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Influence of linseed cake feeding and pasture-based rearing on the fatty acid profile and oxidative status in meat from conventional pigs and Turopolje pigs

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Danijel Žirovec

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List of Abbreviations

Abbreviation	Meaning
Bidist.	Bidistilled
BSA	Bovine serum albumin
BCA	Bicinchoninic acid
GC-FID	Gas chromatography – flame ionization detector
GC-MS	Gas chromatography – mass spectrometry
EGTA	Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid
BHT	Butylated hydroxytoluene
DNPH	2,4-Dinitrophenylhydrazine
HPLC-UV	High performance liquid chromatography – ultraviolet detector
LC-MS	Liquid chromatography – mass spectrometry
RT	Room temperature
PV	Peroxide value
MeOH	Methanol
TCA	Trichloroacetic acid
BHT	Butylated hydroxytoluene
IDF	International Dairy Federation
NF	Normal feed
LPC	Linseed press cake feed
TP	Turopolje-pigs
LR	Land race pigs
ALA	α-linolenic acid
ROS	Reactive oxygen species
EPA	Eicosapentaenoic acid
n6:n3 ratio	Omega-6 to omega-3 ratio
FW	Fresh weight

1 Introduction and hypotheses

Fatty acids play a crucial role in the human body, supporting many important functions — particularly omega-3 fatty acids, which are known for their significant health benefits. Especially, to reduce the possibility of cardiovascular diseases and as modulatory and anti-inflammatory agents, omega-3 fatty acids must be acquired through food and can be found in fish, walnuts or seeds like linseeds or chia seeds. Nevertheless, contemporary as well as traditional dietary patterns typically prioritize the intake of food with less omega-3 fatty acids like meat, carbohydrate-rich foods or milk products (Banaszak et al., 2024). In Austria, meat consumption rose slightly in 2024, increasing by 0.4 kilograms to an average of 58.0 kilograms per capita (without bones and fat). Compared to the previous year, there was a small decline in pork consumption (- 0.1 kg), while poultry consumption increased by 0.5 kilograms (Statistik Austria, 2025).

The efficient utilization of available resources for animal production, food security, and the maintenance of both human and animal well-being is essential for achieving an economically viable, resilient, and sustainable future (Ponnampalam et al., 2021). The biological impacts of oxidative stress and the associated generation of free radicals on farm animal performance, productivity, and product quality can be modulated through targeted dietary interventions. These strategies primarily involve the inclusion of feeds, supplements, and forages enriched with antioxidant compounds. To optimize the efficacy of such approaches, a comprehensive understanding of the biochemical pathways involved in free radical generation is crucial (Ponnampalam et al., 2022).

Pork meat is an important protein source in human diet, rich in vitamins, especially B vitamins, with minerals like zinc and iron. Meat is highly susceptible to various degradation processes. Among these, oxidative reactions and microbial spoilage are the most significant, as they affect lipids, in primary and secondary lipid oxidation products, pigments, proteins, and vitamins, but also the color and meat flavor (Flis et al., 2010). Fatty acids with numerous allyl groups are particularly vulnerable to radical attacks (Belitz H. D. et al., 2008).

To support the fulfillment of consumer demands while increasing the competitiveness of the pork industry by elevating meat quality and lowering production expenses, in recent years, researchers have focused on increasing the nutritional value of pork meat. One strategy involves supplementing pig diets with vitamin E to improve meat color stability and reduce oxidative stress (Hoving-Bolink et al., 1998). Additionally, feeding pigs sources of n-3 polyunsaturated fatty acids, especially those of marine origin rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), increases their concentration in pork. This

enrichment improves the health-related value of pork by enhancing its fatty acid profile (Huang et al., 2021).

To date, there has only been a small amount of studies, for example (Czyż et al., 2021), (Jaturasitha et al., 2002) and (Tognocchi et al., 2023a) where pigs were supplemented using different oils or linseed, to increase the n-3 polyunsaturated fatty acids through feed and monitoring the effect of this supplementation the fatty acid profile, lipid oxidation and protein oxidation. These studies often lack detailed information regarding husbandry practices, feeding regimes, and slaughter conditions (Czyż et al., 2021; Jaturasitha et al., 2002; Tognocchi et al., 2023). There is also not a lot of information, on how the different pig races affect the protein and lipid oxidation, especially in Turopolje-pigs. Nevertheless, the limited research in this area highlights a significant gap in the literature and serves as a central motivation for this master's thesis. It is assumed, that in the meat from pigs fed with linseed press cake, the omega-3 fatty acid levels will rise and that the amount of protein oxidation products and lipid oxidation products will decrease. Additionally, it is expected, that by Turopolje-pigs, due to their stress resistance, the amount of protein oxidation products and lipid oxidation products will decrease. If the research shows expected outcome, it could lead to meat production with more healthy benefits, but also using different pig races to increase the meat quality.

2 Literature review

2.1 Pork

Pork is one of the most commonly consumed red meats globally, representing about 35% of total meat consumption worldwide. Quality and flavor of the meat are key influences on consumer choice. Cultural considerations also play an important role, particularly since pork consumption is prohibited in certain beliefs. Pork meat production is influenced by several factors, including genetic traits, rearing systems, feed composition, but also pre-slaughter and post-slaughter processes (Pandey et al., 2024).

During slaughter, pork is typically segmented into two pork halves (see **Figure 1**). Austrian butchering guidelines require clearly separating major cuts such as the loin, leg, shoulder, and belly (see **Figure 2**). Efficient use of by-products, such as the tail and ham hock, is also ensured (Österreichisches Lebensmittelbuch, 2025).

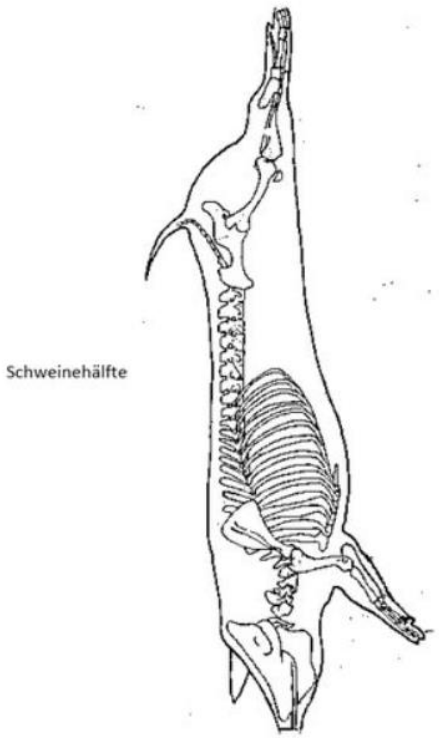


Figure 1: Pork halves (Österreichisches Lebensmittelbuch, 2025)

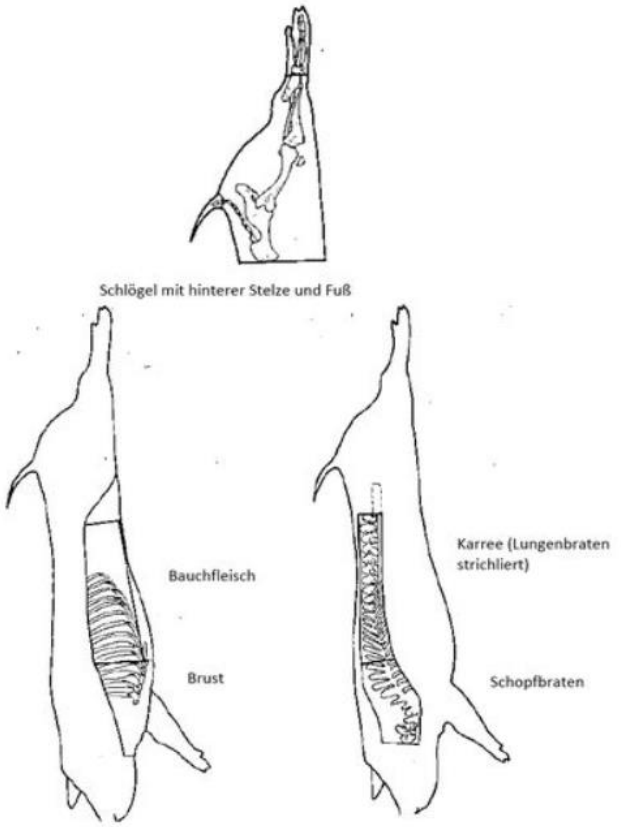


Figure 2: Cuts of pork (Österreichisches Lebensmittelbuch, 2025)

Schlögel mit hinterer Stelze und Fuß: Rear leg with shank and foot; Brust: Breast; Bauchfleisch: Belly meat; Karree: Loin; Schopfbraten: Pork collar

2.1.1 Properties of pork

Pork muscle tissue is composed of several chemical constituents. Water is the major component (about 71.7%), consisting predominantly of free water (≈95%) and a smaller amount of bound water (≈5%). Proteins account for approximately 18.6% and include mainly parapeptone (≈40 – 60%), myogen (≈20 – 30%), and stromal proteins (≈20%). Lipids represent around 5.6% of the tissue and are primarily triacylglycerols (TAG) and phospholipids with unsaturated and saturated fatty acids. Carbohydrates make up about 1.5%, while minerals, especially iron, zinc and selenium, contribute about 0.6%. About 0.01% represent the vitamins, especially Vitamin B_{12} and Vitamin E. The remaining fraction consist of other bioactive compounds, such as nucleotides, carnosine, and creatine (Yi et al., 2023). However, the composition of meat depends on multiple factors, such as species and origin, anatomical cut, and processing conditions (raw or cooked) (Vicente & Pereira, 2024).

Amino acids, a substantial portion of which are essential for human nutrition, represent a key constituent of muscle tissue, but also significantly contribute to taste and flavor of meat.

Pork contains essential amino acids like lysine, methionine, threonine, and tryptophan (Liao et al., 2025).

Pork fat is categorized in three main groups: intermuscular fat (between muscle groups), subcutaneous fat (the outer layer beneath skin), and intramuscular fat (IMF), which infiltrates muscle fiber, and strongly influences flavor, tenderness and juiciness. Additional specialized fat depots include leaf fat (around kidneys) and caul fat (the thin membrane surrounding organs) (Kim et al., 2021). Pork lipids consist mainly of triacylglycerides (TAG), which are used as the energy storage, phospholipids, which are important factors affecting pork flavor and a small amount of cholesterol. Triglycerides are divided in three principal classes of fatty acids: saturated fatty acids, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Excessive intake of saturated fatty acids has been associated with adverse health problems, including obesity, insulin resistance and an increased risk of cardiovascular disease (Yi et al., 2023). In contrast, particularly n-3 PUFA have been recognized for their beneficial effects on human health including regulation of energy metabolism and protection against cardiovascular diseases (Li et al., 2019).

As the source of high-quality protein and several other nutrients, meat has been consumed as a dietary staple for hundreds of years. However, evidence has shown, that consumption of red meat, including pork, is linked to a higher risk of different chronic diseases, especially cardiovascular disease and cancer (Vicente & Pereira, 2024). The International Agency for Research on Cancer (IARC) has classified the consumption of red meat (including pork) as probably carcinogenic to humans (Group 2A), mostly causing colorectal cancer (IARC, 2015.). Moreover, increased intake of red meat has been linked to heightened risk of cardiovascular disease, mostly because of high content of saturated fatty acids, which are known to increase circulating concentrations of low-density lipoprotein (LDL) cholesterol (López-Moreno et al., 2025).

As global population size continues to rise, demand for animal-sourced foods is expected to increase correspondingly, which will also increase the environmental impact. The result of that, are higher greenhouse gas emissions compared to plant-base foods production. Furthermore, meat production is resource intensive, requiring huge amounts of fresh water and foods, but also fossil fuels (Clare et al., 2022).

2.1.2 Pork Loin

The pork loin (longissimus dorsi) is defined as the back of pig (see **Figure 3**), more precisely from the area between the shoulder and the leg, including long back muscle (M. longissimus thoracis et lumborum), and its designation and processing are regulated when used in

special products or as a raw material. It is categorized as a pork cut (loin) and may be marketed either whole or portioned into chops with or without bones. Pork loin is a versatile and flavorful cut, mostly prepared by roasting or grilling (Österreichisches Lebensmittelbuch, 2025).

Pork loin is a lean cut, which contains about 72% water, around 23% Protein and 3-5% fat and is an excellent source of various dietary minerals including zinc, selenium, calcium, potassium, magnesium, sodium and iron. Pork loin is a good source of vitamin B, especially thiamin and niacin, but it also contains a moderate amount of cholesterol (around 70mg/100g) (Ham et al., 2021).

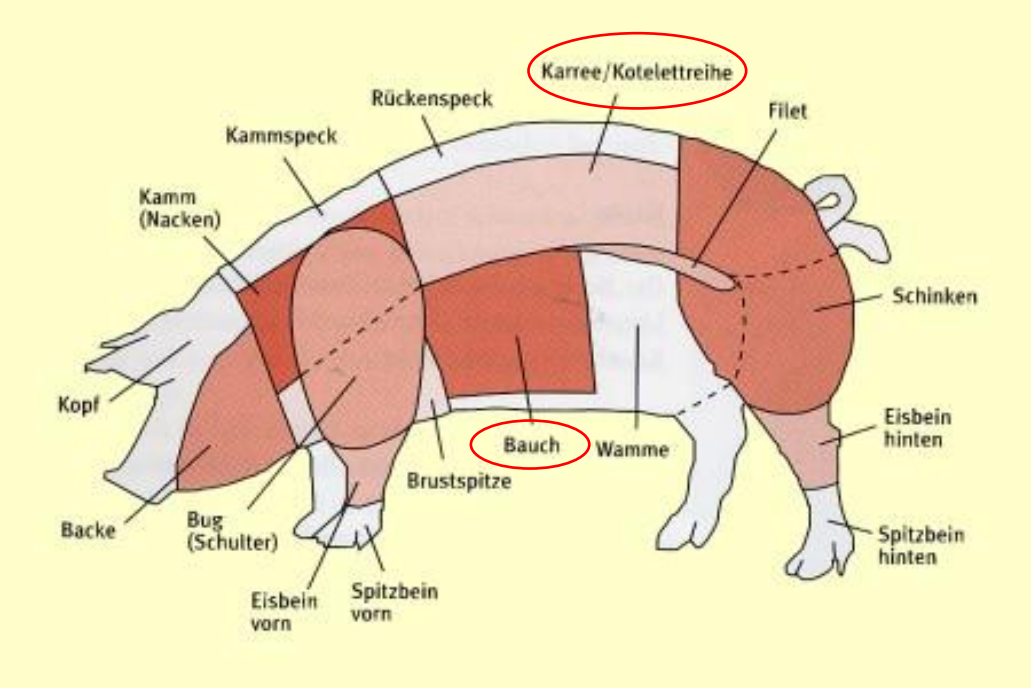


Figure 3: Cuts of pork including pork loin and pork belly (www.lehne.net)

The fatty acid composition of pork loin depends on several factors, including fatness, age, dietary fatty acid composition and the cut, but it is dominated by saturated fatty acids, such as palmitic and stearic acid, and monounsaturated fatty acids, especially oleic acid (Guo et al., 2019). Both fat deposition and fatty acid composition are strongly influenced by genetics and differ significantly among and within breeds (Kasprzyk et al., 2015).

2.1.3 Pork belly

Pork belly is the fatty underside of a pig (see **Figure 3**), consisting of the abdominal wall muscles, including the rectus abdominis and external abdominal oblique with substantial adipose tissue. The pork belly contains some component muscle, but mostly comprising

55% - 60% adipose tissue and this adipose tissue is divided into subcutaneous and intermuscular fat (Lee & Kim, 2023). However, the amount of adipose tissue is strongly influenced by genetics and varies significantly among different breeds. It is important to mention that western markets prefer higher – fat pork belly for processing, whereas South Koreans favor pork belly for roasted preparations (Kang et al., 2024).

Pork belly is rich in B vitamins, particularly thiamin and niacin, but also vitamin E and minerals like phosphorus, zinc and iron. Pork belly skin contains big amounts of collagen, which support skin structure. Due to its high fat content, pork belly is high in calories, saturated fatty acids and cholesterol (approx. 72mg/100g). This can easily increase LDL cholesterol levels, thereby raising the risk of cardiovascular disease. Therefore, pork belly should be eaten in moderation (Choe et al., 2015).

The fatty acid composition of pork belly includes a mix of saturated fatty acids (SFAs), including palmitic acid (C16:0) and stearic acid (C18:0), monounsaturated fatty acids (MUFAs), primarily oleic acid (C18:1), but also polyunsaturated fatty acids (PUFAs) like linoleic acid (C18:2) and linolenic acid (C18:3) (see **Figure 4**). The fatty acid profile varies with breed, diet and sex and oleic acid is generally the most abundant (Oliveira et al., 2015)

Fatty acid	Conventional	Free Range	Organic
Myristoleic acid	0.02 ^{a} ± 0.01	0.02 ^{a} ± 0.00	0.01 ^{b} ± 0.00
Pentadecylic acid	0.06 ^{a,b} ± 0.02	0.06 ^{a} ± 0.01	0.07 ^{b} ± 0.02
Palmitic acid	25.32 ^{a} ± 1.10	25.37 ^{a} ± 0.91	22.98 ^{b} ± 1.86
Palmitoleic acid	2.09 ^{a} ± 0.40	2.10 ^{a} ± 0.29	1.59 ^{b} ± 0.21
Palmitoleic acid	0.01 ^{a} ± 0.00	0.01 ^{a} ± 0.01	0.01 ^{a} ± 0.00
Margaric acid	0.32 ^{a} ± 0.07	0.30 ^{a} ± 0.09	0.40 ^{b} ± 0.11
Heptadecanoic acid	0.23 ^{a} ± 0.05	0.23 ^{a} ± 0.08	0.27^{a} ± 0.08
Stearic acid	13.86 ^{a,b} ± 2.38	14.23 ^{a} ± 1.17	12.78 ^{b} ± 1.44
Trans elaidic acid	0.23 ^{a} ± 0.05	0.22 ^{a,b} ± 0.06	0.18 ^{b} ± 0.05
Oleic acid	36.79 ^{a} ± 3.04	38.93 ^{b} ± 2.05	35.97 ^{a} ± 1.56
Vaccenic acid	2.55 ^{a} ± 0.28	2.63 ^{a} ± 0.32	2.28 ^{b} ± 0.17
Unknown 20.4 min	0.04 ^{a,b} ± 0.01	0.04 ^{a} ± 0.01	0.03 ^{b} ± 0.01
Linoleic acid	14.97 ^{a} ± 3.64	12.58 ^{b} ± 2.17	18.98 ^{c} ± 2.98
Unknown 21.2	0.03 ^{a} ± 0.01	0.04 ^{a} ± 0.01	0.04 ^{a} ± 0.01
Gamma-linolenic acid	0.01 ^{a,b} ± 0.00	0.01 ^{a} ± 0.00	0.01 ^{b} ± 0.00
Alpha-linolenic acid	1.44 ^{a} ± 0.40	1.15 ^{b} ± 0.25	2.02 ^{c} ± 0.34
Eicosenoic acid	0.71 ^{a} ± 0.11	0.83 ^{b} ± 0.11	0.73 ^{a} ± 0.08
Eicosadienoic acid	0.52 ^{a} ± 0.08	0.51 ^{b} ± 0.07	0.70 ^{c} ± 0.10
Unknown 23.3 min	0.03 ^{a,b} ± 0.01	0.03 ^{a} ± 0.01	0.02 ^{b} ± 0.01
Unknown 23.5 min	0.03 ^{a} ± 0.01	0.03 ^{a} ± 0.01	0.03 ^{a} ± 0.01
Dihomo-gamma-linolenic acid	0.09 ^{a} ± 0.02	0.09 ^{a} ± 0.01	0.11 ^{a} ± 0.02
Eicosatrienoic acid + arachidonic acid	0.41 ^{a} ± 0.08	0.37 ^{a} ± 0.06	0.5 ^{b} ± 0.08
Adrenic acid	0.10 ^{a} ± 0.02	0.10 ^{a} ± 0.02	0.11 ^{a} ± 0.03
Docosapentaenoic acid (osbond acid)	0.02 ^{a} ± 0.01	0.01 ^{b} ± 0.00	0.02 ^{a} ± 0.00
Docosapentaenoic acid (clupanodonic acid)	0.12 ^{a} ± 0.03	0.12 ^{a} ± 0.05	0.15 ^{b} ± 0.03
∑ Saturated	39.56 ^{a} ± 2.63	39.95 ^{a} ± 1.48	36.24 ^{b} ± 2.35
∑ Monounsaturated	42.63 ^{a} ± 3.10	44.97 ^{b} ± 2.10	41.03 ^{a} ± 1.58
∑ Polyunsaturated	17.68 ^{a} ± 3.67	14.95 ^{b} ± 2.19	22.60 ^{c} ± 2.98
∑ Unknown	0.13 ^{a} ± 0.02	0.14 ^{a} ± 0.02	0.13 ^{a} ± 0.01
∑ Omega-3	1.97 ^{a} ± 0.41	1.64 ^{b} ± 0.26	2.67 ^{c} ± 0.35

ValVue*Values followed by the same letters in the same row do not differ significantly (p value > 0.05); Values shown for individual fatty acids correspond to the average of 13 samples from the conventional category, 15 samples from the free range category and 13 samples from the organic category; The FAs eicosatrienoic acid and arachidonic acid are reported together because they co-eluted in the chromatographic procedure.

Figure 4: Fatty acid composition of pork belly meat from the categories conventional, free range, and organic expressed as percentage of normalized peak area (Oliveira et al., 2015)

2.1.4 Land race pigs

According to Köck et al., (2009) and The Austrian Pig Breeders Association one of the key traditional breeds in Austria is the land race pig. This breed has strong reproductivity performance, making the ideal for producing healthy litters. They combine good growth rates and meat quality, traits that meet both farmers and consumer expectations. Due to their suitability as breeding stock, land race pigs are frequently used in cross-breeding systems to improve hybrid performance and overall herd productivity. Genetic improvement is based on performance testing, pedigree recording, and breeding values estimation. The Austrian land race population is managed to balance genetic progress, animal health, and adaptability to local farming conditions. In Austria, additionally to land race pigs, Large White and Pietrain are also pedigree breeds used in modern pig breeding programs.

2.1.5 Turopolje-pigs

Originating in the Middle Ages in the Turopolje region of central Croatia, the Turopolje-pig is one of the oldest European fatty-type pig breeds. The breed is well adapted to continental climatic conditions and lowland forest ecosystems. These pigs are highly robust, cold- and disease-resistent, and well suited for outdoor systems. Despite its relatively low reproductive and growth performance, the Turopolje-pig is distinguished by its excellent meat quality traits (Škorput et al., 2024).

However, because of their favorable traits, traditional pig breeds may play an important role in future pig breading programs focused on meat quality. In this context, fatty acid composition is an important factor (see **Table 1**) (Dikić et al., 2002).

Table 1: Fatty acid composition in back fat according to (Dikić et al., 2002)

Fatty acid	Percent of fatty acid in back fat of Turopolje-pigs
C<12:0	0.12
C12:0	0.09
C14:0	1.20
C15:0	0.08
C15:1	0.07
C16:0	22.50
C16:1	2.60
C17:0	0.40
C17:1	0.30
C18:0	11.80

C18:1	46.55
C18:2	11.40
C18:3	0.78
C20:0	0.15
C20:1	0.87
C22:0	0.62
C22:1	0.32
C24:0	0.09
C24:1	0.06

2.2 Regulatory standards in Austrian farming and the use of feed supplements to impact meat quality

In Austria, pork husbandry on organic farms is regulated by EU Organic Regulations 2018/848. These regulations show the minimum requirements for pigs and breeding pigs for indoor and outdoor spaces. For the pigs, the outdoor and indoor spaces depend on the pig´s weight, from 35-50 kg the required indoor area is at least 0.8 m^2 and outdoor areas start at 0.6 m^2 per animal. For the bigger pigs and pigs with piglets the area increases. For pigs with piglets indoor space should be at least 7.5 m^2 and minimum outdoor space 2.5 m^2. The breeding boars required at least 10 m^2 barn space and 8.0 m^2 outdoor area.

On Austrian organic farms, animals must be fed with organic feed (at least 30%). The pig´s outdoor space must be provided with grass or silage. Piglets need to receive natural milk for at least 40 days. Additionally, stressless environment and slaughter should be provided.

Conventional meat (including pork), vegetables or milk in Austria have state-recognized quality mark for food products (AMA-Gütesiegel). This quality mark guarantees transparency regarding origin and high-quality standards. The products come only from Austria and due to strict controls, the production delivers high quality products.

In pork production only “pastus+ AMA-Gütesiegel“ marked food (grain, straw) are used and all the feed must meet the requirements set out in EU Regulations 2017/1017. Pregnant sows must receive enough feed with high crude fibre content (grass, hay or grass silage). Piglets should receive natural milk for at least 28 days. The transport of pigs must be planned and carried out in a way, that the duration of transport is as short as possible. Furthermore, within the AMA-Gütesiegel program, animal welfare is a fundamental requirement, so the health and well-being of the animals must be safeguarded (AMA-Produktionsbestimmungen, 2022)

2.3 Impact of feed supplements on meat quality

Numerous studies have shown that dietary supplementation in pigs has a positive influence on meat quality and nutritional value. As an example, vitamin E supplementation increases the level of α-tocopherol in muscle and fat tissues and has positive effects on electrical conductivity in fresh pork, which is a reliable indicator of meat quality, as it reflects ion mobility in muscle fluids and enables the detection of pale, soft and dry conditions. Vitamin E also improves significantly the antioxidative capacity of stored pork. Herbal extracts like oregano and lemon balm showed beneficial effect on meat color during storage (Bahelka et al., 2011).

Tognocchi et al., (2023) showed that feed including linseed supplementation in pig diets increased the level of n-3 polyunsaturated fatty acids (PUFAs) in the various meat cuts. These results provided a strong basis for development of pig-feeding strategies aimed at producing functional foods. The supplementation with high levels of n-3 PUFAs could in future enable that certain pork cuts meet the “rich in omega-3“ claim.

Linseed press cake, a by-product of linseed oil extraction, is characterized by a rich nutritional profile and a high content of bioactive constituents, particularly alpha-linolenic acid and lignans, which are associated with potential health benefits. Owing to its high protein content, linseed press cake represents a valuable supplement for both animal feed and human nutrition (Talwar et al., 2025). Linseed cake supplementation has shown a positive effect on the levels of several bioactive components in cow´s milk, including milk composition, fatty acid profile, and fat-soluble vitamins in the milk fat fraction (Puppel et al., 2023). Moreover, as a by-product of cold-pressed oil production, the use of linseed press cake is both economical and environmentally sustainable. It also enables the provision of essential nutrients in winter feeding systems comparable to those supplied by pasture grass during summer (Puppel et al., 2023). According to Zhang et al., (2025) dietary flaxseed meal supplementation improves meat tenderness and enhances the alpha-linolenic acid and docosahexaenoic acid contents, as well as catalase activity, in the muscle of Hu lambs.

2.4 Fatty acids in pork

This chapter describes the predominant fatty acids found in pork loin and pork belly.

2.4.1 Stearic acid

Stearic acid (C18:0), also known as octadecanoic acid (see **Figure 5**), is a common saturated fatty acid, which can be found in animal fats as well as in plant fats, including meat, fish, milk, cocoa butter and palm oil. In biosynthetic pathways, stearic acid is formed

through the elongation of palmitoyl-CoA (Shen et al., 2025). Studies have shown, unlike other saturated fatty acids, stearic acid does not increase plasma cholesterol levels and it can even reduce plasma low-density lipoprotein (LDL) levels. Due to these properties, stearic acid has minimal impact on major risk factors with cardiovascular diseases (Shen et al., 2025).

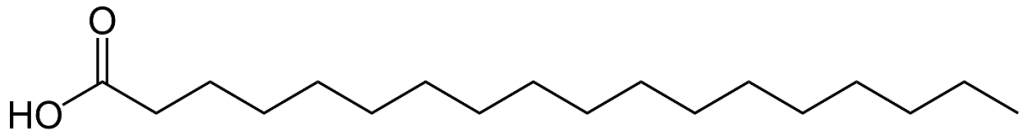


Figure 5: Molecular structure of stearic acid (C18:0)

2.4.2 Palmitic acid

Palmitic acid (C16:0) is the predominant saturated fatty acid in foods (see **Figure 6**). It can be found in high amounts in palm oil, but also in meat, cocoa butter and dairy products. Palmitic acid is mostly provided through dietary intake or produced endogenously from other fatty acids, select amino acids or carbohydrates. It is also an essential component of membrane, secretory and transport lipids, but also playing crucial role in protein palmitoylation and palmitoylated signaling molecules. Together with stearic acid is the most common fatty acid in pork meat. (Innis, 2016).

However, high intake of palmitic acid is often associated with adverse effect on chronic cardiovascular disease, but also, when factors like a sustained positive energy balance, high carbohydrate intake and physical inactivity are present, the regulatory mechanisms that maintain palmitic acid can become impaired. This can contribute to dyslipidemia or hyperglycemia (Carta et al., 2017). Palmitic acid plays a crucial role in fetus development and early infant years, as an essential component of cell membranes and transport lipids (Agostoni et al., 2016).

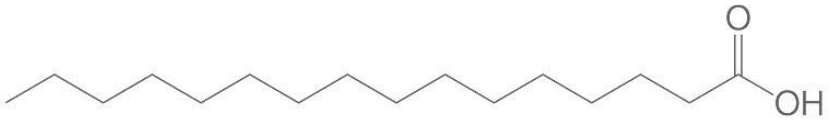


Figure 6: Molecular structure of palmitic acid (C16:0)

2.4.3 Oleic acid

Oleic acid (C18:1n-9) is an omega-9 fatty acid with double a bond at the C9-position (see **Figure 7**). It is monounsaturated and mostly can be found in vegetables oils like olive oil,

but also in animal fats. Oleic acid has positive impacts on various tissues in general, but also some studies showed negative impacts (Karacor & Cam, 2015).

Increased oleic acid levels in membranes contribute to the regulation of membrane lipid structure, which in turn modulates G-protein-mediated signaling. This mechanism has shown positive effects in reducing blood pressure (Terés et al., 2008).

Some papers have also shown that oleic acid and oleic acid-rich oil have reverse insulin inhibitory effects on inflammatory cytokine production (Vassiliou et al., 2009),

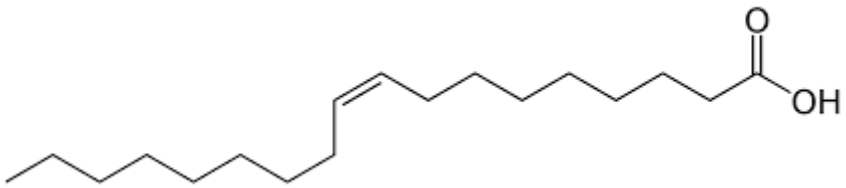


Figure 7: Molecular structure of oleic acid (C18:1)

2.4.4 Linoleic acid

Linoleic acid (18:2 cis-9,12) is an essential omega-6 polyunsaturated fatty acid (see **Figure 8**) that occurs in nuts, seeds and vegetable oils. It is a structural component of cell membranes, biologically active signaling molecules and serves as a direct precursor to bioactive oxidized linoleic acid metabolites. Linoleic acid metabolizes into arachidonic acid, but also pro-inflammatory eicosanoids and endocannabinoids (Choque et al., 2014)

The stability of linoleic acid and its conversion into derivates, such as conjugated linoleic acid (CLA), are of significant interest, as CLA is widely used as a dietary supplement. Conjugated linoleic acid has shown benefits in modulating inflammation, enhancing immune function and preventing cancer. However, the studies of the underlying mechanisms of action and dose-response remain limited (Y. Chen et al., 2025).

Linoleic acid cannot be synthesized, it must be obtained through food. The recommended daily intake for linoleic acid has not been established, but according to Whelan & Fritsche, (2013), the adequate reference intakes 17 g/day for men and 12 g/day for women have been acknowledged.

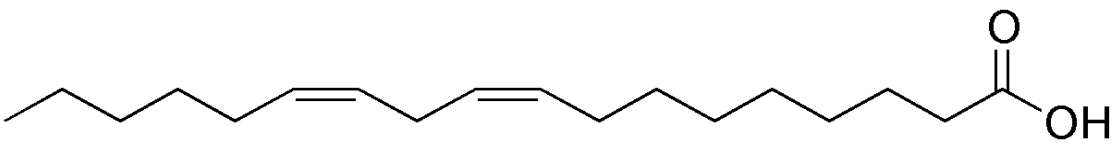


Figure 8: Molecular structure of linoleic acid (C18:2)

2.4.5 α-Linolenic acid

Alpha-linolenic acid (18:3), also known as all-cis-9,12,15-octadecatrienoic acid is an essential omega-3 fatty acid (see **Figure 9**). It is found in plant-based foods such as linseed, green leafy vegetables, and walnuts. Alpha-linolenic acid (ALA) is a metabolic precursor to other omega-3 fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). ALA supports brain function and helps modulate different inflammatory responses. It also protects the cardiovascular system by preventing arrhythmias and improving blood vessel function. ALA cannot be synthesized by the human body, so it needs to be provided through diet (Yuan et al., 2022).

However, because the conversion of ALA to EPA and DHA is limited, ALA shows less health benefits then stearidonic acid (SDA), which can more efficiently be converted to EPA. The conversion of them in the human body is limited (Baker et al., 2016).

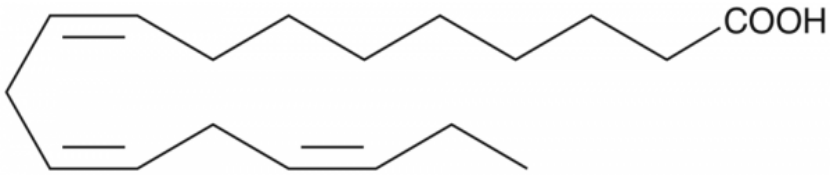


Figure 9: Molecular structure of α-linolenic acid (C18:3)

2.4.6 Arachidonic acid

Arachidonic acid (ARA, C20:4) is an essential omega-6 polyunsaturated fatty acid (see **Figure 10**). ARA plays a crucial role in building bioactive compounds such as prostaglandins, thromboxane and leukotrienes, but also it is an important part of cell membranes, where it helps maintain membrane structure (Martin et al., 2016).

Arachidonic acid and its metabolites have shown their importance in cardiovascular function, different inflammatory disorders, including asthma and arthritis, and possible cancer development. However, arachidonic acid is vital for human health, but only with moderate intake. An imbalanced diet that provides huge amounts of arachidonic acid without counterbalancing nutrients can have negative health effects (B. Wang et al., 2021).

Availability of arachidonic acid is limited and can only be found in different meats, animal products (milk, cheese and eggs) and fish. This can be a problem to various types of vegetarian diets and can lead to ARA deficiency. However, based on the literature, healthy adults are capable of synthesizing ARA from linoleic acid (Weder et al., 2025).

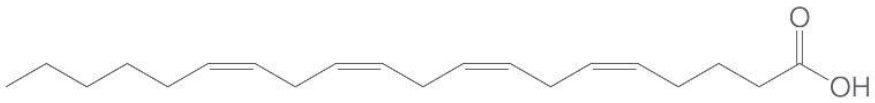


Figure 10: Molecular structure of arachidonic acid (C20:4)

2.4.7 Eicosapentaenoic acid

Eicosapentaenoic acid (EPA, 20:5) is an omega-3 polyunsaturated fatty acid (see **Figure 11**), that can be found in microalgae, fish, and shellfish. But due to environmental and sustainability issues related to wild-caught fish concerns are rising about potential quality and shortages of this essential nutrient (Gu et al., 2021).

Consumption of EPA has been linked to different health benefits such as support for neurological health, regulation of diabetes and protection against cardiovascular disease. These effects arise because EPA serves as a precursor in eicosanoid synthesis (Gu et al., 2021).

Additionally, EPA is a precursor for docosahexaenoic acid (DHA), which is essential for neurological and visual development in infants. However, humans can synthesize EPA and DHA from alpha-linoleic acid, but this process is limited due to lack of desaturase enzymes (Gu et al., 2021).

Pork meat naturally provides very low amounts of eicosapentaenoic acid. Including fish oil in pigs diets increases the amount of omega-3 fatty acids in the meat but also provides health benefits for the animal (Dang & Kim, 2022).

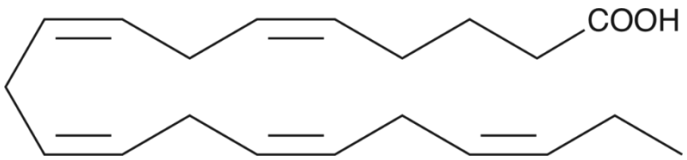


Figure 11: Molecular structure of eicosapentaenoic acid (C20:5)

2.5 Shelf life and oxidation

In the European Union, food shelf life is defined as the time in which food retains its essential characteristics, appearance, organoleptic quality and safety for the consumers. Mostly two different terms are used: “use by“ date, which is applied for foods, that can quickly become unsafe, such as fish and fresh meat, and the “best before“ date, which shows how long a food is expected to keep its nutritional value, texture and taste. However, if the foods have been stored properly and they show no signs of spoilage, the foods may still be safe to eat

even after the “best before“ date. This is all regulated through (EU) No 1169/2011 Regulation. The shelf life of foods varies depending on the product.

There are different factors, that can influence meat quality, but also its shelf life, which is particularly critical, because of the considerable amounts of food waste. Shelf life can be influenced during any stage of processing, packaging, transport and storage. All these factors can be classified in two groups: intrinsic, including physical and chemical damage (such as pH, microbial and water activity) and extrinsic, including storage and packaging conditions (Dittoe et al., 2025).

Meat contains components, such as fats, protein, carbohydrates and vitamins, that are prone to oxidative reactions, making them leading contributor to quality degradation and shortened shelf life in meat products (Kurek et al., 2019). The main factors influencing lipid oxidation in meat are fat content and fatty acid composition, as fatty acids serve as substrates for oxidation processes. Cholesterol, an essential component of animal tissues, is also susceptible to oxidation due to its chemical structure. The formation and concentration of oxidation products depends on multiple factors, including heat, pH, light, oxygen, oxidation time, water activity and the presence of unsaturated fatty acids. Additionally, metals, either as heme-proteins or in free form, are one of the major contributors to lipid oxidation in meat. This is because transition metals, such as iron and copper, act as potent catalysts in various stages of lipid oxidation. Enzymes, such as lipoxygenase plays a key role in the biosynthesis of certain long-chain fatty acids and can abstract hydrogen from the allylic methylene position of polysaturated acids leading to the formation of conjugated dienes (Domínguez et al., 2019). However, by using antioxidants, including tocopherols and polyphenolic compounds the oxidative reactions are actively slowed to preserve meat quality (Kurek et al., 2019).

2.5.1 Lipid oxidation

Lipids play a crucial role in human nutrition and they support numerous functional and regulatory processes in the human body, including development of human tissues. They also supply vital components such as fat-soluble vitamins and essential fatty acids. However, besides their importance, lipids are highly prone to oxidation. Lipid oxidation is a complex process involving multiple interacting mechanisms. It begins, when the unsaturated fatty acids react with molecular oxygen, leading to the formation of hydroperoxides. This reaction is also known as “autoxidation“ that progresses through a chain reaction involving initiation, propagation and termination phases (see **Figure 12**) (Domínguez et al., 2019). Autoxidation is initiated when molecular oxygen is present (triplet ground state) and the bonds of fatty acid are in the single state. Due to this spin restriction,

a direct reaction between fatty acid and oxygen cannot be carried out. For the reaction to proceed, oxygen must be changed to its active form. This is possible through two reactions: conversion to singlet oxygen or formation of reactive oxygen. After the activation, oxygen is able to abstract a hydrogen atom from a fatty acid and form an alkyl radical. The formed radical species react quickly with molecular oxygen forming an extremely reactive peroxy radical. After that, the peroxy radical is able to abstract a hydrogen atom from another fatty acid. This reaction generates a new alkyl radical, which is able to form a new hydroperoxide. This process continues, promoting ongoing chain lipid oxidation. However, it is important to mention that due to possible decomposition of hydroperoxides, and reaction between them, other highly reactive intermediates such as alkoxy, hydroxyl, and peroxy radicals can be formed (Shahidi & Zhong, 2010).

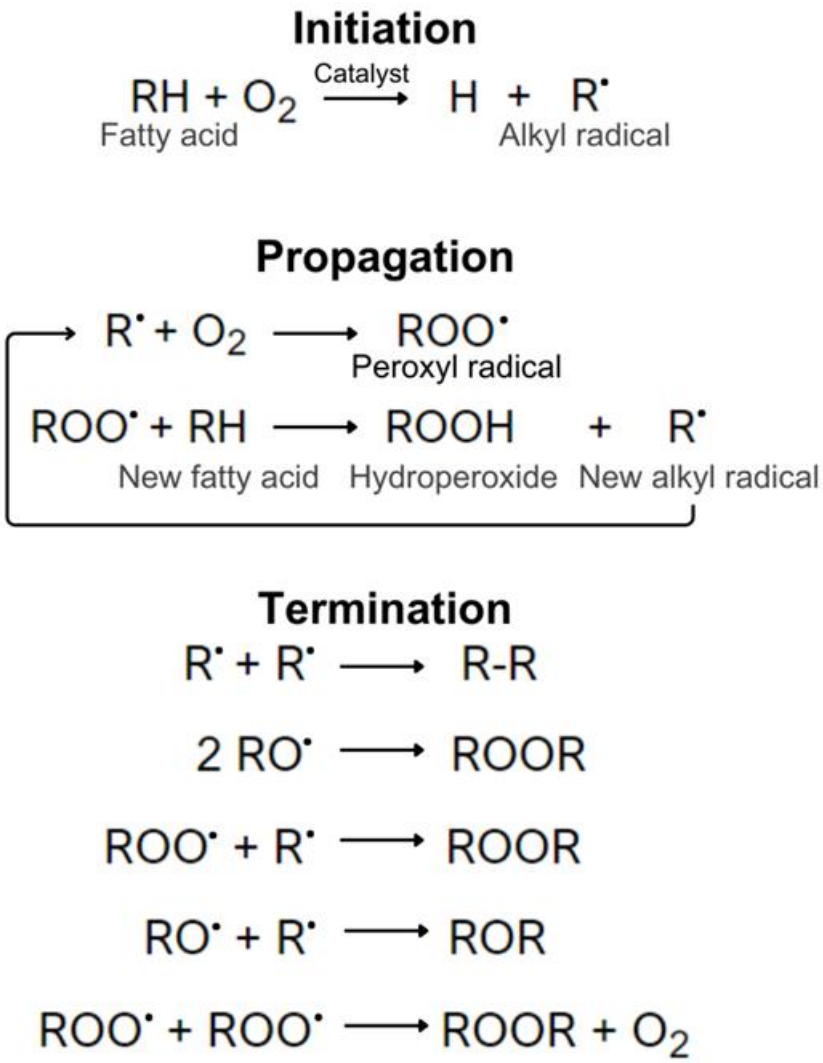


Figure 12: Mechanism of lipid autoxidation (Ganjeh et al., 2024)

Lipids are also significant part of meat and meat products, contributing to flavor, color, texture, juiciness and sensory qualities of a meat. Lipid oxidation can also lead to the loss of essential fatty acids and vitamins in meat. Oxidative reactions can also form a variety of toxic products, such as 4-hydroxy-2-nonenal (HNE) or malondialdehyde (MDA), which are associated with different human health conditions, including inflammation, atherosclerosis and cancer. The industry and research are focused to understand lipid oxidation mechanisms and develop effective strategies to control them (Huang & Ahn, 2019).

2.5.1.1 Primary and secondary lipid oxidation products

The primary lipid oxidation products are hydroperoxides and the quantification of their generation is one of the most used indicators of primary oxidation in meat. In the initial stages of oxidation, hydroperoxide products levels increase because their level of formation is higher than the rate of decomposition. Due to oxidation progress, rapid decomposition of hydroperoxides happens, resulting in a decrease in peroxide value. However, a result of low peroxide value could correspond to either early or advanced levels of oxidation. To obtain a more accurate understanding of oxidation in the sample, it is recommended to monitor changes in peroxide value over time, but it is also useful the measure the oxidative stage at a specific time point during the meat's shelf life (Domínguez et al., 2019). For the detection of primary oxidation products and the quantification of the hydroperoxides in meat different method can be used. All methods are based on oxidative capacity to determine peroxide value. Frequently used techniques include iodometric titration, diene conjugation, and iron oxidation. In iron oxidation methods, where iron(II) ions are oxidized to iron (III) ions by hydroperoxides in the sample. Due to a color reaction, the absorbance and its correlation with the amount of iron (III) ions, can be measured spectrophotometrically leading to the determination of hydroperoxide concentration in foods, especially in meat. However, for this method the lipids from meat must be extracted. The peroxide value can also provide important information about possible further lipid oxidation, when the meat is under influence of other factors like catalysts or additional process, and techniques for measuring secondary oxidation products can be used (Abeyrathne et al., 2021).

The secondary lipid oxidation products such as oxoacids, alcohols, ketonic and aldehydic compounds show a wide range of properties, including volatility, molecular weight and polarity. These substances are produced due to further oxidation of primary oxidation products, but they are more stable and can be responsible for generating unpleasant taste and smell in the meat (Barriuso et al., 2013). Through oxidation of different fatty acids in meat various compounds can develop, including aldehydes such as hexanal and nonanal or alcohols like hexanol and pentanol. Oxidation of alpha-linolenic acid in meat, especially

when enriched through dietary sources, leads to formation of a variety of volatile and non-volatile compounds, including alcohols such as 1-octen-3-ol, 1-penten-3-ol and aldehydes such as pentanal, hexanal und heptanal. Oxidation of linoleic acid in meat primarily generates volatile aldehydes such as hexanal and 2,4-decadienal, which are major contributors to off-flavors (Shahidi & Oh, 2020). One of the most used methods to separate, identify, and quantify secondary oxidation products is gas chromatography with mass spectrometry (GC – MS). This method is able to identify a wide range of volatile substances in meat sample, without long and demanding sample preparation, and it is also suitable for the targeted analysis of specific substances. For the extraction of volatile compounds headspace extraction can be used to eliminate the solvent-delivered peaks, including solid-phase microextraction (SPME), which shows high sensitivity and simplicity (Domínguez et al., 2019).

2.5.2 Protein oxidation

According to Günal-Köroğlu et al., (2025) lipid and protein oxidation play a crucial role in determining the nutritional quality and shelf life of meat. Although lipid oxidation has been widely investigated, the meat proteins have been shown to also be vulnerable to oxidative damage. However, the exact mechanisms between oxygen, amino acids and proteins within the food matrix are not yet fully understood. Amino acids and proteins are influenced by multiple factors, including temperature, water activity, pH, the presence of reactive species (RS•, HO•, ROO•) and the presence of nitrogen species (•NO and $•NO_2$). Primary and secondary lipid oxidation products like hydroperoxides and aldehydes also contribute to protein oxidation. Protein oxidation in meat modifies protein functionality, induces textural alterations, contributes to off-flavor development and reduces overall nutritional value (Alhasawi et al., 2019).

Within food matrices, proteins can undergo oxidation through interactions with phagocytic cells, mitochondria or can be attacked by external reactive oxygen species during processing or storage of meat. For the protein oxidation to occur, the presence of reactive oxygen species (ROS) including hydroxyl radicals, superoxide anions or hydroperoxyl radicals is important, because molecular oxygen in its triplet ground state is relatively unreactive towards proteins and amino acids. Oxidative modifications occur predominantly on amino acid side chains. This includes different chemical reactions including thiol oxidation, hydroxylation, and the formation of carbonyl groups. UV radiation, metal-catalyzed reactions with Fe^{2+} and enzyme-catalyzed reactions including enzymes like oxidase and lactoperoxidase contribute to protein oxidation. Additionally, amino acids, that

are rich in sulfur such as cysteine and methionine, are easily targeted by ROS and can be oxidized forming cysteine disulfide or methionine sulfone (Domínguez et al., 2021).

2.5.2.1 Carbonyl proteins and Schiff bases/Carbonyl-amin adducts

Carbonyl protein formation is widely used as a measurement for protein oxidation and also a general marker of oxidative protein damage. Carbonyl proteins can be generated through four different pathways: fragmentation of the peptide, binding of non-protein carbonyl substances, uninterrupted oxidation of amino acid side chain and reactions with reducing sugars to form Schiff base products. One of the most commonly used methods to test carbonyl proteins is the 2,4-dinitrophenylhydrazine (DNPH) assay. Due to reaction between DNPH and protein carbonyl groups under acidic conditions, hydrazones are formed. Using spectrophotometric measurement at 370 nm hydrazones can be quantified. Primary protein carbonyl products such as alpha-aminoadipic semialdehyde and gamma-glutamic semialdehyde cannot be identified using DNPH due to limitations of the method. In this case HPLC – MS or LC – ESI – MS techniques are the right choice (Günal-Köroğlu et al., 2025).

The reaction of aldehydic products generated during lipid oxidation with amino groups on protein side chains can lead to formation of Schiff bases. Progressive conjugation or crosslinking of Schiff bases and their by-products may result in protein denaturation or polymerization and contribute to off-flavors in meat and meat products. Schiff bases have a characteristic fluorescence, so their quantification can be easily carried out using spectrophotometry at 460 nm (Chelh et al., 2007a).

2.6 Antioxidants

Antioxidants are defined, as the chemical compounds, that are able to significantly delay or inhibit substrate oxidation. They have an ability to neutralize free radicals, break them down, and quench singlet oxygen, but also can work as a synergist with other substances. Antioxidants can naturally occur in foods, or they can be synthetically produced and added to preserve nutritional value of products. However, antioxidants are also very important for the human body, because they protect the body from cell damage (Shahidi, 2000).

The most used antioxidants in food production are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), citric acid, ascorbic acid, tocopherols including alpha-tocopherol, and different phenolic and polyphenolic compounds. Although, spices such as rosemary, black paper and oregano show antioxidant activity and they are commonly used to improve flavor of food products (Shahidi, 2000).

2.6.1 Tocopherols

The molecular structure of tocopherols includes a long carbon side and a chromanol ring. Due to different amount and different position of methyl groups on the ring tocopherols can be produced in various forms known as α-, β-, γ- and δ-tocopherols. Tocopherols are found in various plants, nuts and seeds and due to their oxidative activity often used in food production and human diet (Delgado et al., 2020). Tocopherols, especially alpha-tocopherol are important for human health due to their oxidative characteristics, involvement in anti-inflammatory processes and role in immune activity (Saldeen & Saldeen, 2005).

Alpha-tocopherol is defined as the main form of vitamin E (see **Figure 13**) due to its high level of biological activity. However, the bioavailability of alpha-tocopherol is affected by various factors such as age, gender, diet and genetics (Shahidi, 2000). Vitamin E can be found in animal tissues, where it is primarily stored, but it can only be consumed through diet, because animals cannot synthesize it (M. Chen et al., 2025).

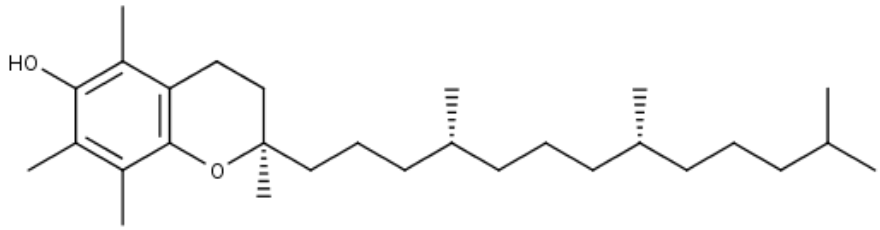


Figure 13: Molecular structure of α-tocopherol

Vitamin E is one of the main fat-soluble antioxidants due to its function as a chain-breaking antioxidant by donating hydrogen atom to peroxyl radicals, changing them into hydroperoxides. The hydroperoxide molecules can continue the oxidative process and produce secondary oxidation products. Alpha-tocopherol regulates protein expression linked to atherogenesis, but also modulates the arachidonic pathway and due to all its properties is essential for meat quality (Traber & Atkinson, 2007).

Additionally, according to Traber & Atkinson (2007) the accumulation of vitamin E in fat and muscle depends on supplement duration and dosage, because of this, different meat cuts can show various levels of vitamin E in their tissues. Furthermore, animals, such as pigs and cows, that consume green pasture for at least 8 weeks have shown an increase of alpha-tocopherols levels in muscle tissues. Vitamin E promotes healthier animal breeding and better nutritional quality of meat and meat products (Traber & Atkinson, 2007).

Gamma-tocopherol exhibits distinct chemical and biological properties that make it particularly effective in specific physiological contexts. It has a strong capability to neutralize reactive nitrogen species, especially peroxynitrite, which is less pronounced in other

tocopherol forms. Gamma-tocopherol exhibits lower antioxidant activity than alpha-tocopherol, as the higher degree of methyl substitution in alpha-tocopherol stabilizes the tocopheroxyl radical and promotes faster reaction with lipid peroxyl radicals (Es-Sai et al., 2025). However, gamma-tocopherol can show higher antioxidative efficacy in lipid systems, since its radical intermediates more readily undergo termination reactions such as dimer formation, thereby limiting chain propagation and reducing potential pro-oxidative effects associated with alpha-tocopherol radicals (Kamal-Eldin & Appelqvist, 1996). Despite many encouraging functions of γ-tocopherol, the complexity of its underlying mechanisms and its interaction with other antioxidants, requires further research (Es-Sai et al., 2025).

2.6.2 Phenolic compounds

Polyphenols are defined as phytochemicals and a diverse class of plant secondary metabolite. They can be classified into four main subgroups: flavonoids, phenolic acids, stilbenes and lignans (see **Figure 14**). The structure of polyphenols are characterized by one or more phenolic parts and the polyphenols mostly occur in their conjugated forms with sugar residues (Ciupei et al., 2024).

Polyphenolic substances can be found in vegetables, fruits, seeds but also in products like tea, beer and wine and thus are an important ingredient in human diet. They play a crucial role for plants, as an antioxidant, contributing to plant pigmentation, and also supporting flavor and color building. They have an ability to scavenge free radicals and to bind metal ions, which is very important in plants, enhancing nutrient uptake by metal-chelating, which helps to reduce Fenton reactions (Bertelli et al., 2021). The polyphenol intake has also shown various benefits for human health such as anti-inflammatory effects, benefits against diabetes, cardiovascular diseases and cancer (Durazzo et al., 2019).

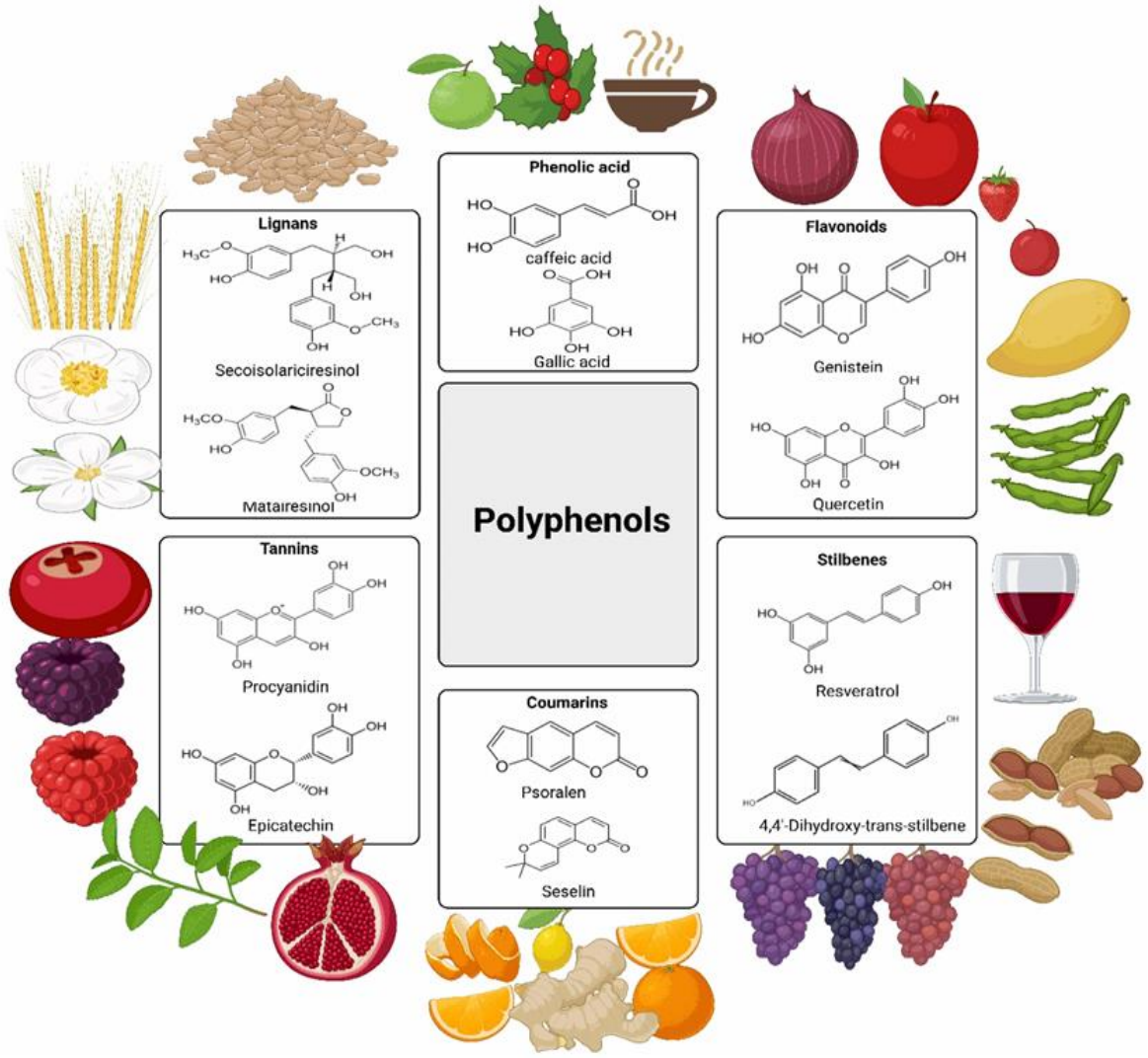


Figure 14: Polyphenols divided in their main subgroups with structures and illustration of the food where they occur (Saad et al., 2025)

Recent research has shown that polyphenols have positive properties in meat and meat production, that can be very important for the meat industry and consumers. Polyphenols act as reducing agents for metmyoglobin. Myoglobin in meat contains Fe(II) that is responsible for meat color. Due to oxidation of Fe(II) to Fe(III), the metmyoglobin formation will occur with brownish-red color. This discoloration is often associated with meat spoilage (Papuc et al., 2017).

Additionally, according to Papuc et al., (2017) polyphenols in meat have shown antimicrobial effects. Use of tannic acid, rosemary ethanolic extract and green tea extract lowered the total viable count of various foodborne bacteria, due to their interaction with structural components of bacteria. As an effective antioxidant and antimicrobial substances, polyphenols are suitable for extending the shelf life of meat and their products.

In the study from Liu et al., (2021) pigs were fed with a polyphenol-rich diet. They showed that polyphenols could be an important feed additive for enhancing growth performance,

antioxidant enzyme activity such as glutathion peroxidase and meat quality in pork. Various phenolic compounds are found in pasture feed such as kaempferol, coumaric acid, caffeic acid and resveratrol that have important properties for animal husbandry (Amrit et al., 2023).

2.6.2.1 Chalcones

Chalcones are major plant secondary metabolites from the flavonoid group and can be found in various edible plants. Most naturally occurring chalcones are polyhydroxilated aromatic compounds (see **Figure 15**) and are considered as the bio precursors for diverse flavonoids. Due to presence of phenolic groups, chalcones exhibit strong radical quenching properties (Rudrapal et al., 2021).

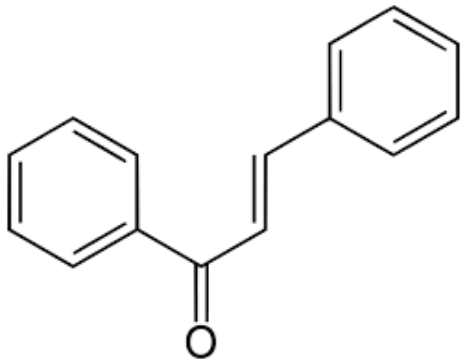


Figure 15: Chemical structure of chalcone

Chalcones, particularly licochalcone A, exhibit antibacterial activity against methicilin-resistant *Straphylococcus aureus (MRSA)* on fresh pork. It can effectively inhibit the growth of MRSA on pork, highlighting their potential to enhance food safety (Zeng et al., 2024).

2.6.2.2 Astragalin

Astragalin is a kaempferol O-glucoside (see **Figure 16**) and belongs to the group of natural flavonoids. It can be found in grapes, wine, green tea and elderflowers. Astragalin is widely recognized for its pharmacological activities, including antioxidant, anti-inflammatory and anticancer effects (Riaz et al., 2018).

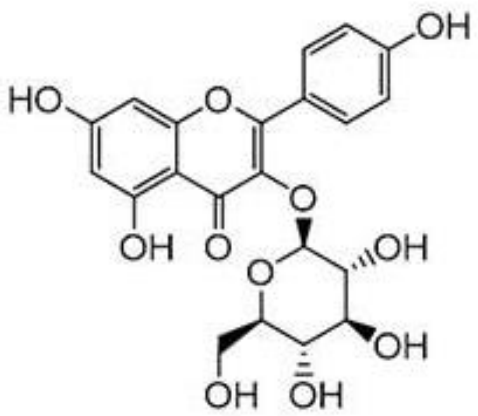


Figure 16: Chemical structure of astragalin

Various plant-based chemical compounds, that are isolated from roots, leaves or seeds contain astragalin. These compounds have shown bioactive potential as the natural antioxidants in meat and meat products, improving shelf life and meat quality (Aminzare et al., 2019).

2.6.2.3 Herbacetin

Herbacetin is a natural flavonoid (see **Figure 17**), which occurs in various plants and seeds such as rose root and flaxseed. Studies describe its antioxidant, antiviral and anticancer properties. It suppresses the expression of various inflammatory markers, protein oxidation and reduces cancer cell viability (Xiao et al., 2012).

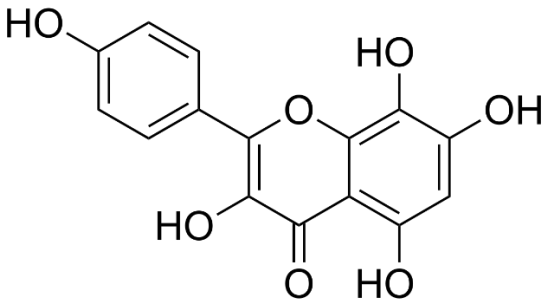


Figure 17: Chemical structure of herbacetin

Flaxseed and byproducts contain high amount of n-3 fatty acids and various polyphenols including herbacetin with important antioxidant activity. These properties enhance the functionality of foods for humans and animals (Panaite et al., 2017).

2.7 Goals of this thesis

The main goal of this thesis was divided into two parts. The first aim was to investigate how the effect of linseed press cake feed in Turopolje-pigs influences the fatty acid profile, protein and lipid oxidation and antioxidant content (tocopherols and polyphenols) in comparison to Turopolje-pigs without linseed press cake supplementation. Due to the existing research gap and the limited information available on the supplementation of pigs with linseed press cake for the enrichment of meat with omega-3 fatty acids and antioxidants, further investigation is required to evaluate its effects on meat quality and the nutritional composition of pork. Such feeding strategies may have a positive impact on agriculture by promoting the utilization of by-products from linseed oil production, enhancing the production of healthier and more oxidatively stable meat, and contributing to more sustainable livestock production systems. In the second part, the main focus was the effect of pig breed on the fatty acid profile, protein and lipid oxidation and antioxidant content (tocopherols and polyphenols) between Turopolje-pigs and landrace pigs, both under conventional conditions. Particular emphasis was placed on the influence of Turopolje-pigs on the oxidative status of the meat, as well as on how such strategies could positively impact agriculture by promoting more sustainable production systems, enhancing biodiversity, and supporting the use of stress- and disease-resistant pig breeds.

To make all these measurements possible, the meat samples were processed into a paste and the fat was extracted. Using the IDF method and GC-MS primary and secondary lipid oxidations products were detected and quantified. Protein oxidation products were examined by the DNPH assay and Schiff bases by fluorescence quantification. For the fatty acid profiles of the meat samples GC-FID was used. Additionally, for the tocopherols content HPLC-UV was applied. Lastly, targeted LC-MS was utilized for the analysis and quantification of polyphenols in all meat samples.

3 Methods and materials

3.1 Samples

In this master's thesis, four different groups of pig meat were tested. In first part of the master's thesis, the focus was on, the effect of linseed press cake feeding between two groups of Turopolje-pigs. The first group consisted of meat from Turopolje-pigs fed additionally with linseed press cake (LPC) and the other group was the meat from Tuopolje-pigs fed with normal feed (NF). Normal feed includes grain feed with 15 % consisting of grass and clover silage, LPC consisted of the normal feed with 10% of the feed replaced by linseed press cake. Both of these groups were raised, fed and slaughtered under organic and stressless farming conditions.

In the other part of the master's thesis the focus was on the effect of pig breed, where the first group was the meat from Turopolje-pigs (TP) and the other group was the meat from landrace pigs from supermarket (LR). The feed included only grain feed without grass or clover silage. Both of these groups were raised, fed and slaughtered in conventional conditions. These conditions fulfill the minimum requirements set by Austrian AMA-Gütesiegel.

In all four groups two different meat cuts were analyzed: loin (Longissimus dorsi) and the belly meat (Ventris caro). The loin of the pig is in the back. The long loin extends from the shoulder to the last ribs, where the short loin begins. Pork belly refers to the well-marbled and ribbed pieces of the rear and lower rib cage of pigs. There were eight samples per group and per cut, except for the NF-group, where the number of samples per cut were three to four. A total amount of fifty-two samples were analyzed.

The groups TP, LPC and NF were the groups of the Turopolje-pigs from local Austrian farms, which is the typical race from the region of the same name in Croatia. The meat was delivered vacuum-packed upon delivery and stored like this for three days at 4 °C until sample preparation.

The group LR represents the conventional landrace pig. The meat for this group was bought from a local supermarket. It was always freshly cut from large pieces, received non-vacuum packed, and stored for three days at 4 °C until processing.

3.2 List of chemicals, devices, software and consumables

Table 2: Chemicals for fat extraction

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
Methanol	CH_4O	≥ 99.9 %	VWR Chemicals	Danger	
Chloroform	$CHCl_3$	≥ 99 %	Carl Roth	Danger	
Sodium sulphate	$(Na)_2SO_4$	≥ 99 %	Sigma-Aldrich		
Nitrogen, compressed	N_2	99.8 %	Linde	Warning	
Argon, compressed	Ar	≥ 99.999 %	Linde	Warning	

Table 3: Chemicals for determination of primary lipid oxidation content

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
Methanol	CH_4O	≥ 99.9%	VWR Chemicals	Danger	
Chloroform	$CHCl_3$	≥ 99 %	Carl Roth	Danger	
Iron-(II)-sulphate heptahydrate	$FeSO_4 * 7 H_2O$	≥ 99 %	Sigma-Aldrich	Warning	
Bariumchloride dihydrate	$BaCl_2$	≥ 99 %	Carl Roth	Danger	
Hydrochloric acid	HCl	37 %	Carl Roth	Danger	

Iron-(III)-chloride	$FeCl_3$	≥ 99.5 %	Carl Roth	Danger	
Hydrogen peroxide	H_2O_2	30 %	VWR Chemicals	Danger	
Ammoniumthiocyanate	CH_4N_2S	≥ 99 %	Sigma-Aldrich	Danger	

Table 4: Chemicals for determination of secondary lipid oxidation

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
Sodium chloride	NaCl	≥ 99.5 %	Carl Roth		
Hexanal, 4 mg/mL	$C_6H_{12}O$	98 %	Sigma-Aldrich	Warning	
Nitrogen, compressed	N_2	99.8 %	Linde	Warning	

Table 5: Chemicals for determination of the fatty acid profile and content

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
Heptadecanoic acid methyl ester	$C_{18}H_{36}O_2$	≥ 99 %	Sigma-Aldrich		
Methanol	CH_4O	≥ 99.9 %	VWR Chemicals	Danger	
Sodium methoxide solution, 0.5 M in MeOH	CH_3ONa	0.5 M	Sigma-Aldrich	Danger	

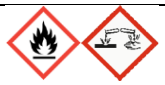
Sodium sulphate	$(Na)_2SO_4$	≥ 99 %	Sigma-Aldrich		
Toluol	$C_6H_5CH_3$	≥ 99.7 %	Fluka Chemika	Danger	
Pyrogallol	$C_6H_6O_3$	≥ 98 %	Sigma-Aldrich	Warning	
Glacial acetic acid	$C_2H_4O_2$	≥ 99 %	Sigma-Aldrich	Danger	
n-Hexane	C_6H_{14}	≥ 99 %	Carl Roth	Danger	
Synthetic air, compressed	N_2 and O_2	80 % and 20 %	Linde	Warning	
Helium, compressed	He	≥ 99.999 %	Linde	Warning	
Nitrogen, compressed	N_2	99.8 %	Linde	Warning	
Methyl palmitate	$C_{17}H_{34}O_2$	≥ 97 %	Sigma-Aldrich		
Methyl stearate	$C_{19}H_{38}O_2$	99 %	Sigma-Aldrich		
Methyl oleate	$C_{19}H_{36}O_2$	99 %	Sigma-Aldrich		
Methyl linoleate	$C_{19}H_{34}O_2$	≥ 98 %	Sigma-Aldrich		
Methyl linolenate	$C_{19}H_{32}O_2$	≥ 99 %	Sigma-Aldrich		
Methyl arachidonic acid	$C_{20}H_{32}O_2$	≥ 99 %	Sigma-Aldrich		
Eicosapentaenoic acid	$C_{20}H_{30}O_2$	≥ 99 %	Sigma-Aldrich		

Table 6: Chemicals for determination of carbonyl protein content

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
Potassium chloride	KCl	≥ 99.5 %	Carl Roth		
Sodium pyrophosphate tetrabasic decahydrate	$Na_4P_2O_7$	≥ 99.9 %	Sigma-Aldrich	Danger	
Tris-Maleate	$C_4H_{11}NO_3$ * $C_4H_4O_4$	≥ 99.5 %	Sigma-Aldrich		
Magnesium chloride	$MgCl_2$	≥ 99 %	Carl Roth		
Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid	$C_{14}H_{24}N_2O_{10}$	≥ 97 %	Sigma-Aldrich		
Trichloroacetic acid	CCl_3COOH	≥ 99 %	Carl Roth	Danger	
2,4-Dinitrophenylhydrazine, moistened with 33 % of H_2O for synthesis	$(NO_2)_2C_6H_3NHNH_2$	99 %	VWR Chemicals	Danger	
Hydrochloric acid	HCl	37 %	Carl Roth	Danger	
Ethanol	C_2H_6O	≥ 99.9 %	VWR Chemicals	Danger	
Ethyl acetate	$C_4H_8O_2$	≥ 99.8 %	Carl Roth	Danger	
Guanidine hydrochloride	CH_5N_3 * HCl	≥ 98 %	Sigma-Aldrich	Warning	
Sodium phosphate monobasic	H_2NaPO_4	≥ 98 %	Sigma-Aldrich		
Nitrogen, compressed	N_2	99.8 %	Linde	Warning	

Table 7: Chemicals for determination of Schiff base/carbonyl-amin adduct content

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
Sodium phosphate monobasic	H_2NaPO_4	100 %	Sigma-Aldrich		
Coomassie Brilliant Blue G-250	$C_{47}H_{48}N_3O_7S_2Na$	100 %	Carl Roth		
Ethanol	C_2H_6O	≥ 99.9 %	VWR Chemicals	Danger	
Phosphoric acid	H_3PO_4	≥ 90 %	Sigma-Aldrich	Danger	
Bovine serum albumin (BSA) standard		≥ 99 %	Thermos scientific		

Table 8: Chemicals for determination of tocopherol content

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
2-propanol	C_3H_8O	100 %	Carl Roth		
Tocol standard	$C_{26}H_{44}O_2$	≥ 95 %	Sigma-Aldrich		
Alpha-tocopherol	$C_{29}H_{50}O_2$	≤ 100 %	Sigma-Aldrich	Warning	
Gamma-tocopherol	$C_{28}H_{48}O_2$	≥ 96 %	Sigma-Aldrich		
Delta-tocopherol	$C_{27}H_{46}O_2$	≥ 90 %	Sigma-Aldrich		
Methanol	CH_4O	≥ 99.9 %	VWR Chemicals	Danger	

Table 9: Chemicals for determination of polyphenolic content

Chemical	Chemical Formula	Purity	Supplier	Signal Word	GHS Hazard Pictogram
Caffeic acid	$C_9H_8O_4$	100 %	Sigma-Aldrich		
Secoisolariciresinol diglucoside	$C_{32}H_{46}O_{16}$	≥ 97 %	Sigma-Aldrich		
Epicatechin	$C_{15}H_{14}O_6$	≥ 90 %	Sigma-Aldrich	Warning	
Kaempferol	$C_{15}H_{10}O_6$	100 %	Sigma-Aldrich	Warning	
Methanol	CH_4O	≥ 99.9 %	VWR Chemicals	Danger	
Butylated hydroxytoluene	$C_{15}H_{24}O$	≥ 90 %	Carl Roth	Warning	
Acetonitrile	C_2H_3N	100 %	VWR Chemicals	Danger	

Table 10: Devices, consumables and tools

Name of Device, consumable or tool	Supplier
Vacuum Pump V-700	Büchi Labortechnik AG
Vacuum Controller V-850	Büchi Labortechnik AG
Vortex	Starlab GmbH
Rotavapor R-210	Büchi Labortechnik AG
Heating Bath B-300 Base	Büchi Labortechnik AG
Mixer B-400	Büchi Labortechnik AG
Concentrator plus	Eppendorf SE

Cheese cloth	DuEco
Pipette tips (10; 200; 1250 and 5000 µL)	Starlab GmbH
Piston pipettes (10; 100; 200; 1000 and 5000 µL)	Eppendorf SE
Multichannel piston pipette (100 µL)	Eppendorf SE
Centrifuge 5418 R	Eppendorf SE
Centrifuge 5810 R	Eppendorf SE
Tecan Spark	Tecan Trading AG
UV/VIS Spectrometer Lambda 35	PerkinElmer
Tubes (15 and 50 mL)	Sarstedt AG & Co. KG
FlexStation 3	Molecular Devices, LLC
Rotizell® Tissue	Carl Roth GmbH + Co. KG
Rotilabo® -precision quartz glass cuvette (0.7 mL)	Carl Roth GmbH + Co. KG
Beaker (100; 250; 500 and 1000 mL)	DWK Life Sciences
Round bottom flask (50 mL)	DWK Life Sciences
Microplate Shaker	VWR International, LLC
Balance (Pioneer)	Ohaus Corp.
Analytical balance (Pioneer)	Ohaus Corp.
Analytical balance	Sarorius Lab Instruments GmbH & Co. KG

Mixer (model: 3531; 230 VAC; 50 Hz; 400 W; KB: 30 s)	Gourmetmaxx
Bemis parafilm	Fisher Scientific GmbH
Magnetic stirrer with heating plate VMS – C7	VWR International, LLC
pH meter 1000 L	VWR International, LLC
Duran bottles (500 and 1000 mL)	DWK Life Sciences
Volumetric flask (100 mL)	DWK Life Sciences
Fume hood	Thulab Laborservice GmbH
Ultrasonic cleaner and water bath	VWR International, LLC
Arium® Pro Ultrapure Lab Water Systems (sartorius)	VWR International, LLC
Rotilabo® safety reaction vials (2.0 mL; PP; colorless)	Carl Roth GmbH + Co. KG
Whatman Nr. 1 filter paper (diam.: 125 mm; circles)	Merck KGaA
Energy Star freezer	VWR International, LLC
Injekt® syringe (2 mL)	B. Braun Melsungen AG
Rotilabo® -syringe filters, PVDF, unster. (pore size: 0,20 µm; diam.: 15 mm)	Carl Roth GmbH + Co. KG
Gas chromatograph GC-2010 Plus	Shimadzu Corp.
Minichiller-NR	Peter Huber Kältemaschinenbau GmbH
Autosampler AOC-20s	Shimadzu Corp.
Autoinjector AOC-20i	Shimadzu Corp.

Gas chromatograph mass spectrometer GCMS-QP2010 Ultra	Shimadzu Corp.
Autosampler AOC-5000 Plus	Shimadzu Corp.
Micro-inserts, borosilicate glass (clear glass; conical 15 mm tip, 0.1 mL)	Carl Roth GmbH + Co. KG
Micro-insert for vials with small opening (0.2 mL; clear glass; 31 x 5 mm; flat bottom)	VWR International, LLC
Autosampler vials for HPLC and GC	Agilent Technologies, Inc.
Rotilabo® -aluminium caps with septum (PTFE/silicone rubber; diam.: 11 mm)	Carl Roth GmbH + Co. KG
Septa (diam.: 8 mm; thickn. 1.3 mm; dur. 45°; silicone white/PTFE red; ND8)	Carl Roth GmbH + Co. KG
Liquid chromatograph mass spectrometer LCMS-8040	Shimadzu Corp.
Column oven CTO-20AC	Shimadzu Corp.
UV/VIS detector SPD-20A	Shimadzu Corp.
Valve unit FCV-20AH$_2$	Shimadzu Corp.
Degasser DGU-20A$_5$	Shimadzu Corp.
Liquid chromatograph LC-20AD	Shimadzu Corp.
Autosampler SIL-20AC HT	Shimadzu Corp.
HPLC Shimadzu LC-20AD (equipped with SIL-20AC autosampler)	Shimadzu Corp.
HPLC detector Nexera X2 diode array detector SPD-M30A	Shimadzu Corp.
HPLC column Shimadzu, Shim-pack, VP-ODS (150 x 4.6 mm)	Shimadzu Corp.

Guard column Shimadzu, Shim-pack, GVP-ODS (10 x 4.6 mm)	Shimadzu Corp.

Table 11: Computer programs for measuring and analysis

Program	**Supplier**	**Usage / method**
Microsoft Excel	Microsoft Corp.	Calculation
GraphPad Prism 9	GraphPad Software	Graph creation
Spark Control V 3.1 SP1	Tecan Trading AG	Data Acquisition & Analysis
PerkinElmer UV WinLab	PerkinElmer	Data Acquisition & Analysis
Shimadzu LabSolution CS	Shimadzu Corp.	Data Acquisition & Analysis
SoftMax Pro 7	Molecular Devices, LLC	Data Acquisition & Analysis

3.3 Meat homogenization and storage

For each group (P1, P2, P4 and P5) a 100g slices of meat of every cut (loin and belly) were acquired. Before it was used for further analysis, the slices were refrigerated at 4 °C for three days.

For the meat homogenization, the meat slice was cut into 1 cm cubes, to make the homogenization faster and easier. The small meat pieces were blended in BÜCHI B-400 Mixer for 10 sec. After the proper homogenization, the meat paste was moved into pre-weighted 50 mL tubes. Lastly, all the tubes with meat paste were purged with argon gas and stored in a – 80 °C freezer until further analysis.

3.4 Extraction of meat fat

For the fat extraction from meat, the *Bligh and Dyer* extraction method described by Pérez-Palacois T. et al. (2008) with some small modifications was used (Pérez-Palacois T., et al.; 2008). The weight of sample taken depended on the pig race and meat cut: for Turoplje-pigs approximately 3 – 5 g of meat paste, for loin from land race pigs 7.5 g of meat paste and for belly from land race pigs approximately 5 g of meat paste was put into 50 mL tubes.

To each tube, 5 mL of chloroform ($CHCl_3$) and 10 mL of methanol (MeOH) were added. After that, the sample was vortexed for 2 min to homogenize the mixture. The samples were centrifuged at RT and 1250 rcf for 10 min. Afterwards the supernatant was filtered using a Whatman filter, into a new 50 mL tube. The remaining liquid was rehomogenized with 15 mL of chloroform, vortexed and centrifuged. The second supernatant was filtered in the first tube to combine the filtrates. The combined filtrate was mixed with 5 mL of bidist. Water and the mixture were vigorously shaken until phase separation. Using a 1 mL pipette the upper aqueous phase was conscientiously removed. To the organic phase 1.5 g of sodium sulfate was added to remove remaining water. After that the mixture was shaken and centrifuged at RT and 3220 rcf for 3 min. The organic phases of the two same samples were transferred into a 50 mL pointed-bottom flask. Using a rotary evaporator the chloroform was evaporated under vacuum (approx. 30 min at 50 mbar). To make the fat solvent-free, the sample was put under a nitrogen stream. The extracted fat was carefully transferred from the flask into a pre-weighed centrifugal tube, purged using argon gas, and stored in a freezer at – 80 °C for further analysis.

3.5 Determination of primary lipid oxidation content using IDF-Assay

The methodology confirmed by Shantha & Decker, (1994) was used for the determination of primary lipid oxidation (Shantha & Decker, 1994). Firstly, the iron-(II)-chloride and iron-(III)-chloride solution were separately prepared. For the preparation of the iron-(II)-chloride solution, 0.5 g of iron-(II)-sulfate heptahydrate was dissolved in 50 mL of bidist. water. In another tube, 0.4 g of barium chloride dihydrate was also dissolved in 50 mL of bidist. water. The barium chloride solution was slowly added to the iron-(II)-sulfate solution while stirring. To the foggy solution 2 mL of 10 M HCl were added and then the solution was filtered through a Whatman filter (change the filter after filtrating half of the mixture). The filtrated solution was stored in a 100 mL volumetric flask, wrapped in tin foil and refrigerated at 4 °C (max. two weeks). The iron-(III)-chloride solution was prepared using 0.5 g of iron-(III)-chloride, which was dissolved in 50 mL of 10 M HCl. After that, 1.5 mL of 30 % hydrogen peroxide was added to the solution and the mixture was boiled in a water bath for 5 min. The solution was cooled down to room temperature and transferred into a 500 mL volumetric flask. At the end, the mixture was filled with bidist. water to the mark. For the analysis the 10 µg/mL iron-(III)-chloride stock solution was prepared using 0.5 mL of the prepared solution, which was pipetted into a 50 mL volumetric flask and filled with a MeOH/chloroform (3:7) solution to the mark. Using this stock solution, different standard solutions were freshly made for before every analysis. The standard solutions were prepared according Table 17. Firstly, the volume of MeOH/chloroform (3:7) was pipetted to

a 15 mL tube and after that the 10 µg/mL iron-(III)-chloride stock solution in different volumes. At the end, the standard solutions were vigorously vortexed. An ammonium thiocyanate solution was made by dissolving 0.3 g of ammonium thiocyanate in 1 mL of bidist. water, freshly. For the sample preparation, 30 – 40 mg of the extracted fat sample were weighed into a 15 mL tube, and 9.9 mL of MeOH/chloroform (3:7) were added and the mixtures were vortexed. For the blank measurement, only 9.9 mL of MeOH/chloroform (3:7) were added to a 15 mL tube. Before the measurement, all the samples and the standards were in water bath for 30 min at 25 °C. For the measurement, 50 µL of the iron-(II)-chloride solution was added to the blank, standard or the sample, and the solution was immediately vortexed. After that, 50 µL of the ammonium thiocyanate solution was added, and the mixtures were quickly vortexed. A timer was started for 5 min and after the reaction time was over, the absorbance of the solution was measured at 500 nm using quartz glass cuvette. All the blanks, standards and samples were measured in triplicates. In the Appendix (see **Figure 56**) one of the standard curve can be seen. Using Equation 1. the peroxide value was determined. Based on the standard deviation of the blank mean a LOD of 0.197 meq O_2/kg fat and a LOQ of 0.648 meq O_2/kg fat was calculated.

Table 12: Preparation of iron-(III)-chloride standards

Conc. $FeCl_3$ [µg/10 mL]	**Vol. $FeCl_3$ [µL]**	**$CHCl_3$/MeOH (7:3) [mL]**
2.0	200	9.7
5.0	500	9.4
7.5	750	9.15
10.0	1000	8.9
15.0	1500	8.4
20.0	2000	7.9

$$PV\ \left[\frac{meq\ O_2}{kg\ sample}\right] = \frac{(A_s - A_B)\cdot\frac{1}{k}}{55.84\cdot E\cdot 2}$$

Equation 1: Calculation of the peroxide value (PV) in meq O_2/kg fat

As/Ab: absorbance of sample or blank at 500 nm; 1/k: inverse slope of the standard curve; 55.84: molecular mass of iron; E: initial weight of the sample in g; 2: factor to convert oxygen into peroxides

3.6 Determination of volatile secondary oxidation products by GC-MS

For the determination of secondary lipid oxidation content, the procedure based on Holler M. et al. (2023) was followed. At the beginning, 1.5 g of meat paste were weighted in a 20 mL GC-MS vials and then 4mL of 25% of NaCl solution were added. All the samples were made in triplicates. A hexanal control sample was also prepared. For the control sample, a 4 mg/mL hexanal stock solution in methanol was diluted 1:10 with 25% of NaCl solution. After that, 1 µL of this standard were added to the 4 mL of 25% NaCl to make a 100 µg/mL hexanal control standard. All the samples were measured by SPME-GC-MS. Using commercial library (NIST, ver. 11.0 library; > 85% match), the produced mass spectra were compared to identify the measured compounds. At the end, the measured peak areas were normalized using the weight of the examined sample to semi-quantify specific volatile compounds.

Table 13: Measurement parameters (GC) for the determination of secondary oxidation products by GC-MS

Parameter	**Setting**		
Column name	Phenomenex Zebron ZB-5 ms (30 m x 0.25 mm x 0.25 µm)		
Injection mode	Splitless		
Carrier gas	Helium		
Column oven temperature	50.0 °C		
Injection temperature	250.0 °C		
Sampling time	2.00 min		
Flow control mode	Linear velocity		
Pressure	53.6 kPa		
Column flow	1.00 mL/min		
Purge flow	3.0 mL/min		
Total flow	4.0 mL/min		
Linear velocity	36.3 cm/sec		
Split ratio	0.0		
Column oven program	Rate	Final temperature [°C]	Hold time [min]
	-	50.0	5.00
	5.00	300.0	17.00
	0.00	0.0	0.00
	0.00	0.0	0.00
Total program time	72.00 min		

Table 14: Measurement parameters (MS) for the determination of secondary oxidation products by GC-MS

Parameter	Setting
Detector voltage	Relative to the tuning result: 0.4 kV
Ion source temperature	230 °C
Interface temperature	305 °C
Threshold	0
Solvent cut time	2 min
Micro scan width	0 u
Start time	2.00 min
End time	72.00 min
Acq. Mode	Scan
Event time	0.20 sec
Scan speed	3333
Start m/z	41.00
End m/z	600.00

3.7 Determination of the fatty acid profile and fatty acid content using GC-FID

Using the method described by Lall R. K. et al. (2009) and the GC-FID technique as described by Grüneis V. et al. (2019), with modifications based on Pignitter M. et al. (2014) the samples were prepared. Firstly, approx. 100 mg of the extracted fat sample was put into a 50 mL tube. For the antioxidants 2 mL of toluene and 100 mg of pyrogallol were added to a sample tube. As the internal standard, 0.5 mL of a 1 % heptadecanoic acid methyl ester (HME) solution in methanol was added. Lastly, 4 mL of a 0.5 M sodium methoxide solution in MeOH were pipetted to the solution. The mixture was shortly vortexed, purged with argon gas and then heated for 12 min in a water bath at 50 °C. After this step, 0.2 mL of glacial acetic acid was quickly added and the samples were put in a refrigerator for 30 min at 4 °C. Later, 5 mL of n-hexane and 5 mL of bidist. water was added and the sample was vortexed for 2 min. After few moments at RT, the two phases were separated and the upper organic phase was attentively transferred into a 15 mL tube. The aqueous phase was additionally mixed with 5 mL of n-hexane and vortexed for 2 min. The second organic upper phase was then combined with first organic upper phase. To the organic phase mixture, a small amount of sodium sulphate was added and the tube was shaken. After that, the sample was filtered through a 0.20 µm syringe filter into a new tube. At the end, the GC vials with 100 µL inserts were filled with the filtrated sample and analyzed using GC-FID. Each sample and standard

were analyzed in technical triplicates. In the table 15 and 16 the measurement parameters are listed. Using methylated fatty acid standards diluted with n-hexane in different concentrations (see **Table 17**) the standard curves were constructed, that can be seen in the Appendix. Using Equation 3 the fatty acid methyl ester content was calculated. LOD and LOQ (see **Table 18**) were calculated based on the standard deviation of the blank mean.

Table 15: Measurement parameters for the determination of the fatty acid content using GC-FID

Parameter	**Setting**
GC column	Phenomenex ZB-WAX Zebron ™ (PEG) capillary column 30 m x 0.25 mm x 0.25 µm
Carrier gas	Helium
Flow	3 mL/min
Temperature of the injector	220 °C
Temperature of the detector	270 °C
Injection volume	1 µL
Split ratio	20

Table 16: Temperature conditions for the determination of the fatty acid content using GC-FID

Heating rate [°C/min]	**Temperature [°C]**	**Hold time [min]**
-	60	2
13	150	0
2	240	0

Table 17: Methylated fatty acid standards and their concentration range

Methylated fatty acid standard	**Concentration range [mg/mL]**
Methyl palmitate	0.025 – 3.0 mg/mL
Methyl stearate	0.025 – 1.0 mg/mL
Methyl linoleate	0.025 – 3.0 mg/mL
Methyl linolenate	0.0025 – 0.3 mg/mL
Methyl oleate	0.25 – 10.0 mg/mL
Methyl arachidonate	0.0025 – 0.3 mg/mL
Methyl eicosapentanoate	0.0025 – 0.3 mg/mL

Table 18: LOD and LOQ based on the standard deviation for the determination of fatty acids measured by GC-FID

Fatty acid		Value [g/100 g fat]
Palmitic acid	LOD	0.0014
	LOQ	0.0043
Stearic acid	LOD	0.0018
	LOQ	0.0054
Oleic acid	LOD	0.0084
	LOQ	0.0255
Linoleic acid	LOD	0.0013
	LOQ	0.0039
α-Linolenic acid	LOD	0.0001
	LOQ	0.0004
Arachidonic acid	LOD	0.0001
	LOQ	0.0004
Eicosapentaenoic acid	LOD	0.0001
	LOQ	0.0004

$$FAME\ \left[\frac{g}{100\ g}\right] = \frac{\frac{Area - d}{k} \cdot 0.012}{E} \cdot \frac{1}{Rec} \cdot 100$$

Equation 3: Calculation of the fatty acid methyl ester (FAME) content in g/100 g fat

*Area: peak area of the respective sample in mAU*min; d: intercept of the standard curve; k: slope of the standard curve; E: initial weight of the sample in g; Rec: Recovery of the internal standard (HME); 0.012 determines the volume of the mixture in L; 100 is the factor to calculate the amount in g/100 g*

3.8 Determination of carbonyl protein content using DNPH assay

For the determination of carbonyl protein content, the procedure based on Armenteros M. et al. (2009) with some modifications was used. Firstly, using DNPH and 2 M HCl a 0.2 % (w:v) solution was prepared. Afterwards, in 50 mL tube 2 g of meat paste were weighted and 20 mL of pyrophosphate buffer (pH 7.4) were added. The pyrophosphate buffer contained 2 mM $MgCl_2$, 2 mM EGTA, 0.1 M KCl, 2 mM $Na_4P_2O_7$, and 10 mM tris-maleate. For 30 sec the mixture was homogenized in mixer and filtered using a cheesecloth. The filtered sample was vortexed and 100 µL of the sample filtrate (in total four sample aliquots and two blank aliquots) were transferred to the reaction tubes. To the sample filtrate, 1 mL of 10 % TCA was added, and the reaction tube was vortexed and centrifuged (RT, 5000 rcf,

5 min). The supernatant was removed, 1 mL of 2 M HCl was added to the two blank aliquots, and 1 mL of the DNPH solution was added to the four sample aliquots. At RT the mixtures were incubated for 1 hour, vortexing every 15 min. Thereafter, to each mixture 1 mL of 10 % TCA was added. Samples were vortexed and centrifuged (RT, 5000 rcf, 5 min). The supernatant was removed and the pellet was washed using 1 mL of an EtOH/ethyl acetate (1:1) solution, followed by vortexing, centrifugation (RT, 10000 rcf, 5 min) and removing the wash solution. This wash strategy was performed three times. Under a nitrogen stream the protein pellet was dried and later dissolved in 1.5 mL of 6 M guanidine hydrochloride in 20 mM sodium hydrogen phosphate (pH 6.5). After mixing, the sample was centrifuged (RT, 5000 rcf, 2 min), and the absorbance was measured at 280 nm and 370 nm in a quartz glass cuvette using a spectrophotometer. At the beginning, a solvent blank (6 M guanidine hydrochloride in 20 mM sodium hydrogen phosphate) was measured. The blanks were prepared and measured in duplicates, while the samples were prepared and measured in quadruplicates. Using equation 3, the carbonyl content was calculated.

$$CC\left[{}^{nmol}/_{mg\ protein}\right] = \frac{Abs_{370} - Abs_{370}(blank)}{22000 \cdot \left[Abs_{280} - \left(Abs_{370} - Abs_{370}(blank)\right)\right] \cdot 0.43} \cdot 10^6$$

Equation 2: Calculation of the carbonyl content CC in nmol/mg protein

Abs_{370}: absorbance of the sample at 370 nm; $Abs_{370}(blank)$: absorbance of the blank at 370 nm; Abs_{280}: absorbance of the sample minus absorbance of the blank at 280 nm; For the molar absorptivity of the hydrazone, ε_{M370} = 22000 and ε_{M276} = 9460, which equals 43 % (factor 0.43) of that at 370 nm

3.9 Determination of tocopherol content by HPLC-UV

Following the method established by Pignitter M. et al. (2014) the determination of tocopherol content was carried out. Initially, α-, γ- and ∂-tocopherol standards were prepared using 2-propanol, creating a concentration range of 0.5 µg/mL to 100 µg/mL (see **Table 19**). Afterwards, in the reaction tube 100 mg of the sample were weighted. Using 1 mL of 2-propanol the sample was dissolved and vortexed thoroughly. As internal standard, 1 µL of a 5 mg/mL tocol standard was added to the samples. After these steps, the mixture was filtered through a 0.40 µm syringe filter. For the measurement 200 µL of the filtrate was transferred into HPLC vials with 200 µL inserts. All blanks, samples and standards were measured in technical triplicates and for the blank 2-propanol was utilized. The measurement parameters are listed in Tables 20 and 21. To determine the α- and γ-tocopherol content, a standard curve was generated, as illustrated in the Appendix. A LOD of 0.55 µg/mL and a LOQ of 0.65 µg/mL for α-tocopherol were determined as well as for γ-tocopherol (LOD= 0.41 µg/mL, LOQ=0.48 µg/mL), based on the standard deviation of the blank mean.

Table 19: Preparation of α-, γ- and ∂-tocopherol standards

Standard	Conc. α-, γ- and ∂-tocopherol standard [µg/mL]	Vol. of standard x [µL]	Vol. 2-propanol [µL]
S1	100	100 µL of 1 mg/mL each stock	700
S2	50	500 µL of S1	500
S3	25	500 µL of S2	500
S4	12.5	500 µL of S3	500
S5	5	100 µL of S4	900
S6	2.5	500 µL of S5	500
S7	1	200 µL of S5	800
S8	0.5	500 µL of S7	500

Table 20: Measurement parameters for the determination of tocopherol content by HPLC-UV

Parameter	Setting
Column	Shimadzu, Shim-Pack, VP-ODS (150 x 4.6 mm)
Guard column	Shimadzu, Shim-Pack, VP-ODS (10 x 4.6 mm)
Column temperature	10 °C
Injection volume sample / standard	80 µL
Run time	38 min
Solvent A	Bidist. water
Solvent B	Methanol
Flow	0.5 mL/min
Detection wavelength	295 nm

Table 21: HPLC gradient solvent program used for the analysis of tocopherols

Time [min]	Solvent A [%]	Solvent B [%]
4	0	100
34	0	100
36	5	95
38	5	95

3.10 Determination of polyphenolic content by LC-MS

Following the method outlined by Branciari R. et al. (2017), with some modifications, the determination of polyphenolic content was carried out. At the beginning, 5 g of meat paste were weighted into a 50 mL tube. To the meat paste 20 mL of a BHT solution (MeOH/H_2O 80:20, containing 20 mg/L BHT) was added. Using the mixer, the mixture was homogenized for 1 min and finally vortexed for 1 min. Afterwards, the sample was centrifuged (RT, 1250 rcf, 10 min) and filtered through a Whatman filter into a 50 mL round bottom flask. Using a rotary evaporator and the water bath at 40 °C the sample was evaporated (50 mbar, 30 min). After the evaporation, the remaining sample was transferred into a reaction tube and the sample was put under a nitrogen stream for 10 min. Afterwards, the sample was put in the freezer at - 80 °C for 15 min. For the evaporation of the remaining parts a freeze dryer was used (- 80 °C, <5 mbar, overnight). The following day, the dried sample was dissolved in 500 – 700 µL of MeOH/H_2O (80:20). After that, the sample was centrifuged (RT, 1250 rcf, 10 min) and transferred into small tubes. At the end, the sample was purged with argon gas, and stored at - 80 °C. For the LC-MS measurements, the defrosted sample was filtered through a 0.20 µm syringe filter. Into HPLC vials containing 200 µL inserts 200 µL of the filtrate were pipetted. Additionally, a 1 mg/mL multi-standard was prepared, from which various standard concentrations were prepared. In order to correct for possible matrix effects different volumes of the multi-standard were added to one of the sample filtrates (see **Table 22**). This was used as an internal standard to determine the concentrations of polyphenols in the samples. The multi-standard included epicatechin, secoisolariciresinol diglucoside, kaempferol and caffeic acid. From the detected peak in the samples, the measured peak area of the standard compound was subtracted and the standard curve was generated. The blanks, standards and the samples were measured in technical triplicates using LC-MS-8040 with the measurement parameters, that are listed in Table 22. The results were normalized based on the initial weight of the meat paste.

Table 22: Preparation of the multi-standards (epicatechin, secoisolariciresinol diglucoside, kaempferol and caffeic acids) in the final sample filtrate

Added standard conc. [µg/5 g meat]	Vol. of final sample filtrate [µL]	Vol. of 1 mg/mL multi-standard [µL]
7	49.5	0.5
14	49.0	1.0
20	48.6	1.4
25	48.2	1.8
50	46.4	3.6
75	44.6	5.4

100	42.9	7.1

Table 23: Measurement parameters for the determination of polyphenols by LC-MS

Parameter	Setting
Column	C18 (Acclaim 120, 2.1 mm x 150 mm, 3 µm, Thermo Fisher Scientific)
Gradient	0 -2 min with and isocratic flow at 10 % B 2 – 8 min from 10 % to 80 % B 8 – 20 min to 100 % B Holding for 1 min 21 – 21.1 min from 100 % to 10 % B 21.1 – 25 min isocratic flow of 10 % B
Mobile phase A	Acetonitrile with 0.1 % formic acid
Mobile phase B	H_2O (LC-MS grade) with 0.1 % formic acid
Flow rate	0.3 mL/min
Injection volume	5 µL
Column temperature	25 °C
MS-Setting	
ESI	Source voltage = 3.5 kV Capillary voltage = 25 V Capillary temperature = 300 °C Sheath gas flow = 45 AU (N_2) Aux gas flow = 10 AU (N_2)
Sample analysis	Negative mode Range of m/z = 100 – 1000
Calibration lock mass	m/z 112.9856 $((HCOOH)_2+Na-2H)^-$

3.11 Determination of Schiff bases / carbonyl-amin adduct content using fluorescence measurement

Following the method outlined by Utrera M. et al. (2012) the determination of Schiff bases was carried out. At the beginning, 2 g of meat were weighted into 50 mL tube. To the meat paste 20 mL of a 20 mM monosodium phosphate buffer (pH 6.5) were added and the sample was mixed for 1 min and at the end filtered using a cheesecloth. After that, the filtrate was centrifuged (RT, 1250 rcf, 10 min), and the sample was diluted with the same

buffer (1:20). Afterwards, 200 µL of the diluted sample was pipetted into an opaque-bottom 96-well plate. A total of 200 µL of the monosodium phosphate buffer was used as a blank. The fluorescence of the samples was then measured with a UV-VIS spectrometer using the parameters detailed in Table 24. Using fluorescence intensity emitted at 460 nm the Schiff base content was determined. All the values were normalized to the protein content. The BCA protein assay was performed using the filtered and diluted samples to measure the protein content. The protein content determination is described in the next section. After that process, the fluorescence intensity normalized by protein was calculated using equation 4.

Table 24: Measurement parameters for measuring the fluorescence intensity of Schiff bases

Parameter	Setting
Excitation wavelength for Schiff bases	350 nm
Measured spectrum	400 – 500 nm
Excitation and emission slid widths	10 nm
Data collection	500 nm/min

$$FI[normalized\ fluorescence\ intensity] = \frac{F - blank}{\left(\frac{x}{avg(x)}\right)}$$

Equation 3: Calculation of the fluorescence intensity (FI) of Schiff bases (normalized by the protein content) in normalized fluorescence intensity

F: fluorescence of the sample; blank: fluorescence of the blank; x: protein content of the sample; avg (x): average of the protein content of the same sample group

3.11.1 Determination of protein content by BCA Assay

The BCA Assay was conducted following the methodology outlined by Cortés-Ríos et al., with some modifications. The diluted filtrates from the Schiff base determination (section 3.11) were used in this assay. The BCA working reagent was prepared using kit preparation guidelines. After that, the BSA standards were generated from 0 to 2000 µg/mL concentration using a BSA stock solution (2000 µg/mL) diluted with a 20 mM monosodium phosphate buffer (pH 6.5). The preparation scheme is shown in table 25. Afterwards, 25 µL of blank, standard and each sample were pipetted into a transparent flat-bottom 96-well plate. The monosodium phosphate buffer was again used as the blank. To each well 200 µL of working solution were added and the plate was covered with sticky paper. The plate was placed on a plate shaker for 30 sec and was incubated for 30 min at 37°C. The samples and blank were pipetted in triplicates, while the standards were pipetted in duplicates. After the reaction time, the sticky paper lid was removed, and the sample

absorbance was measured using a plate reader at a wavelength of 562 nm. Based on the BSA standard curve the protein content was calculated.

Table 25: Preparation of albumin (BSA) standards

Standard	Conc. BSA standard [µg/mL]	Vol. of BSA standard [µL]	Vol. 20 mM monosodium phosphate buffer (pH 6.5) [µL]
A	2000	300 µL of stock	0
B	1500	375 µL of stock	125
C	1000	325 µL of stock	325
D	750	175 µL of B	175
E	500	325 µL of C	325
F	250	325 µL of E	325
G	125	325 µL of F	325
H	25	100 µL of G	400

3.12 Statistical analysis

All experiments were carried out in technical triplicates, except for the DNPH assay, which was conducted in quadruplicates. To maintain data integrity, a Grubbs' outlier test was performed, and any detected outliers were excluded from subsequent analyses. Using the Kolomogorov-Smirnov method, the data were evaluated for both normality and lognormality. Because always two groups were compared with each other, an unpaired parametric t-test was performed, and results with a p-value below 0.05 ($p < 0.05$) were considered statistically significant and marked with an asterisk in the graphs. For the analysis of the fatty acid profile by GC-FID, the quantification of hydroperoxides using the IDF method, and the determination of Vitamin E content by HPLC-UV, the limits of detection (LOD) and quantification (LOQ) were established based on the standard deviation. Based on the S/N, the LOD und LOQ were determined in polyphenols analysis. Results of all analysis are presented as mean ± standard deviation.

4 Results

The results are presented by boxplots with the median. Q1 – Q3 are displayed as the box, while the numerical values in the text are shown as mean ± standard deviation, and are additionally listed in the Appendix.

4.1 Effects of linseed press cake feeding

4.1.1 Primary lipid oxidation products

In **Figure 18** the peroxide value (PV) of LPC loin was 2.1 ± 0.8 meq O_2/kg fat and the PV of NF loin was 1.5 ± 0.4 meq O_2/kg fat. There was no significant difference between LPC and NF loin. Additionally, the comparison between a peroxide value of LPC and NF belly did not show significant difference.

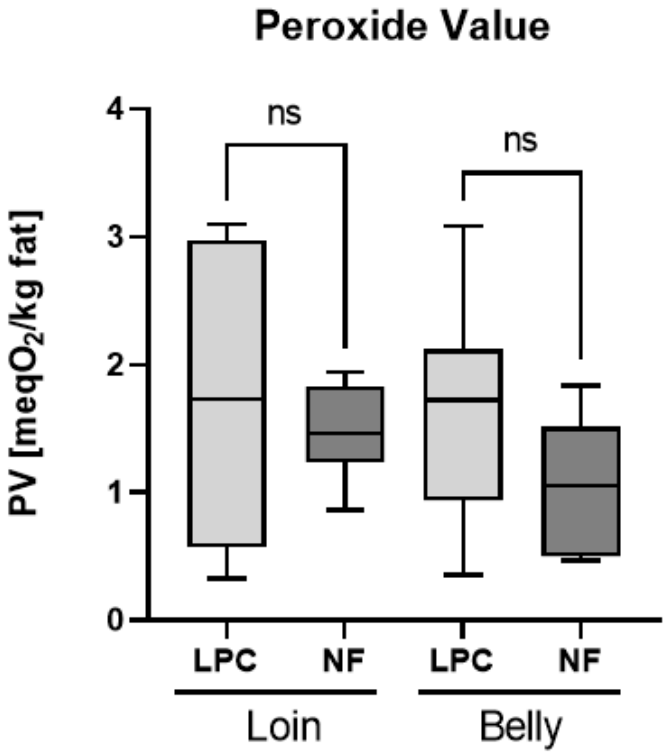


Figure 18: Peroxide value (PV) in meq O_2/kg fat of loin and belly meat in the groups LPC and NF measured by IDF method

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

4.1.2 Secondary lipid oxidation products

Five aldehyeds and four alcohols were detected in samples and are shown in **Figure 19**. The identified aldehydes were hexanal, heptanal, octanal, nonanal and 2-nonenal. The alcohols 1-pentanol, 1-hexanol, 1-heptanol and 1-octanol were detected in the samples. The results are presented as fold change between NF and LPC. Hexanal was the only parameter showing significant difference in NF belly comparing to LPC belly. There was around 2.4 fold more hexanal in NF belly as in LPC belly. The exact values are listed in the Appendix (see **Table 29**).

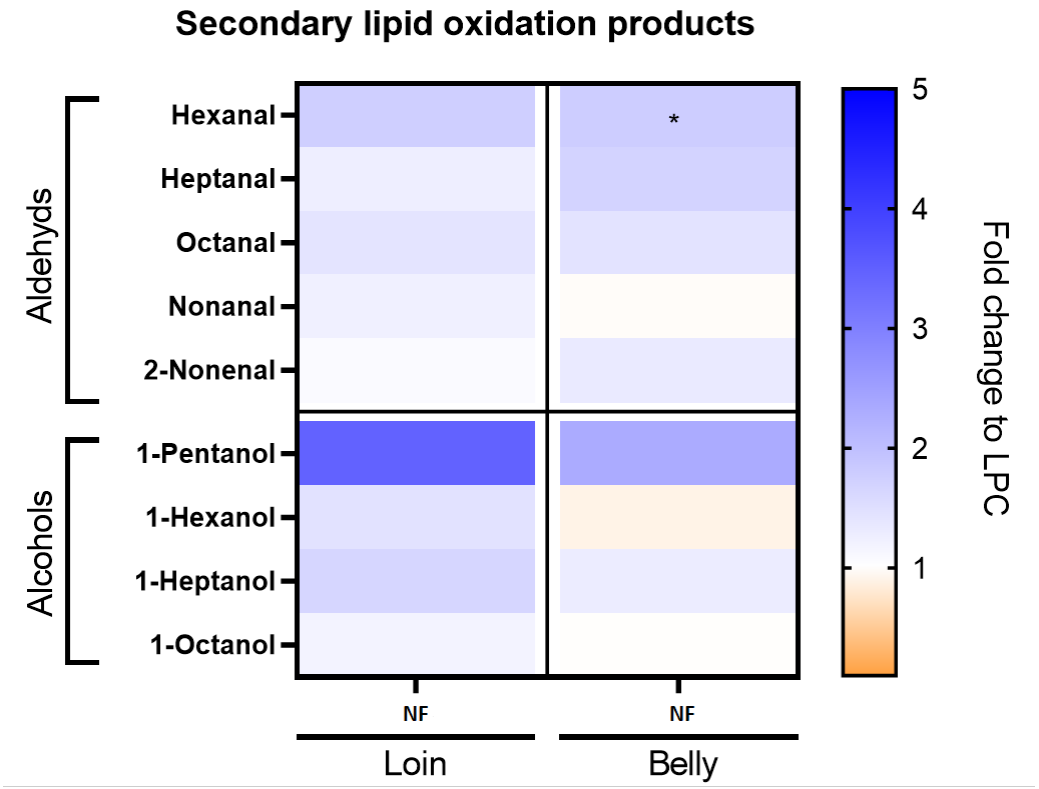


Figure 19: Fold change of normalized peak area of secondary lipid oxidation products in loin and belly meat from NF and LPC measured by GC-MS. The peak area of the detected secondary lipid oxidation products of NF was compared to the peak area of the detected secondary lipid oxidation products of LPC.

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant, based on biological replicates with n= 8 for all groups*

4.1.3 Fatty acid profile and fatty acid content

GC-FID analysis showed that in NF loin there was 21.7 ± 1.25 g/100 g fat palmitic acid, which was significantly higher in comparison to LPC loin with an amount of 17.09 ± 3.36 g/100 g fat palmitic acid (see **Figure 20**). A significant difference in belly samples was not found.

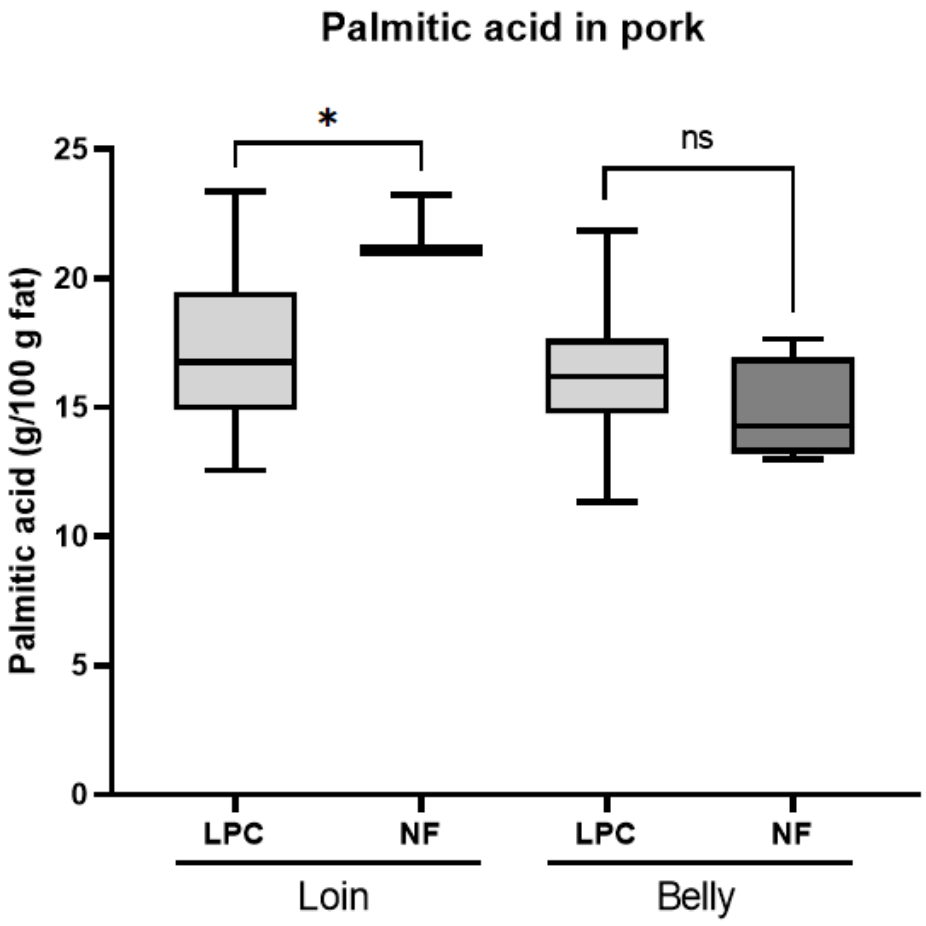


Figure 20: The content of palmitic acid in g/100 g fat of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

In **Figure 21** the significant difference of stearic acid in NF loin was observed with an amount of 12.90 ± 1.62 g/100 g fat stearic acid compared to LPC loin with 10.21 ± 1.73 g/100 g fat stearic acid. A significant difference within belly samples was not detected.

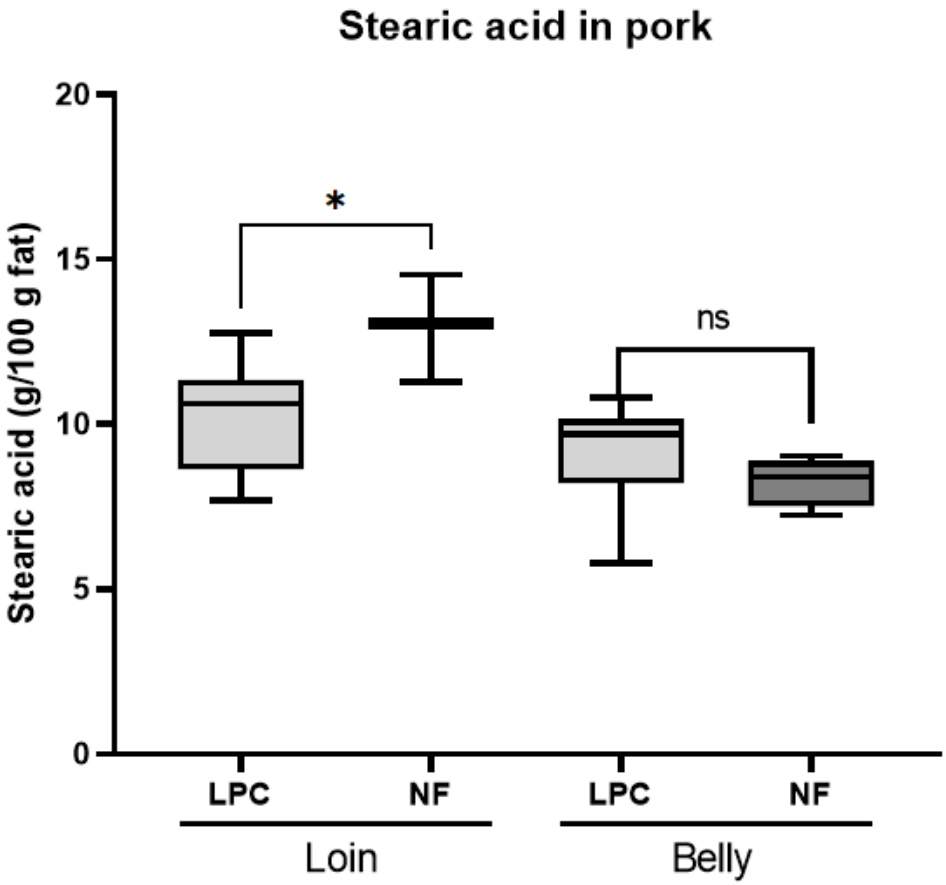


Figure 21: The content of stearic acid in g/100 g fat of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

As shown in **Figure 22** there was no significant difference in oleic acid content between the LPC and NF loin groups. Similar results were obtained for the belly samples.

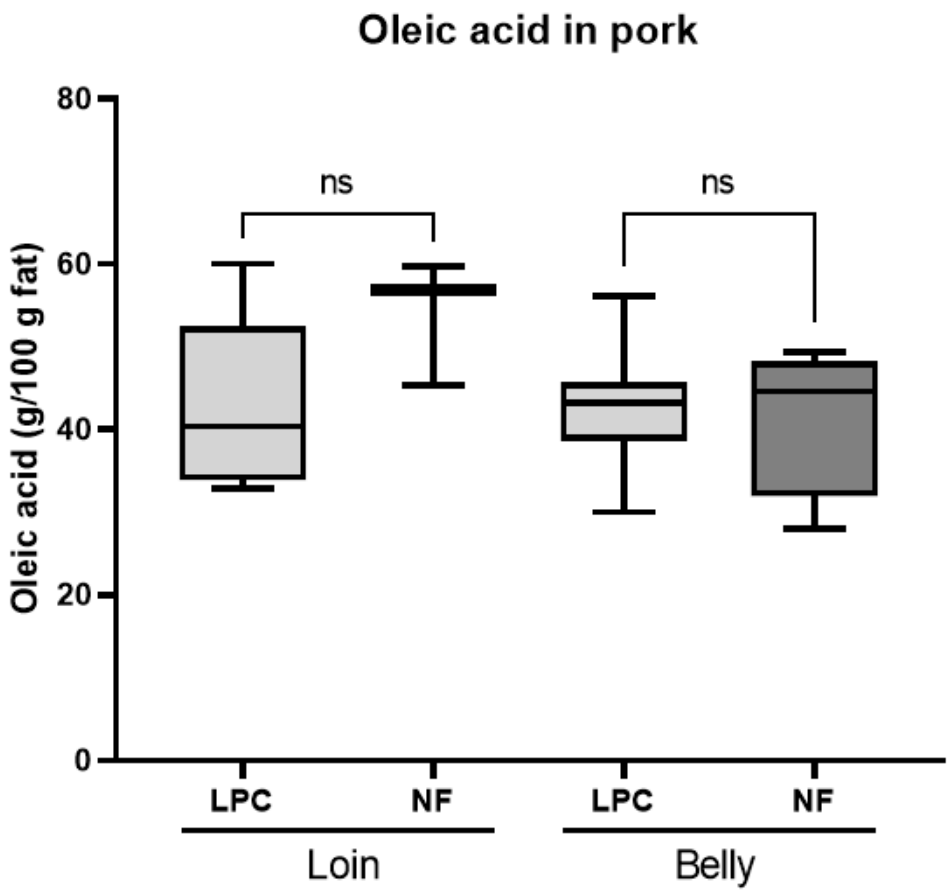


Figure 22: The content of oleic acid in g/100 g fat of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

In **Figure 23** the linoleic acid concentration in LPC and NF between both loin and belly samples was determined. However, a statistical significance between the groups was not found.

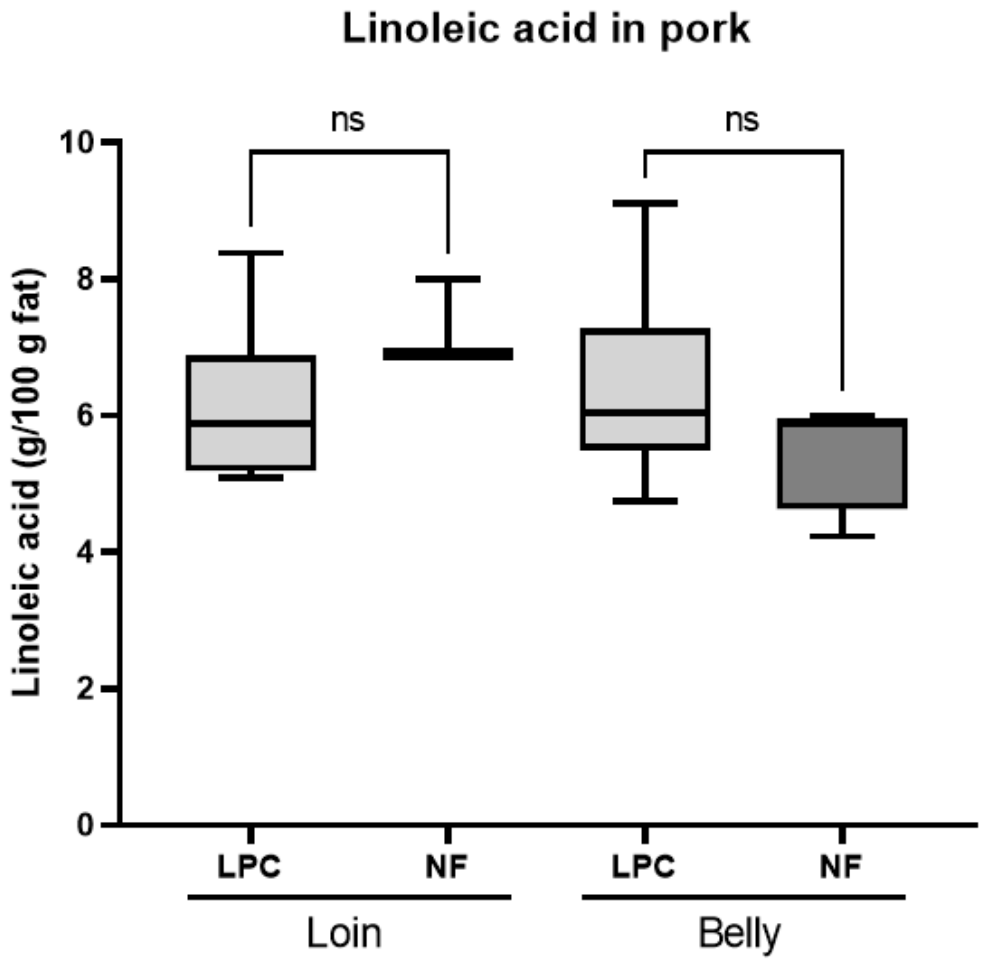


Figure 23: The content of linoleic acid in g/100 g fat of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

In **Figure 24** it can be observed that both LPC groups showed significantly higher content of alpha-linolenic acid compared to the respective NF groups. In loin samples the LPC showed 2.87 ± 0.74 g/100 g fat alpha-linolenic acid compared to NF with only 0.92 ± 0.33 g/100 g fat alpha-linolenic acid. In belly samples the LPC amounted 2.66 ± 0.97 g/100 g fat alpha-linolenic acid compared to NF with only 0.71 ± 0.18 g/100 g fat alpha-linolenic acid.

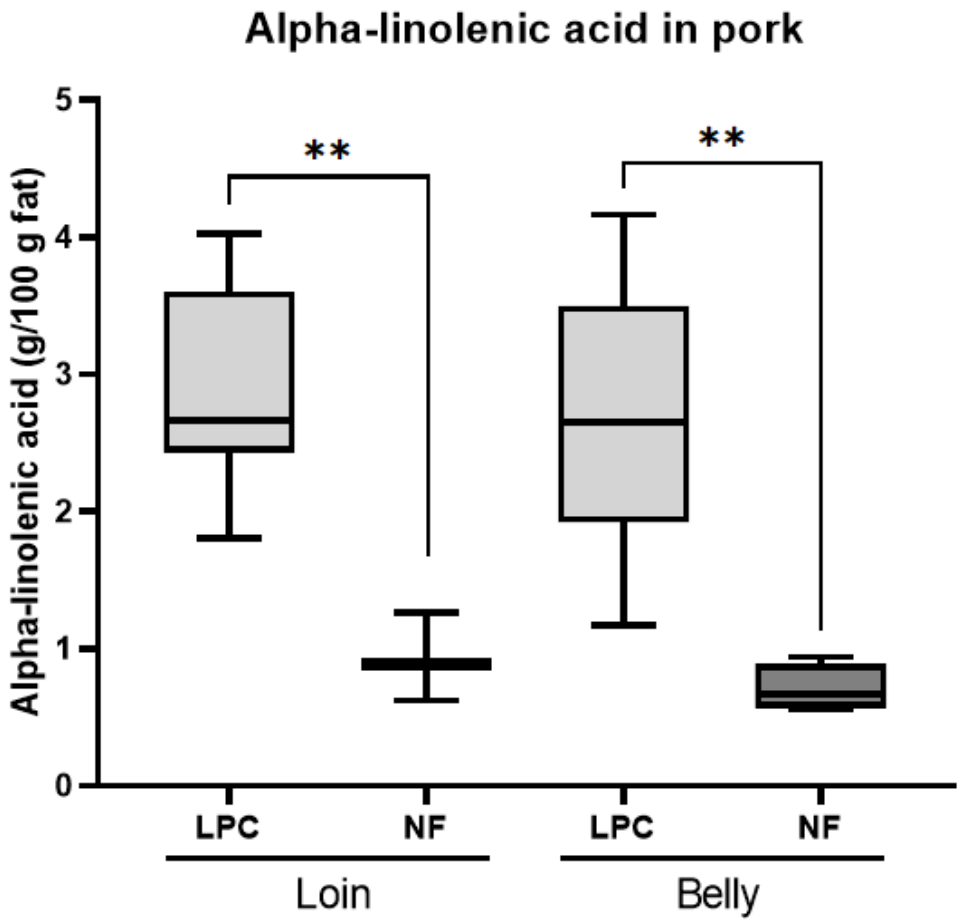


Figure 24: The content of alpha-linolenic in g/100 g fat of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

As shown in **Figure 25** the significant difference of arachidonic acid between loin groups was determined. The average amount of arachidonic acid in NF loin was 0.35 ± 0.02 g/100 g fat arachidonic acid compared to LPC loin with an amount of 0.24 ± 0.06 g/100 g fat arachidonic acid. Additionally, there was no significant difference between the belly groups regarding arachidonic acid.

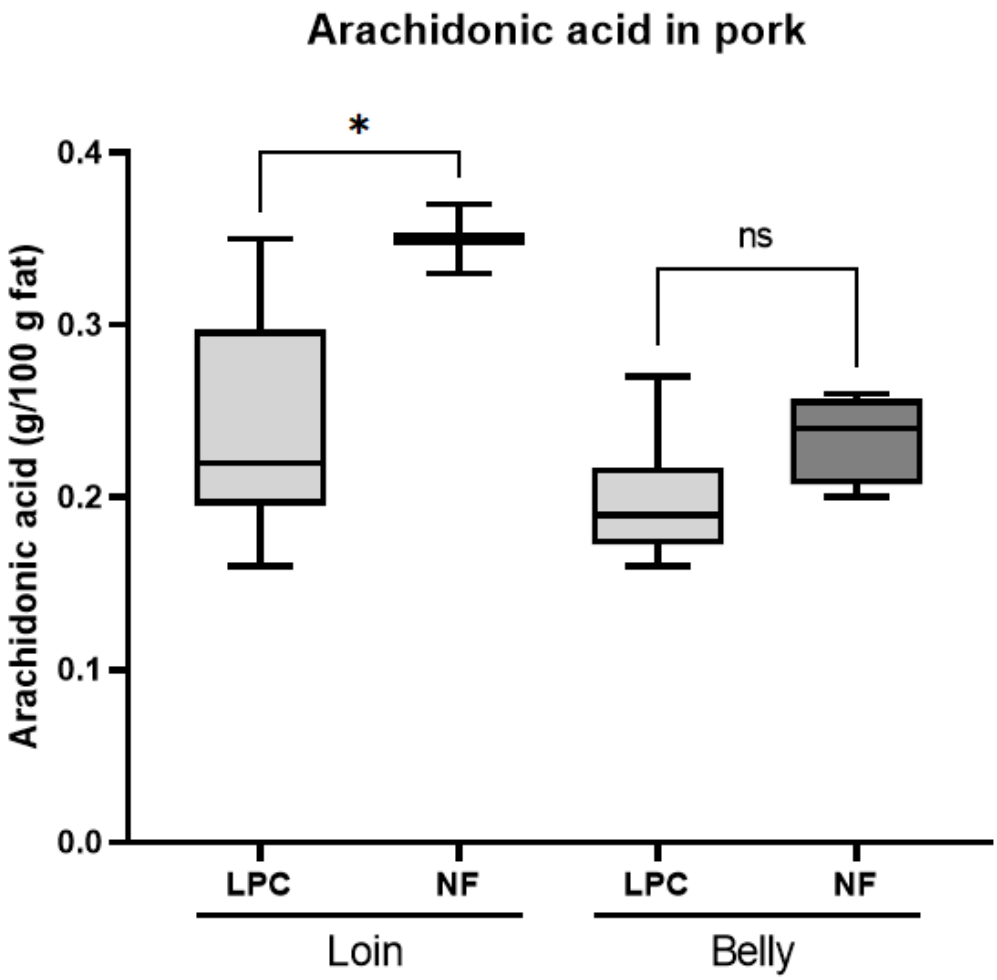


Figure 25: The content of arachidonic acid in g/100 g fat of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

In **Figure 26** it is shown that the eicosapentaenoic acid concentration in loin groups as well as in belly groups did not show significant difference.

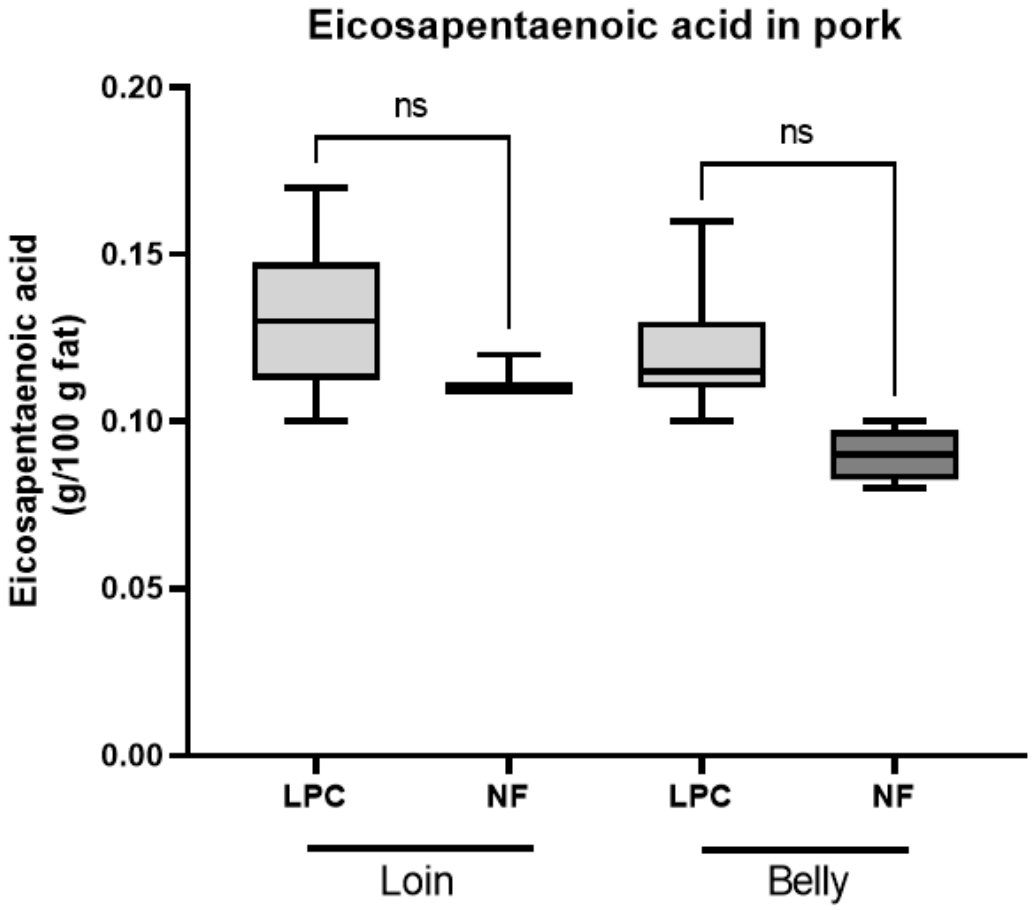


Figure 26: The content of eicosapentaenoic acid in g/100 g fat of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

As shown in **Figure 27** the n6:n3 ratio between loin samples showed significant difference. The NF loin samples showed n6:n3 ratio of 7.75 ± 2.03, while the LPC loin had n6:n3 ratio of 2.87 ± 0.74, which means that n6:n3 ratio of NF loin was nearly three times higher compared to LPC loin. Within the belly groups the n6:n3 ratio of NF belly was 7.33 ± 1.60 in comparison to LPC belly with n6:n3 ratio of 2.57 ± 0.76. Additionally, the highest n6:n3 ratio was found in NF loin samples.

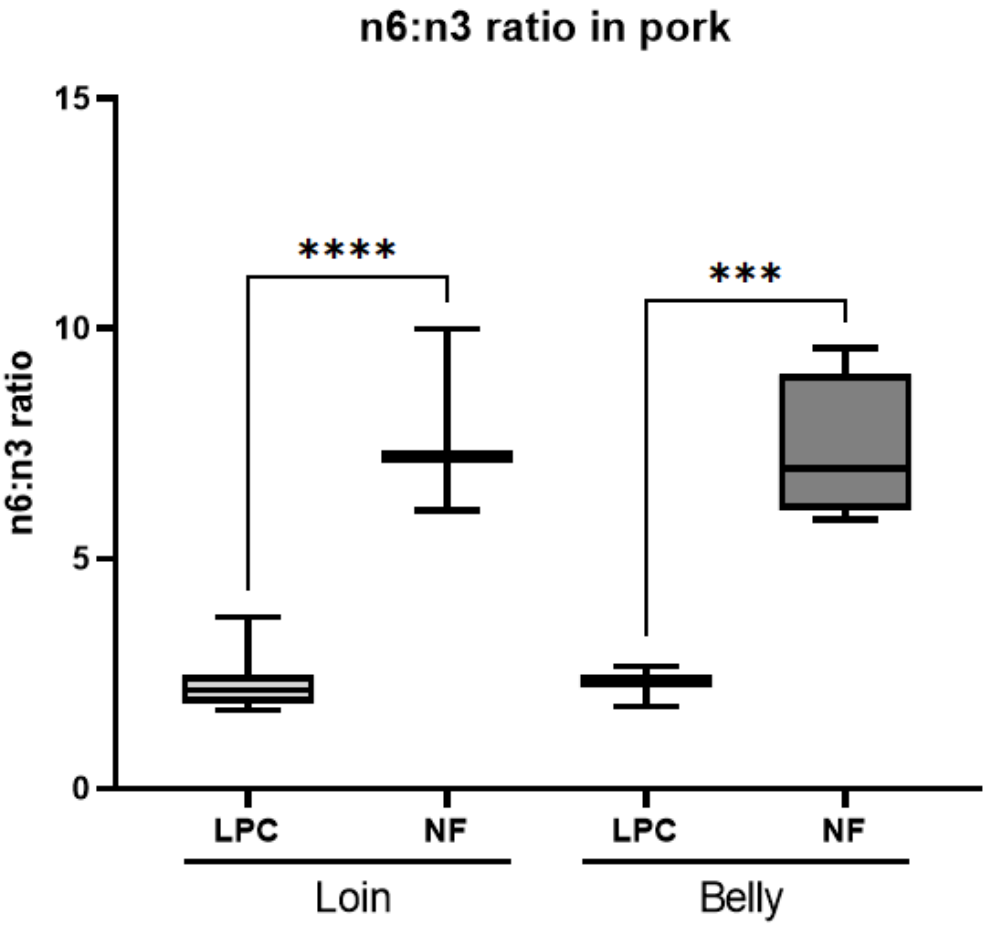


Figure 27: n6:n3 ratio of loin and belly meat in the groups LPC and NF measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for P2 and n= 3-4 for P4*

4.1.4 Carbonyl protein content

In **Figure 28** the carbonyl content of the samples is shown. There was no significant difference between the carbonyl content in loin of LPC and NF as well as between belly groups.

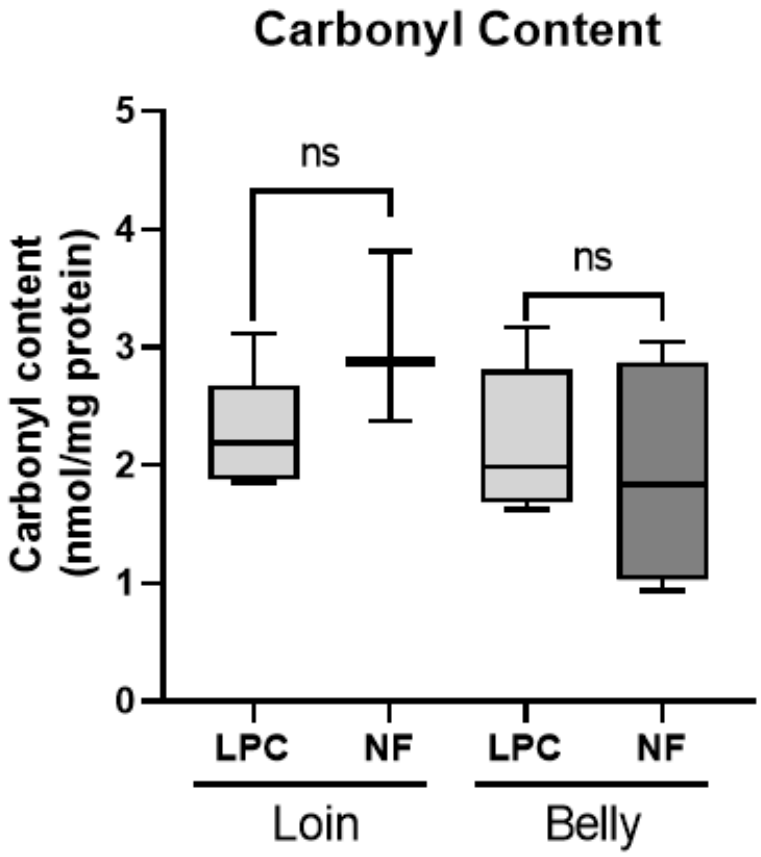


Figure 28: Carbonyl content of loin and belly meat in the groups LPC and NF in nmol/mg protein measured by DNPH assay

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 5 for LPC and n= 3-4 for NF group*

4.1.5 Schiff bases/carbonyl-amin adduct content

As shown in **Figure 29** a significant difference in normalized fluorescence intensity between LPC and NF belly was identified. The normalized fluorescence intensity in LPC belly was 410.06 ± 146.65, which was almost doubled compared to normalized fluorescence intensity in NF belly 258.11 ± 155.42. However, there was no significant difference between loin groups.

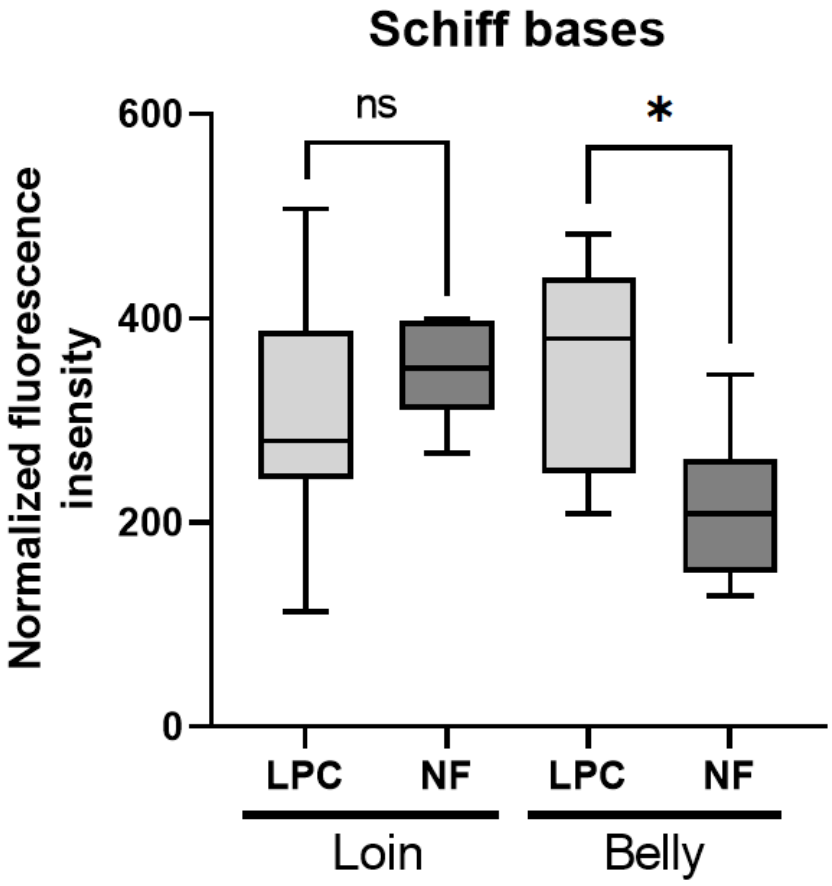


Figure 29: Normalized fluorescence intensity of loin and belly meat in the groups LPC and NF measured by fluorescence spectrometry. The protein content was determined by BCA and the fluorescence intensity was normalized by the protein content.

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for LPC and n= 7-8 for NF group*

4.1.6 Tocopherol content

The α-tocopherol and the γ-tocopherol content of LPC and NF pork in loin and belly was measured. However, the levels of both tocopherols in all samples were below the limit of detection, preventing their detection and quantification in LPC and NF samples. The LOD of α-tocopherol was 0.646 µg/g fresh weight (FW) meat and the LOD of γ-tocopherol was 0.483 µg/g fresh weight meat.

4.1.7 Polyphenol content

As shown in **Figure 30** there was no significant difference in total polyphenol content between LPC and NF loin, but LPC belly had a significantly higher total polyphenol content of 28.7 ± 8.6 µg/g in comparison to NF belly with total polyphenol content of 12.3 ± 2.2 µg/g.

LPC belly had more than double the total polyphenol content levels in comparison to NF belly. In **Figure 31** the most abundant polyphenols in loin are shown. Herbacetin, in LPC loin had the highest concentration of 60.6 ± 14.4 µg/g, while the herbacetin in NF loin amounted 41.5 ± 14.0 µg/g. Additionally, the amount of astragalin in LPC loin was 10.6 ± 4.9 µg/g and in NF loin 5.4 ± 1.0 µg/g. However, there was no significant difference between these samples. In **Figure 32** the concentration of herbacetin in LPC belly was 24.7 ± 8.4 µg/g thereby being significantly higher in comparison to NF belly with herbacetin concentration of only 9.8 ± 4.1 µg/g. The amount of astragalin concentration between LPC and NF belly did not show significant difference.

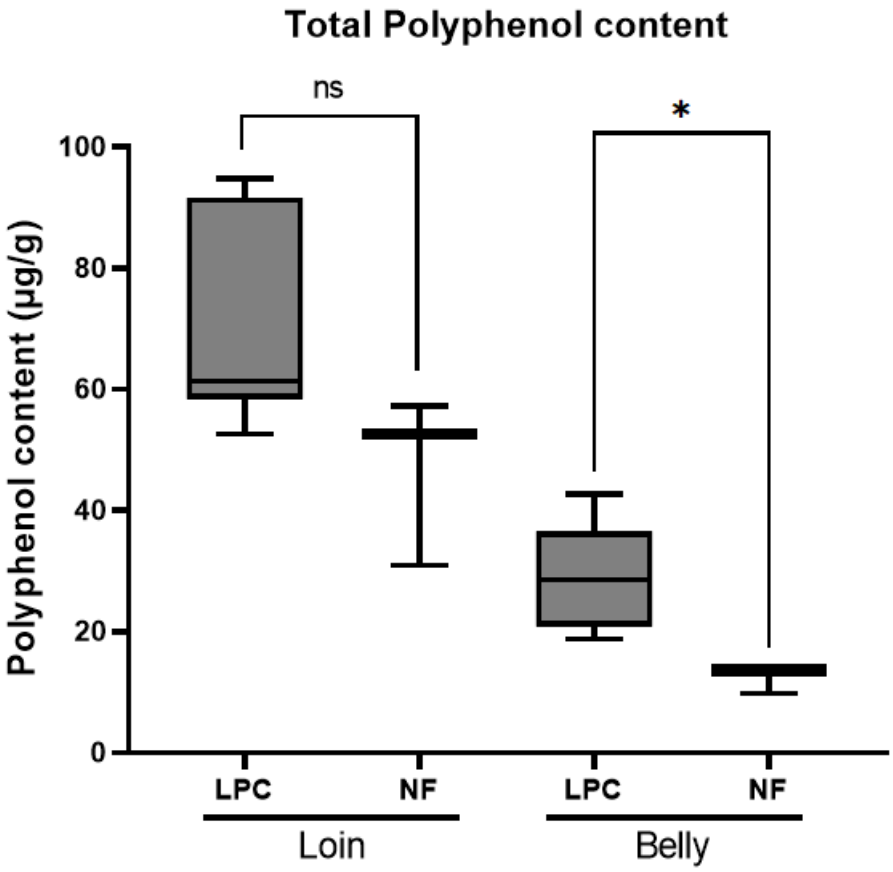


Figure 30: Total polyphenol content of loin and belly meat in the groups LPC and NF in µg/g determined by targeted LC-MS

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for LPC and n= 3 for NF group*

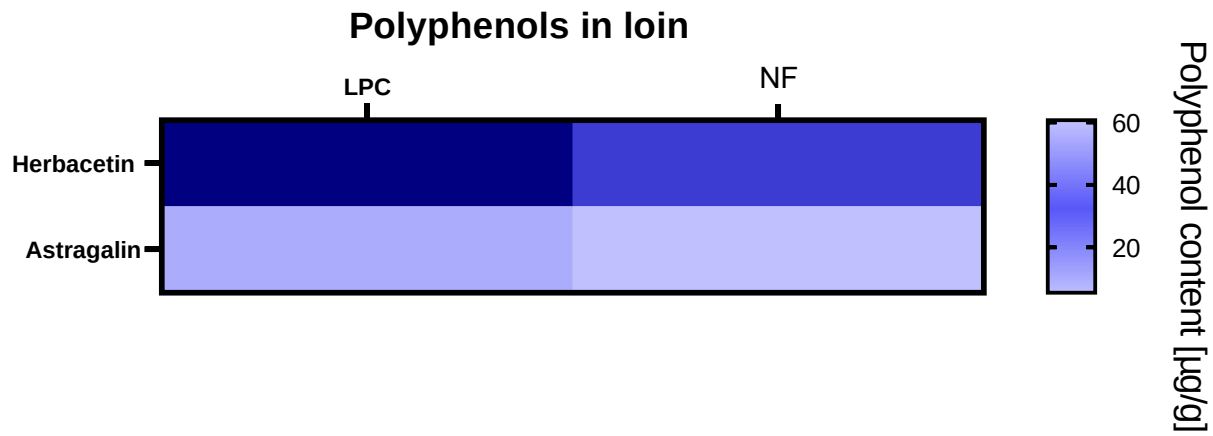


Figure 31: Polyphenol content of loin meat in the groups LPC and NF in µg/g measured by targeted LC-MS

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 (p ≤ 0.05 = *; p ≤ 0.01 = **; p ≤ 0.001 = ***) were considered statistically significant, based on biological replicates with n= 8 for LPC and n= 3 for NF group*

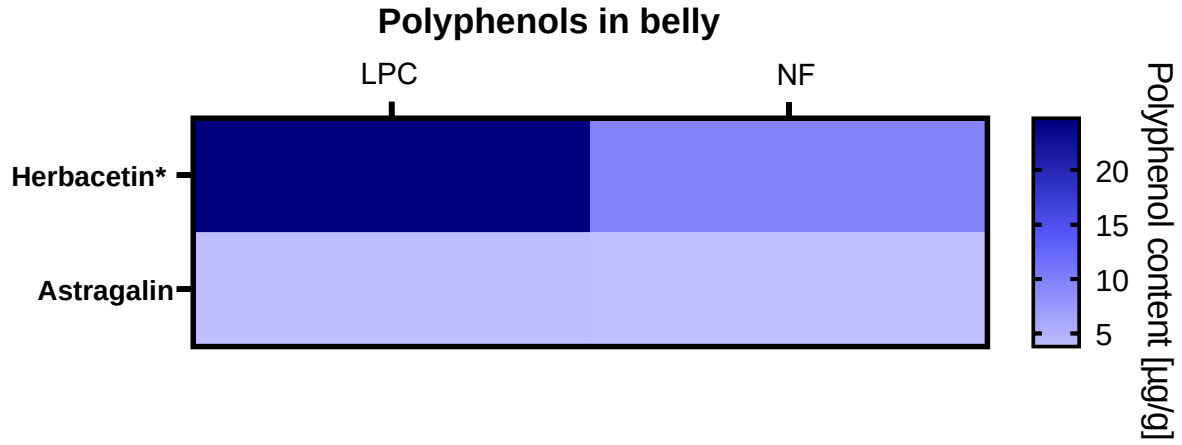


Figure 32: Polyphenol content of belly meat in the groups LPC and NF in µg/g measured by targeted LC-MS

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare LPC and NF loin, as well as LPC and NF belly, and results with a p-value lower than 0.05 (p ≤ 0.05 = *; p ≤ 0.01 = **; p ≤ 0.001 = ***) were considered statistically significant, based on biological replicates with n= 8 for LPC and n= 3 for NF group*

4.2 Effects of pig race

4.2.1 Primary lipid oxidation products

As shown in **Figure 33** the peroxide value (PV) of TP loin was 2.5 ± 1.3 meq O_2/kg fat and the PV of LR loin was 1.8 ± 0.5 meq O_2/kg fat. There was no significant difference between TP and LR loin. Furthermore, the peroxide value between TP and LR belly also did not show significant difference.

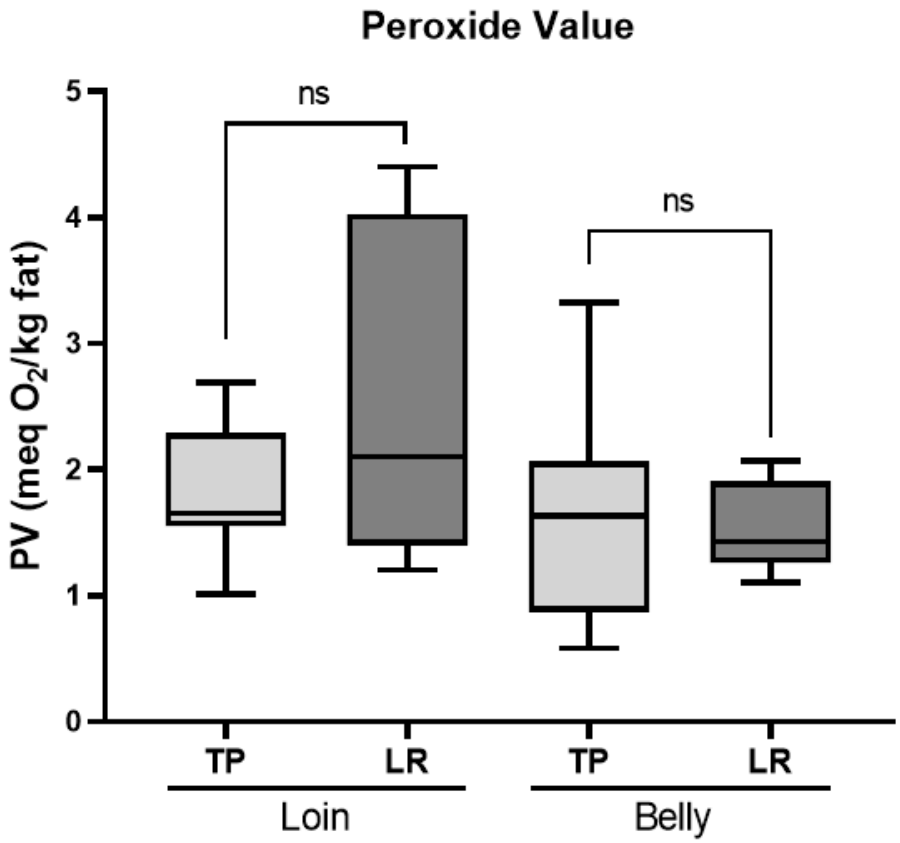


Figure 33: Peroxide value (PV) in meq O_2/kg fat of loin and belly meat in the groups TP and LR measured by IDF method

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups.*

4.2.2 Secondary lipid oxidation products

Eleven aldehydes and six alcohols were detected in samples and shown **in Figure 34**. The identified aldehydes were pentanal, hexanal, heptanal, octanal, nonanal, decanal, 2-heptanal, 2-octanal, 2-nonenal, 2-decanal und 2,4-decadienal. The alcohols 1-pentanol, 1-hexanol, 1-heptanol and 1-octanol, 1-octen-3-ol und 2,3-octandione were detected in the

samples. The result is presented as fold change between LR and TP. There was around 3.0 fold more 2-decanal in LR loin in comparison to TP loin. Additionally, there was around 2.1 fold more octanal and 3.5 fold more 1-octen-3-ol in LR belly in comparison to TP belly. For all these compounds a significant difference was observed between the groups. However, 2,4-decadienal, 1-pentanol and 2,3-octandione were under the LOQ in belly samples.

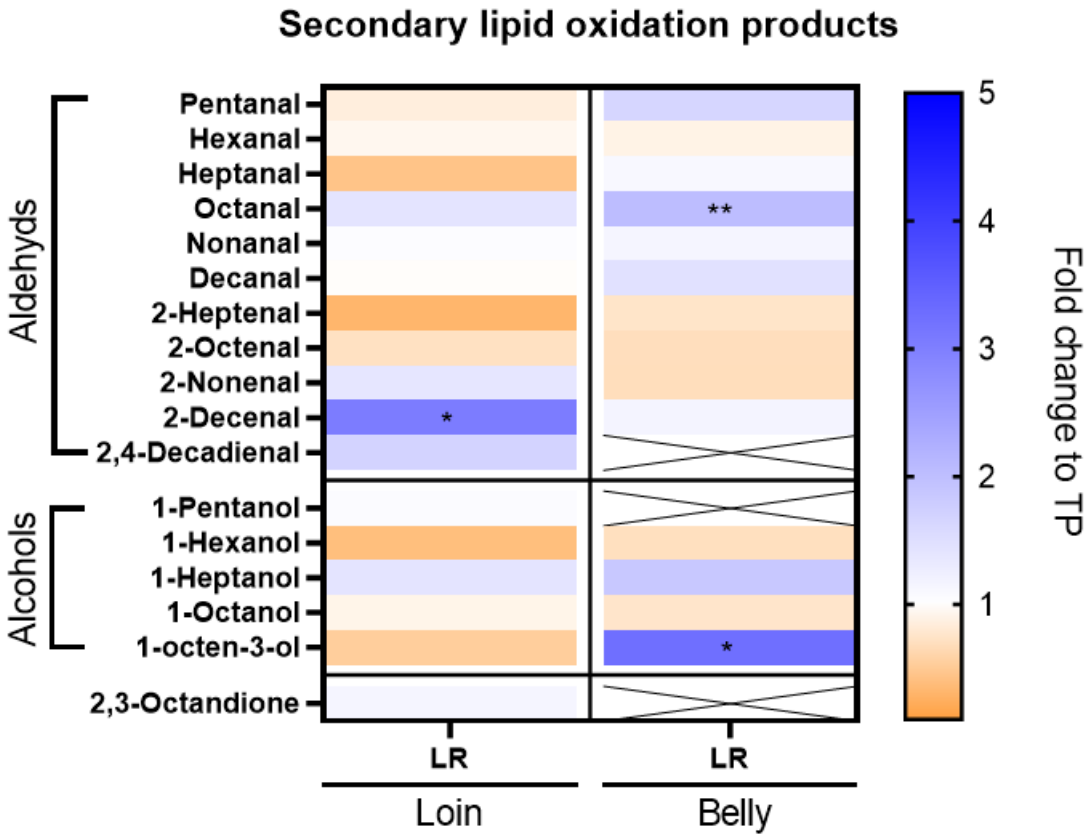


Figure 34: Fold change of normalized peak area of secondary lipid oxidation products in loin and belly meat in the groups LR and TP measured by GC-MS. The peak area of the detected secondary lipid oxidation products of LR was compared to the peak area of the detected secondary lipid oxidation products of TP.

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant based on biological replicates with n= 8 for all groups. A crossed box indicates values below the LOQ.*

4.2.3 Fatty acid profile and fatty acid content

The fatty acid profile and content for the TP and LR groups were also carried out using GC-FID. There was no significant difference of palmitic acid content in TP and NF loin groups as well as in TP and NF belly groups (see **Figure 35**).

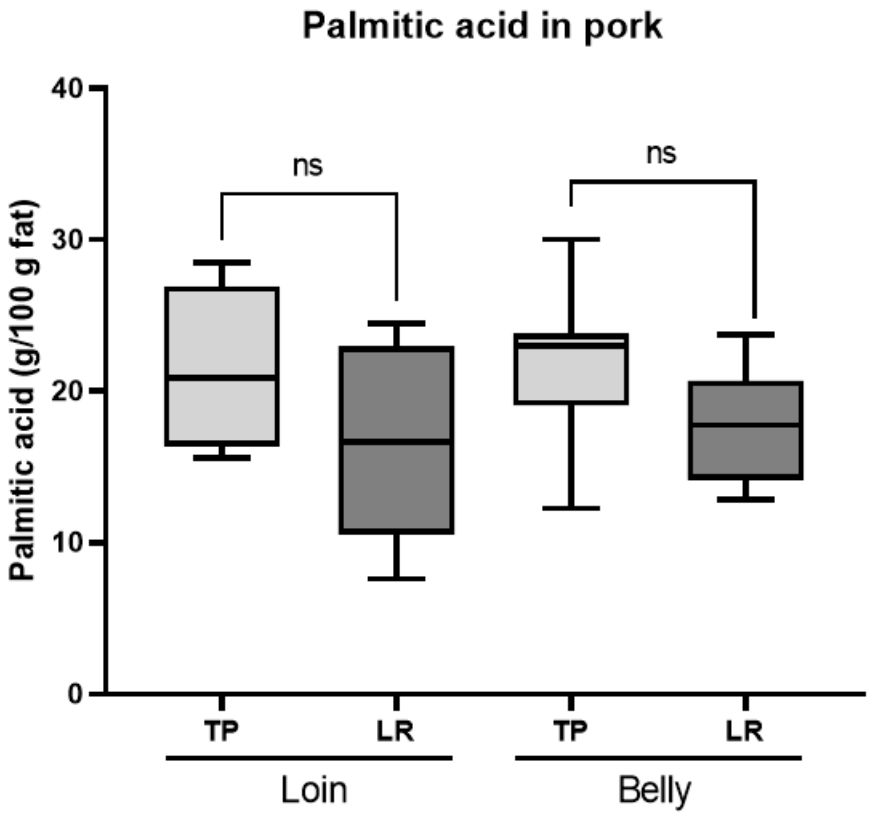


Figure 35: The content of palmitic acid in g/100 g fat of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

In **Figure 36** the stearic acid content in TP and NF loin samples did not show significant difference. Additionally, in TP and NF belly samples no significant difference was found as well.

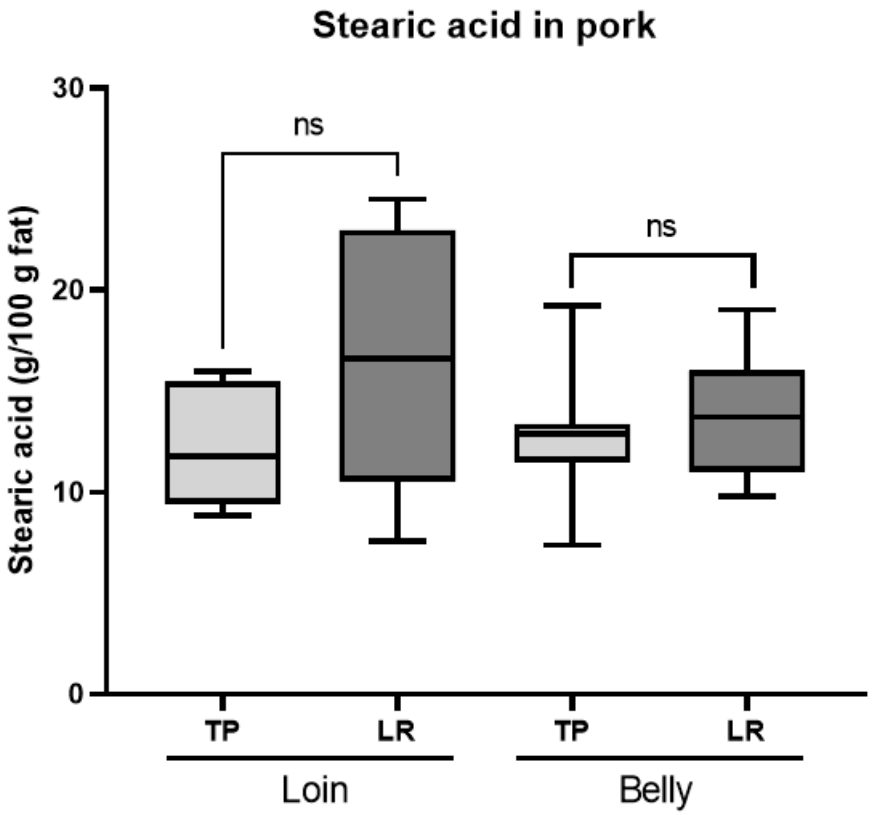


Figure 36: The content of stearic acid in g/100 g fat of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

As shown in **Figure 37** a significantly higher oleic acid content was identified in TP pork belly samples. The amount of oleic acid in TP belly was 48.82 ± 11.27 g/100 g fat compared to LR belly with 34.72 ± 8.54 g/100 g fat oleic acid. However, there was no significant difference within TP and LR loin samples.

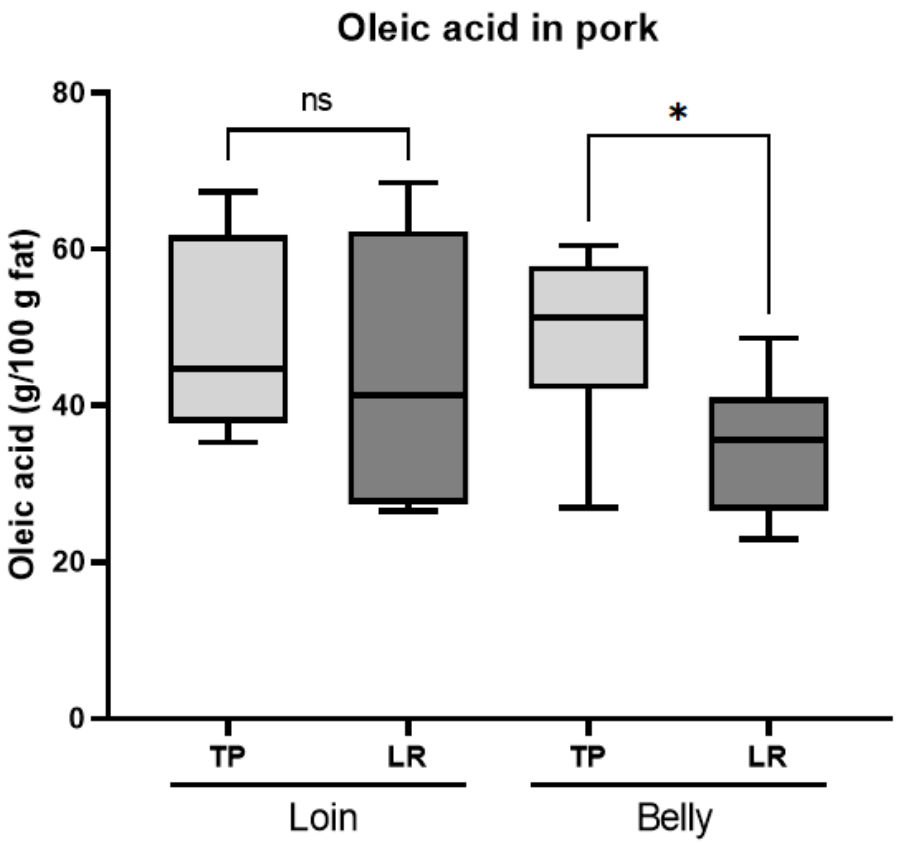


Figure 37: The content of oleic acid in g/100 g fat of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

The **Figure 38** shows the linoleic acid content in all loin and belly samples from TP and LR. However, within TP and LR loin as well as within TP and LR belly no significant difference was obtained.

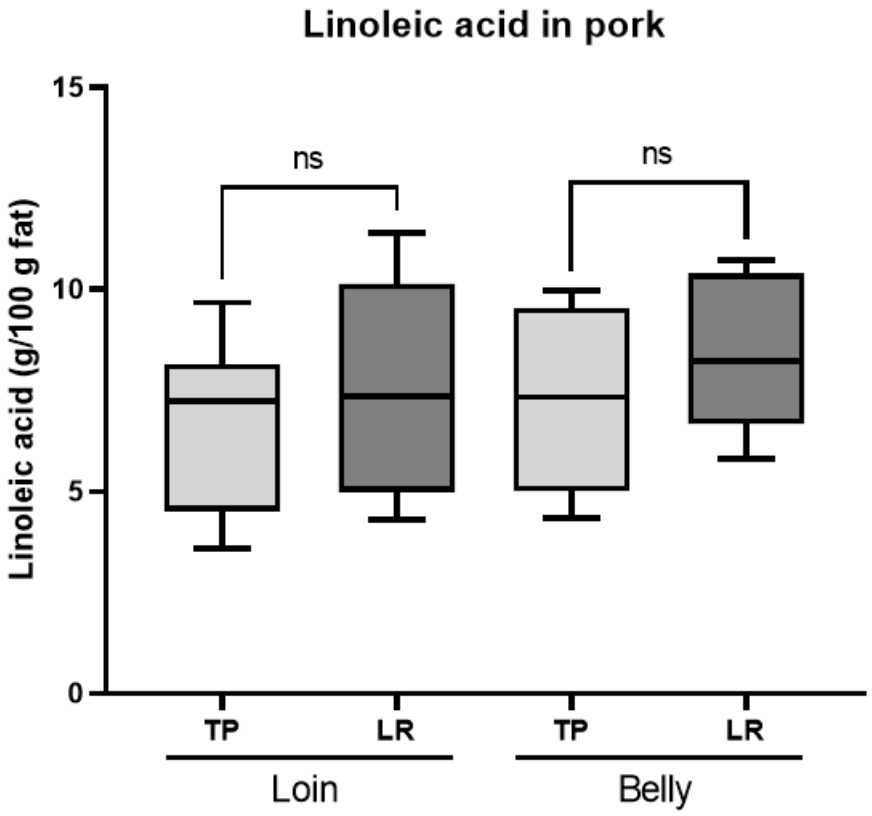


Figure 38: The content of linoleic acid in g/100 g fat of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

Figure 39 shows the significantly higher alpha-linolenic acid in belly from TP (1.82 ± 1.79 g/100 g fat) compared to belly from LR (0.43 ± 0.09 g/100 g fat). In TP and LR loin, both groups showed very low concentration of alpha-linolenic acid, with no significant difference.

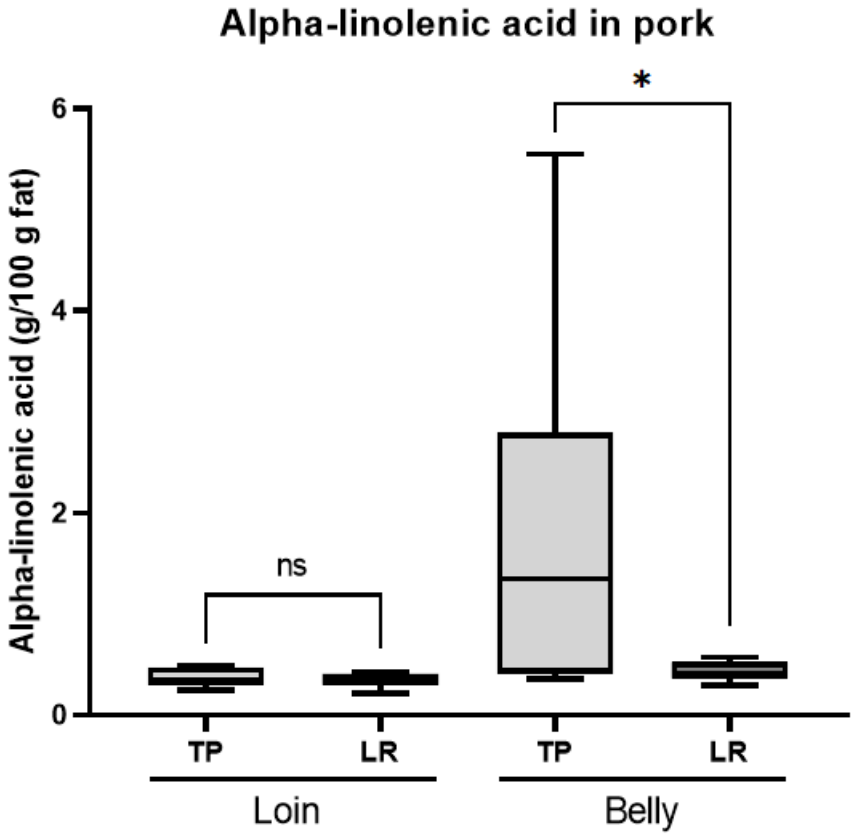


Figure 39: The content of alpha-linolenic acid in g/100 g fat of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

In **Figure 40** arachidonic acid concentration in pork samples are shown. The significant higher arachidonic acid content was found in LR belly (0.47 ± 0.17 g/100 g fat) in comparison to TP belly (0.27 ± 0.12 g/100 g fat). Even higher significant difference was identified in loin samples, with 0.80 ± 0.38 g/100 g fat in LR loin and 0.28 ± 0.05 g/100 g fat in TP loin.

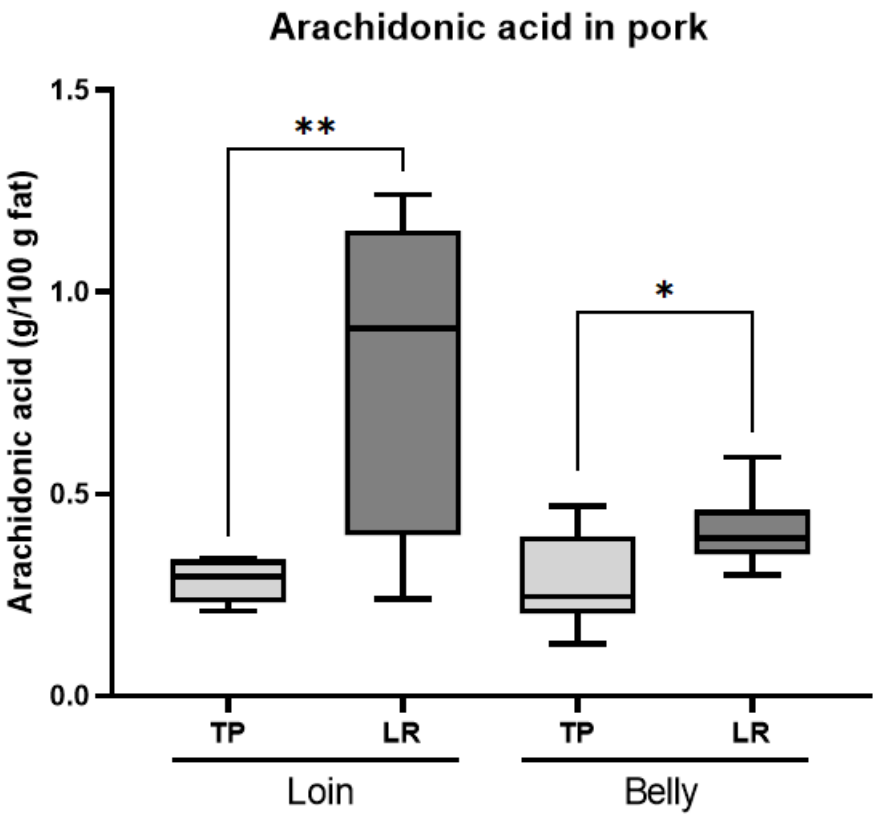


Figure 40: The content of arachidonic acid in g/100 g fat of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

As shown in **Figure 41** there was no significant difference in TP and LR loin as well as in TP and LR belly samples.

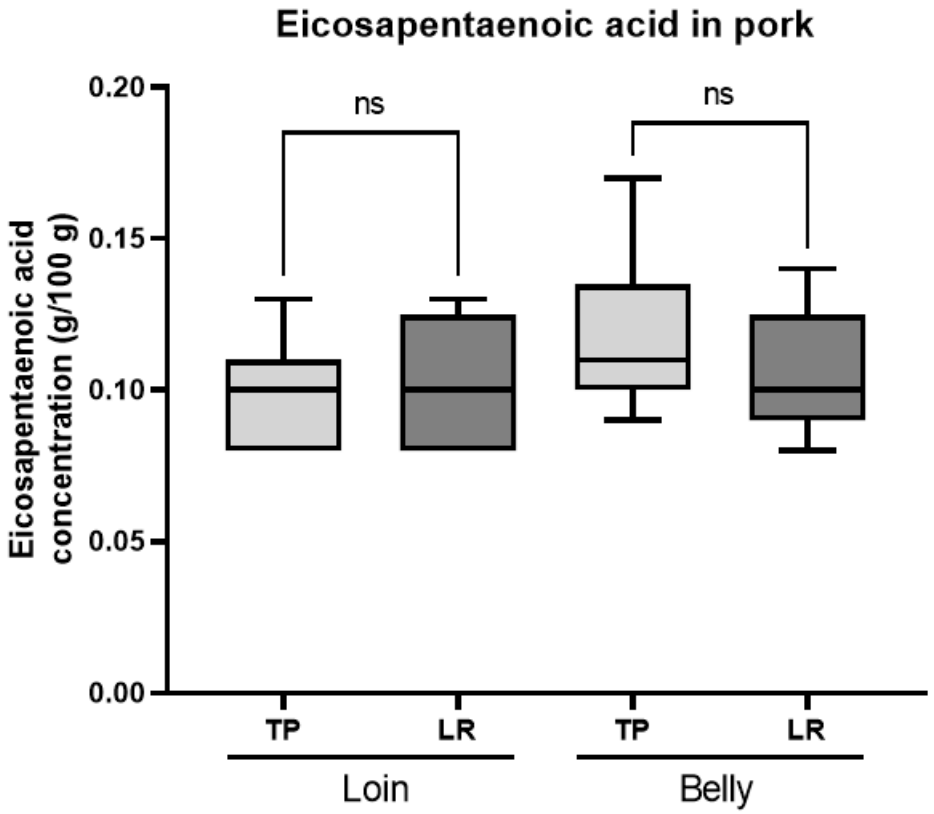


Figure 41: The content of eicosapentaenoic acid in g/100 g fat of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

In **Figure 42** there was no significant difference in the n6:n3 ratio in TP and LR loin samples. However, the n6:n3 ratio between TP and LR belly showed significant difference. The n6:n3 ratio in LR belly (16.97 ± 2.65) was more than two times higher as in the TP belly samples (7.51 ± 5.64).

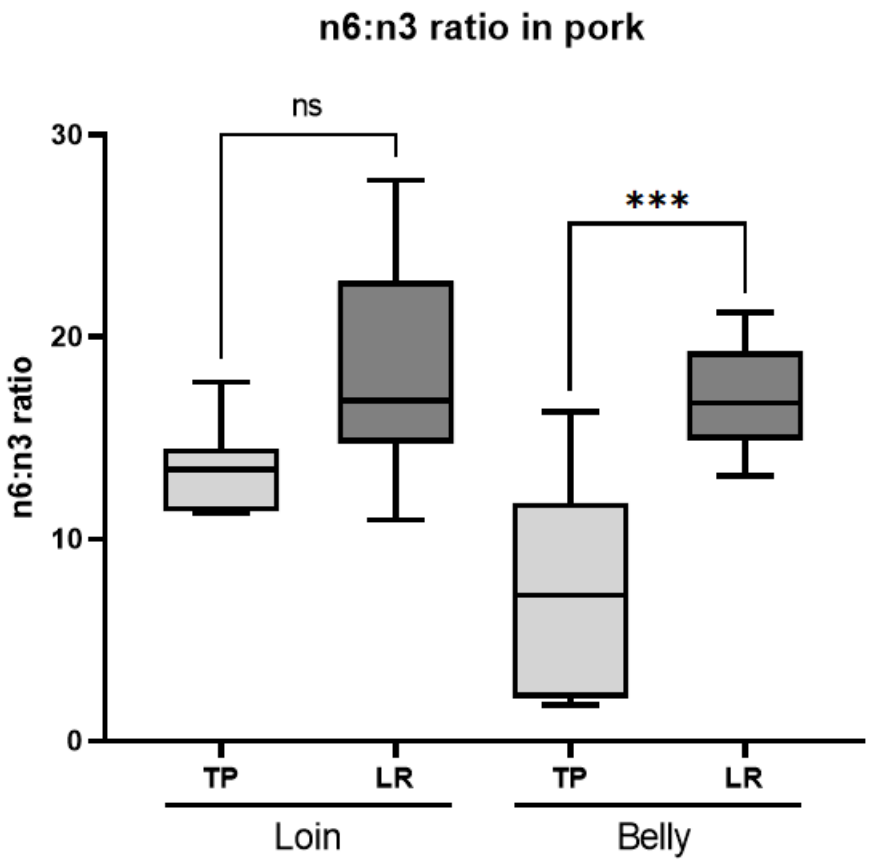


Figure 42: n6:n3 ratio of loin and belly meat in the groups TP and LR measured by GC-FID

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 (p ≤ 0.05 = *; p ≤ 0.01 = **; p ≤ 0.001 = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

4.2.4 Carbonyl protein content

Figure 43 shows the carbonyl protein content in LR and TP loin samples, but also LR and TP belly samples. Between LR and TP loin groups as well as between LR and TP belly groups a significant difference was not identified.

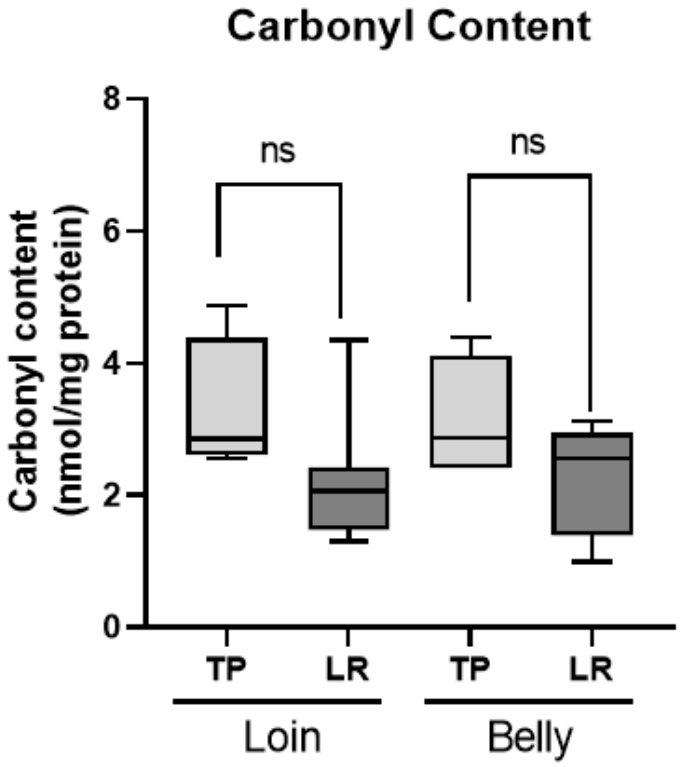


Figure 43: Carbonyl content of loin and belly meat in the groups TP and LR in nmol/mg protein measured by DNPH assay

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

4.2.5 Schiff bases/carbonyl-amin adduct content

As shown in **Figure 44** there was a significantly higher normalized fluorescence intensity in TP loin (319.82 ± 164.25) compared to LR loin (135.32 ± 44.03), resulting in more than two times higher normalized fluorescence intensity in TP loin in comparison to LR loin. In TP and LR belly samples a significant difference was not found.

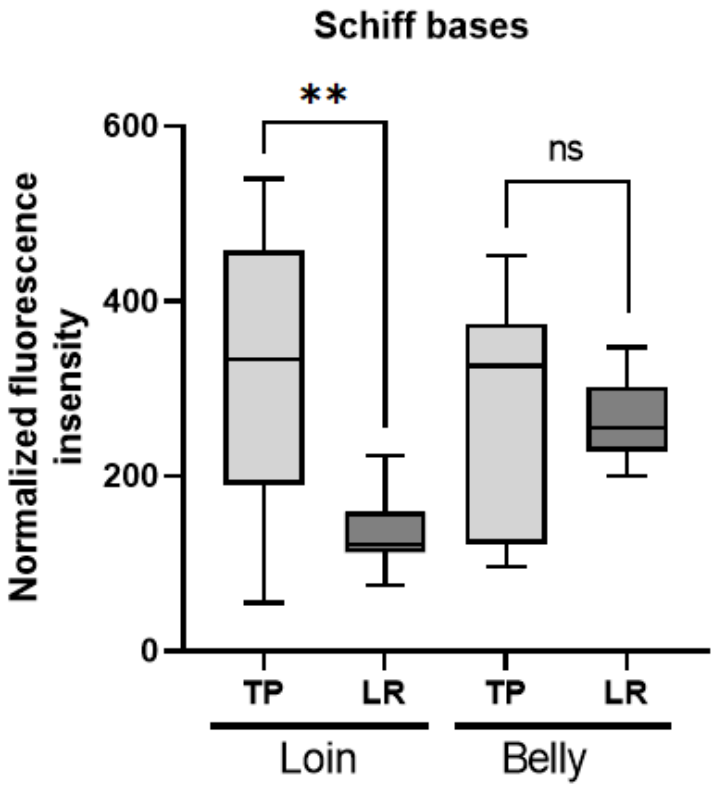


Figure 44: Normalized fluorescence intensity of loin and belly meat in the groups TP and LR measured by fluorescence spectrometry. The protein content was determined by BCA and the fluorescence intensity was normalized by the protein content

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

4.2.6 Tocopherol content

In **Figure 45** it is shown that there is no significant difference in the amount of α-tocopherol between TP and LR loin as well as between TP and LR belly groups. However, γ-tocopherol was found in TP loin in the amount of 0.74 ± 0.34 µg/g FW meat, while all LR loin samples were under the LOD (see **Figure 46**). Additionally, there was no significant difference in γ-tocopherol between TP and LR belly.

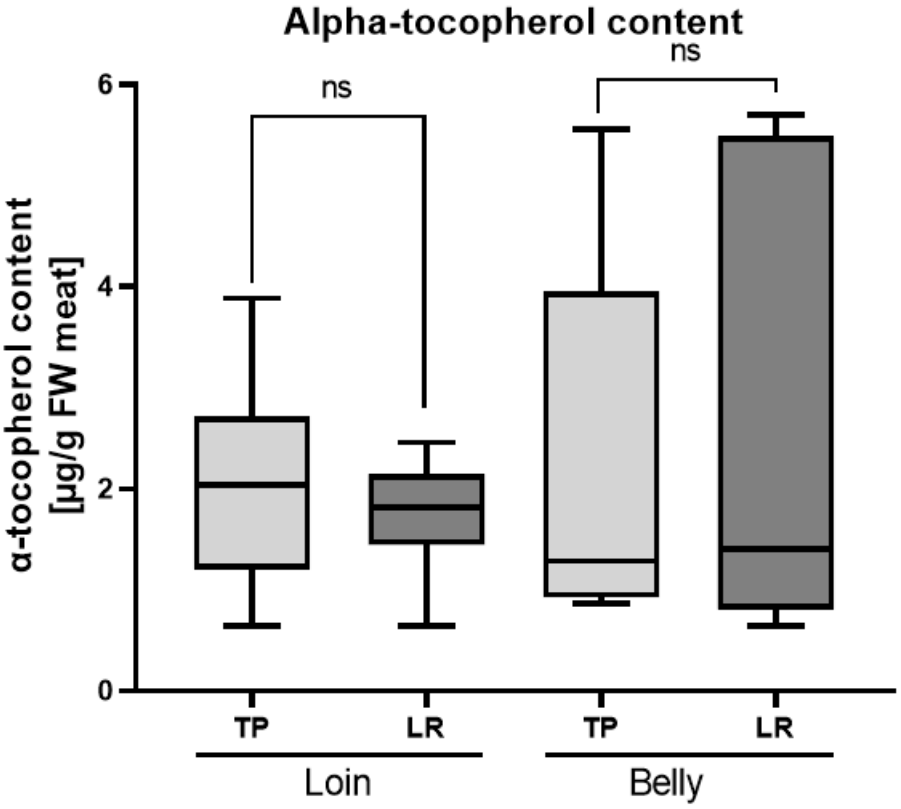


Figure 45: α-Tocopherol content of loin and belly meat in the groups TP and NF in µg/g fresh weight (FW) meat measured by HPLC-UV

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

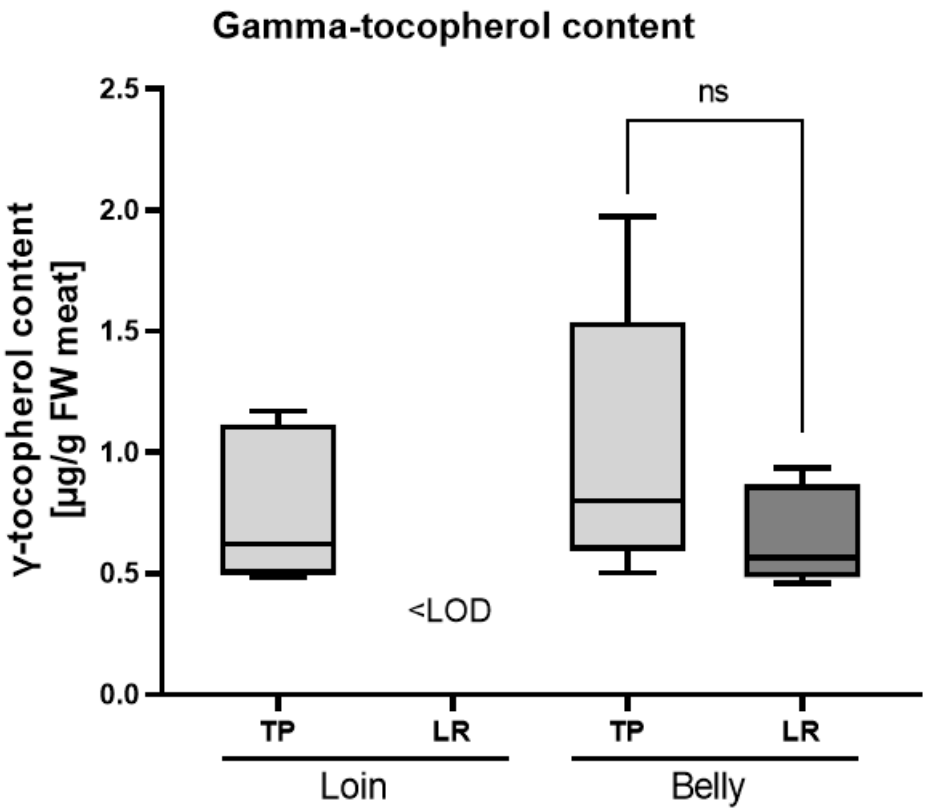


Figure 46: γ-Tocopherol content of loin and belly meat in the groups TP and NF in µg/g fresh weight (FW) meat measured by HPLC-UV

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 ($p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***) were considered statistically significant. Data are presented as boxplots showing the median, with the box spanning Q1-Q3 and whiskers extending from the box, based on biological replicates with n= 8 for all groups*

4.2.7 Polyphenol content

The polyphenols were only able to be detected and quantified in TP loin and belly samples. In the LR loin and belly samples the content of all polyphenols was under the LOD. In the TP loin, the amount of chalcone concentration was 3.04 ± 1.57 µg/g and the amount of chlorogenic acid was 4.55 ± 1.15 µg/g (see **Figure 47**). Additionally, in TP belly samples (see **Figure 48**) chalcone was also identified (1.75 ± 1.49 µg/g), but instead of chlorogenic acid, which was not found, herbacetin was quantified (12.47 ± 6.52 µg/g).

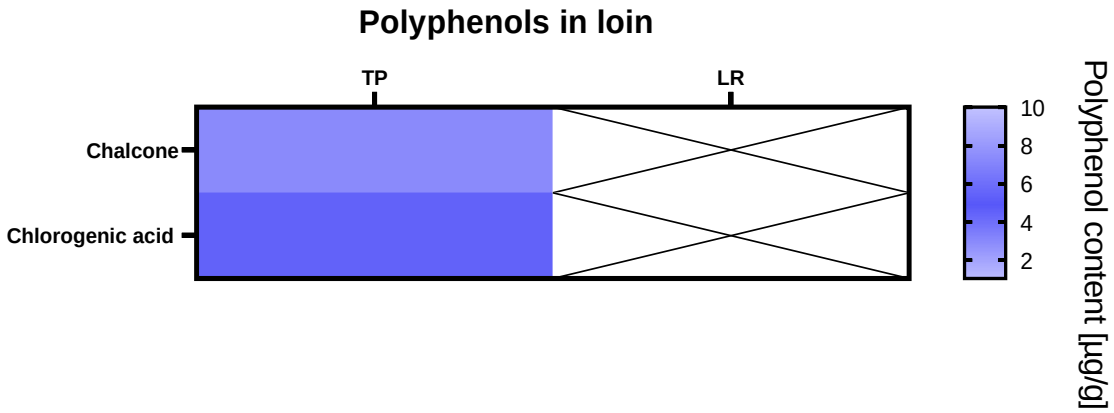


Figure 47: Polyphenol content of loin meat in the groups TP and LR in µg/g measured by targeted LC-MS

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 (p ≤ 0.05 = *; p ≤ 0.01 = **; p ≤ 0.001 = ***) were considered statistically significant, based on biological replicates with n= 8 for all groups*

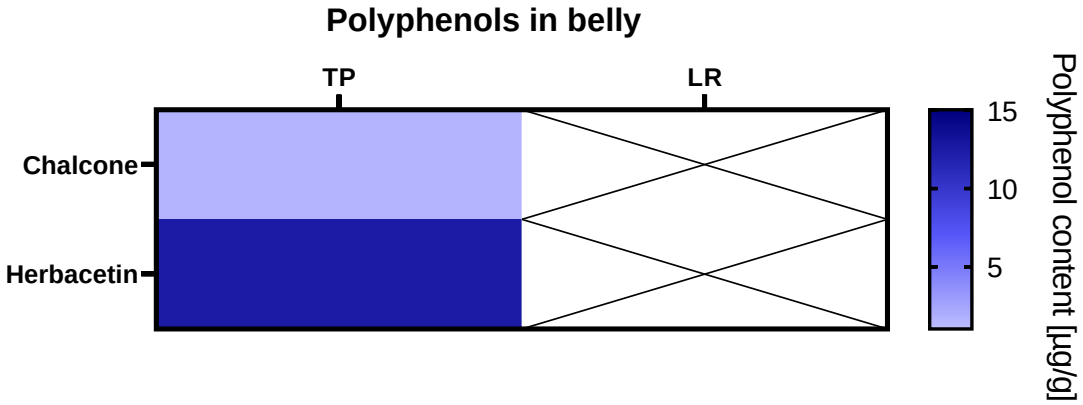


Figure 48: Polyphenol content of belly meat in the groups TP and LR in µg/g measured by targeted LC-MS

*Using a Grubbs outlier-test the outliers were identified. Normality and lognormality were assessed using the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value test. An unpaired parametric t-test was used to compare TP and LR loin, as well as TP and LR belly, and results with a p-value lower than 0.05 (p ≤ 0.05 = *; p ≤ 0.01 = **; p ≤ 0.001 = ***) were considered statistically significant, based on biological replicates with n= 8 for all groups*

5 Discussion

5.1 Fatty acid profile and fatty acid content

Pork fat has a very important role in various pork products by contributing to texture and flavor. Modifying animal feed has been an effective strategy for improving fatty acid composition and quality of pork meat and pork fat. Various kinds of supplementation such as vitamin E or polyphenols can also reduce the lipid and protein oxidation and improve stability of fatty acids (Górska et al., 2024). Due to targeted feeding strategies, there is a possibility to enrich pork meat with n-3 polyunsaturated fatty acids (PUFA). This can be carried out using different feed supplementation such as fish oil, flax seed oil or as in this thesis linseed press cake. However, the effect of the feed supplementation on meat and fatty acid profile can depend on different factors including, duration of the supplementation and the portion of the supplementation (Shimizu & Iwamoto, 2022).

According to Shimizu & Iwamoto, (2022) the linseed supplementation resulted in a significant increase in saturated fatty acids and in n-3 polyunsaturated fatty acids. However, the content of palmitic and stearic acid (see **Figure 20** and **Figure 21**) only significantly increased in NF loin samples. This can occur through an increase in the synthesis under the influence of biologically active substances present in feed additive (Priss et al., 2025). However, research shows that the linseed supplementation has little or no effect on increase of monounsaturated fatty acids (MUFA), especially oleic acid. No significant increase of oleic acid occured between LP and NF samples. Only arachidonic acid in NF loin samples showed significantly higher concentration compared to LPC loin samples (see **Figure 25**) (Tognocchi et al., 2023).

Furthermore, for linseed supplementation the main goal is to increase the content of n-3 polyunsaturated fatty acids. Guillevic et al., (2009) stated that the concentration of alpha-linolenic acid in raw pork chops fed with 4.2% linseed was 4.16 ± 0.23 g/100 g fatty acids compared to control samples with 0.65 ± 0.23 g/100 g fatty acids. In LPC loin samples the concentration of alpha-linoleic acid was 2.87 ± 0.74 g/100 g fat, which is a nearly three times higher concentration compared to NF loin samples (0.92 ± 0.33 g/100 g fat. Additionally, pork belly samples also showed nearly the same increase (see **Figure 24**). The concentration of eicosapentaenoic acid in all LPC and NF loin and belly samples did not show significant increase. This often occurs in pork due to low conversion rates from alpha-linolenic acid to long chain PUFAs including eicosapentaenoic acid (Karolyi et al., 2012). According to Tognocchi et al., (2023) and Karolyi et al., (2012) the linseed supplementation always improves the n6:n3 in various pork cuts, including pork belly and pork chops (loin). The n6:n3 ratio in linseed supplemented pork loin showed the value of

4.6 ± 2.7 compared to non-supplemented pork loin with a value of 17.4 ± 1.5 (Karolyi et al., 2012). In LPC loin samples the n6:n3 ratio were 2.87 ± 0.74 whereas NF loin a value of 7.75 ± 2.03 was detected. Linseed press cake feeding also lowered the saturated fatty acid content in loin samples. Also, the LPC belly samples showed significantly improved n6:n3 ratio. According to Simopoulos, (2002) the optimal n6:n3 ratio ranges from 1:1 to 4:1, showing beneficial effects on human health, especially against chronic diseases, such as cardiovascular disease, autoimmune disease and cancer (Simopoulos, 2002).

However, linseed press cake supplementation resulted in a significant increase in α-linolenic acid content in pork meat. Nevertheless, according to the European Food Safety Authority and EU regulation (EG) Nr. 1924/2006, the requirement for labeling food products as a “source of omega-3 fatty acids” (minimum 0.3 g α-linolenic acid per 100 g and per 100 kcal of food product, including meat) was not achieved. An additional factor to consider is that omega-3 fatty acids are preferentially stored in fatty tissues, whereas consumer preference generally favors lean meat, which may further limit the effective omega-3 content in pork products.

In comparison between TP and LR samples some significant differences in fatty acid profile were also observed. There is a significant higher content of arachidonic acid in LR loin and belly samples compared to TP samples (see **Figure 40**). Arachidonic acid concentration can be reduced using dietary modifications such as limiting linoleic acid or adding omega-3-rich supplements (Šottníková-Zajacová, 2008). Additionally, alpha-linolenic acid in TP belly showed significantly higher value (1.82 ± 1.79 g/100 g fat) compared to LR belly (0.43 ± 0.09 g/100 g fat). This can indicate that this particular meat cut may have greater potential to absorb alpha-linolenic acid, or alternatively, alpha-linolenic acid may have been better protected from oxidation in this tissue (Popova & Nakev, 2019). The n6:n3 ratio in TP belly samples (7.51 ± 5.64) was significantly lower than that observed in LR belly samples (16.97 ± 2.65), primarily due to the higher α-linolenic acid content in TP belly.

Considering that both TP and LR pigs were fed the same conventional diet, it can be concluded that TP pigs exhibit a greater capacity for α-linolenic acid storage, which also appears to be dependent on the pork cut. The α-linolenic acid concentration and the n6:n3 ratio in TP belly samples could also be interpreted critically. The last four TP belly samples showed markedly higher α-linolenic acid concentrations compared to the first four samples, which is reflected in the high standard deviation. In contrast, the α-linolenic acid levels in the first four TP belly samples were more comparable to those observed in LR belly samples. This pattern may indicate that the last four TP pigs had access to feed containing higher amounts of omega-3 fatty acids, such as pasture or green feed. Additionally, the low sample size (n = 8 per group) and the lack of strict control over individual feed intake,

represent further limitations that may have influenced the results. Due to the antioxidants levels, the antioxidative status of these samples was likely improved, which may explain why no significant differences were observed in peroxide values and protein carbonyl content (Yuan et al., 2022).

5.2 Tocopherol content

According to M. Chen et al., (2025), vitamin E is predominantly found in animal tissues, where it cannot be synthesized and must be obtained through the diet. Alpha-tocopherol, the primary form of vitamin E, is particularly beneficial due to its strong antioxidant activity, which helps protect cells and tissues from oxidative damage. Gamma-tocopherol also exhibits strong antioxidant capacity, but due to the limited number of available studies, it is not commonly used as a dietary supplement in animal nutrition (Es-Sai et al., 2025). Supplementation of animal feed with vitamin E leads to increased vitamin E content in muscle tissue during postmortem phase. The level of vitamin E accumulation depends on the muscle type, the administered dose and the duration of supplementation. It contributes to optimal quality of pork meat and meat products (Ilie et al., 2017).

Högberg et al., (2003) demonstrated that no significant differences in the α- and γ-tocopherol concentrations were observed between organic and conventional pork feeding systems. However, muscle tissue from female pigs contained significantly higher levels of α-tocopherol compared with that of castrated males. A possible explanation for the concentrations of both tocopherols being below the limit of detection in the LPC and NF samples is that the 10% linseed press cake may not have provided sufficient vitamin E. In the NF group, it could be suggested that Turopolje-pigs may have had a limited capacity to absorb vitamin E efficiently.

In the TP and LR belly samples, typical concentrations of α- and γ-tocopherols associated with conventionally fed pigs were detected (see **Figure 45**). Pork generally contains only small amounts of vitamin E, typically ranging from 0.1 to 0.3 mg per 100 g. These levels are consistent with conventional feeding practices in the EU, where vitamin E is authorized as a feed additive for all animal species in accordance with current regulations, including the Regulation (EU) 2023/341.

Additionally, in LR loin samples the concentration of gamma tocopherol was under LOD (see **Figure 46**). Rey et al., (2006) explained that gamma-tocopherol accumulates preferentially in the backfat and adipose tissue, especially when animals consume natural diets rich in gamma-tocopherol. This may explain the absence of detectable gamma-

tocopherol in LR loin samples, while measurable concentrations were observed in the LR belly.

5.3 Polyphenolic content

According to Papuc et al., (2017) polyphenols possess beneficial properties for plants, animals and humans. Polyphenols are also highly valuable to the meat industry because of their advantageous effects, including antioxidant and antimicrobial effects. Due to their multiple mechanisms of action, they help preserve the color of meat, inhibit the growth of foodborne bacteria and maintain meat quality. Liu et al., (2021), Papuc et al., (2017) and Amrit et al., (2023) reported that feeding pigs a polyphenol-rich diet may enhance growth performance and increase antioxidant enzyme activity. Such diets can include a variety of seeds, such as linseed or its byproducts (e.g., linseed press cake), as well as different plant oils like rosemary oil or pasture feed.

This study revealed a significantly higher total polyphenol content in LPC belly (28.7 ± 8.6 µg/g) compared with NF belly total polyphenol content (12.3 ± 2.2 µg/g). LPC loin samples showed slightly higher total polyphenol content compared to NF loin, but without significant difference. Based on these results, it can be stated that linseed press cake supplementation positively increased the total polyphenol content. The most abundant polyphenols detected were herbacetin and astragalin, with herbacetin showing its highest concentration in LPC loin (60.6 ± 14.4 µg/g). However, a significant difference in herbacetin concentration was observed in LPC belly (24.7 ± 8.4 µg/g) compared to NF belly (9.8 ± 4.1 µg/g). These results are consisted with the findings of Fliniaux et al., (2014), who reported that the flavonol herbacetin diglucoside is a known constituent of linseed. The results also indicate that polyphenol concentration varies among different meat cuts due to differences in their tissue composition.

Between TP and LR samples, polyphenols were detected exclusively in the TP loin and belly samples, while no polyphenols were identified in the LR samples. In the TP loin, chalcone (3.04 ± 1.57 µg/g) and chlorogenic acid (4.55 ± 1.15 µg/g) were identified. In the TP belly samples, chalcone (1.75 ± 1.49 µg/g) and herbacetin (12.47 ± 6.52 µg/g) were quantified. One possible explanation for these results is that, although both TP and LR pigs were reportedly fed conventional feed without grass feeding or dietary supplements, Turopolje-pigs may possess a greater capacity for polyphenol accumulation or storage compared to landrace pigs, due to their genome differences (Margeta et al., 2006). This potential appears to be dependent on the pork cut, as differences were observed between loin and belly samples.

However, these finding should be considered critically in light of the limitations of the master´s thesis. It cannot be fully excluded that TP pigs had access to green feed, which could have contributed to the higher concentrations observed in TP loin and belly samples. Additionally, the relatively small sample size (n = 8 per group) represents a further limitation. The feed intake per pig was not strictly controlled, meaning that individual differences in feed consumption could have influenced polyphenol levels in the analyzed samples. It is also important to note that the higher polyphenol levels may explain why no significant differences were observed in peroxide values and protein carbonyl content between the TP and NF groups, as the polyphenols (in TP samples) possess antioxidant properties that can inhibit lipid and protein oxidation (Bertelli et al., 2021). However, no polyphenols were found in LR samples, which may have contributed to increased values in secondary oxidation products, possibly due to reduced lipid protection.

5.4 Primary lipid oxidation products

As stated by X. Huang et al., (2024) because of the unsaturated bonds, polyunsaturated fatty acids are more susceptible to oxidation compared to saturated fatty acids. The oxidative stability of pork could also be influenced by different factors such as race, feed, animal husbandry, meat cut and storage. However, in this paper there was no significant difference in the amount of lipid oxidation products in longissimus dorsi (pork loin) of various pig races, including Duroc, Yorkshire and land race pigs.

In **Figure 33** there was no significant difference between Turopolje-pigs (TP) and land race pigs (LR), in both cuts, including pork loin and pork belly. According to these results and the results from X. Huang et al. (2024), there is no meat quality difference referring to primary lipid oxidation between Turopolje-pigs and land race pigs, but also between other races included in the paper. As shown in **Figure 18** there was also no significant difference in primary lipid oxidation products comparing LPC and NF pigs. Nevertheless, there are also the possibility that the primary lipid oxidation products, due to their instability, degradation or further chain reaction, reacted to secondary lipid oxidation products (Domínguez et al., 2019).

Additionally, various antioxidants could have contributed to slowing down lipid oxidation, including alpha- und gamma-tocopherol. According to Houben et al. (1998), the minced meat from pigs fed diet, enriched with vitamin E, showed significantly higher resistance to lipid oxidation than meat from normal diet pigs. In TP and LR samples an increased amount of alpha- und gamma-tocopherol was found, which could help to slow down the oxidation reactions.

In the TP, LPC and NF samples an increased amount of polyphenols was found. Cimmino et al., (2018) explained that due to antioxidant ability of polyphenols, lipid oxidation can be reduced, especially because of the chelating potential, where they are capable of binding iron at the gastrointestinal level and isolate it for absorption.

5.5 Secondary lipid oxidation products

According to Shimizu & Iwamoto, (2022) and Park et al., (2007) hydroperoxides can decompose and produce a wide spectrum of volatile compounds, including aldehydes, ketones, hydrocarbons and alcohols. These substances are named as secondary lipid oxidation products. The production of volatile compounds can be affected by pig race, diet, packaging method, meat cut, storage time, and fatty acid composition.

As stated by Duan et al., (2023) and Wang et al., (2023) the alcohols, including 1-pentanol, 1-octanol, and 1-octen-3-ol are commonly detected in pork meat. These compounds were also found in the present samples (see **Figure 19** and see **Figure 34**) and can be formed through the degradation and oxidation of oleic acid. Aldehydes such as hexanal, octanal, nonenal and decanal are mostly produced by oxidation of PUFAs. Both of these compound groups can influence the meat aroma and smell (García-Márquez et al., 2013). There was around 3.0 fold more 2-decanal, 2.1 fold more octanal and 3.5 fold more 1-octen-3-ol in LR samples compared to TP (see **Figure 34**), that showed a significant difference between samples. This can be explained by different fatty acid composition between TP and LR pigs, especially due to significantly different n6:n3 ratio in TP and LR belly. This suggests that groups with higher concentration of omega-3 fatty acids, such as alpha-linolenic acid, may generate higher levels of alcohols as secondary oxidation products, whereas samples richer in omega-6 fatty acids, may show increased formation of aldehydes (Grebenteuch et al., 2021). Additionally, the pig race can also play a significant role in production of secondary lipid oxidation products, primarily due to differences in fatty acid composition, intramuscular fat, and endogenous antioxidant capacity (Huang et al., 2025). Wang et al., (2023) also explained that 1-octen-3-ol is associated with mushroom-like aroma. It is a key contributor for odor smell but also the typical flavor for cooked meat.

5.6 Carbonyl protein content

As Günal-Köroğlu et al.,(2025) reported, protein oxidation is an important characteristic to investigate the oxidative protein damage, but also the shelf life and quality of meat and meat products. According to Hernández-López et al., (2016) there was a significant effect of dietary avocado on the carbonyl content of cooked and chilled pork loins, resulting in a significantly lower carbonyl protein content compared to normal diet. Other authors reported

no significant effect of antioxidant-rich diets on carbonyl levels in cooked pork, which can also be observed in **Figure 28** between LPC and NF samples (Haak et al., 2008; Botsoglou et al., 2012). Protein oxidation is a complex mechanism, which is not limited to the formation of carbonyl groups. As proteins undergo oxidative stress, secondary reactions can occur, which can be a reason, why no significant difference was observed between all samples (Chelh et al., 2007a).

However, Wu et al., (2025) showed that the polyphenols such as chlorogenic acid and gallic acid demonstrated antioxidant properties that reduced protein carbonylation. Chlorogenic acid was found in TP loin samples, which is a possible reason for why there was no significant increase of carbonyl protein content in TP loin samples.

Popova et al., (2015) stated that vitamin E supplementation can have some effects on protein oxidation in fresh and cooked samples, but significant differences were observed only in one meat cut, so it is uncertain, if vitamin E can be successfully used for increased protein oxidation stability. However, in this study no significant difference between LPC and NF as well as between TP and LR was observed.

5.7 Schiff bases/carbonyl-amin adduct content

Amino acids, which contain amino groups in their side chains, can undergo oxidation leading to the formation of carbonyl groups. Aldehydic by-products of lipid oxidation can also react with amino groups on protein side chains, forming fluorescent Schiff bases. Chemical oxidation by oxygenated radicals promotes the formation of Schiff bases in myofibrils. This can degrade the quality of meat and its nutritional value (Chelh et al., 2007).

According to Adeyemi et al., (2016), protein oxidation is connected with the protein content and amino acid composition, which varies between different meat cuts. There was a significantly higher normalized fluorescence intensity in LPC belly (410.06 ± 146.65) compared to normalized fluorescence intensity in NF belly (258.11 ± 155.42). These results indicate that protein oxidation was more pronounced in the belly samples of pigs fed linseed press cake. Nevertheless, slightly lower protein oxidation, but without significant difference, was observed in LPC loin samples compared to NF loin samples.

Additionally, there was also significantly higher normalized fluorescence intensity in TP loin (319.82 ± 164.25) compared to LR loin (135.32 ± 44.03), where it can be observed, that more protein oxidation occurred in Turopolje-pigs loin samples. Among the belly samples, slightly higher protein oxidation was observed in Turopolje pigs compared with landrace pigs.

In all four pork groups, no significant differences in protein carbonyl content were observed among the individual groups or meat cuts. This may be explained by the fact that protein oxidation does not result solely in the formation of carbonyl protein; rather, oxidative reactions may continue, leading to the formation of Schiff bases (Chelh et al., 2007a).

Another possible explanation is that the protein oxidation may vary among different meat cuts as well as between pig breeds, but due to the limited number of available studies, this cannot be stated with certainty (Adeyemi et al., 2016). Consequently, the reasons why these results do not correlate with carbonyl protein levels remain unclear.

Furthermore, Wu et al., (2025) demonstrated that polyphenols exhibit antioxidant properties that reduce protein carbonylation. Accordingly, slightly lower protein oxidation was observed in LPC loin samples compared with NF loin samples, suggesting that polyphenols may have exerted a protective effect against protein oxidation in the LPC loin.

6 Conclusion

In this master´s thesis, pork loin and pork belly samples from four different experimental groups were analyzed. The research was divided into two main experimental parts. In the first part, both experimental groups consisted of Turopolje-pigs raised under organic farming conditions. One of the groups was additionally supplemented with 10% linseed press cake. The primary objective was to assess the impact of linseed press cake supplementation on the fatty acid profile, antioxidant content, as well as protein and lipid oxidation. It was expected that supplementation with linseed press cake can result in the production of omega-3-enriched meat, thereby enhancing its nutritional value and potentially improving oxidate stability, while also promoting the efficient utilization of by-products from linseed oil production.

The second part of the study focused on the influence of pig breed. In this phase of the study, Turopolje-pigs and land race pigs were compared, with both groups raised under conventional farming conditions and fed standard diets. It was aimed to determine how genetic differences between pig breeds affect the fatty acid composition, antioxidant levels, and the extent of protein and lipid oxidation. It is hypothesized that the Turopolje-breed, owning to its inherent stress resilience, may modulate meat oxidative status and the integration of such breeds into production systems may contribute to sustainable agriculture and promotion of stress- and disease-resistant pig breeds.

Firstly, in the group receiving linseed press cake, a significantly higher content of alpha-linolenic acid was detected compared to the non-supplemented group. This increase positively affected the n6:n3 fatty acid ratio in the supplemented samples. Linseed press cake feeding also had a beneficial effect on reducing saturated fatty acid levels in pork loin. Overall, the results indicate that linseed press cake supplementation had a beneficial effect on the fatty acid profile of the pigs. When comparing Turopolje-pigs with land race pigs, higher amounts of arachidonic acid were observed in land race pigs, which also led to the deterioration of n6:n3 ratio in land race samples. In addition, higher levels of alpha-linolenic acid were found in belly samples. However, significantly higher levels of alpha-linolenic acid were only observed in the last four samples. Therefore, these results should be interpreted with caution.

The comparison of primary and secondary oxidations products between samples supplemented with linseed press cake and those without supplementation showed no significant differences, including peroxide values and levels of carbonyl protein oxidation. These results indicate, although omega-3 fatty acids are generally more prone to oxidation, increased oxidation did not occur in the supplemented samples. Similarly, no significant

differences were observed between Turopolje-pigs and land race pigs with regard to primary oxidation products, peroxide values or carbonyl protein oxidation levels. However, samples from land race pigs exhibited higher amount of certain secondary oxidation products, including octanal, 2-decanal, and 1-octen-3-ol. These compounds do not pose a risk to human health but may contribute to differences in meat odor and influence shelf life.

The results showed that a significant amount of Schiff bases was detected in belly samples from pigs supplemented with linseed press cake. In addition, when comparing Turopolje-pigs and land race pigs, higher levels of Schiff bases were found in loin samples from Turopolje-pigs. These findings indicate that Schiff base content may vary among different meat cuts as well as between pig breeds, and may reflect differences in meat freshness.

Another interesting finding was, that tocopherol levels in the samples supplemented with linseed press cake and non-supplemented samples were below the limit of detection. However, small amounts of α- and γ-tocopherol were detected in samples from both Turopolje- and land race pigs. This is likely attributable to their conventional feed and feed additives, which may slightly increase tocopherol levels in tissues. Furthermore, tocopherols are known to play a role in protecting lipids and protein from oxidative degradation, as supported by the observed results.

Linseed press cake supplementation proved to be an effective source for increasing polyphenol levels in pork samples. Additionally, organic and pasture-based feeding slightly increased polyphenol content. When comparing Tupolje- and land race pigs, no polyphenols were detected in land race pigs, whereas measurable levels were observed in Turopolje-pigs. This may indicate that Turopolje-pigs possess a greater capacity for polyphenol accumulation than landrace race pigs, possibly due to genetic differences.

In conclusion, the results suggest that linseed press cake supplementation positively influences the fatty acid profile, oxidative stability, and overall meat quality. However, the omega-3 content of the meat cuts was insufficient to meet the criteria for labeling pork as a “source of omega-3 fatty acids”. Linseed press cake supplementation also resulted in an increased polyphenol content, which contributed to the protection against oxidative reactions and may improve shelf life. Turopolje-pigs showed indications of a greater capacity for the polyphenol accumulation compared to land race pigs, but this effect was strongly dependent on meat cut and feeding regime, which also influenced tocopherol levels. The observed increase in omega-3 fatty acid concentration in some belly samples from TP should be interpreted with caution, as it may have been influenced by possible feed restrictions. Additionally, higher levels of arachidonic acid and secondary oxidation products

were observed in land race pork compared to Turopolje-pigs. Therefore, further research on Turopolje-pigs is required, including larger sample sizes and strictly controlled diets.

Overall, these finding demonstrate positive progress toward increasing omega-3 fatty acid content in pork, but additional studies are needed to optimize supplementation strategies, including the amount and the duration of omega-3 supplementation, as well as to evaluate meat quality parameters after cooking. Moreover, further investigation into the meat quality characteristics of Turopolje-pigs is warranted due to the limited existing data and their potential suitability for conventional pork production.

7 Abstract

A modern diet is often poor in omega-3 fatty acids despite the fact that these fatty acids are essential components with various benefits for human health. In Austria, pork is the most commonly consumed meat type. This high level of pork consumption underscores the interest in enriching pork meat with omega-3 fatty acids and evaluating other pig breeds for potentially beneficial nutritional properties that could be implemented in conventional production systems.

The increase in omega-3 fatty acids may affect meat quality, as these fatty acids are highly prone to oxidative degradation. However, the presence of antioxidants can help reduce oxidative reactions and protect meat quality. Feeding, slaughter conditions, and pig breed significantly shape the fatty acid profile, the levels of oxidation products, and the content of antioxidants in meat, all of which can greatly affect various quality characteristics. Due to limited research available, this study focused on analyzing pigs supplemented with linseed press cake compared with non-supplemented pigs under organic conditions, as well as comparing different breeds, including Turopolje-pigs and conventional land race pigs under conventional production systems.

In this thesis four groups of pork were investigated. Two groups were raised under organic conditions, with one group supplemented with linseed press cake and the other non-supplemented. The other two groups were raised under conventional conditions, including Turopolje-pigs and land race pigs. For each group, eight samples of pork loin and pork belly were analyzed. The study aimed to evaluate the effects of linseed pressed cake supplementation and pig breed on the fatty acid profile, oxidation products, tocopherol content and polyphenol levels. Samples were analyzed using GC-MS, GC-FID, HPLC-UV, and LC-MS, along with spectrophotometric and fluorescence techniques.

In the first part, the results indicated that the linseed press cake supplementation significantly increased omega-3 fatty acid content, decreased the saturated fatty acid content and improved the n6:n3 fatty acid ratio, without affecting peroxide value or protein carbonyl content. Schiff base levels were significantly higher in supplemented samples and varied by meat cut. Tocopherols were below the LOD in both groups, whereas polyphenols were detected in both groups with higher concentrations observed in linseed press cake-supplemented samples.

In the second part, Turopolje-pigs showed higher omega-3 fatty acid concentrations and lower arachidonic acid levels compared to land race pigs. No significant differences were observed in peroxide value or protein carbonyl content between breeds. However, secondary oxidation products were higher in land race pork. Schiff base levels were higher

in Turopolje-pigs and depended on meat cut. Tocopherols were detected in both groups, whereas polyphenols were only found in samples of Turopolje-pigs.

In conclusion, linseed press cake supplementation improved fatty acid profile, oxidative stability, and overall meat quality of pork. Although omega-3 levels increased, they were insufficient for “source of omega-3 fatty acids” labeling. Turopolje-pigs exihibited a higher capacity for polyphenol accumulation compared with land race pigs, although the effect was strongly influenced by meat cut.

8 Zusammenfassung

Eine moderne Ernährung ist häufig arm an Omega-3-Fettsäuren, obwohl diese Fettsäuren essenzielle Bestandteile mit vielfältigen positiven Wirkungen auf die menschliche Gesundheit sind. In Österreich ist Schweinefleisch die am häufigsten konsumierte Fleischart. Dieser hohe Konsum unterstreicht das Interesse, Schweinefleisch mit Omega-3-Fettsäuren anzureichern und zudem andere Schweinerasse hinsichtlich potenziell vorteilhafter ernährungsphysiologischer Eigenschaften zu evaluieren, die in konventionellen Produktionssystemen umgesetzt werden könnten.

Eine Erhöhung des Omega-3-Fettsäuregehalts kann jedoch die Fleischqualität beeinflussen, da diese Fettsäuren besonders anfällig für oxidative Abbauprozesse sind. Das Vorhandensein von Antioxidantien kann oxidative Reaktionen reduzieren und somit zur Erhaltung der Fleischqualität beitragen. Fütterung, Schlachtbedingungen und Schweinerasse beeinflussen maßgeblich das Fettsäureprofil, die Konzentration von Oxidationsprodukten sowie den Gehalt an Antioxidantien im Fleisch, was wiederum verschiedene Qualitätsmerkmale erheblich beeinflussen kann. Aufgrund der begrenzten Studienlage untersuchte die vorliegende Arbeit Schweine mit Supplementierung von Leinpresskuchen im Vergleich zu nicht supplementierten Tieren unter ökologischen Haltungsbedingungen sowie unterschiedliche Rassen, darunter Turopolje-Schweine und konventionelle Landrasse-Schweine, unter konventionellen Produktionsbedingungen.

In dieser Arbeit wurden vier Gruppen von Schweinefleisch untersucht. Zwei Gruppen wurden unter Bio-Bedingungen gehalten, wobei eine Gruppe mit Leinpresskuchen supplementiert wurde und die andere als nicht supplementierte Kontrollgruppe diente. Die beiden weiteren Gruppen wurden unter konventionellen Bedingungen gehalten und umfassten Turopolje-Schweine sowie Landrasse-Schweine. Von jeder Gruppe wurden jeweils acht Proben aus dem Schweinekarree und dem Bauchfleisch analysiert. Ziel der Studie war es, die Auswirkungen der Supplementierung mit Leinpresskuchen sowie der Schweinerasse auf das Fettsäureprofil, Oxidationsprodukte, den Tocopherolgehalt und die Polyphenolgehalte zu untersuchen. Die Analysen erfolgten mittels GC-MS, GC-FID, HPLC-UV und LC-MS sowie ergänzend durch spektrophotometrische und fluoreszenzbasierte Methoden.

Im ersten Teil der Studie zeigte sich, dass die Supplementierung mit Leinpresskuchen den Gehalt and Omega-3-Fettsäuren signifikant erhöhte, den Gehalt an gesättigten Fettsäuren reduzierte und das n6:n3-Verhältnis verbesserte, ohne jedoch den Peroxidwert oder den Proteincarbonylgehalt zu beeinflussen. Die Schiff-Basen-Werte waren in den supplementierten Proben signifikant erhöht und variierten in Abhängigkeit vom Fleischteil.

Tocopherol lagen in beiden Gruppen unter der Nachweisgrenze (LOD), während Polyphenole in beiden Gruppen nachgewiesen wurden, mit höheren Konzentrationen in den mit Leinpresskuchen supplementierten Proben.

Im zweiten Teil der Studien wiesen Turopolje-Schweine höhere Konzentrationen an Omega-3-Fettsäuren und niedrigere Arachidonsäuregehalte im Vergleich zu Landrasse-Schweinen auf. Hinsichtlich Peroxidwert und Proteincarbonylgehalt wurden keine signifikanten Unterschiede zwischen den Rassen festgestellt. Sekundäre Oxidationsprodukte waren jedoch im Fleisch der Landrasse höher. Die Schiff-Basen-Werte waren bei Turopolje-Schweinen erhöht und vom Fleischteil abhängig. Tocopherole wurden in beiden Gruppen nachgewiesen, während Polyphenole ausschließlich in Proben der Turopolje-Schweine gefunden wurden.

Zusammenfassend verbesserte die Supplementierung mit Leinpresskuchen das Fettsäureprofil, die oxidative Stabilität und die Gesamtqualität des Schweinefleisches. Obwohl die Omega-3-gehalte anstiegen, reichten sie nicht aus, um eine Kennzeichnung als „Quelle von Omega-3-Fettsäuren“ zu ermöglichen. Turopolje-Schweine zeigten eine höhere Fähigkeit zur Polyphenolakkumulation im Vergleich zu Landrasse-Schweinen, wobei dieser Effekt stark vom Fleischteil beeinflusst wurde.

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10 Appendix

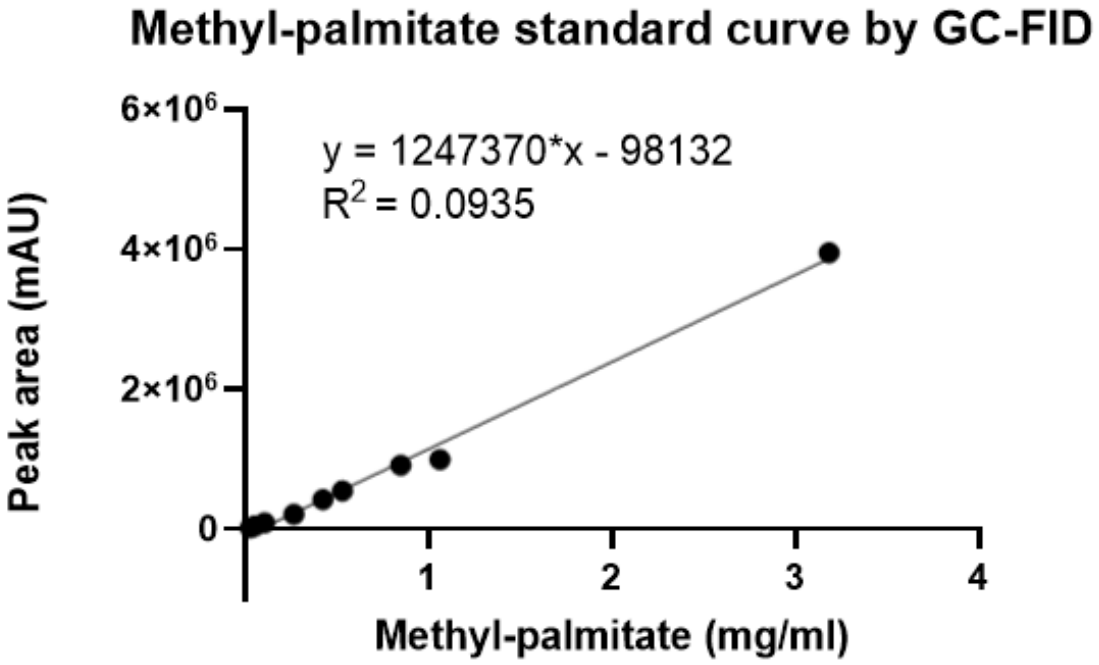

Figure 49: Methyl-palmitate standard curve by GC-FID

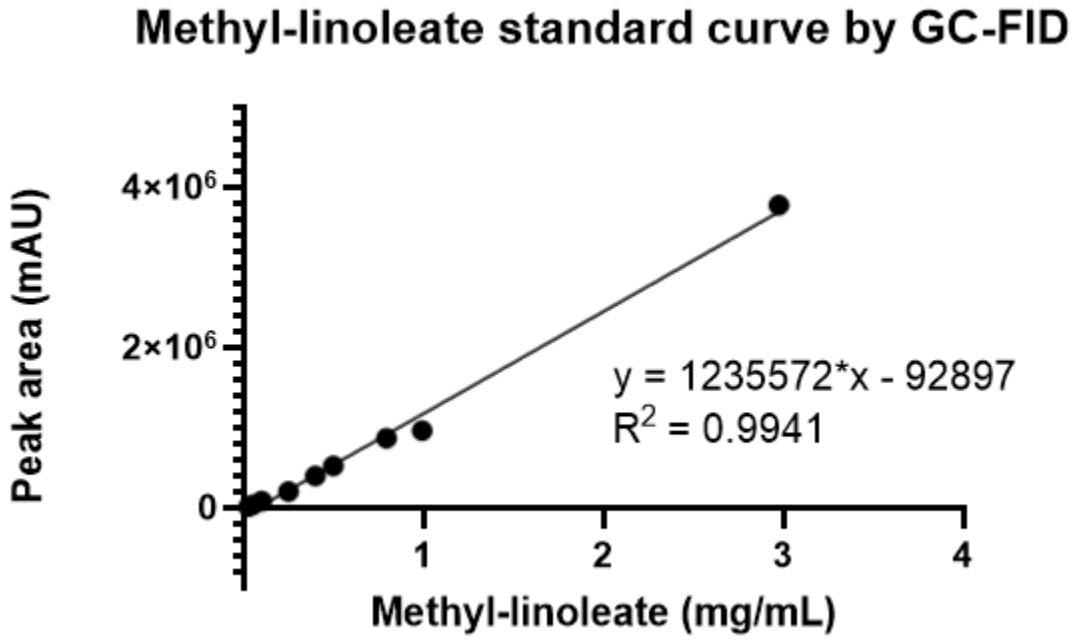

Figure 50: Methyl-linoleate standard curve by GC-FID

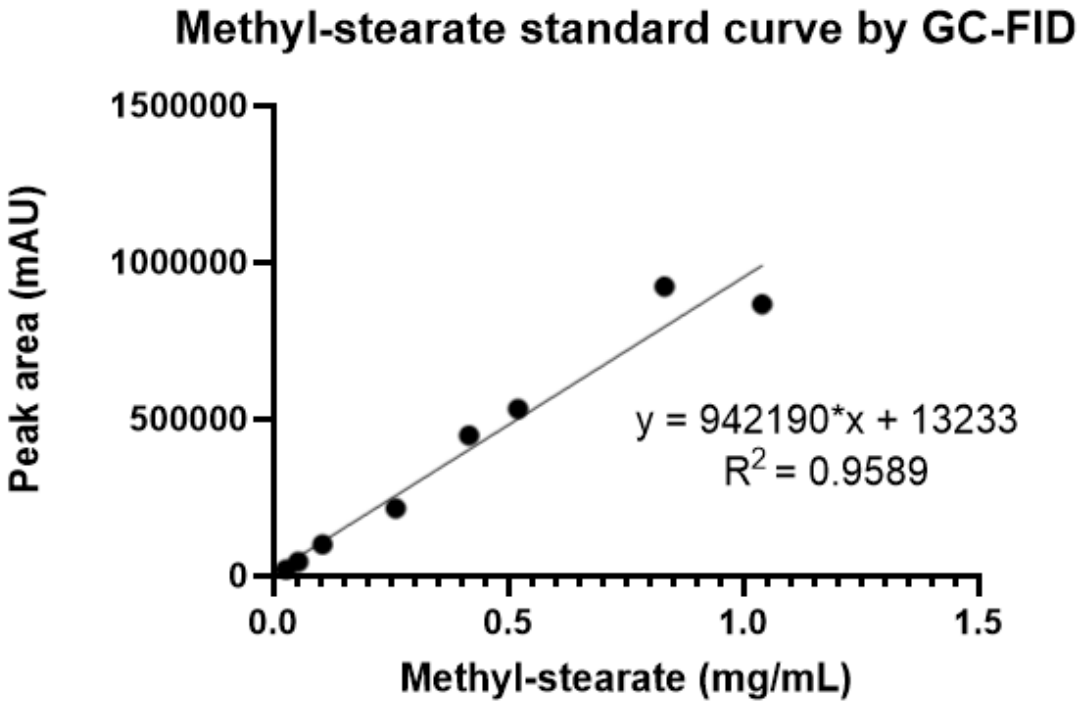

Figure 51:Methyl-stearate standard curve by GC-FID

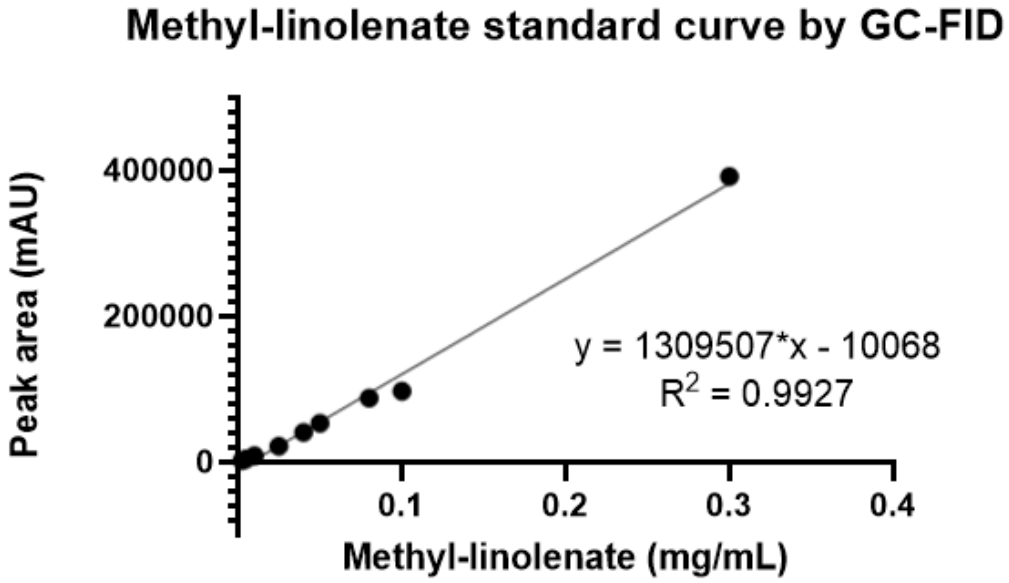

Figure 52: Methyl-linolenate standard curve by GC-FID

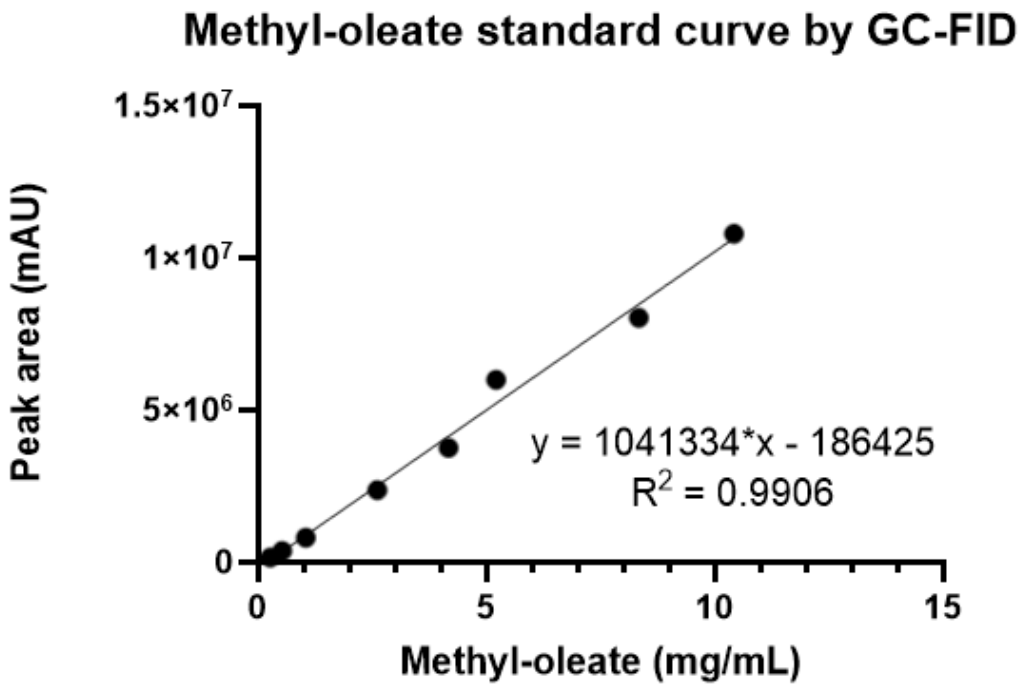

Figure 53: Methyl-oleate standard curve by GC-FID

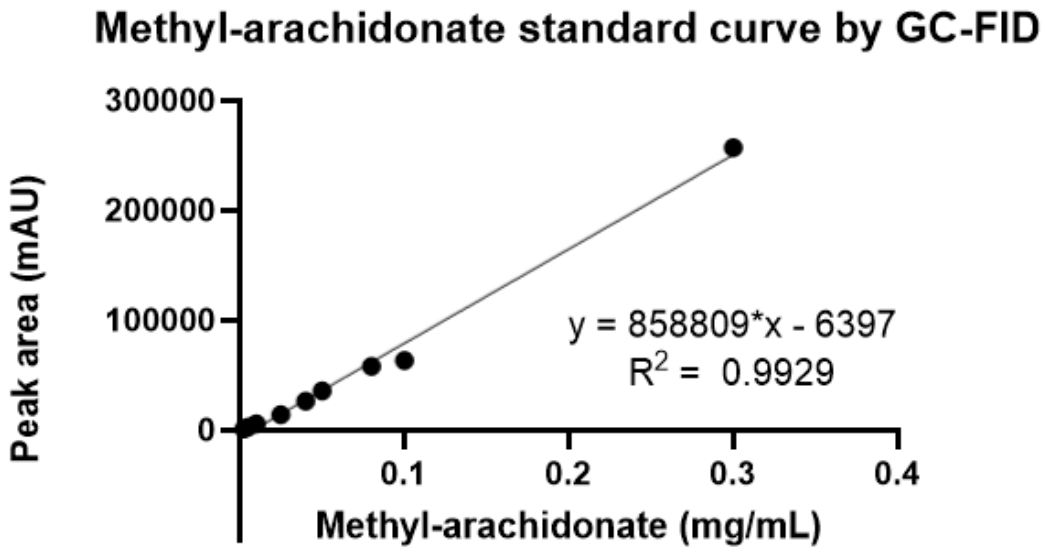

Figure 54: Methyl-arachidonate standard curve by GC-FID

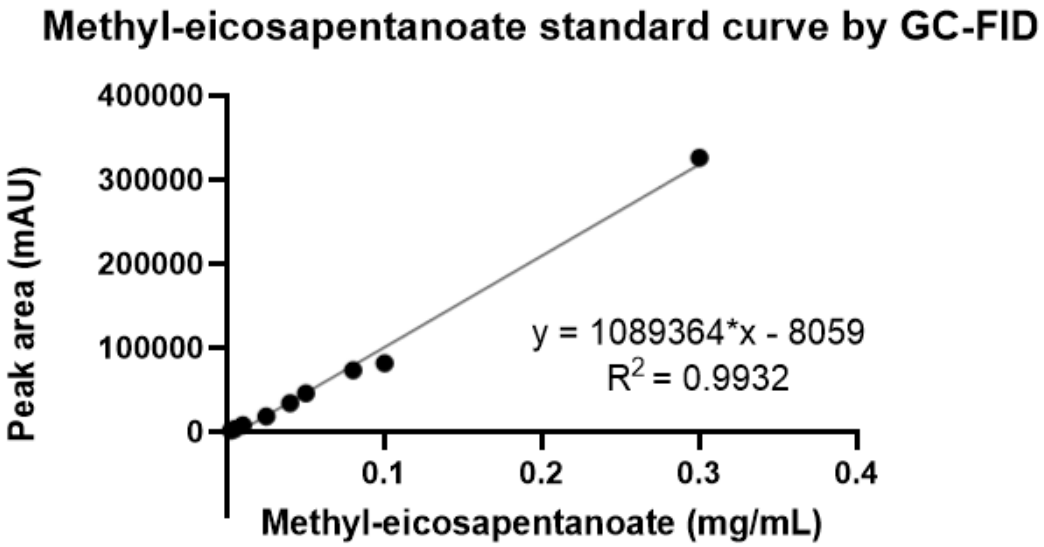

Figure 55: Methyl-eicosapentanoate standard curve by GC-FID

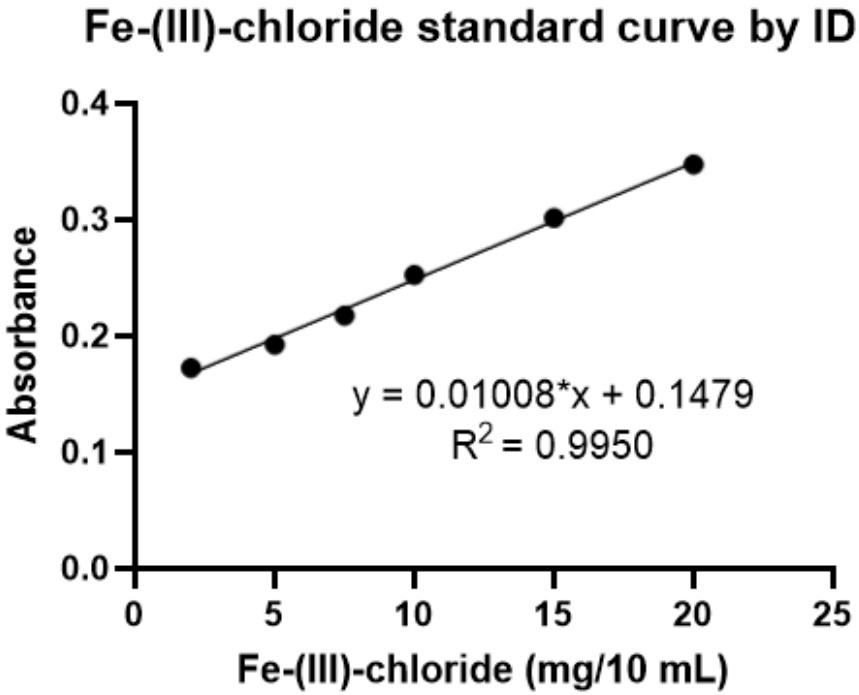

Figure 56: Standard curve for Fe-(III)-chloride by IDF

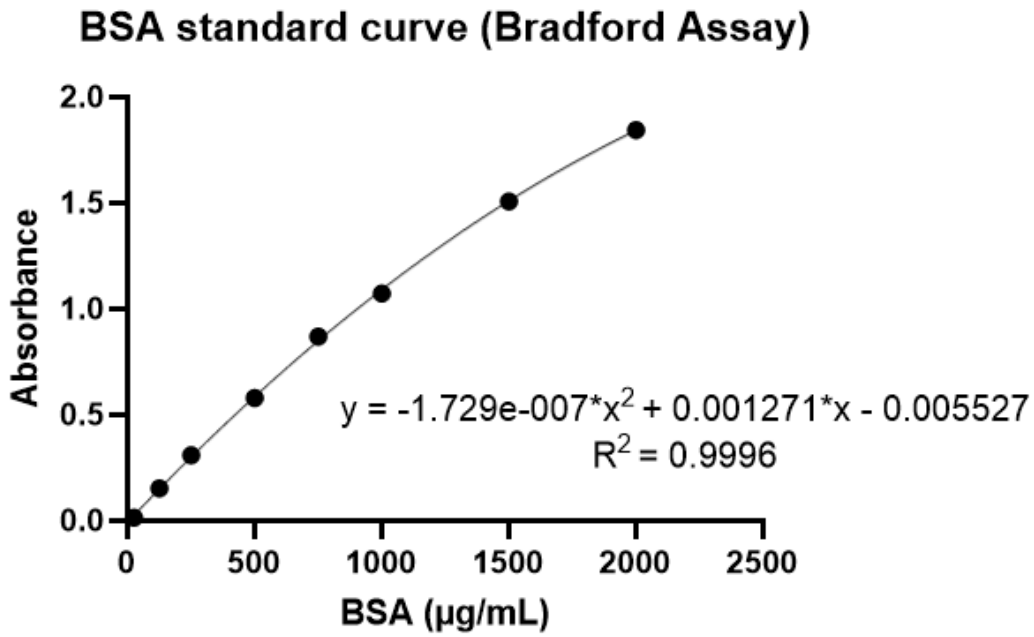

Figure 57: Standard curve for BSA (Bradford Assay)

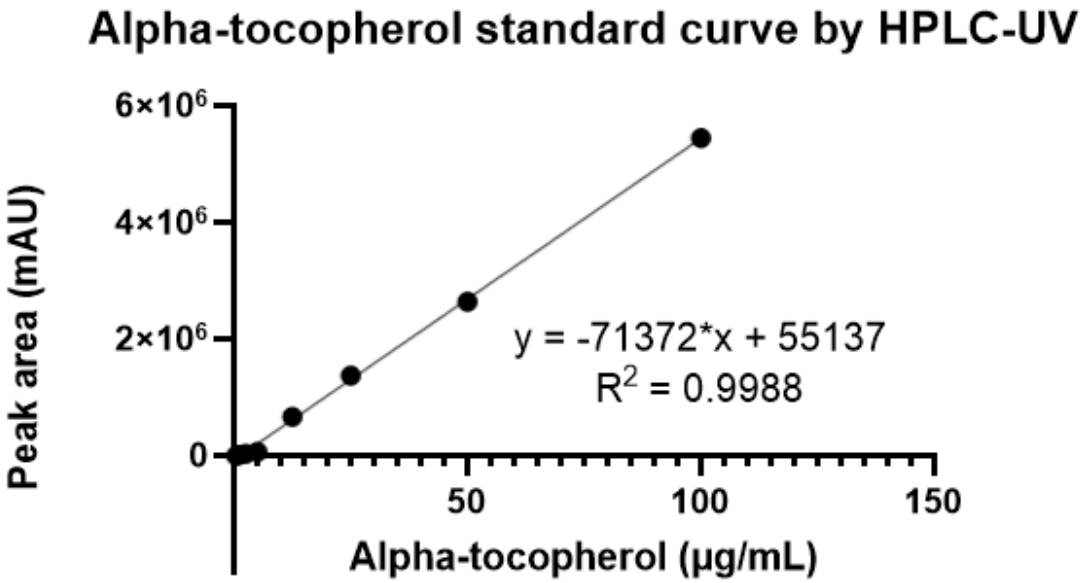

Figure 58: Alpha-tocopherol standard curve by HPLC-UV

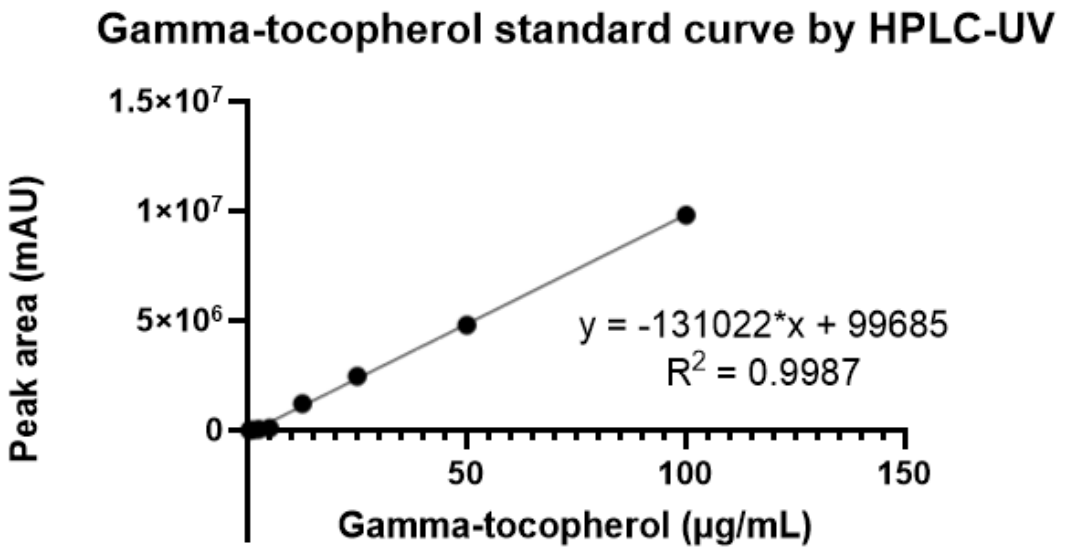

Figure 59: Gamma-tocopherol standard curve by HPLC-UV

Table 26: Average content and standard deviation of fatty acids in g/100 g fat of loin and belly from LPC, NF, TP, and LR analyzed by GC-FID

			Average fatty acid content (g/100 g fat)	**Standard deviation (g/100 g fat)**
LPC	Loin	Methyl palmytate	17.094	3.364
		Methyl stearate	10.212	1.732
		Methyl oleate	42.666	10.110
		Methyl linoleate	6.171	1.152
		Methyl linolenate	2.874	0.740
		Methyl arachidonate	0.242	0.064
		Eicosapentanoic acid	0.121	0.021
		n6:n3	2.290	0.632
	Belly	Methyl palmytate	16.272	2.991
		Methyl stearate	9.156	1.616
		Methyl oleate	42.814	7.454
		Methyl linoleate	6.422	1.361
		Methyl linolenate	2.660	0.970
		Methyl arachidonate	0.199	0.037
		Eicosapentanoic acid	0.119	0.019
		n6:n3	2.568	0.764

NF	Loin	Methyl palmytate	21.770	1.250
		Methyl stearate	12.960	1.620
		Methyl oleate	53.960	7.590
		Methyl linoleate	7.250	0.650
		Methyl linolenate	0.920	0.330
		Methyl arachidonate	0.350	0.020
		Eicosapentanoic acid	0.110	0.010
		n6:n3	7.750	2.030
	Belly	Methyl palmytate	14.800	2.030
		Methyl stearate	8.270	0.750
		Methyl oleate	41.620	9.380
		Methyl linoleate	5.480	0.840
		Methyl linolenate	0.710	0.170
		Methyl arachidonate	0.230	0.030
		Eicosapentanoic acid	0.080	0.010
		n6:n3	7.330	1.600
TP	Loin	Methyl palmytate	21.430	5.208
		Methyl stearate	12.338	2.858
		Methyl oleate	48.549	12.348
		Methyl linoleate	6.690	2.123
		Methyl linolenate	0.913	1.527
		Methyl arachidonate	0.284	0.054
		Eicosapentanoic acid	0.101	0.037
		n6:n3	12.202	4.576
	Belly	Methyl palmytate	21.746	5.123
		Methyl stearate	12.815	3.255
		Methyl oleate	48.815	11.271
		Methyl linoleate	7.237	2.171
		Methyl linolenate	1.822	1.792
		Methyl arachidonate	0.274	0.119
		Eicosapentanoic acid	0.117	0.025
		n6:n3	7.508	5.641
LR	Loin	Methyl palmytate	22.710	9.040
		Methyl stearate	16.310	6.310
		Methyl oleate	44.130	17.160
		Methyl linoleate	7.460	2.650

		Methyl linolenate	0.350	0.080
		Methyl arachidonate	0.800	0.380
		Eicosapentanoic acid	0.100	0.020
		n6:n3	18.310	5.360
	Belly	Methyl palmytate	17.830	3.720
		Methyl stearate	13.780	3.040
		Methyl oleate	34.720	8.540
		Methyl linoleate	8.390	1.980
		Methyl linolenate	0.680	0.720
		Methyl arachidonate	0.470	0.170
		Eicosapentanoic acid	0.110	0.020
		n6:n3	16.970	2.650

Table 27: Average peroxide value (PV) and standard deviation in meq O2/kg fat of loin and belly from LPC, NF, TP, and LR analyzed by IDF

		Average PV (meq O_2/kg fat)	**Standard deviation (meq O_2/kg fat)**
LPC	Loin	2.084	1.400
	Belly	1.645	0.900
NF	Loin	1.467	0.400
	Belly	1.046	0.600
TP	Loin	3.934	6.000
	Belly	1.634	0.900
LR	Loin	2.503	1.300
	Belly	1.529	0.400

Table 28: Peak area of secondary lipid oxidation products of loin and belly from LPC, NF, TP, and LR analyzed by GC-MS and normalized to the weight

		Loin		Belly	
		Average peak area (A.U.)	**Standard deviation**	**Average peak area (A.U.)**	**Standard deviation**
LPC	Hexanal	6958500	882175	5569897	2644749
	1-Pentanol	376279	207968	413162	108758
	1-Hexanol	137049	56333	118480	65740
	Heptanal	433785	236253	188541	104686

	1-Heptanol	52634	21651	42283	14566
	Octanal	125178	59190	63231	29437
	1-Octanol	58744	24681	25007	9650
	Nonanal	267523	94358	151084	40744
	2-Nonenal	46242	26803	13052	8536
	2,4-Decadienal	-	-	-	-
NF	Hexanal	6583067	1288563	5955216	1982992
	1-Pentanol	423984	107961	464747	94898
	1-Hexanol	85791	14797	87545	33776
	Heptanal	197627	52905	162612	65002
	1-Heptanol	28719	2495	37030	7570
	Octanal	63871	8372	55395	22236
	1-Octanol	27945	6201	20191	7110
	Nonanal	168300	15386	122583	34755
	2-Nonenal	21756	7817	13688	6857
	2,4-Decadienal	-	-	-	-
TP	Pentanal	826371	840168	2259635	3155517
	Hexanal	10952465	10067001	18441865	18536366
	Heptanal	728774	885855	1489179	1964456
	Octanal	422795	603416	994400	1827530
	Nonanal	650637	558087	1221508	1366041
	Decanal	19586	13846	124065	196667
	2-Heptenal	313954	448854	452667	500889
	2-Octenal	217538	226466	520898	832531
	2-Nonenal	97589	138508	106236	151602
	2-Decenal	63122	61286	117951	168461
	2,4-Decadienal	77476	99727	-	-
	1-Pentanol	929376	895686	-	-
	1-Hexanol	1960206	2918722	1407073	2054612
	1-Heptanol	191241	242632	434864	619051
	1-Octanol	155973	170207	315072	389688
	1-octen-3-ol	2241379	2234805	3529599	5036444

	2,3-Octandione	1092784	826041	-	-
LR	Pentanal	692651	410597	1188877	670410
	Hexanal	10071931	6284141	16189060	9486123
	Heptanal	418987	323843	619312	278993
	Octanal	308632	170538	459237	196769
	Nonanal	678518	359736	1117443	567689
	Decanal	14554	5846	146877	196728
	2-Heptenal	159970	163393	337259	175407
	2-Octenal	153943	110181	357217	211895
	2-Nonenal	74612	50418	91816	62121
	2-Decenal	192028	107940	183465	144114
	2,4-Decadienal	130050	90029	-	-
	1-Pentanol	980280	393552	-	-
	1-Hexanol	369723	159478	1249035	878856
	1-Heptanol	153513	65342	245713	155705
	1-Octanol	139734	67112	255204	180490
	1-octen-3-ol	1736941	1652633	3157917	2377018
	2,3-Octandione	1263254	649715	-	-

Table 29: Fold change of secondary lipid oxidation products of loin and belly from LPC and NF analyzed by GC-MS

	LPC:NF ratio in loin (fold change)	**LPC:NF ratio in belly (fold change)**
Hexanal	0.997	1.086
Heptanal	2.351	1.318
Octanal	2.089	1.294
Nonanal	1.248	1.351
2-Nonenal	1.981	1.051
1-Pentanol	0.887	0.889
1-Hexanol	1.607	1.588
1-Heptanol	1.933	1.269
1-Octanol	1.637	1.333

Table 30: Fold change of secondary lipid oxidation products of loin and belly from TP and LR analyzed by GC-MS

	TP:LR ratio in loin (fold change)	TP:LR ratio in belly (fold change)
Pentanal	0.838	1.642
Hexanal	0.920	0.878
Heptanal	0.436	1.097
Octanal	1.411	2.032
Nonanal	1.043	1.142
Decanal	0.974	1.457
2-Heptenal	0.303	0.745
2-Octenal	0.708	0.686
2-Nonenal	1.384	0.683
2-Decenal	3.042	1.163
2,4-Decadienal	1.679	-
1-Pentanol	1.055	-
1-Hexanol	0.385	0.697
1-Heptanol	1.416	1.861
1-Octanol	0.896	0.753
1-octen-3-ol	0.533	3.251
2,3-Octandione	1.156	-

Table 31: Average carbonyl content and standard deviation in nmol/mg protein of loin and belly from LPC, NF, TP, and LR analyzed by DNPH assay

		Average carbonyl content (nmol/mg protein)	Standard deviation (nmol/mg protein)
LPC	Loin	2.258	0.507
	Belly	2.194	0.629
NF	Loin	3.016	0.731
	Belly	1.911	0.971
TP	Loin	3.278	1.069
	Belly	3.127	0.941
LR	Loin	2.210	0.960
	Belly	2.267	0.808

Table 32: Average Schiff bases content and standard deviation expressed in normalized fluorescence intensity of loin and belly from LPC, NF, TP, and LR analyzed by fluorescence spectroscopy

		Average Schiff bases content (normalized fluorescence intensity)	Standard deviation (normalized fluorescence intensity)
LPC	Loin	298.973	118.292
	Belly	410.060	209.434
NF	Loin	401.204	146.654
	Belly	258.107	155.420
TP	Loin	215.307	110.577
	Belly	161.616	80.730
LR	Loin	179.546	58.419
	Belly	370.135	66.515

Table 33: Average α-tocopherol content and standard deviation in µg/g FW meat of loin and belly from TP, LR, LPC and NF analyzed by HPLC-UV

		Average α-tocopherol content (µg/g FW meat)	Standard deviation (µg/g FW meat)
TP	Loin	2.018	1.040
	Belly	2.336	1.844
LR	Loin	1.675	0.701
	Belly	2.666	2.341
LPC	Loin	< LOD	
	Belly	< LOD	
NF	Loin	< LOD	
	Belly	< LOD	

Table 34: Average γ-tocopherol content and standard deviation in µg/g FW meat of loin and belly from TP, LR, LPC and NF analyzed by HPLC-UV

		Average γ-tocopherol content (µg/g FW meat)	Standard deviation (µg/g FW meat)
TP	Loin	0.743	0.338
	Belly	1.003	0.551
LR	Loin	< LOD	
	Belly	0.615	0.242
LPC	Loin	< LOD	

	Belly	< LOD
NF	Loin	< LOD
	Belly	< LOD

Table 35: Average total polyphenol content and standard deviation in µg/g of loin and belly from LPC and NF analyzed by LC-MS

		Average total polyphenol content (µg/g)	**Standard deviation (µg/g)**
LPC	Loin	71.141	17.497
	Belly	28.686	8.570
NF	Loin	46.973	14.054
	Belly	12.339	2.234

Table 36: Average polyphenol content and standard deviation in µg/g of loin and belly from LPC, NF, TP and LR analyzed by LC-MS

		Polyphenols	**Average polyphenol content (µg/g)**	**Standard deviation (µg/g)**
LPC	Loin	Herbacetin	60.568	14.432
		Astragalin	10.573	4.916
	Belly	Herbacetin	24.712	8.354
		Astragalin	3.975	0.500
NF	Loin	Herbacetin	41.528	14.028
		Astragalin	5.445	0.974
	Belly	Herbacetin	9.817	4.055
		Astragalin	3.783	0.561
TP	Loin	Chalcone	3.039	1.568
		Chlorogenic acid	4.549	1.152
	Belly	Chalcone	1.756	1.493
		Herbacetin	12.471	6.517
LR	Loin	< LOD		
	Belly	< LOD		