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Oxidative stability, lipid composition and nutritional value of ruminant meat as affected by animal feeding system, sex and common household treatments

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A Paola

"O frati", dissi, "che per cento milia
perigli siete giunti a l'occidente,
a questa tanto picciola vigilia

d'i nostri sensi ch'è del rimanente
non vogliate negar l'esperïenza,
di retro al sol, del mondo sanza gente.

Considerate la vostra semenza:
fatti non foste a viver come bruti,
ma per seguir virtute e canoscenza".

Dante Alighieri

Inferno Canto XXVI, (vv. 112-120)

ACKNOWLEDGMENTS

E' difficile scrivere dei ringraziamenti per molti motivi. Il primo, perchè tantissime persone hanno contribuito a formare la persona che oggi sono ed è impossibile elencarli tutti in una paginetta o poco più (ma loro lo sanno...), il secondo perchè anche con l'enorme gioia di tagliare un traguardo importante come il dottorato, si ha sempre paura di perdere qualcosa, ad esempio gli amici "colleghi" di questi tre anni, i professori nel proficuo rapporto di dare ricevere (in senso lato), l'ambiente stesso di un'esperienza che costituisce una parte di vita fondamentale.

Il lavoro condotto per questa tesi è necessariamente non completo, o meglio, sono molte le cose che si possono affinare e sviluppare. Spero di aver dato delle conferme e di essere stata all'altezza di quanto mi è stato chiesto di fare, di certo l'impegno e la passione da parte mia non sono mancati.

Spero di non aver disturbato troppo nessuno, e spero inoltre di poter continuare in futuro ad applicarmi in tali ambiti e con le stesse persone con le quali ho avuto il piacere e l'onore di lavorare per questa tesi, che magari potrebbe sembrare un tantino avventurosa agli occhi di molti...ma è stata di gran lunga un'enorme soddisfazione che ripeterei fin da ora! Penso che per "contenere" tutto quello che ho imparato non basterebbero tre corsi interi; è sufficiente questo per farmi esprimere una profonda gratitudine verso coloro che mi hanno portato a tale risultato.

ABSTRACT

Ruminant meats have been suffered from a negative health image related to the nature of their lipid fraction, mainly due to the higher content in SFA. Even so, ruminant edible fats are the major natural source of CLA, provide proteins of high biological value and important micronutrients, such as A, group B, D and E vitamins, minerals and essential fatty acids all these components are associated with potential health benefits. Oxidation of lipids in foods is the major biochemical process that cause food quality deterioration, leading to the characteristic, unpalatable odour and flavour called rancidity. In addition to unpalatability, rancidity may give rise to toxic levels of certain compounds like malondialdehyde. In this PhD study UV/VIS absorbance spectroscopy was applied to determine the level of MDA in ruminant meat and its relationship with parameters like feeding, sex, cooking and storage conditions. Beef and lamb meat in this study were analysed. On beef and lamb meat, a study of lipid fraction before and after cooking was carried out to evaluate their chemical composition, fatty acid profile and some nutritional ratio. This manuscript reports the main results obtained in the five activities briefly summarized as follows: 1. effect of sex and cooking on nutritional value and oxidative stability in lamb meat; 2. evaluation of effects of diet on the oxidative stability of lamb meat during storage; 3. effect of sex and cooking on nutritional value and oxidative stability in beef meat; 4. evaluation of effects of diet on lipid oxidative stability and composition of beef meat; 5. effect of type of suckling and cooking on nutritional value and oxidative stability in lamb meat.

TABLES OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

ADI	Adeguate Daily Intake
AH	Antioxidant
AI	Index of atherogenicity
ALA	α-Linolenic acid, C18:3 n-3
ARA	Arachidonic acid, C20:4 n-6
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
CK	Cooked
CLA	Conjugated linoleic acid
DHA	Docosahexaenoic acid, C22:6 n-3
DPA	Docosapentaenoic acid, C22:5 n-3
EPA	Eicosapentaenoic acid, C20:5 n-3
EU	European Union
FAME	Fatty acid methyl esters
FDA	Food and Drug Administration
FID	Flame ionization detector
g	Gram
GC	Gas chromatography
h	Hypocholesterolaemic fatty acids; Hour
H	Hypercholesterolaemic fatty acids
HDL	High density lipoprotein
HPLC	High pressure liquid cromatography
HO•	Hydroxyl Radical
IMF	Intramuscular fat
In	Initiator
IT	Index of thrombogenicity

Kcal	Kilocalorie
LC PUFA	Long-chain polyunsaturated fatty acids
LD	*Longissimus dorsi*
LDL	Low density lipoprotein
LSM	Least squares means
LN	Linoleic acid, C18:2 n-6
M	Molar
MDA	Malondialdehyde
mg	Miligram
ml	Mililitre
μg	Microgram
μl	Microlitre
MUFA	Monounsaturated fatty acids
NP	No-pasture
ns	Not significant
O_2^{-}•	Radical Superoxide
O_2	Singlet oxygen
%	Percent
P	Pasture
PG	Propyl gallate
PUFA	Polyunsaturated fatty acids
R•	Free alkyl radical
RO•	Radical Alkoxyl
ROO•	Radical Peroxyl
ROOH	Lipid Hydroperoxyl
ROS	Reactive Oxygen Species
RW	Raw
s	Second
SEM	Standard error of mean
SFA	Saturated fatty acids
TAG	Triacylglycerols

TBARS	Thiobarbituric acid reactive substances
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TEP	Tetraethoxy-propane
TFA	*Trans* fatty acids
TR	True Nutrient Retention Values
TVA	Trans vaccenic acid, C18:1 *t*11
TXA2	Thromboxane A2
TXA3	Thromboxane A3
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
v	Volume
WCRF	World Cancer Research Fund
WHO	World Health Organization
WHO/FAO	World Health Organization/Food and Agriculture Organization
wt	Weight

INTRODUCTION

The chemical oxidation of lipids is an area of research involving both the quality of the foods and several biological systems. In foods, lipid oxidation is a leading cause of quality changes, involving taste, texture and appearance. It can affect, to some extent, the nutritional value and safety of foods (Eriksson, 1987; Gordon, 2001). Recent studies (Benzie, 1996; Girotti, 1998; Diblock , et al., 1998) have shown that an uncontrolled increase in lipids oxidation involved in the development of many chronic diseases. These factors highlight the importance of understanding the mechanism of oxidation of lipids and to identify factors that influence the reaction pathways of lipid oxidation.

This process is due to several interactions between the food constituents and determines the formation of both desirable and undesirable products; therefore the oxidative reactions that occur in food are one of major source of deterioration that happens during the manufacture, storage, distribution and preparation of foods.

Foods components more susceptible to oxidation, due to their nature, are lipids.

Lipids are important macromolecules in food because they:

- are a source of energy "concentrate" (9 Kcal/g),
- carry fat-soluble vitamins (A,D, E and K),
- provide essential fatty acids,
- are important constituents of cell membranes,
- make foods more palatable giving them flavor and texture,
- confer a sense of satiety.

The oxidation of lipids is promoted and facilitated by the presence of catalytic systems such as light, heat, enzymes, metals and micro-organisms and determines the development of off-flavors and nutritional losses. Lipids may be subjected to different oxidative processes that lead despite a similar impact on foods but proceeding with different chemical and physical mechanisms. Extensive studies have shown that there are at least three different mechanisms through which can occur the process of oxidation of lipids and these are: autoxidation by a free radical chain mechanism, photo-oxidation and enzymatic oxidation; all of these involve some type of free radicals or reactive oxygen species (ROS).

Lipid oxidation products are ubiquitous in foods, although high variation exists in their level. Although these levels are generally low, the problem of lipid oxidation severely compromises the quality of some foods and limits the shelf-life of others (Addis, 1986). The presence of lipids in foods, even at very low level (<1%), making them susceptible to oxidation, especially foods cooked or subjected to high temperatures and successively stored, such as cheeses and meat, oils and food fried. The oxidation of lipids in foods is, in effect, the major non-microbial factor that can affect adversely their quality (Pradhan et al., 2000).

Deleterious quality losses in food caused by lipid oxidation are summarized in Table 1.

Table 1. Consequences of Lipid Oxidation Activity

Loss of flavor
Development of off-flavors (rancidity)
Colour changes:
• Myoglobin (red) ⟶ Metmyoglobin (brown)
• Loss of carotenoid
Accumulation of oxidation products with potentially detrimental health implication
Protein denaturation
Functionality changes
Loss of nutrient value

Experimental animal studies and biochemical investigations lend support to the hypothesis that lipid-oxidation products, ingested with food or produced endogenously, represent a health risk (Estebauer, 1993). Lipid oxidation products are related to atherosclerosis (Estebauer et al., 1993), Alzheimer's disease (Markesbery et al., 1998), cancer (Boyd et al., 1991), inflammation or aging processes (Parker et al., 1977) (Broncano et al., 2009); furthermore, Coronary Artery Disease (CAD) may be in part caused by the consumption of lipid oxidation products.

The complex nature of the oxidation products, their instability, their tendency to interact with the food components and the interferences caused by substances in foods makes difficult to assess the state of oxidation of food lipids. The synergistic use of chemical and physical analytical methods, developed in recent years, has made progress in understanding the lipid oxidation and its influence on food quality.

- PART I -

SCIENTIFIC BACKGROUND AND RESEARCH ISSUE

CHAPTER 1

CHEMISTRY AND MECHANISM OF LIPID OXIDATION

1.1. General

The oxidative deterioration of food lipids involves, primarily, autoxidative reactions which are accompanied by various secondary reactions having oxidative and non-oxidative character (Gray, 1978). The main protagonists involved in the process of lipid autoxidation in foods are unsaturated fatty acids; their oxidative degradation is promoted by oxygen, light, heat, trace metals, peroxide and sometimes by enzymes. The primary products of lipid oxidation are lipid hydroperoxides.

The formation of hydroperoxides of fatty acids can occur through three different mechanisms, namely autoxidation, photo-oxidation and enzymatic oxidation (Frankel, 1998). Under mild reaction conditions hydroperoxides are formed primarily to autoxidation. The process of autoxidation of lipids is a spontaneous free radicals chain mechanism and the main protagonists involved in this process are unsaturated fatty acids and molecular oxygen. Another important pathway for the formation of hydroperoxides is photo-oxidation. In this process the light, in the presence of oxygen and photosensitizer, promotes the oxidation of unsaturated fatty acids (Frankel, 1998, p. 43). In addition, the presence of enzymes such as lipoxygenase, promotes the controlled synthesis of hydroperoxides, which are precursors of products with potential biological activity (Brash, 1999). These three different mechanisms may occur simultaneously. For example, the reaction of autoxidation in the oils could be initiated by hydroperoxides formed during photo-oxidation process in presence of natural photosensitizers such as chlorophylls that lead to the formation of singlet oxygen in presence of visible light (Frankel, 1980; Gunstone, 1984).

Each of these mechanisms produces a characteristic mixture of hydroperoxides. In the introduction of the present thesis the formation of hydroperoxides from oleic, linoleic, linolenic and arachidonic acid are used as examples of the processes of autoxidation that occur in monounsatured, diunsatured, polyunsatered and highly unsatured fatty acids.

1.2. Autoxidation

The main protagonists involved in the process of lipid autoxidation in foods are unsaturated fatty acids and molecular oxygen which react with a spontaneous free radical chain mechanism characterized by three main steps (Gray, 1978; Frankel, 1984; Morrissey et al., 1998):

1. ***Initiation***: in presence of initiators ($In^{\bullet}$) an α-methylenic hydrogen atom is abstracted from an unsaturated fatty acid to form an highly reactive lipid (alkyl) free radical ($R^{\bullet}$);

$$RH + In^{\bullet} \longrightarrow R^{\bullet} + InH$$

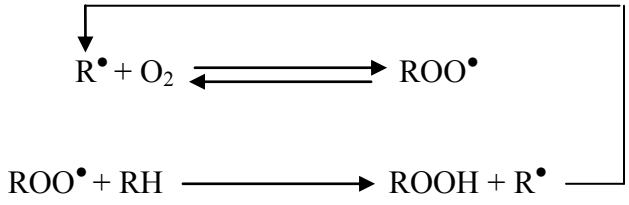
Initiation is frequently attributed in most foods to reaction of fatty acids with reactive oxygen species (ROS) which include both radical and non-radical substances, in fact, the spontaneous reaction of the unsaturated fatty acid with molecular oxygen is highly unlikely due to of electronic state of O_2. The main ROS, produced during normal aerobic metabolism, are summarized in table 2. This reaction is however thermodynamically unfavorable therefore proceeds very slowly without the aid of catalysts which promote and facilitate the process of oxidation (Frankel, 1984).

Table 2. Important reactive oxygen species and lipid aldehydes and their common abbreviations.

Reactive Oxygen Species and Aldehydes	
Radicals	Non radicals
Hydroxyl ($HO^{\bullet}$)	Hydrogen peroxide (H_2O_2)
Superoxide ($O_2^{-\bullet}$)	Singlet oxygen (O_2)
Lipid peroxyl ($ROO^{\bullet}$)	Lipid hydroperoxide (ROOH)
Alkoxyl ($RO^{\bullet}$)	4-Hydroxy-2-hexenal (HHE)
Peroxyl ($ROO^{\bullet}$)	4-Hydroxy-2-nonenal (HNE)

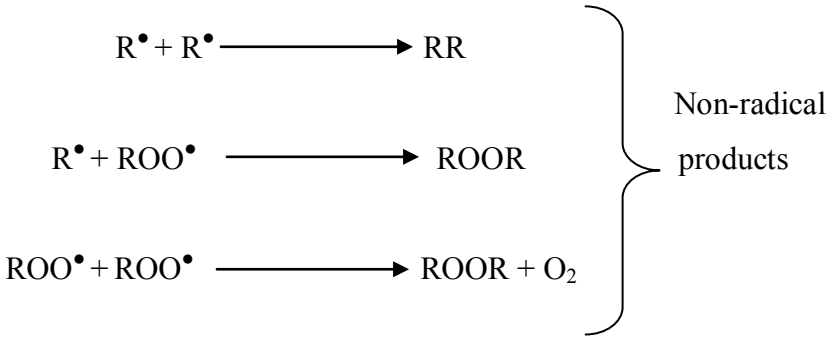
adapted from Halliwell and Gutteridge, 1999

1. ***Propagation***: free alkyl radical, in presence of molecular oxygen, breaks down quickly to form lipid peroxy radical ($ROO^{\bullet}$) able to abstracts hydrogen from another unsaturated fatty acid, this interaction lipid-lipid, that proceeds slowly, resulting in new lipid free radical ($R^{\bullet}$) and lipid hydroperoxide (ROOH) the primary products of autoxidation;



This type of interaction lipid-lipid could continue from10 to 100 times before termination of the process of lipid autoxidation (Gutteridge & Halliwell, 1990). Propagation steps may be more complicated than the simple transfer and addition steps (Porter at al., 1995). Peroxyl radicals are involved in competing reactions, such as β-Scission and cyclization by intramolecular rearrangement (Gardner, 1989).

2. ***Termination***: two free lipid alkyl ($R^{\bullet}$) and/or peroxy ($ROO^{\bullet}$) radicals combine to terminate the process through formation of more stable non-radical products that are not capable of propagating the chain reactions.



Antioxidants (AH) can break this chain reaction by reacting with $ROO^{\bullet}$ to form stable radicals ($A^{\bullet}$) which are either too unreactive or form non-radical products (Frankel, 1984).

The susceptibility to oxidation of fatty acids depends from the number of double bonds present, therefore unsaturated fatty acids presents in lipid of foods are oxidized more easily than saturated fatty acids, a property that is primarily due to the lower activation energy in the initiation of free radical formation (Fig. 1).

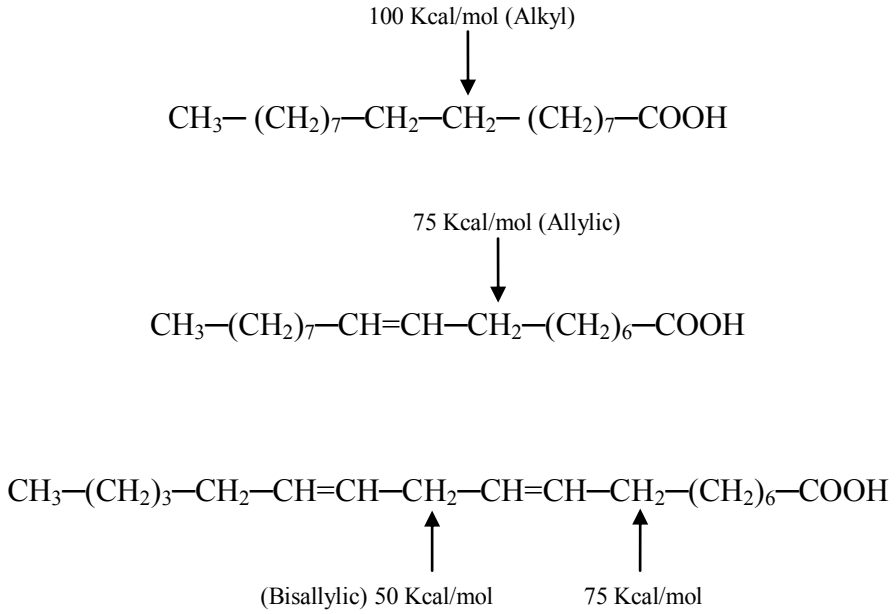


Figure 1. Energy required for hydrogen removal from Stearic, Oleic and Linoleic acids.

Increasing the number of double bonds present in fatty acids is observed an increase of the relative rate of autoxidation and of development of hydroperoxides. The relative rate of autoxidation of oleate (C18:1): linoleate (C18:2): linolenate (C18:3) was reported to be in order of 1:40-50:100 on the basis of the oxygen uptake and 1:12:25 on the basis of peroxide development (Frankel, 1984).

In monounsaturated fatty acids (MUFA), like oleic acid, the abstraction of an hydrogen atom occurs on the carbon atom allylic adjacent to the double bond, in the allylic radical obtained electrons are delocalized through 3-carbon systems and during reaction with oxygen a mixture of *cis* and *trans* allylic hydroperoxides are produced. The mechanisms, as shown in detailed in previous studies (Frankel, 1984; Porter et al., 1995), involved hydrogen atom abstraction at the 8 and 11 position of oleic acid to give two allylic radicals. The complete mechanism of this reaction is presented in figure 2.

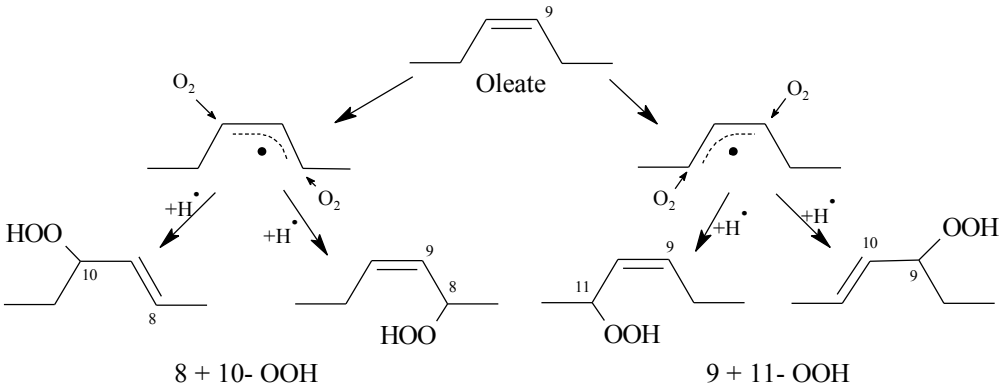


Figure 2. Mechanism of formation of oleic acid hydroperoxides. (Modified from Frankel, 1984).

In the case of polyunsaturated fatty acids (PUFA), like linoleic and linolenic acids, the most labile hydrogen atoms are those of methylene group situated between two consecutive double bonds; after abstraction of hydrogen atom is obtained a bisallylic radical where electrons are delocalized through 5-carbon systems (pentadienyl radical).
Linoleic acid is one of the main fatty acids in lipid membranes and in Low Density Lipoproteins (LDLs), and has the unsaturated bonds between carbons C9 and C10, and between C12 and C13.
The classical mechanism of linoleic autoxidation proceeds by hydrogen abstraction from the bisallylic carbon in position 11 to produce a delocalized pentadienyl radical. Auto-oxidation of linoleic acid followed by oxygen attack at the end positions produces an equal mixture of conjugated 9- and 13-hydroperoxide isomers with the *trans, cis-configuration*, with a concomitant formation of a conjugated diene structure on the adjacent carbons (C10–C13 or C9–C12, respectively). In addition, Schieberle and Grosch (1981) have been found that autoxidation of linoleic acid, as well as 9-OOH and 13-OOH, lead to the formation of a small amount of hydroperoxides with peroxide substitution at the 8, 10, 12 and 14 carbons. In figure 3 is presented a possible mechanism for the formation of these hydroperoxides as suggested by Porter et al. (1995).

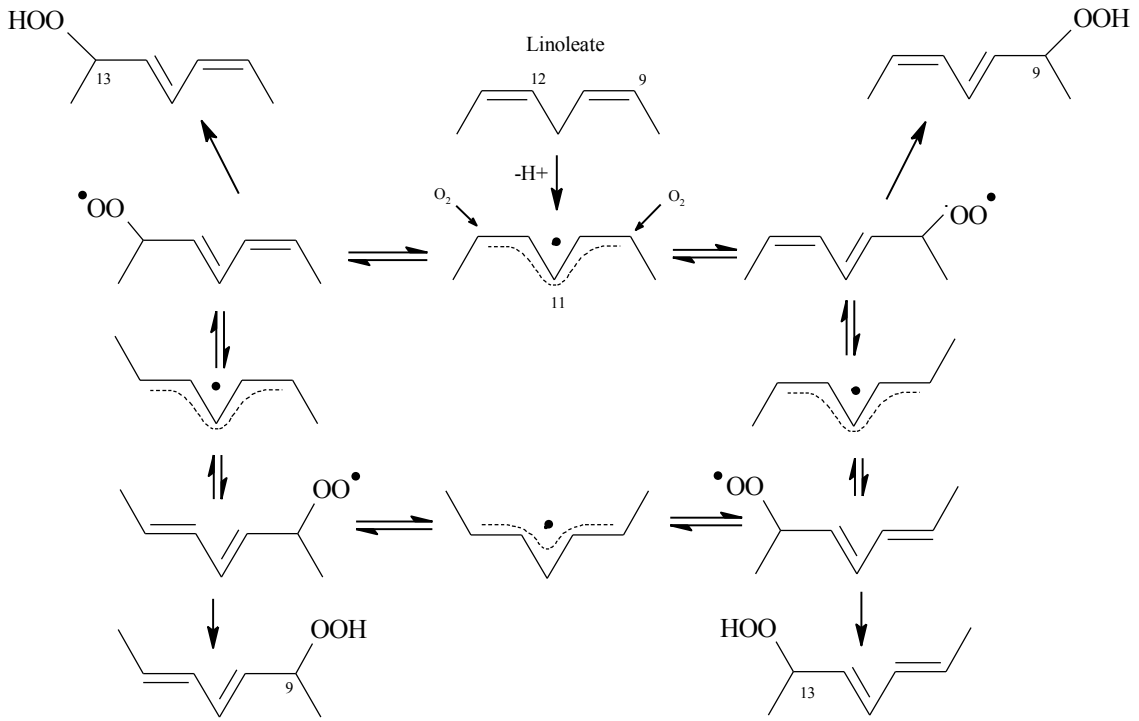


Figure 3. Formation of linoleic acid hydroperoxides. (Modified from Porter et al., 1995).

The formation of hydroperoxides from linolenic acid may be studied using linoleic acid as model compound. Linolenic acid (C18:3 *cis* 9,12,15) contains two separate 1,4 diene system, a C-9 to C-13 system identical with the linoleic acid plus a C-12 to C-16 system. The auto-oxidation of linolenic acid results in the formation of a mixture of 9-, 13-, 12-, and 16- hydroperoxides each as *cis,trans* and *trans,trans* isomers. However, studies (Frankel, 1991) have demonstrated that the 9- and 16-hydroperoxides are formed approximately four times more than 12- and 13-hydroperoxides. This nonuniform distribution is due to the tendency of 12- and 13-hydroperoxyl radical to submit to rapid 1,3-cyclization. These cyclic endoperoxides may decompose under stress condition to form predominantly malondialdehyde (MDA). In figure 4 is presented a probable mechanism of formation of cyclic peroxides and linolenic acid hydroperoxides.

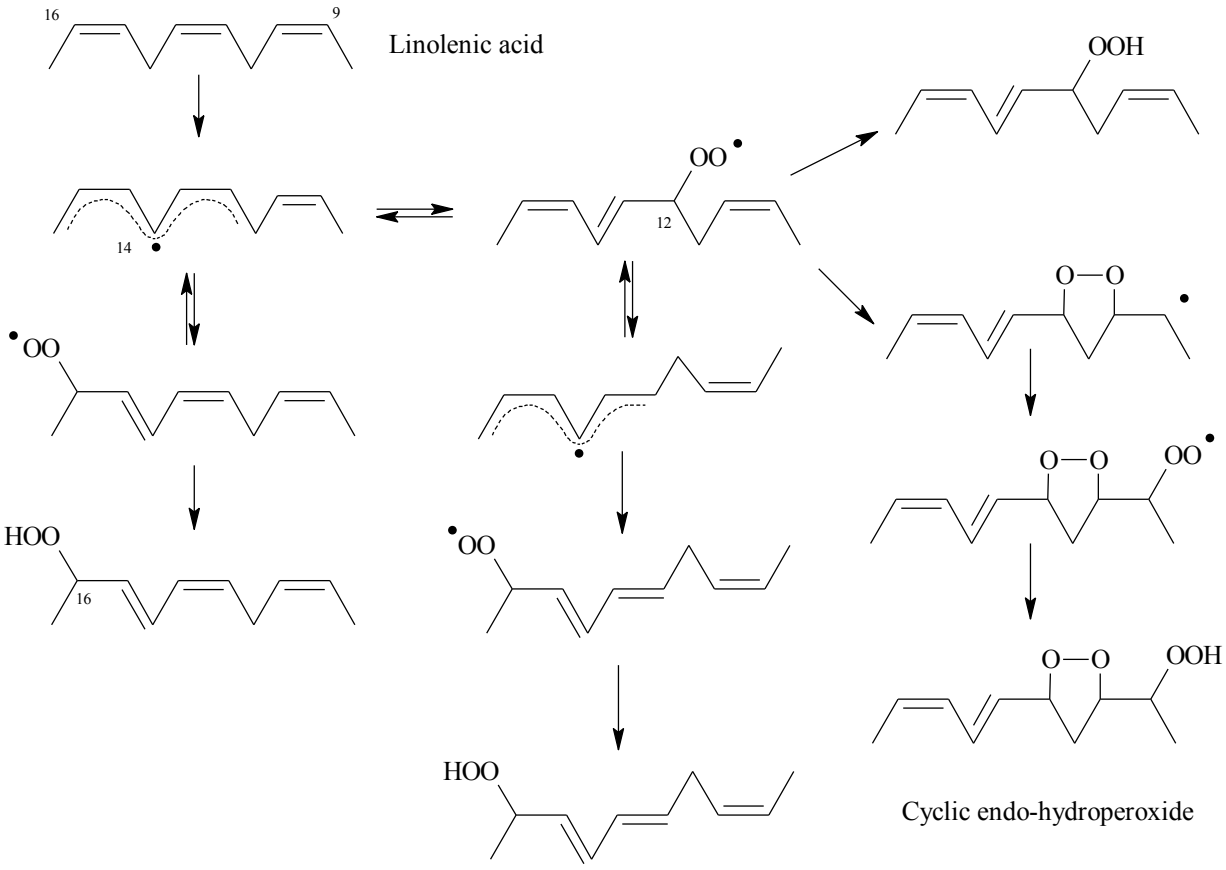


Figure 4. Formation of linolenic acid hydroperoxides (Modified from Gardner, 1989).

Autoxidation of arachidonic acid proceeds by the same mechanisn as linoleic acid. The abstraction of an hydrogen atom occurs at the three bisallylic carbons in position 7-, 10- and 13- and leads to form 3 delocalized pentadienyl radical. Molecular oxygen attacks these intermediates products yields 6 isomeric hydroperoxides with a conjugated diene system and 2 methylene-interrupted double bond. In figure 5 is presented the probable mechanism of autoxidation of arachidonic acid. Like in linolenic acid, the external hydroperoxide isomers, in position 5- and 15-, are formed in relatively higher concentrations that the internal hydroperoxides isomers on the 8-, 9-, 11- and 12- carbon atoms due to their tendency to cyclize (Porter, 1981).

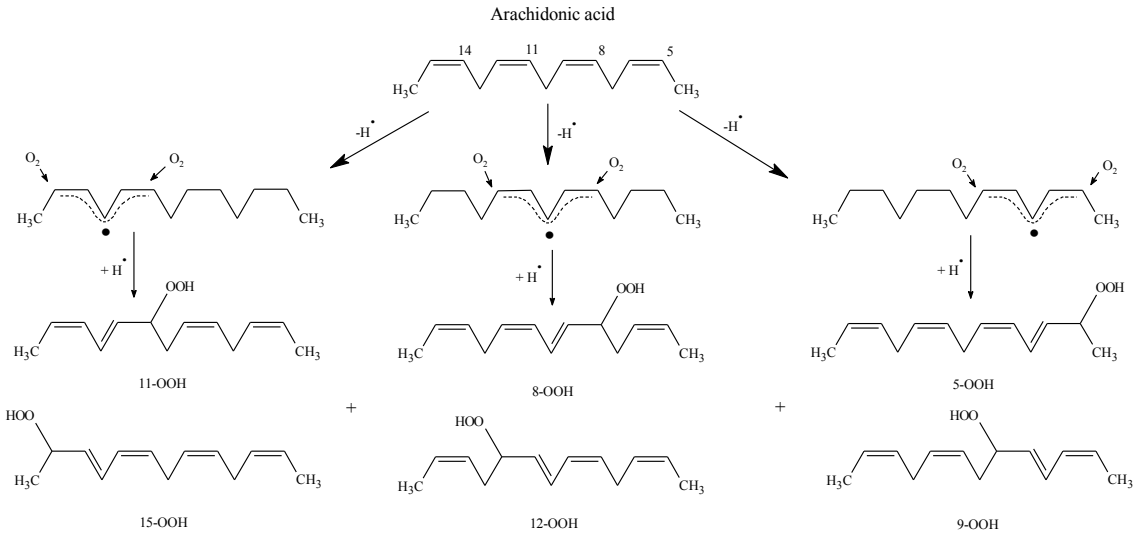


Figure 5. Mechanism of autoxidation of arachidonic acid (from Frankel E.N., 1984).

The autoxidation rate is dependent not only on the degree of the unsaturation of the fatty acid also by the lipid structure. Miyashita and Takagi (1986) have demonstrated that oleic, linoleic and linolenic acid as free fatty acids were autoxidized more rapidly than their corresponding methyl esters. They suggested that free fatty acids are oxidized at higher rate due to the catalytic action of carboxyl groups on the decomposition of a small amount of hydroperoxides formed in the initial stage of autoxidation.

1.3. Photo-oxidation

Photo-oxidation is an alternative route leading to the formation of hydroperoxides instead of the mechanism of autoxidation. This scheme of oxidation involves the photodynamic generation of singlet oxygen ($^1O_2$ state) via a photosensitizer (sens), in the presence of UV-light, and the addition of this to an unsaturated bond in the fatty acid. Several natural photosensitizers such as tetrapyrroles (bilirubin), flavins, chlorophyll, hemoproteins and reduced pyridine nucleotides (NADH) are known to generate singlet oxygen (Girotti A.W., 1990). In addition many photosensitizers are found in food, cosmetics, or are used as drugs.

The two different types of photo-oxidation (Gordon, 2001) are summarized in figure 6:
Type I – an electron or a hydrogen atom is transferred between a sensitizer, in the form of triplet excited, and a substrate (PUFA), producing free radicals or radical ions;
Type II – triplet oxygen ($^3O_2$) can be excited by light to singlet oxygen ($^1O_2$), which reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide (Frankel, 1984) this reaction results in a formation of a trans configuration. By this process, that does not follow a chain reaction involving free radicals, oxygen is activated to singlet state for transfer of energy from the photosensitizer. The resulting singlet oxygen ($^1O_2$) generated in this process is extremely reactive. Linoleate is reported to react at least 1500 times faster with 102 than with the normal oxygen in the triplet ground state ($^3O_2$). This pathway for the formation of hydroperoxides is so rapid that it was indicated as a process capable of initiating free radical autoxidation.

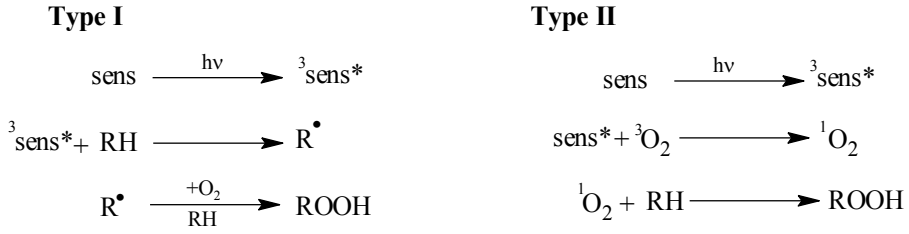


Figure 6. Different mechanisms of photosensitized oxidation of fatty acids.

1.4. Enzymatic oxidation

The third mechanism of oxidation is based on enzymatic activity of lipoxygenase (LOX). Lipoxygenase is a globular protein soluble in water consisting of a single polypeptide chain with a molecular mass of ~75–80 kDa in animals and ~94–104 kDa in plants. It belongs to the class of oxidoreductases and it is therefore capable of producing conjugated hydroperoxides through the oxidation of polyunsaturated fatty acids (Whitaker, 1994), and catalyzes the first reaction of so-called "way of lipoxygenase".

The "way of lipoxygenase" is of fundamental importance in the formation of all the volatile substances responsible for the aroma of fruits and vegetables that, in the case of olive oil, contributes markedly to the characterization and differentiation between different cultivars.

A molecule of LOX contains, at the active site, one iron atom in high spin state Fe 2+ that must be oxidized to Fe 3+, so the oxidation reaction may proceed. This enzyme stereoselectively catalyzes the non-reversible oxidation of PUFA containing the group 1,4-*cis,cis*-pentadiene (─CH=CH─CH_2─CH=CH─, commonly called malonic system), and yields a stereospecific conjugated diene hydroperoxides fatty acid (Tappel, 1963; Galliard, 1989). The sequence of four steps that describes the activity of this enzyme (Fig. 6) has been proposed by Tappel (1961) and completed by Hamberg and Samuelson (1967).

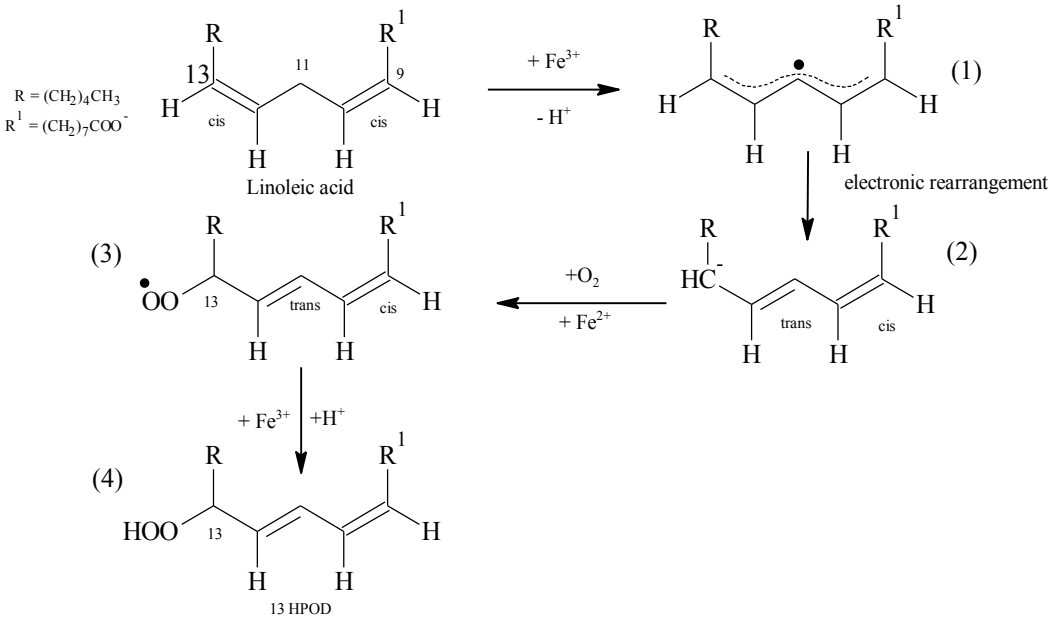


Figure 7. Mechanism of formation of 13-hydroperoxioctadienoic acid (13-HPOD) from linoleic acid by catalytic action of LOX.

The catalytic action of LOX is primarily exerted on the natural isomers of linoleic, linolenic and arachidonic fatty acids.

The iron (Fe) present as a coenzyme in the LOX appears to be involved in electron transfer during oxygen incorporation in unsaturated fatty acids containing the system *cis,cis*-1,4-pentadiene. The oxidized active form of LOX can catalyze the abstraction of stereospecific hydrogen from an active carbon present in specific fatty acids (*e.g.* methylene group C-11 of linoleic acid and linolenic acid) (O'Connor and O'Brien, 1991). It forms a delocalized pentadienyl radical and lipoxygenase is reduced to form Fe 2+ (Gardner, 1988).

1.5. Isomerization of fatty acid hydroperoxides and formation of diene conjugated

The bisallylic radical, formed during oxidation of PUFA, is not stable and undergoes an electronic rearrangement resulting in the shift of double bonds and in a partial isomerization from the *cis* form to the *trans* form. Oxygen attack produces more complex mixture of geometrical and positional conjugated and no conjugated diene hydroperoxides, which are very difficult to analyze qualitatively and quantitatively. The process of isomerization proceeds by a free-radical chain mechanism and the reaction condition modified the equilibrium of the hydroperoxides isomers (Fig. 5).

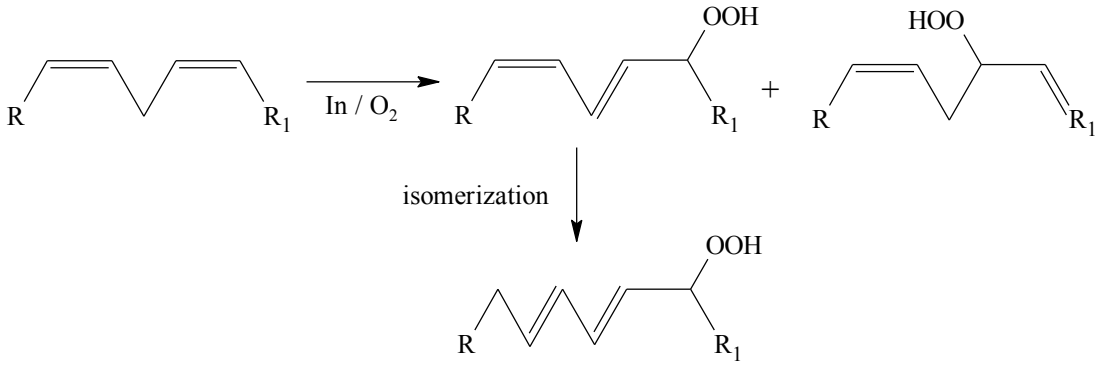


Figure 8. Formation of conjugated and no conjugated diene hydroperoxides from polyunsatured fatty acid by reaction with oxygen.

This fact was confirmed by Porter (1986) who have demonstrated that the *cis,trans* hydroperoxides are formed under kinetic control whereas *trans,trans* hydroperoxides are produced under thermodynamic control.

CHAPTER 2

DECOMPOSITION OF FATTY ACIDS HYDROPEROXIDES

2.1. Formation of secondary products of lipid oxidation through free radical reactions

The primary products of lipid autoxidation are the hydroperoxides (with the general formula ROOH), a class of chemical compounds non-volatile, odorless and tasteless, but relatively unstable and reactive.

Hydroperoxides decompose readily by hemolytic cleavage to form alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals.

$$2\ ROOH \longrightarrow ROO^{\bullet} + RO^{\bullet} + H_2O$$

The radicals formed are highly reactive and decomposed quickly by the homolytic scission of a carbon-carbon bond e/or oxygen-oxygen bond to produce compounds of various chemical nature including aldehydes, ketones, esters, alcohols, hydrocarbons, furans, epoxides and lactones as well as polymers. These compounds are flavor-active, particularly aldehydes, that possess a low threshold values in parts per million or even parts per billion levels, thus they are responsible for the development of warmed-over flavor (WOF), as coined by Tims and Watts (1958), and meat flavor deterioration (MFD) (Drumm and Spanier, 1991).

The possible routes of formation of secondary products of lipid oxidation from alkoxyl and peroxyl radicals are shown in Fig. 9 and Fig. 10.

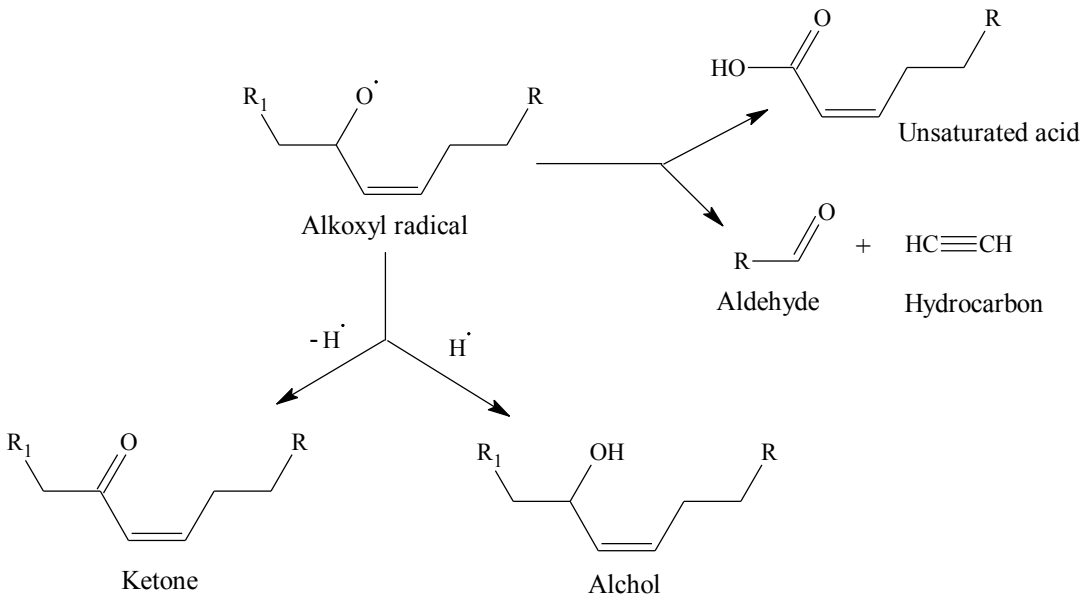


Figure 9. Formation of secondary products of lipids oxidation from alkoxyl radical**.**

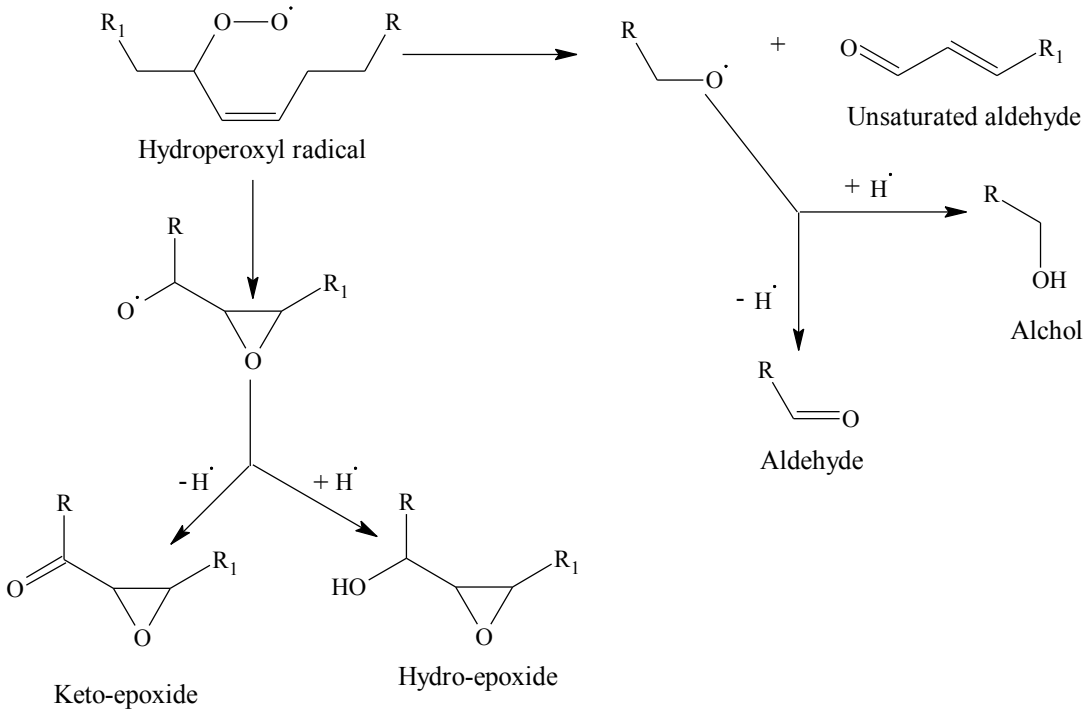


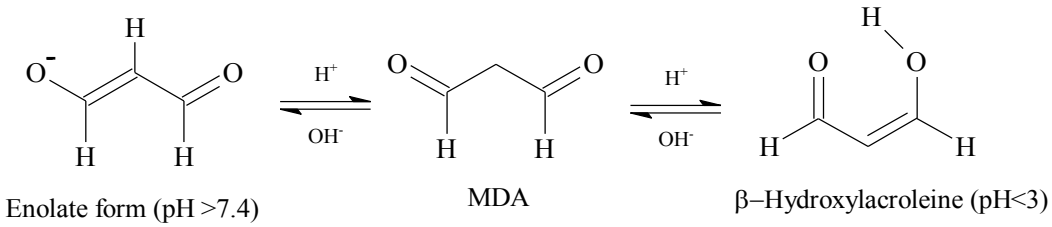
Figure 10. Formation of secondary products of lipids oxidation from hydroperoxyl radical.

Secondary lipid oxidation products contribute to the organoleptic deterioration of foods (color, flavor, taste, texture and safety) loss of nutritional value and their wholesomeness; in addition they are implicated in biological oxidation and may also interact with other foods components and change their functional and nutritional properties.

In conclusion, decomposition of lipid hydroperoxides constitutes a very complicated process and produces a multitude of materials that may have biological effects and cause flavor deterioration in fat-containing foods (Frankel, 1984). The variety of fatty acids present in lipid foods and the random nature of free radical reactions determined the type of secondary lipid oxidation products, among them special attention should be paid to Malondialdehyde (MDA). This compound, formed by degradation of fatty acids with three or more double bonds, is the principal and most studied product of polyunsaturated fatty acid peroxidation and can be considered as the major indicator of the extend of lipid oxidation, both for the early appearance as oxidation occurs and for the sensitivity of the analytical method.

2.2. Malondialdehyde (MDA)

Malondialdehyde (MDA) is a highly reactive three carbon dialdehyde, with carbonyl groups at the C-1 and C-3 position, produced as a byproduct of polyunsaturated fatty acid peroxidation (Janero, 1990) and also, *in vivo*, during arachidonic acid metabolism for the synthesis of prostaglandins (Mamett, 1999). Aldehydes are generally reactive species capable of forming adducts and complexes in biological systems and MDA is no exception although the main species at physiological pH (7.4) is the enolate ion which is of relative low reactivity, under more acidic condition ($pH \leq 4$), β-hydroacroleine is the predominant form that is a very reactive electrophile capable of reacts with a large number of biological molecule.



Enolate form (pH >7.4) MDA β–Hydroxylacroleine (pH<3)

Figure 11. Structural changes of malondialdehyde with the pH.

In conclusion, MDA can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA. The monitoring of MDA levels in foodstuffs and in biological materials can be used as an important indicator of extent of lipid peroxidation in vitro and in vivo for various diseases.

2.3. Formation of malondialdehyde

Peroxidation of the polyunsaturated fatty acids of biological membranes in cells can result in many potential genotoxic products, with malondialdehyde (MDA) being a major example. Different hypothesis about the possible mechanisms of MDA formation, through decomposition of lipid hydroperoxides from PUFA with two or more methylene-interrupted double bonds, have been proposed. Pryor and Stanley (1975) hypothesized that the mechanism of formation of MDA can also be formed as a by-product of arachidonic (C20:4 n-6) acid metabolism in the biosyntesis of prostaglandin involves the formation of prostaglandin-like endoperoxides (Fig. 12).
These non-volatile precursors decompose under stress condition (heat or acids) to form MDA. This mechanism has been confirmed by a subsequent study carried out by Frankel and Neff, (1983). An alternative mechanism, proposed by Esterbauer et al., (1991), is based on successive hydroperoxide formation and β-cleavage of polyunsaturated fatty acids. MDA is then directly formed by β-scission of a 3-hydroperoxyaldehyde or by reaction between acrolein and hydroxyl radicals (Fig. 13).

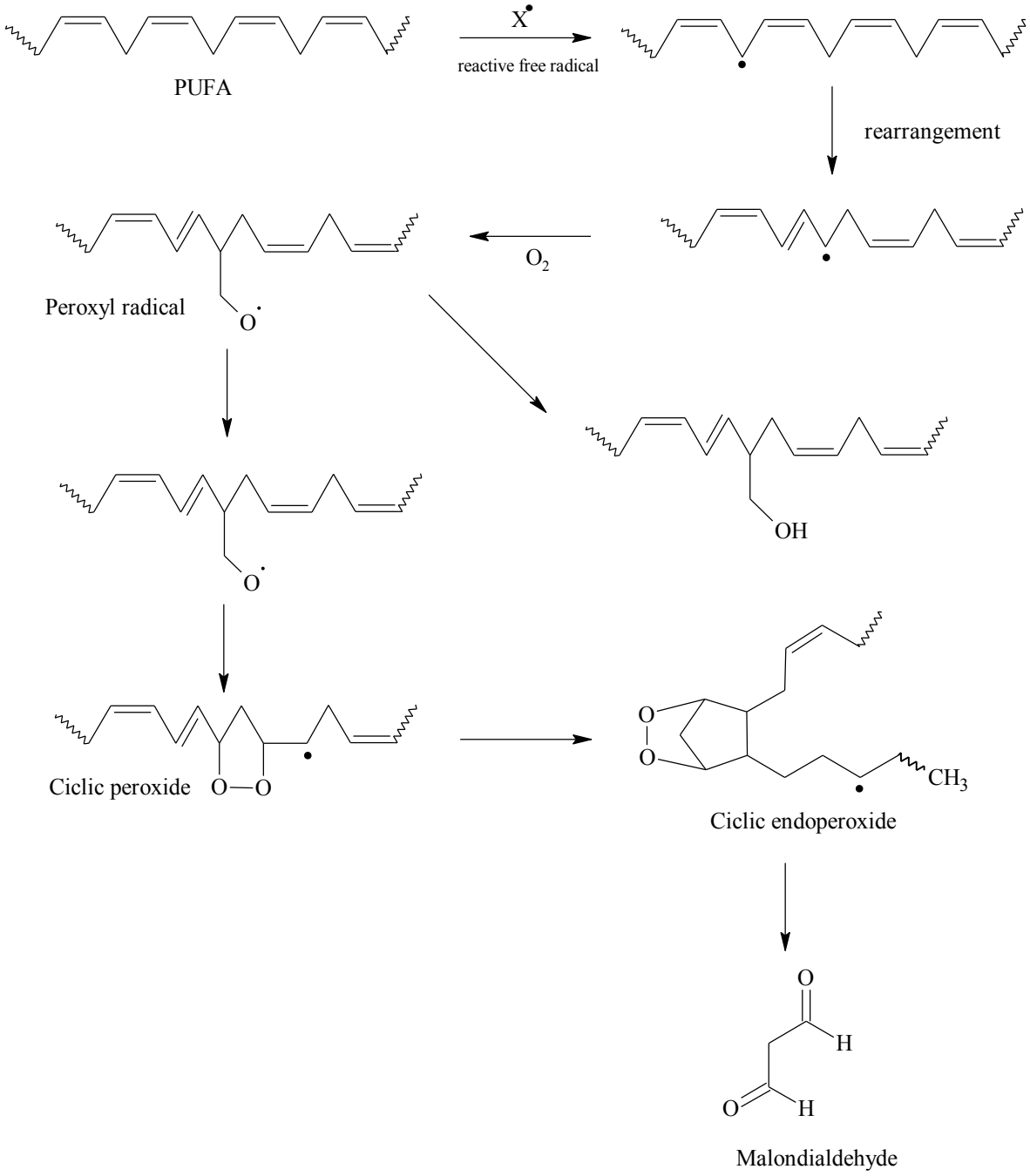


Figure 12. Mechanism of formation of Malondialdehyde from prostaglandin-like endoperoxides (reproduced from Singh et al.,(2001)).

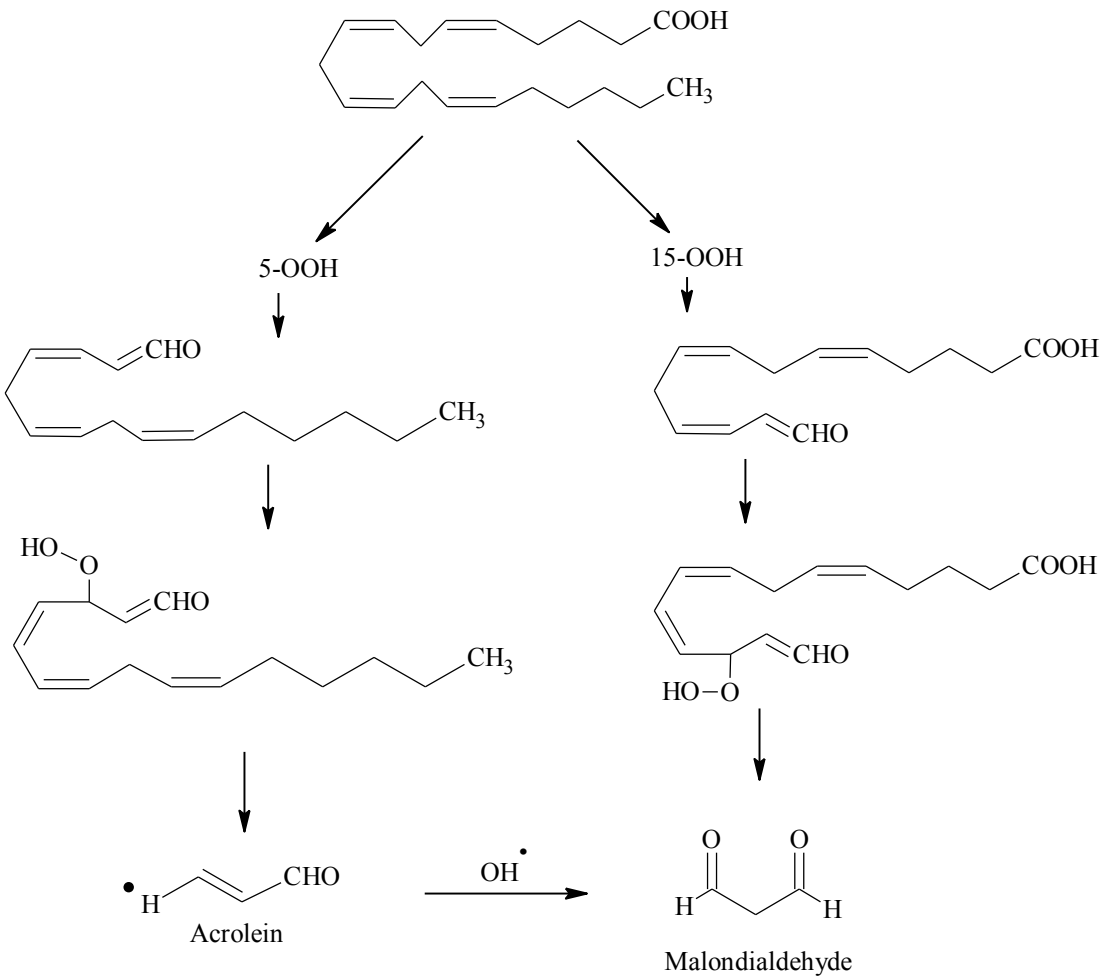


Figure 13. Mechanisms of formation of Malondialdehyde proposed by Esterbauer et al., 1991 from Arachidonic acid.

2.4. Chemical and biological properties

MDA or malondialdehyde is also called malonaldehyde (IUPAC) or propandienale. It has the molecular formula $C_3H_4O_2$ and molecular weight 72.06. The melting point is 72–74°C (IARC, 1985). Highly pure MDA is quite stable under neutral conditions but not under acidic conditions such as those used to prepare it by hydrolysis of its bis(dialkyl)acetal (see Figure .

MDA has been detected in the leaves of pea and cotton plants. It is found in many foodstuffs and can be present at high levels in rancid foods. It has been detected in fish meat, fish oil, rancid salmon oil, rancid nuts, rancid flour, orange juice essence, vegetable oils, fats, fresh frozen green beans, milk, milk fat, rye bread and in raw, cured and cooked meats (United States National Library of Medicine, 1997).

MDA is found in human and animal tissue as an end-product of lipid peroxidation. It is also a side-product of prostaglandin and thromboxane biosynthesis. MDA is present in blood platelets and in serum (IARC, 1985). This aldehyde is a highly toxic molecule and should be considered as more than just a marker of lipid peroxidation. Its interaction with DNA and proteins has often been referred to as potentially mutagenic and atherogenic (Del Rio et al., 2005). Lefevre et. al., (1995) reported that the majority of MDA present in human plasma is bound to protein; this would explain the very low levels of MDA in plasma as measured under standard assay conditions.

This molecule, how reported by Marnette (1999), is able to interact with nucleic acid bases to form several different adducts such as deoxyadenosine (M_1dA), and deoxycytidine (M_1dC) and deoxyguanosine (M_1dG).

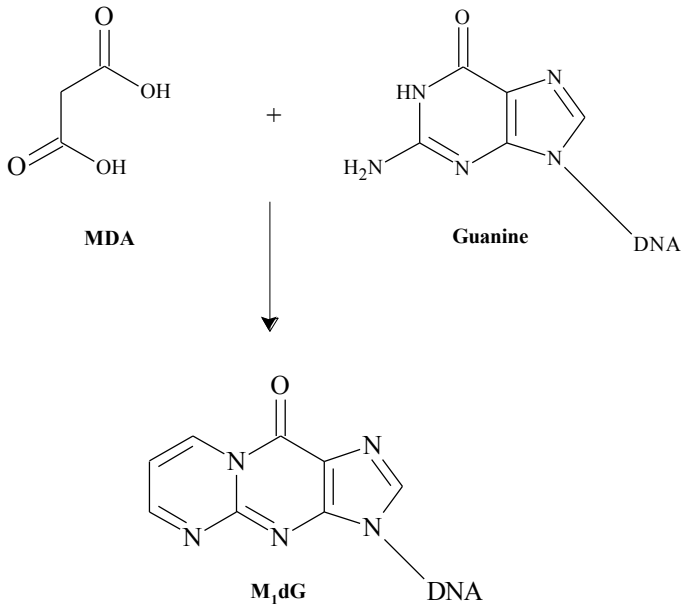


Figure 14. Reaction of MDA with deoxyguanosine in DNA

The major adduct is a pyrimidopurinone (M_1dG) (Figure 14) that has been shown to be mutagenic. MDA and its tautomer β-hydroxyacrolein react with guanine in DNA to form the pyrimidopurinone adduct (M_1dG). When M_1dG correctly paired with cytosine in duplex DNA, M_1dG undergoes a base-catalyzed conversion to an acyclic structure, N_2-OPdG, which is known to be highly mutagenic in bacteria and mammalian cells, inducing both frame shifts and base substitutions (Cline S. D., et al., 2004). All of these potentially genotoxic activities of MDA may lead to mutations and subsequently to cancer.

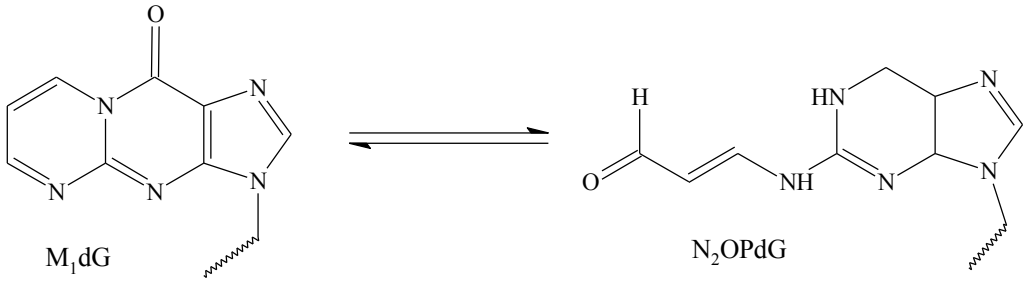


Figure 15. Structure of the main product of malondialdehyde-DNA interaction M1dG in equilibrium with the open form N_2-oxopropenyl-guanosine (N_2OPdG).

The toxicity of MDA in vivo also affects the cardiovascular system, studies show that it reacts with primary amines in lipoproteins (LDL) generating lysine-lysine cross-links. These reaction products were detected in apoB fractions of oxidized LDL and they are responsible of the initiation of the atherosclerotic lesion (Uchida K., 2000).

CHAPTER 3

EFFECTS OF ANTIOXIDANTS ON LIPID OXIDATION

3.1. Generality and mechanisms of action

Antioxidant (AH) is defined as "*any substance that is present in very low concentration compared to that of an oxidisable substrate, can delay or significantly inhibit the oxidation of that substrate"* (Halliwell & Gutteridge, 1989).

Antioxidants (AHs) are additives that play an increasingly prominent role in the conservation and food processing. Their role is the ability to inhibit the development of oxidative rancidity of foods that contain fat and especially meat, dairy products and fried foods. The antioxidants used in foodstuffs are generally added in order to maintain the freshness and prevent browning, rancidity and the development of unpleasant flavors, for so they are particularly important for foods containing large amounts of fats or oils, which are the most vulnerable to the development of oxidative rancidity. Thus, the addition of antioxidants to packaged food products significantly extends their shelf-life, and keeps fresh flavors and aromas as long as possible. The use of antioxidants in food products is governed by regulatory laws of the individual country or by internal standards. Even though many natural and synthetic compounds have antioxidant properties, only a few of them have been accepted as 'generally recognized as safe (GRAS)' substances for use in food products by international bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Community's Scientific Committee for Food (SCF). Toxicological studies are crucial in determining the safety of an antioxidant and also in determining the acceptable daily intake (ADI) levels (Miková, 2001).

There are various types of antioxidants, these can be grouped into two broad categories: natural and synthetic. Natural antioxidants are those found as natural components of fruits, vegetables, cereals and meat, where they play a protective action. Synthetic antioxidants are those created in the laboratory and are widely used in the preservation of food. However, synthetic and natural antioxidants may differ in their levels of performance. Synthetic antioxidants have been shown to have superior efficacy, low cost and more stability in food than the natural ones, nevertheless the suspicion that these may be potentially carcinogenic limiting their use in the food industries. Instead,

natural ones are known to have more beneficial effects on health such as the ability to prevent diseases such as cancer, cardiovascular diseases and those related to aging.

The choice of the type of antioxidants to be used depends mainly on the intrinsic characteristics of the food system, the compatibility of products and regulatory guidelines which govern its use (Giese J., 1996). In conclusion, the antioxidants used in food must be inexpensive, nontoxic, effective at low concentrations, stable and not cause any change in color, taste and smell.

Antioxidants can be also classified by mechanism of action as primary antioxidants and secondary antioxidants. Primary antioxidant or type 1, or chain-breaking antioxidants are free radical acceptors that delay or inhibit the initiation step or interrupt the propagation step of autoxidation. Primary antioxidants react with free radicals, formed during lipid oxidation, giving them a hydrogen atom, this reaction leads to the formation of more stable, non-radical products and radical antioxidants ($A^{\bullet}$) that are more stable and less readily available to further promote autoxidation. Chain-breaking antioxidant reacts directly with the various types of free radicals formed during lipid oxidation, such as peroxy, alkoxy and alkyl radicals, the reactions are briefly summarized in the following equations:

$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$

$$RO^{\bullet} + AH \longrightarrow ROH + A^{\bullet}$$

$$R^{\bullet} + AH \longrightarrow RH + A^{\bullet}$$

The most commonly used primary antioxidants in foods are synthetic compounds. However, a few natural components of food also act as primary antioxidants and are commonly added to foods. Tocopherols and carotenoids are the most commonly used natural antioxidants that have primary antioxidant activity, although the mechanism of carotenoids differs from this of the phenolics.

Secondary antioxidant or type 2, or preventive antioxidants act through numerous possible mechanisms. These antioxidants slow the rate of oxidation by several different actions,but they do not convert free radicals to more stable products. Secondary antioxidants can chelate prooxidant metals and deactivate them (EDTA), replenish hydrogen to primary antioxidants, decompose hydroperoxides to nonradical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers.

These antioxidants are often referred to as synergists because they promote the antioxidant activity of primary antioxidants.

Ascorbic acid is a good example of synergists and represents a truly multifunctional antioxidant, in fact it is able to quench singlet oxygen, reduce free radicals, and remove molecular oxygen in the presence of metal ions, it is also a primary antioxidant.

Antioxidants not only extend the shelf life of the food systems, but reduce the waste of raw materials and nutritional losses, and expand the range of fat that can be used in the food industry (Coppen P.P., 1983).

3.2. Natural antioxidants

Natural antioxidants are found as normal constituents of foods such as fruits, vegetables and meat. There are several common natural antioxidants and the most common are presented in figure 15.

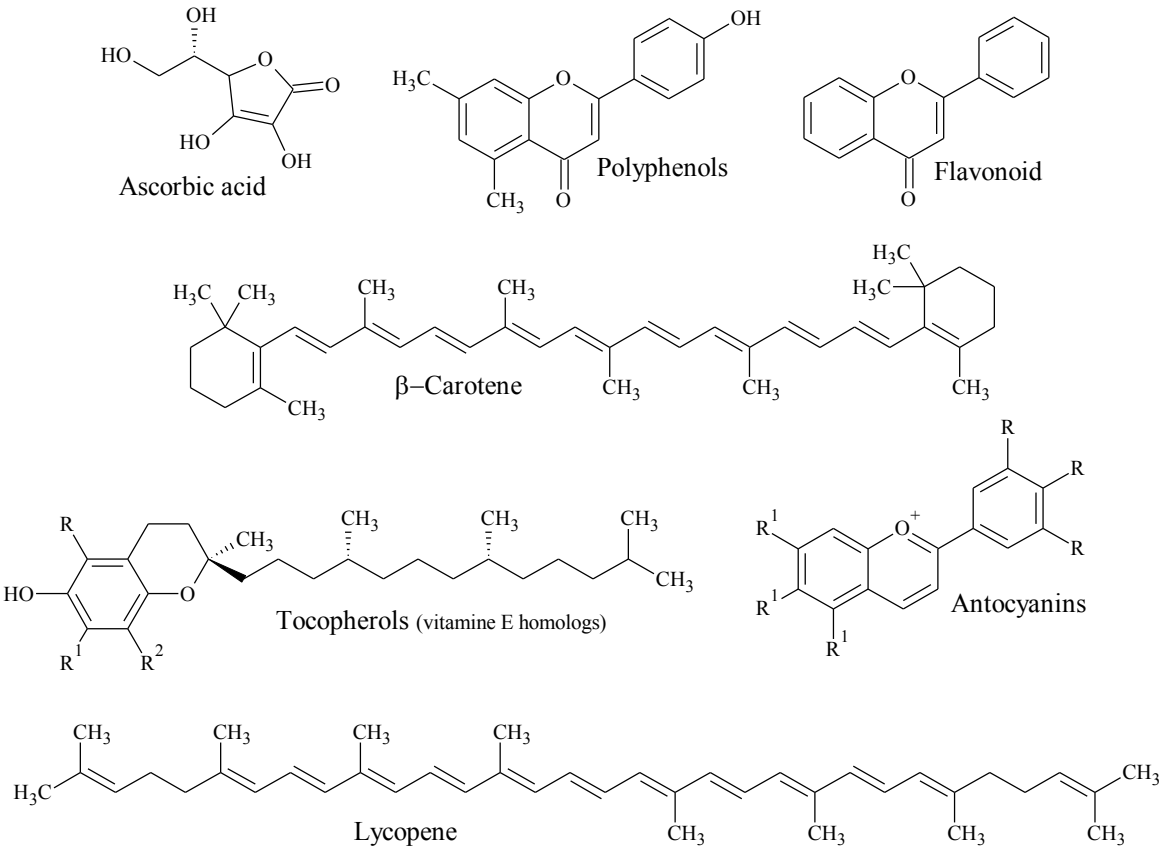


Figure 16. Chemical structures of the major and most common natural antioxidant.

Each natural antioxidant is available in a wide range of natural sources. For each of them the recruitment and the amounts to be taken vary in order to be able to maximize their absorption. Table 3 shows the typical natural sources of the main natural antioxidants.

Table 3. The most common natural antioxidant used as food preservatives, and their natural sources.

Natural antioxidant (name)	Natural source
Vitamine C (Ascorbic acid)	Most fruits (particularly citrus fruits), some vegetables, tomatoes.
Vitamine E / Tocopherols	Cereal grains, broccoli, Brussels sprouts, cauliflower, cooking oils (olive, sunflower, safflower), almonds, hazelnuts.
Beta-Carotene	Vegetables such as kale, red paprika, spinach, parsley, and tomatoes, carrots, sweet potatoes, apricots, papayas.
Flavonoid (a type of Polyphenol)	Potatoes, tomatoes, lettuce, onions, wheat, dark chocolate, Concord grapes, red wine, black tea.
Antocyanin (a type of Flavonoid)	High content in red wines, some in whiskey, sake.
Various Polyphenols (e.g. catechins)	Teas (mainly green, some rooibos), as well as many red/purple hued fruits or vegetables, such as Concord grapes, red cabbage, blueberries, blackberries, açai berries, etc.
Lycopene	Tomatoes, papaya, watermelon, pink grapefruit, guava, the skin of red grapes.
CoQ10 (an antioxidant enzyme)	Wheat bran, fish, organ meats (eg. Chicken liver)

Vitamin C or ascorbic acid is one of the main natural antioxidants that can be taken daily with food. It is a small polar molecule, soluble in water which is found mainly in fruits especially citrus fruits (eg oranges and lemons) but is found in some vegetables such as tomatoes. With extensive laboratory tests has been shown that taking a daily amount of 30 mg of Vitamin C ensures the maximum absorption of 50%, instead if increasing the dose administered the absorption decreases drastically. Therefore it is recommendable, so that it may maximize absorption, consume small doses during the

entire day rather than the consumption in a single administration of high doses (Daniel, 1986).

The tocopherols, which also include vitamin E (α-tocopherol), are natural antioxidants, non-polar and soluble in fats. It mainly come from the vegetable world, where they are distributed abundantly in various products such as seeds of cereal grains, leafy vegetables, seeds and oleaginous fruits and their oils. The bioavailability is about 20-40% of the amount ingested and, as for vitamin C, this percentage decreases with increasing levels of intake. Studies have shown that their bioavailability can be significantly improved if consumed in conjunction with dietary fat.

The β-carotene, a precursor of vitamin A, is a natural antioxidant fat-soluble belonging to the carotenoid family. In nature, carotenoids are found abundantly in vegetables (e.g. kale, carrots, potatoes) and orange-hued fruits (such as apricots and papaya), their bioavailability is considerably low, a daily intake of 1200 mg is needed to achieve a plasma level of 30 mg, and comparison to other natural antioxidants, the level of absorption decreases with increasing of the amount ingested. Lycopene is another fat-soluble antioxidant belonging to the family of carotenoids, but unlike other carotenoids cannot be converted into vitamin A. Lycopene is found mostly in tomatoes and products derived thereof. Furthermore belong to the family of carotenoid the natural extracts of the red hot chili pepper (Capsicum annus) capsanthin and capsicina used in food industry for their antioxidant and flavoring property, which recently have been recognized as a beneficial effect of regularization of the blood circulation, in addition it was found that in regions where its widespread use, diseases such as heart attacks, arteriosclerosis and excess cholesterol is considerably limited. Carotenoids being fat-soluble are best absorbed in presence of dietary fat.

Polyphenols, another great class of natural antioxidants, including flavonoids and anthocyanin, are commonly found in products such as tea (green and black), dark fruit (such as berries, blueberries, blackberries), dark chocolate and red wine. The bioavailability of these compounds is very low and it is estimated around 15-20% of the amount consumed. The absorption increased if the consumed polyphenols are not bound with sugar molecules, therefore polyphenols from tea have major level of absorption to those from dark fruits.

The final common type of natural antioxidant found in foods is the protein CoenzymeQ10 (CoQ10). Coq10 is a small, soluble protein used by animals as a natural antioxidant. Thus, it is found in substantial quantities in food sources such as fish and other meats, particularly organ meats (e.g. Chicken liver), as well as in high-protein plant sources such as wheat bran. The bioavailability of CoQ10 from meat sources and poultry has been seen to be approximately 60%.

Vitamin E, ascorbic acid and β-carotene are chain-breaking antioxidants (Halliwell and Gutteridge, 1989); they are capable of scavenging free radicals and by this, prevent or delay the onset of the lipid peroxidation process (Maraschiello et al., 1999). In the living cells ascorbic acid is located in the cytosol whereas the liposoluble vitamin E and β-carotene are integrated within the cellular plasma membrane (Halliwell and Gutteridge, 1989; Halliwell et al., 1995). Generally, in living cells the original pool of chain-breaking antioxidants, can be reintegrated and sustained through the diet (Thomas, 1995). Instead in food systems, especially in meat, this is impossible due to post-mortem conditions, in the sense that the pool of primary antioxidants presents in the muscle cellules cannot regenerate, restore and repair itself. Buckley and Morrissey (1994) have demonstrate that supplementation of antioxidants in the diet is an effective way to increase the oxidative stability of raw and cooked meat. The accumulation of vitamin E in the muscle cells membranes, as consequence of dietary supplementation, increased lipid oxidative stability of the meat following cooking and storage (Buckley and Morrissey, 1994; Buckley et al., 1995; Liu et al., 1994; Liu et al., 1995; Maraschiello et al, 1998; Yang et al., 2002; Realini et al., 2004).

3.3. Synthetic antioxidants

Synthetic antioxidants are intentionally added to food formulations to inhibit lipid oxidation. A range of synthetic antioxidants are authorized for use in food system and animal feed to protect against oxidation of unsaturated lipids. These include ethoxyquin (EQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), dodecylgallate (DG), octylgallate (OG) and propylgallate (PG), tertiary butylhydroquinone (TBHQ) and Etylendiaminetetraacetic acid (EDTA) (Figure 16).

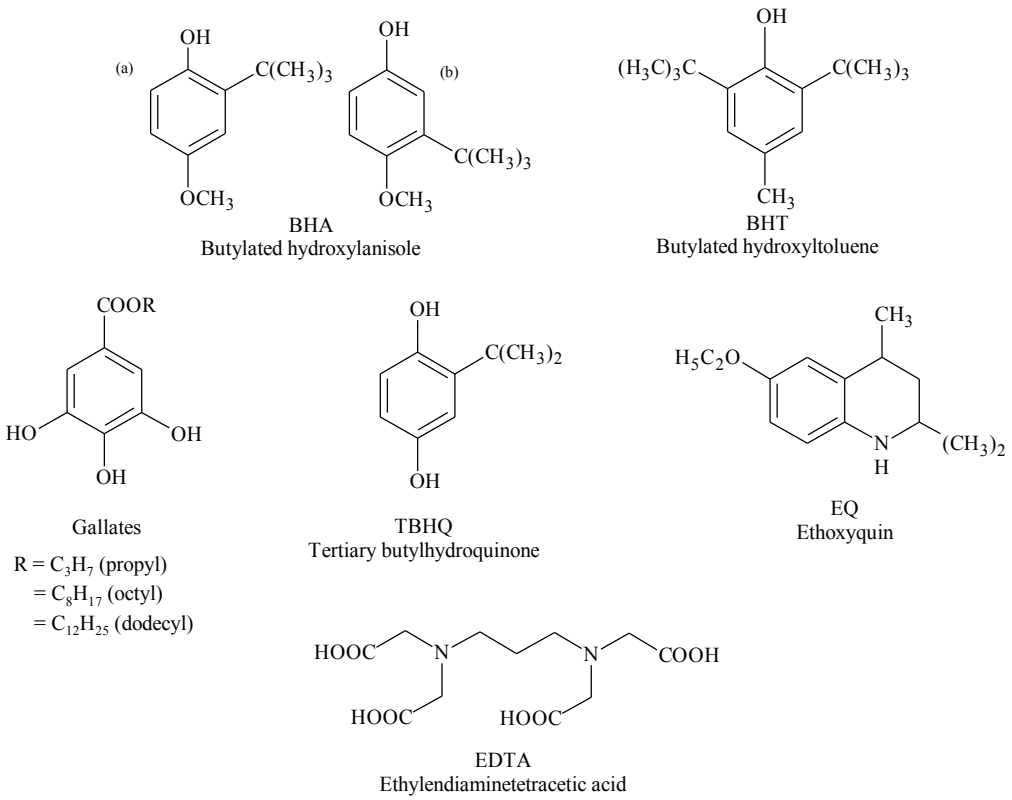


Figure 17. Chemical structure of main food antioxidants of synthetic origin.

The information found in literature relating to synthetic antioxidants are contradictory and their use has been questioned due to their potential health risks and toxicity (Kahl and Kappus, 1993), in fact studies have shown that these products have anti-oxidant and anti-carcinogenic properties for human health, while other researchers have recognized the synthetic antioxidants, despite their antioxidant capacity, as potential carcinogens and tumor promoters (Iverson, 1999;. Botterweck et al 2000). These antioxidants may be used at 200 ppm in bulk oils and at 200 ppm, based on the lipid content of other foods (Shahidi F., 2000). Synthetic antioxidants such as BHA and gallates were introduced in the 1940s when to BHA was recognized the ability to delay the progression of lipid oxidation in oils and when was discovered the antioxidant efficiency of various alkyl esters of gallic acid. Later, in 1954, was approved in the U.S.A. the use of BHT as food antioxidant additive while the commercialization and widespread use of TBHQ began in 1972.

Since 1948 Propyl gallate (PG, E310) has been added to foods containing oils and fats and has been used in the cosmetic industry, in the manufacture of lipsticks, to prevent oxidation.

Butylated hydroxyanisole (BHA, tert-butyl-4-hydroxyanisole) is a synthetic phenolic antioxidant, authorized as a food additive (E320) in the European Union for certain food products including cake mixes, cereal-based snack foods and milk powder, while it cannot used for food application in Japan and a number of other country.

Butylated hydroxytoluene (BHT, 2,6-di-tert-butylp-cresol, E321) is another synthetic phenolic antioxidant authorized as a food additive (individually or in combination with other synthetic antioxidants authorized in food) in the European Union for a range of products including sauces, chewing gum and oils. In contrast to BHT and BHA, EQ (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, E324) is only authorized as a feed additive in the European Union. The use of TBHQ (E319) as a food additive is not allowed in Canada, Japan and the European Union.

Another important synthetic antioxidant is ethylenediaminetetraacetic acid (EDTA, E385) which belongs to the category of chelating agents or metal deactivator. It is able to form complexes with pro-oxidative metal ions such as copper and iron, through a not shared pair of electrons present in its molecular structure, thus binding to the reactive metals leads to a decrease in their levels of free, soluble and reactive. It is mostly used in processed fruits and vegetables, soft drinks, salad dressings, margarine and canned sheelfish. It is banned in Australia and some other countries. The safety of synthetic antioxidants has been evaluated several times by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the former European Commission's Scientific Committee for Food (SCF) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in the case of EQ (Lundebye A.-K. et al., 2010).

The various properties of the important synthetic antioxidants discussed in this section are summarized in Table 4.

Compound name*	Chemical structure / Mechanism of action	Morphology / Solubility	Adeguate Daily Intake (ADI)**	Food and maximum level (mg/kg)***	Country banned in
BHA (butylated hydroxyanisole E320)	Monohydric Phenol Radical scavenger	White waxy flakes. Soluble in fat, insoluble in water	0-0.5 mg/kg bw	Cereals, chewing gum, potato chips, vegetable oils, biscuits, cakes, pastries, sugar, honey, spices, meat products, milk products, etc. (200 mg/kg)**	Japan
BHT (butylated hydroxytoluene E321)	Monohydric Phenol Radical scavenger	White crystalline compound. Soluble in fat, insoluble in water	0–0.125 mg/kg bw	Cereals, chewing gum, potato chips, oils, biscuits, cakes, pastries, beverages, sugar, honey, spices, meat products, milk products, etc.(100 mg/kg)**	None
PG (Propyl Gallate E310)	Polyphenol Radical scavenger	White crystalline powder sparingly. Soluble in water	0-2.5 mg/kg bw	Vegetable oil, meat products, potato sticks, chicken soup base, chewing gum, sugar, honey, spices, milk products, etc. (200 mg/kg)**	None
OG (Octyl gallate E311)	Polyphenol Radical scavenger	White to creamy white crystalline solid. Insoluble in water	0-0.5 mg/kg bw	Oils and fats, cereals, snack foods, dairy produce, sugar, honey, meat products, etc. (200 mg/kg)**	None
DG (Dodecyl gallate)	Polyphenol Radical scavenger	White to creamy white crystalline solid. Insoluble in water	0-0.05 mg/kg bw	Oils and fats, cereals, snack foods, dairy produce, meat products, etc. (200 mg/kg)**	None
TBHQ (tertiary butylhydroquinone E319)	Diphenol Radical scavenger	Beige colored powder. Soluble in fats	0–0.2 mg/kg bw	Milk, milk products like cheese, meat & meat products, chewing gum, fish & fish products, sea food, sugar, honey, spices, etc.	Canada, Japan, European Union
EDTA (Ethylenediaminetetra acetic acid E385)	Polyamino carboxylic acid Metal chelator	Colourless solid. Water-soluble.	0-2.5 mg/kg bw	Salad dressing, margarine, sandwich spreads, soft drinks mayonnaise, processed fruits and vegetables, canned shellfish.	Australian
EQ (Ethoxyquin 324)	Derivative of quinoline Proton donor	Yellow viscous liquid. Soluble in most organic solvents	0-0.005 mg/kg bw	Livestock, aquaculture and pet foods, chilli and paprika powders.	None

Table 4. Additional data of some synthetic antioxidants conditionally permitted in foods.
*Antioxidants are indicated with the specific chemical name and with the EEC number. **Last Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation
***When a combination of gallates, BHA and BHT are used, the individual levels must be reduced proportionally and is variable in function of foodstuffs. bw = body weight

ADIs for widely used antioxidants such as BHA, BHT and gallates have changed over the years mainly because of their toxicological effects in various species. The acceptable daily intake (ADI) established by both JECFA and the SCF for BHA is 0-0.5 mg/kg body weight (bw) (JECFA 1999; Commission of the European Communities 1989). The ADI set by the SCF for BHT is 0.05 mg/kg bw (Commission of the European Communities 1989) on the basis of reproduction, thyroid and haematological effects in a 90-day feeding study in rats. In contrast, the ADI established by JECFAs on the basis of hepatic enzyme induction in a 22-month feeding study in rats is six times higher, namely 0.3mg/kg bw (JECFA 1995).

The application of antioxidants in foods for infants and young children and for particular nutritional needs is regulated by Directive 89/398/EEC. Antioxidants permitted in weaning foods for infants and young children in good health are listed in Table 5.

Table 5. Antioxidants permitted in foodstuffs for infants and young children.

E number	Common name	Foodstuffs	Maximun level
E330	Citric acid	Weaning food	*quantum satis*
E300	L-ascorbic acid	Fruit and vegetable-based drinks, juices and baby foods	0.3g / kg
E301	Sodium L-ascorbate		
E302	Calcium L-ascorbate	Fat-containing cereal-based food including biscuits.	0.2g / kg
E304	L-ascorbic palmitate	Fat containing cereals, biscuits, rusks and baby foods	*100 mg /kg Individually or in combination
E306	Tocopherol-rich extract		
E307	α-tocopherol		
E308	γ-tocopherol		
E309	β-tocopherol		
E322	Lecithin	biscuits and rusks, cereal-based foods, baby foods	10 g / kg

*10 mg/kg for follow-on formulae for infants in good health

The synthesis of novel antioxidants for food use is limited by rising costs of research and development, costs associated with safety assessment, and the time required to obtain regulatory approval of food additives. These restrictions, as well as growing consumer preference for natural food additives, have led foods industry to prefer natural foods as a source of new antioxidants. Food security will not only be an issue of protection, but also one of the maintenance and promotion of health. It also means that the regulatory and judicial system must be prepared to accept this expanded role for food in the matrix of national life and the international market. Major efforts are being made at the international level to obtain maximum coordination in regulation of principles for control and acceptance of new additives (Miková, 2001).

3.4. Enzymatic antioxidants

Living cells have several mechanisms of protection against oxidative processes, including antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px). Catalase and glutathione peroxidase (GSH-Px) are considered the major peroxide-removing enzymes located in the cytosol (Chan & Decker, 1994; Decker & Xu, 1998; Halliwell, et al., 1995). SOD plays an important role in protecting against damage by the superoxide anion radical (Chan & Decker, 1994).

The presence of these enzymatic antioxidants systems is particularly important in foods such as meat and meat products. Information on factors influencing the activity of antioxidant enzymes in meat is limited. Antioxidant enzyme activities differ between meat of different species (Pradhan, Rhee, & Hernández, 2000) and muscle type (Hernández, Park, & Rhee, 2002). Antioxidant enzyme activity could also vary between animals of a single species. Therefore, variations in the activity of these enzymes between different genetic types could lead to differences in oxidative stability of the meat.

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the conversion of two superoxide anions into oxygen and hydrogen peroxide (Bannister et al., 1987), as summarily shown in the following equations.

$$O^{-\bullet} + O^{-\bullet} \xrightarrow{\text{SODs}} O_2 + H_2O_2$$

The benefit here is that hydrogen peroxide is substantially less toxic that superoxide. SOD accelerates this detoxifying reaction roughly 10.000-fold over the non-catalyzed reaction.

SODs enzymes are present in almost all aerobic cells and in extracellular fluids. SODs are metal-containing enzymes that depend on a bound manganese, copper or zinc for their antioxidant activity (Zelko et al., 2002). In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms predominant in cytoplasm. SODs in extracellular fluids contains copper and zinc in its active sites. Interestingly, SODs are inducible enzymes, exposure of bacteria or vertebrate cells to higher concentrations of oxygen results in fact, in rapid increases in the concentration of SOD.

Catalases are enzymes that promote the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani et al.,2004). This protein is localized to peroxisomes in most eukaryotic cells.. Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism (del Río et al., 1992). Here, its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Despite its apparent importance in hydrogen peroxide removal, humans with genetic deficiency of catalase "acatalasemia" suffer few ill effects (Ogata, 1991).

Glutathione peroxidase is a group of enzymes that include glutathione, glutathione reductase, glutathione peroxidases and glutathione *S*-transferases (Meister et al., 1983). This system is found in animals, plants and microorganisms. Glutathione peroxidase (GPx) is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. The metabolic function of this enzyme is vital for cells, as it is part of a mechanism responsible for the metabolism and detoxification of oxygen. It is assumed that GPx can protect DNA from oxidative damage and consequently from mutation leading to neoplastic transformation of cells (Combs & Clark, 1985).

There are at least four different glutathione peroxidase enzymes in animals. Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides (Brigelius-Flohé, 1999). In addition, the glutathione *S*-transferases show high activity with lipid peroxides (Sharma et al., 2004). These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism.

In conclusion, several studies have been demonstrate that the addition of antioxidants to animal feed (Maraschiello et al., 1999) and to meat (Igene et al.,1985; Tang et al., 2001) inhibits lipid oxidation as reflected in decreased thiobarbituric acid-reactive substances (TBArs). Amongst the commercially available antioxidants which are used to inhibit lipid oxidation in food are tocopherols and synthetic phenolic derivatives, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The disadvantage of using tocopherols is that they can act as pro-oxidants at high doses (Frankel, 1998) and furthermore that some authors have reported that BHA and BHT have adverse effects in human cells and organs (Fujisawa et al., 2004; Kang et al., 2005). Even though glutathione peroxidases, like most other enzymes, lose their activity by heating (Lindmark-Månsson et al., 2001; Mei et al., 1994) they may be used in searching for a suitable antioxidant counteracting lipid oxidation.

CHAPTER 4

HEALTH AND NUTRITIONAL IMPLICATION OF LIPID OXIDATION PRODUCTS

4.1. Introduction

Nowadays nutritional and toxicological effects of lipid oxidation in food and the possible implications on human health after exposition to oxidized fats in the diet have attracted much interest. Although primary hydroperoxides and lipid polymers produced during lipid oxidation in food systems are not toxicologically important, toxic effects may be induced by secondary lipid peroxides and cholesterol oxides (Kubow S.,1990). Another major nutritional problem related to lipid oxidation of foods is due to the interactions occurring between the lipid oxidation products with other food components, particularly vitamins and proteins. These interactions result in various and numerous nutritional and sensory effects.

4.2. Effects of oxidized lipids on health

The toxicity of dietary oxidized lipid has been studied evaluating the effects of feeding with highly oxidized fat in experimental animals of various species.

Studies on the possible pathological significance of lipid oxidation products were carried out to monitor in particular the effects on human health of lipid hydroperoxides and secondary products of oxidation especially cholesterol oxides and malondialdehyde. The primary products of lipid oxidation – hydroperoxides – are generally decomposed in the stomach, but it is possible that at low doses they are converted to the corresponding hydroxyl fatty acids that were absorbed and transported to the blood. They can promote thrombosis, accelerate progression of atherosclerosis (Kubow S., 1990) and participate in the development of cancer in human (Addis P.B., 1996).

Recent studies have also demonstrated that the dietary lipid peroxidation products reduce activities of glucose-6-phosphate dehydrogenase and other enzymes involved in fatty acid synthesis in the liver. In addition administration of fats subjected to oxidation lead to cellular damage in various organs, cardiac fibrotic lesions, hepatic bile duct lesions and altered fatty acid composition of tissue lipids (Sanders, 1989; Addis & Warner, 1991; Kubow , 1990; Eder, 1999). This study also reported increased liver and kidney weights in animals fed oxidized oils.

Low molecular products of decomposition of hydroperoxides, such as aldehydes, are absorbed, in free form or conjugated with amino acids, from gastrointestinal tract to plasma and incorporated into liver and muscle. They are very reactive compounds capable of react with protein and DNA to form cross-link bounds that have been show mutagenic and cytotoxic activity. Furthermore, secondary lipid oxidation products, particularly malondialdehyde, may react with the free amino groups of proteins and lipids in the cell membranes to form cross-linked products between the various components of the membrane, which confer increased rigidity to the membrane.

Cholesterol is a molecule with an unsaturated bond at position Δ 5-6 of the sterol nucleus, therefore it is prone to oxidation (Maerker, 1987). The molecule undergoes autoxidation by a free-radical mechanism leading to the formation of hydroperoxides and then to a number of oxidation products, the so-called oxysterols. These oxidation products are found in many commonly-consumed foods and are formed during their manufacture and/or processing. Many different oxysterols (up to 70) have been identified (Schroepfer, 2000). The toxicity of oxidized cholesterols has been demonstrated in several studies. The oxysterols are absorbed more quickly than cholesterol from the intestinal tract and transported into the circulatory system. There is considerable evidence that some oxysterols are powerful artherogenic agents and have also cytotoxic and mutagenic properties (Addis.& Warner, 1991; Osada et al., 1998).

In conclusion, it can affirmer that not a single product but a mixture of lipid oxidation products can occur in daily diet. For this reason foods should be protected in any way to minimize their concentration in foods and eliminate their deleterious effects (Wąsowicz, 2004).

4.3. Interaction of lipid oxidation products with food components

Lipid oxidation in foods is initiated by free radical and/or singlet oxygen mechanisms which generate a series of autocatalytic free radical reactions. These autoxidation reactions lead to the breakdown of lipid and to the formation of a wide array of oxidation products. The nature and proportion of these products can vary widely between foods and depend on the composition of the food as well as numerous environmental factors.

The toxicological significance of lipid oxidation in foods is complicated by interactions of secondary lipid oxidation products with other food components. These interactions could either form complexes that limit the bioavailability of lipid breakdown products or can lead to the formation of toxic products derived from non-lipid sources.

A lack of gross pathological consequences has generally been observed in animals fed oxidized fats. On the other hand, secondary products of lipid autoxidation can be absorbed and may cause an increase in oxidative stress and deleterious changes in lipoprotein and platelet metabolism.

The primary products of lipid autoxidation the hydroperoxides are able to decompose lipid soluble vitamins (vitamin A, E, D and K) or its provitamins (carotenes) and, also water-soluble vitamin C (Gurr, 1988). As a result the intake of these vitamins is decreased, and the protective effects of these antioxidant vitamins are reduced.

Hydroperoxides and several secondary products of lipids oxidation react with proteins to form polymers and complexes involving protein-protein cross-links and lipid-protein cross-links. The overall effect of these reactions is damage to amino acids residues; lysine is particularly susceptible to such degradation. The interaction of lipids oxidation products with protein and others food components has also a significant impact on oxidative and flavor stability and texture during processing, cooking and storage (Frankel, 1998). All degradation that regards protein-containing foods will influence their nutritive value. These reactions also lead to the appearance of brown colour and production of unpleasant odours and taste (Korczak et al., 2000).

The reaction of malonaldehyde with various food constituents was studied (Kwon et al., 1965). In the presence of water, malonaldehyde exists mainly as its non-volatile enolate anion. As such, it can react with amino acids, proteins, glycogen, and other food constituents to form products in which the malonaldehyde exists in bound form. MDA reacts preferentially with histidine, lysine, arginine, tyrosine and methionine. Aqueous malonaldehyde can be converted to its volatile isomer by acidification only but acid and heat are necessary to release malonaldehyde from its bound state in proteins.

Interaction of MDA with carbohydrates could be demonstrated only with glycogen. The amount of MDA bound with glycogen was comparable to that bound by proteins (1.3-2.6 g MDA / mg food components).

The presence of reactive lipid oxidation products in foods needs more systematic research in terms of complexities of food component interactions and the metabolic processing of these compounds.

CHAPTER 5

METHODS FOR THE MEASUREMENT OF LIPID OXIDATION

5.1. Methods for estimating lipid peroxidation

The measurement of the extent of lipid oxidation may be useful to monitor the changes in chemical, physical or organoleptic properties of lipid in foods that occurs during oxidation. Many analytical methods have been developed to measure lipid oxidation products (Halliwell and Chirico, 1993), and procedures of quantitative analysis are being constantly improved.However, there is no uniform and standard method for detecting all oxidative changes in all food systems, and it is necessary to select an adequate and proper method for a particular application (Shahidi et al., 2002). The available common methods to monitor lipid oxidation in foods and biological systems can be classified based on what they measure, for example: losses and changes of unsatured fatty acids, absorption of oxygen, formation of free radicals and formation of primary and secondary products of lipid oxidation. The measurement of different parameters that characterize the oxidation of lipids can thus be performed with different physical, chemical and instrumental methods, in addition to these sensory tests may be useful to provide subjective and, in some cases, objective evaluation of oxidative deterioration in food systems. The measurement of several oxidation products enables assessment at different stage of the oxidative pathway, providing detailed information of this dynamic process of rancidity and oxidative deterioration (Devasagaya T.P.A., 2003). The diversity and abundance of methods used to monitor lipid oxidation reflect the complexity of this issue and confirm the fact that multiple methods should be applied to get the maximum information available (Wąsowicz, 2004). Each method shows both advantages and disadvantages, thus it is important to select the most adequate method, depending on the system under investigation and the state of oxidation itself. The use of two or more methods assessing both primary and secondary oxidation products is highly recommended (Shahidi et al., 2002).Table 3 summarized some of most common available chemical and physical methods for measuring lipid oxidation in foods and biological system.

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Table 6. Mainly chemical methods to detect and measure extend of lipid peroxidation.

Method	What is measured	Remarks
Analysis of fatty acids by GC or HPLC	Loss of unsaturated fatty acids	In foods containing fats or oils, unsaturated fatty acids are the main reactants whose composition changes significantly during oxidation. Changes in fatty acid composition provide an indirect measure of the extent of lipid oxidation. Lipids are extracted from food and subsequently converted into derivatives suitable for chromatographic analysis. Fatty acid methyl esters (FAME) are the derivatives frequently used for determination of fatty acid composition, usually by gas chromatography (GC). Measurement of changes in fatty acid composition is useful for identification of lipid class and fatty acids that are involved in oxidation reactions.
Iodometric determination (titration method)	Lipid peroxides	Reduction of ROOH with KI and measurement of I_2. Titration with thiosulphate ($Na_2S_2O_3$). Useful for bulk lipids (e.g. foodstuff). Method can be applied if other oxidizing agent are absent. Although iodometric titration is the most common method for measurement of peroxides, it suffers from several disadvantages. The procedure is time-consuming and labor-intensive.
Ferric ion complexes (colorimetric method)	Lipid peroxides	Reduction of ROOH with Fe^{2+} and formation of Fe^{3+} complexes. Absorption at 560nm of the blue-purple complex with xylenol orange (FOX method). This method is rapid, inexpensive, and not sensitive to ambient oxygen or light. The FOX method has been successfully adapted to a variety of applications.
Fluorescence	Aldehydes	Aldehydes such as MDA can react with compounds containing primary amino group to form fluorescent conjugate Shiff-Base. Formation of fluorescent products is a minor reaction pathway and has very complex chemistry, but is a highly sensitive method. A fluorescence assay has been successfully used to assess lipid oxidation in muscle foods and biological tissues.

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Table 6. (Continued)

Method	What is measured	Remarks
UV Spectrometry	Conjugate dienes and trienes	Conjugated dienes are typically produced during the formation of hydroperoxides from unsaturated fatty acids due to the rearrangement of the double bonds. The resulting conjugated dienes exhibit an intense absorption at 234 nm; similarly conjugated trienes absorb at 268 nm. An increase in UV absorption theoretically reflects the formation of primary oxidation products in fats and oils. Ultraviolet detection of conjugated dienes is simple, fast, and requires no chemical reagents and only small amounts of samples are needed.
TBA test	TBA reactive substances (TBArs), mainly Malondialdehyde (MDA)	Spectrometry technique. The test material is heated at low pH with thiobarbituric acid (TBA), and the resulting pink chromogen is measured by absorbance at 525-535nm. The TBA test can be performed with various procedures and it is used frequently to assess the oxidative state of a variety of food systems although lack of specificity and sensitivity. Despite its limitations, the TBA test provides an excellent means for evaluating lipid oxidation in foods.
Carbonyls	Total volatile carbonyl compounds	This spectrometry technique is related with off-flavour. Total volatile carbonyl compounds are reacted with 2,4-dinitrophenylhydrazine (DNPH) followed by the reaction of the resulting hydrazones with alkali. The orange colured 2,4-dinitrophenylhydrazone resulting is measured spectrophotometrically. The carbonyl compounds formed during lipid oxidation are an useful indicator of oxidation processes in food system.
p-Anisidine value (p-AnV)	Aldehydes (principally 2-alkenals and 2,4-alkadienals)	It is based on the color reaction of p-methoxyaniline (anisidine) and the aldehydic compounds (55). The reaction of p-anisidine reagent with aldehydes under acidic conditions affords yellowish products that absorb at 350 nm. The p-AnV is a reliable indicator of oxidative rancidity in fats and oils and fatty foods

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In this thesis will be studied in a more detailed way the TBA-test which is one of the most commonly used method to measure the extend of lipid oxidation in meat (Gray J.I.,1978). Although this method has been criticized because of its non-specificity (in addition to MDA other substances, also referred to as TBArs, may react with TBA lead to an overestimate of the pink-complex) and low sensitivity (small concentrations of MDA are not detected) the TBA-test provides an excellent means for evaluating lipid oxidation in foods, especially on a comparative basis.

5.2. 2-Thiobarbituric acid (TBA) test

Determination of the level of peroxides is a technique widely used in the laboratory to assessment the extend of oxidation in food, but due to the transitory and unstable nature of the peroxides and their susceptibility to environmental conditions (eg light and heat) this method is an indicator approximate of the dynamic of the lipid oxidation and results particularly useful in the initial stages of oxidation. A more appropriate indices per valutare the extend of lipid oxidation is the measurement of secondary oxidation products, such as MDA. The most widely used assay for the titration of MDA is a test based on the measurement of the pink fluorescent complex produced upon incubation with 2-thiobarbituric acid (TBA) at low pH and high temperature.

The 2-thiobarbituric acid (TBA) test for the measurement of lipid oxidation was discovered over 50 years ago and is now one of the most extensively used methods to measure the oxidative state of biological and food materials and for monitor the progression of deterioration of fat-containing foods at different stages of oxidation.

The reaction among MDA and two molecules of TBA proceeding by attack of MDA, in the monoenolic form, on the active methylene groups of TBA (Fig. 9) yields a pink TBA-MDA adduct that is measured spectrophotometrically at its absorption maximum at 525–535 nm (Sinnuhuber R.O. et al., 1958).

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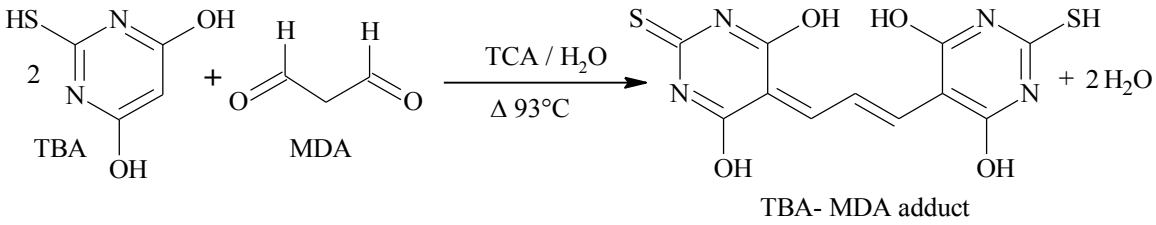

Figure 18. Reaction of 2-thiobarbituric acid (TBA) and malonaldehyde (MDA).

The intensity of colour is directly correlated with the concentration of MDA (Tarlagis et al., 1960) and also with the rancidity (Zipser et al., 1964) and with Warmed Off Flavor (WOF) (Igene et al., 1985). The extend of oxidation is reported as the TBA value and is expressed as milligrams of MDA equivalent for kilogram of samples or as micromoles of MDA equivalents per gram of sample. The quantification of MDA is carried out comparing the absorbance of the MDA-TBA adduct with a standard made from 1,1,3,3 tetraethoxy-propane (TEP) or 1,1,3,3 tetramethoxy-propane (TMP), in fact the MDA can be obtained by acid hydrolysis from TEP or TMP in an equimolecular reaction (Fig. 10).

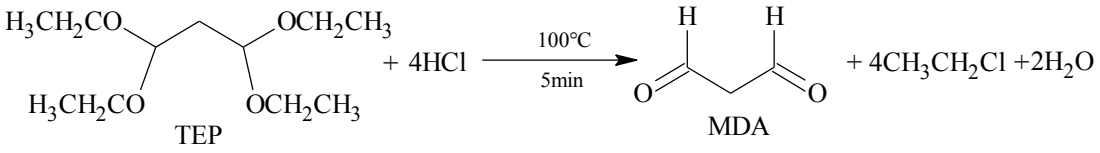

Figure 19. Equimolecular reaction of acid hydrolysis of TEP to obtain MDA.

The speed of the reaction of TBA with MDA depends on the concentration of the TBA solution, temperature and pH (Raharjo et al., 1992).

The TBA test in food can be performed by various procedures, the major types that have frequently been employed are reported subsequently:

a. Direct heating of the sample with TBA solution and subsequent extraction of the pink complex produced (Turner et al., 1954; Sinnhuber and Yu, 1958).
b. Distillation of the food samples and subsequent reaction of a portion of steam distillate with TBA (Tarlagdis et al., 1960; Rhee, 1978; Yamauchi et al., 1982; Ke et al., 1984).

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c. Lipid extraction with organic solvents from food sample and subsequent reaction of extract in acid condition with TBA (Pikul et al., 1983, 1989).
d. Aqueous acid extraction of MDA from food samples followed by acid reaction with TBA (Raharjo et al. 1992; Botsoglou et al., 1994; Maraschiello et al.1999).

Among all these, the method can be considered as the best for estimate the amount of MDA in food samples is the aqueous acid extraction method, because the sample is not subjected to heating as in method (a) is also faster and less consuming than distillation extraction method (b), and its use is recommended for the analysis of a large number of samples in a short time (Pikul et al. 1989).

However, low concentrations of MDA cannot normally be measured unless you use methods such as gas chromatography/mass spectrometry that are more expensive and time consuming (Yeo et al., 1994). Despite improvements in recent years, HPLC is not a widely used technique for the analysis of MDA in foods. In contrast, the chromatographic fractionation and fluorimetric detection of the TBA-MDA complex represent a procedure currently used to measure the concentration of MDA in samples of human blood (Kinter M., 1995) and food (St. Angelo A.J., 1996).

CHAPTER 6

NUTRITIONAL VALUE OF MUSCLE FOODS IN RUMINANT MEAT

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6.1. Meat consumption

Meat from ruminants is an integral part of a habitual balanced diet for many adults living in the western world. It is recognized that in over 2 million years of evolution, humans have been adapted to consuming large quantities of lean meat (Mann, 2000). Recently, several epidemiological studies have associated ruminant and processed meat consumption with the development of two of the major chronic diseases in the Western world: cardiovascular disease (CVD) and colon cancer (Cross et al., 2007; Kontogianni et al., 2008) and this has led to a negative perception of the role of meat in health.

The components of red meat that are considered be responsible for these associations are the fat content, fatty acid composition and the possible formation of carcinogenic compounds such as heterocyclic amines (HCA), as a result of cooking meat at high temperatures (Bingham, Hughes & Cross, 2002). Although many studies documenting the correlation between meat consumption and the development of chronic diseases, the results are not always consistent and there are several methodological issues that may limit these findings. As well as have been assessed the risks to human health associated with the consumption of red meat should be evaluated, in the same way, the many health benefits due to the consumption of red meat, this would be important to give the right information in terms of public health without erroneously demonizing the dietary intake of red meat.

Many factors such as wealth, the volume of livestock production, and socioeconomic status of consumers could explain the higher consumption meat from the western populations (Mann, 2000; Speedy 2003). Other factors which may affect the consumption of meat are sex, age, religion, vegetarian status, day of dietary recall, smoking behavior, education, body mass index (BMI) and total energy intake, as reported by the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (Linseisen et al., 2002). Table 7 shows mean daily intakes of total, red and processed meats among consumers of several European countries as measured by EPIC and NSIFCS (North South Ireland Food Consumption Survey).

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Table 7. Mean daily intakes of total, red and processed meat (g/d) by men and women as measured in several European country.

Country	Total meat		Red meat [a]		Processed meat [b]	
	Men	Women	Men	Women	Men	Women
UK[d]	108.1	72.3	40	24.6	38.4	22.3
Ireland[e]	167.9	106.6	63.9	37.5	30.9	19.9
Greece[d]	78.8	47.1	45.3	25.5	10	5.8
Spain[d]	170.4	99.2	74	37.8	52.8	29.6
Germany[d]	154.6	84.38	52.2	28.6	83.2	40.9
Italy[d]	140.1	86.1	57.8	40.8	33.5	19.6
Denmark[d]	141.1	88.3	69.6	44.1	51.9	25.3
Netherlands[d]	155.6	92.7	63.8	41.1	72.4	37.9

[a] Total meat: pork, beef, veal, lamb/mutton, poultry, game, rabbit, horse, goat, offal and processed meat.
[b] Red meat: beef, veal, pork and lamb/mutton.
[c] Processed meat: ham, bacon, processed meat cuts, minced meat and sausages.
[d] Source: Linseisen et al. (2002). [e] Source: Cosgrove et al. (2005).

Total meat can be divided into three sub-group, namely: red meat (including beef, lamb, veal and pork), white meat (including chicken, game and turkey) and processed meat (including cured and smoked meats; ham, bacon, sausages, hamburgers, salami and tinned meat) (Linseisen et al., 2002).

Data from EPIC studies show that in European country men tend to eat more meat than women, and this trend is also observed within other extra-European countries such as USA and Australia. Also, curiously, this study conducted in ten European countries revealed that Sunday was the day with the highest meat consumption; while meat intake was lowest on Friday. With increasing age the amount of meat consumed decreased significantly, in both women and men. Participants with a higher relative body weight (BMI) ate more meat and meat products than subjects with a low BMI. Also, smoking habit showed an effect on meat intake, with smokers revealing a significantly higher intake of meat than former or never smokers. With increasing educational level and sports activity, the meat intake values decreased.

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In the years have been developed a set of guidelines that provide information about dietary recommended intake levels for red and processed meat, in particular, leaving out the directives of the individual states, the World Cancer Research Fund (WCRF) 1997 report recommended that red meat intakes should be no more than 80 g/d, of which very little should be processed (WCRF, 1997). However after 10 years, the most recent report lowered this limit to 71 g/d or 500 g red meat per week and further emphasized that intakes of processed meat should be completely avoided (WCRF, 2007).

However in the last 20 years in most countries of the Western world has been a reported downward trend for the red meat intake. This is certainly related to public opinion that the consumption of red meat promotes the onset of CVD and cancer of the colon, along with public concerns food security arising from epidemic of bovine spongiform encephalopathy (BSE) which may have led to a lack of confidence by consumers in red meat (Verbeke et al., 2007). It is interesting to note, however, that despite the intake of meat in the UK is declining, the incidence colon cancer has increased significantly, contradicting experimental evidence that indicated the consumption of red meat as a cause of colon cancer (Hill, 2002).

Italy currently, the consumption of meat, on the whole, accounts for 21.5% of food expenditure of households (INEA, 2004) but this percentage has steadily declined in the last 20 years. The meat, in fact, represents one of the products food more symbolic in terms of both socio-economic and cultural and its consumption has always been subject to significant changes, closely related to the different socio-economic scenarios that have occurred in the past in Italy. After considerable growth in meat consumption that has occurred up the 1980s (Ismea, 1993), linked to the increase in economic welfare, then there has been a stabilization mainly determined by healthy and cultural motivations type (Fig. 19). Moreover, there was a propensity to diversify consumption of meat to that poultry or pigs, to the detriment of bovine meat, with the exception of veal, who has been a product that is supported by a particular image.

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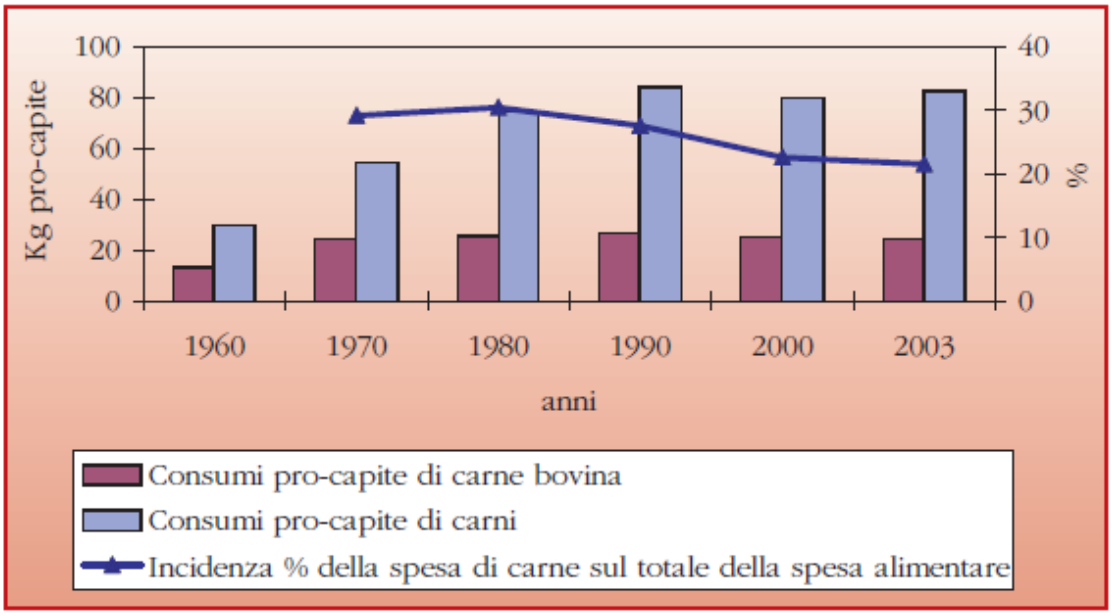

Source: Based on data from ISTAT and INEA. The latest data of per capita consumption of meat is related to 2002

Figure 20. Consumption per capita and percentage incidence of expenditure for the meat in Italy.

A separate mention must be made for the consumption of sheep and goat which is very limited in Italy and showed a negative trend over the past ten years, also in the same period, also reducing domestic production has decreased to 56% the production self-sufficiency. The Italian market for sheep and goat meat shows some peculiar characteristics that first of all it is mainly oriented towards the consumption of suckling lamb, which represents 58% of the total animals slaughtered in the country in year 2002 (Fig. 20. Source: ISTAT 2002), a second aspect concerns the seasonal pattern of demand (ISMEA, 1992). A survey on the consumption of small ruminants meat by the Italian consumers has demonstrated a clear prevalence of those who purchase this food only at major Christian festivities (75.2%), mainly Easter (60%) and Christmas (28%). This seasonality in consumption is particularly marked in northern Italy, while in the South and in the islands the consumption is much greater and is not seasonal but year-round, and it is addressed not only to suckling animals but also to heavy lambs and adult sheep.

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Table 8. Categories of sheep and goat slaughtered in Italy in the year 2002.

	Head (000)	Average live weight (Kg)	Dressing percentage (%)
Suckling lambs	5021.6	13	58.1
Heavy lambs	699.6	25	52.0
Adult sheep	608.4	42	48.7
Kids	500.2	10	61.2
Others	105.4	-	-

Source: Istat, 2002

Despite the Italian diet has remained fairly close to the Mediterranean model until the '60s, it was observed in the years following a sharp Mediterranean-profile departure from the consumption of vegetable protein and fewer (11 to 6%), a significant increase in the consumption of animal protein (2 to 7%), more and more fat, especially animal (7 to 21 %) all at the expense of carbohydrates (59 to 45%) (Processing INRAN on ISTAT and FAOSTAT data, 2011**).**

6.2. Meat quality and muscle lipids

Meat, and other animal foods such as milk and cheese, can make a valuable contribution to the diets due to their high nutritional value. Meat is a concentrated nutrient source essential for optimal growth and development. The importance of meat in the diet is due to the fact that it is highly nutritious foodstuff, it helps to provide proteins of high biological value, that contain all the essential amino acids and its amino-acid composition is complementary to that of cereals and vegetable proteins. It is also a good source of important micronutrients, such as B, D and E vitamins, minerals (e.g. iron, zinc, selenium and phosphorous), essential fatty acids and liver is a very rich source of vitamin A (Biesalski, 2005; Williamson et al., 2005). All these components are known to enhance well-being and health (Williamson et al., 2005; Schönfeldt & Gibson, 2008). However, in the last 10-15 years, these positive attributes have often been overshadowed due to the fact that the meat, and particularly the red meat, is frequently and incorrectly associated with a "negative" image for health because of its high content of fat, especially saturated fat, and specially the consumption of red meat has been commonly associated with the onset of cancer.

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Fat, especially animal fat, has been the subject of much concern and debate because of the association with risks for some chronic diseases when consumed in excess. Current insights and nutritional guidelines suggest the need to consider the quantity as well as the quality of fat in diet (Alfaia, 2009).

Meat is a very complex product whose characteristics can be influenced by a multitude of factors. Knowledge of muscle structure and how the muscle is converted into meat helps us understand what changes affect the nutritional and conformational characteristics of meat. The animal carcass consists of muscle, connective tissue, fat and bone and some 75% water in proportions depending on species, breed, size, age, etc. The composition of muscle (lean meat) of a given species is relatively constant, the largest variable in the composition of the carcass is the amount of fat, this in fact may change for the same species if they have been bred extensively or intensively or if they are wild or domesticated animals.

Fat in meat is present as cellular fat (as phospholipid), intramuscular fat (IF) (or marbling, i.e. within the muscles), intermuscular fat (IMF) (between the muscles), and subcutaneous fat (SF). Fat content varies widely depending on the cut and degree of trimming. The largest amount by fat is in the storage deposits under the skin and around the organs (SF). This constitutes the obvious, visible fat in a piece of meat and can be as much as 40-50% of the total weight in fatty meat or fatty bacon; this adipose tissue is composed largely of triglycerides. Clearly this visible fat can be trimmed off the meat during processing, before cooking or at the table, a growing practice in the western world.

Small streaks of fat are visible between the bundles of muscle fibres, IF, i.e. in the lean part of the meat; this is known as "marbling" and can amount to 4-8% of the weight of lean meat. Marbling fat is an important meat quality trait in relation to juiciness, aroma and tenderness and is the fat depot of most interest in relation to fatty acid composition and human health. It refers to the white flecks or streaks of adipose tissue between the bundles of muscle fibres (Scollan et al., 2006).

There are small amounts of fat within the muscle structure as membrane fat (or structural fats) in amounts varying with the tissue. This can be 1-3% of the wet weight of muscle. Structural fats are largely phospholipids and include long chain fatty acids.

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In conclusion, lipids are important structural and functional components of meat; they have an essential effect on its quality even their concentration is low (Saghir et al., 2005). Lipids in lean meat consist of 2-4% triacylglycerols and 0.8-1% phospholipids, of which 44% are polyunsaturated fatty acids and susceptible to lipid oxidation (Frankel, 1998).

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6.3. Dietary recommendation and nutrient composition of ruminant meat

6.3.1. Dietary recommendation

Today in developed countries the major causes of mortality are related to unhealthy diets and a sedentary lifestyle and the consumption of some dietary fat (e.g. SFAs, TFAs and cholesterol) has been associated with a higher incidence of chronic diseases such as coronary heart disease (CHD), obesity, diabetes type-2, hyperlipidemia and hypertension, artherosclerosis and cancer (McAfee et al., 2010). This has contributed to the development of specific World Health Organization guidelines in relation of fat in the diet in accordance of the widespread consensus that a ‘‘balanced diet’’ has preventive effects on chronic non-deficiency diseases, e.g. obesity, type 2 diabetes, cancer and cardiovascular diseases. (WHO, 2003). These guidelines include the following targets for fat intake: total dietary fat, 15–30% of energy (En%); saturated fatty acids (SFA), <10 En%; n-6 polyunsaturated fatty acids (PUFA n-6), 5–8 En%; n-3 polyunsaturated fatty acids (PUFA n-3), 1–2 En%; trans-fatty acids (TFA), <1 En%. The target for monounsaturated fatty acids (MUFA) is calculated as follows: MUFA = total fat-(SFA + PUFA + TFA) (Vasta et al. 2005). Reducing the intake of SFA (which are known to raise total and low-density lipoprotein (LDL) cholesterol and increasing the intake of n-3 PUFA is particularly encouraged. Among the PUFA n-3, eicosapentaenoic acid (EPA, C20:5 n-3) and docasahexaenoic acid (DHA, C22:6 n-3) have been demonstrated to have important roles in reducing the risk of cardiovascular disease, 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). Since the health implications of fat consumption are determined by the proportions between fatty acids, some recommendations are still made on the basis of specific fatty acid ratios, which are considered to be beneficial for consumer health (Jiménez-Colmenero, 2007). In this respect, the ratio of PUFA to SFA (P/S) should ideally be between 0.4 and 1.0 (British Department of Health, 1994). Since some meats naturally

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have a P:S ratio of around 0.1, meat has been implicated in causing the imbalanced fatty acid intake of today's consumers. The ratio of n-6/n-3 PUFA, in food, is a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack (Enser, 2001). As recommended by Simopoulos (2003), the n-6/n-3 ratio should not exceed 4.0 this ratio in fact appears to have positive effects on membrane fluidity, gene expression, cytokine formation, level and composition of serum lipids and modulation of immune response (British Nutrition Foundation, 1992; Simopoulos, 1996). The recommendation is for a ratio of less than 4 and again some meats are higher than this. (Wood et al., 2003). Excessive amounts of PUFAs n-6 and very high n-6/n-3 PUFA ratios promote pathogenesis of many kinds, including cardiovascular disease (CVD), cancer and inflammatory and autoimmune diseases, whereas increased levels of PUFAs n-3 (and low n-6/n-3 PUFA ratios) exert suppressive effects (Simopoulos, 2002). It was recently demonstrated that the greatest risk factor for arteriosclerosis and ischaemic heart disease is not hypercholesterolaemia or high cholesterol intake but a high n-6/n-3 PUFA ratio (Okuyama & Ikemoto, 1999). For this reason, meats can be manipulated towards a more favorable PUFA/SUFA and n6/n3 ratios. The ratio PUFA/SFA, however, is not an appropriate index to evaluate the nutritional value of fatty acids because it considers all saturated fatty acids equally and does not take into consideration the beneficial effects of monounsaturated fatty acids. In this regard it would be better to use another index, as proposed in several studies (Orellana et al., 2009; Santo-Silva et al., 2002), the ratio of hypocholesterolaemic to hypercholesterolaemic fatty acids (h/H), which is based on the functional effects of different fatty acids on cholesterol metabolism. In order to better assess the synergic action of individual fatty acids and classes of them that can influence the incidence of CHD, Ulbricht and Southgate in the 1991 devised the artherogenicity (AI) and thrombogenicity (TI) index. AI evaluates the risks of atherosclerosis while TI is useful to define the risk of platelet aggregation; so plus the values of these indexes are close to zero as the food is recommended for a healthy diet. In conclusion, nutritional guidelines indicated not only the amount of fat but also the fatty acid composition in order to reduce the potential adverse effects of fats on human health. More specifically, is recommended a lower intake of SFA and TFA as well as a higher PUFA intake

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(especially of n-3 PUFA to comply with an appropriate n6/n3 balance) in order to prevent cardiovascular-type diseases (Griffin, 2008; Sierra et al., 2008).

6.3.2. Nutrient composition

Red meat is long established as an important dietary source of high value protein and essential micronutrients including iron, zinc and vitamin B12, all of which are essential for good health through-out life, but recent researches report that its consumption, due to its relatively high ratio of saturated to unsaturated fatty acids in its lipids, may be a risk factor for the development of vascular and coronary diseases (CVD) (Barton et al., 2007) and colon cancer, these findings have led to a negative perception of the role of red meat in health (McAfee et al., 2010). Recent studies have investigated the risks and benefits associated with consumption of ruminant/red meat and have demonstrated that consuming moderate amounts of lean red meat, as part of a healthy balanced diet, valuably contributes to intakes of essential nutrients and possibly to intakes of long chain (LC) n-3 PUFA and conjugated linoleic acid (CLA) isomers that could be of particular relevance to human health. Meat and other ruminant products such as milk and cheese are dietary sources of CLA (Ritzenthaler et al., 2001). The dominant CLA in ruminant meat is the cis-9, trans-11 isomer, which has being identified as possessing a range of health promoting biological properties including antitumoral and anticarcinogenic activities (De la Torre, Debiton et al., 2006).

Lean muscle contains approximately 75% water. The other main components include protein (approximately 20%), lipids or fat (approximately 5%), vitamins and minerals (often analyzed as ash, approximately 1%), carbohydrate are virtually not contained in muscle foods.

Fat is contained in meat (on dry matter mass) in varying amount, between 10% for lean meat and 25% for most fatty cuts, but its energy value is disproportionately great owing to the high gross caloric value of lipid (9 Kcal/g) compared to protein (4 Kcal/g) (Merrill and Watt, 1973).

Dietary protein is essential for growth, maintenance and repair of the body, and can also provide energy. Protein from foods consists of chains of hundreds to thousands of

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nitrogen-containing amino acids. Some amino acids can be synthesized in the body, whilst others – essential amino acids – cannot.

Therefore, essential amino acids need to be consumed in the diet to maintain good health. Because the pattern of amino acids in other mammal cells is comparable to the pattern in human cells, red meat (and in some cases meat products) is an excellent source of high biological value protein and an important source of the eight essential amino acids for adults (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine and valine) and histidine which is considered to be an additional essential amino acid for children. Red meat contains, on average, 20-24g of protein per 100g (when raw) and cooked red meat contains 27-35g of protein per 100g (cooked weight). As meat is cooked, the water content decreases and the nutrients become more concentrated, therefore the protein content increases. Lean red meat contains a higher proportion of protein than fattier cuts (Williamson et al., 2005). The meat proteins are highly digestible, around 94% compared to the digestibility of 78% in beans and 86% in whole wheat (Bhutta, 1999).

Fat is the richest dietary source of energy (9 Kcal/g vs 4 Kcal/g provided by either protein or carbohydrate), supplies essential bioactive lipid components and antioxidants and also serves as important carrier for nutrients, including fat-soluble vitamins (Vitamins A, D, E and K) and essential fatty acids, but despite being an essential component of the diet it must be consumed in moderation (Kelly, 2001).

Even though many consumers consider the fat as an unpopular constituent of meat often mistakenly referred to as unhealthy; anyway, the fat, and fatty acids that comprise it, present in adipose tissue or muscle, contribute to various aspects of its quality and have an essential role for nutritional value of meat (Wood et al., 2008).

Moreover, fat also provides palatability and determines the culinary qualities of meat (flavour, juiciness and aroma) and texture parameters which are no less important to the consumer, playing a key role in manufacture and cooking processes (Williamson et al., 2005).

The major lipid class in adipose tissue (>90%) is neutral lipids (NL), it consists mainly of triacylglycerols (TAG) serving as energy store and present in the adipocytes that are located along the muscle fibres and in the interfascicular region (DeSmet et al., 2004). TAG consisting of fatty acids and glycerol. The major component of TAG are saturated

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and monounsaturated fatty acids that are present in similar proportion in lean red/ruminant meat (Williamson et al., 2005).

Muscle lipids are composed also of polar lipids (PL) (~10%) and a significant proportion of these (~44%) are constituted by phospholipid located in the cellular and subcellular membranes. In red/ruminant meat long chain (>C18) highly polyunsaturated fatty acids are not esterificated into TAG but preferentially deposited in membranal phospholipid in order to perform its function as a constituent of cellular membranes (Dannenberger et al., 2007). Meat and meat products vary greatly in their fat content according to the animal age, sex, breed, diet, the cut of meat and the degree of trimming (Higgs, 2000; Wood & Enser,1997). The fat content and fat composition is also affected by animal feeding, a fact that is exploited for modification of the meat fatty acid composition, with the relatively best results in single-stomached pigs and poultry (Bolte et al., 2002; Wood & Enser, 1997; Wood et al., 1999). In some country, meat with a low content of fat (less than 10%) is classified as "lean meat", and despite there is no an international definition of lean meat standard seem to be similar in different country (Dietary Guidelines Advisory Committee, 2000; Red Meat and Health Expert Advisory Committee, 2001; Food Advisory Committee, 1990).

6.3.3. Fatty acid composition

From the qualitative point of view is decisive for the structural characteristics and function of the lipid its fatty acid composition, the most common dietary fatty acids have been subdivided into three broad classes according to the degree of unsaturation; saturated fatty acids (SFA) have no double-bonds, monounsaturated fatty acids (MUFA) have one double-bond and polyunsaturated fatty acids (PUFA) have two or more double bonds. In general, these fatty acids have an even number of carbon atoms and have unbranched structures.

The presence of double bonds, the length of the carbon chain, the geometric and positional isomerism affect the nutritional, physical and biological properties of lipids. It should be noted that increasing the number of double bonds lowers the melting point and increases susceptibility to peroxidative phenomena.

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Fatty acids are involved in various ‘‘technological’’ and “sensory” aspects of meat quality. Because they have very different melting points, variation in fatty acid composition has an important effect on firmness or softness of the fat in meat, especially the subcutaneous and intermuscular, but also the intramuscular (marbling) fat. Groups of fat cells containing solidified fat with a high melting point appear whiter than when liquid fat with a lower melting point is present, so fat colour is another aspect of quality affected by fatty acids.

The ability of unsaturated fatty acids, especially those with more than two double bonds, to rapidly oxidise, is important in regulating the shelf life of meat (rancidity and colour deterioration). However, this propensity to oxidise is important in flavor development during cooking (Wood et al., 2003).

Fatty acid composition of ruminat/red meat comprises mainly SFA and MUFA (Valsta et al., 2005) that are present in similar proportion in lean ruminant/red meat (Williamson et al., 2005). Approximately 50% of the intra-muscular fat of beef and lamb is made up of unsaturated fatty acids MUFA, primarily oleic acid (C18:1 c-9) and PUFA, predominantly the essential n-6 and n-3 PUFA linoleic acid (LA, C18:2 n-6) and alpha-linolenic acid (ALA, C18:3 n-3), respectively.

The predominant SFAs in meat are C16:0 (palmitic acid) and C18:0 (stearic acid); there are also minor amounts of C14:0 (myristic acid) and C12:0 (lauric acid). SFAs are well known as hypercholesterolaemic and able to influence plasma cholesterol levels, but they show different effects among the fatty acids. In fact, mystric acid is thought to increase cholesterol levels more potently than palmitic and lauric acids, while stearic acid appears, as well as the short chain SFA (C4:0 and C6:0), to have no effect on cholesterol levels, despite being a SFA (Ulbricht & Southgate, 1992; Higgs, 2000; Williamson et al., 2005).

Even so, dietary recommendation indicate that the intake of SFA should be restricted but include also stearic acid along with the other SFA that rise blood cholesterol. In ruminant meats (beef and lamb) the substantial proportion of SFA is due to the extensive biohydrogenation by the rumen bacterial of dietary unsatured fatty acids (Jenkins et al., 2008).

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The rumen microorganisms are also responsible for the presence of odd-number carbon and branched-chain fatty acids (with *iso*- or *anteiso* structure) in ruminant intramuscular fat (Bas et al., 2003). The interest in these fatty acids is that *iso*-C15:0 and *anteiso*-C15:0 have beneficial effects on the inhibition of cancer cell proliferation (Yang et al., 2000; Yang et al., 2003; Wongtangtintharn et al., 2004).

Branched-chain fatty acids make up of 15 to 20% of total bacterial fatty acids, ad as much of 30% of fatty acids in bacterial phospholipids (Jenkins, 1994). The main function in membranes may be to increase the fluidity of lipids as an alternative to double bonds, which are more liable to oxidation.

MUFAs represent typically between 30 and 40 % of the fat in ruminant meat. These fatty acids occur mainly as *cis* and *trans* isomer of C18:1 and the principal of these is oleic acid (C18:1*cis*9) produced by the Δ^9 desaturation of stearic acid. In a nutritional prospective, human diets rich in *cis*-MUFA have been related to positive health benefits. In fact, C18:1*c*9 is known to have hypocholesterolemic properties reducing LDL-cholesterol levels and increasing high density lipoprotein (HDL)-cholesterol concentration in blood, which result in lower risk of coronary problems (Katan et al., 1994).

Ruminant meat can be a relatively good source of n-6 and n-3 PUFA, approximately 5% of the total fatty acids. The predominant PUFAs in meat are linoleic acid C18:2 n-6 (LA) and α-linolenic acid C18:3 n-3 (ALA) and representing approximately 2-3% of total triacylglycerol fatty acids (Scollan et al., 2006). LA and ALA cannot be synthesized *de novo* by humans and other mammals and for this reason they are referred to as essential fatty acids, but they must be obtained from plants in the diet.

The proportion of PUFA in phospholipids is much higher (45-55% of the total fatty acids) than in TAG containing not only the essential fatty acids LA and ALA but also their longer chain derivatives such arachidonic acid C20:4 n-6 (AA), EPA C20:5 n-3, DPA C22:5 n-3 and DHA C22:6 n-3. In fact LA and ALA can be converted to n-6 and n-3 families of C20 and C22 LC-PUFA by the action of Δ5 and Δ6 desaturase and elongase enzymes (Figure 20).

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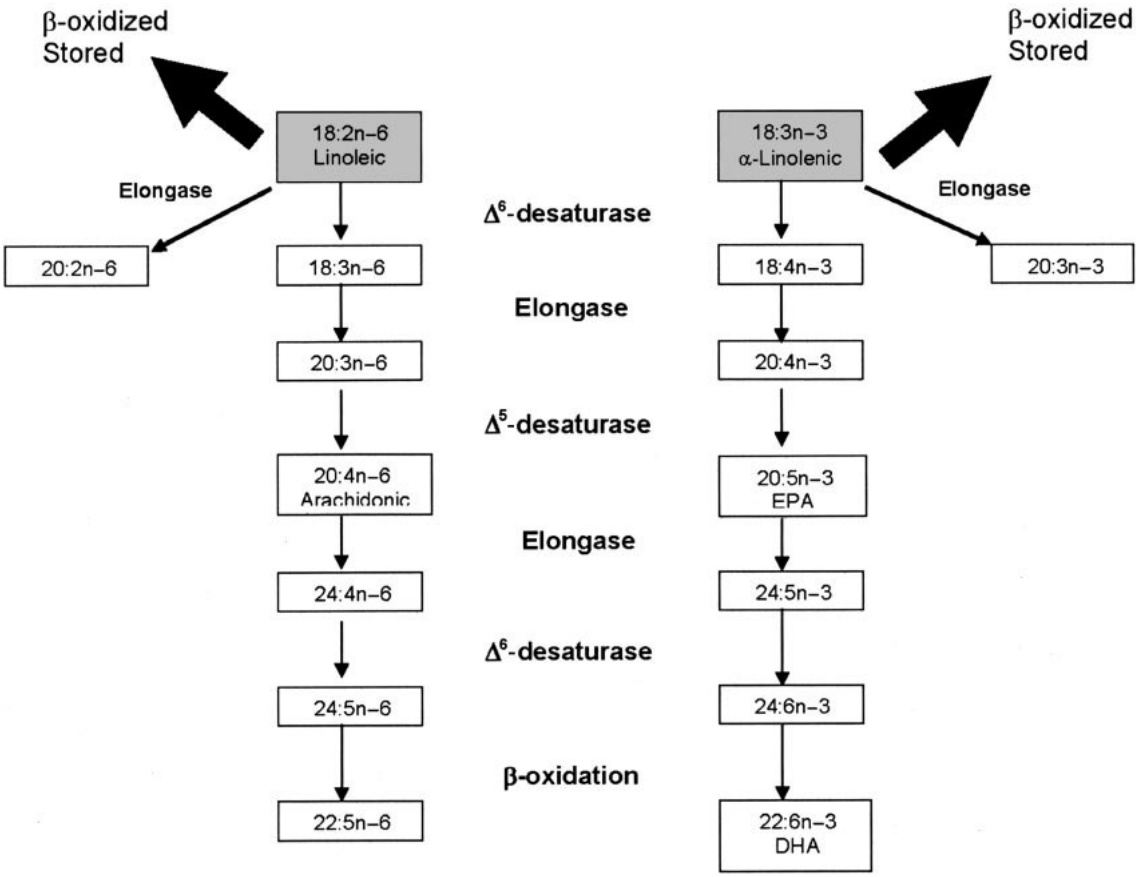

Figure 21. Metabolic pathways for the conversion of dietary linoleic and α-linolenic acids to their longchain polyunsaturated fatty acids (from Bayiln et al., 2007).

The phospholipids play central roles in cell membrane function and the PUFA composition is strictly controlled by a complex enzymatic system, such as Δ^5 and Δ^6 desaturases and elongase lipogenic enzymes, responsible for the conversion of C18:2 n-6 and C18:3n-3 to their longer chain derivatives (Raes et al., 2004; Smith, 2007). In the body LA is metabolized to arachidonic acid (AA) and ALA is converted to EPA and DHA (Simopoulos, 2003). Concentrations of LCn-3 PUFA found in beef and lamb are lower than those within oily fish (0.28 mg and 0.52 mg vs. 19.9 mg/g), but may be a very significance source due to general low fish consumption.

The potential health benefits of omega-3 fatty acids have been widely reported for several conditions including cardiovascular disease, hypertension, atherosclerosis, brain development, diabetes, cancer, arthritis, inflammatory, autoimmune and neurological disorders (Simopoulos, 2000; Gogus and Smith, 2010).

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Red meat is the main dietary source of DPA, which accumulates in mammals but not in oily fish (Givens & Gibbs, 2006). Little research exists on the clinical significance of DPA, but it has been suggested to be inversely related to atherosclerotic risk and risk of acute coronary events in middle-aged men from Finland (Hino et al., 2004; Rissanen et al., 2000). Despite the fact that DPA is not considered in dietary recommendations for LCn-3 PUFA, it is has comparable health benefits to those of EPA and DHA in reducing CVD risk (Howe, Buckley, & Meyer, 2007).

In Western diet, the predominant polyunsaturated fats in food supply are n-6 fatty acids. Among the PUFA, it is the PUFA n-3 which possesses the most potent immunomodulatory activities, and among PUFA n-3, those from fish oil, EPA and DHA, are more biologically potent than ALA. The metabolic products of n-6 and n-3 fatty acids are metabolically distinct and have opposing physiologic function, hence the need for an adequate and balanced ratio between n-6 and n-3 fatty acids essential for maintenance and a normal growth and development When diets are supplemented with n-3 fatty acids, these partially replace the n-6 fatty acids in the membranes of practically all cells (i.e. erythrocytes, platelets, lymphocytes, endothelial cells, hepatic cells, neuronal cells, granulocytes and retinal cells).

Competition between n-6 and n-3 fatty acids occurs in prostaglandin formation (Figure 21), in fact EPA is capable to compete with AA for enzymatic metabolism, in eicoesanoid formation, inducing the production of less inflammatory and chemiotatic derivatives (Simopoulus, 2002). The eicosanoids from AA, the principal membrane of n-6 PUFA, (prostaglandins: PGG_2, PGH_2, PGI_2, thromboxanes: TXA_2, leukotrienis: LTB_4 and lipoxins) are prothrombotic, potent platelets aggregator and vasoconstrictor, proinflammatory and promoter cell proliferation while the eicosanoids derivate from EPA (prostaglandins – PGG_3, PGH_3, PGI_3, thromboxanes – TXA_3, leukotrienis – LTB_5 and lipoxins) are active vasodilatator, inhibitors of platelets aggregation, weak inducer of inflammation and weak chemiotatic agent.

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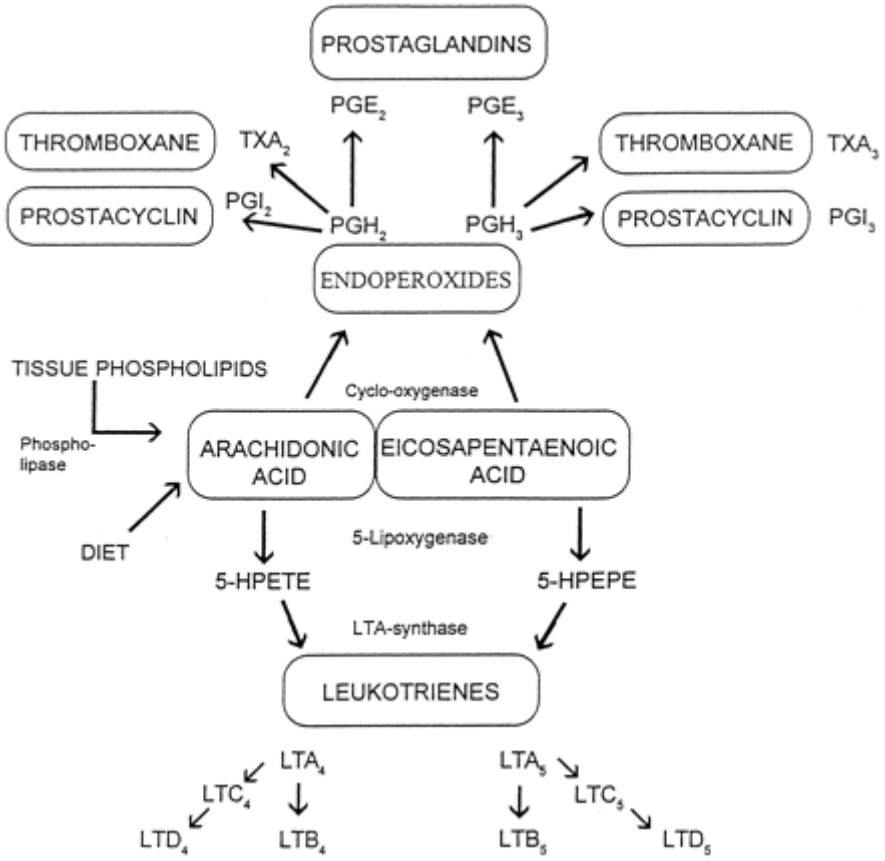

Figure 22. Oxidative metabolism of arachidonic acid end eicosapentaenoic acid by the cyclooxygenase and 5-lipioxygenase pathways. (from Simopoulos, 2002).

In conclusion, LC n-3 PUFA may reduce the risk of cardiovascular disease and some neurological and inflammatory diseases, but these are endogenously synthesized in the human body at low levels considered inadequate to meet their requirements and therefore must be supplemented with the diet.

A host of public health organizations worldwide have issued recommendation on the intake of LC n-6 and n-3 PUFA. In Italy these guidelines were developed by the *Italian Society of Human Nutrition* (SINU) which through the publication of tables has indicated the Nutritional Levels Recommended Daily Intake of Energy and Nutrients for Italian Population (LARN). In the last revision, in 1996, have been added for the first time, the recommended levels of essential fatty acids by age group, divided into n-3 and n-6 polyunsaturated expressed as a percentage of the total energy in the diet that in g/day.

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According to the information provided by SINU, a correct lipid intake should therefore be provided an appropriate balance between fatty acids, precursors and derivatives, of the series n-6 and n-3, which in normal conditions bring well amount of 18:2 n-6 and 18:3 n-3 and small quantities of 18:3 n-6, 18:4 n-3, 20:5 n-e, 20:4 n-6 and 22:6 n-3 (Barzanti et al., 1994). The minimum requirement for adults comes from studies on which it appears that 0.5% of total calories is sufficient to maintain the metabolic integrity. Therefore it is recommended a level of 1-2% of calories as linoleic acid and 0.2-0.5% as n-3 PUFA (Commission of the European Communities, 1993) (Table 9.).
The table also shows that some clarifications are necessary regarding the requirements of pregnant women, infant, children and the elderly. During pregnancy, essential fatty acids and their derivatives play an important role in fetal-placental unit, so an adequate intake is important both in the case of certain diseases, such as gestational hypertension or threatened preterm delivery both to facilitate the fetal growth.

Table 9. Levels of recommended intakes of unsaturated fatty acids of n-6 and n-3 series for Italian population.

Categoria	Age	n- 6		n- 3	
	(years)	% energy	g/die	% energy	g/die
Infants	0,5 - 1	4,5	4	0,2-0,5	0,5
Children	1 - 3	3	4	0,5	0,7
	4 - 6	2	4	0,5	1
	7 - 10	2	4	0,5	1
Males	11 - 14	2	5	0,5	1
	15 - 17	2	6	0,5	1,5
	> 18	2	6	0,5	1,5
Females	11 - 14	2	4	0,5	1
	15 - 17	2	5	0,5	1
	> 18	2	4,5	0,5	1
Pregnant		2	5	0,5	1
Lactating		2	5,5	0,5	1

(source LARN, 1996).

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The availability of adequate amounts of essential fatty acid precursors and derivatives is also important for growth and development of the infant (Simopoulos, 1991).

In particular, AA and DHA acids are necessary for brain and retinal structures, the storage capacity of the fetus and the supply in the infant through breast milk appear to meet the requirements. In the elderly the addition of small amounts of 18: 3 n-6 and 18:4 n-3 and derivatives seems to positively influence the course of the metabolism of PUFA, promoting a more balanced synthesis of eicosaenoids and better physiological activity of cell membranes and of the enzyme systems connected (Barzanti et al., 1994).

The phospholipid fatty acids are less influenced by diet than triacylglycerol fatty acids, but differences in the content of n-6 and n-3 long chain PUFA do occur. For example, meat from animals fed a grass diet have higher concentrations of LC n-3 PUFA in phospholipids and triacylglycerols compared to concentrate feeding (Yang et al., 2002; Dannenberger et al., 2004; Realini et al., 2004). Significantly, grass compared to concentrate feeding not only increased 18:3 n-3 in muscle phospholipid but also EPA, DPA and DHA (Dannenberger et al., 2004; Warren et al., 2002).

In comparison, concentrates rich in 18:2 n-6 lead to higher concentrations of 18:2 n- 6 and associated longer chain derivatives (20:4 n- 6).

Special attention has been given to some unsaturated fatty acids, which have one or more double bonds in the *trans* configuration, instead the *cis* configuration. Trans fatty acids (TFA) are geometrical isomers of cis-unsaturated fatty acids and assume a configuration similar to that of saturated fatty acids. TFA occur naturally in some foods, especially those from ruminant animals, in addition the partial hydrogenation of unsaturated vegetable oils, an industrial process used to extend the deadline of polyunsaturated fatty acids (PUFAs), creates TFA and also removes the critical double bonds in essential fatty acids necessary for their action.

Metabolic studies have shown that TFA render the plasma lipid profile even more atherogenic than how are not able to do saturated fatty acids, not only increasing share similar LDL cholesterol but also reduce the cholesterol associated with high-density lipoprotein (HDL) (Pfeuffer & Schrezenmeir, 2006). Several large cohort studies have shown that intake of *trans* fatty acids increases the risk of coronary heart disease (CHD) and atherosclerosis (Hunter, 2006; Willett, 2006; Prates & Bessa, 2009).

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Most *trans* fats are made from oil provided "hard" through industrial processes. Although it is seeking to reduce or eliminate trans fatty acids from fat marketed and their spread in many parts of the world, fast food restaurants that sell fried foods and or roasted represent a major source and, unfortunately, increasing. Although the dietary recommendation, growing evidence has been show that different TFA have distinct biological effects and that the association between the intake of TFA and chronic disease generally do not apply to TFA of ruminants source.

Intakes of elaidic acid (C18:1 *trans*-9), the major industrially produced *trans*-fat, can negatively affect cholesterol metabolism in humans (Sundram et al., 1997). In contrast, there is emerging evidence that *trans*-vaccenic acid (TVA, C18:1 trans-11), the major trans fatty acid found within red meat and dairy products, has no effect on either total cholesterol or LDL cholesterol concentrations (Chardigny et al., 2008). Therefore, the TFA content of red meat is unlikely to be a contributing factor to risk of CVD. Furthermore, minor concentrations of total trans fatty acids consumed from European diets are not thought to be a cause for concern (Hulshof et al., 1999).

In Italy, based on the indications provided by SINU, intake of trans fatty acids should not exceed 5g/day. The intake of trans fatty acids in the Italian diet is on average only 1.3-2.0 g/day, compared with 5-10 g/day found in countries with high consumption of hydrogenated fats (USA, Canada, Germany, Sweden and UK) (Pizzoferrato & Nicoli, 1994).

Trans fatty acid, present in ruminant meat, arise as intermediates of the ruminal biohydrogenation of dietary unsaturated fatty acids (Fritsche & Steinhart, 1998). TFA comprise about 1–2% of total fatty acids across all types of meat; in ruminant meats they represent 2–4% (Valsta et al., 2005). The contribution of TFA in the human dietfrom the meat and meat products was estimated to be between 10 and 30%, however, the actual evolution tends to favor a replacement and/or decrease, due to constant efforts of the food industry, of TFA content in foods.

TVA, the major trans fatty acid, a biohydrogenation product of C18:2n-6, is converted to conjugated linoleic acid *cis*-9, t*rans*-11 isomer (CLA, C18:2 *c*9,*t*11) which was given the trivial name rumenic acid (RA, Kramer et al., 1998a), which may have potential health benefits.

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The biosynthesis of CLA *c*9,*t*11 isomer occurs in adipose tissue by the action of stearoyl Co-A desaturase (Palmquist et al., 2005; Wood *et al*., 2008), the same enzyme responsible for the production of C18:1*cis*-9 from C18:0. Like C18:1*cis*-9, both C18:1 *trans*-vaccenic and CLA *c*9,*t*11 are at higher proportions in neutral lipid than phospholipid and higher in adipose tissue than muscle; CLA isomers in fact, are usually found esterified in triglycerides.

The synthesis of all the other isomers with conjugated linoleic acid also CLA c9, t11, it is assumed occur in the rumen through the ruminal biohydrogenation of dietary unsaturated C18-PUFAs (Bessa et al., 2007) made by the bacterium *Butyrivibrio fibrosolvens* (Kepler et al., 1966). Nevertheless, synthesis from 18:1 *trans*-vaccenic in tissues is quantitatively the most important contributor to tissue levels (Scollan et al., 2006). In figure 22 is summarized the mechanism involved in the synthesis of cis-9, trans-11 CLA in rumen and in tissue.

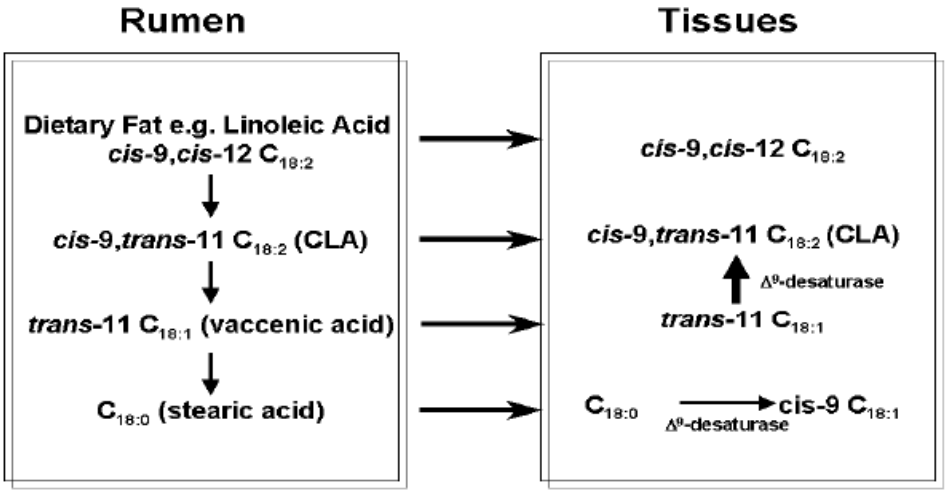

Figure 23. Role of rumen biohydrogenation and tissue Δ^9-desaturase in the prduction of cis-9, trans-11 conjugated linoleic acid in ruminant fat (from Bauman et al., 1999).

The isomer *c*9, *t*11 belongs to a specific group of unsaturated fatty acids commonly known as CLAs, which are a mixture of positional and geometric isomers of linoleic acid, commonly found in beef, lamb and dairy products, characterized by the fact that in their structure is present a system of conjugated double bonds, namely the double bonds

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in CLAs occur on adjacent carbons and are not separated by a methylene group (Figure 21).

The double bonds are located predominantly in positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13, while the geometric configuration of these double bonds can be *trans*/*trans*, *trans*/c*is*, *cis*/t*rans* or *cis*/*cis*.

Although there are 28 different CLA isomers, the main isomer present in natural foods is the CLA *c*9,*t*11 isomer, which accounts for >80% of total CLA isomers in dairy products and 75% of those in beef fats (Fritsche et al., 1999).

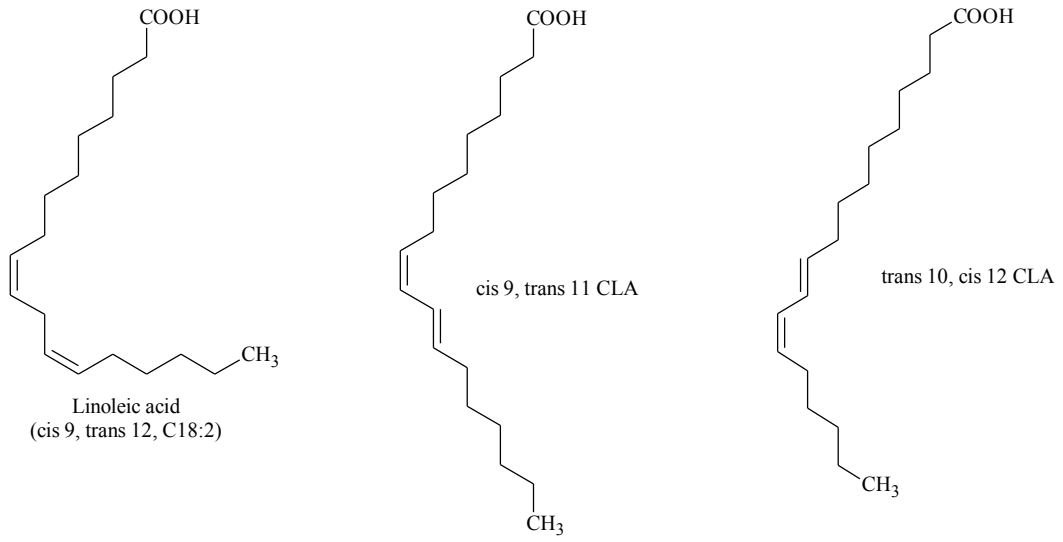

Figure 24. Structures of linoleic acid and the biologically active conjugated linoleic acid (CLA) isomers.

Since their discovery, the CLAs have been extensively studied and researchers have been widely demonstrated, using animal models and cell cultures derived from both men and animals, their various beneficial effects on health and their physiological properties in fact, have been recognized for them, unequivocally, antiadipogenic (Park et al., 1999; Pariza et al., 2001), anticarcinogenic (Belury, 2002; Ip & Banni et al., 1999), antiterathogenic (Lee et al., 1994), antidiabetogenic (Ryder et al., 2001) and anti-inflammatory properties (Yu et al., 2002).

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There are very few or no data regarding optimal dietary intake of CLA in humans. The minimum effective dose-response and optimal dietary intake are still unknown. Knekt et al., (1996) have suggested, using epidemiological data that connect increased consumption of milk with a reduced incidence of breast cancer, that 95 mg CLA / day are sufficient to produce positive effects on reducing impact of breast cancer in women. Ha et al. (1989) have estimated that 3 g/day CLA are needed to promote human health benefits. Ritzenthaler et al. (2001) estimated CLA intakes of 620 mg/day for men and 441 mg/day for women are necessary for cancer prevention. Obviously, all these values represent rough estimates and are mainly based on experiment performed on animal.
However, monitoring the dose-response on the anticarcinogenic effects of CLA in rats, and adjusting it for the metabolic differences between men and rats it can state that a daily intake of 0.8 to 3.0 grams of CLA could provide an important support for human health (Ip et al., 1994; Parrish et al., 2003).
The physiologic and health properties attributed to CLAs are summarized in Table 10.

Table 10. Physiological and health effects of conjugated linoleic acid seen in animals.

Physiological properties
↓ Adipose mass
↑ Lean mass
↑ Saturated/monounsaturated fatty acid ratio
↓ Milk fat synthesis
Health effects
↓ Carcinogenesis
↓ Atherogenesis
↓ Proinflammatory cytokine production
↓ Eicosanoid production
↑ Glucose tolerance

Source: Badinga & Greene, 2006.

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In addition, the National Academy of Sciences (NRC, 1996) publication *Carcinogens and Anticarcinogens in the Human Diet* concluded that ". . . conjugated linoleic acid (CLA) is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals."

The most important sources of CLA in human diets as well known are the products derived from ruminant animals, including milk, dairy products and meat while only traces of CLA may be found in fish or some vegetables. This reiterates the fact that different intakes of CLA were observed in the human diet depending of individual dietary preferences and food selections (ruminant vs. nonruminant sources and plant vs. animal sources).

In conclusion, due to of the presence of this potent anticarcinogen and a lot of many other essential healthier constituent, foods from ruminant animals such as milk, dairy products and meat should be considered functional foods (McGuire and McGuire, 2000).

The definition of functional food was introduced for the first time in Japan in mid-1980s, referring to processed foods containing ingredients that exhibited a role in disease prevention and health promotion, as well as being nutritious. No other country has issued guidelines on these foods and only in the 1994, the Institute of Medicine's Food and Nutrition Board (IOM/NAS, 1994) defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains", but without proposing any directive of law in this regard.

In conclusion, the increased consumer interest to certain foods that, containing physiologically and biologically active components, have a role in improving human health, makes even more contemporary the principle "Let food be thy medicine and medicine be thy food "enunciated by Hippocrates nearly 2,500 years ago, but that is nowadays rediscovering a renewed interest.

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6.3.4. Micronutrient in red meat

Red meat contains a multitude of micro-nutrients necessary for health and human wellness. In accordance with current European legislation whereas 100 g serving of beef, lamb and pork provide half the recommended daily intake (RDA) of vitamin B12 and one-sixth of the RDA of vitamin B6, niacin, phosphorous and zinc, these foods may be considered for these micronutrients a *rich source* and a *source*, respectively, as well as they are important resource of a several dietary fat-soluble vitamins, minerals and trace elements (such as selenium, magnesium, copper and nickel).

Iron is vital for many cellular metabolic processes in the body it is a component of hemoglobin (haem-iron) in the blood and myoglobin in the muscles, where the molecular oxygen binding guarantees its adequate transport and accumulation in the blood.

Therefore, a mild iron deficiency can have a negative impact on health (Gibson & Ashwell, 2002) even before the onset of anemia Although iron is one of the most abundant elements in the Earth's crust, paradoxically, iron deficiency anemia (IDA) is the most common and widespread nutritional disorder in the world (DeMaeyer & Adiels-Tegman, 1985). This nutritional deficiency affects most of the world's population without distinction of socio-economic status and is particularly common among children and young women (Gibson & Ashwell, 2002). Heme iron found in meat is more bioavailable than non-heme iron found in plant sources, and for this reason, the meat eaters have levels of iron higher in the blood than vegetarians and vegans (Cosgrove et al., 2005; Gibson & Ashwell, 2002). Red meat in particular is recognized as a significant source of haem iron than the poultry and fish (Johnston et al., 2007). Small amounts of red meat (<41 g/day) provide 13.1 mg and 15.8 mg/d iron for men and women respectively (Cosgrove et al., 2005) and considering that the RDA recommended for men and women are respectively 10.0-12.0 and 18.0 mg/d (LARN, 1996) is necessary to include meat in the diet for an adequate intakes of iron. However, Gibson and Ashwell (2002) report that consume less than 90 g/d of red meat causes for both men and women a three times greater risk to exhibit an iron deficiency.

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In relation to contribution of zinc, beef and lamb contain 4.1 mg and 3.3 mg/100 g tissue (Chan et al., 1996), as a result, have been classified as *sources* (British Nutrition Foundation, 2002), also the zinc contained in meat is present in a highly bioavailable form. Zinc is associated with the activity of a wide variety of enzymes. Low consumption of red meat (<41 g/d) is estimated to contribute 10.2 mg and 10.6 mg/d of zinc to men and women respectively (Cosgrove et al., 2005). Considering that the RDA for zinc is set at 9.0–10.0 mg and 7.0–9.0 mg (LARN, 1996) for males and females, intakes of red meat at such levels are capable of providing adequate zinc for optimum health. Red meats are also good sources of selenium, providing about 10 μg selenium per 100g of meat, which is about 25% of RDI, although it is likely that selenium values in meat will be significantly affected by animals feeding system, the soil in which the animal feed was grown and the time of the year of sampling (Williamson et al., 2005; Williams, 2007). Selenium is part of the active site of the enzyme glutathione peroxidase (GPx) and this is the best known biochemical role for it.

The metabolic function of GPx enzyme is vital for cells, in fact it is assumed that GPx can protect DNA from oxidative damage and consequently from mutation leading to neoplastic transformation of cells (Combs & Clark, 1985), for so selenium is considered one of the major cellular antioxidant, in *vivo*, that protect against coronary heart disease and cancer. In addition, despite it was always thought that the bioavailability of selenium from vegetable foods was higher than foods of animal origin, recent data show that the meat, raw and cooked, is a source of selenium in a highly bioavailable from (Shi et al., 1994).

As noted previously for many micronutrients, red meat is also an excellent source of fat-soluble vitamins in a highly bioavailable from as well as B vitamins (thiamin, riboflavina, niacin, pantothenic acid, folic acid, B6 and B12), vitamin D, vitamin A and vitamin E. Moderate red meat consumption by men and women has been to contribute to estimated 5.3 μg and 6.5 μg/d of vitamin B12, respectively (Cosgrove et al., 2005). In fact, red meat provides more than two thirds of RDA of vitamin B12 in 100g serving, and for this reason represents the major dietary source of this vitamin in the diet.

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Up to 25% RDA of riboflavin, niacin, vitamin B6 and pantothenic acid can also be provided by 100g of red meat, but compared to pork is a relatively poor source of thiamine (Williams, 2007).

Vitamin D is essential for the development and maintenance of bone tissue, but only a few foods can contribute significantly to its intake and certainly, among these, the meat supplies a useful contribution to its dietary intake. Meat and meat products in fact contribute for 20-22% of RDA of vitamin D. In particular, the vitamin D metabolite 25-hydroxycholecalciferol ($25OHD_3$) is found in significant amounts in meat and liver, and it is better and faster absorbed from the diet, compared with other food systems (Groff et al., 1995). Furthermore, it was hypothesized that components of the meat protein may increase the utilization of vitamin D in humans, particularly when exposure to sunlight is limited. Liver is an excellent source of vitamin A, in the form of retinol, and folate, but the levels in lean meat tissue are low.

For all these vitamins, the amount present in meat can be variable and depend on the age of animals, older animals tend to have higher concentrations, so the levels in beef are generally higher than those in veal, as well as sheep has more than lamb (Williams, 2007), the fat content, in fact being fat-soluble vitamins their concentration will be higher in fattier meat, the composition of the feed consumed, for example vegetable oils are particularly rich in vitamin E and including seed oils in animals' diets will have contributed to an increase in the vitamin E content (Williamson et al., 2005).

In Italy meat and meat products were a valuable source of micronutrients, supplying 47, 48, and 24% of zinc, niacin, and thiamin daily requirements, respectively, and over 10% of iron, copper, selenium, and riboflavin daily average requirement values of the Italian RDAs calculated for the population involved in the Nationwide Nutritional Survey of Food Behavior of the Italian population (INN-CA study) (Turrini et al., 2001).

The daily intakes of total iron and haem iron were 1.65 and 1.13 mg/person/day. Zinc intake was 3.65 mg/person/day. Daily intake of selenium (7.14 μg/person/day) was provided mainly by poultry consumption. Thiamine intake was 228 μg/person/day, and meat products were the main source (110 μg/person/day).

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Riboflavin intake was 136 μg/person/day, with both beef and meat products as the main contributors (40 μg/person/day). Niacin intake was 7.53 mg/person/day, and poultry was the main source (2.28 mg/person/day) (Lombardi-Boccia et al.,2004).

In conclusion, it is clear that red meat is a food rich in nutrients, providing valuable amounts of protein, haem iron, zinc, B vitamins, selenium and retinol, with a higher bioavailability than other food sources (Cosgrove et al, 2005; Davey et al, 2003).

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PART II

EXPERIMENTS

– EXPERIMENT ONE –

Effect of sex and microwave cooking on chemical, fatty acid composition and oxidative stability in suckling Sarda lamb meat produced under traditional rearing system.

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1.1. Abstract

The aim of this work was to study the influence of sex and microwave cooking on the meat quality, fatty acid composition, and lipid oxidation of Sarda suckling lambs. Male (M) and female (F) lambs were bred under the Sardinian traditional rearing system fed mainly ewes' milk but every day they followed their mothers on pasture so they have had the opportunity to eat freely grass for grazing. Lambs M and F were slaughtered at 28 days of age corresponding to 11·4 and 10·9 kg live weight (LW), respectively. After 24 h of refrigeration at 4°C, the thight muscles were dissected from each right half-carcass and split into pieces; a 50 g portion of each sample was cooked in a microwave oven at 650 W for 35 s to a final internal temperature of 75°C. Parameters measured on thight muscles were: ultimate pH (pH_{24}), ash, crude protein, fat, moisture and cooking loss (CKL). Fatty acid composition and TBArs were studied in the intramuscular (IM) fat depots in raw e cooked samples. Data were analyzed by one-way ANOVA with sex as fixed factor. One-way ANOVA was used to compare raw versus cooked samples. Cooking induced significant changes ($P<0.01$) in the values of all nutrient content and energy value in meat from both males and females, while the proximate composition was not influenced by sex for all selected nutrient. There were no differences between sexes in pH, while difference significantly ($p<0.05$) between sexes were found for the values of cooking loss. No significant differences between sexes were observed in the total saturated fatty acids and total unsaturated fatty acids. However, the males of the breed had significantly higher proportions of odd and branched fatty acids (OBCFA) with significantly higher content of pentadecanoic (C15:0) acid than the females in the IM fat depots. The sex of lambs did not modify almost all FA analyzed. The cooking modify several of FA considered. The cooked meat has a significant lower level of SFA, a better value n6/n3 and levels of compared to the raw meat of both gender. Lipid oxidation, expressed as mg MDA/kg muscle, was significantly ($p=0.000$) influenced by sex, cooking and their interaction. The MDA was increased significantly by cooking on both sex. In cooked samples, the MDA was higher in females compared to males ($P<0.01$), probably due to a higher proportion of hight-peroxidisable polyunsaturated fatty acids (HP-PUFA).

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The sex of lambs did not cause substantial changes in FA composition. The absence of significant significantly differences in fatty acid content between males and females showed that at the low slaughter weight, sex had no effect on the nature and composition of fat except for the content of OBCFA. The cooking cause changes in FA profile and lipid oxidation even if the values of MDA detected in cooked samples of both sex are very low and within the limits of rancidity indicated by literature in 1-2 mg MDA/100 g of meat Cooking losses and true retention of nutrient were also evaluated.

1.2. Introduction

Consumption of red meat, due to its excellent nutritional value, is necessary to ensure a healthy and balanced diet (Biesalski, 2005, Willett et al, 1995). The lamb meat, in particular, has a higher content of n-3 polyunsaturated fatty acids (PUFA) (Nudda et al., 2011), that is beneficial to health because it helps control blood cholesterol levels (Wood et al., 1999). The consumption of meat from suckling lamb, with an average finished carcass weight of 8 kg is typical and traditional in some Mediterranean areas such as Sardinia. Sheep-farming is widespread throughout the region of Sardinia and historical findings confirm that the sheep-farming had already existed in the age prenuragic as reported frequently in documents of the Roman period. The famous French geographer , sheep farming is widespread in the whole territory of the region of Sardinia and historical findings confirm that the sheep-farming had already existed in the age prenuragic as reported frequently in documents of the Roman period. the famous French geographer, Maurice Le Lannou, following of his numerous trips in the island wrote in the second half of the 20th century *"Patres paysans et de la Sardaigne"* (1941), in which he described sheep farming as a characteristic of the landscape and as being responsible for the resistance of the cultures in the interior of the island. Traditionally in Sardinia lambs were reared in a totally natural environment featuring open spaces exposed to the intense sunshine, winds and climate of the island territory.

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Feeding consists mainly of the suckling of the lambs by their dams and following their mother on pasture they can freedely grazing grass and other plants typical of the island habitat of Sardinia.

Lamb meat is characterized by high moisture content, low fat levels, pink-pale color, delicate and mild flavor and high tenderness. Usually the meat is cooked before being consumed. Cooking causes many positive effects, such as the increase in taste and aroma, destruction of microorganisms and improve digestibility, but also produces some adverse effects such as formation of undesirable compounds and nutritional losses.

Cooking is in effect the treatment that best promotes and accelerates lipid oxidation in meat, so the cooked meat is more susceptible to oxidative rancidity than raw meat. The oxidation of lipids in foods, and therefore also in meat, is in effect, the largest non-microbial factor that may adversely affect their quality (Pradhan et al., 2000).

The aim of the present study was to compare the effect of microwave cooking on fatty acid composition and oxidative stability of intramuscular fat in thigh muscles in suckling lambs of Sarda sheep breed. The effect of sex were also determined to find possible differences in fatty acid composition and oxidative stability between sucklings male vs. female lambs.

1.3. Materials and methods

1.3.1. Animals and muscle sampling

The study was carried out on a total of twenty-four Sarda breed suckling lambs, bred under the Sardinian traditional rearing system in a dairy farm located in the Sassari province. In this farm, twelve males and twelve females were randomly selected from a larger group of animals. All male and female lambs were reared with their dams on a natural pasture of Northwest Sardinia (Italy), were fed mainly with maternal milk, but every day they followed their mothers on pasture so they have had the opportunity to eat freely grass for grazing. Twins were considered. At 28 days of age lambs were

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slaughtered at live weight of 10.12±0.47 kg and 9.32±0.62 kg for male (M) and female (F), rispectively.

After 24 h of refrigeration at 4°C, the leg muscles (*semitendinosus, semimembranosus and femoral biceps*) were dissected from each right half-carcass, all subcutaneous and visible intermuscular fat were removed and pH was evaluated at 24 h postmortem on each muscle portion using pH meter (Orion Research Inc., model 250A, Boston, MA) equipped with thermometer and a penetrating glass-electrode (Hamilton Company USA, model 238405, Reno, NV).

All samples, still partially frozen, were trimmed of connective and adipose tissue and sliced into 3 square cuts of about 50 grams, one was subjected to the cooking treatment, while the others were left raw as control for determination of nutritional value and lipid oxidation in uncooked meat. The raw and cooked samples used for the determination of chemichal composition and fatty acid content, were lyophilized, minced finelly, stored in a plastic container and frozen (-20°C) until needed for processing. Analyses were performed within 3 months.

The lipid oxidative stability of Sarda suckling lambs meat was evaluated measuring the quantity of MDA in raw and cooked samples by the TBA test. For assay on raw muscles, single portion of about 50 g from each muscle sample was homogenized with a household grinder (La Moulinette, Moulinex, s.p.a., Milan, Italy), for 60 s, placed in an airtight plastic container and stored in a household freezer at -20°C for up to 30 days after slaughtering. The effect of frozen aerobic storage on raw meat was also studied. Raw minced samples from all animals were stored in a household freezer at -20°C , in the dark, and the extending of lipid oxidation were determined after 30, 90, 150 and 180 days of frozen storage. The temperature of -20°C was chosen because it is commonly used for domestic purposes.

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1.3.2. Cooking and preparation of samples

The cooking method selected was microwaving. A Samsung GE82W microwave oven equipped with a revolving plate was selected for microwave cooking, which was performed in a polypropylene (PP) pan suitable for microwave.

After removing the visible intermuscular fat, about 50 g of muscle was cooked at 650 W, for about 35s, to attain a final core temperature of 75°C required to achieve a constant degree of doneness (medium, according to Matthews and Garrison, pp 64-65 (1975)). The internal temperature was measured with a digital thermometer (TAYLOR USA, model 9847N, Oak Brook, IL), insert in the centre of the samples, upon removal from the oven.

Finished samples were allowed to cool at room temperature, dried with filter paper and weighed. Cooking loss was expressed as the percentage of loss related to the initial weight.

After measurement of cooking loss, samples were split into two pieces (~ 25 g): one was used for chemical analysis, while the other was used for evaluating the lipid oxidative stability by quantification of MDA levels by TBA test. The pieces used for chemical analysis were wrapped individually in aluminum foil, vacuum-packed before freezing and storage at –80°C until required for analyses 4–5 days later, while the other pieces were processed for MDA determination the day after the cooking procedure.

1.4. Chemical analyses

Analyses were carried out on RW and CK samples, all reagents were of analytical and liquid chromatographic grade.

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1.4.1. Proximate Composition and Energy Value

Samples were analyzed in duplicate for protein and ash, while the determination of fat and moisture was performed in single. Crude protein content of RW and CK meat was calculated as total nitrogen amount multiplied by a conversion factor of 0.625 per 100 g of meat. The nitrogen content was determined by the Kjeldahl method (AOAC, 1990) using a 2200 Kjeltec ^{TM} automatic distillation unit (Foss Tecator, Höganäs, Sweden).
Moisture was determined on about 50 g of diced RW and CK samples as weight loss after 72 h of freeze-drying. Total ash content was determined on RW and CK meat by ashing the samples in the muffle-furnace at 550 °C for 24 h (AOAC, 1990).
Lipid was extracted from freeze-dried sample following the method of Folch et al. (1957), with some modification, using chloroform-methanol (2:1 v/v) for extraction. Briefly, a quantity of freeze-dried sample (raw or cooked) 1 g (~0,0001 g) was weighted in a 50 mL screw-cap glass centrifuge tube, and 30 mL of previously prepared chloroform-methanol solution (2:1 v/v) were added. The mixture was sonicated for 5 min, shaken vigorously with a vortex for 60 s and then centrifuged for 10 min (1500 x g at room temperature).
The chloroformic phase was filtered through a Whatman No. 541 and 6 mL 0,6% NaCl (wt/v) were added. The tube was centrifuged at 1500 x g for 10 min. The upper methanol-water layer was removed using a water aspirator and discarded; the lower organic phase was poured into a round flat-bottom flask (50 mL), chloroform was removed using a vacuum rotatory evaporator at 40°C (Büchi 461, BÜCHI Labor AG, Flawil, Switzerland) and samples were evapored until dryness. Energy value (kcal) was calculated by multiplying the amount of protein by 4 and the amount of fat by 9 (EEC, 1990).

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1.4.2. Fatty acid content and composition

Lipid extract from each sample (RW or CK) was used for fatty acid determination. The total extracted meat fat from the modified method Folch was recorder gravimetrically. After 24 h in a desiccator, extracted fat was redissolved in hexane and an aliquot of muscle lipid was esterified to obtain the fatty acid methyl esters (FAMEs).

To ~20 mg of total lipid, exactly weighted, were added 0.5 mg of nonadecanoic acid (C19:0) methyl ester (Sigma-Aldrich Inc., St. Louis, MO, USA) as internal standard, and then esterified by cool base-catalyzed methylation using 0.5 M methanolic solution of sodium methoxide (Sigma-Aldrich Inc., St. Louis, MO, USA) according to the standard FIL-IDF procedure (FIL-IDF 1999), the methylation was achieved in 15 min at room temperature.

Separation and quantification of the fatty acid methyl esters was carried out using a gas chromatograph (GC Turbo 3400 *CX*, Varian Inc. Palo Alto, CA) equipped with a flame ionization detector and an automatic sample injector 8200 *CX*, and using a a CP-select CB for Fame fused silica capillary column (100m, 0.32 mm i.d., 0.25 μm film thickness, Varian Inc. Palo Alto, CA). The chromatographic conditions were as follows: initial column temperature 75°C maintaining this temperature for 1 min, programmed to increase at a rate of 8°C/min up to 165°C, held for 35 min, then increasing again at 5.5°C/min up to 210°C, held for 1 min, and finally increased at 3°C/min to final temperature of 240°C and maintaining for 15 min.

The injector and detector were maintained both at 255 °C. Helium was used as carrier gas at a constant flow-rate of 1.0 mL/min, with the column head pressure set at 37.00 psi. The split ratio was 1:100, 1 μL of solution was injected and the run time was 74 min. High purity hydrogen and cromatographic air were supplied to the FID. Retention time and area of each peak were computed using the Varian Star 3.4.1. software.

Nonadecanoic acid methyl ester (C19:0 ME) at 0.5 mg/mL was used as internal standard. Individual fatty acid methyl ester, were identified by comparing their retention times with those of authenticated standards. Fatty acids were expressed as a percentage of total fatty acids identified.

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Individual FAME were identified by comparing them to a standard mixture of FIM-7FAME Mix (Matreya Inc. Pleasant Gap, Pa., USA). Branched saturated fatty acid methyl ester (BCFA) were identified using GLC-110 MIX (Matreya Inc. Pleasant Gap, Pa., USA) as external standard and individual BCFA methyl ester standards, methyl 14-methylpentadecanoate (isoC16:0), methyl 12-methyltetradecanoate (anteisoC15:0), methyl 12-tridodecanoate (isoC14:0) and methyl 14-methylhexadodecanoate (anteisoC17:0) (Matreya Inc. Pleasant Gap, Pa., USA). Polyunsaturated fatty acid methyl esters (PUFA) were identified by comparing relative retention times with isomer mixtures PUFA-1, PUFA-2 and PUFA-3 Animal Source (Supelco, Bellefonte PA) and individual external methyl ester standards, all cis-5,8,11,14,17 C20:5 (EPA), all cis-4,7,10,13,16,19 C22:6 (DHA), all cis-5,8,11,14 C20:4 (ARA), all cis-6,9,12 C18:3, all cis-9,12,15 C18:3, all cis-11,14 C20:2 and all cis-5,8,11 C20:3 (Matreya Inc. Pleasant Gap, Pa., USA).

High purity individual *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA (Matreya Inc., Pleasant Gap PA, USA), CLA mix standard (Nu-Ceck-Prep. Inc. Elysian, Minn., USA), and published isomeric profile (Kramer et al., 2004) were also used to help identify the CLA isomers in meat. The identification of C18:1 isomers was based on individual commercial standard (Supelco, Bellefonte, PA, USA) and on chromatograms published by Kramer et al., (2008).

The isomers of C16:1 and C17:1 were identified using published chromatographic reports in ruminant fats (Alves et al., 2006; Luna et al., 2009 respectively).

Fatty acids were expressed as "normalized" reports (each fatty acid as percentage of total FAME). The proportion of polyunsaturated (PUFA), monounsaturated (MUFA), saturated (SFA) and odd-branched (OBCFA) fatty acid contents and the ratios PUFA/SFA and n-6/n-3 were calculated. The atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht & Southgate (1991): AI=[C12:0+(4 ×C14:0)+C16:0]/[(ΣPUFA)+(ΣMUFA)]; TI=[C14:0+C16:0+C18:0]/[(0.5×ΣMUFA)+(0.5×n-6)+(3×n-3)+(n-3/n-6)]. The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated according to Fernández et al. (2007): h/H=[(sum of C18:1c-9, C18:1c-11, C18:2n-6, C18:3n-6, C18:3n-3, C20:3n-6,C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3 and C22:6n-3)/(sum of C14:0 and C16:0)].

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1.4.3. Measurement of oxidative stability

Lipid oxidative stability was assessed in raw and cooked minced lamb meat by determination of secondary products of lipid oxidation as 2-thiobarbituric acid reactive substances (TBArs) using a modification of the aqueous acid-extraction method described by Raharjo and Sofos (1992). Minced meat (10.0 g) was weighed into a glass test tube, to which butylated hydroxytoluene (BHT) (Carlo Erba Reagenti, Milan, Italy), at a level of 0.15% based on lipid content (Pikul et al., 1983), was added. The sample was homogenized in trichloroacetic acid (TCA) solution (40 ml, 5% w/v) using an Ultraturrax T25 homogenizer (IKA-Labortechnik, Staufen, Germany), transferred into a 50 ml centrifugation tube and centrifuged for 10 min at 5000g for 45 min at +5°C. The supernatant was filtered through Whatman No. 541 filter paper into a 50 ml volumetric flask. TCA (5% aqueous) was used to make up the volume. The filtered supernatant (2 ml) was transferred to a 10 ml screw cap glass Pirex® tube, mixed with 2-thiobarbituric acid (TBA, 40 mM, 2 ml) and heated at 93°C for 20 min to develop the rose-pink color by reaction between malondialdehyde (MDA) and TBA. The absorbance was measured at 525 nm (Cary 50 scan UV-Vis Spectrophotometer - Varian Inc. Palo Alto, CA) against a blank containing TCA (2 ml, 5% w/v) and TBA (2 mL 40 mM). The TBArs values were calculated using an external standard technique from a standard curve (0.2 - 20 μM) of 1,1,3,3-tetraethoxypropane (TEP) and expressed as mg of MDA per kg of muscle. The final conversion of TEP concentration to MDA concentration was accomplished using the following formula (Pikul et al., 1989):

$$TBArs_{(mg\ MDA/Kg\ meat)} = ((A \times m \times 7.063 \times 10^{-6})/E) \times 1000$$

where A is the absorbance of each sample, m is the slope of the calibration curve calculated by dividing the concentration in nM of each standard solutions of TEP with its absorbance, 72.063 is the conversion factor from nmol to mg of MDA (assuming that one mole of TEP from place to one mole of MDA), E is the sample weight equivalent.

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The equivalent (E) for 10 g of sample was 0.4 when a 2 mL aliquot of the original 50 mL filtrate was analyzed. Two replicates were run per sample.

1.4.4. Nutrient retention values

True retention values (TR) for all nutrients were calculated using the following formula (Murphy et al., 1975):
TR (%) = [(nutrient content per g of cooked food × g of food after cooking) / (nutrient content per g of raw food × g of food before cooking)] × 100.

1.4.5. Statistical analysis

Data on meat composition and fatty acid content were analyzed with a GLM procedure using the statistical software Minitab (16, 2009) (Minitab Inc., State College, PA). The model included sex (M v*s* F) and treatment (RW *vs* CK) as fixed effects and their interaction (sex × treatment). One-way ANOVA was used to compare raw versus cooked samples. Lipid oxidation (TBArs values) was processed by the GLM procedure of Minitab (16, 2009) (Minitab Inc., State College, PA). Tukey's test was used to compare mean values.

1.5. Results and discussions

1.5.1. Proximate composition

The separable lean of the muscles analyzed in the raw state (Table 11) shown proximate composition and energy value in line with the range values reported for raw lamb rib loin (or, whenever unavailable, "generic" lamb lean) in food composition table, databases and compositional surveys published in several European countries (Chan et al., 1995; Favier et al.,1995; INRAN, 2000; Souci et al., 2000); in the USA (Nat.Livest.& Meat, 1988; USDA, 2002) and in Australia (Hoke et al., 1999; Williams, 2007). Based on the results obtained for the raw state to the muscle samples used in this

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study can be applied to the definition of "extra-lean meat" as defined by Chan et al. (1995) and U.S. Food and Drug Administartion (1999). No significant differences between sexes were observed in the proximate composition for the raw state similar to that found by Horcada et al., (1998) in males and female suckling Lacha-breed lambs.

Table 11. Proximate composition (g/100g of muscle, except where noted) and energy value of raw and cooked leg muscle (*semitendinosus, semimembranosus and femoral biceps*) from male and female Sarda-breed suckling lamb.

	Sex (SX)								P-value	
	Male				Female					
State (ST)	Raw		Cooked		Raw		Cooked		SX	ST
Trait	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
Moisture	74.81^{a}	0.25	69.06^{b}	0.60	74.43^{a}	0.68	68.70^{b}	0.33	n.s.	**
Protein	20.39^{b}	0.18	24.22^{a}	0.63	20.45^{b}	0.11	25.68^{a}	0.47	n.s.	***
Lipids	2.32	0.14	3.32	0.55	2.29	0.22	3.20	0.34	n.s.	**
Ash	1.21^{ab}	0.03	1.25^{ab}	0.03	1.16^{b}	0.02	1.27^{a}	0.02	n.s.	**
Energy value$^{\#}$	103.7^{b}	0.90	137.2^{a}	8.66	103.8^{b}	0.58	131.5^{a}	3.97	n.s.	***
Cooking loss (%)	—		25.48	1.09	—		29.06	1.28	*	—
pH	5.72	0.03	—		5.73	0.03	—		n.s.	—

Least square means (LSM) in the same row with different superscript letters differ significantly: $p \leq 0.05$.
State × Sex interaction was not statistically different for all traits.
*** $p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant.
SEM, standard error of mean. $^{\#}$ Kcal/100g of muscle

Cooking produced a statistically significant decrease in moisture content and this leads to a significant increase in the protein, fat and ash content which yields a significantly higher energy value, but without significant differences between the sexes. The results obtained are in agreement with those reported in previous studies (Badiani et al., 2004; Maranesi et al., 2005) involving lambs of different breeds, age and slaughtered at different weights without distinction between the sexes and using a different cooking method.

The values obtained for the microwave cooked meat once again confirm that the meat of suckling lamb is considerably lean and only 21-22% energy comes from fat against 78-79% provided by protein this is in line with what is suggested for adults by SINU. Different cooking losses ($p<0.05$) was also observed between sexes despite the

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proximate composition and the energy value were quite similar. There were no differences between sexes in pH values.

The consumption of 100g of cooked thigh muscles from suckling lamb of Sarda-bred satisfied, on average for males and females, 7% of the recommended daily energy ration for an adult involved in moderate activity that leads to a daily consumption of 2000 Kcal. An average serving of cooked thigh muscles of suckling lamb of Sarda-bred is able to provide between 32 and 34% (for males and females, respectively) of the RDA of protein and only between 4.9 and 5.1% (for females and males, respectively) of the RDA of fat recommended for adults of both sexes. Therefore, based on the results obtained, it can be said that the thigh muscles from suckling lamb of Sarda-bred is a lean meat that has a high nutrient density for protein.

1.5.2. Intramuscular fatty acid composition

Data on partial sums (g/100g FAME) of fatty acids, in both males and females, raw and cooked samples are shown in Table 12. The sex of lambs did not modify almost all partial sums of fatty acids considered, except for the content of OBCFA which is significantly higher in males than in females. This result is comparable to that reported by Horcada et al., (1998) which have found in suckling lambs of Lacha bred significant differences between the sexes only for this group of fatty acids while no other significant differences were found on the partial sums of fatty acids. A significant decrease ($p<0.05$) in the content of SFA and OBCFA occurred after cooking for both males and females, while no difference were observed for the relative proportion of TFA, MUFA and PUFA. Regarding the partial sums of PUFA n-3 and PUFA n-6 their values tented to increase ($p=0.07$) after the microvawe cooking for both sexes. Our results were in accordance with the findings reported by Vincent et al., (2004) for Gentile di Puglia male suckling lambs, slaughtered at 45 days of age and fed only with maternal milk. In their research in fact, after cooking the lamb meat in a ventilated electric oven, were observed changes in the fatty acid profile, in particular a decrease in the content of SFA and an increase in the amount of PUFA.

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The ratio n-6/n-3 and PUFA/SFA, which are indices widely used to determine the nutritional value of fat for human health, are present in Table 12. In the human diet, the recommended value for PUFA/SFA ratio should be included between 0.45-0.60 (British Department of Health, 1994), while, the optimal dose or ratio of n-6/n-3 should not exceed the value of 4.0 (Simopoulos, 2002).

In the present study the values obtained regarding these nutritional ratios were not influenced by sex of lambs or cooking treatment with values close to the recommended limit. In order to better assess the effects of fatty acids on human health the artherogenicity (AI) and thrombogenicity (TI) indices were also calculated according to Ulbricht and Southgate (1991). In this study no significant different emerged among sex regards the TI and the AI values, while TI and AI were affected significantly ($p<0.05$ and $p\leq0.01$ respectively) by cooking. The hypocholesterolemic/hypercholesterolemic ratio (h/H) calculated according to Fernández et al. (2007) was resulted not influenced by sex but a its significant increase ($p<0.05$) was observed after cooking due to an higher level of some PUFA fatty acids and a relative decrease of some SFAs in the cooked samples versus the raw samples.

Fatty acid composition (g/100g of FAME) in raw and cooked sample of thigh muscles from Sarda-bred suckling lambs is present in Table 13. In both raw and cooked samples for males and females the predominant fatty acid were, in decreasing order of percentage, oleic (C18:1c9, 33-34%), palmitic (C16:0, 18-20%), stearic (C18:0, 13%) followed by mystric (C14:0, 4-5%) and linoleic (C18:2 n-6, 4-6%) acids, for a total of about 80% FAME. Branched-chain fatty acids plus odd-numbered fatty acid were between 2.5-3.0% of total FAME in both raw and cooked sample for male and female lambs. The levels of the main fatty acids observed in ours study were similar to those reported by other researchers (Badiani et al., 1998; Badiani et al., 2004) in studies performed with lambs of different breeds, slaughtered at different age and subjected to different feeding system and cooking with different method.

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Table 12. Effect of cooking and sex on partial sum of fatty acid (g/100g FAME), nutritional fatty acids ratios and values of TBArs (mg MDA/Kg of meat) of thigh muscle s*emitendinosus, semimembranosus and femoral biceps*) from Sarda-breed suckling lambs.

	Sex (Sx)								P-value	
	Males				Females					
State (St)	Raw		Cooked		Raw		Cooked		St	Sx
Item	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
Partial sums										
Σ SFA	43.27	0.96	40.66	1.20	42.33	1.35	39.29	1.11	*	n.s.
Σ MUFA	42.54	0.55	42.83	0.42	42.21	1.12	42.59	1.23	n.s.	n.s.
Σ TFA	5.56	0.29	5.21	0.28	5.21	0.20	4.95	0.27	n.s.	n.s.
Σ PUFA	13.59	0.72	15.94	1.09	14.84	2.05	17.53	1.87	n.s.	n.s.
Σ PUFA n-3	3.27	0.27	4.01	0.32	3.59	0.59	4.40	0.54	†	n.s.
Σ PUFA n-6	8.11	0.52	9.65	0.73	9.01	1.29	10.84	1.17	†	n.s.
HP-PUFA	5.61	0.49	7.03	0.67	6.28	1.39	7.82	1.24	n.s.	n.s.
Σ OBCFA	3.05^{a}	0.11	2.84^{ab}	0.14	2.77^{ab}	0.10	2.51^{b}	0.09	*	**
Ratios										
PUFA/SFA	0.32	0.02	0.40	0.04	0.37	0.07	0.46	0.07	n.s.	n.s.
MUFA/SFA	0.99	0.03	1.06	0.04	1.00	0.03	1.09	0.04	*	n.s.
n-6/n-3	0.40	0.02	0.42	0.02	0.39	0.02	0.40	0.02	n.s.	n.s.
h/H	1.76	0.11	2.09	0.14	1.85	0.15	2.22	0.17	*	n.s.
AI	0.81	0.05	0.66	0.05	0.77	0.05	0.63	0.05	**	n.s.
TI	1.12	0.06	0.98	0.06	1.10	0.07	0.92	0.05	*	n.s.
Lipid Oxidation										
MDA (mg /Kg meat)	0.42	0.07	2.75	0.16	0.59	0.11	3.63	0.23	***	***

Least square means in the same row with different superscrip letters differ significantly: $p \leq 0.05$
***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant.
FAME, Fatty acids methyl esters; SFA, Saturated Fatty Acids; MUFA, Monounsatured Fatty Acids; PUFA, Polyunsatured Fatty Acids; HP-PUFA, highly peroxidisable; OBCFA, Odd and Branched Fatty Acids.
ΣSFA: sum of C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C22:0, C24:0 and Odd-Branched fatty acids;
ΣMUFA: sum of C14:1, ΣC16:1, ΣC17:1, ΣC18:1
ΣTFA: sum of C16:1*t*9, C18:1*t*4, C18:1*t*6+*t*8, C18:1*t*9, C18:1*t*10, C18:1*t*11, C18:2*t,t* + *c/t,* C18:2*t*8,*c*13.
(*c* is a *cis*-isomer and *t* is a *trans*-isomer)
ΣPUFA: sum of total n-6 and total n-3;
ΣPUFA n-3: sum of C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:5n-3, C22:6n-3.
ΣPUFA n-6: sum of ΣC18:2n-6 *cis*/*trans* isomers, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6, C22:4n-6.
HP-PUFA : sum of PUFA with three or more double bonds.
OBCFA : sum of C13:0, C13:0 *i*, C13:0 *ai*, C14:0 *i*. C15:0 *i*, C15:0 *ai*, C16:0 *i*, C17:0, C17:0 *i*, C17:0 *ai*, Σ C17:1.
(*i* is an *iso*-isomer and *ai* is an *ante-iso*-isomer).
State × Sex interaction was statistically significant only for TBArs ($p \leq 0.001$)

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Table 13. Fatty acid composition (g/100g FAME) of raw and cooked thigh muscle (*semitendinosus, semimembranosus and femoral biceps*) from males and females Sarda-breed suckling lambs.

	Sex (SX)								p-value	
	Males				Females					
State (ST)	Raw		Cooked		Raw		Cooked		ST	SX
Fatty acids	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
C12:0	0.67^{a}	0.07	0.49^{ab}	0.06	0.57^{ab}	0.07	0.42^{b}	0.04	**	n.s.
C14:0	5.48	0.39	4.24	0.40	5.17	0.39	4.11	0.40	**	n.s.
C14:1 c9	0.16	0.02	0.11	0.01	0.16	0.02	0.14	0.02	†	n.s.
C15:0	0.54^{a}	0.03	0.47^{ab}	0.03	0.46^{ab}	0.02	0.38^{b}	0.02	**	**
C16:0	20.61	0.57	19.19	0.58	20.38	0.69	18.82	0.70	*	n.s.
C16:1 *c*9	1.38	0.07	1.26	0.05	1.47	0.11	1.30	0.16	n.s.	*
C16:1 *t*9	0.15	0.01	0.16	0.01	0.13	0.01	0.14	0.01	n.s.	n.s.
C17:0	0.93	0.02	0.91	0.03	0.91	0.03	0.86	0.03	n.s.	n.s.
C17:1 *c*9	0.45	0.01	0.46	0.01	0.48	0.02	0.50	0.03	n.s.	*
C18:0	13.18	0.49	13.67	0.42	13.19	0.45	13.23	0.59	n.s.	n.s.
C18:1 *t*11	2.38	0.16	2.21	0.13	2.15	0.08	2.01	0.12	n.s.	†
C18:1 *c*9	33.89	0.56	34.31	0.47	33.50	1.05	34.26	1.14	n.s.	n.s.
C18:2 n-6 (LN)	4.89	0.36	5.89	0.47	5.52	0.77	6.72	0.68	†	n.s.
C18:3 n-3 (ALA)	1.17	0.07	1.37	0.08	1.24	0.10	1.48	0.10	*	n.s.
CLA c9,t11	1.51	0.09	1.39	0.06	1.46	0.07	1.36	0.09	n.s.	n.s.
C20:4 n-6 (ARA)	1.53	0.17	1.98	0.25	1.79	0.51	2.25	0.49	n.s.	n.s.
C20:5 n-3 (EPA)	0.58	0.06	0.76	0.08	0.71	0.18	0.95	0.18	n.s.	n.s.
C22:4 n-6	0.09	0.01	0.11	0.01	0.09	0.02	0.11	0.02	n.s.	n.s.
C22:5 n-3 (DPA)	0.87	0.08	1.05	0.10	1.00	0.23	1.14	0.21	n.s.	n.s.
C22:6 n-3 (DHA)	0.56	0.07	0.72	0.08	0.55	0.09	0.71	0.08	*	n.s.

Fatty acid are represented in the following manner: the first number indicates the number of carbons, while the second represents the number of double bonds; n-x indicates where the first double bond is located counting from the terminal methyl carbon toward the carbonyl carbon; *c*-x is for *cis*, *t*-x is for *trans* and indicate the configuration of double bond and where the double bond in located counting from the carbonyl carbon toward the terminal methyl carbon.

Least square of means (LSM) on the same row followed by superscript different letters differ significantly ($p \leq 0.05$).

***p≤0.001; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant. SEM, standard error of mean.

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Sex had a minimum impact on the fatty acid profile of suckling lambs in fact significant differents were found only for C15:0 ($p<0.01$), C16:1c9 ($p<0.05$) and C17:1c9 ($p<0.05$) with a higher content in males than in females for C15:0 and a higher level in females versus males for C16:1c9 and C17:c9. These result agreed partially with those presented by Horcada et al., (1998) that had found a significantly higher proportions of pentadecanoic (C15:0) and palmitoleic (C16:1) in suckling female lambs of Lacha bred than in males. Finally the percentage of vaccenic (C18:1t11, VA) acid tended ($p<0.09$) to be higher in males than female.

The cooking treatment affect significantly ($p<0.05$) the content of 8 of the 20 fatty acid selected. In particular, some SFA, namely lauric (C12:0), stearic (C14:0), pentadecenoic (C15:0) ($p<0.01$ for all three FA) and palmitic (C16:0) ($p<0.05$) acids were significantly lower in cooked meat samples than in uncooked meat. In contrast, the percentage of linoleic (C18:2 n-6, LN) acids tented to increase ($p<0.07$) in cooked lamb meat compared to raw meat. Within the PUFA n-3, no appearance cooking effect were observed, except for linolenic (C18:3 n-3, ALA) and docosahexaenoic (C22:6 n-3, DHA) which had a higher significant content ($P<0.05$) in cooked lamb relative to raw meat. These facts can be due to the migration of fatty acids from muscle to other locations, the subcutaneous fat migration into the lean, the loss of volatile fatty acids and the oxidative degradation with specific reference to PUFA. Our findings partially differ from the variations in fatty acid composition of lamb lipids reported by Badiani et al., (2004) which have found not significant differences, except for pentaenoic (C15:0) acid, in the fatty acid profile of cooked meat compared to raw meat. No significant effect of cooking was also reported in other similar studies performed on beef meat (Sarries et al., 2009 and Nayigihugu et al., 2004) and camel meat (Kadimi et al., 2011). However, Juarez et al. (2009) have found great differences, induced by cooking, in fatty acid composition in buffalo meat; in particular they have observed a significant increase in the content of total PUFA and a relevant decrease in the content of SFA after heating. In conclusion, variation in the fatty acid content between raw and cooked meat sample have widely been reported in several studies performed on animals of different species (chicken, beef, pigs, camel, buffalo) but, despite some exceptions, in general the changes observed in the fatty acid profile in cooked meat samples compared

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to uncooked meat are minimum and marginal suggesting that there were not "selective" variation for specific fatty acid.

1.5.3. Fatty acid content and True Retention Values

In order to perform observations of nutritional interest and calculate the True Nutrient Retention Value (TR), the quantitative composition of fatty acids (expressed as mg/100g of edible portion) was determined in both raw and cooked meat (Table 14) (considering males and females together).

As expected, cooking produce an increase in the contents of all fatty acid, in particular, significant differences ($p<0.01$) were observed for the contents of PUFA, PUFA n-3, PUFA n-6 and HP-PUFA. The increase in PUFA after grilling has been also reported by other authors in beef (Scheeder et al., 2001; Correia and Biscontini, 2003) and hamburgers (Rodriguez-Estrada et al., 1997), and it has been related to triglycerides unsaturated fatty acid drip losses (Scheeder et al., 2001; Cobos et al., 2008). Despite, numerically it is possible to observed a substantial increase in the amounts of SFA, MUFA, TFA and OBCFA after cooking, this was not of statistical significance ($p>0.10$) for all traits. Considering the single fatty acids, it was observed that cooking has led to a ponderal increase in the content of all fatty acids considered, with the exception of lauric (C12:0) and mystric (C14:0) acids for which has been observed a substantial, but not statistically significant, decrease of their contents in cooked compared to raw meat. In particular, linoleic (C18:2 n-6, LN), α-linolenic (C18:3 n-3, ALA), arachidonic (C20:4 n-6, ARA) and docosapentaenoic (C22:5 n-3, DPA) acids were significantly higher ($p<0.05$) in cooked meat samples than in uncooked meat control. While, highly significant increase ($p<0.01$) in the amounts of eicosenoic (C20:5 n-3, EPA) and docosahexaenoic (C22:6 n-3, DHA) were found after cooking. The mean total CLA content provided by the raw meat amounted to 25.24 mg/100g of edible portion, after microwave cooking, the content of CLA increase, but without a significant difference, rising to a mean value, between males and females, of 27.18 mg/100g of cooked meat.

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Table 14. Fatty acid content (mg/100g of edible portion) of raw and cooked meat from Sarda-breed suckling lambs

	State (ST)				
	Raw		Cooked		p-value
Partial sums	LSM	SEM	LSM	SEM	
Σ SFA	737.84	48.51	814.50	12.77	n.s.
Σ MUFA	729.23	43.24	859.85	12.92	n.s.
Σ TFA	92.47	6.22	102.73	14.69	n.s.
Σ PUFA	232.99^{b}	13.59	286.03^{a}	18.04	*
Σ PUFA n-3	55.99^{b}	3.93	69.33^{a}	3.07	**
Σ PUFA n-6	140.03^{b}	8.72	174.47^{a}	11.03	**
HP-PUFA	94.91^{b}	6.48	118.65^{a}	4.26	**
Σ OBCFA	50.38	3.37	55.49	8.69	n.s.
Fatty acids					
C12:0	10.26	0.83	9.76	1.83	n.s.
C14:0	92.22	7.45	90.84	17.07	n.s.
C16:0	354.71	24.25	385.90	56.39	n.s.
C18:1 *c*9	580.51	34.88	692.72	10.30	n.s.
CLA c9,t11	25.24	1.67	27.18	3.82	n.s.
C18:2 n-6 (LN)	85.57^{b}	6.13	107.45^{a}	7.07	*
C18:3 n-3 (ALA)	20.38^{b}	1.40	25.32^{a}	2.06	*
C20:4 n-6 (ARA)	25.52^{b}	2.06	31.94^{a}	1.28	*
C20:5 n-3 (EPA)	10.11^{b}	0.90	12.95^{a}	0.46	**
C22:5 n-3 (DPA)	14.81^{b}	1.13	17.39^{a}	0.69	*
C22:6 n-3 (DHA)	9.14^{b}	0.81	11.54^{a}	0.48	**

Least square of means (LSM) on the same row followed by superscript different letters differ significantly ($p \leq 0.05$). ***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant. SEM, standard error of mean.
See footnotes in Tables 12 and 13.

There are very few or no data regarding optimal dietary intake of CLA in humans. The minimum effective dose-response is still unknown. According to animal studies and epidemiological calculations the optimal intake is between 0.095 and 3.5 g/day (Ha et al, 1989; Enser, 1999).

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In conclusion, one serving (100 g) of cooked suckling lamb meat is able to supply an average amount of 286 mg of PUFA n-6, 107 mg of which derived from LN and 32 mg from ARA. The contribution of PUFA n-3 amounted to about 70 mg on 100 g of cooked meat, of which 25 mg was supplied by ALA and about 12 mg by DHA.

Although the contents of PUFA in general, and of n-3 PUFAs in particular are negligible compared to the daily human requirements of n-3 PUFAs as well as recently proposed by EURODIET (2000), at 2000 mg of ALA + 200 mg of very long chain n-3 fatty acids, the consumption of lamb meat into a balanced diet provides a modest intake of fats and despite the limited content of n-3 PUFAs, which makes this food may not be the first dietary choice for the consumer in order to increase the intake of n-3 PUFAs, supplies a good intake of nutrients beneficial to human health, is rich in proteins of high biological value and represents an important source of CLA.

The TR values of selected nutrients and fatty acids calculated in order to evaluate the correct increase or loss of meat components during cooking are present in Table 15.

The differences observed between males and females were not statistically significant for all the nutrients ad fatty acids considered indicating that the meat from the two sexes, subjected to microwave cooking, behaves in the same way during the heat treatment.

The TR values obtained for moisture, and ash were comparable with those reported by Ono et al., (1984) for lambs of two age groups, Hoke et al, (1999) for exported Australian lambs, Badiani et al., (2004) for lamb rib loins from lambs of 5 months of age and by Maranesi et al., (2005) for heavy lamb meat subjected to microwave cooking, while the values calculated for protein and energy were found to be lower in the range of 88-90% and 90-96% respectively, compared to those reported in the sources mentioned above, a result for which there is not ready explanation.

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Table 15. True nutrient retention values (%) for cooked samples of thigh muscle (*semitendinosus, semimembranosus and femoral biceps*) from Sarda-breed suckling lamb.

	Sex (SX)				
	Males		Females		p-value
Selected nutrients	LSM	SEM	LSM	SEM	
Moisture	69.42	1.37	69.09	1.01	n.s.
Protein	88.47	2.04	90.14	1.22	n.s.
Lipid	109.34	17.77	104.49	10.62	n.s.
Ash	76.39	1.94	76.73	1.21	n.s.
Energy	89.75	3.88	96.58	4.44	n.s.
Fatty acids					
Σ SFA	91.77	21.23	77.25	12.68	n.s.
Σ MUFA	97.28	21.22	83.15	12.83	n.s.
Σ TFA	93.82	22.71	77.83	12.23	n.s.
Σ PUFA	100.81	11.63	100.70	14.92	n.s.
Σ PUFA n-3	108.07	15.24	111.90	21.04	n.s.
Σ PUFA n-6	102.63	12.15	102.86	15.25	n.s.

Least square of means (LSM) on the same row followed by superscript different letters differ significantly ($p \leq 0.05$). ***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant. SEM, standard error of mean.
See footnotes in Tables 12.

The TR observed in this study for lipids did not vary much from 100%, which can be accounted for by a mere concentration effect or by a substantial equilibrium between fat migration into the lean and extract of intramuscular fat from the lean. The TR calculated by Hoke et al. (1999) and by Ono et al (1984) were, instead, higher ranging between 115% and 126%. Lipid TR values higher than 100% in lean meat, trimmed of subcutaneous fat, as was in this study, suggest, how explained by previous studies (Smith et al., 1989; Bragagnolo et al., 2003) that negligible levels of subcutaneous and intramuscular fats were been liquefied during cooking and were been absorbed by the lean tissue leads to TR values higher than 100%.

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1.5.4. Lipid oxidation

The TBArs value is widely used as an index of oxidation of muscle lipids at biochemical level. When meat samples were cooked in microwave oven, the mean concentration of MDA (mg/Kg of meat), for both males and females, were significantly increased than corresponding raw sample analyzed before cooking, these results are also presented in Table 12. Lipid oxidation was significantly ($p<0.001$) influenced by sex, cooking and their interaction, and the value of MDA obtained for both sexes revealed that the absolute concentration of MDA generated by cooking is strongly dependent on the initial MDA levels of uncooked meat. It is well known that the rate and extend of lipid oxidation may be strongly influenced by cooking method, rate and final temperature of heat treatment, meat composition, amount of lipids and fatty acid composition. In addition, dietary fats could affect fatty acid composition influencing the oxidative stability of meat (Bou et al., 2001). Therefore, comparison with literature data is difficult due to variety of analytical methods and procedures that do not allows to perform trials under the same experimental conditions, making them incomparable. In meat from male lambs the amount of MDA (mg/Kg of meat) observed (2.75 mg/Kg of meat) is resulted below the critical value of 3 mg/Kg of meat at which rancidity is virtually detected (Wong et al., 1995), while, the meat sample from female lambs, after cooking, have shown levels of MDA slightly higher of the limit of rancidity mentioned above (3.75 mg/Kg of meat). This fact could be related to a slightly higher ponderal content of long chain PUFAs and HP-PUFA in cooked meat from females than males.The effect of frozen storage, in aerobic condition, in household freezer at -20°C for up to 6 months is presented in Figure 24.

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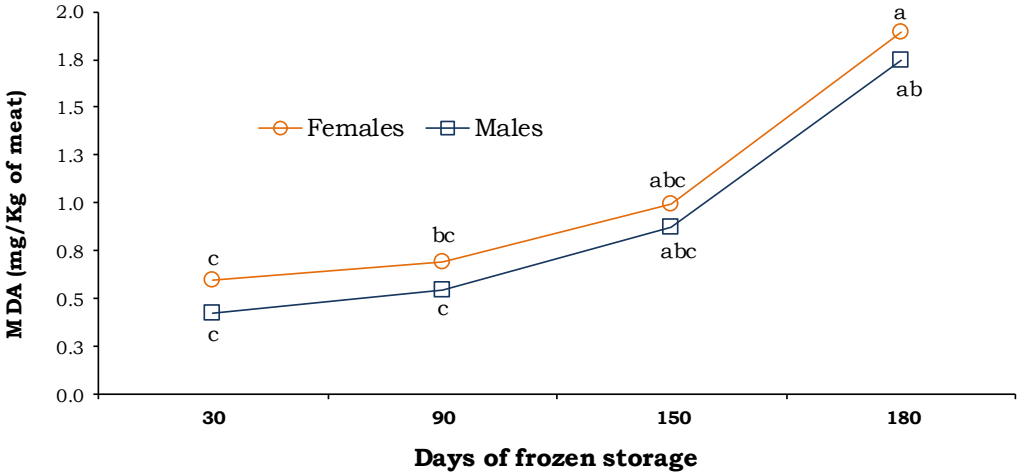

Figure 25. Development of lipid oxidation during frozen storage in suckling lamb meat from males and females.

Duration of storage has affected significantly (p=0.000) the overall concentration of MDA for both males and females, while the effects of sex and the interaction sex × time of storage were not statistically significant. Despite sex did not affect the lipid oxidation during storage, meat from female lambs have shown, for the whole experiment duration, concentration of MDA greater than males. Looking at the Figure 24, it can be seen the increase in the concentration of MDA was constant during the period of freezing in a similar manner in both sexes. This phenomenon appears to be difficult to interpret because the absence of pronounced differences between males and females and therefore requires further, more detailed studies.

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1.6. Conclusion

The trial was designed to characterize the quality and the oxidative stability, before and after microwave cooking, of the intramuscular fat of thigh muscles of male and female suckling lambs taken from a livestock traditional production of the ewes of "Sarda-breed". As a result of the greater level of unsaturation of the intramuscular fat, suckling lamb meat is more suitable from a dietetic point of view than veal or beef, because it has a more favorable ratio between saturated and unsaturated fatty acids. It is fundamental to underline that the sex did not affect fatty acid profile, probably because animals are slaughtered at an early age, when there is still not an important action of sexual hormones. MDA concentration in suckling lamb meat increase after microwave cooking, and these difference were significantly affect by sex, cooking and their interaction. The sex did not significantly affect the lipid oxidative stability of muscle during whole period of frozen storage. Oxidative stability was only affect, significantly, by time of storage in frozen condition, and the final values noted depends on the initial levels of MDA in the fresh meat. The absence of significant differences in the chemical composition, fatty acid content, retention values and oxidative stability between males and females showed that at the low slaughter weight, sex had no effect on the nature and composition of fat.

– EXPERIMENT TWO -

The changes in oxidative stability of Sarda suckling lamb meat, from two different rearing system, during frozen storage.

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2.1. Abstract

Lipid oxidation is the primary cause of loss of meat quality during frozen storage. Raw samples of thight muscles (*semitendinosus, semimembranosus* and *femoral biceps*) from Sarda suckling lambs were frozen at -20°C for up to 6 months and the oxidative changes in their lipids were evaluated by measuring the values of malondialdehyde (MDA) by TBArs test. Samples come from 48 suckling lambs divided into 2 groups with different feeding systems: 24 were raised indoor in stable (group S) and 24 followed their mother outdoor on pasture (group P). Lambs were slaughtered at 28 days of age. After 24h of refrigeration at 4°C, the thight muscles were divided into different pieces (about 250 g each) one, finelly minced, was stored in a household freezer at -20°C for 30, 90, 150 and 180 days, while the other was freeze-dried and analyzed for chemical and fatty acid composition. MDA levels were measured with spectrophotometer and were expressed as mg MDA/kg meat. A frozen storage temperature of -20°C was adopted because it is commonly used for domestic purposes. Data were analyzed using a model including storage, feeding system and their interaction. The MDA levels were significantly influenced by feeding system, storage and their interaction. The increase in the values of MDA was not statistically significant in the S group samples. The values of MDA increased significantly in the P group at 180 days of storage likely due to a higher content of polyunsaturated fatty acids. The results showed that the storage of meat at -20°C for extended periods does not prevent the oxidation process but certainly slows it down. The values of MDA detected in both experimental groups are within the limits of rancidity of about 1-2 mg MDA/100 g of product and 3mg/100g of edible portion indicated by Watts (1962) ad Wong et al., (1995) respectively.

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2.2. Introduction

Food preservation by freezing has proven to be a successful technology to extend the shelf-life of food products that have minimal effect on their sensory qualities. The main degradation process that occurs during frozen storage of the meat is lipid oxidation. Oxidative reactions occurring in the lipid fraction of frozen meat, although slower than at refrigeration or room temperature, tend to increase with a higher fat content in meat, especially when the meat contains a relatively large amounts of polyunsaturated fatty acids (PUFAs). Chemical oxidation of PUFAs generates various oxidation products, expecially malondialdehyde (MDA), that have been associated with off-flavour and off-odour, loss of colour and lower consumer acceptability and may also affect the safety of meat (Pearson et al.,1983). The most common method for measuring MDA values, as marker of lipid oxidation, in food products seems to be the thiobarbituric acid test (TBA-test) which is based on spectrophotometric quantitation of the pink complex formed after reaction of MDA with two molecules of 2-thiobarbituric acid (TBA). A frozen storage temperature of -20°C was adopted because it is commonly used for domestic purposes. Since the development of oxidation process in meat is important, related to its quality and consumer' acceptance, the aim of this study was to examine the change in oxidative stability in Sarda suckling lamb meat, from two rearing system, during six months of frozen storage at -20°C in household freezer,

2.3. Materials and methods

2.3.1. Animals and muscle sampling

Forty-eight Sarda breed suckling lambs were selected from 36 Sarda dairy ewers grazing on natural pasture. At birth the lambs were divided in two groups (24 lambs each): lambs kept indoor in stable during the grazing time of the ewes (group S or reared indoor in stable) and lambs which followed the mother on pasture (group P or reared outdoor on pasture).

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The lambs were weighed and then slaughtered at 28 days of age. The cold carcass weight (CCW, body weight minus blood, skin, viscera, feet, tail) and pH was measured after 24 h of storage at 4 °C. After 24 h of refrigeration the thight muscles (*Semitendinosus*, *Semimembranosus* and *Femoral Biceps*) were excised from the right side of each carcass, and pH was evaluated at 24 h postmortem on each muscle portion using pH meter (Orion Research Inc., model 250A, Boston, MA) equipped with thermometer and a penetrating glass-electrode (Hamilton Company USA, model 238405, Reno, NV).

Muscles were trimmed of external fat and connective tissue. The separable lean dissected from each thigh, which was intended as the edible portion, was split into two parts; a piece of about 50 g were finely diced and homogenized whit a household grinder (La Moulinette, Moulinex, s.p.a., Milan, Italy), was kept in an airtight plastic container and stored in a household freezer, in the dark, at -20°C and extending of lipid oxidation was analyzed after 30, 90, 150 and 180 days of frozen storage. The temperature of -20°C was chosen because it is commonly used for domestic purposes. A second part of the sample (about 50 g) was lyophilised for 72 h (−55 °C and 2.0 hPa) to constant weight, using a lyophilisator LyoLab 3000 (Heto-Holten, Allerød, Denmark), and was maintained exsiccated at room temperature, and analysed within two weeks. The freeze-dried samples, finely grinded, were used to determine moisture, total protein, ash, lipid and fatty acid composition.

2.3.2. Proximate composition

Sample were analyzed for moisture, total ash and total nitrogen in duplicate, while analyses of total lipid were carried out in single. All reagents were of analytical grade.

Moisture was determined as weight loss after 72 h of freeze-drying. Total ash content was quantified on meat by ashing the samples in a muffle furnace at 550 °C for 24 h (Method 920.153, AOAC, 1999). Total protein was calculated from Kjeldahl nitrogen using a 6.25 conversion factor. The nitrogen content was determined by the Kjeldahl method (Method 928.08, AOAC 1997) using a 2200 Kjeltec ^{TM} automatic distillation unit (Foss Tecator, Höganäs, Sweden). Lipid was extracted from 1 g of each freeze-dried sample following the method of Folch et al. (1957), with some modification,

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using chloroform/methanol (2:1, v/v) for extraction. Total lipids were measured gravimetrically and an aliquot of the fat extract (20-25 mg), dissolved in n-hexane, was transferred to a screw-caps amber vials, stored in a frozen (-20°C) and used within 24h for fatty acid analysis.

2.3.3. Fatty acid composition

Lipid extract from each leg sample was used for fatty acid determination. Fatty acids were converted to methyl esters by base-catalysed esterification with sodium methoxide $NaOCH_3$ (0.5 M solution in anhydrous methanol) (Sigma-Aldrich Inc., St. Louis, MO, USA) during 15 min at room temperature, according to the standard FIL-IDF procedure (FIL-IDF 1999). Quantification of FAME was based on the internal standard technique, using methyl-nonadecanoic acid (C19:0ME) as internal standard. Gas chromatography analyses of FAME were performed with an Turbo 3400 *CX* GC (Varian Inc. Palo Alto, CA) gas chromatograph equipped with a flame ionization detector (FID) and Varian 8200 *CX* auto sampler. The FAME were separated on a CP-select CB for FAME (Varian Inc. Palo Alto, CA) capillary column (100 m x 0.32 mm i.d., 0.25 µm film thickness), using a split ratio of 1:100 and helium as carrier gas at flow rate of 1.0 ml/min, the injected sample was 1µL and the total run time was 74 min. Column oven temperature was held at 75°C for 1 min, increased at 8°C/min to 165°C (35-min hold), increased at 5.5°C/min to 210°C (1-min hold), and finally increased at 3°C/min to 240°C and held for 15 min. The injector temperature and the detector temperature were both set at 255°C. High purity helium served as the carrier gas with a pressure of 37 psi. High purity hydrogen and chromatographic air were supplied to the FID. Identification was accomplished by comparing the retention time of peaks from samples with those of FAME standard mixtures and single standard isomers and with values published in the literature (Kramer et al., 1994; Kramer et al., 1998; Alves et al., 2006; Luna et al., 2009).

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Fatty acids were expressed as percentage of total fatty acids identified, and grouped as follows: saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs), trans-fatty acid (TFA), polyunsaturated fatty acids (PUFA), polyunsaturated n-3 fatty acids (PUFA n-3), polyunsaturated n-6 fatty acids (PUFA n-6), highly peroxidisable PUFA (HP-PUFA) (Yang et al.,2002) and odd-branched fatty acids (OBCFA).

To evaluate the nutritional value of meat the ratios of *n*-6/*n*-3, PUFA/SFA, h/H (hypocholesterolaemic/Hypercholesterolaemic) (Fernández et al. 2007) and the artherogenicity (AI) and thrombogenicity (TI) index (Ulbricht & Southgate, 1991), which are nutritional indices widely used for human diet, were calculated and their values are presented in Table 16 .

2.3.4. Measurement of oxidative stability

Lipid oxidative stability was assessed in the frozen, ground lamb meat by determination of secondary products of lipid oxidation as 2-thiobarbituric acid reactive substances (TBArs) using a modification of the aqueous acid-extraction method described by Raharjo and Sofos (1992). Minced meat (10.0 g) was weighed into a glass test tube, to which butylated hydroxytoluene (BHT) (Carlo Erba Reagenti, Milan, Italy), at a level of 0.15% based on lipid content (Pikul et al., 1983), was added. The sample was homogenized in trichloroacetic acid (TCA) solution (40 ml, 5% w/v) using an Ultraturrax T25 homogenizer (IKA-Labortechnik, Staufen, Germany), transferred into a 50 ml centrifugation tube and centrifuged for 10 min at 5000g for 45 min at +5°C. The supernatant was filtered through Whatman No. 541 filter paper into a 50 ml volumetric flask. TCA (5% aqueous) was used to make up the volume. The filtered supernatant (2 ml) was transferred to a 10 ml screw cap glass Pirex® tube, mixed with 2-thiobarbituric acid (TBA, 40 mM, 2 ml) and heated at 93°C for 20 min to develop the rose-pink color by reaction between malondialdehyde (MDA) and TBA. The absorbance was measured at 525 nm (Cary 50 scan UV-Vis Spectrophotometer - Varian Inc. Palo Alto, CA) against a blank containing TCA (2 ml, 5% w/v) and TBA (2 mL 40 mM).

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2.3.5. Standard curve

The TBArs values were calculated using an external standard technique from a standard curve of 1,1,3,3-tetraethoxypropane (TEP) and expressed as mg of MDA per kg of muscle. The stock solution of 1,1,3,3-tetraethoxypropane (0.1 M TEP) was prepared as descrived below. A volume of 240 µl of TEP was accurately pippeted into a 10 ml volumetric flask and hydrolyzed with TCA (5% aqueous solution) to make up the final volume. The TEP stock solution (0.1M) was stored at 4°C in the dark; it was stable for at least 1 week. Ten working solutions, with concentration values of TEP ranging from 0.2-20 µM, were prepared by transferring different volumes of the TEP stock solution in other 25 ml volumetric flasks and diluting to final volume with TCA. The standard curve was constructed by incubating 2 ml of each TEP working solution with 2 ml of **2-**thiobarbituric acid (TBA) 40 mM in a water bath at 93°C for 20 min.

The standard curve obtained is presented in Figure 25.

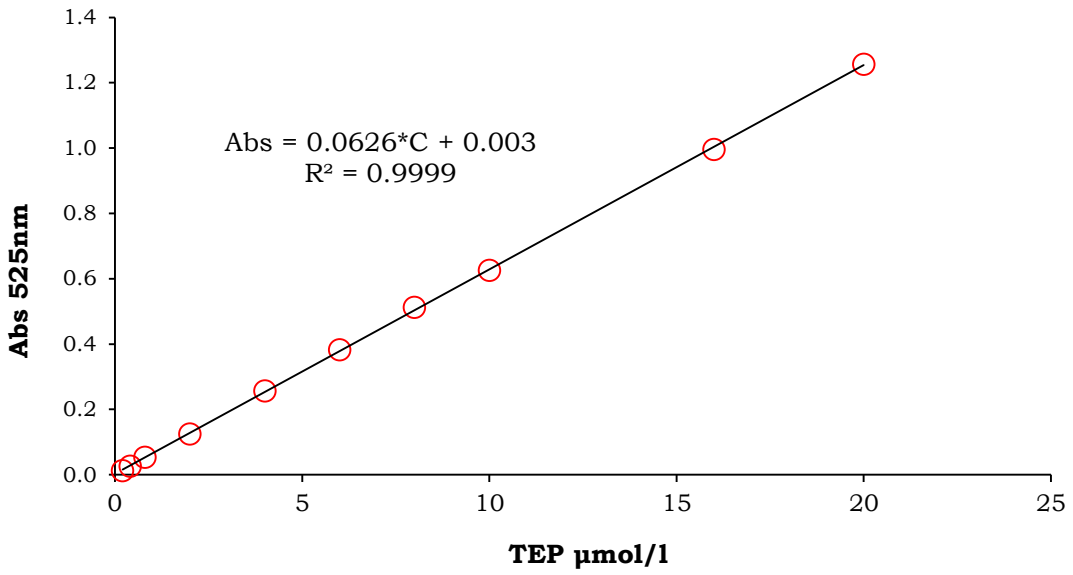

Figure 26. The standard curve of TBA-hydrolyzed TEP adduct (the final concentration of TBA was 40mM, the incubation was conducted at 93°C for 20min).

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The final conversion of TEP concentration to MDA concentration was accomplished using the following formula (Pikul et al., 1989):

$$TBArs_{(mg\ MDA/Kg\ meat)} = ((A \times m \times 7.063 \times 10^{-6})/E) \times 1000$$

where A is the absorbance of each sample, m is the slope of the calibration curve calculated by dividing the concentration in nM of each standard solutions of TEP with its absorbance, 72.063 is the conversion factor from nmol to mg of MA (assuming that one mole of TEP from place to one mole of MA), E is the sample weight equivalent. The equivalent (E) for 10 g of sample was 0.4 when a 2 mL aliquot of the original 50 mL filtrate was analyzed. Two replicates were run per sample.

2.3.6. Statistical analysis

Statistical analysis of data was performed using Minitab (16, 2006) (Minitab Inc., State College, PA). TBArs data were subjected to ANOVA using PROC GLM using a model including time of storage, rearing system and their interaction. Proximate and fatty acid composition were analyzed by Anova one-way with rearing system as the only main effect. Mean values were compared by means of the Tukey's test, p values < 0.05 were accepted as statistically significant in all cases.

2.4. Results and discussions

2.4.1. Chemical and fatty acid composition

The effect of the two different rearing system on chemical composition and partial sums of fatty acid (g/100g of total fatty acid) of the thigh muscles of Sarda suckling lambs is show in Table 16. The principal chemical characteristics determined did not differ between the two rearing system. Only the content of ash tended (p=0.08) to be higher in S group than P group.

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Table 16. Effect of different feeding system on chemical composition (g/100g of muscle) and partial sums of fatty acid (g/100g of total FAME) of thig muscle from suckling lambs of Sarda breed

	Feeding system				
	Stable (S)		Pasture (P)		p-value
Selected nutrients	LSM	SEM	LSM	SEM	
Moisture	74.36	0.27	74.62	0.36	n.s.
Protein	20.33	0.40	20.42	0.11	n.s.
Lipids	2.05	0.16	2.31	0.13	n.s.
Ash	1.25	0.02	1.19	0.02	†
Energy value (Kcal/100g of muscle)	99.77^{b}	2.36	102.47^{a}	0.56	*
pH	5.74	0.02	5.72	0.02	n.s.
Fatty acids partial sums					
Σ SFA	43.26	0.60	42.80	0.81	n.s.
Σ MUFA	42.88	0.58	42.38	0.61	n.s.
Σ TFA	5.45	0.32	5.39	0.18	n.s.
Σ PUFA	13.30	0.67	14.21	1.07	n.s.
Σ PUFA n-3	3.10	0.18	3.43	0.32	n.s.
Σ PUFA n-6	7.98	0.47	8.56	0.69	n.s.
HP-PUFA	5.32	0.33	5.94	0.70	n.s.
Σ OBCFA	3.02	0.05	2.91	0.08	n.s.
Nutritional ratios					
n-6/n-3	2.59	0.08	2.61	0.09	n.s.
PUFA/SFA	0.31	0.02	0.35	0.04	n.s.
h/H	1.68	0.06	1.80	0.09	n.s.
AI	0.82	0.03	0.79	0.04	n.s.
TI	1.14	0.03	1.11	0.05	n.s.

Least square of means (LSM) on the same row followed by superscript different letters differ significantly ($p \leq 0.05$).
***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant. SEM, standard error of mean.
See footnotes in Tables 12 and 13.

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Meat from suckling lambs of Sarda breed showed a good protein content (about 20%) that is in agreement with those reported by Mazzone et al. (2010) in *Longissimus lumborum* of Apennine suckling-lambs slaughtered at 60 days of age and by Osorio et al. (2007) in muscle from Churra suckling lambs slaughtered at an average age of 30 days. The mean fat content range between 2.05 and 2.37%; these values are closed with these previously observed in Sarda suckling lamb (Vacca et al. 2008; Nudda et al., 2009), and similar to these reported by Lanza et al. (2006) in Barbaresca suckling lamb slaughtered at 40 days of age and feeding only with maternal milk (1.93%) and lower than observed by Polidori et al. (2009) in Fabrianese heavy lambs slaughtered at four months of age (4.5%), maybe due to the higher slaughter weight (33 Kg).

Even if the fat and protein contentdid not differ significantly between S and P groups the numerically higher values of fat and protein caused a significantly higher energy value (Kcal/100g of muscle) in P than S lambs.

Rearing system had not significant effect on fatty acids composition. However, lamb meat from group P presented numerically higher contents of total PUFA both from n-6, n-3 compared with animals of group S. The nutritional fatty acid ratios did not differ among the groups. According to current nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFA, the n-6/n-3 ratio should not exceed 4.0 (British Department of Health, 1994). Despite the values of n-6/n-3 ratio determined in this study was lower than recommended values it was comparable with previously observation in suckling lambs meat from Sarda and Barbaresca breed (Nudda et al., 2009; Lanza et al., 2006). Serra et al., (2009) and Velasco et al., (2001) have reported lower values found in Massese and Talaverana breed suckling lambs slaughtered at 14 Kg live weight (1.03 and 1.39, respectively).

The PUFA/SFA ratios were lower than the recommended value of 0.45 (Wood et al., 2003) and comparable with those previously reported for meat from Massese and Italian Merino suckling lamb (Serra et al., 2009; Oriani et al., 2005). Lower values of PUFA/SFA ratio were observed in Barbaresca suckling lambs fed only maternal milk and slaughtered at 40 days of age (Lanza et al., 2006).

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2.4.2. Lipid oxidation

The development of lipid oxidation, during frozen storage, in suckling lamb meat is presented in figure 26. The MDA levels were significantly influenced (p=0.000) by feeding system, storage and their interaction. The increase in the values of MDA was not statistically significant in the S group samples during all 180 days of storage. The values of MDA increased significantly in the P group at 180 days of storage likely due to a higher content percentage of polyunsaturated fatty acids (Table 16).

Although it is difficoult to make comparison of MDA contents between studies due to the different analytical method and procedures used, our results are in line with those reported in several researches; in fact, it was always observed that frozen storage slows the progression of the lipid oxidation process, but does not prevent it. In particular, Pikul et al., (1998) have found that MDA levels, in raw chiken brest, increased continuously during frozen storage, reaching after 6 months of freezing at -18 ° C values about 5 times higher than initial.

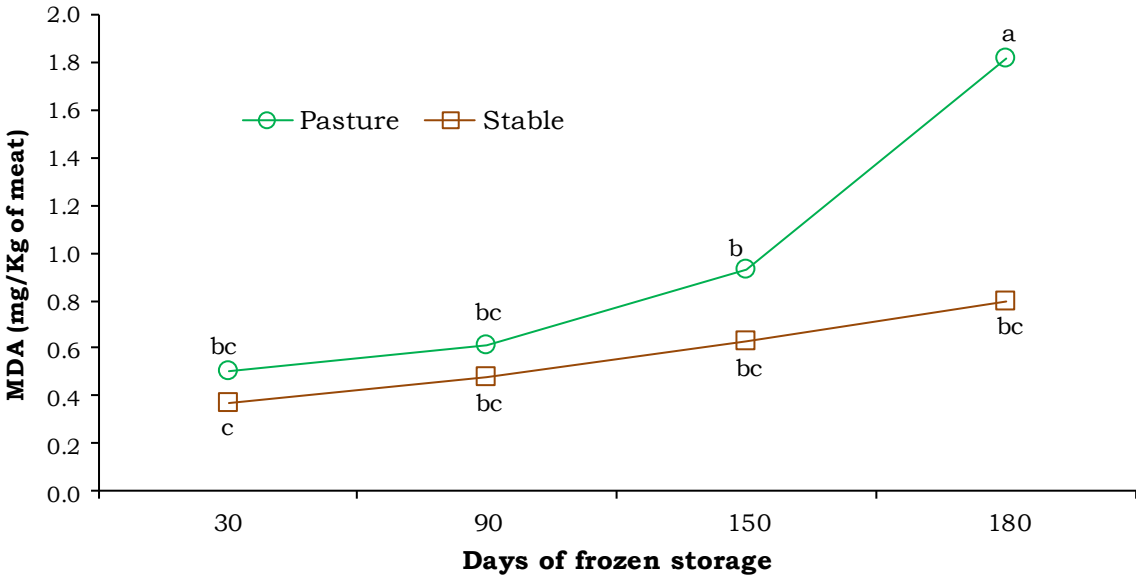

Figure 27. Development of lipid oxidation during six months of frozen storage at -20°C suckling lamb meat from two different rearing system. Points, connected with different letters are statistically different.

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Another study conducted by Hernandez et al., (1999) in longissimus dorsi muscle of pigs subjected to frozen storage for 6 months in vacuum-packed conditions, found that TBArs increased only slightly, confirming that the presence of oxygen can promote and accelerate the oxidation process by enabling the oxygen to react with the components of the muscle (O'Grady et al., 2000).

2.5. Conclusions

The results showed that the storage of meat at -20°C for extended periods does not prevent the oxidation process but certainly slows it down. Values of MDA were always much lower than the consumer sensory threshold for meat flavour deterioration estimated by White et al., (1988), which was 6.3 mg MDA /kg of meat.

This study clearly shows that the oxidative stability of lamb meat depends on diet. The pasture-diet presents some svantages in term of lipid oxidation. Moreover, pasture feeding increases polyunsaturated fatty acids (especially n−3 PUFAs) and conjugated linoleic acid content of the meat, which are beneficial for human health. In conclusion, storage in frozen condition has been proven to be a successful technology to improve the shelf life of meat especially in virtue of the fact that, this commune domestic practice has minimal effects on nutritional and sensory qualities of meat. So considering the meat quality and the dietetic aspect we could recommend the storage in frozen condition in order to preserve the nutritional properties of meat without to alter the organoleptic characteristics.

– EXPERIMENT THREE -

Effect of microwave cooking on proximate composition, fatty acid content and lipid oxidation in trimmed Longissimus Dorsi muscle from Bruno-Sarda young bulls and heifers

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3.1. Abstract

A study was carried out to evaluate the effect of microwave cooking on proximate composition, fatty acid content e lipid oxidation in Longissimus dorsi (LD) muscle from bulls and heifers of grazing Sardo-Bruna cattle. After weaning, 5 bulls and 6 heifers (average 8.5 months of age) were grazed only during the daylight and supplemented with a mixture of concentrate and of meadow hay. Males and females were slaughtered at about 500 and 400 kg live weight an average age of 17 and 15 months, respectively. Sample of LD were taken between 5^{th} and 7^{th} rib, trimmed of external fat and sliced into about 2.5 cm thick chops. Each chop was split in two pieces, one was used raw while the other was cooked in a microwave oven to achieved on internal temperature of 75°C. Chemical and fatty acid analysis were performed on raw and cooked meat as well as the extent of lipid oxidation was determined, after cooking, during 4 days of refrigerated storage and during 2 months of frozen storage, by measuring the content of malondialdehyde (MDA) by the TBA test. The LD from bulls had the lowest content of intramuscular fat than heifers (1.4 vs 2.7, $p=0.07$), while the content of total protein, moisture and ash did not differ between sexes. The raw LD from bulls exhibited higher percentage ($P<0.001$) of Polyunsaturated Fatty Acids (PUFA) and a lower concentration ($p<0.001$) of Monounsaturated Fatty Acids (MUFA) compared to the heifers' muscle. Similar percentages of Saturated fatty Acids (SFA) were found in both sexes. Meat from heifers was characterized by a higher level of CLA and Vaccenic acid compared to that from bulls. Cooking induced significant changes ($p\leq0.01$) in proximate composition for all trait considered except for total fat content ($p\leq0.04$), while, no difference were found in the fatty acid composition in meat after cooking. Lipid oxidation was significantly ($p=0.000$) influenced by sex, cooking and their interaction. Time had significant impact on the MDA level during frozen storage of the LD muscle from bulls and heifers ($p=0.009$ and $p=0.016$ respectively). Lipid oxidation increased significantly ($p<0.001$) in LD muscle from bulls and heifers for both raw and cooked samples across the 4 days of storage period at +4°C, and it was affect by sex and time of refrigeration, while no significant time × sex interaction were observed. in both raw and cooked samples.

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3.2. Introduction

The relative proportion of nutrients and the fatty acid composition of adipose and muscle tissues can be affected by different factors like diet, species, fatness, age/weight, depot site, sex, breed, maintenance and hormones (Rule et al., 1995; Nürnberg et al., 1998; Wood et al., 2008). Gender of beef cattle affects some of the characteristics of meat quality such as tenderness and distribution of fat and protein (Fritsche and Steinhart, 1998). Sex has been widely recognized as one of the ante-mortem factors that contributes to generate variations in the characteristics of beef muscle in fact affect the growth of the animal, the amount of lean mass and fat deposition in the carcass and also the quality of the fat. In general carcass and beef quality are significantly affected by sex. Meat from heifers is more tender, has more intramuscular fat, higher degree of marbling and carcass quality (Frickh et al., 2003; Litwinczuk et al., 2006), better meat quality parameters and sensory characteristics (Frickh et al., 2003; Velik et al., 2008) compared to that of the bulls.

The sex of animals is also an important factor for fatty acid composition due to its effect on carcass fatness (Nürnberg et al., 1998). Several studies, in fact have demonstrate that at equal slaughter weights, males are leaner than females (Enser et al., 1991); the relative concentration of linoleic acid (C18:2 n-6) and PUFA in fat decreases in the order of males > females, whilst the SFA percentage increases (Nürnberg and Ender, 1989). The objective of the present study was to assess the effect of sex on chemical composition, fatty acids composition and lipid oxidation in Longissimus dorsi muscle of beef from Bruno-Sarda heifers and bulls finished on pasture to the age of about 8.5 months. Finally, the influence of microwave cooking on the nutritional value, fatty acid composition and oxidative stability of beef intramuscular fat was also assessed.

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3.3. Materials and methods

3.3.1. Animals, diet and muscle sampling

The study was carried out with 11 animals (5 bulls and 6 heifers) of Bruno-Sarda breed after weaning at the age of 8.5 months the animals were maintained on pasture during only the daylight, supplemented with a mixture of barley-corn meal, a complete mixed feed and meadow hay before and after daily grazing time. The animals were slaughtered at a suitable weight for the breed, an average of 450 kg (536 kg for bulls and 412 kg for heifers), an average age of 16 months (17.0 for bulls and 14.8 for heifers), at a commercial abattoir following EU animal welfare guideline on the 4th June (2 heifers and 1 bull), on the 2nd July (4 heifers) and on 20th (4 bulls) August 2010. The carcasses were aged 4 days at 2-3°C prior to being fabricated into retail cuts. Muscle Longissimus dorsi (LD) was removed from each carcass between 5th and 7th rib, slice into about 2.5 cm thick, vacuum-packaged and stored at -20°C for 24 h until required for analysis. All samples were trimmed of external fat and each slice was taken two sub-samples of about 50 grams, one was used for cooking while the other was left raw.

3.3.2. Preparation of raw and cooked meat

Samples of LD muscle, still partially frozen, were trimmed of the external fat and diced using a knife. Part of the diced meat was minced finely, using a food processor (La Moulinette, Moulinex), and used to monitor lipid oxidation of raw (RW) meat over 2 months of frozen aerobic storage (-20°C), while the remaining diced meat was cooked and used to monitor lipid oxidation in cooked (CK) meat and after 3 days of refrigeration (+4°C) under aerobic conditions. Each raw and cooked sample was also analyzed for chemical and fatty acid composition.

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For study the effect of frozen aerobic storage on raw meat, minced meat was stored in an airtight plastic container and stored in a household freezer, in the dark, at -20°C and extending of lipid oxidation was analyzed 5 days after slaughtering (day 0) and after 30 and 60 days of frozen storage. The temperature of -20°C was chosen because it is commonly used for domestic purposes.

For assay lipid oxidation in cooked meat, 50 g of diced muscle, from each animals, were placed in a polypropylene (PP) pan suitable for microwave and cooked in a microwave oven (Samsung GE82W), equipped with a revolving plate, at 650 W, for about 45s, when a final core temperature of 75°C, required to achieve a constant degree of doneness (well done, according to Matthews and Garrison, pp. 17-18 (1975)), was reached. The internal temperature was measured with a digital thermometer (Orion Research Inc., model 250A, Boston, MA), insert in the center of the samples, upon removal from the oven. Finished samples were allowed to cool at room temperature and dried with filter paper. After cooling, samples were split into two pieces (~ 25 g): one was used for chemical analysis, while the other was used for evaluating the lipid oxidative stability by quantification of TBArs. The pieces used for chemical analysis were wrapped individually in aluminum foil, vacuum-packed before freezing and storage at –80°C until required for analyses 4–5 days later, while the other pieces were processed for TBArs immediately (day 0) and after 3 days of aerobic storage at +4°C.

3.3.3. Chemical analyses and energy value

Each sub-sample of RW and cooked CK meat were analyzed for moisture, total ash and total nitrogen in duplicate, while analyses of total lipid were carried out in single. All reagents were of analytical grade.

Moisture was determined on RW and CK meat by drying samples in an oven for 24 h at 105°C (Method 950.46, AOAC, 1997). Total ash content was quantified on RW and CK meat by ashing the samples in a muffle furnace at 550 °C for 24 h (Method 920.153, AOAC, 1999). Total protein was calculated from Kjeldahl nitrogen using a 6.25 conversion factor. The nitrogen content was determined by the Kjeldahl method

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(Method 928.08, AOAC 1997) using a 2200 Kjeltec ^{TM} automatic distillation unit (Foss Tecator, Höganäs, Sweden).

Extraction and analysis of muscle fatty acids were performed on RW and CK freeze-dried samples. Diced meat (RW and CK) was weighed in a polypropylene (PP) pan, frozen overnight at –80°C, freeze-drying (LyoLab 3000, Heto-Holten, Allerød, Denmark) for 72 h and finely ground in a food processor prior to analysis. Total lipid was extracted from 1 g of each freeze-dried sample following the method of Folch et al. (1957), using chloroform/methanol for extraction. Briefly, a quantity of freeze-dried sample (RW and CK) 1 g (~0,0001 g) was weighted in a 50 mL screw-cap glass centrifuge tube, and 30 mL of previously prepared chloroform-methanol solution (2:1 v/v) were added. The mixture was sonicated for 5 min, shaken vigorously with a vortex for 60 s and then centrifuged for 10 min (1500 x g at room temperature). The chloroformic phase was filtered through a Whatman No. 541 filter paper (Whatman, Ltd., Maidstone, U.K.) and 6 mL 0, 6% NaCl (wt/v) were added. The tube was centrifuged at 1500 x g for 10 min. The upper methanol-water layer was removed using a water aspirator and discarded; the lower organic phase was poured into a round flat-bottom flask (50 mL), chloroform was removed using a vacuum rotatory evaporator at 40°C (Büchi 461, BÜCHI Labor AG, Flawil, Switzerland) and samples were evaporate until dryness. The total extracted meat fat from the modified method Folch was recorder gravimetrically.

After 24 h in a desiccator, extracted fat was redissolved in n-hexane and an aliquot of muscle lipid (20-25 mg) was transferred to a 2mL screw-cap amber-glass vial and used immediately for fatty acid analysis.

Energy value (Kcal) was obtained by multiplying the amount of total protein by the factor of 4 and the amount of total lipid by the factor of 9 (EEC, 1990).

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3.3.4. Fatty acid content and composition

Total lipid extracted from each RW and CK meat samples was used for fatty acid determination. The lipid sample (about 20-25 mg) was esterified by cool base-catalyzed methylation using 0.5 M $NaOCH_3$ in methanol (Sigma-Aldrich Inc., St. Louis, MO, USA) according to the standard FIL-IDF procedure (FIL-IDF 1999); the methylation was achieved in 15 min at room temperature. For quantify the fatty acid methyl esters (FAMEs) nonadecanoic acid (C19:0) methyl ester was used as an internal standard.
FAMEs were identified by gas chromatography using a Turbo 3400 *CX* GC (Varian Inc. Palo Alto, CA) gas chromatograph equipped with a flame ionization detector (FID) and Varian 8200 *CX* auto sampler. Separation was accomplished on a CP-select CB for FAME (Varian Inc. Palo Alto, CA) capillary column (100 m x 0.32 mm i.d., 0.25 μm film thickness). The split ratio was 1:100, the injected sample was 1μL and the total run time was 74 min. Column oven temperature was held at 75°C for 1 min, increased at 8°C/min to 165°C (35-min hold), increased at 5.5°C/min to 210°C (1-min hold), and finally increased at 3°C/min to 240°C and held for 15 min. The injector temperature and the detector temperature were both set at 255°C. High purity helium served as the carrier gas with a pressure of 37 psi. High purity hydrogen and chromatographic air were supplied to the FID. Retention time and area of each peak were computed using the Varian Star 3.4.1. Software. Individual FAME was identified by comparing them to a standard mixture of FIM-7FAME Mix (Matreya Inc. Pleasant Gap, Pa., USA). Branched saturated fatty acid methyl ester (BCFA) were identified using GLC-110 MIX (Matreya Inc. Pleasant Gap, Pa., USA) as external standard and individual BCFA methyl ester standards, methyl 14-methylpentadecanoate (*iso*C16:0), methyl 12-methyltetradecanoate (*anteiso*-C15:0), methyl 12-tridodecanoate (*iso*C14:0) and methyl 14-methylhexadodecanoate (*anteiso*-C17:0) (Matreya Inc. Pleasant Gap, Pa., USA).

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Polyunsaturated fatty acid methyl esters (PUFA) were identified by comparing relative retention times with isomer mixtures PUFA-1, PUFA-2 and PUFA-3 Animal Source (Supelco, Bellefonte PA) and individual external methyl ester standards, all cis-5,8,11,14,17 C20:5 (EPA), all cis-4,7,10,13,16,19 C22:6 (DHA), all cis-5,8,11,14 C20:4 (ARA), all cis-6,9,12 C18:3, all cis-9,12,15 C18:3, all cis-11,14 C20:2 and all cis-5,8,11 C20:3 (Matreya Inc. Pleasant Gap, Pa., USA).

High purity individual *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA (Matreya Inc., Pleasant Gap PA, USA), CLA mix standard (Nu-Ceck-Prep. Inc. Elysian, Minn., USA), and published isomeric profile (Kramer et al., 2004) were also used to help identify the CLA isomers in meat. The identification of C18:1 isomers was based on individual commercial standard (Supelco, Bellefonte, PA, USA) and on chromatograms published by Kramer et al., (2008).

The isomers of C16:1 and C17:1 were identified using published chromatographic reports in ruminant fats (Alves et al., 2006; Luna et al., 2009 respectively). Fatty acids were expressed as percentage of total FAME and as absolute concentration (mg/100g of muscle). The artherogenicity (AI) and thrombogenicity (TI) index (Ulbricht and Southgate, 1991) were also calculated.

3.3.5. Lipid oxidation measurement

The rapid aqueous acid-extraction method described by Raharjo and Sofos (1992) has been chosen for measuring 2-thiobarbituric acid-reactive substances (TBArs), as an indicator of Malondialdehyde (MDA), a major secondary product of lipid oxidation in meat, with some modifications. Briefly, 10 g of ground RW and CK meat was weighted in a glass test-tube, and Butylated hydroxytoluene (BHT) (Carlo Erba Reagenti, Milan, Italy) was added immediately, as antioxidant, at a level of 0.15% based on lipid content (Pikul et al., 1983); then 40 mL of 5% (v/v) aqueous trichloroacetic acid (TCA) (Carlo Erba Reagenti, Milan, Italy) solution was also added to the tube, and homogenization was carry out for 60s at 13.500 rpm using an Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany). The meat slurry was centrifugated at 5000 x g at 4°C for 45 min.

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The supernatant was filtered through a No. 42 Whatman filter paper into a 50 ml volumetric flask. TCA (5% aqueous) was used to make up the volume. A 2 mL aliquot was pippeted into a 10 mL screw-capped test tube, and 2 mL of 40 mM aqueous TBA solution was added. The reaction mixture was incubated for 20 min at 93°C in a water bath under gentle agitation. The tubes were tempered for 60 min at room temperature, the absorbance was measured at 525 nm on a Cary 50 scan UV-Vis Spectrophotometer (Varian Inc. Palo Alto, CA) against a blank consisting of 2 mL 5% aqueous TCA and 2 mL 40 mM TBA.

For the quantitative determination of MDA, calibration curve were prepared using 1,1,3,3,tetraethoxypropane (TEP) (Sigma-Aldrich Inc., St. Louis, MO, USA) with a concentration range of 0.2 - 20 μM ($y = 0.0626x + 0.0030$; $R^2 = 0.9999$).

The blank, the calibration solution and the samples were analyzed the same time. TBArs value was expressed as of mg MDA per kilogram of meat and was calculated from standard curve using the following formula (Pikul et al., 1989):

$$TBArs_{(mg\ MDA/Kg\ meat)} = ((A \times m \times 7.063 \times 10^{-6})/E) \times 1000$$

where A is the absorbance of each sample, m is the slope of the calibration curve calculated by dividing the concentration in nM of each standard solutions of TEP with its absorbance, 72.063 is the conversion factor from nmol to mg of MA (assuming that one mole of TEP from place to one mole of MA), E is the sample weight equivalent. The equivalent (E) for 10 g of sample was 0.4 when a 2 mL aliquot of the original 50 mL filtrate was analyzed. Two replicates were run per sample.

3.3.6. Statistical analysis

Data on meat composition and fatty acid content were analyzed with a GLM procedure using the statistical software Minitab (16, 2009) (Minitab Inc., State College, PA). The model included sex (M *vs* F) and state (RW *vs* CK) as fixed effects and their interaction (sex × state). Lipid oxidation (TBArs values) was processed by the GLM procedure of Minitab (12.2, 1998).

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The model included sex, the days of storage (days 0, 30 and 60 for raw frozen meat and days 0, 2, 4 and 7 for cooked and refrigerated meat) and they interaction (Sex × Time) as fixed effects. A one-way ANOVA was performed to test the effect of the treatment (RW *vs* CK) on the lipid oxidation. Tukey's test was used to compare mean values.

3.4. Results and discussions

3.4.1. Chemical characteristics and energy value of meat

Chemical characteristics and energy value of raw and cooked *Longissimus dorsi* muscle from bulls and heifers of Bruno-Sarda cattle are shown in Table 17.

Sex does not cause any significant difference on the chemical characteristics analyzed and on the energy value of the LD muscle, just the amount of intramuscular fat tends to be higher in females than in males ($p = 0.079$). These results agree with those found by Liotta et al. (2011) for males and females of Cinisara cattle.

The moisture, total protein and ash content detected in the Bruno-Sarda breed was similar compared with other studies performed on different cattle breeds, but on the same muscle. The intramuscular fat content shows instead greater variability compared with other studies. This could be related to factors intrinsic to the breed such as a different rate of deposition of fat, age and sex; but being fat a deposit of energy that the animal use for its own subsistence its concentration may also vary due to extrinsic factors such as feeding conditions and breeding system (for example walking conditions).

The state (RW *vs* CK) affect significantly ($p=0.000$) all chemical characteristics and energy value, except for intramuscular fat ($p=0.040$). Cooking, as reported by Badiani et al. (2002), caused moisture loss resulting in increased nutrient concentration in cooked meat compared to raw meat. No sex × state interaction was detected for all trait in LD muscle.

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Table 17. Effect of sex and cooking on chemical characteristics and energy value of *Longissimus Dorsi* (LD) muscle from bulls and heifers from Bruno-Sarda cattle (g/100g of muscle, except where noted.)

	Sex (SX)								P-value	
	Males				Females					
State (ST)	Raw		Cooked		Raw		Cooked		SX	ST
Trait	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
Moisture	76.02^{A}	0.21	67.12^{B}	0.59	75.09^{A}	0.41	66.98^{B}	0.73	n.s	**
Protein	21.54^{B}	0.46	28.28^{A}	0.90	21.21^{B}	0.46	26.90^{A}	0.92	n.s	**
Lipids	1.42^{b}	0.25	2.86^{ab}	0.27	2.65^{ab}	0.40	3.85^{a}	0.58	†	*
Ash	1.04^{B}	0.01	1.12^{Aa}	0.02	1.03^{Bb}	0.01	1.16^{A}	0.03	n.s	**
Energy value (Kcal/100g of muscle)	98.9^{B}	1.5	138.8^{A}	5.8	108.7^{B}	3.5	142.2^{A}	0.35	n.s	**

Least square means in the same row with different superscript letters differ significantly:
small $0.01 \leq p \leq 0.05$, capital $p \leq 0.01$.
State × Sex interaction was not statistically different for all traits.
** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant
SEM, standard error of mean.

The values obtained in this study for males, were agreed than those reported in the literature for trimmed raw and cooked beef from Alentejano bull (Alfaia et al. 2010) supporting the generally accepted fact that as a result of cooking the moisture content decreases as well as increasing the content of nutrients.

Both RW and CK beef, for both bulls and heifers, according to the Food Advisory Committee (1990) can be considered lean meat. To assess the contribution to the diet of cooked beef meat, from bulls and heifers from Bruno-Sarda breed, 100 g of LD muscle were taken as an average serving. The content of the main nutrients determinated in this study were discussed by referring to the Recommended Daily Allowance (RDA) suggested by the Italian Society of Human Nutrition (SINU, 1989). The energy content of the raw meat was on average lower than 105 Kcal/100g, for bulls and heifers; after cooking the energy content increased ($p<0.001$) resulting in an average value of 140 Kcal/100g.

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In cooked samples the protein supply about of 79% of the total calorie content, the remaining 21% is provided by fat, this is in line with what is suggested for adults by SINU. The consumption of 100g of cooked LD of Bruno-Sarda satisfied, on average for bulls and heifers, 7% of the recommended daily energy ration for an adult involved in moderate activity that leads to a daily consumption of 2000 Kcal. An average serving of cooked LD from Bruno-Sarda cattle is able to provide between 36 and 38% (for bulls and heifers, respectively) of the RDA of protein and only between 4.4 and 5.9% of the RDA of fat recommended for adults of both sexes. Therefore, based on the results obtained, it can be said that the LD muscle from Bruno-Sarda cattle is a lean meat that has a high nutrient density for protein.

3.4.2. Fatty acid composition

The fatty acid profile of LD muscle of bulls and heifers from Bruno-Sarda cattle, both raw and cooked, is reported in normalized terms (each fatty acid as percentage of total FAME) in table 18 are present fatty acid groups, while in table 19 are reported values relative to single fatty acid. There was not significant effect of cooking ($p>0.10$) and no significant interaction between the effects of sex and cooking ($p>0.10$) for any groups (Table 2) or any individual fatty acid (Table 3), these values are similar with those found by Bragagnolo and Rodriguez (2003), Nayigihugu et al. (2004) and Sarriés et al. (2009) which have found that cooking did not cause changes in the relative distribution of fatty acids following cooking in LD beef steaks, but contrast with the results obtained from Alfaia et al. (2010) that show how different cooking methods result in a significant increase in the percentage of SFA and MUFA and a decrease in that of PUFA in beef meat. The major effects found in this study related to the fatty acids composition are due to sex, the meat from bulls in fact, differed significantly ($p<0.001$) from that of heifer for the fatty acid composition of intramuscular fat for all group of fatty acids considered except for total SFA and total OBCFA for which was not detected a statistically significant difference, $p=0.147$ and 0.410 respectively, and for total PUFA n-3 which was slight different between the sexes, $p=0.040$, these values were in line with those found by Karolyi et al. (2009) for bulls and heifers from Simmental cattle.

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Table 18. Fatty acid groups composition (% of total fatty acids) of raw and cooked LD muscle from bulls and heifers of Bruno-Sarda breed.

	Sex (Sx)								P-value	
	Bulls				Heifers					
State (St)	Raw		Cooked		Raw		Cooked		Sx	St
Partial sums	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
Short chain	0.02	0.00	0.02	0.00	0.04	0.01	0.04	0.00	**	n.s.
Medium chain	26.92^{b}	1.09	27.69^{ab}	1.01	29.96^{ab}	0.55	30.46^{a}	0.45	**	n.s.
Long chain	73.06^{a}	1.09	72.28^{ab}	1.01	70.00^{ab}	0.56	69.50^{b}	0.45	**	n.s.
Σ SFA	48.85	1.53	49.84	0.76	47.94	1.09	47.48	0.81	n.s.	n.s.
Σ MUFA	35.92^{b}	2.45	37.08^{b}	2.42	44.25^{a}	0.82	45.18^{a}	0.84	***	n.s.
Σ PUFA	15.23^{a}	2.44	13.09^{ab}	2.10	7.81^{b}	0.39	7.34^{b}	0.45	***	n.s.
Σ PUFA n-3	1.67	0.38	1.43	0.22	1.14	0.11	1.02	0.10	*	n.s.
Σ PUFA n-6	12.66^{a}	2.09	10.71^{ab}	1.90	5.56^{bc}	0.29	5.17^{c}	0.39	***	n.s.
HP-PUFA	3.88	0.75	3.17	0.53	2.35	0.27	2.09	0.24	**	n.s.
Σ CLA	0.80^{b}	0.04	0.85^{bc}	0.03	1.02^{ac}	0.04	1.05^{a}	0.06	**	n.s.
Σ OBCFA	3.56	0.12	3.85	0.12	3.58	0.18	3.58	0.12	n.s.	n.s.
PUFA/SFA	0.32^{a}	0.05	0.26^{ab}	0.04	0.16^{b}	0.01	0.16^{b}	0.01	***	n.s.
MUFA/SFA	0.74^{b}	0.06	0.75^{bc}	0.06	0.93^{ac}	0.04	0.95^{a}	0.03	***	n.s.
n-6/n-3	7.89^{b}	0.85	7.38^{ab}	0.77	5.00^{a}	0.32	5.15^{a}	0.28	***	n.s.
AI	0.55	0.03	0.57	0.02	0.62	0.03	0.62	0.02	*	n.s.
TI	1.58	0.12	1.65	0.06	1.58	0.08	1.57	0.05	n.s.	n.s.
Lipid oxidation										
MDA (mg/Kg meat)	0.07^{c}	0.00	0.52^{b}	0.07	0.10^{c}	0.02	1.07^{a}	0.12	***	***

Least square means in the same row with different superscrip letters differ significantly: $p \leq 0.05$
State × Sex interaction was statistically different only for MDA. SEM, standard error of mean.
***p≤0.001; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant.
CLA, Conjugated Isomer of Linoleic; ΣCLA includes C20:0 and C20:1n-9 that eluted in CLA region. The isomers have been summed because the chromatographic conditions were not adequate to allow accurate individual quantification.
ΣSFA: sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C22:0, C24:0 and Branched fatty acids;
ΣMUFA: sum of C14:1, ΣC16:1, ΣC17:1, ΣC18:1
ΣPUFA: sum of total n-6 and total n-3;
ΣPUFA n-3: sum of C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:5n-3, C22:6n-3.
ΣPUFA n-6: sum of ΣC18:2n-6 (cis/trans isomers, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6 and C22:4n-6.
HP-PUFA : sum of PUFA with three or more double bonds.
OBCFA : sum of C13:0, C13:0 *i*, C13:0 *ai*, C14:0 *i*. C15:0 *i*, C15:0 *ai*, C16:0 *i*, C17:0, C17:0 *i*, C17:0 *ai* and Σ C17:1. (*i* is an *iso*-isomer and *ai* is an *ante-iso*-isomer).
Index of atherogenicity (IA) = $[(12:0) + (4 \times 14:0) + (16:0)] \times [(n\text{-}6 \text{ and } n\text{-}3 \text{ PUFA}) + \text{MUFA})]^{-1}$
Index of thrombogenicity (IT) = $[(14:0) + (16:0) + (18:0)] \times [(0.5 \times \text{MUFA}) + (0.5 \times n\text{-}6 \text{ PUFA}) + (3 \times n\text{-}3 \text{ PUFA}) + (n\text{-}3 \times n\text{-}6^{-1} \text{ PUFA})]^{-1}$ (Ulbricht & Southgate, 1991).

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Fat in LD muscle from bulls had a level of SFA similar to that found in the fat of heifer, a higher content of PUFA and lower content of MUFA. This fact may be related to fatness; heifer in fact had more intramuscular fat than bulls and how demonstrated by De Smet et al. (2002) an increase in carcass fatness was paralleled by an increased content of MUFA, and a decreased content of PUFA in beef meat.

According to some nutritional recommendations the ratios of PUFA/SFA and n-6/n-3, which are indices used to evaluate the nutritional value of fat for human health, were calculated. In the human diet, the recommended value for PUFA/SFA ratio is 0.45-0.65 (British Department of Health, 1994) and lower ratios in the diet as a whole may increase the incidence of cardiovascular disease. Within the PUFA, the optimal dose or ratio of n-6/n-3 varies from 1/1 to 4/1 for so the ratio should not exceed the value of 4.0 (Simopoulos, 2002). In particular, the PUFA/SFA ratios, observed in this study, were lower than the recommended ratio for raw meat from bulls and heifers, 0.32 and 0.16 respectively and these values were not modified following cooking ($p=0.75$). Cooking, also, did not changed significantly the ratio n-6/n-3 for both bulls and heifers ($p=0.753$), although the values observed in this study were higher than those recommended for human health, being average 7.64 for bulls and 5.08 for heifer, due to difference in concentration of PUFA n-6 within the sex.

The ratios mentioned above could not explain completely the effects of fatty acids on human health, in order to better assess these effects is most useful to consider the synergic action of individual fatty acids and class of them in modulating the onset of chronic disease. For these purpose Ulbricht and Southgate in the 1991 devised the artherogenicity (AI) and thrombogenicity (TI) index. AI evaluates the risks of atherosclerosis while TI is useful to define the risk of platelet aggregation; so plus the values of these indexes are close to zero as the food is recommended for a healthy diet. In this study no significant different emerged among sex and state regards the TI, while the value of AT was affected significantly ($p\leq0.01$) by sex but not by cooking.

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Table 19. Fatty acid profile (% of total fatty acids) of raw and cooked LD muscle from bulls and heifers of Bruno-Sarda breed.

	Sex (SX)								p-value	
	Bulls				Heifers					
State (ST)	Raw		Cooked		Raw		Cooked		SX	ST
Fatty acids	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
C14:0	1.41^{b}	0.08	1.54^{b}	0.10	1.94^{a}	0.11	1.98^{a}	0.15	***	n.s.
C14:1 c9	0.13^{b}	0.03	0.15^{b}	0.10	0.24^{a}	0.01	0.27^{a}	0.01	***	n.s.
C15:0	0.36	0.02	0.40	0.02	0.39	0.04	0.38	0.03	n.s	n.s.
C16:0	21.78	0.86	22.07	0.66	23.48	0.40	23.71	0.34	**	n.s.
C16:1 *c*9	1.39^{b}	0.21	1.47^{bc}	0.23	2.04^{ac}	0.09	2.23^{a}	0.08	***	n.s.
C17:0	1.01	0.04	1.06	0.02	0.98	0.04	0.95	0.05	n.s.	n.s.
C17:1 *c*9	0.45	0.04	0.46	0.04	0.52	0.02	0.54	0.02	*	n.s.
C18:0	22.57	1.62	22.84	1.32	19.46	0.74	18.74	0.86	**	n.s.
C18:1 *t*10	0.15^{bc}	0.03	0.20^{ac}	0.03	0.13^{b}	0.03	0.12^{b}	0.03	***	n.s.
C18:1 *t*11	1.39	0.12	1.60	0.12	1.91	0.23	1.96	0.15	*	n.s.
C18:1 *c*9	29.82^{b}	2.38	30.54^{bc}	2.29	37.04^{ac}	0.93	37.61^{a}	0.91	**	n.s.
C18:1 *c*11	1.42	0.04	1.38	0.04	1.25	0.03	1.24	0.03	***	n.s.
C18:2 n-6 (LN)	9.92^{a}	1.72	8.38^{a}	1.57	3.85^{b}	0.30	3.65^{b}	0.16	***	n.s.
C18:3 n-3 (ALA)	0.94	0.22	0.84	0.14	0.59	0.04	0.56	0.04	*	n.s.
CLA c9,t11	0.34^{b}	0.04	0.37^{b}	0.03	0.57^{a}	0.05	0.60^{a}	0.03	***	n.s.
C20:2 n-6	0.10^{a}	0.01	0.09^{a}	0.01	0.05^{b}	0.00	0.05^{b}	0.00	***	n.s.
C20:3 n-6	0.36	0.06	0.28	0.06	0.23	0.02	0.20	0.02	*	n.s.
C20:4 n-6 (ARA)	1.55^{ab}	0.30	1.21^{bc}	0.25	0.79^{bc}	0.10	0.66^{c}	0.13	**	n.s.
C20:4 n-3	0.04^{b}	0.00	0.03^{b}	0.00	0.06^{a}	0.00	0.03^{b}	0.00	**	n.s.
C20:5 n-3 (EPA)	0.18	0.05	0.14	0.03	0.14	0.02	0.11	0.03	n.s.	n.s.
C22:4 n-6	0.16^{ac}	0.03	0.12^{bc}	0.02	0.08^{b}	0.01	0.07^{b}	0.01	***	n.s.
C22:5 n-3 (DPA)	0.46	0.10	0.35	0.07	0.31	0.04	0.26	0.04	†	n.s.
C22:6 n-3 (DHA)	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.00	n.s.	n.s.

Fatty acid are represented in the following manner: the first number indicates the number of carbons, while the second represents the number of double bonds; n-x indicates where the first double bond is located counting from the terminal methyl carbon toward the carbonyl carbon; c-x is for *cis*, t-x is for *trans* and indicate the configuration of double bond and where the double bond in located counting from the carbonyl carbon toward the terminal methyl carbon. Least square of means on the same row followed by different letters differ significantly ($p \leq 0.05$).
State × Sex interaction was not statistically different for all fatty acids.
***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant SEM, standard error of mean.

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The main fatty acid in the intramuscular fat from raw LD muscle of bulls and heifers from Bruno-Sarda cattle were oleic (C18:1*c*9, 30-37%), palmitoleic (C16:0, 22-24%) and stearic (C18:0, 22-19%) acids which represent respectively, 74 and 80% of the total fatty acids; these values, as well as the percentages of the 24 individual fatty acid (FA) selected were not affected by cooking ($p>0.10$).

Although sex determined the major effect on fatty acid profile, agreed with other similar published dates, not all FA analyzed were significantly altered. In particular there were no changes in the concentration of pentadecenoic (C15:0, $p=0.86$), eptadecenoic (C17:0, $p=0.11$), C20:5n-3 (EPA) ($p=0.25$) and C22:6n-3 (DHA) ($p=0.89$) and only slight variation were observed for C22:5n-3 (DPA) ($p=0.09$), these values are in line with those found by Bartoň et al. 2005. The percentages of C14:0, C14:1*c*9, C16:0, C16:1*c*9, C18:1*c*9 were higher ($p\leq0.01$) in the intramuscular fat of heifers than bulls. LD muscle from bulls instead, had higher ($p\leq0.01$) concentrations of C18:0, C18:2n-6 (LN), C18:3n-3 (ALA), C20:4n-6 (ARA) and C22:4n-6. This may be attributable to the higher concentration of intramuscular fat in meat of heifers than bulls which determines an increase of ratio triglycerides/phospholipids, which is associated with increased levels of SFA and MUFA and decrease in those of PUFA.

The percentages of total CLAs, CLA *cis*9,*trans*11 isomer and vaccenic (C18:1*t*11) acid were higher in intramuscular fat from heifers than bulls as reported in previously similar study conducted in beef from different breeds, slaughtered at differencing age and subjected to different feeding system.

3.3.3. Lipid oxidation

Lipid oxidation in meat is one of the reasons for quality degradation after cooking during storage. Refrigeration (2-5°C) and frozen storage are usually the most common household method in order to preserve the freshness and the nutritional quality of fresh meat and meat product. However, many undesirable changes of the meat can occurs during storage at both refrigeration and freezing temperatures due to microbial growth and lipid oxidation, which give rise to reduction of meat quality. Control and monitoring of lipid oxidation during meat storage are important to evaluate as long

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refrigeration and frozen storage may be extended without complying deleterious changes in the palatability of the meat. Lipid peroxidation in fact, is one of the major processes of quality deterioration in stored foods, expecially in muscle tissue. In the current study MDA measuraments by TBA-test, was chosen as representative analysis of secondary lipid oxidation products.

MDA content in raw and cooked beef from bulls and heifers, at 0 days of storage, are presented in Table 18. The values observed did not exceed 1.0 mg MDA/kg of meat and it is resulted lower of the critical value of 3 mg/Kg of meat at which rancidity is virtually detected (Wong et al., 1995). The MDA levels were significantly influenced ($p<0.001$) by cooking, sex and cooking × sex interaction.

The MDA values for the minced raw meat during 4 days of storage at 4°C are presented in Figure 27a. The MDA levels were significantly influenced ($p=0.014$) by sex and time of storage ($p=0.000$), while no significant time × diet interaction ($p=0.903$) were observed.

During storage at 4°C lipid oxidation of minced meat from heifers tended to increase significantly ($p=0.066$) from 0.10 mg/Kg (day 0) to 0.16 mg/Kg (day 2) and after 4 days of storage at 4°C reached 0.42 mg/Kg of meat, with a significant increase ($p=0.000$) compared to MDA level displayed at 2 days of storage. Regarding the minced meat from bulls, that presented a lower, but not significant ($p=0.182$) initial value of MDA (0.07 mg/Kg, day 0) compared to meat from heifers (0.10 mg/Kg). The initial value increased, no significantly, to 0.11mg/Kg of meat after 2 days of refrigerated storage and advanced to 0.38 mg/Kg at the end of storage period (4 days). No sex effect was noted by ANOVA analysis on MDA levels detected in raw meat samples during the whole period of refrigerated storage.

MDA content in cooked LD muscle from bulls and heifers of Bruno-Sarda breed, gradually increased during 4 days of refrigerated storage at 4°C (Figure 27b). MDA levels in cooked meat during refrigered storage were significantly affected by sex and time of storage ($p=0.000$), while no significant interation ($p=0.753$) sex × time was observed.

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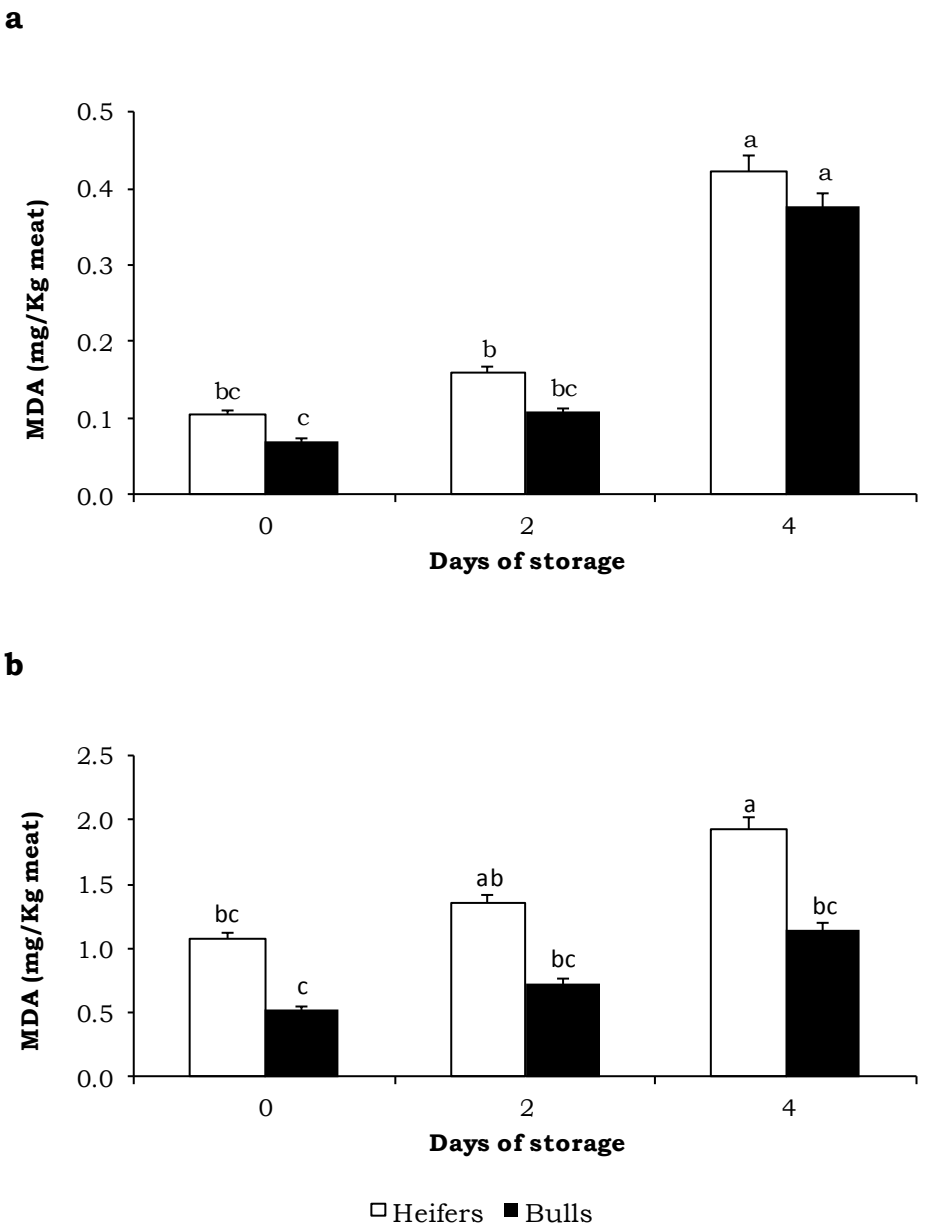

Figure 28. Effect of sex (Heifer *vs* Bulls) and of the time of storage (days 0, 2, 4) on MDA content in raw minced meat (a) and cooked minced meat (b) stored over 4 days at +4°C. Means with different superscript letters are statistically different ($p<0.05$).

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Significant changes ($p<0.05$) were detected in MDA content between day 0 and day 4 for LD meat from bulls (from 0.52 mg MDA/KGg meat to 1.14 mg MDA/Kg meat) and heifers (from 1.07 mg MDA/Kg meat to 1.92 mg MDA/Kg meat). LD muscle from heifers had significantly higher MDA levels than LD muscle from bulls at all stage of storage, with the largest difference being detected at day 4 (bulls 1.14 mg MDA/Kg meat; heifers 1.92 mg MDA/Kg meat; $p=0.029$). Accordingly, the rate of MDA increased was higher in LD muscle from heifers compared to LD muscle from bulls (0.85 mg MDA/Kg meat vs. 0.62 mg MDA/Kg meat).

The development of lipid oxidation during 2 months of frozen storage, for ground meat samples from bulls and heifers of Bruno-Sarda cattle is present in Figure 28.

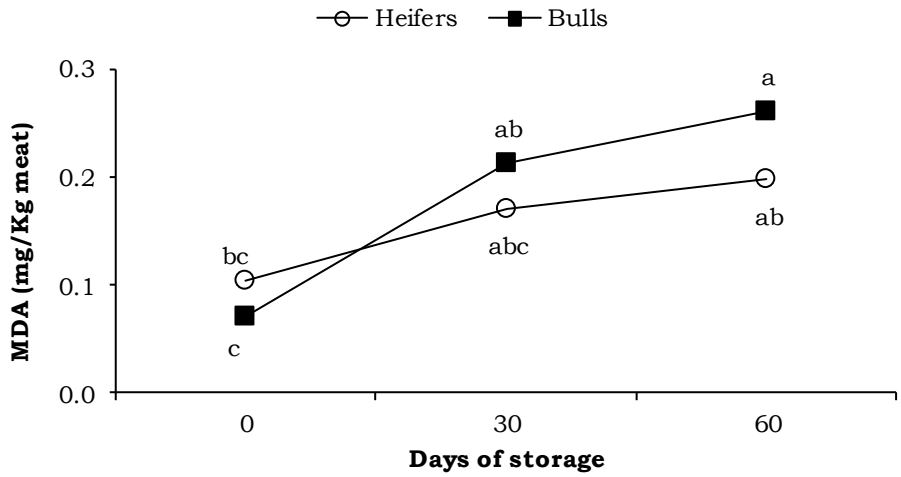

Figure 29. Development of lipid oxidation during two months of frozen storage at -20°C in LD muscle from bulls and heifers. Points connected with different letters are statistically different ($p<0.05$).

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Frozen storage had significant effect (p=0.000) on the MDA content in the LD muscle from both bulls and heifers, while no relevant changes were observed by sex (p=0.300) and sex × time interaction (p=0.214). The amount of MDA increased significantly from 0.07 mg MDA/Kg meat to 0.26 mg MDA/Kg meat (p=0.010) and from 0.11 mg MDA/Kg meat to 0.20 mg MDA/Kg (p=0.008) for LD muscle from bulls and heifers respectively. The accumulation of MDA mainly occurred from day 0 to day 30 of frozen storage, while during the last month of storage until 60 days of freezing, MDA content increased not significantly for both bulls and heifers (p=0.472 and 0.363, respectively). This reveals a certain stability in the evolution of the oxidation process after thirty days from the beginning of freezing for both sexes and confirms the fact that the most delicate phase in the process of freezing is the initial one when can be not yet completely inhibited some chemical and enzymatic processes which is due to the spoilage of meat.

3.4. Conclusions

The effects of sex and cooking on meat quality were studied in 11 Bruno-Sarda cattle (6 heifers and 5 bulls) reared on pasture during the daylight and slaughtered at about 16 months old. The sex of the animal had no effect on chemical characteristics of the meat except for intramuscular fat deposition in fact meat from bulls tended to have lower content of intramuscular fat than heifers. Sex also greatly influenced the differences in FA composition in the intramuscular fat from Bruno-Sarda cattle. The raw LD from bulls exhibited higher percentage PUFA and a lower concentration MUFA compared to the heifers' muscle. Similar percentages of SFA were found in both sexes. Meat from heifers was characterized by a higher level of oleic acid, CLA and vaccenic acid compared to that from bulls which however showed higher concentration of stearic, linoleic and arachidonic acids. As far as human dietectics is concerned, more favourable n-6/n-3 ratio was found in meat from heifers (5.00:1) compared to that from bulls (7.89:1).

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Cooking induced significant changes in proximate composition for all traits considered except for total fat content, while, no difference were found in the fatty acid composition in meat after cooking.

Lipid oxidation was affect by cooking and time of storage in refrigerated and frozen condition, while no difference were found in lipid oxidative stability by sex. The detected differences in beef fat quality between bulls and heifers may be related to the genetic control of animal development and production of sex hormones and their influence the lipid composition. In these sense, based on the results presented here, both in terms of chemical composition, fatty acid profile and lipid oxidative stability, it is concluded that beef of higher quality can be obtained from heifers than from bulls.

- EXPERIMENT FOUR –

Nutritional value, fatty acid content and lipid oxidation in trimmed raw and cooked Longissimus Dorsi muscle from Bruno-Sarda young bulls as affected by different feeding system.

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4.1. Abstract

The effect of microwaving on the composition, oxidative stability and nutritional quality of beef intramuscular fat from cattle fed with two different diet was investigated. *Longissimus Dorsi* (LD) muscle from 10 Bruno-Sarda young bulls reared on pasture lands during only the daylight or indoor in stable without the opportunity to eat grass for grazing was analyzed. Protein, moisture and ash were affect by diet and microwave cooking, while fat content showed no significant differences between finishing system, and heat treatment. Fatty acid composition was affected by diet. Bulls reared on pasture had higher percentage of linolenic (C18:3 n-3), CLA *c*9,*t*11, vaccenic (C18:1 *t*11) and odd-branched (OBCFA) fatty acids, in particular grass-fed bulls have higher proportion of C15:0, C17:0 and C17:1*c*9 than confined bulls. Beef reared indoor tented to have higher percentage of PUFA n-6 ($p<0.10$), in particular significant differences were found in the percentage of arachidonic (C20:4 n-6) acid and C22.4 n-6 ($p<0.05$ and $p<0.001$, respectively). There were not changes in the relative distribution of fatty acids upon microwave cooking (650W for 35s), therefore this cooking method preserves the nutritional value of meat. Cooking and diet have not changed the nutritional ratios considered, except for the n-6/n-3 ratio that was significantly affected by diet, in fact, meat from pasture finished animals had a more favorable n6/n-3 ratio than from confined animals (7.6 vs. 14.5, respectively). These difference are a consequence of the fatty acid composition of diet. Lipid oxidation, expressed as mg MDA/kg muscle, was significantly ($p=0.000$) influenced by cooking for both diets. MDA levels are increased as storage time progressed regardless of the dietary treatment for both raw and cooked meat samples. Time had significant impact on the MDA levels during frozen storage of the meat from confined animals, while no change in oxidative stability was observed in meat from pasture finished animals. Grass-based diets have been shown to enhance total conjugated linoleic acid (CLA), vaccenic (C18:1 *t*11) acid, linolenic (C18:3 n-3) and total odd-branched (OBCFA) fatty acids as well, it contributed to reduce the n-6/n-3 ratio in intramuscular fat and consequently may improve the nutritional quality of beef. In addition, pasture diet contributes natural antioxidants in sufficient amounts and is an efficient way to prevent lipid oxidation in raw and cooked meat from beef.

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The results of the study show that feeding grass cattle can have positive effects on the fatty acid profile and lipid oxidative stability of its meat, therefore, it is necessary to bring the positive benefits of grass-fed beef to the attention of the public, medical profession, producers and consumers.

4.2. Introduction

Many factors affect ruminant meat quality, and among the genetic and environmental factors (diet, weather, slaughtering procedures etc.), feeding plays an important role in the determination of quality. Historically, most of the beef produced until 1940 was from cattle fattened on grass. In the course of 1950, to improve efficiency of beef production, the livestock industry has introduced the high-energy diet with grains resulting in decreased days of feeding and increased the level of marbling of the meat (intramuscular fat: IMF). In addition, consumers in industrialized countries, have become accustomed to the taste of grain-fed beef, generally prefer the flavor and overall palatability of the meat produced with such intensive fattening systems (Wood et al., 2003). However, changes in consumer demand, in addition to the new research on the effects of feed on the nutrient quality of meat, have apostolate and entice farmers to return to pastoral approach to beef production, despite the intrinsic inefficiencies. The research covers three decades suggests that diets based on grass-only can significantly alter the composition of fatty acids and improve the overall content of antioxidants in beef. The influence of nutrition on the fatty acid composition of the adipose and muscle tissues of ruminant has been established in beef (French et al., 2001; Scollan et al., 2003; Raes et al. 2003; Raes et al., 2004b; Dannenberg et al., 2007; Alfaia et al., 2009). In particular previous research has shown that pasture feeding can affect several meat quality characteristics of beef, in particular colour, flavour and fatty acid composition compared to concentrate diet systems (Priolo et al., 2001; Steen et al., 2003; Wood et al., 2003; Varela et al., 2004; Realini et al., 2004; Nuernberg et al., 2005), and that including grass in the diet of dairy and beef cattle increased concentrations of polyunsaturated fatty acids and CLA in muscle compared with beef from grain-fed cattle (Larick & Turner, 1989; Melton et al., 1982; Yang et al., 2002).

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Although recent researches have well recognized the nutritional value and importance of PUFA n-3 and conjugated linoleic acid (CLA) isomers, thanks of their health properties, an increased in the PUFA n-3 concentration lead to reduction of meat oxidative stability (Realini et al., 2004). In fact increasing PUFA n-3 in meat would be expected an increase of the susceptibility of lipids, particularly phospholipids, to oxidative deterioration (Dunne et al, 2011) despite meat from pasture fed-cattle can have high content of α-tocopherol and other anti-oxidants (carotenoids, ascorbic acid and phenolic compounds) originating from naturally occurring compounds present in grasses (Yang et al., 2002). In conclusion, the susceptibility of meat to lipid oxidation depends on the absolute concentration of PUFA and on the balance between the concentration of antioxidants (such as vitamin E) and that of the highly oxidisable substrates (Desclalzo & Sancho, 2008).

Furthermore, heat treatment during cooking process can affect the lipid composition of meat in relation to raw samples especially the fatty acid content, in fact how reported by Rodriguez-Estrada et al., (1997) the heat treatment can lead to changes in the nutritional value of meat and mainly promotes lipid oxidation. The objectives of this study were to compare beef quality, longissimus dorsi fatty acid and oxidative stability in raw and cooked meat samples from cattle finished on pasture during only the daylight or without grazing and verify possible correlation between the factors analyzed.

4.3. Materials and methods

4.3.1. Animals, diet and muscle sampling

Ten young bulls from Bruno-Sarda breed were used in this study. Five young bulls were maintained on pasture during only the daylight (P), supplemented with a mixture of barley-corn meal, a complete mixed feed and meadow hay before and after daily grazing time, while the other 5 animals were kept in the stable without the possibility to eat grass for grazing during the daylight (NP). The animals were slaughtered at approximately 500 kg of body weight at the average age of 18 months.

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The animals were slaughtered at a commercial abattoir following EU animal welfare guideline. The carcasses were aged 4 days at 2-3°C prior to being fabricated into retail cuts. Muscle Longissimus dorsi (LD) was removed from each carcass between 5th and 7th rib, slice into about 2.5 cm thick, vacuum-packaged and stored at -20°C for 24 h until analysis. All samples, still partially frozen, were trimmed of connective and adipose tissue and sliced into 4 square cuts of about 50 grams, one was subjected to the cooking treatment, while the others were left raw as control for determination of nutritional value and lipid oxidation in uncooked meat.

4.3.2. Cooking treatment and preparation of samples

For microwave cooking, one cut of about 50 g was placed in a polypropylene container, suitable for microwave, in the center of the carousel of a microwave oven (Samsung GE82W), set at 650 W. One heating cycle of 45 s was used to attain a final core temperature of 75°C in order to obtain a constant degree of doneness (well done, according to Matthews and Garrison, pp. 17-18 (1975)). Internal temperature was monitored using a digital thermometer (Orion Research Inc., model 250A, Boston, MA), insert into the approximate center of each cut, upon removal from the oven. After cooking the samples were cooled at room temperature (1 h at 20-22°C) dried with a paper towel and split into two pieces (~ 25 g): one was used for chemical analysis, while the other was used for evaluating the lipid oxidative stability by quantification of TBArs. The pieces used for chemical analysis were wrapped individually in aluminum foil, vacuum-packed before freezing and storage at –80°C until required for analyses 4–5 days later, while the other pieces, grinded finely using a food processor (La Moulinette, Moulinex s.p.a., Milan Italy), were processed for TBArs immediately (day 0) and after 2, 4 and 7 days of aerobic storage at +4°C.

Two cuts of the diced raw meat was minced individually, using a food processor (La Moulinette, Moulinex). One was used to monitor lipid oxidation of raw (RW) meat over 2 months of frozen aerobic storage (-20°C), while the other minced cuts was used to monitor lipid oxidation of raw meat over a 1-week period of refrigerated storage (+4°C) under aerobic conditions. For study the effect of frozen aerobic storage on raw meat, minced meat was stored in an airtight plastic container and stored in a household

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freezer, in the dark, at -20°C and extending of lipid oxidation was analyzed 5 days after slaughtering (day 0) and after 30 and 60 days of frozen storage. The temperature of -20°C was chosen because it is commonly used for domestic purposes.

For the conservation study on refrigerated raw diced meat, each grinded subsample was placed in individual airtight plastic container and stored in a household fridge, in the dark, at +4°C. The extend of lipid oxidation in raw refrigerated samples was determined 6 h after grinding (day 0) and after 2, 4 and 7 days of storage.

For determination of muscle and fatty acid composition the remaining raw cuts (about 50 g) were exactly weighted and wrapped individually with a layer of film for food and stored frozen at -80°C until analysis.

4.3.3. Chemical analyses and energy value

Each sub-sample of RW and cooked CK meat were analyzed for moisture, total ash and total nitrogen in duplicate, while analyses of total lipid were carried out in single. All reagents were of analytical grade.

Moisture was determined on RW and CK meat by drying samples in an oven for 24 h at 105°C (Method 950.46, AOAC, 1997). Total ash content was quantified on RW and CK meat by ashing the samples in a muffle furnace at 550 °C for 24 h (Method 920.153, AOAC, 1999). Total protein was calculated from Kjeldahl nitrogen using a 6.25 conversion factor.

The nitrogen content was determined by the Kjeldahl method (Method 928.08, AOAC 1997) using a 2200 Kjeltec ^{TM} automatic distillation unit (Foss Tecator, Höganäs, Sweden).

Extraction and analysis of muscle fatty acids were performed on RW and CK freeze-dried samples. Diced meat (RW and CK) was weighed in a polypropylene (PP) pan, frozen overnight at –80°C, freeze-drying (LyoLab 3000, Heto-Holten, Allerød, Denmark) for 72 h and finely ground in a food processor prior to analysis. Total lipid was extracted from 1 g of each freeze-dried sample following the method of Folch et al. (1957), using chloroform/methanol for extraction. Briefly, a quantity of freeze-dried sample (RW and CK) 1 g (~0,0001 g) was weighted in a 50 mL screw-cap glass

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centrifuge tube, and 30 mL of previously prepared chloroform-methanol solution (2:1 v/v) were added. The mixture was sonicated for 5 min, shaken vigorously with a vortex for 60 s and then centrifuged for 10 min (1500 x g at room temperature). The chloroformic phase was filtered through a Whatman No. 541 filter paper (Whatman, Ltd., Maidstone, U.K.) and 6 mL 0, 6% NaCl (wt/v) were added. The tube was centrifuged at 1500 x g for 10 min.

The upper methanol-water layer was removed using a water aspirator and discarded; the lower organic phase was poured into a round flat-bottom flask (50 mL), chloroform was removed using a vacuum rotatory evaporator at 40°C (Büchi 461, BÜCHI Labor AG, Flawil, Switzerland) and samples were evaporate until dryness. The total extracted meat fat from the modified method Folch was recorder gravimetrically. After 24 h in a desiccator, extracted fat was redissolved in hexane and an aliquot of muscle lipid (20-25 mg) was transferred to a 2mL screw-cap amber-glass vial and used immediately for fatty acid analysis.

Energy value (Kcal) was obtained by multiplying the amount of total protein by the factor of 4 and the amount of total lipid by the factor of 9 (EEC, 1990).

4.3.4. Fatty acid composition

Total lipid extracted from each RW and CK meat samples was used for fatty acid determination. The lipid sample (about 20-25 mg) was esterified by cool base-catalyzed methylation using 0.5 M $NaOCH_3$ in methanol (Sigma-Aldrich Inc., St. Louis, MO, USA) according to the standard FIL-IDF procedure (FIL-IDF 1999); the methylation was achieved in 15 min at room temperature. For quantify the fatty acid methyl esters (FAMEs) nonadecanoic acid (C19:0ME) methyl ester at 0.5mg/ml was used as an internal standard.

FAMEs were identified by gas chromatography using a Turbo 3400 *CX* GC (Varian Inc. Palo Alto, CA) gas chromatograph equipped with a flame ionization detector (FID) and Varian 8200 *CX* auto sampler. Separation was accomplished on a CP-select CB for FAME (Varian Inc. Palo Alto, CA) capillary column (100 m x 0.32 mm i.d., 0.25 μm film thickness). The split ratio was 1:100, the injected sample was 1μL and the total run

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time was 74 min. Column oven temperature was held at 75°C for 1 min, increased at 8°C/min to 165°C (35-min hold), increased at 5.5°C/min to 210°C (1-min hold), and finally increased at 3°C/min to 240°C and held for 15 min. The injector temperature and the detector temperature were both set at 255°C. High purity helium served as the carrier gas with a pressure of 37 psi. High purity hydrogen and chromatographic air were supplied to the FID.

Retention time and area of each peak were computed using the Varian Star 3.4.1. Software. Individual FAME was identified by comparing them to a standard mixture of FIM-7FAME Mix (Matreya Inc. Pleasant Gap, Pa., USA). Branched saturated fatty acid methyl ester (BCFA) were identified using GLC-110 MIX (Matreya Inc. Pleasant Gap, Pa., USA) as external standard and individual BCFA methyl ester standards, methyl 14-methylpentadecanoate (*iso*C16:0), methyl 12-methyltetradecanoate (*anteiso*-C15:0), methyl 12-tridodecanoate (*iso*C14:0) and methyl 14-methylhexadodecanoate (*anteiso*-C17:0) (Matreya Inc. Pleasant Gap, Pa., USA).

Polyunsaturated fatty acid methyl esters (PUFA) were identified by comparing relative retention times with isomer mixtures PUFA-1, PUFA-2 and PUFA-3 Animal Source (Supelco, Bellefonte PA) and individual external methyl ester standards, all cis-5,8,11,14,17 C20:5 (EPA), all cis-4,7,10,13,16,19 C22:6 (DHA), all cis-5,8,11,14 C20:4 (ARA), all cis-6,9,12 C18:3, all cis-9,12,15 C18:3, all cis-11,14 C20:2 and all cis-5,8,11 C20:3 (Matreya Inc. Pleasant Gap, Pa., USA). High purity individual *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA (Matreya Inc., Pleasant Gap PA, USA), CLA mix standard (Nu-Ceck-Prep. Inc. Elysian, Minn., USA), and published isomeric profile (Kramer et al., 2004) were also used to help identify the CLA isomers in meat. The identification of C18:1 isomers was based on individual commercial standard (Supelco, Bellefonte, PA, USA) and on chromatograms published by Kramer et al., (2008).

The isomers of C16:1 and C17:1 were identified using published chromatographic reports in ruminant fats (Alves et al., 2006; Luna et al., 2009 respectively). Fatty acids were expressed as percentage of total FAME (% AG / Tot. FAME). The artherogenicity (AI) and thrombogenicity (TI) index (Ulbricht and Southgate, 1991) were also calculated.

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4.3.5. Lipid oxidation measurement

The rapid aqueous acid-extraction method described by Raharjo and Sofos (1992) has been chosen for measuring 2-thiobarbituric acid-reactive substances (TBArs), as an indicator of Malondialdehyde (MDA), a major secondary product of lipid oxidation in meat, with some modifications. Briefly, 10 g of ground RW and CK meat was weighted in a glass test-tube, and Butylated hydroxytoluene (BHT) (Carlo Erba Reagenti, Milan, Italy) was added immediately, as antioxidant, at a level of 0.15% based on lipid content (Pikul et al., 1983); then 40 mL of 5% (v/v) aqueous trichloroacetic acid (TCA) (Carlo Erba Reagenti, Milan, Italy) solution was also added to the tube, and homogenization was carry out for 60s at 13.500 rpm using an Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany). The meat slurry was centrifugated at 5000 x g at 4°C for 45 min.
The supernatant was filtered through a No. 42 Whatman filter paper into a 50 ml volumetric flask. TCA (5% aqueous) was used to make up the volume. A 2 mL aliquot was pippeted into a 10 mL screw-capped test tube, and 2 mL of 40 mM aqueous TBA solution was added. The reaction mixture was incubated for 20 min at 93°C in a water bath under gentle agitation. The tubes were tempered for 60 min at room temperature, the absorbance was measured at 525 nm on a Cary 50 scan UV-Vis Spectrophotometer (Varian Inc. Palo Alto, CA) against a blank consisting of 2 mL 5% aqueous TCA and 2 mL 40 mM TBA. For the quantitative determination of MDA, calibration curve were prepared using 1,1,3,3,tetraethoxypropane (TEP) (Sigma-Aldrich Inc., St. Louis, MO, USA) with a concentration range of 0.2 - 20 μM ($y = 0.0626x + 0.0030$; $R^2 = 0.9999$).
The blank, the calibration solution and the samples were analyzed the same time. TBArs value was expressed as of mg MDA per kilogram of meat and was calculated from standard curve using the formula (Pikul et al., 1989):

$$TBArs_{(mg\ MDA/Kg\ meat)} = ((A \times m \times 7.063 \times 10^{-6})/E) \times 1000$$

where A is the absorbance of each sample, m is the slope of the calibration curve calculated by dividing the concentration in nM of each standard solutions of TEP with its absorbance, 72.063 is the conversion factor from nmol to mg of MDA (assuming that one mole of TEP from place to one mole of MDA), E is the sample weight

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equivalent. The equivalent (E) for 10 g of sample was 0.4 when a 2 mL aliquot of the original 50 mL filtrate was analyzed. Two replicates were run per sample.

4.3.6. Statistical analysis

Data on meat composition and fatty acid content were analyzed with a GLM procedure using the statistical software Minitab (16, 2002) (Minitab Inc., State College, PA). The model included diet (P *vs* NP) and treatment (RW *vs* CK) as fixed effects and their interaction (diet × treatment).

Lipid oxidation (TBArs values) was processed by the GLM procedure of Minitab (16, 2002). The model included diet, days of storage (days 0, 30 and 60 for raw frozen meat and days 0, 2, 4 and 7 for cooked and refrigerated meat) and they interaction (diet × time) as fixed effects. A one-way ANOVA was performed to test the effect of the treatment (RW *vs* CK) on the lipid oxidation. Tukey's test was used to compare mean values. All statistical tests were performed for a significance level $p<0.05$.

4.4. Results and discussions

4.4.1. Proximate composition and energy value

Proximate composition (g/100 g of muscle) and energy value (Kcal/100g of muscle) in *Longissimus dorsi* muscle from Bruno-Sarda cattle before and after the cooking treatment were presented in Table 20. The different feeding regimes caused large differences in proximate composition in particular moisture and protein content were significantly affect ($p<0.001$) by diet, cookind and their interaction.

There were no significant differences in fat content between finishing systems (Table 20), likely because the slaughter age was the same and the energy content of the finishing diets was similar. Regarding the fat content its value tented to increase ($p=0.07$) after the microvawe cooking for both feeding regimes.

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Table 20. Effect of diet and cooking on proximate composition and energy value of Longissimus Dorsi (LD) muscle from young bulls from Bruno-Sarda cattle (g/100g of muscle, except where noted).

	Diet (D)								P-value		
	Pasture (P)				No-pasture (NP)						
State (ST)	Raw		Cooked		Raw		Cooked		D	ST	D×ST
Traits	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
Moisture	76.02^{A}	0.21	67.12^{A}	0.59	75.66^{B}	0.29	64.66^{C}	0.41	**	**	**
Protein	21.54^{C}	0.46	28.28^{B}	0.90	21.48^{C}	0.19	32.02^{A}	0.11	**	**	**
Lipids	1.42	0.25	2.86	0.27	1.50	0.17	2.06	0.20	n.s.	†	n.s.
Ash	1.04^{B}	0.01	1.12^{AB}	0.02	1.05^{B}	0.02	1.20^{A}	0.03	†	**	n.s.
Energy value (Kcal/100g of muscle)	98.93^{B}	1.5	138.87^{A}	5.8	99.43^{B}	2.05	146.79^{A}	1.87	n.s.	**	n.s.

Least square means in the same row with different superscript letters differ significantly: $p \leq 0.05$
** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant
SEM, standard error of mean.

Our results were in accordance with the findings reported by Alfaia et al., (2010) in *Longissimus lumborum* muscle from Alentejano young bulls, slaughered at approximately 600 Kg of live body and fed on concentrate or pasture, in this study no significant difference ($p>0.05$) between animal diets and cooking treatment were found. The values of total lipids contend determined in our study (1.42-1.50% for raw meat and 2.06-2.86% for cooked meat) were generally lower than those reported in previusly researches for trimmed beef in food composition table published in several European countries (Chan et al., 1995; Favier et al.,1995; INRAN, 2000; Souci et al., 2000) in the USA (USDA, 2009) and in Australia (Hoke et al., 1999; Williams, 2007).

In conclusion, in accordance with the Food Advisory Committee (1990) (<5% of fat) guidelines and USDA Food Safety and Inspection Service (FSIS) definition (no more than 10% fat), both raw and cooked meat sample are considered lean meat.

The state (RW *vs* CK) affect significantly ($p=0.000$) ash content and energy value, while these traits were not been different statistcally between the feeding regimes. As reported by Badiani et al. (2002), cooking caused moisture content decrease resulting in increased nutrient concentration, particulary in the protein content, and a consequent rise in energy values in cooked meat compared to raw meat.

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The values obtained for the microwave cooked meat once again confirm that the meat of young bulls from Bruno-Sarda bred is considerable lean meat whereas only 13-19% energy (for NP and P respectively) comes from fat against 81-87% (for P and NP respectively) provided by protein this results to be in accordance with the guidelines suggested for adults by SINU.

The consumption of 100g of cooked LD muscle from young bulls of Bruno-Sarda bred satisfied, on average, 7.% of the recommended daily energy ration for an adult involved in moderate activity that leads to a daily consumption of 2000 Kcal.

An average serving of cooked LD muscles muscle from young bulls of Bruno-Sarda bred is able to provide between 38 and 43% (for P and NP diet, respectively) of the RDA of protein and only between 3.2 and 4.4 % (for NP and P diet , respectively) of the RDA of fat recommended for adults of both sexes. Therefore, based on the results obtained, it can be said that the LD muscles muscle from young bulls of Bruno-Sarda bred is a very lean meat that has a high nutrient density for protein.

4.4.2. Intramuscular fatty acid composition

Data on partial sums (% of total FAME), in both raw and cooked samples for the two different feeding system, are show in Table 21.

There was not significant effect of cooking e no significant interaction between the effect of diet and cooking for any partial sums of fatty acids or for any single fatty acid selected (Table 22). These results were in accordance with Sarriés et al., (2009), who no found changes in the fatty composition in Longissimus dorsi muscle from Charolais crossbred heifers subjected to cooking in conventional oven. Similar findings have been found by Bragagnolo and Rodriguez (2003) in the fatty acid profile of raw and cooked beef steaks, and by Nayigihugu et al. (2004) in Longissimus dorsi muscle from forage-fed Angus x Gelbvieh crossbred beef cattle subjected to cooking on flame-broiled. Instead Alfaia et al., (2010) observed significant changes in the fatty acid composition in *Longissimus lumborum* muscle from Alentejano young bulls, slaughered at approximately 600 Kg of live body and fed on concentrate or pasture, subjected to three different cooking methods, while Scheeder et al., (2001) found slight changes in fatty acid composition during grilling of beef patties.

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Table 21. Effect of cooking and diet on partial sums of fatty acids (g/100g of total FAME) and nutritional ratios of LD muscle from Bruno-Sarda bulls (n=10).

	Diet (D)								P-value	
	Pasture				No-Pasture					
State (St)	Raw		Cooked		Raw		Cooked		D	St
Partial sums	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
Σ SFA	48.85	1.53	49.84	0.76	47.29	1.50	47.07	1.20	†	n.s.
Σ MUFA	35.92	2.45	37.08	2.42	35.77	1.34	36.45	1.35	n.s.	n.s.
Σ PUFA	15.23	2.44	13.09	2.10	16.95	1.45	16.48	1.80	n.s.	n.s.
Σ PUFA n-3	1.67	0.38	1.43	0.22	1.13	0.17	1.00	0.13	†	n.s.
Σ PUFA n-6	12.66	2.09	10.71	1.90	15.05	1.29	14.71	1.70	†	n.s.
HP-PUFA	3.88	0.75	3.17	0.53	4.27	0.66	3.74	0.52	n.s.	n.s.
Σ CLA	0.80^{a}	0.04	0.85^{a}	0.03	0.65^{b}	0.02	0.65^{b}	0.03	**	n.s.
Σ OBCFA	3.56^{a}	0.12	3.85^{a}	0.12	2.86^{b}	0.10	2.90^{b}	0.16	**	n.s.
Nutritional ratios										
PUFA/SFA	0.32^{a}	0.05	0.26^{ab}	0.04	0.36	0.04	0.35	0.04	n.s.	n.s.
MUFA/SFA	0.74^{b}	0.06	0.75^{bc}	0.06	0.76	0.04	0.78	0.04	n.s.	n.s.
n-6/n-3	7.89^{b}	0.85	7.38^{b}	0.77	14.03^{a}	1.35	14.89^{a}	1.08	**	n.s.
AI	0.55	0.03	0.57	0.02	0.51	0.02	0.50	0.01	*	n.s.
TI	1.58	0.12	1.65	0.06	1.54	0.06	1.54	0.08	n.s.	n.s.
Lipid oxidation										
MDA (mg /Kg meat)	0.07^{b}	0.00	0.52^{a}	0.07	0.23^{b}	0.04	0.56^{a}	0.10	n.s.	***

Least square means in the same row with different superscrip letters differ significantly: $p \leq 0.05$
State × Diet interaction was not statistically different for all item. SEM, standard error of mean.
***p≤0.001; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant.
FAME, Fatty acids methyl esters; SFA, Saturated Fatty Acids; MUFA, Monounsatured Fatty Acids; PUFA, Polyunsatured Fatty Acids; OBCFA, Odd and Branched Fatty Acids.
CLA, Conjugated Isomer of Linoleic; ΣCLA includes C20:0 and C20:1n-9 that eluted in CLA region.
The isomers have been summed because the chromatographic conditions were not adequate to allow accurate individual quantification.
ΣSFA: sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C22:0, C24:0 and Branched fatty acids.
ΣMUFA: sum of C14:1, ΣC16:1, ΣC17:1, ΣC18:1,
ΣPUFA: sum of total n-6 and total n-3; HP-PUFA: Highly peroxidable-PUFA, calculated as the sum of PUFA with three or more double bounds (Yang et al., 2002).
ΣPUFA n-3: sum of C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:5n-3, C22:6n-3.
ΣPUFA n-6: sum of ΣC18:2n-6 (LN) cis/trans isomers, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6 and C22:4n-6. Other PUFA : sum of C18:4 n-2. C18:3 n-9 and C20:3 n-9.
OBCFA : sum of C13:0, C13:0 *i*, C13:0 *ai*, C14:0 *i*. C15:0 *i*, C15:0 *ai*, C16:0 *i*, C17:0, C17:0 *i*, C17:0 *ai* and ΣC17:1. (*i* is an *iso*-isomer and *ai* is an *ante-iso*-isomer).
Index of atherogenicity (IA) = $[(12:0) + (4 \times 14:0) + (16:0)] \times [(n\text{-}6 \text{ and } n\text{-}3 \text{ PUFA}) + \text{MUFA})]^{-1}$
Index of thrombogenicity (IT) = $[(14:0) + (16:0) + (18:0)] \times [(0.5 \times \text{MUFA}) + (0.5 \times n\text{-}6 \text{ PUFA}) + (3 \times n\text{-}3 \text{ PUFA}) + (n\text{-}3 \times n\text{-}6^{-1} \text{ PUFA})]^{-1}$ (Ulbricht & Southgate, 1991).

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The concentration of monounsaturated (MUFA), polyunsaturated (PUFA) and highly-peroxidizable PUFA (HP-PUFA) were not significantly different between no-pasture finished bulls and pasture finished bulls. Alfaia et al., (2009) in crossbred steers, Realini et al., (2004) in Hereford steers and Descalzo et al., (2005) in crossbred steers have found , instead, that MUFA increased in meat from grain-finished cattle when compared to that of grass-fed cattle; while the content of PUFA was higher in grass-fed cattle relative to grain-finished cattle.

Regarding the concentration of SFA and PUFA n-3 these tend to be higher ($p=0.08$ and $p=0.06$, respectively) in bulls finished on pasture compared to those confined. These values are closed with these previously observed by Realini, et al. (2004) in Hereford steers finished on pasture or concentrate (49.08% and 47.62%, respectively) and similar to these reported by Leheska, et al. (2008) in mixed bred cattle, and Garcia et al. (2008) in Angus steers that have found higher significant proportion of SFA in grass-fed cattle versus grain-fed cattle. However, several recent studies have observed that grass-fed beef have lower percentages of SFA than grain-fed beef (Yang et al., 2002; Descalzo, et al., 2005; Noci et al., 2005). There was no significant influence of feeding system on the sum of PUFA n-6 their proportion infact tended ($p=0.09$) to be higher in bulls finished on pasture compared to confined bulls. Our results according to values found by Garcia et al. (2008) in Angus steers and Ponnampalam, et al. (2006) in Angus steers that have observed higher concentration of PUFA n-6 in grain-fed cattle relative to grass-fed cattle. In the contrary, Nuernberg, et al. (2005) in Simmental bulls, Alfaia, et al. (2009) in crossbred steers and Descalzo, et al. (2005) in crossbred steers have observed that cattle finished on pasture showed higher content of PUFA n-6 compared to concentrate finished cattle. The percentage concentrations of CLA and OBCFA (0.85% and 3.85%, respectively) found in muscle of animals on the grass-based system were significantly higher ($p<0.01$) compared to percentages observed in confined bulls (0.65% and 2.90%, respectively).

Cooking and diet have not changed the nutritional ratios considered, except for the n-6/n-3 ratio that was significantly affected by diet, in fact, meat from bull finished on pasture had a more favorable n6/n-3 ratio compared to bulls reared indoor (7.6 vs. 14.5, respectively).

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Table 22. Effect of cooking treatment and diet on fatty acid composition (g/100g FAME) of LD muscle from Bruno-Sarda bulls (n=10).

	Diet (D)								p-value	
	Pasture				No-Pasture					
State (ST)	Raw		Cooked		Raw		Cooked		D	ST
Fatty acids	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM		
C14:0	1.41^{b}	0.08	1.54^{b}	0.10	1.38	0.07	1.32	0.05	n.s.	n.s.
C14:1 c9	0.13^{b}	0.03	0.15^{b}	0.10	0.16	0.03	0.17	0.02	n.s.	n.s.
C15:0	0.36	0.02	0.40	0.02	0.33	0.02	0.33	0.02	*	n.s.
C16:0	21.78	0.86	22.07	0.66	20.98	0.58	21.00	0.46	n.s.	n.s.
C16:1 *c*9	1.39^{b}	0.21	1.47^{bc}	0.23	1.38	0.11	1.40	0.09	n.s.	n.s.
C17:0	1.01^{ab}	0.04	1.06^{a}	0.02	0.90^{b}	0.03	0.91b	0.04	***	n.s.
C17:1 *c*9	0.45	0.04	0.46	0.04	0.37	0.02	0.39	0.01	*	n.s.
C18:0	22.57	1.62	22.84	1.32	21.90	1.30	21.71	1.38	n.s.	n.s.
C18:1 *t*10	0.15^{b}	0.03	0.20^{b}	0.03	0.35a	0.03	0.42a	0.05	†	†
C18:1 *t*11	1.39^{ab}	0.12	1.60^{a}	0.12	0.91^{c}	0.10	0.95^{bc}	0.11	***	n.s.
C18:1 *c*9	29.82^{b}	2.38	30.54^{bc}	2.29	30.00	1.25	30.37	1.21	n.s.	n.s.
C18:1 *c*11	1.42	0.04	1.38	0.04	1.44	0.05	1.45	0.04	n.s.	n.s.
C18:2 n-6 (*LN*)	9.92	1.72	8.38	1.57	11.43	0.87	11.47	1.31	n.s.	n.s.
C18:3 n-3 (*ALA*)	0.94	0.22	0.84	0.14	0.52	0.05	0.50	0.03	*	n.s.
CLA *c*9,*t*11	0.34	0.04	0.37	0.03	0.26	0.02	0.25	0.01	**	n.s.
C20:2 n-6	0.10	0.01	0.09	0.01	0.13	0.01	0.12	0.02	*	n.s.
C20:3 n-6	0.36	0.06	0.28	0.06	0.45	0.07	0.40	0.07	n.s.	n.s.
C20:4 n-6 (*ARA*)	1.55	0.30	1.21	0.25	2.20	0.39	1.89	0.29	*	n.s.
C20:4 n-3	0.04	0.00	0.03	0.00	0.03	0.00	0.02	0.00	†	n.s.
C20:5 n-3 (*EPA*)	0.18	0.05	0.14	0.03	0.12	0.04	0.09	0.03	n.s.	n.s.
C22:4 n-6	0.16^{c}	0.03	0.12^{bc}	0.02	0.32^{ab}	0.05	0.29^{a}	0.04	***	n.s.
C22:5 n-3 (*DPA*)	0.46	0.10	0.35	0.07	0.39	0.08	0.32	0.06	n.s.	n.s.
C22:6 n-3 (*DHA*)	0.02	0.00	0.02	0.00	0.04	0.01	0.03	0.01	n.s.	n.s.

Fatty acid are represented in the following manner: the first number indicates the number of carbons, while the second represents the number of double bonds; n-x indicates where the first double bond is located counting from the terminal methyl carbon toward the carbonyl carbon; c-x is for *cis*, t-x is for *trans* and indicate the configuration of double bond and where the double bond in located counting from the carbonyl carbon toward the terminal methyl carbon. Least square of means on the same row followed by different letters differ significantly ($p \leq 0.05$).
State × Diet interaction was not statistically different for all fatty acids.
***p≤0.001; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant

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In the present study, microwave cooking (650W for 35s), did not exercise any significant effect on fatty acid composition, while the different feeding regimes caused large differences in the fatty acid composition of intramuscular fat of Longissimus muscle as shown in Table 22, therefore this cooking method preserves the nutritional value of meat. The effect of cooking on fatty acid composition of meat has been reported with contradictory results due to different animal species, meat cuts, cooking methods and temperatures (Ono et al., 1985; Scheeder et al., 2001, Sarries et al., 2009, Gerber et al., 2009). However the effect of cooking dependents pricipally on type of meat and fat content (Rhee, 2000). This is in agreement with Sarries et al. (2009) who reported no changes in the relative distribution of fatty acids of beef Longissimus muscles after cooking at 140°C for 30 mins. However, Gerber et al. (2009) reported that the total saturated, unsaturated and polyunsaturated fatty acids of beef muscles decreased significantly by grilling, while Alfaia et al. (2009) have observed that the content of 16 of the 34 fatty acids analyzed in Longissimus lomborum muscle was significatly influenced by three different methods of cooking (boiling, grilling and microwaving). Statistical differences in individual fatty acids occurring between diets for some of the all fatty acids chosen. The predominant SFAs, in increasing order of percentage, present in the intramuscular fat of LD muscle were, in both finishing system, myristic (C14:0, 1.32-1.54%), palmitic (C16:0, 21-22%) and stearic (C18:0, 21.9-22.9%) acids. SFA influence plasma cholesterol, and C16:0 is less potent than C14:0 (Williamson et al., 2005), while in this regard C18:0 may be considered as neutral (Yu et al., 1995). Linoleic (C18:2 n-6, 11.5-9.9 %) and α-linolenic (C18:3 n-3, 0.50-0.94%) acids are the main PUFA while oleic acid (18:1*c*9, 30-31%) is the most prominent MUFA, with the remainder of the MUFA detected mainly as cis and trans isomers of 18:1 and palmitoleic (C16:1*c*9, 1.4-1.5%) acid. These results agreed with the findings of Raes et al. (2003); Realini et al. (2004) and Varela et al. (2004) in the intramuscular of steers and heifers. Levels of C15:0 and C17:0 were higher in bulls finished on pasture, as also reported Varela et al. (2004) and Garcia, et al. (2008) in Angus steers.

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The finishing diet has affected the proportion of α-Linolenic (C18:3 n-3) acid with an higher significant ($p<0.05$) percentage in grass-fed bulls compared to bulls reared confined in stable. Similar results have been previously found by Nuernberg, et al. (2005) in Simmental bulls, Garcia, et al. (2008) in Angus steers and Varela et al. (2004) in Rubia Gallega bred. This may be due to the fact that C18:3 is the major fatty acid present in grass (Garton, 1960). Although a high proportion of this fatty acid is hydrogenated in the rumen and transformed into C18:0, a significant amount escapes the rumen to be absorbed intact in the small intestine (Sañudo et al., 2000). There was a higher concentration of linoleic (C18:2 n-6) acid in bulls finished confined in stable (NP) compared with bulls fattened on the grazing system (P), although no significant differences ($p>0.10$) were observed. This is probably because linoleic acid is the principal fatty acid present in cereal grains (Barnes, 1983) and concentrates. As observed in Table 22, pasture finished animals presented a larger content of CLA *cis*-9, *trans*-11($p<0.01$) and vaccenic (C18:1 *t*11) acid ($p<0.001$) relative to confined animals as reported by Nuernberg et al. (2005) in Simmental bulls and Dannenberger et al. (2003). Increased dietary forage promotes ruminal biohydrogenation of the predominant fatty acid in pasture (C18:3n-3) also leads to production of C18:1*trans*-11 and ultimately to CLA in tissue. French et al. (2000) showed that inclusion of pasture compared with grass silage or concentrates in the diets of finishing steers increased the proportion of CLA, increased the PUFA/SFA ratio, and decreased the n-6/n-3 ratio in fat from Longissimus muscle of steers grazed pasture for 85 days. In addition Noci et al. (2005) in Longissimus muscle from crossbred Charolais heifers have demonstrate that the extent of the alteration in the fatty acid profile due to grazing is a function of the duration of grazing before slaughter.

Rumenic acid distribution is not similar to other PUFA, as it was independent of fat location (Santos-Silva et al., 2003) and according to Banni et al. (2001) in rat livers was incorporated priority in neutral lipids. In ruminant muscles, it is know that CLA is mainly associated to the triacylglycerol fraction (Bauchart et al., 2005) which is related to the content of fat in tissues (Ashes et al., 1992).

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Statistical differences in individual fatty acids were also found between diets for, arachidonic (C20:4 n-6) acid ($p<0.05$), C20:2 n-6 ($p<0.05$) and C22:4 n-6 ($p<0.001$), and their percentage were always higher in animals confined versus animals reared on pasture. It was also observed in our experiment that the muscles of grass-fed bulls had higher percentages of long chain highly unsaturated PUFA n-3 faty acids, namely: C20:5 n-3 (EPA), C22:5 n-3 (DPA) and C22:6 n-3 (DHA) than bulls confined but the differences were not significant ($p>0.10$). These results were according to those of Yang et al. (2002) which showed that all of the highly unsaturated fatty acids C18:3 n-3, C20:5 n-3, and C22:5 n-3 were significantly more abundant in the total lipids of the muscles from pasture fed cattle. For Wood et al. (2003), these results are due to the predominance of C18:3 n-3 (the n-3 series precursor) in grass lipids and C18:2 n-6 (the n-6 series precursor) in most other plants and seeds.

In conclusion, the results showed that the lipid components of beef meat could be altered by the feeding system, in particular diet induces change in functional fatty acids such as C18:3 n-3, CLA *c*9,t11, and C18:1 t11; moreover pasture diet contributed to reduce the n-6/n-3 ratio in intramuscular fat and consequently may improve the nutritional quality of beef.

4.4.3. Lipid oxidation

Lipid oxidation is one of the most important changes that occurs during food storage and processing. How demonstrated by Nuernberger et al., (2006) the oxidative process in meat depends by the PUFA content, as well as by the balance between anti- and pro-oxidant compounds. The determination of TBARS is widely used as an index of lipid oxidation (Rey et al., 2001), and this assay measures the quantity of MDA, which is an product of degradation of peroxidized polyunsaturated fatty acids.

MDA content in raw and cooked beef from bulls submitted to two different feeding systems, at 0 days of storage, are presented in Table 21. The values observed did not exceed 1.0 mg MDA/kg of meat and it is resulted lower of the critical value of 3 mg/Kg of meat at which rancidity is virtually detected (Wong et al., 1995). The MDA levels were significantly influenced by cooking ($p<0.001$), while no significant effect of diet and time × diet interaction were observed.

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Lipid oxidation as presented in Figure 29, increased significantly for both raw and cooked samples, as storage time progressed.

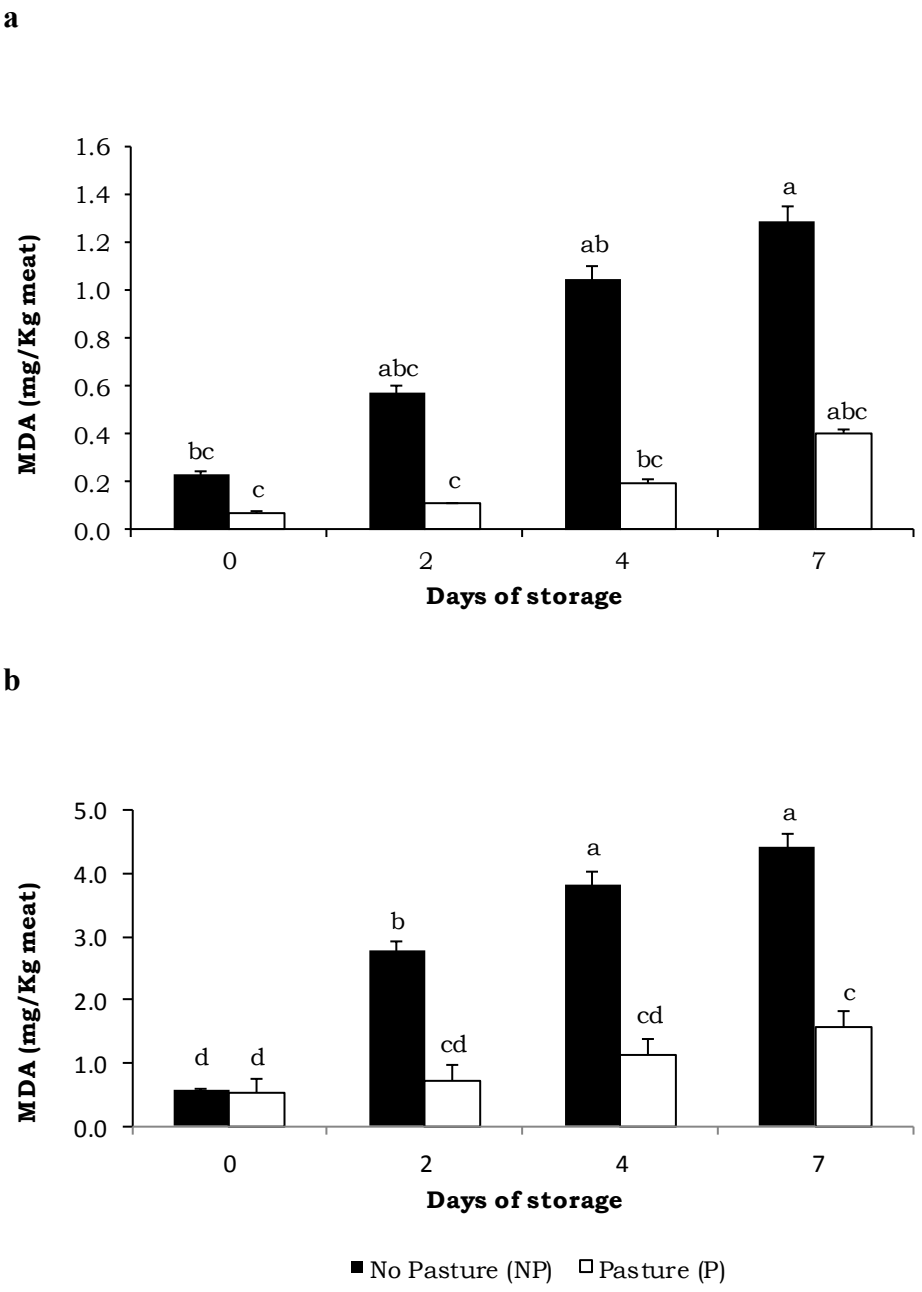

Figure 30. Effect of the dietary treatment (P, NP) and the time of storage (days 0, 2, 4, 7) on MDA levels of raw minced meat (a) and cooked minced meat (b) stored at +4°C over 7 days. Mean value assigned different superscripts differ significantly ($p \leq 0.05$).

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Figure 29a shows lipid oxidation values, determined as mg of MDA equivalents per Kg of meat, for raw minced meat from Longissimus dorsi muscle in P and NP group at different storage time during 7 days of refrigeration at 4°C. The MDA levels were significantly influenced ($p=0.000$) by feeding system and time of storage, while no significant time × diet interaction were observed. Considering separately the dietary treatment, lipid oxidation increased significantly ($p=0.040$) in raw meat from bulls in NP group across the 7 days of storage period, while no significant difference ($p>0.05$) were observed in raw meat from bulls in P group during the7 days of refrigeration. No effect of the diet on lipid oxidation was counted for the difference in MDA levels observed at the different storage time. There was a general tendency ($p<0.10$) toward significance at 2, 4 and 7 days of storage, and the values of MDA measured in meat from bulls fed grass (P) were ponderal lower, compared with bulls finished confined in stable (NP) although no significant differences ($p>0.10$) were observed.

Figure 29b shows the effect of diet (P or NP) and time of storage (days 0, 2, 4 and 7) on lipid oxidation values, determined as mg of MDA equivalents per Kg of meat, for cooked minced meat from Longissimus dorsi muscle stored over 7 days of refrigeration at 4°C. The MDA levels were significantly influenced by feeding system, time of storage, and their interaction ($p<0.001$). Lipid oxidation increased significantly ($p<0.01$) within the first 4 days of storage in meat from bulls in the NP group. However, after 7 days of storage, there was only a slight but not significant increase ($p>0.05$) in the content of MDA in the NP group. In fact the value observed after 7 days of refrigeration (1.29 mg MDA/Kg meat) was statistically similar to the value noted after 4 days of storage (1.05 mg MDA/Kg meat). In the P group lipid oxidation increased slower, in fact, no significant differences ($p>0.05$) in MDA content were detected within the first 4 days of storage. After 7 days, meat from animals in the P group reached higher ($p<0.05$) MDA values (0.39 mg MDA/Kg meat) compared to those found on day 0 (0.07 mg MDA/Kg meat). Any other significant difference ($p>0.05$) were instead noted in the levels of MDA in meat from P group, in fact the value reached at 7 days (0.39 mg MDA/Kg meat) was similar, despite ponderal values are objectively very different, to those observed at 2 and 4 days of refrigeration (0.10 and 0.19 mg MDA/Kg meat, respectively).

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A pronounced effect of diet on the lipid oxidative stability has been counted for the differences in the values of MDA found at different times of refrigeration. Only on day 0 the content of MDA in all samples of raw and cooked muscles, was not affected by the dietary treatments ($P>0.05$), while after 2, 4 and 7 days of storage, meat from bulls in the P treatment had lower MDA content ($p<0.001$) compared to those in the NP group.

The lower MDA levels observed in meat from grass-fed bulls than in meat from animals confined in stable are in agreement with several studies showing the protective effect of grass-based feeding system as compared to concentrate-based diets (Descalzo et al., 2005; Gatellier et al., 2005; Santé-Lhoutellier et al., 2008). The lack of a significant difference between diets, during the 7 days of refrigeration of raw samples could be related to the fact that meat has been minced before analysis, in fact, as noted by Realini et al. (2004), when measured on minced muscle TBArs values were higher in meat of grazing animals. This is due to, as suggested by the author, that the adipose tissue of grazing animals have greater concentration of n-3 PUFAs compared to that of animals finished on concentrated in fact it is known that mincing increases the susceptibility to oxidation that generally is so further enhanced in substrates such as polyunsaturated fatty acids easily oxidizable.

The development of lipid oxidation during 2 months of frozen storage, for ground meat samples from Bruno-Sarda young bulls are present in Figure 30. The MDA contents were significantly influenced ($p=0.008$) by time of frozen storage, while no significant effect by diet and time × diet interaction were observed. Oxidation of samples was significantly influenced by time of storage ($p=0.009$) only in meat from bulls in the P group, while MDA values tended to increase ($P=0.072$) during the whole period in meat from animals in the NP group, although nonsignificantly. Besides, no effect of diet ($p>0.10$) on the lipid oxidative stability has been observed for the differences in the values of MDA found at different times of frozen storage.

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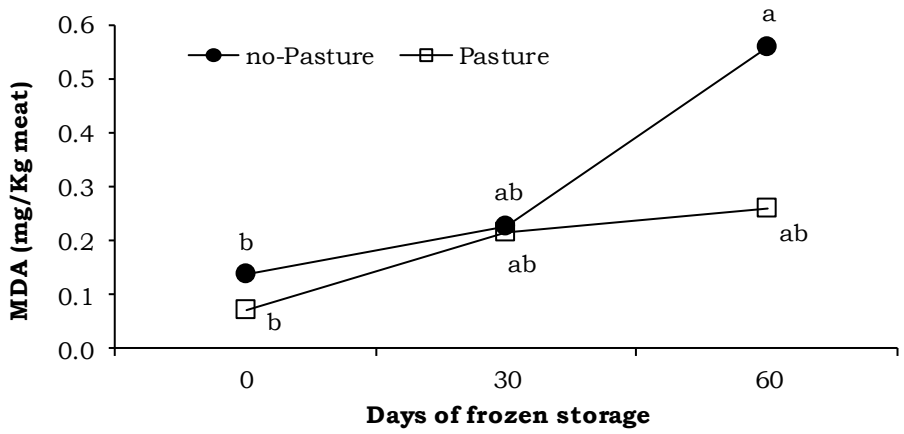

Figure 31. Levels of MDA (mg/Kg of muscle) during two months of frozen storage at -20°C of LD muscle of bulls from different feeding system. Points connected with different letters are statistically different ($p<0.05$).

In conclusion, in the condition of observation carried out in this experiments the dynamic of lipid oxidative process shown influence of time only in the meat from P group, while during the whole period of frozen storage no significant differences were found in the levels of MDA in the meat from NP group. Lipid oxidation was not affect by dietary treatment in the course of frozen storage remained, for both experimental group, below the threshold for detection of rancid odor, however it was higher in the NP group than P group, although nonsignificantly.

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4.5. Conclusions

This study on Bruno-Sardacattle showed that a pasture finishing diet presented some advantages on meat quality, fatty acid composition and lipid oxidation. Moreover, pasture feeding increases polyunsaturated fatty acids (especially PUFAs n-3) and conjugated linoleic acid content of the meat, as well, it contributed to reduce the n- 6/ n-3 fatty acids ratio in intramuscular fat and consequently may improve the nutritional quality of beef which are beneficial for human health.

Microwave cooking (650W for 35s) did not produce adverse effects on nature and / or content of the fatty acid in meat preserving the nutritional values of the meat. Therefore, the changes observed in fatty acid composition of LD muscle were caused exclusively by the different dietary treatment. Oxidation markers derived from lipid oxidation, as MDA content was higher in confined than in grass-fed bulls. A plausible explanation is that pasture diet improved an overall antioxidant and redox status to fresh meat when compared to a grain-finishing diet. Therefore, pasture diet contributes natural antioxidants in sufficient amounts and is an efficient way to prevent lipid oxidation in fresh meat.

Our results suggest that the negative image atribuited to meat from beef due to its highly saturated nature can be overcome by improving the acidic profile of intramuscular fat through diet, particularly grass-fed animal present a fatty acids composition interesting for human health.

- EXPERIMENT FIVE –

Effect of type of suckling on chemical, fatty acid composition and oxidative stability in raw and cooked Sarda lamb meat.

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5.1. Abstract

This study was carried out in order to evaluate the effect of type of suckling on chemical, fatty acid content and oxidative stability in raw and cooked meat from suckling lambs raised under mothers or reared by artificial suckling. Meat samples were taken from twenty-four Sarda suckling lambs from two groups of animals, one group was subjected to natural rearing, and fed only with maternal milk (NR, n=12), while the other was hand reared with milk substitute (AR, n=12). Lambs were slaughtered at 28 days of age. After 24 h of refrigeration at 4°C, the thigh muscle were dissected from each right half-carcass and split into pieces, one of which was used raw while the other was cooked in a microwave oven (650W, 35s) until an internal temperature of 75°C was reached. Chemical and fatty acid analysis were performed on raw and cooked meat as well as the extent of lipid oxidation was determined, after cooking and during 6 months of frozen storage, by measuring the content of malondialdehyde (MDA) by the TBA test. Cooking loss and true nutrient retention values (TR) were also determinate. The fatty acid composition of lamb meat was affect by rearing system. Thigh muscles of natural reared lambs (NR) had significantly higher content of SFA, MUFA, TFA and OBCFA ($p<0.001$). PUFA and PUFA n-6 content was higher ($p<0.001$), in artificially reared (AR) lamb meat leading to a higher n-6/n-3 ratio ($p<0.001$). The cooked meat has a significant lower level of SFA, a higher percentages of PUFA, PUFA n-3 and PUFA n-6 compared to the raw meat of both rearing system. Cooking produced significant increases in the content (mg/100g meat) of all fatty acids considered, but without any significant differences between NR and AR lamb groups. The type of suckling may be responsible for a significant effect in oxidative stability of raw suckling lamb meat during frozen storage Thus, meat from artificial reared suckling lambs may become more stable to oxidation compared to meat from natural reared suckling lambs. Lipid oxidation, expressed as mg MDA/kg muscle, was significantly ($p<0.001$) influenced by cooking for both rearing system.

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5.2. Introduction

Lamb and goat meat, as well as small ruminant dairy products (e.g. cheese and yogurt), are traditional food consumed in many Mediterranean countries (Capatti & Montanari, 1999; Simopoulos & Sidossis, 2000). Consumer demand is addressed mainly toward meat from lightweight lambs (8-12 Kg), these young animals, slaughtered 30–45 days after birth, traditionally raised with their dams and fed exclusively with breast milk from birth until slaughter (Vergara et al, 1999; Sañudo et al, 2006). The meat of the suckling lamb is tender and juicy, characterized by a pearly white pale pink color, also presents smooth texture and possesses a distinctive flavor (Gorraiz et al., 2000).

Meat from suckling lamb meet consumer demand especially in specific periods (Christmas, Easter), and their consumption in Mediterranean countries, such as in Italy, is predominantly regional (Turrini et al., 2001), which means a small market in Europe. Despite that these animals reared exclusively on breast milk provide to the consumer a high quality product and a certain economic relevance in these areas (Sañudo et al. 1998), to increase milk availability for cheese production and to allow the survival of lambs rejected or that cannot suckle their dams, artificial rearing of lambs with milk replacers has been developed in the dairy sheep production systems (Congiu, 1982; Morbidini et al., 1998; Lanza et al., 2005).

As amply demonstrated by several studies, the fatty acid composition of meat can be modified by diet (Antogiovanni et al., 2003; Raes et al., 2004; Dunne et al., 2011), this unfortunately occurs only limited in the meat of ruminants due to of the extensive biohydrogenation of unsaturated fatty acids that occurs in the rumen. A separate discussion should be done about that for suckling animals, because the milk-fed lambs are to be considered "functional monogastric "due to a poorly developed rumen what protects unsaturated fatty acids from biohydrogenation (Lanza et al., 2006); then the composition of fatty acids in muscle of pre-ruminant animals reflects the dietary intake and quality of fat (Bas et al., 2000; Napolitano at al., 2002 Vincenti et al., 2004).

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The aim of this study was to evaluate the effects of suckling on chemical and fatty acid composition and oxidative stability of raw and cooked lamb meat. To obtain this was evaluated meat quality of lambs fed exclusively with maternal milk as compared to lambs fed exclusively with a milk replacer.

5.3. Materials and methods

5.3.1. Animals and muscle sampling

The experiment, lasting 4 weeks, was performed on 24 male and female Sarda lambs divided into two experimental groups of 12 subjects each. Twins were considered. One group of twelve animals was subjected to natural rearing, and fed only with maternal milk for the whole duration of the experiment (group NR or natural rearing). Whereas, the second group of twelve animals was separated from their dams, 18–24 h after parturition housed in a separate straw-bedded pen and fed exclusively with artificial milk (group AR or artificially reared lambs). Lambs of group AR were offered a milk substitute (crude protein 24%, crude fat 24%, crude fiber 0%, and ash 7%) for the whole experimental period. The milk replacer used in our trial included, as reported in commercial label, milk products, cereal products, a mixture of vegetable oils, mineral and vitamin. Individual milk samples were taken before the morning suckling and stored at −20 °C for subsequent fatty acid analysis.

The lambs were slaughtered at 28 days of age. At 24 h *post-mortem* the carcasses were halved into sides and from the right side the thigh muscles (*Semitendinosus*, *Semimembranosus* and *Femoral Biceps*) were excised, vacuum-packaged and stored at -20°C for 24 h until analysis. Ultimate pH was assessed in the thigh muscles after 24 h of storage at 4 °C using a pH meter (Orion Research Inc., model 250A, Boston, MA) equipped with a penetrating glass electrode (Hamilton Company USA, model 238405, Reno, NV) and thermometer.

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All samples, still partially frozen, were trimmed of connective and adipose tissue and sliced into 3 square cuts of about 50 grams; one was subjected to the cooking treatment, while the others were left raw as control for determination of nutritional value and lipid oxidation in uncooked meat.

5.3.2. Cooking treatment and preparation of samples

For microwave cooking, one cut of about 50 g was placed in a polypropylene container, suitable for microwave, in the center of the carousel of a microwave oven (Samsung GE82W), set at 650 W. One heating cycle of 45 s was used to attain a final core temperature of 75°C in order to obtain a constant degree of doneness (medium, according to Matthews and Garrison, pp. 64-65 (1975)). Internal temperature was checked with a digital thermometer (Orion Research Inc., model 250A, Boston, MA), insert into the approximate center of each cut, upon removal from the oven. After cooking the samples were cooled at room temperature (1 h at 20-22°C) dried with a paper towel and split into two pieces (~ 25 g): one was used for chemical analysis, while the other was used for evaluating the lipid oxidative stability by quantification of TBArs. Total cooking losses (% initial raw mass) was also determinate.

The pieces used for chemical analysis were wrapped individually in aluminum foil, vacuum-packed before freezing and storage at –80°C until required for analyses 4–5 days later, while the other piece diced and finely ground with 30s bursts using a food processor (La Moulinette, Moulinex s.p.a., Milan Italy), was processed for TBArs immediately.

One cut of diced raw meat was minced individually, using a food processor (La Moulinette, Moulinex) and used to monitor lipid oxidation of raw (RW) meat over 6 months of frozen storage (-20°C) under aerobic conditions. For study the effect of frozen aerobic storage on raw meat, minced meat was stored in an airtight plastic container and stored in a household freezer, in the dark, at -20°C and extending of lipid oxidation was analyzed 30 days after slaughtering (day 30) and after 60, 90, 120 and 180 days of frozen storage. The temperature of -20°C was chosen because it is commonly used for domestic purposes.

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For determination of proximate and fatty acid composition in lean muscle the remaining raw cuts (about 50 g) were exactly weighted and wrapped individually with a layer of film for food and stored frozen at -80°C until analysis.

5.3.3. Chemical analyses and energy value

Each sub-sample of RW and cooked CK meat were analyzed for moisture, total ash and total nitrogen in duplicate, while analyses of total lipid were carried out in single. All reagents were of analytical grade.

Moisture was determined on RW and CK meat as weight loss after 72 h of freeze-drying. Total ash content was quantified on RW and CK meat by ashing the samples in a muffle furnace at 550 °C for 24 h (Method 920.153, AOAC, 1999). The nitrogen content was determined by the Kjeldahl method (Method 981.10, AOAC 2000) using a 2200 Kjeltec ^{TM} automatic distillation unit (Foss Tecator, Höganäs, Sweden). Total protein was calculated from Kjeldahl nitrogen using a 6.25 conversion factor.

Extraction and analysis of muscle fatty acids were performed on RW and CK freeze-dried samples. Diced meat (RW and CK) was weighed in a polypropylene (PP) pan, frozen overnight at –80°C, freeze-drying (LyoLab 3000, Heto-Holten, Allerød, Denmark) for 72 h and finely ground in a food processor prior to analysis. Total lipid was extracted from 1 g of each freeze-dried sample following the method of Folch et al. (1957), using chloroform/methanol (2:1 v/v) for extraction. Briefly, a quantity of freeze-dried sample (RW and CK) 1 g (~0,0001 g) was weighted in a 50 mL screw-cap glass centrifuge tube, and 30 mL of previously prepared chloroform-methanol solution (2:1 v/v) were added. The mixture was sonicated for 5 min, shaken vigorously with a vortex for 60 s and then centrifuged for 10 min (1500 x g at room temperature). The chloroformic phase was filtered through a Whatman No. 541 filter paper (Whatman, Ltd., Maidstone, U.K.) and 6 mL 0, 6% NaCl (wt/v) were added. The tube was centrifuged at 1500 x g for 10 min. The upper methanol-water layer was removed using a water aspirator and discarded; the lower organic phase was poured into a round flat-bottom flask (50 mL), chloroform was removed using a vacuum rotatory evaporator at 40°C (Büchi 461, BÜCHI Labor AG, Flawil, Switzerland) and samples were evaporate

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until dryness. The total extracted meat fat from the modified method Folch was recorder gravimetrically. After 24 h in a desiccator, extracted fat was redissolved in hexane and an aliquot of muscle lipid (20-25 mg) was transferred to a 2mL screw-cap amber-glass vial and used immediately for fatty acid analysis.

Energy value (Kcal) was obtained by multiplying the amount of total protein by the factor of 4 and the amount of total lipid by the factor of 9 (EEC, 1990).

5.3.4. Fatty acid content and composition

Total lipid extracted from each RW and CK meat samples was used for fatty acid determination. The lipid sample (about 20-25 mg) was esterified by cool base-catalyzed methylation using 0.5 M $NaOCH_3$ in methanol (Sigma-Aldrich Inc., St. Louis, MO, USA) according to the standard FIL-IDF procedure (FIL-IDF 1999); the methylation was achieved in 15 min at room temperature. For quantify the fatty acid methyl esters (FAMEs) nonadecanoic acid (C19:0ME) methyl ester at 0.5mg/ml was used as an internal standard.

FAMEs were identified by gas chromatography using a Turbo 3400 *CX* GC (Varian Inc. Palo Alto, CA) gas chromatograph equipped with a flame ionization detector (FID) and Varian 8200 *CX* auto sampler. Separation was accomplished on a CP-select CB for FAME (Varian Inc. Palo Alto, CA) capillary column (100 m x 0.32 mm i.d., 0.25 μm film thickness). The split ratio was 1:100, the injected sample was 1μL and the total run time was 74 min. Column oven temperature was held at 75°C for 1 min, increased at 8°C/min to 165°C (35-min hold), increased at 5.5°C/min to 210°C (1-min hold), and finally increased at 3°C/min to 240°C and held for 15 min. The injector temperature and the detector temperature were both set at 255°C. High purity helium served as the carrier gas with a pressure of 37 psi. High purity hydrogen and chromatographic air were supplied to the FID.

Retention time and area of each peak were computed using the Varian Star 3.4.1. Software. Individual FAME was identified by comparing them to a standard mixture of FIM-7FAME Mix (Matreya Inc. Pleasant Gap, Pa., USA). Branched saturated fatty acid methyl ester (BCFA) were identified using GLC-110 MIX (Matreya Inc. Pleasant Gap,

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Pa., USA) and individual BCFA methyl ester standards, methyl 14-methylpentadecanoate (*iso*-C16:0), methyl 12-methyltetradecanoate (*anteiso*-C15:0), methyl 12-tridodecanoate (*iso*-C14:0) and methyl 14-methylhexadodecanoate (*anteiso*-C17:0) (Matreya Inc. Pleasant Gap, Pa., USA) as external standards.

Polyunsaturated fatty acid methyl esters (PUFA) were identified by comparing relative retention times with isomer mixtures PUFA-1, PUFA-2 and PUFA-3 Animal Source (Supelco, Bellefonte PA) and individual external methyl ester standards, all *cis*-5,8,11,14,17 C20:5 (EPA), all *cis*-4,7,10,13,16,19 C22:6 (DHA), all cis-5,8,11,14 C20:4 (ARA), all *cis*-6,9,12 C18:3, all *cis*-9,12,15 C18:3, all *cis*-11,14 C20:2 and all *cis*-5,8,11 C20:3 (Matreya Inc. Pleasant Gap, Pa., USA). High purity individual *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA (Matreya Inc., Pleasant Gap PA, USA), CLA mix standard (Nu-Ceck-Prep. Inc. Elysian, Minn., USA), and published isomeric profile (Kramer et al., 2004) were also used to help identify the CLA isomers in meat. The identification of C18:1 isomers was based on individual commercial standard (Supelco, Bellefonte, PA, USA) and on chromatograms published by Kramer et al., (2008).

The isomers of C16:1 and C17:1 were identified using published chromatographic reports in ruminant fats (Alves et al., 2006; Luna et al., 2009 respectively). Fatty acids partial sums were expressed as percentage of total FAME and as absolute concentration (mg/100g of muscle). The proportion of polyunsaturated (PUFA), monounsaturated (MUFA), saturated (SFA) and odd-branched (OBCFA) fatty acid contents and the ratios PUFA/SFA and n-6/n-3 were calculated. The atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht & Southgate (1991): AI=[C12:0+(4 ×C14:0)+C16:0]/[(ΣPUFA)+(ΣMUFA)]; TI=[C14:0+C16:0+C18:0]/[(0.5×ΣMUFA)+(0.5×n-6)+(3×n-3)+(n-3/n-6)]. The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated according to Fernández et al. (2007): h/H=[(sum of C18:1c-9, C18:1c-11, C18:2n-6, C18:3n-6, C18:3n-3, C20:3n-6,C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3 and C22:6n-3)/(sum of C14:0 and C16:0)].

The quantitative fatty acid composition (expressed as mg/100g of meat) was determined in both raw and cooked meat in order to permit observations of nutritional interest and calculating the true nutrient retention values according to the cooking method applied.

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5.3.5. Lipid oxidation measurement

The rapid aqueous acid-extraction method described by Raharjo and Sofos (1992) has been chosen for measuring 2-thiobarbituric acid-reactive substances (TBArs), as an indicator of Malondialdehyde (MDA), a major secondary product of lipid oxidation in meat, with some modifications. Briefly, 10 g of ground RW and CK meat was weighted in a glass test-tube, and Butylated hydroxytoluene (BHT) (Carlo Erba Reagenti, Milan, Italy) was added immediately, as antioxidant, at a level of 0.15% based on lipid content (Pikul et al., 1983); then 40 mL of 5% (v/v) aqueous trichloroacetic acid (TCA) (Carlo Erba Reagenti, Milan, Italy) solution was also added to the tube, and homogenization was carry out for 60s at 13.500 rpm using an Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany). The meat slurry was centrifuged at 5000 x g at 4°C for 45 min.

The supernatant was filtered through a No. 1 Whatman filter paper (Grau et al., 2000) into a 50 ml volumetric flask. TCA (5% aqueous) was used to make up the volume. A 2 mL aliquot was pippeted into a 10 mL screw-capped test tube, and 2 mL of 40 mM aqueous TBA solution was added. The reaction mixture was incubated for 20 min at 93°C in a water bath under gentle agitation. The tubes were tempered for 60 min at room temperature, the absorbance was measured at 525 nm on a Cary 50 scan UV-Vis Spectrophotometer (Varian Inc. Palo Alto, CA) against a blank consisting of 2 mL 5% aqueous TCA and 2 mL 40 mM TBA. For the quantitative determination of MDA, calibration curve were prepared using 1,1,3,3,tetraethoxypropane (TEP) (Sigma-Aldrich Inc., St. Louis, MO, USA) with a concentration range of 0.2 - 20 μM ($y = 0.0626x + 0.0030$; $R^2 = 0.9999$).

The blank, the calibration solution and the samples were analyzed the same time. TBArs value was expressed as of mg MDA per kilogram of meat and was calculated from standard curve using the formula (Pikul et al., 1989):

$$TBArs_{(mg\ MDA/Kg\ meat)} = ((A \times m \times 7.063 \times 10^{-6})/E) \times 1000$$

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where A is the absorbance of each sample, m is the slope of the calibration curve calculated by dividing the concentration in nM of each standard solutions of TEP with its absorbance, 72.063 is the conversion factor from nmol to mg of MDA (assuming that one mole of TEP from place to one mole of MDA), E is the sample weight equivalent. The equivalent (E) for 10 g of sample was 0.4 when a 2 mL aliquot of the original 50 mL filtrate was analyzed. Two replicates were run per sample.

5.3.7. True retention of nutrients

True retention values (TR) for all nutrients were calculated using the following formula (Murphy et al., 1975):
TR (%) = [(nutrient content per g of cooked food × g of food after cooking) / (nutrient content per g of raw food × g of food before cooking)] × 100.

5.3.6. Statistical analysis

Data on meat composition and fatty acid profile were analyzed with a GLM procedure using the statistical software Minitab (16, 2002) (Minitab Inc., State College, PA). The model included rearing system (NR *vs.* AR) and state (RW *vs.* CK) as fixed effects and their interaction (rearing system × state). A one-way ANOVA was performed to test the effect of rearing system on fatty acid content ad TR values. Lipid oxidation (TBArs values) was processed by the GLM procedure of Minitab (16, 2002). The model included rearing system, days of storage (days 30, 90, 150 and 180 days) and they interaction (rearing system × time) as fixed effects. A one-way ANOVA was performed to test the effect of the treatment (RW *vs.* CK) on the lipid oxidation. Tukey's test was used to compare mean values. Significance was defined at $p<0.05$. Values were expressed as means ± standard error of mean (SEM).

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5.4. Results and discussions

5.4.1. Chemical composition and energy value

The chemical composition of the suckling lamb meat according to the rearing system and cooking is shown in table 23.

Table 23. Chemical composition (g/100g of meat) of raw and cooked thigh muscle (*semitendinosus, semimembranosus and femoral biceps*) from Sarda suckling lambs reared on natural (NR) and artificial (AR) systems

	Rearing system (RS)								P-value		
	Natural reared (NR)				Artificial reared (AR)						
State (ST)	Raw		Cooked		Raw		Cooked		RS	ST	RS ×ST
Traits	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
Moisture	74.19^{b}	0.45	68.48^{c}	0.34	76.32^{a}	0.38	68.89^{c}	0.36	***	***	*
Protein	19.95^{b}	0.63	26.33^{a}	0.45	19.66^{b}	0.15	26.18^{a}	0.45	n.s.	***	n.s.
Lipids	2.08	0.30	2.73	0.33	1.88	0.13	2.33	0.13	n.s.	*	n.s.
Ash	1.31^{a}	0.02	1.23^{b}	0.01	1.25^{ab}	0.01	1.33^{a}	0.03	n.s.	n.s.	***
Energy value $^{\#}$	96.22^{b}	4.66	125.0^{a}	2.73	94.82^{b}	1.79	125.3^{a}	2.41	n.s.	***	n.s.
Cooking loss§	—		28.04^{b}	0.97	—		31.18^{a}	1.15	*	–	–
pH	5.75	0.03	—		5.76	0.03	—		n.s.	–	–

Least square means in the same row with different superscript letters differ significantly: $p \leq 0.05$
***p≤0.001; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant
SEM, standard error of mean. $^{\#}$Energy value: Kcal/100g of meat. §Cooking loss: % percentage

Only the moisture content was significantly affect by the rearing system ($p<0.001$), its levels in the raw meat samples in AR group were higher than in NR group these results differ from findings by Osorio et al. (2008) who found in *longissimus dorsi* muscle and leg meat no effect of rearing lambs with either maternal milk or milk replacer. While Lanza et al. (2006) found that meat from the artificial reared group tended to be more moist ($P<0.10$) and showed a significantly lower ($P<0.001$) fat content compared to meat from the natural reared group.

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The type of rearing system affected meat cooking loss, which ranged from 28.04% to 31.18% (in NR and AR group, respectively), these results differ from findings by Vincenti et al. (2004) and Osorio et al. (2008) which have found that the type of diet administered to lambs did not affect meat cooking loss. The pH of thigh muscle was close to 5.75 for both NR and AR samples (Table 22). Other authors also found no effect of rearing lambs with either maternal milk or milk replacer on meat pH (Lanza et al., 2006; Napolitano et al., 2006).

Cooking significantly reduced ($p<0.001$) moisture content in meat sample for both rearing system, furthermore the cooking process affect significantly ($p<0.001$) the protein concentration of meat regardless of the rearing system; these results were also obtained by Vicenti et al. (2004). The lipid content was significantly ($p<0.05$) increased following cooking for both the rearing system, our findings differ from results present by Vincenti et al. (2004) who found that the fat content decrease significantly after cooking for both artificial and natural suckling. The presence of ashes was similar in both group, although its level in the NR group decreased after cooking, while in the AR group cooking treatment led to an increase in the ash content, a significant Rearing system × State interaction ($p<0.001$) were observed for this traits. The state (RW *vs.* CK) affect significantly ($p<0.001$) the energy value, while this trait was not affected statistically by rearing system. As reported by Badiani et al. (2002), cooking caused moisture content decrease resulting in increased nutrient concentration, particularly in the protein and fat content, and a consequent rise in energy values in cooked meat compared to raw meat.

5.4.2. Fatty acid profile

Table 24 shows the fatty acid group content (g/100g of FAME) in both raw and cooked samples of thigh muscle from Sarda suckling lambs reared on natural (NR) and artificial (AR) systems. In general, the fatty acid profile of raw meat reflects that of the milks administrated, and it was significantly ($p<0.001$) affect by rearing system for all the partial sums selected.

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Table 24. Effect of cooking and rearing system on partial sums of fatty acids (g/100g of total FAME) and nutritional ratios of thigh muscle from Sarda suckling lambs.

	Rearing system (RS)								P-value		
	Natural reared (NR)				Artificial reared (AR)						
State (St)	Raw		Cooked		Raw		Cooked		RS	St	RS ×St
Item	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
Partial sums											
Σ SFA	44.33^{a}	0.60	38.96^{b}	0.63	34.83^{c}	0.77	32.89^{c}	0.62	***	**	*
Σ MUFA	43.27^{a}	0.69	42.4^{ab}	0.90	40.05^{b}	0.90	39.47^{b}	0.90	***	n.s.	n.s.
Σ TFA	4.94^{a}	0.14	4.65a	0.09	0.32^{b}	0.13	0.24b	0.09	***	n.s.	n.s.
Σ PUFA	11.84^{a}	0.43	18.08^{a}	1.44	24.79^{c}	1.34	27.27^{b}	1.44	***	***	n.s.
Σ PUFA n-3	2.92^{b}	0.16	5.30^{a}	0.31	2.75^{b}	0.18	3.14^{b}	0.31	***	**	***
Σ PUFA n-6	6.79^{c}	0.28	10.34^{b}	1.11	21.25^{a}	1.17	23.23^{a}	1.11	***	***	n.s.
HP-PUFA	4.72^{b}	0.26	8.86^{a}	0.75	9.28^{a}	0.74	10.09^{a}	0.75	***	***	*
Σ OBCFA	3.09^{a}	0.07	2.51^{b}	0.03	0.46^{c}	0.06	0.43^{c}	0.29	***	***	***
Ratios											
PUFA/SFA	0.27^{c}	0.01	0.47^{b}	0.06	0.72^{a}	0.05	0.84^{a}	0.06	***	***	n.s.
MUFA/SFA	0.98^{b}	0.03	1.10^{a}	0.02	1.15^{a}	0.03	1.20^{a}	0.02	***	***	n.s.
n-6/n-3	2.36^{b}	0.07	2.01^{b}	0.30	7.86^{a}	0.31	7.69^{a}	0.30	***	n.s.	n.s.
h/H	1.62^{c}	0.06	2.27b	0.11	2.57^{ab}	0.12	2.88^{a}	0.11	***	***	n.s.
AI	0.85^{b}	0.03	0.59^{a}	0.03	0.63^{a}	0.04	0.51^{a}	0.03	***	***	†
TI	1.19^{a}	0.03	0.86^{b}	0.03	0.84^{b}	0.03	0.77^{b}	0.03	***	***	***
Lipid Oxidation											
MDA (mg /Kg meat)	0.35^{b}	0.03	1.72^{a}	0.13	0.14^{b}	0.01	1.97^{a}	0.14	n.s.	***	*

Least square means in the same row with different superscrip letters differ significantly: $p \leq 0.05$
***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant.
FAME, Fatty acids methyl esters; SFA, Saturated Fatty Acids; MUFA, Monounsatured Fatty Acids; PUFA, Polyunsatured Fatty Acids; HP-PUFA, highly peroxidisable; OBCFA, Odd and Branched Fatty Acids.
ΣSFA: sum of C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C22:0, C24:0 and Odd-Branched fatty acids;
ΣMUFA: sum of C14:1, ΣC16:1, ΣC17:1, ΣC18:1
ΣTFA: sum of C16:1*t*9, C18:1*t*4, C18:1*t*6+*t*8, C18:1*t*9, C18:1*t*10, C18:1*t*11, C18:2*t,t* + *c/t,* C18:2*t*8,*c*13.
(*c* is a *cis*-isomer and *t* is a *trans*-isomer)
ΣPUFA: sum of total n-6 and total n-3;
ΣPUFA n-3: sum of C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:5n-3, C22:6n-3.
ΣPUFA n-6: sum of ΣC18:2n-6 *cis*/*trans* isomers, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6, C22:4n-6.
HP-PUFA : sum of PUFA with three or more double bonds.
OBCFA : sum of C13:0, C13:0 *i*, C13:0 *ai*, C14:0 *i*. C15:0 *i*, C15:0 *ai*, C16:0 *i*, C17:0, C17:0 *i*, C17:0 *ai*, Σ C17:1.
(*i* is an *iso*-isomer and *ai* is an *ante-iso*-isomer).

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In particular, meat obtained from lambs reared with milk substitute (AR) showed a markedly lower amount of SFA, MUFA, TFA and OBFA compared to lambs fed the maternal milk (NR), our results were similar to those found by Napolitano et al. (2002) in Comisana lambs, Lanza et al. (2006) in Barbaresca lambs and by Vincenti et al. (2004) in Gentile di Puglia male lambs. The content of iso- and anteiso-methyl branched fatty acid increased in lambs subjected to natural rearing because these fatty acids are components of rumen bacterial derivation (Harfoot, et al.,, 1988) and they are synthesized by chain elongation of branched-chain precursors (Garton, 1977). Since young milk-fed lambs are to be considered "functional monogastrics"their intramuscular fatty acid composition could be modified by the different fatty acid profile of milk substitute as compared to that of natural milk (Napolitano et al., 2002). Similarly, the content of unsaturated trans fatty acid (TFA) seems to reflect milk fatty acid composition, in fact meat of natural reared lambs showed a higher content of TFA than that of artificially reared animals (4.94 vs. 0.32, $p<0.001$). Conversely, the AR group showed a higher ($p<0.001$) proportion of PUFA than natural rearing (24.79 vs. 11.84). In particular, within the PUFA a higher content of PUFA n-6 was found lambs fed the artificial milk (21.25 vs. 6.79), whereas no differences were observed in the proportion of PUFA n-3 in the raw state (2.92 vs. 2.75 for NR and AR, respectively) according to Vincenti et al. (2004). These results are likely to be associated to the different fatty acid composition of milk, in fact artificial milk is rich in linoleic (C18:2 n-6) acid due to a large vegetable oils inclusion.

The greater level of PUFA n-6 found in the AR group determined a higher n-6/n-3 ratio compared to NR group (7.86 v. 2.36, respectively) which, consequently, may present a lower risk of heart disease.

Cooking changed the fatty acid profile of meat in different way; in particular for both rearing system a statistically relevant reduction of the concentration of SFA and OBCFA was recorded ($p<0.01$ and $p<0.001$, respectively), whereas the percentage of PUFA, PUFA n-3, PUFA n-6 as well as the proportion of HP-PUFA were increased, regardless rearing system, following cooking. Consequently, the ratio n-6/n-3 was not affect by heat treatment.

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These results differ from findings by Vincenti et al. (2004) which have found that the cooking process did not affect significantly any fatty acid and nutritional parameters considered. However, Juarez et al., (2009) have found great differences, induced by cooking, in fatty acid composition in buffalo meat; in particular they have observed a significant increase in the content of total PUFA and a relevant decrease in the content of SFA after heating. As suggested by Scheeder et al. (2001) unsatured fatty acids were less affect by cooking because they belonged to the membrane structure to a greater higher extent than SFA. Thus, the proportional change in fatty acid composition may be explained by drip loss containing mainly TAG of adipose tissue with relative more SFA (Ramamutri, 1986).

In conclusion, from a dietetic point of view, a natural suckling lamb feeding regime is considered more suitable in order to provide a favorable intramuscular fatty acid composition than a feeding system based on artificial milk.

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5.4.3. Fatty acid content and true retention values

The fatty acid profile of cooked thigh muscle from suckling lamb, subjected to two different rearing system, is reported quantitatively (mg/100g of meat) in Table 25.

The quantitative fatty acid composition (fatty acid expressed in mg/100g of meat) was determinate in cooked meat in order to permit observations of nutritional interest and calculate the true nutrient retention values of the principal nutrient and the fatty acids.

Cooking produced significant increases in the content of all fatty acids considered, but without any significant differences between NR and AR lamb groups (data not shown).

The proportion of total energy derived from SFA, MUFA and PUFA amounted, for NR and AR groups, to means values of 5.9-3.5%, 6.5-4.2% and 2.5-2.8% respectively; moreover within MUFA, the energy fraction contributed by oleic (C18:1 *c*9) was clearly predominant for both rearing system (~85%).

Total SFA contents were approximately 1.7 times higher ($p<0.05$) in natural reared lambs than in artificial reared lamb meat. This was particularly evident in relation to palmitic acid (C16:0) myristic acid (C14:0) and stearic acid (C18:0) their values in fact were numerically higher in NR group compared to AR group, although differences statistically significant were observed only for C 18:0 ($p<0.001$). Total MUFA were 1.5 times lower ($p<0.05$) in AR group than NR group and within MUFA, the content of principal fatty acids palmitoleic (C16:1 *c*9) acid and oleic (C18:1*c*9) acid were numerically higher in lambs fed with maternal milk than in lambs fed with milk substitute, although the values did not differ significantly ($p>0.10$).

Total PUFA, PUFA n-3, and PUFA n-6contents were higher in lamb meat from NR group as compared to lamb meat from AR group ($p<0.001$). The very long chain PUFA n-3 , namely EPA (C22:5 n-3), DPA (C22:5 n-3) and DHA (C22:6 n-3), were presented in contents significantly higher ($p<0.001$) in meat from lambs reared under their mother, in particular, the amounts of these fatty acids were 2.5, 1.8 and 2.2 times higher compared to lambs reared with milk substitute.

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Table 25. Fatty acid content (mg/100g meat) of cooked thigh muscles from **suckling lamb meat**.

	Rearing System (RS)				
	Natural rearing (NR)		Artificial rearing (AR)		p-value
Partial sums	LSM	SEM	LSM	SEM	
Σ SFA	824.63	124.53	482.21	44.26	*
Σ MUFA	905.38	141.62	582.06	54.26	*
Σ TFA	101.10	17.00	3.04	0.82	***
Σ PUFA	352.92	27.74	380.51	11.12	n.s.
Σ PUFA n-3	99.95	6.38	43.03	1.87	***
Σ PUFA n-6	206.23	18.36	325.11	10.58	***
Σ OBCFA	54.06	8.98	6.22	0.59	***
Fatty acids					
C12:0	7.07	1.48	15.15	3.12	*
C14:0	80.24	16.24	54.57	9.00	n.s.
C15:0	7.00	1.59	1.32	0.14	**
C15:0 *ai*	2.81	0.62	0.26	0.05	***
C16:0	395.33	62.30	270.19	24.15	†
C16:1 c9	27.04	5.47	17.47	2.86	n.s.
C17:0	18.71	2.88	2.75	0.21	***
C17:0 *ai*	10.05	1.83	0.76	0.10	***
C18:0	284.87	36.88	132.61	8.37	***
C18:1 *t*11	44.94	6.66	0.46	0.46	***
C18:1 *c*9	724.55	113.70	527.62	50.14	n.s.
CLA c9,*t*11	29.14	4.41	1.67	0.31	***
C18:2 n-6 (LN)	121.55	10.97	233.29	8.54	***
C18:3 n-3 (ALA)	33.51	2.83	11.87	0.83	***
C20:4 n-6 (ARA)	43.81	3.27	74.20	2.81	***
C20:5 n-3 (EPA)	20.75	1.49	8.08	0.83	***
C22:4 n-6	2.54	0.19	6.53	0.48	***
C22:5 n-3 (DPA)	26.73	1.63	14.61	0.78	***
C22:6 n-3 (DHA)	16.71	1.07	7.43	0.41	***

Least square of means (LSM) on the same row followed by superscript different letters differ significantly ($p \leq 0.05$).
***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant. SEM, standard error of mean.
See footnotes in Tables 12 and 13.

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Proportion of linolenic (C18:3 n-3) acid was significantly higher (about 3 times) in the intramuscular fat of lambs fed maternal milk under. Contrarily, the proportion of linoleic (C18:2 n-6), arachidonic (C20:4 n-6) and C22:4 n-6 acids were higher (233.29, 74.20 and 6.53mg/100g of meat, respectively) AR group than in NR group (121.5, 43.81 and 2.54 mg/100g, respectively). The mean of CLA c9, *t*11 content provided by the cooked meat amounted to 29.14 mg/100g of edible portion, in lambs fed with maternal milk, while in artificial reared lambs the content of CLA c9, *t*11 decreased significantly ($p<0.001$) rising to a mean value, of 1.67 mg/100g of cooked meat. Similar results were observed in the content of trans-vaccenic (C18:1 *t*11) acid that resulted about 100 times higher in NR group compared to AR group (44.94 vs 0.46 mg/100g of edible portion). These results according to Osorio et al. (2007) are likely to be associated to the different fatty acid composition of milk, in fact comparing the two milk sources, the following relevant facts have been repeatedly reported (Napolitano et al., 2002; Lanza et al., 2006; Osorio et al., 2007). Most differences between both milk sources have been found (data not shown) for odd and branched fatty (OBCFA) contents, which were from 3 to 10 times higher in ewe's milk fat. Higher percentages of SFA and lower of both MUFA and PUFA have been found in ewe's milk fat. Artificial milk fat is richer in linoleic (C18:2 n-6) acid and n-6/n-3 rate showed differences too. In addition, principal CLA isomers were almost undetectable in the substitute compared to ews's milk as well as trans-vaccenic (C18:1, t11, TVA) acid.

In conclusion, one serving (100 g) of cooked suckling lamb reared under their mother meat is able to supply an average amount of 206.23 mg of PUFA n-6, 121.55 mg of which derived from LN and 43.81 mg from ARA. The contribution of PUFA n-3 amounted to about 100 mg on 100 g of cooked meat, of which 33.51 mg was supplied by ALA and about 64.19 mg by very long chain PUFA n-3 namely EPA (C22:5 n-3), DPA (C22:5 n-3) and DHA (C22:6 n-3) (20.75, 26.73 and 16.71 mg/100g of edible portion, respectively). Moreover, as reported above, 100g of cooked lamb meat provide a significant amount of CLA *c*9,*t*11 equivalent to 29.14 mg/100g of edible portion.

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Contrarily, one serving (100 g) of cooked suckling lamb reared in artificial condition and fed only with milk substitute is able to supply an average amount of 325.11 mg of PUFA n-6, 233.29 mg of which derived from LN and 74.20 mg from ARA. The contribution of PUFA n-3 amounted to about 43 mg on 100 g of cooked meat, of which 11.87 mg was supplied by ALA and about 30.12 mg by very long chain PUFA n-3 namely EPA (C22:5 n-3), DPA (C22:5 n-3) and DHA (C22:6 n-3) (8.08, 14.61 and 7.43 mg/100g of edible portion, respectively). Moreover, 100g of cooked lamb meat provide a negligible amount of CLA *c*9,*t*11 compared to lambs from NR group, equivalent to 1.64 mg/100g of edible portion. In conclusion, fatty acid content in suckling lamb intramuscular fat is depented by content of fat and it is strongly affect by the type of rearing and by milk fat composition. Changes in fatty acid composition of commercial artificial are required in order to make fat composition of milk substituite meat more similar to that of ewes' milk in order to improve intramuscular fatty acid composition of artificial reared lambs from a point of view nutritional and healthy.

The concentration of food components, as observed by Bragagnolo et al. (2003), can increase or decrease during cooking due to loss of moisture, which causes concentration of the components in cooked food, or absorption of moisture that leads to a diluition of the food components after cooking. In order to evaluate true increase or loss/degradation of food components during cooking the true nutrient retention (TR) values were determined (Table 26). In this study TR varied from 87 to 105% for total lipids during microwaving, for AR and NR group, respectively, and there was no significant difference between the two rearing system. Lieterature data for TR for beef and lambs lipids vary remarkable, and depends by type of meat (total cut or lean portion), type of muscle, type of cooking, degree of doneness and weight of the cooked portion, from 96 to 102% in lamb rib-loins (Maranesi et al., 2005), from 98 to 107% in beef LD (Bragagnolo et al., 2003), from 91 to 160% in beef steck (Harris et al., 2002),

These variability can be explained by the presence of undetectable levels of subcutaneous and intermuscular fat that liquefied during cooking and subsequently infiltrate into the lean tissue causing a TR values higher than 100%.

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Table 26. True nutrient retention values (TR%) for cooked samples of thigh muscle from Sarda-breed suckling lambs

	Rearing System (SX)				
	Natural reared (NR)		Artificial reared(AR)		p-value
Selected nutrients	LSM	SEM	LSM	SEM	
Moisture	69.23[a]	1.03	62.19[b]	1.33	***
Protein	95.47	2.88	94.82	1.97	n.s.
Lipid	105.02	11.88	87.41	4.53	n.s.
Ash	67.44[b]	1.59	77.19[a]	2.76	**
Energy	96.33	2.05	91.85	3.11	n.s.
Fatty acids					
Σ SFA	85.79	10.12	85.70	10.55	n.s.
Σ MUFA	97.14	12.73	88.39	10.50	n.s.
Σ TFA	92.28	11.79	91.32	24.63	n.s.
Σ PUFA	153.92[a]	23.27	92.40[b]	3.10	*
Σ PUFA n-3	185.02[a]	30.12	94.35[b]	3.27	**
Σ PUFA n-6	153.72[a]	24.43	91.14[b]	3.16	*
Σ OBCFA	78.82	9.05	89.10	13.14	n.s.

Least square of means (LSM) on the same row followed by superscript different letters differ significantly ($p \leq 0.05$).
***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.10$; n.s. not significant. SEM, standard error of mean.
See footnotes in Tables 12

The results of ANOVA (Table 26) show that protein, lipid and energy retentions were not affected by rearing system, while the type of suckling had a significant effect ($p<0.001$) on the moisture and ash ($p<0.01$) retentions. Water was the nutrient of greatest loss (retention >62%) during cooking, while protein, for both experimental groups, was the most retained nutrient at >94%. Ash retention varied significantly between the two rearing system, howevere there was not practical nutritional significance of this result.

Table 26 shows that rearing system had an effect on PUFA, PUFA n-3 and PUFA n-6 retention ($p<0.05$, $p<0.01$ and $p<0.05$, respectively), in fact, retention values of these fatty acid groups were higher >153% in NR group compared to AR group in which the retention values for these fatty acid groups were always less than 100.

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Our results contrast to what was previously found by Maranesi et al., (2005) lamb in rib-loins and Alfaia et al., (2010) in *longissimus lomborum* muscle from Alentejo young bulls which have observed that the values for the partial sums of SFA, MUFA and TFA were higher than 100%, whereas for the sums of PUFA the values were found to be generally lower (from 72.2 to 109.2%), assuming that the higher values of TR for SFA, MUFA and TFA are likely due to the greater extractability of lipids from the cooked tissue, while for PUFA values below or around 100% suggesting a little loss or degradation of these fatty acids during cooking. These observed differences followed no clear scheme and cannot be explained.

5.4.4. Lipid oxidation

Secondary lipid oxidation was studied by the TBArs test, which is an index of malonaldehyde (MDA) concentration. MDA is one of the main end-products of lipid oxidation. The development of MDA during 6 months of frozen storage in thigh mucle from Sarda suckling lambs is shown in Figure

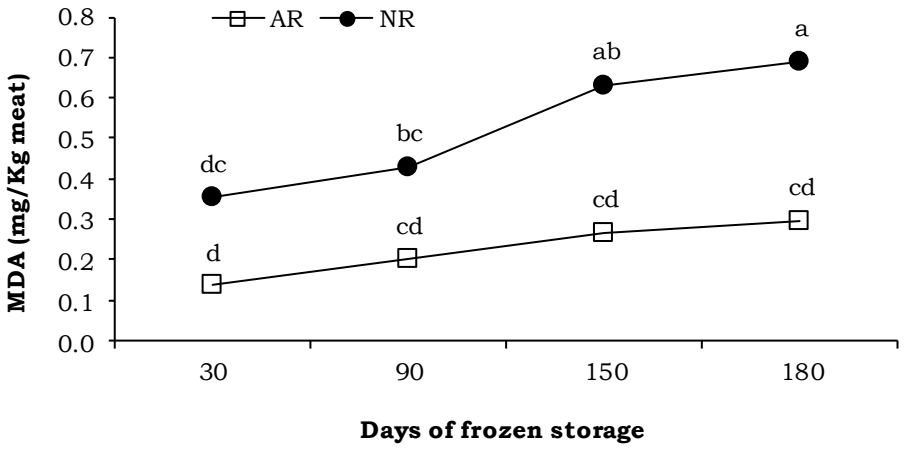

Figure 32. Levels of MDA (mg/Kg of muscle) during six months of frozen storage at -20°C of thigh muscles from Sarda suckling lambs reared under two different feeding regimes. Points connected with different letters are statistically different ($p<0.05$).

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The MDA levels were significantly influenced ($p<0.001$) by rearing system and time of storage while no effect rearing system × time of storage interaction was observed. The increase in the values of MDA was not statistically significant in the AR group samples during all 180 days of storage. The amount of MDA increased significantly in the NR from 0.35 mg MDA/Kg meat to 0.63 mg MDA/Kg meat ($p<0.010$) after 150 days of storage while during the last month of storage until 180 days of freezing, MDA content increased in meat from NR group, up to the value of 0.69 mg MDA/Kg meat but in a way not statistically significant ($p>0.10$).

Lipid-oxidative stability of meat depends on an antioxidative and pro-oxidative balance, with vitamin E content and fatty acid composition being two primary factors (Morrisey et al., 1998). Sevaral studies (Macit et al., 2003; Lauzurica et al., 2005) in fact, have demonstrate that lipid oxidative stability is clearly improved by increasing levels of vitamin E in meat. On the other hand, lipid oxidation rate has been directly correlated with the degree of unsaturation of meat fatty acids (Morrisey et al., 1998). Osorio et al.(2008) have foud that concentrations of Retinol and δ-, γ-, and α-tocopherol were significantly higher in meat from lambs fed with artificial milk due to higher amount of those vitamins (especially γ- and α-tocopherol) in the milk replacer than in the ewe's milk because retinol and tocopherols are added as ingredients in the artificial milk. In the light of these scientific evidence, despite the greater proportion of HP-PUFA in the meat of lambs fed with milk substitute may be explained the extreme oxidative stability of this meat during 6 months storage.

When meat samples were cooked in microwave oven, the mean concentration of MDA (mg/Kg of meat), for both NR and AR group, were significantly increased than corresponding raw sample analyzed before cooking, these results are also presented in Table 24. Lipid oxidation was significantly ($p<0.001$) influenced by cooking and cooking × rearing system interaction ($p<0.05$), while the type of suckling did not affect the oxidative stability. So it's clear that during cooking the oxidative stability of meat in the AR group is lost, in fact as observed by Igene et al. (1979) cooking deactivates the antioxidant defense system presents in muscle food leads to the denaturation of protein and disruption of membranes causing the disorganization of cell structure and release of pro-oxidant metal ions that interacting with PUFAs leads to the development of lipid oxidation.

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5.5. Conclusions

Differences in meat quality between Sarda suckling lambs reared exclusively on natural milk (NR) or on artificial milk (AR) were remarkable. Intramuscular fat of lambs fed with ewe's milk presented more favorable n- 6/n -3 ratio due to lower percentages of linoleic and PUFA n-6 fatty acids and higher content of α-linolenic and other very long chain PUFA n-3 fatty acids than lambs fed with milk substitute. Despite higher proportions of PUFA, meat from lambs fed milk replacer showed a lower CLA content than meat from natural reared lambs. In conclusion, a milk-feeding regime exclusively based on artificial milk adversely affected the dietetic value of lamb meat compared to a natural rearing system, reducing the level of desirable fatty acids such as n-3 series and CLA.

Regarding lipid stability, meat from lamb fed with milk substitute shown lower oxidation rates compared with meat from lambs fed with maternal milk. This difference would be particularly important when meat is subjected to prolonged periods of frozen storage. This fact may be related to the different composition of milk source (ewe 'milk vs. artificial milk), in fact artificial milk containing appropriate levels of antioxidants that can be responsible for significant variation in oxidative stability of fresh suckling lamb meat from artificial reared compared to meat from natural reared. This stability is lost during cooking due to the heat treatment caused deactivation of the antioxidant defense system present in muscle food, so no difference was observed in the levels of MDA after cooking between the two different types of suckling.

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REFERENCES

Abuja, P.M., Albertini, R. 2001. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clinica Chimica Acta,* 306, 1–17.

Addis P.B. 1986. Occurrence of lipid oxidation products in foods. *Food Chem. Toxic*., 48, 2075-2081.

Addis P.B., Warner G.J.. 1991. The potential health aspects of lipid oxidation products in food. *In*: Free Radicals and Food Additives (eds. O.I. Auroma, B Halliwell) Taylor and Francis, London, pp 77-119.

Alfaia C.M.M., Alves S.P., Martins S.I.V., Costa A.S.H., Fontes C.M.G.A., Lemon J.P.C., Bessa R.J.B., Pretes 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.P., 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 methods on fatty acids, conjugated isomer of linolenic acid and nutritional of beef intramuscular fat. *Meat Science*, 84, 769-777.

Antongiovanni M., Buccioni A., Petacchi F., Secchiari P., Mele M., Serra A. (2003). Upgrading the lipid fraction of foods of animal origin by dietary means: rumen activity and presence of trans fatty acids and CLA in milk and meat. *Italian Journal of Animal Science*. 2, 255-268.

A.O.A.C. (1990). Meat and meat products, in *Official Methods of Analysis, 15th Ed*., Association of Official Analytical Chemists, vol.2, Washington DC, U.S.A, pp. 931-948.

A.O.A.C. (2000). Meat and meat products, in *Official Methods of Analysis, 17th Ed*. Gaithersburg, MD : Association of Official Analytical Chemists.

.

Badiani A., Nanni N.,. Gatta P.P, Bitossi F., Tolomelli B. and Manfredin M. (1998). Nutrient content and retention in selected roasted cuts from 3-month-old ram lambs. *Food Chemistry*, 61 pp. 89–100.

Badiani A., Stipa S., Bitossi F., Gatta P.P., Vignola G. and Chizzolini R. (2002). Lipid composition, retention and oxidation in fresh and completely trimmed beef muscles as affected by common culinary practices. *Meat Science*, 60**,** pp. 169–186.

Badiani A., Montellato L., Bochicchio D., Anfossi P., Zanardi E. and Maranesi M. (2004) Select nutrient contents, fatty acid composition, including conjugated linoleic acid, and retention value in separable lean from lamb rib loins as affect by externalfats and cooking method. *Journal of Agricultural and Food Chemistry,* 52, pp. 5187-5194.

Bannister J., Bannister W., Rotilio G. 1987. Aspects of the structure, function, and applications of superoxide dismutase. *CRC Crit. Rev. Biochem* 22 (2), pp. 111–180.

Barnes, P. J. (1983). *Lipids in cereal technology*. London: Academic Press.

Barton, L., Marounek, M., Kudrna, V., Bures, D., & Zahradkova, R. (2007). Growth performance and fatty acid profiles of intramuscular and subcutaneous fat from Limousin and Charolais heifers fed extruded linseed. *Meat Science*, 76(3), pp. 517-523.

Barzanti V., Battino M., Baracca A., Cavazzoni M., Cocchi M., Noble R., Maranesi M., Turchetto E., Lenaz G. (1994). The effect of dietary lipid changes on the fatty acid composition and function of liver, heart and brain mitochondria in the rat at different ages. *British Journal of Nutrition*. 71, pp. 193-202.

Bas P., Morand-Fehr P. (2000). Effect of nutritional factors on fatty acid composition of lamb fat deposits. *Livest. Prod. Sci*. 64, pp. 61-79.

Bass P.D., Engle T.E., Belk K.E., Chapman P.L., Archibeque S.L., Smith G.C., Tatum J.D. (2010). Effects of sex and short-term magnesium supplementation on stress responses and longissimus muscle quality characteristics of crossbred cattle. *Journal of Animal Science*, 88, pp. 349-360.

.

Bauman, D.E., Baumgard, L.H., Corl, B.A., & Griinari, J.M. (1999). Biosynthesis of conjugated linoleic acid in ruminants. In *Proceedings of the American Society of Animal Science,* pp. 1–5. 21– 23 July 1999, Indianapolis, Indiana.

Baylin A., Ruiz-Narvaez E., Kraft P., and Campos H. (2007). α-Linolenic acid, Δ^6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. *Am. J. Clin. Nutr.* 85, pp. 554–560.

Bragagnolo N., Rodriguez-Amaya D.B. (2003). New data on the total lipid, cholesterol and fatty acid composition of raw an grilled beef *longissimus dorsi*. *Archivios Latinosamericanos of Nutricion*, 53 (3), pp. 312-319

Belury MA. (2002). Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. *J. Nutr.* 132, pp. 2995 –2998.

Benzie, I.F.F. (1996). Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *Int. J. Food Sci. Nutr.* 47, pp. 233-261.

Bessa, R.J.B., Alves, S.P., Jerónimo, E., Alfaia, C.M., Prates, J.A.M., & Santos-Silva, J. (2007). Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acid in lambs. *European Journal of Lipid Science and Technology*, 109(8), pp. 868–878.

Bhattacharya, A., Banu, J., Rahman, M., Causey, J., & Fernandes, G. (2006). Biological effects of conjugated linoleic acids in health and disease: review. *Journal of Nutritional Biochemistry*, 17, pp. 789-810.

Bhutta Z. 1999. Protein: digestibility and availability. In: *Encyclopedia of Human Nutrition*. M Sadler, J Strain and B. Caballero (Editors). San Diego: Academic Press, pp. 1646-1656.

Biesalski, H. K. 2005. Meat component of a healthy diet- are there any risks or benefits if meat is avoided in the diet? *Meat Science*, 70, pp. 509–524.

Bognar A. 1998. Comparative study of frying to the other cooking techniques. Influence on the nutritive value. *Grasas y Aceites*, 49, pp. 250-260.

Botterweck A.A.M., Verhagen H., Goldbohm R.A., Kleinjans J., van den Brandt P.A. (2000). Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort Study. *Food Chem Toxicol*. 38, pp. 599–605.

.

Bou R., Guardiola F., Grau A., Grimpa S., Manich A., Barroeta A. et al. (2001). Influence of dietary source, a-tocopherol, and ascorbic acid supplementationon sensory quality of dark chiken meat. *Poultry Science*, 80, 800-807.

Brash, A.R. (1999). Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *J. Biol. Chem*. 274, pp. 23679-23682.

British Department of Health. (1994). Nutritional aspect of cardiovascular disease. Report on health and social subject no.46. HMSO:London.

British Department of Health. (1992). Metabolism of unsaturated fatty acids-nutritional and physiological significance. London, UK: Chapman and Hall.

Brigelius-Flohé R. (1999). Tissue-specific functions of individual glutathione peroxidases. *Free Radic. Biol. Med*. 27 (9–10), pp. 951–965.

Buckley D.J., Morrissey P.A. (1994). Vitamin E and meat quality. *Proc. Nutr. Soc*. 53 (2), pp. 289-295.

Buckley D.J., Morrissey P.A., Gray J.I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *J. Anim. Sci*. 75, pp. 3122-3130.

Calder, P.C. (2004). N-3 Fatty acids and cardiovascular disease evidence explained and mechanisms explored. *Clinical Science* (London), 107, pp. 1–11.

Chardigny, J. M., Destaillats, F., Malpuech-Brugere, C., Moulin, J., Bauman, D. E., Lock, A. L., et al. (2008). Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular risk factors in healthy subjects? Results of the trans fatty acids collaboration (TRANSFACT) study. *American Journal of Clinical Nutrition*, 87(3), pp. 558–566.

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

Chan W., Brown J., Lee S.M:, Buss D.H. (1995). Meat, poultry and game. McCance and Widdowson's The Composition of Foods, 5th Suppl., 5th ed.; The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food: Cambridge, U.K., pp 4, 30-53, 136, 144.

.

Chelikani P., Fita I., Loewen P. (2004). Diversity of structures and properties among catalases. *Cell. Mol. Life Sci*. 61 (2), pp. 192–208

Cline S.D., Riggins J.N., Tomaletti S., Mamett L.J., Hanawalt P.C. (2004). Malondialdehyde adducts in DNA arrest transcription by T7 RNA polymerase and mammalian RNA polymerase II. Proc Natl Acad Sci USA. 101, pp. 7275-7280.

Cobos A., Veiga A. and Díaz O. (2008). Chemical and lipid composition of deboned pieces of dry-cured pork forelegs as affected by desalting and boiling: the effects of vacuum 236 packaging. *Food Chemistry* 106, pp. 951–956.

Correia R.T.P. and Biscontini T.M.B. (2003) Influencia da dessalga ecozimento sobre a composic¸ao química e perfil de ácidos 239 graxos de charque e jerked beef. *Cienc Tecnol Alimentos*. 23, pp. 38–42.

Combs, G.F., & Clark, L.C. (1985). Can dietary selenium modify cancer risk?. *Nutrition Reviews*, 43, pp. 325–331.

Commission of the European Communities. (1989). Reports of the Scientific Committee for Food, 22nd Series. Brussels (Belgium): ECSC-EEC-EAEC.

Congiu F. (1982). Allevamento del capretto con latte artificiale. *Informatore Zootecnico*, 9, pp. 40-49.

Coppen P.P. (1983). The use of antioxidants. In: *Rancidity in Foods* (J. C. Alenn and R. J. Hamilton, eds.). Applied Science Publishers, New York, pp. 67.

Cross, A. J., Leitzmann, M. F., Gail, M. H., Hollenbeck, A. R., Schatzkin, A., & Sinha, R. (2007). A prospective study of red and processed meat intake in relation to cancer risk. *PLos Medicine*, 4(12), p. 1973.

Cosgrove, M., Flynn, A., & Kiely, M. (2005). Consumption of red meat, white meat and processed meat in Irish adults in relation to dietary quality. *British Journal of Nutrition*, 93, pp. 933–942.

Daniel, J.W. (1986). Metabolic aspects of antioxidants and preservatives. *Xenobiotica* 16, pp. 1073-1078.

.

Dannenberger, D., Nuernberg, G., Scollan, N., Schabbel, W., Steinhart, H., Ender, K., et al. (2004). Effect of diet on the deposition of n-3 fatty acids, conjugated linoleic- and C18:1trans fatty acid isomers in muscle lipids of German Holstein bulls. *Journal of Agriculture and Food Chemistry*, 52, pp. 6607–6615.

Dannenberg D., Nuernberg G., Scollan N., Ender K., Nuernberg K. (2007). Diet alters the fatty acid composition of individual phospolipids classes in beef muscle. J. *Agric. Food Chem*. 55, pp. 452-460.

Davey, G. K., Spencer, E. A., & Appleby, P. N. (2003). EPIC-Oxford: Lifestyle characteristics and nutrient intakes in a cohort of 33,883 meat-eaters and 31,546 non-meat-eaters in the UK. *Public Health Nutrition*, 6(3), pp. 259–269.

del Río L., Sandalio L., Palma J., Bueno P., Corpas F. (1992). Metabolism of oxygen radicals in peroxisomes and cellular implications. *Free Radic. Biol. Med*. 13 (5), pp. 557–580.

De la Torre A., Debiton E., Juaneda P., Durand D., Chardigny J. M., Barthomeuf C., et al. (2006). Beef conjugated linoleic acid isomers reduce human cancer cell growth even associated with other beef fatty acids. *Br. J. Nutr*., 95, pp. 346–352.

DeMaeyer, E. M., & Adiels-Tegman, M. (1985). The prevalence of anaemia in the world. *World Health Statistics Quarterly*, 38, pp. 302–316.

DeSmet S., Raes K., Demeyer D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: a review. *Anim. Res*. 53, pp. 81-98.

Descalzo A, Insani EM, Biolatto A, Sancho AM, Garcia PT, Pensel NA (2005). Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science*, 70, pp.35-44.

Descalzo A.M., & Sancho A.M. (2008). A rewiew of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Science*. 79, pp. 423-436.

Devasagayam T.P.A., Boloor K.K., Ramasarma T. (2003). Methods for estimating lipid peroxidation: An analysis of merits and demerits. *Indian J. Biochem. Biophys*., 40, pp. 300-308.

.

Diplock, A.T., Charleux, J.-L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Stahl, W. and Vina-Ribes, J. (1998). Functional food science and defence against reactive oxidative species. *British J. Nutr*. 80, pp. S77-S112.

Drumm T.D. Spanier A.M. (1991). Changes in the content of lipid autoxidation and sulfur-containing compounds in cooked beef during storage. *J. Agric. Food Chem*. 39, pp. 336–343.

EEC (1990). Council directive (EEC) No. 496/90 on nutrition labelling for foodstuff. *Official Journal of the European Communities* L 276, pp. 40-44.

Eder K. (1999). The effects of a dietary oxidized oil on lipid metabolism in rats. *Lipids*, 34, pp. 717-725.

Enser M. (1991). Animal carcass fats and fish oils. In: J.B. Rossel and J.L.R. Pritchard (Eds.), *Analysis of oilseeds, fats and fatty foods*. Elsevier Appl. Sci., London, 329-394.

Enser, M., Scollan, N.D., Choi, N.J., Kurt, E., Hallett, K., & Wood, J.D. (1999). Effect of dietary lipid on the content of conjugated linoleic acid (CLA) in beef muscle. *Animal Science*, 69, pp. 143-146.

Enser, M., Scollan, N., Gulati, S., Richardson, I., Nute, G., & Wood, J. (2001). The effects of ruminally-protected dietary lipid on the lipid composition and quality of beef muscle. Proceedings of the 47th International Congress of Meat Science and Technology, 1, pp. 12–13.

Eriksson, C.E. 1987. Oxidation of lipids in food systems. In: *Autoxidation of Unsaturated Lipids*. H.W.-S. Chan (ed.). Academic Press Inc., London, pp. 207-231.

Esterbauer H, Schaur RJ, Zollner H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.*, 11 pp. 81-128.

Esterbauer H. 1993. Cytotoxicity and genotoxicity of lipid-oxidation products. Am. J. Clin. Nutr. 57(suppl): pp. 779S-786S.

Esterbauer H., Wäg G., Puhl H. 1993. Lipid peroxidation and its role in atherosclerosis. British Medical Bulletin, 49 (3), pp. 566–576.

.

EURODIET. (2000). *Nutrition and diet for healthy lifestyles in Europe: Science and policy implications*. Core report of the EURODIET project steering committee. Available: http//eurodiet.med.ouc.gr. Accessed September 26, 2002.

FIL-IDF. International Dairy Federation. 1999. Milk fat. Preparation of fatty acid methyl esters. Standard 182:1999. IDF, Brussels, Belgium.

Folch, J., Lees, M., & Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry. 226, pp. 497–509.

Frankel, E.N. 1980. Lipid oxidation. Prog. Lipid Res. 19, pp. 1-22.

Frankel, E.N. 1991. Recent advances in lipid oxidation. J. Sci. Food Agric. 54, pp. 495-511.

Frankel E.N.. 1984. Lipid oxidation: mechanisms, products and biological significance. Journal of the America Oil Chemists' Society. 61, pp. 1908-1916.

Frankel E.N.. 1998. Free radical oxidation. *In*: Lipid Oxidation. The Oily Press, Scotland, pp. 23-41.

Frankel, E.N. 1998. *Lipid Oxidation*. The Oily Press Ltd, Dundee.

Frankel E.N., Neff W.E.. 1983. Formation of malonaldehyde from lipid oxidation products. Biochim Biophys Acta. 754, pp. 264-70.

French P., O'Riordan E.G., Monahan F.J., Caffrey P.J., Vidal M.; Mooney M.T., Ttoy D.J., Moloney A.P. (2000). Meat quality of steers finished on autumn grass, grass silage, concentrate-based diets. *Meat Science.* 56, pp. 173-180

French P., Stanton C., Lawless F., O'Riordan E.G., Monahan F. J., Caffrey P.J and 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, pp. 2849–2855.

Frickh J.J., Steinwidder A., Baumung R. (2003). Effect of ration, sex and slaughter weight on meat quality of Simmental cattle. *Zuchtungskunde*. 75(1), pp. 16-30.

.

Fritsche S, .Steinhart H. (1998). Differences in natural steroid hormone patterns of beef from bulls and steers. Journal of Animal Science 76, pp. 1621-1625.

Fritsche, J.R.R., Steinhart, H. 1998. Analysis, occurrence, and physiological properties of *trans* fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA) – a review. *Fett/Lipid* 100(6), pp. 190-210.

Fritsche J.R.R., Steinhart H. (1999). Formation, contents, and estimation of daily intake of conjugated linoleic acid isomers and trans-fatty acids in foods. *Advances in Conjugated Linoleic Acid Research*, 1, pp. 378 – 96.

Fujisawa, S., Kadoma, Y., & Yokoe, I. (2004). Radical-scavenging activity of butylated hydroxytoluene (BHT) and its metabolites. *Chemistry and Physics of Lipids*, 130, pp. 189−195.

Galliard T. 1989. Rancidity in cereal products. *In*: Allen JC, Hamilton RJ, eds. Rancidity in foods. New York: Elsevier Science Publishing Co. pp. 141–145.

Gardner, H.W. (1989). Oxygen radical chemistry of polyunsaturated fatty acids. *Free Rad. Biol. Med.* 7, pp. 65-86.

Garton G.A. (1960). Fatty acid composition of the lipids of pasture grasses. *Nature* (London), 187, pp. 511–512.

Garton G.A. (1977). Fatty acid metabolism in ruminants. *In Biochemistry of lipids II*, (Vol. 14) (pp. 337–370). Baltimore: Goodwin.

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, pp. 175–186.

Gerber N., Scheeder M.R.L. and Wenk C. (2009). The influence of cooking and fat trimming on the actual nutrient intake from meat. *Meat Science*, 81, pp. 148-154.

Gibson, S., & Ashwell, M. (2002). The association between red and processed meat consumption and iron intakes and status among British adults. *Public Health Nutrition*, 6(4), pp. 341–350.

.

Giese. J. (1996). Antioxidants: Tools for preventing lipid oxidation. *Food Technology*. 50, p.72.

Girotti A.W. (1990). *Photochem. Photobiol*. 51 p. 497.

Girotti, A.W. (1998). Lipid hydroperoxide generation, turnover, and effector action in biological systems. *J. Lipid Res*. 39, pp. 1529-1542.

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. *British Nutrition Foundation, Nutrition Bulletin*, 31, pp. 104–110.

Gordon, M.H. (2001). The development of oxidative rancidity in foods. In: *Antioxidants in Food. Practical applications*. J. Pokorny, N. Yanishlieva and M. Gordon (eds.),. Woodhead Publishing Ltd, Cambridge, pp. 7-21

Gorraiz, C., Beriain, M.J., Chasco, J., Iraizoz, M. (2000). Descriptive analysis of meat from young ruminants in Mediterranean systems. *J. Sens. Stud.* 15, pp. 137–150.

Grau A., Guardiola F., Boatella J., Barroeta A., Codony R. (2000). Measurement of 2-thiobarbituric acid values in dark chicken meat throught derivative spectrophotometry: influence of various parameters. Journal of Agricoltural and Food Chemistry, 48, pp. 155-1159.

Gray, J. I. (1978). Measurement of Lipid Oxidation. A Review. *J. Am. Oil Chem. SOC.* 55, pp. 539-546.

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, pp. 7-62.

Gunstone, F.D. (1984). Reaction of oxygen and unsaturated fatty acids. *J. Am. Oil Chem. Soc.* 61, pp. 441-447.

Gutteridge J.M.C., Halliwell B. (1990). The measurement and mechanism of lipid peroxidation in biological systems. *Trends Biochem. Sci.* 15, pp. 129–135.

Ha, Y.L., Grimm, N.K., & Pariza, M.W. (1989). Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. *Journal of Agricultural and Food Chemistry*, 37, pp. 75-81.

.

Halliwell, B. and Gutteridge, J.M.C. (1989). Free Radicals in Biology and Medicine. (2nd ed.). Oxford University Press: New York.

Halliwell B., Chirico S. (1993). Lipid peroxidation: its mechanisms, measurement and significance. *Am. J. Clin. Nutr.* 57 (suppl.7), pp. 15S-25S.

Halliwell B., Gutteridge J.M.C. (1999). Free Radicals in Biology and Medicine (3rd ed.). Oxford, UK: Oxford University Press.

Halliwell B., Murcia M.A., Chirico S., Aruoma O.I. (1995). Free radicals and antioxidants in food and in vivo: what they do and how they work. *Crit. Rev. Food Sci. Nutr.* 35 (1/2), pp. 7-20.

Hamberg M., Samuelson B. (1967). On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. *J.Biol.Chem.* 242, 5329.

Hernández, P., Navarro J., Todra F. (1999). Effect of frozen storage on lipid and lipolytic activicties in the longissimus dorsi muscle of the pigs. *Z Lebensm Untersuch Forsch A*. 208, pp. 277-285.

Hernández, P., Park, D.K., & Rhee, K.S. (2002). Chloride salt type/ionic strength, muscle site and refrigeration effects on antioxidant enzymes and lipid oxidation in pork. *Meat Science.* 61, pp. 405–410.

Harfoot C.G., Hazlewood G.P., Hobson P.N. (1988). Lipid metabolism in the rumen. *In The rumen microbial ecosystem* (pp. 285–322). London: Elsevier Science Publications Ltd.

Harris K.B., Harberson T.J., Savell J.W., Cross H.R., & Smith S.B. (1992). Influences of quality grade, external fat level, and degree of doneness on beef steak fatty acids. *Journal of Food Composition and Analysis*, 5, pp. 84-89.

Higgs J. (2000). The changing nature of red meat: 20 years of improving nutritional quality. *Trends in Food Science and Tecnology*, 11, pp. 85-95.

Hill, M. (2002). Meat, cancer and dietary advice to the public. *European Journal of Clinical Nutrition*, 56(1), pp. S36–S41.

Hino, A., Adachi, H., Toyomasu, K., Yoshida, N., Enomoto, N., Hiratsuka, A., et al. (2004). Very long-chain n-3 fatty acid intake and carotid athersclerosis. An epidemiological study evaluated by ultrasonography. *Atherosclerosis*, 176, pp. 145–149.

.

Hoke I.M., Buege D.R., Ellefson W., Maly E. (1999). Nutrient and related food composition of exported Australian lamb cuts. Journal of Food Composition and Analysis, 12, pp. 97-109.

Howe, P., Buckley, J., & Meyer, B. (2007). Long-chain omega-3 fatty acids in red meat. *Nutrition and Dietetics*, 64(4),pp. S135–S139.

Hunter, J.E. 2006. Dietary *trans* fatty acids: review of recent human studies and food industry responses. *Lipids*, 41, pp. 967-992.

Igene J.O., Yamauchi K., Pearson A.M., Gray J.I., Aust S.D. (1985). Evaluation of 2-thiobarbituric acid reactive substabces in relation to warmed-off flavor development in cooked chicken. *J. Agric. Food Chem.,* 33, pp. 364-367.

IOM/NAS (1994). Opportunities in the nutrition and food sciences. In P.R. Thomas & R. Earl (Eds.), (p. 109). Institute of Medicine/National Academy of Sciences, Washington, DC: National Academic Press.

Ip, C., Scimeca, J.A., & Thompson, H.J. (1994). Conjugated linoleic acid: a powerful anticarcinogen from animal fat sources. *Cancer*, 74,pp. 1050-1054.

Ip C, Banni S, Angioni E, et al. (1999). Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J. Nut.*, 129, pp. 2135 –2142.

ISMEA (1992) Composizione e dinamica dei consumi di carni ovi-caprine in Italia. Arti Grafiche S.Marcello - Roma.

ISMEA (1993), Carne, una vera rivoluzione nei consumi, "*ISMEA Informazioni*", vol. 9, n. 1, pp. 36-42.

Iverson F. 1999. In vivo studies on butylated hydroxyanisole. *Food Chem. Toxicol*. 37, pp. 993–997.

Janero DR. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 9:pp. 515-540.

Jenkins, T.C. 1994. Regulation of lipid metabolism in the rumen. *Journal of Nutrition*, 124,pp. 1372S–1376S.

.

Jenkins, T.C., Wallace, R.J., Moate, P.J. & Mosley, E.E. 2008. Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem: review. *Journal of Animal Science*, 86, pp. 397-412.

Jiménez-Colmenero, F. 2007. Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meats fats: review. Trends in Food Science and Tecnology, 18, pp. 567-578.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1995. Forty-fourth Report of the Joint FAO/WHO Meeting on Food Additives. Technical Report Series Number 859. Geneva (Switzerland): JECFA.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1999. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives JECFA. WHO Food Additives Series Number 42. Geneva (Switzerland): JECFA.

Joint Meeting on Pesticide Residues (JMPR). 2005. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Geneva (Switzerland): JMPR.

Johnston, J., Prynne, C. J., Stephen, A. M., & Wadsworth, M. E. J. (2007). Haem and non-haem iron intake through 17 years of adult life of a British Birth Cohort. *British Journal of Nutrition*, 98, pp. 1021–1028.

Kahl, R., Kappus, H.. 1993. Toxicity of synthetic antioxidants BHA and BHT in comparison with natural antioxidants vitamin E. *Zeitschrift fur Lebensmittel-Untersuchung und –Forschung* 196, pp. 329–338.

Kang, H. G., Jeong, S. H., Cho, J. H., Kim, D. G., Park, J. M., & Cho, M. H. (2005). Evaluation of estrogenic and androgenic activity of butylated hydroxyanisole in immature female and castrated rats. *Toxicology*, 213, pp. 147−156.

Katan, M.B., Zock, P.L., & Mensink, R.P. (1994). Effects of fats and fatty acid on blood lipids in humans: an overview. *American Journal of Clinical Nutrition*, 60, pp. 1017-1022.

Karolyi, D., Đikić, M., Salajpal, K., Jurić, I. 2009. Fatty acid composition of muscle and adipose tissue of beef cattle. 2009. Italian Journal of Animal Science 8, Suppl.3, pp. 264-266.

.

Kelly C. (2001). Dietary fat and cardiovascular disease. *In Health Professionals (Nº. 1).* (pp. 1−24). France: INRA.

Kepler, C., Hirons, K. P., McNeill, J. J., & Tove, S. B. (1966). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrovibrio fibrisolvens*. *Journal of Biology and Chemistry*, 241, pp. 1350–1354.

Kinter M., (1995). Analytical technologies for lipid oxidation products analysis. *Journal of Chromatography B*, 671, pp. 223-236.

Kramer, J.K.G., Parodi P.W., Jensen R.G.,. Mossoba M.M, Yurawecz M.P., and Adlof R.O. (1998). Rumenic acid: a proposed common name for the major conjugated linoleic acid isomer found in natural products. *Lipids* 33, 835.

Kramer, J. K., Hernandez, M., Hernandez, C. C., Kraft, J., Dugan, M. E. (2008). Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. *Lipids*, 43, pp. 259-273.

Kontogianni, M. D., Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C., & Stefanadis, C. (2008). Relationship between meat intake and the development of acute coronary syndromes: The CARDIO2000 case–control study. *European Journal of Clinical Nutrition*, 62, pp. 171–177.

Kwon, T.W., Menzel, D.B., Olcott, H.S. (1965). Reactivity of malonaldehyde with foods costituents. *Journal of Food Science*, 30, pp. 808-813.

Kubow S. (1990). Toxicity of dietary lipid peroxidation products.. *Trend Food Science and Technoogy.* 1, pp. 67-71

Lanza M., Bella M., Priolo A., Barbagallo D., Galofaro V., Landi C. (2006)**.** Lamb meat quality as affected by natural or artificial milk feeding regimen. *Meat Science*, 73, pp. 313-318.

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, pp. 2282-2293.

Lauzurica S., De la Fuente J., Díaz M.T., Álvarez I., Pérez C., Cañeque, V. (2005). Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. Meat Science 70, pp. 639–646.

.

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, pp. 55–377.

Lee K.N., Kritchevsky D., Pariza M.W. (1994). Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis*, 108

Lindmark-Månsson, H., Chen, J., Paulsson, M., Aldén, G., Ren, B., Ladenstein, R., et al. (2001). The effect of storage and heat treatment on glutathione peroxidase in bovine milk and whey. *International Dairy Journal*, 11, pp. 71−81.

Linseisen, J., Kesse, E., Slimani, N., Bueno-de Mesquito, H. B., Ocke, M. C., Skeie, G.,et al. (2002). Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: Results from 24-hour dietary recalls. *Public Health Nutrition*, 5(6B), pp. 1243–1258.

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

Liu Q., Scheller K.K., Schaefer D.M., Arp S.C., Williams S.N. (1994). Dietary α-tocopheryl acetate contributes to lipid stability in cooked beef. *Journal of Food Science.* 59 (2) pp. 299-290.

Litwinczuk Z., Florek M., Pietraszek K. (2006). Physico-chemical quality of meat from heifers and young bulls of the Black-and-White (BW) variety of Polish Holstein-Friesian breed, and commercial BW crossbreds sired by Limousine and Charolaise bulls. Anim. Sci. Pap. Rep. 24, pp. 179-186.

Lombardi-Boccia G., Lanzi S., Lucarini M., Di Lullo G. (2004). Meat and meat products consumption in Italy: contribution to trace elements, heme iron and selected B vitamins supply. *Int J Vitam Nutr Res.* 74(4), pp. 247-251.

Lundebye A.-K., Hove H., Mååge A.,. Bohne V.J.B & Hamre K. (2010). Levels of synthetic antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in fish feed and commercially farmed fish. *Food Additives & Contaminants: Part A,* 27:12, pp. 1652-1657

Macit M., Aksakal, V., Emsen E., Irfan Aksu M., Karaoglu M., Esenbuga, N. (2003). Effects on vitamin E supplementation on performance and meat quality traits of Morkaraman male lambs. Meat Science, 63, pp. 51–55.

.

Maerker G.. 1987. Cholesterol autoxidation. Current status. *Journal American Oil Chemistry Society.* 64, pp. 7-392.

Maranesi M., Bochicchio D., Montellato L., Zaghini A., Pagliuca G., Badiani A. (2005). Effect of microwave cooking or broiling on selected nutrients contents, fatty acid patterns and true retention values in separable lean from lamb rib-loins, with emphasis on conjugated linoleic acid. *Food Chemistry*. 90, pp. 207-218.

Maraschiello C., Sárraga C., García Regueiro J.A. (1999=. Glutathione Peroxidase Activity, TBARS, and α-Tocopherol in meat from chickens fed differents diets. *J. Agric. Food Chem.* 47, 867-872.

Markesbery W.R., Lovell M.A. (1998). Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiology of Aging*, **19** 1, 33–36.

Marnett LJ. (1999). Lipid peroxidation-DNA damage by malondialdehyde. *Mutat Res*. 424, 3-95.

Marnett LJ. (1999). Generation of mutagens during arachidonic acid metabolism. *Cancer Metastas Rev.* 13, 303-308.

Matthews R.H., Garrison Y.J. (1975). *Food yield summarized by different stages of preparation*. Agriculture Handbook 102; U.S. Department of Agriculture. Washington, DC, pp 64-65.

Mazzone G., Giammarco M., Vignola G., Sardi L., Lambertini L. (2010). Effects of the rearing season on carcass and meat quality of suckling Apennine light lambs. *Meat Science*, 86, 474–478.

McGuire, M.A., and M.K. McGuire. (2000). Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. *Proc. Am. Soc. Anim. Sci.* [online publication].

Mei, L., Crum, A.D., & Decker, E.A. (1994). Development of lipid oxidation and inactivation of antioxidant enzymes in cooked pork and beef. *Journal of Food Lipids*, 1, 273−283.

Meister A., Anderson M.E. (1983). Glutathione. *Annual Review of Biochemistry* 52, 711–60.

.

Melton S.L., Black J.M., Davis G.W., Backus W.R. (1982). Flavor and selected chemical characteristics of ground beef from steers backgrounded on pasture and fed corn up to 140 days. *Journal of Food Science*, 47, 699-704.

Melton, S. L. (1983). Methodology for Following Lipid Oxidation in Muscle Foods. *Food Technol.,* 37(7), 105-111, 116.

Merril A.L., Watt B.K. (1973). Energy value of foods: basis and derivation: Human Nutrition Research Branch, Agricultural Research Service, United States Department of Agriculture. *Agricultural Handbook* No.74.

Mikova K. (2001) The regulation of antioxidants in food, J. Pokorny, N. Yanishlieva, M. Gordon, Editors , *Antioxidants in food*, CRC press LLC, Boca Raton, USA, pp. 267–283.

Miyashita, K. and Takagi, T. (1986). Study on the oxidative rate and prooxidant activity of free fatty acids. *J. Am. Oil Chem. Soc.* 63, 1380-1384.

Morrissey P.A., Sheehy P.J.A., Galvin K., Kerry J.P. & Buclkey D.J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49, S73-S86.

Murphy, E.W., Criner P.E., Gray B. C. (1975). Comparison of Methods for Calculating Retentions of Nutrients in Cooked Foods. *J. Agric. Food Chem.* 23, 1153-1157.

Napolitano, F., Cifuni, G. F., Pacelli, C., Riviezzi, A. M., & Girolami, A. (2002). Effect of artificial rearing on lamb welfare and meat quality. *Meat Science, 60*, 307–315.

Noci F., Monahan F. J., French P., and Moloney A. P. (2005). The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: Influence of the duration of grazing. *Journal of Animal Science*. 83, pp. 1167–1178

NRC. (1996). Carcinogens and Anticarcinogens in the Human Diet. National Academy Press, Washington, DC. Ogata M. 1991. *Acatalasemia. Hum. Genet.* 86 (4), 331–340.

Nudda A., Mele M., Serra A., Manca M. G., Boe R., Secchiari P. (2009). Comparison of fatty acid profile in lamb meat and baby food based on lamb meat. *Italian Journal of Animal Science*, 8(Suppl. 2), pp.525-527.

.

Nudda A., McGuire m.K., Battacone G., Manca M-G-, Boe R., and Pulina G. (2011). Documentation of fatty acid profile in lamb meat and lamb-based infant food. *Journal of Food Science*, 76, (2), pp. 43-47.

Nuernberger K.; Dannenberg D.; Nuernberger G.; Ender K.; Voigt K.., Scollan N.D., Wood J.D., Nute G.R., Richarson R.I. (2005). Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of *longissimus* muscle in different cattle breeds. *Livestock Production Science*, 94 pp. 137–147

Nuernberger K., Kuechenmeister U., Nuernberger G., Hartung M., Dannenberger D., & Enser K. (2006). Effect of storage and grilling on fatty acids in muscle of pigs fed plant oils. *European Journal of Lipid Science and Technology*, 108, pp. 554-560.

Nürnberg, K., Wegner, J., Ender, K. (1998). Factors influencing fat composition in muscle and adipose tissue of far animals. Livestock Production Science, 56, pp-145-156.

O'Grady M.N., Monahan F.J., Burke R.M., Allen P. (2000). The effect of oxygen level and exogenous a-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. *Meat Science*, 55, pp. 39-45.

Okuyama, H., & Ikemoto, A. (1999). Needs to modify the fatty acid composition of meats for human health. In: Proceeding of the 45th International Congress of Meat Science and Technology (Vol. II) pp. 638-640.

OnoK., Berry B.W., Johnson H.K., Russek E., Parker C.F., Chaill V.R., Althouse P.G. (1984). Nutrient composition of lamb of two age group. *Journal of Food Science*, 49, pp. 1233-1239.

Ono K., Berry B.W. and Paroczay E. (1985). Contents and retention of nutrients in extra lean, lean and regular ground beef. *Journal of Food Science*, 50, pp. 701-706.

Orellana C., Peña F. García, 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, pp. 57-64.

.

Oriani G., Maiorano G., Filetti F., Di Cesare C., Manchisi A., Salvatori G. (2005). Effect of age on fatty acid composition of Italian Merino suckling lambs. *Meat Science*, 71, pp. 557–562.

Osorio M.T., Zumalacàrregui J.M., FigueiraA., Mateo J. (2007 a). Fatty acid composition in subcutaneous, intermuscular and intramuscular fat deposits of suckling lamb meat: Effect of milk source. *Small Ruminant Research*, 73, pp. 127–134.

Osorio M.T., Zumalacárregui J.M., Figueira A., Mateo J. (2007 b). Physicochemical properties of perirenal and omental fat from suckling lamb carcasses evaluated according to the type of milk source. *Small Ruminant Research*, 72, 111–118.

Osorio M.T., Zumalacárregui A., Figueira A., Cabeza E.A., & Mateo, J. (2008). Effect of rearing system on some meat quality traits and volatile compounds of suckling lamb meat. *Small Ruminant Research*, 78, pp. 1–12

Palmquist, D.L., Lock, A.L., Shingfield, K.J., & Bauman, D.E. (2005). Biosynthesis of conjugated linoleic acid in ruminants and humans. *Advances in Food and Nutrition Research*, 50, pp. 79–217.

Park Y., Storkson J.M., Albright K.J., Liu W., Pariza M.W. (1999). Evidence that the trans-10, cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids,* 34, pp. 235– 41.

Pariza M.W., Park Y., Cook M.E. (2001). The biologically active isomers of conjugated linoleic acid. *Prog. Lipid Res,*. 40, pp.283–98.

Parrish, F.C. Jr., Wiegand, B.R., Beitz, D.C., Ahn, D.U., Du, M., & Trenkle, A.H. (2003). Use of dietary CLA to improve quality of animal-derived foods. In J-L Sébédio, W.W. Christie, & R. Adlof (Eds.), *Advances in Conjugated Linoleic Acid Research*, Vol. 2., pp. 189–217. Champaign, IL, USA: AOCS Press.

Pfeuffer, M., & Schrezenmeir, J. (2006). Impact of *trans* fatty acids of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk. *International Dairy Journal*, 16, pp. 1383-1388.

Pikul J., Leszczynski D.E., Kummerow F.A. (1983). Elimination of sample autoxidation by butylated hydroxytoluene additions before thiobarbituric acid assay for malonaldehyde in fat from chicken meat. *J. Agric. Food Chem.,* 31, pp. 1338-1342.

.

Pikul J., Leszczynski D.E., Kummerow F.A. (1989). Evaluation of three modified TBA method for measuring lipid oxidation in chiken meat. *J. Agric. Food Chem.,* 37, pp. 1309-1313.

Pizzoferrato L. & Nicoli S. (1994) Caratterizzazione della componente lipidica di diete di riferimento. In: *Agrobiotecnologie nei processi di valorizzazione dei prodotti e sottoprodotti agricoli. R.A.I.S.A. Sottoprogetto 4*. vol II, Consiglio Nazionale delle Ricerche, Roma.

Polidori P., Ortenzi A., Lebboroni G. (2009). Meat quality of Fabrianese heavy lambs. *Italian Journal of Animal Science*, vol. 8 (Suppl. 2), 572.

Porter, N. A., Caldwell, S. E., Mills, K. A. (1995). Mechanims of free radical oxidation of unsaturated lipids. *Lipids*. 30 (4), pp. 277-290.

Pradhan, A.A., Rhee, K.S., & Hernández, P. (2000). Stability of catalase and its potential role in lipid oxidation in meat. Meat Science. 54, pp. 385−390.

Prates, J.A.M., & Bessa, R.J.B. (2009). *Trans* and *n*-3 fatty acids. In Nollet, L.M.L., & Toldrá, F. (Eds.), *Handbook of Muscle Foods Analysis*, Vol. 1., pp. 399-417. Boca Raton (Fl, EUA): CRC Press − Member of Francis & Taylor Group.

Priolo, A., Micol, D.; Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research.* 50, pp. 185–200.

Pryor WA, Stanley JP. (1975). A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation (Letter). *J. Org. Chem.,* 40, pp. 3615-3617.

Raes, K., Balcan, A., Dirinck, P., Winne, A. De Clayes, E., Demeyer, D., De Smet, S. (2003). Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat Science*. 65, pp. 1237-1246

Raes, K., De Smet, S., & Demeyer, D. (2004)a. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. *Animal Feed Science and Technology*, 113, pp. 119–221.

.

Raes K., Haak L., Balcaen A., Claeys E., Demeyer D., De Smet S. (2004)b. Effect of linseed feeding at similar linoleic acid levels on the fatty acid composition of double-muscled Belgian Blue Young bulls. *Meat Science*, 66, pp. 307-315.

Raharjo S., Sofos J. N., Schimdt G. R. (1992). Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C18 method for measuring lipid peroxidation in beef. *J. Agric. Food Chem.,* 40, pp. 2182-2185.

Ramamutri K. (1986). Effect of cooking, freezing, and frozen storage on the fatty acid profile pattern of ground beef. Lebensmittel-Wissenchaftund-Technologie, 19 (6), pp. 477-481.

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 acids composition, and quality of Uruguayan beef. *Meat Science,* 66, pp. 567-577.

Rhee, K.S. (2000), Fatty acids in meats and meat products, in Chow, C.K. (Ed.), *Fatty Acids inFoods and their Health Implications*, Marcel Dekker, New York, NY.

Rissanen, T., Voutilainen, S., Nyyssönen, K., Lakka, T.A., & Salonen, J.T. (2000). Fishoil derived fatty acids, docosahexaenoic acid and docosapentaenoic acid, and the risk of acute coronary events: The Kuopio Ischaemic Heart Disease Risk Factor Study. *Circulation*, 102, pp. 2677–2679.

Rodriguez-Estrada M.T., Penazzi G., Caboni M.F., Bertacco G. & Lercker G. (1997). Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Science*, 45 (3), pp. 365-375.

Rule D.C., Smith S.B., Romans J.R., (1995). Fatty acid composition of muscle and adipose tissue of meat animals. In: S.B. Smith and D.R. Smith (Eds.), *The biology of fat in meat animals*. Champaign, Illinois, 144-165.

Ryder J.W., Portocarrero C.P., Song X.M., et al. (2001). Isomer-specific antidiabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. *Diabetes*, 50, pp. 1149–1157.

.

Saghir S., Wagner K.-H., Elmadfa I. (2005). Lipid oxidation of beef fillets during braising with different cooking oil. *Meat Science*, 71, pp. 440-445.

Sanders T.A.B. (1989). Nutritional aspects of rancidity. In: *Rancidity in Foods* (eds. J.C. Allen, R.J. Hamilton) Elservier, London, pp. 125-139.

Santos-Silva, J., Bessa, R.J., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock*, 77, pp. 187-194.

Sañudo, C., Alfonso, M., San Juli´an, R., Thorkelsson, G., Valdimarsdottir, T., Zygoyiannis, D., Stamataris, C., Piasentier, E., Mills, C., Berge, P., Dransfield, E., Nute, G.R., Enser, M., Fisher, A.V., 2006. Regional variation in the hedonic evaluation of lamb meat from diverse production systems by consumers in six European countries. *Meat Science*, 75, pp. 610–621.

Sañudo C., Sanchez A., Alfonso M. (1998): Small ruminant production systems and factors affecting lamb meat quality. *Meat Science*, 49, pp. S29‑S64.

Sante´-Lhoutellier V.,. Engel E, Gatellier Ph. (2008). Assessment of the influence of diet on lamb meat oxidation. *Food Chemistry*, 109, pp. 573–579

Sarriés M.V., Murray B.E., Moloney A.P., Troy D., Beriain M.J. (2009). The effect of cooking on the fatty acid composition of longissimus muscle from beef heifers fed rations designed to increase the concentration of conjugated linoleic acid in tissue. *Meat Science*, 81, pp. 307-312.

Scheeder M.R.L., Casutt M.M., Roulin M., Escher F., Dufey P.A. and Kreuzer, M. (2001). Fatty acid composition, cooking loss and texture of beef patties from meat of bulls fed different fats. *Meat Science*, 58, pp. 321–328.

Schieberle, P. and Grosch, W. (1981). Detection of monohydroperoxides with unconjugated diene systems as minor products of the autoxidation of methyl linoleate. *Z. Lebensm. Unters. Forsch*. 173, pp. 199-203.

Schroepfer G.J. (2000). Oxysterols: Modulators of cholesterol metabolism and other process. *Physiol Rev.* 80, pp. 361-563.

.

Schönfeldt, H.C., & Gibson, N. (2008). Changes in the nutrient quality of meat in an obesity context: review. *Meat Science*, 80pp. 0-27.

Scollan, N.D,; Enser, M., Gulati, S.K., Richarson, R.I., Wood, J.D. (2003). Effects of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle. British Journal of Nutrition 90, pp. 709-716

Shahidi,F. (2000). Antioxidants in food and food antioxidants. *Nahrung.* 44, Nr. 3,pp. 158-163.

Shahidi F., Wanasundara U.N., in C. C. (2002). Akoh and D. B. Min, eds., *Food Lipids: Chemistry, Nutrition and Biotechnology*, Marcel Dekker, Inc., New York, pp. 465-487.

Sharma R., Yang Y., Sharma A., Awasthi S., Awasthi Y. (2004). Antioxidant role of glutathione S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. *Antioxid. Redox Signal* 6 (2),pp. 289–300.

Shi, B. and Spallholz, J.E. (1994) Selenium Peroxidase is Highly Available as Assessed by Liver Glutathione Peroxidase Activity and Tissue Selenium. *British Journal of Nutrition* 72,pp. 873-881

Sierra, V., Aldai, N., Castro, P., Osoro, K., Coto-Montes, A., & Oliván, M. 2008. Prediction of the fatty acid composition of beef by near infrared transmittance spectroscopy. Meat Science, 78,pp. 248-255.

Simopoulos A.P. (1991). Omega-3 fatty acids in health and disease and in growth and delelopment. *Am. J. Clin. Nutr.*, 54,pp. 438-63.

Simopoulos A.P. (1996). Omega-3 fatty acids. Part II: Epidemiological aspects of omega-3 fatty acids in disease states. In Spiller (Ed.): *Handbook of lipids in human nutrition*, pp. 75-89. Boca Raton, FL: CRC Press.

Simopoulos A.P. (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition.* 21 (6), pp. 495-505.

Simopoulos A.P. (2003). Importance of the ratio of omega-6/omega-3 essential fatty acids: Evolutionary aspects. *World Review of Nutrition Diet*. 92, pp. 1-22.

.

Sinnhuber R.O., Yu I.C., Yu T.C. (1958). Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. *Food Res.,* 23, pp. 624-634.

Sinnhuber R.O., Yu T.C. (1958) 2-thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. *Food Tech*., 12,pp. 9-12.

Smith, W.L. (2007). Nutritionally essential fatty acids and biologically indispensable cyclooxigenases. *Trends in Biochemical Sciences*, 33(1),pp. 27-37.

Speedy, A.W. (2003). Global production and consumption of animal source foods. *Journal of Nutrition*, 133, pp. 4048S–4053S.

St. Angelo A.J. (1996). Lipid oxidation in foods. *CRC Crit. Rev. Food Sci. Nutr*., 36, pp. 75-224.

Steen, R.W.J., Lavery, N.P.; Kilpatrick, D.J. (2003). Effects of pasture and high concentrate diets on the performance of beef cattle, carcass composition at equal growth rates, and fatty acid composition of beef. *N. Z. J. of Agri. Res.* pp. 46 69 - 81

Sundram K., Ismail A., Hayes K.C., Jeyamalar R. & Pathmanathan R. (1997). Trans (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. *Journal of Nutrition*, 127(3), pp. 514S–520S.

Tang S., Kerry J.P., Sheehan D., Buckley D.J. & Morrissey P.A. (2001). Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish to lipid oxidation. *Food Research International*, 34,pp. 651−657.

Tappel A.L. (1961). Biocatalysts: lipoxydase and hematin compounds. In: *Lundberg W.O. (Ed)*. *Autoxidation and Antioxidants*, Vol.1, 325.

Tappel A.L. (1963). Lipoxigenase. In: *Enzymes*. Boyer P.D., Lardy H., Myrback K. (eds); 2nd ed.Academic Press: NY. Vol B., 275.

Tarladgis B.G., Watts B.M. (1960). Malonaldehyde production during the controlled oxidation of pure, unsaturated fatty acids. *J. Am. Oil Chem. Soc.,* 37, pp. 403-406.

.

Tarladgis B.G., Watts B.M. Younathan M.T., Dugan L.Jr. (1960). A destellation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.,* 37, pp. 44-48.

Thomas M.J. (1995). The role of free radicals and antioxidants: how do we know that they are working? *Crit. Rev. Food Sci. Nutr.* 35 (1/2), pp. 21-39.

Tims M.J., Watts B.M. (1958). Protection of cooked meats with phosphates. *Food Technol.*, 12 (5), pp. 240-243.

Turrini A., Saba A., Perrone D., Cialfa E. and D'Amicis A. (2001). Original Communication Food consumption patterns in Italy: the INN-CA Study 1994-1996. *European Journal of Clinical Nutrition*. 55,pp. 571-588

Uchida K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radic Biol Med.*, 28,pp. 1685-96.

Ulbricht, T.L.V., Southgate, D.A.T. (1991). Coronary heart disease: seven dietary factors. *Lancet*, 338, pp. 985-992.

U.S. Food and Drug Administration. *The Food Label*; FDA Backgrounded, p.12, available at http://www.cfsan.fda.gov/~dms/fdnewlab.htlm.

USDA. U.S. *Department of Agriculture Nutrient Database for Standard Reference*, release 15; Nutrient Data Laboratory, 2002; available at http://www.nal.usda.gov/fnic/foodcomp/Data/SR15/sr15htlm, accessed Aug 20, 2002.

USDA. U.S. *Department of Agriculture Nutrient Database for Standard Reference*, release 1.0; USDA Nutrient Data Set for Retail Beef Cuts, 2009; available at http//www.ars.usda.gov/nutrientdata.

Wąsowicz E., Gramza A., Hęś M., Jeleń H.H., Korczak J., Malecka M., Mildner-Szkudlarz M., Rudzińska M., Samotyja U., Zawirska-Wojtasiak R. (2004). Oxidation of lipids in food. *Pol. J. Food Nutr. Sci.,* 13/54, SI 1, pp. 87-100.

Vacca G.M., Carcangiu V., Dettori M.L., Pazzola M., Mura M.C., Luridiana S., Tilloca G. (2008). Productive performance and meat quality of Mouflon x Sarda and Sarda x Sarda suckling lambs. *Meat Science*, 80, pp. 326–334.

.

Varela, A., Oliete, B., Moreno, T., Portela, C.; Monserrat, L., Carballo, J.A., Sanchez, L. (2004). Effect of pasture finishing on meat characteristics and intramuscular fatty acid profile of steers of the Rubia Gallega breed. *Meat Science,* 67, pp. 515-522

Velik M., Steinwidder A., Frickh J.J., Ibi G., Kolbe-Romer A. (2008). Effect of ration, sex and breed on carcass performance and meat quality of cattle from suckler cow systems. *Zuchtungskunde*, 80, pp. 378-388.

Verbeke, W., Frewer, L. J., Scholderer, J., & De Brabander, H. F. (2007). Why consumers behave as they do with respect to food safety and risk information. *Analytica Chimica Acta*, 586, pp. 2–7.

Vergara, H., Molina, A., Gallego, L., (1999). Influence of sex and slaughter weight on carcass and meat quality in light and medium weight lambs produced in intensive systems. *Meat Science,* 52, pp. 221–226.

Vincenti A., Colonna M.A., Ragni M., Toteda F. (2004). Effect of type of suckling and polyunsaturated fatty acid use on lamb production. 2. Chemical and fatty composition of raw and cooked meat. Italian Journal of Animal Science. 3, pp. 81-91.

World Health Organization (WHO). (2003). Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consultation. *WHO technical report series* 916, Geneva.

Willett, W.C. 2006. *Trans* fatty acids and cardiovascular disease – epidemiological data. *Atherosclerosis Supplements*, 7, pp. 5-8.

Willett W. C., Sacks F., Trichopoulou G., Drescher G., Ferro-Luzzi A., Helsing E., et al. (1995). Mediterranean diet pyramid: A cultural model for healthy eating. *American Journal of Clinical Nutrition*, 61, pp. 1402 S–1406S.

Williams, P.G, Nutritional composition of red meat. (2007). *Nutrition & Dietetics*. 64 (Suppl. 4), pp. 113-119.

Williamson C.S., Foster, R.K., Stanner, S.A., & Buttriss, J.L. (2005). Red meat in the diet: review. *Nutrition Bulletin*, 30, pp. 323-355.

.

Whitaker J. R. (1994). Principles of enzymology for the food sciences (second edition). Marcel Dekker, Inc. 27, pp. 579-589.

Wood J.D., & Enser M. (1997). Factors influencing fatty acids in meat and the role of anti-oxidants in improving meat quality. *British Journal of Nutrition*, 78, pp. S49–S60.

Wood J.D. Richardson R.I. Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., et al. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, 66 (1), pp. 21-32.

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

Wongtangtintharn, S., Oku, H., Iwasaki, H., & Toda, T. (2004). Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. *Journal of Nutritional Science and Vitaminology,* 50, pp. 137−143.

Wong, D.W.S. (1989). Lipids. In: *Mechanism and Theory in Food Chemistry*; Van Nostrand Reinhold: New York. pp 1-47.

World Cancer Research Fund/American Institute for Cancer Research. (1997). *Food, nutrition, physical activity and the prevention of cancer: A global perspective.* Washington, DC: American Institute for Cancer Research.

World Cancer Research Fund/American Institute for Cancer Research. (2007). *Food, nutrition and the prevention of cancer: A global perspective.* Washington, DC: American Institute for Cancer Research.

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

Yang P.Y., Collin P:, Madden T., Chan D., Sweeney-Gotsch B., McConkey D. et al (2003). Inhibition of proliferation of PC3 cellsby the branched-chain fatty acid, 12-methyltetradecanoic acid, is associated with inhibition of 5-lipoxygenase. *Prostate*, 55, pp. 281-291.

.

Yang Z.H., Liu S.P., Chen H., Huang M., Zheng J.P. (2000). Induction of apoptotic cell death and in vivo growth inhibition of human cancer cells by saturated branched-chain fatty acid, 13-methiltetradecanoic acid. *Cancer Research*, 60, pp. 505-509.

Yu Y, Correll PH, Vanden Heuvel JP. (2002). Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. *Biochim. Biophys. Acta*; 1581, pp. 89– 99.

Yu S., Derr J., Etherton T. D., & Kris-Etherton P. M. (1995). Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monosaturated fatty acids are hypocholesterolemic. *American Journal of Clinical Nutrition*, 61, pp. 1129–1139.

Zelko I., Mariani T., Folz R. (2002). Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med*., 33 (3), pp. 337–349.