“The only time you’ll find succes before work is in the dictionary”

(J. W. Pence)

“Een wetenschapper is een bijzondere vogel: eerst broedt hij en vervolgens legt hij zijn ei”

(Ferwerda)

“Le savant n'est pas l'homme qui fournit les vraies réponses; c'est celui qui pose les vraies questions"

(C. Lévi-Strauss)

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IMPACT OF ANIMAL DIET AND MEAT PREPARATION ON FATTY ACID COMPOSITION AND OXIDATIVE STABILITY OF PORK

Thesis submitted in fulfillment of the requirements for the degree of
Doctor (PhD) in Applied Biological Sciences

Dutch translation of the title:

Invloed van voedersamenstelling en vleesbereiding op de vetzuursamenstelling en de oxidatieve stabiliteit van varkensvlees

Haak L. 2007. Impact of animal diet and meat preparation on fatty acid composition and oxidative stability of pork. PhD dissertation, Ghent University, Ghent, 228p.

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NOTATION INDEX

AA	arachidonic acid (C20:4n-6)
AA	ascorbic acid
AF	animal fat
AP	ascorbyl palmitate
AT	α-tocopherol
ATA	α-tocopheryl acetate
BCFA	branched-chain fatty acids
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
CA	carnosic acid
CAT	catalase
CCH	cooked cured ham
CLA	conjugated linoleic acid
CVD	cardiovascular disease
DCH	dry cured ham
DHA	docosahexaenoic acid (C22:6n-3)
DPA	docosapentaenoic acid (C22:5n-3)
EC	epicatechin
ECG	epicatechin gallate
EDTA	ethylenediaminetetracetic acid
EGC	epigallocatechin
EGCG	epigallocatechin gallate
EPA	eicosapentaenoic acid (C20:5n-3)
FAME	fatty acid methyl ester
FO	fish oil
FS	fermented sausage
G	gallic acid
GSH	glutathione
GSH-Px	glutathione peroxidase
HDL	high density lipoprotein
IMFA	intramuscular fatty acid

IV	iodine value
KCl	potassium chloride
LA	linoleic acid
LDL	low density lipoprotein
LO	linseed oil
LT	longissimus thoracis
Mb	deoxymyoglobin
MDA	malondialdehyde
MetMb	metmyoglobin
MUFA	monounsaturated fatty acids
NaCl	sodium chloride
NO	nitric oxide
OxyMb	oxymyoglobin
PG	propyl gallate
PL	phospholipids
POV	peroxide value
PUFA	polyunsaturated fatty acids
R/RE	rosemary extract
ROS	reactive oxygen species
SD	standard deviation
SFA	saturated fatty acids
SH	thiol
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
TBHQ	tert-butylhydroquinone
TE	tea extract
TG	triacylglycerols
TRC	total reducing capacity
UV	ultraviolet light
WOF	warmed-over flavour
α-LNA	α-linolenic acid
γ-LNA	γ-linolenic acid (C18:3n-6)

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INTRODUCTION

INTRODUCTION

Over recent years, food production from farm animals evolved from producer-driven to consumer-oriented. Interest in meeting consumers' concern for food quality and health has increased, and food has become the primary vehicle along the road to optimal health and wellness. Formerly, the formulation of farm animal diets was mainly based on meeting the animal's requirements for energy and protein to optimize growth performance and carcass lean content, and on supplying sufficient minerals and vitamins to prevent deficiency symptoms. Apart from this, current nutritional strategies focus on further improving meat quality. These strategies have emphasized the alteration of the nutritional profile and the improved oxidative stability of meat. Hereby, an increased content of health-promoting long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) and a reduced oxidation in the animal tissues and products by dietary antioxidant supplementation are two main issues. Oxidation processes are one of the major causes of deterioration in food for human consumption: besides reduced sensory attributes, they are responsible for losses in texture, appearance and nutritional value.

The animal's diet represents an effective technology to alter the nutritional and oxidative quality of muscle foods (especially for intact roasts and steaks where *post mortem* use of additives is difficult). In addition, since product composition is altered biologically, it is more label-friendly as no declarations of additives are required. Moreover, this approach provides a possible way of realizing added value for the producer. In some cases, animal nutritional approaches to improve the oxidative stability of muscle foods are more effective than *post mortem* addition of antioxidants (e.g. for α-tocopherol that is preferentially deposited in the lipid membrane systems where it is most needed). However, in non-intact muscle foods, the cost-benefit ratio of alternative approaches (*i.e.* by direct addition to the food matrix or by dietary means) for enhancing specific nutrients should be analysed case by case. The effect of culinary processing (e.g. pan-frying) is another factor with a considerable impact on the final composition and quality of a food item.

In this PhD research, nutritional strategies to improve the fatty acid profile of pork were investigated. Besides, the effect of pan-frying in different culinary fats on the fatty acid profile of pork, as it is consumed, was tested. Since it is extremely important to maintain an acceptable oxidative stability at the same time, the effect of dietary supplementation or *post mortem* addition of antioxidants or antioxidant cocktails was tested.

In chapter 1, strategies to alter the fatty acid profile of pork are discussed. Also, a general overview of oxidation processes and their determinants in meat is given. However, a detailed description of the chemistry of oxidation is out of scope of this dissertation.

In chapter 2A, an experiment in which the fatty acid profile of pork was altered by dietary means is described. Simultaneously with a change in the fatty acid profile, an altered oxidative status of the meat (products) was expected and therefore, the influence on consumer acceptance of this enriched pork was investigated. In chapter 2B, the alteration of the pork fatty acid composition by culinary practice (pan-frying) was assessed. As the oxidative status of meat depends on the balance of antioxidants and prooxidants, an improved oxidative stability can be obtained by increasing antioxidant levels in the meat. This can be achieved either by dietary delivery (chapter 3A) or by *post mortem* addition of antioxidants during processing (chapter 3B). In chapter 4, some general conclusions on the experiments are made and the future prospects to improve the nutritional and sensory quality of pork are discussed. Also, a proposal is done to estimate the health impact of n-3 enriched products of non-ruminant origin.

Overview of the different chapters

Chapter 1. Dietary approaches to improve pork quality

Chapter 2. Alteration of pork fatty acid profile

2A. Improving the pork fatty acid profile by dietary strategies

Redrafted after part of Haak, L., De Smet, S., Fremaut, D., Van Walleghem, K., and Raes, K. (2007). Fatty acid profile and taste panel evaluation of pork as influenced by duration and time of dietary linseed or fish oil supplementation. Journal of Animal Science (revised version submitted).

2B. Alteration of pork fatty acid profile by culinary practice (pan-frying)

Redrafted after Haak, L., Sioen, I., Raes, K., Van Camp, J., and De Smet, S. (2007). Effect of pan-frying in different culinary fats on the fatty acid profile of pork. Food Chemistry, 102, 857-864.

Chapter 3. Effect of antioxidant supplementation on the oxidative stability of pork

3A. Effect of dietary antioxidant supplementation on the oxidative stability of pork

Redrafted after Haak, L., Raes, K., Smet, K., Claeys, E., Paelinck, H., and De Smet, S. (2006). Effect of dietary antioxidant and fatty acid supply on the oxidative stability of fresh and cooked pork. Meat Science, 74, 476-486.

and after Haak, L., Raes, K., Van Dyck, S., and De Smet, S. (2007). Effect of dietary rosemary and alpha-tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. Meat Science (accepted).

3B. Effect of exogenous antioxidant addition on the oxidative stability of pork

Redrafted after Haak, L., Raes, K., and De Smet, S. (2007). Effect of plant phenolics, tocopherol and ascorbic acid on oxidative stability of pork patties. (in preparation).

Chapter 4. General discussion and future prospects

CHAPTER 1

DIETARY APPROACHES TO IMPROVE PORK QUALITY

CHAPTER 1

DIETARY APPROACHES TO IMPROVE PORK QUALITY

1. MEAT QUALITY

The concept 'meat quality' is multi-factorial in nature and is comprised of many complementary attributes. The criteria consumers associate with the quality of meat are nutritional value, wholesomeness, freshness, leanness, juiciness, taste, and tenderness (Grunert, 1997). In this dissertation, the following quality attributes are dealt with: wholesomeness (fatty acid composition as a parameter of the health value) and sensory quality (shelf-life, flavour, and colour). Attributes such as process characteristics, safety, convenience, environmental concern, and animal welfare are outside the scope here. Consumers are increasingly quality-orientated and an understanding of the factors that influence product quality is a prerequisite to produce high quality pork to meet market requirements. The possibilities to improve intrinsic pork quality attributes are numerous and pertain to nutrition, selection, pre- or peri-slaughter handling of the pig, and post-slaughter handling of the carcass. In this thesis, the impact of two aspects of pork nutrition - fatty acids and antioxidants - on pork quality is investigated. Moreover, since meat is subject to preparation before consumption, the impact of *post mortem* processing must be taken into account. Here, the effect of pan-frying in culinary fat and *post mortem* addition of antioxidants is studied.

2. IMPROVED FATTY ACID PROFILE OF PORK

2.1 Fatty acids: classification, nomenclature and sources

Intramuscular lipids are composed of polar lipids, mainly phospholipids (PL) located in the cell membranes, and neutral lipids consisting mainly of triacylglycerols (TG) in the adipocytes.

The intramuscular lipid fraction, and more specifically its fatty acid composition, is highly important from a nutritional point of view. Fatty acids consist of the elements carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxyl group (-COOH) at one end. Fatty acids are subdivided according to their chain-length and the number of double bonds.

2.1.1 Fatty acid classification

Subdivision according to chain length and branching

Most fatty acids have an even number of carbon atoms. Chain lengths range from 2 to 80 carbon atoms but more commonly from 12 to 24. Fatty acids with a chain length from 2 to (4 or) 6 are called short-chain, from (6 or) 8 to 10 medium-chain, and from 12 to 24 long-chain fatty acids. Odd-chain fatty acids are quantitavely less common in nature and are either linear or branched. Branched-chain fatty acids (BCFA) are usually mono-methyl substituted fatty acids. They usually have either an iso-structure (methyl group at the penultimate carbon atom counting from the terminal methyl group) or an anteiso-structure (methyl group on the third carbon from the end) (Figure 1.1). BCFA have a lower melting temperature as compared to the corresponding linear fatty acids because of the hindrance by the side groups.

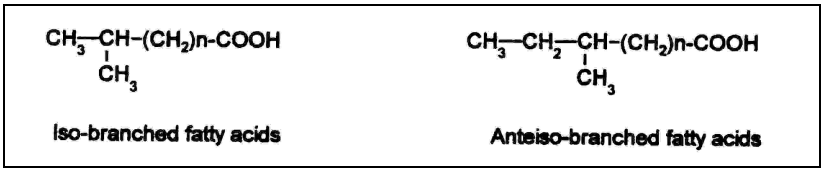

Figure 1.1. The structure of iso and anteiso fatty acids

Subdivision according to number of double bonds

Saturated fatty acids (SFA)

SFA do not contain any double bonds along the chain, and are therefore "saturated" with hydrogen. This means that all carbons (apart from the carboxylic acid group [-COOH]) contain as many hydrogens as possible, *i.e.* the methyl end contains 3 hydrogens (CH_3-) and each carbon within the chain contains 2 hydrogens (CH_2-). They have the general formula: $CH_3(CH_2)_nCOOH$ (Figure 1.2). As a result of the straight chain, SFA can be packed together very tightly, allowing living organisms to store chemical energy very densely.

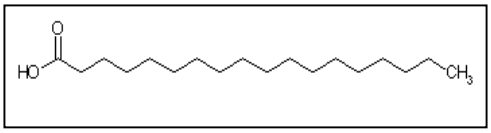

Figure 1.2. The structure of a saturated fatty acid (C18:0)

Unsaturated fatty acids

When double bonds are present, fatty acids are said to be unsaturated. The two carbon atoms in the chain bound to either side of the double bond can occur in a *cis* (Z) or *trans* (E) configuration (Figure 1.3).

In a *cis* configuration, the hydrogens on the two carbons of the double bond are on the same side of the bond. The double bond causes the chain to bend and restricts the conformational freedom of the fatty acid. The more double bonds in the *cis* configuration, the less flexible and the more bent the chain is. When fatty acids are part of a PL bilayer, or of TG in lipid droplets, *cis* bonds limit their ability to be closely packed and consequently affect the melting temperature of the membrane or the fat.

In a *trans* configuration, by contrast, the next two hydrogen atoms are bound to opposite sides of the double bond. As a result, *trans* bonds do not cause the chain to bend much, and their shape and physical properties are similar to straight SFA.

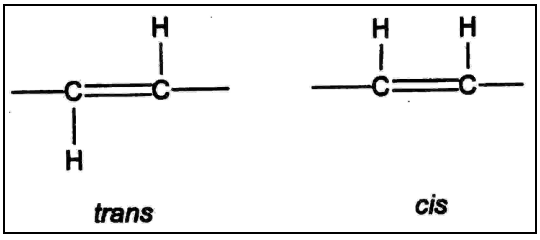

Figure 1.3. *Cis* and *trans* configuration of unsaturated fatty acids

In most naturally occurring unsaturated fatty acids, double bonds are in the *cis* configuration. After all, most desaturases involved in the biosynthesis of unsaturated fatty acids in humans and mammals, exclusively produce *cis* double bonds (Ledoux et al., 1999). *Trans* fatty acids can be the result of processing (e.g. hydrogenation of oils and fats and oil deodorisation).

Unsaturated fatty acids can be divided in two groups: monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). MUFA contain only one double bond (Figure 1.4).

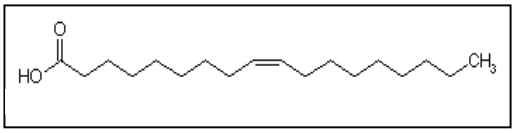

Figure 1.4. The structure of a monounsaturated fatty acid (c9 C18:1)

PUFA have 2 to 6 double bonds, generally in the *cis* configuration (Figure 1.5). The double bonds are generally separated by a single methylene group (methylene-interrupted unsaturation). Some uncommon PUFA have two adjacent double bonds separated by more than one methylene group, and are named polymethylene-interrupted fatty acids.

Conjugated linoleic acid (CLA) refers to a family of many isomers of linoleic acid (LA) (at least 13 are reported), in which the double bonds are conjugated, *i.e.* there is no saturated carbon between two adjacent double bonds. They are primarily found in ruminant products where they are produced by micro-organisms in the fore-stomach. Some isomers of CLA are also produced in the intermediary animal metabolism from non-conjugated fatty acids. One such example is vaccenic acid (t11 C18:1), which is converted to c9t11 CLA by Δ-9-desaturase activity. The most important CLA isomers are the c9t11 and the t10c12 isomer.

In mammals, desaturases only introduce new double bonds between C10 and the carboxyl group. In plants, however, new double bonds can be introduced towards the methyl end. This gives rise to 3 important PUFA families: the n-9, n-6 and n-3 families with their respective precursor fatty acids: oleic acid, linoleic acid and α-linolenic acid (α-LNA).

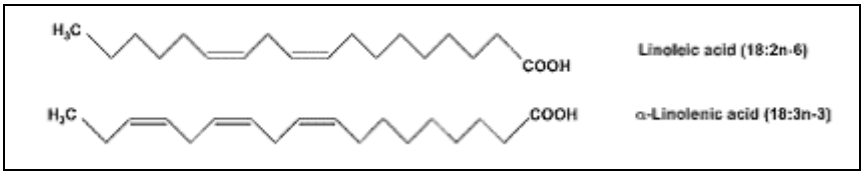

Figure 1.5. Structures of linoleic and α-linolenic acid, the simplest n-6 and n-3 polyunsaturated fatty acids

2.1.2 Fatty acid nomenclature

Fatty acids are most commonly expressed by their trivial names e.g. 'linoleic acid'.The shorthand nomenclature of fatty acids include the number of carbon atoms and double bonds (e.g. C18:1 or 18:1). Each double bond can be either in a *cis*- or *trans*- conformation and in a different position with respect to the ends of the fatty acid. In the shorthand nomenclature, there are two different ways to make clear where the double bonds are located within the hydrocarbon chain:

- *delta*-x or Δx: the double bond is located on the *x*th carbon-carbon bond, counting from the carboxyl terminus. In case of a molecule having more than one double bond, the notation is, for example Δ9,Δ12.
- *omega*-x or ω-x or n-x: A double bond is located on the xth carbon-carbon bond, counting from the ω end (methyl end), determining the metabolic family.

Thus, linoleic acid is named in the shorthand nomenclature C18:2n-6 or C18:2ω6. This component has 18 carbon atoms, 2 double bonds and 6 carbon atoms from the last double bond to the terminal methyl group.

According to the International Union of Pure and Applied Chemists (IUPAC) terminology, straight-chain fatty acids are named after the parent hydrocarbon while the final '-e' is changed to '-oic'. The exact structure is given by their systematic name '*cis*-9, *cis*-12-octadecadienoic acid'.

2.1.3 Dietary fatty acid sources

Short-chain SFA (C4-C10) are mainly found in milk fat, whereas long-chain SFA (>C12) are most common in animal fats and plant oils. SFA with more than 18 carbons are less common.

Odd-chain fatty acids are synthesised by many micro-organisms and are produced to a lesser degree in animal tissues when propionic acid is the precursor for fatty acid synthesis (Christie, 1989; Chesworth et al., 1998). BCFA are typical for most G^+ and some G^- micro-organisms where they account for 15 to 20% of fatty acids (O'Kelly and Spiers, 1991). These BCFA are found in ruminant products as a result of the digestion and absorption of lipids from rumen micro-organisms during the duodenal passage. However, BCFA do not (or very little) occur in products of monogastric animals.

Because LA and α-LNA are synthesized by plants, plant tissues and oils tend to be good sources of these fatty acids. For example, green plant tissues are especially rich in α-LNA acid, which typically comprises approximately 55% of the fatty acids present. However, these tissues are not rich in fat, therefore limiting their potential contribution to the intake of these fatty acids in humans. In contrast, plant oils used in cooking (e.g. corn oil, sunflower oil and rapeseed oil) and margarines made from such oils make a significant contribution to the intakes of these fatty acids. Most plant oils are much richer in LA than α-LNA and so intake of the former is much greater in most Western populations. Animal feed sources rich in α-LNA include vegetable oils obtained from flaxseed or linseed, walnuts, and soybean or canola.

The long-chain PUFA however, are consumed in relatively small amounts in most Western diets. The main dietary source of arachidonic acid (AA; C20:4n-6) is meat. A good dietary source of long-chain n-3 PUFA (eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) is fish (oil) or marine algae. Fish can be classified into lean fish that store fat as TG in the liver (e.g. cod) or 'fatty' ('oily') fish that store fat as TG in the flesh (e.g. mackerel, herring, salmon and tuna). Different oily fish (and thus different fish oils) contain different amounts of long-chain n-3 PUFA. In the absence of fatty fish or fish oil consumption, α-LNA is the by far the major dietary n-3 fatty acid. Recent studies have revealed that conversion of α-LNA into its long-chain derivatives is not at all efficient in adult humans, especially males (see further). This means that consuming the long-

chain derivatives themselves (*i.e.* EPA and DHA), is by far the easiest way to increase their amounts in human tissues.

2.2 Nutritional and health value of fatty acids

2.2.1 Nutritional value of fatty acids

Fatty acids provide a highly efficient energy storage, storing double energy for their weight as compared to carbohydrates and proteins (37.7 vs. 16.7 kJ/g respectively). The animal body can produce all but two of the fatty acids it needs. These two, LA and α-LNA, must be supplied in the food and are called essential fatty acids (EFA). In the body, these EFA are the parent components of the n-6 and n-3 fatty acid series respectively. Their long-chain metabolites are primarily used to produce hormone-like substances that regulate a wide range of functions (see below).

It is now evident that *in vivo* synthesis of EPA and DHA from dietary α-LNA is very limited in adult humans, especially in men (Burdge et al., 2003) leading to the concept that these n-3 PUFA should be classified as semi-essential.

2.2.2 Health value of fatty acids

Industrialised countries and increasingly also developing countries are confronted with a large incidence of several chronic diseases, that are at least partly mediated by dietary factors (WHO/FAO, 2003). As the incidence of obesity is increasing worldwide, concern is with excess energy intake and more specifically with total fat intake. Moreover, not only the fat quantity leaves a lot to be desired, also the fat quality in terms of fatty acid composition urges upon the need for changing our eating pattern.

After all, within the scope of disease prevention and health promotion, the fatty acid composition of the diet is of primordial importance (Williams, 2000). The main concerns about dietary fatty acids for human health refer to cardiovascular disease (CVD) and cancer. For both, evidence suggests links with either total or type of fat in the diet (Baghurst, 2004).

Characteristics determining the health value of fatty acids are chain length and amount, position (n-3, n-6, n-9) and geometrical configuration (*cis*, *trans*) of the double bonds and the position of the fatty acids in the lipid molecule (sn-position).

Saturated fatty acids

The cholesterol increasing effect of fatty acids in the diet was said to be mainly attributed to lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) (as well for total cholesterol as for the LDL-cholesterol fraction (Low Density Lipoprotein)). Fats, however, also affect HDL-cholesterol (High Density Lipoprotein), and more recently, the ratio of total to HDL-cholesterol was proposed as a more specific marker of CVD than is LDL cholesterol (Mensink et al., 2003). For lauric acid it was found that it greatly increases total cholesterol, but much of its effect is on HDL cholesterol.

The quantitatively most important SFA, stearic acid (C18:0), neither increases total cholesterol, nor LDL-cholesterol (Williams, 2000). This neutral effect of stearic acid can be ascribed to its fast conversion to oleic acid (c9 C18:1), a fatty acid neutral towards cholesterol (Bonanome and Grundy, 1988) or to its inability to lower LDL receptors on the cel membranes. This is in contrast with other SFA lowering the amount of LDL receptors and thus preventing the uptake of LDL by the cells and consequently increasing its concentration in plasma.

Next to increasing the CVD risk, a high SFA intake is associated with an increased risk of cancer of the colon, prostate or breast (Chow, 1992; Williams, 2000).

Monounsaturated fatty acids

MUFA lower the TG level in the blood and decrease LDL-cholesterol without affecting HDL-cholesterol (Kris-Etherton et al., 1999), thus reducing the risk of CVD.

Trans MUFA, however, negatively influence CVD risk. Hereby, *trans* isomers of palmitoleic acid (c9 C16:1) have a higher impact than those of oleic acid. Concerning the relative harm of the different *trans* isomers of oleic acid (elaïdic acid (t9 C18:1) and vaccenic acid (t11 C18:1)), disagreement remains (Precht and Molkentin, 1995; Ledoux et al., 1999). However, in the recent dietary recommendations for Belgium (Voedingsaanbevelingen voor België, 2006), *trans* MUFA are not counted among MUFA.

Polyunsaturated fatty acids

In the body, LA and α-LNA are the parent components of the n-6 and n-3 fatty acid series respectively. Their pathway of conversion to long-chain fatty acids is described first. Their long-chain metabolites are primarily used to produce hormone-like substances that

regulate a wide range of functions. In this respect, the benefits (and risks) to human health of consuming greater quantities of long-chain n-3 PUFA are summarized further.

PUFA metabolism towards eicosanoid mediators

The simplest members of the n-6 and n-3 fatty acid families are LA and α-LNA respectively. Animals lack Δ12- and Δ15-desaturase to insert an n-6 and n-3 double bond into fatty acids. Hence, LA and α-LNA can only be formed in plants. Although mammalian cells cannot synthesize LA and α-LNA, they can metabolize them by further desaturation and elongation (Figure 1.6). Overall, the efficiency and conversion rate of LA and α-LNA towards EPA and especially DHA is low (less than 10%). After all, high intakes of α-LNA are needed to increase concentrations of C20 and C22 n-3 PUFA (Cunnane et al., 1993; Pawlosky et al., 2003; Burdge and Calder, 2005a,b). This low efficiency and conversion rate of α-LNA in humans, requires the intake of EPA and DHA as such.

LA can be converted into γ-linolenic acid (γ-LNA; C18:3n-6) by Δ6-desaturase and then γ-LNA can be elongated to dihomo-γ-LNA (C20:3n-6). Dihomo-γ-LNA can be desaturated further by Δ5-desaturase to yield AA. Using the same series of enzymes as used to metabolize n-6 PUFA, α-LNA is converted into EPA. In monogastric animals, the pathway of desaturation and elongation occurs mainly in the liver. It is evident from the pathway shown in Figure 1.6 that there is competition between the n-6 and n-3 fatty acid families for metabolism. The Δ6-desaturase reaction is rate limiting in this pathway. In case of comparable amounts of substrates, the metabolism of the n-3 series is preferred to that of the n-6 series (Kinsella et al., 1990; Brenner, 1997). Although the preferred substrate for Δ6-desaturase is α-LNA, the metabolism of n-6 fatty acids is quantitatively the most important because LA is much more prevalent than α-LNA in most human diets. Further conversion of EPA into DHA involves addition of two carbons to form DPA (docosapentaenoic acid; C22:5n-3), two further carbons to produce C24:5n-3 and desaturation at the Δ6 position to form C24:6n-3. Then two carbons are removed from C24:6n-3 by limited β-oxidation to yield DHA. Retroconversion of DHA to EPA is also possible (Sprecher et al., 1995).

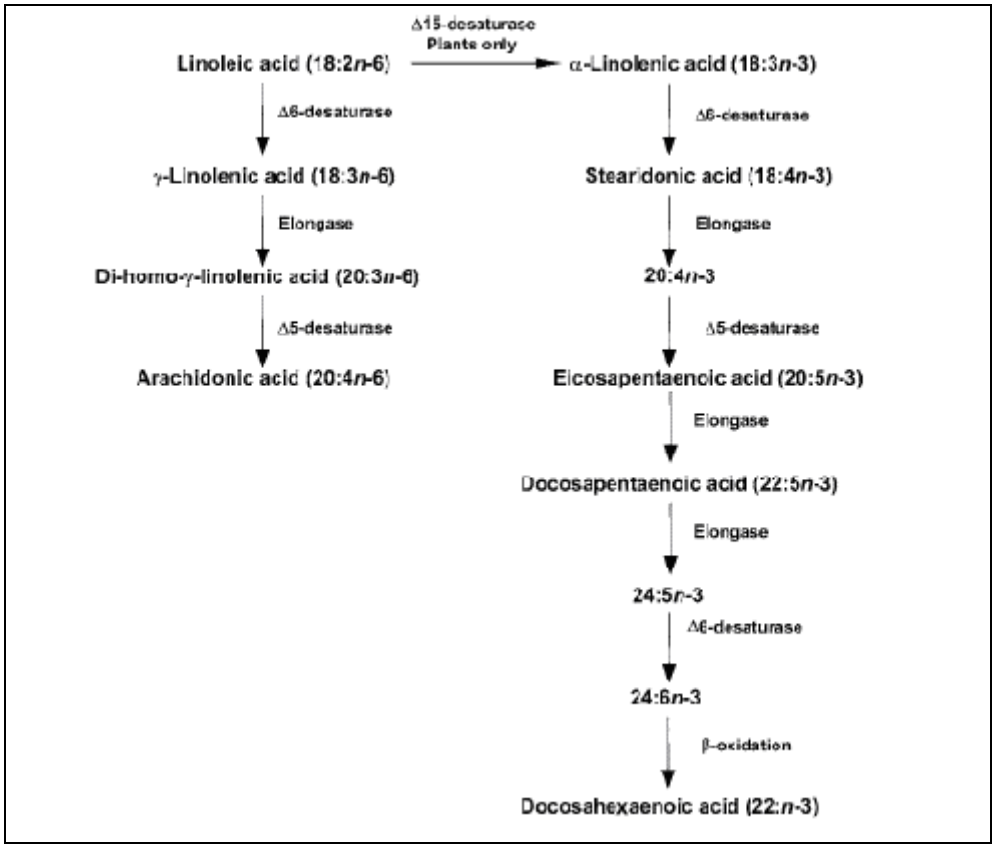

Figure 1.6. Pathway of the conversion of linoleic and α-linolenic acids into their long-chain derivatives (Chesworth et al., 1998)

Health benefits of n-3 PUFA

The long-chain n-3 PUFA, EPA and DHA, exert positive effects on human health showing beneficial cardiovascular, anti-thrombotic, anti-inflammatory and immuno-suppressive properties (see review Narayan et al., 2006). As a result of the robust evidence in their favour, a number of recommendations to increase intake of EPA and DHA have been made.

Lower cardiovascular disease risk (CVD)

The relationship between dietary fat type and intake and the risk of CVD is strong and consistent as seen from a wide body of data (Kris-Etherton et al., 2001, 2002; WHO/FAO, 2003; Von Schacky, 2004). Consumption of fatty fish, fish oils and long-chain n-3 PUFA reduces the risk of CVD, also in secondary prevention studies in patients post-myocardial infarction. The following mechanisms most likely explain the primary and secondary cardiovascular protection afforded by long-chain n-3 PUFA consumption.

Long-chain n-3 PUFA may protect against both the pathological processes leading to CVD (*i.e.* atherosclerosis), and the acute events (e.g. thrombosis followed by myocardial infarction or stroke). They favourably affect a number of factors involved in the development of atherosclerosis. For example, they lower fasting and post-prandial plasma TG concentrations (see reviews e.g. Harris, 1996; Williams, 1997; Roche, 1999) and raise HDL-cholesterol levels (Kromhout et al., 1995; Sorensen et al., 1998; Von Schacky et al., 1999; Lichtenstein and Schwab, 2000; Laidlaw and Holub, 2003). Long-chain n-3 PUFA have been shown to decrease production of chemoattractants, growth factors and adhesion molecules which could down-regulate processes leading to leucocyte and smooth muscle migration into the vessel wall intima. A recent study by Thies et al. (2003) suggests that they might also act to stabilize advanced atherosclerotic plaques, perhaps through decreasing inflammatory processes within the vessel wall (as described below) (Calder, 2003; Yaqoob, 2003), which are now recognized to be a major contributory factor in atherosclerosis (Ross, 1993, 1999; Glass and Witztum, 2001). Since the vulnerability of the plaque to rupture rather than the degree of atherosclerosis is the primary determinant of thrombosis-mediated acute cardiovascular events (Plutzky, 1999), it is likely that these n-3 PUFA decrease thrombosis and thus protect towards both fatal and non-fatal cardiovascular events.

Long-chain n-3 PUFA also have a small, but significant, hypotensive effect (Geleijnse et al., 2002). These fatty acids cause endothelial relaxation and promote vascular compliance (Chin et al., 1993; McVeigh et al., 1994; Goode et al., 1997; Tagawa et al., 1999), which might be related to increased nitric oxide production (Harris et al., 1997).

Finally, modest intake of n-3 PUFA could reduce the risk of primary cardiac arrest by electrical stabilization of myocardial membranes reducing susceptibility to ventricular arrhythmias and consequently risk of sudden death (McLennan, 1993; Leaf and Kang, 1997).

There has been considerable interest in examining whether increasing intake of α-LNA itself would lead to improvements in the risk factors for CVD. High intakes of α-LNA can beneficially affect a number of CVD risk factors including LDL-cholesterol (e.g. Zhao et al., 2004) but it seems likely that, despite the low efficiency of conversion, these effects are mainly due to increased synthesis of EPA. A number of reviews on this topic (e.g. Burdge and Calder, 2005a,b, 2006) conclude that the principal biological role of α-LNA is as a precursor for EPA synthesis.

Brain and neural development

Both long-chain n-3 and n-6 PUFA are important for neural development (especially of the fetus and the newborn) (Innis, 1991; Makrides et al., 1996). There appears to be a high requirement for DHA in the last trimester of pregnancy and the first 3 months of life with the fetus and newborn being dependent on the maternal supply of DHA. Especially in populations that tend to have a lower background intake of long-chain n-3 PUFA, increased maternal long-chain n-3 PUFA intake during pregnancy may produce beneficial effects (Smuts et al., 2003).

An important issue still is the adequacy of α-LNA in infant food as a precursor for DHA, in particular for preterm infants where DHA was found to improve visual acuity and neuro-mental development (Salem et al., 1996).

Anti-inflammatory effects

The key link between long-chain n-3 PUFA and inflammation is that the eicosanoid family of inflammatory mediators is generated from 20 carbon PUFA liberated from cell membrane PL. The membrane PL of inflammatory cells taken from humans consuming Western-type diets typically contain approximately 20% of fatty acids as AA, whereas the proportions of other 20 carbon PUFA such as the n-6 PUFA dihomo-γ-LNA and the n-3 PUFA EPA are typically about 2% and <1% of fatty acids, respectively (Calder, 2006). Thus, AA is usually the dominant substrate for eicosanoid synthesis. Eicosanoids include prostaglandins (PGs) and prostacyclines (PCs), thromboxanes (TXs), leukotrienes (LTs), and hydroxyeicosatetraenoic acids (HETEs).

The AA-derived eicosanoids have inflammatory actions in their own right and also regulate the production of other mediators including inflammatory cytokines.

Increased consumption of EPA and DHA decreases the amount of AA in cell membranes and so available for eicosanoid production. Thus, n-3 PUFA decrease production of AA-derived eicosanoids and increase production of alternative and less potent eicosanoid mediators (Calder, 2006). Although their action in antagonising AA metabolism is a key anti-inflammatory effect of n-3 PUFA, these fatty acids have a number of other anti-inflammatory effects that might occur downstream of altered eicosanoid production or might be independent of this. These fatty acids also decrease the production of the classic pro-inflammatory cytokines by monocytes and T lymphocytes (tumour necrosis factor, interleukin-1, and interleukin-6) and the cell surface expression of adhesion molecules involved in inflammatory

interactions between leukocytes and endothelial cells. This interaction leads to leukocyte infiltration into sites of inflammatory activity. Recent studies have identified a novel group of mediators, termed E-series resolvins, formed from EPA that appear to exert anti-inflammatory actions (Serhan et al., 2000).

Apart from that, Calder (2001) reported that high levels of either α-LNA or EPA + DHA decrease chemotaxis of neutrophils and monocytes, production of reactive oxygen species by neutrophils and monocytes, and T lymphocyte proliferation.

These beneficial effects of dietary n-3 PUFA may be of use as a therapy for acute and chronic inflammation (rheumatoid arthritis) and for disorders which involve an inappropriately activated immune response (auto-immunity). Evidence for beneficial effects of long-chain n-3 PUFA in rheumatoid arthritis is strong and there is less strong evidence for benefit in Crohn's disease, ulcerative colitis and psoriasis and among some adult asthmatics (Calder, 2001).

Health risks of n-3 PUFA

In spite of their health benefits, one must be cautious in recommending PUFA rich diets given the possible deleterious health effects of their lipoperoxidation products (Williams, 2000). Since consumption of n-3 PUFA can be associated with a higher susceptibility to *in vitro* oxidizability of LDL, this should be accompanied by adequate amounts of antioxidants such as α-tocopherol (Muggli, 1994). However, the established beneficial effects of plant and marine n-3 PUFA on other risk factors for CVD likely outweigh any increased risk from oxidative changes (de Deckere et al., 1998).

Also, some positive associations of cancer with per capita use of PUFA, and specifically LA, are reported (Prentice and Sheppard, 1990; Sasaki et al., 1993). The sensitivity of PUFA towards oxidation, may play a role in carcinogenesis (Welsch, 1987; Fang et al., 1996).

Dietary recommendations

The following recommendations relate to healthy adult subjects (Voedingsaanbevelingen voor België, 2006) and are expressed as % of energy-intake (Table 1.1). Some groups, especially those at risk of developing cardiovascular disease, can have more specific needs.

Table 1.1. Dietary fatty acids recommendation for healthy subjects (Voedingsaanbevelingen voor België, 2006)

Total fat	< 30-35
SFA	< 10 (intake not imperative)
MUFA	> 10
PUFA	5,3 – 10,0
n-3 PUFA α-LNA EPA+DHA	1,3 – 2,0 > 1 > 0,3
n-6 PUFA LA AA	4 – 8 > 2 -
Trans fatty acids	< 1 (ideally 0)
Cholesterol	<300mg/day (intake not imperative)

Some indices used in relation to human health considerations are the P/S (PUFA/SFA) and n-6/n-3 ratio (see below). However, de Deckere et al. (1998) prefers separate recommendations for α-LNA, marine n-3 PUFA and LA. The n-6/n-3 ratio is not useful for characterising foods or diets because plant and marine n-3 PUFA show different effects, and because a decrease in n-6 PUFA intake does not produce the same effects as an increase in n-3 PUFA intake.

P/S: (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0)

>0.7 (SFA=C14:0+C16:0+C18:0) (Voedingsaanbevelingen voor België, 2003)

>0.45 (SFA=C12:0+C14:0+C16:0+C18:0) (Department of Health, 1994)

n-6/n-3: n-6/n-3 PUFA (long-chain PUFA (C20-C24) included)

<5 (Voedingsaanbevelingen voor België, 2003)

<4 (Department of Health, 1994)

2.3 Enrichment of pork with n-3 PUFA

Mantzioris et al. (2000) proposed that n-3 PUFA enriched foods would provide a means to achieve desired biochemical effects of n-3 PUFA without the ingestion of supplements, or a change in dietary habits. In pork, PUFA may be introduced by manipulation of animal feed or by enrichment with PUFA during processing.

2.3.1 Dietary strategies

Fat depots of monogastric animals include intra- and intermuscular fat, as well as abdominal (e.g. perirenal, omental) and subcutaneous adipose tissue (e.g. backfat). The fatty acid composition differs between various tissues and their location in the body, and there is additional variability between similarly located muscles or subcutaneous fat depots. Of the fat depots mentioned here, both intramuscular fat and backfat of pigs considerably contribute to the total fat intake from meat products in the Western diet.

Intramuscular lipids are composed of polar lipids, mainly PL located in the cell membranes, and neutral lipids consisting mainly of TG in the adipocytes that are located along the muscle fibres and in the interfascicular area. A small amount of TG is also present as cytosolic droplets in the muscle fibres (Gandemer, 1999). The content of PL in the muscle is relatively independent of the total fat content and varies between 0.2 and 1% of muscle weight. However, the content of muscle TG is strongly related to the total fat content and varies from 0.2% to more than 5% (Sinclair and O'Dea, 1990; Fernandez et al., 1999; Gandemer, 1999). PL are particularly rich in PUFA (LA, α-LNA, AA, EPA and DHA), whereas TG contain much lower amounts of PUFA (mainly LA and α-LNA). Because PL are membrane components, their PUFA proportion is strictly controlled in order to maintain membrane properties (Webb et al., 1998).

Meat fatty acid content and composition is mainly influenced by dietary factors (fat content and fatty acid composition), and to a lower extent by maturity, sex and genetic factors (e.g. species, breed and fatness). Growth and fattening of meat animals is associated with an accumulation of TG into existing adipocytes, first in subcutaneous and later in intramuscular fat. Fat deposition in the pig's carcass is determined by *de novo* fatty acid synthesis and the uptake of exogenous fatty acids (Vernon and Flint, 1988). The fatty acids synthesized by the pig are mostly SFA (principally palmitic and stearic acid) and MUFA (oleic acid) (Metz and

Dekker, 1981); those from the diet are more often PUFA (principally LA and α-LNA). Deposition of PUFA occurs only if they are included in the diet.

Pigs that are leaner (dependent on genetics, sex, maturity or energy intake) usually synthesize less amounts of fatty acids endogenously and the relative proportion of fatty acids from the diet on total deposited fatty acids is higher. The result is that leaner pigs usually have more unsaturated fat (Pettigrew and Esnaola, 2001). With increasing fatness, the contents of SFA and MUFA increase faster than does the content of PUFA, resulting in a decrease in the relative proportion of PUFA and consequently in the P/S ratio (Cameron and Enser, 1991; Riley et al., 2000). The dilution of PL with TG and the differences in fatty acid composition of these fractions explain this decrease in the P/S ratio. The fat level also influences the n-6/n-3 PUFA ratio, due to the difference of this ratio in polar and neutral lipids. However, these effects are much smaller than the effects that can be achieved by dietary means (Enser et al., 2000).

Moreover, fatty acids absorbed from the diet, especially PUFA, specifically inhibit endogenous synthesis of fatty acids, inflating the effect of dietary fatty acid composition on body fatty acid composition (Pettigrew and Esnaola, 2001). Therefore, in our genetically lean pig lines, it is possible to manipulate the composition of body fat quite dramatically by selection of polyunsaturated dietary fats.

Therefore, the majority of studies attempting to achieve fatty acid profiles in meat that correspond better to current human nutrition guidelines (more n-3 PUFA), have dealt with animal feeding (see reviews e.g. Faustman, 1993; Nürnberg et al., 1998; Jakobsen, 1999; Raes et al., 2004). In the n-3 PUFA enrichment of pork, the feed oil source can deliver either EPA and DHA as such as in fish oil, or under the form of the precursor fatty acid α-LNA as in linseed (oil). Apart from the nature of the oil source, the duration and level of its supplementation are of crucial importance and can be varied to determine the optimal supplementation strategy (see reviews e.g. Wood et al., 2003; Raes et al., 2004). Warnants et al. (1999) concluded that maximal feeding effects with essential fatty acids can be achieved within 40 days, with half of the effect occurring within 2 weeks. This was confirmed by Kouba et al. (2003).

2.3.2 Fortified meat products by post mortem addition of n-3 PUFA

An alternative approach to increase the intake of n-3 PUFA, is the enrichment of frequently consumed foods with vegetable or marine oils (so called selected nutrient

fortification) (Kolanowski and Laufenberg, 2006). Modern food technology makes it possible to fortify various food products (e.g. bread, spreadable fats, dairy products, pasta, ice-cream, milkshakes or instant concentrates, and meat) in such a way that makes them indistinguishable from non-fortified foods. Such applications are not easy because the addition of fish oil for example, introduces stability problems especially with regard to odour and taste to most food products (Gibney, 1997; Kolanowski et al., 1999). Successful incorporation of PUFA into foods requires stabilized PUFA protected from oxidation and from undesirable interactions with other ingredients. One way to avoid these problems is to add the unsaturated oils to short shelf-life food products. These problems can often be minimized by refining and deodorizing the oils, and by applying various antioxidant strategies (Garg et al., 2006). Another way is to convert fish oil to powder by microencapsulation, a technique that changes the form of fish oil from liquid to powder and protects the highly sensitive n-3 PUFA from oxidation, thereby maintaining their acceptability and shelf-life (Lovegrove et al., 1997). The most suitable to such enrichment seem to be food products that are not strongly heated, briefly stored, and packed without access to light and oxygen.

Linseed oil has been used to change PUFA/SFA and n-6/n-3 PUFA ratios in meat (products) (Ansorena and Astiasaran, 2004; Valencia et al., 2006; Pelser et al., 2007). Fish oil has been used in various forms and levels to enrich different food products with long-chain n-3 PUFA, including some meat derivatives (Kolanowski and Laufenberg, 2006). Some marine algae produce DHA-rich oil, and this algal oil has been used to produce meat products (Lee et al., 2006). No specific problems have generally been reported in connection with oxidative stability in meat based products formulated with healthier lipid profiles.

Studies on improving the fatty acid composition of pork usually do not take into account the influence of culinary processing, which often includes a heat treatment and a fat addition. Pan-frying is a common way of culinary preparation of pork in Western countries. During pan-frying, exchange of fatty acids between the food item and the culinary fat takes place (Nawar, 1984). Research by Candela et al. (1998) for deep-fried poultry, pork and sardines, by Ramirez et al. (2005) for deep-fried pork, by Sioen et al. (2006a) for pan-fried fish (salmon and cod), and by Haak et al. (2007) for pan-fried pork indicate that the fatty acid composition of the fried food tends to be similar to the one of the culinary fat used. This reflects the development of a fatty acid gradient equilibrium between the culinary fat and the samples being fried.

2.4 Increased PUFA and pork quality

While improving the nutritional quality of pork by increasing the n-3 PUFA content, also the sensory (shelf-life and rancidity) and technological quality (firmness) may be affected. Both are strictly related to the fat quality which is in turn determined by the fatty acid composition and the oxidative stability of the fat. The colour (whiteness) of fat is important in some countries but will not be considered here (Ringkob, 2003).

Oxidative processes lead to the degradation of lipids, proteins (including pigments), and nucleic acids (mainly DNA) and are one of the primary mechanisms of quality deterioration in meat and meat products. They cause loss of flavour (off-odours), colour and nutritive value, limit the shelf-life of meat and meat products and may lead to the formation of toxic components (Kanner, 1994). The appearance of fresh retail meat is a major determinant of its appeal to consumers and, consequently, sales of the product. The oxidative stability of meat depends upon the balance of anti- and prooxidants and the composition of oxidation substrates including PUFA, cholesterol, proteins and pigments (Bertelsen et al., 2000). Given their health-promoting effect, many efforts have been made to increase the n-3 PUFA concentration in pig tissues by dietary manipulation (Raes et al., 2004). However, the goal is hampered by the higher susceptibility of the n-3 fatty acids towards oxidative deterioration in pig meat (Monahan et al., 1992b; Nurnberg et al., 1999). This has been identified as a major problem in previous work using α-LNA-rich oilseeds or fish oils to enrich pork with n-3 PUFA above certain concentrations (Romans et al., 1995a,b; Overland et al., 1996; Leskanich et al., 1997; Wood et al., 2003). In addition to fishy or rancid taints resulting from feeding fish oils, there is also the possibility of alterations in flavour as a result of increasing α-LNA relative to LA, the major PUFA in pork. However, normal eating quality parameters are not always adversely affected by using an α-LNA-enriched diet as demonstrated by Sheard et al. (2000).

Cameron and Enser (1991) showed that correlations between the concentration of specific fatty acids and eating quality traits were generally weak. However, correlations involving PUFA and palatability scores were generally negative and those for the SFA were generally positive suggesting that the higher the degree of unsaturation in the intramuscular fat, the greater the incidence of abnormal flavours.

For preventing the negative quality effects of high PUFA diets in pork, Warnants et al. (1996) proposed a maximum threshold of 18 g PUFA/kg feed. Moreover, for concentrations

of α-LNA below 3% of total fatty acids, adverse effects on meat quality in terms of shelf-life and flavour are usually absent (Wood et al., 2003). Because of its relatively high concentration in conventional feedstuffs and fat sources used in pig diets, a PUFA of major concern is LA. To prevent problems occurring, dietary specifications in Europe generally include a maximum inclusion level for LA which is commonly set at around 1.6% of the diet for finisher rations. A measure of the degree of unsaturation of fats, both dietary and within the body, is the iodine value (IV), with higher values indicating a greater proportion of unsaturated fats. Threshold levels for body fat composition for soft fat problems have been established by Barton-Gade (1987), setting a fairly rigid standard of a maximum body fat IV of 70. Boyd et al. (1997) suggested a less stringent IV threshold of 74 and dietary LA maximum of 2.1%.

Most dietary fats are more unsaturated than the TG the pig synthesizes endogenously, so addition of fat to the diet usually increases the unsaturation. The softness of fat is directly proportional to the amount of unsaturated fatty acids in the fat depot. Moreover, the lower content of TG in the adipocytes of lean pigs, contributes to the lower firmness of the fat. Soft fat is of major concern to the meat processor because it can cause significant problems during cutting, grinding and slicing operations and can result in lower processing yields and reduced value.

A specific challenge when increasing the tissue concentration of n-3 PUFA, is to counteract the increased susceptibility of fortified muscle food products to oxidation. Strategies to counter the undesirable effects of n-3 enrichment, include limiting the inclusion levels of n-3 PUFA sources, decreasing the duration of feeding, altering the feeding phase, and including high levels of antioxidant(s) (cocktails) along with high-quality ingredients (less prooxidative minor components) (Decker, 1998; Jakobsen, 1999; Gonzalez-Esquerra and Leeson, 2001; Kamal-Eldin and Yanishlieva, 2002).

3. IMPROVED OXIDATIVE STABILITY OF MUSCLE FOODS

3.1 Oxidative processes in muscle foods

3.1.1 Oxidation and free radical formation

In a stable atom, electrons usually orbit in pairs around the nucleus. A molecule that has an unpaired electron (represented by a dot next to the chemical structure, e. g. $A^{\cdot}$), is unstable and is referred to as a free radical or oxidizing agent. As a rule, a radical needs to pair its unpaired electron with another, and will react with another molecule by "stealing" an electron. This process is called oxidation.

Damaging free radicals can be produced in our bodies both from normal (essential) body processes and from sources that originate outside of the body. Some sources of free radicals that originate inside the body are the following: respiration and energy production, immune function and normal enzyme or chemical reactions. Oxygen is the primary oxidant in metabolic reactions designed to obtain energy from the oxidation of a variety of organic molecules. Therefore, while an essential chemical of life, the oxygen molecule produces free radicals (reactive oxygen species (ROS)) in the body resulting in oxidative damage (see below). Oxygen is a two-edged sword, both helping and hurting us. This has been referred to as the "paradox of aerobic life" (Davies, 1995).

Radicals are also produced by processes other than normal metabolism - by exposure to ultraviolet (UV) light and other forms of ionizing radiation, smoking and other pollutants, herbicides and pesticides, and are even found in certain types of food (e. g., deep-fat fried foods) (Møller et al., 1996; Papas, 1999).

Oxidative stress has been defined as a disturbance in the equilibrium status of prooxidant/antioxidant systems in intact cells. This definition implies that intact cells have prooxidant/antioxidant systems that continuously generate and detoxify oxidants during normal aerobic metabolism. When additional oxidative events occur, the prooxidant systems outbalance the antioxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids. Such damage has been linked to a number of pathological conditions (e.g. aging, atherogenesis and carcinogenesis). Mild, chronic oxidative stress may alter the antioxidant systems by inducing or repressing proteins that participate in these

systems, and by depleting cellular stores of antioxidant materials such as glutathione and vitamin E.

Radicals of oxygen, carbon, nitrogen, and sulfur comprise the variety of reactive species that can constitute oxidative stress to cells. Also, non-radical species such as singlet oxygen ($^1O_2$*) and hydrogen peroxide (H_2O_2) are strong oxidants. Figure 1.7 shows some ROS and the complexity of their origin. Factors such as the site of production, the availability of transition metals, and the action of enzymes determine the fate of each radical species and its availability for reaction with cellular molecules.

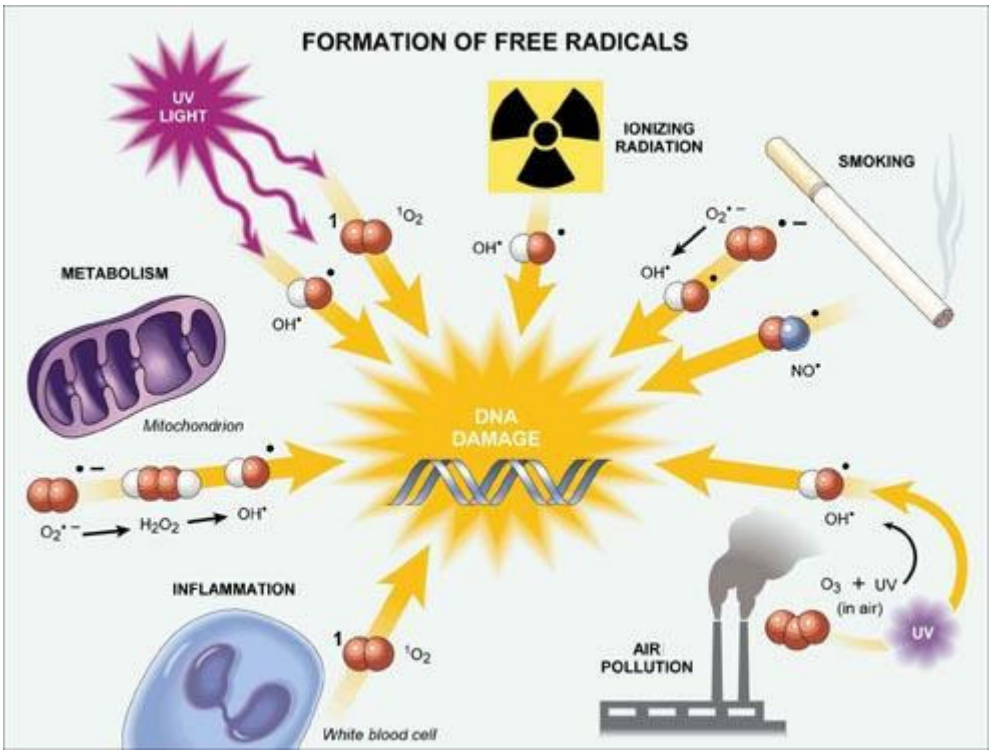

Figure 1.7. The origin of reactive oxygen species (http://www.lef.org/magazine/graphics/freerad.jpg)

3.1.2 Effects of oxidants on macromolecules

Lipid oxidation

Lipid oxidation is known to proceed by a free radical chain reaction involving initiation, propagation and termination (Figure 1.8). During the initiation stage, a radical (e.g. hydroxyl radical, $HO^{\cdot}$) reacts with another molecule (e.g. PUFA, LH), turning this last into an alkyl radical ($L^{\cdot}$). In the propagation stage, a chain reaction of radical formation is begun. In case of lipid oxidation, $L^{\cdot}$ could react with ground-state oxygen ($^3O_2$) to produce a peroxyl radical ($LOO^{\cdot}$), and this step is called peroxidation. The peroxyl radical again is highly

reactive, and can react with another organic substrate in a chain reaction. In the final step, the termination, a radical pairs its unpaired electron by reacting with a second radical. Both radicals are neutralized and the chain reaction is terminated.

This type of chain reaction is common in the oxidative damage of fatty acids and other lipids, and demonstrates why radicals such as $HO^{\cdot}$ can cause much more damage than one might have expected. Once the lipid molecule takes up oxygen, it usually breaks apart into smaller molecules, such as pentanal, hexanal, and 2,4-decadienal, which have the off-odours and flavours we recognize as warmed-over flavour (WOF). These substances are extremely volatile and perceptible in very low concentrations (ppb) (Vega and Brewer, 1994). They are retained in the lipid phase until reheating (Mottram and Edwards, 1983). In intact cells, this oxidation seriously impairs membrane function.

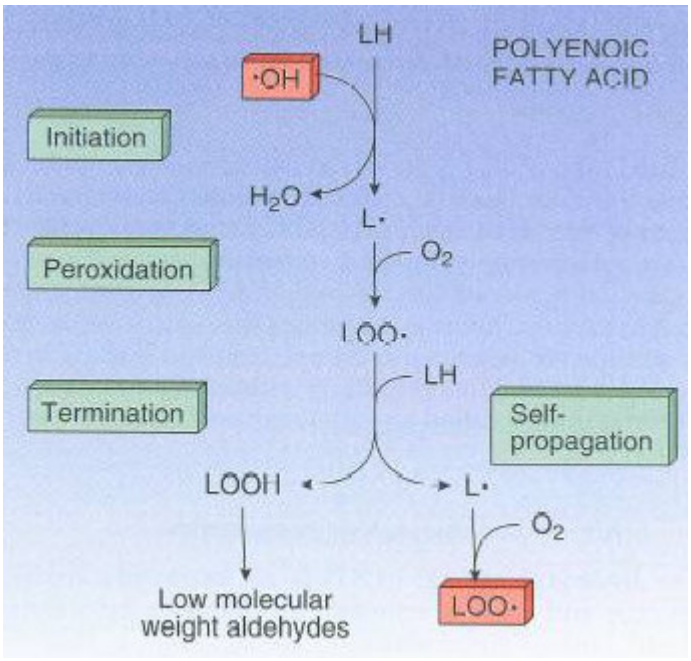

Figure 1.8. The free radical chain reaction during lipid oxidation

Protein oxidation

Proteins and nucleic acids (mainly DNA) are highly susceptible to oxidative damage, particularly where sulfur-containing amino acids or metal binding sites are found. Oxidative damage of proteins results in degradation, fragmentation, formation of disulfides or sulfoxides, or cross-linking of DNA to protein (a form of damage particularly difficult for the cell to repair). Although all cells have some capability of repairing oxidative damage to proteins and DNA, excess damage can cause mutations or cell death.

A specific type of protein oxidation is pigment (colour) oxidation which is discussed more in detail below because of its significant importance to meat quality.

Colour oxidation

Colour is produced when light in the visible range (400-700 nm) is perceived by our eyes. Pigments are molecules which absorb some of the wavelengths from the light that illuminates an object. The absorbed wavelengths, we don't see; the reflected wavelengths produce the colour.

Fresh meat colour

The colour of fresh meat is influenced by the concentration and chemical nature of the heme pigments present (Govindarajan, 1973). These are determined by intrinsic factors such as breed, genotype, gender, age, type of muscle, and dietary supplementation with antioxidants. Extrinsic factors such as pre/post-slaughter handling and slaughter procedure, influence the pH decline and the ultimate pH, and thus the muscle structure and colour (Offer et al., 1989). Furthermore, storage time and conditions (temperature, atmosphere, light etc.) are important (Faustmann and Cassens, 1990; Renerre, 1990; Sellier and Monin, 1994; Rosenvold and Andersen, 2003b).

In most meats, myoglobin is the main heme pigment, although hemoglobin may be present in significant concentrations and mitochondrial cytochrome c to some extent as well (Hamm, 1975; Ledward, 1992). Myoglobin is distributed uniformly throughout the muscle in the sarcoplasmic fraction (Govindarajan, 1973; Swatland, 1984), and its role is to store and to facilitate the diffusion of oxygen from the capillaries into the intracellular structures, where the oxygen is used for oxidative processes (Stryer, 1981; Ledward, 1992). Hemoglobin, which is contained in the red blood cells, serves as the oxygen carrier in blood (Stryer, 1981).

Myoglobin is a compact globular protein consisting of globin and an iron containing heme group, Fe-protoporphyrin, which is the chromophore of myoglobin (Stryer, 1981) (Figure 1.9). The characteristic colours of myoglobin upon interaction with light depend on the redox state of the iron atom and on the ligand bound to the chromophore (Govindarajan, 1973; Hamm, 1975) (Table 1.2). Hemoglobin is composed of four globin molecules with four heme groups. The colour of meat can be discussed focusing on myoglobin, as the different redox states of myoglobin and hemoglobin have nearly identical colour characteristics.

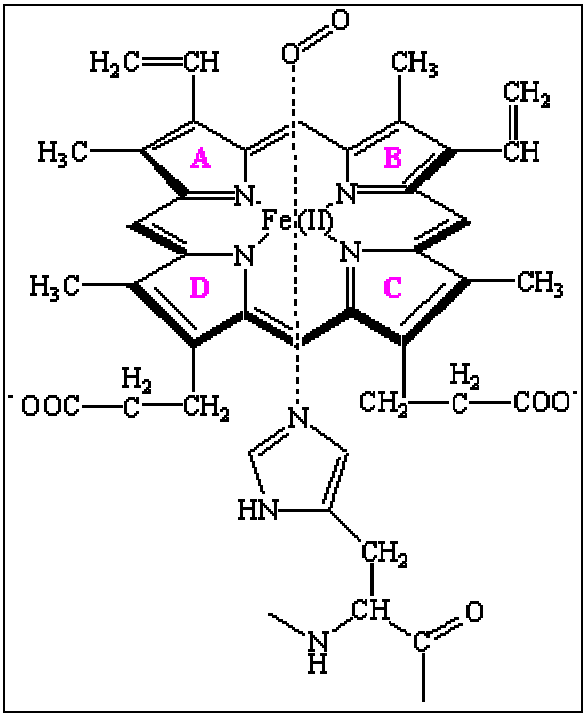

Figure 1.9. The chemical structure of myoglobin

(http://chemed.chem.purdue.edu/genchem/topicreview/bp/1biochem/graphics/13.gif)

In both hemoproteins, the iron atom binds to the four nitrogens in the centre of the protoporphyrin ring and can form two additional bonds, one on either side of the heme plane, the fifth and sixth coordinating positions. The iron atom can be in several redox states with the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) states being most important in relation to fresh meat colour. The fifth coordinating position is bound to histidine on the globin, and the sixth is free for binding to different small ligands such as O_2, H_2O, OH^-, NO and CO (Hamm, 1975; Stryer, 1981). Oxygen can only be bound to myoglobin in the ferrous redox state, whereas H_2O is bound in the ferric redox state at physiological pH and below (Govindarajan, 1973). The different myoglobin species in fresh meat are shown in Table 1.2.

Table 1.2. Myoglobin species in fresh meat

Myoglobin species	Oxidation state	Ligand	Colour
Deoxymyoglobin (Mb)	Fe^{2+}	none	purple
Oxymyoglobin (OxyMb)	Fe^{2+}	O_2	bright cherry-red
Metmyoglobin (MetMb)	Fe^{3+}	H_2O (pH<8)	brown

The colour cycle of fresh meat is reversible and dynamic with constant interconversion of mainly the three species by oxygenation, oxidation and reduction: deoxymyoglobin (Mb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb) (Figure 1.10).

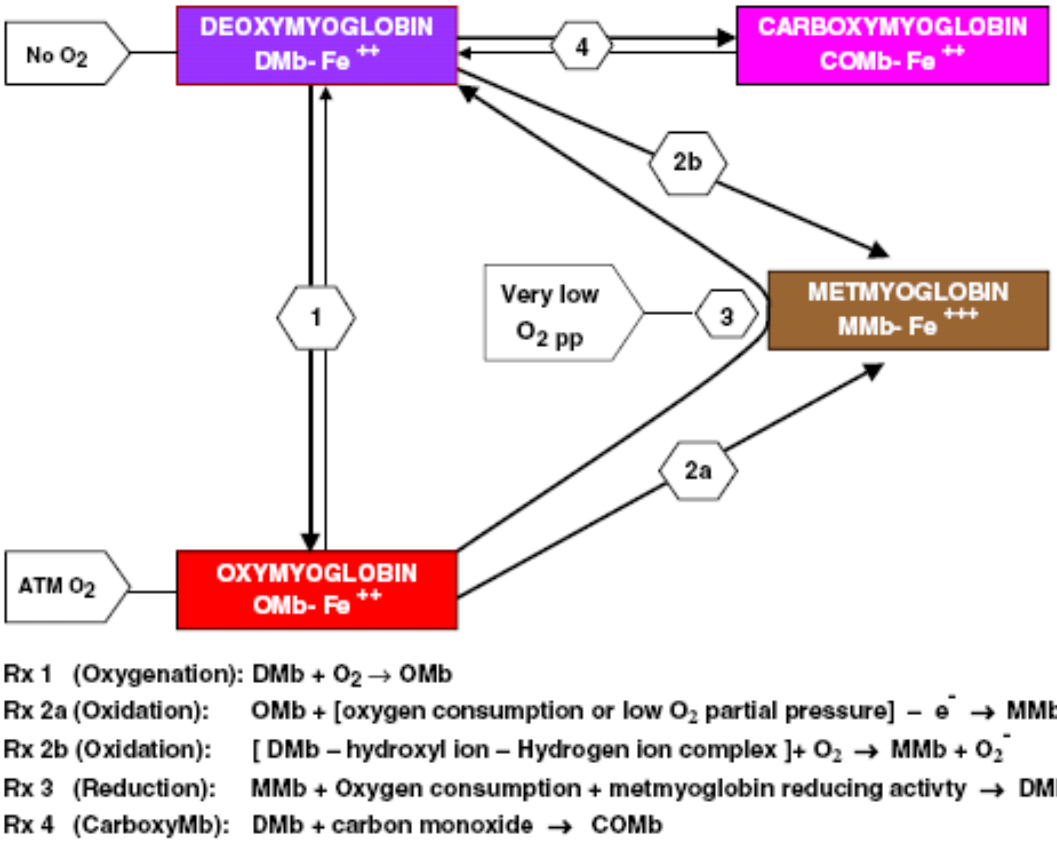

Figure 1.10. Visible myoglobin redox interconversions on the surface of meat (Mancini and Hunt, 2005)

Oxygenation - When meat is freshly cut, the myoglobin is in the purple reduced form Mb. On exposure to air, myoglobin combines with oxygen to form the bright cherry-red OxyMb, a reaction known as blooming (Govindarajan, 1973; Giddings, 1977; Ledward, 1992). The reaction is reversible with oxygen partial pressure determining the partition between the two species (Giddings, 1974). The meat surface blooms to the bright cherry-red colour within minutes of exposure to air (Ledward, 1992) and with time, the small layer of OxyMb spreads downwards into the meat (Feldhusen et al., 1994). Blooming is more efficient under conditions that increase oxygen solubility and discourage enzymic activity (oxygen-consuming enzymes), *i.e.* at low temperatures and low pH values (*post mortem* conditions) (Ledward, 1992). This results in a more pronounced and faster blooming when meat has been

aged for several weeks in vacuum prior to exposure to air (Feldhusen et al., 1994). MetMb reducing activity may also be involved in the blooming process.

Oxidation - The ferrous species Mb and OxyMb oxidise to ferric MetMb upon which the oxygenation ability is lost. OxyMb is more stable to oxidation compared with Mb (Govindarajan, 1973; Stryer, 1981). The development of MetMb at the meat surface depends essentially on the myoglobin oxidation rate, enzymic MetMb reduction and oxygen consumption rate (Renerre, 1990), and in some cases, microbial growth.

The rate of oxidation increases with increasing temperature (Brown and Mebine, 1969), low pH (Gotoh and Shikama, 1974) and low oxygen pressure (George and Stratmann, 1952). The oxidation rate has a maximum at an oxygen partial pressure of 1 mm Hg, and the rate decreases when the oxygen partial pressure increases up to 30 mm Hg. Above 30 mm Hg the oxygen pressure has little or no effect on the rate of oxidation (George and Stratmann, 1952). A few millimeters below the meat surface, a brown layer develops due to the accelerated formation of MetMb (Govindarajan, 1973; Giddings, 1977), as the partial pressure of oxygen in this region is in the optimum range for formation of MetMb (Figure 11). The main discolouration process proceeds from this MetMb layer. Under the MetMb layer is the purple colour characteristic of Mb (Figure 1.11).

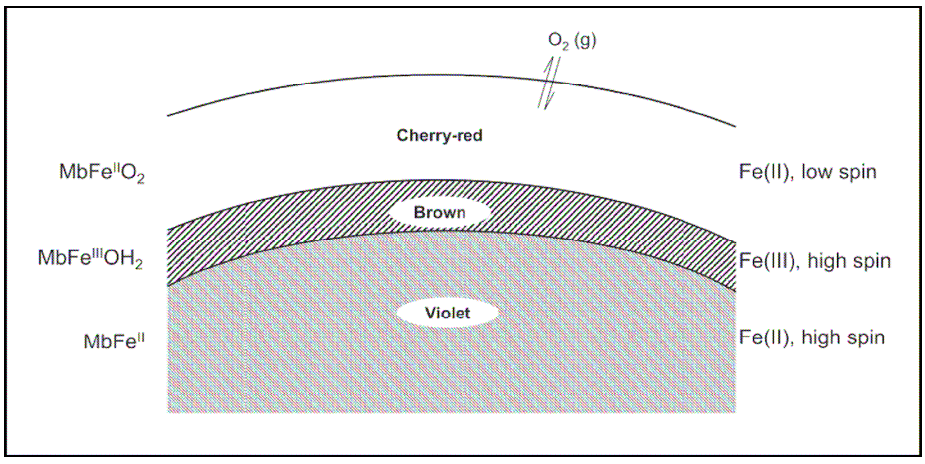

Figure 1.11. Schematic illustration of myoglobin forms in layers of fresh meat in equilibrium with atmospheric oxygen at the surface showing ligand coordinated and iron atom spin state (Møller and Skibsted, 2006)

Reduction - A key ingredient in meat colour life is MetMb reduction, a process that requires NADH as a reductor and which is capable of reducing MetMb to its ferrous state. Loss of reducing activity in meat during storage as observed in porcine muscle by Zhu and Brewer (1998), is due to a combination of factors including depletion of required substrates and co-factors, pH-induced denaturation of the enzymes and ultimately complete loss of structural integrity and functional properties of the mitochondria (Giddings, 1974).

Cooked meat colour

When meat is heated, the various forms of myoglobin are denatured: the globin portion of myoglobin is denatured and the iron of the heme ring is oxidized to the ferric state. The resulting cooked meat pigment is a ferric complex (Giddings, 1977) which has a tan-brown colour and is generally called denatured globin hemichrome. The Maillard browning reaction also contributes to the colour of cooked meat, especially when the surface of the meat is dry and high temperatures are achieved.

Cured meat colour

The basic colour reaction during curing is one in which Mb or OxyMb is oxidized to MetMb and at the same time nitrite (NO_2^-) is reduced to nitric oxide (NO) (Watts, 1957; Sebranek and Kipe, 1985). Typically, the slightly acid pH of *post mortem* muscle (5.5 - 6.0) and reducing agents in brines (sodium salts of ascorbate or its isomer, erythorbate) favour this process. NO binds to myoglobin to provide the pigment variously termed nitric oxide myoglobin, nitrosomyoglobin, or nitrosylmyoglobin (NOMb). It is not entirely clear what intermediate components exists in the transition from MetMb and NO to NOMb. It has been proposed that NO and MetMb combine to form nitrosylmetmyoglobin (NOMetMb) prior to the reduction of ferric iron, leading to NOMb (MacDougall et al., 1975; Killday et al., 1988). Upon heating, the dark red NOMb turns to the pink nitrosylhemochrome commonly associated with cooked cured meats (also termed nitric oxide hemochrome or nitrosohemochrome). The globin portion of the molecule is detached during heating, and the second NO attaches to the denatured protein rather than to the heme portion of the molecule (Killday et al., 1988). The nitrosylhemochrome is thus a mononitrosyl complex and carries a reduced ferrous iron.

Colour change results from decomposition of nitrosylhemochrome and is accelerated by light and to a negligible degree by heat. Watts (1957) outlined two types of

nitrosylhemochrome decomposition, the first being the dissociation of NO from the heme portion of the molecule followed by oxidation of both NO and ferrous iron. This reaction results in gray-brown hemichromogen (denatured MetMb), and is reversible. Residual nitrites in the presence of added or natural reductants can ensure retention of nitrosylhemochrome. The second decomposition reaction is that in which the porphyrin ring of heme is destroyed after formation of hemichromogen. This is often termed the "greening reaction", because of the resulting green-gray colour of the meat. This reaction is not reversible, even in the presence of nitrite and reducing agents. Watts (1957) states that ring destruction can occur rapidly due to peroxides of hydrogen or fat which may result from lipid oxidation, bacterial action, or ionizing radiation in the presence of oxygen. Attention to packaging, particularly vacuum packaging in wrappers impermeable to oxygen, is the best defense against colour fading. Attention to packaging and storage conditions means lower levels of residual nitrite are required to maintain acceptable product quality (Sebranek and Kipe, 1985).

Interaction of colour and lipid oxidation

Several studies have shown that lipid oxidation products can promote colour oxidation and vice versa although the strength of the relationship between these two aspects of shelf-life is sometimes low (Mercier et al., 1995).

Several mechanisms have been proposed to explain lipid oxidation induced catalysis of colour oxidation. It has been suggested that free radicals generated during lipid oxidation may initiate colour oxidation (Yin and Faustman, 1994). More recently, the products of lipid oxidation have been shown to contribute to colour oxidation (Chan et al., 1997). Lipid oxidation is an oxygen-consuming process, and colour oxidation is markedly affected by oxygen level, whereby low oxygen tensions favour MetMb formation (Ledward, 1970). It is possible that lipid oxidation lowers dissolved oxygen to levels conducive to colour oxidation, but to date, in a system containing lipids and OxyMb, it has not been conclusively shown whether OxyMb oxidation occurs first and then catalyzes lipid oxidation or vice versa (O'Grady et al., 2001).

Some theories explaining the opposite interaction exist as well. Colour oxidation releases the transition metals from the porphyrine structure and this free heme has a higher peroxidase activity compared to protein bound heme (Grinberg et al., 1999; Baron and Andersen, 2002). The redox activities involved in the discolouration of meat affect the oxidative stability of the lipid fraction of the product, as iron redox cycling seems to initiate

peroxidation and formation of low-molecular weight components responsible for off-flavours and rancidity (Jensen et al., 1998a; Møller and Skibsted, 2006). The interaction between meat discolouration and lipid oxidation in meat involves the brown MetMb, which forms several prooxidative myoglobin species during the catalytic cycle following reaction with H_2O_2 and other peroxides (Figure 1.12). MetMb also takes part in the propagation of lipid peroxidation by cleavage of lipid hydroperoxides (LOOH) (Skibsted et al., 1998). H_2O_2 is central in triggering of processes leading to oxidative rancidity in fresh meat, as $O_2^{\cdot-}$ formed during oxidation of OxyMb yields H_2O_2, either spontaneously or mediated by superoxide dismutase (SOD, see below). In addition, H_2O_2 may also be produced in significant quantities as a result of growth of the catalase-negative lactic acid bacteria on the meat surface. Transition metal redox couples such as Fe^{3+}/Fe^{2+} or Cu^{2+}/Cu^{1+} have standard reduction potentials allowing catalytic decomposition of LOOH to form LO• radicals (see reaction under 'hydroxyl radical' above), and can thereby initiate the chain reaction characteristic for lipid oxidation. Mb reacts with H_2O_2 in a two-electron process in which a hypervalent perferrylmyoglobin (•MbFeIV=O) is formed. This initial form then undergoes reduction to produce ferrylmyoglobin (MbFeIV=O) (Irwin et al., 1999). Both of these hypervalent myoglobin species are known to initiate lipid peroxidation by hydrogen abstraction from fatty acids (Giulivi and Cadenas, 1994).

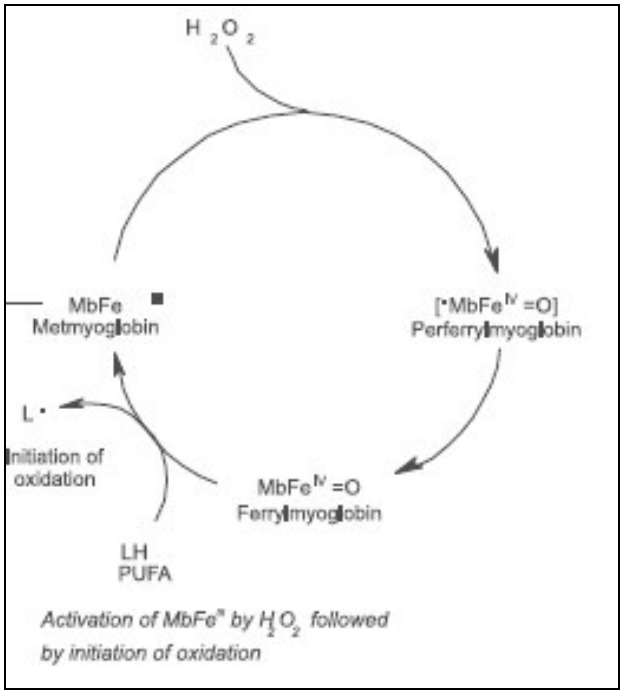

Figure 1.12. Prooxidative cycle of Mb activated by H_2O_2 (Møller and Skibsted, 2006)

3.1.3 Factors influencing the oxidative stability of muscle foods

The oxidative stability of muscle foods is dependent on the concentrations, composition and activity of prooxidants, antioxidants and reaction substrates (e.g., PUFA). The balance between these three factors, determines the oxidative stability of muscle foods.

Antioxidants

An antioxidant is a substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate.

Skeletal muscle is an excellent example of a biological tissue that contains a multicomponent antioxidant system that is biphasic, being found in both aqueous and lipid environments. This system includes components that scavenge free radicals, control prooxidant catalysts, inactivate ROS, inactivate photoactivated sensitizers, and quench secondary lipid oxidation products. The most effective antioxidant is dependent on the specific molecules causing the oxidative stress, *i.e.*, superoxide anion, lipid peroxides, hydroxyl radical, etc., and the cellular or extracellular location of the source of these molecules. A more detailed description of antioxidants is given in section 3.2 Antioxidants. Increasing antioxidant protection can occur either by increasing cellular antioxidants (endogenous antioxidants and antioxidant enzymes) or by application of exogenous antioxidants (processing).

Prooxidants

A variety of substances and processes can trigger oxidation in meat, and are therefore said to be prooxidative (e.g. heat, light, transition metals, ROS or oxidizing enzymes). However, many prooxidative muscle food components contribute to desirable properties by acting as nutrients (e.g. Fe, Cu) or pigments (e.g. myoglobin).

Oxidative reactions in muscle foods are accelerated by *heating* which releases protein-bound iron (an active catalyst for lipid oxidation), activates heme-containing proteins, disrupts cellular membrane systems bringing the lipid substrates (PL) and catalysts in closer proximity, and inactivates antioxidant enzymes (Igene et al., 1979; Chen et al., 1984).

Light, especially UV light, can add enough energy to induce the oxidation reaction. It appears to photosensitize meat pigments and to elevate oxygen to a high-energy state, increasing its ability to participate in oxidation (Jadhav et al., 1996). The spectrum of light

becomes important to the WOF problem because meat products, even pre-cooked, frozen items, may be displayed at retail for hours or days. Carotenoids can be used to inactivate $^1O_2$* and photoactivated sensitizers.

The use of *sodium chloride* (*NaCl*) is common in meat products at concentrations of 5–20 g kg^{-1} for its effects on sensory, functional and preservation properties (prevention of colour oxidation). However, addition of NaCl increases the prooxidant activity of iron (Kanner et al., 1991), and promotes lipid oxidation in meat products, leading to the formation of off-flavours. Replacement of NaCl by potassium chloride (KCl) is an effective means for reducing NaCl-catalyzed lipid oxidation in processed meats (Zipser et al, 1964; Rhee et al., 1983).

Transition metals can react directly with lipids by reducing the amount of energy required for the formation of a free radical, and catalyze the decomposition of the lipid hydroperoxide resulting in production of additional free radicals. The most lipid prooxidative metals are transition metals able to undergo a single electron transfer during a change in oxidation state (e.g., iron and cupper) (Jadhav et al., 1996). These can be supplied by water, processing equipment and spices. Control of transition metals can be accomplished by chelators.

The relative roles of *heme and non-heme iron* as catalysts of lipid oxidation in meat have been the subject of much debate (Johns et al., 1989). Zipser et al. (1964) concluded that heme iron in the ferric state, induced oxidation in uncured, cooked pork. Greene and Price (1975) concluded that both heme and non-heme iron are capable of catalyzing lipid oxidation in meats. They surmised that heme pigments may be more active catalysts when iron is in the ferric state, while non-heme iron may be a more active catalyst in the ferrous state. Love (1983), in reviewing the role of heme iron in lipid oxidation of red meat, stated that both valence states can influence oxidation. However, it should be noted that free heme per se has a higher oxidative activity compared to protein bound heme (Grinberg et al., 1999).

The interior of whole muscle cuts contains very little *oxygen*, however, mechanical manipulation (grinding, chopping, mechanical deboning, chunking and forming, mixing and tumbling) can introduce oxygen into the meat, ultimately promoting oxidation and WOF if the product is pre-cooked and stored (Sato and Hegarty, 1971; Pearson et al., 1977; Kanner, 1994). Physical means of delaying the onset of WOF include oxygen exclusion by the use of technologies such as vacuum tumbling and vacuum stuffing prior to cooking, and vacuum packaging of cooked products prior to storage.

ROS consist of radical and non-radical oxygen species than can induce severe oxidation. They are discussed in a previous section (3.1. Oxidation: definition and chemistry).

Microbes change the pH and produce components which react with heme pigments to produce other colours. Catalase-negative bacteria produce H_2O_2 resulting in a green colour and rancid flavours.

Madhavi and Carpenter (1993) found that surface MetMb accumulation, MetMb reductase activity and oxygen consumption rate (OCR) were affected by *muscle fibre type*. The influence of muscle fibre type on OCR is due to differences in muscle metabolism (oxidative/glycolytic) as shown in studies on beef (Lanari and Cassens, 1991). Oxidative muscles show greater MetMb accumulation, lower MetMb reductase activity and greater oxygen consumption than glycolytic muscles. It is reasonable to assume that with an increase in meat respiration (e.g. OCR), there is likely greater increase in oxidative processes (Morrissey et al., 1998) and hence lower colour stability. However, Zhu and Brewer (1998) found that this assumption was not valid for abnormal meat (PSE, DFD). Muscle fibre type might also influence other factors, such as the amount of PL and their fatty acid composition, the myoglobin content and so influence oxidative stability (Andres et al., 2001).

3.2 Antioxidants

3.2.1 Antioxidant mechanisms and classification

Antioxidant classification

Antioxidants can be subdivided in several ways: by chemical structure, hydrophilicity/lipophilicity, natural or synthetic origin, nutrients/non-nutrients, enzymatic/non-enzymatic, endogenous/diet-derived, and by mechanism of action. In the section below, the antioxidant mechanisms of action are discussed in more detail.

According to the chemical structure, antioxidants can be categorized as follows: ascorbic acid (AA; vit C), tocopherols (mainly AT; vit E), carotenoids, polyphenols (e.g. flavonoids). Flavonoids are subdivided in flavones, flavanones, flavonols, anthocyanidins, catechins, isoflavones and chalcones (Hollman, 1997). Some examples of hydrophilic antioxidants are AA, uric acid, glutathione (GSH) and of lipophilic antioxidants are AT, carotenoids, ubiquinones. Some synthetic antioxidants are butylated hydroxyanisole (BHA),

butylated hydroxytoluene (BHT), propyl gallate (PG), ethoxyquin, and tert-butylhydroquinone (TBHQ). On the contrary, the following antioxidants have a natural character: AT, polyphenols (flavonoids), carotenoids, and AA. Some antioxidants are classified as essential nutrients (the vitamins AT and AA, and trace elements, e.g. selenium (Se)) and must be provided in the diet; others are non-nutrients (antioxidant phytochemicals, e.g. polyphenols).

Mechanisms of action

Antioxidants protect components from oxidation by sacrificing themselves to the oxidation process. Antioxidant mechanisms can be triple: chain-breaking, preventive, and repairing. Their effectiveness is concentration-dependent and also depends on which free radical is involved, how and where it is generated, and where the target of damage is. Thus, while in one particular system an antioxidant may protect against free radicals, in other systems it could have no effect at all. Moreover, in certain circumstances, an antioxidant may act as a prooxidant that generates toxic oxygen species. In general, more hydrophilic antioxidants are better in stabilizing bulk oil than oil-in-water emulsions, while the activity of lipophilic antioxidants follows the opposite trend (the "antioxidant paradox" by Halliwell (2000)).

Chain-breaking antioxidants

Chain-breaking antioxidants can react rapidly with free radicals to yield stable products. They interrupt the propagation reaction and are classified as primary antioxidants. They have a low redox potential and can reduce peroxyl or other radicals. Primary antioxidants include phenolic components such as AT, BHT, BHA, TBHQ, flavonoids, etc.

Preventive antioxidants

These antioxidants block the process of oxidation by preventing or reducing free radicals (e.g. β-carotene as $^1O_2$* quencher). Such antioxidants can prevent an oxidation chain from ever setting in motion and are classified as secondary antioxidants. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) prevent oxidation by reducing the rate of chain initiation. They can also prevent oxidation by complexing transition metals (citric acid, ethylenediaminetetracetic acid (EDTA), some polyphenols, and metal chelating proteins: transferrin, ferritin, ceruloplasmin, albumin, and

lactoferrin) (Miller et al., 1993; Halliwell, 1996; Chaudière and Ferrari-Iliou, 1999). Preventive antioxidants can also act by scavenging oxygen (e.g. AA) or by converting hydroperoxides to non-radical species.

Repairing damaged parts of molecules

This level consists of lipolytic, proteolytic and other enzymes (e.g. DNA repair enzymes) (Yu, 1994; Halliwell, 1996). Together, they destroy oxidized lipids (that are constituents of cell membranes), degrade oxidized protein, and repair oxidized DNA.

Action of some important antioxidants

Vitamin E refers to a family of related components (tocopherols and tocotrienols) that have polar hydroxylated aromatic rings (chromanol rings) and non-polar isoprenoid side chains. AT, the most widely available isomer, has the highest biopotency. The molecule is lipophilic and resides in cell membranes where the chromanol ring may be at the surface of the membrane and the isoprenoid chain inserted into the non-polar bilayer. AT acts as a chain-breaker and because of its unique position in the cell, it safeguards cell membranes from damage by free radicals. AT also protects the lipids in LDL from oxidation.

Ascorbic acid scavenges oxygen in an aqueous environment (cytoplasm) and works synergistically with AT to quench free radicals, hereby regenerating the reduced (stable) form of AT. The ascorbate radical formed in this process, is subsequently reduced by GSH or by a specific AA reductase enzyme. Thus, a specific attack on membranes results in the participation of at least three different antioxidants. This is called antioxidant recycling and is shown in Figure 1.13. Moreover, AA can form a stable complex with prooxidant metals. However, AA can be prooxidative at low concentrations.

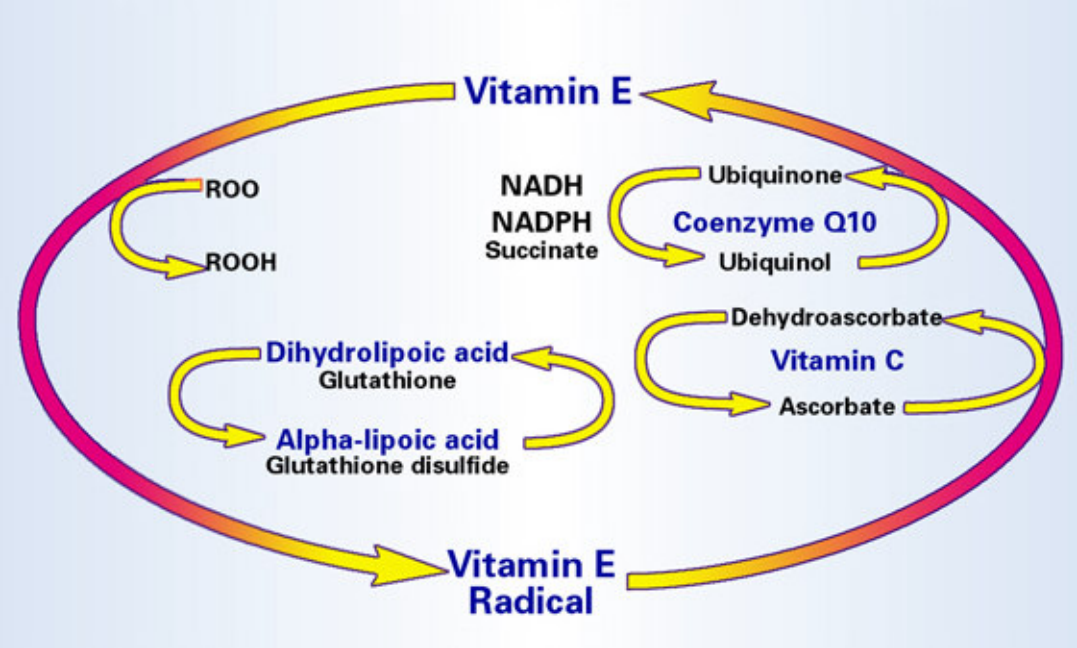

Figure 1.13. The antioxidant regeneration of vitamin E (http://www.vrp.com/graphics/lipoic.fig.1.jpg)

Selenium (Se) is a trace element, a mineral needed in only very small quantities, but without which one could not survive. It forms the active site of GSH-Px. There are 2 major sources of Se, organic Se, mainly in the form of selenomethionine, and inorganic Se, mainly selenite or selenate. Similar to Se, the minerals manganese, zinc and cupper are trace elements that form an essential part of SOD. Fe is required for CAT activity.

Antioxidant enzymes

The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are made in the body in response to the presence of certain free radicals and their amount is controlled by specific redox-sensitive genes. SOD is present in cell cytoplasm (copper-zinc enzyme) and in mitochondria (manganese enzyme) and catalyzes the dismutation of superoxide anion ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2) and oxygen ($^3O_2$) (Figure 1.14). CAT is usually found in peroxisomes, except in cells that do not contain these organelles where it is a cytoplasmic enzyme (e.g. in erythrocytes). GSH-Px is a cytoplasmic and mitochondrial enzyme. Both CAT and GSH-Px are important in H_2O_2 detoxification and work simultaneously with GSH to reduce H_2O_2 and ultimately produce water (H_2O) (Figure 1.14). Glutathione disulfide (GSSG) is subsequently reduced by GSH reductase, using NADPH as the reductant.

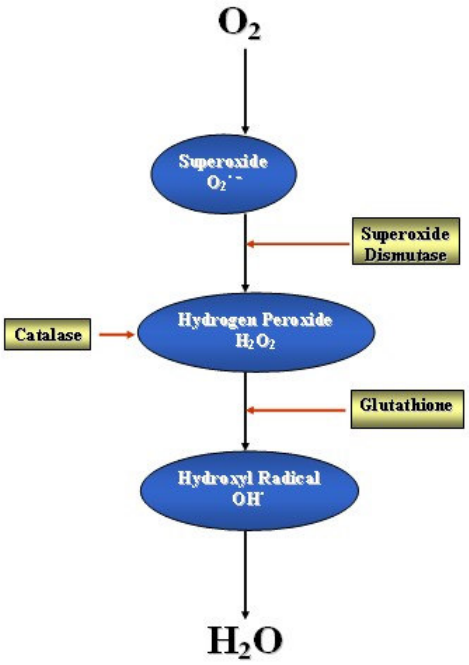

Figure 1.14. The reduction of reactive oxygen species by endogenous antioxidants

In addition to vitamins, minerals, and enzymes, there appear to be many other components with antioxidant properties. Among them are coenzyme Q10 (or ubiquinone) and uric acid (a product of DNA metabolism). Additionally, substances in plants called phytochemicals are being investigated for their antioxidant activity and health-promoting potential (e.g. flavonoids).

3.2.2 Endogenous versus exogenous supplementation of antioxidants and their potential for improving muscle quality

Dietary strategies to influence the antioxidant composition of muscle

The quality and stability of fresh meat as an ingredient for the preparation of several meat products is of crucial importance. For fermented sausage it was shown that the level of lipid oxidation at the end of the manufacturing process strongly depends on the initial stability of the meat and lard (Demeyer, 2001). Moreover, *post mortem* addition of antioxidants to fresh meat and some meat products (e.g. artisanal ham) is sharply restricted or even forbidden. Therefore, preserving or enhancing endogenous antioxidant systems may be a more desirable

method for improving colour and lipid stability in meat. This can be achieved by dietary antioxidant supplementation, especially with AT. Obviously, the response to dietary antioxidant supplementation will depend on the level fed and the time of feeding.

It is generally accepted that the oxidative stability of muscle lipids is dependent on the AT concentration in the tissues (Buckley and Morrissey, 1992; Sheldon et al., 1997), which in turn is dependent on the concentration of α-tocopheryl acetate (ATA) in the feed (Wen et al., 1997). AT, fed above dietary requirement levels (e.g. 200mg/kg feed), consistently protects against lipid oxidation in fresh pork and pork products (Asghar et al., 1991; Buckley et al., 1995; Jensen et al., 1997b, 1998b; O'Sullivan et al., 1998; Lauridsen et al., 1999b; Morrissey et al., 2000). In contrast, the results obtained in relation to colour stability of pork, have been inconclusive (Faustman and Wang, 2000). Some studies have shown improved colour stability after AT supplementation (Asghar et al., 1991; Monahan et al., 1992a). However, several studies do not find any effect of AT supplementation on the colour stability of pork (Cannon et al., 1996; Jensen et al., 1997b; Zanardi et al., 1999; Rosenvold and Andersen, 2003a). More recent studies show limited effect on pork colour (Hasty et al., 2002; Geesink et al., 2004; Swigert et al., 2004). Jensen et al. (1997b) speculated that the beneficial effect of added AT on muscle colour depends on the relative muscle AT level in controls. Where these are above a critical level (3.5mg/g), no beneficial effect is seen. Where the level is low, for example, in the study of Asghar et al. (1991), supplementation to the critical level improves colour retention.

Although AA is a potent biological antioxidant, the effect of its dietary supplementation is questionable (and not as effective as dietary AT). Endogenous AA improved colour and lipid stability less than exogenous AA (Mitsumoto, 2000).

Nowadays, there is a strong interest in isolating antioxidants from natural sources because of the numerous applications for the protection of animals and their products against oxidation (Wenk, 2003). Amongst those, rosemary has been reported to possess antioxidant properties when administered in the diet (Lopez-Bote et al., 1998; Basmacioğlu et al., 2004). Also, dietary tea catechins have been shown to be an effective alternative to AT in chickens (Tang et al., 2000, 2002). In addition, the use of cocktails of antioxidants could have a superior effect compared to single antioxidants. Two or more antioxidants together can act synergistically, *i.e.* they can limit oxidation to a higher extent than the sum of the contributions from each single antioxidant (Duthie, 1999; McCarthy et al., 2001).

Exogenous addition of antioxidants to meat

Synthetic antioxidants such as BHA, BHT, PG and TBHQ have been widely used in the meat industry. However, rising consumer resistance towards the use of synthetic additives, has increased interest in naturally occurring substances (Pokorny, 1991). A list of permitted food antioxidants can be found in the European Directive 95/2/EC.

In the meat industry, the antioxidants AA and AT have been widely considered for extending the retail display life of meat. AA has since long been known for protecting the colour of raw red meat during storage (Greene et al., 1971). However, depending on its concentration, AA either promoted or inhibited lipid oxidation in muscle foods (Yen et al., 2002). In contrast to the strong antioxidant activity of AT when supplemented via the animal diet, it was less effective in controlling lipid oxidation when applied to meat and fish products *post mortem* (Mitsumoto et al., 1993). The main reason for this is that AT is not positioned sufficiently close to the subcellular membrane to fully execute its antioxidative effect.

A variety of extracts of fruits, herbs, vegetables, cereals and other plant material rich in antioxidant substances have been commercialized for food and nutraceutical applications (Shahidi, 2000; Pellegrini et al., 2003). The antioxidant properties of these plant extracts are dedicated to a cocktail of active components, which could act individually as well as in synergism (McCarthy et al., 2001). Several studies have demonstrated that their *post mortem* supplementation to pork, improved its oxidative stability (Madsen et al., 1996; McCarthy et al., 2001; Tang et al., 2001a,b; Rey et al., 2005; Sebranek et al., 2005; Martinez et al., 2006).

CHAPTER 2

ALTERATION OF PORK FATTY ACID PROFILE

CHAPTER 2A

IMPROVING THE PORK FATTY ACID PROFILE BY DIETARY STRATEGIES

Redrafted after part of Haak, L., De Smet, S., Fremaut, D., Van Walleghem, K., and Raes, K. (2007). Fatty acid profile of pork as influenced by duration and time of dietary linseed or fish oil supplementation. Journal of Animal Science (revised version submitted).

CHAPTER 2A

FATTY ACID PROFILE AND TASTE PANEL EVALUATION OF PORK AS INFLUENCED BY DURATION AND TIME OF DIETARY LINSEED OR FISH OIL SUPPLEMENTATION

Abstract - In this experiment, the effect of duration and time of feeding n-3 PUFA sources on the fatty acid composition and sensory characteristics of the *longissimus thoracis* was investigated. Linseed (L) and fish oil (F), rich in α-linolenic acid (α-LNA) and eicosapentaenoic and docosahexaenoic acid (EPA and DHA) respectively, were supplied equivalent to a level of 1.2% of oil, either during the whole fattening period or only during the first (P1; 8 weeks) or second (P2; 6 to 9 weeks till slaughter) fattening phase. In the basal diet (B), only animal fat was used as supplementary fat source. Three dietary groups were supplied the same fatty acid source during both fattening phases, *i.e.*, group BB, LL, and FF. For the other 4 dietary groups, the fatty acid source was switched after the first phase (groups BL, BF, LF, and FL; the first and second letter indicating the diet in P1 and P2 respectively). All diets (rich in linoleic acid (LA)) were based on barley, wheat, and soybean meal and were fed *ad libitum*. Crossbred pigs (n = 154; Topigs 40 x Piétrain) were randomly allotted to 7 feeding groups. Twelve animals per feeding group were selected based on average live weight. The LT was analysed for fatty acid composition; lipid stability (thiobarbituric acid-reactive substances, TBARS) and colour stability (a* value, % of myoglobin pigments) were determined on the LT after illuminated chill storage up to 8 days. For groups BB, LL and FF, meat was grilled and 3 types of meat products (fermented sausage, cooked and dry cured ham) were prepared and evaluated by an untrained consumer panel. The α-LNA, EPA, and docosapentaenoic acid (DPA) incorporation was independent of the duration of linseed feeding (respectively 1.24, 0.54, and 0.75% of total fatty acids for group LL). Supplying fish oil during both phases resulted in the greatest EPA and DHA proportions (1.37 and 1.02% of total fatty acids) ($P<0.05$), but the content of DPA was not affected. The proportion of DHA was greater when fish oil was administered during P2 as compared to P1 ($P<0.05$). There was no effect of diet on meat ultimate pH and drip loss, nor on lipid or colour oxidation. Except for the dry cured ham, meat products from pigs fed on this level of fish oil were generally less

appreciated by the consumer panel compared to meat products from animals fed on the basal or linseed diet ($P<0.05$). However, the eating quality differences were not pronounced.

Key Words: fish oil, linseed, omega-3 fatty acids, oxidative stability, pork, taste panel

INTRODUCTION

Long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), exert positive effects on human health (see review Narayan et al., 2006). Increasing their intake may be most easily achieved by supplementing the human diet with encapsulated fish oil or microalgae or by increasing the consumption of fish. However, as the intake of fatty fish is low in Western societies, the consumption of LC n-3 PUFA from terrestrial animal products (meat, eggs) may be important (Howe et al., 2006). Givens et al. (2006) and De Henauw et al. (2007) argued that enrichment of meat products with n-3 PUFA by dietary means could help bridging the gap between their recommended and actual intake. Hereby, the feed oil source is important and delivering either the LC n-3 PUFA as such as in fish oil, or under the form of precursor fatty acid (FA) (α-linolenic acid; α-LNA) as in linseed (oil). Apart from the nature of the oil source, the duration and level of its supplementation are of crucial importance and can be varied to determine the optimal supplementation strategy (see review Wood et al. (2003) and Raes et al. (2004)).

A more unsaturated fatty acid profile may limit the shelf-life and palatability of meat as PUFA are more prone to oxidation. This has been identified as a major problem in previous work using α-LNA-rich oilseeds or fish oils to enrich pork with n-3 PUFA above certain concentrations (Romans et al., 1995b; Overland et al., 1996; Leskanich et al., 1997; Wood et al., 2003). However, only when concentrations of α-LNA approach 3% of TG or PL are there any adverse effects on meat quality, defined in terms of shelf-life and flavour (Wood et al., 2003). Oxidation may be manifest as off-odours and flavours resulting from lipid oxidation or as an impaired meat colour.

In this experiment, we assessed the importance of the time and duration of linseed and fish oil supplementation to enhance the incorporation of n-3 PUFA in pork. As it was aimed to alter the fatty acid profile without affecting the oxidative stability or consumer acceptability, colour and lipid oxidation was also measured and an untrained consumer taste panel was carried out on fresh and processed meat products.

MATERIALS AND METHODS

Experimental setup and sampling

Crossbred pigs (n = 154; Topigs 40 sow (Helvoirt, The Netherlands) x Piétrain sire) at a mean (SD) live weight of 36.4 (4.5) kg were randomly allotted to 7 feeding groups. Each group was housed in 2 pens of 11 animals, and was fully balanced according to sex (barrow-gilt). The trial lasted for 14–17 weeks and consisted of 2 phases (P1: 8 weeks (till approximately 70 kg); P2: 6 to 9 weeks (till approximate slaughter weight of 100 kg)), in which the feed fat source differed depending on the dietary group. Pigs were fed *ad libitum*. All diets were based on barley, wheat, and soybean meal. The dietary fat was adjusted to 4% (on as-fed basis) by the addition of rendered animal fat. Diets were formulated for an equal energy supply (2225 kcal/kg as-fed) and a minimum linoleic acid (LA) content of 0.9%. In the basal diet (B), only animal fat was used as supplementary fat source. In diets L and F, linseed (group L; α-LNA supply) or fish oil (group F; EPA and DHA supply) were added respectively to provide 1.2% of oil at the expense of animal fat. Three dietary groups were supplied the same fatty acid source during both fattening phases, *i.e.*, group BB, LL, and FF. For the other 4 dietary groups the fatty acid source was switched after the first phase (groups BL, BF, LF, and FL; the first and second letter indicating the diet in P1 and P2 respectively). A graphical presentation of the dietary groups is given in Figure 2A.1. The composition and the fatty acid profile of the experimental diets are given in Tables 2A.1 and 2A.2 respectively.

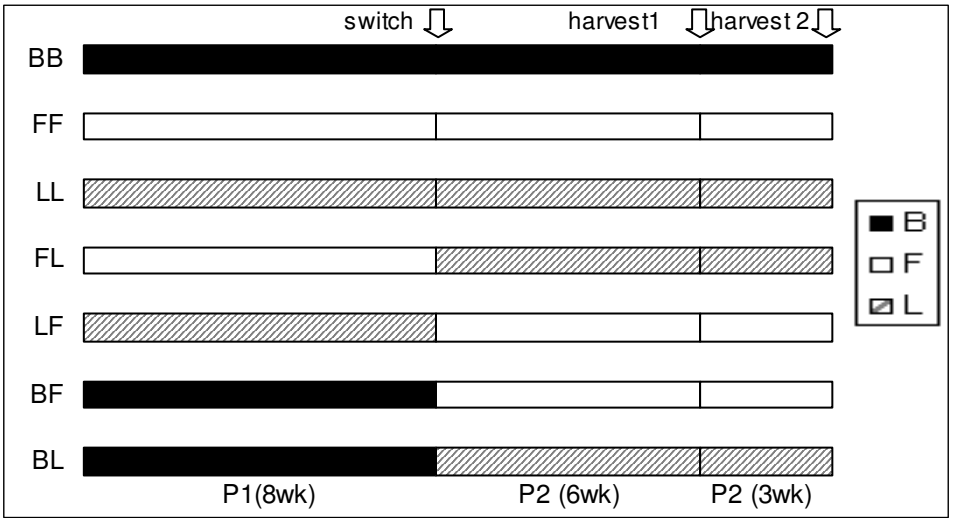

Figure 2A.1. Graphical presentation of the experimental design

Table 2A.1. The ingredient composition of the experimental diets (% as-fed)

	Basal B	Linseed L	Fish oil F
Omega-3 plus[1]	-	-	6
Linseed (crushed)	-	3	-
Barley	22	22	22
Wheat	23	23	23
Wheat gluten	12.5	9.5	8.6
Cassava	17	17	17
Soybean meal 42% CP	10	10	11
Soybean meal 47	5.2	2.8	3.5
Rendered animal fat	2.1	1.3	0.96
Ca-carbonate 38%	1.1	1.1	1.1
Peas	5.8	9.1	5.5
Vitamin-mineral premix[2]	1	1	1
DL-Methionine	0.05	0.05	0.05
L-Lysine HCl	0.17	0.20	0.20
L-Threonine	0.04	0.06	0.05

[1] Omega-3 Plus (Trouw Nutrition, Gent, Belgium) contained 20% of refined fish oil
[2] Vitamin-mineral premix contained 100 ppm vitamin E (all-rac α-tocopheryl acetate)

Table 2A.2. The fatty acid profile of the experimental diets (g/100g FAME)

	Basal B	Linseed L	Fish oil F
C12:0	0.28	0.16	0.32
C14:0	1.19	0.76	2.15
C16:0	20.2	16.9	17.5
C18:0	8.90	6.94	5.77
C16:1c	1.54	1.15	2.51
C18:1c9	26.0	22.4	21.2
C18:1c11	1.76	1.39	1.78
C20:1	0.55	0.41	1.29
C18:2n-6	28.5	27.5	25.5
C18:3n-3	3.41	15.9	3.79
C20:5n-3	-	-	2.31
C22:6n-3	-	-	3.53

The experiment was carried out according to the guidelines of the ethical committee of Ghent University (Belgium). Animals were harvested in 2 groups with a 3-week interval in a commercial abattoir following electrical stunning (Westvlees, Belgium). Per slaughter day, 6 animals from each feeding group (3 barrows and 3 gilts) closest to the average group live weight were selected. Mean (SD) live weight at slaughter and cold carcass weight was 97.1 (6.1) kg and 78.6 (5.1) kg respectively. The *longissimus thoracis* (LT; starting from the seventh rib, left carcass side) was sampled 24 h *post mortem* (day 0) and sliced into 2.5 cm thick chops. One chop was immediately vacuum packed and stored at –18°C until fatty acid analysis. The other chop was used for fresh meat quality assessment (lipid and colour oxidation). In addition, 4 animals from the BB, LL, and FF feeding group were randomly selected for the preparation of grilled meat (GM; from LT), as well as for cooked cured ham (CCH; ham left carcass side), dry cured ham (DCH; ham right carcass side), and fermented sausage (FS) according to standard recipes and manufacturing procedures in a butchery school

(KTA, Diksmuide, Belgium). The fermented sausage was prepared by using one third of meat and backfat respectively from the experimental animals, and one third of beef.

Carcass and meat quality measurements

At the start of each phase and before slaughter, pigs were weighed individually. Carcass lean content was assessed by means of a Giralda Choirometer PG 200 apparatus. At 24 h *post mortem*, pH in the LT (6^{th}- to 7^{th}-rib region; Knick Portamess 654 with Schott N5800A electrode) and PQM (PQM-I Kombi (INTEK, GmbH)) in the LT and the ham (*Semimembranosus*) were measured.

Percentage drip loss of meat was determined by measuring the amount of fluid lost from a 2.5 cm-thick loin chop that was suspended for 48 h in a chill cabinet at 4°C.

For colour measurements, meat samples were overwrapped in an oxygen permeable polyethylene film (oxygen transmission rate > 1000 $cm^3/m^2/24h$). On day 0 the measurement was done after 30 min of blooming (in the film at 4°C) and then daily until 8 days of illuminated chill storage (fluorescent light, 900 lux, 4°C). Reflectance spectra (every 10 nm between 400 and 700 nm) and colour coordinates (CIE L*a*b* colour system 1976) were assessed using a HunterLab Miniscan Minolta XE plus spectrocolorimeter (light source of D65, standard observer of 10°, 45°/0° geometry, 1 in. light surface, white standard). The results were expressed as lightness (L*), redness (a*), yellowness (b*), hue value ($\tan^{-1}$ b*/a*), and saturation index (SI) ($(a^{*2} + b^{*2})^{1/2}$). By means of reflectance values at specific wavelengths, the percentage of the different forms of myoglobin (oxymyoglobin (OxyMb), deoxymyoglobin (Mb), and metmyoglobin (MetMb)) were calculated according to the method of Krzywicki (1979), modified by Lindahl et al. (2001).

For lipid oxidation analysis, the same samples as for colour measurements were used. Lipid oxidation was assessed by thiobarbituric acid-reactive substances measurement (TBARS) using the distillation method as described by Tarladgis et al. (1960) after adding the strong antioxidant butylated hydroxytoluene (BHT) and was expressed as µg malondialdehyde (MDA)/g meat. Lipid oxidation of LT samples was measured in duplicate after 8 days of storage.

Fatty acid analysis

Feed and meat samples were extracted using chloroform/methanol (2/1; vol/vol) (modified after Folch et al., 1957). Fatty acids were methylated as described by Raes et al. (2001) and analysed by gas chromatography (HP6890, Brussels, Belgium) on a CP-Sil88 column for fatty acid methyl esters (FAME) (100 m x 0.25 mm x 0.2 µm; Chrompack, The Netherlands). Peaks were identified based on their retention times, corresponding with standards (NuChek Prep., IL, USA; Sigma, Bornem, Belgium). Individual and total i.m. fatty acid content were quantitated after addition of the internal standard C19:0.

Taste panel

A taste panel was organised on the occasion of the agricultural fair Agriflanders (Ghent Expo, Belgium, 2005). Visitors of the fair were invited to taste 1 of 3 meat products (fermented sausage, cooked and dry cured ham) and were given a brief explanation about the aim and setup of the experiment. The participants (n = 830) were asked to score meat pieces for taste preference, taste intensity, and overall preference on a 1 to 5-scale (1 = dislike, weak; 5 = like, strong). Because of the impossibility of grilling meat at the fair, a supplementary taste panel, however much smaller, was organised at the laboratory to have taste panel data on grilled pork. For the GM taste panel evaluation, 30 people participated. All meat samples were served under red light to mask possible colour differences. The participants received 4 coded samples of 1 meat product (1,5 * 1,5 * 1,5 cm), 1 sample for each feeding group (= 3 samples) and a fourth sample as a replicate of 1 of the other 3 samples. The latter was done to test for the consistency of scoring. Data from panellists having tasted less than 3 pieces or not having tasted the replicates have been omitted. Also, panellists not scoring consistently (sum of the differences for scoring the 3 characteristics for the replicates being 7 or more), were excluded from statistical analysis. A total of 2644 observations (76.8%) were retained for the statistics. The contribution of the observations for DCH, CCH, FS, and GM, was 30.1, 29.3, 36.8, and 3.8% respectively.

Statistics

For all analyses performed on the fresh meat, a GLM with the fixed factors feeding group and gender was used. The interaction term was not significant ($P>0.05$). In a preliminary statistical analysis, slaughter group was added as a factor but was omitted in the

final statistical model because of lack of significance. Contrast analysis was performed to detect differences between feeding groups. The effect of the fatty acid profile of the diets given during both feeding phases was tested by the contrasts LL vs. BB, LL vs. FF, and BB vs. FF. The effect of duration of supplementation of linseed and fish oil was assessed by the following contrasts respectively: LL vs. (BL and FL) and FF vs. (BF, LF, and FL). The significance of the time of fish oil supplementation was tested by contrasting LF vs. FL. Effects of the feed fatty acid profile on the taste panel evaluations were analysed by One-Way ANOVA for each type of meat product separately using the Bonferroni *post hoc* comparison of means test. The analyses were performed using the statistical software package S-Plus for Windows (version 6.0).

RESULTS

Performance and carcass characteristics

Diet did not influence ADG (637.3 and 675.8 g/day in P1 and P2 respectively) or feed efficiency (on average 379 and 320 g gain/kg feed in P1 and P2 respectively). The mean (SD) carcass lean content was 59.6 (2.42)%, and was generally not different between feeding groups, except for a lower carcass lean content in group FF as compared to the groups FL and LL (58.3% for FF and 60.4% and 60.7% for FL and LL) ($P<0.05$).

Fatty acids

There was no difference for the total i.m. fatty acid content between the feeding groups (mean (SD) 1.41 (0.22) g/100g meat) with the lowest and greatest value for groups BL and FF respectively (Tables 2A.3 to 2A.5).

Table 2A.3 shows the effect of supplying a single fatty acid source during the whole fattening period on the fatty acid profile of the LT muscle (groups BB, LL, and FF). The total n-6 PUFA and LA proportion was similar in groups BB and LL. The basal diet gave rise to a greater arachidonic acid (C20:4n-6; AA) proportion as compared to linseed feeding ($P<0.05$). The proportion of total as well as individual n-6 PUFA was lower in the FF group as compared to the LL and BB groups ($P<0.05$) (except for LA being lower, however non-significantly ($P>0.05$), in FF as compared to LL).

The α-LNA proportion was greatest in the meat of group LL ($P<0.05$). The supply of α-LNA in this group also increased the proportion of EPA and DPA as compared to the basal feeding group ($P<0.05$). However, the DHA proportion did not differ between BB and LL. The total and LC n-3 PUFA proportion was greatest for group FF ($P<0.05$). The fish oil diet resulted in a 6-fold increased deposition of EPA and DHA in the LT as compared to the basal diet and a 3- and 5-fold increase of EPA and DHA respectively as compared to the linseed feeding. In the FF group, the DPA level was greater compared to group BB ($P<0.05$).

The effect of duration and time of linseed or fish oil supplementation on the fatty acid profile of the LT muscle is shown in Tables 2A.4 and 2A.5. The α-LNA incorporation was similar when feeding linseed during both phases or only during P2. For EPA and DPA, there was no difference between feeding the basal or linseed diet in P1 in combination with linseed

in P2. Feeding fish oil continuously as compared to fish oil feeding only in 1 phase, resulted in greater EPA and DHA proportions ($P<0.05$), but the proportion of DPA was not affected. When fish oil was fed either during P1 or P2, only the proportion of DHA was influenced, being greater when fish oil was administered during P2 ($P<0.05$).

Barrows had the greatest i.m. fatty acid content, together with the greatest total and individual SFA proportions (C12:0, C14:0, C16:0, and C18:0) ($P<0.05$). Only the proportion of C17:0 was greater for gilts (data not shown) ($P<0.05$). The proportions of the major MUFA, C18:1c9 and C16:1c, were greater for barrows (data not shown) ($P<0.05$). Total and individual n-6 (except for C22:4n-6) and n-3 PUFA were greater for gilts ($P< 0.001$) (Table 2A.6).

Table 2A.3. Fatty acid profile (g/100g FAME) of the LT muscle as influenced by a single fatty acid source during the whole fattening period (n = 12)

	BB[1]	LL[1]	FF[1]	SEM	LL-BB	LL-FF	BB-FF
C18:2n-6	11.2	10.4	8.84	2.35			*
C20:4n-6	2.90	2.38	1.65	0.77	*	*	*
C22:4n-6	0.39	0.29	0.15	0.16		*	*
n-6	15.1	13.7	11.1	3.24		*	*
C18:3n-3	0.55	1.24	0.47	0.40	*	*	
C20:5n-3	0.22	0.54	1.37	0.45	*	*	*
C22:5n-3	0.47	0.75	0.82	0.23	*		*
C22:6n-3	0.14	0.18	1.02	0.37		*	*
n-3	1.38	2.72	3.68	0.99	*	*	*
SFA[2]	34.5	34.0	35.5	2.06		*	
MUFA[3]	44.5	44.6	45.6	3.00			
PUFA[4]	16.8	16.7	15.1	3.67			
n-6/n-3[5]	11.0	5.23	3.03	2.64	*	*	*
P/S[6]	0.35	0.35	0.27	0.09		*	*
Total, g/100g	1.38	1.49	1.79	0.54			*

[1] B, L, F: basal, linseed and fish oil diet respectively, first and second letter indicating diet in phase 1 and 2 respectively
[2] SFA = C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0
[3] MUFA = C14:1 + C16:1t + C16:1c + C17:1 + C18:1
[4] PUFA = n-6 + n-3 + C20:2n-6
[5] n-6/n-3 ratio was calculated as (C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6)/(C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)
[6] P/S = (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0)

Table 2A.4. Fatty acid profile (g/100g FAME) of the LT muscle as influenced by duration of linseed supplementation and oil source during P1 (n = 12)

	LL[1]	BL[1]	FL[1]	SEM	LL-BL	LL-FL
C18:2n-6	10.4	11.6	10.6	2.35		
C20:4n-6	2.38	3.04	2.26	0.77	*	
C22:4n-6	0.29	0.46	0.23	0.16	*	
n-6	13.7	15.7	13.7	3.24		
C18:3n-3	1.24	1.23	1.12	0.40		
C20:5n-3	0.54	0.57	0.97	0.45		*
C22:5n-3	0.75	0.74	0.91	0.23		*
C22:6n-3	0.18	0.22	0.65	0.37		*
n-3	2.72	2.76	3.66	0.99		*
SFA[2]	34.0	33.0	34.0	2.06		
MUFA[3]	44.6	43.4	43.7	3.00		
PUFA[4]	16.7	18.8	17.6	3.67		
n-6/n-3[5]	5.23	5.79	3.74	2.64		*
P/S[6]	0.35	0.40	0.35	0.09		
Total, g/100g	1.49	1.08	1.32	0.54	*	

[1] B, L, F: basal, linseed and fish oil diet respectively, first and second letter indicating diet in phase 1 and 2 respectively
[2] SFA = C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0
[3] MUFA = C14:1 + C16:1t + C16:1c + C17:1 + C18:1
[4] PUFA = n-6 + n-3 + C20:2n-6
[5] n-6/n-3 ratio was calculated as (C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6)/(C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)
[6] P/S = (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0)

Table 2A.5. Fatty acid profile (g/100g FAME) of the LT muscle as influenced by duration and time of fish oil supplementation (n = 12)

	FF[1]	LF[1]	BF[1]	FL[1]	SEM	BF-FF	LF-FF	FL-FF	LF-FL
C18:2n-6	8.84	9.82	9.94	10.6	2.35			*	
C20:4n-6	1.65	2.04	2.45	2.26	0.77	*		*	
C22:4n-6	0.15	0.19	0.20	0.23	0.16				
n-6	11.1	12.5	13.0	13.7	3.24			*	
C18:3n-3	0.47	0.77	0.50	1.12	0.40		*	*	*
C20:5n-3	1.37	1.09	1.04	0.97	0.45	*	*	*	
C22:5n-3	0.82	0.83	0.71	0.91	0.23				
C22:6n-3	1.02	0.78	0.82	0.65	0.37	*	*	*	*
n-3	3.68	3.47	3.07	3.66	0.99	*			
SFA[2]	35.5	35.5	34.9	34.0	2.06			*	*
MUFA[3]	45.6	44.0	44.6	43.7	3.00				
PUFA[4]	15.1	16.3	16.4	17.6	3.67			*	
n-6/n-3[5]	3.03	3.63	4.25	3.74	2.64	*		*	
P/S[6]	0.27	0.30	0.30	0.35	0.09			*	
Total, g/100g	1.79	1.53	1.29	1.32	0.54	*		*	

[1] B, L, F: basal, linseed and fish oil diet respectively, first and second letter indicating diet in phase 1 and 2 respectively
[2] SFA = C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0
[3] MUFA = C14:1 + C16:1t + C16:1c + C17:1 + C18:1
[4] PUFA = n-6 + n-3 + C20:2n-6
[5] n-6/n-3 ratio was calculated as (C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6)/(C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)
[6] P/S = (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0)

Table 2A.6. Fatty acid profile (g/100g FAME) of the LT muscle as influenced by gender (n = 42)

	Barrow	Gilt	P	SEM
C18:2n-6	9.27	11.4	< 0.001	2.35
C20:4n-6	2.08	2.69	< 0.001	0.77
C22:4n-6	0.26	0.28	0.546	0.16
n-6[1]	12.1	15.0	< 0.001	3.24
C18:3n-3	0.79	0.89	0.036	0.40
C20:5n-3	0.74	0.91	0.008	0.45
C22:5n-3	0.68	0.82	0.001	0.23
C22:6n-3	0.48	0.60	< 0.001	0.37
n-3[2]	2.68	3.22	< 0.001	0.99
Total, g/100g	1.58	1.23	0.001	0.54

[1]n-6 = C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6
[2]n-3 = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3

Meat quality traits

Mean (SD) drip loss was 4.6 (0.7)% and was not influenced by a particular diet. pH after 24 h *post mortem* was 5.7 (0.04) and was not different between groups. PQM measured in the loin and ham was 5.1 (0.3) and 6.7 (1.0) respectively and was also not influenced by the dietary treatments.

For the colour of fresh LT expressed as L*, a*, and b* values (and SI and hue values calculated from those data), groups were not different throughout the storage period (data not shown). Up to day 3 of chill storage, colour stability expressed as MetMb% and OxyMb% was not different between groups. From day 3 until the end of the chill storage, group LL behaved different, although not significantly, as compared to the other groups having lower MetMb% and greater OxyMb% (Figure 2A.2).

Lipid oxidation values as assessed by the TBARS test were not influenced by dietary treatment or gender. On day 8 of storage, TBARS values were lower than 0.2 µg MDA/g meat (data not shown).

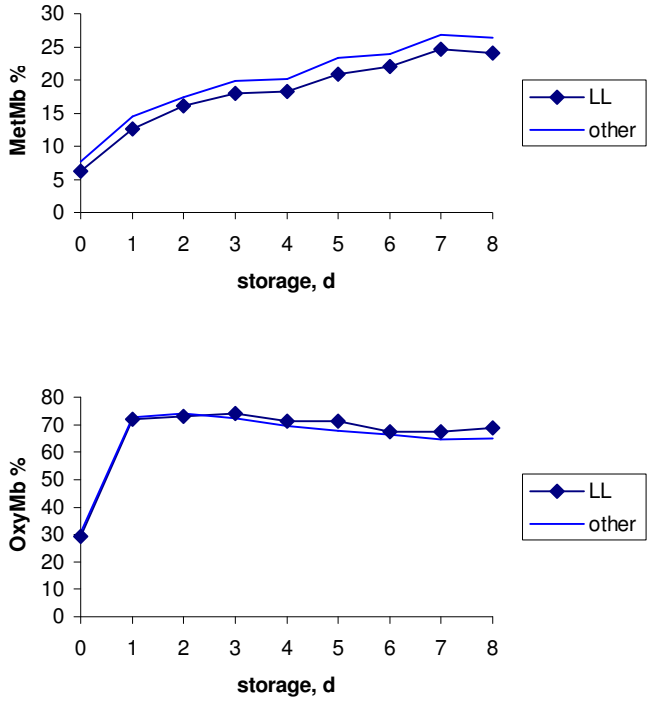

Figure 2A.2. % of metmyoglobin (MetMb) (upper panel) and oxymyoglobin (OxyMb) (lower panel) in the *longissimus thoracis* during chill storage as influenced by feeding group (◆ for the LL group: linseed diet in both phases; - for the mean of the other groups)

Taste panel

For the CCH, the scores for all characteristics were greater for group BB > group LL > group FF ($P<0.001$). For the taste intensity and taste preference of the FS, group BB again scored higher compared to group FF ($P = 0.004$ and $P<0.001$ respectively), whereas the score for the LL group was intermediate. Overall preference for the FS was in the order of group BB > group LL > group FF ($P<0.001$). For the DCH, the taste characteristics did not reveal significant differences between the groups. For the GM, scores for all taste characteristics were greater for the BB group compared to the FF group, whereas results for the LL group were intermediate ($P<0.05$) (Table 2A.7).

Table 2A.7. Scores for taste characteristics as influenced by feeding oil source

	BB[1]	LL[1]	FF[1]	SEM	P
cooked cured ham (n = 776)					
taste preference	3.65[a]	3.30[b]	2.98[c]	1.14	0.001
taste intensity	3.62[a]	3.14[b]	2.94[c]	1.06	< 0.001
overall preference	3.58[a]	3.22[b]	2.99[c]	1.12	< 0.001
fermented sausage (n = 972)					
taste preference	3.29[a]	3.13[a]	2.88[b]	1.16	< 0.001
taste intensity	3.38[a]	3.20[ab]	3.14[b]	1.09	0.004
overall preference	3.35[a]	3.09[b]	2.88[c]	1.2	< 0.001
dry cured ham (n = 796)					
taste preference	3.23	3.29	3.26	1.19	0.868
taste intensity	3.31	3.31	3.31	1.14	0.964
overall preference	3.15	3.24	3.22	1.17	0.768
grilled meat (n = 100)					
taste preference	3.75[a]	3.34[ab]	3.17[b]	1.16	0.025
taste intensity	3.58[a]	3.10[ab]	3.06[b]	1.09	0.039
overall preference	3.73[a]	3.31[ab]	3.06[b]	1.16	0.004

[1] B, L, F: basal, linseed and fish oil diet respectively, first and second letter indicating diet in phase 1 and 2 respectively

[a,b,c] Within a row, means without a common superscript letter differ (P<0.05)

DISCUSSION

Performance and carcass characteristics

Average daily gain and feed efficiency were within normal ranges for fattening pigs and were not influenced by diet. This is consistent with literature recommending a maximum of 5% of fish oil in the diet to prevent depression of feed intake and growth of the pigs (Van Oeckel and Boucque, 1992).

Fatty acids

The lack of effect of dietary fatty acid source on the i.m. fatty acid content accords with other work on pigs receiving diets differing in total fat and (or) fatty acid composition (Allee et al., 1972; St. John et al., 1987; Rhee et al., 1988; Leskanich et al., 1997; Scheeder et al., 2000).

The slightly lower dietary LA supply in the FF group resulted in a significantly lower n-6 PUFA proportion in the meat. In case of similar dietary supply of n-6 fatty acids (groups LL and BB), an increased n-3 PUFA supply in the LL group was not accompanied by a decreased deposition of n-6 fatty acids, except for AA. These results were partly in agreement with Riley et al. (2000) where an equal amount of LA together with increasing levels of dietary α-LNA did not induce differences in total n-6 fatty acids deposited in the LT. However, in contrast to our study, no differences in any of the individual n-6 PUFA were found by Riley et al. (2000). The greater AA proportion in meat of the animals on the basal diet compared to the linseed or fish oil diet confirms the inhibiting effect of higher α-LNA proportions on elongation and desaturation of LA to its long-chain metabolites and the competition between AA and LC n-3 PUFA for incorporation into PL. The increased supply of α-LNA in group LL yielded a significant increase in the proportion of EPA and DPA as compared to group BB. However, increasing DHA proportion in meat was only achieved when fish oil was included in the animals diet. Hence, this study supports other literature findings that specific dietary supply of DHA is needed to increase the DHA content of pork (Romans et al., 1995a,b; Ahn et al., 1996; Riley et al., 2000; Raes et al., 2004). The DHA formation seems to be strictly metabolically regulated and cannot be substantially influenced by dietary supply of the precursors. The desaturation and elongation chain of n-3 fatty acids

seems to block at the level of DPA. On the other hand, Enser et al. (2000) observed an increased i.m. DHA level in pork by feeding linseed at a level of 4 g α-LNA/kg feed. However, the increase was small (0.38 and 0.45 g/100 g of total fatty acids for the control and linseed fed group, respectively). Also, incorporation of α-LNA and elongation to EPA and DPA was more efficient in their research compared to our study. Recently, also Kralik et al. (2006) found that adding rapeseed oil to pigs' diets (at a level of 3% and 6%) increased the DHA content in muscle tissue, which in turn supports the fact that pigs can synthesize DHA *in vivo*.

The total and LC n-3 PUFA proportion was significantly greater for group FF > group LL > group BB. It seems that the incorporation efficiency of EPA and DHA from fish oil into muscle was much higher than the one of α-LNA from linseed. A level of 2.31% EPA and 3.53% DHA in the lipid fraction of the fish oil diet resulted in 1.37% and 1.02% of EPA and DHA respectively in the muscle lipid fraction, whereas 16% α-LNA in the lipid fraction of the linseed diet only resulted in 1.24% α-LNA in the muscle lipid fraction. This can be explained by 3 factors. First, there might be an effect of the matrix in which the fatty acids were embedded, leading to a potentially higher digestibility of the fatty acids in the fish oil compared to those in crushed linseed. Secondly, β-oxidation is faster for α-LNA compared to its long-chain products which are more selectively incorporated into PL within permanent cell structures (Leyton et al., 1987). When a fatty acid is preferentially oxidized for energy, this could account for low incorporation rates observed with this particular fatty acid. Thirdly, after absorption α-LNA has to compete with LA for incorporation and desaturation and elongation to its long-chain metabolites in the tissues (Mohrhauer and Holman, 1963).

The increased level of DPA after fish oil supplementation as compared to the basal diet, is in accordance with Lauridsen et al. (1999a). However, other studies consistently showed an increased EPA and DHA deposition, but no effect on the DPA concentration in pigs fed fish oil diets (Irie and Sakimoto, 1992; Morgan et al., 1992; Leskanich et al., 1997). DPA can be formed during elongation of EPA or by retroconversion of DHA (Sprecher et al., 1995).

The α-LNA incorporation was similar when feeding linseed during both phases or only during P2. This indicates that, in case of linseed supplementation during P2, the α-LNA proportion remained unchanged irrespective of the oil source during the previous phase (P1). Also, for EPA and DPA there was no difference between feeding the basal or linseed diet in P1 in combination with linseed in P2. It seems that by supplying linseed for 6 to 9 weeks

before slaughter at the level in this experiment, the saturation plateau for the synthesis of the long-chain metabolites was reached.

The greatest EPA and DHA proportions were obtained when fish oil was fed during both phases. When supplied during either P1 or P2, the proportion of DHA was greatest with fish oil feeding in P2. To our knowledge, the alternating supply of fish oil and linseed (oil) has only been studied in fish (Bell et al., 2004). Feeding fish oil in at least 1 phase caused the n-6/n-3 ratio to be conform the recommendation of < 5 by Voedingsaanbevelingen voor België (2003). The n-6/n-3 ratio is known to be highly influenced by the fatty acid composition of the diet. The range of the P/S ratio was narrow (0.27 to 0.4) and for all groups lower than the recommended minimal value of 0.7 by Voedingsaanbevelingen voor België (2003).

Meat quality traits

Despite the significant effects of dietary fat source on the i.m. fatty acid composition, none of the measured meat quality traits (pH, drip loss, colour, lipid oxidation) was significantly different between dietary treatments. The reason for the distinct colour stability for group LL from day 3 on is unclear. For monitoring the negative quality effects of high PUFA diets in pork, several thresholds have been suggested for the maximum level of PUFA in feed. Warnants et al. (1996) proposed the threshold for feed to be 18g PUFA/kg feed. For all the feeds in this experiment, PUFA were below this level. Feeding linseed at a similar level to our trial did not induce negative effects on pork quality as seen by Sheard et al. (2000). However, it should be kept in mind that these threshold levels are dependent on other factors besides the PUFA levels in the feed, e.g. duration of supplementation, dietary antioxidant levels, storage and processing conditions of the meat, muscle and animal differences.

Taste panel

Overall, organoleptic quality differences between feeding groups were small and the general appreciation was good, on average 3 or higher on a scale of 1 to 5. This is in agreement with several investigations indicating that deleterious organoleptic changes can be limited even in the presence of high tissue PUFA levels (Irie and Sakimoto, 1992; Morgan et al., 1992; Leskanich et al., 1994). Also Leskanich et al. (1997) found that organoleptic evaluation of cooked chops and sausages revealed no major differences in sensory quality for

different treatments including fish oil. Also, a maximum fishmeal inclusion of 10% (8 to 10% crude fat) has been suggested for fresh pork (Valaja et al., 1992). However, the meat products from the fish oil fed animals were generally scored lowest in our study. This lower appreciation might be partly due to Belgian consumers not being used to taste such n-3 PUFA enriched meat products and thus reflect the influence of eating habits. The lack of effect of diet on sensory evaluation of DCH was unexpected.

Although no effect of diet on TBARS values of the chill stored meat was observed in our study, untrained panellists apparently distinguished meat products from fish oil fed pigs compared to meat from pigs fed on other dietary fat sources. Similarly, Bryhni et al. (2002) failed to observe a link between TBARS values and sensory evaluation scores in meat from fish oil fed pigs. However, contrary to our research, fish oil in the diet (0.4%) increased TBARS values of loin without affecting sensory evaluation scores.

IMPLICATIONS

The effects of duration and time of feeding a specific fat source on the muscle fatty acid composition is dependent on the fatty acids considered. For the deposition of α-linolenic acid and its conversion to long-chain metabolites in muscle following the supply of linseed, only the last phase before slaughter was determinant. When supplying fish oil, the greatest eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) proportions were found in case of a continuous supply throughout the fattening period, and levels of DHA but not EPA were lower when fish oil was fed during the first fattening phase followed by linseed feeding before slaughter. Neither meat quality traits (drip loss, pH,…), nor lipid or colour oxidation were influenced by dietary oil source or duration of supplementation. Nevertheless, meat products from pigs fed on this level of fish oil (1.2%) were less appreciated by an untrained taste panel compared to meat products from animals fed on the basal or linseed diet. However, the eating quality differences were not pronounced.

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CHAPTER 2B

ALTERATION OF PORK FATTY ACID PROFILE BY CULINARY PRACTICE

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CHAPTER 2B

EFFECT OF PAN-FRYING IN DIFFERENT CULINARY FATS ON THE FATTY ACID PROFILE OF PORK

Abstract - This study was set up to determine how pan-frying either without culinary fat or with different culinary fats (polyunsaturated fatty acids (PUFA) enriched culinary fat, olive oil and margarine) affects the fatty acid (FA) composition of pork. The meat samples (*longissimus thoracis* (LT)) originated from pigs fed different dietary fat sources (animal fat, soybean oil or linseed oil) and thus had a different fatty acid composition before frying. Pan-frying resulted in considerable increases in the meat total fatty acid content, although this was not always significant and highly variable despite standardisation of the frying process. The fatty acid composition of the pan fried meat tended to become similar to that of the culinary fat used, and the extent of changes in the content of a particular fatty acid was relative to the fatty acid gradient from the culinary fat to the meat. However, this was also dependent on the culinary fat used, since frying in olive oil appeared to affect the fatty acid composition of the meat more than frying in the other culinary fats. Differences in fatty acid composition of meat resulting from different animal feeding treatments remained unchanged after pan-frying without fat, they became smaller after frying in margarine and PUFA enriched culinary fat, whereas frying in olive oil largely masked the initial fatty acid profile differences. Long-chain PUFA (LCPUFA) in the meat were not significantly lost by the frying process, but their proportion was influenced by the uptake of the culinary fat.

Key Words: pan-frying, culinary fat, fatty acids, pork

INTRODUCTION

The regular consumption of polyunsaturated fatty acids (PUFA), and more specifically n-3 PUFA, is considered to be important because of their roles in the prevention of numerous diseases common to Western populations, including cardiovascular and inflammatory disorders, cancer and stroke (Din et al., 2004; Kris-Etherton et al., 2003; Ruxton et al., 2004). For this reason, many efforts have been made to increase the n-3 PUFA concentration in pig tissues by dietary manipulation (see review e.g. Raes et al., 2004; Wood et al., 2003). However, the goal is hampered by the higher susceptibility of the n-3 fatty acids (FA) towards oxidative deterioration in pig meat (Monahan et al., 1992b; Nurnberg et al., 1999) with a possible reduced sensory and nutritional quality of such foods and because of processing problems due to 'soft' fat. Studies on improving the fatty acid composition of pork usually do not take into account the influence of culinary processing, which often includes a heat treatment and a fat addition. Pan-frying is a common way of culinary preparation of pork in Western countries. During pan-frying, exchange of fatty acids between the food item and the culinary fat takes place (Nawar, 1984; Ramirez et al., 2005; Sioen et al., 2006a), and this exchange might differ according to the culinary fat used. In addition, PUFA are subject to oxidation during heating. The n-3 long-chain PUFA (n-3 LCPUFA) (e.g. EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3)) are the most heat-labile and oxidation-sensitive fatty acids. Therefore, a determination of the extent of their decrease during pan-frying is important because this might reflect a reduction of the nutritional value of n-3 LCPUFA enriched meat.

In this study, it was aimed to investigate how pan-frying, either without or with a culinary fat affected 1) the fatty acid composition of the pan fried pork and 2) the differences in fatty acid profile of the pork as achieved by different feeding strategies.

MATERIALS AND METHODS

Samples

The meat used for this experiment originated from a pig trial in which the effect of the dietary fat source (soybean oil (SO): C18:2n-6 rich, linseed oil (LO): C18:3n-3 rich or animal fat (AF): rich in SFA) (added at 5g/kg weight) on the fatty acid composition of the meat was investigated. The trial lasted for 16 weeks and the mean start weight of the pigs was 30 (SD 8.5) kg. Pigs were slaughtered at a mean (SD) live weight of 121 (14) kg. The carcasses had a mean (SD) meat yield of 61.7 (4.4)%. pH-drop was normal (*i.e.* mean (SD) pH_{40min}=6.15 (0.25) and pH_{24h}=5.53 (0.10)). The LT muscle (*longissimus thoracis*; 6-14th rib) of 2 animals of each group was sampled 24h *post mortem*, cut into 2.5 cm thick slices, vacuum packed and stored at -18 °C until the moment of pan-frying or fatty acid analysis.

The choice of culinary fats was conditioned by their claimed fatty acid composition, *i.e.* PUFA enriched culinary fat, and olive oil and a margarine being high in monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) respectively. The fatty acid composition of the culinary fats is given in Table 2B.1.

Frying procedure

The thawing of the meat took 1h during which the meat was turned every 5 minutes to prevent the thawing moisture from being located at 1 side of the meat and thus preventing a homogeneous heat transfer through the sample. Meat slices were cut into pieces of 5x5 cm before pan-frying. For all the frying experiments preheated Tefal frying pans (Ø = 20 cm) were used. After each frying process, the pans were cleaned with a scraper and a kitchen paper to recover the fat as much as possible and were washed with detergent. During the frying process, the temperature was monitored continuously and only samples with a final core temperature of 70-72 °C were withdrawn for analysis.

The amount of culinary fat used for frying was exactly 10% of the weight of the raw meat. At the start of the frying process, the meat slice was fried for 1 minute on each side and was subsequently turned every 30 seconds until the desired core temperature of 70-72 °C was reached. Both meat and fat were weighed after frying and stored at -18 °C until fatty acid analysis. For each treatment (combination of one of four culinary fat treatments and one of

three animal feeding backgrounds), the pan-frying process was repeated until 4 samples with the desired final core temperature were obtained.

Analytical procedures

Before extraction of the total lipids for fatty acid analysis, all samples were minced. Extraction of the total lipids was done using chloroform/methanol (2/1; v/v) according to the method of Folch et al. (1957) and methylated as described by Raes et al. (2001). Nonadecanoic acid (C19:0) was used as internal standard. The fatty acid methyl esters (FAME) were analysed by gas chromatography as described by Raes et al. (2001). Briefly, a GC HP 6890 (Agilent, Belgium) with a CP-Sil88 column for FAME (100 m x 0.25 mm x 0.20 µm) (Chrompack, The Netherlands) was used with an injector temperature of 250 °C, a detector temperature of 280 °C, H_2 as carrier gas and the following temperature program: 150 °C for 2 min, followed by an increase of 1.5 °C/min to 200 °C, then 5 °C/min to 215 °C. Peaks were identified by comparing the retention times with those of the corresponding standards (Sigma, Belgium).

Recoveries

For each fatty acid, the recovery was calculated as follows:

$$\%\,\text{recovery} = 100 \cdot (C_F \cdot g_F + C_{fatres} \cdot g_{fatres}) / (C_R \cdot g_R + C_{fat} \cdot g_{fat})$$

C_R and C_F is the concentration of the fatty acid (g/100g) in the raw sample (R) and the fried sample (F) respectively and C_{fat} and C_{fatres} is the concentration of the fatty acid (g/100g) in the culinary fat before (fat) and after pan-frying (fatres) respectively. These concentrations are multiplied by the mass of the raw or fried sample (g_R and g_F) and the mass of the culinary fat before or after pan-frying (g_{fat} and g_{fatres}).

Statistical analyses

Within feeding group and type of culinary fat, the effect of frying on the change in individual fatty acid content was evaluated using a paired t-test ($P<0.05$). The effects of type of culinary fat and feeding group on the fatty acid composition of the meat samples after frying were compared separately by one-way analysis of variance (ANOVA), because the interaction between feeding group and culinary fat was highly significant. Comparison of

means was done by the *post hoc* Duncan test. Statistical analyses were performed using SPSS 10.0 software package (SPSS Inc., USA).

RESULTS AND DISCUSSION

Fatty acid profile of fresh culinary fats

In Table 2B.1 the fatty acid composition of the culinary fats is shown. In all tables only the major fatty acids are listed, representing more than 90% of total fatty acid content of the samples.

Table 2B.1. Fatty acid composition of the different fresh and fried culinary fats (g/100g of product) (n=2)

	analysed fresh			analysed fried		
	PUFA enriched	olive oil	margarine	PUFA enriched	olive oil	margarine
SFA	8	8	25	5	7	17
MUFA	28	52	31	19	40	22
PUFA	38	19	12	24	3	9
n-6	34	14	10	21	2	7
n-3	4	4	3	2	0.2	2
Total	75	80	69	49	50	49

SFA=C12:0+C14:0+C16:0+C17:0+C18:0+C20:0+C22:0
MUFA=C14:1+C16:1c+C16:1t+C17:1+C18:1t9+C18:1t11+ C18:1c9+C18:1c11
n-6=C18:2n-6+C18:3n-6+C20:3n-6+C20:4n-6+C22:4n-6
n-3=C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3
PUFA=n-6+n-3+C20:2n-6

Olive oil was rich in MUFA and margarine was rich in SFA. The n-6 PUFA content was markedly higher in the PUFA enriched culinary fat compared to the other culinary fats. In all culinary fats the n-3 PUFA content was equal.

Fatty acid profile of culinary fats after frying

The pan-frying process altered the fatty acid content and composition of the culinary fats (Table 2B.1). This might be important for the fatty acid intake profile in case the residual fats are consumed together with the fried meat.

For all types of culinary fat, the fatty acid content was reduced to a level of about 50g/100g of culinary fat. The fat absorption in the meat together with oxidation of the fatty acids during pan-frying, and uptake of water from the meat during frying could be responsible for these changes in fatty acid content.

The fatty acid composition of olive oil had profoundly changed after pan-frying compared to the other culinary fats, *i.e.* only 25% of the initial PUFA proportion and moreover hardly anything of the initial n-3 PUFA proportion remained. Together with this decline in PUFA, the SFA and MUFA proportion increased. For the other culinary fats, the fatty acid composition did not change as profoundly by the frying process.

Fatty acid composition of the raw meat as affected by animal feeding

The fatty acid content of the raw LT of the three feeding groups is shown in Table 2B.2 to 2B.5. Quantitatively, the most important fatty acids in raw LT were oleic acid (C18:1c9), palmitic acid (C16:0), stearic acid (C18:0) and linoleic acid (C18:2n-6). In the meat of the two animals of the SO group, a considerably lower total fatty acid content was found as compared to the meat of the other groups. This should not be interpreted as an effect of the feeding oil, but is due to random animal variability. For this study, two animals were randomly selected from a larger group of animals, wherein the average meat fatty acid content was similar across feeding groups (1.21, 1.24 and 1.31g/100g for the AF, LO and SO group respectively). The SFA proportion was similar for the three dietary groups (36-38% of FAME), the proportion of MUFA was significantly lower for the SO group (25% compared to 35-36% in other groups) and the proportion of PUFA was significantly higher in meat of the SO fed animals (27% compared to 12-15% in the other groups) (Figure 2B.1-2B.3). The lower total fatty acid content, together with a higher LA supplementation via the feed for the SO group, explains the higher AA (arachidonic acid, C20:4n-6) proportion in the meat.

The n-6/n-3 PUFA ratio in the meat was 9, 2 and 11 for the SO, LO and AF group respectively, and was thus only for the LO group below the recommended value of 5 (Voedingsaanbevelingen voor België, 2003).

Fatty acid composition of fried meat

A) Pan-frying without fat

The fatty acid profile of the pork samples after pan-frying without fat is shown in Table 2B.2. Pan-frying without fat resulted in an increase in the absolute amount of each of the reported fatty acids as a consequence of water loss during the frying process. PUFA, and in particular LCPUFA, were still detectable after the frying process. This reflects an incomplete oxidation of the LCPUFA during frying, if at all there is a measurable loss of fatty acids due to oxidation. It also implies that there is no selective leaching of these LCPUFA out of the meat. This is in accordance with the finding that modifications in fatty acid composition of fried samples are due to the exchange between the neutral lipids and free fatty acids of the frying oils and the meat. The PL fraction of the meat, in which LCPUFA are mainly present, remained unchanged after frying due to the fact that this lipid fraction makes up the cell membrane structure in which fatty acids are not easily exchanged (Ramirez et al., 2005).

Table 2B.2. Mean values (standard deviation) (mg/100g product unless stated otherwise) and recoveries (%) of the fatty acid content in pork fried without fat (n=2 and 4 before and after frying respectively) (ND: not detected)

Fatty acid	Pork AF				Pork SO				Pork LO			
	Before frying	After frying	P	Recovery (%)	Before frying	After frying	P	Recovery (%)	Before frying	After frying	P	Recovery (%)
C12:0	1.6 (0.2)	3.1 (0.9)	0.024	151.4 (35.2)	0.4 (0.2)	0.9 (0.4)	0.079	201.1 (37.6)	1.9 (0.9)	1.8 (0.9)	0.488	83.1 (20.4)
C14:0	24.8 (1.9)	43.6 (8.9)	0.022	133.4 (23.4)	5.2 (2.4)	7.5 (4.3)	0.215	132.5 (19.5)	18.4 (7.1)	25.4 (14.6)	0.118	116.7 (37.3)
C16:0	453.2 (39.7)	725.3 (122.3)	0.022	118.3 (12.1)	148.2 (48.5)	214.7 (97.0)	0.199	135.0 (16.8)	410.8 (134.7)	548.8 (279.6)	0.121	111.5 (34.3)
C18:0	208.0 (10.1)	291.6 (58.4)	0.092	99.0 (4.4)	79.4 (30.5)	122.7 (63.6)	0.168	140.4 (20.1)	202.0 (55.3)	276.9 (115.8)	0.092	114.5 (33.6)
C18:1c9	636.0 (79.5)	1129.1 (285.3)	0.023	133.9 (25.1)	170.8 (67.3)	253.9 (136.4)	0.203	135.7 (20.5)	595.6 (221.6)	812.2 (462.9)	0.127	114.2 (36.6)
C18:2n-6	157.9 (14.2)	252.5 (39.8)	0.039	123.9 (24.4)	130.4 (23.7)	176.3 (42.4)	0.193	128.9 (22.1)	159.7 (3.4)	223.3 (55.6)	0.119	108.8 (29.6)
C18:3n-3	6.3 (1.2)	10.2 (2.3)	0.104	139.2 (54.1)	4.3 (0.9)	5.3 (1.8)	0.461	119.8 (28.6)	51.1 (7.1)	75.9 (27.6)	0.104	119.2 (38.2)
C20:4n-6	26.1 (2.0)	45.9 (11.3)	0.031	126.6 (27.3)	33.0 (7.4)	45.8 (9.0)	0.073	127.7 (6.5)	23.0 (2.1)	34.7 (7.7)	0.091	116.8 (33.6)
C20:5n-3	2.2 (1.9)	3.0 (2.2)	0.632	68.3 (24.8)	1.6 (0.4)	1.7 (0.3)	0.695	101.4 (39.7)	15.8 (1.2)	18.9 (4.1)	0.214	93.2 (20.6)
C22:5n-3	4.2 (0.4)	6.7 (1.6)	0.027	117.0 (24.1)	6.1 (1.6)	6.9 (1.6)	0.338	99.1 (11.2)	13.5 (2.0)	17.4 (3.5)	0.252	98.9 (28.7)
C22:6n-3	1.3 (0.4)	2.6 (1.1)	0.101	118.8 (26.8)	2.2 (0.8)	2.4 (1.0)	0.566	98.3 (9.0)	2.4 (0.6)	3.3 (0.5)	0.148	102.4 (25.6)
n-6/n-3	11.18	12.00			8.89	9.58			2.20	2.03		
Total (g/100g)	1.79 (0.15)	2.92 (0.57)	0.026		0.67 (0.17)	0.97 (0.40)	0.174		1.70 (0.48)	2.28 (1.07)	0.118	

B) Pan-frying in different culinary fats

The fatty acid content of the pork samples before and after pan-frying in the different culinary fats is shown in Table 2B.3 to 2B.5. Frying in the three culinary fats resulted in an increased total fatty acid content of the meat (g/100g LT), irrespective of the feeding group. This increase was not significant for the meat fried in olive oil, due to high variations in fatty acid uptake of the meat (Table 2B.4). The increase of the fatty acid content in the meat fried in the PUFA enriched culinary fat or the margarine was significant and may be explained by two mechanisms, *i.e.* the loss of water during frying and the absorption of culinary fat. The increase in the absolute amount of EPA, DPA and DHA after frying can only be explained by water loss during frying, because none of the culinary fats contains any of these fatty acids.

The absorption of culinary fat, and especially fatty acids of the culinary fat into the meat was different for the three culinary fats used. As can be seen in Figure 2B.1, frying in PUFA enriched culinary fat caused a decline in the proportion of SFA, while the proportion of MUFA remained unchanged and the proportion of PUFA markedly increased. Particularly the proportion of linoleic (C18:2n-6) and linolenic acid (C18:3n-3) was increased. Compared to the other frying processes, frying in the PUFA enriched culinary fat resulted in the highest proportion of PUFA and the highest P/S ratio in the meat.

Pan-frying in olive oil resulted in a decline in the proportion of SFA and an increased proportion of MUFA in the fried meat, while the effect on PUFA was variable (Figure 2B.2). The proportion of oleic acid (C18:1c9) in meat was highest after pan-frying in olive oil (Table 2B.4). Pan-frying in margarine hardly changed the fatty acid profile of the meat. It caused a slight decline in the proportion of SFA and PUFA, while the proportion of MUFA slightly increased (Figure 2B.3). A switch in the profile of the SFA was observed after pan-frying in margarine, *i.e.* the proportion of lauric (C12:0) and myristic acid (C14:0) was increased whereas the proportion of palmitic (C16:0) and stearic acid (C18:0) was decreased (Table 2B.5).

The results indicate that the fatty acid composition of the fried meat tended to be similar to the one of the culinary fat that was used. This reflects the development of a fatty acid gradient equilibrium between the culinary fat and the samples being fried. These results agree with findings previously reported by Sioen et al. (2006a) for pan fried fish (salmon and cod), by Candela et al. (1998) for deep-fried poultry, pork and sardines and by Ramirez et al. (2005) for deep-fried pork.

After frying and irrespective of the type of culinary fat used, differences in fatty acid profile of the meat obtained by animal feeding strategies could still be observed.

Table 2B.3. Mean values (standard deviation) (mg/100g product unless stated otherwise) and recoveries (%) of the fatty acid content in pork fried in PUFA enriched culinary fat (n=2 and 4 before and after frying respectively) (ND: not detected)

Fatty acid	PUFA enriched (g/100g)	Pork AF				Pork SO				Pork LO			
		Before frying	After frying	P	Recovery	Before frying	After frying	P	Recovery	Before frying	After frying	P	Recovery
C12:0	0.04 (0.0002)	1.6 (0.2)	2.8 (0.6)	0.030	57.2 (30.5)	0.4 (0.2)	1.0 (0.3)	0.013	70.4 (27.0)	1.9 (0.9)	2.0 (0.3)	0.736	87.9 (5.6)
C14:0	0.06 (0.001)	24.8 (1.9)	37.9 (8.7)	0.075	102.4 (26.1)	5.2 (2.4)	8.4 (2.7)	0.052	91.2 (7.0)	18.4 (7.1)	26.6 (6.9)	0.073	109.8 (12.9)
C16:0	4.15 (0.08)	453.2 (39.7)	696.0 (141.9)	0.056	90.0 (22.7)	148.2 (48.5)	247.1 (43.4)	0.019	74.2 (18.0)	410.8 (134.7)	639.9 (89.4)	0.047	106.2 (5.6)
C18:0	3.06 (0.04)	208.0 (10.1)	306.6 (58.7)	0.060	67.5 (19.8)	79.4 (30.5)	137.7 (29.8)	0.017	66.1 (17.4)	202.0 (55.3)	317.5 (17.4)	0.057	96.8 (2.5)
C18:1c9	24.6 (0.62)	636.0 (79.5)	1164.2 (248.5)	0.029	81.8 (38.6)	170.8 (67.3)	428.4 (81.0)	0.003	60.2 (26.2)	595.6 (221.6)	1072.4 (114.6)	0.018	97.8 (8.8)
C18:2n-6	33.2 (0.59)	157.9 (14.2)	472.8 (87.1)	0.006	26.8 (27.2)	130.4 (23.7)	379.7 (42.3)	0.002	52.6 (26.4)	159.7 (3.4)	593.4 (152.1)	0.037	90.5 (10.2)
C18:3n-3	3.85 (0.04)	6.3 (1.2)	35.6 (8.0)	0.004	22.3 (26.4)	4.3 (0.9)	28.0 (4.3)	0.002	46.9 (24.4)	51.1 (7.1)	125.6 (12.3)	0.019	90.1 (10.4)
C20:4n-6	ND	26.1 (2.0)	49.2 (3.3)	0.001	150.9 (17.6)	33.0 (7.4)	49.1 (11.4)	0.008	127.9 (6.7)	23.0 (2.1)	38.9 (14.9)	0.188	151.5 (34.3)
C20:5n-3	ND	2.2 (1.9)	1.8 (0.5)	0.682	113.9 (75.0)	1.6 (0.4)	2.5 (0.6)	0.007	203.9 (31.7)	15.8 (1.2)	25.3 (6.4)	0.138	139.7 (27.3)
C22:5n-3	ND	4.2 (0.4)	6.3 (1.3)	0.049	60.5 (13.7)	6.1 (1.6)	8.0 (2.1)	0.151	64.8 (10.9)	13.5 (2.0)	22.8 (5.8)	0.052	134.5 (57.0)
C22:6n-3	ND	1.3 (0.4)	2.2 (0.5)	0.008	179.7 (87.5)	2.2 (0.8)	3.1 (1.4)	0.055	207.3 (189.4)	2.4 (0.6)	4.3 (1.7)	0.104	191.0 (98.1)
n-6/n-3	8.62	11.18	9.63			8.89	9.56			2.20	3.42		
Total (g/100g)	75.0 (1.37)	1.79 (0.15)	3.20 (0.61)	0.027		0.67 (0.17)	1.51 (0.12)	0.001		1.70 (0.48)	3.32 (0.10)	0.026	

Table 2B.4. Mean values (standard deviation) (mg/100g product unless stated otherwise) and recoveries (%) of the fatty acid content in pork fried in olive oil (n=2 and 4 before and after frying respectively) (ND: not detected)

Fatty acid	Olive oil (g/100g)	Pork AF				Pork SO				Pork LO			
		Before frying	After frying	P	Recovery (%)	Before frying	After frying	P	Recovery (%)	Before frying	After frying	P	Recovery (%)
C12:0	ND	1.6 (0.2)	1.5 (1.1)	0.879	921.4 (492.3)	0.4 (0.2)	3.1 (3.0)	0.185	878 (502)	1.9 (0.9)	4.1 (1.2)	0.053	109 (36.5)
C14:0	0.04 (0.01)	24.8 (1.9)	21.2 (18.5)	0.714	475.8 (513.2)	5.2 (2.4)	49.3 (51.3)	0.192	1911.1 (2125.3)	18.4 (7.1)	42.3 (18.6)	0.035	194.7 (51.9)
C16:0	4.57 (0.11)	453.2 (39.7)	552.5 (361.0)	0.605	124.9 (72.9)	148.2 (48.5)	941.2 (817.1)	0.154	258.2 (134.6)	410.8 (134.7)	924.0 (366.6)	0.031	140.3 (36.1)
C18:0	2.18 (0.04)	208.0 (10.1)	283.4 (159.6)	0.408	111.9 (62.0)	79.4 (30.5)	424.7 (356.6)	0.162	212.5 (120.1)	202.0 (55.3)	437.2 (165.2)	0.037	126.3 (33.5)
C18:1c9	44.8 (0.52)	636.0 (79.5)	1180.4 (665.2)	0.179	68.6 (36.2)	170.8 (67.3)	1707.4 (1371.4)	0.117	116.3 (27.4)	595.6 (221.6)	1787.1 (699.8)	0.020	86.3 (51.7)
C18:2n-6	12.8 (0.25)	157.9 (14.2)	266.3 (53.1)	0.036	50.4 (31.5)	130.4 (23.7)	335.5 (125.5)	0.034	55.6 (22.7)	159.7 (3.4)	332.3 (55.6)	0.010	31.9 (14.0)
C18:3n-3	4.13 (0.09)	6.3 (1.2)	50.6 (54.1)	0.206	38.2 (39.3)	4.3 (0.9)	13.2 (7.2)	0.077	28.7 (24.0)	51.1 (7.1)	101.0 (34.5)	0.048	22.7 (7.62)
C20:4n-6	0.16 (0.003)	26.1 (2.0)	40.2 (4.6)	0.022	94.5 (13.7)	33.0 (7.4)	64.4 (5.4)	0.010	114.0 (12.6)	23.0 (2.1)	42.6 (6.8)	0.019	89.7 (16.7)
C20:5n-3	ND	2.2 (1.9)	12.3 (12.0)	0.154	196.5 (136.9)	1.6 (0.4)	3.2 (0.4)	0.016	34.9 (15.0)	15.8 (1.2)	17.7 (10.9)	0.752	50.1 (39.9)
C22:5n-3	ND	4.2 (0.4)	13.9 (8.8)	0.108	83.1 (53.8)	6.1 (1.6)	10.6 (1.2)	0.014	52.3 (7.86)	13.5 (2.0)	21.4 (2.3)	0.016	73.4 (10.3)
C22:6n-3	ND	1.3 (0.4)	3.1 (1.2)	0.025	259.9 (145.9)	2.2 (0.8)	4.8 (0.5)	0.010	189.1 (68.8)	2.4 (0.6)	4.0 (0.7)	0.084	132.7 (51.2)
n-6/n-3	3.14	11.18	4.37			8.89	14.29			2.20	2.62		
Total (g/100g)	79.6 (12.1)	1.79 (0.15)	2.71 (1.46)	0.277		0.67 (0.17)	4.00 (3.08)	0.126		1.70 (0.48)	4.17 (1.48)	0.091	

Table 2B.5. Mean values (standard deviation) (mg/100g product unless stated otherwise) and recoveries (%) of the fatty acid content in pork fried in margarine (n=2 and 4 before and after frying respectively) (ND: not detected)

Fatty acid	margarine (g/100g)	Pork AF				Pork SO				Pork LO			
		Before frying	After frying	P	Recovery (%)	Before frying	After frying	P	Recovery (%)	Before frying	After frying	P	Recovery (%)
C12:0	6.73 (0.21)	1.6 (0.2)	99.3 (20.9)	0.003	38.3 (33.0)	0.4 (0.2)	65.0 (41.5)	0.053	13.8 (14.2)	1.9 (0.9)	42.3 (15.8)	0.014	65.6 (10.1)
C14:0	2.73 (0.10)	24.8 (1.9)	87.9 (14.3)	0.004	50.3 (32.6)	5.2 (2.4)	35.0 (19.0)	0.045	17.6 (18.9)	18.4 (7.1)	42.4 (20.2)	0.030	75.2 (8.06)
C16:0	11.0 (0.41)	453.2 (39.7)	994.8 (136.7)	0.005	77.7 (18.8)	148.2 (48.5)	358.4 (143.8)	0.044	52.7 (2.79)	410.8 (134.7)	626.5 (367.6)	0.126	84.5 (14.9)
C18:0	3.93 (0.14)	208.0 (10.1)	406.5 (49.4)	0.006	84.3 (19.4)	79.4 (30.5)	171.0 (98.4)	0.112	55.7 (10.6)	202.0 (55.3)	285.9 (140.8)	0.112	84.0 (13.7)
C18:1c9	27.0 (0.98)	636.0 (79.5)	1696.8 (292.1)	0.005	82.2 (26.2)	170.8 (67.3)	573.5 (248.6)	0.033	98.5 (45.0)	595.6 (221.6)	1042.5 (670.3)	0.113	80.9 (14.8)
C18:2n-6	9.31 (0.40)	157.9 (14.2)	433.9 (53.4)	0.003	129.1 (126.7)	130.4 (23.7)	279.2 (93.2)	0.069	38.9 (8.13)	159.7 (3.4)	294.0 (62.9)	0.028	75.4 (11.2)
C18:3n-3	2.55 (0.10)	6.3 (1.2)	48.6 (10.8)	0.004	76.4 (52.4)	4.3 (0.9)	31.6 (15.9)	0.044	19.6 (10.5)	51.1 (7.1)	91.7 (37.2)	0.068	73.5 (14.8)
C20:4n-6	0.12 (0.004)	26.1 (2.0)	57.1 (4.7)	0.001	130.1 (6.9)	33.0 (7.4)	50.0 (8.5)	0.041	89.4 (23.5)	23.0 (2.1)	37.4 (6.9)	0.047	111.0 (26.0)
C20:5n-3	ND	2.2 (1.9)	2.9 (0.2)	0.488	263.2 (185.5)	1.6 (0.4)	2.7 (0.8)	0.057	129.2 (38.4)	15.8 (1.2)	21.6 (3.5)	0.038	224.3 (27.3)
C22:5n-3	ND	4.2 (0.4)	9.2 (3.4)	0.051	93.0 (30.9)	6.1 (1.6)	8.1 (1.3)	0.037	64.3 (10.7)	13.5 (2.0)	19.9 (2.7)	0.078	90.6 (22.9)
C22:6n-3	ND	1.3 (0.4)	4.8 (4.3)	0.220	335.9 (384.9)	2.2 (0.8)	3.0 (1.1)	0.168	113.2 (40.2)	2.4 (0.6)	3.8 (0.1)	0.029	117.0 (26.1)
n-6/n-3	3.70	11.18	7.21			8.89	7.17			2.20	2.46		
Total (g/100g)	69.0 (2.65)	1.79 (0.15)	4.36 (0.62)	0.005		0.67 (0.17)	1.79 (0.71)	0.044		1.70 (0.48)	2.81 (1.19)	0.048	

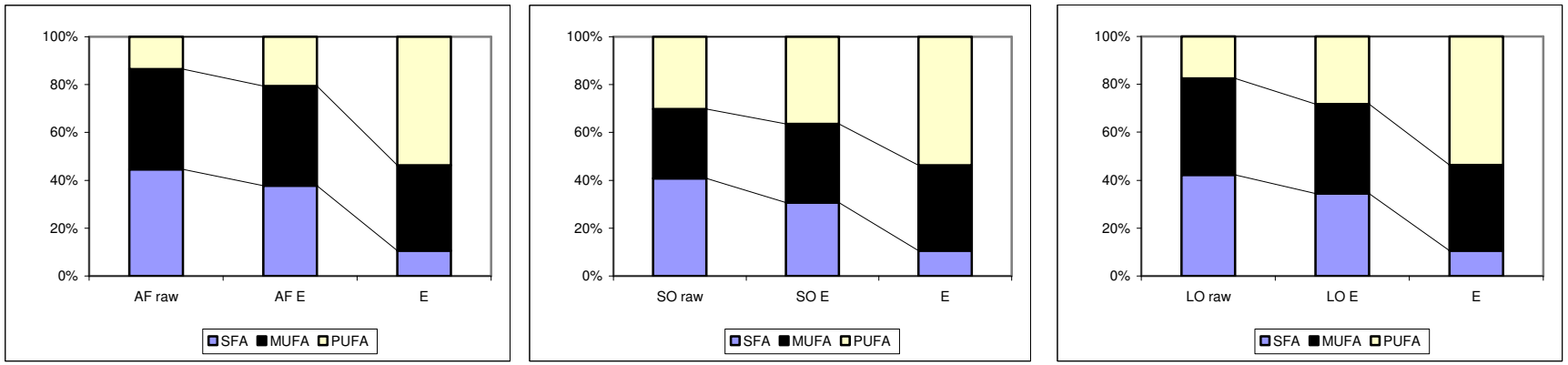

Figure 2B.1. SFA, MUFA and PUFA proportions of pork fried in PUFA enriched culinary fat (E) (left to right: raw meat, fried meat, culinary fat)

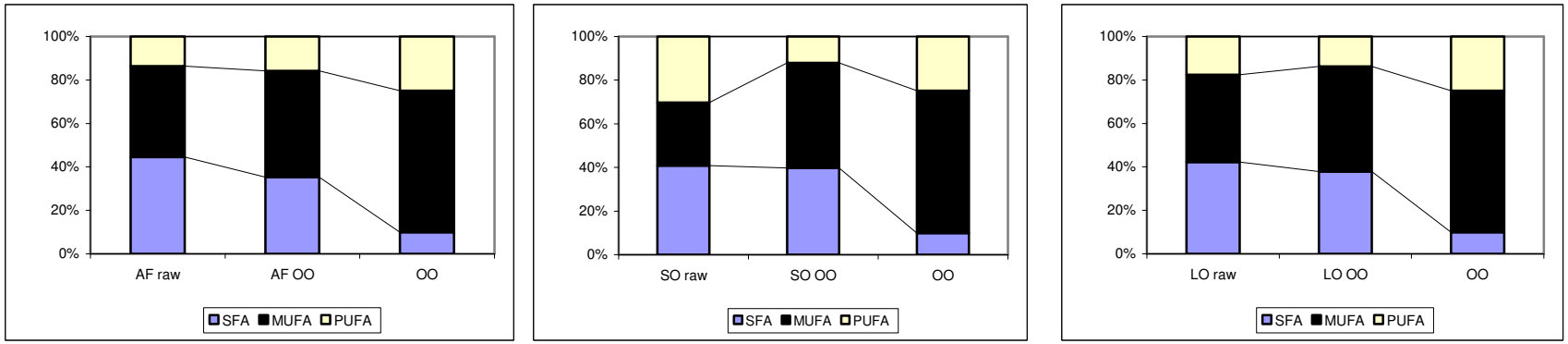

Figure 2B.2. SFA, MUFA and PUFA proportions of pork fried in olive oil (OO) (left to right: raw meat, fried meat, culinary fat)

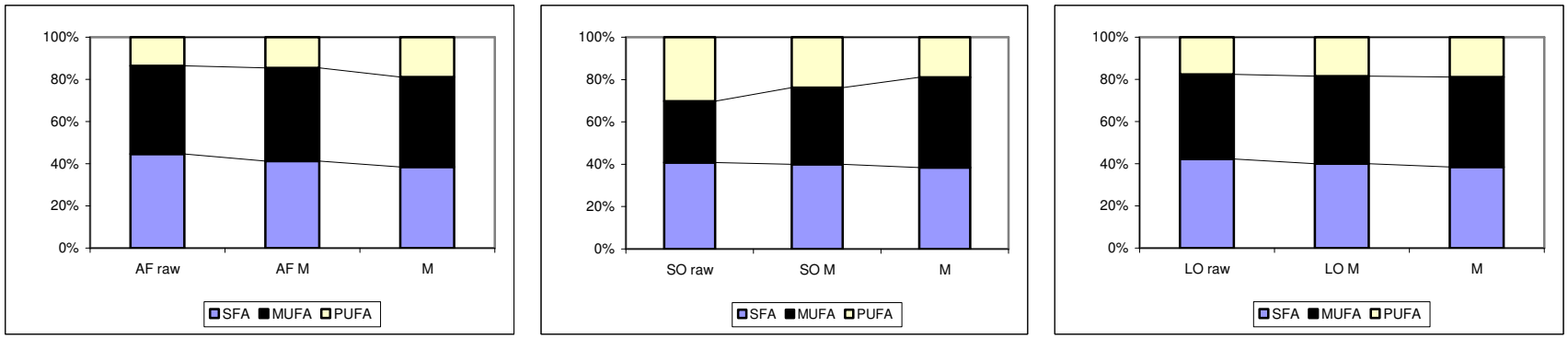

Figure 2B.3. SFA, MUFA and PUFA proportions of pork fried in margarine (M) (left to right: raw meat, fried meat, culinary fat)

Recoveries of fatty acids

The weight losses of the meat samples due to frying in the different culinary fats were not dependent on the type of culinary fat and, moreover, were equal to the weight loss after frying without fat (± 22% of the raw meat weight). This weight loss may consist of mainly water losses. However, the mass recovery of the fats after frying differed very much between the different culinary fats (96%, 121% and 91% for PUFA enriched culinary fat, olive oil and margarine respectively). A mass recovery for olive oil exceeding 100% means that olive oil retained more water lost by the meat during frying compared to the other culinary fats.

In Table 2B.2 to 2B.5 the recoveries of the individual fatty acids after the frying process are presented. This recovery was subject to a large variation. The recoveries below 100% can be explained by fat losses and analytical errors. The fat losses can be caused by oil spraying during the frying process, by incomplete recovery of the fat residue in the pan after the frying process or by oxidation processes. Accurate recoveries are more difficult to obtain when analysing relatively low fatty acid concentrations. Another factor causing variability in the recoveries is due to inherent small differences in fatty acid content between those meat slices that were used for fatty acid analysis of the raw meat and those used for frying and analysing the fatty acid profile after frying. Although originating from the same animal and the same muscle, small anatomical differences cannot be excluded. Recoveries of fatty acids after frying without fat are usually closer to 100% and the variability in these recoveries is smaller compared to the recoveries obtained after frying in the culinary fats, which illustrates the additional errors caused by fat losses in case of using a culinary fat. It is clear that errors are cumulating in the recoveries as they are calculated here, and that it is difficult to deduce any meaningful interpretation.

CONCLUSION

The fatty acid composition of the pan fried meat tended to become similar to that of the culinary fat. The extent of the increase or decrease of a particular fatty acid during frying was relative to the fatty acid gradient from the culinary fat to the meat. Pan-frying without fat is preferable when aiming at a lower fat uptake in the human diet and preserved the differences in initial fatty acid profile of the meat obtained from different feeding strategies. Frying in the PUFA enriched culinary fat increased the PUFA proportion in the meat but had a negative effect on the n-6/n-3 ratio for the SO and LO meat. Frying in margarine gave rise to a significantly higher proportion of the atherogenic lauric (C12:0) and myristic acid (C14:0), although resulting in a better n-6/n-3 ratio compared to frying in PUFA enriched culinary fat. After frying and irrespective of the type of culinary fat used, differences in fatty acid profile of the meat obtained by animal feeding strategies could still be observed. LCPUFA were not significantly lost by the frying process, but their proportion was influenced by the uptake of the culinary fat.

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CHAPTER 3

EFFECT OF ANTIOXIDANT SUPPLEMENTATION ON THE OXIDATIVE STABILITY OF PORK

CHAPTER 3A

EFFECT OF DIETARY ANTIOXIDANT SUPPLEMENTATION ON THE OXIDATIVE STABILITY OF PORK

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and

Haak, L., Raes, K., Van Dyck, S., and De Smet, S. (2007). Effect of dietary rosemary and alpha-tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. Meat Science (accepted).

CHAPTER 3A1

EFFECT OF DIETARY ANTIOXIDANT AND FATTY ACID SUPPLY ON THE OXIDATIVE STABILITY OF FRESH AND COOKED PORK

Abstract - The effect of dietary oil (linseed or soybean oil) and antioxidant treatment (α-tocopheryl acetate (ATA; 40 ppm) versus a cocktail (AOC; 200 ppm): α-tocopheryl acetate + rosemary + citric acid + gallic acid) on colour, lipid and protein oxidation of fresh and processed pork was investigated. No effect of oil source on different parameters of oxidation was seen. No effect of antioxidant treatment on colour stability of fresh *longissimus thoracis* (LT) or cooked cured ham (CCH) was observed. For both antioxidant treatments, lipid oxidation in fresh LT and CCH was well controlled during display. However, lipid oxidation increased significantly in pre-frozen uncured cooked meat under aerobic conditions. No unambiguous effect of antioxidant treatment on protein oxidation was observed. There seemed to be no clear link between colour, protein and lipid oxidation. At the dose used in this study, no additional or synergistic effects of the extra components of the AOC on the different oxidation parameters was found.

Key Words: antioxidant, fatty acid, pork

INTRODUCTION

Oxidative processes lead to the degradation of lipids and proteins (including pigments) and are one of the primary mechanisms of quality deterioration in meat and meat products. They cause loss of flavour, colour and nutritive value and limit the shelf-life of meat and meat products (Kanner, 1994). The appearance of fresh retail meat is a major determinant of its appeal to consumers and, consequently, sales of the product. The oxidative stability of meat depends upon the balance of anti- and prooxidants and the composition of oxidation substrates including polyunsaturated fatty acids (PUFA), cholesterol, proteins and pigments (Bertelsen et al., 2000). Currently, there is considerable emphasis on increasing the dietary n-3 fatty acids as they are claimed to be health promoting (Ruxton et al., 2004) and could counteract some of the unfavourable physiological effects of the abundance of n-6 fatty acids, the major PUFA class in our diet. For this reason, many efforts have been made to increase the n-3 fatty acid concentration in pig tissues by dietary manipulation (Raes et al., 2004). However, the goal is hampered by the higher susceptibility of the n-3 fatty acids towards oxidative deterioration in pig meat (Monahan et al., 1992b; Nurnberg et al., 1999) with a possible reduced sensory and nutritional quality of such foods. Therefore, not only the oil source and the inclusion level but also adequate supplementation with antioxidants must be considered when formulating PUFA-rich pig diets (Jakobsen, 1999; Kamal-Eldin and Yanishlieva, 2002).

Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA), can inhibit lipid oxidation in feed but they exhibit toxic properties (Kahl and Kappus, 1993) resulting in strict regulations over their use in foods. These findings, together with increased resistance to the use of synthetic additives, have increased interest in the antioxidant properties of naturally occurring substances (Pokorny, 1991; Gordon, 1996). In meat quality studies, α-tocopherol (AT) received considerable attention in recent years (Lee et al., 1998). AT is generally regarded as an acceptable supplement and is the primary lipid soluble antioxidant in biological systems which acts by disrupting the chain of lipid oxidation in cell membranes, thus preventing the formation of lipid hydroperoxides (Buckley et al., 1995). When incorporated into

animal diets as α-tocopheryl acetate (ATA), it is a highly effective antioxidant to extend the shelf-life of foods from animal origin (Faustmanet al., 1989; Gray et al., 1996; Jensen et al., 1998b). Proper physiological placement of AT in membranes is, however, as crucial as its concentration for providing optimal stability (Mitsumoto et al., 1993; Lauridsen et al., 1997). Thus, dietary delivery of ATA is recommended as the most efficacious means for obtaining the colour stabilising effect (Faustman et al., 1998). Accordingly, nowadays there is a strong tendency towards isolating organic antioxidants from natural sources as alternatives so a major field of application of herbs is the protection of animals and their products against oxidation (Wenk, 2003). Amongst those, rosemary has been reported to possess antioxidative properties when administered in the diet (Basmacioğlu et al., 2004; Lopez-Bote et al., 1998). In addition, the use of cocktails of antioxidants could have a superior effect compared to single antioxidants. Two or more antioxidants together can act synergistically, *i.e.* affect oxidation to a higher extent than the sum of the contributions from each single antioxidant (Duthie, 1999; McCarthy et al., 2001).

The objective of this study was to gain a better understanding of the effects of dietary modification of the oil source (linseed oil, rich in C18:3n-3 versus soybean oil, rich in C18:2n-6) and of the antioxidant source on oxidation of fresh and cooked pork. The effect of ATA was tested individually as well as in combination with rosemary and other antioxidative components which might act as secondary supporters of the primary antioxidant AT (ATA versus a cocktail (AOC) consisting of ATA, rosemary, citric acid and gallic acid). Instead of focussing on one oxidation parameter, the oxidative stability was assessed by measuring colour, lipid and protein oxidation, and by an estimate of the total reducing capacity.

MATERIALS AND METHODS

Experimental setup

Twenty four crossbred pigs (boar x sow line Rattlerow Seghers, Belgium) with a mean (SD) live weight of 26.5 (1.78) kg were randomly assigned to 8 feeding groups. The experiment was organized in a 4*2*2 factorial design including 4 different oil combinations, 2 different antioxidant sources and the factor gender (barrow/sow). The experiment was carried out during a 16 weeks feeding period, divided in 2 periods of 8 weeks (phase 1 and 2). The diets were grain-based and formulated to an equal energy and protein supply. The vitamin-mineral supplement contained no synthetic antioxidants (e.g. BHT, BHA, ethoxyquin). In the diets, 2% of linseed or soybean oil was included. The oils were refined and no antioxidants were added to stabilise the oils. According to the oil source, the animals were divided into 4 groups: 2 groups were continuously supplied with one oil source, *i.e.* soybean oil (group SS) or linseed oil (group LL) and 2 groups where the oil source was switched after the first phase (group SL and LS). Within each oil source treatment, one group was supplemented with 40 ppm α-tocopheryl acetate (ATA) (Rovimix E-50 Adsorbate, DSM, Deinze, Belgium) and the other group with 200 ppm of an antioxidant complex (AOC) (40 ppm ATA + 40 ppm citric acid + 64 ppm rosemary + 56 ppm gallic acid; Kemin Europa NV, Herentals, Belgium). The antioxidants were added to the oils. Fatty acid composition of the experimental diets is shown in Table 3A1.1. All pigs were fed *ad libitum*.

Table 3A1.1. Fatty acid composition of experimental diets

	phase 1 (8 weeks)		phase 2 (8 weeks)	
g/100g fatty acids	S	L	S	L
C14:0	0.70	0.77	1.41	1.38
C16:0	14.8	13.0	14.5	12.2
C18:0	3.22	3.44	3.46	3.36
C18:1	21.5	20.3	20.4	18.6
C18:2n-6	49.2	33.5	48.2	34.6
C18:3n-3	5.32	22.8	4.83	21.7
Sum (g/100g feed)	4.43	4.02	3.70	3.49

S: soybean oil; L: linseed oil

During the experiment one animal of the SS group supplemented with the AOC had to be slaughtered due to feet problems. At the end of the experiment, the animals were electrically stunned, slaughtered and exsanguinated at the slaughterhouse of the Department of Animal Production (Ghent University, Belgium). Before sampling, carcasses were chilled for 24 h. Mean (SD) live weight at slaughter and cold carcass weight was 110 (14.6) kg and 86.1 (8.02) kg respectively and was not different between treatments. Mean (SD) pH in *longissimus thoracis* (LT) (6-7th rib) at 40 min and 24 h *post mortem* was 5.98 (0.15) and 5.46 (0.07) respectively and was not different between treatments.

Samples

Fresh meat: The LT (starting from the 7th rib, left carcass side) was sampled at 24 h *post mortem* (day 0) and sliced into 2.5 cm thick chops which were then either wrapped in an oxygen permeable polyethylene film and placed in an illuminated chill cabinet (illuminance of 900 lux, temperature 3°C) for 8 days to perform oxidative stability measurements or immediately vacuum packed and stored at -18°C for future fatty acid analysis.

Cooked meat after frozen storage: A 2.5 cm thick chop of the LT was taken 24 h *post mortem* and stored in a vacuum bag at -18°C. After 10 months of frozen storage,

samples were thawed and cooked in a water bath at 70°C for 40 min. The cooked samples were wrapped in an oxygen permeable polyethylene film and placed in the illuminated chill cabinet for 7 days.

Cooked cured ham (CCH) manufacturing: From each feeding group, 2 fresh hams were cured and cooked in 2 different batches on 2 consecutive days, resulting in a total of 16 hams. At 48 h *post mortem*, 3 muscles from the left hind leg (*rectus femoris, biceps femoris* and *semimembranosus*) were excised and removed of skin, tendons and external fat tissue. The muscles were pumped to 112% of their green weight, using a multi-needle brine injector (Rühle PR15). The brine solution was composed of 1.89% nitrite curing salt, 0.5% dextrose, 0.1% ascorbate and 0.1% flavourings. Samples were cooked in an oven at 70°C until a core temperature of 67°C was reached. After cooling, a 2.5 cm thick chop of the ham was taken and wrapped in an oxygen permeable polyethylene film and placed in the illuminated chill cabinet for 7 days.

Subcutaneous fat: At 24 h *post mortem*, subcutaneous fat was taken at the same height of the samples taken in the LT and was stored vacuum-packed at -18°C.

Analyses

Colour measurements

Reflectance spectra, every 10 nm, between 400 and 700 nm and colour coordinates (CIE L*a*b* colour system 1976) were measured on fresh LT samples every day until day 8 of storage with a HunterLab Miniscan spectrocolorimeter (light source of D65, standard observer of 10°, 45°/0° geometry, 1 in. light surface, white standard). On day 0, the measurement was done 30 min after cutting. For the CCH, colour measurements were performed more frequently during the first hours after cutting (30 min, 2 h, 3 h and 4.5 h), in view of the fading of the cooked cured pigment during the first hours of display. Until 7 days of storage, L*a*b* values were measured daily and reflectance was measured every two days.

The results were expressed as lightness (L*), redness (a*), yellowness (b*), hue value ($\tan^{-1}$ b*/a*) and saturation index or chroma ($(a^{*2} + b^{*2})^{1/2}$). By means of reflectance values, the percentage of the different forms of myoglobin (oxymyoglobin

(OxyMb), deoxymyoglobin (Mb) and metmyoglobin (MetMb)) was calculated according to the Krzywicki method (1979), modified after Lindahl et al. (2001). The ratio R650/R570 was calculated to estimate the concentration of the cooked cured pigment nitrosylhemochrome (NH). By considering this concentration, fading of the CCH colour was followed (Erdman and Watts, 1957).

Lipid oxidation

Lipid oxidation was assessed by TBARS (2-thiobarbituric acid-reactive substances) measurement using the distillation method as described by Tarladgis et al. (1960) and expressed as µg MDA (malondialdehyde) /g tissue. Lipid oxidation of fresh LT samples was measured after 2, 5 and 8 days of storage in the chill cabinet. After 8 months of frozen storage, the LT samples that had been stored in the illuminated chill cabinet for 8 days were again analysed for TBARS on day 0 (immediately after thawing) and on day 8 of storage in the chill cabinet. Cooked pre-frozen LT samples and CCH samples were both analysed after 1 and 7 days of storage in the chill cabinet. Subcutaneous fat was analysed after 8 months of frozen storage on day 0 and again after 10 months of frozen storage and display at 23°C for 3 days.

Protein oxidation

Protein oxidation analyses (by carbonyl and thiol method) were performed on the raw LT and the CCH samples. The same samples as used for the TBARS measurement were used.

The measurement of protein carbonyls following their covalent reaction with DNPH was done according to Mercier et al. (1998). This assay involves the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH), which leads to the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone product. The total carbonyl content, expressed as nmol DNPH incorporated/mg protein was quantified by a spectrophotometric assay at 370 nm.

In addition, protein oxidation was assessed by measuring free thiol groups (Batifoulier et al., 2002). This method relies on the incubation of a meat homogenate with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), also called Ellman's reagent, followed by a

spectrophotometrical measurement at 412 nm of the free thiol groups (nmol SH-groups/mg protein).

Total reducing capacity (TRC)

Different methods have been described in literature as an overall estimate of the antioxidative capacity of tissue or blood samples. One of those is the method described by Lee et al. (1981) which relies on the reducing power of meat and implies the reduction of ferric (Fe^{3+}) to ferro (Fe^{2+}) ions. We used this method as an additional measure of the oxidative status.

The decline in concentration of ferric ions can be followed spectrophotometrically at 420 nm. TRC is expressed as the difference in absorbance between 1mM of $K_3Fe(CN)_6$ and the meat sample where $K_3Fe(CN)_6$ has been added to the same concentration. The higher this difference, the higher the reducing power of the meat. TRC was measured on LT samples of day 2 and 8 and on CCH samples of day 1 and 7.

Fatty acid composition

Before extracting the lipids from the muscle using chloroform/methanol (2/1; v/v) (modified after Folch et al. (1957)), samples were minced. Fatty acids were methylated as described by Raes et al. (2001) and analysed by gas chromatography (HP6890, Brussels, Belgium) on a CP-Sil88 column for FAME (100 m x 0.25 mm x 0.2 µm; Chrompack, The Netherlands). Peaks were identified based on their retention times, corresponding with standards (NuChek Prep., IL, USA; Sigma, Bornem, Belgium).

α-tocopherol content

The AT content was determined according to the method of Desai (1984) on fresh LT samples of day 2, on CCH samples of day 1 and on fat tissue. After saponification and hexane extraction, all samples were analysed by reversed phase HPLC (Supelcosil LC 18 (25 cm*4.6mm*5µm) column) fitted with UV detection (292 nm). The eluting solvent was methanol/water (97/3; v/v) at a flow rate of 2ml/min. The AT content of the tissue was determined by comparison of peak areas with those obtained for a standard solution of dl-AT. The results are expressed as µg AT/g tissue.

Statistical analysis

Data were analysed using Paired Samples T-test for the effect of frozen storage. The General Linear Model procedure was used for testing the effect of the fixed factors oil and antioxidant source and gender and their 2-way interaction terms (SPSS for Windows, version 11.0).

RESULTS

Results of analyses for CCH always refer to the *rectus femoris* unless otherwise stated. Data of other muscles in the CCH are not shown because of similarity in the results.

For all analyses (except for fatty acid profile of fresh LT), data for the effect of gender are not shown and only discussed in the section 'results' when significant.

Colour oxidation (Tables 3A1.2 and 3A1.3)

From day 0 to day 1, the a* value of the meat increased significantly and then afterwards remained unaltered, with the exception of day 4 where the a* value was significantly lower compared to all the other days ($P<0.001$). Only on day 0 for the fresh meat, a significant effect of oil source (higher for SS and SL) and gender (higher for barrows) ($P<0.05$) on the a* value was observed. The b^* value of fresh meat was only significantly higher for group SS compared to the other oil groups on day 0 ($P<0.05$). Besides, no effect of gender, antioxidant or oil source was observed on the L^*, a^* and b^* values during display of fresh meat and CCH (data of L* and b* values not shown).

During the display of fresh meat, a significant decline in hue value from day 0 to day 1 and from day 5 to day 6 was noted ($P<0.001$). No effect of antioxidant source, oil source or gender was observed on the hue value of the fresh meat. In the CCH, the hue value increased significantly up to day 2, followed by a non significant increase from day 2 to day 3 and by a significant increase from day 3 to day 4, and remained unchanged until day 7 ($P<0.001$). In the *semimembranosus* of the ham, gilts had a significantly increased hue value on day 0, 1 and 4 ($P<0.05$) and also on day 5 there was a similar trend. Oil or antioxidant source did not influence the hue value for the hams.

Only on day 0, there was a significant effect of gender ($P<0.01$) and oil source on chroma for the fresh meat, while antioxidant source had no effect. No clear effects of antioxidant source, oil source or gender were observed on the chroma for the CCH.

In the fresh meat, antioxidant source had no effect on the % of the different myoglobin forms (data only shown for MetMb%). Oil source had a significant effect on MetMb% from day 0 to day 2 on, with the highest % for the SS group ($P<0.05$).

Throughout the display period of the fresh meat, barrows had the lowest Mb% and the highest MetMb% ($P<0.05$ in case of significance).

The predominant pigment responsible for the pink colour of pasteurised cured meat products is nitrosylhemochrome. For the CCH, none of the factors antioxidant source, oil source or gender had a considerable influence on the oxidation of this cooked cured meat pigment. Next to the wavelengths needed to calculate nitrosylhemochrome (as mentioned in the methods section), the CCH spectrum did not reveal differences between the treatments.

A significant increase for ΔR was observed from day 0 to day 1 ($P<0.05$) which corresponds to blooming of myoglobin at this time and the accompanying increase in redness (a*) towards day 1. No significant differences were noted for the ΔR value as caused by the oil or antioxidant treatment. Gender had a significantly higher ($P<0.01$) ΔR value on day 0 and 1 for barrows which corresponds with the higher a* value and the higher OxyMb% in the meat for barrows on those days.

Table 3A1.2. Influence of antioxidant source on colour parameters

Fresh meat *longissimus thoracis*																
	a*				hue				chroma				MetMb%			
day	ATA	AOC	P	SEM	ATA	AOC	P	SEM	ATA	AOC	P	SEM	ATA	AOC	P	SEM
d0	6.5	6.5	0.987	0.26	67.1	66.5	0.578	0.66	16.5	16.2	0.463	0.29	15.7	15.6	0.910	0.55
d1	9.0	9.6	0.398	0.43	62.1	60.9	0.481	0.78	19.1	19.8	0.336	0.27	18.4	17.3	0.308	1.12
d4	6.8	7.5	0.512	0.62	63.5	61.9	0.582	1.83	15.2	15.8	0.402	0.45	25.8	25.3	0.786	1.46
d8	7.8	8.3	0.603	0.65	54.7	52.4	0.504	2.16	13.4	13.6	0.770	0.43	34.0	33.9	0.941	1.40
Cooked cured ham *rectus femoris*																
	a*				hue				chroma				NH%			
h	ATA	AOC	P	SEM	ATA	AOC	P	SEM	ATA	AOC	P	SEM	ATA	AOC	P	SEM
0.5h	12.8	11.8	0.547	1.43	33.9	38.4	0.477	4.58	15.5	15.0	0.608	0.91	2.27	2.19	0.927	0.42
3h	8.7	8.0	0.410	0.67	45.9	50.2	0.417	4.03	12.5	12.5	0.416	0.05	1.81	1.81	0.937	0.04
d1	7.1	6.2	0.203	0.43	56.2	61.7	0.143	3.97	12.8	12.8	0.909	0.23	1.75	1.70	0.731	0.53
d4	5.9	5.2	0.236	0.39	60.7	63.9	0.271	2.20	12.0	11.7	0.629	0.22	-	-		
d7	5.2	5.2	0.947	0.45	62.1	63.3	0.565	2.29	11.2	11.7	0.044	0.16	1.47	1.41	0.045	0.002

- no data available on that day

ATA: α-tocopheryl acetate; AOC: antioxidant cocktail

Table 3A1.3. Influence of oil source on colour parameters

Fresh meat *longissimus thoracis*																								
	a*						hue						chroma						MetMb%					
day	SS	SL	LS	LL	P	SEM	SS	SL	LS	LL	P	SEM	SS	SL	LS	LL	P	SEM	SS	SL	LS	LL	P	SEM
d0	8.4^{b}	6.3^{ab}	5.4^{a}	5.9^{a}	0.016	0.26	63.3	66.9	69.3	67.6	0.058	0.66	18.7^{b}	15.9^{a}	15.1^{a}	15.6^{a}	0.012	0.29	18.1^{b}	15.5^{a}	14.4^{a}	14.5^{a}	0.042	0.55
d1	10.2	9.4	8.4	9.2	0.622	0.43	60.5	61.0	63.1	61.4	0.761	0.78	20.6	19.4	18.6	19.1	0.40	0.27	21.8^{b}	17.0^{a}	16.8^{a}	15.8^{a}	0.021	1.12
d4	7.1	7.4	6.6	7.4	0.918	0.62	63.3	61.7	63.4	62.2	0.949	1.83	15.8	15.6	14.8	15.7	0.808	0.45	30.5	24.6	23.9	23.4	0.133	1.46
d8	7.7	8.1	7.9	8.5	0.905	0.65	55.4	53.2	53.0	52.5	0.928	2.16	13.5	13.5	13.1	13.9	0.817	0.43	37.7	33.7	32.6	31.8	0.194	1.40
Cooked cured ham *rectus femoris*																								
	a*						hue						chroma						NH%					
h	SS	SL	LS	LL	P	SEM	SS	SL	LS	LL	P	SEM	SS	SL	LS	LL	P	SEM	SS	SL	LS	LL	P	SEM
0.5h	12.4	11.8	12.4	12.4	0.978	1.43	36.8	38.5	35.5	34.9	0.971	4.58	15.4	15.0	15.2	15.1	0.968	0.91	2.24	1.87	2.34	2.30	0.832	0.42
3h	8.5	8.3	8.1	8.5	0.979	0.67	47.9	48.6	47.4	48.7	0.998	4.03	12.6^{ab}	12.7^{ab}	12.0^{a}	12.9^{b}	0.030	0.05	1.81	1.67	1.87	1.83	0.756	0.04
d1	7.2	8.2	6.4	5.5	0.147	0.43	58.3	50.9	58.8	63.7	0.138	3.97	13.6	13.0	12.4	12.4	0.11	0.23	1.76	1.93	1.61	1.71	0.789	0.53
d4	6.1	6.3	4.6	5.4	0.196	0.39	61.1	57.8	66.0	62.0	0.326	2.20	12.7	11.8	11.4	11.6	0.107	0.22	-	-	-	-		
d7	6.1	5.7	4.4	5.0	0.254	0.45	57.9	61.2	65.9	65.2	0.283	2.29	11.6	11.8	10.7	11.9	0.058	0.16	1.48	1.54	1.37	1.42	0.055	0.002

- no data available on that day

[a,b,c]different superscripts denote significant differences between groups (P<0.05)

S: soybean oil

L: linseed oil

Lipid oxidation (Tables 3A1.4 and 3A1.5)

For all meat samples, there was no significant effect of oil source or gender on TBARS values, which were very low throughout the whole storage period. On day 2, the ATA treatment had the lowest TBARS content in fresh meat ($P<0.05$). In contrast, towards the end of the storage period, lipid oxidation was significantly reduced by the AOC treatment compared to the ATA treatment, both for the fresh LT samples on day 8 ($P<0.05$) and for the CCH on day 7 ($P<0.05$). Only after cooking the pre-frozen meat, TBARS values were markedly increased to an average value of 3.42 and 9.63 μg MDA/g tissue on day 1 and day 7 of chill storage respectively. Lipid oxidation of the pre-frozen subcutaneous fat was not affected by oil source or antioxidant treatment. Barrows had significantly lower TBARS compared to gilts immediately after freezing (day 0) ($P<0.05$) whereas no effect of gender was seen after being stored at 23°C for 3 days.

Table 3A1.4. Influence of antioxidant source on TBARS value (µg MDA/g tissue)

day	AT	AOC	P	SEM
Fresh meat *longissimus thoracis*				
d2	0.16	0.23	0.013	0.001
d5	0.27	0.22	0.252	0.004
d8	0.41	0.22	0.032	0.01
Meat long-term frozen storage *longissimus thoracis*				
d0 (12 months)[1]	0.10	0.17	0.185	0.005
d6 (12 months)	0.90	1.00	0.498	0.06
d0 sample d8[2] (8 months)	0.43	0.42	0.947	0.05
d6 sample d8 (8 months)	2.48	1.52	0.165	0.97
Pre-frozen cooked meat *longissimus thoracis*				
d1 (10 months)	3.51	3.80	0.529	0.52
d7 (10 months)	9.22	10.36	0.283	2.51
Cooked cured ham				
d1	0.17	0.15	0.298	0.0005
d7	0.38	0.25	0.038	0.004
Subcutaneous fat				
d0 (8 months)	0.13	0.17	0.364	0.004
d3 23°C (10 months)	3.03	1.65	0.210	2.59

ATA: α-tocopheryl acetate; AOC: antioxidant cocktail

[1] analysis after 12 months of frozen storage at –18°C

[2] TBARS on d0 of the sample that had been stored in the chill cabinet for 8 days prior to 8 months of frozen storage at –18°C

Table 3A1.5. Influence of oil source on TBARS value (µg MDA/g tissue)

day	SS	SL	LS	LL	P	SEM
Fresh meat *longissimus thoracis*						
d2	0.23	0.22	0.13	0.19	0.058	0.001
d5	0.23	0.29	0.20	0.25	0.317	0.004
d8	0.19	0.42	0.34	0.32	0.240	0.01
Meat long-term frozen storage *longissimus thoracis*						
d0 (12 months)[1]	0.13	0.16	0.09	0.16	0.609	0.005
d6 (12 months)	1.37	0.82	0.92	0.69	0.076	0.06
d0 sample d8[2] (8 months)	0.51	0.52	0.28	0.39	0.565	0.05
d6 sample d8 (8 months)	2.04	2.59	1.95	1.41	0.376	0.97
Pre-frozen cooked meat *longissimus thoracis*						
d1 (10 months)	4.11	3.92	2.67	3.92	0.255	0.52
d7 (10 months)	11.6	9.53	7.60	10.4	0.232	2.51
Cooked cured ham						
d1	0.15	0.17	0.15	0.18	0.300	0.0005
d7	0.30	0.30	0.34	0.32	0.653	0.004
Subcutaneous fat						
d0 (8 months)	0.15	0.16	0.17	0.13	0.817	0.004
d3 23°C (10 months)	0.21	2.65	2.72	3.78	0.164	2.59

S: soybean oil; L: linseed oil

[1] analysis after 12 months of frozen storage at -18°C

[2] TBARS on day 0 of the sample that had been stored in the chill cabinet for 8 days prior to 8 months of frozen storage at -18°C

Protein oxidation (Tables 3A1.6 and 3A1.7)

During chill storage of the fresh LT, there was an initial significant decline in thiol groups for both antioxidant treatments (P<0.01) which was followed by a significant increase towards day 8 for the AOC (P<0.001) and a non-significant increase for the AT treatment. For the AT treatment, the amount of thiol groups of fresh LT samples on day 8 of chill storage was not significantly lower compared to day 2. For the CCH a significant decline in thiol groups over days was observed (P<0.01). No effect of gender, antioxidant source or oil combination was observed on the thiol groups.

During the chill storage period, there was a significant increase in carbonyl groups for the fresh LT samples (P<0.001), whereas for the hams no significant effect of time was observed. There was no effect of oil source on the content of carbonyl groups for both fresh LT samples and hams. On day 8 for the fresh meat, the carbonyl content was significantly (P<0.01) higher for the AOC treatment compared to the ATA treatment. For the CCH on day 7 the AOC significantly lowered the carbonyl content. On day 8, gilts had a significantly (P<0.01) higher carbonyl content compared to barrows.

Table 3A1.6. Influence of antioxidant source on protein oxidation measured by carbonyl groups (nmol DNPH incorporated/mg protein) and thiol groups (nmol SH-groups/mg protein)

day	carbonyl				thiol			
	ATA	AOC	P	SEM	ATA	AOC	P	SEM
Fresh meat *longissimus thoracis*								
d2	1.65	1.83	0.427	0.14	75.2	79.7	0.163	1.90
d5	1.79	1.59	0.569	0.22	68.1	65.3	0.311	1.72
d8	2.32	4.66	0.003	0.32	73.2	69.1	0.353	2.69
Cooked cured ham								
d1	2.30	1.54	0.332	0.42	34.3	33.0	0.634	3.21
d7	1.99	1.29	0.036	0.16	24.5	23.9	0.927	2.17

ATA: α-tocopheryl acetate; AOC: antioxidant cocktail

Table 3A1.7. Influence of oil source on protein oxidation measured by carbonyl groups (nmol DNPH incorporated/mg protein) and thiol groups (nmol SH-groups/mg protein)

day	carbonyl						thiol					
	SS	SL	LS	LL	P	SEM	SS	SL	LS	LL	P	SEM
Fresh meat *longissimus thoracis*												
d2	2.08	1.71	1.49	1.68	0.483	0.14	76.7	77.1	79.0	77.0	0.955	1.90
d5	1.51	1.97	1.51	1.77	0.651	0.22	64.4	67.5	66.9	68.1	0.768	1.72
d8	2.56	3.70	3.87	3.83	0.368	0.32	71.5	73.4	66.9	72.8	0.665	2.69
Cooked cured ham												
d1	2.32	1.80	1.99	1.52	0.743	0.42	35.0	30.8	31.5	35.9	0.870	3.21
d7	1.24	1.60	1.61	2.10	0.173	0.16	22.9	24.7	22.3	27.1	0.626	2.17

S: soybean oil; L: linseed oil

Total Reducing Capacity (TRC) (Table 3A1.8)

No effect of gender, oil source or antioxidant source on TRC was observed besides the fact that the ATA treatment significantly lowered TRC of fresh meat on day 2 ($P<0.05$). For the CCH it was not possible to determine TRC because all the samples seemed to be 100% reducing (value for TRC of about 0.90 compared to 0.30 in fresh LT).

Table 3A1.8. Total reducing capacity of fresh LT

day	antioxidant source			oil source					
	ATA	AOC	P	SS	SL	LS	LL	P	SEM
d2	0.23	0.28	0.011	0.31	0.24	0.24	0.24	0.054	0.001
d8	0.26	0.28	0.187	0.29	0.27	0.27	0.25	0.395	0.001

ATA: α-tocopheryl acetate; AOC: antioxidant cocktail
S: soybean oil; L: linseed oil

α-tocopherol (AT) (Table 3A1.9)

No effect of antioxidant source, oil source or gender was found on the AT content in fresh meat, CCH or subcutaneous fat samples. The AT values in the different samples were strongly and positively correlated.

Table 3A1.9. α-tocopherol content in subcutaneous fat (subc), *longissimus thoracis* (LT) and cooked cured ham (CCH) (µg /g tissue)

	antioxidant source			oil source					
	ATA	AOC	P	SS	SL	LS	LL	P	SEM
subc	3.71	2.54	0.515	3.21	2.77	2.20	4.32	0.763	7.68
LT	0.75	0.67	0.503	0.68	0.69	0.66	0.81	0.603	0.03
CCH	1.23	0.93	0.209	1.14	1.03	1.01	1.12	0.939	0.07

ATA: α-tocopheryl acetate; AOC: antioxidant cocktail
S: soybean oil; L: linseed oil

Fatty acid profile (Table 3A1.10)

The main groups of PUFA in fresh LT are expressed in g/100g of total FAME (fatty acid methyl esters).

There was a significant effect of oil source on the n-3 PUFA proportion in the meat, which was significantly higher for the LL group compared to the SS group. The SL and LS group had an intermediate proportion of n-3 PUFA.

A significant effect of gender ($P<0.05$) was observed on the PUFA profile. Antioxidant source did not have a significant effect on the fatty acid profile of the intramuscular fat: there was only a trend for a higher n-3 PUFA proportion in the fresh meat for the AOC treatment.

Table 3A1.10. Fatty acid profile (g/100g FAME) of fresh LT

	gender			ao source			oil source					
	barrow	gilt	P	ATA	AOC	P	SS	SL	LS	LL	P	SEM
n-6	14.3	19.4	0.031	15.4	18.3	0.189	21.6	16.0	17.0	12.8	0.071	10.3
n-3	2.76	3.64	0.038	2.85	3.55	0.099	1.62[a]	3.63[bc]	2.98 [ab]	4.58 [c]	0.004	0.34

[a,b,c]different superscripts denote significant differences between groups (P<0.05)
ATA: α-tocopheryl acetate; AOC: antioxidant cocktail
S: soybean oil; L: linseed oil

DISCUSSION

Colour oxidation

For both antioxidant treatments, values of the different colour parameters during storage of fresh meat are within the normal range.

A significantly higher a* value on day 0 for animals of the SS group, combined with a higher MetMb% for those animals from day 0 to day 2 on, corresponds with the findings of Mercier et al. (1998) for turkey. In that study, animals fed soybean oil had a higher haeminic iron content than birds fed rapeseed oil or tallow and showed a higher susceptibility to oxidative reactions.

When considering the colour of the CCH, irrespective of the treatment, a quick fading was observed during the first hours after cutting. Exposure of this pigment to light even at very low oxygen levels promotes its oxidation, which imposes a dull greyness to the meat surface. One hour after cutting, the ratio of the cooked cured colour was still characteristic for an excellent cured colour (2.2 on average), while two hours later this ratio already declined to a value that reflects a less intense cured colour (1.7 on average) (Hunt and Kropf, 1984). This, together with the lack of effect of antioxidant treatment on colour parameters, suggests that supplementation of 40 ppm ATA (as treatment in se or as part of the AOC) during this 16-weeks period is sufficient for stabilization of colour during the meat storage period. This corresponds with literature where no clear negative effects on colour oxidation were seen when low doses of antioxidants (60 ppm or lower) were compared to higher doses when supplemented during a similar long period (from weaning on or from about 40 kg on until slaughter) (Hoving-Bolink et al., 1998; Jensen et al., 1998a; Mason et al., 2005).

This also implicates that the supplemental components of the AOC (citric acid, rosemary and gallic acid) are of minor importance for colour stabilisation and consequently have no additional or synergistic effect on colour stability at this dose. Another possibility could be that the antioxidant components other than AT had already been used up in the feed during oxidation. In addition, we do not have an idea about the rate of absorption and incorporation of these components in muscle tissue. Furthermore,

the high colour stability of the glycolytic muscle used in this experiment (LT) probably reduced the amount of antioxidant needed to stabilize the colour of the fresh meat.

The hue value for fresh meat and CCH decreased respectively increased towards the end of the storage period. For the fresh meat, this evolution is unexpected because the hue value of meat normally increases (decreased redness) with the accumulation of MetMb during storage. The lower hue value on the last days of storage for the fresh meat is mainly caused by the low b* value on those days.

Lipid oxidation

Although the PUFA profile (especially fatty acids with 3 or more double bonds) of the meat was different between the different oil groups, the TBARS value was not influenced. It is well known that, to decrease oxidative processes, AT is a highly-efficient antioxidant in cell membranes, acting as a chain-breaker as also demonstrated in chicken meat (Sheehy et al., 1993) and pork (Monahan et al., 1992b) during storage. The excellent antioxidative effect of AT in pork was also observed in this experiment as lipid oxidation in fresh meat, measured as TBARS values, was relatively low throughout the whole display period for all the treatments (<0.4 µg MDA/g meat). Although on day 2 ATA seemed more efficient to inhibit lipid oxidation in fresh meat, the TBARS value of the AOC group remained unaltered during 8 days of chill storage whereas the ATA treatment could not overcome an increase in TBARS value (even though the latter was still below the threshold level of acceptance for fresh meat). For raw porcine muscle, this threshold value was reported to be 0.50 µg MDA/g tissue by Lanari et al. (1995) or 1.0 µg MDA/g tissue by Buckley and Connolly (1980). Also a significantly lower TBARS value on day 7 for the CCH samples was noticed for the AOC group. These results could indicate that AOC is superior in inhibiting lipid oxidation compared to single ATA. However, this difference between antioxidant treatments could no longer be observed after frozen storage. The superior effect of dietary administration of an antioxidant cocktail was also observed for turkey by Botsoglou et al. (2003) who found that a combination of AT with oregano essential oil was superior to treatments with either AT or oregano oil individually.

TBARS values of meat samples displayed for 8 days and analysed before and after freezing for 8 months, showed a strong correlation (r=0.662) and were not significantly different. This last observation is probably due to the fact that, during frozen storage, primary oxidation products accumulate and are only converted after thawing into secondary oxidation products which can then be measured by the TBARS method. This is also confirmed by the observation that for a similar duration, the display of pre-frozen meat, resulted in a higher TBARS increase compared to the display of fresh meat. These results imply that, for none of both antioxidant treatments, oxidation is stopped during freezing.

Heating could affect many factors involved in lipid oxidation. Heat disrupts muscle cell structure, inactivates antioxidative enzymes and releases oxygen from oxymyoglobin. Also, the level of free iron greatly increases during cooking (Rhee et al., 1987) and high temperature decreases the activation energy for oxidation and breaks down hydroperoxides to free radicals, which propagate lipid peroxidation. Heating seemed to be very much prooxidative for the pre-frozen meat samples as measured by the high TBARS values which, from day 1 on, exceed the threshold for the appearance of rancidity off-flavours in cooked meat of 1.0 µg MDA/g tissue (Igene et al., 1979). Consequently, it can be said that there is a rapid onset of rancidity in cooked meat during refrigerated storage. However, it has to be mentioned that this meat, prior to heating, had been stored frozen for 10 months and this fact probably also contributed to the large increase in TBARS value.

After heating, lipid oxidation is not as serious a problem for cured meat as it is for uncured meat. Curing of meat results in meat products which are rather stable against lipid oxidation. When nitrite and ascorbate have been added during the curing process, it seems that lipid oxidation after heating is inhibited strongly as also confirmed by our results. In this study, nitrite was injected in the ham at a level of 108 ppm and residual nitrite is at maximum 20 ppm. When comparing with an experiment of Dineen et al. (2001) with a similar level of nitrite in the curing process and with either 10 or 1000 ppm of ATA in the feed, TBARS values in our trial were even less than the ones for the highest AT level. However, AT concentrations in the muscle for our trial were far less beyond the ones obtained by the 1000 ppm addition and approached the one of the 10

ppm addition. A possible explanation for this could be the fact that in the trial of Dineen et al. (2001), analyses were done on thin slices of ham instead of 2.5 cm thick chops in our experiment. Secondly, this might point to the excellent antioxidative role of nitrite and ascorbate even at these moderate levels.

Next to its antioxidative effect, addition of nitrite leads to an attractive colour of meat products and exhibits anti-microbial activity. The mechanism by which nitrite delays or prevents the peroxidation of meat lipids is a complex process (Igene et al., 1985; Morrissey and Tichivangana, 1985).

Protein oxidation

Although proteins in muscle cells are also targets for oxidation *in vivo*, much less is known about radical attack on proteins, particularly during meat maturation. In the present study, little difference in thiol or carbonyl groups was observed with time, suggesting a low oxidation of proteins during storage. The increase in thiol groups from day 5 to day 8, which was significant for the AOC, was not as expected and is difficult to explain.

According to the antioxidant treatment, no unambiguous effect on protein oxidation was observed, although the AOC reduced lipid oxidation in the fresh meat. According to the carbonyl method, AT seems more efficient in inhibiting protein oxidation in the fresh meat, whereas the results in CCH seemed to suggest the opposite. The reason for this is unknown. To our knowledge, little research has been done on protein oxidation in CCH. From these results, protein and lipid oxidation seem not to be related. This is in contrast with the rare results in literature for beef (Mercier et al., 1995) and turkey (Batifoulier et al., 2002), although on pork no literature data are available to our knowledge. However, both carbonyl production and loss of thiol groups are limited to only one group of amino acids and might not be representative for the whole protein oxidation phenomena. There are numerous different types of protein oxidative modifications and there is no single universal marker for protein oxidation. For this reason, it would be advisable to use more markers to understand better the effect of diet on the mechanism of protein oxidation.

The lower absolute number of thiol groups in CCH compared to that of fresh meat (30 vs. 70 nmol thiol/mg protein respectively), can be caused by the fact that meat innate reducing systems and in particular thiol groups can participate in the reduction of nitrosometmyoglobin to nitrosomyoglobin which takes place during the formation of the cooked cured pigment. Also, nitrite disappears in a reductive process mediated by electron donors such as thiol groups (Lee et al., 1981).

TRC

In this method, the combined activity of the different anti- and prooxidants in the meat is measured. For the CCH it was not possible to determine TRC because all the samples seemed to be 100% reducing. This might be caused by the ferric reducing activity of the residual ascorbic acid in the sample that originated from the curing agent.

α-tocopherol

Medical and food research nowadays is highlighting the superior effect of a cocktail of antioxidants compared to just one single component. For the AOC, apart from the AT component, two other classes of antioxidants, notably polyphenols and organic acids were represented. Nevertheless, AT content in the different samples was not affected by antioxidant treatment and consequently there seems to be no sparing effect of these supplementary antioxidants of the AOC. This is in contrast with Botsoglou et al. (2003) where supplementation of oregano oil to a basal diet with 30 ppm ATA increased the retention of α-tocopherol in turkey.

When comparing oil source treatments with varying degrees of unsaturation (induced in this study by soybean or linseed oil), no effect on AT deposition was found. This is in line with studies on broiler meat where also fat sources with a different degree of unsaturation were used (Lin et al., 1989b; Asghar et al., 1990). Some authors found that the content of AT in muscle was lower when the dietary MUFA/PUFA ratio increased (Lopez-Bote et al., 2003). However, other authors showed a lack of effect (Monahan et al., 1992b; Rey et al., 2001).

Using the linear equation between AT intake and muscle deposition for pigs as described by Jensen et al. (1998b) (*mg AT/kg LD = (-3.0) + 4.0 (log (dietary AT (mg/kg))*; R^2=0.85), an AT content of 0.8mg/kg muscle is predicted for a 40 ppm ATA supplementation. This value corresponds quite well with the one observed in our study.

CONCLUSIONS

No effect of oil source on different parameters of oxidation was seen. Also no effect of antioxidant treatment on colour stability of fresh LT or CCH was observed. For both antioxidant treatments, lipid oxidation in fresh LT and CCH was well controlled during display and was inhibited most by the AOC. However, lipid oxidation increased significantly in pre-frozen uncured cooked meat under aerobic conditions. No unambiguous effect of antioxidant treatment on protein oxidation was observed. ATA seemed more efficient in reducing protein oxidation in fresh LT, whereas for CCH, results suggested the opposite. There seemed to be no clear link between colour, protein and lipid oxidation. At the dose used in this study, no additional or synergistic effects of the extra components of the AOC on the different oxidation parameters was found.

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CHAPTER 3A2

EFFECT OF DIETARY ROSEMARY AND ALPHA-TOCOPHERYL ACETATE ON THE OXIDATIVE STABILITY OF RAW AND COOKED PORK FOLLOWING OXIDIZED LINSEED OIL ADMINISTRATION

Abstract - The effect of a 2% dietary administration to pigs of oxidized linseed oil (targeted level of 150 mEq O_2/kg oil after heating at 50°C and exposure to air for 3-4 days following addition of 10 ppm $CuSO_4$), either or not in combination with antioxidants, on the oxidative stability of raw and cooked pork during illuminated chill storage was assessed. Apart from a control group without antioxidants, the antioxidant treatments were: 40 ppm α-tocopheryl acetate, 40 ppm rosemary extract, 40 ppm rosemary extract + 2 ppm gallic acid, and 40 ppm α-tocopheryl acetate + 40 ppm rosemary extract. A total of 20 ppm of α-tocopheryl acetate (ATA) was added to all diets in order to meet the physiological requirement of the animals. The antioxidant treatments did not exert any effect on colour and protein oxidation. Lipid oxidation was only decreased by dietary ATA when comparing the ATA supplemented groups combined versus the control treatment group for raw meat but not for cooked meat. This was due to a higher content of α-tocopherol in the meat and subcutaneous fat. The results on lipid oxidation suggested a lack of antioxidant effect for the rosemary extract. No evidence for a synergistic effect of the antioxidant combinations could be observed at the doses applied.

Key Words: alpha-tocopherol, antioxidant, oxidized oil, oxidative stability, pork, rosemary

INTRODUCTION

Oxidative processes are one of the primary mechanisms of quality deterioration in meat and meat products as they cause loss of flavour, colour and nutritive value and consequently limit their shelf-life (Kanner, 1994). The oxidative stability of meat and meat products depends upon the balance of anti- and pro-oxidants and the composition of oxidation substrates including polyunsaturated fatty acids (PUFA), cholesterol, proteins and pigments (Bertelsen et al., 2000). Antioxidants are added to the feed for several reasons: to protect it from oxidative deterioration and to improve the antioxidant status of animal tissues and thus prevent or reduce oxidation, both in vivo and postmortem. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA), can exhibit toxic properties (Kahl & Kappus, 1993), resulting in strict regulations over their use in foods. These findings, together with increased consumer resistance towards the use of synthetic additives, have increased interest in the antioxidant properties of naturally occurring substances (Gordon, 1996; Pokorny, 1991). In meat quality studies, α-tocopherol (AT) has received considerable attention in recent years (Morrissey, Kerry & Galvin, 2003). AT is generally regarded as an acceptable supplement. It is the primary lipid soluble antioxidant in biological systems which acts by disrupting the chain of lipid oxidation in cell membranes, thus preventing the formation of lipid hydroperoxides (Buckley, Morrissey & Gray, 1995). When included in animal diets as α-tocopheryl acetate (ATA), it is a highly effective antioxidant to extend the shelf-life of foods from animal origin (Faustman, Cassens, Schaefer, Buege, Williams & Scheller, 1989; Gray, Gomaa & Buckley, 1996; Jensen, Lauridsen & Bertelsen, 1998). Nowadays, there is a strong interest in isolating antioxidants from natural sources because of the numerous applications for the protection of animals and their products against oxidation (Wenk, 2003). Amongst those, rosemary has been reported to possess antioxidant properties when administered in the diet (Basmacioğlu, Tokuşoğlu & Ergül, 2004; Lopez-Bote, Gray, Gomaa & Flegal, 1998). In addition, the use of cocktails of antioxidants could have a superior effect compared to single antioxidants. Two or more antioxidants together can act synergistically, *i.e.* they can limit oxidation to a higher

extent than the sum of the contributions from each single antioxidant (Duthie, 1999; McCarthy, Kerry, Kerry, Lynch & Buckley, 2001).

A study conducted in the past at our laboratory showed little or no effect of the composition of the dietary antioxidant source (single vs. cocktail) on the oxidative stability of meat from pigs that were fed diets differing in the degree of unsaturation of the fat source (Haak, Raes, Smet, Claeys, Paelinck & De Smet, 2006). Feeding oxidized fat to the animals could be another model for studying the effect of dietary antioxidants. Oxidized fat in the animals' feed is a source of oxidative stress in vivo which could possibly lower the antioxidant status of tissues and enhance the formation of oxidation products in meat postmortem. In literature, studies are available showing a reduced oxidative status of the animal or the meat after feeding highly oxidized fats (e.g. Engberg, Lauridsen, Jensen, Jakobsen, 1996; Jensen, Engberg, Jakobsen, Skibsted & Bertelsen, 1997). De Rouchey et al. (2004) stated that peroxide values below 40 mEq O_2/kg fat and a p-anisidine value below 10.6 do not affect growth rate or digestibility in young pigs. In industrial practice, generally one does not use fats in animal feed manufacturing with peroxide values above 10-20 mEq O_2/kg oil.

In this experiment, the effect of dietary administration of an oxidized unsaturated oil (linseed oil), either or not in combination with antioxidants (α-tocopherol, rosemary, gallic acid), on the oxidative stability (colour, lipid and protein oxidation) of raw and cooked pork during illuminated chill storage (900 lux, 4°C) was assessed.

MATERIALS AND METHODS

Experimental setup and sampling

Crossbred pigs (n = 50; Piétrain sire x crossbred dam (French Landrace x (Large White x English Landrace))) at a mean (sd) live weight of 25.8 (1.6) kg were randomly assigned to 5 feeding groups, each fully balanced according to sex. The animals were housed on a private farm (Biervliet, Oudekapelle, België) and were fed *ad libitum*. A 3-phase feeding system was applied: 31 d in phase 1 (P1), 35 d in phase 2 (P2) and 49 or 56 d (two slaughter days) in phase 3 (P3), corresponding with a mean (sd) live weight of 46.9 (2.8), 75.0 (5.8) and 113.0 (6.1) kg at the end of the consecutive phases. The diet contained 20 ppm all-rac ATA (basic physiological requirement) and was free of any other synthetic antioxidant. The dietary composition is shown in Table 1 and the fatty acid composition of the experimental diets is presented in Table 2. All diets contained 2% of oxidized linseed oil (targeted level of 150 mEq O_2/kg oil). Non-stabilised linseed oil was purchased from Mosselman (Ghlin, Belgium). Before the start of the trial, one batch of linseed oil was oxidized by Kemin AgriFoods Europe (Herentals, Belgium) by heating (50°C ± 5°C) and exposure to air (1 bar) for 3-4 days after addition of 10 ppm of $CuSO_4$. The oxidized oil was kept frozen (-20°C) until the start of P2 and P3 of the experiment.

The antioxidant treatments were: 1) no added antioxidants (C), 2) 40 ppm α-tocopheryl acetate (E; DSM, Deinze, Belgium), 3) 40 ppm of rosemary extract containing 7.2% of phenolic diterpenes and flavonoids (R; Kemin AgriFoods Europe, Herentals, Belgium), 4) 40 ppm rosemary extract + 2 ppm gallic acid (RG), 5) 40 ppm α-tocopheryl acetate + 40 ppm rosemary extract (ER). The antioxidants were mixed with the oxidized linseed oil, before the oil was included in the batches of feed. It is well known that α-tocopheryl acetate as such is not active in the feed because the acetate ester blocks the active phenol group. In the fourth treatment, rosemary extract was combined with 2 ppm of gallic acid (RG) because of the possible protective effect of the latter on the active components of the rosemary extract. The rosemary extract and gallic acid were protected against oxidation during feed storage by encapsulation in small sphere particles of palm oil following spray-cooling of a mixture of these antioxidants and a high melting point

palm fat. These fat particles were mixed in the liquid linseed oil only shortly before the feed manufacturing. The supplier guaranteed that this process protects the antioxidants to be released from the particles during feed storage. The premix was supplied by DSM (Deinze, Belgium) and was unaltered throughout the experiment.

The animals were slaughtered in a private slaughterhouse (Braems NV, Herzele, Belgium) and were stunned by means of carbon dioxide. Per feeding group, 1 barrow and 1 gilt deviating most from the mean live weight, were left out for sampling of meat, resulting in 8 animals per treatment for oxidative stability measurements.

The *M. longissimus thoracis* (LT; starting from the 7^{th} rib, left carcass side) was sampled at 24 h postmortem (day 0) and sliced into 2.5 cm thick chops. Three chops were cooked in a water bath at 70°C for 40 min (in an impermeable plastic bag). These cooked chops together with three raw chops were then wrapped in an oxygen permeable polyethylene film (oxygen transmission rate > 1000 $cm^3/m^2/24h$) and placed in an illuminated chill cabinet (fluorescent light, 900 lux, 4°C) for 2, 5 or 8 days to perform oxidative stability measurements. Two raw chops were immediately vacuum-packed and stored at -18°C for future fatty acid and α-tocopherol analysis. Also, subcutaneous fat was taken at the same height of the LT samples and was stored vacuum-packed at -18°C.

Table 3A2.1. The ingredient composition of the experimental diets (%)

	P1	**P2**	**P3**
Barley	21.50	6.00	7.00
Wheat	27.43	35.00	35.00
Maize	10.00	1.50	1.70
Wheat middlings	3.00	-	-
Rye flour	-	12.00	12.00
Wheat gluten feed	5.50	11.00	13.00
Peas	6.00	8.00	8.00
Sugar cane molasses	-	1.00	1.00
Toasted soybeans	5.00	-	-
Soybean meal	15.50	20.00	17.00
Linseed oil	2.00	2.00	2.00
DL-Methionine	0.07	-	-
L-Lysine HCl	0.20	-	-
L-Threonine	0.10	-	-
Monocalcium phosphate	0.20	-	-
Vitamin-mineral premix[1]	3.00	3.00	3.00
Benzoic acid	-	-	0.30

[1]contained 20 ppm all-rac α-tocopheryl acetate

Table 3A2.2. The fatty acid profile (mean (SD)) of the experimental diets in the 3 phases (g/100g FAME)

	P1	P2	P3
C12:0	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)
C14:0	0.11 (0.01)	0.11 (0.01)	0.12 (0.02)
C16:0	11.26 (0.41)	10.81 (0.55)	11.24 (0.40)
C18:0	3.11 (0.05)	2.98 (0.09)	2.95 (0.03)
C16:1	0.18 (0.02)	0.17 (0.02)	0.18 (0.04)
C18:1c9	17.40 (0.12)	15.19 (0.16)	15.14 (0.19)
C18:1c11	1.06 (0.02)	0.80 (0.25)	0.91 (0.05)
C18:2n-6	38.44 (0.34)	34.42 (0.48)	34.94 (1.25)
C18:3n-3	21.68 (0.86)	29.14 (0.47)	28.35 (1.40)

Oxidative stability assessment of the feeds

The peroxide value (POV) of the feeds in the 3 phases was determined by iodometric titration according to Gray (1978) after a 3- or 6-weeks storage at 25°C.

The oxygen bomb method was used for rapid assessment of the oxidative stability of the feeds (Kemin AgriFoods Europe). A glass sample holder was filled with 50 g of the feed and was placed in a stainless steel cylinder also referred to as bomb (Seta 2652). The system was closed and purged with oxygen. A pressure transducer (Seta 2631) was installed on top of the cylinder. The oxygen bomb with 3.45 bar oxygen pressure was then immersed in an oil bath at 98°C. The pressure was monitored for 30 h using a Seta Autoxidation Control Unit (Seta 2629). The pressure decrease measured with this technique, is a direct indication of the oxidation rate.

Carcass and meat quality measurements

At the start of each phase and before slaughter, pigs were weighed individually. Carcass lean content was assessed by means of a CGM apparatus (Sydel, Lorient, France). At 40 min and 24 h postmortem, pH was measured in the LT (6^{th}-7^{th} rib region;

Knick Portamess 654 with Schott N5800A electrode) and conductivity was measured in the LT and in the ham (*M. semimembranosus*) with a PQM meter (PQM-I Kombi; INTEK, GmbH).

The colour measurements were done on raw meat samples on day 0 following a 30 min bloom period (in film) and from then on daily until 8 days of illuminated chill storage (fluorescent light, 900 lux, 4°C). Reflectance spectra (every 10 nm between 400 and 700 nm) and colour coordinates (CIE L*a*b* colour system 1976) were assessed using a HunterLab Miniscan Minolta XE plus spectrocolorimeter (light source of D65, standard observer of 10°, 45°/0° geometry, 1 in. light surface, white standard). The results were expressed as lightness (L*), redness (a*), yellowness (b*), hue value ($\tan^{-1}$ b*/a*) and saturation index (SI) ($(a^{*2} + b^{*2})^{1/2}$). By means of reflectance values at specific wavelengths, the percentage of the different forms of myoglobin (oxymyoglobin (OxyMb), deoxymyoglobin (Mb), and metmyoglobin (MetMb)) were calculated according to the method of Krzywicki (1979), modified by Lindahl, Lundström and Tornberg (2001). Since samples were taken away for lipid and protein oxidation assessment, the number of samples per animal on which colour measurements were performed changed over the storage period. From d0 to d2 the measurements on 3 meat chops were averaged, from d3 to d5 the average of 2 chops was calculated, whereas from d6 to d8 results were derived from the measurement on 1 chop per animal.

Lipid and protein oxidation of raw and cooked LT samples was measured after 2, 5 and 8 days of illuminated chill storage. Lipid oxidation was assessed by 2-thiobarbituric acid-reactive substances-measurement (TBARS) using the distillation method as described by Tarladgis, Watts and Younathan (1960) and was expressed as µg malondialdehyde (MDA)/g meat.

Protein oxidation was assessed by determining the increase in carbonyl content and the decrease in free thiols during storage. The measurement of protein carbonyls following their reaction with 2,4-dinitrophenylhydrazine (DNPH) was done according to Mercier, Gatellier, Viau, Remignon and Renerre (1998). The total carbonyl content, expressed as nmol DNPH incorporated/mg protein, was quantified by a spectrophotometric assay at 370 nm. The assessment of free thiol groups was done according to Batifoulier, Mercier, Gatellier and Renerre (2002). This method relies on the

incubation of a meat homogenate with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), followed by a spectrophotometrical measurement of the free thiol groups at 412 nm (expressed as nmol SH-groups/mg protein).

Fatty acid analysis

Feed and raw LT samples were extracted using chloroform/methanol (2/1; v/v) (modified after Folch, Lees and Stanley (1957)). Fatty acids (FA) were methylated and analysed by gas chromatography (HP6890, Brussels, Belgium) on a CP-Sil88 column for fatty acid methyl esters (FAME) (100 m x 0.25 mm x 0.2 µm; Chrompack, The Netherlands), as described by Raes, De Smet and Demeyer (2001). Peaks were identified based on their retention times, corresponding with standards (NuChek Prep., IL, USA; Sigma, Bornem, Belgium).

α-tocopherol content

The AT content of raw LT and subcutaneous fat samples was determined according to the method of Desai (1984). After saponification and hexane extraction, all samples were analysed by reversed phase HPLC (Supelcosil LC 18 (25 cm x 4.6 mm x 5 µm) column) fitted with UV detection (292 nm). The eluting solvent was methanol/water (97/3; v/v) at a flow rate of 2ml/min. The AT content of the tissue was determined by comparison of peak areas with those obtained for a standard solution of dl-α-tocopherol. The results are expressed as µg α-tocopherol/g tissue.

Statistical analysis

A General Linear Model with the fixed factors diet and gender was used. The interaction term diet x gender was not significant and was not included in the model. Duncan was used as *post hoc* comparison of means test. The analyses were performed using the statistical software package SPSS for Windows (version 11.0).

RESULTS

Fatty acid composition and oxidation assessment of the feed

The oxidation of the feed as assessed by the oxygen pressure decrease in the oxygen bomb is shown in Figure 1. There was no difference between the curves, and thus no difference in the rate and degree of oxidation of the feeds. The mean (sd) POV-value of feed samples from the different feeding phases after a 3- and 6-weeks storage at 25°C was 35.8 (10.8) and 37.6 (9.9) mEq O_2/kg fat respectively. No difference in POV value was observed between the different feeds.

The FA composition of the feeds in the different phases is shown in Table 2. Within each phase, the total FA content and the FA profile were similar for all the test feeds. The total FA content was 4.4, 3.5 and 3.3 g FA/100g feed in P1, P2 and P3 respectively. The lower total FA content in P2 and P3 feeds resulted in a higher C18:3n-3 (α-linolenic acid) proportion compared to P1 feeds.

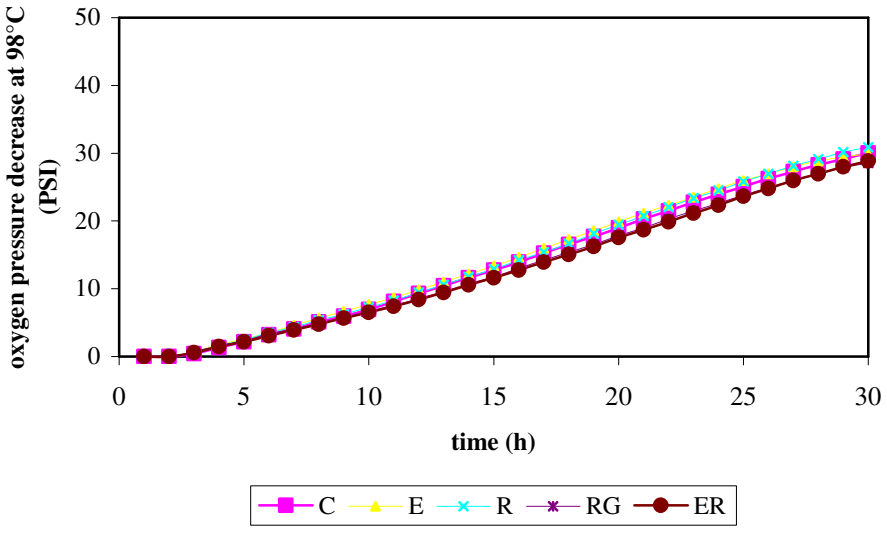

Figure 3A2.1. Oxygen pressure decrease for the different feeds in the oxygen bomb

Animal performances and muscle pH

Zootechnical parameters (growth, slaughter weight, lean meat percentage), and postmortem pH values were within the normal range for pork and were not influenced by diet. Diet did not affect feed intake. The average daily gain was 677.2, 794.9 and 676.7 g/d in P1, P2 and P3 respectively. pH in the loin and the ham 40 min after slaughter was 6.1 and 6.2 respectively, both declining to 5.6 after 24 h.

From P2 on, barrows showed a significantly higher growth rate, resulting in a higher weight of these animals at the start of P3 and a higher slaughter weight (113.9 and 107.7 kg for barrows and gilts respectively) ($P<0.01$). The carcass lean content was noticeably higher in gilts as compared to barrows (62.8 and 57.1% respectively) ($P<0.001$).

LT muscle and subcutaneous fat measurements

Fatty acid composition of the LT muscle

The total intramuscular fatty acid content (IMFA) (mg/100g meat) and the proportion of the main fatty acid classes of the LT (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-6 and n-3 polyunsaturated fatty acids (PUFA); g/100g FAME) for the different groups are shown in Table 3. The total IMFA content was higher in treatment R compared to all other treatments ($P<0.05$), partly due to one pig with a very high IMFA content. The other groups where rosemary was added (RG and ER) did not display a higher IMFA content. No important effect of diet on the proportions of the main FA classes or the individual FA in the meat were detected (data not shown). For the R group, C22:6n-3 was significantly lower ($P<0.05$) and there was a trend towards a lower C22:4n-6 proportion. Barrows had a higher, although not significant, IMFA content compared to gilts. The proportion of SFA, and particularly C16:0 and C18:0, was higher for barrows ($P<0.05$).

Table 3A2.3. Total fatty acid content (mg FAME/100g muscle) and fatty acid profile (g/100g FAME) of the meat as influenced by feeding group (n = 8)

	C	E	R	RG	ER	P_{gender}	P_{group}	SEM
Total	1316 [a]	1403 [a]	2081 [b]	1287 [a]	1278 [a]	0.443	0.031	96.6
SFA	35.84	35.46	36.77	34.85	35.01	0.011	0.270	0.322
MUFA	40.76	41.50	44.78	41.85	40.74	0.912	0.057	0.496
n-6	13.83	13.65	10.52	13.65	14.37	0.326	0.084	0.485
n-3	4.83	4.96	4.15	5.10	5.20	0.203	0.196	0.152
PUFA	18.94	18.91	14.92	19.03	19.85	0.277	0.106	0.638

SFA=C12:0+C14:0+C16:0+C17:0+C18:0+C20:0+C22:0
MUFA=C14:1+C16:1+C17:1+C18:1c9+C18:1c11+C20:1
n-6=C18:2n-6+C18:3n-6+C20:3n-6+C20:4n-6+C22:4n-6
n-3= C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3
PUFA= n-6+n-3+C20:2n-6
[a,b] Different superscripts denote significant differences between groups (P<0.05)

α-tocopherol content of the LT muscle and the subcutaneous fat

The AT content of the LT muscle and the subcutaneous fat (μg/g) is shown in Table 4. The E and ER groups showed a significantly higher AT content (P<0.001) in the LT muscle compared to the other groups. When the E and ER groups were combined versus the other groups, the difference in AT content in the subcutaneous fat was also significant (P<0.01). Between the E and ER groups, the AT content was not different. Gender did not influence the AT content of either intramuscular or subcutaneous fat.

Table 3A2.4. Effect of feeding group on the α-tocopherol content in the meat and the subcutaneous fat (µg /g) (n = 8)

	C	E	R	RG	ER	P_{gender}	P_{group}	SEM
LT	1.55 [a]	2.58 [b]	1.25 [a]	1.55 [a]	2.67 [b]	0.556	<0.001	0.120
fat	2.52	3.44	2.21	2.66	3.32	0.341	0.106	0.173

[a,b] Different superscripts denote significant differences between groups (P<0.05)

Oxidation assessment of the LT muscle

Throughout the storage period, all parameters of colour and colour stability were within the regular range for pork (mean (sd) values for a*, MetMb% and OxyMb% on d8 were 8.2 (1.9), 29.5 (6.8), 63.9 (14.6) respectively). Neither feeding group nor gender did influence these colour parameters.

The TBARS values (µg MDA/g LT) as an indication of lipid oxidation of raw and cooked meat throughout storage are shown in Table 5. Lipid oxidation in the raw meat was significantly reduced by ATA, when comparing the ATA supplemented groups combined versus the control group (P<0.05 for t-test (E and ER) vs. C). For the cooked meat on d2 and d5, the TBARS value was lowest for group ER, followed by group E (P<0.05). However, these TBARS values were not significantly different from the control group.

For the raw meat on d5 and d8, the mean TBARS value for group RG was significantly higher than for the control. Also, group R showed a higher TBARS value than the control group for the raw meat, however this difference was not significant. For the cooked meat, groups R and RG showed higher TBARS values than the control group, but again both groups were not significantly different from the control group. The TBARS value for group ER compared to group E was higher in the raw meat and lower in the cooked meat, but here also the difference was not significant. Gender did not influence lipid oxidation as measured by TBARS.

The content of carbonyl groups did not increase significantly when storing raw or cooked meat from d2 to d5, however, the increase was significant towards d8 (P<0.001

for t-test between d5 and d8). No effect of feeding group on carbonyl groups in raw or cooked meat was observed (Table 6). On d8 after cooking, barrows displayed a lower carbonyl content compared to gilts ($P<0.05$). In the raw meat, thiol groups decreased significantly up to d8 ($P<0.001$), whereas in the cooked meat a significant decrease between d2 and d5 was followed by a significant increase towards d8 ($P<0.001$). No effect of feeding group on thiol groups of raw or cooked meat was found (Table 7). The significant effect of gender on thiol groups in raw meat on d5 ($P<0.01$) and cooked meat on d2 ($P<0.05$) was in the opposite direction.

Table 3A2.5. Effect of feeding group on TBARS value of raw and cooked meat throughout storage (µg MDA/g) (n = 8)

	C	E	R	RG	ER	P_{gender}	P_{group}	SEM
				raw meat				
d2	0.26	0.21	0.31	0.34	0.20	0.093	0.293	0.026
d5	0.42 [ab]	0.26 [a]	0.51 [bc]	0.66 [c]	0.31 [ab]	0.105	0.007	0.041
d8	0.71 [ab]	0.41 [a]	0.94 [bc]	1.19 [c]	0.57 [ab]	0.334	0.001	0.068
				cooked meat				
d2	10.19 [ab]	9.22 [a]	11.15 [ab]	12.43 [b]	8.80 [a]	0.025	0.031	0.435
d5	15.01 [abc]	14.71 [ab]	16.56 [bc]	16.85 [c]	14.43 [a]	0.920	0.031	0.311
d8	18.64	18.01	19.21	20.38	18.35	0.752	0.137	0.312

[a,b,c] Different superscripts denote significant differences between groups ($P<0.05$)

Table 3A2.6. Effect of feeding group on carbonyl groups of raw and cooked meat throughout storage (nmol DNPH incorporated/mg protein) (n = 8)

	C	E	R	RG	ER	P_{gender}	P_{group}	SEM
				raw meat				
d2	1.58	1.78	1.57	1.51	1.67	0.031	0.811	0.076
d5	1.62	1.81	1.83	1.86	1.65	0.476	0.539	0.054
d8	2.59	2.61	2.78	2.76	2.82	0.451	0.860	0.077
				cooked meat				
d2	3.19	2.99	3.07	3.51	3.02	0.593	0.480	0.099
d5	3.33	3.14	3.67	3.29	3.53	0.865	0.650	0.115
d8	3.69	3.51	3.32	3.82	3.43	0.018	0.329	0.088

Table 3A2.7. Effect of feeding group on thiol groups of raw and cooked meat throughout storage (nmol SH/mg protein) (n = 8)

	C	E	R	RG	ER	P_{gender}	P_{group}	SEM
				raw meat				
d2	102.2	99.74	95.99	94.33	97.70	0.890	0.481	1.432
d5	83.81	75.81	83.06	79.83	81.02	0.001	0.158	1.267
d8	66.51	69.72	70.50	69.71	67.38	0.875	0.863	1.292
				cooked meat				
d2	65.12	67.51	64.25	68.37	66.70	0.018	0.931	1.689
d5	54.15	44.64	47.33	46.69	45.74	0.590	0.438	1.682
d8	59.95	53.52	53.82	51.86	53.50	0.404	0.427	1.399

DISCUSSION

Oxidation of the feed and animal performances

The primary objective of this research was to examine the *in vivo* effect of dietary antioxidants. All feeds, regardless of antioxidant treatment, had similar oxidation rates in the oxygen bomb. This finding was to be expected, since the antioxidants were incorporated in the feed in such a way to assure their stability during feed storage. Only during digestion, α-tocopherol, rosemary and gallic acid, were expected to be released from their protective matrix. In accordance with the results from the oxygen bomb, the POV values indicate that the formation of primary oxidation products was not different between the feeds. It should be realised that the POV values of the feeds after 3 and 6 weeks of storage at 25°C were lower than the values at the end of the oxidation process since the rate of degradation of hydroperoxides during storage may probably have been much higher than their formation.

The experimental diets did not impair feed intake nor animal growth. This implicates a lack of effect of the oxidized oil at this supplementation level on the palatability and digestibility of the feed. This is in accordance with De Rouchey et al. (2004). It seems that oxidation of fatty acids does not prevent their digestion and absorption because chylomicrons within the lymphatic system are adept in the transport of lipid peroxides (Aw, Williams & Gray, 1992). However, some conflicting results are reported on this topic. For example, oxidized fats as components of animal diets have likewise been shown to impair growth in broilers, dogs and mink (Borsting, Engberg, Jakobsen, Jensen & Andersen, 1994; Engberg et al., 1996; Lin et al., 1989; Turek, Watkins, Schoenlein, Allen, Hayek & Aldrich, 2003).

Meat oxidative stability measurements

From the results, it seems that the supply of antioxidants in a diet based on oxidized linseed oil did not alter the meat fatty acid profile and by no means the profile of

the more oxidation-sensitive polyunsaturated fatty acids. This lack of dietary effect on the meat fatty acid profile was in line with the unaltered fatty acid profile of the feed between the groups. The reason for the higher IMFA content for the R group is unclear since rosemary extract added via the feed is not known to increase the IMFA content. Moreover, this is unlikely to be a consistent effect as the other groups where rosemary was added at the same level (RG en ER), did not display a higher IMFA content.

Only the groups where ATA was supplemented on top of the physiological requirement (E and ER), presented a significantly higher intramuscular and subcutaneous AT content compared to the other groups. The AT content in the subcutaneous fat was similar to a previous study at our laboratory where the same ATA content was supplied via the feed (Haak et al., 2006). However, the AT content in the LT muscle was much lower in the current experiment, although the IMFA content was comparable in both trials. The reason for this lower AT content, specifically in the muscle tissue, is unknown.

There was no preserving effect of rosemary (R) on AT in the muscle since the AT content did not differ between the E and ER groups. Moreover, the AT content after R or RG supplementation was not different from the C group where no supplementary antioxidants were supplied. This implies that rosemary did not exert an effect on the AT incorporation at the current levels of supplementation. The lack of sparing or synergistic effect of supplementary dietary antioxidants corresponds with the findings in our previous trial (Haak et al., 2006). This is in contrast with results of Botsoglou, Grigoropoulou, Botsoglou, Govaris and Papageorgiou (2003) for turkey, where supplementation of oregano oil to a basal diet with 30 ppm ATA increased the retention of AT.

TBARS values of the raw meat clearly indicated that groups with extra ATA supplementation (E and ER) were best protected from lipid oxidation. Here again, it could be noticed that the administration of oxidized oil, even without antioxidants, did not induce a high level of oxidation. The observed lack of antioxidant effect for the rosemary extract is contradictory to its well-known antioxidant properties. Furthermore, for the combination of rosemary extract and gallic acid, a significant lipid pro-oxidant effect could be seen in the raw meat. When added to the meat post mortem, herbs and spices and their extracts have been well documented in terms of their ability to increase

shelf-life of pork, beef, and poultry products through their antioxidant capacities (El-Alim, Lugasi, Hóvári & Dworschák, 1999; Govaris, Botsoglou, Papageorgiou, Botsoglou & Ambrosiadis, 2004; Liu, Booren, Gray & Crackel, 1992; Sebranek, Sewalt, Robbins & Houser, 2005; Tanabe, Yoshida & Tomita, 2002). These reports, however, demonstrate the exclusive use of these compounds as additives to minced meat during further processing or after cooking, or as surface treatments of whole cuts prior to storage. Very little published work describes the use of these compounds to influence product attributes when incorporated in the diets of meat-producing animals. Lopez-Bote et al. (1998) noted a slightly enhanced oxidative stability in meat from chickens fed rosemary and sage oleoresins. However, failing to observe a response in pigs, it was suggested that the effect of spice oil feeding may not translate between species, and that further investigation is warranted. Besides, recent studies have described the complexity associated with the use of herbs or plant extracts as inhibitors of oxidative reactions (Kähkönen et al., 1999; Zheng & Wang, 2001). According to Kähkönen et al. (1999), Rietjens et al. (2002), Skerget, Kotnik, Hadolin, Hras, Simonic and Knez (2005), Wong, Hashimoto and Shibamoto (1995), and Yen, Chen and Peng (1997), the effect of plant phenolics on the oxidative stability is affected by the oxidation conditions and lipid characteristics of the system and the presence of tocopherols and other active substances leading to antioxidant or prooxidant effects. Moreover, antioxidants might behave differently in different matrices (depending on the polarity), a phenomenon often referred to as the ‘polar paradox’ (Frankel, 1998).

Neither for the raw nor for the cooked meat, any effect of antioxidant on protein oxidation as measured by carbonyl or thiol groups could be detected. Moreover, in contrast to findings of Batifoulier et al. (2002), Estévez and Cava (2004), and Mercier, Gatellier and Renerre (1995), there seemed to be no linkage between protein and lipid oxidation here. As expected, carbonyl groups increased and thiol groups decreased as a result of oxidation brought about by storage and the cooking process. For thiol groups however, the increase in the cooked meat from d5 to d8 was unexpected since thiol groups should decrease as oxidation is progressing during storage. However, this corresponds to our finding in an earlier experiment where thiol groups increased significantly during storage of raw meat from d5 to d8 (Haak et al., 2006).

CONCLUSIONS

In this experiment, the dietary administration of single antioxidants or antioxidant combinations (α-tocopheryl acetate and rosemary extract (+ gallic acid)) did not exert any effect on colour or protein oxidation in pork. Only lipid oxidation was decreased by supplementary α-tocopheryl acetate in the diet. This was associated with a higher content of α-tocopherol in the meat and the subcutaneous fat. No antioxidant effect of rosemary extract was observed in this experiment. Moreover, the results failed to provide evidence for a synergistic effect of either α-tocopherol and rosemary or rosemary and gallic acid.

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CHAPTER 3B

EFFECT OF EXOGENOUS ANTIOXIDANT ADDITION ON THE OXIDATIVE STABILITY OF PORK

Redrafted after Haak, L., Raes, K., and De Smet, S. (2007). Effect of plant phenolics, tocopherol and ascorbic acid on oxidative stability of pork patties. (in preparation).

CHAPTER 3B

EFFECT OF PLANT PHENOLICS, TOCOPHEROL AND ASCORBIC ACID ON OXIDATIVE STABILITY OF PORK PATTIES

Abstract - The effect of rosemary extract (RE), green tea extract (TE), tocopherol, trolox, ascorbic acid (AA) and ascorbyl palmitate (AP), at levels of 50 to 200 ppm of antioxidant components, on the oxidative stability of fresh, frozen and cooked pork patties during illuminated chill storage was investigated. Individual major components of RE and TE, carnosic acid and epicatechins respectively, and minor components of TE, theobromine and caffeic acid (TB+CAF), were also tested. Colour (CIE L*a*b*), lipid (TBARS) and protein oxidation (thiol groups) were measured. RE, TE, AP, tocopherol and trolox equally inhibited lipid oxidation in fresh and frozen patties, whereas for cooked patties RE was most effective. At the doses applied here, AA stimulated lipid oxidation. No effect of antioxidant dose in the range of 50 to 200 ppm was found for fresh and frozen patties, whereas for cooked patties higher doses of RE and TE more efficiently prevented lipid oxidation. Protein oxidation was hardly influenced by antioxidant treatment. Colour stability decreased as follows: tocopherol, AA and AP> RE and TE> trolox. Antioxidant properties of the extracts and their major antioxidant components were comparable. Furthermore, TB+CAF showed considerable antioxidant activity despite the low dose applied here.

Key Words: ascorbic acid, green tea, meat, oxidative stability, rosemary, tocopherol

INTRODUCTION

Antioxidants are added to meat products during processing to delay oxidation. Oxidative processes in meat and meat products during storage or cooking lead to the degradation of colour pigments, lipids and proteins which, in turn, can contribute to the deterioration in flavour, texture, colour and nutritional value of the meat (Decker et al., 1998). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) have been widely used in the meat industry. However, rising consumer resistance towards the use of synthetic additives, has increased interest in naturally occurring substances (Pokorny, 1991). Moreover, given the mounting body of science continuing to report on their antioxidant potential, a variety of extracts of fruits, herbs, vegetables, cereals and other plant material rich in antioxidant substances have been commercialized for food and nutraceutical applications (Shahidi, 2000 and Pellegrini et al., 2003). Several studies have demonstrated that their *post mortem* supplementation to pork, improved its oxidative stability (McCarthy et al., 2001; Rey et al., 2005; Martinez et al., 2006). The antioxidant properties of these plant extracts are dedicated to a cocktail of active components, which could act individually as well as in synergism (Halliwell, 2000). Among those, are the frequently studied rosemary (*Rosmarinus officinalis*) and green tea (*Camellia sinensis*) extracts (RE and TE respectively), that have been shown to improve the oxidative stability of meat products (Madsen et al., 1996; Sebranek et al., 2005; Martinez et al., 2006). The major active antioxidant components of RE and TE belong to the polyphenols and are known as phenolic diterpenes (carnosic acid (CA) and carnosol) and epicatechins (epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC)) respectively. The total polyphenol content of plant extracts however can be highly variable and depends on the extraction solvent and the plant variety used (e.g. Gramza et al. (2006) for TE). Therefore, it is important to compare antioxidant extracts based on their active component levels instead of on a w/w basis (weight of substance by weight of meat). By doing so, the superiority of certain antioxidants over others can be properly assessed, and differences can then only be explained by qualitative and not quantitative differences in antioxidant components present.

In the meat industry, the antioxidants ascorbic acid (AA) and α-tocopherol have been widely considered for extending the retail display life of meat. AA has since long been known for protecting the colour of raw red meat during storage (Greene et al., 1971). However, depending on its concentration, AA either promoted or inhibited lipid oxidation in muscle foods (Yen et al., 2002). The lipid antioxidant α-tocopherol has demonstrated strong antioxidant activity in a wide range of meats when supplemented as α-tocopheryl acetate via the animal diet (Higgins et al., 1998; Kerry et al. 1998). However, when applied to meat and fish products as α-tocopherol, it was less effective in controlling lipid oxidation (He and Shahidi, 1997; Higgins et al., 1998).

The purpose of this work was to investigate the effectiveness of natural antioxidants and antioxidant extracts (tocopherol, AA, RE and TE) in delaying oxidation (of colour, lipids and proteins) in fresh, frozen and cooked pork patties. All antioxidant(s) (extracts) were added on the basis of their active component content (as specified by the manufacturer) in order to effectively compare antioxidants.

MATERIALS AND METHODS

Solvents, standards and plant extracts

Hydrochloric acid and methanol were analytical grade and purchased from Sigma-Aldrich (Bornem, Belgium). Butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), tris(hydroxymethyl)aminomethane (TRIS), ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich (Bornem, Belgium) as the available standards. Mixed tocopherol (30% purity; R,R,R-α,β,γ,δ-tocopherol isomers 5-15%, 0-5%, 60-70%, and 20-30% respectively; tocopherol), ascorbic acid (>99% purity; AA) and ascorbyl palmitate (>98% purity; AP) were kindly donated by DSM Nutritional Products (Deinze, Belgium). All other antioxidative components (trolox, CA, EGCG, EGC, ECG, EC; theobromine (TB) and caffeic acid (CAF)) were purchased from Sigma-Aldrich (Bornem, Belgium) as the available standards. Rosemary extract (Stabiloton® OS; 17% CA; RE) and green tea extract (46% tea catechins: 50.0% EGCG, 30.4% EGC, 15.2% ECG, 4.4% EC; 0.75% TB and 7.5% CAF; TE) were a gift from RAPS (Beringen, Belgium).

Preparation of patties

Frozen and vacuum packed pork meat (*longissimus thoracis*) and fat (both originating from a previous experiment at our laboratory) were weighed in a ratio 2/1 (w/w), chunked, minced through a 10-mm steel plate using a Grindomix GM 200 mincer (Retsch, Pakistan) and held overnight at 0°C. Calculated quantities of antioxidant(s) (extracts) were first blent with sodium chloride (NaCl, added at 1% (w/w)) and added to the meat before final grinding. To the control patties, only NaCl was added. During preparation, the internal temperature of the patties was monitored by a probe thermometer and did not exceed 4°C. For the control and each of the antioxidant treatments, meat mixes of 160g were prepared and formed into 4 patties (40 g each) using a round shaped mould of 55 mm internal diameter and 20 mm height. The patties were

wrapped in an oxygen permeable polyethylene film and held under refrigerated (4°C) display conditions (illumination of 900 lux) for 8 days.

Experimental design

In experiment I, the first objective was to test the antioxidant action of 4 commercially available antioxidants: AA, tocopherol, RE and TE. Secondly, it was aimed at comparing the antioxidant action of tocopherol and its water soluble analogue trolox, and of AA and its lipid soluble analogue AP. Hereby, 2 supplementation levels (100 and 200 ppm of antioxidant components) were tested for all previously mentioned antioxidant treatments. Thirdly, RE and TE were compared to their major antioxidant components, CA and tea catechins respectively (EGCG, EGC, ECG and EC were added proportionally to their specified occurrence in the TE), at a level of 100 ppm. Carnosol was not added because of its low and unspecified content in the extract and the lack of a stable standard. A last treatment consisted of adding TB+CAF, two minor components of TE, in a dose equal to the one obtained (18 ppm) by adding TE up to the desired catechin level of 100 ppm. The preparation of the patties was repeated twice in time and for every repetition, control patties were prepared. Half of the patties were placed in the illuminated chill cabinet to perform oxidative stability measurements on the fresh patties (fresh) and the other half were immediately vacuum-packed and stored at -18°C (4 months) for oxidative stability measurements after freezing and thawing (frozen).

In experiment II, for AA, RE and TE, the dose effect was examined more closely. Therefore, each of these antioxidants were added at a level of 50, 100, 150 and 200 ppm of antioxidant components. The preparation of the patties was repeated thrice in time and again for every repetition, control patties without antioxidant added were prepared. Half of the patties were placed immediately in the illuminated chill cabinet (fresh) and the other half were cooked in a water bath at 70°C for 40 min (in an impermeable plastic bag) (cooked) before illuminated chill storage and the performance of oxidative stability measurements.

Analyses

Colour and colour stability

For experiment I, colour stability was measured on day 0 (d0; after a 30 min bloom period), day 4 and day 8 of illuminated chill storage for both fresh and frozen patties. For experiment II, colour stability was measured on day 0, 1, 2, 4 and 8 of illuminated chill storage for the fresh patties. For the cooked patties, colour stability was not measured. Colour coordinates (CIE L*a*b* colour system (1976)) and reflectance spectra (every 10 nm between 400 and 700 nm) were assessed using a HunterLab Miniscan Minolta XE plus spectrocolorimeter (light source of D65, standard observer of 10°, 45°/0° geometry, 1 in. light surface, white standard). The results were expressed as lightness (L*), redness (a*), yellowness (b*), hue value ($\tan^{-1}$ b*/a*) and chroma ((a^{*2} + b^{*2})$^{1/2}$). By means of reflectance values at specific wavelengths, the percentage of the different forms of myoglobin (oxymyoglobin (OxyMb), deoxymyoglobin (Mb), and metmyoglobin (MetMb)) were calculated according to the method of Krzywicki (1979), modified by Lindahl et al. (2001).

Lipid oxidation

Lipid oxidation was assessed by 2-thiobarbituric acid-reactive substances measurement (TBARS) using the distillation method as described by Tarladgis et al. (1960) and was expressed as μg MDA/g meat. This method estimates MDA, a secondary lipid oxidation product that together with TBA forms a coloured complex, which is determined spectrophotometrically at 532 nm. For both experiments, lipid oxidation was measured after 4 and 8 days of illuminated chill storage. For the frozen patties of experiment I, a supplementary assessment of lipid oxidation was done on day 1 after thawing.

Protein oxidation

Protein oxidation was assessed by measuring thiol groups using the method of Ellman (1959) with slight modifications (Batifoulier et al., 2002) and was expressed as

nmol thiol groups/mg protein. This method relies on the incubation of a meat homogenate with DTNB, followed by a spectrophotometrical measurement of the thiol groups at 412 nm. Protein oxidation was only measured for experiment I, on the same samples and days as for the TBARS measurement.

Statistical analysis

For experiment I, contrast analysis following ANOVA was performed to compare antioxidants or to detect differences between antioxidant extracts and their pure antioxidant components. Also, the effect of antioxidant dose was tested by contrast analysis. Data were analysed separately per sampling day. For all analyses performed in experiment II, a General Linear Model procedure with fixed factors antioxidant and dose was used. The interaction term was not significant and therefore not included in the model. The Bonferroni *post hoc* comparison of means test was used to compare antioxidants and polynomial contrast analysis was performed to compare doses. All analyses were performed using the statistical software package S-Plus for Windows (version 6.0).

RESULTS

Experiment I

The antioxidant action of AA, tocopherol, RE and TE is shown in Figures 3B.1, 3B.2 and 3B.3 for colour, lipid and protein oxidation respectively. No difference in a*-values between AA, tocopherol, RE and TE was found immediately after preparing the patties (d0 fresh). However, subsequently, tocopherol and RE addition resulted in the highest and lowest a*-value respectively ($P<0.05$). Tocopherol and AA significantly lowered MetMb% compared to the control (mean MetMb% on day 8 for fresh patties 42.4, 44.1 and 47.3 respectively) ($P<0.05$), whereas TE and RE did not significantly decrease MetMb% (45.3 and 44.9 respectively). Of all antioxidant treatments, RE showed the highest OxyMb% and the lowest Mb% ($P<0.05$). Throughout the storage period, TBARS values were higher after AA addition and lower after tocopherol, RE or TE addition compared to control patties ($P<0.05$) (Figure 3B.2). Overall, TE and RE inhibited lipid oxidation to the same extent, whereas tocopherol was less efficient ($P>0.05$). The antioxidant treatment did not influence protein oxidation as measured by thiol groups except for day 8 after frozen storage, when protein oxidation was lower (thiol groups were higher) for tocopherol, RE and TE as compared to the control group and AA ($P<0.05$). Hereby, TE was superior to RE, whereas tocopherol was intermediate. There was no difference in thiol groups between the control and the AA group (Figure 3B.3).

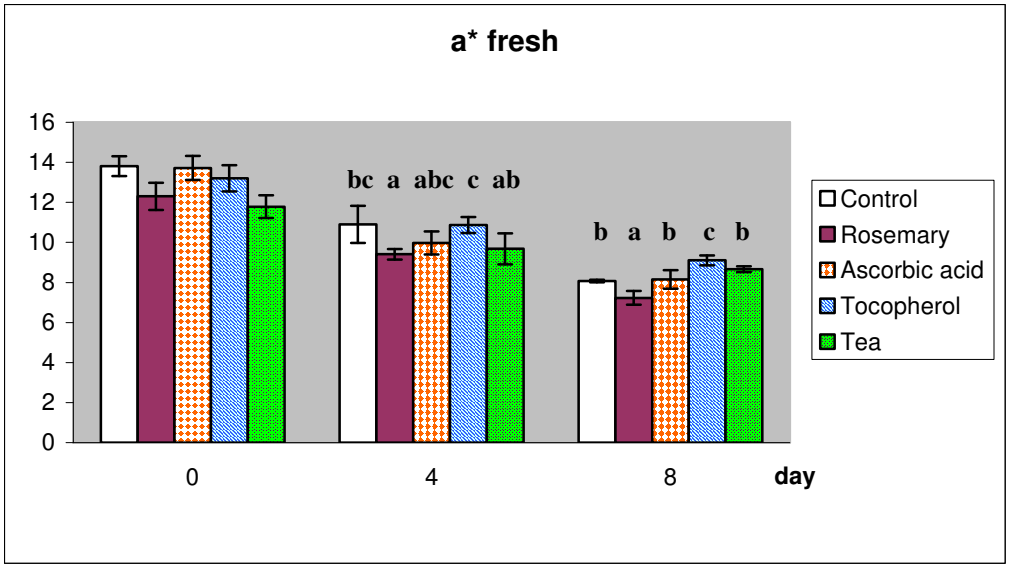

Figure 3B.1. a*-value during illuminated chill storage of fresh patties after addition of ascorbic acid, tocopherol, rosemary and tea extract (within one sampling day, bar charts with different superscripts are significantly different at $P<0.05$)

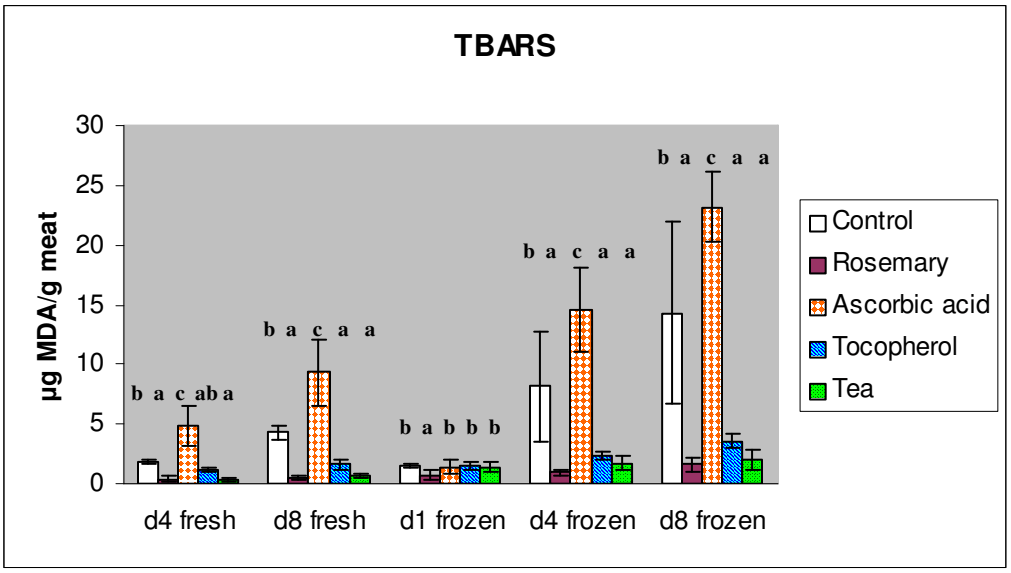

Figure 3B.2. TBARS value (µg MDA/g meat) of fresh and frozen patties during illuminated chill storage after addition of ascorbic acid, tocopherol, rosemary and tea extract (within one sampling day, bar charts with different superscripts are significantly different at P<0.05)

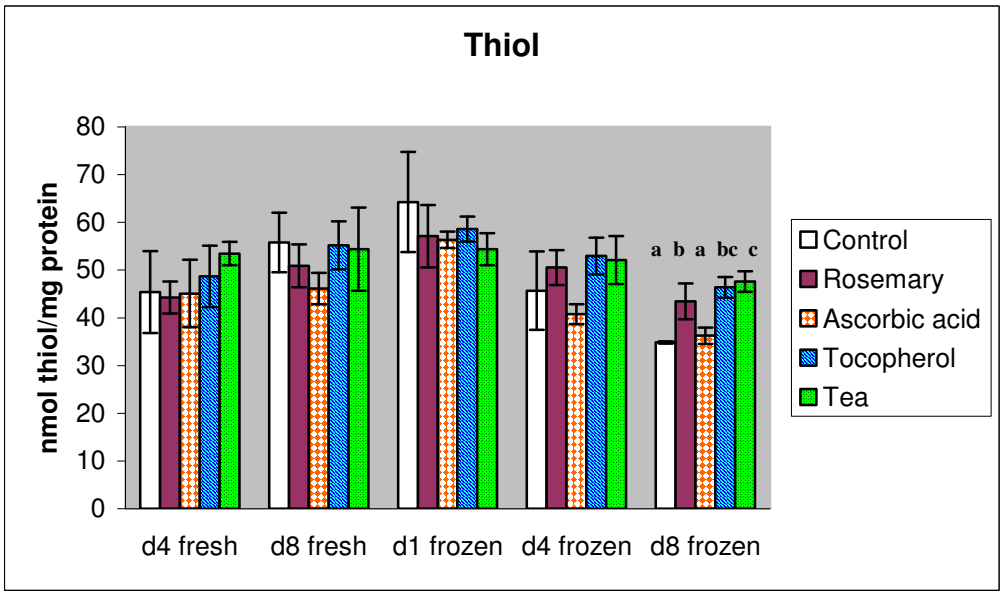

Figure 3B.3. Thiol groups (nmol thiol/mg protein) of fresh and frozen patties during illuminated chill storage after addition of ascorbic acid, tocopherol, rosemary and tea extract (within one sampling day, bar charts with different superscripts are significantly different at P<0.05)

Regarding the antioxidant action of the water and lipid soluble analogues of tocopherol and AA (trolox and AP respectively), the results were as follows: throughout the experiment, AP resulted in an a*- value equivalent to tocopherol and thus higher than AA. The trolox addition resulted in a much lower a*-value than tocopherol, even lower than the control ($P<0.05$). AA, AP and tocopherol inhibited the formation of MetMb as compared to the control ($P<0.05$ for the frozen patties and on day 8 for the fresh patties), whereas trolox had the opposite effect ($P<0.05$). The OxyMb% did not differ between AA and AP, whereas it was much lower for trolox than for tocopherol and the control ($P<0.05$). TBARS values for the AP treatment were lower than for the control ($P<0.05$ after frozen storage), and thus also lower than for AA (Figure 3B.4). Trolox inhibited lipid oxidation more than tocopherol ($P<0.05$ on day 1 after frozen storage) except for day 8 after frozen storage (Figure 3B.4). Furthermore on day 8 after frozen storage, AP and tocopherol lowered protein oxidation as compared to AA and trolox respectively ($P<0.05$) (34.8, 36.3, 45.1, 46.4, and 37.1 nmol thiol/mg protein for control, AA, AP, tocopherol and trolox respectively).

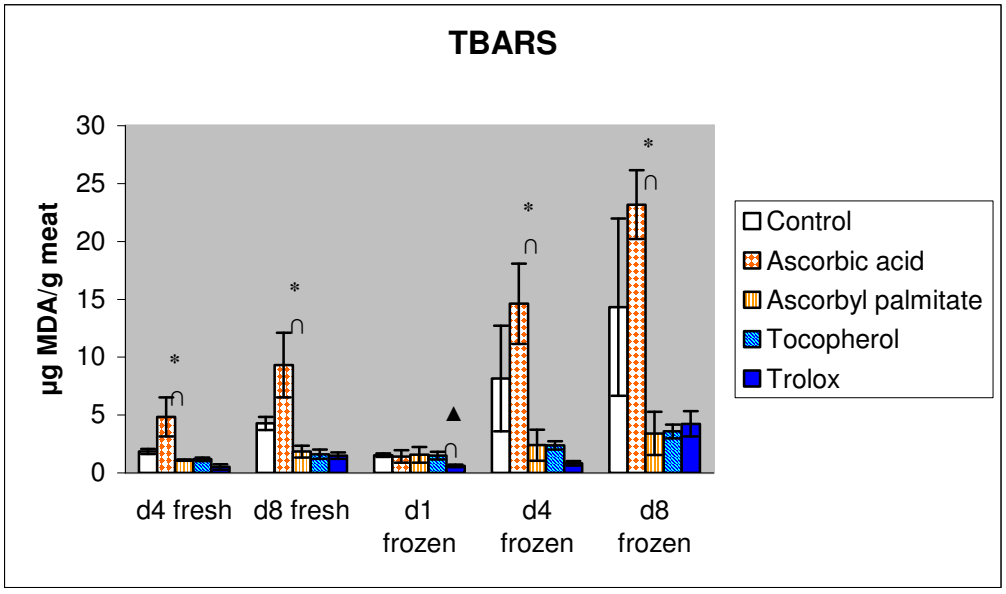

Figure 3B.4. TBARS value (µg MDA/g meat) of fresh and frozen patties during illuminated chill storage for tocopherol vs. trolox and ascorbic acid (AA) vs. ascorbyl palmitate (AP) (within one sampling day, * or ▲ indicate a significant difference ($P<0.05$) between AA and AP or tocopherol and trolox respectively)

The antioxidant dose did not influence the extent of oxidation considerably. Only a few differences were noticed. For AA, the MetMb% was lower for the 200 ppm than for the 100 ppm dose, whereas for RE and trolox the opposite was found (e.g. for fresh patties on day 8 mean MetMb% for 100 and 200 ppm was 45.4 and 42.7, 43.8 and 45.9, 60.2 and 63.0 for AA, RE and trolox respectively). Only for AP after frozen storage, there was a dose effect on lipid oxidation, with the highest TBARS value for 200 ppm ($P<0.05$; e.g. mean value for frozen patties on day 8 was 2.36 and 4.46 μg MDA/g meat for 100 and 200 ppm respectively). Furthermore, there was hardly any effect of the antioxidant dose on thiol groups. Only for RE on day 8 after frozen storage, thiol groups were lower for the 200 ppm as compared to the 100 ppm dose (46.1 vs. 40.8 nmol thiol groups/mg protein) ($P<0.05$). Given the lack of significance for the interaction between antioxidant treatment and dose, it can be said that the anti- or prooxidant character of a specific antioxidant was unaltered, independent of dose.

The comparison between the antioxidant extracts and their major antioxidant components, did not reveal differences in their antioxidant action (see Figures 3B.5 and 3B.6 for lipid oxidation results for TE and RE respectively). The minor components of TE, TB+CAF, resulted in markedly lower TBARS values as compared to the control treatment ($P<0.05$). However, for the fresh and the frozen patties on day 8, they inhibited lipid oxidation to a smaller extent as compared to the TE or the catechins. Nevertheless, this effect was not significant due to the high variability encountered.

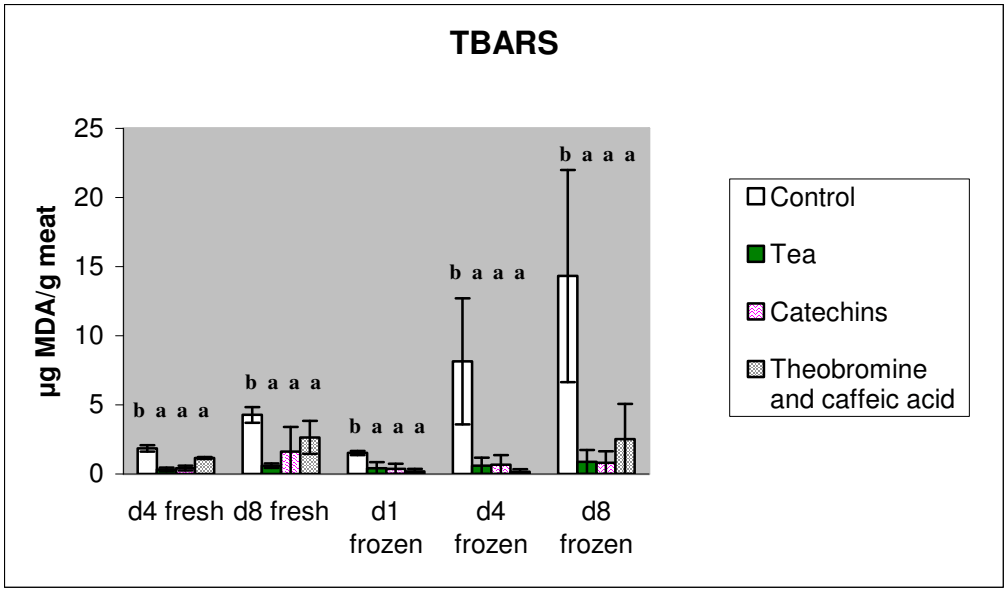

Figure 3B.5. TBARS value (μg MDA/g meat) of fresh and frozen patties during illuminated chill storage for tea extract vs. its major antioxidant components (within one sampling day, bar charts with different superscripts are significantly different at $P<0.05$)

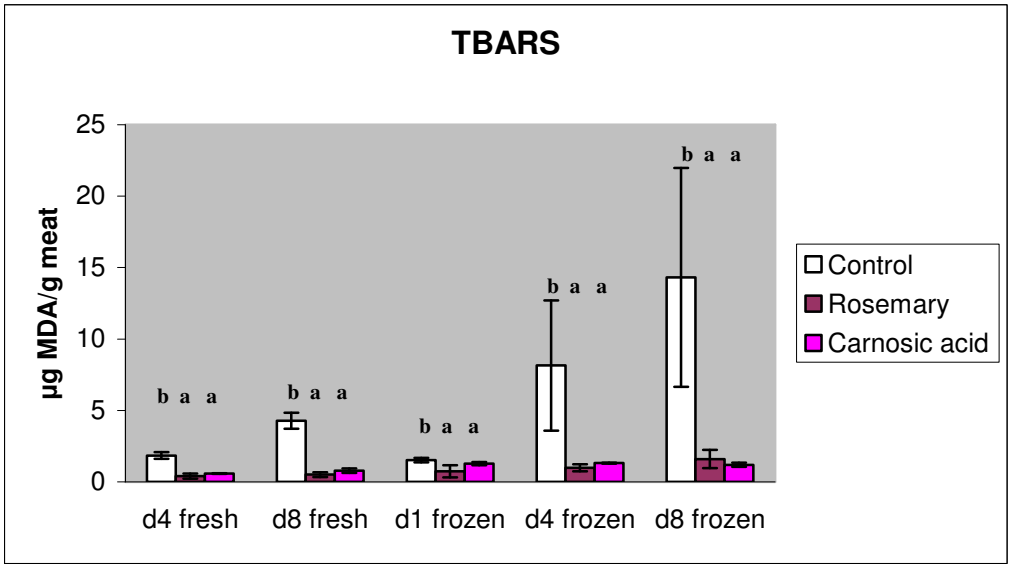

Figure 3B.6. TBARS value (μg MDA/g meat) of fresh and frozen patties during illuminated chill storage for rosemary extract vs. its major antioxidant components (within one sampling day, bar charts with different superscripts are significantly different at $P<0.05$)

Experiment II

There was no effect of antioxidant treatment on the a*-value of the patties, except for d0 where the a*-value was lowest for TE, intermediate for RE and highest for AA and the control (P<0.05) (Figure 3B.7). For RE, Mb% was lowest throughout storage and OxyMb% was highest up to day 2 of storage (P<0.05; data not shown). On day 1 and day 2, MetMb% was highest for AA (P<0.05; data not shown). No important dose or interaction effect was found for the colour parameters.

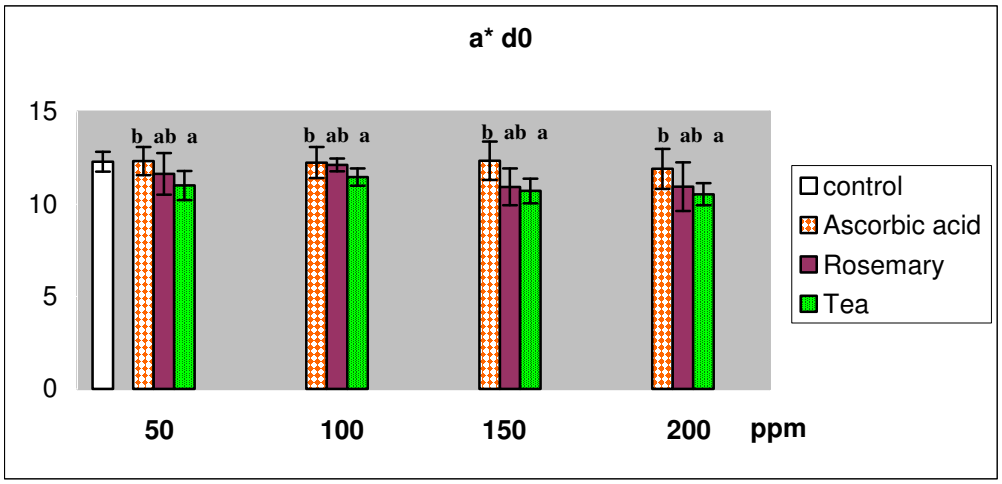

Figure 3B.7. a*-value of fresh patties (d0) after addition of ascorbic acid, rosemary and tea extract at doses of 50-200 ppm (within one dose, bar charts with different superscripts are significantly different at P<0.05)

For the fresh patties, TBARS values were much higher when AA was added compared to RE or TE (P<0.001; data for day 4 in Figure 3B.8). For the cooked patties, TBARS values were highest for AA, lower for TE and lowest after RE addition (P<0.05; data for day 4 in Figure 3B.8). Only after cooking, there was a linear effect of antioxidant dose on lipid oxidation (P<0.05 and P<0.001 for day 4 and day 8 respectively) with decreasing TBARS values for increasing doses of RE and TE, and increasing TBARS values for increasing doses of AA.

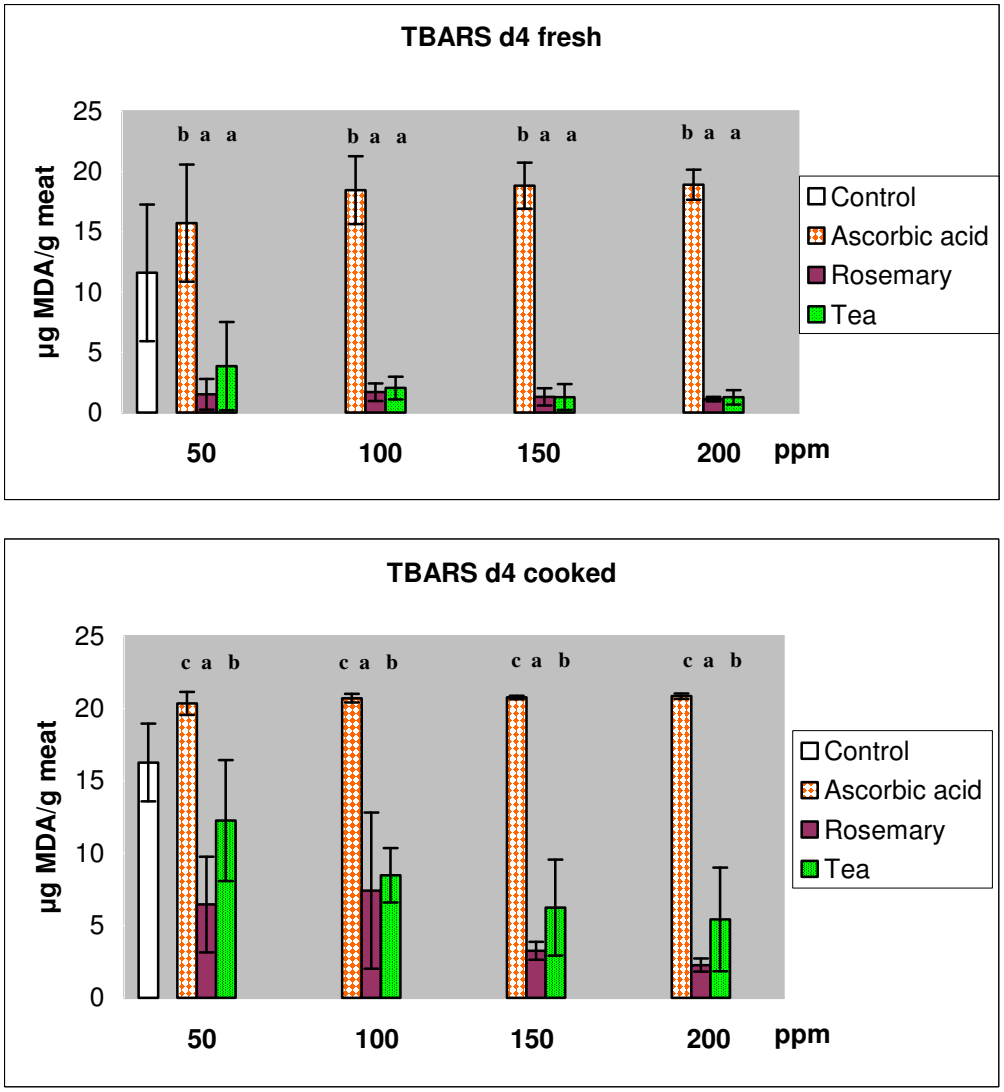

Figure 3B.8. TBARS value (µg MDA/g meat) of fresh and cooked patties after 4 days of illuminated chill storage after addition of ascorbic acid, rosemary and tea extract at doses of 50-200 ppm (within one dose, bar charts with different superscripts are significantly different at $P<0.05$)

DISCUSSION

Tocopherol and AP resulted in the best colour stability (a*-value, MetMb%) whereas trolox negatively influenced colour of the patties. The effect of AA on colour stability was inconclusive. AA inhibited the formation of MetMb and was most effective at the highest dose in our first experiment, however, this could not be confirmed in the second experiment. The behaviour of AA as an antioxidant in terms of colour oxidation, was reported by Mitsumoto et al. (1991). There was no outspoken effect of the extracts or their individual components on colour of the patties. For TE, this was in line with results of Martinez et al. (2006) for fresh pork sausages, whereas contrary to our results showing the lowest a*-value for RE, these authors found an improvement of colour by RE.

Tocopherol, RE and TE strongly inhibited lipid oxidation, whereas AA clearly showed a lipid- prooxidant character in pork patties. Tocopherol and trolox showed a comparable lipid antioxidant activity. This was somewhat unexpected, given the different isomerical configuration of the analogues -α for trolox vs. α, β, γ and δ for tocopherol-favouring the in vitro antioxidant activity of the last (Dziezak, 1986). The prooxidant character of AA was reverted to antioxidant when it was added as its lipid soluble analogue AP, however only in the lowest dose (100ppm). The increased TBARS value for the highest dose of AP indicated a dose-dependent transition towards a lipid prooxidant character as reported by Yen et al. (2002) for AA. The prooxidant character of AA seen for lipid oxidation did not appear in terms of protein oxidation, and as already mentioned, neither for colour oxidation. For cooked patties, RE was a more effective inhibitor of lipid oxidation compared to TE. For stabilizing pork lard and chicken fat, Chen and Chan (1996) concluded that RE was less effective than TE. Although a different antioxidant composition and content of the extracts might be responsible for opposite outcomes in different studies, to our view this also stresses the need to test antioxidants in the food matrix they will be applied in.

Given the lack of difference in the oxidation inhibiting effect of either the extracts (RE and TE) or their major antioxidant components, it can be said that the antioxidant properties of the extracts can be attributed to these individual components. For tea catechins, this is consistent with Tang et al. (2001a,b), reporting on their excellent lipid

oxidation inhibiting effect. Despite their low dose, the minor antioxidant components of TE, TB+CAF, clearly lowered lipid oxidation and might therefore together with the catechins contribute to the global oxidation inhibiting effect of the extract. The lack of dose effect for the antioxidants in fresh and frozen patties, implies that there was no supplementary lipid oxidation inhibiting effect of any of the antioxidants above the level of 100 ppm. After cooking however, higher antioxidant doses (as measured up to 200 ppm) more effectively inhibited oxidation.

Throughout the storage period of the fresh patties, there was no indication for a protein oxidation inhibiting effect of the added substances. Moreover, only at the end of the display period of the frozen patties, it was shown that AP and tocopherol were more effective inhibitors of protein oxidation compared to their water soluble analogues and only then, the antioxidant activity of tocopherol, RE and TE as shown for lipid oxidation, was apparent. This poor protein oxidation inhibiting effect of RE (added at 0.04%) corresponds with findings for cooked pork patties by Salminen et al. (2006). However, it is in contrast to Estevez et al. (2005) stating that the addition of rosemary essential oil (0.6g/kg) in refrigerated frankfurters inhibited protein oxidation. For TE, no study on the protein oxidation inhibiting effect in meat products could be found to compare with. Furthermore, Vuorela et al. (2005) reported that polyphenolic plant components were excellent inhibitors of protein oxidation in cooked pork patties. Another explanation might be that more severe protein oxidation was only setting in late during storage of the frozen patties, so that only then differences between antioxidant treatments became clear.

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CHAPTER 4

GENERAL DISCUSSION AND FUTURE PROSPECTS

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Alteration of pork fatty acid profile

As mentioned above, there is great interest in improving the fatty acid profile of pork to better meet nutritional recommendations. In this work, the effects of animal dietary strategies and culinary practices were examined. These effects will be briefly compared here.

Improving the pork fatty acid profile by dietary strategies

It was shown in this work that the fatty acid profile of pork could be beneficially affected by moderate levels of linseed and fish oil (1.2 % oil or equivalent in the diet) as sources of α-linolenic acid (α-LNA) and the long-chain n-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) respectively. The effect of duration and time of feeding these oil sources (either during the whole fattening period or only during the first or second fattening phase) on the muscle fatty acid composition, varies with the fatty acids considered. When supplying linseed, only the last phase before slaughter was determinant for the deposition of α-LNA and the conversion to its long-chain metabolites EPA and DHA. The greatest EPA and DHA proportions were found for a continuous fish oil supply throughout the fattening period. However, when fish oil was fed during the first fattening phase followed by linseed before slaughter, levels of DHA but not EPA were lower. Neither meat quality traits (drip loss, pH,…), nor lipid or colour oxidation were influenced by dietary oil source or duration of supplementation at this level, even though fish oil supplementation increased the levels of EPA and DHA, and it is well known that these fatty acids are more prone to oxidation. Nevertheless, except for dry cured ham, meat products from pigs fed on this level of fish oil (cooked cured ham, sausage and grilled meat) were less appreciated by a consumer panel compared to meat

products from animals fed on a linseed or basal diet (where animal fat was used as supplementary fat source). However, the eating quality differences were not pronounced.

Alteration of pork fatty acid profile by culinary practice

Studies on improving the fatty acid composition of pork usually do not take into account the influence of culinary processing, which often includes a heat treatment and a fat addition. The significant effect of pan-frying in different culinary fats on the fatty acid profile of pork, clearly emphasizes the need for an integrated approach of dietary practice and *post mortem* processing in the attempt to produce meat that contributes to a valuable and wholesome diet.

The fatty acid composition of the pan-fried meat tended to become similar to that of the culinary fat. The extent of increase or decrease of a particular fatty acid during frying was relative to the fatty acid gradient from the culinary fat to the meat. Pan-frying without fat is preferable when aiming at a lower fat intake in the human diet and preserving the differences in initial fatty acid profile of the meat obtained by different feeding strategies. Frying in a commercial PUFA-enriched culinary fat (higher n-6 PUFA), increased the PUFA proportion in the meat and decreased the SFA proportion but can negatively affect the n-6/n-3 ratio dependent on the fatty acid profile of the meat. Frying in margarine shifted the SFA composition towards a significantly higher proportion of the atherogenic lauric (C12:0) and myristic acids (C14:0), although resulting in a better n-6/n-3 ratio compared with frying in PUFA-enriched culinary fat. Pan frying in olive oil resulted in a decline in the proportion of SFA and an increased proportion of MUFA (particularly oleic acid (C18:1c9)), while the effect on PUFA was variable. After frying, and irrespective of the type of culinary fat used, differences in fatty acid profile of the meat obtained by animal feeding strategies could still be observed. Although LCPUFA are said to be the most heat-labile and oxidation-sensitive fatty acids, they were not significantly lost by the frying process and had thus not explicitly been subject to oxidation during heating. Their proportions however, were influenced by the uptake of the culinary fat.

The fatty acid content and profile of the culinary fat was altered significantly by the frying process and although consuming the residual culinary fat is not advisable in order to limit the fat intake, this might be important for the fatty acid intake profile.

Contribution of this n-3 PUFA enriched meat to a more healthy diet:

In the following paragraph, the contribution of meat fatty acids following different nutritional strategies to nutritional recommendations is estimated. Calculations were done assuming a daily consumption of 100 g of fresh meat and were based on fatty acid data from chapter 2 (Table 4.1). The importance of the frying process on contributions to recommendations can be seen from Table 4.1.

For α-LNA, it can be seen that meat hardly contributes to its recommended intake. After all, the main sources of α-LNA in the diet are vegetable oils and cereals.

Considering fresh meat from pigs fed fish oil throughout the whole fattening period, intakes of EPA and DHA would approximate 36 mg. A daily intake of 200 mg of EPA and DHA has been recommended by the Department of Health (1994). In Belgium, a daily amount of EPA plus DHA above 0.3 % of total energy intake is recommended, which is equal to 867 and 667 mg for an adult man or woman respectively (calculating with 2600 and 2000 kcal/day). As seen from Table 4.1, when considering meat from the fish oil group as the most enriched meat, its contribution is only 4.1 and 5.4 % of the Belgian recommended intake for men and women respectively, whereas it accounts for 18 % of the recommendation by the Department of Health (1994). However, this is nearly 4- and 7-fold the contribution by meat of animals fed on a linseed or basal diet respectively.

During the evolution of recommendations for n-3 fatty acids, guidelines for EPA and DHA have been at the forefront as their benefits have been extensively studied. On the contrary, DPA has been left aside since science has not yet come to an understanding of it's exact health benefit.

This fresh meat does not comply with the recently approved nutrition claims for n-3 fatty acids by the European Food Safety Authority (EFSA, 2005). They include 'n-3 fatty acid source' and 'high in n-3 fatty acids'. For these claims, food must contain more than 15 % or 30 % respectively of the daily recommended nutritional intake (RNI; set at

2g/day for an adult male) per 100g/100ml/100kcal. Although meat of the fish oil group was highest in total n-3 fatty acid content as compared to meat of the linseed and basal group, the content of n-3 fatty acids was only 55mg/100g meat, which is only 2.75 % of the daily RNI of 2g and thus far out of scope of both nutrition claims mentioned here. However, some anomalies arise from these claims. Some foods qualify although a typical serving provides little n-3 PUFA (e.g. radish, safflower oil, butter). These anomalies arise because the reference food quantity (100 g, 100 mL, 100 kcal) is not linked to the typical intake of the food. Moreover, a distinction should be made between α-LNA and EPA or DHA, given their different physiological properties.

Dietary recommendations depend much on whether they have been set either to achieve a nutrient adequacy resulting in the prevention of a fatty acid deficiency, or to prevent and treat chronic diseases. The calculated contribution is highly dependent on the recommended value considered and so varies from almost negligible to substantial.

Based on a 2-days food diary, Sioen et al. (2006b) concluded that the Belgian population showed a large deficit for α-LNA and n-3 PUFA. The mean intake of α-LNA, EPA, DPA, and DHA was only 0.6%, 0.04%, 0.01 %, and 0.06% of energy (E) respectively. Therefore, given our current eating patterns, it seems difficult to comply with the recommendations. Since meat was found to be one of the main sources of long-chain PUFA, apart from fish, seafood, and eggs, even moderate improvements of their fatty acid composition can be valuable. It is therefore obvious that the decision on whether the consumption of this 'enriched' meat could valuably contribute to the needs of the human diet in terms of n-3 fatty acids, must be made independent of the compliance with health claims.

Increasing the fish oil level in the feed could be a means to further optimize the meat fatty acid profile. Nevertheless, considering the threshold of 18 g PUFA/kg feed for monitoring negative quality effects of high PUFA diets in pork (Warnants et al., 1996), a higher dietary supplementation of PUFA was not investigated in the present study.

Table 4.1. Contribution of fresh meat and meat fried in PUFA enriched culinary fat to a more healthy diet

	fish oil	**linseed**	**basal**	**linseed fried[1]**
EPA+DHA				
% FAME[2]	2.39	0.72	0.36	
mg/100g meat[3]	35.9	10.8	5.4	29.6
% intake vs RNI[4] Belgium 0.3%E man (866.7mg/day)	4.1	1.2	0.6	3.4
% intake vs RNI Belgium 0.3%E woman (666.7mg/day)	5.4	1.6	0.8	4.4
% intake vs RNI Dep of Health (1994) (200mg/day)	17.9	5.4	2.7	14.8
α-LNA				
% FAME	0.47	1.24	0.55	
mg/100g meat	7.1	18.6	8.3	125.6
% intake vs RNI Belgium 1%E man (2.89g/day)	0.2	0.6	0.3	4.3
% intake vs RNI Belgium 1%E woman (2.22g/day)	0.3	0.8	0.4	5.7
% intake vs RNI Uauy-Dagach et al.(1992) (700mg/day)	1.0	2.7	1.2	17.9
n-3				
% FAME	3.68	2.72	1.38	
mg/100g meat[3]	55.2	40.8	20.7	178
% intake vs RNI 'n-3 source' EFSA (300mg/day)[5]	18.4	13.6	6.9	59.3
% intake vs RNI 'high in n-3' EFSA (600mg/day)[6]	9.2	6.8	3.45	29.7
% intake vs RNI Belgium 1.3%E man (3.76g/day)	1.47	1.09	0.55	4.74
% intake vs RNI Belgium 1.3%E woman (2.89g/day)	1.91	1.41	0.72	6.16

[1] meat from linseed fed animals after pan-frying in 'PUFA enriched' culinary fat (Haak et al., 2007)
[2] Fatty Acid Methyl Esters
[3] 1.5% and 3.3% fatty acid content for fresh and fried meat respectively
[4] Recommended Nutritional Intake
[5] 15% of RNI (with RNI set at 2g/day for an adult male)
[6] 30% of RNI

Effect of antioxidants on the oxidative stability of pork

Effect of dietary antioxidant supplementation

The effect of dietary antioxidant supplementation on the oxidative stability of meat has been reported for several individual antioxidants. Two or more antioxidants together can act synergistically, *i.e.* protect against oxidation to a higher extent than the sum of the contributions from each single antioxidant (Duthie, 1999; McCarthy et al., 2001). Therefore, the use of antioxidant cocktails in the diet was tested for a possible superior effect compared to single antioxidants.

Firstly, α-tocopheryl acetate (ATA; 40 ppm) was tested individually and as part of a cocktail in which it was combined with components that might act as secondary supporters of the antioxidant action (200 ppm; consisting of ATA, rosemary, citric acid and gallic acid). No effect of antioxidant treatment on colour stability of raw meat or cooked cured ham was observed. For both antioxidant treatments, lipid oxidation in raw meat, cooked cured ham and pre-frozen raw meat was well controlled and only towards the end of the display period, the antioxidant cocktail more strongly inhibited lipid oxidation. However, during display of pre-frozen uncured cooked meat, lipid oxidation increased significantly. It should be mentioned that this frozen storage period of 10 months before retail display is extraordinary long and not common in practice.

No unambiguous effect of antioxidant treatment on protein oxidation was observed as ATA seemed more efficient in reducing protein oxidation in raw meat, whereas for cooked cured ham the opposite was seen.

The results suggest that supplementation of 40 ppm ATA during a 16-weeks period is sufficient for stabilization of meat during the storage period which corresponds with literature (Hoving-Bolink et al., 1998; Jensen et al., 1998; Mason et al., 2005). Moreover, the AT content in musle or subcutaneous fat tissue was not affected by antioxidant treatment. Based on these results, it can be said that the supplemental components of the cocktail (citric acid, rosemary and gallic acid) have no additional or synergistic effect at the doses applied here or that they are partly used during oxidation processes in the feed.

Although in this experiment, the PUFA profile of the meat (especially fatty acids with 3 or more double bonds) was different between animals fed linseed oil or soybean oil, the oxidative stability (including TBARS value) was not influenced. Consequently, feeding oxidized linseed oil (targeted level of 150 mEq O_2/kg oil) to the animals was used as another strategy for studying the effect of dietary antioxidants as a more intense trigger for oxidation could reveal distinct properties of the antioxidants.

Oxidized fat in the animals' feed is a source of oxidative stress *in vivo* which could possibly lower the antioxidant status of tissues and enhance the formation of oxidation products in meat *post mortem* (e.g. Engberg et al., 1996; Jensen et al., 1997). However, the administration of oxidized oil, even without antioxidants supplementary to the basic physiological level for ATA (20 ppm), apparently did not induce a high level of oxidation in our experiment.

The supplementary dietary administration of single antioxidants (ATA or rosemary extract at 40 ppm) or antioxidant combinations (ATA and rosemary extract each at 40 ppm; rosemary extract at 40 ppm and gallic acid at 2 ppm), did not affect colour or protein oxidation in pork. Lipid oxidation was only decreased by supplementary ATA in the diet. This was associated with a higher content of AT in the meat and the subcutaneous fat. No antioxidant effect of rosemary extract was observed in this experiment. On the contrary, the results on lipid oxidation suggested a prooxidant effect of the rosemary extract. It is hypothesized that the more polar antioxidants have a negative impact on the oxidative stability of the meat. Although gallic acid and the polar components in rosemary are powerful antioxidants in lipid systems, they may behave differently in a matrix that also includes water and proteins. This phenomenon is often referred to as the 'polar paradox' (Frankel, 1998). The negative effect of rosemary and gallic acid suggests that the antioxidants are deposited in the meat but most probably in a hydrophilic environment where they are able to increase the prooxidant potential of trace metals, rather than in the lipophilic compartments of the meat where they could delay oxidation more efficiently. Again, the results of this experiment failed to provide evidence for a synergistic effect of either AT and rosemary or rosemary and gallic acid.

As, apart from AT, we did not determine the antioxidants or their metabolites in the muscle tissue, we do not have an idea about their extent of absorption and

incorporation in muscle tissue. This must be kept in mind before drawing conclusions on the activity of antioxidants after dietary supplementation. Besides, as herbs or plant extracts consist of several components in a variety of concentrations, some complexity in the interpretation is associated with their use as inhibitors of oxidative reactions (Kähkönen et al., 1999; Zheng and Wang, 2001; Rietjens et al., 2002; Skerget et al., 2005; Wong et al., 1995; Yen et al., 1997). The effect of plant phenolics on the oxidative stability is affected by the oxidation conditions and lipid characteristics of the system and the presence of other active substances leading to antioxidant or prooxidant effects. Furthermore, the relative antioxidant action of the components can differ depending on the oxidation parameter measured.

Effect of exogenous antioxidant addition

In this experiment, the effect of rosemary extract (RE), green tea extract (TE), mixed tocopherol, trolox, ascorbic acid (AA) and ascorbyl palmitate (AP), at levels of 50 to 200 ppm of antioxidant components, on the oxidative stability of fresh, frozen and cooked pork patties during illuminated chill storage was investigated. Individual major components of RE and TE, carnosic acid and epicatechins respectively, and minor components of TE, theobromine and caffeic acid (TB+CAF), were also tested. Tocopherol and AP resulted in the best colour stability of patties, whereas RE and TE did not exert a colour stabilizing effect, and trolox negatively influenced colour. The effect of AA on colour was variable. Protein oxidation was only poorly influenced by antioxidant treatment. The results showed that all added substances, except for AA, are powerful lipid antioxidants when added to pork patties. Only in cooked patties, RE was more effective against lipid oxidation than TE. The lipid antioxidant action of tocopherol and its water soluble analogue trolox was comparable. The prooxidant activity of AA towards lipid oxidation could be overcome – and even reverted - by adding its lipid soluble analogue AP. However, this was only effective at 100 ppm as there was a transition towards a lipid prooxidative character at the higher dose.

For fresh and pre-frozen pork patties, there was no supplementary lipid oxidation inhibiting effect of any of the antioxidants above a level of 100 ppm. After cooking however, it was seen that RE and TE were more efficient in preventing lipid oxidation at

higher doses. The lipid and protein antioxidant properties of the extracts and their major antioxidant components (RE vs carnosic acid and TE vs tea catechins) were comparable. The minor antioxidant components of TE, TB+CAF, clearly lowered lipid oxidation and might therefore together with the catechins contribute to the global oxidation inhibiting effect of the extract.

Future prospects

There are two promising nutritional strategies for improving the quality of pork: the beneficial effect of added antioxidants on the oxidative stability and shelf-life, and the improved fatty acid composition (n-3 PUFA, specifically EPA and DHA) following dietary supplementation. Pork improved by dietary intervention has a natural character and can improve the human diet without requiring a shift in eating pattern. Generally speaking, most of the approaches to improve quality will increase production costs. However, to achieve widespread adoption of these feeding strategies in the pork production chain, the added value should be allocated to the levels of the chain where it was created (e.g. primary producers). This contrasts with the current trend of decreasing producers' share in consumer expenditures for livestock based food products.

Apart from awaiting an economic incentive, these developments require further attention to decrease the variability in outcome. Furthermore, for n-3 enriched pork products, it would be advisable to estimate its contribution to a more balanced human intake of fatty acids. This could be done by developing a model relating the fatty acid composition of pork products to the fatty acid composition of the feed. In combination with an estimation of the human intake of these products, this will allow us to calculate the contribution of n-3 enriched pork to the total human intake and thus assess the relevance of the efforts made by the primary producers. For non-intact muscle foods, the cost-benefit ratio of alternative approaches (*i.e.* by direct addition to the food matrix or by dietary means) for enhancing specific nutrients should be analysed case by case.

For antioxidants, further research is needed into the synergistic action of antioxidant cocktails, as well added via the feed as *post mortem*. These antioxidants should be tested in the food matrix they will be applied in. Moreover, their dose-dependent evaluation is necessary since the dose can determine the anti- or prooxidant character of a component.

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SUMMARY-SAMENVATTING

SUMMARY

To increase the quality and health value of pork, there is currently great interest in increasing the content of health-promoting long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) and reducing oxidation in animal tissues and animal products by dietary supplementation with fatty acid sources and antioxidants. These issues were examined in the present work. Several feeding trials with slaughter pigs were performed, and meat of these animals was analysed for its fatty acid composition and oxidative stability.

In chapter 2A, the effect of duration and time of feeding n-3 PUFA sources on the fatty acid composition and sensory characteristics of the *longissimus thoracis* (LT) was investigated. Linseed (L) and fish oil (F), rich in α-linolenic acid (α-LNA) and eicosapentaenoic and docosahexaenoic acid (EPA and DHA) respectively, were supplied equivalent to a level of 1.2 % of oil, either during the whole fattening period or only during the first (P1; 8 weeks) or second (P2; 6 to 9 weeks till slaughter) fattening phase. In the basal diet (B), only animal fat was used as supplementary fat source. Three dietary groups were supplied the same fatty acid source during both fattening phases, *i.e.*, group BB, LL, and FF. For the other 4 dietary groups, the fatty acid source was switched after the first phase (groups BL, BF, LF, and FL; the first and second letter indicating the diet in P1 and P2 respectively). For groups BB, LL and FF, meat was grilled and 3 types of meat products (fermented sausage, cooked and dry cured ham) were prepared and evaluated by an untrained consumer panel.

It was shown that the fatty acid profile of pork could be beneficially affected by this moderate level of linseed and fish oil. The effect of duration and time of feeding these n-3 PUFA sources on the muscle fatty acid composition varied with the fatty acids considered. When supplying linseed, only the last phase before slaughter was determinant for the deposition of α-LNA and the conversion to its long-chain metabolites EPA and DHA. The greatest EPA and DHA proportions were found for a continuous fish oil supply throughout the fattening period. When fish oil was fed during the first fattening phase followed by linseed before slaughter, levels of DHA but not EPA were lower. Simultaneously with a change in the fatty acid profile, an altered oxidative status of the meat (products) was expected and therefore, the oxidative stability and the influence on

consumer acceptance of this enriched pork was investigated. Neither meat quality traits (drip loss, pH,…), nor lipid or colour oxidation were influenced by dietary oil source or duration of supplementation at this level. Nevertheless, except for dry cured ham, meat products from pigs fed on this level of fish oil (cooked cured ham, sausage and grilled meat) were less appreciated by the consumer panel compared to meat products from animals fed on a linseed or basal diet. However, the eating quality differences were not pronounced.

Studies on improving the fatty acid composition of pork usually do not take into account the influence of culinary processing, which often includes a heat treatment and a fat addition. A study was set up to determine how pan-frying either without culinary fat or with different culinary fats (PUFA enriched culinary fat, olive oil and margarine) affects the fatty acid composition of pork (chapter 2B). The meat samples (LT) originated from pigs fed different dietary fat sources (animal fat, soybean oil or linseed oil) and thus had a different fatty acid composition before frying.

The fatty acid composition of the pan-fried meat tended to become similar to that of the culinary fat. The extent of increase or decrease of a particular fatty acid during frying was relative to the fatty acid gradient from the culinary fat to the meat. After frying, and irrespective of the type of culinary fat used, differences in fatty acid profile of the meat obtained by animal feeding strategies could still be observed. Although long-chain PUFA are said to be the most heat-labile and oxidation-sensitive fatty acids, they were not significantly lost by the frying process as measured by GC analysis and had thus not explicitly been subject to oxidation during heating.

The significant effect of both dietary fat source and pan-frying on the fatty acid profile of pork, clearly emphasizes the need for an integrated approach of dietary practice and *post mortem* processing in the attempt to produce meat that contributes to a valuable and wholesome diet. Given our current eating patterns, it seems difficult to comply with nutritional recommendations for n-3 PUFA. Since meat was found to be one of the main sources of long-chain PUFA in our diet (Sioen et al., 2006b), even moderate improvements of its fatty acid composition could be valuable.

Oxidation processes are one of the major causes of deterioration in food for human consumption: besides reduced sensory attributes, they are responsible for losses in

texture, appearance and nutritional value. As the oxidative status of meat depends on the balance of antioxidants and prooxidants, an improved oxidative stability can be obtained by increasing antioxidant levels in the meat. This can be achieved either by dietary delivery (chapter 3A) or by *post mortem* addition of antioxidants during processing (chapter 3B).

The effect of dietary antioxidant supplementation on the oxidative stability of meat has been reported for several individual antioxidants (e.g. α-tocopherol (AT)). Two or more antioxidants together can act synergistically, *i.e.* protect against oxidation to a higher extent than the sum of the contributions from each single antioxidant. Therefore, the use of antioxidant cocktails in the diet was tested for a possible superior effect compared to single antioxidants (chapter 3A). The use of α-tocopheryl acetate (ATA; 40 ppm) was tested individually and as part of an antioxidant cocktail (200 ppm; consisting of ATA, rosemary extract (RE), citric acid and gallic acid). The results suggest that supplementation of 40 ppm ATA during a 16-weeks period is sufficient for stabilization of meat during the storage period. Moreover, the AT content in muscle or subcutaneous fat tissue was not affected by antioxidant treatment. Based on these results, it can be said that the supplemental components of the cocktail (citric acid, RE and gallic acid) have no additional or synergistic effect at the doses applied here or that they are partly used during oxidation processes in the feed.

Although in this experiment, the PUFA profile of the meat (especially fatty acids with 3 or more double bonds) was different between animals fed linseed oil or soybean oil, the oxidative stability (including TBARS value) was not influenced. Consequently, oxidized linseed oil (targeted level of 150 mEq O_2/kg oil) was fed to the animals as another strategy for studying the effect of dietary antioxidants (chapter 3A). After all, a more intense trigger for oxidation could reveal distinct properties of the antioxidants.

Supplementary to a basic physiological requirement of 20 ppm ATA, the dietary administration of single antioxidants (ATA or RE at 40 ppm) or antioxidant combinations (ATA and RE each at 40 ppm; RE at 40 ppm and gallic acid at 2 ppm), did not affect colour or protein oxidation in pork. Lipid oxidation was only decreased by supplementary ATA in the diet. This was associated with a higher content of AT in the meat and the subcutaneous fat. No antioxidant effect of RE was observed in this experiment. On the

contrary, the results on lipid oxidation suggested a prooxidant effect of the RE. It is hypothesized that the more polar antioxidants have a negative impact on the oxidative stability of the meat. Although gallic acid and the polar components in RE are powerful antioxidants in lipid systems, they may behave differently in a matrix that also includes water and proteins ('polar paradox'). Again, the results of this experiment failed to provide evidence for a synergistic effect of either AT and RE or RE and gallic acid.

In chapter 3B, the effect of *post mortem* antioxidant addition (50 to 200 ppm of antioxidant components) on the oxidative stability of pork patties was investigated Rosemary extract (RE), green tea extract (TE), mixed tocopherol, trolox, ascorbic acid (AA), and ascorbyl palmitate (AP) were tested. Individual major components of RE and TE, carnosic acid and epicatechins respectively, and minor components of TE, theobromine and caffeic acid (TB+CAF), were also tested. Tocopherol and AP resulted in the best colour stability of patties, whereas RE and TE did not exert a colour stabilizing effect, and trolox negatively influenced colour. The effect of AA on colour was variable. Protein oxidation was only poorly influenced by antioxidant treatment. The results showed that all added substances, except for AA, are powerful lipid antioxidants when added to pork patties. Only in cooked patties, RE was more effective against lipid oxidation than TE. The lipid antioxidant action of tocopherol and its water soluble analogue trolox was comparable. The prooxidant activity of AA towards lipid oxidation could be overcome – and even reverted - by adding its lipid soluble analogue AP. However, this was only effective at 100 ppm as there was a transition towards a lipid prooxidative character at the higher dose. For fresh and pre-frozen pork patties, there was no supplementary lipid oxidation inhibiting effect of any of the antioxidants above a level of 100 ppm. After cooking however, it was seen that RE and TE were more efficient in preventing lipid oxidation at higher doses. The lipid and protein antioxidant properties of the extracts and their major antioxidant components (RE vs. carnosic acid and TE vs. tea catechins) were comparable. The minor antioxidant components of TE, TB+CAF, clearly lowered lipid oxidation and might therefore together with the catechins contribute to the global oxidation inhibiting effect of the extract. From this experiment it was seen that the relative antioxidant effect of antioxidants depends on the oxidation parameter assessed and varies according to the applied dose and hydrophilic/lipophilic character.

SAMENVATTING

In het kader van de optimalisatie van de kwaliteit en de gezondheidswaarde van varkensvlees, is er vandaag de dag een grote belangstelling voor het verhogen van de inhoud van gezondheidsbevorderende langketen n-3 polyonverzadigde vetzuren (n-3 PUFA) en voor het reduceren van oxidatie in dierlijke weefsels en producten door aangepaste supplementatie met respectievelijk vetzuren en antioxidanten via het voeder. Dit werk heeft zich hoofdzakelijk toegespitst op deze thema's. Verschillende voederproeven met varkens werden uitgevoerd en vlees van deze dieren werd geanalyseerd naar vetzuursamenstelling en oxidatieve stabiliteit.

In hoofdstuk 2A, werd het effect van de duur en het tijdstip van het voederen van n-3 PUFA bronnen op de vetzuursamenstelling (VZ samenstelling) en de sensorische karakteristieken van de *longissimus thoracis* (LT) onderzocht. Lijnzaad (L) en visolie (F), respectievelijk rijk aan α-linoleenzuur (α-LNA) en eicosapentaeenzuur en docosahexaeenzuur (EPA en DHA), werden hierbij gesupplementeerd equivalent aan een 1.2 % olie hoeveelheid, hetzij tijdens de volledige afmestperiode ofwel enkel tijdens de eerste (P1; 8 weken) of tweede (P2; 6 tot 9 weken tot slachten) afmestfase. In het basisvoeder (B), werd enkel dierlijk vet gebruikt als aanvullende vetbron. Drie voedergroepen werden continu dezelfde vetzuurbron gesupplementeerd tijdens beide afmestfasen, d.w.z. groepen BB, LL, en FF. In de andere 4 voedergroepen, werd de vetzuurbron omgewisseld na de eerste fase (groepen BL, BF, LF, en FL; de eerste en laatste letter respectievelijk het voeder in P1 en P2 aanduidend). Van de groepen BB, LL en FF, werd vlees gegrild en 3 types vleesproducten (salami, kookham en droog gezouten ham) werden bereid en geëvalueerd door een consumenten panel.

Uit deze proef blijkt dat het vetzuurprofiel van varkensvlees positief kan beïnvloed worden door deze gematigde niveau's van lijnzaad en visolie. Het effect van de duur en het tijdstip van voederen van deze n-3 PUFA bronnen op de VZ samenstelling van spierweefsel varieert per vetzuur. Bij lijnzaad supplementatie was de laatste afmestfase bepalend voor de depositie van α-LNA en de conversie naar de langketen metabolieten EPA en DHA. De hoogste EPA en DHA aandelen werden bekomen na continue supplementatie van visolie over de volledige afmestfase. Visolie supplementatie

tijdens de eerste afmestfase gevolgd door lijnzaad voor het slachten, resulteerde in lagere DHA levels, terwijl dit geen verlaging van EPA veroorzaakte vergeleken met een continue visolie supplementatie. Samen met een gewijzigd vetzuurprofiel, werd een veranderde oxidatieve status van het vlees (en de vleesproducten) verwacht en daarom werden de oxidatieve stabiliteit en de consumenten appreciatie van dit aangerijkte varkensvlees onderzocht. Bij deze supplementatieniveau's, werden noch de kwaliteitskenmerken van het verse vlees (vochtverlies, pH, …), noch de vet- of kleuroxidatie na bewaring beïnvloed door het type oliebron in het voeder of de supplementatieduur. Desondanks, behalve voor droog gezouten ham, werden vleesproducten (kookham, salami en gegrild vlees) na visolie supplementatie minder positief beoordeeld door het consumentenpanel vergeleken met vleesproducten van dieren uit de lijnzaadgroep of uit de groep met het basisvoeder, ook al waren de verschillen in eetkwaliteit slechts gering.

Doorgaans wordt bij het onderzoek naar een gewijzigde VZ samenstelling van varkensvlees, het effect van bereiding (in de keuken) over het hoofd gezien. Dit laatste betreft doorgaans het verhitten al dan niet onder toevoeging van een vetstof. In dit werk werd een experiment uitgevoerd ter bepaling van de invloed van bakken (zonder bakvet of met verschillende bakvetten: PUFA aangerijkt bakvet, olijfolie en margarine) op de VZ samenstelling van varkensvlees (hoofdstuk 2B). Het vlees voor deze proef (LT spier) was afkomstig van varkens gevoederd op basis van verschillende vetbronnen (dierlijk vet, soja- of lijnzaad olie) en had bijgevolg een verschillende VZ samenstelling voor het bakken.

De VZ samenstelling van het gebakken vlees evolueerde naar die van het gebruikte bakvet. De mate van toename of afname van een welbepaald vetzuur tijdens het bakken was proportioneel met de vetzuur gradiënt tussen het bakvet en het vlees. Na bakken en onafhankelijk van het type bakvet, waren de verschillen in VZ samenstelling veroorzaakt door een aangepast voeder nog steeds waarneembaar. De langketen PUFA, hoewel gewoonlijk zeer hitte-labiel en sterk onderhevig aan oxidatie, gingen niet verloren door het bakproces en waren dus niet expliciet onderhevig aan oxidatie gedurende dit proces.

Het significant effect van zowel de vetbron in het voeder als van het bakken in verschillende bakvetten op de VZ samenstelling van varkensvlees, benadrukt duidelijk de nood voor een geïntegreerde benadering van voederstrategie en behandeling *post mortem* bij de productie van vlees dat bijdraagt tot een waardevol en gezond dieet. In het licht van onze huidige voedingsgewoonten, lijkt het moeilijk te voldoen aan de voedingsaanbevelingen voor n-3 PUFA. Met vlees als een van de voornaamste bronnen van langketen PUFA in ons dieet (Sioen et al., 2006b), kan zelfs een matige verbetering van de VZ samenstelling ervan waardevol zijn.

Oxidatieprocessen zijn een van de belangrijkste oorzaken van kwaliteitsafname in onze voeding: naast een verminderde smaak en uitzicht, zijn deze verantwoordelijk voor het verlies van textuur en voedingswaarde. De oxidatieve status van vlees is afhankelijk van de balans van anti- en pro-oxidantia. Een verbeterde oxidatieve stabiliteit kan bijgevolg bekomen worden door de levels van antioxidantia in het vlees te verhogen. Dit laatste is mogelijk hetzij door aanbreng via het voeder (hoofdstuk 3A) hetzij door *post mortem* toevoeging van antioxidantia gedurende processing (hoofdstuk 3B).

Het gunstige effect van supplementatie van antioxidantia via het voeder op de oxidatieve stabiliteit van vlees werd reeds aangetoond voor verschillende individuele antioxidantia (bv. α-tocoferol (AT)). Echter, twee of meerdere antioxidantia tesamen kunnen synergetisch werken, d.w.z. oxidatie in sterkere mate verhinderen dan de som van de contributies door de individuele antioxidantia. Daarom werd het gebruik van antioxidant cocktails vergeleken met dat van individuele antioxidantia om een mogelijk superieur effect van de cocktails te onderzoeken (hoofdstuk 3A). α-tocoferyl acetaat (ATA; 40 ppm) werd getest individueel en als deel van een antioxidant cocktail (200 ppm; bestaande uit ATA, rozemarijn extract (RE), citroenzuur en galluszuur). Hieruit bleek dat supplementatie van 40 ppm ATA gedurende 16-weken volstaat ter stabilisatie van het vlees gedurende bewaring. Bovendien was de AT inhoud in spierweefsel of subcutaan vet niet verschillend voor de antioxidant behandelingen. Op basis van deze bevindingen blijkt dat de extra componenten van de antioxidant cocktail (citroenzuur, RE en galluszuur) geen additieve noch synergetische effecten hebben bij de hier toegevoegde

doses. Een andere mogelijke verklaring is dat ze reeds gedeeltelijk werden opgebruikt tijdens oxidatieprocessen in het voeder.

Ondanks de variaties in het PUFA profiel van het vlees na supplementatie met lijnzaad olie of sojaboon olie via het diervoeder (vnl. variatie in vetzuren met 3 of meer dubbele bindingen) was er geen variatie m.b.t. de oxidatieve stabiliteit (incl. TBARS waarde). Daarom, als alternatieve strategie om het effect van antioxidantia toegevoegd via het voeder te bestuderen, werd geoxideerde lijnzaad olie (150 mEq O_2/kg olie) gesupplementeerd aan de dieren (hoofdstuk 3A). Een sterkere stimulatie van de oxidatie zou immers andere eigenschappen van de antioxidantia kunnen aantonen.

Bovenop een basaal fysiologisch niveau van 20 ppm ATA in het voeder, was er geen effect van extra supplementatie van individuele antioxidantia (ATA of RE aan 40 ppm) of van antioxidantia cocktails (ATA en RE elk aan 40 ppm; RE aan 40 ppm en galluszuur aan 2 ppm), op de kleur- of eiwitoxidatie van het varkensvlees. Vetoxidatie was enkel verlaagd door extra ATA in het voeder. Dit ging gepaard met een hogere inhoud van AT in het vlees en subcutaan vet. Er werd geen antioxidatief effect van RE waargenomen in dit experiment. Integendeel, de resultaten van de vetoxidatie suggereren eerder een pro-oxidatief effect van RE. Hoewel galluszuur en de polaire componenten van het RE optreden als krachtige antioxidantia in lipofiele systemen, kunnen deze zich anders gedragen in een matrix die ook opgebouwd is uit water en eiwitten (o.a. vlees) (‘polaire paradox’). Opnieuw was er geen bewijs voor een synergetische werking van AT en RE of van RE en galluszuur.

In hoofdstuk 3B, werd het effect onderzocht van *post mortem* toevoeging van natuurlijke antioxidanten (50-200 ppm antioxidatieve componenten) op de oxidatieve stabiliteit van hamburgers (op basis van varkensvlees). Hierbij werden rozemarijn extract (RE), groene thee extract (TE), een mix van tocoferol isomeren, trolox, ascorbinezuur (AA) en ascorbyl palmitaat (AP) getest. Individuele hoofdcomponenten van RE en TE, carnosinezuur en epicatechines respectievelijk, alsook minor componenten van TE, theobromine en caffeïnezuur (TB+CAF), werden getest. Tocoferol en AP resulteerden in de beste kleurstabiliteit voor de hamburgers, terwijl RE en TE geen kleur stabiliserend effect vertoonden, en trolox kleur zelfs negatief beïnvloedde. Het effect van AA op de kleur was variabel. Eiwitoxidatie werd nagenoeg niet beïnvloed door de antioxidant

behandeling. Alle toegediende antioxidanten, met uitzondering van AA, waren effectief in het verlagen van vetoxidatie in hamburgers. Enkel in gekookte hamburgers, was de vetoxidatie inhiberende activiteit van RE hoger dan die van TE. De antioxidant activiteit van tocoferol en diens wateroplosbaar analoog trolox m.b.t. vetoxidatie was vergelijkbaar. De pro-oxidatieve activiteit van AA m.b.t. vetoxidatie kan vermeden worden, en zelfs omgekeerd, door het toevoegen van zijn vetoplosbaar analoog AP. Dit laaste effect geldt echter enkel voor doses tot 100 ppm, aangezien er bij hogere doses opnieuw een transitie is naar een pro-oxidatief karakter m.b.t. vetoxidatie. Voor verse en pre-diepgevroren hamburgers, was er geen extra vetoxidatie inhiberend effect voor elk van de antioxidanten boven een dosis van 100 ppm. Na koken echter, zijn hogere doses van RE en TE efficiënter ter preventie van vetoxidatie. De vet- en eiwitoxidatie inhiberende eigenschappen van de extracten en hun voornaamste antioxidatieve componenten (RE vs. carnosinezuur en TE vs. thee catechines) waren vergelijkbaar. De minor antioxidatieve componenten van TE, TB+CAF, hadden duidelijk een verlagend effect m.b.t. vetoxidatie en dragen daarom wellicht samen met de catechines bij tot de globale antioxidant activiteit van de extracten. Uit dit experiment bleek opnieuw dat de relatieve antioxidant activiteit van antioxidanten afhankelijk is van de beschouwde oxidatieparameter en varieert naargelang de dosis en het hydrofiele/lipofiele karakter van de component en de matrix.

CURRICULUM VITAE

CURRICULUM VITAE

BEKNOPT OVERZICHT

Lindsey Haak (°Geraardsbergen, 8 juli 1980) behaalde het diploma hoger secundair onderwijs (Latijn-wiskunde) aan het Sint-Catharinacollege te Geraardsbergen in 1998. In 2003 studeerde zij met grote onderscheiding af als Bio-ingenieur in de Landbouwkunde aan de Faculteit Bio-ingenieurswetenschappen van de Universiteit Gent. Sinds september 2003 werkt ze als doctoraatsbursaal aan de Vakgroep Dierlijke Productie op het project 'Natuurlijke antioxidantia in veevoeders en dierlijke voedingsmiddelen voor de gezondheid van mens en dier' gefinancierd door het Bijzonder Onderzoeksfonds (UGent). Haar onderzoeksactiviteiten betreffen de effecten van voedersamenstelling en vleesbereiding op de kwaliteit en functionaliteit van varkensvlees. Zij is auteur en co-auteur van verschillende publicaties in internationale tijdschriften en nam deel aan verschillende nationale en internationale congressen. Zij droeg bij aan het onderwijs van de vakgroep voor de praktische oefeningen van enkele opleidingsonderdelen en de begeleiding van afstudeerwerken in het kader van de opleidingen 'Bio-ingenieur in de Landbouwkunde', 'Industrieel Ingenieur Landbouw en Voedingstechnologie', 'Master in Food Science', en 'Graduaat Voedings- en Dieetkunde'. In 2006 behaalde ze het diploma van Geaggregeerde voor het Secundair Onderwijs (AILO opleiding).

WETENSCHAPPELIJKE PUBLICATIES EN PRESENTATIES

1. Artikels gepubliceerd in internationale tijdschriften opgenomen in de 'Science Citation Index' (A1 publicaties)

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2. Artikels ingediend voor publicatie in internationale tijdschriften opgenomen in de 'Science Citation Index' (A1 publicaties)

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Lindsey
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