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Monitoring of volatile substances in the meat

Master's thesis

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Declaration

I, Many Eath, hereby declare that this thesis entitled **"Monitoring of volatile substances in the meat"** is my own work, and all the sources have been quoted and acknowledged by means of complete references.

In Prague, April 27, 2017

Many Eath

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Abstract

Production of volatile organic compounds is often considered as an important factor affecting food quality. Chemical analysis of these is not always simple, especially in the meat. One of the more advanced ways to analyze these substances is to use the SPME-GC-MS combination. This technique makes it possible to monitor the changes in the chemical composition of volatile compounds from meat under different storage conditions (temperature, time). In this work, SPME-GC-MS technique monitored the volatile compounds profiles from fresh pork samples, stored at 25 °C and 8 °C for 1, 3, 5 and 7 days. Chemical structures of the compounds were determined from their mass spectra or by comparison of their Kovats indices. Chemical analysis has shown the presence of many compounds, especially aldehydes and alcohols, then ketones, acids, terpenic substances, hydrocarbons and others. These compounds are produced by biochemical processes during ageing of meat and also by action of microorganisms. The present study will give basic information on the acceptability of the meat under certain storage conditions and open door for more future research on meat aroma and flavor. The method can be used to study volatile substances from different types of meat, along with post-mortem biochemical changes, and can be used in food analysis.

Keywords: volatile compounds, pork, storage conditions, SPME, GC-MS

Abstrakt

Produkce těkavých organických sloučenin je často považována za důležitý faktor ovlivňující kvalitu potravin. Chemická analýza těchto látek v mase není, ale vždy jednoduchá. Jednou z modernějších možností, jak tyto látky analyzovat je použití kombinace SPME-GC-MS. Tato technika umožňuje sledovat změny v chemické složení těkavých látek z masa za různých skladovacích podmínek (teplota, čas). V této práci byly pomocí techniky SPME-GC-MS, sledovány profily těkavých látek ze vzorků čerstvého vepřového masa, dále pak po skladovány při teplotě 25 °C a 8 °C po dobu 1, 3, 5 a 7 dnů. Jednotlivé složky byly identifikovány z hmotnostního spektra a srovnáním Kováčových indexů. Chemická analýza prokázala přítomnost mnoha sloučenin, především aldehydů a alkoholů, dále pak ketonů, kyselin, terpenických látek, uhlovodíků a další, které jsou produkovány biochemickými procesy během zrání, popř. kažení masa a také činností mikroorganismů. Předložená studie nabízí základní informace o použitelnosti masa za určitých skladovacích podmínek a ukazuje možnost dalších výzkumů aromatických látek masa. Uvedená metoda může být použita ke studiu těkavých látek z různých druhů a typů masa, spolu s tím biochemických změn během zrání masa a může být využita při analýze potravin.

Klíčová slova: těkavé látky, vepřové maso, skladovací podmínky, SPME, GC-MS

Contents

List of tables

List of figures

List of abbreviations

ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
CoA	coenzyme A
CP	creatine phosphate
DED	direct extraction device
DH	dynamic headspace
DHE	dynamic headspace extraction
EPA	environmental protection agency
FAD	flavin-adenine dinucleotide
GC-MS	gas chromatography and mass spectrometry
GTP	guanosine triphosphate
HPLC	high-performance liquid chromatography
HS	headspace
IM	identification method
IMF	intramuscular fat
KI	Kovats index
LC	liquid chromatography
MAP	modified atmosphere
MSD	mass spectrometer detector
MW	molecular weight
NAD	nicotinamide adenine dinucleotide
NIH	national institutes of health
NIST	national institute of standards and technology
P&T	purge and trap
QMF	quadrupole mass filter
RF	radio frequency
RT	retention time
SD	Strecker degradation
SDE	simultaneous distillation extraction
SPME	solid-phase microextraction

1. Introduction

While meat is one of the main sources for human nutrition, pork is often referred to as one of the red meat types which has been widely produced due to its low cost of production. Over the last few decades, pork has become more favored by consumers in the industrialized nations and thought to be healthier to eat (Warriss, 2000). Correspondingly, the idea of storing meat in the refrigerator is getting more popular owing to the workload of working people these days. Refrigerator has become an important appliance that can keep our food safe because of its capacity to slow down bacterial growth causing food poisoning (Foodsafety, 2010). During refrigerated storage, it is well known that meat becomes tenderer. This commonly happens because of the proteolytic changes occurring in the architecture of the myofibril and its associated proteins (Huff-Lonergan, 2010).

The generation of volatile compounds in meat and meat products has been widely studied due to its role of flavour in the overall acceptability (Mottram, 1998). Soncin et al. (2007) and Estévez et al. (2003) have worked on volatile fraction in raw meat of pork and under refrigerated storage by using solid-phase microextraction (SPME), which is an inexpensive, easy and rapid technique (Brunton et al., 2001) offering chemical data closely related to olfactory assessment (Pawliszyn, 1997), coupled with gas chromatography and mass spectrometry (GC-MS). However, there still lacks research working on the monitoring and comparison of volatile substances in pork at room temperature and under refrigeration by using the aforementioned technique. This has led to the idea of commencing this study.

2. Literature Review

2.1 Nutritional value of meat

Meat has played an important part in human diet because of its great source of protein and main source of certain vitamins and minerals (Mottram, 1991; Biesalski, 2005). According to Warriss (2000) and Smil (2013), meat contains abundant amount of protein whose composition matching closely to our own proteins and is rich in vitamin B, vitamin A, iron, copper, zinc, and selenium. Meat is one of the best sources of dietary iron because it supplies this essential mineral as haem iron, which is easily absorbed in the upper small intestine and helps absorb non-haem iron present in plant food, and even small amount of meat consumption can help prevent anemia (Bender, 1992; NZ Nutrition Foundation, 2016). Iron deficiency is one of the well-known public health problems which globally affects 1.62 billion people or more than a fifth of all humanity (WHO, 2008), and, even more tragically, in developing countries, it impairs brain development of approximately half of all children and is associated with every fifth maternal death (Micronutrient Initiative, 2009). Please refer to table 1 for the nutritional value of some meats.

Table 1: Nutritional value of lean beef, lamb, and pork

Nutritional value	Lean beef	Lean lamb	Lean pork
Protein (%)	20	21	21
Fat (%)	5	9	7
Energy content (kJ/100g)	517	679	615
Iron (mg/100g)	2.1	1.6	0.9
Zinc (mg/100g)	4.3	4.0	2.4
Thiamine-B1 (mg/100g)	0.07	0.14	0.89
Riboflavin-B2 (mg/100g)	0.24	0.28	0.25
Niacin-B3 (mg/100g)	5.2	6.0	6.2
Pyridoxine-B6 (mg/100g)	0.32	0.25	0.45
Cobalamin-B12 (µg/100g)	2	2	3

(Source: McCance and Widdowson, 1997)

2.2 Types of meat

From the aspect of food legislation, meat is referred to as postmortem component originating from live animals which is suitable for human consumption (Kauffman, 2012; MAFF, 2016) and can be categorized as follows:

Pork : meat from pig (very young/5-7 months-old) – fairly soft consistency, fine fibred with pale pink, pink or whitish-grey colour, and interspersed and entwined with fat.

Beef : The most important categories are:

- Young bull (18-22 months, live weight > 300 kg) – fine fibred, well-marbled.
- Cow (> 2 years, already calved) – medium red to brown red, moderately fine to coarse fibred, yellow fat, marbled.
- Heifer (15-24 months, not calved) – red, fine fibred, white fat.

Poultry: The two main categories are:

- Dark meat – geese, ducks, and pigeons.
- Light meat – chickens, turkeys, and peacocks.

Colour varies based on age, breed, and body part (breast meat is light, thighs and drumsticks are dark). Poultry fat tends to become rancid due to its high content of unsaturated fatty acids.

Game : Wild game can be classified based on fur-bearing animals:

- Deer – antelope, caribou, elk, and white-tailed deer.
- Wild boars – wild pigs.
- Other wild game – hare, rabbit, badger, beaver, and bear.
- Birds or fowl – heathcock, partridge, pheasant, snipe, etc.

The meat of wild game consists of fragile fibres with a firm consistency and colour ranging from red to red-brown.

Variety meats : meat of various animal organs – tongue, heart, liver, kidney, spleen, brains, retina, intestines, tripe, bladder, pork crackling, cow udders, etc. Many of these variety meats, such as liver, kidney or heart, are highly-valued foods because they contain vitamins, trace elements as well as high quality protein (Belitz et al., 2009).

Table 2: Proportional value of carcass meat and by-products from cattle, hogs, and lambs

Carcass meat and by-products	Cattle (%)	Pig (%)	Lamb (%)
Carcass meat	34	52	32
Bones	16	17	18
Organs	16	7	10
Skin and attached fat	6	6	15
Blood	3	3	4
Fatty tissues	4	3	3
Horns, hoofs, feet, and skull	5	6	7
Abdominal and intestinal contents	16	6	11

(Source: Goldstrand, 1988)

Table 3: Composition of different cuts of meat

Meat	Cut of meat	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Cal/100g
Beef	Chuck	18.6	65	16	0.9	220
	Flank	19.9	61	18	0.9	250
	Loin	16.7	57	25	0.8	290
	Rib	17.4	59	23	0.8	280
	Topside	19.5	69	11	1.0	180
	Rump	16.2	55	28	0.8	320
Pork	Ham	15.2	53	31	0.8	340
	Loin	16.4	58	25	0.9	300
	Shoulder	13.5	49	37	0.7	390
	Spare rib	14.6	53	32	0.8	350
Lamb	Breast	12.8	48	37	0.7	380
	Leg	18.0	64	18	0.9	240
	Loin	18.6	65	16	0.7	220
	Rib	14.9	52	32	0.8	360
	Shoulder	15.6	58	25	0.8	300

(Source: Sawyer, 1975)

2.3 Types of muscle

In living animals, there are three types of muscle whose functions are very important to maintain life. They are smooth, cardiac, and skeletal muscles. While smooth muscle locates in the skin and walls of tubular organs with their associated glands, cardiac muscle is found only in the heart (Kisia and Onyango, 2005; Hill and Olson, 2012). Distinct from the aforementioned, skeletal muscle is the most abundant type in the animal body where its own tissue contributes generally between 50-70% of carcass weight and most of the value (Weaver, 2012). The others are connective tissue, nerve tissue, and vascular tissue (National Institutes of Health, 2016). In this type of muscle, contractile, structural, and regulatory proteins are well arranged into a distinct striated pattern. Also, it serves as a storage for lipids and contains considerable quantity of extracellular fluids, mainly water (Kauffman, 2012). Refer to table 4 for the characteristics of each muscle type and figure 1 for the structure of a skeletal muscle.

Table 4: Characteristics of muscle types

Muscle type	Striated	Nuclei per muscle fibre or cell	Nervous control
Skeletal	Yes	Many	Voluntary
Cardiac	Yes	1-2	Involuntary
Smooth	No	1	Involuntary

(Source: Weaver, 2012)

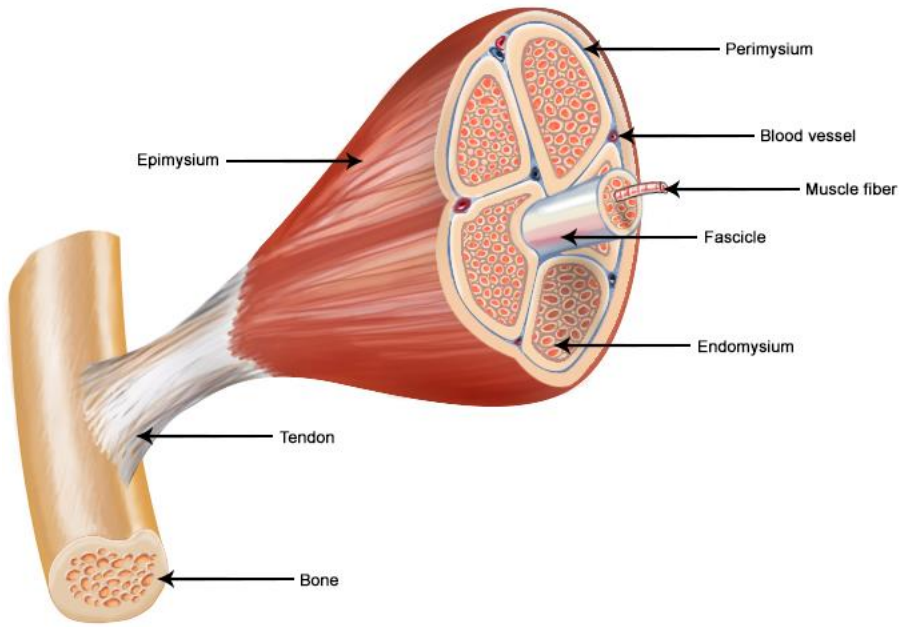

Figure 1: Structure of a skeletal muscle (Source: National Institutes of Health, 2016)

Because of the proportion of fibres, muscle varies in colours ranging from white to red. Fibres can be classified on the basis of colour as red with higher content of myoglobin and numbers of capillaries and mitochondria, white with low amounts of myoglobin, and intermediate with intermediate properties (Moody and Cassens, 1968; Warriss, 2000); and of metabolic characteristics or contraction speed as type I for slow-twitch oxidative, type IIA for fast-twitch oxidative, and type IIB for fast-twitch glycolytic (Pearson and Young, 1989; Body Building, 2007).

Skeletal muscle has a great number of fibres. Each fibre, which is enclosed with connective tissue, has more than 1,000 parallel myofibrils embedded in sarcoplasm (Toldrá and Reig, 2012). When examining each myofibril, we can see dark lines known as Z-lines where the distance between the two consecutive called a sarcomere containing more than 65 proteins (Fraterman et al., 2007). Myofibrils are the contractile machinery of the cell and comprise thick and thin filaments, partly overlapped and giving rise to alternating dark (A band) and light (I band) areas (Huff-Lonergan, 2010).

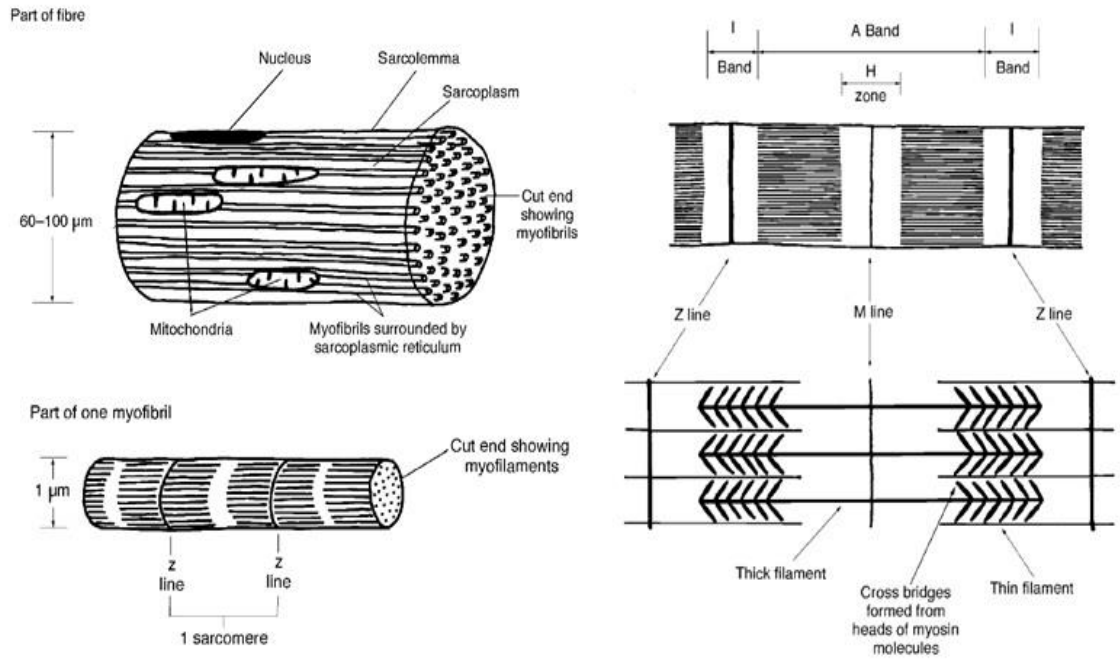

Figure 2: Schematic representations of a fibre and fibril with its banding pattern and structure (Source: Warriss, 2000)

2.4 Muscle composition

Muscle composition varies considerably, and the accumulation of lipid is the most influential on this variation. According to the U.S. Department of Agriculture (2008), the largest constituent of mammalian muscle is water (65-80%) followed by protein (16-22%), lipid (1-13%), carbohydrate (0.5-1.5%), non-protein nitrogenous compounds (1-2%), and other non-protein substances; minerals, vitamins, etc. (0.5-1%). Additionally, there is an inverse relationship between the percentage of protein and moisture and that of fat, meaning, meats with high content of fat have lower amount of moisture and protein (Toldrá and Reig, 2012). Refer to figure 3 for fresh muscle composition and table 5 for the approximate composition of pork muscle *Longissimus Dorsi*.

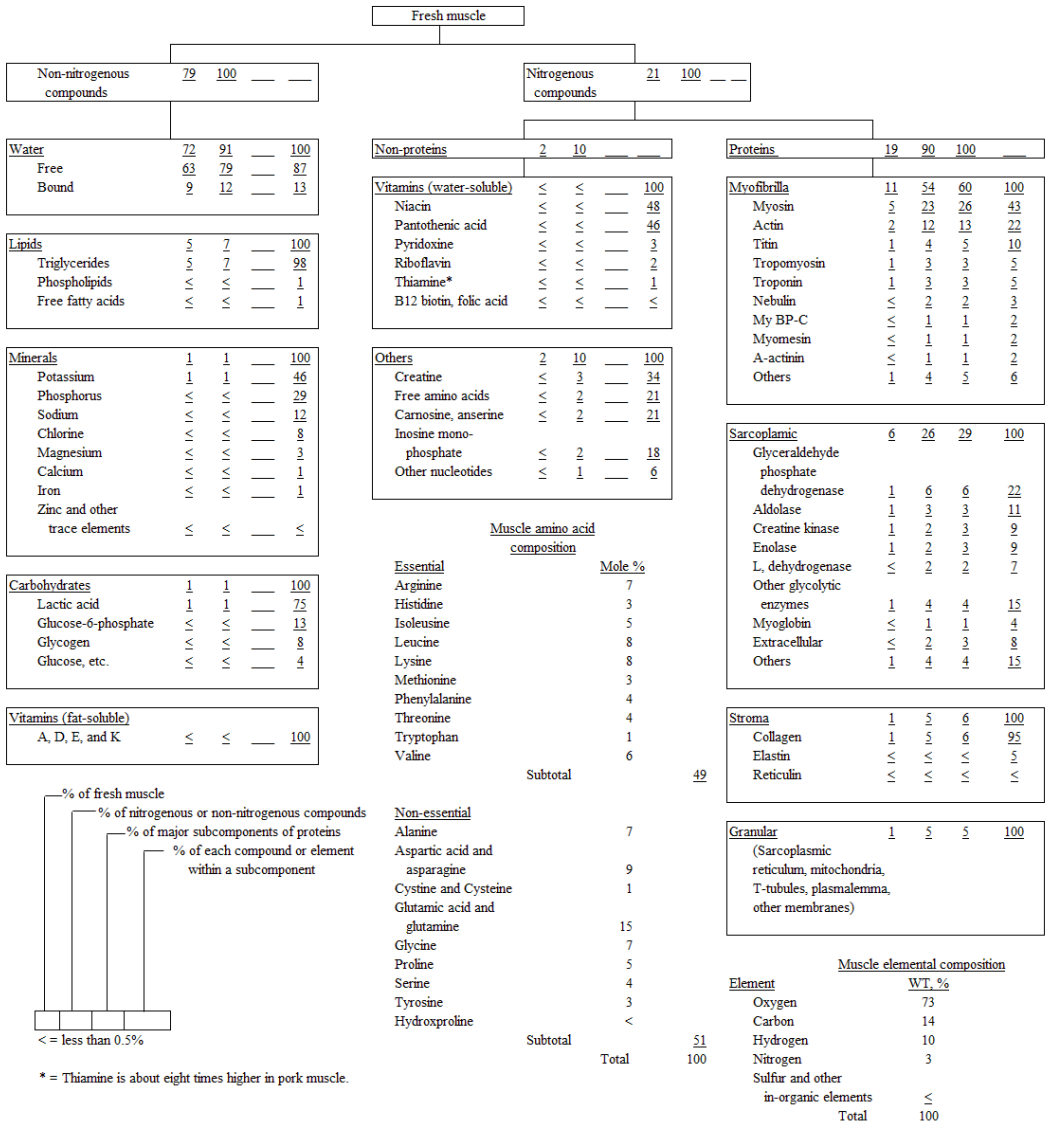

Figure 3: Fresh muscle composition (Source: Kauffman, 2012)

Table 5: The approximate composition of pork muscle *Longissimus Dorsi*

	Average
Gross composition (g/100g)	
Moisture	74.5
Protein	21.4
Lipid	2.7
Carbohydrate	0.5
Ash	0.9
Proteins (g/100g)	
Myofibrillar	9.5
Sarcoplasmic	9.1
Connective	3.0
Lipids (g/100g)	
Phospholipids	0.586
Triglycerides	2.12
Free fatty acids	0.025
Some minor compounds (mg/100g)	
Cholesterol	46.1
Haem content	400
Dipeptides	347.6
Free amino acids	90.2

(Source: Aristoy and Toldrá, 1998; Hernández et al., 1998; Toldrá, 1999, unpublished)

2.5 Biochemistry of meat

2.5.1 Biochemical processes in meat pre-slaughter

As long as the animal is alive, energy in the form of ATP is required for muscle contraction and relaxation. To form ATP in pre-slaughter muscle tissue, glucose is primarily used while fat or proteins are only utilized when no more carbohydrates available. When needed for energy, glycogen, the muscular sugar and a polysaccharide stored in muscle tissue and liver, is readily broken down to glucose which then undergoes glycolysis producing pyruvate as the end point. To enter Krebs cycle, also known as the tricarboxylic acid cycle or citric acid cycle, pyruvate is then transformed into acetyl CoA with the help of enzyme pyruvate dehydrogenase. Unlike glucose, proteins and fat are transformed directly into activated acetic acid or acetyl CoA. During Krebs cycle (figure 4), free hydrogen atoms are obtained producing reduced coenzymes NADH and $FADH_2$ which contain more energy than their non-reduced state, and this energy is used in the last step of oxidative phosphorylation to synthesize ATP from ADP. During oxidative

phosphorylation, reduced coenzymes NADH and $FADH_2$ are oxidized with the support of oxygen, and since hydrogen is split into protons and electrons in this last step, electrons are passed from one carrier to another which is known as the electron-transfer chain. For the entire process of rebuilding ATP, 36 molecules are obtained in total per molecule of glucose; 2 molecules of ATP from glycolysis, 2 molecules of GTP from the Krebs cycle, and 32 molecules of ATP from oxidative phosphorylation (Feiner, 2006).

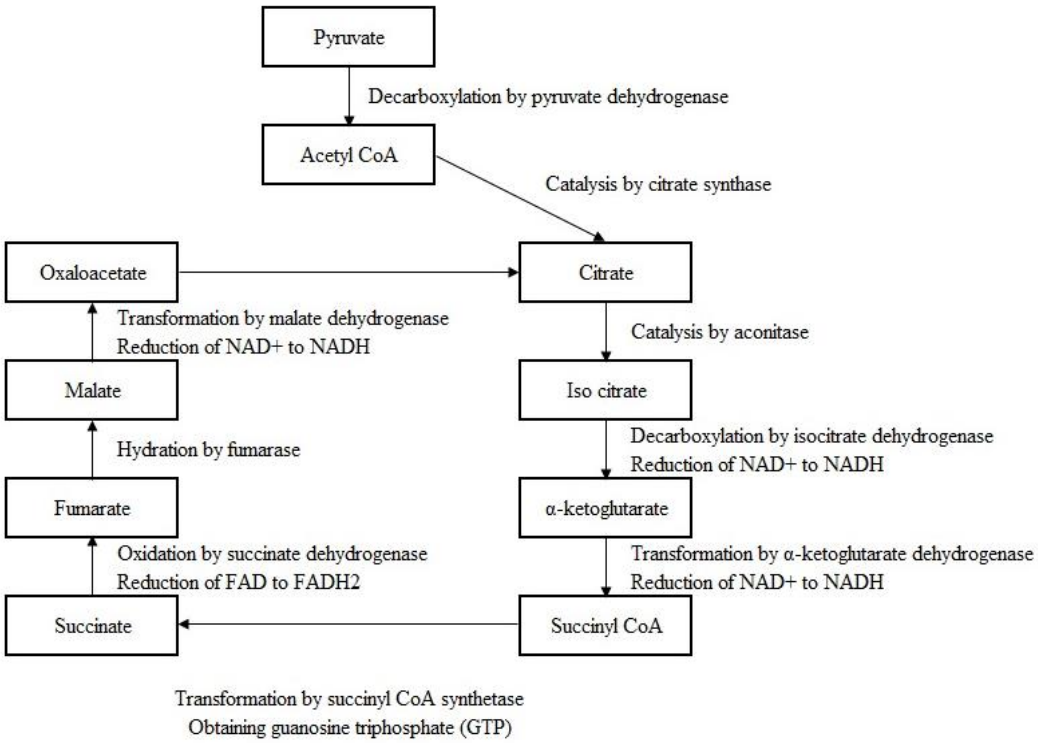

Figure 4: The Krebs cycle (Source: Feiner, 2006)

2.5.2 Biochemical processes in meat post-slaughter

Once the animal is slaughtered, oxygen concentration in the muscle will start decreasing rapidly. At this point, whether oxygen is present or not, glycolysis still takes place by converting glycogen into pyruvate as in the muscle of living animals (figure 5). However, due to the unavailability of oxygen, the pyruvate obtained is no longer transformed into acetyl CoA resulting no activated acetic acid enters the Krebs cycle to produce reduced coenzymes, and oxidative phosphorylation will never happen. Instead, pyruvate catalyzed by the enzyme lactate dehydrogenase is reduced mainly to lactic acid which is not carried back to the liver as in live animals, and increases steadily after slaughter resulting in the decrease of pH in muscle tissue. Under anaerobic glycolysis, only 3 molecules of ATP, but 38 molecules of lactic acid are obtained from one molecule

of glucose, and as the concentration of ATP drops below 1µmol per gram of muscle tissue, actin and myosin are bound together to form the actomyosin complex leading to the rigor mortis (figure 6). Then meat enters another stage called maturing or ripening, and is tenderized by enzymes released from pH value decline (Feiner, 2006). Refer to figure 7 for the chemical and physical changes in pig muscle postmortem.

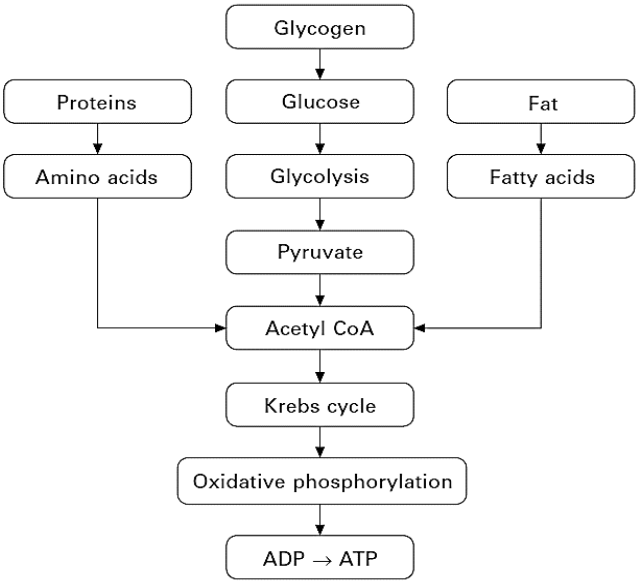

Figure 5: The process of obtaining ATP under aerobic conditions (Source: Feiner, 2006)

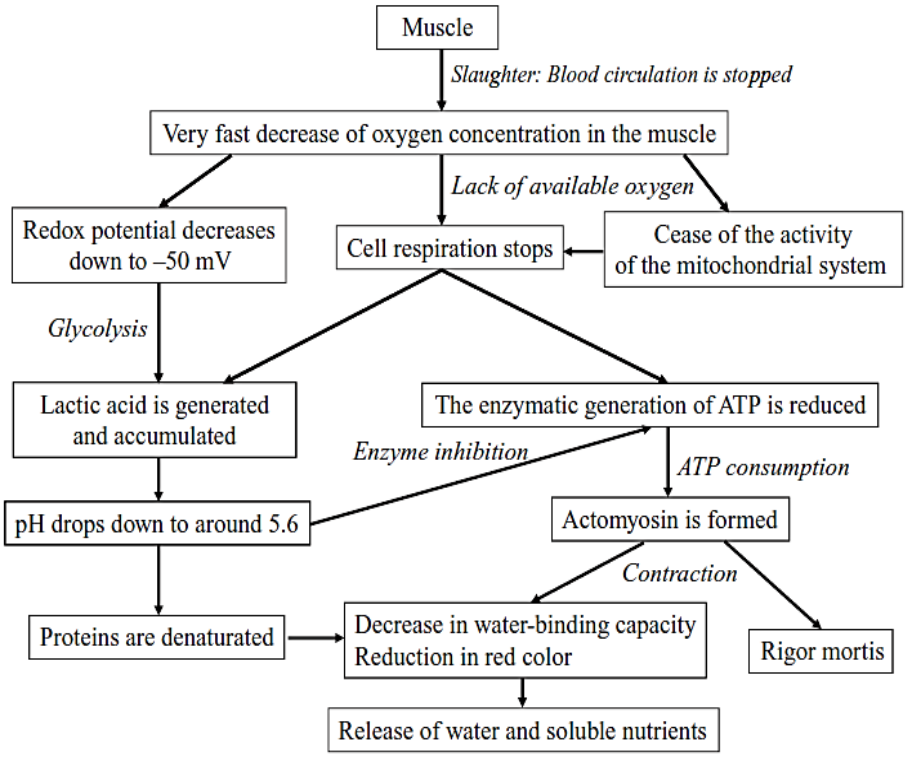

Figure 6: Summary of main changes during conversion of muscle to meat (Source: Toldrá and Reig, 2012)

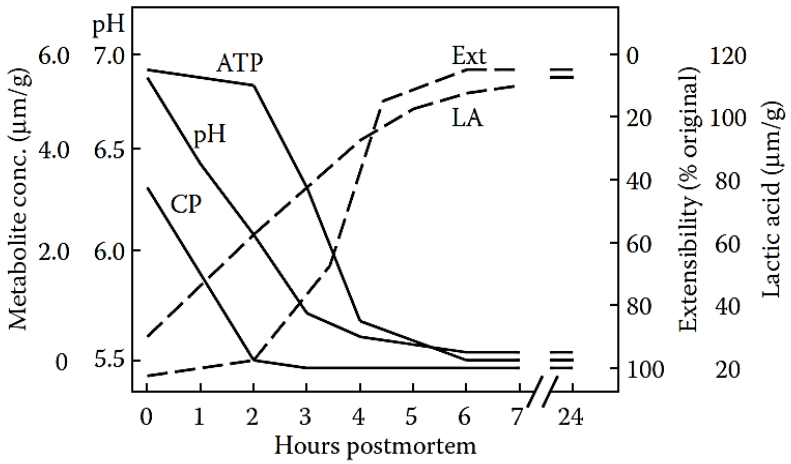

Figure 7: Chemical and physical changes in pig muscle postmortem (Source: Greaser and Guo, 2012)

2.6 Storage life and spoilage of meat

Due to its biological composition, fresh meat is a highly perishable product (Lambert et al., 1991). To prevent or delay quality deterioration, meat needs to be packaged and stored properly. As stated by Gill and Harrison (1989), pork muscle cut *longissimus dorsi* gets spoilt after 2 weeks under vacuum packaging and 5.5 weeks under CO_2 at 3°C by *Brochothrix thermosphacta*. However, the acidity of the meat and the structure of the muscular tissue also play an important role in meat spoilage (Berkel et al., 2004). As defined by Berk (2013), spoilage is any process contributing to the deterioration of the safety, sensory quality or nutritional value of food and can be classified as microbial, enzymatic, chemical, and physical.

2.7 Volatile compounds in meat

Immediately after slaughter, post-mortem biochemical processes start leading to the generation of a large number of volatiles such as acids, aliphatic aldehydes, ketones, and alcohols, and the formation of some others such as nitrogen- and sulfur-containing compounds. According to Mottram (1998), this happens as the lipid fraction of meat, particularly phospholipids, undergoes autoxidation phenomena. To Vinauskiene et al. (2002), the production of volatile compounds is also associated with meat deterioration during refrigerated storage while breed and farming system should not be neglected (Elmore et al., 1999; Cameron et al., 2000). However, lipids are possibly the most important precursor among different others (Gray et al., 1996; Ahn et al., 1997).

Table 6: Main reactions of secondary aroma-active compound formation

Reactions	Main classes of compounds produced
Hydrolysis	Acids, alcohols
Oxidation	Aldehydes, ketones, hydrocarbons, lactones
Pyrolysis	Heterocycles, hydrocarbons
Fermentation	Esters, acids, acetals
Decarboxylation	Amines, hydrocarbons, ketones
Strecker degradation	Aldehydes, heterocycles
Maillard reactions	Heterocycles, aldehydes

(Source: Davidek et al., 1990)

Table 7: Main reactions producing hydrocarbons

Precursor	Type of reaction
Terpenes	Enzymatic reactions
Fatty acids, esters	Oxidation reactions
Various aromatic, alicyclic substances	Pyrolysis
Secondary alcohols, sterols	Dehydration
Carboxylic acids	Decarboxylation

(Source: Davidek et al., 1990)

Table 8: Main reactions leading to aldehyde formation

Precursor	Type of reaction
Unsaturated lipids	Autoxidation and lipoxygenase-catalyzed oxidation
Amino acids	Strecker degradation, oxidative deamination
Saccharides	Non-enzymatic browning reactions, reverse aldolization
Phenolic substances	Enzymatic reactions
Primary alcohols, cyclitols	Free-radical or enzymatic oxidations
Acetals	Hydrolysis
Hydroxy acids	Decarboxylation

(Source: Davidek et al., 1990)

Table 9: Formation of heterocyclic aroma compounds

Precursors	Types of reaction	Products
Monosaccharides, oligosaccharides	Pyrolysis (caramelization, roasting) Maillard reactions	Furans and pyrans
Amino acids, peptides, proteins	Pyrolysis (frying, roasting) Strecker degradation Maillard reactions	Pyrroles, pyrazines, piperazines, imidazoles
Fatty acids, lipids	Oxidation	Furans and pyrans
Sulphur-containing amino acids	Pyrolysis (frying, roasting)	Thiophens, thiazoles, thiolans, trithians

(Source: Davidek et al., 1990)

2.7.1 Lipid oxidation in meat

Lipid oxidation, which is also known as peroxidation or autoxidation, causes quality deterioration of stored meat and meat products (Min and Ahn, 2005; Ladikos and Lougovois, 1990). Lipid oxidation is associated with flavour deterioration (development of rancidity or warmed-over flavour), loss of colour (redness), loss of nutritional value, functional property changes, or the formation of toxic compounds, all of which influence consumer acceptance of the meat (Addis, 1986; Frankel, 1984). Oxidation can occur through auto-oxidation (which can occur in the dark and at room temperature through the production of free radicals), photo-oxidation (occurs in the light when certain sensitisers are present) and also can be induced by enzymes (such as lipase). The mechanisms have been reviewed in detail by Saxby (1993) and Hamilton (2003). In animal tissues, the main unsaturated fatty acids are oleic, linoleic, linolenic, and arachidonic (Ladikos and Lougovois, 1990), all of which susceptible to oxidation. The other factors such as processing and storage conditions of meat, antioxidants and additives, or pro-oxidants (free iron) could also affect lipid oxidation development in meat. Considered as a major catalyst for the initiation step of lipid oxidation, iron in meat is found in hemoglobin and myoglobin, iron-containing enzymes, and transferrin (Min and Ahn, 2005).

2.7.2 Strecker degradation

The Strecker degradation (SD) plays several roles in the formation of flavour compounds in processed foods. Primarily, it is the major pathway for conversion of amino

acids into structurally related aldehydes of significant flavour value. Also, the SD provides a relatively low energy route for mobilizing amino acids' nitrogen and sulfur to form ammonia, hydrogen sulfide and many flavour-significant S/N/O-containing heterocyclic compounds. Finally, the SD provides a reduction mechanism for conversion of dicarbonyls into acyloins thereby opening the door to still more diverse flavour compound formation (Rizzi, 1999).

2.7.3 Maillard reaction

The Maillard reaction, which is also known as browning reaction, is one of the most important flavour-producing reactions in cooking. The chemical mechanisms involve the condensation of the carbonyl group of the reducing sugar with the amino compound to give a glycosylamine. During thermal processing, this breaks down to various sugar dehydration and degradation products. These compounds then interact with other reactive components such as amines, amino acids, aldehydes, hydrogen sulphide, and ammonia, and it is these reactions which provide the basis for the colours and aromas which characterize cooked foods (Mottram, 2007).

Because the generation of volatile compounds in meat do influence the flavour, a lot of researches have been conducted on both cooked and raw meat. Focusing on the studies of volatile compounds in raw meat of pork, two main articles were reviewed; one was done by Estevez et al. (2003) and the other by Soncin et al. (2007). In 2003, the analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS was studied. The results showed that methyl alcohols and ketones (such as 2-ethyl-hexan-1-ol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol, and 3-hydroxy-butan-2-one) were the most representative in refrigerated meat (Table 10) due to the degradation of carbohydrates and proteins together with the Strecker degradation pathway while lipid-derived volatiles were the most abundant in cooked meat and refrigerated cooked meat. The other study conducted in 2007 was the preliminary study of the volatile fraction in the raw meat of pork, duck, and goose. Among the three species, different chemical compositions were detected. While alcohols, ketones, and 2-pentylfuran were found in pork (Table 12), aldehydes and hexanoic acid were detected in duck. These showed that endogenous compounds derived from lipid peroxidation were predominant in pork and duck. Distinct from the two aforementioned, carbon disulphide and a contaminant (p-dichlorobenzene) were found predominant in the goose.

Olmo et al. (2014) studied the effect of high-pressure-processing and modified-atmosphere-packaging on the volatile compounds and odour characteristics of sliced ready-to-eat "lacón", a cured-cooked pork meat product. The results indicated that in vacuum-packaged "lacón", levels of esters, alcohols, acids, and benzenic compounds increased until day 120 while ketones and sulphur compounds peaked on day 60 and declined afterwards. In modified-atmosphere-packaged "lacón", the levels of easters, sulphur compounds, and alcohols were lower, and the levels of hydrocarbons were higher than those in vacuum-packaged "lacón". In high-pressure-processing "lacón", the levels of acids, alcohols, esters, and sulphur compounds were lower, and the levels of aldehydes were higher than those in vacuum-packaged "lacón". Kang et al. (2013) evaluated the effects of high pressure processing on fatty acid composition and volatile compounds in Korean native black goat meat. The results showed that the 9,12-octadecadienoic acid and octadecanoic acid, well-known causes of off-flavours, were detected, and the volatile compounds in the meat were affected by high pressure processing. Acevedo et al. (2012) studied on modeling volatile organic compounds released by bovine fresh meat using an integration of SPME and databases. Experimental data indicated that post-mortem glycolysis is related with the release of volatile compounds in fresh meat. Therefore, the proposed technique could be used to study possible sources of biochemical compounds in meat. Vasta et al. (2011) worked on the volatile profile of *longissimus dorsi* muscle of heifers fed pasture, pasture silage or cereal concentrate: implication for dietary discrimination. The results revealed that some aldehydes, ketones, and furans derived from lipid oxidation were affected by the treatments. While skatole, 3-undecanone, cuminic alcohol, 1-butanol, and 2-methyl were accounted mostly for this dietary discrimination, germacrene D, a terpenoid, was a maker of grass feeding. Ventanas et al. (2008) analysed volatile compounds of Iberian dry-cured loins with different intramuscular fat (IMF) contents using SPME-DED. The results demonstrated that the amount of the main chemical families identified (hydrocarbons, aldehydes, alcohols, ketones, and acids) increased with ripening time. In the headspace of high IMF loins, higher content of some volatile compounds derived from lipid oxidative reactions and others from the degradation of certain amino acids were displayed. Therefore, IMF content could influence both the generation of volatile compounds and the transfer of such compounds from the product matrix to the headspace.

Table 10: Volatile compounds (AU × 10^6) detected in the headspace of raw meat and refrigerated meat from lean and Iberian pigs

compounds	raw meat		refrigerated meat		RT	IM
	lean	Iberian	lean	Iberian		
acetic acid	7.59	4.13	8.31	5.65	7.95	MS
butanoic acid	0.93	0.53	0.00	0.00	16.78	MS, KI
octanoic acid	1.09	0.00	1.82	1.61	28.04	MS
nonanoic acid	3.09	5.74	1.84	1.82	30.23	MS, KI
butane-2,3-dione	0.00	0.00	4.72	5.54	6.42	MS
3-hydroxy-butan-2-one	0.88	0.00	70.72	64.32	14.06	MS
heptan-2-one	0.28	0.08	5.60	2.42	20.80	MS, KI
octan-2-one	0.00	0.00	0.76	0.57	23.85	MS, KI
pentanal	0.00	0.00	2.61	0.75	12.29	MS, KI
hexanal	2.17	3.80	5.11	4.46	17.44	MS, KI
heptanal	0.00	0.00	0.16	0.50	21.17	MS, KI
octanal	3.87	1.75	2.29	3.68	24.21	MS, KI
oct-(*E*)-2-enal	0.00	0.00	0.71	0.98	25.72	MS, KI
nonanal	15.38	9.74	13.33	16.97	26.85	MS, KI
decanal	0.00	0.00	1.53	2.14	29.22	MS
dec-(*E*)-2-enal	0.00	0.00	3.50	2.06	30.5	MS, KI
undec-(*E*)-2-enal	0.00	0.00	2.24	2.69	32.62	MS, KI
3-methyl-butan-1-ol	0.00	0.00	7.43	23.31	14.35	MS, KI
2-methyl-butan-1-ol	0.00	0.00	0.98	8.20	14.55	MS, KI
pentan-1-ol	0.00	0.00	0.00	5.04	15.89	MS, KI
butane-2,3-diol	0.00	0.00	17.19	5.73	17.22	MS
hexan-1-ol	0.69	6.16	6.29	7.31	20.01	MS, KI
heptan-1-ol	6.89	4.37	2.52	4.84	23.19	MS, KI
oct-1-en-3-ol	1.62	2.01	2.52	2.39	23.5	MS, KI
2-ethyl-hexan-1-ol	70.81	7.96	232.19	17.33	24.87	MS
octan-1-ol	9.01	8.07	4.18	3.83	25.92	MS, KI
2-phenyl-ethanol	0.00	0.00	1.74	0.68	27.35	MS
dodecan-1-ol	0.00	0.00	1.26	1.19	34.62	MS, KI
acetic acid ethyl ester	0.00	0.00	3.94	3.80	7.36	MS
1,1,2,2-tetrachloro-ethane	4.82	0.00	0.00	0.00	17.98	MS
nona-(E,E)-1,3-diene	0.00	0.00	1.15	0.00	21.98	MS
2,2,4,6,6-pentamethyl-heptane	9.61	2.30	0.00	0.00	23.99	MS
decane	7.77	1.33	1.07	0.84	24.07	MS, KI
limonene	11.87	1.43	1.11	1.14	25.14	MS, KI
dimethyl-sulfide	0.00	0.00	1.19	0.95	15.00	MS, KI
dihydro-furan-2-one	0.53	1.63	0.00	0.78	21.73	MS
2-pentyl-furan	0.83	2.27	0.76	2.78	23.94	MS, KI
pyridine	2.08	2.50	0.00	0.00	15.09	MS
methyl-benzene	5.07	4.65	4.03	5.66	16.09	MS, KI
1,3-dimethyl-benzene	7.91	5.82	2.08	11.74	20.24	MS, KI

(Source: Estévez et al., 2003)

Table 11: Volatile compounds found in fresh and marinated pork with different packaging atmosphere (air, MAP, and vacuum)

compounds	fresh (n=27)				marinated (n=27)			
	H	A/V	A/MAP	V/MAP	H	A/V	A/MAP	V/MAP
2,4-dimethyl-hexane	0.18	ns	ns	ns	3.19	ns	ns	ns
3-methyl-hexane	0.70	ns	ns	ns	0.97	ns	ns	ns
2,5-dimethyl-hexane	0.03	ns	ns	ns	1.34	ns	ns	ns
2,4-dimethyl-heptane	1.10	ns	ns	ns	0.95	ns	ns	ns
2-propanone	3.56	ns	ns	ns	1.76	ns	ns	ns
2-octene	3.43	ns	ns	ns	7.16	ns	ns	ns
ethyl ester acetic acid	0.17	ns	ns	ns	6.10	ns	ns	ns
2-butanone	1.19	ns	ns	ns	1.86	ns	ns	ns
2-(1-methylethoxy) -1-propanol	8.51	ns	ns	$p < 0.05$	1.90	ns	ns	ns
2-hydroxypropanoic acid ethyl ester	0.68	ns	ns	ns	2.04	ns	ns	ns
2-pentanone	2.35	ns	ns	ns	1.48	ns	ns	ns
pentanal	1.75	ns	ns	ns	1.19	ns	ns	ns
acetic acid ethenyl ester	3.28	ns	ns	ns	0.58	ns	ns	ns
chloroform	1.55	ns	ns	ns	1.45	ns	ns	ns
2-butanol	1.58	ns	ns	ns	6.36	ns	ns	ns
ethyl ester butanoic acid	0.23	ns	ns	ns	3.11	ns	ns	ns
3,5,5-trimethyl-1-hexene	0.69	ns	ns	ns	9.36	ns	$p < 0.01$	ns
dimethyl disulphide	0.83	ns	ns	ns	0.39	ns	ns	ns
hexanal	0.13	ns	ns	ns	1.17	ns	ns	ns
2-beta-pinene	0.00	ns	ns	ns	14.38	ns	$p < 0.05$	$p < 0.001$
2-nitrobutane	0.00	ns	ns	ns	5.36	ns	ns	ns
3-carene	0.00	ns	ns	ns	0.02	ns	ns	ns
heptanal	0.11	ns	ns	ns	2.25	ns	ns	ns
dl-limonene	0.00	ns	ns	ns	3.75	ns	ns	ns
3-methyl-1-butanol	6.92	ns	ns	ns	8.65	ns	ns	$p < 0.05$
2-pentyl-furane	0.71	ns	ns	ns	1.66	ns	ns	ns
ethyl ester hexanoic acid	3.01	ns	ns	ns	2.04	ns	ns	ns
1-(methylphenyl)-ethanone	7.41	ns	ns	$p < 0.05$	4.12	ns	ns	ns
octanal	0.07	ns	ns	ns	2.51	ns	ns	ns
1-hexanol	2.57	ns	ns	ns	0.89	ns	ns	ns
nonanal	1.79	ns	ns	ns	1.45	ns	ns	ns
1-heptanol	0.27	ns	ns	ns	1.63	ns	ns	ns
decanal	0.84	ns	ns	ns	0.57	ns	ns	ns
2-ethyl-1-hexanol	0.00	ns	ns	ns	4.71	ns	ns	ns
1-octanol	0.02	ns	ns	ns	3.25	ns	ns	ns
4-methyl-1-(1-methylethyl) -3-cyclohexen-1-ol	2.43	ns	ns	ns	1.00	ns	ns	ns
2-decenal	0.05	ns	ns	ns	0.55	ns	ns	ns

(Source: Garcia-Marquez et al., 2013)

Table 12: Volatile compounds detected in pork

compounds	RT	Sample 1	Sample 2	Sample 3	Sample 4
Alcohols					
1-pentanol	10.40[a]	9.2[b]	n.d.	n.d.	10.1
1-hexanol	11.80-12.33	50.9	4.1	18.5	25.0
1-heptanol	13.81-13.83	3.7	n.d.	n.d.	1.3
1-octanol	15.37	2.4	n.d.	n.d.	n.d.
1-octen-3-ol	13.73-13.80	10.1	4.8	8.5	12.3
2-phenylethanol	19.92-19.95	3.4	4.3	6.0	n.d.
3-methyl-1-butanol	9.53	n.d.	48.0	n.d.	n.d.
Ketones					
3-hydroxybutanone	10.94-11.03	18.2	7.3	65.7	36.1
2-nonanone	12.80	n.d.	29.6	n.d.	n.d.
Heterocyclic compound					
2-pentylfuran	9.94	n.d.	n.d.	n.d.	13.1
Total unknown		2.1	1.9	1.3	2.1

[a] Single value (if it is the same for the four samples) or extreme values (if different).

[b] Percent of the total area of the detected compounds.

(Source: Soncin et al., 2007)

Table 13: Volatile compounds identified in raw pork

compounds	RT	IM	evidence in the literature
acetone	1.93	MS, KI	Overland et al. (2011); Moon et al. (2006)
pentanal	3.19	MS, KI	Overland et al. (2011); Calkins and Hodgen (2007); Moon et al. (2006)
hexanal	5.26	MS, KI	Overland et al. (2011); Soncin et al. (2007); Moon et al. (2006)
heptanal	7.72	MS, KI	Overland et al. (2011); Moon et al. (2006)
2-pentylfuran	8.64	MS, KI	Soncin et al. (2007); Hodgen (2006); Moon et al. (2006)
pentanol	9.58	MS, KI	Soncin et al. (2007); Moon et al. (2006)
octanal	10.19	MS, KI	Moon et al. (2006)
tridecane	10.50	MS, KI	Moon et al. (2006); Yuan et al. (2006)
2,3-octanedione	10.84	MS, KI	Moon et al. (2006); Yuan et al. (2006)
hexanol	11.75	MS, KI	Soncin et al. (2007); Moon et al. (2006)
nonanal	12.13	MS, KI	Overland et al. (2011); Soncin et al. (2007); Moon et al. (2006)
1-octen-3-ol	13.17	MS, KI	Ba et al. (2012); Baruth and Ternes (2011); Soncin et al. (2007); Moon et al. (2006); Yuan et al.(2006)
heptanol	13.39	MS, KI	Soncin et al. (2007); Moon et al. (2006); Yuan et al. (2006)
octanol	15.21	MS, KI	Soncin et al. (2007); Moon et al. (2006); Arnold and Senter (1998)
2-octen-1-ol	15.96	MS, KI	Calkins and Hodgen (2007)
hexanoic acid	18.95	MS, KI	Soncin et al. (2007); Moon et al. (2006); Yuan et al. (2006)
heptanoic acid	20.16	MS, KI	Calkins and Hodgen (2007)
octanoic acid	21.59	MS, KI	Calkins and Hodgen (2007)

(Source: Gasior and Wojtycza, 2016)

2.8 Gas chromatography and mass spectrometry

Gas chromatography and mass spectrometry (GC-MS) is the combination of two powerful analytical techniques, which can be further explored in many excellent books (Hübschmann, 2008; McLafferty and Turecek, 1993; McMaster, 2008; Sparkman and Penton, 2011). While the gas chromatography separates the components of a mixture in time, the mass spectrometer does provide information that aids in the structural identification of each component (Larsen et al., 1996). GC-MS has been extensively used to analyse complex organic and biochemical mixtures (Skoog et al., 2007) in the fields of environmental science, forensics, health care, medical and biological research, health and safety, the flavour and fragrances industry, food safety, packaging, and many others (Penton et al., 2011). Please see figure 8 for a schematic diagram of a GC-MS system.

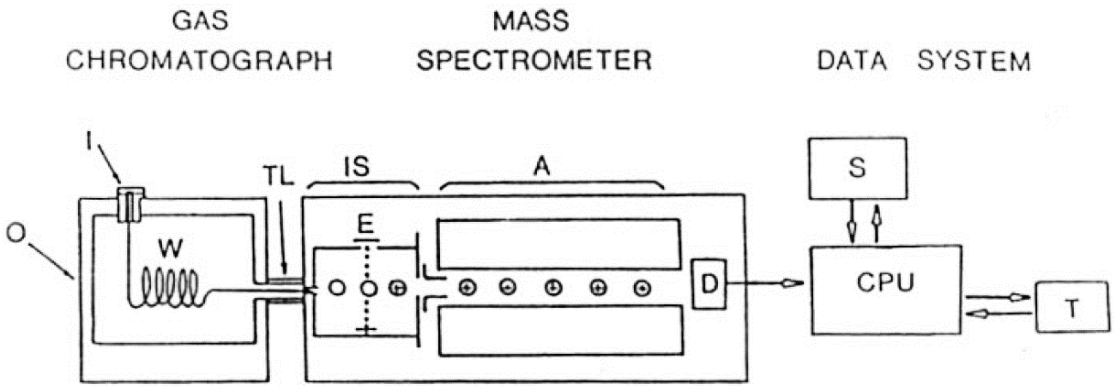

Figure 8: A schematic diagram of a GC-MS system (Source: Ministry of Environment, 1991) (O=Oven, I=Injector, W=WCOT Column, TL=Transfer Line, IS=Ion Source, E=Electron Beam, A=Analyser, D=Detector, CPU=Central Processing Unit, T=Terminal, S=Data Storage Device)

Gas chromatography is a widely applied technique involving the distribution or partitioning of a compound between a mobile phase and a stationary phase. In GC, the mobile phase is a carrier gas, usually helium, nitrogen, hydrogen or argon, and the stationary phase is an immobile, high molecular weight liquid which is deposited on or chemically bonded to the inner walls of a long capillary tubing called a chromatographic column. One of the most important characteristics of the GC column is its resolution or the ability to separate components with very similar distribution constants between the two phases (Stashenko and Martinez, 2014). During analysis, sample is swept through

the column by a stream of carrier gas. Components in the sample are then separated from each other based on the amount of time consumed to pass through the column – called retention time which depends mainly on their chemical structure (Hussain and Maqbool, 2014). Please see figure 9 for a gas chromatogram of a premium grade petrol.

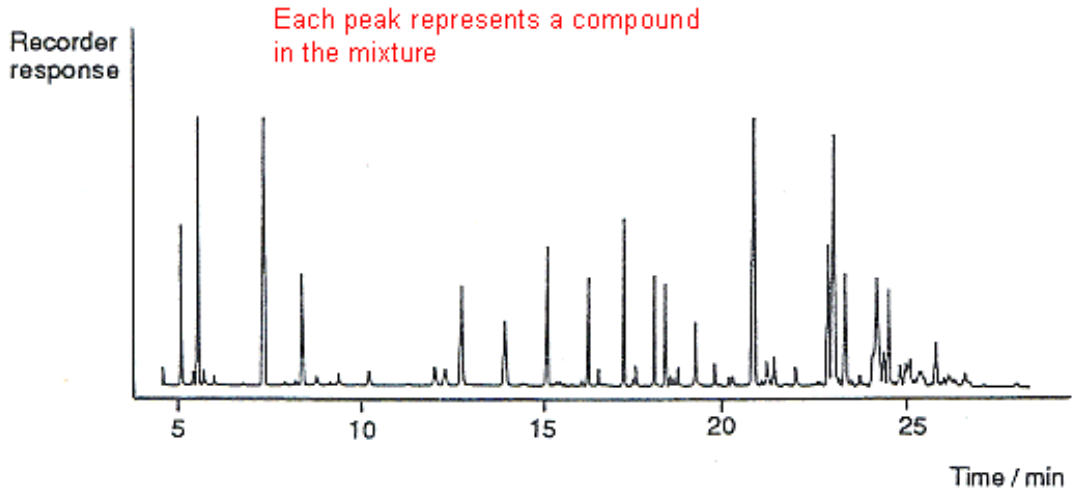

Figure 9: A gas chromatogram of a premium grade petrol (Source: Gas – Lipid Chromatography, 2017)

Column bleed is the normal background signal generated by the column stationary phase. Because column bleed originates from the phase, the level of bleed is proportional to the amount of phase in the column. Therefore, thicker film columns bleed more than thinner film ones. While column bleed levels may differ from column to column, all columns bleed to a certain extent. The higher the temperature, the higher the level of bleed and, at a constant temperature, the level of true column bleed should remain constant (Kinesis, 2017).

Mass spectrometry is one of the most used techniques to obtain the fingerprint of the molecule called a mass spectrum in food science. Once molecules enter the source chamber of the mass spectrometer, they will be bombarded by electrons. Because of the energy transferred during this process, ionization and fragmentation will happen. Ions will be detected by an extremely sensitive device called an electron multiplier after travelling across the analyser section where they are separated according to their mass-to-charge ratio (m/z). By plotting the abundance of ions detected versus their m/z, a mass spectrum is obtained. Like a fingerprint, the mass spectrum of a compound consists of a bar graph representation of the m/z of the ions and their abundance normalized to the

most abundant ion (base peak) which can be used to identify the original organic structure. To obtain a positive identification of the sample component, the GC retention time of a sample component and its mass spectrum are used to match with those of a standard reference compound analysed under the same conditions (Ministry of Environment, 1991). Please see figure 10 for a mass spectrum of methyl 5-oxo stearate (octadecanoate).

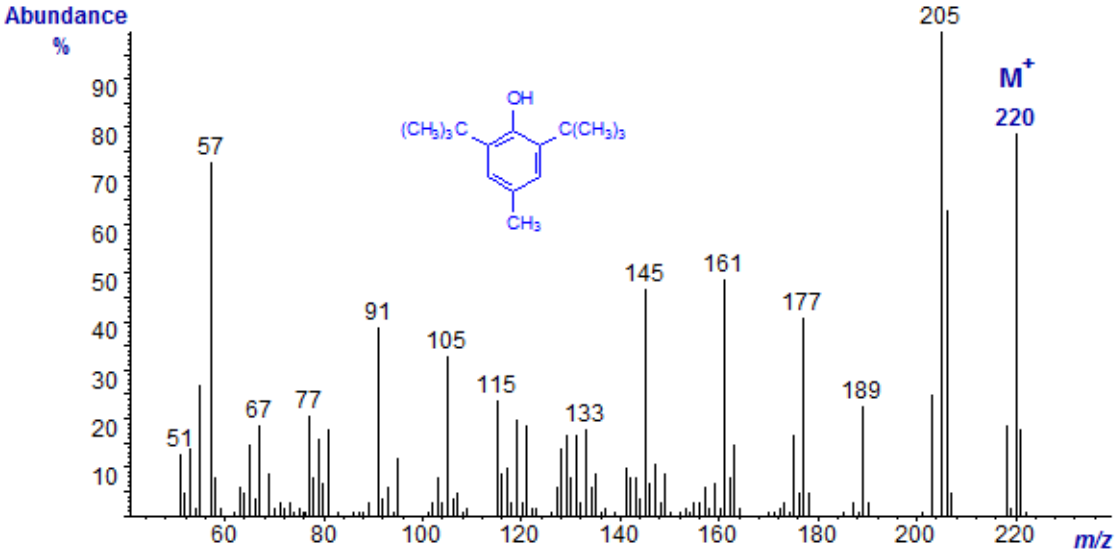

Figure 10: A mass spectrum of methyl 5-oxo stearate (octadecanoate) (Source: AOCS Lipid Library, 2014)

Quadrupole mass analyser, which is also known as quadrupole mass filter (QMF) or quadrupole mass spectrometer, is one type of mass analyser used in mass spectrometry and a key component of a modern mass spectrometer. A QMF uses direct current and alternating current electric fields to analyse positive or negative ions by mass to charge ratio. A QMF consists of 4 parallel rods spaced equidistantly, the ratio of the rod radius to the radius of the inscribed circle is 1.148. Opposite pairs of rods are electrically connected. Typical rod diameters are between 5 and 12 mm with rod lengths between 100 and 200 mm. The frequency of the alternating current component of the electric field is typically in the range 1 to 10 MHz (Comsol, 2017). There are two types of QMF; single and triple QMF. Mass range, sensitivity, abundance sensitivity, resolution, and transmission are determined by the quadrupole mass filter rod size and the RF operating frequency. Increasing the RF operating frequency increases the sensitivity, abundance sensitivity, resolution, and the high energy ion transmission while increasing the

quadrupole rod size increases ion transmission. Also, decreasing the quadrupole rod size or the RF operating frequency increases the mass range (HennikerScientific, 2017).

Vasta et al. (2007) worked on mass spectrometry analysis of volatile compounds in raw meat for the authentication of the feeding background of farm animals. According to univariate and multivariate data treatments performed on virtual-DH-MS fingerprints, volatile fraction of the meat was associated with the type of feeding of the living animals. By using DH-GC-MS, 33 volatile compounds among the 204 detected in the muscle enabled us to discriminate the feeding type of the lambs. In 2012, Fischer et al. determined boar taint compound skatole in meat juice by using stable isotope dilution analysis – direct immersion-SPME-GC-MS. The results revealed that the proposed technique is suitable for such a research.

2.9 Extraction methods

Regarding the extraction of volatiles from meat, some methods have been implemented worldwide by researchers based on time, money, sample, and solvent. Choosing a suitable extraction technique is very important since its efficiency could impact the chemical identification dramatically (Lin, 2014). Compared with other extraction methods such as dynamic headspace extraction (DHE), simultaneous distillation extraction (SDE), and purge and trap (P&T), solid-phase microextraction (SPME) is one of the most significant developments in sample preparation (Xu et al., 2016). Please see figure 11, 12 and 13 for a schematic diagram of DHE, SDE and P&T.

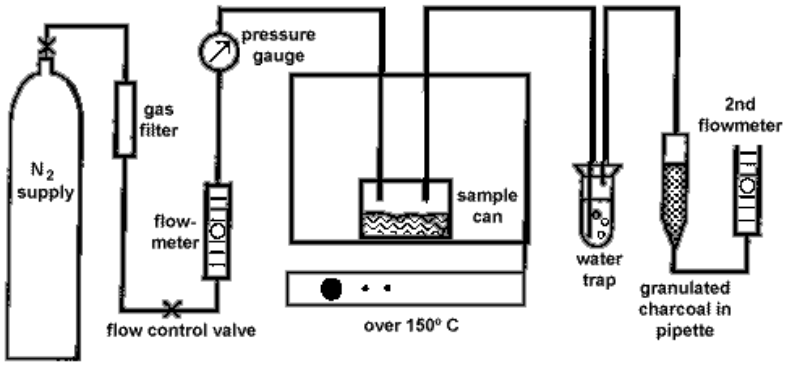

Figure 11: A schematic diagram of DHE equipment (Source: Cafe and Stern, 1989)

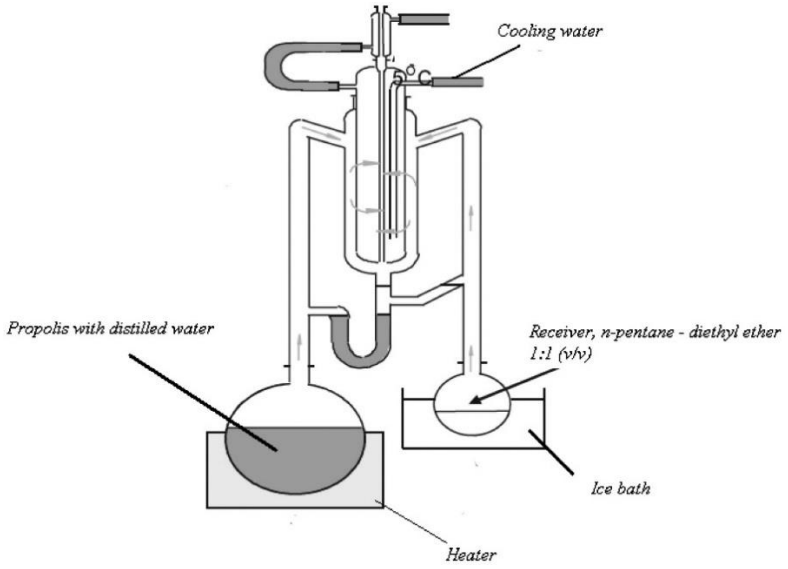

Figure 12: A schematic diagram of Likens-Nickerson apparatus for distillation – extraction of volatiles (Source: Bankova et al., 2016)

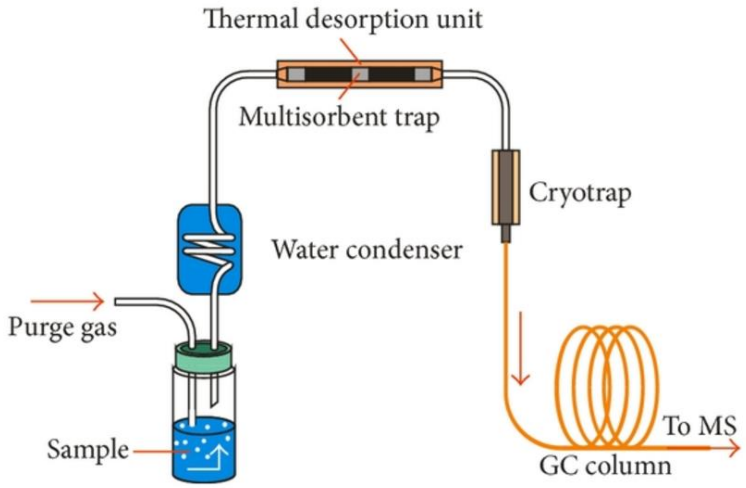

Figure 13: A schematic diagram of P&T technique (Source: ResearchGate, 2017)

SPME is a simple, sensitive, rapid, and solvent-free extraction method of analytes from gaseous, liquid, and solid samples (Merkle et al., 2015) which can be routinely used in combination with GC-MS (Vas and Vékey, 2004). SPME has been demonstrated to be a useful technique to extract volatile compounds from meat and meat products on account of its high flexibility and one-step combination of sampling, isolation, concentration, and enrichment. However, coating material, the core of SPME technique and could directly determine the sensitivity and selectivity of the method, should be taken into account. Sorbent materials such as carbon materials, mesoporous nanomaterials, nano inorganic oxides, ionic liquids, molecular imprinting polymers, and mesoporous organicinorganic

hybrid materials are usually designed with larger specific surface area or controllable pore size (Xu et al., 2016). Each SPME fibre type is limited by the number of injections. Over usage of it might lead to fibre bleed resulting contamination from siloxanes (Elmore, 2014).

The efficiency of SPME is not only influenced by fibre stationary phase, but also other chromatographic parameters. The two critical parameters that should not be neglected and might impact the extraction efficiency are extraction temperature and time. With the increasing extraction time before the equilibrium, the total analyte concentration increases while increased temperature can transfer more energy to analytes, mainly the high molecular weight compounds, from liquid to headspace. Once the high molecular weight compounds are adsorbed by the fiber, the low molecular weight compounds will be hardly adsorbed particularly at the higher temperature. Therefore, the recovery of semi-volatile compounds is usually higher than volatile compounds when high temperature and longer sampling time are adopted in volatile extraction. On the other hand, high temperature will accelerate the equilibrium so as to shorten the required extraction time, but as a result, analyte degradation may occur (Perestrelo et al., 2011). Please refer to figure 14 for a schematic diagram of a commercial SPME device and its types; and table 14-15 for SPME fibres and their temperature and conditioning recommendations for GC use and pH guidelines.

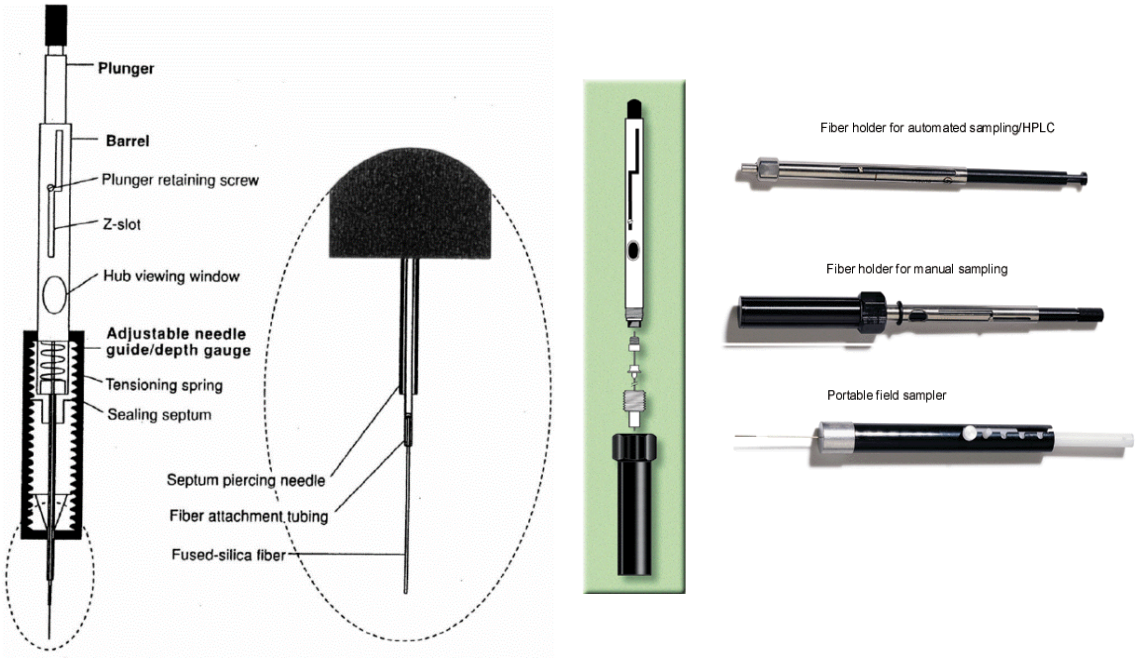

Figure 14: A schematic diagram of a commercial SPME device and its types (Source: Sigma-Aldrich Co., 1999; Vas and Vékey, 2004)

Table 14: Summary of commercially available SPME fibres

Fibre coating	Film thickness (µm)	Polarity	Coating method	Hub description	Technique	Compounds to be analysed
Polydimethylsiloxane (PDMS)	100	Non-polar	Non-bonded	Red-plain	GC/HPLC	Volatiles
	30	Non-polar	Non-bonded	Yellow-plain	GC/HPLC	Non-polar semivolatiles
	7	Non-polar	Bonded	Green-plain	GC/HPLC	Medium- to non-polar semivolatiles
Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)	65	Bipolar	Cross-linked	Blue-plain	GC	Polar volatiles
	60	Bipolar	Cross-linked	Brown-notched	HPLC	General purpose
	65[a]	Bipolar	Cross-linked	Pink-plain	GC	Polar volatiles
Polyacrylate (PA)	85	Polar	Cross-linked	White-plain	GC/HPLC	Polar semivolatiles (phenols)
Carboxen/Polydimethylsiloxane (CAR/PDMS)	75	Bipolar	Cross-linked	Black-plain	GC	Gases and volatiles
	85[a]	Bipolar	Cross-linked	Lt. Blue-plain	GC	Gases and volatiles
Carbowax/Divinlybenzene (CW/DVB)	65	Polar	Cross-linked	Orange-plain	GC	Polar analytes (alcohols)
	70[a]	Polar	Cross-linked	Yellow-green plain	GC	Polar analytes (alcohols)
Carbowax/Templated Resin (CW/TPR)	50	Polar	Cross-linked	Purple-notched	HPLC	Surfactants
Divinlybenzene/Carboxen/PDMS (DVB/CAR/PDMS)	50/30[a]	Bipolar	Cross-linked	Gray-notched	GC	Odours and flavours

[a] Stableflex type is on a 2 cm length fibre.

(Source: Vas and Vékey, 2004; Sigma-Aldrich Co., 1999)

Table 15: Temperature and conditioning recommendations for GC use and pH guidelines

Fibre	Film Thickness (µm)	pH	Maximum Temperature (°C)	Recommended Operating Temp. (°C)	Conditioning Temperature (°C)	Time (Hrs.)
PDMS	100	2-10	280	200-280	250	0.5
	30	2-11	280	200-280	250	0.5
	7	2-11	340	220-320	320	1
PDMS/DVB	65	2-11	270	200-270	250	0.5
PA	85	2-11	320	220-310	300	2
CAR/PDMS	75	2-11	320	250-310	300	1-2
CW/DVB	65	2-9	260	200-250	220	0.5
DVB/CAR/PDMS	50/30	2-11	270	230-270	270	1

(Source: Sigma-Aldrich Co., 1999)

Argyri et al. (2015) evaluated meat spoilage through the evolution of volatile compounds in the spoilage of minced beef. The volatile compounds of meat were isolated HS-SPME with a 50/30 µm DVB/CAR/PDMS fibre. They found that the HS-SPME-GC-MS analysis provided useful information about a great number of volatile metabolic compounds detected during meat storage. Rivas-Canedo et al. (2011) compared DHE and SPME as extraction methods for analyzing volatile profile in cooked beef. They found out that SPME with a 50/30 µm DVB/CAR/PDMS fibre was more efficient in extracting substances such as 1-alcanols, ethyl esters and acids. Rivas-Canedo et al (2012) assessed the effect of high-pressure treatment on the volatile compounds of low-acid fermented sausage "espetec" and sliced cooked pork shoulder by comparing DHE and SPME methods. SPME was found out to be a more efficient extraction method for a large number of chemical families, especially fatty acids. Watkins et al. (2012) used SDE and SPME techniques to extract volatile compounds in heated beef and sheep fats. A 50/30 µm DVB/CAR/PDMS fibre was used for extracting compounds. More than 100 compounds were characterized in the volatile profiles of the two techniques which differences were observed. Acevedo et al. (2012) used GC-MS-SPME with four SPME fibres to measure volatile organic compounds of bovine fresh meat samples. The 65 µm PDMS/DVB and 50/30 µm DVB/CAR/PDMS were found out to be the most suitable fibres for extracting volatiles from beef. Liu et al. (2006) investigated volatile compounds of traditional Chinese Nanjing water-boiled salted duck during its stages of processing by

using HS-SPME coupled with GC-MS. A 75 µm CAR/PDMS SPME fibre was selected for extraction based on the previous research (Liu et al., 2004). The results showed that the most volatiles identified were degradation products of fatty acids, which were considered to be the typical flavour of duck meat. In 2007, another study on the flavour profiles of traditional Chinese Nanjing marinated duck was conducted by comparing three different extraction techniques; SPME with a CAR/PDMS fibre, P&T using Tenax-TA absorbent, and SDE. Results indicated that SPME method was better than P&T method, and SPME with SDE method may well complement each other. Ma et al. (2013) optimized HS-SPME for GC-MS analysis of aroma compounds in cooked beef. The results suggested that for optimal concentration, HS-SPME should be carried out for 25 minutes at 40 ^{o}C with 10 minutes equilibrium time. Please refer to figure 15 for SPME procedure for GC and LC.

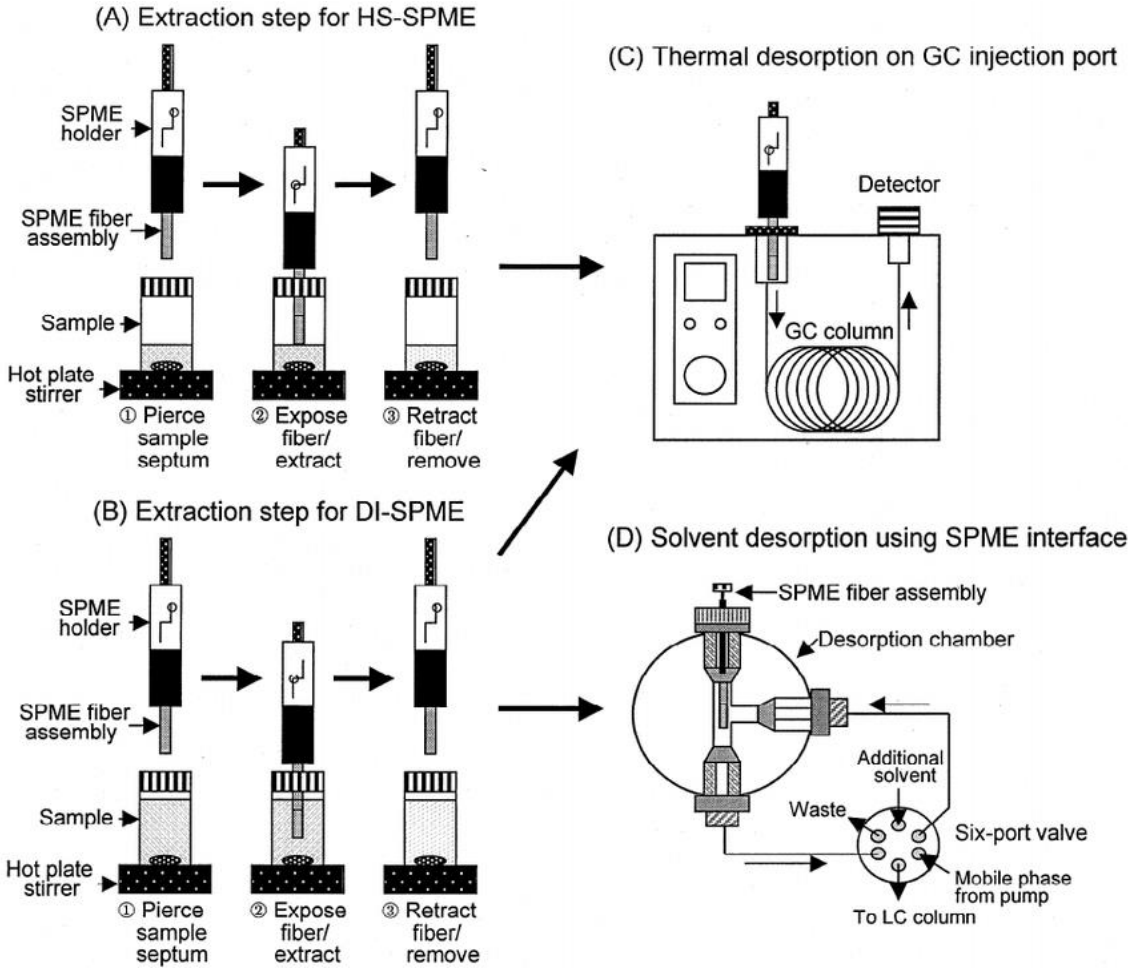

Figure 15: SPME procedure for GC and LC (Source: Vas and Vékey, 2004)

3. Hypotheses and Objectives

There are a lot of researches working on the volatile compounds in cooked meat and meat products. However, there still lacks information in the scientific papers on raw meat, particularly pork. The generation of volatile substances in meat is believed to be associated with lipid fraction undergoing autoxidation phenomena and deterioration during storage. Pork muscle cut will take longer time to get spoiled under vacuum package and refrigeration. Therefore, my hypothesis was that the chemical composition of volatile compounds would change, under different storage conditions, and these changes will be related to aging, later with decomposition of meat and with activity of microorganisms.

The aims of the research were mentioned as follows:

- To monitor volatile substances and their composition under various conditions;
- To confirm of the usability of the method for analysis of volatile substances from meat;
- To recommend good storage condition and duration of pork.

4. Methodology

4.1 Sample and preparation

Organic pork shoulder of Prestice black-pied sow was obtained from the carcass after being slaughtered at 80-180 kg live weight and an age of 8-10 months in Biofarma Sasov's slaughterhouse.

Raw meat was frozen at -18 ^{o}C for 2 weeks before being prepared by being freed from visible fat and plastic contaminants; and cut into 9 pieces (50 g weight) which were then treated as follows:

storage condition	fresh	1 day	3 days	5 days	7 days
room temperature	1	1	1	1	1
refrigeration		1	1	1	1

After being treated, samples were vacuum packaged and kept frozen at -80 ºC until required for analysis.

4.2 SPME analysis

Representative meat samples were left to defrost for 10-15 minutes before being homogenized in an electrical meat grinder for approximately 1 minute. Three aliquots of 2 g from each treatment were placed in 4 ml vials. Each replicate was immersed in a 37 ºC bath for 10 minutes before exposing a 50/30µm DVB/CAR/PDMS SPME fibre to the headspace equilibrated for another 30 minutes. Please see figure 16 for the SPME needle used for the whole study.

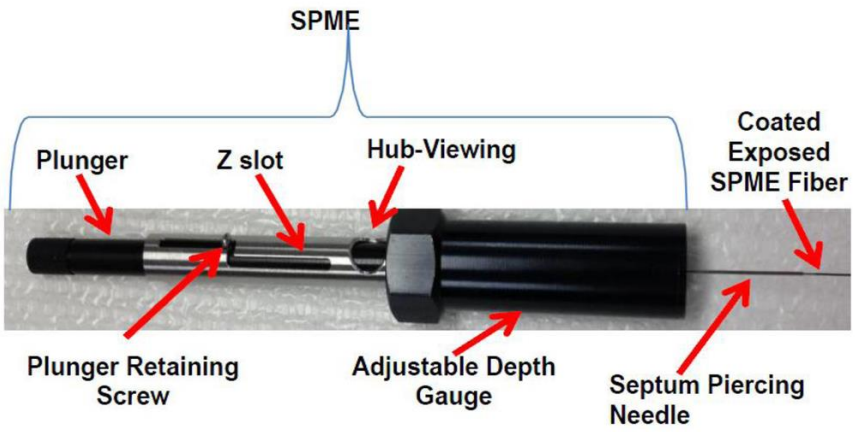

Figure 16: Fibre holder for manual sampling

4.3 GC-MS analysis

Prior to analysis each day, the SPME fibre was preconditioned at 250 ºC, and the blank measurement was done for 45 minutes totally. Analyses were performed on the Agilent 7890B/5977A GC/MSD System (figure 17) using a 5% phenyl methyl silox HP-5 column (30 m x 250 µm x 0.25 µm). The carrier gas was helium with a flow of 1 ml/min at 270 ºC. The SPME fibre was desorbed and maintained in the injection port at 250 ºC during the whole chromatography run (28 minutes). The injector port was in the splitless mode. The temperature program was isothermal for 5 min at 45 ºC and then raised to 250 ºC at a rate of 10 ºC/min and to 300 ºC at 20 ºC/min. *n*-Alkanes (Sigma R-8769) were run under the same conditions to calculate the Kovats Index (KI) values for the compounds.

The MSD transfer line temperature was 270 ºC with ionization energy of 70 eV and collected data at a rate of 1 scan/s over a range of *m/z* 30-600. Data were treated by MassHunter Workstation Software Qualitative Analysis Version B.07.00. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH library version 2.2 and by comparison of KI. The identifications of some volatile compounds were only performed by using MS data because the retention index was unavailable.

Figure 17: The Agilent 7890B/5977A GC/MSD System (Source: Agilent Technologies, 2013)

4.4 Data analysis

All data were saved and calculated in Microsoft Excel 2013 and analysed in SPSS Win 19 software by using independent sample t-test and one-way ANOVA. Duncan was used once $P < 0.05$ in order to compare the differences.

5. Results

Based on GC-MS data obtained from volatile compounds analysis of fresh, room temperature, and refrigerated meat samples, 34 compounds, categorized into 8 classes, were detected in the extracts in total. All of them were tentatively identified (good match of MS and coincidence of KI).

In fresh meat, only 12 compounds were identified, being the most abundant: acetic acid, hexanal, and methoxy-phenyl-oxime. As we can see in Table 16 and 17, compounds of aldehydes and terpenes classes were the most detectable. In comparison with room temperature and refrigerated meat, the level of methoxy-phenyl-oxime was the highest with significant difference ($P < 0.05$).

In room temperature meat, 34 volatiles were detected, being the most abundant: acids (acetic acid), ketones (butanone), aldehydes (hexanal and 2-phenylacetaldehyde), and alcohols (ethanol, 3-methyl-butan-1-ol, butanediol, and hexan-1-ol). Compared with fresh meat, the number of volatile substances detected doubled, meaning, the longer meat is stored, the more compounds generated. The quantity of most volatiles fluctuated over time while others gradually increased (butanone, pentanal, 2-phenylacetaldehyde, hexadecanal, ethanol, and heptan-1-ol) and decreased (guanidineacetic acid). Surprisingly, 2,4-dimethyl-hexane was the only compound detected on day 7 under room temperature condition. Please refer to Table 16 for details.

In refrigerated meat, 29 volatiles were detected, being the most abundant: acids (acetic acid), aldehydes (hexanal), and alcohols (ethanol and hexan-1-ol). Compared with fresh meat, the number of volatile substances detected also doubled. However, the number was still lower than the one detected in the room temperature meat due to the absence of some volatiles (butanone, 3-methyl-butan-1-ol, 2-methyl-butan-1-ol, 2-phenyl-ethanol, and 2,4-dimethyl-hexane). The quantity of most compounds fluctuated over time while 3 substances (acetic acid, 2-phenylacetaldehyde, and ethanol) gradually increased, and 2 volatiles (6-methyl-5-hepten-2-one and hexadecanal) were detected on day 7. Dissimilar to the room temperature meat, the quantity of terpenes (cymene and γ-terpinen) were statistically significant ($P < 0.01$). Please refer to Table 17 for details.

Table 16: Volatile compounds (AU × 10^6) detected in fresh and room temperature meat

compounds	MW	fresh	1dRT	3dRT	5dRT	7dRT	*p* value	SEM	RT	IM
					acids					
acetic acid	60	0.79^{b}	2.89^{b}	8.82^{a}	10.45^{a}	9.81^{a}	0.000	1.12	4.11	MS
guanidineacetic acid	117	0.00^{b}	0.05^{a}	0.03^{a}	0.03^{a}	0.00^{b}	0.002	0.01	13.48	MS
					ketones					
butanone	72	0.00^{c}	0.00^{c}	0.00^{c}	1.83^{b}	7.40^{a}	0.000	0.77	3.00	MS
3-hydroxy-butan-2-one	88	0.00^{b}	0.00^{b}	0.60^{a}	0.10^{b}	0.00^{b}	0.000	0.07	5.76	MS
octanedione	142	0.08^{b}	0.29^{a}	0.10^{b}	0.23^{a}	0.00^{b}	0.001	0.03	11.34	MS, KI
6-methyl-5-hepten-2-one	126	0.00^{b}	0.00^{b}	0.12^{b}	0.43^{a}	0.15^{b}	0.000	0.05	11.42	MS, KI
					aldehydes					
3-methyl-butanal	86	0.00^{b}	0.08^{ab}	0.31^{ab}	0.48^{a}	0.42^{a}	0.040	0.07	3.57	MS
pentanal	86	0.00^{b}	0.20^{b}	0.23^{b}	0.39^{b}	1.09^{a}	0.003	0.11	4.24	MS
hexanal	100	1.23^{b}	6.15^{a}	3.38^{b}	7.89^{a}	1.79^{b}	0.000	0.73	6.83	MS
heptanal	114	0.00^{c}	0.14^{b}	0.27^{a}	0.35^{a}	0.12^{b}	0.000	0.03	9.5	MS, KI
2-heptenal	112	0.22	0.22	0.17	0.20	0.13	0.586	0.02	10.79	MS, KI
benzaldehyde	106	0.00	0.02	0.26	0.23	0.25	0.115	0.04	10.9	MS, KI
octanal	128	0.07^{d}	0.21^{bc}	0.32^{b}	0.48^{a}	0.13^{cd}	0.000	0.04	11.73	MS, KI
2-phenylacetaldehyde	120	0.05^{c}	0.31^{bc}	0.50^{bc}	1.59^{b}	4.95^{a}	0.000	0.51	12.58	MS, KI
nonanal	142	0.23^{c}	0.38^{bc}	0.53^{ab}	0.55^{a}	0.28^{c}	0.004	0.04	13.61	MS, KI
hexadecanal	240	0.00^{b}	0.00^{b}	0.16^{a}	0.16^{a}	0.19^{a}	0.000	0.02	22.99	MS, KI
					alcohols					
ethanol	58	0.00^{e}	0.55^{d}	7.90^{c}	8.03^{b}	9.70^{a}	0.000	1.10	2.20	MS
3-methyl-butan-1-ol	88	0.00^{c}	0.00^{c}	1.76^{b}	5.13^{a}	2.00^{b}	0.000	0.51	5.09	MS
2-methyl-butan-1-ol	88	0.00^{c}	0.00^{c}	0.18^{c}	1.14^{a}	0.54^{b}	0.000	0.12	5.23	MS
pentan-1-ol	88	0.00^{b}	0.58^{a}	0.37^{a}	0.49^{a}	0.59^{a}	0.016	0.07	6.02	MS
butanediol	90	0.00^{b}	0.02^{b}	0.04^{b}	4.42^{a}	0.90^{b}	0.002	0.51	7.02	MS
hexan-1-ol	102	0.00^{c}	2.63^{b}	2.89^{b}	2.86^{b}	4.24^{a}	0.000	0.37	8.72	MS, KI
heptan-1-ol	116	0.00^{c}	0.26^{b}	0.42^{ab}	0.44^{ab}	0.48^{a}	0.002	0.05	11.06	MS, KI
octen-1-ol	128	0.08^{c}	0.76^{b}	0.75^{b}	1.14^{a}	0.81^{b}	0.000	0.10	11.26	MS
octan-1-ol	130	0.00^{b}	0.28^{a}	0.38^{a}	0.31^{a}	0.27^{a}	0.000	0.04	12.97	MS, KI
2-phenyl-ethanol	122	0.00^{b}	0.00^{b}	0.18^{b}	0.82^{a}	0.13^{b}	0.000	0.09	13.88	MS, KI
					furans					
2-pentylfuran	138	0.00^{c}	0.09^{b}	0.06^{bc}	0.20^{a}	0.09^{b}	0.001	0.02	11.5	MS, KI
					hydrocarbons					
2,4-dimethyl-hexane	114	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.56^{a}	0.000	0.06	6.81	MS
dimethyldecane	170	0.00^{b}	0.03^{b}	0.14^{a}	0.00^{b}	0.12^{a}	0.000	0.02	12.76	MS
undecane	156	0.02^{b}	0.04^{b}	0.12^{a}	0.02^{b}	0.06^{b}	0.001	0.01	13.55	MS, KI
					terpenes					
cymene	134	0.27	0.14	0.26	0.19	0.07	0.782	0.05	12.19	MS, KI
γ-terpinen	136	0.10	0.12	0.15	0.13	0.11	0.934	0.02	12.84	MS, KI
					others					
dimethyl-sulphide	62	0.00^{e}	0.21^{d}	2.31^{a}	1.40^{b}	0.75^{c}	0.000	0.23	2.60	MS
methoxy-phenyl-oxime	151	4.22^{a}	1.45^{b}	1.39^{b}	1.61^{b}	1.11^{b}	0.024	0.38	9.85	MS

Table 17: Volatile compounds (AU × 10^6) detected in fresh and refrigerated meat

compounds	MW	fresh	1dR	3dR	5dR	7dR	*p* value	SEM	RT	IM
					acids					
acetic acid	60	0.79^{bc}	0.54^{c}	1.60^{b}	3.49^{a}	3.83^{a}	0.000	0.38	4.11	MS
guanidineacetic acid	117	0.00^{c}	0.09^{b}	0.21^{a}	0.14^{b}	0.09^{b}	0.001	0.02	13.48	MS
					ketones					
butanone	72	0.00	0.00	0.00	0.00	0.00	n/a	0.00	3.00	MS
3-hydroxy-butan-2-one	88	0.00	0.00	0.13	2.24	1.40	0.285	0.40	5.76	MS
octanedione	142	0.08^{d}	1.17^{b}	2.12^{a}	0.82^{bc}	0.44^{cd}	0.000	0.19	11.34	MS, KI
6-methyl-5-hepten-2-one	126	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.09^{a}	0.000	0.01	11.42	MS, KI
					aldehydes					
3-methyl-butanal	86	0.00^{b}	0.02^{ab}	0.03^{ab}	0.00^{b}	0.04^{a}	0.040	0.01	3.57	MS
pentanal	86	0.00^{b}	0.16^{ab}	0.32^{a}	0.23^{a}	0.31^{a}	0.024	0.04	4.24	MS
hexanal	100	1.23^{c}	13.90^{a}	15.74^{a}	7.20^{b}	7.51^{b}	0.000	1.45	6.83	MS
heptanal	114	0.00^{b}	0.33^{a}	0.22^{ab}	0.11^{ab}	0.28^{a}	0.040	0.04	9.5	MS, KI
2-heptenal	112	0.22^{b}	0.37^{a}	0.30^{ab}	0.10^{c}	0.12^{c}	0.001	0.03	10.79	MS, KI
benzaldehyde	106	0.00^{c}	0.00^{c}	0.03^{b}	0.06^{a}	0.02^{b}	0.000	0.01	10.9	MS, KI
octanal	128	0.07^{b}	0.18^{ab}	0.16^{ab}	0.10^{ab}	0.21^{a}	0.040	0.02	11.73	MS, KI
2-phenylacetaldehyde	120	0.05^{c}	0.05^{c}	0.12^{b}	0.17^{b}	0.42^{a}	0.000	0.04	12.58	MS, KI
nonanal	142	0.23^{c}	0.81^{a}	0.78^{a}	0.50^{b}	0.43^{b}	0.000	0.06	13.61	MS, KI
hexadecanal	240	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.04^{a}	0.000	0.00	22.99	MS, KI
					alcohols					
ethanol	58	0.00^{e}	0.83^{d}	3.90^{c}	4.13^{b}	6.60^{a}	0.000	0.64	2.20	MS
3-methyl-butan-1-ol	88	0.00	0.00	0.00	0.00	0.00	n/a	0.00	5.09	MS
2-methyl-butan-1-ol	88	0.00	0.00	0.00	0.00	0.00	n/a	0.00	5.23	MS
pentan-1-ol	88	0.00^{b}	0.52^{ab}	1.06^{a}	0.87^{a}	0.99^{a}	0.009	0.12	6.02	MS
butanediol	90	0.00^{b}	0.03^{b}	0.03^{b}	1.15^{a}	0.75^{a}	0.003	0.14	7.02	MS
hexan-1-ol	102	0.00^{c}	0.43^{c}	5.42^{b}	8.79^{a}	5.77^{b}	0.000	0.92	8.72	MS, KI
heptan-1-ol	116	0.00^{c}	0.05^{c}	0.30^{b}	0.56^{a}	0.29^{b}	0.000	0.06	11.06	MS, KI
octen-1-ol	128	0.08^{d}	1.31^{bc}	2.14^{a}	1.80^{ab}	0.94^{c}	0.000	0.20	11.26	MS
octan-1-ol	130	0.00^{c}	0.25^{b}	0.50^{a}	0.60^{a}	0.30^{b}	0.000	0.06	12.97	MS, KI
2-phenyl-ethanol	122	0.00	0.00	0.00	0.00	0.00	n/a	0.00	13.88	MS, KI
					furans					
2-pentylfuran	138	0.00^{b}	0.09^{ab}	0.14^{a}	0.08^{ab}	0.05^{ab}	0.040	0.02	11.5	MS, KI
					hydrocarbons					
2,4-dimethyl-hexane	114	0.00	0.00	0.00	0.00	0.00	n/a	0.00	6.81	MS
dimethyldecane	170	0.00^{b}	0.12^{a}	0.09^{a}	0.00^{b}	0.11^{a}	0.000	0.01	12.76	MS
undecane	156	0.02^{c}	0.18^{a}	0.18^{a}	0.08^{b}	0.15^{a}	0.000	0.02	13.55	MS, KI
					terpenes					
cymene	134	0.27^{c}	1.12^{bc}	1.78^{ab}	2.18^{a}	1.14^{bc}	0.005	0.20	12.19	MS, KI
γ-terpinen	136	0.10^{c}	0.55^{a}	0.50^{ab}	0.36^{ab}	0.29^{bc}	0.007	0.05	12.84	MS, KI
					others					
dimethyl-sulphide	62	0.00^{e}	1.20^{b}	1.53^{a}	0.36^{d}	0.77^{c}	0.000	0.15	2.60	MS
methoxy-phenyl-oxime	151	4.22^{a}	2.73^{b}	1.47^{c}	0.52^{c}	0.70^{c}	0.000	0.39	9.85	MS

Table 18: Comparison between room temperature and refrigerated meat for day 1 and 3

compounds	MW	1 day		*p* value	3 days		*p* value	RT	IM
		RT	R		RT	R			
acids									
acetic acid	60	2.89	0.54	0.011	8.82	1.60	0.002	4.11	MS
guanidineacetic acid	117	0.05	0.09	0.195	0.03	0.21	0.007	13.48	MS
ketones									
butanone	72	0.00	0.00	n/a	0.00	0.00	n/a	3.00	MS
3-hydroxy-butan-2-one	88	0.00	0.00	n/a	0.60	0.13	0.023	5.76	MS
octanedione	142	0.29	1.17	0.011	0.10	2.12	0.000	11.34	MS, KI
6-methyl-5-hepten-2-one	126	0.00	0.00	n/a	0.12	0.00	0.162	11.42	MS, KI
aldehydes									
3-methyl-butanal	86	0.08	0.02	0.000	0.31	0.03	0.027	3.57	MS
pentanal	86	0.20	0.16	0.654	0.23	0.32	0.378	4.24	MS
hexanal	100	6.15	13.90	0.000	3.38	15.74	0.014	6.83	MS
heptanal	114	0.14	0.33	0.320	0.27	0.22	0.427	9.5	MS, KI
2-heptenal	112	0.22	0.37	0.078	0.17	0.30	0.061	10.79	MS, KI
benzaldehyde	106	0.02	0.00	0.118	0.26	0.03	0.288	10.9	MS, KI
octanal	128	0.21	0.18	0.743	0.32	0.16	0.035	11.73	MS, KI
2-phenylacetaldehyde	120	0.31	0.05	0.000	0.50	0.12	0.001	12.58	MS, KI
nonanal	142	0.38	0.81	0.008	0.53	0.78	0.011	13.61	MS, KI
hexadecanal	240	0.00	0.00	n/a	0.16	0.00	0.000	22.99	MS, KI
alcohols									
ethanol	58	0.55	0.83	0.000	7.90	3.90	0.000	2.20	MS
3-methyl-butan-1-ol	88	0.00	0.00	n/a	1.76	0.00	0.019	5.09	MS
2-methyl-butan-1-ol	88	0.00	0.00	n/a	0.18	0.00	0.000	5.23	MS
pentan-1-ol	88	0.58	0.52	0.814	0.37	1.06	0.074	6.02	MS
butanediol	90	0.02	0.03	0.184	0.04	0.03	0.529	7.02	MS
hexan-1-ol	102	2.63	0.43	0.002	2.89	5.42	0.005	8.72	MS, KI
heptan-1-ol	116	0.26	0.05	0.000	0.42	0.30	0.307	11.06	MS, KI
octen-1-ol	128	0.76	1.31	0.018	0.75	2.14	0.001	11.26	MS
octan-1-ol	130	0.28	0.25	0.587	0.38	0.50	0.113	12.97	MS, KI
2-phenyl-ethanol	122	0.00	0.00	n/a	0.18	0.00	0.001	13.88	MS, KI
furans									
2-pentylfuran	138	0.09	0.09	1.000	0.06	0.14	0.035	11.5	MS, KI
hydrocarbons									
2,4-dimethyl-hexane	114	0.00	0.00	n/a	0.00	0.00	n/a	6.81	MS
dimethyldecane	170	0.03	0.12	0.001	0.14	0.09	0.190	12.76	MS
undecane	156	0.04	0.18	0.007	0.12	0.18	0.041	13.55	MS, KI
terpenes									
cymene	134	0.14	1.12	0.014	0.26	1.78	0.001	12.19	MS, KI
γ-terpinen	136	0.12	0.55	0.005	0.15	0.50	0.023	12.84	MS, KI
others									
dimethyl-sulphide	62	0.21	1.20	0.000	2.31	1.53	0.000	2.60	MS
methoxy-phenyl-oxime	151	1.45	2.73	0.137	1.39	1.47	0.933	9.85	MS

Table 19: Comparison between room temperature and refrigerated meat for day 5 and 7

compounds	MW	5 days		*p* value	7 days		*p* value	RT	IM
		RT	R		RT	R			
acids									
acetic acid	60	10.45	3.49	0.024	9.81	3.83	0.001	4.11	MS
guanidineacetic acid	117	0.03	0.14	0.031	0.00	0.09	0.057	13.48	MS
ketones									
butanone	72	1.83	0.00	0.000	7.40	0.00	0.000	3.00	MS
3-hydroxy-butan-2-one	88	0.10	2.24	0.342	0.00	1.40	0.201	5.76	MS
octanedione	142	0.23	0.82	0.067	0.00	0.44	0.014	11.34	MS, KI
6-methyl-5-hepten-2-one	126	0.43	0.00	0.001	0.15	0.09	0.399	11.42	MS, KI
aldehydes									
3-methyl-butanal	86	0.48	0.00	0.177	0.42	0.04	0.021	3.57	MS
pentanal	86	0.39	0.23	0.171	1.09	0.31	0.071	4.24	MS
hexanal	100	7.89	7.20	0.746	1.79	7.51	0.006	6.83	MS
heptanal	114	0.35	0.11	0.000	0.12	0.28	0.169	9.5	MS, KI
2-heptenal	112	0.20	0.10	0.008	0.13	0.12	0.774	10.79	MS, KI
benzaldehyde	106	0.23	0.06	0.023	0.25	0.02	0.044	10.9	MS, KI
octanal	128	0.48	0.10	0.003	0.13	0.21	0.234	11.73	MS, KI
2-phenylacetaldehyde	120	1.59	0.17	0.041	4.95	0.42	0.004	12.58	MS, KI
nonanal	142	0.55	0.50	0.609	0.28	0.43	0.066	13.61	MS, KI
hexadecanal	240	0.16	0.00	0.000	0.19	0.04	0.115	22.99	MS, KI
alcohols									
ethanol	58	8.03	4.13	0.000	9.70	6.60	0.000	2.20	MS
3-methyl-butan-1-ol	88	5.13	0.00	0.000	2.00	0.00	0.012	5.09	MS
2-methyl-butan-1-ol	88	1.14	0.00	0.005	0.54	0.00	0.003	5.23	MS
pentan-1-ol	88	0.49	0.87	0.214	0.59	0.99	0.021	6.02	MS
butanediol	90	4.42	1.15	0.073	0.90	0.75	0.726	7.02	MS
hexan-1-ol	102	2.86	8.79	0.023	4.24	5.77	0.063	8.72	MS, KI
heptan-1-ol	116	0.44	0.56	0.397	0.48	0.29	0.055	11.06	MS, KI
octen-1-ol	128	1.14	1.80	0.156	0.81	0.94	0.414	11.26	MS
octan-1-ol	130	0.31	0.60	0.022	0.27	0.30	0.535	12.97	MS, KI
2-phenyl-ethanol	122	0.82	0.00	0.026	0.13	0.00	0.061	13.88	MS, KI
furans									
2-pentylfuran	138	0.20	0.08	0.102	0.09	0.05	0.441	11.5	MS, KI
hydrocarbons									
2,4-dimethyl-hexane	114	0.00	0.00	n/a	0.56	0.00	0.006	6.81	MS
dimethyldecane	170	0.00	0.00	n/a	0.12	0.11	0.826	12.76	MS
undecane	156	0.02	0.08	0.012	0.06	0.15	0.020	13.55	MS, KI
terpenes									
cymene	134	0.19	2.18	0.012	0.07	1.14	0.049	12.19	MS, KI
γ-terpinen	136	0.13	0.36	0.135	0.11	0.29	0.012	12.84	MS, KI
others									
dimethyl-sulphide	62	1.40	0.36	0.000	0.75	0.77	0.091	2.60	MS
methoxy-phenyl-oxime	151	1.61	0.52	0.354	1.11	0.70	0.157	9.85	MS

For day 1, 8 volatile compounds (butanone, 3-hydroxy-butan-2-one, 6-methyl-5-hepten-2-one, hexadecanal, 3-methyl-butan-1-ol, 2-methyl-butan-1-ol, 2-phenyl-ethanol, and 2,4-dimethyl-hexane) were not detected in either room temperature or refrigerated meat. The dominant compounds in this storage length were acetic acid, hexanal, hexan-1-ol, and methoxy-phenyl-oxime. Interestingly, hexanal was as high as 6.15 and 13.90 in room temperature and refrigerated meat respectively. Acetic acid and hexan-1-ol was 2.89 and 2.63 in room temperature meat, but less than 1.00 each in refrigerated one. Among the 26 volatiles detected, the quantity of 2 compounds (guanidineacetic acid and methoxy-phenyl-oxime) was not significantly different in both conditions of storage (Table 18).

For day 3, 32 volatiles were detected except butanone and 2,4-dimethyl-hexane. Hexanal with the increased amount was still the dominant compound in refrigerated meat. In this storage length, acetic acid was abundant in the room temperature meat since the amount had tripled up to 8.82. Remarkably, the amount of 3 compounds (octanedione, octen-1-ol, and cymene) was still higher while that of the other 2 (ethanol and dimethyl-sulphide) was lower in the refrigerated meat. 3-methyl-butan-1-ol, which was absent on day 1, was detected in the room temperature meat during this storage time (Table 18).

For day 5, 2,4-dimethyl-hexane was still not detectable, and dimethyldecane disappeared due to its small amount from day 3. In this storage length, the amount of acetic acid was still higher in room temperature meat while there was no significant difference for that of hexanal in both storage conditions. Interestingly, the amount of ethanol, 3-methyl-butan-1-ol, and 2-methyl-butan-1-ol kept increasing in room temperature meat whereas the later 2 was still absent in refrigerated meat. Butanone, which had not been detected since day 1, was present only in room temperature meat contrasting with the amount of hexan-1-ol which almost doubled in refrigerated meat (Table 19).

For day 7, all 34 compounds were detected. Surprisingly, 2,4-dimethyl-hexane, which had not been detected since day 1, was generated during this storage condition. In room temperature meat, acetic acid (9.81) was still the dominant volatile followed by ethanol (9.70) and butanone (7.40). Hexanal (7.51), however, was abundant in the refrigerated meat followed by ethanol (6.60) and acetic acid (3.83). 3-methyl-butan-1-ol and 2-methyl-butan-1-ol, which were not detected in the refrigerated meat on day 5, were still not detectable during this period of storage (Table 19).

6. Discussion

The presence of acetic acid and hexanal in fresh Prestice black-pied meat of this research is similar to the results found on Iberian and lean meat by Estévez et al. (2003), but contrasting with those found on Large White meat by Soncin et al. (2007). Pig breed and animal diet might be the main causes of this occurrence. The amount of hexanal, the indicator of lipid oxidation (Chambers and Koppel, 2013) and meat flavour deterioration; off-flavours and odors (Shahidi and Pegg, 1994), was quite high. This really reflected on the frozen fresh meat which was reported susceptible to lipid oxidation (Love and Pearson, 1971). Lactic acid bacteria which may give a sour note might be the precursor of acetic acid present in the meat (Montel et al., 1998). According to Luck and Jager (1997), acetic acid can be produced synthetically by oxidation of acetaldehyde or low hydrocarbons, and all customary biological processes are based on the ability of acetobacter to oxidize ethanol to acetic acid. Methoxy-phenyl-oxime, which is considered as meat freshness indicator found in redspot swimming crab and prawn (Zhang et al., 2010), was also identified with very high amount. Nonanal which is believed to give rancid flavour note and be produced by anti-oxidant material from bacteria was also present in the fresh meat. IMF in each pig breed not only has the effects on eating quality traits but also can be influential in the generation of volatile compounds, therefore, low fat content meat could be related to loss in flavour development (Cameron et al., 2000). Due to low fat content of 1.93% in Large White pork (Jukna et al., 2013), only 10 compounds were detected in the study conducted by Soncin et al. (2007) while more substances were found on Iberian pork with the average fat content of 3.68% (Serra et al., 1998) in the research done by Estévez et al. (2003). Because Prestice black-pied pig breed contains average fat content of 2.67% (Matousek et al., 2016), not more than 34 volatile compounds were discovered in this study.

In the room temperature meat, the presence of acetic acid and hexanal was still seen but with fluctuating trend. According to Dickens et al. (1994), the visual appearance and microbiological quality of the meat at room temperature might be influenced by the high concentration of acetic acid. Butanone, ethanol, and dimethyl-sulphide, which have been found as markers of chicken breast spoilage confirmed by Mikš-Krajnik et al. (2015), were detected with higher amount. 3-methyl-butan-1-ol and 2-methyl-butan-1-ol, which also contributed to the spoilage of the meat, appeared from day 3 because of amino

acid metabolism by alcohol dehydrogenase while butanediol is generated from pyruvate catabolism (Montel et al., 1998). Alcohols such as pentan-1-ol and hexan-1-ol have been reported as being produced by reduction of corresponding aldehydes derived from lipid oxidation (Garcia et al., 1991; Loury, 1972). Ketones and hydrocarbons, the breakdown products of hydroperoxides, also appeared during this period of time but with only small amount. The presence of 2-pentylfuran, which is an oxidation product from linoleic and other n-6 fatty acids and has already been discovered in meat (Nonaka et al., 1967; Greenberg, 1981), was also found. Therefore, hydrolysis and oxidation of lipid/fatty acids seemed to be the main cause of volatile generation during room temperature storage.

Changes in volatile compounds during refrigerated storage can indicate chemical, enzymatic, and microbial deterioration in meat (Vinauskiene et al., 2002) while desirable meat flavour is achieved by cooking (Mottram, 1998). In general, raw meat refrigeration caused a small increase in oxidation-derived aldehydes and a large increase in methyl alcohols and ketones generated from branched chain amino acids and pyruvate catabolism. Compared with the study conducted by Estévez et al. (2003), the results were similar with most abundant substances (acetic acid, 3-hydroxy-butan-2-one, hexanal, pentan-1-ol, butanediol, hexan-1-ol, and dimethyl-sulphide). The existence of 3-hydroxy-butan-2-one, which has been reported to be a meat aging indicator (Mottram, 1998), was not significant in this study. However, its amount was quite high compared with that of the room temperature. The amount of octanedione, which is believed to be the cause of warmed-over flavour and lipid-derived volatile, was high under refrigeration condition and disagreed with the result illustrated by Estévez et al. (2003). The striking concentration of hexanal under this storage condition showed that the refrigerated meat had undergone lipid oxidation (Love and Pearson, 1971). According to Estévez et al. (2002), enzymatic activity was the most important cause of meat deterioration during refrigeration while oxidation phenomena had a secondary role.

7. Conclusion

The GC-MS analysis of volatile substances developed by raw meat appeared to clearly differentiate meat stored in different conditions and gave information on the acceptability of the meat. This research indicated that storage condition and duration does influence the number of volatile compounds generated and their composition while animal breed and diet should not be neglected. From the point of view of the production of volatile substances, it can be inferred from the results that storing Prestice black-pied pig meat for 1 day at room temperature and 3 days in the refrigerator is good for consumption. The amount of acetic acid, which is produced by lactic acid bacteria and negatively affects the colour, texture and quality of the meat, was not quite high compared with the fresh meat, but the microbial analysis was not done.

The results revealed in this study will give basic ideas of volatile compounds generated under various storage conditions and open door for more future research. Although the analytical data were obtained from only 1 individual animal, the usefulness of SPME analysis to monitor volatile compounds generated in meat, under different storage conditions, has been confirmed. Conducting a complete research on this topic by including more individuals and pig breeds, the analysis of fat content, fatty acid composition, and the monitoring of volatile substances in cooked meat after storing under various conditions will further explain what the meat has undergone until consumption and respond to the limitations of this study. By estimating volatile compounds in raw meat, the presence of undesired substances could be monitored, and meat products unsuitable for processing could be avoided.

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