Environmental Aspects of Proteinous Bioplastic

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Master of Science(Technology) in Materials and Processing

at

The University of Waikato

by

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THE UNIVERSITY OF WAIKATO

Te Whare Wānanga o Waikato

April, 2010

Abstract

During the processing of Novatein Thermoplastic Protein (NTP), volatile compounds are released which are odourous and potentially harmful. For NTP to be manufactured on a commercial scale the odour that currently arises during production must be minimised.

Literature revealed that blood components are thermally unstable under oxidising conditions and in addition, the presence of iron in bloodmeal, may catalyse the formation of various odourous compounds.

The objectives of this work were to establish an odour profile for bloodmeal and pre-extruded NTP (PNTP) and determine the effect of chemically treating bloodmeal on the resulting odour profile. In addition, the potential for NTP to be degraded by composting and effect of prolonged exposure to UV radiation was investigated.

It was found that the initial odour of bloodmeal was caused by putrefaction during the storage of whole blood prior to drying as well as the formation of other, less odourous, volatile compounds derived from lipids and proteins during drying.

The odour profile of pre-extruded NTP (PNTP) was found to change after chemical treatment of bloodmeal. Chemical treatments successfully minimised or changed the odour of the bloodmeal. Peracetic acid treatment successfully removed the characteristic "bloodmeal" odour resulting in decolourised bloodmeal with a mild vinegar odour. In general, removing odour from the PNTP could not be achieved without the addition of 10-20 wt% activated carbon or greater than 20 wt% natural zeolite.

Additionally, for NTP to be marketed as biodegradable as well as bio-derived plastic, it must be shown to degrade in the environment at a rate comparable with cellulose. NTP was found to undergo substantial deterioration and polymer degradation within 12 weeks of composting, for plasticised samples up to 47 % dry mass loss was observed. However, exposure to 12 weeks of ultra-violet radiation, lead to embrittlement of the plastic with very little polymer degradation occurring.

Acknowledgements

Firstly, I would like to thank my family, particularly my parents Colin and Wendy Hicks, for supporting me in every way imaginable throughout what has been an exciting and challenging experience. You have taught me so much; to love myself, aim high, work hard and take pride in achieving my goals. Without you both, this would never have been possible.

To my sister Briarley, thank you for photographing the composting and UV degraded samples professionally and providing me with hours of entertainment during much needed breaks throughout the year.

To my friends, both within university and abroad, thank you so much for the never ending support you have given me, this year has been challenging for many reasons, thank you for being so understanding.

I would also like to thank my academic supervisor, Dr. Johan Verbeek for his endless support and enthusiasm throughout this endeavour, and co-supervisor Dr. Alan Langdon for his guidance within the chemistry department. It has been wonderful working with you both.

Thank you to Aaron Low for the preparation of sodium chlorite and peracetic acid treated bloodmeal samples and Kingston Masters for help with the FTIR analysis, this was greatly appreciated.

Thank you also to the Composites group, for kindly easing me into a new area of science, for your support and for always being around when no-one else could be found.

Finally, I would like to thank the academics and technical support staff at the University of Waikato. The time you have spent helping me throughout this research is hugely appreciated, I have learned a lot from you all.

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1 Introduction

There is an overwhelming environmental driving force for biodegradable plastic production. Worldwide, approximately 140 million tonnes of petroleum-based synthetic polymer materials are produced each year. Despite their widespread use, their resistance to chemical and microbial degradation have lead to their accumulation in the environment [1, 2].

Recently, there has been a renewed interest in using natural polymers such as polysaccharides, lipids and proteins from plant and animal resources to produce bioplastics [3-6].

Bioplastics have several advantages over conventional plastics derived from petroleum. Typically, natural processes such as hydrolysis and oxidation are able to degrade the bioplastic resulting in lower carbon dioxide emissions. In addition, the starting materials can be obtained from renewable resources or in some cases from what would typically be the "waste" by-products of industrial processes.

In New Zealand, approximately 80,000 tonnes of raw blood is collected annually from rendering and slaughterhouse operations [7]. For environmental reasons blood is often dried and sold as bloodmeal, a low-cost protein supplement or fertiliser, or processed into high value products from the plasma fraction.

It has recently been shown that, due to its high protein content, bloodmeal can be used as a feedstock for producing proteinous bioplastic [7]. Novatein, a company established from research undertaken at the University of Waikato, has patented the thermoplastic derived from bloodmeal [8], herein known as Novatein Thermoplastic Protein (NTP).

During the processing of NTP, volatile compounds are released which are odourous and potentially harmful. Thus, for the bioplastic to be manufactured on a commercial scale the odour that currently arises during production must be minimised.

Additionally, to be marketed as biodegradable as well as bio-derived, NTP must be able to degrade in the environment at a rate comparable with cellulose. This

thesis therefore has two objectives, odour profiling and investigation of environmental degradation, more specifically:

1. Odour Profiling
    - Develop a method of analysis for identification of volatile compounds
    - Establish an odour profile for bloodmeal and NTP and determine the source of odour formation
    - Determine what effect various chemical treatments of bloodmeal have on the resulting bloodmeal and the resulting bioplastic odour profile
2. Environmental Degradation

- Investigate potential for NTP with and without plasticiser to be degraded in a composting environment
- Determine what effect composting has on the structural and chemical integrity of the bioplastic as degradation is occurring
- Investigate potential for NTP with and without plasticiser to be degraded in direct sunlight
- Determine what effect exposure to ultra-violet light has on the structural and chemical integrity of the bioplastic as degradation is occurring

The work is based on the proprietary formulation to produce Novatein Thermoplastic Protein. Bloodmeal and the required additives were not extruded to exclude the effect additional thermal treatment would have on the odour profiling. However, for composting and UV irradiation samples were extruded and injection moulded.

1.1 Proteinous Bioplastic

1.1.1 Proteins and Amino acids

Proteins have commonly been used as raw material for producing edible packaging and other materials, and could eventually replace conventional plastic packaging derived from petroleum [5].

Proteins are natural polymers consisting of different combinations of amino acids formed via condensation reactions of amino acids, as shown in Figure 1 [9]. Three classes of proteins can be distinguished based on their tertiary structure, these are: globular or pseudo-globular proteins, membrane proteins and fibrous proteins [10].

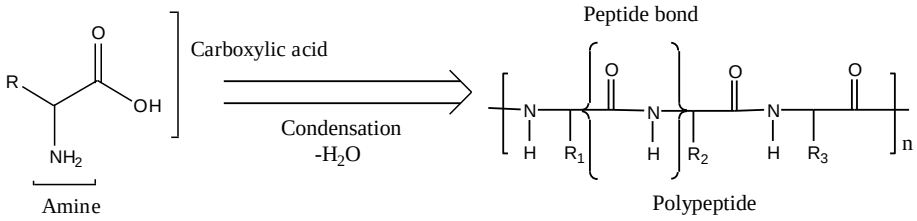


Figure 1: General structure of an amino acid and polypeptide

The primary structure of a protein is its specific sequence of amino acids (Figure 2). Intermolecular interactions between nearby amino acid side chains lead to stability of the macromolecule. These interactions include dipole-dipole interactions (polar and non-polar), ionic bonding or repulsion, hydrogen bonding and covalent disulfide linkages [7]. These interactions give the protein its secondary structure such as random coils, pleated sheets and alpha helices.

A protein's tertiary structure is determined by the interaction of, for example, alpha helices with pleated sheets, and is a 3-dimensional representation of atomic positions. Its structure is determined by taking on the most thermodynamically favourable conformation, known as the native state. Some proteins such as haemoglobin are comprised of many polypeptide chains yielding a quaternary structure.

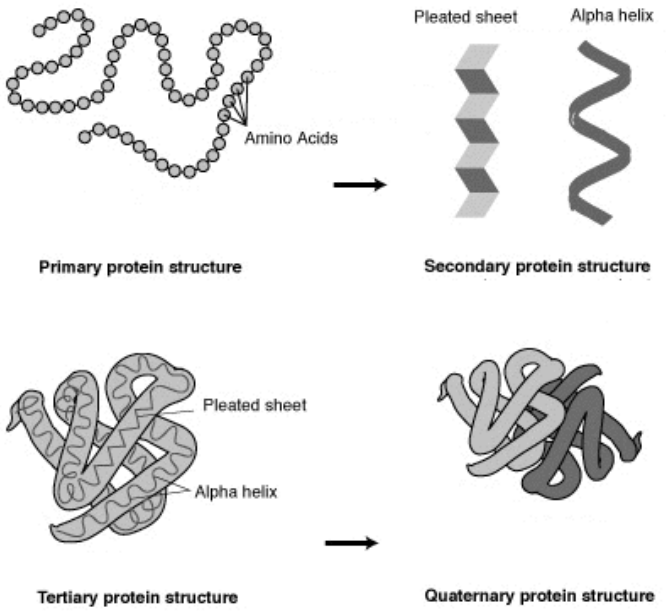


Figure 2: Representation of protein structure. Image sourced from [11].

The secondary and tertiary structure of a protein in its native state can be disrupted or destroyed by altering the non-covalent or hydrogen bonding interactions between the side chains of consecutive amino acids in the polypeptide. In the case of proteins with quaternary structure, this too may be altered. The process of unfolding the protein chain is known as denaturing and can be reversible or irreversible [12].

Due to the relatively weak intramolecular interactions (such as hydrogen bonding with a bonding energy of 4 $kJmol^{-1}$) stabilising the protein in its native state, denaturing is observed when environmental conditions such as temperature, pH and ionic strength are altered [9]. Commonly used denaturants include acetic acid, trichloroacetic acid, urea, guanidinium chloride and lithium perchlorate. When developing plastic materials from natural protein polymers, denaturants must be used to expose non-polar cysteine amino acid residues which can later form other disulfide bridges influencing the plastic's mechanical properties [7].

Over 20 amino acids have been identified using acid hydrolysis of proteins. All amino acids are primary amines with the exception of proline, and all are chiral with the exception of glycine. Amino acids are classified by their side chains as hydrophilic (charged or uncharged) or hydrophobic. In Figure 3, the structures of the most common amino acids are shown. In addition, other amino acids can be

synthesised in-vitro such as ornithine from arginine and hydroxylated derivatives of proline.

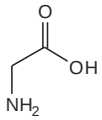
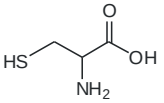
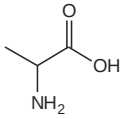
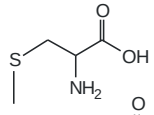
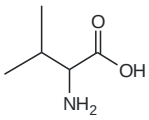
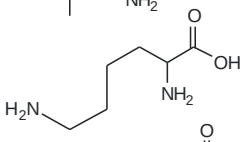
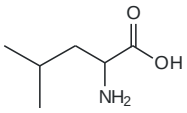
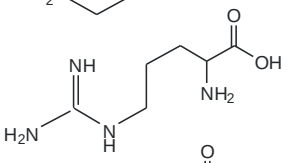
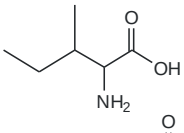
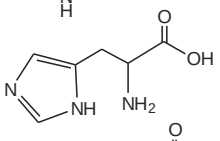
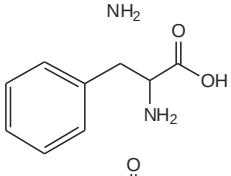
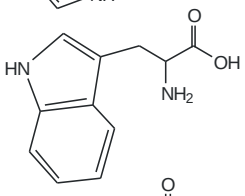
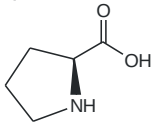
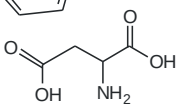
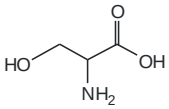
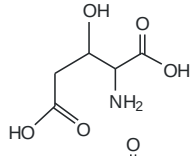
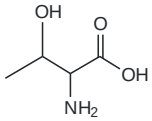
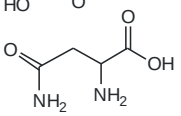
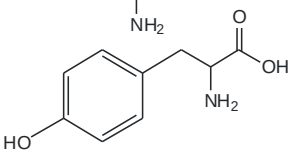
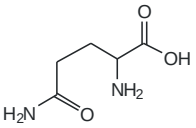
Name		Formula	Name		Formula
Glycine	Gly		Cysteine	Cys	
Alanine	Ala		Methionine	Met	
Valine	Val		Lysine	Lys	
Leucine	Leu		Arginine	Arg	
Isoleucine	Ile		Histidine	His	
Phenylalanine	Phe		Tryptophan	Trp	
Proline	Pro		Aspartic Acid	Asp	
Serine	Ser		Glutamic Acid	Glu	
Threonine	Thr		Asparginine	Asn	
Tyrosine	Tyr		Glutamine	Gln	

Figure 3: Twenty amino acids isolated from proteins

Blood composition

Whole bovine blood is comprised of 80.9 wt% water, 17.3 % protein, 0.23 % lipid, 0.07 % carbohydrate and 0.62 % minerals [13]. Blood can be divided into two main fractions: cellular mass (35-40 v%) which is mainly red blood cells and plasma (65-70 v%) in which the red blood cells are suspended [13]. A summary of the components found in whole blood and its fractions are shown (Table 1).

Table 1: General chemical composition of whole blood and its fractions (wt%). Data retrieved from [13].

Component	Whole Blood	Plasma	Red Blood Cells
Water	80	90.8	60.8
Salts	0.9	0.8	1.1
Fat	0.2	0.1	0.4
Albumin	2.8	4.2	-
Globulin	2.2	3.3	-
Fibrinogen	0.3	0.4	-
Haemoglobin	10	-	30
Other	1.1	0.4	2.6

Bovine blood plasma contains 7.9 wt% protein, composed of albumins (4.2 %), globulins (3.3 %) and fibrinogen (0.4 %) [13, 14], however, over 27 different proteins and protein fragments have been identified [15].

The predominant protein type in bovine plasma is albumin. Albumin is responsible for maintaining osmotic pressure and transporting other molecules such as hormones and fatty acids throughout the body. Immunoglobins regulate the immune system and fibrinogen aids in coagulation [16].

Haemoglobin is the predominant protein found in red blood cells, it is the globular protein responsible for the transportation of oxygen throughout the body. Glycophorins, a type of glycoprotein, also makes up a small portion of red blood cells [17, 18]. A glycoprotein is a protein that has mono- or poly- saccharides linked to the protein chain via glycosidic bonds to either a hydroxyl or amine group on the side chain of an amino acid. They are found in erythrocyte

membranes and are responsible for binding sugars and maintaining a hydrophilic membrane coating to inhibit adverse interaction with other cells or vessel walls.

The high protein content of blood has caused considerable interest in its potential use as food additives such as emulsifying agents [19]. For blood proteins to be used for human consumption the blood must be collected in a hygienic manner. This is not always practical due to its high expense; even if it were practical not all animals are healthy enough to be used for this purpose. As a result, a large quantity of blood is produced which must be disposed of carefully due to its high biological and chemical oxygen demand. Hence, slaughterhouse and rendering plants typically dispose of the blood via incineration, to produce bloodchar or drying, to produce bloodmeal.

The presence of proteins, lipids and carbohydrates in raw blood implies that the blood should be preserved in order to minimise microorganism mediated degradation of lipids and putrefaction of proteins during storage and transport that lead to the formation of odourous volatile compounds.

1.1.2 Bloodmeal

Bloodmeal is a reddish-brown granular powder produced by drying whole blood collected during slaughterhouse operations. The composition of the dry weight fraction of whole bovine blood and commercial bloodmeal is given in Table 2 [20]. It was shown that commercial bloodmeal contains 7.0 - 12.2 wt% water, and of the dry weight contained 72.3 – 96.6 wt% crude protein [20]. The quantity of haem iron present in bloodmeal is 2273.0 mg/kg [20].

Table 2: Chemical composition of bloodmeal (wt% in dry matter) [20].

Component	**Bloodmeal**	
	Mean	Variation
Organic Matter	92.9	73.8 - 97.8
Crude Protein	92.5	72.3 - 96.6
N-free Extract	3.3	0 - 10.7
Crude Fat	1.2	0 - 5.9
Crude Fibre	0.9	0.4 - 1.0
Crude Ash	5.3	2.0 - 15.6

Crude protein content is dependent on the drying method employed as some methods such as open air drying and ring drying induce more damage to the blood proteins than others, leading to variation in the protein and amino acid composition of bloodmeal [20].

Fresh whole blood has almost no odour and the same is true if it is dried immediately. In a healthy animal, blood is sterile, but when bled out during slaughtering and rendering operations, bacterial contamination may occur [14]. For medical purposes, whole blood must be stored between 2 ^{o}C and 6 ^{o}C to minimise bacterial growth [21]. Inedible blood from these operations is usually strained to remove debris and then dried immediately, however, sometimes it is collected and stored in large containers for later processing. Due to the large quantity of water and nutrients present in the raw blood, it forms an ideal media for bacterial proliferation.

Bloodmeal Production

Immediately after collection, whole blood begins to coagulate. Coagulation occurs due to proteins forming insoluble complexes with free calcium ions. To inhibit coagulation, anticoagulants such as ethylenediaminetetraaceticacid disodium salt (EDTA), oxalic acid or anhydrous sodium citrate are added to the blood [13, 22]. Addition of anticoagulants is not a common practice in the rendering and slaughterhouse industries as the coagulation of blood is typically induced prior to dewatering. There is currently no evidence to suggest coagulation influences thermal, oxidative or putrefactive deterioration of the blood proteins.

During storage and transportation of whole blood, oxygen is rapidly consumed by facultative and aerobic bacteria. Once oxygen is consumed, blood begins to undergo putrefaction, the decomposition of proteins by anaerobic bacteria. Putrefaction results in the formation of odorous amine compounds such as putrescine and cadaverine. To prevent the onset of putrefaction some bloodmeal manufacturers add formic acid, sodium chloride and unslaked lime, 3 % sulfuric acid or potassium metabisulfite to the blood during collection [20].

Blood is often aged to induce coagulation at lower temperatures thereby minimising the irreversible degradation of the protein caused at higher temperatures [23]. However, blood does not need to be aged prior to drying, as coagulation at lower temperature can be achieved if free calcium ions are introduced into the blood (as 1 % calcium chloride) prior to processing [24].

There are a number of ways to produce bloodmeal; generally, prior to dewatering, whole blood is strained to remove debris, [25] and coagulated by direct steam injection at 90 ^{o}C [26]. The coagulant is separated using a centrifuge decanter to remove up to 50 % of the water and the coagulant is passed into a rotating drum drier.

The rotating drum is heated to a surface temperature between 120 ^{o}C and 175 ^{o}C to evaporate the water present in the coagulant but prevent destruction and over-drying of the plasma content of the blood [27]. The drum is rotated at speeds sufficient for the rapid removal of dried blood with a residence time of no more than two minutes [27]. After drying, bloodmeal is hammer milled before being bagged at a moisture content of 2-4 wt%.

Some of the amino acids which make up proteins are not thermally stable above 110 ^{o}C. The four least stable amino acids are asparagine, glutamine, cysteine and aspartic acid [28], which in addition to other amino acids, can undergo deamidation/deamination to release ammonia gas. During thermal processing denaturing of the heat-labile proteins may occur, however, solubility and other properties of the blood can be retained if the blood is dried below 80 ^{o}C [25].

Another issue pertaining to the thermal instability of proteins is the Maillard reaction (Figure 4), an umbrella term for non-enzymatic condensation reactions between the α-amino group of amino acids, peptides, proteins or free ammonia with the carbonyl group of reducing sugars.

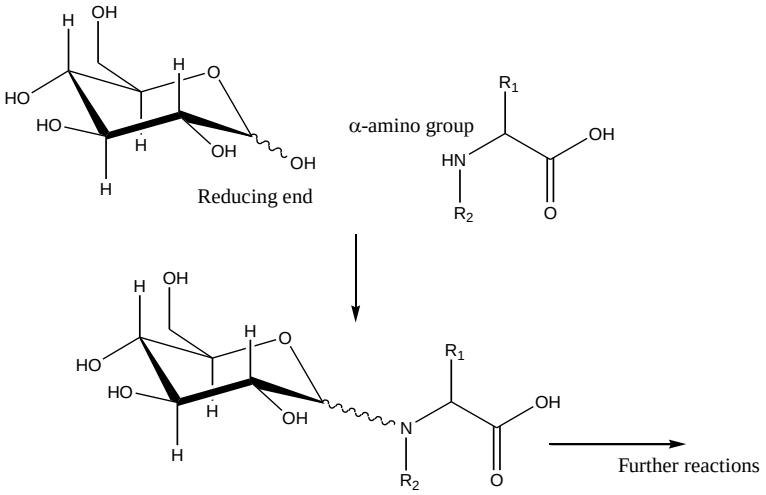


Figure 4: Maillard reaction occurring between a reducing sugar and an amine

The Maillard reaction is known to occur during the drying of whole egg or yolk due to free glucose present. In China, egg proteins were de-sugared prior to drying using microbial fermentation to ensure minimal loss of solubility and palatability of the protein due to the Maillard reaction or reaction of glucose with phospholipids [29].

Alternatively, low temperature spray drying methods for commercial production of animal blood-products, prevent heat coagulation and Maillard reactions from occuring [30].

Maillard reactions are responsible for the formation of furans and nitrogen containing compounds which give cooked meat its aroma and flavour [28]. There are many various nitrogen-containing heterocyclic compounds such as pyrazines, pyridines, and pyrroles that can be formed, depending on the amino acid and sugar involved in the reaction.

Amino acids such as asparagine, glutamine, lysine, arginine, histidine and tryptophan all contain two nitrogen atoms which may contribute to the formation of nitrogen-containing heterocylics. It has been shown that furans are formed in majority when aspartic acid reacts with glucose while nitrogen-containing heterocyclic compounds are the major products when asparagine reacts with glucose [31].

Other odourous volatile compounds are formed from the oxidative breakdown of unsaturated lipids and free fatty acids [32]. Unsaturated fatty acids such as linoleic acid and linolenic acid are present in whole blood as cholesterol or triglyceride esters associated with the cell membrane and plasma [33]. Oxidation of lipids occurs via a free radical reaction causing the formation of odourous volatile compounds [34]. These oxidative reactions can proceed at both ambient and elevated temperatures [32].

It can then be assumed that a large variety of reactions may take place during bloodmeal processing due to the thermal and oxidative instability of proteins, carbohydrates and lipids. The oxidative reactions of the organic compounds in blood that proceed during drying and bloodmeal storage lead to the formation of odourous compounds. In addition to the putrefaction products formed during the storage of whole blood, these oxidative reactions lead to an odourous bloodmeal product.

Bloodmeal Storage

Bloodmeal is a microbiologically stable compound when stored in dry conditions [22]. Like all meals from animal origin, bloodmeal relies on having a low moisture content and water activity for preservation. Commercial blood meal typically has a moisture content of 5-8 wt% and a water activity of 0.3-0.45 [35].

Fungal growth will not occur on a substrate with a moisture content less than 12 % or a water activity less than 0.65 [36]. In addition, it is well known that single celled organisms, such as bacteria, require much higher moisture content than fungi for growth. Although some strains of bacteria and fungi have been known to live in low moisture conditions, it is unlikely that significant protein degradation would occur without the presence of water.

Volatile fatty acids (organic acids) can be produced in two ways: auto-oxidation of lipids and deamination of the various amino acids [37]. It is unlikely that deamination would occur without the presence of water. However, auto-oxidation of free fatty acids could occur during the storage of bloodmeal, and has been observed in aging parchments from goat, sheep or calf skin [38]. Auto-oxidation

is limited to unsaturated fatty acids which are formed via hydrolysis of the ester bonds between the fatty acid chain and the triglyceride or cholesterol moiety to which it is attached. These esters are the lipids found in the cell membrane of blood cells.

The products of the auto-oxidation of fatty acids include volatile fatty acids, aldehydes, ketones, alcohols and aliphatic hydrocarbons which may contribute to the bloodmeal's odour.

1.1.3 Processing Novatein Thermoplastic

To thermoplastically process proteins in bloodmeal, additives are used to ensure the proteins are stabilised in their denatured form, have sites where intra- and intermolecular bonding may occur after extrusion and the glass transition temperature is low enough for the resulting mixture to be processed.

Water is used to dissolve the additives prior to extrusion guaranteeing uniform dispersion throughout the bloodmeal. Water is an ideal plasticiser for proteins. Like other hydroxylated plasticisers, it inhibits the strong interactions that occur between the amino acid side chains of proteins that lead to brittle materials [7]. In addition to water, triethylene glycol (TEG) is added to further plasticise the material.

Thermoplastic processing requires that the proteins are denatured. Urea interrupts the interaction between the protein and water molecules by preferentially binding to the surface of the protein. The change in the proteins electrostatic environment results in a partially unfolded protein. It is important that the proteins are denatured to expose disulfide bonds for reductive cleavage [39]. This allows new cross-links to be formed during extrusion.

As a result of denaturing, changes in haemoglobin may occur where the haem moiety is released and may form free iron ions capable of catalysing lipid oxidation or interacting with the added reducing agent. The addition of a reducing agent is required to break disulfide bonds which prevent the formation of a flowable melt during thermal processing [7]. At room temperature, a reducing

agent is required to induce homolytic cleavage of disulfide bonds such as those of cystine to produce a thiol (R-SH) group [40].

Sodium sulfite is used to break cystine bonds (disulfide bonds) in proteins via a sulfonate ($-S-SO_3^-$) derivative (Figure 5) [41, 42]. This reaction is known to occur most favourably at pH 9 [43] and 30- to 40-fold excess sodium sulfite is required to force the equilibrium to favour the sulfonate derivative as the reaction is reversible when the sulfite ions are consumed in oxidative conditions [42].

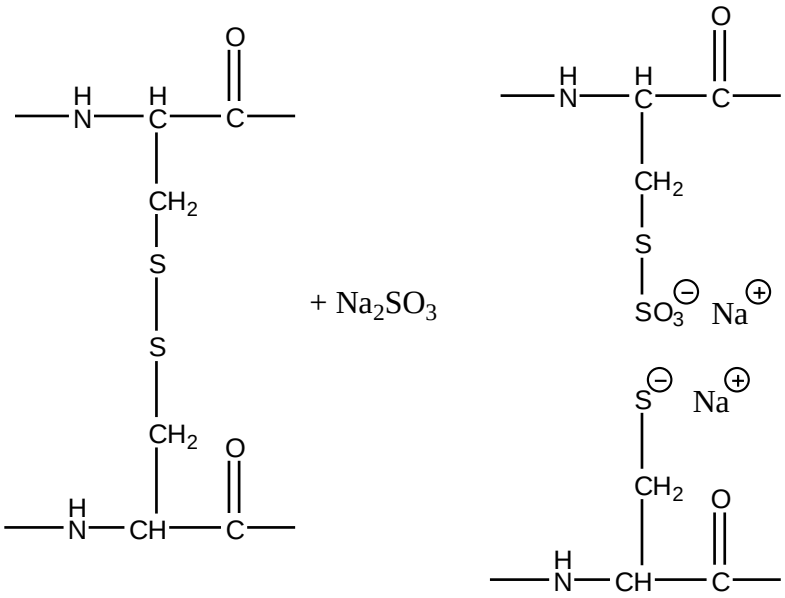


Figure 5: Cleavage of a disulfide bond to yield sulfonate and sulfyl derivatives using sodium sulfite. Oxidising agents such as cupric ions or o-iodosobenzoate are used to ensure cystine converts to a sulfonate derivative.

Cleaving the disulfide bond leads to lower viscosity making the mixture easier to process [44]. The formation of the sulfyl group occurs simultaneously, which can be oxidised to form a new disulfide bridge in oxidative conditions [42]. Sulfite is a strong nucleophile which reacts with proteins by substitution at electrophilic positions [45].

When proteins are denatured, and then reduced, they unravel further forming random coils [46]. The addition of an anionic surfactant (such as sodium dodecyl sulphate (SDS)) leads to a higher degree of order because SDS forms a complex with proteins to give more uniform hydrodynamic shapes (rods or ellipsoids) [46]. This occurs due to SDS preventing hydrophobic and electrostatic interactions between protein chains, making it a powerful denaturant and stabiliser of the denatured form.

The pre-extruded Novatein thermoplastic (PNTP) is then thermoplastically processed as per the patented method, (Appendix 1), into a polymer with material properties similar to that of low density polyethylene [7].

Section A: Odour Profiling

2 Odours arising from NTP processes

Generally an odourant is a volatile, hydrophobic and low molecular weight (<300Da) organic compound that can be perceived by the sense of olfaction. The search to underpin the relationship between odourant compounds and the olfactory response that they have has been explored extensively [47].

Research carried out by Zwaardemaker (1895), Henning (1916), Crocker and Henderson (1927) and Dyson (1938) established the now accepted concept that volatile molecules fit into specific receptors to give certain olfactory sensations [47, 48]. From this "lock and key" concept many attempts to explain stimulation of the receptors have been suggested, currently, it is thought that each odourant molecule activates a variety of receptor types simultaneously and each receptor type responds to a range of odourants.

Over 350 active odourant receptor types have been identified in humans, from which up to 10000 different odourant molecules could be discerned [49]. It can then be concluded that stimulation of receptors by an odourant and consequently olfactory perception is complex, making the determination of key malodourous compounds arising from biological materials difficult.

2.1 Odour formation during NTP processing

Due to the chemical composition of blood and how it is handled, bloodmeal has a characteristic odour. There are several stages in which odourous volatile organic compounds are formed:

1. Prior to bloodmeal production

   Raw blood is often stored prior to drying which may lead to putrefaction reactions via microbial action.

2. Thermal degradation during drying and storage

   Fatty acids may thermally degrade during drying or become oxidised during storage of bloodmeal which may lead to malodours.

3. Side reactions during NTP production

These mechanisms are further explained in the following sections.

2.1.1 Protein degradation

The decomposition of proteins can occur in both aerobic (decay) and anaerobic (putrefaction) environments [50]. The rancid and putrid odours associated with degrading blood can be classified into three main groups:

- Amines and nitrogen containing compounds
- Sulfurous compounds
- Organic acids.

These odourous volatile compounds are formed via bacteria-mediated deamidation, deamination and decarboxylation reactions of the amino acids in aerobic and anaerobic environments.

The decomposition of the proteins begins with protease enzymes produced by bacteria present in the medium. Protease enzymes can be classified into two

subgroups: proteinases and peptidases. Proteinases hydrolyses the peptide bonds of the proteins to form polypeptides after which peptidase enzymes hydrolyse the peptide bonds of polypeptides to form amino acids [50]. Breakdown products (amino acids) arising from the action of protease enzymes are probably odourless [50].

Decay

Decay is the process of aerobic decomposition of proteins, where amino acids that were formed via hydrolysis undergo oxidative deamination to form the corresponding carboxylic acids with one less carbon (Figure 6).

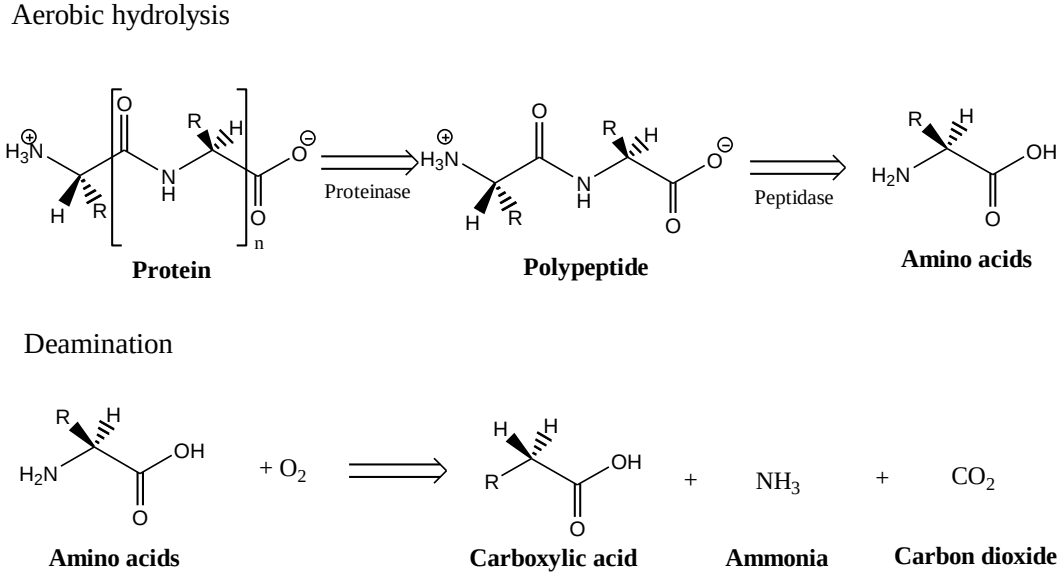


Figure 6: Aerobic hydrolysis of proteins and peptides to amino acids. The amino acids are deaminated or deamidated to form a carboxylic acid with one less carbon, carbon dioxide and ammonia.

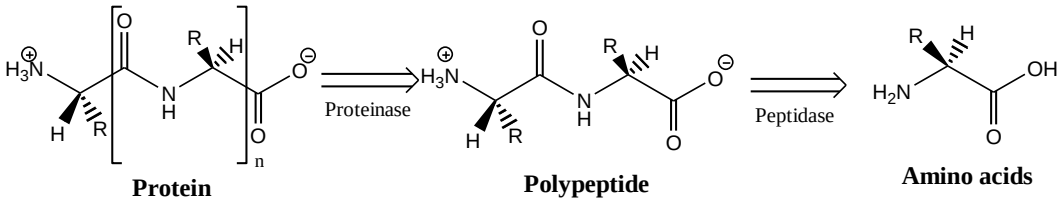
Putrefaction

Putrefaction is the process of anaerobic decomposition of proteins. Amino acids formed via protein hydrolysis are able to undergo two types of reactions within a bacterial cell, mediated by intercellular or respiratory enzymes which result in rancid smelling compounds (Figure 7) [50]:

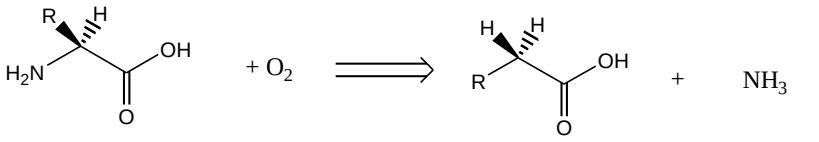
- Decarboxylation leading to the formation of the amino acid's corresponding amine with one less carbon and carbon dioxide.

- Deamination or deamidation leading to the formation of the amino acid's corresponding carboxylic acid and ammonia.

Anaerobic hydrolysis



Deamination



Decarboxylation

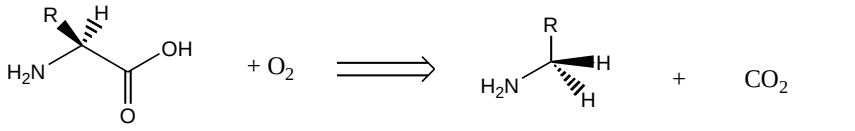


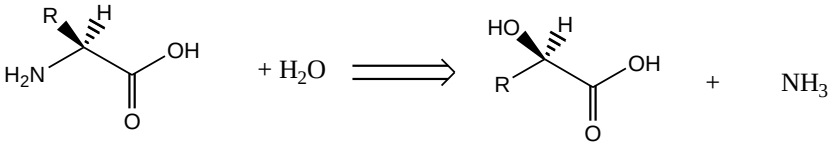
Figure 7: Anaerobic hydrolysis of proteins and peptides to amino acids. The amino acids undergo deaminatation or deamidation to form a carboxylic acid and ammonia. Amino acids are also able to undergo decarboxylation to form an amine with one less carbon and carbon dioxide.

In addition to the production of odourous carboxylic acid compounds, deamination of the sulfur containing amino acids (sulfate reduction) can occur [37]. Deamination of methionine leads to the formation of mercaptans and deamination of cysteine leads to the formation of sulfides.

The products of deamination and decarboxylation of amino acids include amines, mercaptans, sulphides, indole, 2-methyl indole, phenol, 4-methyl phenol, carboxylic acids, carbon dioxide, ammonia and others. Odourous compounds such as those named above are found in swine and poultry farms, rendering plants and other agricultural operations dealing with proteinous materials [37, 51-53].

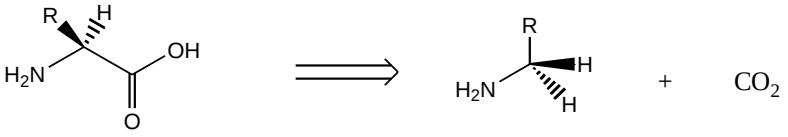
The following reactions as outlined by Salle (1943) are able to account for the formation of non-volatile and volatile compounds which comprise the putrid odours released during storage of the whole blood prior to drying.

1. Hydrolytic deaminisation to form a hydroxyl acid



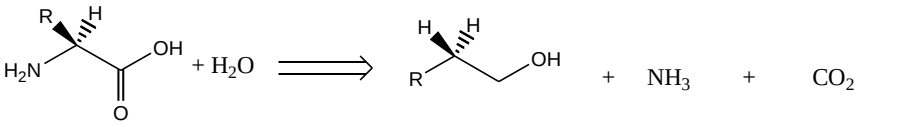
Amino acid **Hydroxyl acid** **Ammonia**

2. Decarboxylation or elimination of CO_2 resulting in an amine as shown on previous page



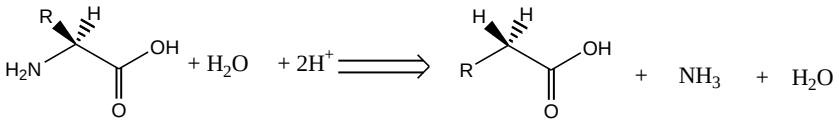
Amino acid **Amine** **Carbon dioxide**

3. Deaminisation and decarboxylation forming a primary alcohol with one less carbon.



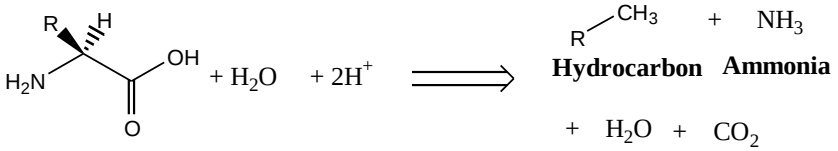
Amino acid **Primary alcohol** **Ammonia** **Carbon dioxide**

4. Deaminisation and reduction forming a carboxylic acid



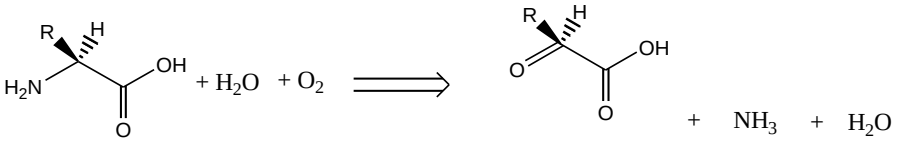
Amino acid **Carboxylic acid** **Ammonia** **Water**

5. Deaminisation, decarboxylation and reduction forming a hydrocarbon



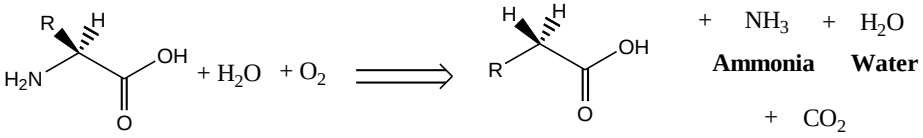
Amino acid **Hydrocarbon** **Ammonia** **Water** **Carbon dioxide**

6. Deaminisation and oxidation forming an α-keto acid



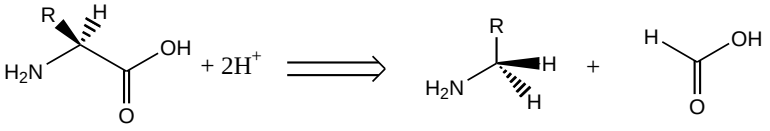
Amino acid **α-keto acid** **Ammonia** **Water**

7. Deaminisation, decarboxylation and oxidation forming a carboxylic acid with fewer carbons



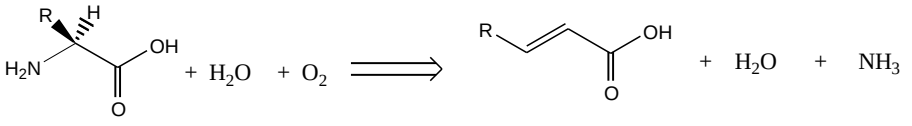
Amino acid **Carboxylic acid** **Ammonia** **Water** **Carbon dioxide**

8. Reduction and elimination of formic acid to form an amine with one less carbon



Amino acid **Amine** **Formic acid**

9. Deaminisation and desaturation at the α-β-linkage forming an unsaturated carboxylic acid



Amino acid **Unsaturated carboxylic acid** **Water** **Ammonia**

10. Anaerobic decomposition resulting in the formation of hydrogen

These putrefactive reactions are known to occur in several industrial operations. Amines have been identified in many animal by-product meals, including bloodmeal, formed in the raw animal by-product by the action of micro-organisms

that contain peptidase and decarboxylase enzymes prior to drying [54-56]. Amines are infamous for the odour they impart, often described as fishy or putrid.

Sulfur containing compounds such as methyl mercaptan, are also known for their distinct odour, typically described as rotten and are often formed during putrefactive degradation of proteins [37]. A selection of commonly observed odourous compounds is given in (Table 3). For a comprehensive list of common odours and how they arise during various agricultural and industrial operations see Appendix 2.

Both amines and sulfurous compounds have relatively low odour thresholds, as a result their detection by conventional analytical methods can be difficult without extraction or derivitisation prior to analysis.

In addition to putrefaction induced degradation leading to the formation of odourous compounds, oxidation of lipids may also result in the formation of similar compounds.

Table 3: Odour characteristics of volatile compounds identified in industrial processes.

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in
Sulfur Compounds				
Hydrogen Sulfide H_2S	[57] Sewer, Fecal	[58]0.00047	[58]0.0047	[37]Meat Rendering, meat meal [51]Swine operation
Dimethyl Disulfide $(CH_3)_2S_2$	[58]Decaying Vegetables,	[51]0.0123	[59]0.0056	[37]Meat rendering, meat meal. [51]Swine operation
Methyl Mercaptan CH_3SH	[58]Decaying Cabbage, [37]Potato-like	[58], [51]0.0011	[58]0.0021	[37]Meat rendering [51]Swine operation
Alcohols				
Butanol $CH_3(CH_2)_3OH$	[51]fusel oil	[51]0.490		[37]Meat rendering [51]Swine operation
Hexanol $CH_3(CH_2)_5OH$		[51]0.0437		[37]Meat rendering [51]Swine operation
Heptanol $CH_3(CH_2)_6OH$		[51]0.0251		[37]Meat rendering [51]Swine operation
Phenol C_6H_5OH	[51] Phenolic	[51]0.110		[51] Swine operation
4-methyl phenol $CH_3C_6H_5OH$	[5] Phenolic	[51]0.00186		[51]Swine operation
Aldehydes				
Hexanal $CH_3(CH_2)_4CHO$	[57] grassy, [37]Fruity, sour, sharp, pungent, almond	[57]0.0073-0.0129 [51]0.0138	-	[37]Meat Rendering [51]Swine operation
2 –methyl butanal $CHOCH_2(CH_3)CH_2$-CH_3	Fatty	-	-	[37] Meat Rendering [12] Swine operation
3-methyl butanal $CHOCH_2CH_2(CH_3)$-CH_3	Fatty	[60]0.00015-0.0023 [51]0.00224	-	[37]Meat Rendering, meat meal [51]Swine operation
Benzaldehyde C_6H_5CHO	[51]Almond	[51]0.0417		[51]Swine operation
Ketones				
Acetone CH_3COCH_3	[37]Sweet	[37]0.46	-	[37]Meat meal
Pentanone $CH_3CO(CH_2)_2CH_3$	[37]Dairy, cheese, mushroom	[51]1.55		[37]Meat Rendering [51]Swine operation
Hexanone $CH_3COCH_2CH_2$ CH_2CH_3	[37]Dairy, cheese, mushroom	[51]0.166		[37]Meat Rendering [51]Swine operation
Heptanone $CH_3COCH_2CH_2CH_2$ CH_2CH_3	[37]Dairy, cheese, mushroom	[57] 0.14-0.28		[37]Meat Rendering [51]Swine operation
Acetophenone $C_6H_5COCH_3$	[51]Orange Blossom	[51]0.363		[51]Swine operation

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in
Carboxylic Acids				
Acetic acid CH_3COOH	[51]Pungent, Sour	[57] 0.02-0.48	[51]0.2	[50]Putrefaction [51]Swine operation
Propanoic acid CH_3CH_2COOH	[37]Dairy, sour, rancid,	[57] 0.03-0.16		[37]Meat Rendering [51]Swine operation
Butanoic acid $CH_3CH_2CH_2COOH$	[37]Dairy, sour, rancid,	[57]0.0039		[37]Meat Rendering [51]Swine operation
2-methyl propanoic acid $CH_3CH_2(CH_3)COOH$	Sour, cheesy, butter	[51]0.0195		[50]Putrefaction [51]Swine operation
Pentanoic acid $CH_3(CH_2)_3COOH$	Putrid, sour, sweaty, rancid	[57]0.0048		[37]Meat Rendering [51]Swine operation
3-methyl butanoic acid $CH_3CH_2(CH_3)CH_2$ -COOH	[57]body odour Sour, sweaty feet	[57]0.00036-0.0025		[50]Putrefaction [51]Swine Operation
4-methyl pentanoic acid $CH_3CH_2CH_2CH_2(CH_3)$ – COOH	Pungent cheese	[51]0.0155		[51]Swine operation
Nitrogen Compounds				
Ammonia NH_3	[58]Sharp, Pungent	[58]0.037	[58]47	[37]Meat rendering [51]Swine operation
Methyl amine CH_3NH_2	[58]Putrid, Fishy	[58]0.021 [51]0.0186	[58]0.021	[50]Putrefaction [51]Swine operation
Ethyl amine $CH_3CH_2NH_2$	[58]Ammonia-cal	[58] 0.83 [51]0.324	[58] 0.83	[50]Putrefaction [51]Swine operation
Propyl amine $CH_3CH_2CH_2NH_2$	Ammonia-cal	[51]0.011	-	[37]Putrefaction [51]Swine operation
Tyramine $OH\text{-}(C_6H_5)CH_2CH_2NH_2$	Mild meaty, dirty, phenolic	-	-	[50]Putrefaction
Trimethyl-amine $N(CH_3)_3$	Fishy, rancid, sweaty, fruity	[37]0.046 [51]0.0024	-	[37]Meat rendering, meat meal [50]Putrefaction [51]Swine operation
Putrescine $NH_2(CH_2)_4NH_2$	[58]Putrid	[58]0.0037	-	[50]Putrefaction
Cadaverine $NH_2(CH_2)_5NH_2$	[58]Putrid, decaying flesh	-	-	[50]Putrefaction
Histamine $(C_3H_2N_2H)CH_2CH_2\text{-}NH_2$	Fishy	-	-	[50]Putrefaction
Indole C_8H_6NH	[58]Fecal	[51]0.000032	-	[50]Putrefaction [51]Swine operation
2-methyl indole C_9H_8NH	[58]Fecal	[58]0.0012 [51]0.00056	[58], [59]0.47	[50]Putrefaction [51]Swine operation
Furans				
2-pentyl furan $C_9H_{14}O$	[37]Sweet, bitter, almond like Green, earthy, beany	[57]0.016-0.048	-	[37]Meat meal [51]Swine operation

2.1.2 Lipid Oxidation

Fatty acids present in bovine blood are attached to triglycerides, cholesterol esters or phospholipids (Figure 8) and can be either saturated or unsaturated fatty acids and comprise part of the cell membrane of blood cells [33]. The two most abundant unsaturated fatty acids found in plasma and red blood cells are oleic and linoleic acid (Figure 9).

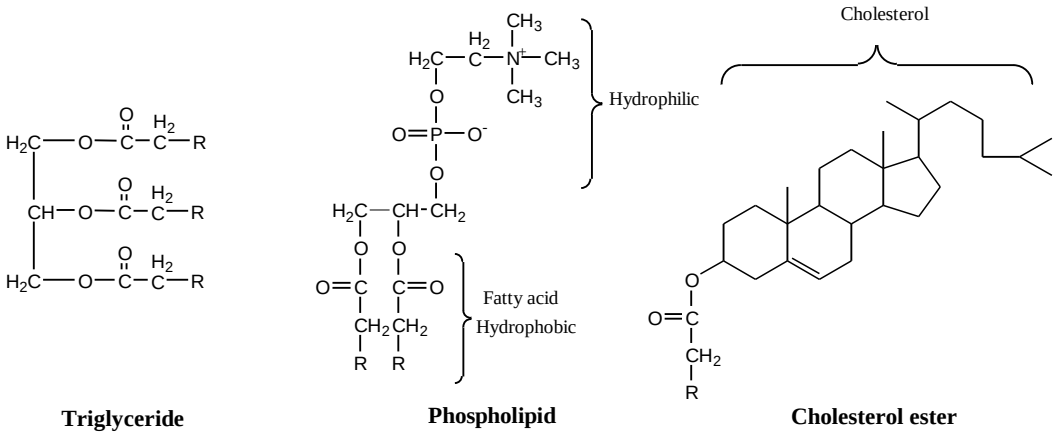


Figure 8: Lipids present in bovine blood.

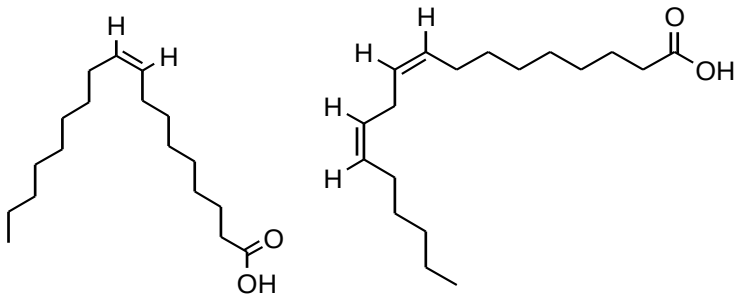


Figure 9: Unsaturated fatty acids, oleic acid (C18:1) and linoleic acid (C18:2).

Free fatty acids may be formed via deamination and desaturation at the α-β-linkage of peptides linked to a fatty acid. Alternatively, hydrolytic cleavage of the ester bonds between a fatty acid chain and the glycerol or cholesterol to which it is attached in whole blood can also lead to free fatty acids [61]. Hydrolytic cleavage may occur during storage of whole blood due to enzymatic activity or thermally induced hydrolysis or oxidation during drying.

Auto-oxidation of unsaturated free fatty acids such as oleic, linoleic and linolenic acid results in the formation of malodourous volatile organic compounds such as C_5-C_9 ketones [32], benzaldehyde, 2-penytl furan and C_1-C_9 aldehydes [34]. In addition, a hydroperoxide lyase enzymes are known to produce 1-octen-3-ol from the oxidative cleavage of linoleic acid [62].

Auto-oxidation is initiated by the loss of a hydrogen radical in the presence of trace metals such as iron(III), light or heat. The reaction mechanism involves initiation, propagation and termination:

Initiation: $RH \rightarrow R^{\cdot}$ (1)

The radical ($R^{\cdot}$) can then react with oxygen to form peroxy radicals ($ROO^{\cdot}$) which can react with other compounds to form hydroperoxides (ROOH) [63]:

Propagation: $R^{\cdot} + O_2 \leftrightarrow ROO^{\cdot}$ (2)

$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$ (3)

Termination: $ROO^{\cdot} + ROO^{\cdot} \rightarrow$ non radicals (4)

Lipid hydroperoxides are the primary products of lipid auto-oxidation, are very reactive and undergo a variety of oxidation reactions yielding secondary products such as aliphatic alcohols, aldehydes, ketones and hydrocarbons which are responsible for flavour deterioration in meats [63]. The hydroperoxides are also known to cause oxidative damage to saturated fatty acids at temperatures greater than 60 ^{o}C, leading to the formation of methyl ketones such as 2-heptanone, 2-nonanone and 2-decanone [64].

Auto-oxidation is observed during aging of raw and cooked meat and is initiated by inorganic iron, which is released from haem when haemoglobin is denatured during cooking or through enzymatic activity [65]. In the same study, it was found that chelating the free iron with EDTA significantly reduced lipid oxidation [65].

2.1.3 Sulfite Side-reactions

Thermoplastic processing of bloodmeal requires the addition of excess sodium sulfite. Sulfite ions are known to form sulfite and sulfate radical anions, where the sulfate radicals are capable of interacting with methionine in the presence of transition metal ions and oxygen to form methyl mercaptan [66], [67].

Methyl mercaptan formation is thought to be initiated by the production of sulfite radicals produced from the interaction of sulfite ions with free transition metal ions and oxygen. The sulfite radicals then react with oxygen and other transition metal ions to form the peroxysulfate radical ($SO_5^{\cdot-}$) and sulfate radical ($SO_4^{\cdot-}$) anions.

The sulfate radical anion has an oxidising power similar to that of the hydroxyl radical and may induce the formation of methyl mercaptan in a similar manner to the hydroxyl radical (Figure 10 and Figure 11).

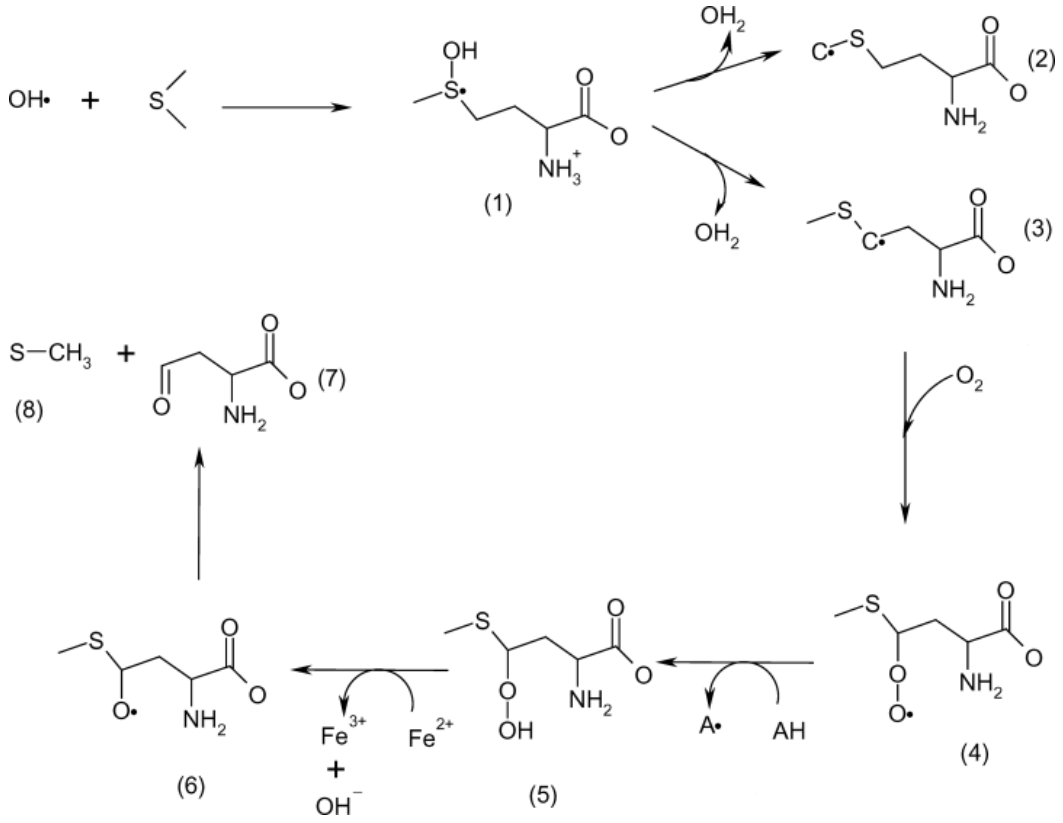


Figure 10: Proposed mechanism for hydroxyl radical reaction with -S in methionine and methionine containing peptides and proteins. (1) hydroxyl sulfunyl radical, (2) and (3) α-(alkylthio)alkyl radicals, (4) peroxy radical, (5) hydroperoxy compound, (6) oxy radical (7) unreactive α-keto product and (8) methyl mercaptan. Retrieved from [66].

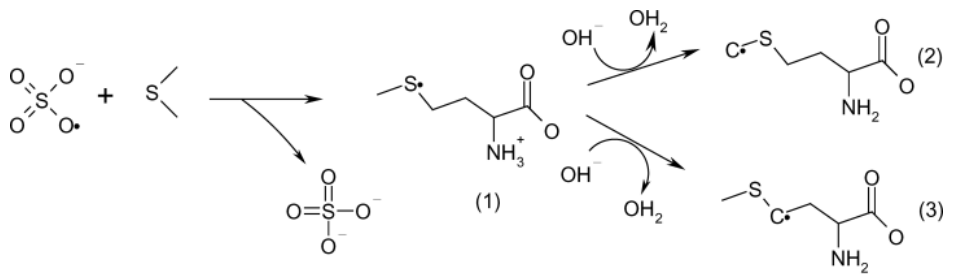


Figure 11: Proposed mechanism for sulfate radical anions reacting with methionine and methionine containing peptides and proteins. Retrieved from [66].

Sulfite ions and methionine alone do not undergo a reaction to form methyl mercaptan, but in the presence of transition metal ions the formation of methyl mercaptan occurs spontaneously. Studies show as little as 4 mmol methionine, and 3.2 mmol sulfite ions in the presence of 17 μmol Fe^{2+} or Fe^{3+} leads to the formation of methyl mercaptan (made up as a 25mL solution at pH 7.8, 22 ^{o}C) [66].

A comparison of the above reaction with that occurring in the NTP process, it is plausible to suggest that methyl mercaptan is being formed:

- Commercial bloodmeal contains 2273.0 mg/kg haem iron (4071 μmol Fe per 100 g bloodmeal)
- Commercial bloodmeal contains 1.10 wt% methionine [68] (~7 mmol methionine per 100 g bloodmeal)
- 3 g of sodium sulfite is required per 100 g bloodmeal (~23.8 mmol sulfite per 100 g bloodmeal)

In the NTP reaction using 100 g bloodmeal, there is seven times the quantity of sulfite ions available to form sulfate radical anions and up to two times the quantity of methionine. During the drying of blood, and during the denaturing of bloodmeal proteins in the NTP process, part of the haem iron may be released as inorganic iron. With the quantity of methionine present in the NTP reaction, approximately 17-34 μmol of $Fe^{2+/3+}$ is required to catalyse a reaction, ie. less than 0.5 % of the haem iron in bloodmeal being released during NTP process would provide catalysis for the formation of methyl mercaptan. Due to the low odour

threshold of methyl mercaptan, given as 0.0011 ppm (Table 3), its formation may cause a major change in the odour profile of bloodmeal.

2.1.4 Identifying Odourous Compounds

There are several methods which are employed to identify and quantify odourous compounds in samples. A list of compounds associated with malodour from agricultural industries, their odour thresholds and how they arise, have been given in Appendix 2. The two most commonly used methods of analyte separation during analysis are liquid chromatography (LC) or gas chromatography (GC). The type of analyte to be identified is especially important when developing an extraction method.

All extraction methods are based on polar or non-polar interactions between the solvent and the analytes, the analytes will be extracted into the phase that they are more miscible in. Extraction into a solvent allows for easy identification using LC or if the extract is heated, using headspace GC. Due to the number of steps required to carry out an extraction and concentrate the analytes for analysis solvent extraction methods can be time consuming and error prone. Solvent extraction is ideal for sparingly volatile compounds such as amines, these often require derivitisation prior to analysis to reduce their polarity and increase their volatility thereby improving chromatographic separation [69-71].

Other compounds that often require derivitisation due to their high polarity and low volatility include phenols, organic acids and organometallics [70]. More recently odour extraction for identification and quantification is carried out using solid phase micro-extraction (SPME) where the analyte in the sample is concentrated on the fibre allowing identification of most compounds without derivitisation.

2.2 Solid Phase Micro-extraction

Solid phase micro-extraction (SPME) is a rapid, solvent-free extraction technique developed by Pawliszyn and co-workers in 1989. Generally, SPME uses a one centimeter long, fused silica fibre coated with an extracting phase such as a liquid

polymer or solid sorbent and is mounted in a modified syringe to absorb or adsorb organic compounds [72]. Depending on the fibre coating, both non-volatile and volatile analytes can be extracted from liquid or gas phase samples.

In contrast to conventional extraction methods, such as liquid-liquid extraction and solid-phase extraction, SPME is an equilibrium method which depends on analyte partitioning between the sample and the SPME fibre (Figure 12) [73].

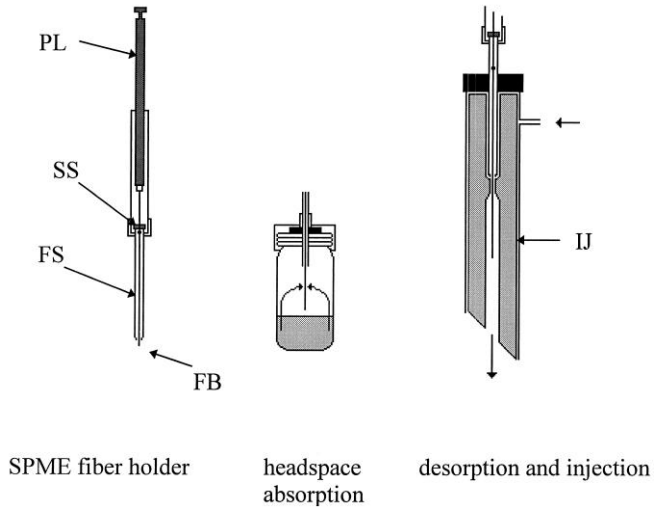


Figure 12: SPME adsorption/ absorption of analytes from sample headspace and desorption in the GC inlet. FB=coated fused silica fibre, FS=syringe needle, SS=sealing septum, PL=plunger; IJ=GC inlet. Image sourced from [74].

One of the major advantages of SPME is that it samples, isolates and concentrates the analytes of interest in one step. Following extraction, the SPME fibre is inserted into the injection port of a separating instrument for direct analysis, minimising analyte losses due to multiple-step processes. Since its commercialisation in the early 1990s, SPME has been successfully applied to the sampling and analysis of environmental samples, food, pharmaceuticals and various odour issues [75].

When sampling an aqueous or solid sample containing non-volatile or volatile organic compounds the sample is placed in a vial and sealed with a cap that contains a septum. The sheath of the SPME syringe is pushed through the septum and the fibre is immersed into aqueous samples or exposed to the headspace over

the liquid or solid sample until equilibrium is reached between the analyte partitioned in the fibre coating and the analyte present in the sample matrix [72].

After extraction, the SPME fibre is inserted into the injection port of a GC (or modified high performance liquid chromatography inlet) where the analytes are thermally desorbed and transferred to the capillary column to be separated and analysed in the same manner as conventional chromatographic analysis [73].

The amount of analyte extracted onto the fibre, when at equilibrium, is proportional to its initial concentration in the sample matrix. The time required to achieve equilibrium is dependent upon the partition coefficient of each of the analytes between the fibre and the sample matrix and also the thickness of the fibre coating [73].

The temperature to which a sample is heated will depend on the volatility of the analytes and the thermal stability of the sample. The required headspace volume and sample size will depend on the concentration of the analytes in the sample, and the sample volume.

For headspace analysis, temperature is a factor that influences the extraction efficiency, however, for the purpose of obtaining reproducible results consistent timing and sampling conditions are more important than obtaining equilibrium [76].

There are many types of fibres available with varying polarities and coating thicknesses (Table 4).

Table 4: Commonly used comercial SPME fibres.

Fibre Coating	Film Thickness (µm)
Polydimethylsiloxane (PDMS)	100
PDMS	30
PDMS	7
PDMS/Divinylbenzene (PDMS/DVB)	65
Polyacrylate	85
PDMS/Carboxen (PDMS/CAR)	75
DVB/CAR/PDMS	50/30
Carbowax®/DVB	70

The type of fibre employed will largely depend on the analytes. Several fibre materials are available with varying polarities and coating thicknesses. The analytes are absorbed in the fibre coating in the case of polydimethylsiloxane (PDMS) and polyacrylate coatings while are adsorbed onto the surface of PDMS blends that contain carboxen or divinylbenzene. PDMS-carboxen blended fibre coating allows for both adsorption and absorption due to the sorbent containing micropores [70].

The coating thickness influences equilibration time with the sample analytes where the thinnest film coating is typically used to reduce extraction times [70]. For volatile flavour and odour analysis, most fibres will be coated in polydimethylsiloxane (PDMS) resin and may also contain blends with divinylbenzene or carboxen to improve compound selectivity. All fibres must be conditioned prior to sample extraction (0.5-4 h) to ensure they are thoroughly clean.

2.2.1 Gas Chromatography Mass Spectrometry

Gas chromatography (GC) is a method used to separate thermally stable volatile compounds. A sample is injected into the inlet port and is flushed onto the column using an inert gas (mobile phase) such as helium or nitrogen. It uses either a packed or capillary column to separate analytes based on each compounds interaction with the column relative to the stationary phase [77].

When the sample enters a capillary column, the various compounds are focused at the beginning of the column due to the low column temperature. As the temperature of the column is increased, the van der Waals forces and hydrogen bonding interaction between the compounds and the column's stationary phase (a silicone-based polymeric resin coating) is overcome. Because each analyte has different physio-chemical properties it interacts with the column to different extents and forms the basis of separation. The time it takes each analyte to move through the column will depend on the flow rate of the mobile phase and temperature of the column.

GC/MS Sample Injection

There are two common sample injection methods: split and splitless. When the SPME fibre is injected into the inlet the volatile compounds are thermally desorbed, flushed onto the column and refocused as a band due to the cooler temperature (Figure 13) [78].

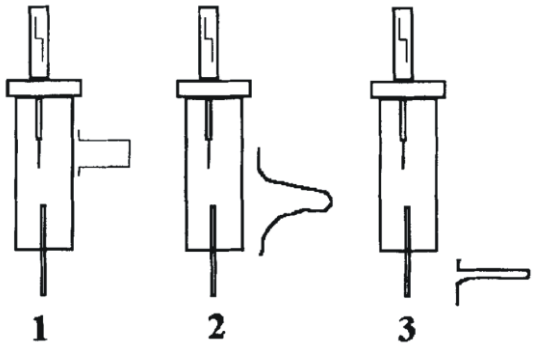


Figure 13: SPME Desorption: Shows distribution of analytes 1. Before desorption, 2. During desorption and 3. Focused on the column. Image from [78].

Split injections only allow a portion of the sample to be flushed onto the column and the rest is purged from the inlet [77]. Due to the smaller amount of sample entering the column, the peaks obtained are symmetrical and well separated. The major drawback of using split injection is known as analyte discrimination, where the most volatile compounds are flushed onto the column and the less volatile compounds may be swept to waste.

For analysis of samples with low concentration of analytes or small solvent volumes as involved in SPME, splitless injections are typically used. Splitless injection forces all of the volatilized sample analytes onto the column [77]. This ensures the results are representative of the sample. However, the drawback of splitless injection is a longer time required for the sample analytes to be forced onto the column, which may lead to peak broadening in the earlier eluted compounds.

Focusing analytes

Focusing the compounds at the head of the column is paramount and can be difficult when using a splitless injection. It is important to have the inlet temperature high enough to quickly volatilize all compounds as they are flushed onto the column in order to obtain sharp peaks (Figure 14) [73].

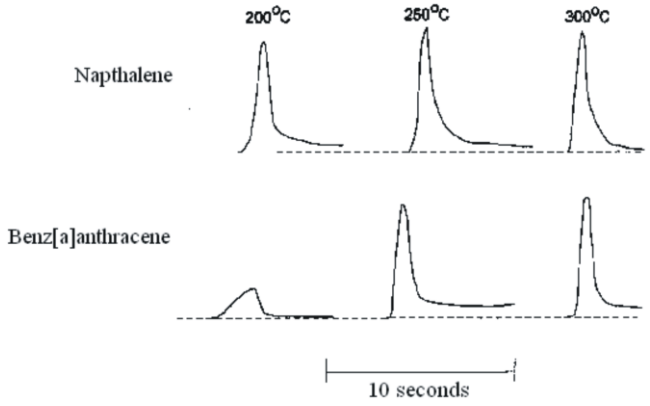


Figure 14: Comparison of real-time desorption profiles of naphthalene and benz-[a]anthracene using 7μm SPME fibres at 200, 250 and 300°C. Image adapted from [73].

For SPME a narrow-bore inlet liner should be used to minimize peak broadening. Narrow inlet liners have a smaller volume and taking less time to flush the volatilized analytes onto the column. The most ideal method of focusing the compounds at the inlet of the column is to use cryogenic cooling to cool the column below 0 ^{o}C (Figure 15) and use a higher column film thickness [73]. When the volatile compounds encounter the low temperature diffusion is slowed down, and slowly increasing the temperature of the column allows the compounds to be separated efficiently.

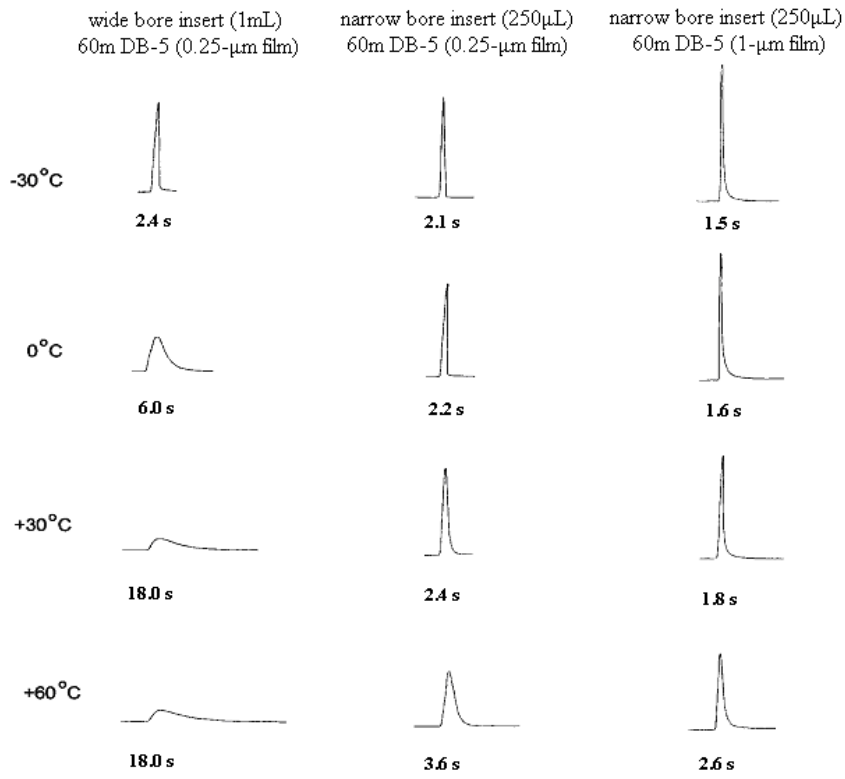


Figure 15: Effect of inlet-liner volume, cryogenic cooling temperature and column-film thickness on the chromatographic peaks observed for benzene desorbed from a 100µm PDMS fibre at 300°C. Image adapted from [73].

2.2.2 GC/MS Column Conditions

There are two types of GC columns used: packed and capillary. For analysis of odourous volatile compounds non-polar or low to medium polarity capillary columns are often used [51, 79-82].

Temperature is the most important factor affecting separation of compounds in the column. There are two ways in which the column can be temperature controlled: isothermally or using a gradient temperature profile. Isothermal conditions can be useful for identifying non-polar compounds that have similar physio-chemical properties, but often requires longer separation times. If used at a moderate oven temperature, high temperature boiling compounds will elute in a broad peak, conversely at high oven temperature, low temperature boiling compounds will not be retained at all [77]. Thus for highest retention efficiency and chromatographic resolution, thermal gradients are used, increasing sample throughput at the same time by lowering the total run time [77].

Mass spectrometry and detection

Upon elution from the capillary column, the analyte compounds enter the ionisation source where the compound is ionised. The ions are then accelerated into the mass separator where they are discriminated by their mass to charge ratio into an ion beam [77]. The ion beam is then detected by its ion current and plotted by the MS software as a total ion chromatogram. Each peak in the total ion chromatogram has a corresponding mass spectrum, a "fingerprint" unique to the fragmentation of the parent compound which aids identification.

Identification of compounds in the total ion chromatogram can be carried out using the mass spectra obtained for each peak and the NIST mass spectral library, a database of mass spectra for a large variety of compounds. Using the NIST mass spectra library and the elution times from the total ion chromatogram the compounds are able to be identified. The NIST identification is carried out after background subtraction in the total ion chromatogram.

2.3 Removing Odourous Compounds from NTP

Many methods exist for the mitigation of malodour in the home, workplace or in industries. Malodours can be altered by the addition of pleasant smelling compounds to mask the unpleasant odour, however, the most effective method of odour mitigation is to remove the odourous compound. This can be achieved by adsorbing or chemically altering the compound so that it is no longer odourous. Some methods currently used to remove odour include:

- Aqueous extraction
- Acid/Alkaline treatment
- Oxidation
- Physical Adsorption

This section discusses the principles involved in removing odourous compounds from bloodmeal using methods commonly employed to remove odours from solid materials.

2.3.1 Aqueous Extraction

Odourous volatile compounds need to be sparingly water soluble in order to be detected by the sense of olefaction. Aqueous extraction methods have been developed to remove the beany-odour from soy protein isolate, and generally involve steam injection to extract the odours from protein suspended in water [83, 84]. In principle a similar method could be applied to all proteinous materials, providing the temperature of the protein mixture does not exceed the thermal denaturing temperature. This method assumes that odours are only particle associated, completely water soluble, and that exhaustive extraction can be achieved at relatively low temperatures.

In the soy protein manufacturing process, there are unsaturated lipid compounds that remain bound to the protein [84]. These lipids can undergo auto-oxidation during further processing and storage to cause off-flavours and odours. Water vapour stripping employing sub- or super-atmospheric pressure is a common process used in a variety of patents for the removal of malodourous and off-flavour components in soy protein materials [85, 86].

Flash evaporation involves ejecting a heated slurry of protein and steam under pressure into a retention chamber before instantly reducing the pressure [84]. As a result a portion of the water containing the off-flavours is removed. Aqueous extraction of odourous compounds requires a temperature (93-204 ^{o}C) higher than that for thermal denaturing of proteins to minimise carbonyl binding to proteins [84].

To exhaustively extract odourous compounds from a material at a lower temperature using aqueous extraction, the extraction step must be carried out multiple times. The low removal efficiency of carbonyl compounds at lower temperatures (caused by reduction in pressure) is caused by an increase in the binding of the carbonyl compounds to the protein [87].

2.3.2 Acid/alkaline treatment

Odourous compounds such as amines and mercaptans are only sparingly soluble in water. By adjusting the pH of the aqueous solvent used for extraction, these compounds can be made soluble in the aqueous phase, and therefore be removed by decanting.

In acidified solution, amines become protonated, making them more miscible. Amines, such as putrescine, are found in bloodmeal and can be extracted using 0.1-1.0 $molL^{-1}$ hydrochloric acid [56]. It is thought that treating the bloodmeal with hydrochloric acid will extract the amines and may reduce its overall odour.

In alkaline solution, mercaptans become water soluble mercaptides. Mercaptans are commonly found in proteinous materials such as bloodmeal and can be extracted using sodium hydroxide [88]. It is thought that any mercaptans present in bloodmeal as a result of putrefaction could be made soluble and removed using sodium hydroxide, thus reducing the bloodmeal's overall odour.

Regardless of the potential of acid or alkaline treatment on minimising the overall odour of bloodmeal, it is well known that slight changes in pH influence the tertiary structure of the protein, as it is often employed as a denaturant. The problem with exposing the proteins in bloodmeal to changes in pH is the potential for irreversible denaturing of the protein in addition to potential loss of water soluble proteins present. These changes in protein composition as a result of pH change may cause major changes in the mechanical properties of the bioplastic.

2.3.3 Oxidation

Oxidation of organic compounds is a known method to reduce malodour. Several examples include the oxidation of odourous compounds in air from rendering, swine and poultry operations using electrically activated oxygen, ozone treatment, incineration and bio-filters [52, 53].

Some of the compounds known to be oxidised to form odourless products include sulfides, disulfides, mercaptans, aldehydes, esters, ammonia, phenols, alcohols,

indoles and skatoles [52]. Ozone treatment is an effective means of removing odourous compounds, however, in high humidity conditions it reacts with water to form hydrogen and oxygen, eliminating the ozone before it can react with the odourants [52].

Hydrogen peroxide has been used to oxidise odourous sulfides in wastewater facilities [89], and other common bleaching agents such as peracetic acid and sodium chlorite have also been used to manage odour associated with various waste streams [90, 91].

Peracetic acid is an equilibrium mixture of peracetic acid, water, hydrogen peroxide and acetic acid. It is known to be a stronger oxidising agent than hydrogen peroxide, possibly because of the presence of two oxidising agents in equilibrium, as shown by the standard reduction potentials given in Table 5 [92]. The chlorite ion is also a strong reducing agent, and in solution with pH > 7 it is reduced to the chloride ion or chlorite ion [93].

Hydrogen peroxide and peracetic acid are typically not used for the treatment of odour due to their high cost. However, they are both environmentally friendly products and have high oxidation efficiency.

Table 5: Standard reduction potentials for common oxidising agents.

Reduction reaction	E^o (V)
Peracetic acid $CH_3C(O)CO\text{-}OH + 2H^+ + 2e \rightarrow H_2O + CH_3COOH$ $H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O$	1.81[a]
Hydrogen peroxide $H_2O_2 + 2H^+ 2e \rightarrow 2H_2O$	1.76[b]
Chlorite $ClO_2^- + H_2O + 2e \rightarrow Cl^- + 2OH^-$ (pH>7)	0.890[c]
Chlorite $ClO_2^- + H_2O + 2e \rightarrow ClO^- + 2OH^-$ (pH>7)	0.681[c]

Data sourced from: a= [94], b= [95] and c= [93].

2.3.4 Physical Adsorption

Absorption or adsorption is a common technique which has been used to minimise malodour in the home, workplace, industrial waste treatment facilities and for

treating other waste emissions. For adsorption to be effective in odour removal, the sorbent must have a high surface area per unit mass and high degree of porosity to maximise the number of electrostatic interactions (such as Van der Waals and hydrogen bonding) that can occur between the odourant compounds and the sorbent.

Odour removal can be achieved using activated alumina, bauxite, activated carbon or forms of zeolite [96]. Physical adsorption is a reversible process and over time the lower molecular weight odourous compounds are displaced by the adsorption of heavier molecular weight compounds. As a result, the ability of the sorbent to remove a mixture of odourous compounds depends on the adsorption capacity of the least easily adsorbed compound and may become less effective over time.

Zeolites, are aluminosilicate materials which have pores of differing sizes which depend on their crystal structure [97]. Natural zeolites are polar sorbents [98] and have been used as heavy metal scavengers, ion exchange media, soil conditioners, ammonium ion scavengers and in biofilters to remove odours [99], [100], [101], [53], [102].

Over 40 types of natural zeolite have been discovered but only 11 are sold commercially, each with different porosity and ion exchange capacities [103]. There are three common types of natural zeolite used for ion exchange and adsorption, found to have a specific surface area of up to 1100 m^2g^{-1} [104]. The zeolite clinoptilolite has been shown to control nuisance odours such as acetic acid, butanoic acid, 3-methyl butanoic acid, indole and skatole that occur in poultry manure storage [105].

Activated carbon is a non-polar sorbent and has a specific surface area of 800-1200 m^2g^{-1} and 35-40 % porosity [98]. Due to its ability to adsorb up to 40 % of its own mass, it is often used to remove odourous compounds, particularly hydrogen sulfide, during sewerage treatment [106].

3 Experimental

The purpose of this work was to assess the odour profile of bloodmeal. In order to identify the VOCs which arise from bloodmeal, a suitable method of extraction and analysis required developing, from which all subsequent treated samples could be analysed for comparison.

3.1 VOC extraction method development

There are many variables that could influence the extraction and analysis procedure of volatile compounds from a solid sample. In order to develop a suitable method, consideration is given to the SPME extraction parameters (fibre type, sample size, extraction temperature and time) as well as the conditions used for GC/MS.

The purpose of the work presented in this chapter was to develop a suitable method for extracting and analysing the VOCs present in bloodmeal using SPME.

Equipment

Clear glass 10 mL SPME headspace vials and septa were purchased from Supelco (Sigma Aldrich, Bellefonte, PA, USA).

SPME holder and SPME fibres in "Fibre Kit 1 and 4" were purchased from Supelco (Sigma Aldrich, Bellefonte, PA, USA). For extraction the 100μm polydimethylsiloxane (PDMS), 85μm polyacrylate (PA), 75 μm PDMS/carboxen (PDMS/CAR) and 65μm PDMS/divinylbenzene (PDMS/DVB) were used. The position of the fibre within the SPME holder was set to 1.2 on the vernier gauge for extraction of VOCs.

GC/MS analysis was performed using HP 6890 series GC system (Agilent Technologies, Little Falls, DE, USA) on a Phenomenex™ 30 m×0.25 mm I.D., 0.25 μm d_f ZB-5 capillary column (Agilent Technologies, Little Falls, DE, USA).

Compounds were tentatively identified based on the probability that it's spectrum matched that of the suggested compound from the NIST Mass Spectral Library. However, compounds identified with low probability have been included in the results if their mass spectra resembled the suggested compound.

Materials

Bloodmeal sourced from Taranaki Byproducts.

GC–MS Conditions

Capillary GC analyses were done on a Phenomenex™ 30 m×0.25 mm I.D., 0.25 μm d_f ZB-5 column. Desorption of the SPME fibre was accomplished by thermal desorption in the back inlet at 250 ^{o}C for 2 minutes, with the fibre set to 3.8 on the vernier gauge on the holder during desorption. The GC/MS run was commenced using a pulsed splitless injection at 15 psi as soon as the fibre was injected to ensure all analytes enter the column simultaneously. A glass inlet liner of 1 mm I.D. was used in the injection port. The oven was held at 40 ^{o}C for 5 minutes and ramped at 8 ^{o}C/min to 200 ^{o}C and held for 15 minutes, unless otherwise indicated. Helium was used as carrier gas. The HP 5973 mass spectrometric detector was operated in the scan mode (*m*/*z* 40–300), MS Quad operated at 150 ^{o}C and MS source at 230 ^{o}C.

After initial investigation of the extraction parameters, the column was replaced. The HS-SPME extraction parameters and GC/MS conditions were then re-evaluated using a new Phenomenex™ 30 m × 0.25 mm I.D., 0.25 μm d_f ZB-5 column from the same batch as the initial column. However, the new column installed could no longer detect dimethyl disulfide and some compounds were identified with lower probability using the NIST spectral library.

3.1.1 Headspace-solid phase micro-extraction

Fibre Type

A range of fibre coatings are available, each with specific application areas. Initially, fibre coatings appropriate for the analysis of volatile components were

selected. According to the selection criteria used by Sigma Aldrich, this corresponds to Fibre kits 1 and 4 of which the following choices were used:

- 100 μm polydimethylsioxane (PDMS), used to extract volatile compounds of low to medium polarity.
- 85 μm polyacrylate (PA), suitable for adsorption of semi-volatile polar compounds.
- 75 μm PDMS/carboxen (PDMS/CAR), used for extraction of low molecular weight compounds.
- 65 μm PDMS/divinylbenzene (PDMS/DVB) fibre for volatile amines and nitroaromatic compounds.

Prior to sampling, all fibres were conditioned as per manufacturer's guidelines outlined in Table 6. A sample size of 2.0 g bloodmeal in a 10 mL headspace vial was used for fibre selection. The sample was heated to 75 ^{o}C for 1 hour to obtain equilibrium. An extraction time of 5 minutes was used followed by GC analysis.

Table 6: Temperature and Conditioning Recommendations for GC Use and pH Guidelines. Data from Supelco.

Fibre Coating	Film Thickness (μm)	pH	Maximum Temperature (^{o}C)	Recommended Operating Temperature (^{o}C)	Conditioning Temperature (^{o}C)	Conditioning Time (Hrs)
PDMS	100	2-10	280	200-280	250	0.5
Polyacrylate	85	2-11	270	220-300	280	1
Carboxen/PDMS	75	2-11	320	250-310	300	1
PDMS/DVB	65	2-11	340	200-270	250	0.5

The final fibre choice for the analysis of VOCs was based on a comparison between the number of peaks identified and their resolutions in the total ion chromatogram (TIC), obtained via GC/MS.

Sample Size

Bloodmeal sample masses of 1.0, 2.0 and 4.0 g were heated to 75 ^{o}C to determine which sample size yielded the highest peak abundance with good resolution. An extraction time of 5 minutes was used for the PDMS/DVB fibre, using the same GC conditions as for fibre selection.

Extraction temperature

Extraction temperatures of 50 ^{o}C, 75 ^{o}C and 90 ^{o}C were used for 2.0 g bloodmeal samples to determine which yielded the largest number of analyte peaks, with the best possible resolution. A sample size of 2.0 g was used, being extracted for 5 minutes using the PDMS/DVB fibre, using the same GC conditions as above.

Extraction time

Extraction times of 1 minute, 5 minutes and 20 minutes were used to determine ideal extraction time. Extraction was carried out on 2.0 g sample at 75 ^{o}C using the PDMS/DVB fibre, using the same GC conditions as above.

3.1.2 Results and Discussion

The development of an appropriate HS-SPME sampling method and GC/MS method were investigated, the results of which is described in the following sections.

Fibre Type

A summary of the compounds identified from the TICs using various SPME fibres is given in Table 7. The TIC for each fibre used is shown Figure 17, Figure 16, Figure 19 and Figure 18.

Only four compounds were identified using the PDMS fibre. The small range of compounds extracted from the bloodmeal headspace suggests the fibre would not be ideal for the analysis of a wide range of volatile compounds as would be found in animal waste by-products.

One peak was found at R_t= 18.85 minutes using the PA fibre. This compound was tentatively identified as indole. Indole is a semi-volatile compound known for its fecal odour. No other compounds were detected using this fibre indicating that if other semi-volatile compounds are present in the bloodmeal headspace, they are low in quantity, or are too polar to be retained by the GC capillary column used.

The PDMS/CAR fibre allowed identification of several peaks with relatively poor resolution in the first 10 minutes, showing a large siloxane derived peak at R_t=3.0 minutes presumably an artifact of the fibre. Because a large number of the compounds of interest (short chain carboxylic acids) are slightly polar they are the first compounds to elute from the column, the lack of resolution in the first 13 minutes of the analysis may strongly hinder the identification of these compounds.

Table 7: Compounds extracted from bloodmeal using various fibres.

Compound	PDMS	PA	PDMS/CAR	PDMS/DVB
acetone			X	
heptanal				X[b]
pentanoic acid				X
4-methyl pentanoic acid				X[a]
6-methyl heptanone			X	X[a]
benzaldehyde			X[a]	
phenol			X[b]	X[b]
2-pentyl furan	X[a]		X[a]	X[c]
acetophenone			X[c]	X[a]
4-methyl phenol	X[a]		X[a]	X[a]
2-nonanone			X	X
nonanal			X[c]	X
2-decanone	X		X[a]	X
decanal				X[b]
indole		X[c]	X[b]	X
indole derivative	X[b]			X[b]
Number of compounds identified	4	1	11	14
Chromatography	Good	Good	Reasonable	Reasonable

Note: **a**= detected with NTIS <50 % probability, **b**= detected with NTIS <30 % probability, **c**= detected with NTIS <10 % probability.

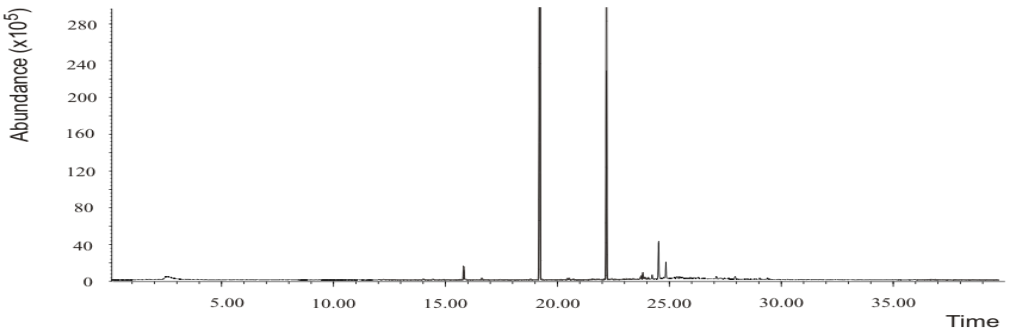


Figure 16: TIC of bloodmeal headspace using PDMS fibre.

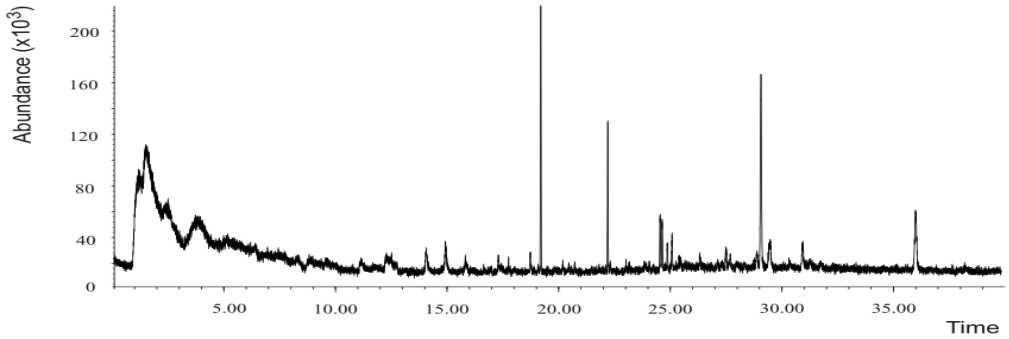


Figure 17: TIC of bloodmeal headspace using PA fibre.

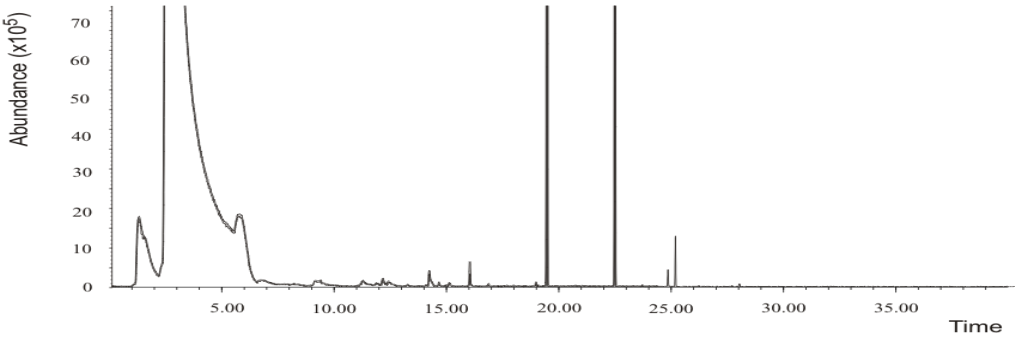


Figure 18: TIC of bloodmeal headspace using PDMS/CAR fibre.

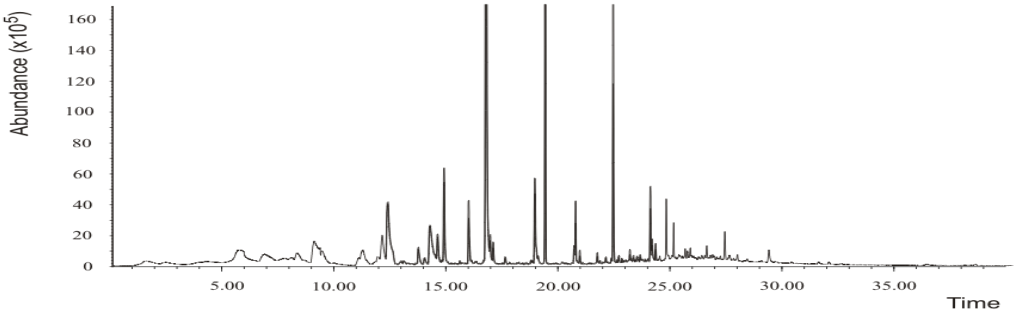


Figure 19: TIC of bloodmeal headspace using PDMS/DVB fibre.

The compounds of interest were identified between R_t = 1-15 minutes and from 17-23 minutes. Other compounds identified were likely to be artifacts of the fibre. Due to the largest number and range of compounds being identified using the PDMS/DVB fibre, it was chosen for the analysis of modified bloodmeal samples to identify the odour causing volatile compounds.

Sample Size

A summary of the compounds identified from the TIC using various sample sizes is given (Table 8). The TICs obtained are shown in Figure 20 and Figure 21.

Table 8: Compounds extracted from different sample sizes of bloodmeal using the PDMS/DVB fibre.

Compound	1.0 g BM	2.0 g BM	4.0 g BM
Hexanal	X[c]		X[c]
Heptanal	X[b]	X[b]	X[a]
pentanoic acid		X	
4-methyl pentanoic acid		X	
6-methyl heptanone		X[a]	X
Benzaldehyde			
Phenol		X[b]	
2-pentyl furan		X[c]	X[b]
Acetophenone		X[a]	X
4-methyl phenol	X	X[a]	X
2-nonanone	X	X	X
Nonanal	X	X	X
2-decanone		X	
Decanal	X[c]	X[b]	X[a]
Indole	X[a]	X	X[b]
indole derivative	X[c]	X[b]	X[b]
Number of compounds identified	8	14	11
Chromatography	Good	Reasonable	Poor

Note: **a**= detected with NTIS <50 % probability, **b**= detected with NTIS <30 % probability, **c**= detected with NTIS <10 % probability.

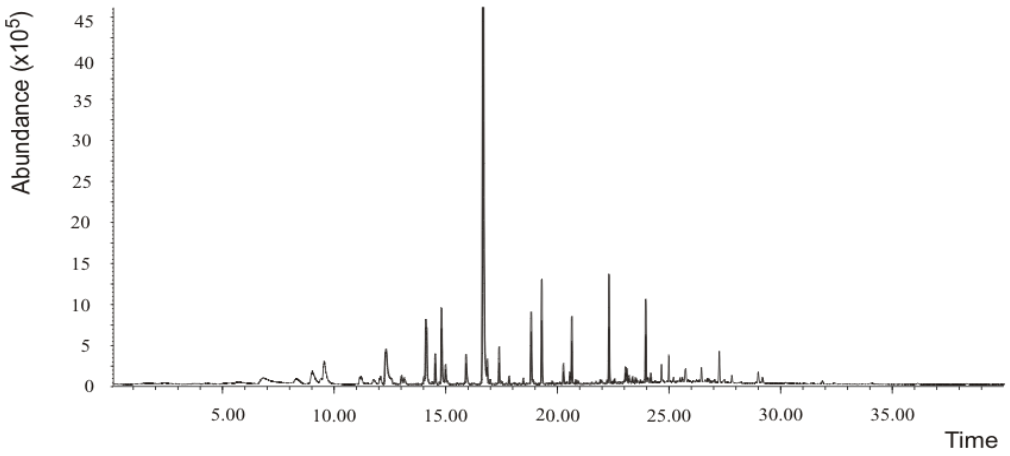


Figure 20: TIC of 1.0 g bloodmeal headspace using PDMS/DVB fibre.

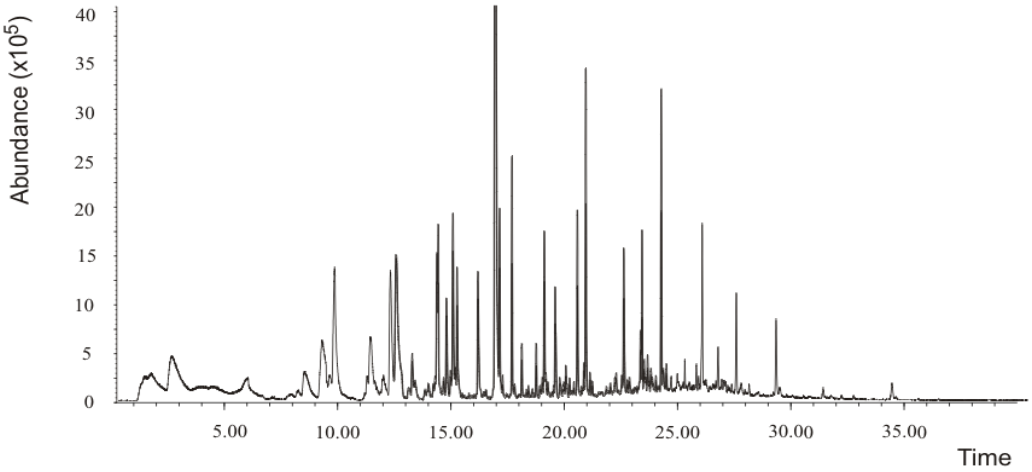


Figure 21: TIC of 4.0 g bloodmeal headspace using PDMS/DVB fibre.

The largest number of compounds identified, while still obtaining reasonable chromatographic resolution was achieved using 2.0 g bloodmeal. Using 4.0 g bloodmeal lead to a highly saturated chromatogram with poor resolution, making identification of the eluting compounds difficult. Based on the TIC for each sample size used, a sample size of 2.0 g was chosen for further analysis as it allowed the easiest identification of the largest range of compounds based on peak size and separation.

Extraction temperature

A summary of the compounds identified from the TIC using various extraction temperatures is given in Table 9. The TICs obtained are shown in Figure 22 and Figure 23. Based on the number of compounds identified and the chromatograph obtained an extraction temperature of 75 ^{o}C was chosen for the systematic development of HS sampling and GC/MS analysis parameters.

Table 9: Compounds extracted from 2.0 g bloodmeal at various temperatures using the PDMS/DVB fibre.

Compound	50 ^{o}C	75 ^{o}C	90 ^{o}C
hexanal			
heptanal		X[b]	
pentanoic acid		X	
4-methyl pentanoic acid		X	
hexanoic acid			X[b]
6-methyl heptanone		X[a]	
benzaldehyde	X[c]		X[c]
phenol		X[b]	
2-pentyl furan		X[c]	X[b]
acetophenone	X[c]	X[a]	X[c]
4-methyl phenol	X	X[a]	X
2-nonanone		X	X
nonanal	X[a]	X	X
2-decanone		X	
decanal	X[c]	X[b]	X[a]
2,5-dione, 3-ethyl-4-methyl-1-H-pyrrole			X
indole	X	X	X
indole derivative	X[c]	X[b]	
Number of compounds identified	7	14	10
Chromatography	Good	Reasonable	Reasonable

Note: **a**= detected with NTIS <50 % probability, **b**= detected with NTIS <30 % probability, **c**= detected with NTIS <10 % probability.

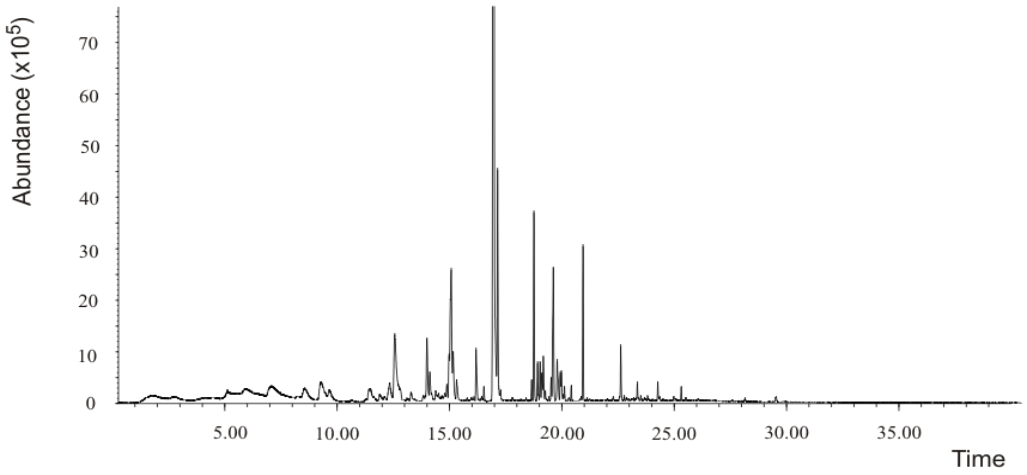


Figure 22: TIC of extraction carried out at 50 ^{o}C on headspace of 2.0 g bloodmeal using PDMS/DVB fibre.

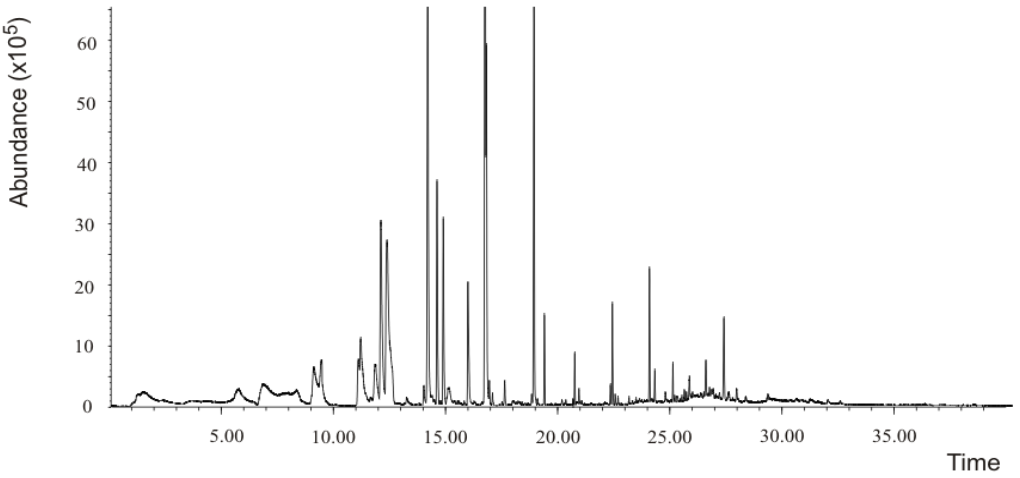


Figure 23: TIC of extraction carried out at 90 ^{o}C on headspace of 2.0 g bloodmeal using PDMS/DVB fibre.

Extraction 50 ^{o}C on 2.0 g bloodmeal yielded the smallest quantity of peaks, which were difficult to identify with any certainty and was dismissed for further investigation. Extraction at 90 ^{o}C also yielded a small quantity of peaks, however this extraction temperature was dismissed due to the possibility of thermal degradation of the protein leading to the formation of pyrroles.

Based on the TIC for each extraction temperature, for the determination of compounds arising from treated bloodmeal samples, an extraction temperature of 75 ^{o}C was chosen for further analysis. This temperature allowed the easiest identification of the largest range of compounds based on peak size and separation,

without the opportunity for sample degradation which would ensue at higher temperatures.

Extraction time

A summary of the compounds identified from the TIC using various extraction times is given in Table 10. The TICs obtained are shown in Figure 24 and Figure 25. An extraction time of 5 minutes was chosen to finalise the development of HS sampling due to the larger number of compounds identified and better chromatography obtained.

Table 10: Compounds extracted from 2.0 g bloodmeal for various lengths of time using the PDMS/DVB fibre.

Compound	1 minute	5 minutes	20 minutes
butanoic acid			X
hexanal			
heptanal		X[b]	X[b]
pentanoic acid		X	
4-methyl pentanoic acid		X	
hexanoic acid			
6-methyl heptanone	X[a]	X[a]	
benzaldehyde			X[c]
phenol		X[b]	X[c]
2-pentyl furan	X[c]	X[c]	X[b]
acetophenone		X[a]	X[a]
4-methyl phenol	X	X[a]	X
2-nonanone	X	X	X
nonanal	X	X	X
2-decanone	X	X	
decanal		X[b]	X[a]
indole	X[a]	X	X
indole derivative	X[b]	X[b]	X[b]
Number of compounds identified	8	14	12
Chromatography	Good	Reasonable	Poor

Note: **a**= detected with NTIS <50 % probability, **b**= detected with NTIS <30 % probability, **c**= detected with NTIS <10 % probability.

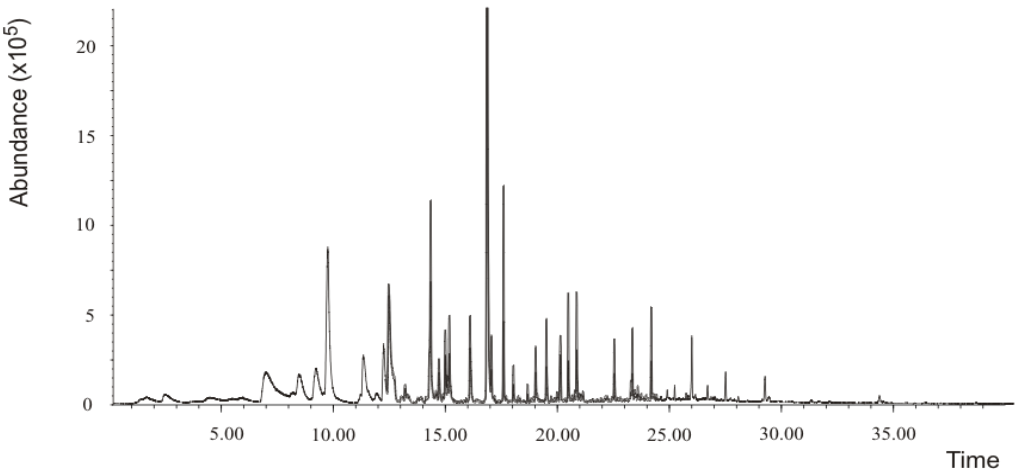


Figure 24: TIC of 1 minute extraction carried out at 75 ^{o}C on headspace of 2.0 g bloodmeal using PDMS/DVB fibre.

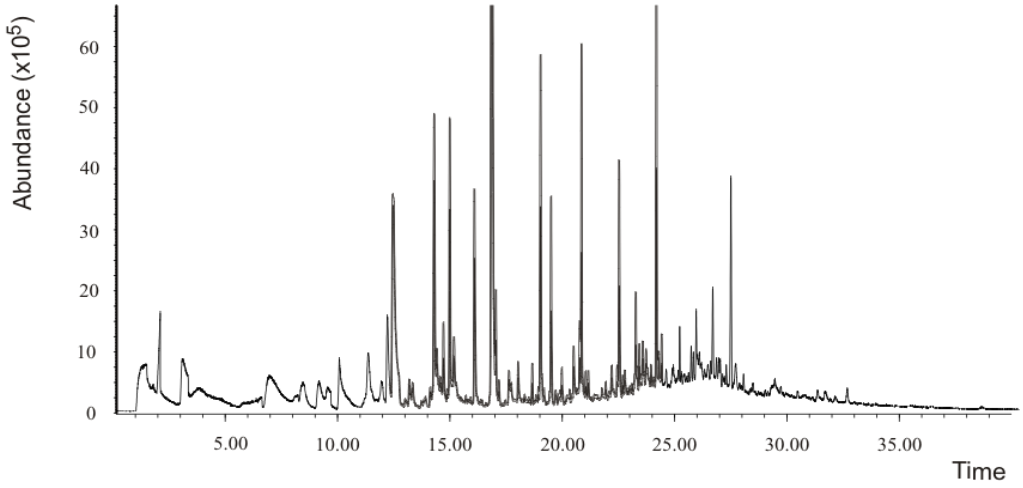


Figure 25: TIC of 20 minute extraction carried out at 75 ^{o}C on headspace of 2.0 g bloodmeal using PDMS/DVB fibre.

An extraction time of 1 minute yielded the smallest quantity of compounds. No organic acids were detected in the first 10 minutes of the GC run, and very few other peaks of interest were observed and was dismissed from further investigation. Whereas using an extraction time of 20 minutes yielded a larger number of peaks, however, the chromatogram obtained was highly saturated and poorly resolved making identification of other compounds present difficult. In addition the extraction time was too long for routine analysis, and was dismissed from further investigation.

For the purpose of this investigation an extraction time of 5 minutes was used in order for extraction to be time efficient and ensure the extract was representative of the sample.

Optimising GC conditions

A summary of the compounds identified from the TIC using two temperature programs is given in Table 11. The TIC obtained for 5 ^{o}C/minute temperature program is shown in Figure 26.

Table 11: Compounds extracted from 2.0 g bloodmeal using the PDMS/DVB fibre and different temperature programs for separation of the extract.

Compound	5 ^{o}C/minute	8 ^{o}C/minute
propanoic acid	X	
heptanal		X[b]
pentanoic acid		X
4-methyl pentanoic acid		X
hexanoic acid		
6-methyl heptanone	X	X[a]
benzaldehyde		
phenol	X	X[b]
2-pentyl furan	X[c]	X[c]
acetophenone		X[a]
4-methyl phenol	X[a]	X[a]
2-nonanone	X	X
nonanal	X[a]	X
2-decanone	X	X
decanal		X[b]
indole	X[b]	X
indole derivative	X[b]	X[b]
Number of compounds identified	10	14
Chromatography	Poor	Reasonable

Note: **a**= detected with NTIS <50 % probability, **b**= detected with NTIS <30 % probability, **c**= detected with NTIS <10 % probability.

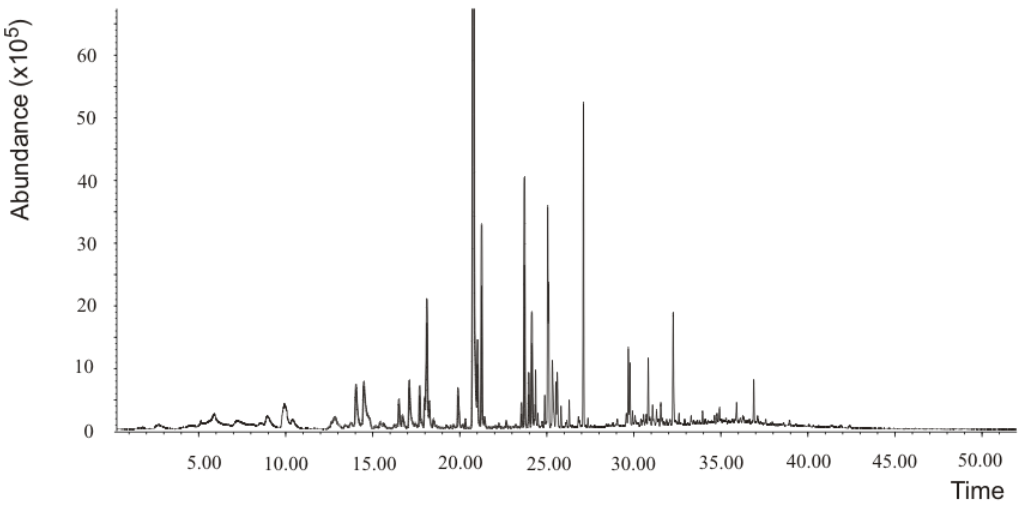


Figure 26: TIC of 5 minute extraction carried out at 75 ^{o}C on headspace of 2.0 g bloodmeal using PDMS/DVB fibre. GC analysis carried out using 5 ^{o}C/minute ramp from 40 ^{o}C.

Using a temperature program of 5 ^{o}C/minute yielded ten compounds, seven of which could be identified tentatively with >30 % probability (Table 11). The chromatogram obtained was not well resolved, possibly caused by slow diffusion of the analytes through the column due to the lower temperature gradient, making identification of other compounds present in the sample difficult. In addition, the analysis took longer to complete and was dismissed from further investigation.

A temperature program of 8 ^{o}C/minute yielded thirteen compounds which were identified tentatively with >30 % probability and the chromatogram obtained was reasonably well resolved (Figure 19). A heating rate of 8 ^{o}C/minute was chosen for compound separation in the GC/MS as it gave better peak separation and was more time efficient.

3.1.3 Summary

Appropriate conditions for HS-SPME-GC/MS are required to identify a maximum number of compounds with good chromatographic resolution. The following conditions were found to satisfy this requirement: Powdered samples were used for analysis with a sample mass of 2.0 g The samples were heated to 75 ^{o}C for one hour to ensure thermal equilibrium. The 65 μm PDMS/DVB fibre was used for extraction of the volatile compounds from the sample headspace. For sampling the fibre was inserted into the vial at 1.0 on the vernier gauge on the SPME holder. An extraction time of 5 minutes was used for the concentration of the volatile compounds from the headspace onto the fibre.

A glass wide bore inlet liner of 1 mm I.D. was used in the GC injection port. Desorption of the analytes was accomplished by thermal desorption in the back inlet of a 6890 Agilent GC (Agilent Technologies, Little Falls, DE, USA) with the SPME fibre set to 3.8 on the vernier gauge on the holder.

The injection was carried out at 250 ^{o}C for 2 minutes via pulsed splitless injection (15 psi) for 5 minutes. The GC run was commenced immediately after the fibre was inserted to ensure all analytes enter the column simultaneously. The fibre was removed from the injection port after desorption to prevent bleeding of any remaining analyte, causing shadow peaks.

GC were done using a capillary 30 m×0.25 mm I.D., 0.25 μm d_f ZB-5 column (Agilent Technologies). The oven was held at 40 ^{o}C for 5 minutes and then ramped at 8 ^{o}C/min to 200 ^{o}C and held for 15 minutes. Helium was used as carrier gas. The Agilent 5973 mass spectrometric detector was operated in the scan mode (*m*/*z* 40–300), MS Quad operated at 150 ^{o}C and MS source at 230 ^{o}C.

3.2 Odour Profiling

The purpose of this work was to assess the odour profile of bloodmeal used and bloodmeal treated using various chemicals. The impact of treatment on bloodmeal and PNTP odour profiles were assessed qualitatively, using HS-SPME-GC/MS and also supplementing these results with sensory panel analysis. The treatment methods used to remove odour from bloodmeal included aqueous extraction, physical absorption, acid/alkaline treatment and oxidation. Materials used in the various treatment methods are listed in Table 12.

Materials

Table 12: Materials used for odour profiling and odour removal from bloodmeal and PNTP.

Material	Grade	Supplier	Comments
Bloodmeal	Agricultural	Taranaki Byproducts	ρ = 1300 kg/m^3, sieved to 710 μm
Bovine red blood cells	Food	Taranaki Byproducts	
Sodium dodecyl sulphate	Technical	Sigma-Aldrich	
Sodium sulfite	Analytical	BDH Lab Supplies	
Urea	Agricultural	Agrinutrients-Ballance	
37 % Hydrochloric acid	Technical	Asia Pacific Specialty Chemicals Ltd	
Sodium hydroxide	Analytical	Ajax Finechem	Pellets
6 % Hydrogen peroxide	Technical	Ajax Finechem	
5 % Peracetic acid	Technical	Solvay	Commercially sold as Oxystrong®
Sodium chlorite	Technical	Ajax Finechem	
Natural zeolite	Technical	Blue Pacific Minerals Ltd	Sieved to 710 μm
Activated charcoal	Technical	Ajax Laboratory Chemicals	Sieved to 710 μm
Distilled water			

Equipment

Standard laboratory equipment was employed for the treatment of bloodmeal under acidic, alkaline and oxidising conditions and also for the preparation of PNTP with added adsorbent material. For aqueous extraction using steam or humid air, a small-scale fluidised bed styled reactor was assembled (Figure 27).

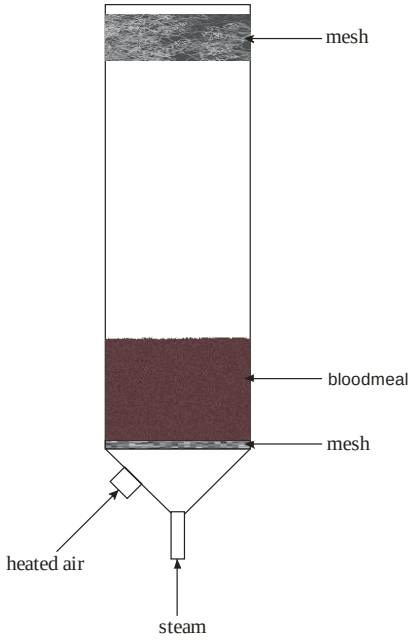


Figure 27: Fluidised bed apparatus for extraction of malodour from bloodmeal. For humid air treatment the heated air was passed through condensed water at the steam inlet (closed).

Analysis

Odour was qualitatively assessed by smelling the treated samples and compared to bloodmeal. HS-SPME and GC/MS analysis were performed according to the method outlined in Section 3.1.3. The methodology followed for sensory panel analysis is outlined in Appendix 3.

3.2.1 Degradation Pathways

During the handling of blood to produce bloodmeal, various chemical changes may occur and were discussed in section 2.1.

To determine the source of VOCs in bloodmeal, bloodmeal and PNTP were analysed and compared with the VOCs identified from freshly dried, thermally degraded and biologically degraded red blood cells as well as PNTP prepared from dry red blood cells (Table 13).

Table 13: Sample preparation for preliminary investigation of bloodmeal and PNTP odour profiles.

Sample	Preparation
Bloodmeal (BM)	-
PNTP	As per patent, Appendix 1.
Red blood cells (RBC)	Fresh red blood cells.
Dry red blood cells (DRBC)	500 mL fresh red blood cells oven dried in open air container at 100 ^{o}C for 24 hours.
PNTP prepared from red blood cells (RBCPNTP)	Oven dried fresh red blood cells were prepared and used instead of bloodmeal according to the NTP process (Appendix 1).
Thermally degraded red blood cells (RBC120^{o}C)	2.0 g fresh red blood cells were placed inside sealed 10 mL headspace vial and heated to 120 ^{o}C for 1 hour.
Biologically degraded red blood cells (PRBC)	50 mL of fresh red blood cells were placed inside air-free sealed container without light for one week at room temperature.

3.2.2 Odour removal

In order to remove malodours from bloodmeal, treatment methods, as discussed earlier in Section 2.3, were employed according to the methods outlined in Table 14.

Table 14: Treatment method employed for removal of from bloodmeal and PNTP.

Treatment Type	Chemical	Method
Aqueous Extraction	Steam	A fluidised bed was created for treating 100 g bloodmeal by allowing a flow of heated compressed air (45-65 ^{o}C) through the column. The temperature of the fluidised bed was controlled to not exceed 85 ^{o}C. Every 10 minutes steam (158 ^{o}C) was passed through for 20-30 seconds, allowing the mixture to cool before the next steam injection. After one hour the bloodmeal was washed from the column with water, decanted and oven dried at 80 ^{o}C for 24 hours.
	Humid air	A fluidised bed was created for treating 100 g bloodmeal by allowing a flow of humidified compressed air (45-65 ^{o}C) through the column for three hours. The bloodmeal was washed from the column with water, decanted and oven dried at 80 ^{o}C for 24 hours.

Treatment Type	Chemical	Volume	Bloodmeal (g)	Reaction time	Method
Acid	1.0 molL^{-1} HCl	100 mL	50 g	1 hour	Mixtures were stirred vigourously for 1 minute every 10 minutes. After reaction, mixtures were diluted with 400 mL water, decanted, filtered and washed with 200 mL water. The bloodmeal was oven dried at 80 ^{o}C for 24 hours.
Alkaline	0.5 molL^{-1} NaOH				
Oxidation	1.0 molL^{-1} H_2O_2	100 mL	50 g	1 hour	Mixture placed on stirrer. After reaction, solution was decanted, filtered and washed with 200 mL water. The bloodmeal was oven dried at 80 ^{o}C for 24 hours.
	1.5 wt% Peracetic acid	150 g	50 g	2-5 minutes	Mixtures were constantly stirred, filtered and washed with 150 mL water. The bloodmeal was freeze-dried overnight.
	5 wt% Peracetic acid	150 g	50 g	2-5 minutes	
	5 wt% $NaClO_2$				

Treatment Type	Chemical	Method
Physical Adsorption	Natural zeolite Activated carbon	PNTP was prepared as in Appendix 1, after which 5, 10 and 25 wt% of adsorbent material (relative to mass bloodmeal) was added and mixed until it appeared homogenously dispersed.

4 Results and Discussion

4.1 Degradation Pathways

In this section results from experiments relating to the origin of Novatein Thermoplastic Protein's (NTP) odour is presented. Tests were carried out using bloodmeal that was prepared according to the patented formulation (Appendix 1) and was not extruded prior to testing. Extrusion was excluded to eliminate the effect of post processing on bloodmeal and to allow comparison with red blood cells and dried red blood cells that cannot be extruded. Pathways that could lead to formation of malodour were identified earlier, of which thermal degradation, lipid oxidation and putrefaction are most important.

The odour profile of bloodmeal was compared to:

- pre-extruded NTP (PNTP) and PNTP prepared from dried RBC in order to identify compounds that form as a result of the protein denaturing process (RBCNTP)

- red blood cells, dried RBC and RBC exposed to high temperature (RBC120) to identify compounds that form as a result of thermal degradation and lipid oxidation

- putrefied RBC to identify microbial breakdown products (PRBC)

A total of twenty volatile compounds were identified from the various bloodmeal compositions tested, and are summarised in Table 15. Tentative identification was based on the mass spectra matching those found in the spectral library with a minimum of 45 % certainty. The results indicate there are several reaction mechanisms leading to odorous compound formation. From the table it is clear that dimethyl disulfide, hexanal and 2-pentyl furan are present in bloodmeal and all but the putrefied specimens tested. It can therefore be concluded that the denaturing process does not lead to the formation of new compounds. This phenomenon has been further explored in subsequent sections.

Table 15: Volatile compounds identified from blood products using HS-SPME-GC/MS.

Compound	BM	PNTP	RBC	Dry RBC	(RBC)NTP	RBC120°C	PRBC
Compounds found in most samples							
dimethyl disulfide	X	X	X	X	X	X	
hexanal	X	X	X	X	X	X	X
2-pentyl furan	X	X	X	X	X	X	
Compound formed as a result of thermal degradation							
2-heptanone	X	X		X	X		
6-methyl-2-heptanone	X	X		X	X		
2-nonanone	X	X		X	X	X	
nonanal	X	X		X	X	X	
2-decanone	X	X		X	X	X	
Compounds formed as a result of bacterial and/or thermal degradation							
3-methyl butanoic acid	X	X		X	X	X	X
dimethyl trisulfide						X	X
dimethyl tetrasulfide						X	
Compounds formed as a result of lipid auto-oxidation							
heptanal	X	X					
benzaldehyde	X	X					
Compounds from the action of putrefactive bacteria							
2-methyl butanoic acid							X
phenol	X	X					X
4-methyl phenol	X	X					X
indole	X	X					X
methyl indole	X	X					X
Unclassified compounds							
1-octen-3-ol	X	X					
tributylamine				X	X		

X= compound present. Bloodmeal (**BM**), pre-extruded NTP (**PNTP**), red blood cells (**RBC**), dried red blood cells (**Dry RBC**), red blood cells dervived PNTP ((**RBC)NTP**), putrid red blood cells (**PRBC**) and red blood cells thermally degraded at 120°C (**RBC120°C**).

Three types of degradation reactions were indentified that lead to formation of odorous VOCs, and are mostly preventable during the production of bloodmeal. These were auto-oxidation of lipids during storage, thermal degradation during drying and bacterial putrefaction.

In order to explore the effect of thermal degradation, the odour profile of fresh, dried and thermally degraded red blood cells were compared to that of bloodmeal. Outlined in red, compounds such as 2-heptanone, 6-methyl-2-heptanone, nonanal, 2-nonanone and 2-decanone are only found in dried blood product samples and are probably a result of lipid oxidation or thermal degradation at high temperatures.

Only dimethyl tri- and tetra- sulfide were identified in thermally degraded red blood cells (RBC120°C) that were not also found in bloodmeal (shown in grey). This suggests that severe thermal degradation of blood proteins does not occur during the manufacture of bloodmeal.

It appears that heptanal and benzaldehyde are caused by auto-oxidative degradation of lipids during storage, as they are only present in bloodmeal and NTP (shown in blue). However, hexanal, another product of lipid auto-oxidation, is also formed at low temperature (<75^{o}C).

Putrefactive breakdown products (shown in green), such as 2-methyl-butanoic acid, phenol, 4-methyl phenol, indole and methyl indole are found only in bloodmeal, PNTP and putrefied red blood cells. Indole and methyl indole (possibly skatole) were tentatively identified at only 25% probability due to the mass spectra of these compounds closely resembling other indole derivatives.

3-methyl butanoic acid was not observed in unprocessed red blood cells. Although it is expected to be identified in putrefied blood, it is also known to be produced via auto-oxidation of lipids, suggesting that some putrefaction may lead to its formation prior to bloodmeal production or during the drying process.

The absence of 2-pentyl-furan from putrid red blood cells is probably caused by poor chromatographic resolution due to peak overlap occurring with the broadly eluting phenol peak, as the compound is known to occur from the auto-oxidation of unsaturated fatty acids.

Although the SPME fibre is used routinely for amine extraction, no amines were detected using this method. This may indicate that they are present in low quantities or are unable to be detected without derivitisation, using the non-polar GC capillary column.

An unusual observation is that 1-octen-3-ol is only identified in bloodmeal and PNTP (shown in yellow). It was thought to be formed from auto-oxidation of unsaturated fatty acids such as linoleic acid. However, it was not detected in thermally degraded red blood cells. For this reason it could be assumed that 1-

octen-3-ol is formed via an alternative route, possibly by enzymatic oxidation of linoleic acid in the presence of bacteria or through auto-oxidation during long-term storage of bloodmeal. However, it is more likely that is was not observed in other samples due to poor chromatographic resolution the column. After the column was replaced, 1-octen-3-ol was detected in red blood cells as well (Table 16). Also shown in yellow, is tributylamine, whose formation is unknown, and could be a result of contamination.

From the GC/MS results, it is probable that the initial odour of bloodmeal is a result of protein and lipid degradation caused by biological and thermal deterioration of the blood throughout the manufacturing process. This is supported by sensory panel analysis which suggested that dried red blood cells had a slightly lower odour intensity compared to bloodmeal (Figure 28).

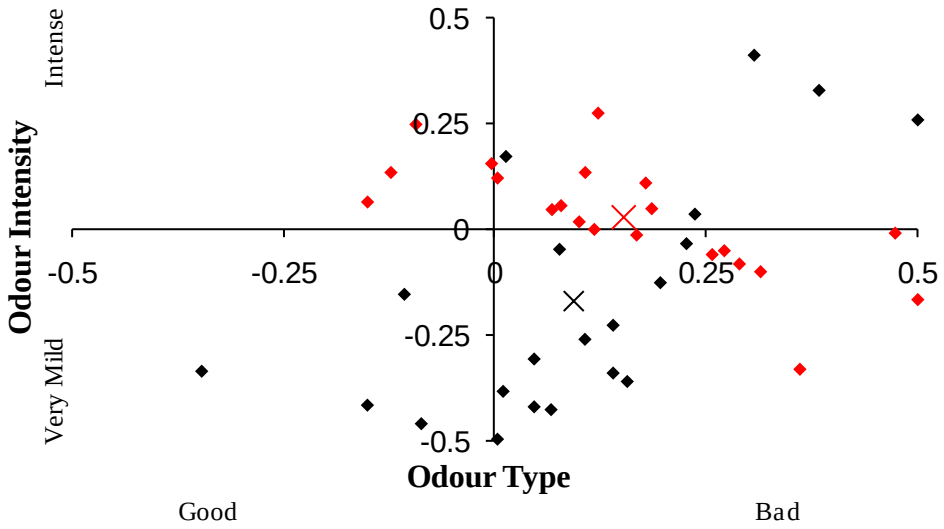


Figure 28: Odour characteristics of bloodmeal (red) and red blood cells (black). "X" represent average results.

4.2 Odour removal

Before the effect of chemical treatment on odour removal can be determined, a detailed understanding of the VOCs arising from bloodmeal must be obtained. At this point the column was replaced with new column of exactly the same specifications. This lead to some of the compounds listed in the previous section not being identified. Analysis were performed in duplicate, but not all compounds were identified in both. In addition, results obtained from HS-SPME-GC/MS were compared with the data provided from the sensory panel analysis.

4.2.1 Bloodmeal

The compounds identified from bloodmeal and PNTP, identified between the duplicate analysis are shown in Table 16. Compounds which have not been identified in red blood cells, are outlined with grey boxes and correspond to compounds formed solely due to bloodmeal manufacturing.

Comparison of the VOCs identified in the bloodmeal with those from the PNTP mixture shows 14 VOCs common to both; heptanal, 6-methyl-2-heptanone, benzaldehyde, 1-octen-3-ol, phenol, 2-pentyl furan, acetophenone, 4-methyl phenol, 2-nonanone, nonanal, 2-decanone, decanal, indole and methyl indole.

Five of these compounds are common to all blood related samples; 6-methyl-2-heptanone, benzaldehyde, 2-nonanone, nonanal and decanal. Despite the difficulties detecting hexanal using the new column, it too can also be assumed to be common to all blood related samples. All of these aldehydes or ketones are known to be caused by the auto-oxidation of free unsaturated fatty acids, as discussed in Section 2.1, and are often found in meat industries (Table 3). Of these compounds only decanal was not identified in red blood cells, probably a result of poor chromatography.

Three alcohols were identified from bloodmeal and red blood cells: 1-octen-3-ol, phenol and 4-methyl phenol. 1-octen-3-ol could be caused by enzyme mediated oxidative cleavage of linoleic acid [107], however, its origin is unsure as it is observed in red blood cells which should have had little enzyme activity while

they were stored at low temperature. Phenol and 4-methyl phenol are found only in bloodmeal and are known to occur in the meat industry, caused by putrefaction of the amino acid tyrosine [108] (Table 3).

Table 16: Compounds extracted from bloodmeal, red blood cells and their corresponding bioplastic mixtures using PDMS/DVB fibre.

Compound	BM	PNTP	RBC	(RBC)PNTP
Alcohols				
1-octen-3-ol	X[c]	X[a]	X[a]	
phenol	X[b]	X[b]		
4-methyl phenol	X	X[a]		
Aldehydes				
hexanal	X	X[b]	X[c]	
heptanal	X[b]	X[a]	X[a]	
benzaldehyde	X[a]	X		X
nonanal	X	X[a]	X	
decanal	X	X[x]		
Ketones				
2-heptanone		X	X[b]	X[a]
6-methyl 2-heptanone	X[a]	X	X	X[b]
acetophenone	X[a]	X		
2-nonanone	X	X	X	X
2-decanone	X	X	X	
Carboxylic acids				
4-methyl-pentanoic acid	X[a]			
Nitrogen Containing Compounds				
indole	X[a]	X[a]	X[a]	X[a]
methyl indole	X[b]	X[b]		
Furans				
2-pentyl furan	X[b]	X	X	X[b]

Note: **a**= detected with NIST <50 % probability, **b**= detected with NIST <30 % probability, **c**= detected with NIST <10 % probability. **x**= eluted peak but was unable to be identified using NIST spectral library. Outlined in grey boxes, compounds that were not identified in the samples, as they result from bloodmeal processing.

There are other ketones found in bloodmeal: 2-heptanone, acetophenone and 2-decanone. Acetophenone, is known to be present in aging meat, and is thought to be formed during thermal treatment via oxidation of fatty acids [109, 110]. Similarly, 2-heptanone and 2-decanone are formed by thermal oxidation of fatty acids, as mentioned in Section 2.1.

Only one carboxylic acid was identified in bloodmeal. 4-methyl pentanoic acid has been found in swine farms (Table 3) and may result from putrefaction of proteins. This is supported by its absence in red blood cells.

Bloodmeal was found to contain putrefaction products of tryptophan, namely indole and methyl indole. These putrefactive compounds are also known to occur in meat industries. Methyl indole was absent in RBC suggesting that less putrefaction occurred.

Finally, 2-pentyl furan, another compound caused by lipid oxidation and known to occur in meat industries (Table 3) was found in both bloodmeal and red blood cells, which confirms it is caused by lipid oxidation prior to drying.

Detection and identification of some compounds was difficult due to the low peak resolution in the TIC at the beginning of the run (Figure 29). In particular, the identification of hexanal (R_t=5.9 minutes) was ambiguous due to its elution occurring in close proximity to or during the elution of a siloxane compound, an artifact of the PDMS/DVB fibre. For this reason hexanal could only be identified if it was present at high concentration and could not be detected in the bloodmeal due to peak overlap.

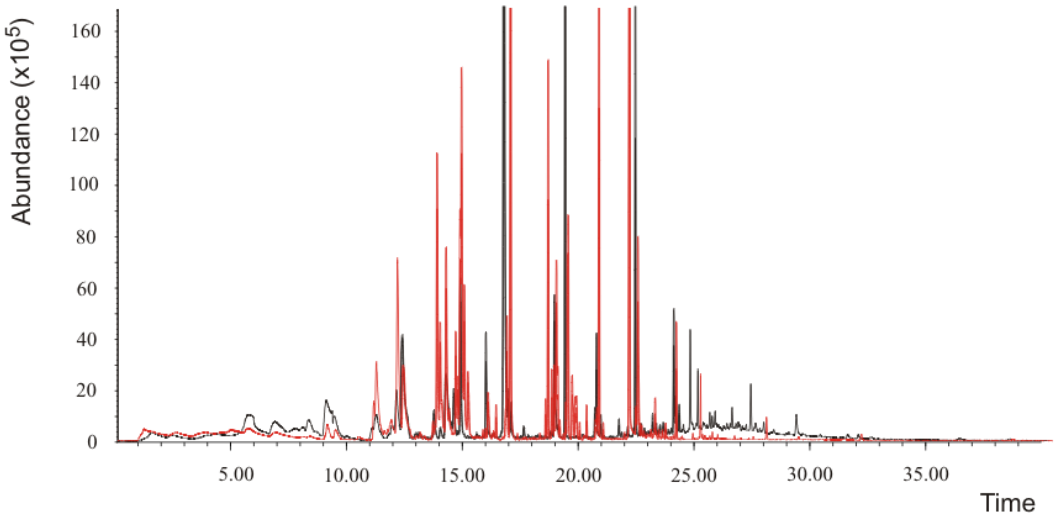


Figure 29: TIC of bloodmeal (black) and PNTP (red).

Similarly, 2-heptanone (R_t=9.1 minutes) was difficult to identify and could not be detected in the bloodmeal samples due to peak overlap. The compound was detected in the PNTP, probably due to the increased concentration giving a more substantial peak area with better resolution.

For comparison, red blood cells were dried and subjected to the same analysis. Due to the age of the red blood cells used for this analysis, indole is detected, and possibly 1-octen-3-ol for the same reason, however indole is typically not found in fresh red blood cells (see previous chapter). These red blood cells were used for sensory panel analysis.

Due to the large variation in the absolute area obtained by integration of the TIC for bloodmeal and PNTP, it is difficult to determine what occurs when the bioplastic mixture is prepared. It appears that upon formation of the PNTP a significant increase in the overall quantity of VOCs occurs, probably due to the denaturing of the proteins and subsequent plasticisation of the random coils causing VOCs trapped in the protein chains to be released.

Although trapped VOCs are likely to escape when the proteins are denatured and plasticised, it does not explain why upon addition of the reducing agent the mixture has such an intense unpleasant odour, as the compounds identified via HS-SPME appear to be the same for both bloodmeal and its corresponding bioplastic mixture. This observation is verified by the sensory panel assessment (Figure 30), and a similar trend is observed with red blood cells (Figure 31).

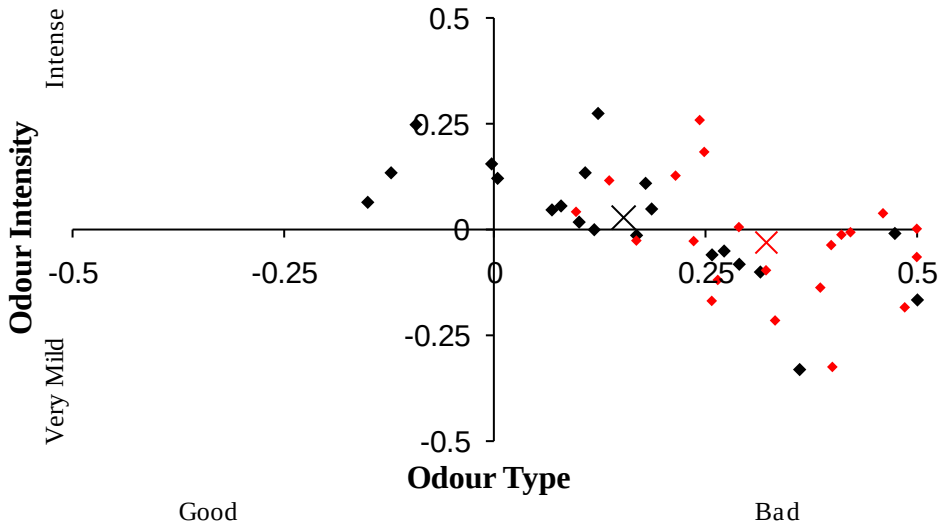


Figure 30: Odour characteristics of bloodmeal (black) and PNTP (red), labels X, represent average result.

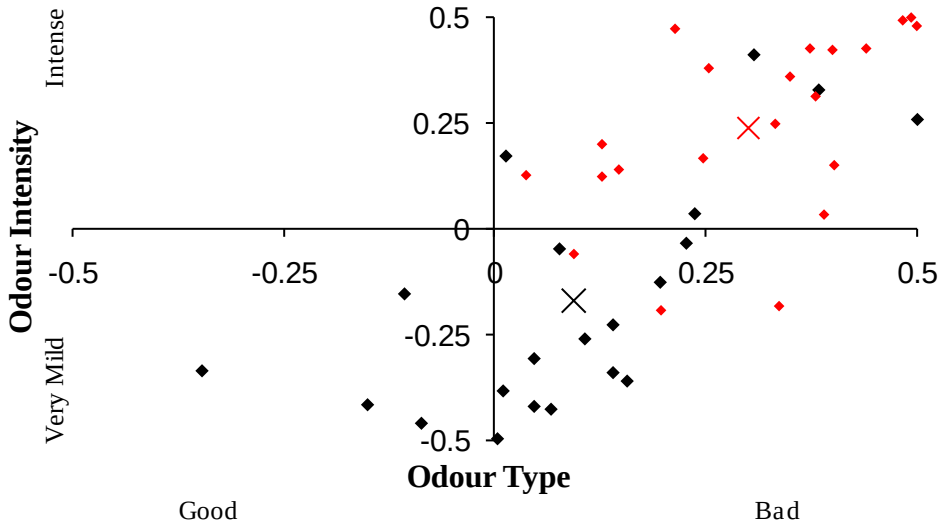


Figure 31: Odour characteristics of red blood cells (black) and red blood cells PNTP (red) labels X, represent average result.

Despite the large variation in the perceived odour type and intensity, there is a definite increase in the odour intensity once the bioplastic mixture is prepared, and the overall odour is significantly more offensive. This trend is also observed for red blood cells.

However, due to the inability to detect mercaptans and amines via GC/MS it is not possible to determine exactly what the culprit odour is responsible for the large change in odour type and intensity when the bioplastic mixture is prepared. It is clear that the addition of the reducing agent plays a major role in the development of the intense unpleasant odour.

4.2.2 Odour Removal

Qualitative Observations from bloodmeal treatments

The influence of various types of chemical treatments on the odour profile of bloodmeal were determined by comparing the HS-SPME-GC/MS results obtained for bloodmeal and PNTP, and comparing them with those obtained after treatment. The results given herein were obtained after the GC column was replaced, as mentioned in the previous chapter.

Observations during treatment and sample preparation are summarised in Table 17.

Table 17: The observed efficiency of each odour treatment on the odour profile and physical appearance of PNTP.

Method	Chemical	Observation
Aqueous extraction	Steam	After treatment there was no change in the odour type but it had milder intensity. Once dried, it smelled the same as bloodmeal with a milder intensity. When the denaturant and reducing agent were added, it was soapy in appearance and did not absorb the water added. The resulting PNTP had an intense fecal and sulphurous odour.
	Humid air	After treatment there was no noticeable change in odour type but it had lower intensity. Once dried, it had no discernable odour, but once rewet the odour was similar to dry untreated blood meal. When the denaturant and reducing agent were added, it did not absorb all of the water added. The resulting mixture had a similar odour to that resulting of PNTP.
Acid/ alkaline treatment	Hydrochloric acid	After treatment the odour type had improved and had a lower intensity. Once dried it had no discernable odour. When the denaturant and reducing agent were added, it appeared similar in consistency to PNTP. The resulting mixture had a smell similar to that resulting from PTNP.
	Sodium hydroxide	After treatment the odour type was the same but had higher intensity. Once dried it had no discernable odour. It appeared crystalline, having a shiney surface and was very consolidated (requiring grinding), indicative of cross-linking. When the denaturant and reducing agent were added , it did not absorb all of the water added and appeared soapy. The PNTP had a much more intense odour than that resulting from untreated PTNP.
Oxidation	Hydrogen peroxide	After treatment there was no noticeable change in odour type but it had higher intensity. Once dried, it had a very mild odour. When the denaturant and reducing agent were added , it formed a mixture similar in appearance to PNTP but with an odour much more intense.
	1.5 % Peracetic acid	After treatment there was an acetic acid odour associated with the slurry. Once dried, it still had an acetic acid odour. When the denaturant and reducing agent were added, it formed a mixture with a similar consistency to PNTP, and smelled similar to burnt hair.
	5 % Peracetic acid	After treatment there was a strong acetic acid odour associated with the slurry. Once dried, it had a strong acetic acid odour. When the denaturant and reducing agent were added, it formed a mixture similar in consistency to PNTP, and smelled similar to burnt hair and vinegar.
	5 % Sodium chlorite	After treatment the slurry had a chlorine odour associated it. Once dried, no odour associated with bloodmeal could be discerned, although there was still a mild chlorine odour. When the denaturant and reducing agent were added, the mixture was similar in appearance to PNTP and had an odour similar to burnt hair.
Physical adsorption	Natural zeolite	PNTP containing 25 wt% had a faint odour associated with it. After one week at room temperature, the odour had returned and smelled identical to PNTP prepared without natural zeolite.
	Activated carbon	PNTO containing 25 wt% had no odour associated with it. After one week at room temperature the odour had partially returned and smelled similar to PNTP prepared without activated carbon, but slightly less intense.

The results of both aqueous extraction methods suggest that this type of treatment to remove odour will not be effective on bloodmeal, as it is clear the odourous compounds are not water miscible, and was therefore dismissed.

4.2.3 Acid/Alkaline treatment

Hydrochloric acid extraction

It is known that hydrochloric acid extracts amines, and in addition it can be successfully used to extract other compounds. The TIC obtained for HCl treated bloodmeal is similar to that of bloodmeal and similarly for their PNTP (Figure 32 and Figure 33).

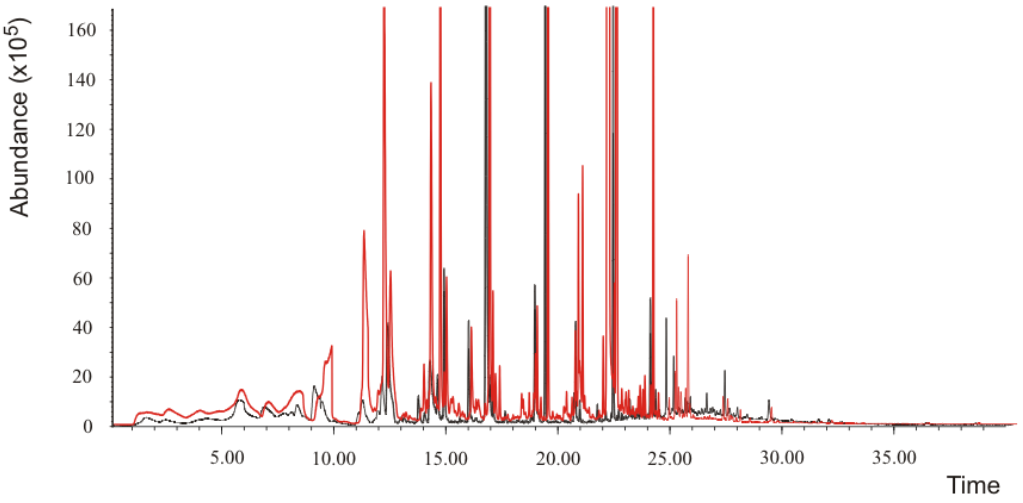


Figure 32: TIC of bloodmeal (black) and hydrochloric acid treated bloodmeal (red).

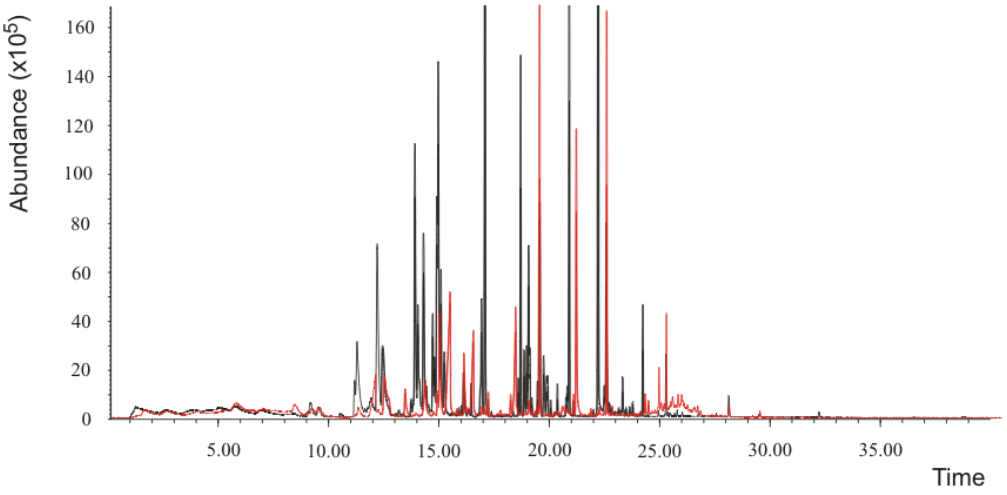


Figure 33: TIC of PNTP (black) and hydrochloric acid treated PNTP (red).

A summary of the identified compounds are given in Table 18. In black boxes are newly identified compounds after treatment, in grey boxes are compounds removed by treatment.

It appears HCl treatment allows the identification of new compounds, typically organic acids and esters: acetic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, pentanedioic acid diethylmethyl ester, 2-ethyl hexanoic acid, nonanoic acid and 2-n-octyl furan. Some of these compounds also appear in the PNTP after HCl treatment, in addition to 3-methyl butanoic acid and octanoic acid. The two esters are only found in one of the HCl treated bloodmeal samples, making identification of its possible origin difficult.

The origin of formation of 2-octyl furan is unknown. It is possible that it may have been formed via acid hydrolysis of any unhydrolysed lipids attached to proteins present in the bloodmeal during the HCl treatment.

Hexanal, heptanal, 1-octen-3-ol, phenol, indole and methyl indole are not identified in any of the HCl treated samples. However, upon formation of PNTP, heptanal can be identified. This is possibily caused by plasticising the proteins, allowing trapped heptanal which were not extracted, to be released.

In addition to the partial removal of compounds formed from putrefaction, the HCl treatment also lead to the removal of the amines, indole and methyl indole.

Despite the formation of many volatile organic acids after HCl treatment, it appears to have improved the overall perceived odour compared with untreated bloodmeal (Figure 34). This indicates that the odourous compounds are partially removed by extraction with HCl, such as the putrefaction compounds in bloodmeal (phenol, indole and methyl indole).

Table 18: Compounds extracted from hydrochloric acid treated bloodmeal and its PNTP using PDMS/DVB fibre.

Compound	BM	PNTP	HCl	(HCl)PNTP
Alcohols				
1-octen-3-ol	X[c]	X[a]		
Phenol	X[b]	X[b]		
4-methyl phenol	X	X[a]	X	
Aldehydes				
Hexanal		X[b]		
Heptanal	X[b]	X[a]		X
Benzaldehyde	X	X	X	X
Nonanal	X	X[a]	X[a]	X
Decanal	X	X[x]	X[b]	X[a]
Ketones				
2-heptanone		X		X[b]
6-methyl 2-heptanone	X[a]	X	X[b]	X
Acetophenone	X[a]	X	X[b]	
2-nonanone	X	X	X	X
2-decanone	X	X	X	
Carboxylic Acids				
acetic acid			X	
butanoic acid			X	
3-methyl butanoic acid				X
pentanoic acid			X	
hexanoic acid			X[a]	X
heptanoic acid			X[b]	X
2-ethyl-hexanoic acid			X	X
octanoic acid				X
nonanoic acid			X	
Nitrogen Containing Compounds				
Indole	X[a]	X[a]		
methyl indole	X[b]	X[b]		
Furans				
2-pentyl furan	X[b]	X	X[b]	X
2-n-octyl furan			X	
Esters				
pentanedoic acid diethylmethyl ester			X	
hexanoic acid diethylmethyl ester			X	

Note: **a**= detected with NIST <50 % probability, **b**= detected with NIST <30 % probability, **c**= detected with NIST <10 % probability. **x**= detected peak but was unable to be identified using NIST spectral library. In black boxes are newly identified compounds after treatment, in grey boxes are compounds removed by treatment.

Upon conversion of HCl treated bloodmeal to the corresponding PNTP, the odour was found to be more offensive than the PNTP (Figure 35). A similar problem is observed when the bloodmeal is treated with sodium hydroxide.

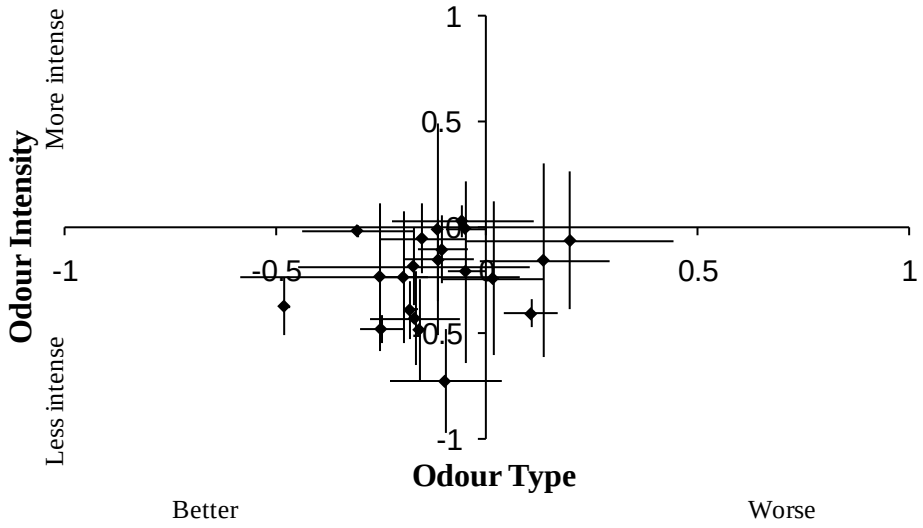


Figure 34: Odour characteristics of HCl treated bloodmeal relative to bloodmeal (0,0).

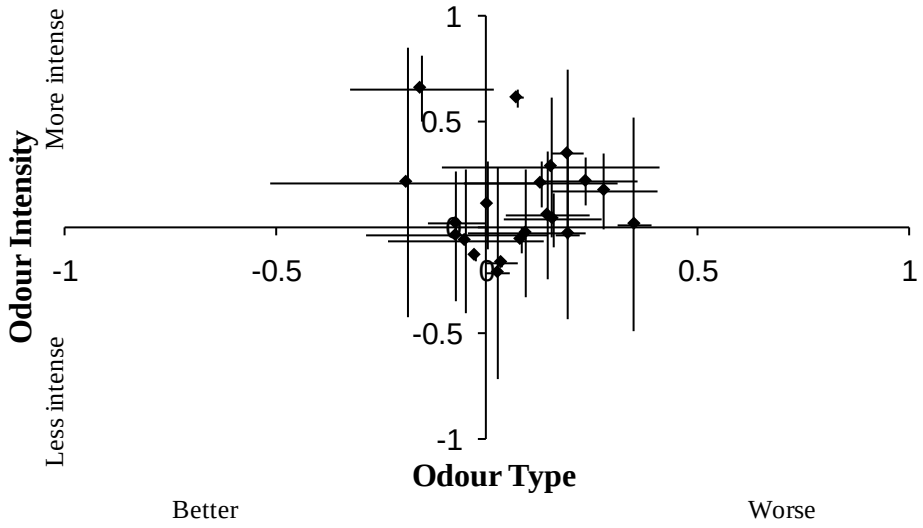


Figure 35: Odour characteristics of HCl treated PNTP relative to PNTP (0,0).

Sodium hydroxide extraction

It is known that sodium hydroxide is used to extract sulfides and mercaptans. Very little changes in the identified VOCs were observed with sodium hydroxide

treatment. The TIC from the treated bloodmeal appears to be in similar proportions to that obtained with no treatment (Figure 36), whereas the initial part of the TIC for NaOH treated PNTP appears much higher in concentration (Figure 37).

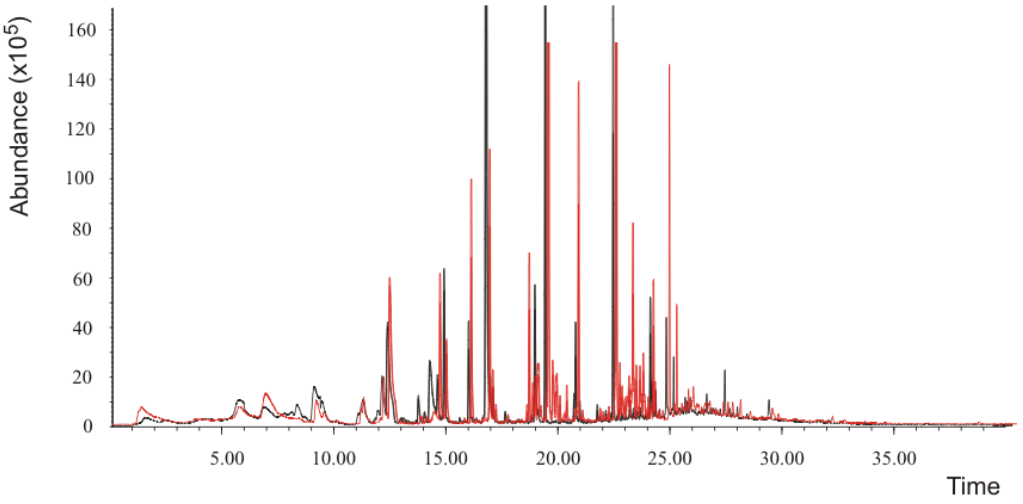


Figure 36: TIC of bloodmeal (black) and sodium hydroxide treated bloodmeal (red).

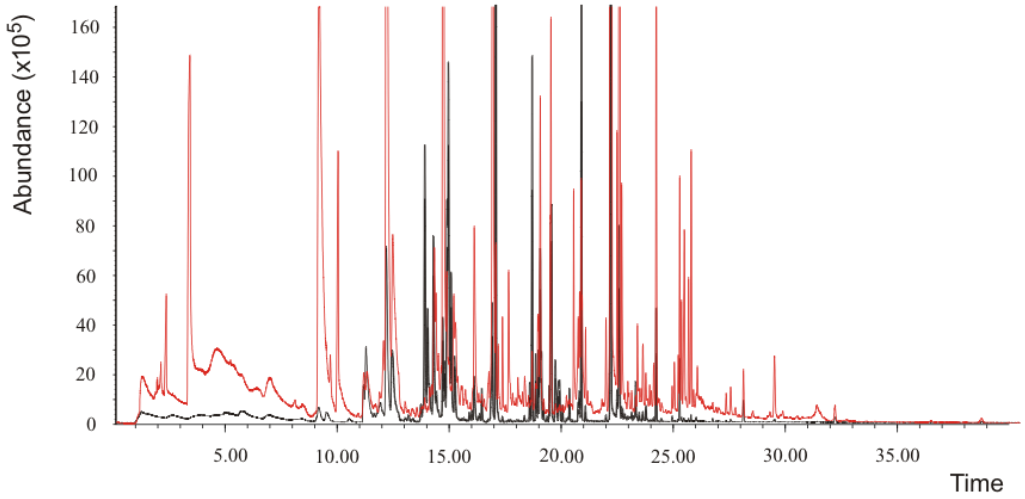


Figure 37: TIC of PNTP (black) and sodium hydroxide treated PNTP (red).

After treatment, hexanal and 2-pentyl furan can no longer be detected, and only two new compounds are detected: 2-octanone and octanal, observed in both the NaOH treated bloodmeal and its corresponding PNTP. A summary of the compounds identified are given in Table 19. The black boxes indicate new

compounds present after treatment and in grey boxes are compounds removed by treatment.

Table 19: Compounds extracted from sodium hydroxide treated bloodmeal and PNTP using PDMS/DVB fibre.

Compound	BM	PNTP	NaOH	(NaOH)PNTP
Alcohols				
1-octen-3-ol	X[c]	X[a]	X[x]	X[c]
Phenol	X[b]	X[b]	X[c]	X
Octanol			X[b]	
4-methyl phenol	X	X[a]	X[a]	X
Aldehydes				
Hexanal		X[b]		
Heptanal	X[b]	X[a]	X	
Benzaldehyde	X	X	X[a]	X
Nonanal	X	X[a]	X	X[x]
Decanal	X	X[x]	X[a]	X[x]
Ketones				
2-heptanone		X	X	X
6-methyl 2-heptanone	X[a]	X	X	X[b]
2-octanone			X	X[b]
Acetophenone	X[a]	X	X[b]	X[a]
2-nonanone	X	X	X	X
2-decanone	X	X	X	X
Nitrogen Containing Compounds				
Indole	X[a]	X[a]	X[a]	X[a]
methyl indole	X[b]	X[b]	X[c]	X
Furans				
2-pentyl furan	X[b]	X		

Note: **a**= detected with NIST <50 % probability, **b**= detected with NIST <30 % probability, **c**= detected with NIST <10 % probability. **x**= detected peak but was unable to be identified using NIST spectral library. In black boxes are newly identified compounds after treatment, in grey boxes are compounds removed by treatment.

Only one pair of replicate NaOH bloodmeal treatments allowed detection of 2-heptanone, heptanal, 6-methyl-2-heptanone, 1-octen-3-ol, phenol and octanal, indicating that the extraction may not be efficient for their total removal from bloodmeal. However, the treatment appears to be effective for removing hexanal and 2-pentyl furan, known odourous compounds in the meat industry (Table 3).

The odour of the NaOH treated bloodmeal and the corresponding PNTP is similar to that of untreated bloodmeal (Figure 38 and Figure 39). Although the results vary for each panel member, it is implied that this is not an effective method for

removing the initial bloodmeal odour or having any major impact on the resulting odour of PNTP.

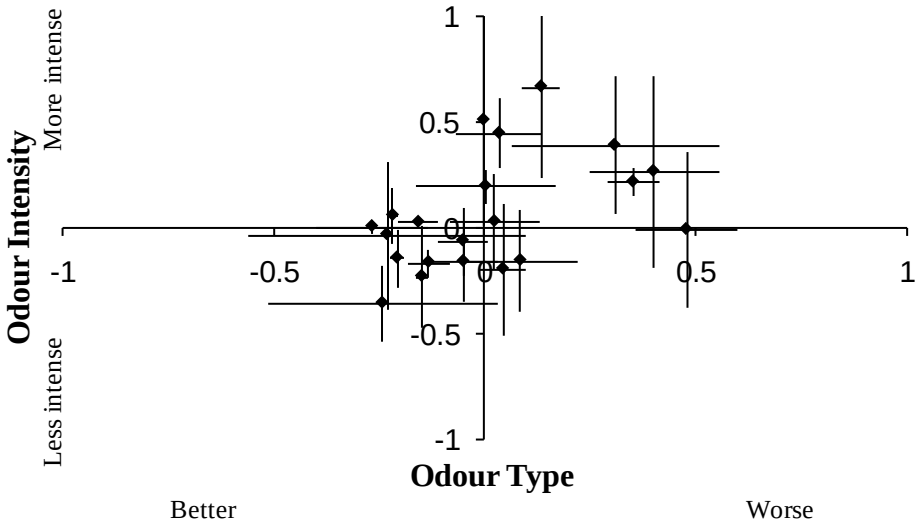


Figure 38: Odour characteristics of NaOH treated bloodmeal relative to bloodmeal (0,0).

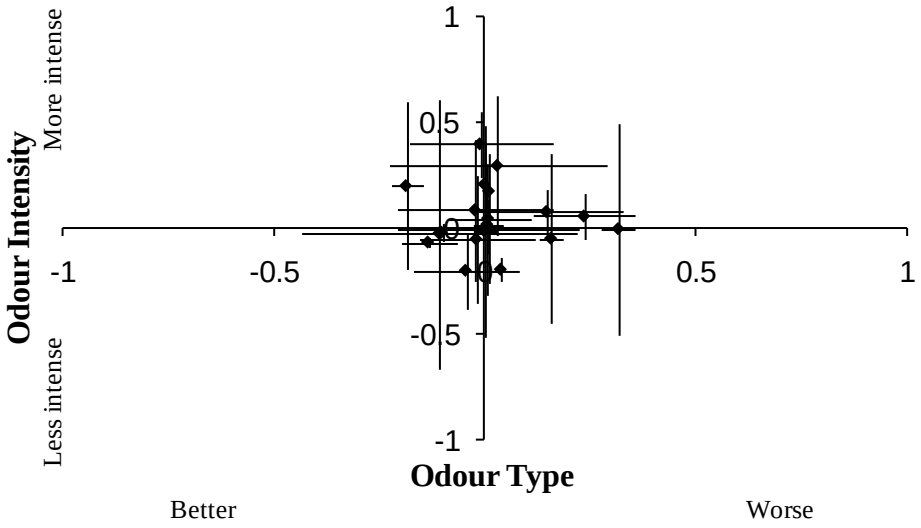


Figure 39: Odour characteristics of NaOH treated PNTP relative to PNTP (0,0).

Summary

It appears that extraction of odourous volatile compounds can be achieved with treatment using hydrochloric acid but not sodium hydroxide. However, treatment

using hydrochloric acid causes the formation of many other volatile compounds which may have adverse effects when health and safety concerns are considered.

The apparent ineffectiveness at removing many of the volatile compounds may still lie in the fact this is an aqueous method. Although, acidic conditions appear to solubilise alcohols and amines, removal of aldehydes and ketones is difficult. Dissolving these compounds becomes more difficult as the chain length increases, which makes the compound more non-polar. Complete removal of these compounds may require a non-polar solvent.

4.2.4 Oxidation of Odourous Compounds

Hydrogen Peroxide

Hydrogen peroxide is a strong oxidizing agent, and is often used to remove odourous compounds. The TIC from the treated bloodmeal appears to be much less concentrated compared to that obtained with no treatment (Figure 40), whereas the initial part of the TIC for H_2O_2 treated PNTP appears much higher in concentration (Figure 41).

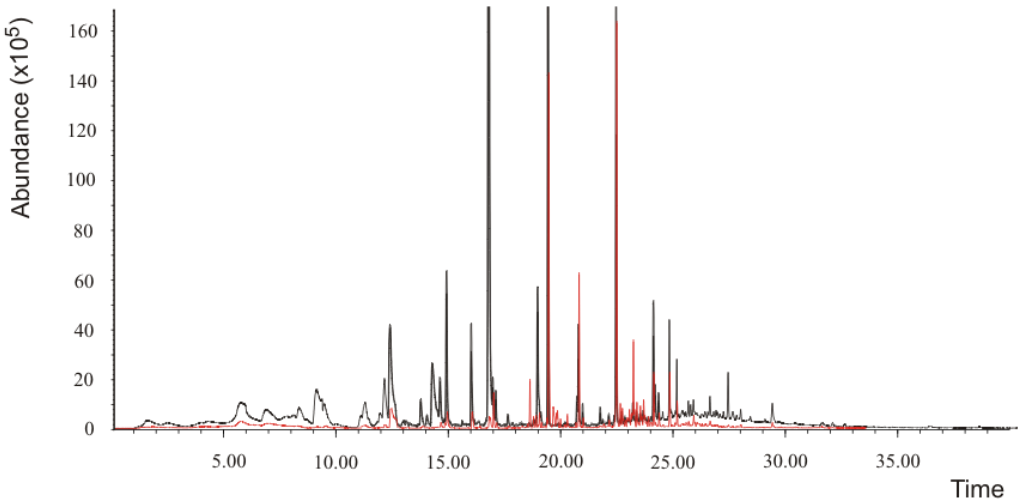


Figure 40: TIC of bloodmeal (black) and hydrogen peroxide treated bloodmeal (red).

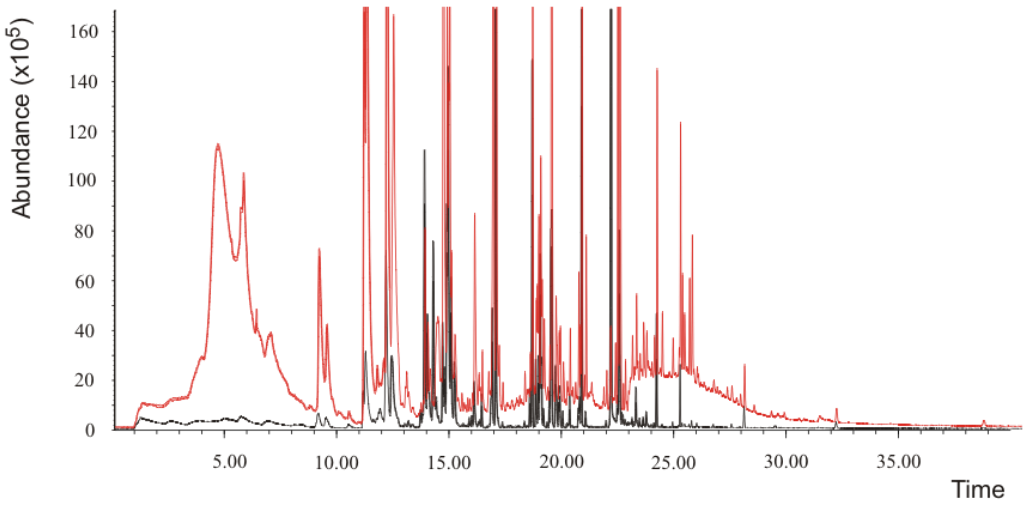


Figure 41: TIC of PNTP (black) and hydrogen peroxide treated PNTP (red).

Due to the high abundance of ions in the first 10 minutes of the GC analysis for H_2O_2 derived PNTP only 2-heptanone could be identified. From the peak area there appears to be almost a ten-fold increase in the quantity of 2-heptanone and heptanal for H_2O_2 treated bloodmeal and its corresponding PNTP compared with those of untreated bloodmeal (Table 20). The black boxes indicate new compounds present after treatment and in grey boxes are compounds removed by treatment.

There were several new compounds that were identified after treatment with H_2O_2. The origin of tetrazole 5-amine is unknown. Pentanedioc (glutaric) acid diethylmethyl ester and hexanoic (caproic) acid diethylmethyl ester may be a result of contamination as they were not detected in any other sample and it is unlikely that dilute hydrogen peroxide would be able to facilitate esterification of the aldehydes without the presence of a catalyst. In addition, 2-n-octyl furan was formed, presumably from oxidation of fatty acids.

After treatment, 1-octen-3-ol, phenol, 4-methyl phenol, indole and methyl indole were no longer observed. However, 4-methyl phenol, indole and methyl indole were present in the resulting PNTP indicating that they were oxidised initially, but significant quantities were also trapped in the protein chains, and when plasticised, were able to become volatile. Hexanal is also removed after H_2O_2 treatment, presumably by oxidation.

Table 20: Compounds extracted from hydrogen peroxide treated bloodmeal and PNTP using PDMS/DVB fibre.

Compound	BM	PNTP	H_2O_2	(H_2O_2)PNTP
Alcohols				
1-octen-3-ol	X[c]	X[a]		
Phenol	X[b]	X[b]		
4-methyl phenol	X	X[a]		X
Aldehydes				
Hexanal		X[b]		
Heptanal	X[b]	X[a]		X
Benzaldehyde	X	X	X	X
Nonanal	X	X[a]	X	X
Decanal	X	X[x]	X	X[b]
Ketones				
2-heptanone		X	X	X
6-methyl 2-heptanone	X[a]	X	X	X
Acetophenone	X[a]	X	X[c]	X
2-nonanone	X	X	X	X
2-decanone	X	X	X	X
Nitrogen Containing Compounds				
tetrazole-5-amine				X[a]
Indole	X[a]	X		X[a]
methyl indole	X[b]	X[b]		X[b]
Furans				
2-pentyl furan	X[b]	X	X[c]	X
2-n-octyl furan				X
Esters				
pentanedioic acid diethylmethyl ester			X	
hexanoic acid diethylmethyl ester			X	

Note: **a**= detected with NIST <50 % probability, **b**= detected with NIST <30 % probability, **c**= detected with NIST <10 % probability. **x**= detected peak but was unable to be identified using NIST spectral library. In black boxes are newly identified compounds after treatment, in grey boxes are compounds removed by treatment. Compounds which may be a result of contamination are indicated by boxes with dashed lines.

The odour of the treated bloodmeal was found to be much less offensive than untreated bloodmeal (Figure 42). However no major changes in odour characteristics were observed for the PNTO prepared from H_2O_2 treated bloodmeal, although there may be a slight improvement to the overall intensity relative to that of the PNTP (Figure 43).

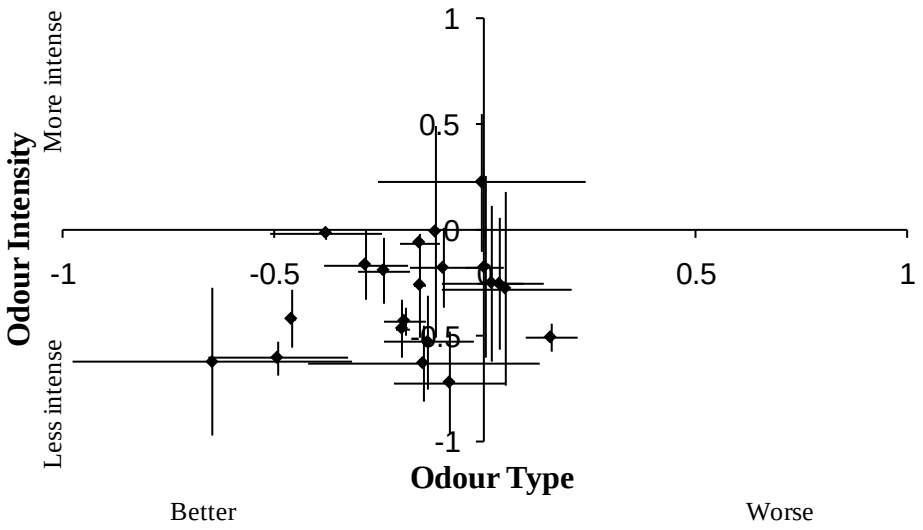


Figure 42: Odour characteristics of H_2O_2 treated bloodmeal relative to bloodmeal (0,0).

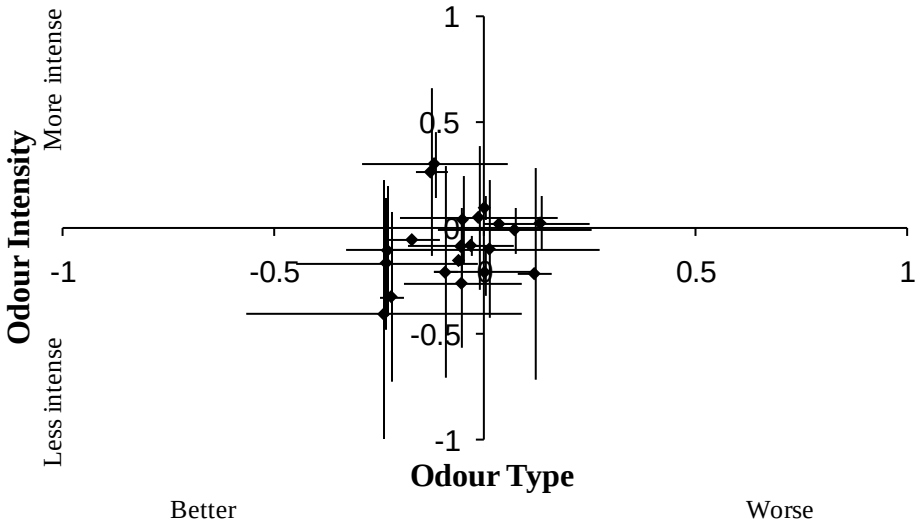


Figure 43: Odour characteristics of H_2O_2 treated PNTP relative to PNTP (0,0).

These results suggest that the treatment of bloodmeal with H_2O_2 is effective for oxidising the compounds responsible for bloodmeal's odour, but has no affect on the resulting PNTP.

1.5 % and 5 % Peracetic acid

Peracetic acid is another commonly used oxidizing agent. Aside from the large acetic acid peak (R_t=2.0 minutes), benzaldehyde peak (R_t=11.2 minutes) and nonanal peak (R_t=14.8 minutes) the TIC from the treated bloodmeal appears to be much less concentrated compared with that obtained with no treatment (Figure 44). In contrast the bioplastic mixture shows a severe increase in concentration during the initial part of the TIC compared with untreated bloodmeal (Figure 45).

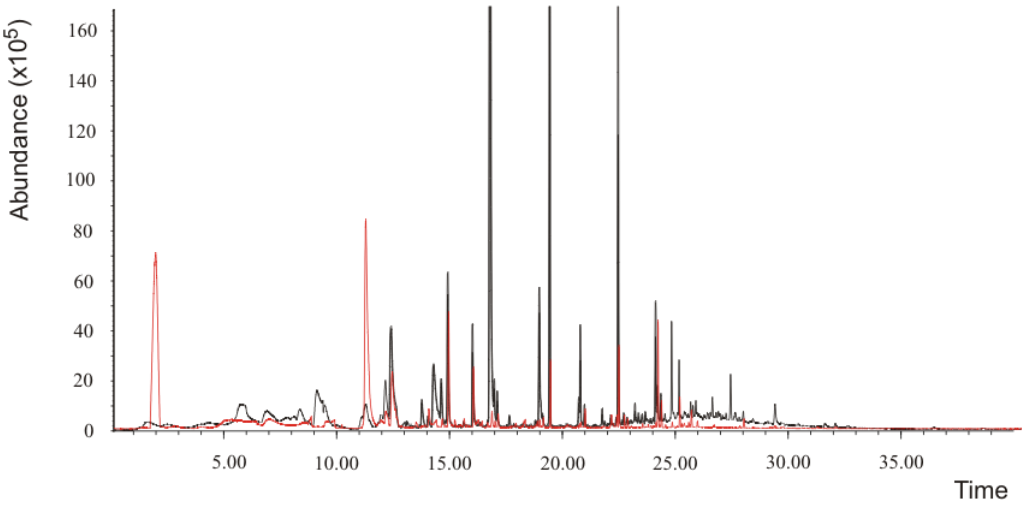


Figure 44: TIC of bloodmeal (black) and 1.5 % peracetic acid treated bloodmeal (red).

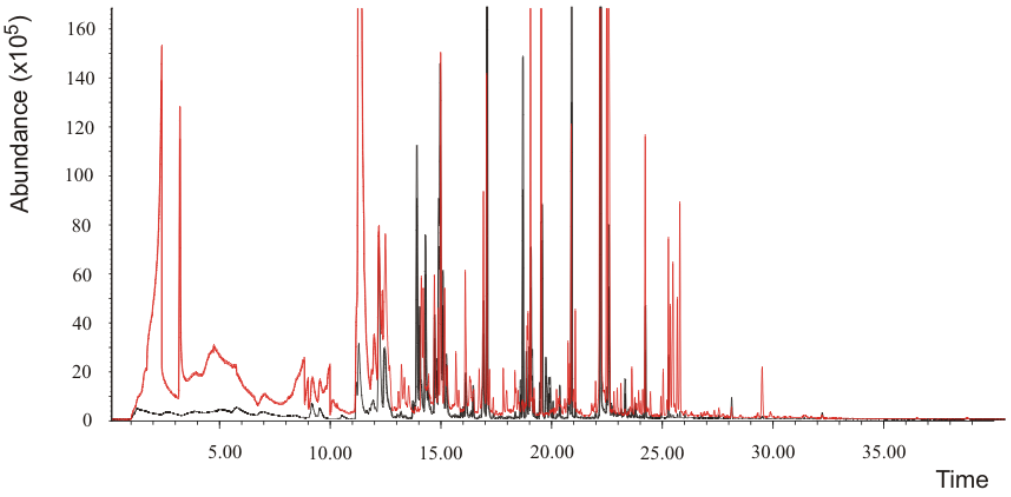


Figure 45: TIC of PNTP (black) and 1.5 % peracetic acid treated PNTP (red).

Treatment with 5 % peracetic acid yielded a similar response, shown in Figure 46 and Figure 47. There was a large quanity of acetic acid detected, but due to its

high saturation, was removed during TIC background subtraction. The large benzaldehyde and nonanal peaks were still observed. Overall, the TIC from the treated bloodmeal appears to be much less concentrated compared with that obtained with no treatment, which severely increased in concentration during the initial part of the TIC of the PNTP.

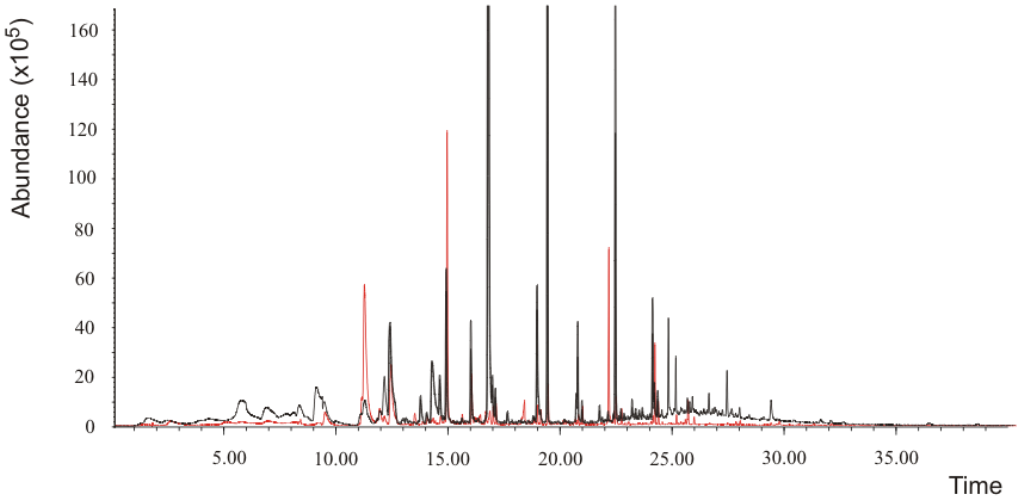


Figure 46: TIC of bloodmeal (black) and 5 % peracetic acid treated bloodmeal (red).

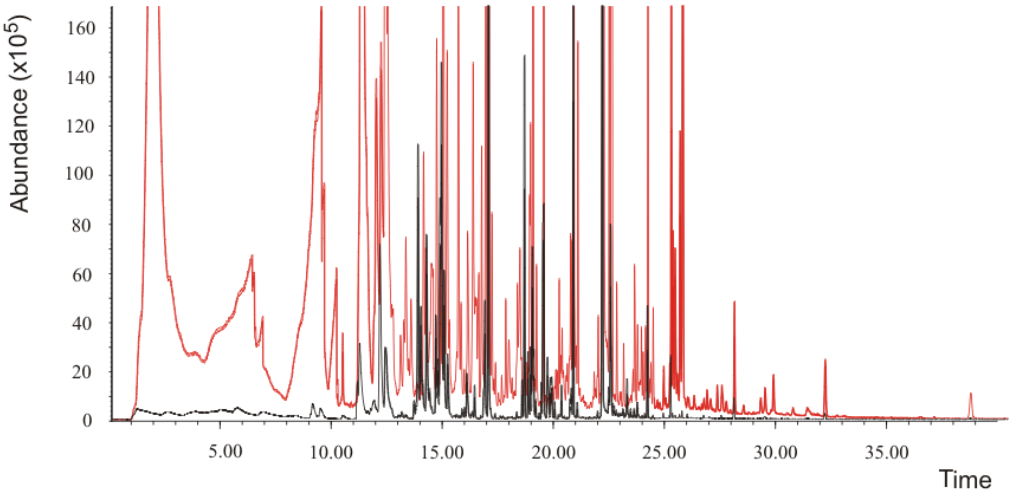


Figure 47: TIC of PNTP (black) and 5 % peracetic acid treated PNTP (red).

Numerous other volatile compounds were identified after treatment with peracetic acid: 3-methyl- and 2-methyl butanoic acid, pentanoic acid, 2-nitro phenol, octanoic acid and nonanoic acid were also detected (Table 21). The black boxes

indicate new compounds present after treatment and in grey boxes are compounds removed by treatment.

Table 21: Compounds extracted from 1.5 and 5 % peracetic acid treated bloodmeal and PNTP using PDMS/DVB fibre.

Compound	BM	PNTP	1.5%PA	(1.5%PA)PNTP	5%PA	(5%PA)PNTP
Alcohols						
1-octen-3-ol	X[c]	X[a]				
Phenol	X[b]	X[b]				
4-methyl phenol	X	X				
Aldehydes						
Hexanal		X[b]				
Heptanal	X[b]	X[a]	X		X	
Benzaldehyde	X	X	X	X	X	X
Octanal					X	
Nonanal	X	X[a]	X	X[a]	X	X
Decanal	X	X[x]	X	X[b]	X	X[a]
Ketones						
2-heptanone		X	X[a]	X[b]		
6-methyl 2-heptanone	X[a]	X	X	X	X	X
Acetophenone	X[a]	X	X	X	X[a]	X
2-nonanone	X	X	X	X[a]	X	X
2-decanone	X	X	X	X		X
Carboxylic Acids						
Acetic acid			X	X	X	X
3-methyl butanoic acid			X	X	X	X
2-methyl butanoic acid			X	X	X	X
Pentanoic acid			X	X	X	X
Heptanoic acid				X[a]		
Octanoic acid			X[b]			
Nonanoic acid			X		X	
Nitrogen Containing Compounds						
2-nitro phenol			X		X	
Indole	X[a]	X[a]				
Methyl indole	X[b]	X[b]				
Furans						
2-pentyl furan	X[b]	X	X[a]	X[c]	X	X[x]

Note: **a**= detected with NIST <50 % probability, **b**= detected with NIST <30 % probability, **c**= detected with NIST <10 % probability. **x**= detected peak but was unable to be identified using NIST spectral library. In black boxes are newly identified compounds after treatment, in grey boxes are compounds removed by treatment.

After treatment with 1.5 % peracetic acid, the resulting PNTP gave rise to heptanoic acid, but 2-methyl propanamide, 2-nitro phenol, octanoic acid and nonanoic acid were not observed. This is probably a result of poor resolution in the TIC caused by the high concentration of the other compounds eluting around the same retention times.

The 1.5 % and 5 % peracetic acid treatments yielded significantly larger quantities of benzaldehyde, 2-nonanone and nonanal. This is indicative of oxidation of benzyl alcohol, 2- and 1-nonanol, compounds which have not been detected using this method of analysis.

In addition, oxidation using 5 % peracetic acid appears to be more effective at oxidising the volatile compounds. Heptanal is found after treatments with both 1.5 and 5 % peracetic acid, shown by the grey areas in Table 21. This compound along with octanal, are intermediate products in the oxidation of heptanol and octanol, which have not been identified. Therefore the detection of octanal in 5 % peracetic acid treated bloodmeal suggests is has higher oxidation efficiency that 1.5 %, as expected due to its higher concentration.

It was observed that oxidation using peracetic acid removed 1-octen-3-ol, phenol, 4-methyl phenol, indole and methyl indole. Because it was capable of removing all of the 4-methyl phenol, indole and methyl indole, it is more effective at removing volatile organic compounds than hydrogen peroxide

Overall, the majority of the VOCs present were found to be organic acids, and the other compounds were similar to those from bloodmeal. The presence of a large quantity of acetic acid causes the odour associated with the treated bloodmeal to resemble vinegar, but has a much less offensive odour than the odour characteristic of bloodmeal. Due to the subjectivity of the sensory panel, the results obtained are difficult to interpret due to the large variation of results (Figure 48 and Figure 49), this was also the case for treatment with 5 % peracetic acid (Figure 50 and Figure 51). The only agreement between all panel members being that the odour of treated bloodmeal was more intense.

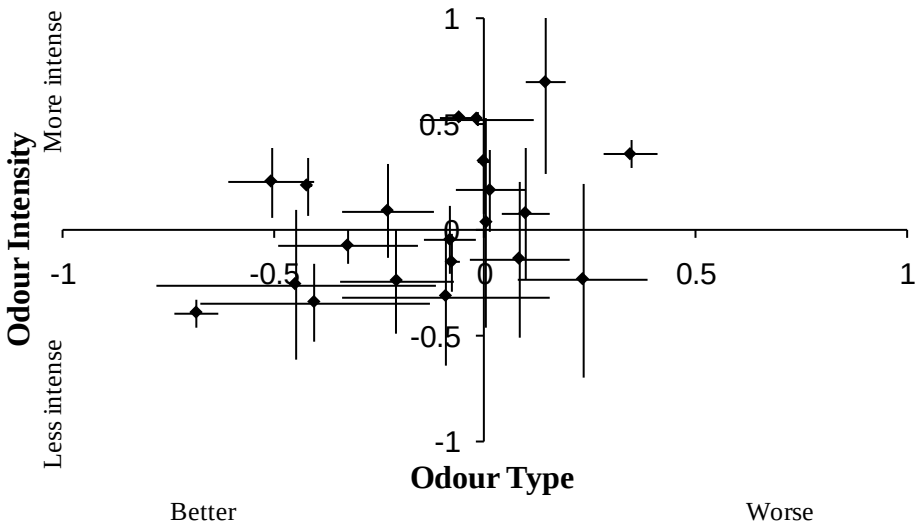


Figure 48: Odour characteristics of 1.5 % peracetic acid treated bloodmeal relative to bloodmeal (0,0).

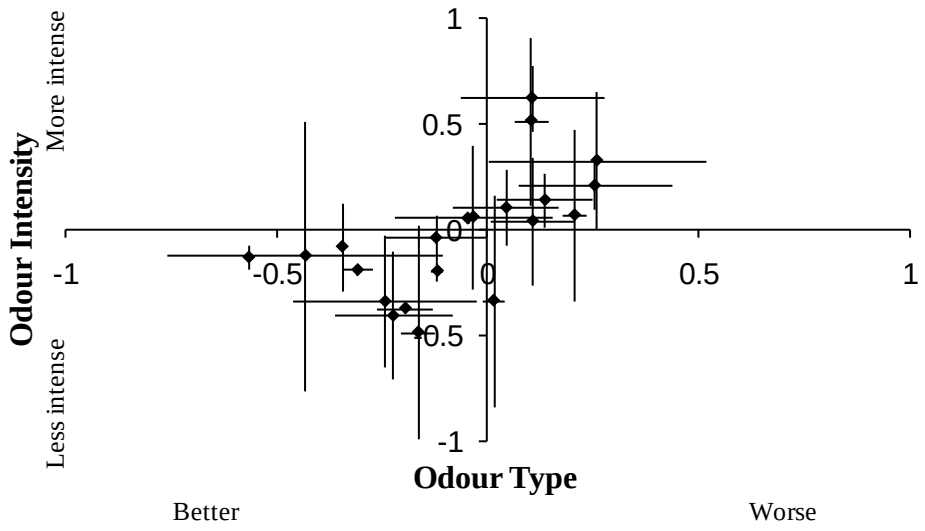


Figure 49: Odour characteristics of 1.5 % peracetic acid treated PNTP relative to PNTP (0,0).

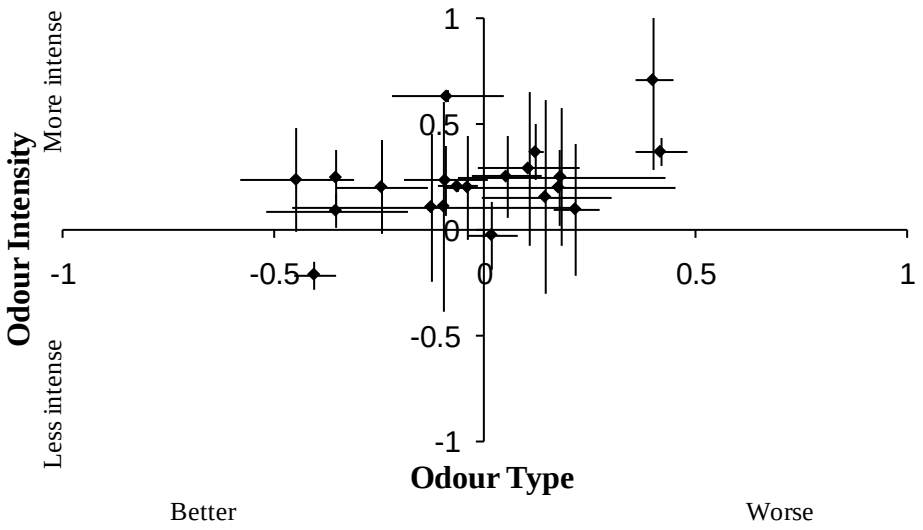


Figure 50: Odour characteristics of 5 % peracetic acid treated bloodmeal relative to bloodmeal (0,0).

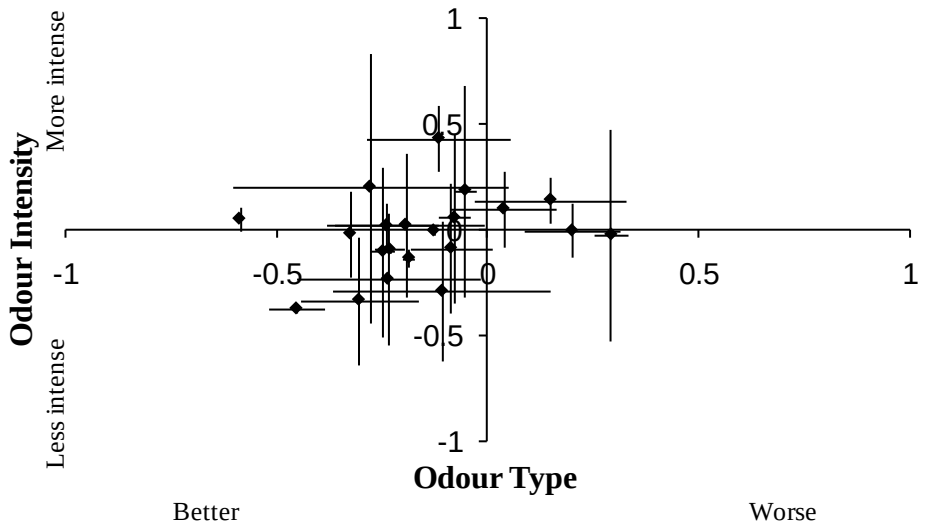


Figure 51: Odour characteristics of 5 % peracetic acid treated PNTP relative to PNTP (0,0).

The formation of many organic acids confirms that peracetic acid is the strongest form of oxidation, and was found to be capable of completely oxidising all compounds in bloodmeal that were a result of putrefaction. The acetic acid by-product significantly impacts the resulting odour, and will need to be removed if this method of odour reduction is to be employed.

5 % Sodium chlorite

Oxidation using sodium chlorite yielded the most information about the impact of volatile compounds on the odour profile. From the TIC of the sodium chlorite treated bloodmeal it was observed that it had a larger quantity of VOCs present relative to untreated bloodmeal, and the same is true for its resulting PNTP (Figure 52 and Figure 53). A summary of the compounds identified are given in Table 22. The black boxes indicate new compounds present after treatment and in grey boxes are compounds removed by treatment.

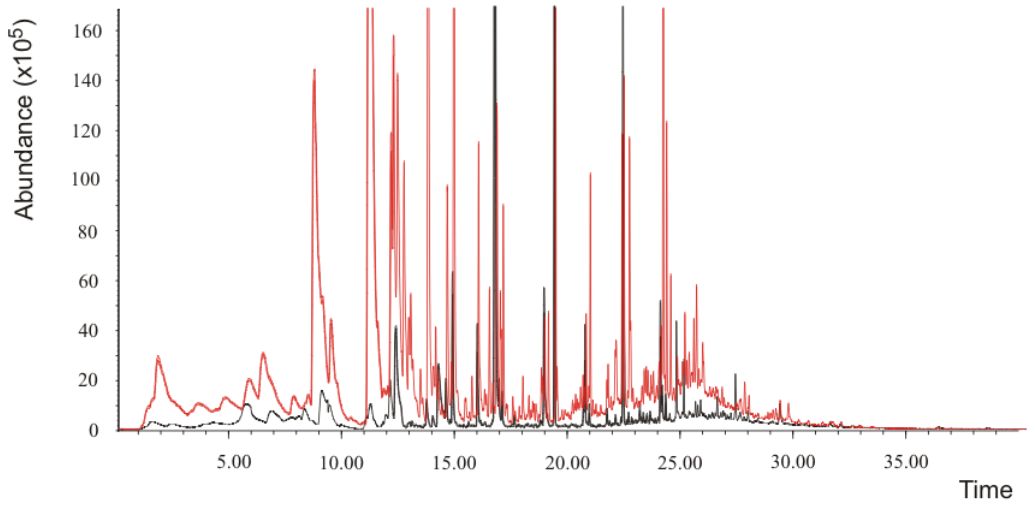


Figure 52: TIC of bloodmeal (black) and sodium chlorite treated bloodmeal (red).

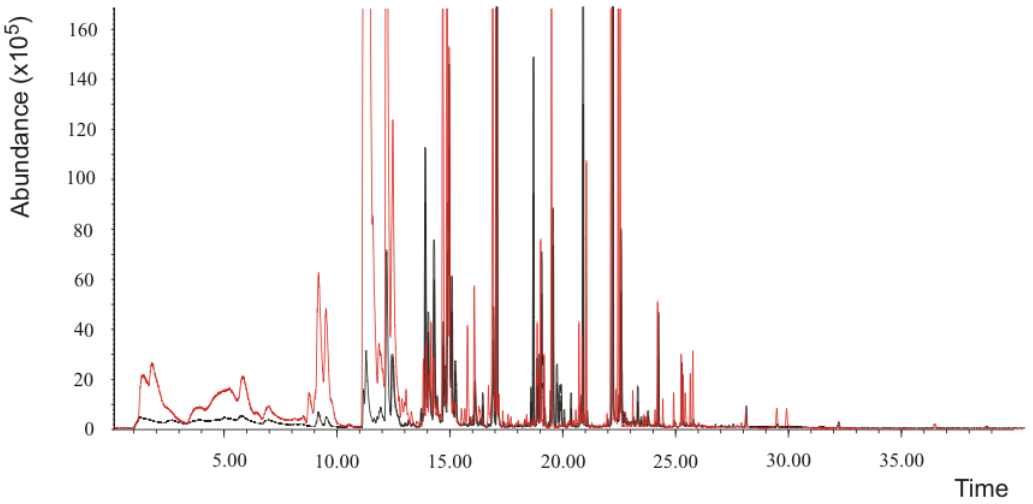


Figure 53: TIC of PNTP (black) and sodium chlorite treated PNTP (red).

Table 22: Compounds extracted from acidified sodium chlorite treated bloodmeal and PNTP using PDMS/DVB fibre.

Compound	BM	PNTP	$NaClO_2$	$(NaClO_2)$PNTP
Alcohols				
1-octen-3-ol	X[c]	X[a]		
Phenol	X[b]	X[b]		
Octanol			X[b]	X[c]
4-methyl phenol	X	X	X[c]	X[a]
Aldehydes				
Hexanal		X[b]	X[c]	X[x]
Heptanal	X[b]	X[a]		X
Benzaldehyde	X	X	X	X
Octanal			X	
Nonanal	X	X[a]	X	X
Decanal	X	X[x]	X	X[a]
Ketones				
2-heptanone		X	X[x]	X[x]
6-methyl 2-heptanone	X[a]	X	X	X
Acetophenone	X[a]	X	X[s]	X[a]
2-nonanone	X	X	X	X
2-decanone	X	X		
Nitrogen Containing Compounds				
Indole	X[a]	X[a]		
methyl indole	X[b]	X[b]		
Furans				
2-pentyl furan	X[b]	X	X	X
Esters				
butyl acetate			X	X[x]
butyl butanoate			X	X[x]
hexyl acetate			X	
4-octen-1-yl acetate			X[a]	
heptan-2-yl acetate			X[a]	
3-methyl, butyl butanoate			X[a]	X[b]
2,3-dimethyl butyl propanoate			X	X
4-hexen-1-yl butanoate			X[a]	

Note: **a**= detected with NIST <50 % probability, **b**= detected with NIST <30 % probability, **c**= detected with NIST <10 % probability. **x**= detected peak but was unable to be identified using NIST spectral library. In black boxes are newly identified compounds after treatment, in grey boxes are compounds removed by treatment.

From the relative areas of the peaks eluted, the most apparent increase in quantity was observed for hexanal (R_t=5.8 minutes), 2-heptanone (R_t=9.1 minutes), heptanal (R_t=9.5 minutes), 6-methyl-2-heptanone (R_t=11.0), benzaldehyde

(R_t=11.2), 2-pentyl furan (R_t=12.1 minutes), 2-nonanone (R_t=14.5 minutes), nonanal (R_t=14.8) and decanal (R_t=17.0 minutes), all products of oxidation.

It can also be observed that there is a variety of esters present such as butyl acetate, hexyl acetate, 4-octen-1-yl acetate, 2-heptanyl acetate, butyl butanoate, 3-methyl butyl butanoate, 2,2-dimethyl butyl propanoate and 4-hexen-1-yl butanoate.

The presence of these esters in both replicates of the treated bloodmeal suggests they are not a result of contamination. The source of the esters is unknown, however, it is postulated that during the treatment of the bloodmeal ester formation may be initiated by the formation of alkyl chlorides from alcohols or via the condensation reaction of carboxylic acids with alcohols present in the bloodmeal. However, no organic acids were identified in bloodmeal or the sodium chlorite treated bloodmeal indicating they may never have been formed, or that if they were formed, were readily converted to other products.

Alkyl chlorides are non-volatile compounds formed from reaction between alcohols and free chloride ions, catalysed by acidic conditions [111]. Free chloride ions are produced from the reduction of chlorite ions [93], and acidic conditions are produced via the dissociation of carboxylic acids formed from the oxidation of aldehydes by chlorite ions, effectively lowering the pH [112].

The pH of the bloodmeal treatment system will not be strongly acidic, because organic acids are only weak acids, so there will be only a small quantity of free hydrogen ions present. However, there may be sufficient quantities to cause catalysis resulting in the formation of alkyl chlorides, which undergo nucleophilic addition and elimination reactions with alcohols to form corresponding esters. The elimination product of these reactions is hydrochloric acid, which could provide further catalysis for the production of alkyl chlorides.

The TIC identified only one putrefaction product after treatment, 4-methyl phenol. This indicates that the other putrefaction products were oxidised and removed from bloodmeal. Although the sodium chlorite treated bloodmeal appears to have higher concentrations of the majority of the VOCs identified compared to

untreated bloodmeal alone, its odour was found to be less offensive (Figure 54). Upon conversion to the corresponding PNTP, no significant difference in odour was observed when compared with that from untreated bloodmeal (Figure 55).

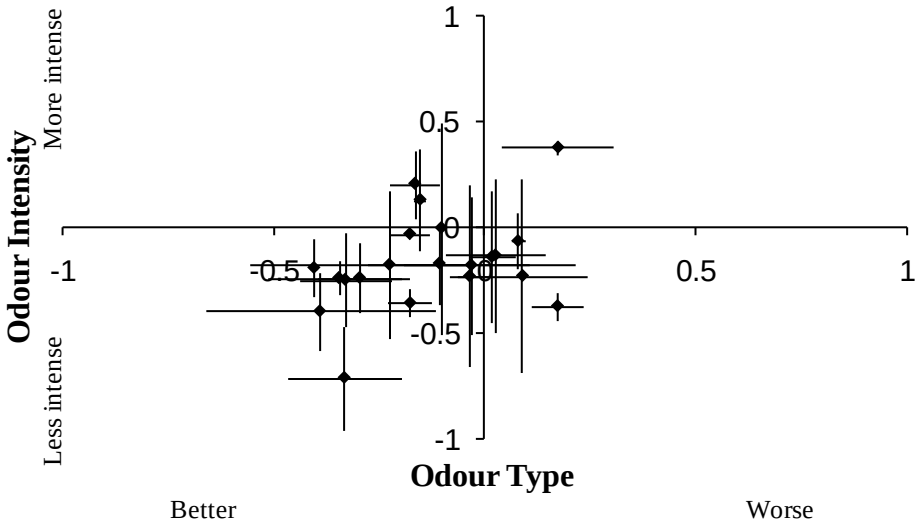


Figure 54: Odour characteristics of sodium chlorite treated bloodmeal relative to bloodmeal (0,0).

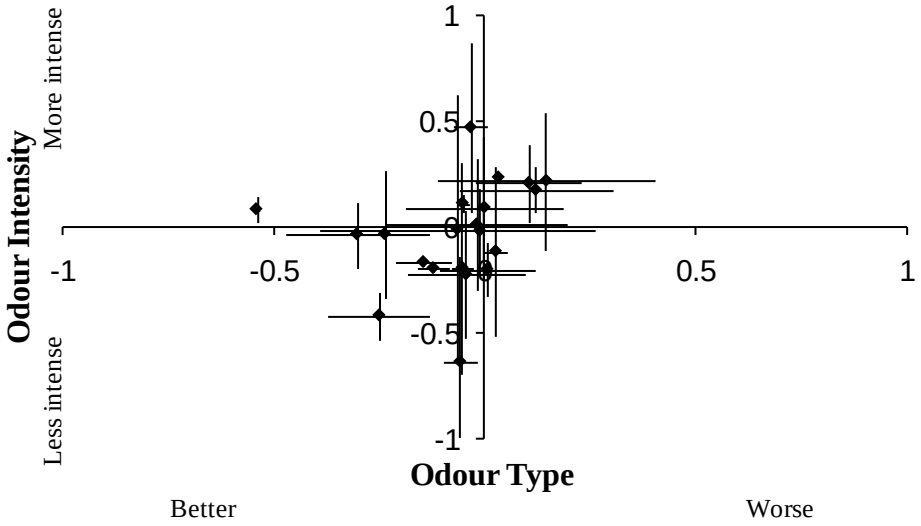


Figure 55: Odour characteristics of sodium chlorite treated PNTP relative to PNTP (0,0).

As expected from literature review, sodium chlorite was the weakest oxidising agent employed for removal of odourous compounds. It was found to cause the formation of significant quantites of aldehydes and even esters, without the production of more heavily oxidised organics such as carboxylic acids. The chlorite oxidation was only capable of removing the putrefactive products indole and methyl indole.

Despite large increases in the quantity of aldehydes and esters in the treated bloodmeal there appears to be an improvement to the overall odour. Because the treated bloodmeal was deemed to be virtually odourless by the sensory panel members, it can be concluded that the aldehydes and esters have no impact on the odour of the treated bloodmeal, and consequently that aldehydes probably have no contribution to the characteristic odour of bloodmeal either.

From this it can be concluded that the major contributors to the characteristic odour of bloodmeal are the compounds produced via putrefaction of the blood proteins when whole blood is stored prior to drying.

Summary

It appears that oxidation using oxidising agents with different standard reduction potentials, leads to differences in the number and types of VOCs removed. In general, alcohols such as 1-octen-3-ol and phenol can be oxidised by all of the oxidising agents employed. However, 4-methyl phenol is only removed when peracetic acid is used, which has the highest standard reduction potential.

Aldehydes such as heptanal, nonanal and decanal not to undergo oxidation reactions unless in the presence of peracetic acid, indicating they require a strong oxidising agent. However, these aldehydes are still found in the PNTP resulting after treatment. The same difficultly with miscibility of aldehydes and ketones is observed in this series of oxidative treatments, as the method still relies on water to extract the oxidised compounds.

Despite a large variety of esters being formed from some oxidation treatments, oxidation appeared to remove the putrefactive compounds, which are the cause the characteristic odour of bloodmeal.

4.2.5 Physical Adsorption of Odourous Compounds

Absorption or adsorption techniques are known to remove odourous compounds. The addition of sorbents such as activated carbon and natural zeolite to PNTP was shown to improve the perceived odour significantly.

Natural Zeolite

The addition of natural zeolite appears to decrease the odour intensity compared to PNTP only when it is present in a quantity of 25 wt%. The apparent perceived odour type improves with addition of greater than 10 wt% (Figure 56, Figure 57 and Figure 58).

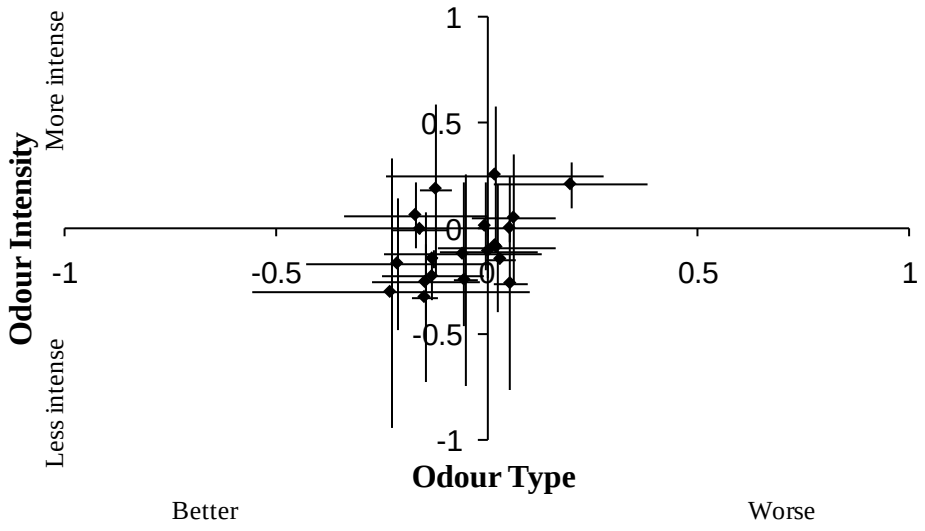


Figure 56: Odour characteristics of PNTP with addition of 10 wt% natural zeolite relative to PNTP (0,0).

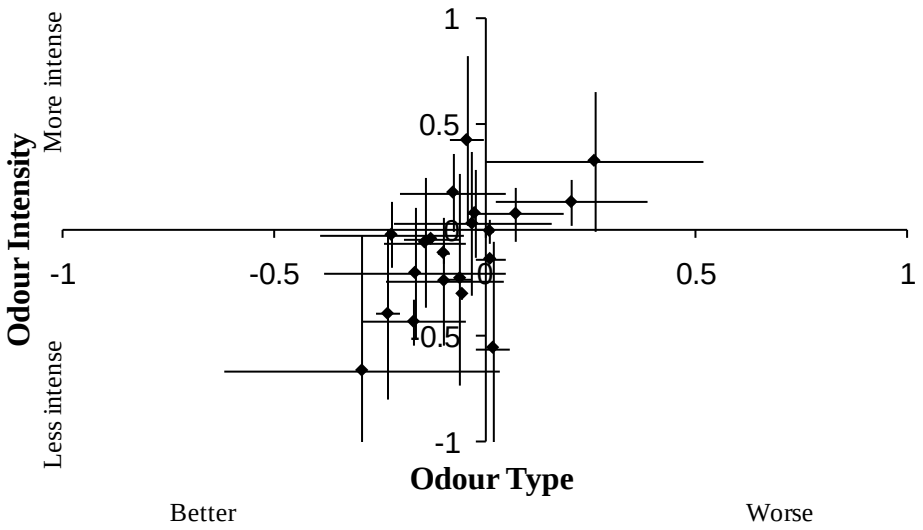


Figure 57: Odour characteristics of PNTP with addition of 15 wt% natural zeolite relative to PNTP (0,0).

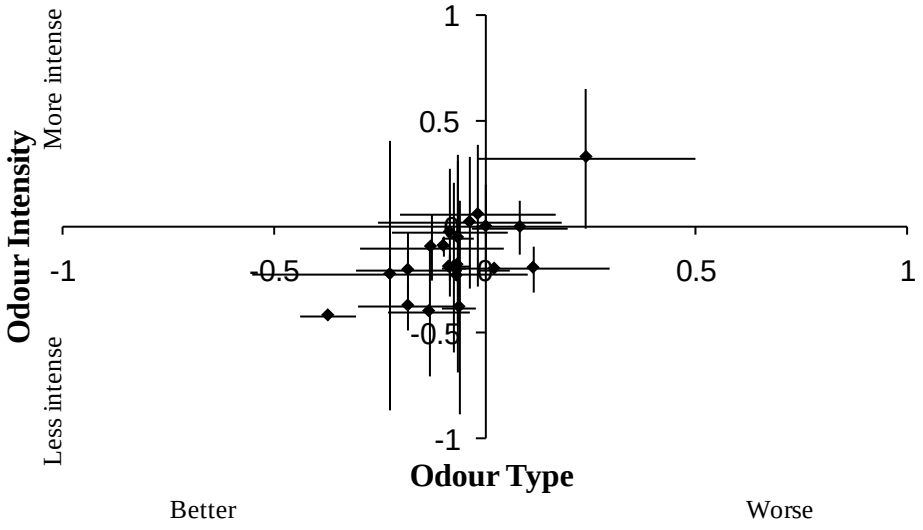


Figure 58: Odour characteristics of PNTP with addition of 25 wt% natural zeolite relative to PNTP (0,0).

In conclusion, it appears natural zeolite is not an effective means of minimising odour associated with the PNTP.

Activated Carbon

The addition of activated carbon appears to dramatically decrease the odour intensity compared to PNTP. This decrease in the odour intensity and improved odour type is observed for quantities of 10 wt% of more (Figure 59, Figure 60 and Figure 61).

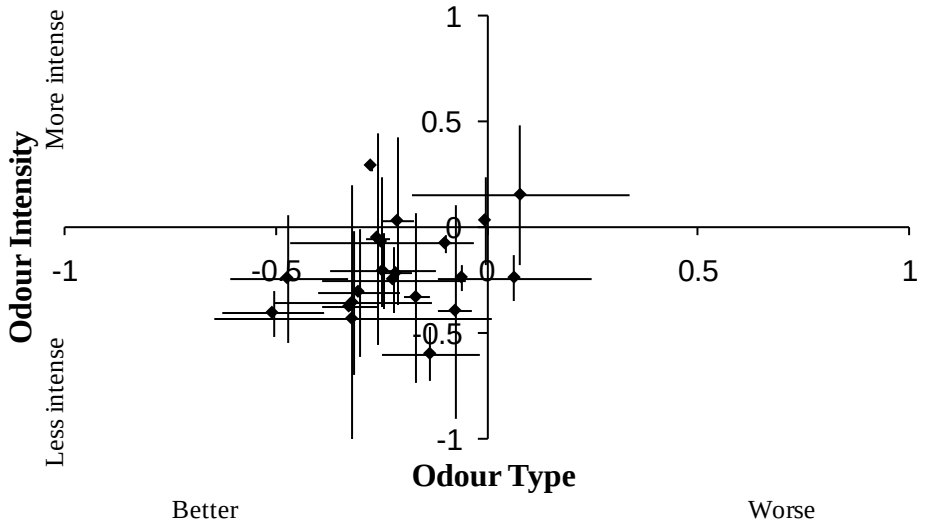


Figure 59: Odour characteristics of PNTP with addition of 10 wt% activated carbon relative to PNTP (0,0).

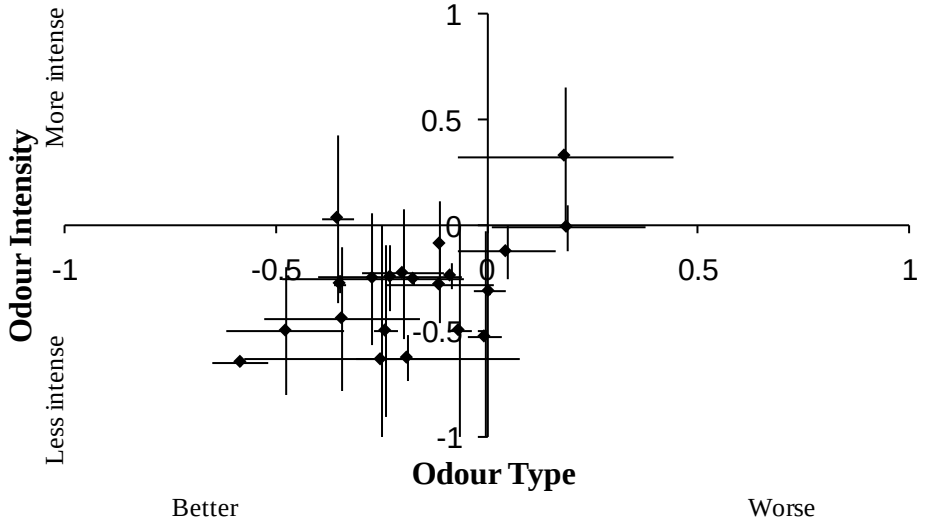


Figure 60: Odour characteristics of PNTP with addition of 15 wt% activated carbon relative to PNTP (0,0).

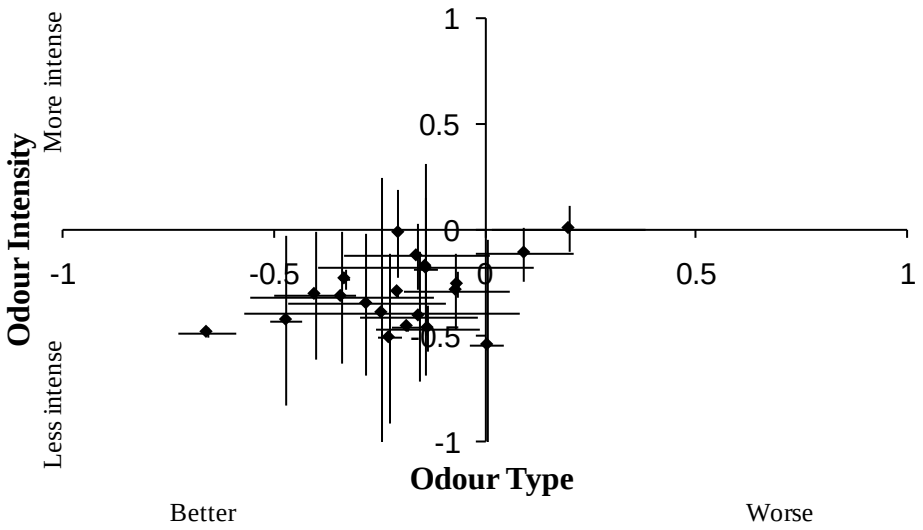


Figure 61: Odour characteristics of PNTP with addition of 25 wt% activated carbon relative to PNTP (0,0).

It can then be concluded that the addition of 10 wt% activated carbon improves the odour quality to a greater extent than that of 25 wt% natural zeolite, and is therefore a plausible means of improving the PNTPs' odour characteristics.

4.2.6 Quick Summary of VOCs identified

Chemical treatments had various effects on the compounds identified in the treated bloodmeal. A summary of all identified compounds is shown in Table 23. It can be observed that alcohols and aldehydes can be minimised or removed from bloodmeal after treatment with HCl or an oxidising agent. In addition, various new compounds were observed, typically carboxylic acids and esters.

All bloodmeal samples regardless of treatment employed were found to contain 6-methyl 2-heptanone, benzaldehyde, acetophenone, 2-nonanone and decanal as shown in red in the table. These compounds are all known to occur from lipid oxidation and may or may not be thermally induced.

Table 23: Compounds identified from bloodmeal and bloodmeal treatments

Compound	BM	DN BM	HCl	DN HCl	NaOH	DN NaOH	H_2O_2	DN H_2O_2	1.5 %PA	DN 1.5 %PA	5 %PA	DN 5 %PA	$H^+/NaClO_2$	DN $H^+/NaClO_2$
Alcohols														
1-octen-3-ol	X	X			X	X								
Phenol	X	X			X	X								
Octanol													X	X
2-nitro phenol									X		X			
4-methyl phenol	X	X	X		X	X		X					X	X
Aldehydes														
Hexanal	X	X											X	X
Heptanal	X	X		X				X	X		X		X	X
Benzaldehyde	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Octanal											X		X	
Nonanal	X	X	X	X	X	X	X	X	X	X	X	X	X	X
decanal	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ketones														
2-heptanone		X		X		X	X	X	X	X			X	X
6-methyl-2-heptanone	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2-octanone					X	X								
Acetophenone	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2-nonanone	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2-decanone	X	X	X	X	X	X		X	X	X	X	X		
Carboxylic acids														
Acetic acid			X						X	X	X	X		
Butanoic acid			X	X										

Compound	BM	DN BM	HCl	DN HCl	NaOH	DN NaOH	H_2O_2	DN H_2O_2	1.5 %PA	DN 1.5 %PA	5 %PA	DN 5 %PA	$H^+/NaClO_2$	DN $H^+/NaClO_2$
Butyl acetate													X	X
3-methyl butanoic acid				X					X	X	X	X		
2-methyl butanoic acid									X	X	X	X		
Pentanoic acid			X						X	X	X	X		
Hexanoic acid			X	X										
Heptanoic acid			X	X						X				
2-ethyl-hexanoic acid			**X**	**X**										
octanoic acid				X					X					
nonanoic acid			X						X		X			
Nitrogen Containing Compounds														
Tetrazole 5-amine							X							
Indole	**X**	**X**			X	X		X						
2-methyl indole	**X**	**X**			X	X		X						
Furans														
2-pentyl furan	**X**	**X**	X	X			X	X	X	X	X	X	X	X
2-n-octyl furan			X					X						
Esters														
Butyl butanoate													X	X
Hexyl acetate													X	
4-octen-1-yl acetate													X	
3-methyl butyl butanoate													X	X
2, 3-dimethyl butyl propanoate													X	X
pentanedioic acid diethylmethyl ester			X											

Compound	BM	DN BM	HCl	DN HCl	NaOH	DN NaOH	H_2O_2	DN H_2O_2	1.5 %PA	DN 1.5 %PA	5 %PA	DN 5 %PA	$H^+/NaClO_2$	DN $H^+/NaClO_2$
4-hex-en-1-yl butanoate													X	X
hexanoic acid diethylmethyl ester							X							

5 Conclusions and Recommendations

It is evident that the initial odour associated with bloodmeal is a direct result of the collection and storage of raw blood prior to drying. All compounds identified as a result of odour profiling have all been identified in meat industries and are known to be caused by deterioration of lipids and proteins. Putrefaction products are known to cause malodour, the identification of 3-methyl butanoic acid, 2-methyl butanoic acid, (Section 4.1), in addition to phenol, 4-methyl phenol, indole and methyl indole (Section 4.2.1) are indicative of putrefaction occurring during the storage of whole blood. Based on the limitations of the HS-SPME-GC/MS analysis of the bloodmeal samples, the possibility of other putrefaction products such as putrescine, cadaverine and methyl mercaptan being present in bloodmeal cannot be discounted.

In addition to the formation of putrefaction products during storage, thermal decomposition of lipids also occurs, as shown in Section 4.1. The results suggest that oxidation of lipids occurs at high temperatures leading to the formation of aldehydes, ketones and furans. Although these compounds are found in bloodmeal, they may in fact have no impact on the overall odour profile of bloodmeal compared with products that result from putrefaction. This is evident from the GC/MS analysis of red blood cells which despite containing the same ketones, aldehydes and furans that were found in bloodmeal (Section 4.2.1), the sensory panel determined that the overall odour of red blood cells was less offensive than that of bloodmeal.

Five compounds were identified which were common to all blood related samples. The formation of 6-methyl-2-heptanone, benzaldehyde, 2-nonanone, nonanal and decanal may occur from the thermal degradation of proteins or lipids or may be caused by their dissociation from the protein chain. Their persistence after oxidative treatment of bloodmeal suggests they are formed thermally, so although they in some cases they may be oxidised, once the sample was re-heated, more were formed. This implies that their presence after chemical treatment may be a result of oven drying or heating the sample for HS-SPME-GC/MS analysis.

After analysis of all treatment methods, it can be concluded that bloodmeal odour can be minimised by removing indole and methyl indole which result from putrefaction, as observed in hydrochloric acid and oxidation treated samples.

From the sensory panel assessment, it was concluded that the addition of the reagents required to process PNTP lead to a major change in the odour quality. It was observed from these results that the odour was found to be much more unpleasant than the bloodmeal it was derived from.

Although HS-SPME-GC/MS analysis yielded results showing that the identified VOCs in bloodmeal increased in quantity in the PNTP due to plasticisation of the proteins, it alone was unable to determine whether these compounds cause the dramatic change in the odour type and intensity.

After oxidative treatment of bloodmeal using sodium chlorite, it was observed that there was a significant increase in the concentration of many aldehydes and ketones, products of oxidation, relative to bloodmeal. Because of this, it would be expected that the odour would be described as more intense. However, what is observed in the sensory panel assessment indicates that the overall odour type and intensity was improved with sodium chlorite treatment. This leads to the obvious conclusion that the putrefactive compounds identified via HS-SPME-GC/MS are the cause of odour in bloodmeal. However, the reason the odour of PNTP is more offensive and intense is still unknown.

Due to the inability to detect amines or mercaptans, it is difficult to discern what does cause this dramatic change in odour quality in PNTP. However, it is evident that it is caused or catalysed by the addition of the sodium sulfite reducing agent.

It can then be concluded that strong oxidation using peracetic acid was effective at completely altering the odour associated with the bloodmeal, resulting in a decoloured product with a vinegar odour. However, no chemical treatment was successful at minimising the odour of PNTP, suggesting its odour is formed via a different mechanism. Although physical adsorption appears to be an effective means of minimising the odour initially. A minimum of 10 wt% activated carbon

appears to be the most effective. However, due to the displacement of lower molecular weight compounds by higher molecular weight compounds over time, this method had limited success.

The major limitation of the investigation of volatile compounds arising from PNTP throughout processing includes the inability to detect amines or mercaptans. To determine the causes of the odour from PNTP the HS-SPME-GC/MS method should be amended to detect amines and sulfurous compounds. This can be achieved by derivitisation techniques or by applying cryogenic cooling to the GC capillary column and possibly the employment of a polar column. In addition, the VOCs identified should be quantified by use of an internal standard. Quantification can be aided by cryogenic cooling and the use of a narrow bore inlet liner allowing improved chromatographic resolution, as the eluted peaks would be sharp and symmetrical, easing integration.

Due to the subjective nature of sensory panel analysis, the perception of odour changes is difficult to assess. Each panel member had a different perception of what an unpleasant odour is and in some cases panel members were unable to differentiate between a change in odour type and a change in odour intensity. In future studies an electronic nose should be employed

Prior to the processing of PNTP the inorganic iron present upon denaturing of the blood proteins should be removed or chelated to minimise catalytic reactions that may occur upon addition of the reducing agent sodium sulfite. Conversely, the method should be adapted to use less sodium sulfite or a different reducing agent to determine the effect on overall odour.

Section B: Environmental Degradation

Environmental impact is a primary concern when it comes to the disposal of many materials. This investigation was carried out to understand what would occur when NTP is exposed to sunlight or disposed of via industrial composting. The objective of this section is to evaluate the mechanisms by which proteins degrade upon exposure to environmental conditions and to identify factors that influence degradation rates.

6 Composting

Subjecting proteinous materials to environments where bacteria are present, such as composting, is known to result in structural changes to the proteins present [113]. The composting process involves the controlled biological decomposition and stabilisation of organic materials in the presence of oxygen to form a humus-like material with the production of carbon dioxide, biomass and heat [114, 115].

$$\text{Organic Matter} + \text{Microorganisms} + O_2 \longrightarrow H_2O + CO_2 + \text{compost} + \text{heat}$$

The main contributors to the breakdown of organic materials during composting are bacteria and fungi. The process of composting is comprised of three phases, shown in Figure 62:

- Mesophilic phase
- Thermophilic phase
- Maturation phase

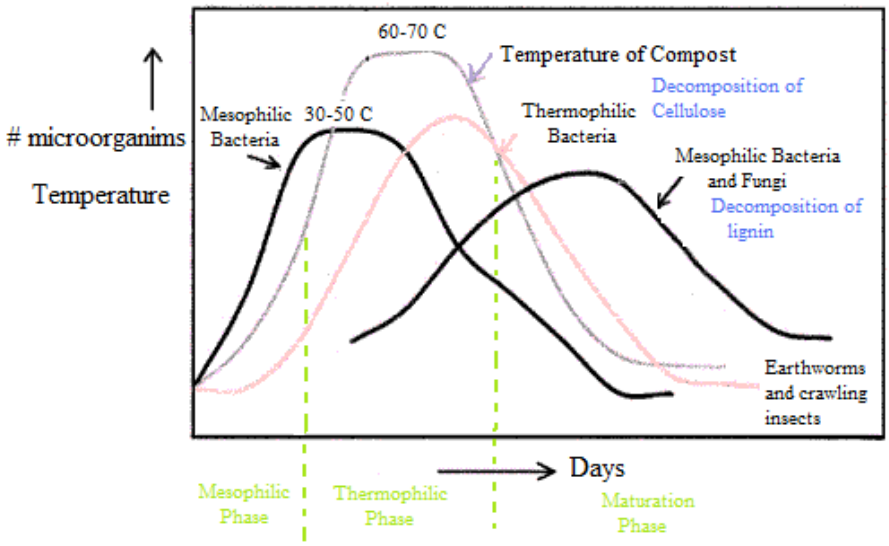


Figure 62: Growth phases of mesophilic and thermophilic bacteria and fungi over time. Image adapted from [116]. In purple, the overall temperature of the compost is shown, in pink, is the number of thermophilic bacteria present and in black, are the types and number of mesophilic micro-organisms present.

These phases are classified by the type of bacteria present and the physio-chemical properties of the compost during that stage [117].

The role of micro-organisms present in the compost pile is to break down organic compounds dissolved in the moisture fraction. The organic matter present in a compost pile is typically comprised of carbohydrates (such as cellulose, hemicelluloses and pectin), proteins, lipids and lignin (Figure 63).

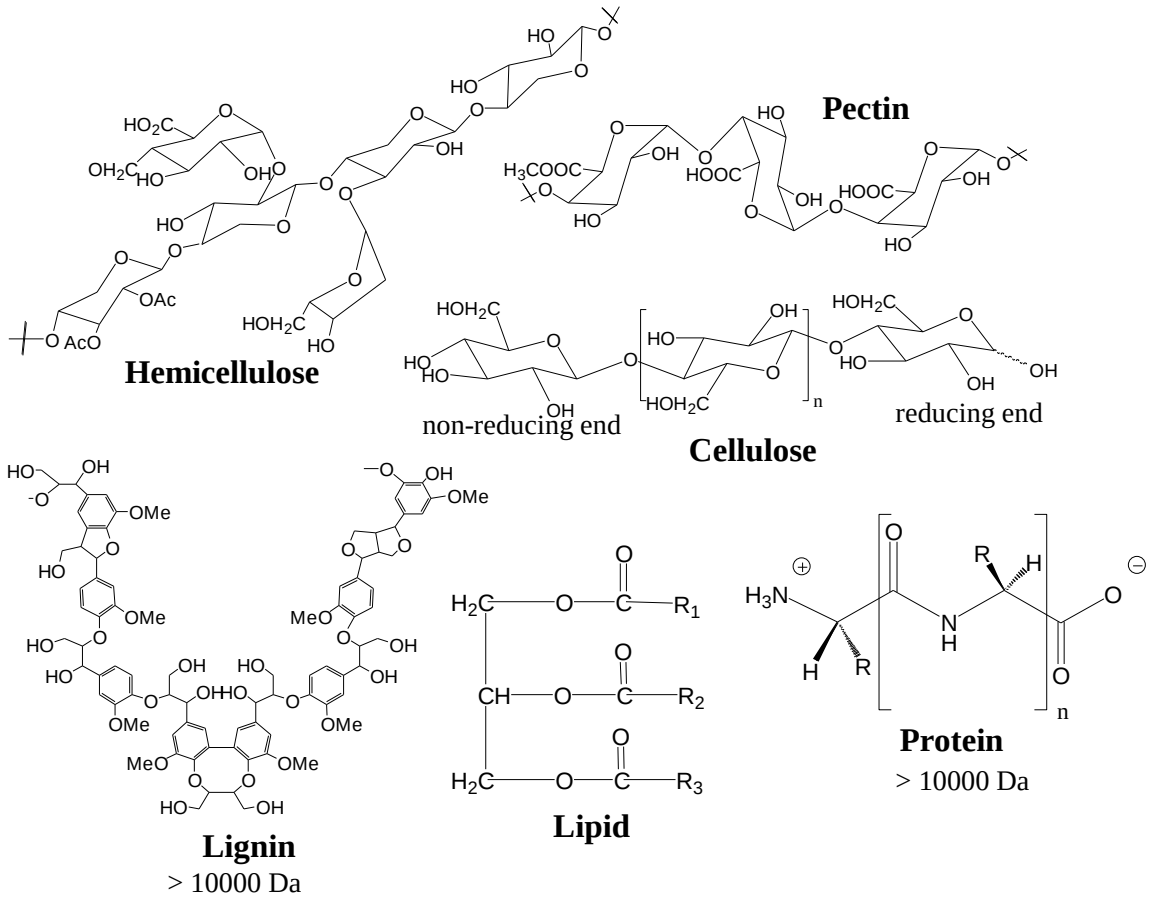


Figure 63: Organic compounds present in compost.

Due to the size of these macromolecules, a large proportion of available organic material is insoluble. Micro-organisms are able to solubilise the molecules by producing exoenzymes to break down the organic compounds until they become soluble.

The complex nature of the substrate necessitates the micro-organisms to produce a large array of specialised enzymes in order to degrade the organic material. The more complex the substrate, the more specialised the enzyme system must be, and requires the micro-organisms to work synergistically to break down the organic compounds into smaller molecules. These molecules can then be catabolised within the microbial cells [114].

Mesophilic Phase

Initially, the compost pile is colonised by mesophilic bacteria and fungi. They break down soluble and easily oxidisable compounds such as monosaccharides,

starch and fats. This occurs via hydrolase enzymes, producing aliphatic or aromatic organic acids through an exothermic reaction mechanism. Some of the energy released is used by the micro-organisms to fix cell damage and for reproduction, but the rest is lost as heat, causing the temperature within the pile to rise [59, 114, 117, 118]. Accumulation of the organic acids produced during the mesophilic phase leads to a drop in pH from 7 to about 5-5.5 [115].

As the temperature of the compost pile increases, the rate at which mesophilic bacteria reproduce increases dramatically. Due to the increasing population of micro-organisms present, the temperature rises quickly, as a result exothermic reactions, and can last from several hours to a few days [115]. This increase in temperature leads to the growth of thermophilic bacteria.

Thermophilic Phase

During the initial stages of the thermophilic phase the organic acids are decarboxylated to form carbon dioxide [117]. Occurring simultaneously, proteins are broken down by protease and peptidase enzymes into the various amino acids, which then undergo deamination to form corresponding α–keto acids and ammonia [114]. The decarboxylation of organic acids and deamination of amino acids causes the pH of the compost to rise to 8-9 [117].

As the temperature rises above 45 ^{o}C, thermophilic bacteria and fungi take over the degradation process entirely. This phase is characterised by oxidation reactions and the microbial breakdown of aromatic and organic acid compounds, causing the temperature to increase further, and can last from a few weeks to several months [115, 117]. At this point the pH of the pile is approximately neutral and anaerobic bacteria may become active as oxygen is rapidly consumed.

The composting process begins as thermophilic temperatures are reached (>45 ^{o}C) and the maximum rate of microbial metabolism is established. Natural selection favours micro-organisms that can thrive at higher temperatures. At about 70 ^{o}C, heat is lost to the environment at the same rate as heat is generated by metabolism processes, and the compost pile will remain at this temperature until all easily oxidisable compounds are consumed [119].

However, in commercial facilities, the peak temperature is ideally maintained between 55-65 ^{o}C by mixing and aeration, to ensure there is diversity of the thermophilic species of bacteria present, optimising the degradation process [117].

After the easily degradable carbon sources have been consumed, less reactive compounds such as cellulose, hemicelluloses and lignin are degraded and partially transformed into humus [114].

Maturation Phase

After the thermophilic bacteria and fungi have consumed the remaining easily degradable carbohydrates the compost begins to cool and becomes stable. This is known as the maturation phase where the pH stabilises and the pile is colonised by new mesophilic species of bacteria and fungi which further humify the compost. At this point the compost pile now contains a large variety of crawling insects and worms [115].

During maturation, the actinomycetes become the predominant biological group giving the maturing compost its characteristic white/grey surface colour typically seen on aging compost piles, and are responsible for the breakdown of lignin and cellulose [120].

Humus is the end product of the humification process where complex carbohydrates such as cellulose are derived into inert humic compounds of various chemical structures [114]. The chemical pathway to form humus from organic material is complex involving series of degradative and condensation steps, and result in inert carbon compounds [121].

Under ideal conditions, the composting process can be completed within a month [118, 119]. However, industrial composting typically takes between 6 – 8 months. The degradation process is limited by factors affecting the biological cycles of micro-organisms such as aeration and moisture content. Aeration is often difficult to carry out ideally due to odour restrictions in the facility's resource consent, which limit turning the compost to only often enough to prevent anaerobic conditions being established, as the odour arising from aerobic composting is

deemed offensive by companies and home owners surrounding the facility [122]. However, when anaerobic conditions are established, degradation of organic material is slowed, and the odour generated is much more offensive.

Composting Plastic

Synthetic polymers are typically more stable in oxidising conditions, and their rapid appearance in the environment has not allowed micro-organisms sufficient time to evolve to be capable of producing the enzymes that would be required to metabolise them. Therefore, their resistance to chemical and microbial degradation, has lead to their accumulation in the environment, and recently biodegradable plastics such as polycaprolactone, have been developed [123].

Natural polymers are able to undergo chemical and microbial degradation to form carbon dioxide and water, and in the case of proteins, forming ammonia also. Degradation is initiated by degrading the polymer into it's monomer units, which can then be mineralised, such processes occur during composting. However, not all natural polymers degrade at the same rate, and some compounds such as lignin may take years to degrade [124, 125]. As a result, although plastics may be capable of undergoing biodegradation, the rate at which they degrade will determine whether it can be deemed a "biodegradable plastic".

Several standards are available for determining the rate at which plastic undergoes aerobic biodegradation during composting. Most of the international standards measure the rate of degradation by measuring CO_2 generated during controlled composting in sealed reaction vessels inoculated with bacteria from mature compost [126].

There is only one standard commonly used for determining whether a plastic can be deemed biodegradable in an industrial composting facility. According to ATSM D6400-04 "Standard Specification for Compostable Plastics" a plastic sample must lose 60 % of its mass within 6 months (24 weeks), degrading at a rate comparable with cellulose. The standard does not stipulate the size requirement for the plastic sample to be tested, only that the plastic must have no adverse effect on the quality of the mature compost. The size of the plastic

sample exposed to composting conditions is known to directly impact the rate of degradation, dependant on the available surface for reactions to proceed [113].

Proteinous based plastics are known for their compostability [115]. Quantifying the degradation that has occurred for a compostable material can be difficult when considering mass loss only. During the process, disintegration of the material makes distinguishing fragments from the compost matrix difficult and cleaning the retrieved samples also becomes an issue [113]. Careful sieving of the compost that the samples are removed from is required to ensure that the entire sample is accounted for, however this is not a guarantee that very small pieces will be found. For this reason it is often preferable to perform a simulated composting process measuring evolved CO_2 [113].

Mass loss alone does not indicate that a material has undergone chemical degradation. To determine the extent to which it has occurred can be achieved by analysing changes in solubility, thermal properties and protein structure [113]. These types of measurements can be carried out using thermogravemetric analysis and Fourier transform infra-red spectroscopy, as discussed later in this section.

7 Ultra-violet radiation

High energy radiation and ultra-violet (UV) light are known to cause structural damage to proteins and lipids. UV light is defined as having a wavelength between 10-400 nm, and is divided into three regions; UVA 320–400 nm, UVB 280–320 and UVC 200–280 nm [127] (Figure 64).

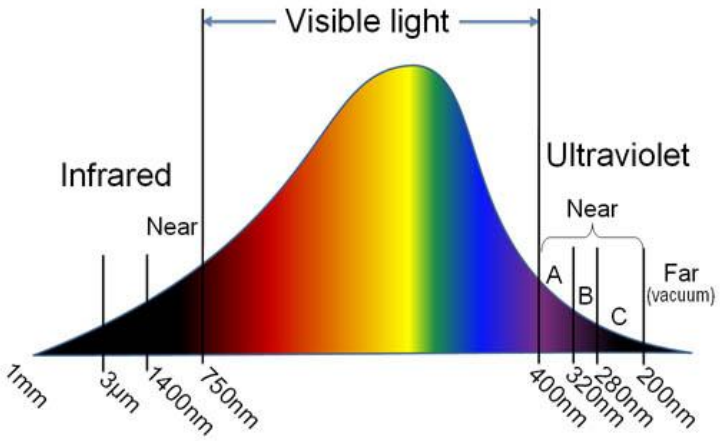


Figure 64: Infrared –ultra violet-visible light spectrum. Image sourced from: [128].

The ozone layer absorbs the majority of UVB and all UVC radiation [127]. Therefore, 90-99 % of the solar radiation that reaches the surface of the earth is comprised of UVA and 1–10 % is comprised of UVB. It is well known that UVB and UVC light have the highest photo-degradation kinetics for unsaturated compounds such as proteins and lipids. However, for investigating environmental photo-degradation of proteins, the effect of UVA light is more relevant due to its higher contribution to the radiation in sunlight.

The effect of UV light on wool [129-133], silk [130, 134], and hair [135-137], has been covered extensively. Prolonged exposure to UV radiation was found to result in photo-bleaching or photo-yellowing in wool and silk depending on whether it was exposed to UVB or UVA light. In addition, hair exposed to UV

radiation was found to cause peptide bond cleavage [138] and embrittlement of the fibers [139].

In proteins there are two major pathways for photochemical oxidation to proceed:

1. Direct absorption

   The protein directly absorbs UV light to generate an excited state (singlet or triplet states) or radicals as a result of photo-ionisation.

2. Indirect oxidation

   Energy is transferred from nearby or protein-bound chromophores (such as haem) to the ground state molecular triplet oxygen ($^3O_2$) [140]. The succeeding reactions of the excited state molecular singlet oxygen ($^1O_2$) with protein components can then occur.

Compounds that absorb UV/visible light are known as chromophores. The absorption of UV light by a molecule causes the excitation of its outer electrons in the groundstate, to the higher energy unoccupied anti-bonding orbital. This only occurs in compounds containing dative, double or triple bonds, such as those present in aromatic compounds [141].

Macromolecules such as proteins are able to absorb UV light due chromophores present in the form of the aromatic amino acid side chains. The strongest amino acid chromophores are cysteine, cystine, histidine, tyrosine, tryptophan and phenylalanine [140]. In addition to amino acid side chains, porphyrin structures such as haem are also able to absorb UV light, contributing to the facilitation of photo-degradation.

The absorption of UV light with wavelengths greater than 230 nm is not significant for the other non-aromatic amino acids, and peptide bonds only absorb weakly between 210-220 nm. Due to their weak absorption of UV light, it can be assumed the photochemical reactions of proteins is predominantly related to the side chains of the amino acids [140].

Most proteins only weakly absorb UV light above 290 nm. Aromatic amino acids are not very efficient at producing singlet oxygen from their excited state, although oxidised derivatives of these amino acids are more efficient.

Despite the ability for singlet oxygen to be formed by amino acid side chains, the presence of bound chromophores makes the formation of $^1O_2$ much more efficient [140]. As such, the exposure of porphyrins such as haem to UV radiation results in an excited state which is able to efficiently transfer energy to $^3O_2$ due to its higher absorption of UV light at wavelengths above 290 nm.

Exposure of proteins to UV light can have various effects on its composition and structure. Investigation into the photo-degradation of structural proteins has shown that prolonged exposure to UV radiation can alter the structure of the protein by causing cleavage of peptide and disulfide bonds or creating new cross-links between lysine and free carboxyl groups [137].

In addition, quantity of the amino acids present in the protein may change due to oxidation, for example, oxidation of phenylalanine to form tyrosine [138] and cystine to form cysteic acid [137]. Cysteic acid is formed by the photo-initiated scission of cystine's C-S bond, yielding S-sulfonic acid, which is then photo-degraded to form cysteic acid [139]. Increased cysteic acid content has been observed for structural proteins found in wool [129], hair [142] and silk after exposure to UV.

It has been shown that a decrease in lysine content may be caused by cross-linking. Oxidation of the α-carbon on the peptide backbone occurred in both wool and hair, leading to the formation of carbonyl and amide groups [139]. These free carbonyl groups are then able to form new intra- and intermolecular cross-links with other protein amino groups such as that of lysine, leading to embrittlement of the fibre [139].

In addition, exposure to UV radiation causes heating, which may lead to the formation of Maillard products. This could occur due to the presence of free carboxyl groups, possibly formed via free radical induced peptide cleavage of the

protein, which may then be able to undergo Maillard reactions with nearby amine groups leading to the formation of odourous cyclic compounds [143]. The Maillard reaction is known to proceed at both high and low water activity, and is generally favoured for longer reaction time and at higher temperatures [144].

In summary, UV degradation can lead to:

- Cleavage of peptide bonds
- Cleavage of disulfide cross-links
- Oxidation of cystine and phenylalanine
- Cross-linking between lysine and free carboxyl groups on nearby proteins
- Formation of Maillard products from exposure to heat during UV irradiation

These processes can result in a material that is more brittle and possibly more odourous than the starting material.

8 Experimental

8.1 Composting

To evaluate the compostability of NTP, 30 specimens (~3 mm depth, 160 mm length and 12 mm width) with and without plasticiser were prepared by injection moulding, as indicated in Appendix 1. Prior to testing the specimens were conditioned at 23 ^{o}C, 50 % relative humidity for one week before being weighed.

To expose the samples to the composting process, a frame was made (540 x 500 x 150 mm) with 2 mm mesh on the bottom, to prevent loss of disintegrated material. The box was covered with 15 mm hexagonal mesh to ensure the contents of the box remained in place when being removed from the pile and to provide aeration. The samples were then placed in the box lined and covered with green-waste as shown in Figure 65, before placing in the centre of a large green-waste pile at Hamilton Organic Recycling Centre, an industrial composting facility (Figure 66).

Figure 65: Box containing NTP samples for exposure to composting process.

Figure 66: Newly prepared compost pile comprised of freshly mulched green-waste.

The compost pile was turned for aeration once per month and approximately 0.5 tonne of water was added to the compost pile every 2-4 weeks as deemed appropriate by the facilities manager to maintain the pile's moisture content between 40-55 wt%. The temperature was measured fortnightly to ensure thermophilic conditions; the average temperature was 69 ^{o}C.

Over 12 weeks five samples with and without plasticiser were removed fortnightly for further analysis. After removing the samples they were photographed, weighed, reconditioned at 23 ^{o}C, 50 % relative humidity for one week and reweighed. Immediately following a part of each sample was weighed and placed in a furnace at 80^{o} C for 72 hours and reweighed to determine moisture content. Remaining parts of the samples were freeze dried and subjected to a Fourier Transform Infra-red (FTIR) spectroscopy analysis, thermogravimetric analysis and tested for changes in solubility.

8.2 UV radiation

To evaluate the impact of exposing NTP to UVA radiation, 30 specimens (~3 mm depth, 160 mm length and 12 mm width) with and without plasticiser were prepared by injection moulding, as indicated in Appendix 1. Prior to testing, the specimens were conditioned at 23 ^{o}C, 50 % relative humidity for one week before being weighed.

To expose the specimens to UV radiation, they were secured in a QUV Accelerated Weathering Tester (Figure 67).

Figure 67: QUV accerated weathering tester used for exposing NTP to UVA radiation.

A UVA-340 nm lamp was used as it most closely resembles natural sunlight [145], and was re-calibrated every 500 hours to 0.58 W/m^2 irradiance, with a black body temperature of 55 ^{o}C. The specimens were rotated through the accelerated weathering chamber every two days, and were turned over to expose the other side of the specimen once each week.

Over 12 weeks five specimens, with and without plasticiser, were removed fortnightly for further analysis. After removing, each specimen was photographed and weighed. After storage at 4 ^{o}C for 3-4 days, the samples were tensile tested and the remains freeze-dried and subjected to a Fourier Transform Infra-red (FTIR) spectroscopy and thermogravimetric analysis.

8.3 Analysis

There are many ways of investigating potential physical and chemical changes to NTP as a result of exposure to either UV or biodegradation.

Mass Loss

Measuring the change in mass of a sample during composting and UV weathering is the simplest means of determining the extent of which degradation has occurred. Changes in mass are observed when water content has varied through absorption, when hydrolysis has occurred or as a result of water diffusing out. In addition, low molecular mass compounds such as urea and triethylene glycol, may leach out of the material observed by mass loss.

The molecular mass of proteins will decrease during degradative processes, due to metabolic processes or UV radiation causing peptide bond cleavage. The lower molecular mass fragments formed as a result of degradation may diffuse out, or be metabolised by micro-organisms in the case of composting, to form carbon dioxide, ammonia and water, both leading to an observed mass loss in the sample.

Finally, when the material disintegrates, even with careful retrieval of all visually distinguishable sample fragments, pieces of sample may remain in the compost that can not be found, contributing to mass loss.

Moisture content can be calculated using different methods and are shown in Equations 1-6.

Composting

$$\text{Initial moisture content} = \frac{m - m_i}{m} \times 100 \quad \text{...(1)}$$

$$\%\ \text{Total mass change} = \frac{m_f - m_i}{m_i} \times 100 \quad \text{...(2)}$$

$$\%\ \text{Mass change} = \frac{m_{re} - m_i}{m_i} \times 100 \quad \text{...(3)}$$

$$\%\ \text{Water absorbed} = \frac{m_f - m_{final\ dry}}{m_{final\ dry}} \quad \text{...(4)}$$

$$\%\ \text{Dry Mass Change} = \frac{m_{initial\ dry} - m_{final\ dry}}{m_{initial\ dry}} \times 100 \quad \text{...(5)}$$

m = Mass of unconditioned NTP sample

$\mathbf{m}_i$ = Mass of conditioned NTP sample

$\mathbf{m}_f$ = Mass of NTP sample immediately following composting

$\mathbf{m}_{re}$ = Mass of NTP sample following composting after reconditioning at 23 ^oC at 50 % relative humidity

$\mathbf{m}_{final\ dry}$ = Mass of NTP sample following composting after oven drying at 80 ^oC for 24 hours

$\mathbf{m}_{initial\ dry}$ = Theoretical dry mass of initial conditioned NTP sample

UV Radiation

$$\%\,\text{Total mass change} = \frac{m_f - m_i}{m_i} \times 100 \qquad \text{...(6)}$$

$\mathbf{m}_i$ = Mass of conditioned NTP sample

$\mathbf{m}_f$ = Mass of NTP sample immediately following UV irradiation

Mass changes were determined once for each sample removed from the degradation process. The result was averaged for the samples removed each fortnight. One standard deviation was used as the error.

Solubility

Solubility of a protein derived bioplastic is a good indicator for the extent of cross-linking [7], and will also include soluble additives required for processing, such as urea and triethylene glycol, as they too are soluble. Solubility in water can indicate the type of interactions that are present; peptide and protein moieties that are un-associated will dissolve. This is a characteristic observation for reduced molecular interaction due to increased protein degradation.

Solubility data will aid determining the extent to which protein oxidation has occurred during composting. As the amount of protein being converted to peptides and free amino acids increases, so too should the solubility of the material, however, the composting process requires water to dissolve the nutrients so the bacteria can assimilate them, so any product resulting from polymer degradation may no longer be present in the material being analysed.

$$\text{Solubility} = \frac{m_{initial\ dy} - m_{final\ dry}}{V} \qquad ...(7)$$

$\mathbf{m}_{\text{final dry}}$ = Mass of dry NTP sample after solubility test

$\mathbf{m}_{\text{initial dry}}$ = Theoretical dry mass of initial conditioned NTP sample

Solubility tests were carried out by immersing 0.5-1.5 g of granulated sample in a beaker containing 20 mL of distilled water for 24 hours. The undissolved material was collected and dried in an oven at 80 ^{o}C for 24 hours. The change in mass between the dry mass (as determined by calculating initial moisture content) and the dry mass after immersion in water was used to determine the materials' solubility (Equation 7). Solubility was determined each fortnight, by determining the solubility for each specimen removed, and presenting the result as the average, with one standard deviation as the error.

Fourier Transform Infra-red Spectroscopy

Any structure that contains covalent bonding will absorb infra-red light, and leads to absorption of IR light at frequencies characteristic of the chemical bonds present.

Infrared analysis is usually performed using mid-infrared light between 4000- 400 cm^{-1} (λ= 2.5-25μm). The vibrational frequency and the probability of absorption depend on the strength and polarity of the molecular bonds, which are affected by intra- and intermolecular interactions [146]. This characteristic of FTIR has caused it to become a routine tool in the analysis of polymeric materials [147].

The amount of IR light that is absorbed increases with increasing polarity of the vibrating bond. The electron density within a bond and thus its polarity is influenced by neighbouring groups within a molecule by inductive and resonance effects (electron withdrawal/donation via σ-bonding or π-bonding respectively), the resonance effect is the reason for amide groups absorbing at lower wavenumber than carbonyl group of carboxylic acids [146].

A major problem with using IR light to characterise protein structure is that the spectrum of larger molecules is comprised of many overlapping bands, and as a result important structural information may be hidden under broad, featureless absorption bands [148].

The general frequencies at which various functional groups absorb IR light are shown in (Table 24), and are useful for the interpretation of FTIR spectra.

Table 24: Characteristic infrared absorption bands

Functional group	Wavenumber (cm^{-1})	Comments
Alcohol	3500-3100[a]	**O-H** stretch, free primary yields strong broad peak, secondary or tertiary –OH yields strong sharp peak.[a] Intermolecular bonding lowers wavenumber.[b]
Ether	1175-1050[a]	**C-O** stretch, aliphatic ether yields strong peak.
Carboxyl	1740-1660[a]	**C=O** stretch observed in ketones, aldehydes and carboxylic acids.
Primary amine	3500-3200[a]	**N-H** stretch for long chain aliphatic amines (3400-3200 cm^{-1}) and for short chain aliphatic amines (3500-3300 cm^{-1}) two moderate peaks, sometimes overlapping observed. Secondary peak appears at 1640-1560 cm^{-1} due to N-H bend in primary amines only, variable in seconday amines.
Secondary amine	3500-3100[a]	**N-H** stretch one moderate peaks observed
Sulfur	2600-2410[a]	**S-H** stretch [a]
	925-820[a]	**S-H** bend[a]
	710-570[c]	**C-S** stretch[c]
Sulfonic acid	1250-1150[a]	**S=O** stretch
	1070-1010[a]	**S=O** bend
	600-700[a]	**(S)-OH** bend
Disulfide	2860[c]	**(S)-CH_2** asymmetric stretch[c]
	1425[c]	**(S)-CH_2** bend[c]
	500[c]	**S-S** stretch[c]

NB: Data sourced from: **a** = [77], **b** = [149], **c** = [150].

During irradiation with IR light, the polypeptide backbone of proteins absorbs IR radiation exciting the vibrational modes of the amide bonds. There are nine characteristic IR absorption regions for amides observed in peptides as shown in Table 25. These regions typically require deconvolution of the FTIR spectrum of proteins due to complex peak overlapping.

Table 25: IR absorption regions of carboxyl and amide functional groups

Absorption Type	Wavenumber (cm^{-1})	Comments
Amide A	3300 (3310-3270)[a]	-**N-H** stretching vibration
Amide B	3100 (3100-3030)[a]	-**N-H** stretching vibration
Amide I	1700-1600 (1650)[a]	-**C=O** stretching vibration
Amide II	1575-1480 (~1550)[a]	-**C-N** stretching vibration, -**N-H** bending vibration
Amide III	1301-1229 (1400-1200)[a]	-**C-N** stretching vibration, -**N-H** bending vibration
Amide IV	767-625	**O=C-N** bending vibration
Amide V	800-640	Out of plane **-N-H** bending vibration
Amide VI	606-537	Out of plane –**C=O** bending vibration

NB: Data obtained from [148], values in parenthesis, a = [146]

The Amide A band is between 3310-3279 cm^{-1} as a result of the N-H stretching vibration, and is unaffected by the conformation of the polypeptide backbone [146]. The Amide I vibration does not significantly change with the type of amino acid side chain; it depends on the secondary structure of the peptide backbone enabling it to be used for secondary structure analysis of proteins. The Amide I band arises primarily from the C=O stretching vibration with minor contribution from the out-of-phase C-N stretch, the C-C-N deformation and the N-H in-plane bend [146, 151].

The Amide II band is the out-of-phase combination of the N-H in-plane bend with the C-N stretch with small contributions from the C=O in-plane bend and the C-C and N-C stretching vibration [146, 151]. This band is largely unaffected by the type of side chain present making it useful for secondary structure analysis of proteins.

The Amide III band is the in-phase combination of the N-H bending and the C-N stretching vibrations with minor contributions from the C=O in-plane bending and the C-C stretch. In proteins, the composition of this band is complex due to its dependence on side chain structure [146].

Using standard FTIR, changes in these amide regions can be observed, but without deconvolution it is not possible to determine exactly what relative contribution each functional group has had to cause the differences in absorbance through the region due to changes in protein conformation.

FTIR has been used to observe the effect of anti-oxidants on peptide cleavage in proteins [138]. FTIR is a suitable technique for the analysis of dehydrated proteins to determine secondary structure from the Amide I and II regions in the IR spectrum [151]. FTIR also yields information regarding polymer degradation over time by observing changes in the absorbance and peak shifts in the spectrum which are caused by deterioration of the proteins and formation of oxidation products, both which can be investigated using conventional peak allocations.

Freeze-dried and powderised samples were prepared as KBr discs (using 1 mg sample to 100 mg KBr) approximately two third of the KBr mixture was pressed at 10 tons pressure using vacuum to minimise water absorption during pressing. FTIR analysis was performed using a Perkin Elmer spectrophotometer, employing 16 scans at 4 cm^{-1} resolution from 4000-400 cm^{-1}. The background was scanned prior to each sample scan and automatically subtracted from the spectrum by the FTIR software.

Thermogravimetric Analysis

A polymer's behaviour under thermal conditions is determined by the strength of intermolecular interactions and bonding within the material. Thermogravimetric analysis (TGA) measures the rate of mass loss of a material as a function of time or temperature under controlled atmospheric conditions, giving important information regarding the materials thermal stability.

There are four major degradation steps typically observed during TGA of plasticised proteins [7]:

- vaporisation of water
- decomposition of plasticiser
- weak bond cleavage
- strong bond cleavage yielding inorganic matter (pyrolysis)

The onset temperature of thermal degradation for polymers will increase with the increasing number of cross-links. This is due to the increased stability caused by strong intermolecular interaction. When a protein is degraded, undergoing peptide bond cleavage, disulfide bond cleavage and changes in tertiary structure, it becomes less thermally stable.

Vaporisation of water and plasticiser will always occur at the same temperature. Weak bond cleavage will also occur at the same temperature, the contribution to total mass the lower molecular mass compounds have may vary. The major indicator of degradation occurring in a polymer, is an observed decrease in the temperature at which strong bond cleavage occurs to yield inorganic matter. This process is known as pyrolysis, an exothermic reaction, which is the complete combustion of organic material. When the molecular mass of a polymer is decreased due to bond cleavage, it becomes easier to oxidise, resulting in a decrease of the temperature required for pyrolysis to occur. To ensure pyrolysis of all organic material the temperature must exceed 520 ^{o}C [152]. In this respect,

TGA is an effective method of determining the extent of polymer degradation and was employed to determine the extent of protein degradation in NTP after composting and UV weathering.

Thermogravimetric analysis was carried out using a DTA-TGA analyser (SDT 2960, TA Instruments, New Castle, Delaware, USA). The DTA-TGA analyser measures thermogravemetric changes and differential thermal analysis (DTA) simultaneously, giving changes in mass as temperature increases and calorimetric information so that phase transitions can be determined [153]. The analysis was carried out from room temperature to 800 ^{o}C at a rate of 10 ^{o}C/minute. A sample size of 5-10 mg was used throughout.

Tensile Testing

Tensile testing is used to determine the mechanical properties of a material. In relation to polymer degradation, a material's mechanical properties are useful for assessing the extent of degradation.

UV radiation typically causes changes in the mechanical properties of proteinous materials, these changes could be a result of:

- cross-linking leading to higher modulus and less elongation
- chain scission causing a decrease in ultimate strength and lower elongation
- embrittlement leading to decreased toughness

Tensile tests were carried out on NTP after exposure to UV radiation to determine the extent and cause of degradation. From these tests, ultimate strength, modulus of elasticity and toughness were obtained. The modulus of elasticity was calculated using the secant tangent modulus method, according to ATSM D638-08. Three tensile test specimens, that had not been exposed to UV radiation were prepared with and without plasticiser to compare overall changes to mechanical properties caused by prolonged exposure. The specimens were conditioned at 23 ^{o}C and 50 % relative humidity for one week, and were stored at 4 ^{o}C in sealed plastic bags for four weeks prior to testing.

The samples were tested using a crosshead speed of 5 mm/minute on the Instron model 4204. Intact specimens exposed to UVA radiation were removed from the weathering chamber, weighed and photographed then stored at 4 ^{o}C in sealed plastic bags for three-four days before being tested.

9 Results and Discussion

9.1 Composting

9.1.1 Visual Observations

During the first four weeks samples containing plasticiser immediately showed signs of surface pitting, shape deformation and rounded edges. Despite their dry appearance, all samples felt damp to touch. Samples without plasticiser started showing minor surface cracking but no surface pitting after two weeks, having very minor shape deformation (Figure 68).

After six weeks plasticised samples had begun to disintegrate with only one sample intact. The samples showed major surface cracking, pitting and shape deformation. The edges were also rounded and easily disintegrated when rubbed. In contrast, the samples without plasticiser were removed intact; showing minor surface cracks, surface pitting, minor shape deformation and smoothed off edges and corners.

After eight weeks the plasticised samples were removed in pieces, with major surface cracking and pitting. The edges of the samples were rounded and the ends of the samples were thinner. The samples without plasticiser were removed intact, with surface cracks and pitting but little shape deformation. The edges of the samples were smoother and corners more rounded than those removed at Week 6.

After ten weeks plasticised samples were removed in smaller pieces, showing major deterioration. Samples without plasticiser were still intact, showing little change in appearance.

By the end of twelve weeks samples with plasticiser were removed in small pieces (1-3 cm^2) the samples were spongy in texture and the edges were easily fragmented when touched. However, samples without plasticiser were still intact showing signs of deterioration similar to that for plasticised samples after ten weeks exposure. All photographs of samples are given in Appendix 4.

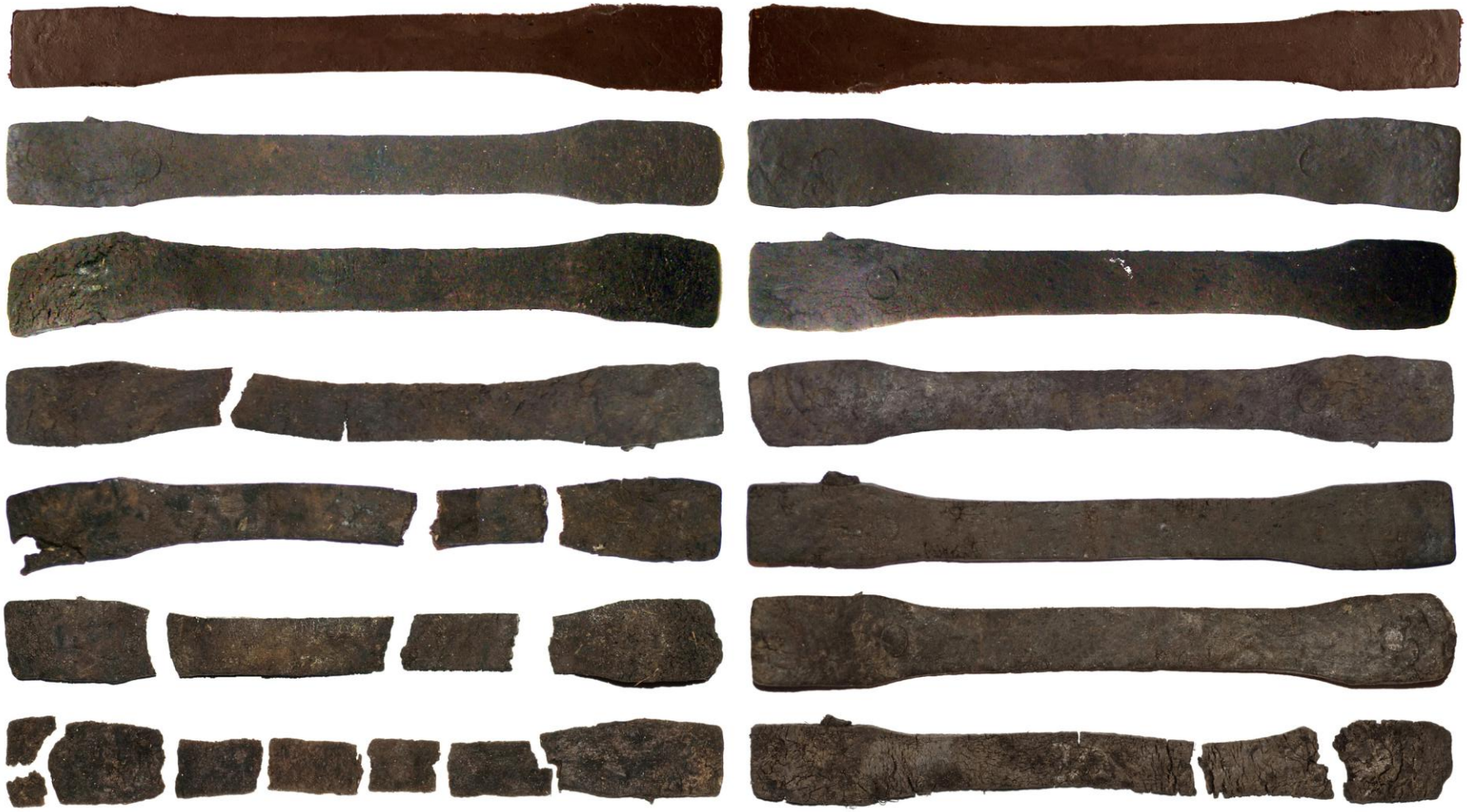


Figure 68: NTP samples with (TEG) and without plasticiser (WOP) removed throughout the composting process. From the top, weeks 0 - 12.

9.1.2 Mass Loss

During the initial 8-10 weeks of the composting process an increase in mass is observed after the samples are removed from the compost pile (Figure 68). Plasticised samples increased their initial mass by a maximum of 17 % as observed after two weeks whereas samples without plasticiser increased in mass by up to 41 % as observed after four weeks.

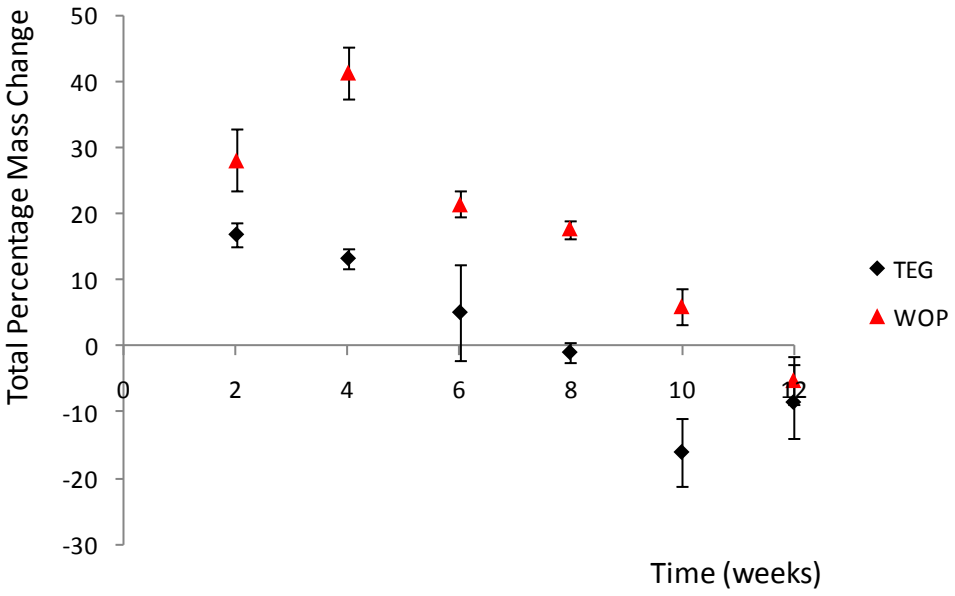


Figure 68: Mass change of NTP removed immediately after composting as a percentage of the original mass (Equation 2).

The observed increase in mass is attributable to the absorption of water and hydrolysis reactions occurring during the composting process. As the proteins are hydrolysed and the plastic is further plasticised, there is an increase in the number of sites available for water to be adsorbed. The increased number of sites available for adsorption is caused by protein chains swelling and the material becoming more porous due to microbial enzymatic catabolism of the material.

The large difference in the amount of water physically adsorbed in samples without plasticiser may be caused by the larger number of sites available for adsorption. Samples containing the plasticiser will have fewer sites available for sorption due to the presence of TEG between protein chains.

Based upon the errors calculated for mass loss after composting and conditioning at 23 ^{o}C, 50 % relative humidity, degradation appears to occur in two phases (Figure 69). The initial loss of easily solubilised compounds probably occurs in the first four weeks of the composting process, carried out by mesophilic and thermophilic bacteria, accounting for 15 % of the mass for samples containing plasticiser and 13 % of the mass for samples without plasticiser.

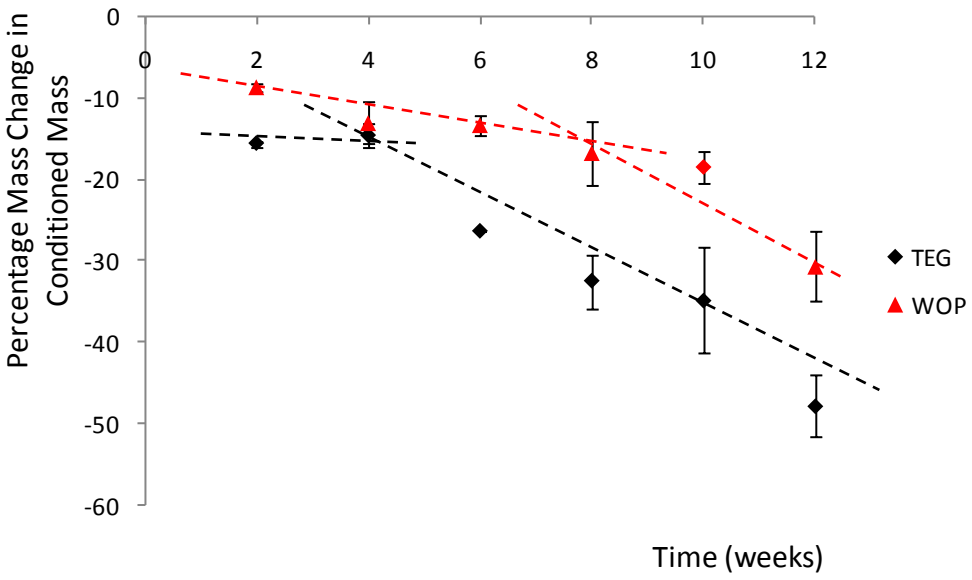


Figure 69: Mass change of NTP after conditioning at 23 oC, 50 % relative humidity as a percentage of the original mass (Equation 3).

Between weeks four and six of the composting process the samples containing plasticiser appear to undergo a rapid increase in the rate of degradation to yield 48 % mass loss after twelve weeks.

A similar trend is observed for samples without plasticiser. There is a steady loss in mass in the eight weeks. The rate rapidly increases between weeks eight and twelve to yield 31 % mass loss.

Comparison of the samples with and without plasticiser indicates that during weeks' ten to twelve external factors are increasing the rate of degradation, such as moisture content and the micro-organism's population size. Therefore the

rapid increase in degradation rate is likely to be caused by the population of thermophilic bacteria present in the compost pile reaching its maximum and the rate of catabolism accordingly.

During the composting process, easily solubilised compounds will be dissolved in the moisture content of the compost pile to be assimilated by the bacteria present. A dramatic decrease in solubility is observed after two weeks of composting (Figure 70). This is caused by the loss of urea and other soluble compounds such as triethylene glycol present in NTP to the moisture content of the compost pile. The decrease in solubility for NTP containing TEG is higher than that observed for NTP without plasticiser due to the initial loss in TEG in addition to unreacted urea. After two weeks the trend observed for changes in solubility is the same for NTP with and without plasticiser indicating after the initial decrease in solubility very little change is observed, within error limits.

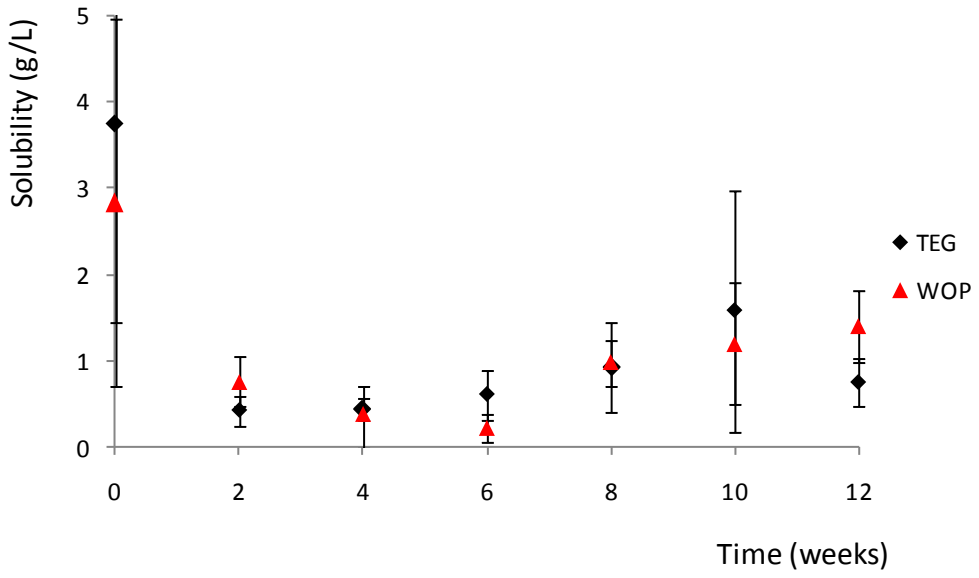


Figure 70: Solubility of NTP throughout the composting process (Equation 7).

Despite a large standard deviation, the graph does suggest a slight increase in solubility that would indicate some degradation has taken place.

There appears to be no trend in the amount of water absorbed relative to its solubility, shown in Figure 71 and Figure 72.

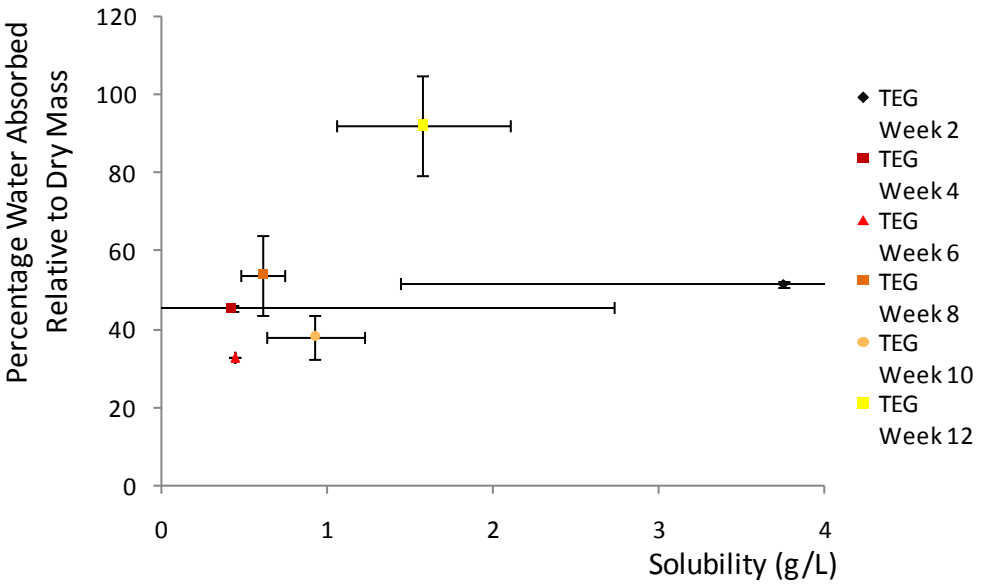


Figure 71: Percentage water absorbed relative to solubility of TEG NTP throughout the composting process.

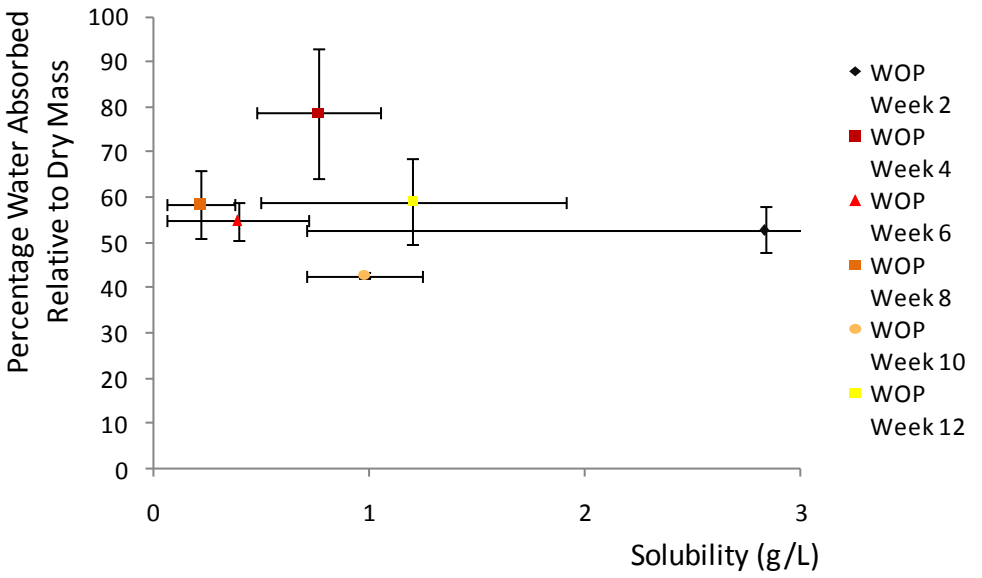


Figure 72: Percentage water absorbed relative to solubility of WOP NTP throughout the composting process.

Although water present in the sample will significantly influence the degradation of the bioplastic, for the purpose of determining total degradation, the mass loss of the bioplastic is discussed in terms of dry mass (Figure 73).

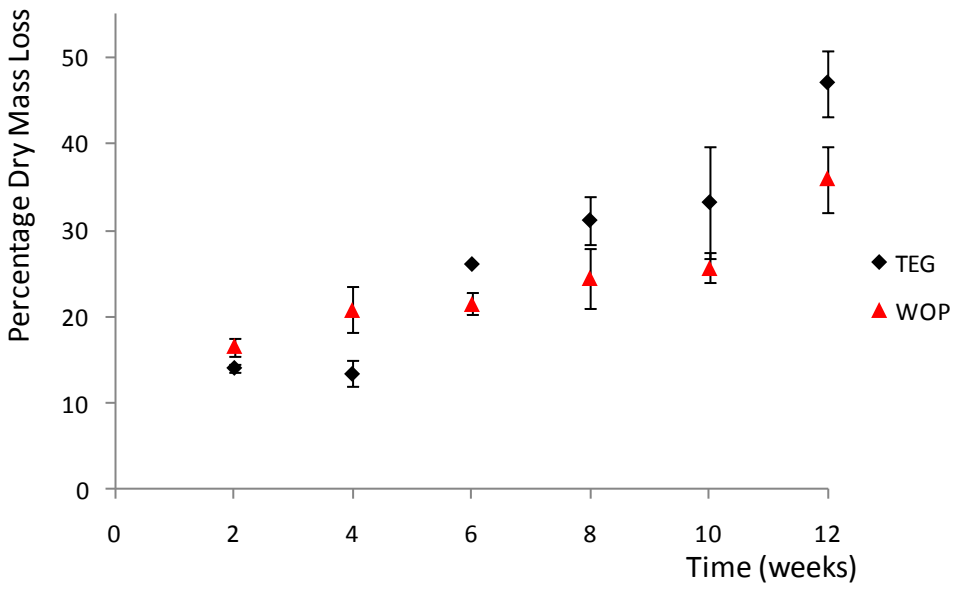


Figure 73: Percentage dry mass loss for NTP with and without plasticiser (Equation 5).

It was observed that plasticised NTP showed 47 ± 4 % dry mass loss during twelve weeks of composting, comparatively NTP without plasticiser showed 36 ± 4 % dry mass loss. It may be possible that the approximate 10 % higher dry mass loss observed for plasticised NTP is could be due to the loss of the 10 wt% TEG used in the NTP as there is no other significant difference within the error limits.

Significant deterioration in NTP samples was observed throughout the twelve week composting process and was confirmed by mass loss data. To determine the extent of degradation of the proteins, FTIR and TGA data was then obtained.

9.1.3 FTIR

The FTIR spectra obtained for plasticised and unplasticised NTP samples showed three significant changes after twelve weeks of composting (Figure 74, Figure 75, Figure 76 and Figure 77):

- Appearance of secondary peak at 3340 cm^{-1} (labelled a)
- Increase in the absorbance of the peak at 1552 cm^{-1} (labelled b)
- Loss of definition and absorbance of peaks in the region 1050-1190 cm^{-1} observed in TEG samples (labelled c)

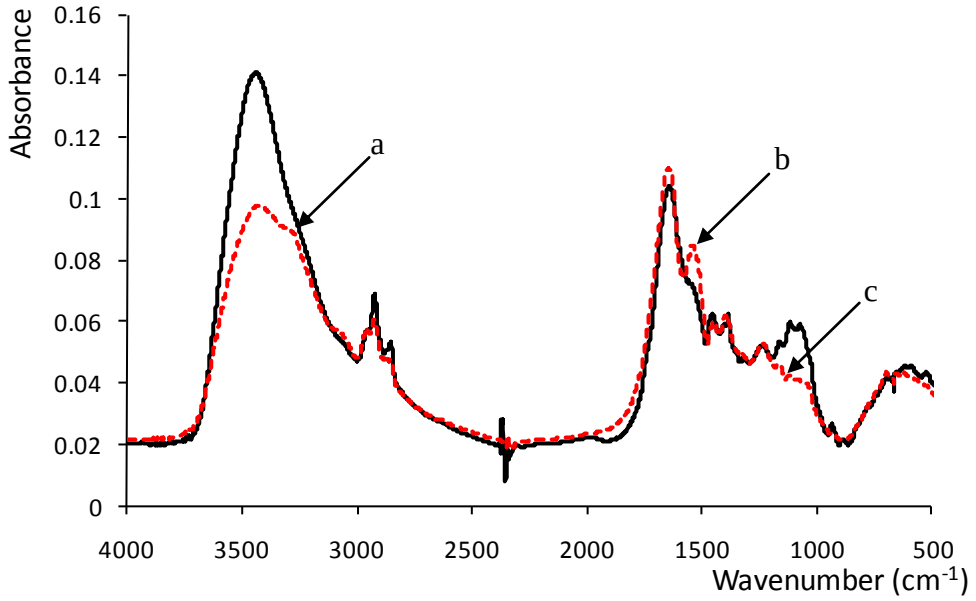


Figure 74: FTIR spectrum of NTP containing TEG after exposure to industrial composting for twelve weeks. Without exposure (black), after exposure (dashed).

The appearance of a shoulder peak at 3340 cm^{-1} suggests the formation of primary amine stretches, shown by point a in Figure 74. Increases in absorbance and broadening of the peak in the region 3500-3100 cm^{-1} can indicate the presence of water, however, because the sample was freeze-dried and the KBr disc prepared under vacuum to prevent water being incorporated, the possibility of the peak change being caused by water can be discounted. In a proteinous material, primary amines are formed as a result of hydrolytic cleavage of its peptide bonds

to give amine groups. This peak is also observed for NTP without plasticiser, confirming that during composting, the protein backbone has undergone hydrolytic cleavage.

There is also a substantial increase in the absorbance of the peak at 1552 cm^{-1}, shown by point b. This peak increase may be due to increased number of amide N-H in-plane bending vibrations, a result of peptide cleavage forming primary amine groups. Generally, this would also result in a corresponding peak at 1590-1650 cm^{-1}, however, a specific peak in this region is not observed in the spectra of NTP due to the complex nature of infrared absorption in proteinous materials, for this change to be observed deconvolution would be required.

Another observation, is the loss of the definition and absorbance of peaks in the region 1050-1190 cm^{-1}, as indicated by point c in Figure 75. In the TEG sample prior to composting, the cause of the multiple peaks in this region could be due to peak overlay with the C-O stretches of triethylene glycol. Therefore the loss of peaks in this region, leaving no specific bands may be due to loss of triethylene glycol in the plasticised samples due its high solubility, as these peaks are absent in NTP samples without plasticiser (Figure 76 and Figure 77).

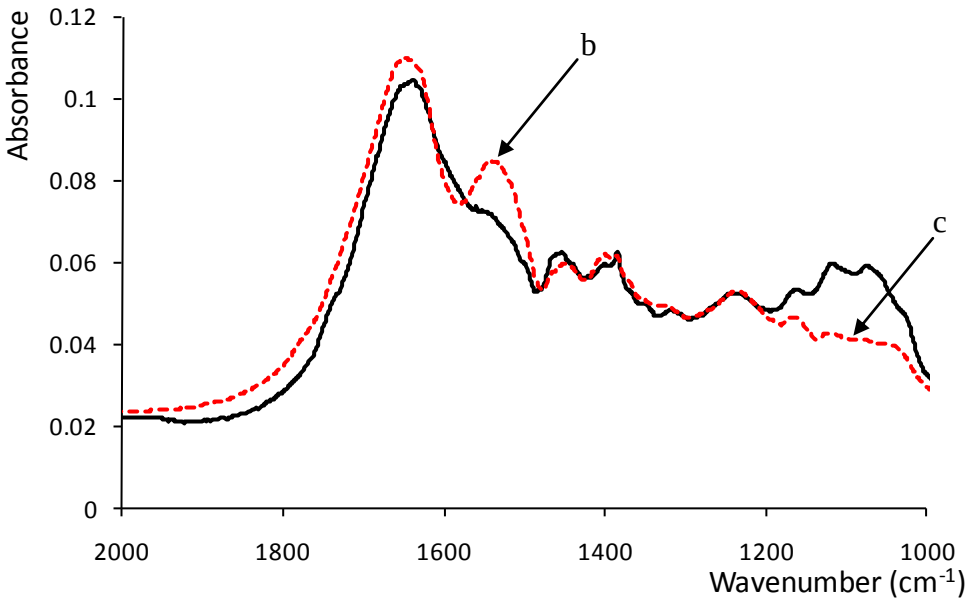


Figure 75: FTIR spectrum 2000-1000 cm^{-1} of NTP containing TEG after exposure to industrial composting for twelve weeks. Without exposure (black), after exposure (dashed).

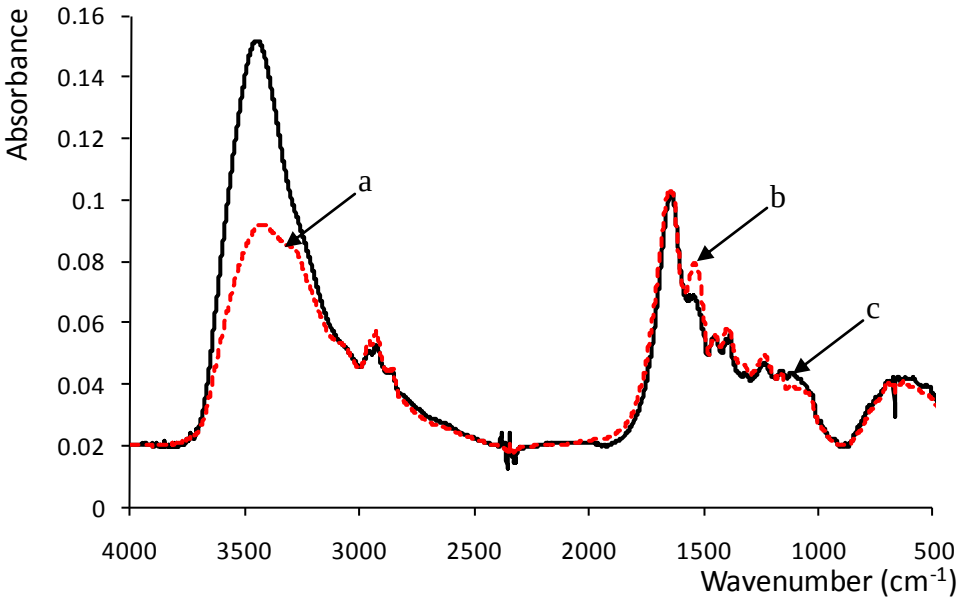


Figure 76: FTIR spectrum of NTP without plasticiser after exposure to industrial composting for twelve weeks. Without exposure (black), after exposure (dashed).

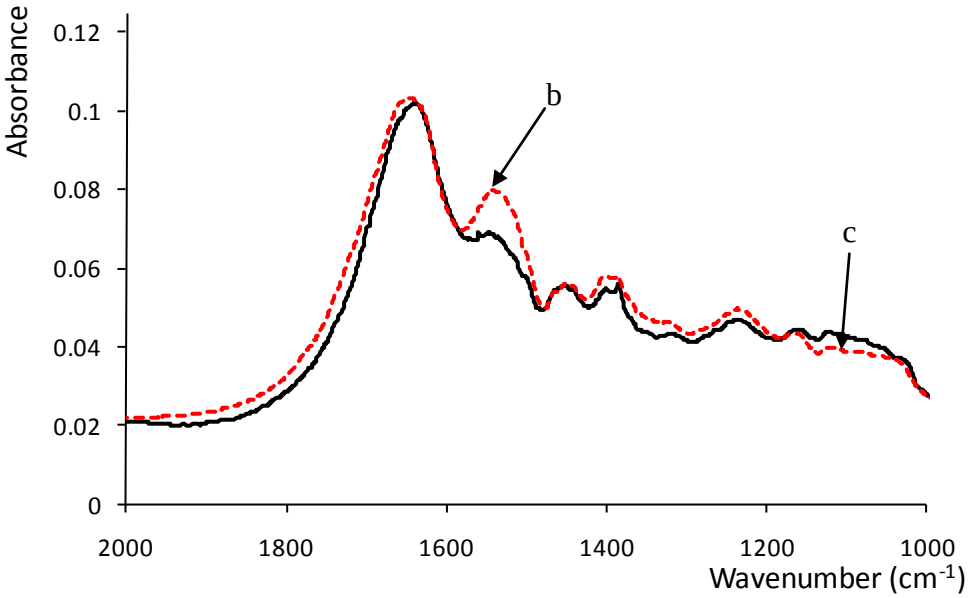


Figure 77: FTIR spectrum 2000-1000 cm^{-1} of NTP without plasticiser after exposure to industrial composting for twelve weeks. Without exposure (black), after exposure (dashed).

In summary, the FTIR results indicated NTP undergoes hydrolytic peptide cleavage during the composting process and the plasticiser had diffused out from NTP.

9.1.4 Thermogravimetric Analysis

There are four zones representing the various stages of thermal decomposition for plastics: 1. vaporisation of water, 2. decomposition of plasticiser, 3. weak bond cleavage and 4. strong bond cleavage, yielding inorganic matter (pyrolysis).

Microbial metabolism of NTP during composting leads to changes in the intra- and intermolecular bonding of proteins and loss of low molecular mass compounds that are soluble. These changes in NTP can be observed with TGA, as they result in changes in of the material's thermal stability.

Both samples with and without plasticiser both show several major changes in their thermal behaviour as a result of exposure to the composting process (Figure 78 and Figure 79):

- Low molecular mass compounds such as triethylene glycol and urea are not present after six weeks (labelled a)

- Smaller chain length peptides may have been removed by degradation, leading to increased thermal stability in Zones 3 and 4 after six weeks exposure (labelled b_1 and b_2)

- Protein degradation to smaller peptides has occurred, observed by a reduction in pyrolysis temperature as shown by the shift in exothermic peak in Zone 4 (labelled c)

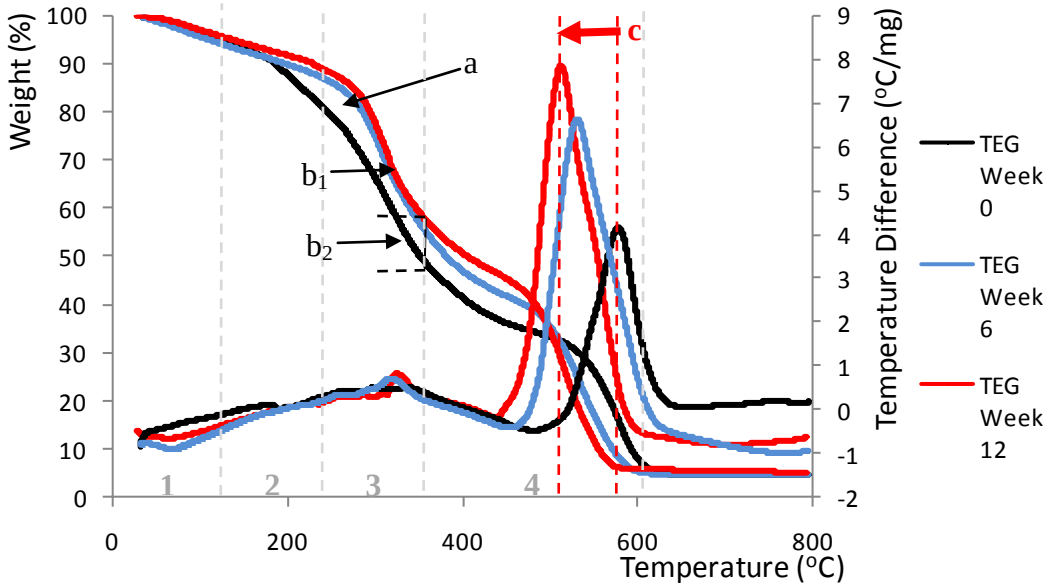


Figure 78: Thermogravemetric traces of TEG-NTP, with 0, 6 and 12 weeks exposure to industrial composting. The primary y-axis represents the percentage sample weight remaining as the temperature is increased and the seconday y-axis shows the exotherm expressed as temperature difference.

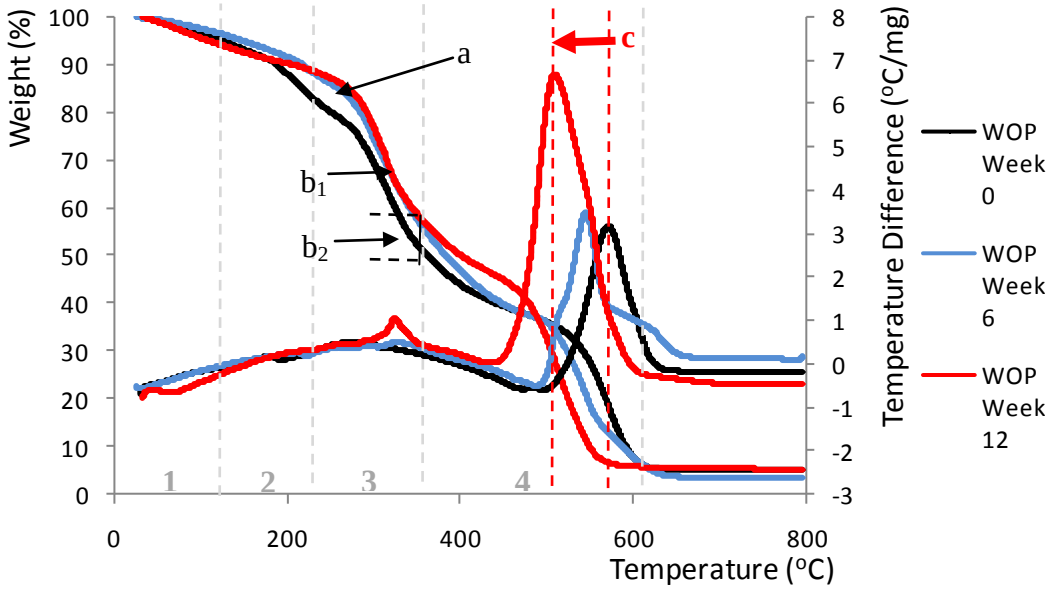


Figure 79: Thermogravemetric traces of WOP-NTP, with 0, 6 and 12 weeks exposure to industrial composting.

It is evident from zones 2 and 3, that after six weeks exposure to composting, a smaller proportion of low molecular mass compounds are present in the remaining

sample, (labelled a). This is observed for NTP with and without plasticiser, suggesting urea, in addition to triethylene glycol, has dissolved or been metabolised within the first six weeks of composting.

The thermal stability in zone 3 is initially lower for NTP prior to composting (labelled b_1) this is consistent with the lower % mass loss observed for NTP at the beginning of zone 4 (labelled b_2). After degradation the thermal stability has increased, possibly caused by the microbial metabolism removing the shorter peptide chains from NTP in the thermophilic stages of the composting process.

A decrease in exothermic peak temperature is observed for NTP as composting time increased (labelled c). The temperature at which pyrolysis occurs is influenced by the strength and number of covalent bonds present. Less covalent bonding is involved in lower molecular mass compounds such as polypeptides, making them less thermally stable than the protein they originated from. Therefore, the reduction in pyrolysis temperature with increasing composting time indicates the proteins in NTP are being degraded to lower molecular mass compounds, confirming that hydrolytic cleavage of peptide bonds and/or disulfide bonds has occurred due to microbial metabolism.

9.1.5 Summary

Composting causes many changes to the physical and chemical structure of NTP. Within weeks, physical deformation and deterioration of the samples is observed, followed by cracking, pitting and within twelve weeks, disintegration.

After two weeks exposure to composting conditions, there is a large decrease in the solubility of NTP, followed by a slight increase. This is indicative that the plasticiser and urea have dissolved or been metabolised by the mesophilic bacteria and are no longer present.

This is confirmed by TGA, after both six and twelve weeks' exposure a dramatic reduction in the quantity of low molecular mass compounds present in the remaining sample is observed. After twelve weeks exposure, the apparent loss of triethylene glycol from NTP observed through changes in solubility and thermal

stability is supported by the disappearance of ether peaks, from TEGs C-O stretching vibrations (1050-1190 cm^{-1}), in the FTIR spectra of TEG-NTP. In addition, the loss of 10 wt% TEG may explain the approximately 10 % lower observed mass loss for the remaining dry mass of NTP without plasticiser.

Degradation of the components in NTP appear to occur in two stages. Mass loss results all indicate the possibility for two different reaction rates, the first rate corresponding to the loss of low molecular mass compounds, either being dissolved or metabolised, followed by a more rapid rate occurring for the loss of higher molecular mass compounds. This model is consistent with literature review of the composting process, where soluble, low molecular mass compounds are consumed first by mesophilic bacteria and fungi, followed by hydrolysis and deamination of the higher molecular mass proteins by thermophilic bacteria as the temperature of the compost exceeds 45 ^{o}C.

Hydrolytic cleavage of the proteins in NTP is shown to occur after six weeks of exposure. The FTIR results show two peaks that have altered after composting, 3340 and 1552 cm^{-1}, caused by the formation of primary amines, a result of peptide bond cleavage. This interpretation is supported by TGA which showed a reduction in the pyrolysis temperature of NTP after composting. A decrease in the temperature at which pyrolysis of an organic material occurs can only be caused by a reduction in covalent bonding. In a protein, thermal stability could be reduced due to peptide bond cleavage, however, cleavage of disulfide bonds cannot be ruled out as possible contributing factor.

After twelve weeks exposure to composting conditions, plasticised NTP had undergone 47 ± 4 % dry mass loss, and NTP without plasticiser had undergone 36 ± 4 % dry mass loss. This reduction in mass can be attributed to disintegration, reduced molecular mass of proteins and loss of low molecular mass soluble compounds such as urea and plasticiser.

9.2 UV weathering

9.2.1 Visual Observations

During the first four weeks samples containing plasticiser immediately showed signs of surface pitting, shape deformation and rounded edges. Despite their dry appearance, all samples felt damp to touch. Samples without plasticiser started showing minor surface cracking but no surface pitting after two weeks.

Within two weeks of exposure to UVA radiation, the surface of the NTP samples appeared glossy, and felt sticky to touch. In addition, the samples had an odour similar to burnt baking and the surface was dark, almost black in colour.

With increasing exposure, very little change in appearance occurred. It is possible that the dark colour of the NTP after exposure to UVA was caused by the oxidation of iron in the haem moiety. In addition, the formation of Maillard products, indicated by the odour of burnt baking, may also contribute (Figure 81).

As exposure time increased, the width of the samples decreased. This observation was more noticeable for NTP samples that contained TEG than those without plasticiser. This decrease in sample size may be caused by loss of low molecular mass, relatively volatile compounds such as water and triethylene glycol caused by prolonged exposure to 55 ^{o}C.

Turning and removing the samples from the accelerated weathering tester became more difficult as exposure time increased as the samples became fragile, and within six-eight weeks exposure some samples were broken during removal. All photographs of samples are given in Appendix 4.

Figure 81: NTP samples with (TEG) and without plasticiser (WOP) removed after increasing exposure to UVA radiation. From the top, weeks 0 - 12.

9.2.2 Mass Loss

There appears to be no significant mass loss over the duration of the exposure to UVA (Figure 80). Assuming 10 wt% water in the initial conditioned NTP samples), it is probable that the loss in mass is due to water evaporation. This appears to have occurred more rapidly in the samples containing TEG plasticiser, possibly due to an easier diffusion path caused by increased chain mobility.

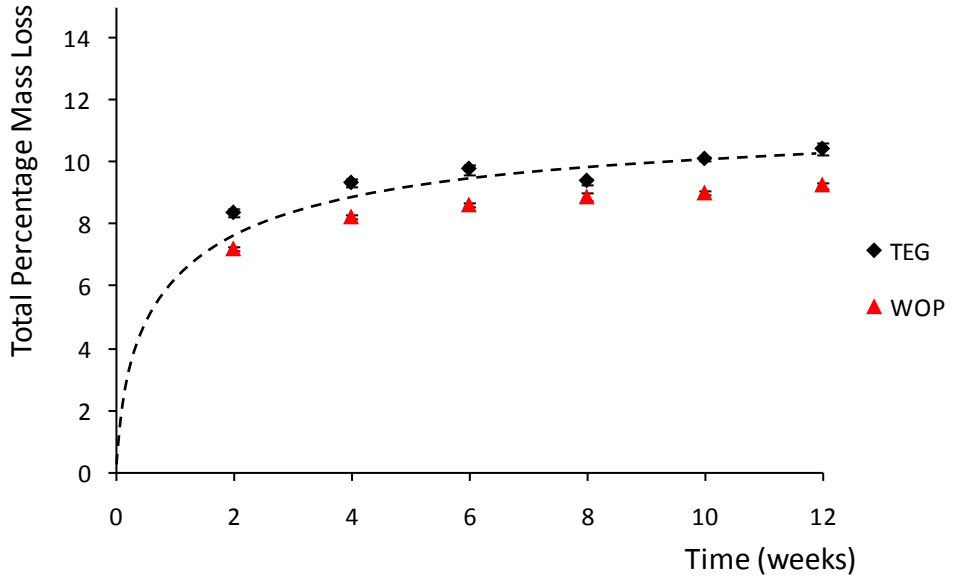


Figure 80: Mass change of NTP removed immediately after exposure to UVA radiation as a percentage of the original mass (Equation 6).

Plasticised samples typically have higher equilibrium moisture content therefore would show a slight increase in mass loss after exposure.

9.2.3 FTIR

There are very few differences in the FTIR spectra obtained for NTP samples after exposure to UVA radiation:

- Increased absorbance at 3480 cm^{-1}, observed only in NTP containing TEG (labelled a)
- Decreased absorbance at 1557 cm^{-1}, observed only in NTP containing TEG (labelled b)
- Decrease in the absorbance and number of peaks in the 1050-1150 cm^{-1} region (labelled c) possibly due to loss of triethylene glycol in the plasticised samples

In this case it is difficult to discern whether the 3480 cm^{-1} peak absorbance has increased due to the formation of new free –COOH or non-hydrogen bonded primary amines, caused by peptide bond cleavage or caused by formation of hydrogen bonded alcohol groups via oxidative reactions, or a combination of all three types of reaction (Figure 81).

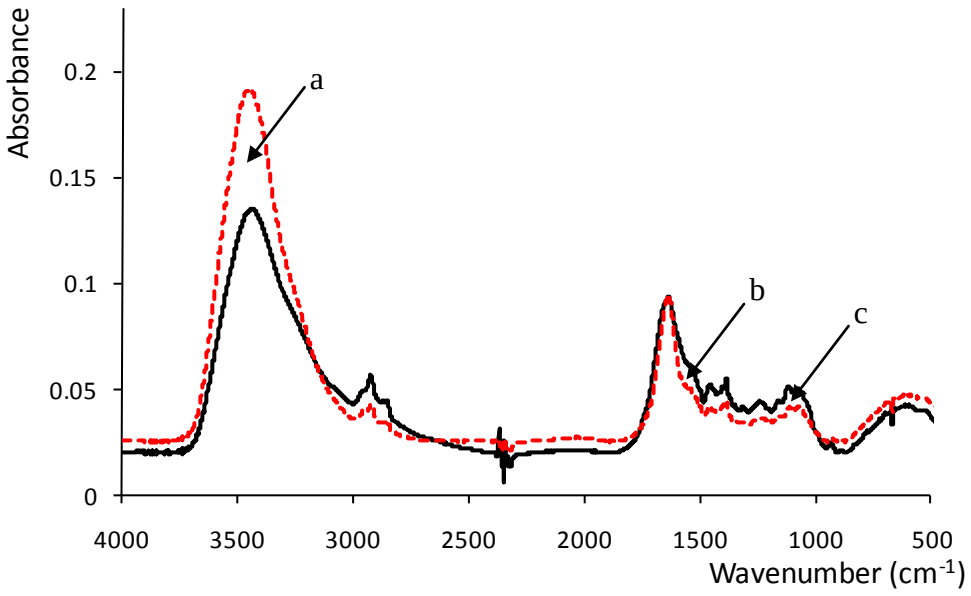


Figure 81: FTIR spectrum of NTP containing TEG after exposure to twelve weeks UVA radiation. Without exposure (black), after exposure (dashed).

In addition, there is a notable decrease in absorbance at 1557 cm^{-1} (Figure 82), seen as a shoulder peak in NTP prior to UV exposure (labelled b). The decrease in this peaks absorbance in NTP containing plasticiser may originate from loss of secondary amide N-H in-plane bends. This would be observed when the quantity of N-H bonds is decreased through the amide nitrogen being incorporated into other nitrogen containing compounds, such as cyclic nitrogenous compounds. This possibility cannot be discarded, as Maillard reactions may be occurring in the weathering chamber due to the high temperature (55 ^{o}C).

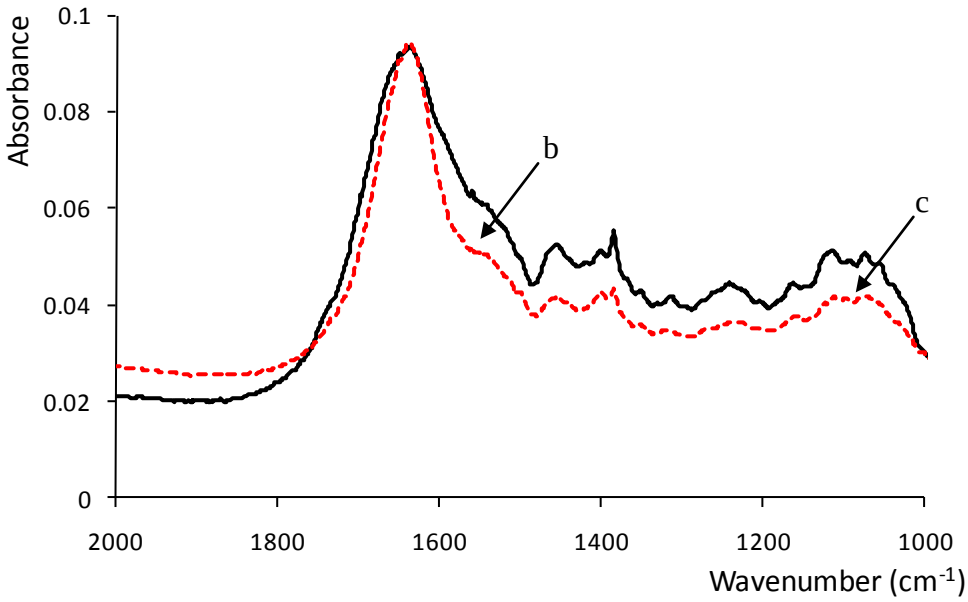


Figure 82: FTIR spectrum 2000-1000 cm^{-1} of NTP containing TEG after exposure to twelve weeks UVA radiation. Without exposure (black), after exposure (dashed).

Literature also suggests that exposure to UV light results in the homolytic cleavage of the disulfide group in cystine, resulting in $–S^{\cdot}$ free radicals [136]. Generally, a decrease in peak absorbance associated with the S-S and C-S bands is observed upon extended exposure to UV radiation [155]. The S-S bands are typically observed around 500 cm^{-1} for S-S stretch, 1425 cm^{-1} for (S-)CH_2 asymmetric stretch, and 2860 for (S-)CH_2 bends [150]. There appears to be no significant changes in these regions after UV exposure for 12 weeks, therefore no comment can be made as to whether disulfide cross-linking has increased or decreased during exposure based on the FTIR spectra obtained.

There appears to be decrease in the absorbance and number of peaks in the 1050-1150 cm^{-1} region (labelled c), which may be due to loss of triethylene glycol in the plasticised samples due to long term exposure to the radiation at 55 ^{o}C causing the plasticiser to evaporate. However, the cause of the bands at 1123 cm^{-1} and 1080 cm^{-1} in NTP without plasticiser cannot be identified Figure 83 and Figure 84.

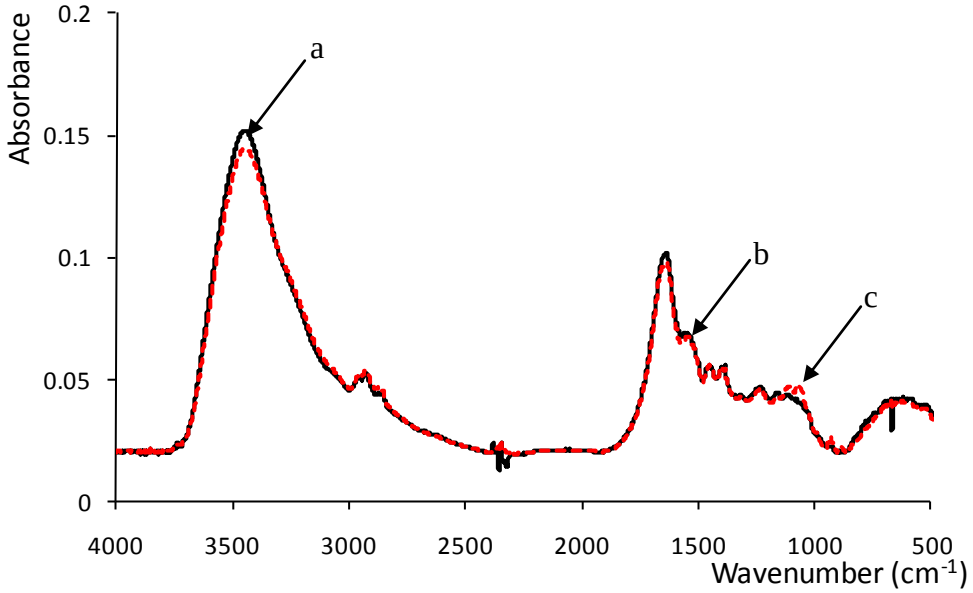


Figure 83: FTIR spectrum of NTP without plasticiser after exposure to twelve weeks UVA radiation. Without exposure (black), after exposure (dashed).

In contrast to NTP containing TEG, there appears to be no significant change at 1557 cm^{-1} (labelled b) in the NTP without plasticiser. The cause of this cannot be discerned.

Due to the multi-component nature of proteins, their FTIR spectra are difficult to analyse without deconvolution, however, based on the spectra obtained, it can be concluded that very little degradation in the chemical structure occurred after exposure to UVA, however, the possibility of formation of Maillard products cannot be ruled out.

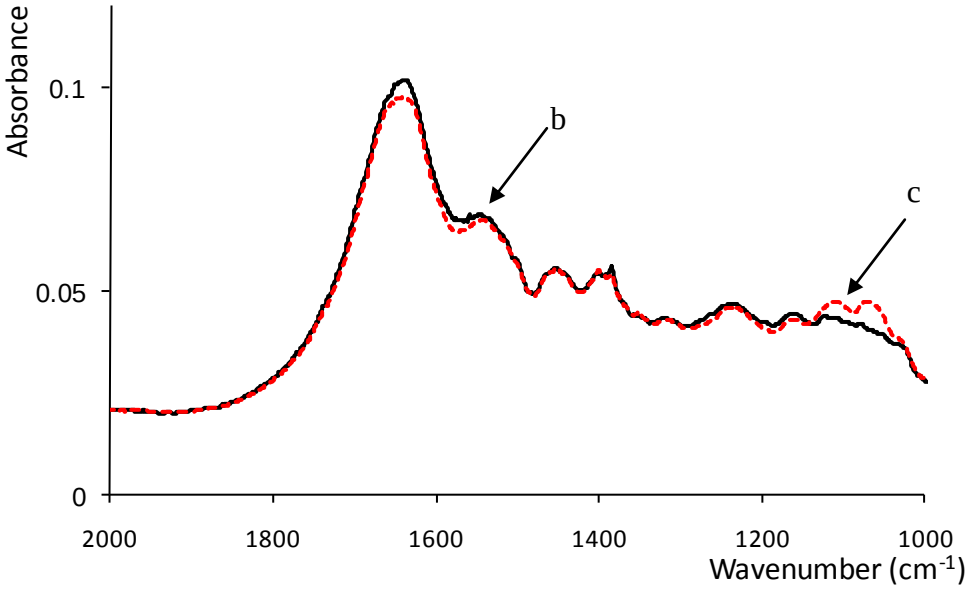


Figure 84: FTIR spectrum 2000-1000 cm^{-1} of NTP without plasticiser after exposure to twelve weeks UVA radiation. Without exposure (black), after exposure (dashed).

9.2.4 Thermogravimetric Analysis

As previously mentioned, there are four zones representing the various stages of thermal decomposition for plastics: 1. vaporisation of water, 2. decomposition of plasticiser, 3. weak bond cleavage and 4. strong bond cleavage, yielding inorganic matter (pyrolysis). After exposure, only one change in the thermogravimetric anaysis of NTP is observed, a reduction in the quantity of low molecular mass compounds (labelled a) which appears larger for TEG than WOP, indicating that the plasticiser has diffused during exposure.

It is known that UV degradation of proteins can result in the cleavage of disulfide and peptide covalent bonds. After exposure to UVA radiation, the samples with and without plasticiser show no significant change in thermal stability with increasing exposure to UVA (labelled b), Figure 85 and Figure 86.

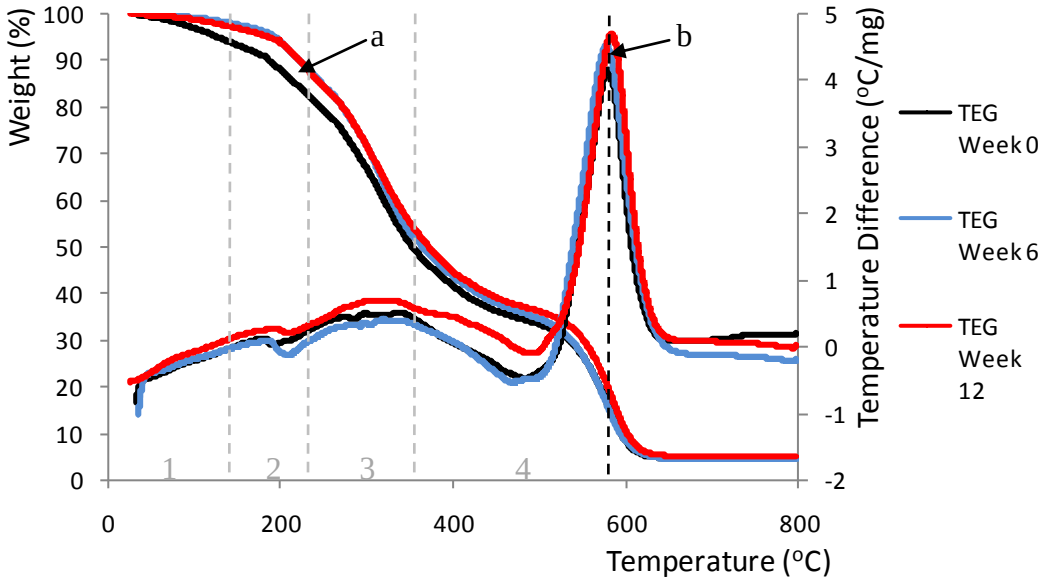


Figure 85: Thermogravemetric traces of TEG-NTP, with 0, 6 and 12 weeks exposure to UVA radiation.

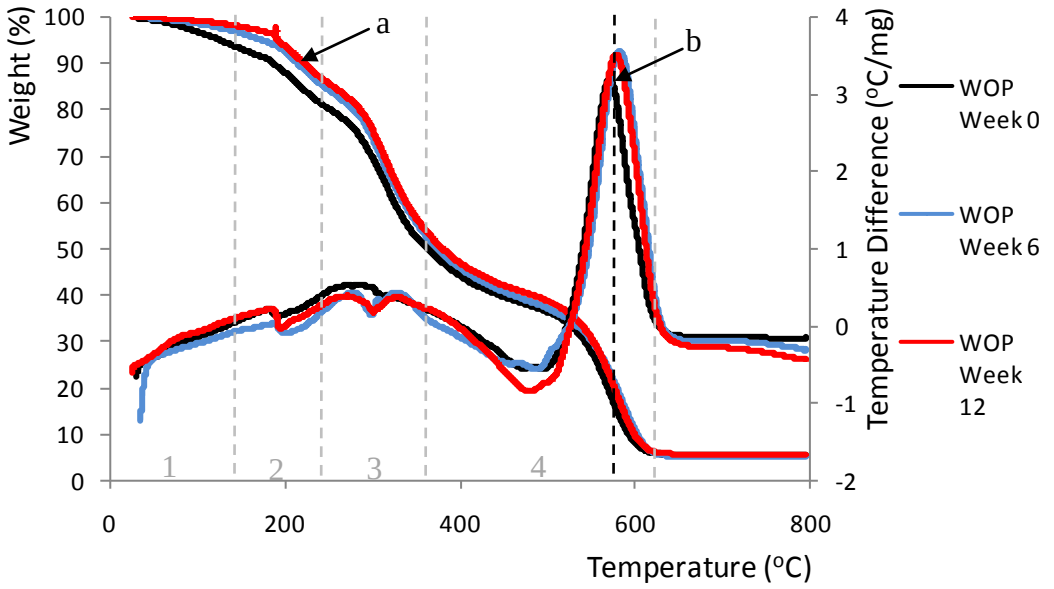


Figure 86: Thermogravemetric traces of WOP-NTP, with 0, 6 and 12 weeks exposure to UVA radiation.

When compared to the major changes observed for NTP exposed to composting conditions, it can be concluded that proteins in NTP are stable against UVA radiation. TGA shows that no significant changes in covalent bonding has occurred during exposure through cleavage of disulfide or peptide bonds,

confirming also, that the mass loss observed earlier in this section was caused solely by loss of water and plasticiser.

9.2.5 Mechanical Properties

Upon exposure of NTP to UVA radiation for six weeks there was an approximately five-fold increase in the secant modulus for samples with triethylene glycol plasticiser and an approximately two-fold increase for samples without plasticiser (Table 26). The difference in the increase in secant modulus for WOP and TEG after exposure is expected to be larger for TEG due to the plasticisation leading to a higher level of elastic deformation initially compared with WOP (Figure 87).

Table 26: Mechanical properties of NTP after exposure to UVA 340 nm radiation.

Time (Weeks)	TEG				WOP			
	σ (MPa)	**E (MPa)**	**Strain %**	**Toughness (MPa/m^2)**	**σ (MPa)**	**E (MPa)**	**Strain %**	**Toughness (MPa/m^2)**
0	12.8 (1.9)	892.1 (204.7)	3.9 (0.7)	2720 (921)	26.0 (4.3)	2052.5 (267.9)	1.6 (0.3)	2320 (884)
6	18.8 (3.5)	5442.7 (404.2)	0.35 (0.07)	350.8 (132.8)	14.6 (4.2)	5731.7 (451.4)	0.24 (0.07)	197.4 (97.3)
12	13.7 (4.5)	5519.5 (279.6)	0.25 (0.09)	250.6 (205.2)	11.3 (3.1)	6730.3 (630.1)	0.16 (0.04)	99.1 (48.6)

NTP containing triethylene glycol (TEG), NTP without triethylene glycol (WOP). Standard deviation of the results used for the absolute error, is given in parenthesis.

Initially, there is also a dramatic decrease in the toughness of both TEG and WOP which within error limits does not change over the latter six weeks. It appears that embrittlement is consistent for both TEG and WOP, within error limits, possibly because after this time both plasticiser and water have almost entirely evaporated (Figure 88). Elongation shown as strain %, decreases after six weeks as decreases further after twelve weeks for NTP with or without plasticiser (Figure 89). This is also consistent with what would be expected for a plastic material becoming embrittled.

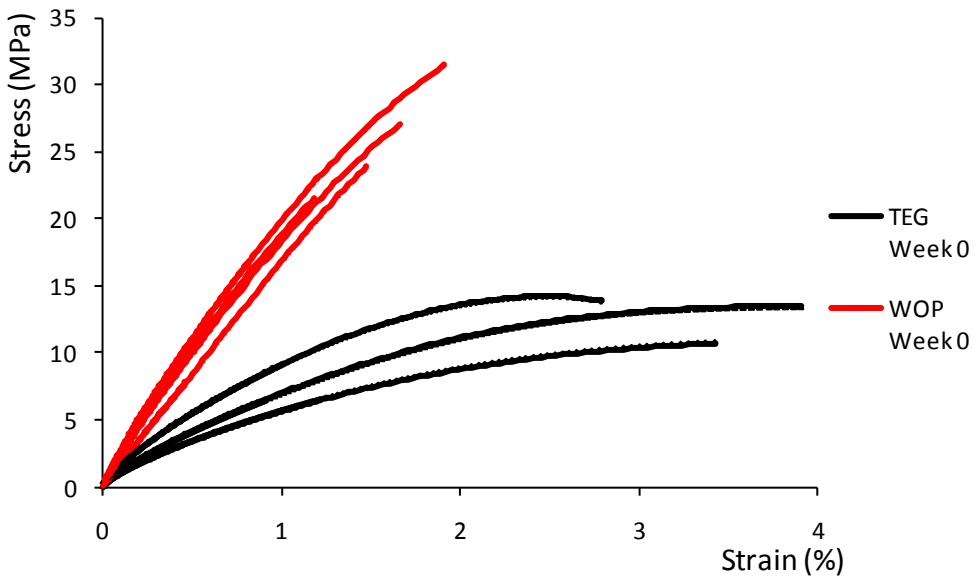


Figure 87: Stress-strain curve for NTP with (TEG) and without plasticiser (WOP).

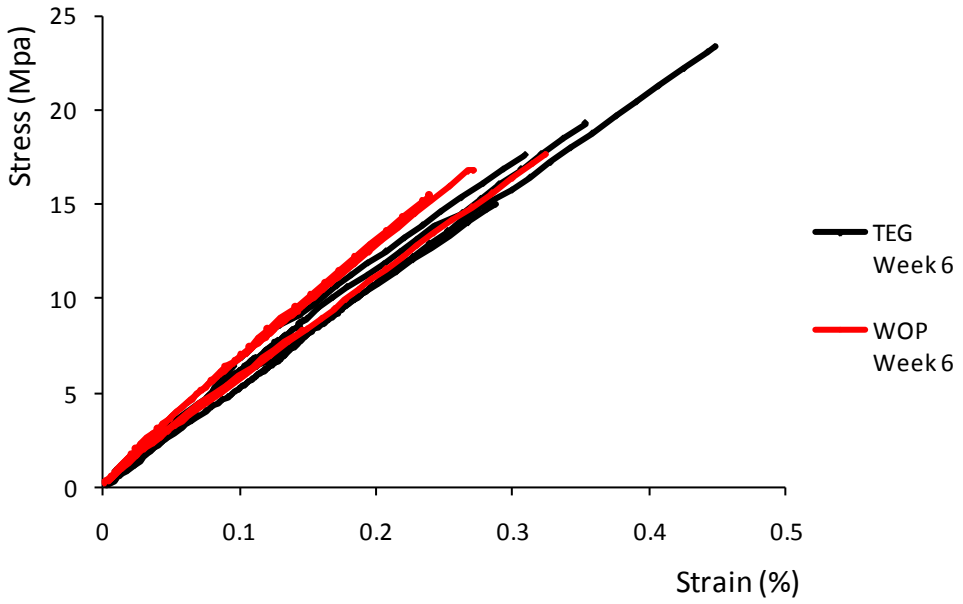


Figure 88: Stress-strain curve for NTP with (TEG) and without plasticiser (WOP) after six weeks exposure to UVA radiation.

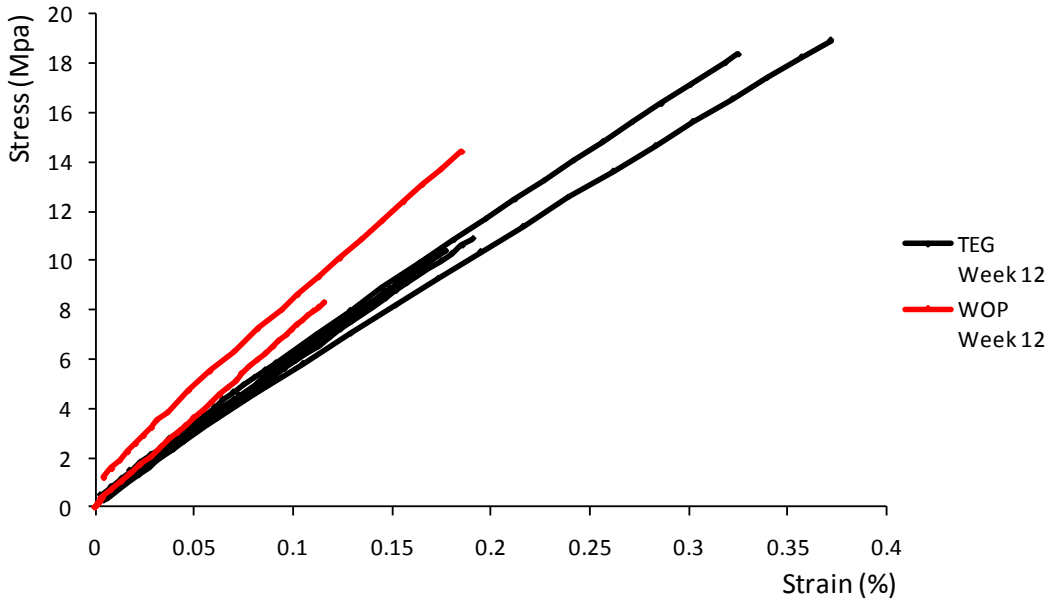


Figure 89: Stress-strain curve for NTP with (TEG) and without plasticiser (WOP) after twelve weeks exposure to UVA radiation.

The error of these results do not allow determination of whether significant changes in the maximum applied stress occurs upon exposure of NTP containing TEG to UVA radiation, although NTP without plasticiser appears to tolerate a significantly lower maximum applied stress after twelve weeks exposure.

In summary, these results are all indicative of plasticiser and water evaporating during exposure to UVA. In addition, it was shown that all NTP samples have undergone significant embrittlement within six weeks of exposure to UVA.

9.2.6 Summary

Based on changes in appearance and mass loss data, it can be concluded that NTP undergoes mass loss consistent with loss of plasticiser and water. This is confirmed by TGA analysis showing loss of low molecular mass compounds from NTP after exposure to UVA for six weeks.

Visual observation suggests the formation of Maillard products resulting from exposure to 55 ^{o}C for extended periods of time. This was not able to be confirmed using FTIR analysis.

Significant changes in the materials mechanical properties are observed, showing that embrittlement of NTP occurs within six weeks of exposure to UVA radiation. This is most probably caused solely by the loss of water and plasticiser, as TGA analysis shows that there are no major differences in the thermal stability of NTP as would be expected if cross-linking or peptide bond cleavage were occurring, the other two most prominent influences on a material's mechanical properties.

In conclusion, the results indicate that NTP is resistant to structural protein changes, but is susceptible to embrittlement caused by prolonged exposure to UVA radiation. Although proteins are able to undergo substantial UV induced degradation, NTP may be relatively UV stable, shown from TGA results, due to its characteristic dark pigmentation, which is known to enhance the stability of polymeric and proteinous materials [156, 157].

10 Conclusions and Recommendations

The impact of composting and UVA irradiation of NTP on the resulting physical and chemical structure of the plastic, are very different. Composting causes much more intense physical changes to NTP compared to UVA exposure. Within weeks of composting, physical deformation and deterioration of the samples was observed, followed by cracking, pitting and within twelve weeks, disintegration. UVA radiation only appeared to cause the formation of a sticky film, which had a characteristic burnt odour on the surface of NTP within two weeks and no other significant change occurred with further exposure.

Consistent to both composting and UVA radiation, within six weeks little or no low molecular mass compounds remained. In composting, this is caused by diffusion or metabolism of the compounds by micro-organisms, whereas for UVA irradiation, this is probably caused by evaporation and possibly formation of Maillard products. The impact of losing low molecular mass compounds such as water and plasticiser resulted in embritlement of NTP, as shown for the UVA irradiated samples.

Composting also appeared to cause much more significant changes to the chemical structure of NTP. During the composting process, NTP was shown to undergo microbial mediated protein hydrolysis forming lower molecular mass peptides, and causing a reduction in the solubility of NTP after two weeks exposure. This suggested that low molecular mass compounds have been metabolised first, consistent with literature review. Conversely, exposure to UVA radiation resulted in no discernible change in the chemical structure of the proteins in NTP, suggesting that the proteins present in NTP are UV stabilised, possibly by the dark pigmentation of the material.

Microbial degradation of NTP during composting appears to occur in two stages, indicated by changes in mass occurring at two different rates, the first corresponding to the loss of low molecular mass compounds followed by a higher rate occurring for the loss of higher molecular mass compounds during

thermophilic degradation of proteins, consistent with literature review of the composting process.

After twelve weeks exposure to composting conditions, plasticised NTP had undergone 47 ± 4 % dry mass loss, and NTP without plasticiser had undergone 36 ± 4 % dry mass loss. The difference in the quantity of mass loss between NTP with and without plasticiser may be due to the presence of 10 wt% triethylene glycol. The total change in mass of NTP is attributed to disintegration, reduced molecular mass of proteins and loss of low molecular mass soluble compounds such as urea and plasticiser.

NTP exposed to UVA radiation appears to undergo mass loss consistent with diffusion of plasticiser and water out of the material, confirmed by changes in mechanical properties that indicate the material is embrittled with increasing exposure and no observed change in the thermal behaviour of the material during TGA, which would be expected if changes in chemical structure, such as disulfide or peptide bond cleavage had occurred. Visual observation also suggests the formation of Maillard products resulting from exposure to 55 ^{o}C, but could not be confirmed.

In conclusion, the results indicate that NTP is UVA stable, as it was shown to be resistant to structural protein changes. However it is susceptible to embrittlement caused by loss of water and plasticiser resulting from prolonged exposure to UVA radiation.

For the purpose of determining the compostability and UVA weathering potential of NTP to determine its degradability in different environments, a replicate simulation should be carried out over a larger period of time with a full mass balance analysis to account for both CO_2 generated and other reaction products.

The major drawback to the industrial composting investigation of NTP was the fact it had to be stopped after twelve weeks. The experiment was designed to last for 24 weeks to determine whether NTP could be called a compostable bioplastic using ATSM standards, however, the weather conditions at the time of the

experiment caused the compost pile to become anaerobic between week twelve and fourteen, after which, no further degradation was observed.

To determine compostability over a longer period of time, it is proposed that it should be carried out using a small-scale closed system compost simulation. This is to control external conditions which may influence microbial activity and to also measure CO_2 generated as the process occurs. In addition, a toxicology analysis should be carried out to determine the impact NTP has on the compost generated.

The major limitation regarding the experiment for NTP exposure to UVA radiation to determine the effect it has on degradation, was that additional water was not incorporated into the investigation, it is known that water plays a major role in the facilitation of photo-induced oxidation of proteins and its impact should be determined in future research.

For determining the impact NTP will have if disposed into the natural environment, a full UV weathering simulation should be carried out to determine the influence humidity and UVA exposure time have on the overall breakdown of the material.

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Appendix 1

Novatein Thermoplastic Protein (NTP) Preparation

Materials

Bloodmeal ρ = 1300 kg/m^3 Sieved to 710µm (Taranaki Byproducts), technical grade sodium dodecyl sulfate (Biolab), analytical grade sodium sulfite (BDH Lab Supplies), agricultural grade urea (Agrinutrients-Balance), analytical grade triethylene glycol (BDH Lab Supplies), distilled water.

Equipment

ThermoPrism TSE-16-TC twin screw extruder, BOY 15-S injection moulder.

Method

1. To a beaker containing 60 g distilled water, 3 g sodium sulfite, 3 g sodium dodecyl sulfate and 10 g urea were added and stirred continuously on a stirrer/hotplate until the solution was 40-50 ^{o}C.
2. The hot solution was added to 100 g bloodmeal in a mixer.
3. The PNTP was blended for 2-3 minutes and then checked for homogeneity by manual mixing.
4. Addition of 10 g triethylene glycol to the mixture
5. The PNTP was further blended for 5-10 minutes until completely homogenous.
6. The PNTP was then processed via extrusion using temperatures 70, 100, 100, 120 ^{o}C from feed to die.
7. The extrudate was then injection moulded using temperatures 100, 100, 120 ^{o}C from feed to mould, with the mould pre-heated to 60 ^{o}C.

Appendix 2

Table 27: Odour Characteristics associated with rendering plants, slaughterhouses and animal by-product meals.

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in	Decomposition product of	Bacteria responsible
Sulfur Compounds						
Hydrogen Sulfide H_2S	[57] Sewer, Fecal	[58]0.00047	[58]0.0047	[37]Meat Rendering, meat meal [51]Swine operation	[37],[50]Reduction or deamination of sulfur containing amino acid cysteine.	[37]Pseudomonas putifaciens, Pseudomonas mephitica
Dimethyl Disulfide $(CH_3)_2S_2$	[58]Decaying Vegetables,	[51]0.0123	[59]0.0056	[37]Meat rendering, meat meal. [51]Swine operation	[37]Reduction or deamination of sulfur containing amino acid methionine.	[37]Lactobacillus helveticus, Achrobacter sp., Pseudomonas putifaciens, Pseudomonas fluorescens, Pseudomonas perolens, Bacillus sp., Ballcilus subtilis.
Methyl Mercaptan CH_3SH	[58]Decaying Cabbage, [37]Potato-like	[58], [51]0.0011	[58]0.0021	[37]Meat rendering [51]Swine operation	[37], [50]Reduction or deamination of sulfur containing amino acids cysteine.	[37]Achrobacter sp., Pseudomonas putifaciens, Pseudomonas fluorescens, Pseudomonas perolens
Aldehydes						
Hexanal $C_6H_{12}O$	[57] grassy, [37]Fruity, sour, sharp, pungent, almond	[57]0.0073-0.0129 [51]0.0138	-	[37]Meat Rendering [51]Swine operation	[158], [159] Lipid oxidation	[37]Candida utilis, Streptococcus spp., Lactic acid bacteria

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in	Decomposition product of	Bacteria responsible
2 –methyl butanal $CHOCH_2(CH_3)CH_2CH_3$	Fatty	-	-	[37] Meat Rendering [12] Swine operation	[160]Leucine	[37]Candida utilis, Streptococcus spp., Lactic acid bacteria
3-methyl butanal $CHOCH_2CH_2(CH_3)$ -CH_3	Fatty	[60]0.00015-0.0023 [51]0.00224	-	[37]Meat Rendering, meat meal [51]Swine operation	[160]Isoleucine	[37]Candida utilis, Streptococcus spp., Lactic acid bacteria
Benzaldehyde C_6H_5CHO	[51]Almond	[51]0.0417		[51]Swine operation		[37]Acinetobacter calcoaceticus
Ketones						
Acetone CH_3COCH_3	[37]Sweet	[37]0.46	-	[37]Meat meal	-	[37]Peniciilium sp.
Butanone $CH_3COCH_2CH_3$	[37]Dairy, cheese, mushroom	[51]7.76		[37]Meat Rendering [51]Swine operation	[160]β-oxidation of fatty acids by microorganisms [61]Thermal oxidation of saturated fatty acids	[37]Peniciilium sp.
Pentanone $CH_3COCH_2CH_2CH_3$	[37]Dairy, cheese, mushroom	[51]1.55		[37]Meat Rendering [51]Swine operation	[160]β-oxidation of fatty acids by microorganisms [61]Thermal oxidation of saturated fatty acids	[37]Peniciilium sp.
Hexanone $CH_3COCH_2CH_2$ CH_2CH_3	[37]Dairy, cheese, mushroom	[51]0.166		[37]Meat Rendering [51]Swine operation	[160]β-oxidation of fatty acids by microorganisms [61]Thermal oxidation of saturated fatty acids	[37]Peniciilium sp.
Heptanone $CH_3COCH_2CH_2CH_2$ CH_2CH_3	[37]Dairy, cheese, mushroom	[57] 0.14-0.28		[37]Meat Rendering [51]Swine operation	[160]β-oxidation of fatty acids by microorganisms [61]Thermal oxidation of saturated fatty acids	[37]Peniciilium sp.

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in	Decomposition product of	Bacteria responsible
Acetophenone $C_6H_5COCH_3$	[51]Orange Blossom	[51]0.363		[51]Swine operation	[61]Unknown	
Carboxylic Acids						
Formic acid CHOOH	[51]Pungent, formyl, sour	[51]28.2	-	[50]Putrefaction [51]Swine operation	[37], [50]Deamination of Aspartic Acid, Cysteine, Histidine, Threonine, Valine, Serine, Glutamic Acid	
Acetic acid CH_3COOH	[51]Pungent, Sour	[57] 0.02-0.48	[51]0.2	[50]Putrefaction [51]Swine operation	[37], [50]Deamination of Alanine, Asparagine, Aspartic Acid, Cysteine, Glutamic Acid, Glycine, Histidine, Valine, Lysine, Proline, Glutamine, Arginine, Serine, Threonine	-
Propanoic acid CH_3CH_2COOH	[37]Dairy, sour, rancid,	[57] 0.03-0.16		[37]Meat Rendering [51]Swine operation	[37], [50]Deamination of Alanine, Serine, Aspartic Acid, Cysteine, Glutamic Acid, Proline, Arginine, Threonine	[37]Streptoccus thermophilis, Propionibacterium freudenreichii, Propionibacterium acidiproprionic
Butanoic acid $CH_3CH_2CH_2COOH$	[37]Dairy, sour, rancid,	[57]0.0039		[37]Meat Rendering [51]Swine operation	[37], [50]Deamination of Glutamic Acid, Histidine, Glutamine, Serine, Threonine	[37]Clostridium acetobutylicum, Clostridium butyricum, Clostridium beijerinckii, Clostridium botulinum
2-methyl propanoic acid $CH_3CH_2(CH_3)COOH$	Sour, cheesy, butter	[51]0.0195		[50]Putrefaction [51]Swine operation	[37], [50]Deamination of isoleucine, leucine, valine	-

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in	Decomposition product of	Bacteria responsible
Pentanoic acid $CH_3(CH_2)_3COOH$	Putrid, sour, sweaty, rancid	[57]0.0048		[37]Meat Rendering [51]Swine operation	[37],[50]Deamination of Isoleucine, Leucine, Proline, Arginine, Serine,Threonine, Valine	-
3-methyl butanoic acid $CH_3CH_2(CH_3)CH_2$ -COOH	[57]body odour Sour, sweaty feet	[57]0.00036-0.0025		[50]Putrefaction [51]Swine Operation	[37], [50]Deamination of Isoleucine, Leucine and Valine	-
Hexanoic acid $CH_3(CH_2)_4COOH$	Sour, fatty, sweaty, cheesy	[57]0.0126		[37]Meat Rendering [51]Swine operation	[158]Lipid Oxidation	-
4-methyl pentanoic acid $CH_3CH_2CH_2CH_2(CH_3)$ -COOH	Pungent cheese	[51]0.0155		[51]Swine operation	-	-
Nitrogen Compounds						
Ammonia NH_3	[58]Sharp, Pungent	[58]0.037	[58]47	[37]Meat rendering [51]Swine operation	Deamination of amino acids	-
Methyl amine CH_3NH_2	[58]Putrid, Fishy	[58]0.021 [51]0.0186	[58]0.021	[50]Putrefaction [51]Swine operation	[37], [50]Decarboxylation of Glycine	-
Ethyl amine $CH_3CH_2NH_2$	[58]Ammonia-cal	[58] 0.83 [51]0.324	[58] 0.83	[50]Putrefaction [51]Swine operation	[37], [50]Decarboxylation of Alanine	-
Propyl amine $CH_3CH_2CH_2NH_2$	Ammonia-cal	[51]0.011	-	[37]Putrefaction [51]Swine operation	[37]Decarboxylation of Glutamic Acid, Histidine, Glutamine, Arginine	
Isobutyl-amine $(CH_3)_2CHCH_2NH_2$	Fishy	-	-	[50]Putrefaction	[37], [50]Decarboxylation of Valine	-
Ethanol amine $CH_2(OH)CH_2NH_2$	Ammonia-cal	-	-	[50]Putrefaction	[50]Decarboxylation of Serine	-
Phenylethyl-amine $(C_6H_5)CH_2CH_2NH_2$	Fishy	-	-	[50]Putrefaction	[37], [50]Decarboxylation of Phenylalanine	-

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in	Decomposition product of	Bacteria responsible
Tyramine $OH\text{-}(C_6H_5)CH_2CH_2NH_2$	Mild meaty, dirty, phenolic	-	-	[50]Putrefaction	[37], [50]Decarboxylation of Tyrosine	-
Trimethyl-amine $N(CH_3)_3$	Fishy, rancid, sweaty, fruity	[37]0.046 [51]0.0024	-	[37]Meat rendering, meat meal [50]Putrefaction [51]Swine operation		[37]Pseudomonas fragi, Shewanella putifaciens
Putrescine $NH_2(CH_2)_4NH_2$	[58]Putrid	[58]0.0037	-	[50]Putrefaction	[37], [50]Decarboxylation of Asparagine, Aspartic Acid, Cysteine, Isoleucine, Leucine, Methionine, Proline, Arginine, Serine, Threonine, Valine	-
Cadaverine $NH_2(CH_2)_5NH_2$	[58]Putrid, decaying flesh	-	-	[50]Putrefaction	[37], [50]Decarboxylation of Lysine	-
Piperidine $C_5H_{11}NH$	Sweet, floral, animal	-	-	[50]Putrefaction	[37]Decarboxylation of Lysine	-
Pyrrolidine C_4H_8NH	Ammonia-cal, egg	-	-	[50]Putrefaction	[37]Decarboxylation of Asparagine, Aspartic Acid, Cysteine, Isoleucine, Leucine, Methionine, Proline, Arginine, Serine, Threonine, Valine	-
Histamine $(C_3H_2N_2H)CH_2CH_2NH_2$	Fishy	-	-	[50]Putrefaction	[37]Decarboxylation of Histidine	[37]Lactobacillus sp.
Tryptamine $C_{10}H_{12}N_2$	-	-	-	[50]Putrefaction	[37], [50]Decarboxylation of Tryptophan	-

Name/Formula	Characteristic Odour	Odour Threshold (ppm)	Recognition Threshold (ppm)	Present in	Decomposition product of	Bacteria responsible
Indole C_8H_6NH	[58]Fecal	[51]0.000032	-	[50]Putrefaction [51]Swine operation	[37], [50]Decarboxylation of Tryptophan	-
Skatole C_9H_8NH	[58]Fecal	[58]0.0012 [51]0.00056	[58], [59]0.47	[50]Putrefaction [51]Swine operation	[37], [50]Decarboxylation of Tryptophan	-
Furans						
2-pentyl furan $C_9H_{14}O$	[37]Sweet, bitter, almond like Green, earthy, beany	[57]0.016-0.048	-	[37]Meat meal [51]Swine operation	[158]Lipid Oxidation	-
Alcohols						
Butanol $CH_3(CH_2)_3OH$	[51]fusel oil	[51]0.490		[37]Meat rendering [51]Swine operation		
Pentanol $CH_3(CH_2)_4OH$		[51]0.468		[37]Meat rendering [51]Swine operation		
Hexanol $CH_3(CH_2)_5OH$		[51]0.0437		[37]Meat rendering [51]Swine operation		
Heptanol $CH_3(CH_2)_6OH$		[51]0.0251		[37]Meat rendering [51]Swine operation		
Phenol C_6H_5OH	[51] Phenolic	[51]0.110		[51] Swine operation		
4-methyl phenol $CH_3C_6H_5OH$	[5] Phenolic	[51]0.00186		[51]Swine operation		

Appendix 3

Sensory Panel Analysis

To supplement the results obtained from HS-SPME-GC/MS analysis and to determine the overall impact of the treatment methods employed to minimise odour a sensory panel analysis was carried out. The panel consisted of 25 members who were asked to rank the odour of treated bloodmeal and treated PNTP relative to untreated bloodmeal and PNTP. This method was developed in a similar manner to the sampling technique outlined in ASTM E1885, "Standard Test Method for Sensory Analysis—Triangle Test"

PNTP was prepared using the treated bloodmeal and physical sorbents outlined in text. The samples were placed in sealed 50 mL polyethylene containers with 10 g of bloodmeal that resulted from each treatment and 10 g of PNTP resulting from the treated bloodmeal.

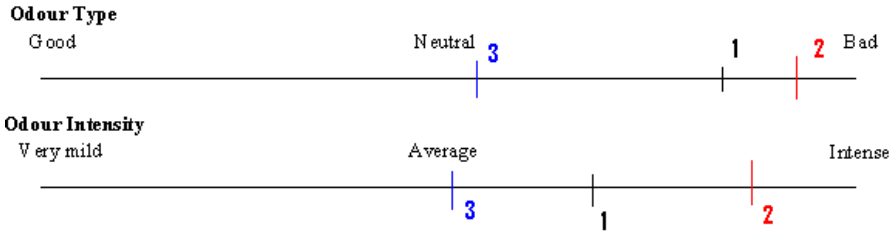
Samples:

Control 1: Polyethylene, 2: Bloodmeal, 3: PNTP

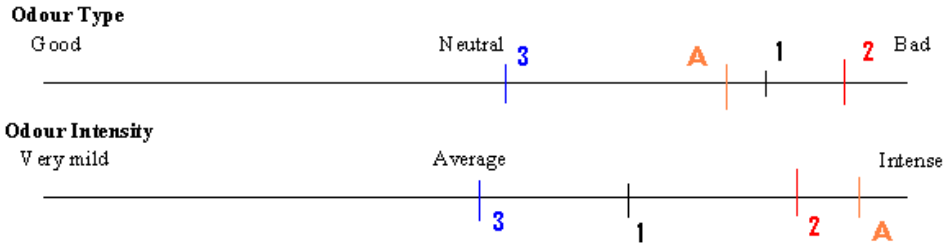
Samples: A: $NaClO_2$, B: 1.5 % peracetic acid, C: HCl, D: Red blood cells, E: H_2O_2, F: 5 % peracetic acid, G: NaOH, H: bloodmeal, I: Polyethylene/lilac, J: RBC-PNTP, K: NaOH PNTP, L: 25 wt% B-cyclodextrin PNTP, M: 25 wt% natural zeolite PNTP, N: 10 wt% activated carbon PNTP, O: $NaClO_2$ PNTP, P: 5 % peracetic acid PNTP, Q: HCl PNTP, R: Polyethylene/lilac, S: 10 wt% natural zeolite PNTP, T: H_2O_2 PNTP, U: 25 wt% activated carbon PNTP, V: 15 wt% activated carbon PNTP, W: 15 wt% natural zeolite PNTP X: polyethylene, Y: PNTP, Z: 1.5 % peracetic acid PNTP.

Method:

The panel were first asked to rank the controls by smelling the controls 1, 2 and 3 and placing a mark on an unnumbered line to represent their perception of the odour type and intensity of the controls, as shown below:



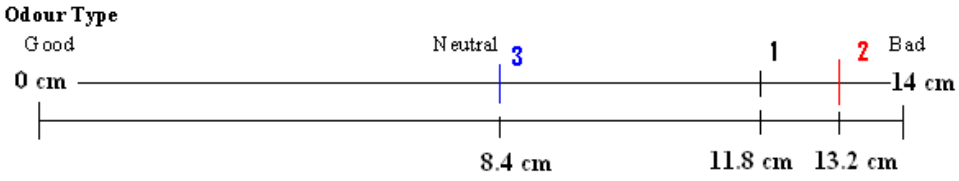
The panel were asked to smell the sample and comparing it to the smell of the controls, and after doing so, use the markings that were made on the scales for the controls to aid ranking the samples, as shown below:



After smelling the sample, the panel member was asked to smell coffee before carrying on to the next sample. This was repeated until all samples A-Z were analysed.

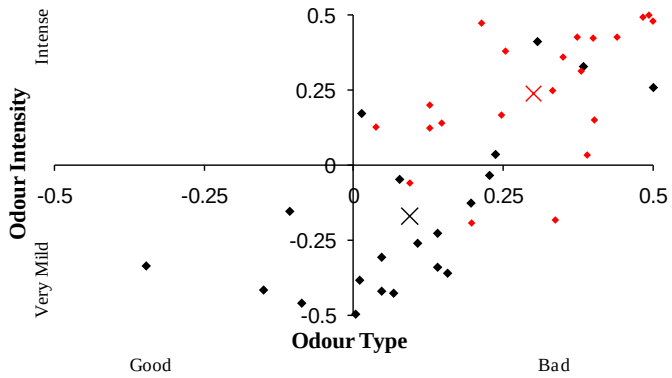
Analysis of results:

Once all the samples were analysed, the total length of each scale was measured, and the marking for each control (1, 2 and 3) and sample (A-Z) was determined as a proportion of the length, which can be plotted as shown below:



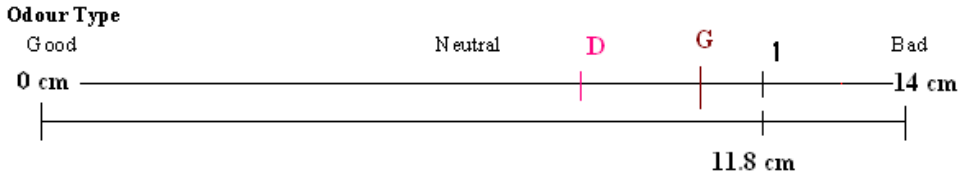
1 = bloodmeal = 11.8/14

2 = PNTP = 13.8/14



The relative improvement of the treatment relative to the corresponding control was determined by the difference in size of each allocated marking, and the error

associated with each result was determined by difference between the external and internal control, as shown below:

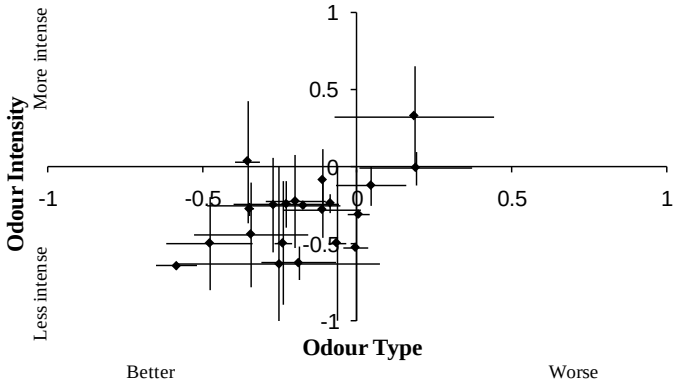


1 = bloodmeal = 11.8/14

G = Bloodmeal Internal control = 10.6/14

D = Activated carbon 15 wt% = 9.2/14

Each proportion determined by difference and the error associated with that result for each panel member, was determined for odour type and intensity for every sample assuming the external control to be no improvement, (0,0), as shown below:



Based on the relative improvement of odour type and intensity, the effectiveness of treatment type on the overall odour profile of bloodmeal and PNTP was determined.

Appendix 4

Compost Week 2 (TEG)

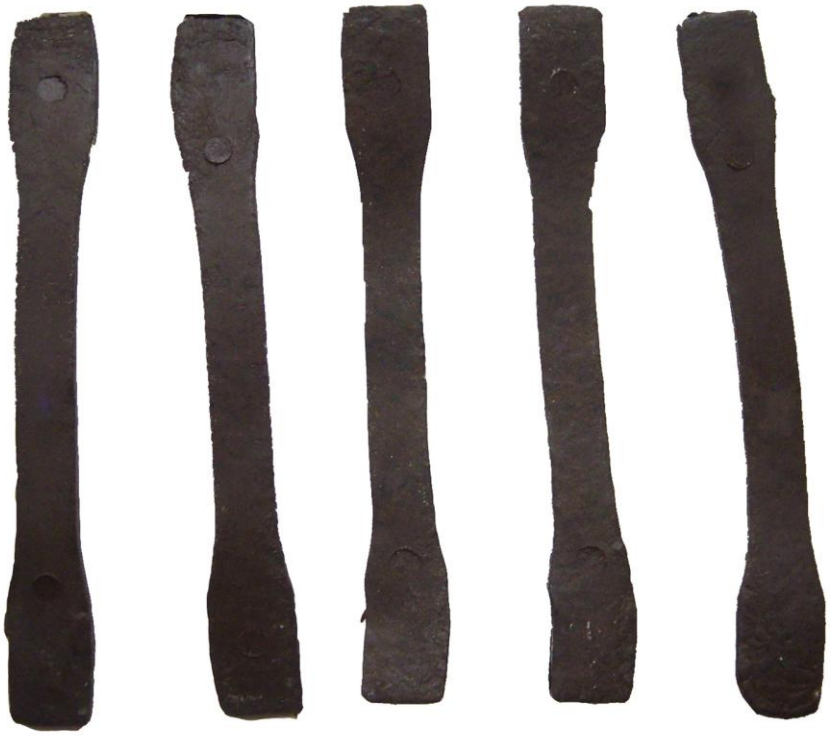
Compost Week 4 (TEG)

Compost Week 6 (TEG)

Compost Week 8 (TEG)

Compost Week 10 (TEG)

Compost Week 12 (TEG)



Compost Week 2 (WOP)

Compost Week 4 (WOP)

Compost Week 6 (WOP)

Compost Week 8 (WOP)

Compost Week 10 (WOP)

Compost Week 12 (WOP)

UV Week 2 (TEG)

UV Week 4 (TEG)

UV Week 6 (TEG)

UV Week 8 (TEG)

UV Week 10 (TEG)

UV Week 12 (TEG)

UV Week 2 (WOP)

UV Week 4 (WOP)

UV Week 6 (WOP)

UV Week 8 (WOP)

UV Week 10 (WOP)

UV Week 12 (WOP)