chapter 39 pp. 13). Gaithersburg, MA, USA: Association of Official Analytical Chemists International.

Arnold, R.N., Arp, S.C., Scheller, K.K., Williams, S.N. & Schaefer, D.M. (1993). Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Science*, 71, 105-118.

Bailey, A.J. (1985). The role of collagen in the development of muscle and its relationship to eating quality. *Journal of Animal Science*, 60, 1580–1587.

Bailey, A.J. (1988). Connective tissue and meat quality. In: Proceedings of the 34th International Congress of Meat Science and Technology. Copenhagen, Denmark, 20-25 August 1988, pp. 152-160.

Bailey, A.J. & Light, N.D. (1989). Connective tissue in meat and meat products. London e New York: Elsevier Applied Science.

Bandman, E. (1994). *Quimica de los tejidos animales*. In Price, J. F. & Schweigert, B. S. Ciencia de la carne e de los productos carnicos. Zaragoza, Editoria Acribia S. A.

Banovic, M., Grunert, K.G., Barreira, M.M., & Fontes, M.A. (2010). Consumers' quality perception of national branded, national store branded, and imported store branded beef. *Meat Science*, 84, 54-65.

Becker, T. (2000). Consumer perception of fresh meat quality: A framework for analysis. *British Food Journal*, 102, 158–176.

Belitz, H.D., Grosch, W. & Schieberle, P. (2009). Food Chemistry. (4th Edition). Munich, Germany: Springer.

Belury, M. (2002). Inhibition of Carcinogenesis by Conjugated Linoleic Acid: Potential Mechanisms of Action. *Journal of Nutrition*, 132, 2995-2998.

Bendall, J.R. (1973). *The biochemistry of rigor mortis and coldcontracture*. In Proceedings of the 19th European Meeting of Meat Research Workers (pp.1–27), Paris, France.

Berge, P., Ertbjerg, P., Larsen, L.M., Astruc, T., Vignon, X., & Moller, A.J. (2001). Tenderization of beef by lactic acid injected at different times post mortem. *Meat Science*, 57, 347–357.

Bernués, A., Olaizola, A. & Corcoran, K. (2003). Extrinsic attributes of red meat as indicators of quality in Europe: an application for market segmentation. *Food Quality and Preference,* 14, 265-276.

Bertram, H.C., Purslow, P., & Andersen, H.J. (2002). Relationships between meat structure, water motility, and distribution: A low-field nuclear magnetic resonance study. *Journal of Agricultural and Food Chemistry*, 50, 824–829.

Bessa, R. J. B., Santos-Silva, J., Ribeiro, J. M. R. & Portugal, A. V. (2000). Retículo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. *Livestock Production Science*, 63, 201-210.

Bessa, R.B., Alves, S.P., Figueiredo, R., Teixeira, A., Rodrigues, A., Janeiro, A., Costa, M.C., Santos-Silva, J. & Prates, J.A.M. (2006). Discrimination of production system and origin of animal products using chemical markers. In: J.M.C. Ramalho Ribeiro, A. E.M. Horta, C. Mosconi, & A. Rosati (Eds.), *Animal products from the Mediterranean area*, EAAP Publication No. 119 (pp. 231-240). The Netherlands: Wageningen Academic Publishers.

Biesalski, H.K. (2005). Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? *Meat Science*, 70, 509-524.

Bjørneboe, A., Bjørneboe, G.E.A. & Drevon, C.A. (1990). Absorption, transport and distribution of vitamin E. *Journal of Nutrition*, 120, 233–242.

Blundell, J.E., & MacDiarmid, J.I. (1997). Fat as a risk factor for overconsumption: satiation, satiety, and patterns of eating. *Journal of the American Diet Association,* 97, S63–S69.

Botsoglou, N.A., Fletouris, D.J., Papageorgiou, G.E., Vassilopoulos, V.N., Mantis, A.J., & Trakatellis, A.G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry,* 42, 1931-1937.

Bottinelli, R. & Reggiani, C. (2000). Human skeletal muscle fibres: molecular and functional diversity. *Progress in Biophysics & Molecular Biology*, 73, 195-262.

Bouton, P.E. & Harris, P.V. (1981). Changes in the tenderness of meat cooked at 50–65 ºC. *Journal of Food Science*, 46, 475–478.

Brake, N.C. & Fennema, O.R. (1999). Glass transition values of muscle tissue. *Journal of Food Science*, 64, 10–15.

Bremer, J. (1983). Carnitine - Metabolism and functions. *Physiological Reviews*, 63, 1420-1480.

Brewer, M. S. &, Harbers, C. A. Z. (1991). Effect of packaging on color and physical characteristics of ground pork in long-term frozen storage. *Journal of Food Science, 52*, 363-366.

British Department of Health (1994). *Nutritional Aspects of cardiovascular disease. Report on Health and Social Subjects No 46*. London: HMSO.

Broschat K.O. (1990). Tropomyosin prevents depolymerization of actin filaments from the pointed end. *Journal of Biological Chemistry*, 265, 21323–21329.

Burlingame, B., Nishida, C., Uauy, Uauy, R. & Weisell, R. (2009). Fats and fatty acids in human nutrition: Introduction. *Annals of Nutrition & Metabolism,* 55, 1-3.

Burson, D.E. & Hunt, M.C. (1986). Heat-induced changes in the proportion of types I and III collagen in bovine Longissimus dorsi. *Meat Science,* 17, 153-160.

Caine, W.R., Aalhus, J.L., Best, D.R., Dugan, M.E.R., & Jeremiah, L.E. (2003) Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Science*, 64, 333-339.

Calder, P.C. (2004). n- 3 Fatty acids and cardiovascular disease evidence explained and mechanisms explored. Clinical Science (London), 107, 1–11.

Calkins, C.R. & Hodgen, J.M. (2007). A fresh look at meat flavor. *Meat Science,* 77, 63-80.

Carlsson, L. & Thornell L.E. (2001). Desmin-related myopathies in mice and man. *Acta Physiol. Scand.* 171, 341–48.

Chambaz, A., Scheeder, M.R.L., Kreuzer, M., & Dufey, P.A. (2003). Meat quality of Angus, Simmental, Charolais and Limousin steers compared at the same intramuscular fat content. *Meat Science*, 63, 491-500.

Cheng, Q. & Sun, D.W. (2008). Factors affecting the water holding capacity of red meat products: A review of recent research advances. *Critical Reviews In Food Science and Nutrition*, 48, 137-159.

Chizzolini, R., Zanardi, E., Dorigoni, V. & Ghidini, S. (1999). Calorific value and cholesterol content of normal and low fat meat and meat products. *Food Science & Technology*, 10, 119-128.

Choi, Y.M., Ryu, Y.C., Kim, B.C., 2007. Influence of myosin heavy- and light chain isoforms on early postmortem glycolytic rate and pork quality. *Meat Science* 76, 281–288.

Choi, Y. M. & Kim, B. C. (2009). Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livestock Science*, 122, 105-118.

Christie, W.W., Sébédio, J.L., & Juanéda, P. (2001). A practical guide to the analysis of conjugated linoleic acid (CLA). *Inform,* 12, 147-152.

Claeys, E., De Smet, S., Demeyer, D., Geers, R. & Buys., N. (2001). Effect of rate of pH decline on muscle enzyme activities in two pig lines. *Meat Science*, 57, 257–263.

Clark, K.A., McElhinny, A.S., Beckerle, M.C. & Gregorio, C.C. (2002). Striated muscle cytoarchitecture: An intrincate web of form and function. *Annual Review of Cell Development and Biology*, 18, 637-706.

Collomb, M., Sieber, R., & Bütikofer, U. (2004). CLA isomers in milk fat from 12 cows fed diets with high levels of unsaturated fatty acids. *Lipids,* 39, 355-364.

Costa, P., Roseiro, L.C., Partidário, A., Alves, V., Bessa, R.J.B., Calkins, C.R., & Santos, C. (2006). Influence of slaughter season and sex on fatty acid composition, cholesterol and α-tocopherol contents on different muscles of Barrosã-PDO veal. *Meat Science,* 72, 130-139.

Costa, P., Roseiro, L.C., Bessa, R.J.B., Padilha, M., Partidário, A., Almeida, J.M., Calkins, C.R., & Santos, C. (2008). Muscle fiber and fatty acid profiles of Mertolenga-PDO meat. *Meat Science,* 78, 502-512.

Council Regulation (EEC) nº 2081/92 of 14 July 1992 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Official Journal* L 208, 24/07/1992 p. 0001-0008.

Culler, R.D., Parrish, F.C., Jr., Smith, G.C., & Cross, H.R. (1978). Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. *Journal of Food Science*, 43, 1177–1180.

Daniel, Z., Wynn, R., Salter, A. & Buttery, P. (2004). Differing effects of forage and concentrate diet on the oleic acid content of sheep tissues: the role of stearoyl-CoA desaturase. *Journal of Animal Science,* 82, 747-758.

Dannenberger, D., Nuernberg, G., Scollan, N., Schabbel, W., Steinhart, H., Ender, K., Nuernberg, K. (2004). Effect of diet on the deposition of n-3 fatty acids, conjugated linoleic- and C18:1*trans* fatty acid isomers in muscle lipids of German Holstein bulls. *Journal of Agriculture and Food Chemistry*, 52, 6607–6615.

Dannenberger, D., Nuernberg, K., Nuernberg, G., Scollan, N., Steinhart, H. & Ender, K. (2005). Effect of pasture vs. concentrate diet on CLA isomer distribution in different tissue lipids of beef cattle. *Lipids,* 40, 589-598.

Danowski, B.A., Imanaka-Yoshida, K., Sanger, J.M., Sanger, J.W. (1992). Costameres are sites of force transmission to the substratum in adult rat cardiomyocytes. *J. Cell Biol.* 118:1411– 20.

Davey, C.L., & Gilbert, K.V. (1974). Temperature-dependant toughness in beef. *Journal of Science Food and Agriculture*, 25, 931.

Davis, G.W., Smith, G.C., Carpenter, Z.L., Dutson, T.R., & Cross, H.R. (1979). Tenderness variations among beef steaks from carcasses of the same USDA quality grade. *Journal of Animal Science*, 49, 103–114.

De Caterina, R., Liao, J. K., & Libby, P. (2000). Fatty acid modulation of endothelial activation. *American Journal of Clinical Nutrition, 71*, 213S–223S.

Decker, E.A., Livisay, S.A., & Zhou, S. (2000). Mechanisms of endogenous skeletal muscle antioxidants: chemical and physical aspects. In E. A. Decker, C. Faustman, & C. Lopez-Bote (Eds.), *Antioxidants in muscle foods* (pp. 25–60). New York: Wiley Interscience.

De La Torre, A., Gruffat, D., Durand, D., Micol, D., Peyron, A., Scislowski, V. & Bauchart, D. (2006). Factors influencing proportion and composition of CLA in beef. *Meat Science,* 73, 258-268.

Delgado, E.F., Geesink, G.H., Marchello, J.A., Goll, D.E., & Koohmaraie, M. (2001). The calpain system in three muscles of normal and callipyge sheep. *Journal of Animal Science*, 79, 398–412.

Demarquoy, J., Georges, B., Rigault, C., Royer, M.C., Clairet, A., Soty, M., Lekounoungou, F. & Le Borgne, F. (2004). Radioisotopic determination of L- Carnitine content in foods commonly eanten in western countries. *Food Chemistry*, 86, 137-142.

Descalzo, A.M., Insani, E.M., Biolatto, A., Sancho, A.M., Garcia, P.T., Pensel, N.A. & Josifovich, J.A. (2005). Influence of pasture or gain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science*, 70, 35-44.

De Smet, S., Claeys, E., Buysse, G., Lenaerts, C., & Demeyer, D. (1998). Tenderness measurements in four muscles of Belgian Blue normal and double-muscled bulls. In Proceedings of the 44th International Congress of Meat Science and Technology (pp. 288–298), 30 August–4 September 1998, Barcelona, Spain.

Destefanis, G., Barge, M. T., Brugiapaglia, A., & Tassone, S. (2000). The use of principal component analysis (PCA) to characterize beef. *Meat Science, 56,* 255-259.

Destefanis, G., Barge, M. T., Brugiapaglia, A., & Dal Molin, E. (2008). Relationship between beef consumer tenderness perception and Warner-Bratzler shear force. *Meat Science*, 78, 153-156.

Dias L.G., Correia, D.M., Sá-Morais, J., Sousa, F., Pires, J.M., & Peres, A.M. (2008). Raw bovine meat fatty acids profile as an origin discriminator. *Food Chemistry,* 109, 840-847.

Díaz, M.T., Alvarez, M.T., De la Fuente, J., Sañudo, C., Campo, M.M., Oliver, M.A., Font i Fournols, M., Montossi, F.,San Julián, R., Nute, G.R. & Cañeque, V. (2005). Fatty acid compositon of meat from typical production systems of Spain, United Kingdom, Germany and Uruguay. *Meat Science*, 71, 256-263.

Djenane, D., Martínez, L., Escalante, A.S., Beltrán, J.A. & Roncalés, P. (2004). Antioxidant effect of carnosine and carnitine in fresh beef steaks stored under modified atmosphere. *Food Chemistry*, 85, 453-459.

Dolezal, H.G., Smith, G.C., Savell, J.W. & Carpenter, Z.L. (1982). Comparison of subcutaneous fat thickness, marbling and quality grade for predicting palatability of beef. *Journal of Food Science*, 47, 397-405.

Doreau, M. & Ferlay, A. (1994). Digestion and utilisation of fatty acids by ruminants. *Animal Feed Science and Technology*, 45, 379-396.

Dransfield, E. (1994). Optimisation of tenderisation, ageing and tenderness. *Meat Science*, 36, 105-121.

Duizer, L.M., Gullett, E.A. & Findlay, C.J. (1996). The relationship between sensory time-intensity, physiological electromyography and instrumental texture profile analysis measurements of beef tenderness. *Meat Science*, 42, 215-224.

Eichhorn, J.M., Bailey, C.M., & Blomquist, G.J. (1985). Fatty acid composition of muscle and adipose tissue from crossbred bulls and steers. *Journal of Animal Science,* 61, 892-904.

Elmadfa, I. & Kornsteiner, M. (2009). Fat and fatty acids requirements for adults. *Annals of Nutrition & Metabolism,* 55, 56-75.

Elmore, J.S., Mottram, D.S., Enser, M., & Wood, J.D. (1999). Effect of polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of Agricultural and Food Chemistry*, 47, 1619–1625.

Elmore, J.S., Warren, H.E., Mottram, D.S., Scollan, N. D., Enser, M., Richardson, R.I., & Wood, J. D. (2004). A comparison of the aroma volatiles and fatty acid compositions of grilled beef muscle from Aberdeen Angus and Holstein-Friesian steers fed diets based on silage or concentrates. *Meat Science, 68*, 27-33

Enser, E. (2001). The role of fats in human nutrition. In B Rossel (Eds.), *Animal carcass fats*. *Oils and fats* (Vol.2, pp. 77-122). Leatherhead, England: Leatherhead Publishing.

Essén-Gustavsson, B., Karlsson, A., Lundstrom, K., & Enfält, A.C. (1994). Intramuscular fat and muscle fibre lipid contents in halothane-gene-free pigs fed high or low protein diets and its relation to meat quality. *Meat Science*, 38, 269–277.

European Food Safety Authority (2010). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. *EFSA Journal, 8*, 1461.

Eurostat (2009). Gross human apparent consumption of main food items per capita. Database food: From farm to fork statistics. <http://epp.eurostat.ec.europa.eu/portal/page/portal/food/data/database>.

Eyre D.R., Paz, M.A., Gallop, P.M. (1984). Cross-linking in collagen and elastin. *Annual Review of Biochemistry*, 53, 717-748.

Eyre, D.R. & Wu, J.J. (2005). Collagen cross-links. *Top Current Chemistry*, 247, 207-229.

Faustman, C., Cassens, R.G., Schaefer, D.M., Buege, D.R., Williams, S.N., & Scheller, K.K. (1989). Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation on vitamin E. *Journal of Food Science,* 54, 858–862.

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

Fenemma, O. R. (2007). Food Chemistry. In S. Damodam, Parkin, Q., Fenema, O. R. (Eds., 4th edition). New York, USA: CRS Press

Fernandez, M. L. & West, K. L. (2005). Mechanisms by which dietary fatty acids modulate plasma lipids. *Journal of Nutrition*, 135, 2075-2078.

Ferreira, F. A. G. & Graça, M. E. S. (1985). Tabela da composição dos alimentos portugueses. Instituto Nacional da Saúde Dr. Ricardo Jorge. Lisboa, Portugal.

Field, R.W. & Faber, J.J. (1970). Biophysical Analysis of the Mechanical Properties of the Sarcolemma. *Canadian Journal of Physiology and Pharmacology*, 48, 394–404.

Fiems, L.O., De Campeneere, S., De Smet, Van de Voorde, G., Vanacker, J.M. & Boucqué, Ch.V. (2000). Relationship between fat depots in carcasses of beef bulls and effect on meat colour and tenderness. *Meat Science*, 56, 41-47.

Fisher, A.V., Enser, M., Richardson, R.I., Wood, J.D., Nute, G.R., Kurt, E., Sinclair, L.A. & Wilkinson, R.G. (2000). Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. *Meat Science,* 55, 141-147.

Fontes, M.A., Lemos, J.P.C., Banovic, M., Monteiro, A.C.G., Lúcio, C., Duarte, F., Fraústo da Silva, M., & Barreira, M.M. (2006). Is beef differentiation a real source of competitiveness? A combination of procedures to achieve an answer. *In proceedings of the "Competitiveness in Agriculture and the Food Industry: US and EU Perspectives", USDA and AIEA2 International meeting*. 15-16 June, Bologna, Italy.

Fraterman, S., Zeiger, U., Khurana, T. S., Wilm, M., & Rubinstein, N. A. (2007). Quantitative proteomics profiling of sarcomere associated proteins in limb and extraocular muscle allotypes. *Molecular & Cellular Proteomics*, 6, 728–737.

French, P., Stanton, C., Lawless, F. O'Riordan, E.G. Monahan, F.J., Caffrey, P.J. & Moloney, A.P. (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science,* 78, 2849-2855.

Fritsche, S., Rumsey, T.S., Yurawecz, P., Ku, Y., & Fritsche, J. (2001). Influence of growth promoting implants on fatty acid composition including conjugated linoleic acid isomers in beef fat. *European Food Research and Technology,* 212, 621-629.

Fukuzawa, K., Inokami, Y., Tokumura, A., Terao, J., & Suzuki, A. (1998). Rate constants for quenching singlet oxygen and activities for inhibiting lipid peroxidation and carotenoids and a-tocopherol in liposomes. *Lipids*, 33, 751–756.

Fung, Y. C. (1981). In Biomechanics. Mechanical properties of living tissues (pp. 433). New York, Heidelberg, Berlin: Springer-Verlag.

Gabinete de Planeamento e Políticas (2007a). Inquérito aos agrupamentos gestores de produtos com nomes protegidos DOP/IGP/ETG, 2006 e 2007 (pp. 11-14). Lisboa: Ministério da Agricultura, Desenvolvimento Rural e Pescas.

Gabinete de Planeamento e Políticas (2007b). Anuário Pecuário 2006/2007. Lisboa, Portugal.

Gabinete de Planeamento e Políticas (2009). Anuário Pecuário 2008/2009. Lisboa, Portugal.

Galland, S., Le Borgne, F., Georges, B., & Demarquoy, J. (2001). Carnitine, cellular and molecular aspects. *Recent Research and Development in Lipids*, 5, 1–14.

Gatellier, P., Mercier, Y., Juin, H. & Renerre, M. (2005). Effect of finishing mode (pasture or mixed diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. *Meat Science*, 69, 175-186.

Gault, N.F.S. (1985). The relationship between water-holding capacity and cooked meat tenderness in some beef muscles as influenced by acidic conditions below the ultimate pH. *Meat Science*, 15, 15–30.

Geay, Y., Bauchart, D., Hocquette, J.F. & Culioli, J. (2002). Valeur diététique et qualités sensorielles des viandes de ruminants. Incidence de l'alimentation des animaux. *INRA Production Animales*, 15, 37-52.

Geesink, G.H., Mareko, M.H.D., Morton, J.D. & Bickerstaffe, R. (2001). Effects of stress and high voltage electrical stimulation on tenderness of lamb *m. longissimus*. *Meat Science* 57, 265–271.

George, M.H., Tatum, J.D., Bekk, K.E. & Smith, G.C. (1999). An audit of retail beef loin steak tenderness conducted in eight U.S. cities. *Journal of Animal Science*, 77, 1735-1741.

Gil, M., Serra, X., Gispert, M., Oliver, M.A., Sañudo, C., Panea, B., Olleta, J.L., Campo, M., Oliván, M., Osoro, K., Garcís-Cachán, M.D., Cruz-Sagredo, R. & Piedrafita, J. (2001). The effect of breed-production systems on the myosin heavy chain 1, the biochemical characteristics and the colour variables of *Longissimus thoracis* from seven Spanish beef cattle breeds. *Meat Science, 58*, 181-188.

Gill, J.L., Matika, O., Williams, J.L., Worton, H., Wiener, P., & Bishop, S.C. (2010). Consistency statistics and genetic parameters for taste panel assessed meat quality traits and their relationship with carcass quality traits in a commercial population of Angus-sired beef cattle. *Animal* 4, 1-8.

Givens, D.I. & Gibbs, R. A. (2006). Very long chain n-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal derived foods to increase intake. *Nutrition Bulletin, 31*, 104-110.

Glitsch, K. (2000). Consumer perceptions of fresh meat quality: Cross-national comparison. *British Food Journal*, 102, 177–194.

Goll D.E., Thompson, V.F., Taylor, R.G., Christiansen, J.A. (1992). Role of the calpain system in muscle growth. *Biochimie,* 74, 225–237.

Goll, D.E., Thompson, V.F., Taylor, R.G., Ouali, A., & Chou, R.G.R. (1999). The calpain system in muscle tissue. In K. K. W. Wang, & P. W. Yuen (Eds.), Calpain: pharmacology and toxicology of calcium-dependent protease (pp. 127–160). Philadelphia: Taylor & Francis.

Gondret, F., Mourot, J., & Bonneau, M. (1998). Comparison of intramuscular adipose tissue cellularity in muscles differing in their lipid content and fibre type composition during rabbit growth. *Livestock Production Science*, 54, 1–10.

Grau, A., Guardiola, F., Boatella, J., Barroeta, A., & Codony, R. (2000). Measurement of 2-thiobarbituric acid values in dark chicken meat through derivative spectrophotometry: influence of various parameters. *Journal of Agricultural Food Chemistry, 48,* 1155–1159.

Gray, J. I., & Gomma, E.A., & Buckley, D.J. (1996). Oxidative quality and shelf Life of meats. *Meat Science*, 43, S111±S123.

Griffin, B. A. (2008). How relevant is the ratio of dietary n- 6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. *Current Opinion in Lipidology*, 19, 57-62.

Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V. & Bauman, D.E. (2000). Conjugated linoleic acid is synthesized endogenously in lacting dairy cows by Δ^9 desaturase. *Journal of Nutrition*, 130, 2285-2291.

Grundy, S. M. (1987). Monounsaturated fatty acids, plasma, cholesterol and coronary heart disease. *The American Journal of Clinical Nutrition*, 45, 1168.

Grunert, K.G., Larsen, H.H., Madsen, T.K. & Baadsgard, A. (1996). Market orientation in food and agriculture. Boston: Kluwer Academic Publishers.

Guerreiro, L. (2001). Marketing PDO (products with geographical denominations of origin) and PGI (products with geographical identities). In L. Frewer, E. Risvik & H. Schifferstein (Eds.), *Food, people and society. A European perspective of consumers' food choices* (pp. 281-296). Heidelberg: Springer Verlag.

Hannula, T. & Puolanne, E. (2004). The effect of cooling rate on beef tenderness: The significance of pH at 7ºC. *Meat Science*, 67, 403-408.

Harper, G.S. (1999). Trends in skeletal muscle biology and the understanding of toughness in beef. *Australian Journal of Agricultural Research,* 50, 1105–1129.

Harper, G.S. & Pethick, D.W. (2004). How Might Marbling Begin? *Australian Journal of Experimental Agriculture,* 44, 653–662.

Harris, W. S. (2007). International recommendations for consumption of long-chain omega-3 fatty acids. *Journal of Cardiovascular Medicine, 8*, S50–S52.

Haugen, J.E., & Kvaal, K. (1998). Electronic nose and artificial neural network. *Meat Science*, 49, S273-S286.

Hedricks, H. B., Paterson, J. A., Matches, A. G., Thomas, J. D., Morrow, R. E., Stringer, W. C., & Lipsey, R. J. (1983). Carcass and palatability characteristics of beed produced on pasture, corn silage or corn grain. *Journal of Animal Science, 57*, 791-801.

Hermansson, A.M. & Langton, M. (1988). Filamentous structures of bovine myosin in diluted suspensions and gels. *Journal of Science Food and Agriculture*, 42, 355–369.

Herring, H.K., Cassens, R.G. & Briskey, E.J. (1965). Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. *Journal of Food Science*, 30, 1049–1054.

Hocquette, J.F., & Gigli, S. (2004) The challenge of quality. In J. F. Hocquette & S. Gigli (Eds.). *Indicators of milk and beef quality*. (pp.13-22). Netherlands: Wageningen Academia Publishers.

Hocquette, J. F., Gondret, F., Baéza, E., Médale, F., Jurie, C., & Pethick, D. W. (2010). Intramuscular fat content on meat-producing animals: development, genetic and nutritional control, and identification of putative markers. *Animal, 4,* 303-319.

Hofmann, K. (1994). What is meat quality? *Meat Focus International,*2, 73–82.

Hollander, D. (1981). Intestinal absorption of vitamins A, E, D, and K. *Journal of Laboratory and Clinical Medicine*, 97, 449–462.

Honikel, (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49, 447-457.

Horgan, D.J., Jones, P.N., King, N.L., Kurth, L.B. & Kuypers, R. (1991). The relationship between animal age and the thermal stability and cross-link content of collagen from five goat muscles. *Meat Science*, 29, 251–262.

Howe, P., Meyer, B., Record, S., & Baghurst, K., (2006) Dietary intakes of long chain ω-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition, 22*, 47–53.

Huff-Lonergan, E., & Lonergan, S. M. (1999). Postmortem mechanisms of meat tenderization: The roles of the structural proteins and the calpain system. In Y. L. Xiong, C. T. Ho, & F. Shahidi (Eds.), *Quality attributes of muscle foods* (pp. 229–251). New York: Kluwer Academic/Plenum Publishers.

Huff-Lonergan, E. & Lonergan, S. (2005). Mecanisms of water holding capacity of meat: The role postmortem biochemical and structural changes. *Meat Science*, 71, 194-204.

Huff-Lonergan, E., Zhang, W. & Lonergan, S.M. (2010). Biochemistry of post-mortem muscle – Lessons on mechanism of meat tenderization. *Meat Science*, 86, 184-195.

Huffman, K.L., Miller, M. F, Hoover, L. C.,. Wu,, C. K., Brittin, H. C. & Ramsey, C. B. (1996). Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. *Journal Animal Science*, 74, 91-97.

Huidobro, F. R., Miguel, E., Blázquez, B. & Onega, E. (2005). A comparison between two methods (Warner-Bratzler and texture profile analysis) for testing either raw meat or cooked meat. *Meat Science*, 69, 527-536.

Hunsley, R. E., Vetter, R. L., Kline, E. A., & Burroughs, W. (1971). Effects of age and sex on quality, tenderness and collagen content of bovine longissimus muscle. *Journal Animal Science, 33*, 933-938.

Hunt, (2009). Literature of the post-graduate Advanced Meat Science Course. 12-16th August 2009, Copenhagen, Denmark.

Hur, S. J., Park, G. B. & Joo, S. T. (2007). Formation of cholesterol oxidation products (COPs) in animal products. *Food Control*, 18, 939-947.

Hwang, I.H. & Thompson, J.M. (2001). The interaction between pH and temperature decline early postmortem on the calpain system and objective tenderness in electrically stimulated beef longissimus dorsi muscle. *Meat Science*, 78, 167-174.

Instituto do Desenvolvimento Rural e Hidráulica, (2006). *Produtos Tradicionais com Nomes Protegidos – Apuramento de 2004* (pp. 12-16). Lisboa: Ministério da Agricultura, Desenvolvimento Rural e Pescas.

Insani, E.M., Eyherabide, A., Grigioni, G., Sancho, A.M., Pensel, N.A. & Descalzo, A.M. (2008). Oxidative stability and its relationship with natural antioxidants during refrigerated retail display of beef produced in Argentina. *Meat Science*, 79, 444-452.

Jeremiah, L.E., Tong, A.K.W., Gibson, L.L. (1991). The usefulness of muscle colour and pH for segregating beef carcasses into tenderness groups. *Meat Science*, 30, 97-114.

Jeremiah, L.E., Dugan, M.E.R., Aalhus, J.L., & Gibson L.L. (1993). Assessment of the relationship between components and palatability of major beef muscles and muscles groups. *Meat Science. 63*, 1013-1019.

Jeremiah, L.E., Gibson, L.L., Aalhus, J.L. & Dugan, M.E.R. (2003). Assessment of palatability attribute of the major beef muscles. *Meat Science*, 65, 949-958.

Jin J.P., Wang K. 1991. Nebulin as a giant actin-binding template protein in skeletal muscle sarcomere. Interaction of actin and cloned human nebulin fragments. *FEBS Lett.*281:93–96

Jones, B.K. & Tatum, J.D. (1994). Predictors of beef tenderness among carcasses produced under commercial conditions. *Journal of Animal Science*, 72, 1492-1501.

Jowitt, R. (1979). In P. Sherman (Ed.), Food texture and rheology (pp. 143–155). London: Academic Press.

Junqueira, L.C. & Carneiro, J. (2004). Histologia Básica. 10ª Edição. Ed. Guanabara Koogan, S.A.

Kanner, J. (1992). Mechanism of nonenzymic lipid peroxidation in muscle foods. In A. J. St. Angelo (Ed.), Lipid oxidation in food (pp. 55–73). ACS Symposium Series 500, Washington, DC.

Katan, M.B., Zock, P.L. & Mensink, R.P. (1994). Effects of fats and fatty acids on blood lipids in humans: an overview. *American Journal of Clinical Nutrition, 60*, 1017S–1022S.

Kemp, C.M., Sensky, P.L., Bardsley, R.G., Buttery, P.J. & Parr, T. (2010). Tenderness- An enzymatic view. *Meat Science*, 86, 248-256.

Kerry, J.P., Buckley, D.J. & Morrissey, P.A. (2000). Improvement of oxidative stability of beef and lamb with vitamin E. *Meat Science*, 56, 61-66.

Kim, N.K., Lim, J.H., Song, M.J., Kim, O.H., Park, B.Y., Kim, M.J. ,Hwang, I.H. & Lee, C.S. (2008). Comparisons of longissimus muscle metabolic enzymes and muscle fiber types in Korean and western pig breeds. *Meat Science*, 78, 455–460.

Kirschke, H., Wiederanders, B., Bromme, D., & Rinne, A. (1989). Cathepsin S from bovine spleen. Purification, distribution, intracellular localization and action on proteins. *Biochemical Journal*, 264, 467–473.

Klont, R. E., Barnier, V. M. H., van Dijk, A., Smulders, F. J. M., Hoving-Bolink, A. H., Hulsegge, B. & Eikelenboom, G. (2000). Effects of rate of pH fall, time of deboning, aging period, and their interaction on veal quality characteristics. *Journal of Animal Science*, 78, 1845-1851.

Knüttel-Gustavsen, S., & Harmeyer, J. (2007). The determination of L-carnitine in several food samples. *Food Chemistry, 105*, 793-804.

Koohmaraie, M., Whipple, G., Kretchmar, D.H., Crouse, J.D., Mersmann, H.J. (1991). Post-mortem proteolysis in Longissimus muscle from beef, lamb and pork carcasses. *Journal of Animal Science*, 69, 617-624.

Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderisation processes of meat. *Meat Science*, 43 (S), 193-201.

Koohmaraie, M., Kent, M.P., Shackelford, S.D., Veiseth, E. & Wheeler, T.L. (2002). Meat tenderness and muscle growth: is there any relationship? *Meat Science*, 62, 345-352.

Koohmaraie, M. & Geesink, G.H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on calpain system. *Meat Science*, 74, 34-43.

Kopp, J., & Bonnet, M. (1987). Stress–strain and isometric tension measurements in collagen. In A. M. Pearson, T. R. Dutson, & A. J. Bailey (Eds.). Advances in meat research collagen as a food (Vol. 4, pp. 163–185). New York, USA: An Avi Book -Vaan Nostrand Reinhold Company.

Kovanen, V. & Suominen, H. (1989). Age- and training-related changes in the collagen metabolism of rat skeletal muscle. *European Journal of Applied Physiology and occupational physiology*, 58,765–771.

Koide, T. & Nagata, K. (2005). Collagen Biosynthesis. *Top Current Chemistry*, 247, 85–114.

Kristensen, L., & Purslow, P.P. (2001). The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. *Meat Science*, 58, 17–23.

Kropf, D. H. (1980). Effects of retail display conditions on meat color. In Proceedings of the 33rd annual reciprocal meat conference (pp. 15–32), 22-25 June 1980, Indiana, USA.

Kumar, N. & Singhal, O.P. (1991). Cholesterol oxides and atherosclerosis. A review. *Journal of the Science of Food and Agriculture*, 55, 497-510.

Kuypers, R., Tyler, M., Kurth, L.B. & Horgan, D. J. (1994). The molecular location of Elrich Chromogen and pyridinoline cross-links in bovine perimysial collagen. *Meat Science*, 37, 67-89.

Larick, D.K. & Turner, B.E. (1989). Influence of finishing diet on the phospholipid composition and fatty acid profile of individual phospholipids in lean muscle of beef cattle. *Journal of Animal Science*, 67, 2282- 2293.

Lawless, H. T., & Heymann, H. (2010). Texture Evaluation. In: D. R. Heldman (Ed.) *Sensory Evaluation of Food: Principles and Practices*, Food science text series (2nd ed.) (pp. 259-282). New York, USA: Springer.

Lawrie, R. A. (1998). The eating quality of meat. In: R. A. Lawrie (Ed), *Meat Science* (6^{th} ed) (pp. 212-257). Oxford, England: Pergamont Press.

Leaf, A., Xiao, Y.F., Kang, J.X., & Billamn, G.E. (2003). Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. *Pharmacology and Therapeutics*, 98, 355–377.

Leat, W.M.F. (1982). Factors affecting the fatty acid composition of depot fats of cattle and other ruminants. In: H. De Boer & J. Martin. (Eds.). *Patterns of Growth and Development in Cattle*. The Hague, Netherland: Martinus Nijhoff.

Lee, S.H., Joo, S.T. & Ryu, Y.C. (2010). Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality. *Meat Science*, 86, 166-170.

Leedle, R. A., Leedle, J. A. Z., & Butine, M. D. (1993). Vitamin E is not degraded by ruminal microorganisms: Assessment with ruminal contents from a steer fed a high-concentrate diet. *Journal of Animal Science, 71*, 3442–3450.

Lefaucheur, L. (2010). A second look into fibre typing – Relation to meat quality. *Meat Science*, 84, 257-270.

Lepetit, J., Grajales, A. & Favier, R. (2000). Modelling the effect of sarcomere length on collagen thermal shortening in cooked meat: consequence on meat toughness. *Meat Science*, 54, 239-250.

Lepetit, J. (2007). A theoretical approach of the relationships between collagen content, collagen cross-links and meat tenderness. *Meat Science*, 76, 147-159.

Lepetit, J. (2008). Collagen contribution to meat toughness: Theoretical aspects. *Meat Science*, 80, 960-967.

Lewis, G.J., & Purslow, P.P. (1991). The effect of marination and cooking on the mechanical properties of intramuscular connective tissue. *Journal of Muscle Foods*, 2, 177–195.

Lewis, Jr., P.K., Rakes, L.K.Y., Brown, H.G., Brown Jr., A.H., Johnson, Z. & Brown, C.J. (1993). Effects of maturation rate, marbling and cooking on the fat, cholesterol and lipid phosphorus composition of muscle. *Journal of Muscle Foods*, 4, 41-56.

Lichtenstein, A.H., Kennedy, E., Barrier, P., Danford, D., Ernst, N.D., Grundy, S.M., Leveille, G.A., Van Horn, L., Williams, C.L., and Booth, S.L. (1998). Dietary fat consumption and health. *Nutrition Reviews* 56, S3–S19.

Lichtenstein, A.H. (2006). Thematic review series: patient-oriented research. Dietary fat, carbohydrate, and protein: effects on plasma lipoprotein patterns. *Journal of Lipid Research,* 47, 1661–1667.

Light, N. D., Champion, A. E., Voyle, C. & Bailey, A. J. (1985). The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. *Meat Science*, 13, 137–149.

Lillford, P. J. (2011). The importance of food microstructure in fracture physics and texture perception. *Journal of Texture Studies*, 42, 130-136.

Listrat, A., Levieux, A., & Lepetit, J. (2007). Muscle, Age and sex effects on total pyridinoline cross-links content and beef tenderness. In Proceedings 53th international congress of meat science and technology (pp. 247–248). Beijing, China.

Liu, A., Nishimura, T. & Takahashi, K. (1996). Relationship between structural properties of intramuscular connective tissue and toughness of various chicken skeletal muscles. *Meat Science*, 43, 43-49.

Lloyd, J. B., & Mason, R. W. (1996). Biology of the lysosome. In Subcellular biochemistry (vol. 27). New York: Plenum Press.

Lukoyanova N., VanLoock M.S., Orlova A., Galkin V.E., Wang K. & Egelman E.H. (2002). Each actin subunit has three nebulin binding sites. Implications for steric blocking. *Current Biology,* 12, 383–88

Luño, M., Roncalés, P., Djenane, D., & Beltran, J.A. (2000). Beef shelf life in low O2 and high CO2 atmospheres containing different low CO concentrations. *Meat Science*, 55, 413-419.

Maggioni, D., Marques, J. A., Rotta, P. P., Perotto, D., Ducatti, T., Vicentainer, J. V., & Prado, I. N. (2010). Animal performance and meat quality of crossbred young bulls. *Livestock Science,* 127, 176-182.

Maher, S.C., Mullen, A.M., Moloney, A.P., Buckley, D.J., & Kerry, J.P. (2004) Quantifying the extent of variation in the eating quality traits of the M. Longissimus dorsi and M. Seninenbranosus of conventionally processed Irish beef. *Meat Science*, 66, 351-360.

Malenfant, P., Joanisse, D.R., Theriault, R., Goodpaster, B.H., Kelley, D.E., & Simoneau, J.A. (2001). Fat content in individual muscle fibers of lean and obese subjects. *International Journal of Obesity*, 25, 1316–1321.

Mancini, R.A. & Hunt, M.C. (2005). Current research in meaty colour: A Review. *Meat Science*, 71,100-121.

Maughan, C., Tansawat, R., Cornforth, D., Ward, R., & Martini, S. (2011). Development of a beef flavor lexicon and its application to compare the flavor profile and consumer acceptance of rib steaks from grass or grain fed cattle. *Meat Science* MESC-05453.

May, S.G., Dolezal, H.G., Gill, D.R., Ray, F.K. & Buchanan, D.S. (1992). Effect of days fed, carcass grade traits, and subcutaneous fat removal on postmortem muscle characteristics and beef palatability. *Journal of Animal Science*, 70, 444-453.

McCormick, R.J. (1994). The flexibility of the collagen compartment of muscle. *Meat Science*, 36, 79-91.

McCormick, R.J. (1999). Extracellular modifications to muscle collagen: Implications for meat quality. *Poultry Science*, 78, 785-791.

McElhinny AS, Kolmerer B, Fowler VM, Labeit S, Gregorio CC. 2001. The N-terminal end of nebulin interacts with tropomodulin at the pointed ends of the thin filaments. *The Journal of biological chemistry,* 276,583–92.

Melody, J.L., Lonergan, S.M., Rowe, L.J., Huiatt, T.W., Mayes, M.S. & Huff-Lonergan, E. (2004). Early post-mortem biochemical factors influence tenderness and water holding capacity of three porcine muscles. *Journal of Animal Science*, 82, 1195-1205.

Melton, S.L., Amiri, M., Davis, G.W. & Backus, W.R. (1982). Flavor and chemical characteristics of ground beef from grass, forage-grain and grain finishes steers. *Journal of Animal Science,* 55, 77-87.

Melton, S.L. (1990). Effects of feeds on flavour of red meat: a review. *Journal of Animal Science*, 68, 4421-4435.

Mensink, R.P. (2005). Effects of stearic acid on plasma lipid and lipoproteins in humans. *Lipids, 40*, 1201-1205.

Meullenet, J.F.C., Carpenter, J.A., Lyon, B.G., & Lyon, C.E. (1997). Bi-cyclical instrument for assessing texture profile parameters and its relationship to sensory evaluation of texture. *Journal of Texture Studies, 28*, 101-118.

Mikami, M., Whiting, A.H., Taylor, M.A.J., Maciewicz, R.A. & Etherington, D.J. (1987). Degradation of myofibrils from rabbit, chicken and beef by cathepsin L and lysosomal lysates . *Meat Science*, 21, 81-97.

Miles, C.A., Avery, N.C., Rodin, V.V. & Bailey, A.J. (2005). The increase in denaturation temperature following cross-linking of collagen is caused by dehydration of the fibres. *Journal of Molecular Biology*, 346, 551–556.

Möller, A.J. & Iversen, P. (1993). Elements in the concept of pig meat quality. *Proceedings of the 44th Annual Meeting of the European Association of Animal Production*, 16-19 August 1993, Aarhus, Denmark.

Monin, G. (1991). Facteurs biologiques des qualités de la viande bovine. *INRA Production Animale*, 4, 151-160.

Monsón, F., Sañudo, C., & Sierra, I. (2005). Influence of breed and ageing time on the sensory meat quality and consumer acceptability in intensively reared beef. *Meat Science*, *71,* 471-479.

Monteiro, A.C.G. (2003). Qualidade da carne de bovinos produzidos em sistema de pastoreio: efeito da castração e da duração do período de acabamento. Dissertação para obtenção do grau de mestre em Ciência e Engenharia de Alimentos. Universidade Técnica de Lisboa, Portugal.

Monteiro, A. C. G., Navas, D. & Lemos, J. P. C. (2005). Effect of castration and age on beef characteristics of animals produced on pasture. *Revista Portuguesa de Engenharia Zootécnica*, Ano XII, Nº2.

Monteiro, A.C.G., Santos-Silva, J., Bessa, R.J.B., Navas, D. & Lemos, J.P.C. (2006). Fatty acid composition of intramuscular fat of bulls and steers. *Livestock Science, 99*, 13-19.

Monteiro, A.C.G., Fontes, M.A., Lemos, J.P.C. & Prates, J.A.M. (2008). Quantification of L-Carnitine, cholesterol, tocopherols and β-carotene in three types of meat commercialized in Portugal: Carnalentejana-PDO, Undifferentiated and Imported. XVI National Congress of Biochemistry. 22-25 October, S. Miguel, Azores (p. 213).

Monteiro, A. C. G., Barreto, A. S., Silva, M. F., Fontes, M. A., & Lemos, J. P. C. (2009). Characterization of three types of certified meat commercialized in Portugal. In Proceedings of the 9º Congresso de Química dos Alimentos. 29 April- 2 May 2009. Angra do Heroísmo, Terceira, Portugal.

Moreno, T., Varela, A., Oliete, B., Carballo, J.A., Sánchez, L. & Montserrat, L. (2006). Nutritional characteristics of veal from weaned and unweaned calves: discriminatory ability of the fat profile. *Meat Science, 73*, 209-217.

Moreno, T., Keane, M.G., Noci, F. & Moloney, A.P. (2008). Fatty acid composition of *M. Longissimus dorsi* from Holstein-Frisian steers of the New Zealand and European/American descendent and from Belgian Blue × Holstein-Frisian steers, slaughtered at two weights/ages. *Meat science, 78,* 157-169.

Morrissey, P.A., Buckley, D.J., & Galvin, K. (2000). Vitamin E and the oxidative stability of pork and poultry. In E. Decker, C. Faustman, & C. L. Lopez-Bote (Eds.), Antioxidants in muscle foods (pp. 263–287). New York: Wiley Interscience.

Morrison, E.H., Mielche, M.M., & Purslow, P.P. (1998). Immunolocalisation of intermediate filament proteins in porcine meat. Fibre type and muscle-specific variations during conditioning. *Meat Science*, 50, 91–104.

Mottram, D.S. (1987). Lipid oxidation and flavor in meat and meat products. *Food Science and Technology Today* 1, 159-162.

Mottram, D.S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, 62, 415-424.

Mutungi, G., Purslow, P. & Warkup, C. (1996). Influence of temperature, fibre diameter and conditioning on the mechanical properties of single muscle fibres extended to fracture. *Journal of the Science of Food and Agriculture*, 72, 359-366.

Myer, R.O., Johnson, D.D., Knauft, D.A., Gorbet, D.W., Brendemuhl, J.H. & Walker, W.R. (1992). Effect of feeding high-oleic-acid peanuts to growing-finishing swine on resulting carcass fatty acid profile and on carcass and meat quality characteristics. *Journal of Animal Science, 70*, 3734-3741.

Napolitano, F., Braghieri, A., Piasentier, E. Favotto, S., Naspetti, S. & Zanoli, R. (2007). Effect of information about organic production on beef liking and consumer willingness to pay. *Food Quality and Preference*, 21, 207-212.

Neti, G., Novak, S. M., Thompson, V. F., & Goll, D. E. (2009). Properties of easily releasable myofilaments: Are they the first step in myofibrillar protein turnover? American Journal of Physiology. Cell Physiology, 296, C1383–C1390.

Ngapo, T.M., Berge, P. Culioli, J., Dransfield, E., De Smet, S. & Claeys, E. (2002). Perimysial collagen crosslinking and meat tenderness in belgian blue double muscle cattle. *Meat Science*, 61, 91-102.

Nishimura, T., Liu, A., Hattori, A. & Takahashi, K. (1998). Changes in mechanical strength of intramuscular connective tissue during post-mortem aging of beef. *Journal of Animal Science*, 76, 528-532.

Norma Portuguesa 1224 (2002). Carnes, derivados e produtos cárneos. Determinação do teor de matéria gorda livre. CT35 (IQA).

Norma Portuguesa 1987 (2002). Carnes derivados e produtos cárneos. Determinação do teor de L-Hidroxiprolina. Método de Referência.

Norma Portuguesa 1612 (2006). Carnes, derivados e produtos cárneos. Determinação do teor de azoto total. Método de referência (2ª Edição).

Nuernberg, K., Dannenbrger, D., Nuernberg, G., Ender, K., Voigt, J., Scollan, N.D., Wood, J.D., Nute, G.R. & Richardson, R.I. (2005a). Effect of grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. *Livesstock Production Science,* 94:137- 147.

Nuernberg, K., Nuernberg, G., Ender, K., Dannenberger, D., Schabbel, W., Grumbach, S., Zupp, W. & Steinhart, H. (2005b). Effect of grass vs. concentrate feeding on the fatty acid profile of different fat depots in lambs. *European Journal of Lipid Science and Technology*, 107, 737–745.

Oddy, V.H., Harper, G.S., Greenwood, P.L. & McDonagh, M.B. (2001). Nutritional and developmental effects on the intrinsic properties as muscle as they relate to the eating quality of beef. *Australian Journal of Experimental Agriculture*, 41, 921-942.

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R., & Purslow, P. (1989a). The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstructure*, 8, 151.

Offer, G., Elsey, J., Parson, N., Cousin, A., & Knight, P. (1989b) In: *Basic Interactions of Water and Foods.* Le Maguer, M., Powrie, W. Eds Marcel Dekker, New York.

Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: effects of chilling regime and rate and extent of glycolysis. *Meat Science*, 30, 157-184.

O'Halloran, G.R., Troy, D.J. & Buckley, D.J. (1997). The relationship between early post-mortem pH and the tenderisation of beef muscles. *Meat Science*, 45, 239-251.

Olson, D.G., Parrish Jr, F.C., & Stromer, M.H. (1976). Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *Journal of Food Science*, 41, 1036-1041.

Olson, R.E. (1998). Discovery of the Lipoproteins, Their Role in Fat Transport and Their Significance as Risk Factors. *Journal of Nutrition*, 128, 439S-443S.

Orellana, C., Peña, F., García, A., Perea, J., Martos, J., Domenech, V., & Acero, R. (2009). Carcass characteristics, fatty acid composition, and meat quality of Criollo Argentino and Bradford steers raised on forage in a semi-tropical region of Argentina. *Meat Science, 81,* 57-64.

Ouali, A. (1999). Structure and biochemistry of muscle as related to meat texture. Proceedings of the 14th European Symposium on the Quality of Poultry Meat. Bologna, Italy, 19-23 September, pp. 91-121.

Owens, F.N., Gill, D.R., Seerist, D.S., Coleman, S.W. (1995). Review of some aspects of growth and development of feedlot cattle. *Journal of Animal Science,* 73, 3152–3172.

Page, J. K., Wulf, D. M., & Schwotzer, T. R. (2001). A survey of beef muscle color and pH. *Journal of Animal Science*, *79*, 678-687.

Palmquist, D.L. (1988). The feeding value of fats. In E. R. Orskov (Ed.), *Feed Science: World Animal Science (disciplinary approach B4)* (pp. 293-311). Amsterdam: Elsevier Publishers.

Palmquist, D.L., St-Pierre, N., & McClure, K.E. (2004). Tissue fatty acid profiles can be used to quantify endogenous rumenic acid synthesis in lambs. *Journal of Nutrition, 134*, 2407-2414.

Pariza, M. W., (2004). Perspective on the safety and effectiveness of conjugated linoleic acid. American Journal of Clinical Nutrition, 79, 1132S-1136S.

Parr, T., Sensky, P. L., Scothern, G. P., Bardsley, R. G., Buttery, P. J., Wood, J. D., Warkup, C. (1999). Relationship between skeletal muscle specific calpain, p94/calpain 3. Journal of Animal Science, 77, 661–668.

Pearson, A. & Young, R.B. (1989). Muscle and meat biochemistry. Food Science and Technology. A Series of Monographs. San Diego, USA: Academic Press, Inc.

Perry, D., Thompson, J.M., Hwang, I.H., Butchers, A. & Egan, A.F. (2001). Relationship between objective measurements and taste panel assessment of beef quality. *Australian Journal of Experimental Agriculture*, 41, 981-989.

Pike, M.M., Ringkob, T.P., Beekman, D.D., Koh, Y.O. & Gerthoffer, W.T. (1993). Quadratic relationship between early-post-mortem glycolytic rate and beef tenderness. *Meat Science*, 34, 13-26.

Polkinghorne, R., Thompson J.M., Watson, R., Gee, A. & Porter, M. (2008). Evolution of the Meat Standards Australia (MSA) beef grading system. *Australian Journal of Experimental Agriculture, 48*, 1351-1359.

Pollack, G.H. (2003). The role of aqueous interfaces in the cell. *Advances in Colloid and Interface Science*, 103, 173–196.

Ponte, P.I.P, Prates, J.A.M., Crespo, J.P., Crespo, D.G., Mourão, J. L., Alves, S.P., Bessa, R.J.B., Chaveiro- Soares, M.A., Ferreira, L. M. A., & Fontes, C.M.G.A. (2008). Improving the lipid nutritive value of poultry meat through the incorporation of a dehydrated leguminous-based forage in the diet for broiler chicks. *Poultry Science, 87*, 1587-1594.

Prates, J. A. M. (2000). Contribuição para o estudo do papel da actividade péptido hidrolásica (EC 3.4) na maturação da carne refrigerada: Estudo Experimental no coelho (*Oryctolagus cuniculus L.*). Dissertação de Doutoramento. Faculdade de Medicina Veterinária. Universidade Técnica de Lisboa. Lisboa.

Prates, J. A. M., Quaresma, M. A. G., Bessa, R. J. B., Fontes, C. M. G. A., & Alfaia, C. M. P. M. (2006). Simultaneous HPLC quantification of total cholesterol, tocopherols and b-carotene in Barrosã-PDO veal. *Food Chemistry, 94*, 469-477.

Prates, J. A. M., & Bessa, R. J. B. (2009). Trans and n-3 fatty acids. In L. M. L. Nollet & F. Toldrá (Eds.), *Handbook of Muscle Foods Analysis* (pp. 399–417). Boca Raton, EUA: CRC Press – Member of Francis & Taylor Group.

Priolo, A., Micol, D., Agabriel, J., Prache, S., & Dransfield, E. (2002). Effect of grass or concentrate feeding systems on lamb carcass and meat quality. *Meat Science*, 62, 179-185.

Puolanne, E. & Halonen, M. (2010). Theorical aspects of water holding in meat. Meat Science, 86, 151-165.

Purchas, R. W., (1990). An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. Meat Science, 27, 129-140.

Purchas, R.W., Busboom, J.R. & Wilkinson, B.H.P. (2006). Changes in the forms of iron and in concentrations of taurine, carnosine, coenzyme Q10, and creatine in beef longissimus muscle with cooking and stimulated stomach and duodenal digestion. *Meat Science*, 74, 443-449.

Purslow, P.P. (1989). Strain-induced reorientation of an intramuscular connective tissue network: Implications for passive muscle elasticity. *Journal of Biomechanics*, 22, 21–31.

Purslow, P.P. & Trotter, J.A. (1994). The morphology and machanical properties of endomysium in series-fibred muscles: variations with muscle length. *Journal of Muscle Research and Cell Motility*, 15, 229-308.

Purslow, P.P. (1999). The intramuscular connective tissue matrix and cell-matrix interactions in relation to meat toughness. In Proceedings of the 45th international congress of meat science and technology, Yokohama, Japan (pp. 210–219).

Purslow, P.P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, 70, 435-447.

Raes, K., De Smet, S., & Demeyer, D. (2001). Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. *Animal Science, 73*, 253-260.

Ratnayake, W.M.N. & Galli, C. (2009). Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: A background review paper. *Annals of Nutrition & Metabolism,* 55, 8-43.

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M., & De Mattos, D. (2004). Effect of pasture vs. Concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*, 567-577.

Regulation (EC) nº 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Official Journal* L 93, 31/03/2006 p. 0012-0031.

Renand, G., Picard, B., Touraille, C., Berge, P. & Lepetit, J. (2001). Relationship between muscle characteristics and meat quality traits of young Charolais bulls. *Meat Science*, 59, 49-60.

Renerre, M. (1986). Influence da facteurs biologiques et technologiques sur la couleur de la viande. *Bulletin Technologique C. R. Z. V. Theix, INRA, 65*, 41-45.

Renerre, M. (2000). Oxidative processes and myoglobin. In E. Deker, C. Faustman, & C. J. Lopez-Bote (Eds.), *Antioxidants in muscle foods* (pp. 113–133). New York, NY: John Wiley.

Resconi, V.C., Campo, M.M., Font i Furnols, M., Montossi, F., & Sañudo, C. (2010). Sensory quality of beef from different finishing diets. *Meat Science, 86*, 865-869.

Rigault, C., Mazué, F., Bernard, A., Demarquoy, J. & Le Borgne, F. (2008). Changes in L-carnitine content of fish and meat during domestic cooking. *Meat Science*, 78, 331-335.

Robelin, J. (1990). Différenciation croissance et développement cellulaire du tissu musculaire. *INRA Production Animales*, 3, 253-263.

Robelin, J. & Casteilla, L. (1990). Différenciation, Croissance et développement du tissu adipeux. *INRA Production Animales*, 3, 243-252.

Robert, N., Briant, M., Taylor, R. & Briand, Y. (1999). The effect of proteosome on myofibrilar structure in bovine skeletal muscle. *Meat Science*, 51, 149-153.

Röhrle, F.T., Maloney, A.P., Black, A., Osorio, M.T., Sweeney, T., Schmidt, O., & Monahan, F.J. (2011). α-Tocopherol stereoisomers in beef as an indicator of vitamin E supplementation in cattle diets. *Food Chemistry,* 124, 935-940.

Ross, C. F. (2009). Sensory science at human-machine interface. *Trends in Food Science and Technology*, *20*, 63-72.

Rucklidge, G.J., Milne, G., McGaw, B., A Milne, E., & Robins, S.P. (1992). Turnover rates of different collagen types measured by isotope ratio mass-spectrometry. *Biochemical Biophysical Acta*, 1156, 57–61.

Rule, D. C., MacNeil, M. D., & Short, R. E. (1997). Influence of sire growth potential, time on feed, and growing finishing strategy on cholesterol and fatty acid composition of the ground carcass and *longissimus lumborum* muscle of beef steers. *Journal of Animal Science, 75*, 1525-1533.

Ryu, Y.C. & Kim, B.C. (2005). The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig *longissimus dorsi* muscle. *Meat Science,* 71, 351-357.

Ryu, Y.C., Choi, Y.M. & Kim, B.C. (2006). Variations in metabolite contents and protein denaturation of the *longissimus dorsi* muscle in various porcine quality classifications and metabolic rates. *Meat Science,* 71, 552-529.

Safari, E., Fogarty, M.M., Ferrier, G.R., Hopkins, D.L. & Gilmore, A. (2001). Diverse lamb genotype. III. Eating quality and the relationship between its objective measurement and sensory assessment. *Meat science*, 57, 153-159.

Sahin, S., & Sumnu, S. G. (2006). Rheological properties of foods. In: D. R. Heldman (Ed.), *Physical properties of foods*. Food science text series. (1st ed.) (pp. 39-106). New York USA, Springer.

Saido, T.C., Sorimachi, H., & Suzuki, K. (1994). Calpain: new perspectives in molecular diversity and physiological- pathological involvement. *FASEB Journal*, 8, 814–822.

Santos-Silva, J., Bessa, R.J.B. & Santos-Silva, F. (2002). Effect of genotype, system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Production Science*, 77, 187-194.

Savell, J. W. & Cross, H. R. (1988). The role of fat in the palatability of beef, pork, and lamb. In: NRC (Ed.), *Designing foods. Animal product options in the marketplace*. (pp. 345-355). Washington D.C., USA: National Academy Press.

Savell, J.W., Mueller, S.L. & Baird, B.E. (2005). The chilling of carcasses - a review. *Meat Science*, 70, 449-459.

Scheeder, M.R.L., Casutt, M.M., Roulin, M., Escher, F., Dufey, P.A., & Kreuzer, M. (2001). Fatty acid composition, cooking loss and texture of beef parties from meat of bulls fed different fats. *Meat Science*, 58, 321–328.

Schmid, A. (2011). The role of meat fat in the human diet. *Critical Reviews in Food Science and Nutrition, 51*, 50-66.

Schnettler, B., Vidal, R., Silva, R., Vallejos, L. & Sepúlveda, N. (2009). Consumer willingness to pay for beef meat in a developing country: The effect of information regarding country of origin, price and animal handling prior to slaughter. *Food Quality and Preference*, 20, 156-165.

Scollan, N. D. (2003). Strategies for optimizing the fatty acid composition of beef. *Iger Innovations*, 41-45.

Scollan, N.D., Hoquette, J.F., Nuernberg, K., Dannenberger, D., Richardson, I. & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipid and their relationship with meat quality. *Meat Science*, 74, 17-33.

Seideman, S.C., & Koohmaraie, M. (1987). Factors associated with tenderness in young beef. *Meat Science*, 20, 281–291.

Sentandreu, M.A., Coulis, G. & Ouali, A.(2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science & Technology*, 13, 400-421.

Sepúlveda, W., Maza, M.T. & Mantecón, A.R. (2008). Factors that affect and motivate the purchase of quality-labelled beef in Spain. *Meat Science, 80*, 1282-1289.

Serra, X., Gil, M., Gispert, M., Guerrero, L., Oliver, M.A., Sañudo, C., Campo, M.M., Panea, B., Olleta, J.L., Quintanilla, R. & Piedrafita, J. (2004). Characterisation of young bulls of Bruna dels Pirineus cattle breed (selected from old Brown Swiss) in relation to carcass, meat quality and biochemical traits. *Meat Science*, 66, 425-436.

Serra, X., Guerrero, L., Guàrdia, M.D., Gil, M., Sañudo, C., Panea, B., Campo, M.M., Olleta, J.L., Piedrafita, J., Graciá-Cachán, J. & Oliver, M.A. (2008). Eating quality of young bulls from three Spanish beef breed-production systems and its relationships with chemical and instrumental meat quality. *Meat Science*, 79, 98-104.

Shackelford, S.D., Koohmaraie, M., & Savell, J.W. (1994). Evaluation of longissimus dorsi muscle pH at three hours post mortem as a predictor of beef tenderness. *Meat Science*, 37, 195–204.

Shackelford, S. D., Koohmaraie, M., & Wheeler, T. L. (1995). Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from Bos indicus and Bos taurus cattle. *Journal of Animal Science, 73*, 3333-3340.

Shackelford, S. D., Wheeler, T., & Koohmaraie, M. (1997). Repeatability of tenderness measurements in beef round muscles. *Journal of Animal Science*, 75, 2411-2416.

Shackelford, S. D., Wheeler, T. & Koohmaraie, M. (1999). Evaluation of slice shear force as an objective method for assessing beef longissimus tenderness. *Journal of Animal Science, 77*, 2663-2699.

Shahidi, F. 1998. Flavor of muscle foods: an overview. In F. Shahidi (Ed.) *Flavor of Meat, Meat Products, and Seafoods*. London, UK: Blackie Academic and Professional.

Sharp, A. & Offer, G. (1992). The mechanism of formation of gels from myosin molecules. *Journal of the Science of Food and Agriculture*, 58, 63-73.

Shimada, K., Sakuma, Y., Wakamatsu, J., Fukushima, M., Sekikawa, M., Kushida, K., & Mikami, M. (2004). Species and muscle differences in L-carnitine levels in skeletal muscles based on a new simple assay. *Meat Science, 68*, 357-362.

Shorthose, W.R. & Harris, P.V. (1991). Effects of growth and composition on meat quality. *Advances in Meat Science,* 7, 515–549.

Sierra, V., Guerrero, L., Frenández-Suárez, V., Martínez, A., Castro, P., Osoro, K., Rodríguez-Colunga, M.J., Coto-Montes, A., & Oliván, M. (2010). Eating quality of beef from biotypes included in the PGI „Ternera Asturiana" showing distinct physicochemical charcateristics and tenderization pattern. Meat Science, 86, 343-351.

Silva, J.A., Patarata, L,. & Martins, C. (1999). Influence of ultimate pH on bovine meat tenderness during ageing. *Meat Science*, 52, 453-459.

Silver, F.H., Kato, Y.P., Ohno, M. & Wasserman, A.J. (1992). Analysis of mammalian connective tissue: relationship between hierarchical structures and mechanical properties. *Journal of Long-Term Effects of Medical Implants*, 2, 165-98.

Simmons, N.J., Daly, C.C., Cummings, T.L., Morgan, S.K., Johnson, N.V. & Lombard, A. (2008). Reassenssing the principles of electrical stimulation. *Meat Science*, 80, 110- 122.

Simões, J.A. & Lemos, J.P.C. (2005). "Efeito do sexo, da idade e da escolaridade dos consumidores na avaliação da tenrura da carne de bovino". Congresso de Ciências Veterinárias 2005., Estação Zootécnica Nacional, Vale de Santarém, Portugal, 13-15 Outubro 2005.

Skibsted, L.H., Mikkelsen, A., Bertelsen, G. (1998). Lipid-derived off-flavours in meat. In F. Shahidi (Ed.), *Flavor of meat, meat products and seafoods*. (pp. 217–256). London, UK: Blackie Academic and Professional.

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

Smulders, F.J.M., Marsh, B.B., Swartz, D.R., Russell, R.L., Hoenecke, M.E. (1990). Beef tenderness and sarcomere length. *Meat Science*, 28, 349-363.

Snowden, J.M., Bouton, P.E., & Harris, P.V. (1977). Influence of constraint during heating and cooling on the mechanical properties of collagenous tissue. *Journal of Food Science*, 42, 890–894.

Sorimachi, H., Toyama-Sorimachi, N., Saido, T.C., Kawasaki, H., Sugita, H., Miyasaka, M., Arahata, K., Ishiura, S. & Suzuki, K. (1993). Muscle-specific calpain, p94, is degraded by autolysis immediately after translation, resulting in disappearance from muscle. *Journal of Biological Chemistry*, 268, 10593–10605.

Stabursvik, E. & Martens, H. (1980). Thermal denaturation of proteins in post rigor muscle tissue as studied by differential scanning calorimetry. *Journal of Science Food and Agriculture*, 31, 1034–1042.

Statistical Analysis System (2004). *SAS/STAT 9.1 user's guide*. Cary, NC, USA: SAS Institute Inc..

Steenkamp, J.B. (1997). Dynamics in Consumer Behaviour with Respect to Agricultural and Food Products. In Wieringa, B., Tilburg, A. van, Grunert, K., Steenkamp, J.-

B. E. M. and Wedel, M. (eds). *Agricultural Marketing and Consumer Behaviour in a Changing World*. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Steenkamp, J.B.E.M. (1990). Conceptual model of the quality perception process. *Journal of Business Research*, 21, 309–333.

Stelzleni, A.M. & Johnson, D.D. (2008). Effect of days on concentrate feed on sensory off-flavor score, off-flavor descriptor and fatty acid profile for selected muscle from cull beef cows. *Meat Science, 79*, 382-393.

Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference*, 13, 215-225.

Tabilo, G., Flores, M., Fiszman, S., & Toldrá, F. (1999). Postmortem meat quality and sex affect textural properties and protein breakdown of dry cured ham. *Meat Science*, 51, 255-260.

Taylor, M.A.J. & Etherington, D.J. (1991). The solubilization of myofibrillar proteins by calcium ions. *Meat Science,* 29, 211-219.

Taylor, M.A.J., Geesink, G.H., Thompson, V.F., Koohmaraie, M. & Goll, D.E. (1995). Is Z-disk degradation responsible for postmortem tenderization? *Journal of Animal Science,* 73, 1351-1367.

Télles, J. A. R. (2004). Características bioquímicas del músculo, calidad de la carne y de la grasa de conejos seleccionados por velocidad de crecimiento. Tese Doctoral. Bellaterra, Barcelona: Facultat de Veterinària, Universitat Autònoma de Barcelona.

Thompson, J.M., Perry, D., Daly, B., Gardner, G.E., Johnston, D.J. & Pethick, D.W. (2006). Genetic and evironmental effects on the muscle structure response *post-mortem*. *Meat Science*, 74, 59-65.

Tornberg, E. (1996). Biophysical aspects of meat tenderness. *Meat Science*, 43,S171-S191.

Tornberg, E. (2005). Effects of heat on meat proteins – Implications on structure and quality of meat products. *Meat Science*, 70, 493-508.

Touraille, C. (1991). Qualités organoleptiques des viandes. Calidad de la canal y de la carne en ruminantes. Saragoça, Espanha.Policopiado.

Trinick, J. (1991). Elastic filaments and giant proteins in muscle. *Current Opinion in Cellular Biology*, 3, 112-119.

Turk, B., Turk, D., & Turk, V. (2000). Lysosomal cysteine proteases: more than scavengers. *Biochimica Biophysica Acta*, 1477, 98–111.

Usha, R., & Ramasami, T. (2000). Effect of pH on dimensional stability of rat tail tendon collagen fiber. *Journal of Applied Polymer Science*, 75, 1577–1584.

Valsta, L.M., Tapanainen, H. & Männistö, S. (2005). Meat fats in nutrition – a review. *Meat Science*, 70, 525-530.

Vatansever, L., Kurt, E., Enser, M., Nute, G.R., Scollan, N.D., Wood, J.D., & Richardson, R.I. (2000). Shelf life and eating quality of beef from cattle of different breeds given diets differing in n-3 polyunsaturated fatty acid composition. *Animal Science, 71*, 471-482.

Vaz, F.M., & Wanders, R.J. (2002). Carnitine biosynthesis in mammals. *Biochemistry Journal, 361*, 417–429.

Veland, J.O., & Torrisen, O.J. (1999). The texture of Atlantic salmon (salmo solar) muscle as measured instrumentally using TPA and Warner-Bratzler shear test. *Journal of the Science of Food and Agriculture*, 79, 1737-1746.

Verbecke, W. & Viaene, J. (1999). Beliefs, attitude and behaviour towards fresh meat consumption in Belgium: empirical evidence from a consumer survey. *Food Quality and Preference*, 10, 437-445.

Verbeke, W. (2001). The emerging role of traceability and information in emandoriented livestock production. *Outlook on Agriculture*, 30, 249–256.

Verbecke, W. & Ward, R.W. (2006). Consumer in terest in information cues denoting quality, traceability and origin: An application of ordered probit models to beff labels. *Food Quality and Preference*, 17, 453-467.

Verbecke, W., Van Wezemael, L., Barcellos, M. D., Kugler, J. O., Hocquette, J. F., Ueland, O. & Grunert, K. G. (2010). European consumers‘ interest in a beef quality guarantee insights from a qualitative study in four EU countries. *Appetite*, 54, 289-296.

Vestergaard, M., Therkildsen, M., Henckel, P., Jensen, L.R., Andersen, H.R., & Sejrsen, K. (2000). Influence of feeding intensity, grazing and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. *Meat Science*, 54, 187-195.

Wandel, M., & Bugge, A. (1996). Environmental concern in consumer evaluation of food quality. *Food Quality and Preference*, 8, 19–26.

Warner, R. D. (1989). Objective description of meat and fat colour in beef carcasses. In: L. E. Brownlie, W. J. A. Hall & S. U. Fabiansson (Ed.), *The automated measurement of beef*. (pp. 145-154). Sidney, Austrália, The Australian Mear and Livestock Corporation.

Warner, R.D., Greenwood, P L., Pethick, D.W. & Ferguson, D.M. (2010). Genetic and environmental effects on meat quality. *Meat Science*, 86, 171-183.

Warren, H.E., Scollan, N.D., Nute, G.R., Hugues, S.I., Wood, J.D. & Richardson, R I. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. II: Meat stability and flavour. *Meat Science, 78*, 270-278.

Warriss, P.D. (2000). Meat Science: An introdutory text (1st Ed.). Wallingford, USA: CABI Publishing.

Watson, R., Gee, A., Polkinghorne, R. & Porter, M. (2008). Consumer assessment of eating quality – development of protocols for Meat Standards Australia (MSA) testing. *Australian Journal of Experimental Agriculture*, 48, 1360–1367.

Weaver, A.D., Bowker, B. C., & Gerrard, D.E. (2008). Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle. *Journal of Animal Science*, 86, 1925−1932.

West, J., Young, O.A., & Agnew, M.P. (1997). Levels of α-tocopherol in beef from New Zealand pastures. In *Proceedings of the 43rd International Congress of Meat Science and Technology*, Auckland, Australia (pp. 350-351).

Wezemael, L.V., Verbecke, W., Kugler, J.O., Barcellos, M.D., & Grunert, K.G. (2010). European consumers and beef safety: Perceptions, expectations and uncertainty reduction strategies. *Food Control, 21*, 835-844.

Wheeler, T.L., Cundiff, L.V. & Koch, R.M. (1994). Effect of marbling degree on beef palatability in Bos taurus and Bos indicus cattle. *Journal of Animal Science*, 72, 3145-3151.

Wheeler, T.L., Shackelford, S.D., & Koohmaraie, M. (1999). Tenderness classification of beef: III. Effect of the interaction between end point temperature and tenderness on Warner-Bratzler shear force of beef longissimus. *Journal of Animal Science*, 77, 400-407.

Wheeler, T.L., Shackelford, S.D., & Koohmaraie, M. (2007). Beef longissimus slice shear force measurement among steak locations and institutions. *Journal of Animal Science*, 85, 2283-2289.

Wierbicki, E., Cahill, V.R., Kunkle, L.E., Klosterman, E.W. & Deatherage, F.E. (1955). Effect of castration on biochemistry and quality of beef. *Journal of Agricultural Food and Chemistry, 3,* 244-248.

Williams, C.M. (2000). Dietary fatty acids and human health. *Annals of Zootechnology*, 49, 165–180.

Williams, C.M., & Burdge, G. (2006). Long-chain n-3 PUFA: plant v. marine sources. *Proceedings of the Nutrition Society*, 65, 42–50.

Wolf, G. (1984). Multiple functions of vitamin A. *Physiological Review*, 64, 873–937.

World Health Organization (2003). Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical report series no. 916. World Health Organization: Geneva.

Wood, J.D. (1990). Consequences for meat quality of reducing carcass fatness. In: Wood, J. D. e Fisher, A. V. (Eds.), *Reducing fatness in meat animals*. London, UK: Elsevier Applied Sci. Publishers Ld.

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R. & Enser, M. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, 66: 21-32.

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

Wright, B. A., & Wiederhorn, N. M. (1951). Studies concerned with the structure of collagen. I. An X-ray investigation of the denaturation of collagen. *Journal of Polymer Science*, 7, 105–120.

Wright, D.J., & Wilding, P. (1984). Differential scanning calorimetric study of muscle and its proteins: myosin and its subfragments. *Journal of Science Food and Agriculture*, 35, 357–372.

Wulf, D.M. & Wise, J.W. (1999). Measuring muscle color on beef carcasses using the L*a*b* color space. *Journal of Animal Science*, 77, 2418-2427.

Xiong, Y.L., Mullins, O.E., Stika, J.F., Chen, J., Blanchard, S.P., & Moody, W.G. (2007). Tenderness and oxidative stability of post-mortem muscles from mature cows of various ages. *Meat Science*, 77, 105−113.

Xue, H., Mainville, D., You, W., & Nayga Jr., R. M. (2010). Consumer preference and willingness to pay for grass-fed beef: Empirical evidence in-store experiments. *Food Quality and Preference,* 21, 857-866.

Yang, A., Brewster, M.J., Lanari, M.C. & Tume, R.K. (2002). Effect of vitamin E supplementation on a-tocopherol and b-carotene concentrations in tissues from pasture- and grain-fed cattle. *Meat Science*, 60, 35-40.

Yates, L.D., Dutson, T.R., Caldwell, J., & Carpenter, Z.L. (1983). Effect of temperature and pH on the post-mortem degradation of myofibrillar proteins. *Meat Science*, 9, 157-179.

Zeece, M.G., Woods, T.L., Keen, M.A., & Reville, W.J. (1992). Role of proteinases and inhibitors in postmortem muscle protein degradation. Proceedings of the Reciprocal Meat Conference, Colorado State University, 45, 51–61.

Zembayashi, M. (1994). Effects of nutritional planes and breeds on intramuscular-lipid deposition in *M. longissimus dorsi* of steers. *Meat Science,* 38, 367–374.

Zembayashi, M., Nishimura, K., Lunt, D.K., & Smith, S.B. (1995). Effect of breed type and sex of the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. *Journal of Animal Science*, 73, 3325-3332.