Quality of Muscle Foods: Effects of Feed and Storage Time

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Abstract

The overall aim of this thesis was to investigate the effects of feed and storage time on meat quality.

To study the effect of feed, chicken were fed seven steam-pelleted or nonpelleted rapeseed (RS) diets with different RS inclusion levels (0, 80, 160, and 240 g/kg) for 34 days, and the dietary effects on meat yield, fatty acid composition in meat, and the expression of lipid metabolism-related genes in liver were determined. Feeding with steam-pelleted diets with high inclusion of RS (160 or 240 g/kg) significantly increased the content of n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) in meat, without compromising the meat yield and total fatty acid content. The increased intake of α-linolenic acid enabled higher conversion into n-3 LC-PUFA. Therefore, feeding broiler chicken steam-pelleted diet with high RS inclusion can be a feasible strategy to increase human intake of n-3 LC-PUFA without compromising meat yield.

The effect of storage time on meat color, microbial growth, and lipid oxidation was studied on beef steaks from *Longissimus thoracis et lumborum* muscles overwrapped with polyvinyl chloride (PVC) film and stored at 4°C for 0, 4, 7, and 10 days. Microbial spoilage of beef occurred at day 10 as indicated by the total viable count exceeding the threshold of 7 $\log_{10}$ CFU/cm^2. *Pseudomonas spp.* and *Brochothrix spp.* were the prevailing bacteria species when meat was spoiled. Moreover, Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis showed the successive changes in the incidences of different bacteria over time. At day 10, the *a** and Chroma decreased and hue angle increased significantly, indicating meat discoloration. Concentrations of spermine, tyramine, and cadaverine were found to be potential spoilage indicators of meat freshness. Lipid oxidation occurred only slightly, which could be attributed to the high content of α-tocopherol in meat. In contrast, the degree of lipid lipolysis increased with storage time as reflected by the increase in the relative content of free fatty acids and decrease in relative content of triacylglycerols.

Keywords: n-3 LC-PUFA, rapeseed, discoloration, microbial growth, biogenic amine, lipolysis, lipid oxidation

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Dedication

To my parents and wife

Content

List of Publications 7

Abbreviations 9

1 Introduction 11
1.1 Meat quality 11
1.2 Meat and the n-3 LC-PUFA 12
1.2.1 The n-3 LC-PUFA and recommended intake 12
1.2.2 Chicken as a source of n-3 LC-PUFA 15
1.2.3 The n-3 and n-6 metabolism in chicken 15
1.3 Meat quality deterioration during storage 16
1.3.1 Meat discoloration 16
1.3.2 Microbial activity 18
1.3.3 Lipid oxidation 19
1.3.4 Methods to study meat quality deterioration 20

2 Aims 23

3 Material and Methods 25
3.1 Experimental design and sample collection 25
3.1.1 Paper I 25
3.1.2 Paper II, III and IV 26
3.2 Analysis methods 26
3.2.1 Lipid extraction and fatty acid analysis 26
3.2.2 Lipid class composition of total lipids 27
3.2.3 Gene expression analysis 27
3.2.4 Meat color 27
3.2.5 Enumeration of bacterial populations and identification of isolates 28
3.2.6 T-RFLP analysis 28
3.2.7 Biogenic amine analysis 29
3.2.8 Static headspace gas chromatography (SHS-GC) analysis of volatile compounds 30
3.2.9 Determination of α-tocopherol 31
3.2.10 Statistical analysis 31

4 Summary of results 33
4.1 Changes in muscle FA composition and hepatic gene expression in chicken fed different RS diets 33
4.2 Microbial growth and BAs formation in beef stored at 4°C 35
4.3 Meat discoloration and its association with microbial growth in beef stored at 4°C 37
4.4 Lipid oxidation in beef stored at 4°C 38

5 Discussion 39
5.1 Changes in muscle FA composition and hepatic gene expression in chicken fed different RS diets 39
5.2 Meat discoloration in beef stored at 4°C 42
5.3 Microbial growth and biogenic amine formation in beef stored at 4°C 43
5.4 Lipid lipolysis and oxidation in beef stored at 4°C 45

6 Future research 47

References 49

Acknowledgments 57

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Li, S.**, Schiller Vestergren, A., Wall, H., Trattner, S. & Ivarsson, E. (2016). Feeding steam-pelleted rapeseed affects expression of genes involved in hepatic lipid metabolism and the fatty acid composition of chicken meat (manuscript).

II **Li, S.**, Zamaratskaia, G., Roos, S., Båth, K., Meijer, J., Borch, E. & Johansson, M. (2015). Inter-relationships between the metrics of instrumental meat color and microbial growth during aerobic storage of beef at 4°C. *Acta Agriculturae Scandinavica Section A-Animal Science* (accepted, DOI:10.1080/09064702.2015.1072579).

III **Li, S.**, Johansson, M., Vidanarachchi, J. K., Pickova, J. & Zamaratskaia, G. (2016). Determination of biogenic amines in beef stored aerobically by high performance thin layer chromatography densitometry (manuscript).

IV **Li, S.**, Pickova, J., Vidanarachchi, J. K., Johansson, M. & Zamaratskaia, G. (2016). Lipolytic and oxidative changes in fresh beef during aerobic storage at 4°C (manuscript).

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The contribution of Shengjie Li to the papers included in this thesis was as follows:

I Participated in the planning of the experimental work together with supervisors. Participated in the collection of samples. Was responsible for the laboratory analysis, the statistical evaluation of the results, and writing the manuscript.

II Participated in the planning of the experimental work together with supervisors. Participated in the collection of samples and was responsible for the laboratory analysis, the statistical evaluation of the results, and writing the manuscript.

III Was responsible for the planning of the experimental work. Performed the laboratory analysis. Was responsible for the statistical evaluation of the results and for writing the manuscript.

IV Participated in the planning of the experimental work together with supervisors. Performed the laboratory work. Was responsible for the statistical evaluation of the results and for writing the manuscript.

Abbreviations

AA	Arachidonic acid (20:4n-6)
ALA	α-linolenic acid (18:3n-3)
BA	Biogenic amine
DeoxyMb	Deoxymyoglobin
DHA	Docosahexaenoic acid (22:6n-3)
DPA	Docosapentaenoic acid (22:5n-3)
EPA	Eicosapentaenoic acid (20:5n-3)
ESO	Ephemeral spoilage organisms
FA	Fatty acid
FFA	Free fatty acid
GC	Gas chromatography
LA	Linoleic acid (18:2n-6)
LC-PUFA	Long chain omega-3 polyunsaturated fatty acid
MetMb	Metmyoglobin
MUFA	Monounsaturated fatty acid
n-3	Omega-3
n-6	Omega-6
OxyMb	Oxymyoglobin
PUFA	Polyunsaturated fatty acid
RS	Rapeseed
SFA	Saturated fatty acid
SHS	Static headspace
TAG	Triacylglycerol
TBARs	2-thiobarbituric acid reactive substances
TLC	Thin layer chromatography
T-RFLP	Terminal restriction fragment length polymorphism

1 Introduction

Meat is generally defined as the flesh of animals used as food, including mammals, birds, reptiles, fish, and various invertebrates (Warriss, 2000; Lawrie & Ledward, 2006). However, the important meat-producing species remain domestic cattle, sheep, pigs, and poultry (Warriss, 2000). Meat is one of the important components of human diet. Nowadays, meat quality is gaining increasing attention because it influence not only consumers' purchasing decisions but also their eating satisfaction and well-being.

1.1 Meat quality

Meat quality is a general concept consisting of different aspects of its properties and perceptions (Warriss, 2000). From the consumer's point of view, the most important meat quality attributes are visual appearance (e.g. color, amount of fat, water losses), eating quality (e.g. tenderness, juiciness, flavour) and wholesomeness (e.g. microbiological safety and nutritional quality).

At the point of sale, consumers cannot evaluate the odor or feel the texture of meat without opening the packages. Therefore, meat color becomes the most important factor influencing consumers' purchasing decisions. A bright cherry-red color is commonly used by consumers as an indicator of beef freshness and wholesomeness (Mancini & Hunt, 2005). Shift in color during storage from cherry-red to brown leads to discrimination and rejection by consumers (Faustman *et al.*, 2010). Surface-discolored whole meat cuts are ground to low-value products or are discarded often well before microbial safety is compromised, leading to huge revenue losses and wastage of valuable food (Suman & Joseph, 2013).

Meat tenderness is considered as the most important trait influencing consumer's eating satisfaction; however variation in meat tenderness,

particularly in beef, is still one of the most critical quality problems encountered by meat industry (Li *et al.*, 2012). To improve meat tenderness, beef cuts are stored either packed or unpacked in a cooler with specific temperature and humidity for a period of time, and this process is defined as meat tenderization. During this process, proteolysis of critical myofibrillar and cytoskeletal proteins occurs (Ouali *et al.*, 2013).

Nowadays, consumers are becoming more aware of the effect of food on their health. Meat provides human with valuable nutrients, such as protein, vitamins, various minerals, and essential fatty acids. In particular, meat and meat products are one of the major sources of long chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Jankowski *et al.*, 2012). EPA and DHA provide well-known beneficial effects to human health. These benefits mainly include a reduced risk of cardiovascular diseases (CVD), a role in development of human brain and antiinflammatory properties (Howe *et al.*, 2006; Calder, 2014). Because the endogenous biosynthesis of the n-3 LC-PUFAs in human body is limited, they are regarded as important dietary compounds (Givens & Gibbs, 2008) and recommendations for intake are listed in Table 1.

The microbial growth in meat matrix leads to decomposition of carbohydrates and proteins, and to the release of malodorous metabolites and slime formation on the meat surface, which ultimately makes meat undesirable for consumption. The microorganisms that lead to meat spoilage can be bacteria, molds and yeasts (Warriss, 2000).

In the retail marketplace, shelf-life is designated to indicate the quality of meat that remains acceptable to consumers. In Sweden, best-before-date is commonly used to indicate the best quality of meat, after which meat might still be edible for some time. However, there is no scientific data on how long after best-before-date meat can still be stored without negative affecting the product and, in turn, the consumer. During storage, the main factors leading to meat deterioration are microbial activity and lipid oxidation.

1.2 Meat and the n-3 LC-PUFA

1.2.1 The n-3 LC-PUFA and recommended intake

Lipids can be classified as acyl residue or neutral-polar, with the latter being more commonly used. Neutral lipids such as fatty acids (FA), monoacylglycerols, diacylglycerols (DAG), triacylglycerols (TAG), wax esters and sterols, function as energy stores. Triacylglycerols have a glycerol molecule backbone, where three molecules of FA are esterified to hydroxyl

groups of glycerol in the *sn*-1, *sn*-2, and *sn*-3 positions (Figure 1). In addition, the dietary FA composition can influence TAG more than phospholipids (PL). Free fatty acids (FFA) are results of catabolic or anabolic processes. Phospholipids belong to polar lipids and have a common backbone of phosphatidic acid, which is esterified to choline, ethanolamine, serine, or inositol to form phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, or phosphatidylinositol respectively (Olsen & Henderson, 1989) (Figure 1).

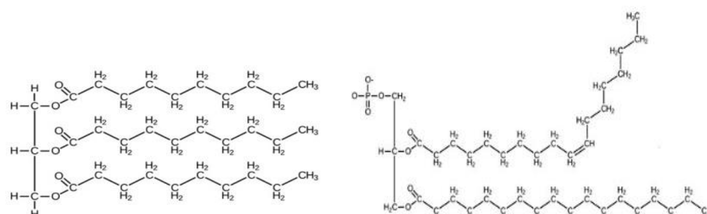

Figure 1. Chemical structure of triacylglycerol (TAG) (left) and phospholipid (PL) (right).

FA is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Fatty acids without double bond are known as saturated FA (SFA), those with only one double bond as monounsaturated FA (MUFA), and those with two or more double bonds as polyunsaturated FA (PUFA). PUFA are further divided into n-3 FA and n-6 FA depending on the position of the first double bond counting from the methyl end of the carbon chain. For n-3 FA, the first double bond closest to the methyl end is on carbon number three, and it is on carbon number six for n-6 (Figure 2). The n-3 LC-PUFA are a family of PUFA containing 20 or more carbons and three or more double bonds, including EPA, docosapentaenoic acid (DPA) and DHA (Figure 2).

To date, there is no dietary reference intake (DRI) for EPA and DHA although many international authorities and expert groups have issued dietary recommendations for them for healthy adults (Table 1). In particular, the European Food Safety Agency (EFSA) has proposed a recommended daily intake of 250 mg/day EPA and DHA for adults because this intake level is negatively related to cardiovascular diseases (CVD) risk in a dose-dependent way up to 250 mg/day (1–2 servings/week of oily fish) in healthy populations (EFSA Panel on Dietetic Products Nutrition and Allergies, 2010). Recent evidence suggests that DPA is just as important as EPA or DHA for delivering the health benefits associated with n-3 LC-PUFA (Howe *et al.*, 2007; Kaur *et al.*, 2011). However, current recommendations on the intake of n-3 LC-PUFA do not include DPA.

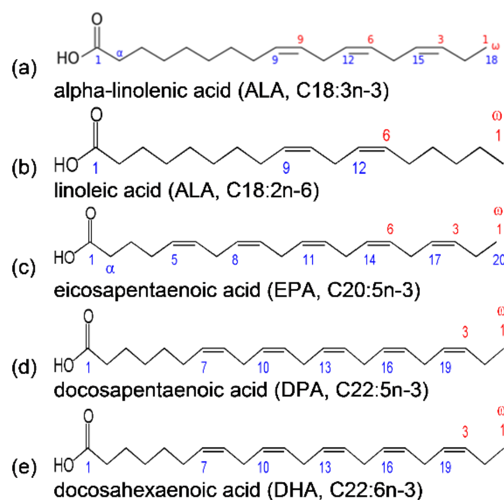

Figure 2. Chemical structure of some important polyunsaturated fatty acids

Besides the intake of EPA and DHA, the balance between n-6 and n-3 FA is important for human health. Excessive amounts of n-6 PUFAs and a very high n-6/n-3 ratio in human diet promote pathogenesis of many diseases, including cardiovascular diseases, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 FAs (a lower n-6/n-3 ratio) exert suppressive effects (Simopoulos, 2006). Humankind has historically consumed a diet with an n-6:n-3 ratio of 1:1 to 4:1; however, the current western diet has been estimated to have a ratio of n-6:n-3 of 15-20:1 (Simopoulos, 2011). The World Health Organization recommends an n-6 to n-3 ratio of less than 5:1 (FAO/WHO, 1994).

Table 1. *Recommended daily intakes of EPA and DHA for healthy adults*

Organization	EPA and DHA recommendation
Academy of Nutrition and Dietetics	≥ 500 mg/day
American Heart Association	≈ 500 mg/day
US Department of Agriculture	≥ 250 mg/day
International Society for the Study of Fatty Acids and Lipids	≥ 500 mg/day
European Food Safety Agency	≥ 250 mg/day
World Health Organization	≥ 250 mg/day

1.2.2 Chicken as a source of n-3 LC-PUFA

Fish are known to be a major source of n-3 LC-PUFA; however, young adults (18–24 years) rarely eat fish (Givens & Gibbs, 2008). The possible reasons are the aversion to eating fish and fear about the presence of harmful environmental pollutants in fish (Givens & Gibbs, 2008; Tur *et al.*, 2012). According to a survey done in Australia, 43% of the n-3 LC-PUFA consumed by adults was estimated to originate from meat, poultry, and game compared with 48% from fish and seafood (Howe *et al.*, 2006).

The lipid composition of edible tissues of monogastric animals, such as poultry and pig, can be relatively easily manipulated by altering the dietary lipid composition (Rymer & Givens, 2005; Ma *et al.*, 2015). Therefore, poultry meat enriched with n-3 PUFAs has been proposed to have the potential to increase our dietary intake of EPA and DHA (Givens & Gibbs, 2008; Bhalerao *et al.*, 2014). Levels of EPA and DHA in poultry meat can be increased by different strategies. Use of fish oil concentration higher than 1 to 2% in poultry diet can create organoleptic problems in meat (Hargis & Van Elswyk, 1993). Therefore, investigations into terrestrial alternatives with lower cost and high sustainability have been promoted in recent decades, e.g. linseed and rapeseed. A major obstacle with polyunsaturated fatty acids is their sensitivity to oxidation; however this can be overcome using antioxidants, e.g. addition of vitamin E, in the diets of poultry (Givens & Gibbs, 2008).

1.2.3 The n-3 and n-6 metabolism in chicken

In chicken, dietary lipids are digested and absorbed from intestine and transported in blood to liver and other different tissues in the form of portomicrons consisting of TAG, cholesterol, and a protein component (Hermier & Suppl, 1997). Liver is the main site of fatty acid synthesis for chicken, accounting for 95% of the *de novo* FA synthesis, and it is generally assumed that almost all the fat that accumulates in chicken adipose tissue is synthesized in the liver or derived from the diet (Zhang *et al.*, 2013). Because the *de novo* biosynthesis pathway to produce ALA (18:3n-3) and LA (18:2n-6) is lacking in higher vertebrates, these FAs are essential, and must be provided in their diets.

Once taken up by liver cells, ALA and LA are further metabolized to form LC-PUFA (Figure 3). LA is converted to 18:3n-6, 20:3n-6 and further by desaturation and elongation to form arachidonic acid (AA, 20:4n-6). AA can be further metabolized to docosapentaenoic acid (22:5n-6). ALA is converted to 18:4n-3 and 20:4n-3 to form EPA using the same series of enzymes as those used to synthesize AA. EPA is further metabolized to DHA. Since conversion of n-3 and n-6 fatty acids share the same series of enzymes, competition exists

between the n-3 and n-6 fatty acid families for metabolism, with an excess of one causing a significant decrease in the conversion of the other (Schmitz & Ecker, 2008). Furthermore, in the biosynthesis of n-3 LC-PUFA from ALA, one of the two rate-limiting steps is the desaturation to EPA, which is determined by the activity of Δ5 desaturase encoded by the FADS1 gene (Cho *et al.*, 1999). The Δ6 desaturase encoded by the FADS2 gene is regarded as another rate-limiting step in n-3 LC-PUFA biosynthesis pathway, which is responsible for the desaturation of ALA to 18:4n-3 (Zheng *et al.*, 2004). The final step of DHA synthesis occurs in peroxisomes through a single round of β-oxidation of 24:6n-3 and acyl-coenzyme-A oxidase 1 (ACOX1), which is the rate-limiting enzyme for peroxisomal fatty acid β-oxidation (Ding *et al.*, 2003; Wanders, 2004; Dyall, 2015).

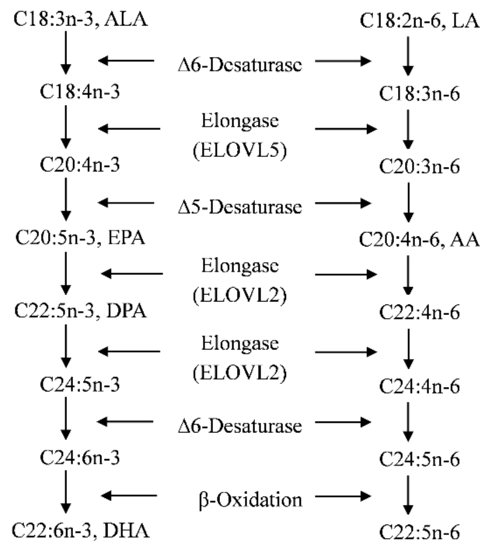

Figure 3. The n-3 and n-6 fatty acid elongation and desaturation

1.3 Meat quality deterioration during storage

1.3.1 Meat discoloration

Meat color is mainly influenced by the concentration of haem pigments, particularly myoglobin, the chemical state of myoglobin, and the physical characteristics of the meat. In general, meat discoloration is associated with the oxidation of myoglobin and the accumulation of metmyoglobin (Insausti *et al.*, 1999; Mancini & Hunt, 2005). Myoglobin is a sarcoplasmic hemeprotein

consisting of a globin (protein) moiety and a heme prosthetic group. The heme group is composed of an iron atom which is bound within a protoporphyrin ring by four of the iron atom's six coordination sites and the sixth coordination site can bind to oxygen, water, carbon monoxide, or other small ligands. In fresh meat, myoglobin can exist in any of the three redox states: deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb). DeoxyMb and OxyMb are in a ferrous (Fe^{2+}) state, and provide purple-red or bright-red color, respectively. The sixth coordinate of the heme iron is occupied by an oxygen molecule in OxyMb, whereas no ligand is bound with the heme iron in DeoxyMb. The oxidation of the ferrous forms of myoglobin to a ferric state leads to the formation of brown MetMb. MetMb has a water molecule bound at the sixth coordinate of the ferric heme and is incapable of binding oxygen (Suman & Joseph, 2013).

During cold storage, the rate of MetMb accumulation on meat surface is determined by many endogenous factors (pH, muscle type, animal age, breed, sex, and diet) and exogenous factors (temperature, oxygen availability, light, microbial growth, and packaging) (Jeong *et al.*, 2009). Of the endogenous factors, muscle types received significant attention. On the basis of color stability, beef muscles have been categorized as color-stable (e.g., *Longissimus lumborum*) and color-labile (e.g., *Psoas major*). Microbial growth is one of the factors that affects color stability and causes discoloration (Mancini & Hunt, 2005), and its possible role is in the reduction of the oxygen level in the surface tissue leading to the formation of MetMb (Robach & Costilow, 1961). Besides oxygen consumption, bacteria can also lead to meat discoloration by producing hydrogen peroxide and hydrogen sulfide, which can lead to greening discoloration on fresh meat surfaces (Robach & Costilow, 1961; Faustmani, 1990). Recent evidence suggested that the reactive products of lipid oxidation, e.g. aldehydes and ketones, compromise meat color by accelerating myoglobin oxidation. The mechanism behind such meat discoloration could be the adduction of aldehydes at proximal and distal histidines, which coordinates the stability of heme and exposes the heme group to an oxidizing environment, leading to increased myoglobin oxidation (Suman & Joseph, 2013).

Redness (a*), color intensity (Chroma) and hue angle are good indicators of meat discoloration, and as discoloration occurs, values for a* and Chroma decrease while hue angle increases (Owen & John, 2001). Particularly, hue angle is useful to indicate shifts in color overtime toward discoloration and larger values indicate less red color (AMSA, 2012).

1.3.2 Microbial activity

Microbial growth

Apart from physical damage, lipid oxidation, and color change, the other spoilage symptoms are attributed to the growth of microorganisms to an unacceptable level (Ercolini *et al.*, 2011). For fresh meat that has an ultimate pH of about 5.6, enough glucose and other simple carbohydrates are present at the meat surface to support about 8 $\log_{10}$ CFU/cm^2 (Jay *et al*, 2006). When glucose is exhausted, microorganisms switch to metabolize amino acids as energy source. At this point, the first indication of spoilage in fresh meat, which is the production of off-odors, becomes apparent (Gill, 1976). A cut-off value of 7 $\log_{10}$ CFU/cm^2 has been suggested as an indicator for microbiological spoilage under aerobic conditions (Ellis & Goodacre, 2001; Peng *et al.*, 2011).

Due to its high water activity and abundant nutrients, fresh meat is an excellent ecological niche for the growth of various bacteria. *Pseudomonas spp.*, *Enterobacteriaceae*, *Brochothrix thermosphacta,* and lactic acid bacteria have been considered to be major contributors to meat spoilage (Nychas *et al.*, 2008). These microorganisms, which can eventually become dominant in spoilage flora, have been designated as Ephemeral Spoilage Organisms (ESOs). Different packaging methods and storage conditions have been found to affect microbial association such as ESOs and consequently the spoilage process (Doulgeraki *et al.*, 2012).

Biogenic amine formation

Biogenic amines (BAs) are nonvolatile low-molecular-weight nitrogenous bases, mainly derived through decarboxylation of amino acids due to tissue activity or microbial enzymes. BAs are naturally present in living organisms, and their formation can also occur during food processing and storage as a result of microbial activities, either spoilage or fermentation. BAs can be classified into three categories according to their chemical structures: aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine), and heterocyclic (histamine, tryptamine). Amines including putrescine, cadaverine, spermine and spermidine are indispensable components of living cells and are important in nucleic acid function and protein synthesis and probably in the stabilization of membranes (Santos, 1996).

High intake levels of BAs, particularly histamine and tyramine, may be hazardous to human health due to effect on the cardiovascular and nervous systems. Histamine and tyramine are considered most toxic for human health, and their toxic action are known as “histamine poisoning” and “cheese

reaction". Although cadaverine and putrescine are not considered toxic individually, they can enhance the effect of histamine and tyramine by interacting with amino oxidases and interfering with the detoxifying mechanism (EFSA Panel on Biological Hazards, 2011). Furthermore, both cadaverine and putrescine can react with nitrite in foods to form carcinogenic nitrosamines (Al Bulushi *et al.*, 2009). BAs are thermo-stable and not significantly affected by normal cooking or other food processing procedures, which makes food assurance in respect to BAs more challenging.

Moreover, BAs are considered as possible indicators of freshness and quality of food. The most prevalent BAs in meat and meat products are tyramine, cadaverine, putrescine, and histamine (Ruiz-Capillas & Jimenez-Colmenero, 2004; Stadnik & Dolatowski, 2010). Most studies on BAs in meat products are generally focused on fermented products, and much less literature is available in relation to other types, such as fresh or cooked meat (Ruiz-Capillas & Jimenez-Colmenero, 2004). Due to its high water activity and abundant nutrients, fresh meat is an excellent ecological niche for the growth of bacteria possess with amino acid decarboxylase activity, *Enterobacteriaceae*, *Pseudomonas spp.* and certain *Lactobacilli*, *Enterococci* and *Staphylococci spp.*(Rokka *et al.*, 2004; Sirocchi *et al.*, 2013).

1.3.3 Lipid oxidation

Lipid oxidation is considered as a primary cause of quality deterioration of meat and meat products, which leads to off-flavor and off-odor development, discoloration, texture change, and drip loss. Moreover, lipid oxidation also causes losses of nutritional values, and generates compounds that can be detrimental to human health (Min & Ahn, 2005). Lipid oxidation in food is a complex chain of reactions whereby unsaturated fatty acids react with molecular oxygen via free radical mechanisms (Ross & Smith, 2006). In this process, primary products (hydroperoxides) are produced, which then readily give rise to secondary oxidation products, such as aldehydes, ketones, epoxides, hydroxy compounds, oligomers and polymers, and most of these secondary oxidation products produce undesirable sensorial and biological effects (Barriuso *et al.*, 2013).

Lipid oxidation depends to a larger extent on its fatty acid composition because PUFAs are more susceptible to oxidation processes (Halliwell & Chirico, 1993). As a result of lipid oxidation, the fatty acid composition of raw beef may be altered during prolonged storage. On the other hand, α-tocopherol is the most active fat soluble antioxidant naturally present in fresh meat, which can retard lipid oxidation and lead to a delay in the deterioration of meat quality and longer shelf life. Much research has been done on the effect of the

dietary supplementation of α-tocopherol on the reduction of oxidative processes in meat (Descalzo *et al.*, 2005; Álvarez *et al.*, 2008; González-Calvo *et al.*, 2014).

The onset of rancidity occurs in cooked meat during short-term refrigerated storage, commonly described as warmed-over flavor, and it also develops in ground raw meat exposed to air or oxygen (Gray *et al.*, 1996).

1.3.4 Methods to study meat quality deterioration

Typically, the identification and characterization of ESOs in meat involved enumeration of bacteria by plating on different solid media, followed by identification of their isolates. However, this culture-based approach is time-consuming, expensive, and labor-intensive, which is not suitable for characterization of a large number of samples. Furthermore, selective media and conditions may distort the results since some food-associated bacteria species cannot be cultured using standard laboratory media. In recent years, several powerful molecular techniques have been developed and applied to produce reliable data on microbial association of meat spoilage (Doulgeraki *et al.*, 2012). Terminal restriction fragment length polymorphism (T-RFLP), as a robust and powerful technique, has been widely applied in various environments for bacterial community characterization (Clement *et al.*, 1998). T-RFLP is a culture-independent method, which is based on the bacterial 16S rRNA gene amplification and restriction digestion of PCR-amplified fragments. For meat research, it has been applied to investigate the bacterial communities of minced meat packed in modified atmosphere packaging and concluded that T-RFLP, as a relatively rapid and inexpensive method, can be used to screen a large number of communities to investigate the response of community structure to intrinsic and extrinsic factors and storage time (Nieminen *et al.*, 2011). More recently, the microbial diversity in food has been studied using high-throughput sequencing (HTS) after direct nucleic acid extraction from the samples (Ercolini, 2013), which can overcome the drawbacks of the T-RFLP method. However, considering the high cost of HTS analysis, it might be useful to use T-RFLP as a screening method of many samples to follow major changes, and such initial screening could select samples for an HTS approach which allows a comprehensive view on both the identities and abundances of all bacterial groups (genera) in the samples.

Thin layer chromatography (TLC), gas chromatography (GC), capillary electrophoresis (CE), and high performance liquid chromatography (HPLC) have been proposed for the determination of BAs, among which HPLC-based methods are the most popular (Önal, 2007; Bedia Erim, 2013). However, HPLC-based methods, though precise and sensitive, require sophisticated

instrumentation and high operational cost, time and technical skills. On the other hand, TLC densitometry methods are rapid, high-throughput and less expensive, and can be effectively used in regulatory food agencies and meat industry (Shalaby, 1999; Jeya Shakila *et al.*, 2001). These methods have been successfully applied to quantify putrescine, cadaverine, spermidine, histamine and tyramine in fish and fishery products (Jeya *et al.*, 2001), and putrescine, cadaverine, histamine and tyramine in wine (Romano *et al.*, 2012). Nevertheless, this TLC method has not yet been used to detect BAs in fresh meat, especially for simultaneous detection of the eight common BAs. The majority of developed methods were limited to analyzing five major biogenic amines. Although a double development method (Lapa-Guimarães & Pickova, 2004) has been developed to separate nine amines, it would also double the operational time and amount of solvent used for developing. As an enhancement form of TLC techniques, high performance thin layer chromatography (HPTLC) enables high resolution and more accurate quantification, which might enable separation of several BAs in one run.

The 2-thiobarbituric acid-reactive substances (TBARs) method has been widely used to monitor lipid oxidation in raw and cooked muscle foods, because it is easy to operate and relatively fast. However, this method has been largely criticized due to its lack of specificity. More recently, volatile secondary products derived from lipid oxidation were monitored as markers for lipid oxidation using gas chromatography combined with static headspace (SHS) sampling (Barriuso *et al.*, 2013). In relation to lipid oxidation, hydrolysis of triglycerides can also be a highly indicative parameter for lipid deterioration and the subsequent enhanced susceptibility to oxidation (Barriuso *et al.*, 2013).

2 Aims

The overall aim of this thesis was to investigate the effects of feed and storage time on meat quality. The specific aims were to:

- Study the dietary effect of steam-pelleted and nonpelleted rapeseed diets with different inclusion levels on the fatty acid composition of chicken meat and on the expression of lipid metabolism-related genes in liver (Paper I).
- Investigate the association between changes in color and microbial growth in beef stored aerobically at 4°C for 10 days (Paper II).
- Characterize dynamic changes in microbiota composition in beef during storage (Paper II).
- Evaluate the presence of different biogenic amines as indicator of beef freshness (Paper III).
- Examine the lipolytic and oxidative changes in beef during chilled aerobic storage for 10 days (Paper IV).

3 Material and Methods

3.1 Experimental design and sample collection

3.1.1 Paper I

A total of 280 unsexed one-day-old chickens (Ross 308) were randomly divided into 35 pens (1.50 × 0.75 m), which in turn were randomly assigned into seven dietary treatments, giving five replicates per group. Chickens were fed one of seven experimental diets (Table 2) for 34 days: six different wheat-soybean meal based diets either in nonpelleted or steam-pelleted form supplemented with 80, 160, and 240 g/kg RS and one nonpelleted wheat-soybean meal based diet without RS supplementation as control.

Table 2. *Steam pelleting process and RS inclusion level for different chicken diets*

	Steam pelleted	RS inclusion level (g/kg)
Control	No	0
RS8N	No	80
RS8P	Yes	80
RS16N	No	160
RS16P	Yes	160
RS24N	No	240
RS24P	Yes	240

Abbreviations: nonpelleted RS diets with RS inclusion level at 80 g/kg (RS8N), 160 g/kg (RS16N) and 240 g/kg (RS24N), steam-pelleted diets with RS inclusion level at 80 g/kg (RS8P), 160 g/kg (RS16P) and 240 g/kg (RS24P).

Chickens (n=5) were electrically stunned and bled by cutting the carotid artery and the external jugular vein. After bleeding, birds were placed in a scalding tank with an average temperature of 60°C for 2 min, and immediately defeathered using an automatic turning defeathering machine. Carcasses were then eviscerated. Liver samples for gene expression analysis were collected,

snap-frozen in liquid nitrogen and stored at -80°C until analysis. Carcass parts, breast (*Pectoralis major and Pectoralis minor*) with ribs and drumstick, were removed and weighed, and then muscle samples for fatty acid composition analysis and liver samples for gene expression were taken and stored at -80°C until analysis.

3.1.2 Paper II, III and IV

Six heifers of Hereford breed (22-23 months, 252 ± 24 kg) were slaughtered on the same day according to the national standard procedures at a Swedish commercial slaughterhouse. On day 6 of post mortem, *Longissimus thoracis* et *lumborum* (LTL) was removed from the 11th rib to the last lumbar vertebrae (about 55 cm) from one side of each carcass. On day of 7 post mortem, each LTL was cut into 8 steaks (approximately 5.5-cm-thick slices) after removal of fat tissue. Steaks were wrapped with oxygen-permeable PVC film (NORM PACK 11545-1, Teampac AB, Tyresö, Sweden) and put onto paper plates. Steaks were randomly divided into four groups consisting of two steaks from each LTL (12 steaks per group). The four groups were randomly assigned to one of four storage times (0, 4, 7, 10 days). At corresponding storage time, meat color and pH were measured (Paper II). Samples for microbiological analysis (Paper II), biogenic amine analysis (Paper III) and lipid lipolysis and oxidation (Paper IV) were taken and stored at -80 °C until analyses.

3.2 Analysis methods

3.2.1 Lipid extraction and fatty acid analysis

Total lipids (Paper I and IV) from diets, chicken breast and drumstick meat, and beef were extracted according to the method of Hara & Radin (1978). Fatty acid methyl esters (FAMEs) were prepared according to Appelqvist (1968), and analyzed by GC using a CP-3800 GC instrument (Varian AB, Stockholm, Sweden) equipped with a fused silica capillary column BPX 70 (50 m, 0.25 mm inner diameter, 0.25 μm film thickness, SGE, Austin, TX, USA) according to the method described by Fredriksson *et al*. (2007). Peaks were identified by comparing their retention times with those of corresponding fatty acids in the standard mixture GLC-68 A (Nu-chek Prep, Elysian, MN, USA). Peak areas were integrated using Galaxie chromatography software version 1.9 (Varian AB, Stockholm, Sweden). Fatty acids were quantified using an internal standard, methyl-15-methylheptadecanoate (Larodan Fine Chemicals AB, Malmö, Sweden).

3.2.2 Lipid class composition of total lipids

Lipid class composition was evaluated using high performance thin layer chromatography (HPTLC) according to previously described by Olsen & Henderson (1989) with minor modifications. Extracted total lipids (4 μg/μL; Paper IV) were applied to precoated glass silica gel 60 (without F) HPTLC plates (20×10 cm; 0.20-mm layer; Merck, Darmstadt, Germany) using Camag TLC sampler 4 (Camag) and separated in an automatic developing chamber (Camag ADC2) using mobile phase hexane:diethyl ether:acetic acid (85:15:1, v/v/v). Phosphomolybdic acid in ethanol was used for derivatization. After drying plates were scanned at 650 nm using a Camag TLC scanner 3. Phospholipids, diacylglycerols, triacylglycerols, cholesterol, sterol esters, and free fatty acid were identified using an external C18–5 TLC standard (Nu-Chek Prep Inc.).

3.2.3 Gene expression analysis

Gene expression in liver (Paper I) was investigated by quantitative real-time PCR using an array of target genes coding for enzymes involved in lipid homeostasis. Total RNA was isolated from liver using SV Total RNA Isolation System and the first strand cDNA was synthesized using a High-Capacity cDNA Archive kit. Real-time PCR analysis was carried out using a Fast SYBR® Green Master Mix (Applied Biosystems) with the StepOnePlusTM Real-time PCR System (Applied Biosystems). A melt curve analysis was performed after each run to ensure that only a single product was amplified. On each plate samples from all treatments were analyzed in triplicate for one target gene, the reference gene (NADPH) and the nontemplate control. The ΔC_T for each sample was calculated per plate by subtracting the C_T value for the reference gene (NADPH) from the C_T for the target gene. The relative expression ($\Delta\Delta C_T$) was calculated by subtracting the ΔC_T values for the group fed control diet from the ΔC_T values for each experimental diet and presented using the term $2^{-\Delta\Delta C_T}$ and reported as arbitrary fold change units (Livak & Schmittgen, 2001).

3.2.4 Meat color

Meat color (Paper II) was measured using a Minolta CM-600d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) with an 8-mm-diameter measuring aperture, a D65 illuminant, a 10° standard observer and CIE L*, a*, b* color scale. Measurements were made directly on oxygen-permeable PVC film that covered the original contaminated surface. Measurements were averaged over four random nonoverlapping zones of each steak. The Minolta instrument recorded reflectance values in the range of 360

nm to 740 nm with 10-nm intervals. The relative content of deoxymyoglobin (Mb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb) were calculated as described before (Krzywicki, 1982). Chroma was calculated as $(a^{*2}+b^{*2})^{1/2}$ and hue angle as arctangent (b*/a*) according to the American Meat Science Association protocols (AMSA, 2012).

3.2.5 Enumeration of bacterial populations and identification of isolates

Meat samples were placed into a stomacher bag and homogenized with 100 mL of sterile 0.1% peptone water in a masticator blender (easyMIX, AES CHEMUNEX, France) for 2 min at room temperature. Total viable count (TVC), *Enterobacteriacea* count (ENT), lactic acid bacteria (LAB), molds count (MOL) and yeasts count (YEA) were enumerated according to Li et al. (2013). Results were expressed as $\log_{10}$ CFU/cm^2 and data were adjusted to 0.01 for statistical analysis when a value was below 1.0.
To identify the dominant bacterial species in spoiled beef, five spoiled beef samples were chosen according to their spoilage status (total viable count exceeding 7.0 $\log_{10}$ CFU/cm^2), and their bacterial colonies were grown on Plate Count Agar (Oxoid). Nineteen colonies from each sample were randomly isolated and identified by amplification and sequencing of 16S rRNA genes according to Willing et al. (2009). Colony PCRs were performed using the broad-range bacterial primers Bact-8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 926r (5'-CCGTCAATTCCTTTRAGTTT-3') and by the same PCR program as mentioned below in the T-RFLP analysis. PCR products were purified and sequenced from Bact-8F. Sequences were compared with sequences in the GenBank database using standard nucleotide BLAST at NCBI (http://www.ncbi.nlm.nih.gov).

3.2.6 T-RFLP analysis

DNA was directly extracted from meat samples according to the protocol described by the manufacturer of PowerFood Microbial DNA Isolation Kit (MoBio, Solana Beach, CA). The 16S rRNA gene regions were amplified from the isolated DNA with broad-range bacterial primers Bact-8F labeled with 6-carboxyfluorescein in the 5' end and 926r. Thermocycling was conducted with a model 9700 Gene Amp PCR system (Applied Biosystems, Foster City, CA) starting with an initial denaturing step at 95°C for 5 min. A total of 30 cycles consisting of 30 s at 95°C, 30 s at 55°C and 60 s at 72°C followed by a final primer extension step at 72°C for 10 min. The amount and size of PCR products were confirmed by agarose gel electrophoresis. PCR products were digested by HaeIII restriction enzyme (GE Healthcare, Uppsala, Sweden), at 37°C for 2 h. Digested fragments were separated on an ABI3730XL DNA

analyzer (Applied Biosystems) at Uppsala Genome Center, Rudbeck Laboratory, Uppsala University. Sizes of the terminal restriction fragments (TRFs) were determined by comparison with the internal GS ROX-500 standard (Applied Biosystems). Peak Scanner software (Applied Biosystems) was used to analyze the fragment data. Relative abundance of each TRF was determined by dividing the area of the peak of interest by the total areas of the peaks within the following threshold values: lower threshold, 28 bp; upper threshold, 500 bp and a fluorescent threshold of 50. A threshold for the relative abundance was applied at 1% and only TRFs with higher relative abundances were included in the remaining analysis, while from the replicate T-RFLP profiles only peaks that occurred in both replicates were included in the analysis. The heat map of TRF data was visualized using MultiExperiment Viewer (MeV 4.9), which was used to show the incidences of dominant TRFs (namely dominant spoilage bacteria) over time for each sample. Microbial diversity, defined by richness and evenness of TRF was measured by Simpson's diversity index of the T-RFLP data.

To identify the bacterial species corresponding to the dominant TRFs, A clone library was constructed by inserting the PCR products into the TOPO TA plasmid vector (Invitrogen, Carlsbad, CA, USA), followed by transformation into competent cells of *Escherichia coli* (TOP10 chemically competent, Invitrogen). Ninety-six inserts were PCR amplified using vector primers M13r and M13f (Invitrogen) and the same thermal cycling program was used as mentioned above. The PCR products were analyzed by T-RFLP analysis as described above. Clones with representative TRFs, which appeared in the T-RFLP analysis of the beef samples were chosen for sequencing and sequences were compared with those in the GenBank database using standard nucleotide BLAST at NCBI (http://www.ncbi.nlm.nih.gov).

3.2.7 Biogenic amine analysis

Biogenic amines were extracted and derivatized by modifying the method described by Lapa-Guimarães and Pickova (2004). A precisely weighed 2.5 g sample was homogenized with 5 mL 5% TCA using an Ultra-Turrax homogenizer, and centrifuged at 1200 x *g* for 5 min at 18°C. This extraction process was repeated twice with 2.5 mL 5% TCA. The supernatant collected was combined and volume was brought up to 12.5 mL with 5% TCA. Afterwards, the extract was filtered through Whatman No.1 filter paper. One millilitre (1 mL) of filtrate was mixed with 1 mL saturated $NaHCO_3$ solution, 400 µL NaOH (2 M), and 1 mL dansyl chloride (5 mg/mL in acetone), and the mixture was incubated in the dark at 40°C for 1 h. Afterwards, 0.5 mL glycine solution (0.1 g/mL) was added and incubated at 40°C for 20 min to remove

residual dansyl chloride. After dansylation, the residual acetone was evaporated under a nitrogen stream, and 3 mL distilled water was added. The extraction of dansyl amines was repeated twice with 2 mL diethyl ether, and the combined diethyl ether extracts were evaporated to dryness under a nitrogen stream. The residue was dissolved in 1 mL ethyl acetate for HPTLC separation.

Using an automated TLC sampler, 3 µL ethyl acetate containing dansyl amines were applied at 2 cm from the edge of the HPTLC plates in 4-mm bands. Plates were developed in the twin trough chamber with 20 mL development solvent (chloroform-diethyl ether-triethylamine; 4:1:1, v:v:v) in each trough. The chamber was presaturated with a saturation pad for 1 h. After development, plates were dried in an oven at 40°C for 10 min and scanned at 330 nm and fluorescence emission was measured using a K400 optical filter.

3.2.8 Static headspace gas chromatography (SHS-GC) analysis of volatile compounds

The volatile lipid oxidation products analysis was performed with a headspace autosampler (CTC analytics, MH 01-00B) attached to a gas chromatographic system (Varian CP-3800, Sweden), which was equipped with a 30 m*0.320 mm inner diameter, 1.00 µm film DB-5 fused capillary column (Agilent Technologies, USA) and a flame ionization detector (FID).

Three grams of meat samples were sealed into a 20 mL headspace vial (Chromacol, USA) with 7 mL deionized water (Millipore Synergy 185, Germany) and agitated in the static headspace auto sampler at a constant temperature of 95°C for 15 min. An aliquot of the vial's gas phase was introduced by a heated 120°C syringe (1.0 mL, SGE, Australia) into the carrier gas stream (helium) which carried it onto the column. Column temperature was increased linearly with 40°C during 3 min, followed by an increase of 5°C per min until 180°C; injector and FID temperatures were set at 250°C and 270°C, respectively. Flow rate of the helium carrier gas was 1.5 mL/min with an inlet pressure of 10 psi and a split injection ratio of 1:1. Blank vials were run between each sample to clean the column and alleviate carry-over effects.

Individual volatile compounds were tentatively identified by comparing their relative GC retention times with those of commercially available standards. To quantify the volatile lipid oxidation products in the meat samples, 20 µL of internal standard (4-methyl-2-pentanone) from working solution (100 ng/µL) was added into 7 mL deionized water in a headspace vial with 3 g of meat sample. Peak areas of internal standard and volatile compounds were integrated using Galaxie chromatography data system software version 1.9 (Varian AB, Sweden).

3.2.9 Determination of α-tocopherol

The α-tocopherol was analyzed by HPLC according to the method described by Azadmard-Damirchi & Dutta (2008). The column used was LiChroCART 250-4 (Merck KgaA, Darmstadt, Germany) packed with LiChrosphere 100 NH2, particle size 5 μm. The α-tocopherol was detected at a wavelength of 294 and 320 nm for excitation and emission, respectively. The isocratic mobile phase was a mixture of n-heptane/tert-butylmethylether/tetrahydrofuran/methanol (79:20:0.98:0.02, v:v:v:v) at a flow rate of 1.2 mL/min. The α-tocopherol was quantified using an external standard method with reference samples of tocopherols (Sigma-Aldrich, Steinheim, Germany).

3.2.10 Statistical analysis

Statistical analyses were carried out using Statistical Analysis System (Version 9.3, SAS Institute, Cary, NC, USA). In Paper I, least squared means (LSM) and standard error (SE) were calculated. One way ANOVA of GLM procedure was used to compare the dietary effects and option probability of differences (PDIFF) was used for multiple comparison. In Paper II, III and IV, the average values calculated from the duplicates of the same animal were used for analysis. Least squared means (LSM) and standard error (SE) were calculated. The MIXED procedure was used with storage time as fixed factor and animal as random factor. The option probability of differences (PDIFF) was used for multiple comparisons. In Paper II and IV, Spearman correlation procedure was used to calculate correlation coefficients between investigated parameters.

4 Summary of results

4.1 Changes in muscle FA composition and hepatic gene expression in chicken fed different RS diets

Chicken were fed six steam-pelleted or nonpelleted rapeseed (RS) diets with different RS inclusion levels (80, 160 and 240 g/kg), and a control diet (nonpelleted diet with no RS included). The contents of SFA, MUFA and PUFA in breast muscle were not influenced by the different dietary treatments. Compared with the control birds, only birds fed steam-pelleted diet with 240 g/kg of RS showed significantly higher PUFA and lower SFA in the drumstick muscle (Table 3 and 4).

The n-3 PUFA content in both muscles was highest in birds fed steam-pelleted diets with high content of RS (160 or 240 g/kg), whereas lowest levels were found in birds fed the control diet or nonpelleted diet with 80 g/kg of RS. In particular, the contents of n-3 LC-PUFA, DPA and DHA in both muscles increased significantly in a graded manner with increasing dietary level of RS, with highest levels in group RS16P and RS24P, and lowest levels in the control group. The EPA contents in both muscles were higher in birds fed RS diets compared with the control group. Furthermore, at high RS inclusion levels (160 and 240 g/kg), steam-pelleted diets significantly increased the contents of n-3 PUFA, n-3 LC-PUFA, DPA and DHA in both muscles compared with birds fed with nonpelleted diets with the same level of RS inclusion. The n-6 PUFA content in breast muscle was not affected by dietary treatments whereas the content in drumstick was higher in birds fed RS16P or RS24P diet compared with control. Birds fed RS24N and RS24P diet had higher AA content in both muscles compared with the control group birds. In both muscles, the n-6/n-3 ratio decreased with increasing level of RS inclusion, and birds fed steam-pelleted diets had lower value of n-6/n-3 ratio than birds fed nonpelleted diets with the same RS inclusion level (Table 3 and 4).

Compared with the control diet, the nonpelleted RS diets decreased (P<0.05) chicken body weight, weights of breast and drumstick with increasing inclusion level of RS. In contrast, chickens fed the steam-pelleted RS diets had similar body weight, breast and drumstick weight compared with chickens fed the control diet. The feed intake (d 34) was significantly reduced in chickens fed the nonpelleted diets RS8N and RS24N compared with chickens fed the control. However, chickens fed steam-pelleted RS diets had similar feed intake to chickens fed the control diet.

Table 3. *Fatty acid composition of breast muscle in chicken fed seven different diets (mg/100 g fresh weight, n=5; least square means)*

	Control	RS8N	RS8P	RS16N	RS16P	RS24N	RS24P
20:5n-3	1.8^{c}	3.9^{ab}	3.6^{ab}	3.9^{a}	4.4^{a}	4.1^{a}	3.0^{b}
22:5n-3	3.5^{f}	4.7^{ef}	6.1^{de}	6.5^{cd}	10.9^{ab}	8.5^{bc}	12.7^{a}
22:6 n-3	3.5^{e}	4.1^{de}	5.5^{cd}	5.9^{bc}	8.1^{b}	7.0^{bc}	13.5^{a}
SFA	212.6	150.5	147.0	174.4	149.4	212.7	143.0
MUFA	261.3	190.8	184.1	272.2	248.7	306.2	234.6
PUFA	153.2	127.6	136.7	150.4	187.0	182.0	173.7
n-3 LC-PUFA	8.9^{e}	12.7^{d}	15.2^{cd}	16.3^{cd}	23.5^{ab}	19.6^{bc}	29.2^{a}
n-3	15.5^{e}	18.3^{de}	23.4^{cd}	28.0^{c}	42.5^{ab}	31.7^{bc}	44.9^{a}
n-6	137.6	109.1	113.1	122.2	144.1	150.1	127.8
n-6/n-3	8.8^{a}	5.9^{b}	4.8^{c}	4.3^{d}	3.4^{e}	4.7^{cd}	2.8^{f}
Total FA	656.5	492.5	478.9	617.8	595.2	731.9	586.2

Abbreviations: SFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids, n3, n-3 polyunsaturated fatty acids, n6, n-6 polyunsaturated fatty acid, n-3 LC-PUFA, EPA (C20:5n-3)+DPA(C22:5n-3)+DHA (C22:6n-3); total FA, total fatty acid. nonpelleted RS diets with RS inclusion level at 80 g/kg (RS8N), 160 g/kg (RS16N) and 240 g/kg (RS24N), steam-pelleted diets with RS inclusion level at 80 g/kg (RS8P), 160 g/kg (RS16P) and 240 g/kg (RS24P).
Different letters in the same row indicates significant differences at *P*<0.05.

Compared with the control group, the mRNA levels of genes involved in fatty acid oxidation ACOX1 and in desaturation FADS1 were significantly higher in birds fed the diet RS24P or in birds fed RS16P and RS24P diet, respectively. No significant dietary effect was observed in transcript levels for the other genes investigated (L-CPT1, L-FABP1, FADS2, FTO, LPL and SREBP2).

Table 4. *Fatty acid composition of drumstick muscle in chicken fed seven different diets (mg/100g fresh weight, n=5; least square means)*

	Control	RS8N	RS8P	RS16N	RS16P	RS24N	RS24P
20:5n-3	2.0^{c}	4.8^{ab}	4.2^{b}	5.4^{ab}	5.8^{a}	5.4^{ab}	4.8^{ab}
22:5n-3	6.8^{d}	9.9^{cd}	11.1^{c}	11.1^{c}	18.7^{ab}	15.2^{b}	19.5^{a}
22:6n-3	4.9^{d}	6.3^{cd}	7.8^{c}	7.5^{c}	11.7^{b}	11.3^{b}	17.8^{a}
SFA	545.0^{a}	556.2^{a}	493.9^{a}	570.4^{a}	499.7^{a}	581.2^{a}	361.3^{b}
MUFA	693.4	768.2	711.4	924.6	947.0	923.7	817.3
PUFA	352.1^{b}	341.8^{b}	369.7^{b}	392.4^{b}	546.9^{a}	435.1^{b}	561.5^{a}
n-3 LC-PUFA	13.6^{d}	21.0^{c}	23.1^{c}	24.0^{c}	36.2^{ab}	31.9^{b}	42.1^{a}
n-3	30.7^{d}	43.8^{cd}	54.9^{bc}	63.1^{bc}	106.3^{a}	71.5^{b}	120.1^{a}
n-6	320.4^{b}	296.4^{b}	314.1^{b}	328.8^{b}	439.0^{a}	362.3^{ab}	440.6^{a}
n-6/n-3	10.3^{a}	6.5^{b}	5.7^{c}	5.2^{d}	4.1^{e}	5.0^{d}	3.6^{f}
Total FA	1607.2	1658.7	1579.7	1894.4	1993.4	1952.3	1741.4

Abbreviations: SFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids, n3, n-3 polyunsaturated fatty acids, n6, n-6 polyunsaturated fatty acid, n-3 LC-PUFA, EPA (C20:5n-3)+DPA(C22:5n-3)+DHA (C22:6n-3); total FA, total fatty acid. nonpelleted RS diets with RS inclusion level at 80 g/kg (RS8N), 160 g/kg (RS16N) and 240 g/kg (RS24N), steam-pelleted diets with RS inclusion level at 80 g/kg (RS8P), 160 g/kg (RS16P) and 240 g/kg (RS24P).
Different letters in the same row indicates significant differences at $P<0.05$.

4.2 Microbial growth and BAs formation in beef stored at 4°C

Storage time affected microbial growth when *Longissimus* muscle overwrapped with polyvinyl chloride (PVC) film was stored at 4°C for 10 days. The microbial counts except molds count significantly increased from day 0 to day 10 of storage (Table 5). Total viable counts increased over time from 2.1 at day 0 to 8.4 $\log_{10}$ CFU/cm^2 at day 10. Yeast was not detectable in day-0 samples, but increased from day 4 to 10 (from 1.2 to 4.3 $\log_{10}$ CFU/cm^2). *Enterobacteriaceae* count was not detected until day 7, and on day 10 it reached 2.8 $\log_{10}$ CFU/cm^2. Lactic acid bacteria count decreased during the first seven days of storage from 1.8 to 0.8 $\log_{10}$ CFU/cm^2 and increased significantly by day 10 to 2.7 $\log_{10}$ CFU/cm^2.

To identify the dominant bacteria responsible for meat spoilage, both T-RFLP analysis and traditional colony identification by 16S rRNA sequencing were used. Furthermore, T-RFLP analysis was also used to study the dynamic changes in incidences of different bacteria occurring over time. For the traditional colony identification (Figure 4), 70% of the colonies identified corresponded to the genus *Pseudomonas* (*P. fragi/P. psychrophila/P. libanensis/P.cedrina*), followed by the genus *Brothothrix* (26%; *B. thermosphacta/B. campestris*). In addition, 3% were identified as *Carnobacterium gallinarum/C. maltaromaticum.*, and 1% as *Acinetobacter lwoffi/A. venetianus*. For the T-RFLP analysis (Table 6), TRF 37 was presented

in each sample with an average abundance of 33% on day 0. After four days of storage, TRF 310 dominated in the T-RFLP profiles in most of the samples while TRF 33 was only dominant in four samples. TRF 33 and 310 were the dominant TRFs on day 7 and 10. TRF 88 was present with similar abundance (4.1-6.8%) in each sample during storage. TRF 200 was detected mainly in meat samples from day 7 and 10 with a relative abundance of 3%.

Table 5. *Microbial counts (log_{10} CFU/cm^2) of beef overwrapped with PVC film stored in air for 10 days at 4°C (Least square means)*

	Day 0	Day 4	Day 7	Day 10
TVC	2.1^d	4.2^c	6.2^b	8.4^a
ENT	0.3^b	0.5^b	1.1^b	2.8^a
LAB	1.8^b	1.0^b	0.8^c	2.7^a
MOL	0.6^a	0.2^a	0.3^a	0.01^a
YEA	0.3^d	1.2^c	2.8^b	4.3^a

Abbreviations: TVC, total viable count; ENT, *Enterobacteriaceae* count; LAB, Lactic acid bacteria count; MOL, mold count; YEA, yeast count.
Data below 1.0 indicate below the detection limit 1 log_{10} CFU/cm^2. Different letters within the same row indicate significant differences (*P*<0.05).

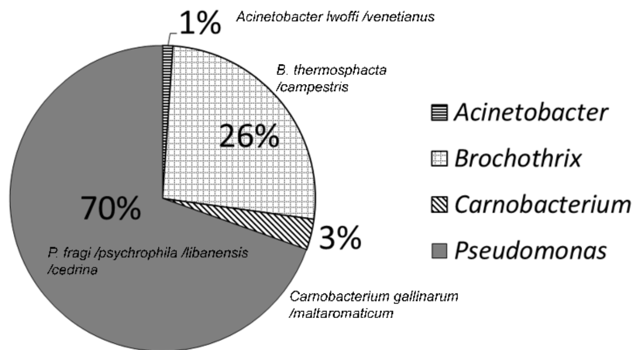

Figure 4. Characterization of microbiota on spoiled beef by colony analysis

Table 6. *Changes in the relative abundance (%) of major TRFs during storage*

TRF length	% maximum identity	Putative identity	Day 0	Day 4	Day 7	Day 10
33	98	*Pseudomonas psychrophila/B. libanensis*	7.0^b	14.8^b	39.4^a	45.6^a
37	-	unknown	33.0^a	12.2^b	2.4^{bc}	1.4^c
88	-	unknown	6.8^a	5.6^{ab}	4.9^{ab}	4.1^b
200	99	*Acinetobacter lwoffii*	0.4^b	0.5^b	2.6^a	2.9^a
310	99	*Brochothrix thermosphacta/B.campestris*	11.9^b	48.8^a	47.1^a	43.3^a

Different letters within the same row indicate significant differences (P<0.05).

Agmatine, spermine and spermidine were detected in all samples, and spermidine was not quantified because it was out of linear range of quantification. The most abundant BA in fresh beef samples was spermine (15.3 mg/kg at day 0), and it decreased significantly from day 4 to day 7 (15.8 to 12.7 mg/kg). Tyramine was not detected until day 10 and it was detected in 7 out of 12 day-10 samples with a highest value of 8.0 mg/kg fresh weight. Among those 7 meat samples, 4 samples also contained cadaverine. In addition, among the tyramine-positive day-10 samples, only 1 meat sample was detected with putrescine (4.0 mg/kg). The content of spermine was negatively correlated to total viable count (r=-0.60, $P<0.001$), *Enterobacteriaceae* count (r=-0.47, $P<0.001$), and the relative abundance of *Pseudomonas spp.* (r=-0.35, $P<0.05$) and *Acinetobacter* (r=-0.48, $P<0.001$). The cadaverine content was positively correlated with *Enterobacteriaceae* count (r=0.39, $P<0.01$) and with total viable count (r=0.41, $P<0.01$). TYR was correlated with both total viable and *Enterobacteriaceae* counts (r=0.58, $P<0.001$; r=0.54, $P<0.001$).

4.3 Meat discoloration and its association with microbial growth in beef stored at 4°C

Storage time significantly affected color traits, such as *a**, hue angle, and Chroma value. The *a** remained constant during the first seven days and decreased significantly at day 10, while hue angle increased ($P<0.05$) from day 7 to 10. The Chroma value reached a maximum at day 4, and then decreased to a level below that at day 0. The relative content of MetMb increased over time, accompanied by a decrease in OxyMb between day 7 and day 10. The relative content of DeoxyMb on day 0 and day 10 was higher compared to day 4 and day 7.

Total viable count was negatively correlated to the *a** (-0.61), *b** (r=-0.59), Chroma (r=-0.63) and OxyMb (r=-0.66), and positively correlated to hue angle (r=0.49), DeoxyMb (r=0.57) and MetMb content (r=0.60). Similar directions, with variable strengths, of correlations were observed for *Enterobacteriaceae*, lactic acid bacteria, yeast, the relative abundance of *Pseudomonas* and *Acinetobacter*. Among these correlations, much higher correlation strengths were observed among *Enterobacteriaceae* and *a** (r=-0.72), Chroma (r=-0.73) and hue angle (r=0.74). In contrast, TRF37 positively correlated to the *a** (0.46), *b** (r=0.47), Chroma (r=0.46) and OxyMb (r=0.43), and negatively correlated to hue angle (r=-0.35) and MetMb content (r=-0.48).

4.4 Lipid oxidation in beef stored at 4°C

Storage time significantly affected the technological lipid quality and thereby lipid class composition and concentrations of volatile secondary lipid oxidation products, but not the fatty acid composition, α-tocopherol content and TBARS values. The percentage of free fatty acid (FFA) increased significantly from 3.6% at day 0 to 6.7% at day 10. The percentage of the major lipid class, TAG, decreased significantly from day 4 (64.7%) to day 10 (60.7%) and the percentage of DAG increased significantly from day 4 (2.3%) to day 10 (3.0%). Hexanal, pentanal, heptanal, octanal, and nonanal were detected in all beef samples stored under refrigerated conditions. The content of hexanal, heptanal and octanal decreased ($P<0.05$) over time. Furthermore, TBARS values were negatively correlated with α-tocopherol content in meat, but were positively correlated with concentrations of the volatiles octanal ($r=0.74$), nonanal ($r=0.67$) and hexanal ($r=0.70$).

5 Discussion

5.1 Changes in muscle FA composition and hepatic gene expression in chicken fed different RS diets

Chicken fed diets containing high level of RS showed increased content of n-3 PUFA in breast and drumstick meat. In particular DPA and DHA increased whereas n-6, MUFA, SFA and total fatty acid content changed only slightly. The steam-pelleting process, enabling high inclusion levels (16% or 24%) of RS in chicken feed, did not negatively affect chicken meat yield as reflected in analysis of breast and drumstick mass.

The ALA contents in breast and drumstick increased significantly when high levels of rapeseed (160 or 240 g/kg) were included in the diets. This can be explained by the significant increase of ALA content in these diets. RS contains about 40% oil, which is an excellent source of ALA (Ajuyah *et al.*, 1991). The ALA content in the edible tissues of poultry can be readily increased by increasing ALA levels in the diet, especially for meat with skin, and dark meat (Rymer & Givens, 2005). Furthermore, the current study showed a significant dose-dependent increase in DPA and DHA contents in both muscles with increasing inclusion level of ALA in chicken diets. Compared to chickens fed the control diet, those fed RS diets either in steam-pelleted form or in nonpelleted form had higher level of EPA in breast and drumstick. This is readily explained by the existing desaturation and elongation pathway in chicken to produce DHA from ALA. As the RS inclusion level increased, more ALA would be ingested and absorbed by birds and subsequently converted to longer chain metabolites (DPA and DHA). These observations agree well with a previous study which showed that a feeding diet with 5% RS oil, increased levels of EPA and DPA in broiler chicken breast, compared with birds consuming a diet with 5% soybean oil (Nyquist *et al.*, 2012).

At the RS inclusion level of 160 and 240 g/kg, birds fed steam-pelleted diets displayed similar levels of ALA and LA in breast, higher levels of ALA and LA in drumstick, and a higher level of n-3 LC-PUFA (in particular DPA and DHA) in both muscles, although the steam-pelleted diets contained relatively less ALA and LA compared to the nonpelleted diets with the same RS level. Moreover, the n-6/n-3 ratio was lower in both muscles from birds fed steam-pelleted diets compared with birds fed the nonpelleted diets. These differences could be explained by the increase in feed intake observed and possible improvement of fat availability for steam-pelleted diets. The feed intake in birds fed nonpelleted RS diets was significantly lower than that of birds fed steam-pelleted diets at the same inclusion level. The steam-pelleting process has been shown to enhance the digestibility of lipids (Shen *et al.*, 1983). As a result, it could be deduced that birds fed these steam-pelleted diets absorbed more ALA, which then could be metabolized to LC-PUFA through chain elongation and desaturation. To the best of our knowledge, this is the first study to show that at high inclusion levels of RS, the steam-pelleting process increases chicken meat nutritional value in the form of n-3 LC-PUFA content and n-6/n-3 ratio.

The current western diet has been estimated to be deficient in n-3 FAs with a high ratio (15-20) of n-6 to n-3 FA (Simopoulos, 2006). Excessive amounts of n-6 PUFAs and a very high n-6/n-3 ratio, promote pathogenesis of many diseases, including cardiovascular disease, cancer, inflammatory and autoimmune diseases, whereas increased levels of n-3 FAs (a lower n-6/n-3 ratio) exert suppressive effects (Simopoulos, 2008). As shown in this study, feeding chicken with steam-pelleted RS diet can be a good strategy to increase human intake of n-3 PUFAs, in particular n-3 LC-PUFA. Birds fed the steam-pelleted diet with 24% RS inclusion provide the most nutritious meat in terms of n-3 PUFA content, particularly n-3 LC-PUFA content and n-6/n-3 ratio. These birds had the highest content of n-3 PUFAs in the meat, with 45 mg/100 g fresh meat in breast and 120 mg/100 g in drumstick, and these levels were about three times higher than control birds fed the nonpelleted diet. Moreover, consuming a 100 g portion of this breast or drumstick meat would provide consumers with 29 mg or 42 mg n-3 LC-PUFA, respectively, compared to 9 or 14 mg from birds fed the control diet. Meanwhile, the n-6/n-3 ratios in this breast and drumstick meat were 2.8 and 3.6, respectively, which were also better than meat from birds fed the control diet (8.8 for breast and 10.3 for drumstick).

In this study, weights of breast with ribs and drumstick were measured to reflect meat yield of chicken carcass. Independent of RS inclusion level, birds fed steam-pelleted diets had similar meat yield compared with control birds,

whereas meat yields in birds fed nonpelleted diets were compromised as RS inclusion level increased. This is in agreement with changes in chicken body weight observed, showing that increasing inclusion levels did not influence live weight at day 34 when birds were fed steam-pelleted diet, but the live weight was reduced with increasing inclusion level when fed nonpelleted diets. This might be due to the decrease in feed intake for birds receiving the nonpelleted feed compared to chicken fed the steam-pelleted diet at the same RS inclusion level. Similarly, Summers *et al.* (1982) reported that feeding 17.5% full-fat canola seed to broiler chickens resulted in depressed fat digestibility and body weight gain. When fed high level of RS, growth performance of broilers can be affected by degradation products of glucosinolate in the seeds through interference with thyroid gland function and decreased feed palatability (McCurdy, 1990; Khajali & Slominski, 2012). Another explanation could be attributed to the reduced energy use efficiency from rapeseed in nonpelleted form due to a lower oil availability. The lower availability has been suggested to result from the oil-encapsulating effect of the cell wall polysaccharides (Lee *et al.*, 1991). On the other hand, the steam-pelleting process can improve the fat availability in RS, possibly due to the efficient pulverization effect of this process (Shen *et al.*, 1983; Salmon *et al.*, 1988; Barekatain *et al.*, 2015).

The hepatic expression of the FADS1 gene was upregulated in birds fed pelleted RS16 and RS24 diets compared with the control. This result agrees with a previous study where an ALA-enriched diet, containing 75% RS oil of total fat as rape seed oil, increased the expression of hepatic FADS1 in salmon compared with the control fed diet containing 100% of fat as fish oil (Jordal *et al.*, 2005). The biosynthesis of n-3 LC-PUFA from ALA includes a series of desaturation, elongation, and ultimately β-oxidation steps (Dyall, 2015). One of the two rate-limiting steps in this pathway is the desaturation of eicosatetraenoic acid (20:4n-3) to EPA, catalyzed by the Δ5 desaturase encoded by the FADS1 gene (Cho *et al.*, 1999). The formation of n-3 LC-PUFA depends on the amount of substrate and removal of the subsequent products. Increased desaturase gene expression could be influenced by either increased dietary substrate concentration or removal or absence of the products (Mirshekar *et al.*, 2015). Hence, the increase of hepatic FADS1 transcript levels observed in the current study could be ascribed to the increased dietary ALA content in the RS diets. On the other hand, the higher level of FADS1 transcripts in RS diets fed birds could, at least in part, account for the higher EPA content in chicken meat observed in birds fed RS16P and RS24P diets compared with birds fed the control diet. Compared to the control diet, the RS24P diet upregulated the transcript levels of ACOX1 in chicken liver. The final step in the pathway of DHA synthesis occurs in peroxisomes through a

single round of β-oxidation of tetracosahexaenoic acid (24:6n-3) (Wanders, 2004; Dyall, 2015). Acyl-coenzyme-A oxidase (ACOX1) is the rate-limiting enzyme for this peroxisomal fatty acid β-oxidation (Ding *et al.*, 2003). The increased hepatic expression of ACOX1 in birds fed RS24P diet may be attributed to the increase in feed intake and possible improvement of fat availability for steam-pelleted diets. Consequently, the higher levels of ALA might trigger a higher expression of ACOX1 leading to the increased formation of DHA in birds fed the steam-pelleted diets, compared to birds fed the control diet.

5.2 Meat discoloration in beef stored at 4°C

Redness (a*), color intensity (Chroma) and hue angle are good indicators of meat discoloration, and as discoloration occurs, values for a* and Chroma decrease while hue angle increases (Owen & John, 2001). In particular, hue angle is useful to indicate shifts in color over time toward discoloration where larger values indicate less red (AMSA, 2012). In the current study, a* and Chroma decreased significantly from day 7 to day 10, accompanied by a significant increase in hue angle, thus indicating meat discoloration at day 10. Meat discoloration is associated with the oxidation of myoglobin and the accumulation of metmyoglobin (Insausti *et al.*, 1999; Mancini & Hunt, 2005; Ercolini *et al.*, 2011). In this study, the relative content of metmyoglobin increased significantly throughout the storage time and reached 35% level at day 10, while the oxymyoglobin content decreased significantly from day 7 to 10.

Spearman rank correlation analysis showed that total viable count was associated with color parameters such as a* (r=-0.61), Chroma (r=-0.63) and hue angle (r=0.49) that are good indicators of meat discoloration. Stronger correlations between *Enterobacteriaceae* count and meat discoloration (such as a*, Chroma and hue angle; r=-0.72, r=-0.73 and r=0.74, respectively) were observed. The relative abundances of aerobic bacteria (e.g. dominant *Pseudomonas spp.* and *Acinetobacter spp.*) were positively correlated with those discoloration indicators. Hence, microbial spoilage of meat as indicated by the total aerobic count and meat discoloration occurred simultaneously for naturally contaminated beef overwrapped with PVC film under aerobic storage, indicating that microbial growth and meat discoloration may not be totally independent effects for meat overwrapped with PVC film during aerobic storage. This association may be explained by the fact that microbial growth, in particular aerobic *Pseudomonas spp.*, facilitates metmyoglobin formation through the reduction of the oxygen level at meat surface

(Faustmani, 1990; Motoyama *et al.*, 2010). Furthermore, growth of some members of *Enterobacteriaceae*, such as *Hafnia alvei* and *Serratia liquefaciens*, was associated with the greening discoloration of meat, probably due to the production of hydrogen sulphide by these two microorganisms (Stanbridge & Davies, 1998).

Reactive lipid oxidation products could accerate myoglobin oxidation through the adduction of aldehydes at proximal and distal histidines, exposing the heme group to an oxidizing environment (Suman & Joseph, 2013). In the same set of the samples, the content of volatile aldehydes, e.g. hexanal, heptanal and octanal decreased significantly over time. This significant decrease may be partly attributed to their interaction with myoglobin, which consequently facilitates the oxidation of myoglobin.

5.3 Microbial growth and biogenic amine formation in beef stored at 4°C

Commonly, the discrimination of spoiled meat is based on the values of total viable counts (TVC) (Ellis *et al.*, 2002), and a cut-off value of 7 $\log_{10}$ CFU/cm^2 has been suggested as an indicator index for microbiological spoilage under aerobic conditions (Ellis & Goodacre, 2001; Jay *et al.*, 2006; Peng *et al.*, 2011). In this study, the total viable count exceeded this threshold at day 10. Comparing to TVC, other microbiological counts such as yeast, *Enterobacteriaceae*, and lactic acid bacteria, remained at lower levels. However, ENT count increased significantly, from below detection limit to 2.8 $\log_{10}$ CFU/cm^2 at day 10, when meat was regarded as spoiled as indicated by the TVC. Members of *Enterobacteriaceae* are successful colonizers of wet environments in the structural and work surface in abattoirs and this group is commonly present in fresh and frozen beef, pork and related meat products (Doulgeraki *et al.*, 2012). However, in terms of numbers, they do not contribute to the microbial associations related with meat spoilage under chilled aerobic storage (Nychas *et al.*, 1998).

In this study, T-RFLP analysis combined with cloning and sequencing techniques was applied to characterize the microbial diversity and to find the trend changes of the incidences of different bacteria occurring over time. T-RFLP is a relatively rapid and inexpensive method and suitable for characterization of a large number of samples. As a comparison, colony identification by 16S rRNA sequencing was also applied to complement the results obtained from T-RFLP analysis. Both approaches showed that *Pseudomonas spp.* and *Brochothrix spp.* were the prevailing species from beef overwrapped with PVC film under aerobic conditions. *P. fragi / P.*

psychrophila / P. libanensis / P. cedrina and *B. thermosphacta / B. campestris* were identified in this study. However, only the major bacteria were detected by T-RFLP approach in the present study, and other bacteria with low prevalence were not detected, such as bacteria from genus *Enterobacteriaceae*. As previously reported, *Pseudomonas spp.* are always the dominant spoilage bacteria under aerobic conditions irrespective of meat sources owing to their capability to degrade glucose and amino acids and ability to rapidly attach to and grow on meat surfaces compared with other spoilage bacteria (Ercolini *et al.*, 2006; Nychas *et al.*, 2007). Three species of *Pseudomonas*, *P. fragi*, *P. fluorescens,* and *P. lundensis* are the most important in this respect (Nychas *et al.*, 2008). *B. thermosphacta* is also an important colonizer of meat, and can grow in aerobic and low oxygen atmosphere, such as a poor vacuum pack or modified atmosphere pack that contains a low level of oxygen). *B. thermosphacta* contributed to meat spoilage by producing a number of compounds, acetoin, methylbutyric, isovaleric acid, lactic acid, carbon dioxide and ethanol, under aerobic conditions (Papadopoulou *et al.*, 2012).

Furthermore, T-RFLP analysis in this study also showed the successive changes in the incidences of different bacteria over time. Initially, the bacteria colonizing the beef surface were highly diverse, but they then became more specific towards the end of the storage period. The major changes in microbial diversity occurred during the first seven days of storage. After four days, *Brochothrix spp.* successfully competed with other bacteria and prevailed on the surface. At day 7, when meat started to become discolored, *Pseudomonas spp*. became the dominant species with *Brochothrix spp.* in almost equal proportions. Similarly, Ercolini *et al.* (2011) reported that *B. thermosphacta* prevailed during the early stages of storage of beef in air, while *Pseudomonas spp.* took over during further storage.

The shelf-life of fresh meat is generally assessed by monitoring the microbial growth or sensory changes during storage. However, these methods are time-consuming and costly, and alternative methods are warranted. In this regard, BAs may serve as potentially better indicators of meat spoilage because the determination of BAs can be completed more easily in a short time. A significant decrease in spermine content was observed after day 4 in this study. Similarly, decreased spermine content during storage has been observed in chicken ascribed to its use as a source of nitrogen by bacteria present in meat (Silva & Glória, 2002). Furthermore, these authors have suggested an index for the evaluation of meat freshness based on the ratio of spermidine/spermine levels in chicken. In addition, spermine together with spermidine is considered as powerful antioxidants (Kalač, 2006); hence the oxidation of spermine during aerobic storage of meat could also lead to the decrease of spermine in meat.

Tyramine and cadaverine have already been suggested as spoilage indexes of fresh beef packed in aerobic atmosphere with biopolymers (Galgano *et al.*, 2009). In the current study, tyramine was detected in seven out of twelve day-10 samples, and the total viable count for all those samples exceeded the spoilage cut-off value.

5.4 Lipid lipolysis and oxidation in beef stored at 4°C

Lipid lipolysis occurred over time during aerobic storage of beef wrapped with PVC film, which was reflected by changed lipid class composition with increased relative content of FFA and decreased relative content of TAG. Lipolysis is one of the main processes of lipid degradation during the processing of meat and meat products, in which endogenous and bacterial lipases or phospholipases could be involved (Gandemer, 2002). Because FFAs are more sensitive to autoxidation than esterified fatty acids, the released FFAs could accelerate lipid oxidation (Alasnier *et al.*, 2000). In particular, PUFAs are more prone to autoxidation than saturated fatty acids (Halliwell & Chirico, 1993). Therefore, fatty acid composition in meat could be altered as a result of lipid oxidation. However, storage time did not affect the relative content of SFA, MUFA or PUFA or each specific fatty acid in this study. On the other hand, the α-tocopherol level in the beef remained unchanged with an average value of 3.4 µg/g meat. The α-tocopherol concentration in beef for optimum protection against lipid and pigment oxidation during 7 days of aerobically refrigerated storage was in the range 3.0 to 3.5 mg/kg meat (Álvarez *et al.*, 2008). Hence, the high α-tocopherol content in beef observed in the present study seems to prevent PUFA from oxidation. Moreover, TBARS values in beef did not increase over time. Moreover, the content of aldehydes, e.g. hexanal, heptanal and octanal, decreased significantly over time. Pignoli et al. (2009) reported that TBARS values did not increase after 8 h of further storage when adequate antioxidants were added to turkey meat, whereas a significant decrease of hexanal concentration was observed. This reduced volatility of hexanal can be ascribed to the interactions between the aldehyde and the amine and sulfhydryl groups of muscle proteins or to bacterial consumption of these compounds (Pignoli *et al.*, 2009; Bianchi *et al.*, 2010). This also leads to a conclusion that protein oxidation products are of interest when measuring oxidation in meat.

6 Future research

The current thesis showed that the n-3 LC-PUFA content in chicken meat could be improved through feeding diet with high level of steam-pelleted rapeseed. However, its level may not be comparable with chicken fed fish oil or fishmeal. Other terrestrial alternatives, such as linseed, need to be investigated.

In the current thesis, meat quality deterioration with regard to discoloration, microbial growth and lipid oxidation were characterized for beef overwrapped with PVC film stored in aerobic conditions. The overall aim of this project was to identify markers for the storage-time dependent changes in meat quality. Results indicated that changes in instrumental color and concentration of some biogenic amines may be useful as indicators for meat spoilage. However, the use of these parameters as indicators for meat spoilage may be unreliable and inconsistent because meat color can be affected by various factors other than storage time and the fact that tyramine don't appear until the very late stage of storage period by when meat was likely to be already spoiled. Although decrease in spermine content is correlated with total viable count, it is not possible to set an exact value for spermine content below which meat can be regarded as spoiled. An attempt was made to use a proteomic approach to identify bacterial proteins as markers in the preliminary study (unpublished). However, the overabundance of meat protein compared with proteins of bacteria origin made this method unsuccessful. Therefore, more studies are warranted to develop novel methods for the rapid online detection of meat spoilage. Until now a few advanced methods have shown some promising results, such as multispectral imaging technology, Fourier transform Raman spectroscopy, Raman spectroscopy, and fingerprinting of volatile microbial metabolites. The use of these methods in the evaluation of meat spoilage is mainly based on the bacterial counts, but bacterial counts are not always consistent with sensory rejection of consumers. Thus, future studies on

evaluation of biomarkers of meat freshness should also involve sensory evaluation.

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