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DEVELOPMENT OF POLYANILINE AS A SENSOR FOR FOOD QUALITY AND
SPOILAGE DETECTION

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Declaration

This is a declaration to certify that no portion of the work referred to in this these has been submitted in support of an application for another degree or qualification of this or any other university, institute of learning.

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Abstract

This thesis describes the research that has been completed for the application of polyaniline as a food quality indicator.

It has been reported by WRAP (Waste and Resources Action Programme) that in the UK alone, a third of all purchased food items are thrown away regardless of the quality or condition. It has also been reported by DEFRA (Department for Environment Food and Rural Affairs) that the food manufacturing and processing industry is one of the UK's largest producers of land filled waste.

At present, the available technology for food freshness determination is largely based on use-by dates which are often recognised as unreliable - or qualitative time temperature indicators (TTIs); which can be costly and do not give an actual measurement of bacterial activity. It is anticipated that the technology produced from this research will give a viable and low cost solution to help minimize preventable food waste from consumers - as well as improving food industry process efficiency, especially in the field of food supply chain management.

The sensors being developed employ a conducting polymer film as a sensor which reacts with volatiles evolving from selected food products (salmon and herring). As food biochemically degrades, the concentrations and volumes of these gases change, and this has been studied by analytical techniques such as gas chromatography and SIFT-MS. Food spoilage has also been followed by the growth and identification of specific spoilage bacteria. The sensor exhibits a number of quantifiable physical changes when exposed to differing volatile mixtures produced by the food stuffs. These physical properties include colour and conductivity changes which are distinctive and easily measurable.

Correlations have been shown between increases in microbial activity and the change in conductivity of the films. These sensors will be able to inform consumers more reliably when the food is safe to consume as well as providing the food industry with more information on traceability and stock conditions of fresh meat and fish. In the context of the catering industry, these sensors will also aid in the decreasing the number of reported cases of food poisoning by observing - in real-time - the condition and safety of food.

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Nomenclature

Abbreviations

dc	Direct Current
ac	Alternating Current
S	Siemens
E/e	Voltage in ac
I/i	current in ac
X	Reactance
PANI	Polyaniline
GCMS	Gas Chromatography Mass spectrometry
SIFT-MS	Selected Ion Flow Tube Mass Spectroscopy
RFID	Radio Frequency Identification
v	Velocity
μ	Growth rate/micro
λ	Lag time
CFU	Colony forming unit
τ	time
TMA	Trimethyl amine
DMA	Dimethyl amine
TVB-N	Total volatile basic nitrogen
FQI	Food quality indicator
TTI	Time temperature indicator/integrator

Chapter 1

Research Rationale

1 Research Rationale

This EngD project involves collaboration between Timestrip and Cranfield University to develop innovative electronic and optical quality control sensors for use in time critical applications. The focus on this EngD is to produce a sensor that can be used in the food retail industry to indicate the freshness of food products. This sensor will react either with certain gases/volatiles – or to specific volatile conditions (acidity/alkalinity) associated with degrading food. The measurement of the reaction of the sensor to these volatiles will be correlated with information and studies on the population of bacteria present on the food. The sensor that has been studied within this research has been the conducting polymer polyaniline. The polymer is interrogated to follow the change in its optical, resistance and/or impedance properties when exposed to degrading food samples.

Organic conducting polymers such as polypyrrole, polyaniline or polyacetylene are defined as polymers with spatially extended π -bonding systems obtained by electropolymerisation or chemical oxidation (Hush, 2003; Trojanowicz, 2003) and which allow electrical conductivity in at least one redox state or by chemical doping. Over the last 25 years the electrical properties of these materials have been studied intensively. This has given rise to a range of useful applications in various areas of chemical analysis, gas sensors and conductive thin – layered coatings. Recognition for this work was marked with the 2000 Nobel Prize in chemistry being awarded to Shirakawa, MacDiarmid and Heeger for their discovery and subsequent research on halogen derivatives of polyacetylene in 1977 (Shirakawa et al., 1977). It is estimated that the total number of papers on conducting polymers up to the year 2002 stood at 40,000 papers and there has still been significant growth to date (Li et al., 2009). At present the amount of reference material available from ScienceDirect on conducting polymers stands at 43,801 articles. Figure 1.1 shows the extent of research carried out on polyaniline and the pattern of growth of reference material over the last 20 years.

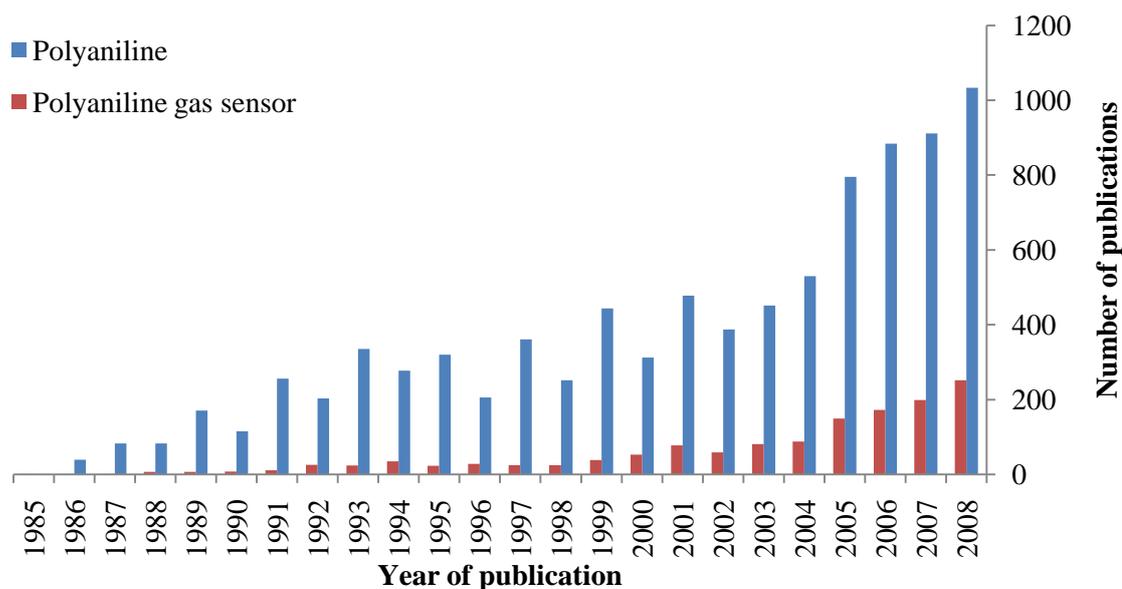


Figure 1.1 The annual number of publications on the subject of "polyaniline" incorporating all applications and "polyaniline gas sensor" as provided by the search engine of ScienceDirect.

Polyaniline was first prepared in 1862 (Letheby H., 1862), however, it was not until the 1980s and 1990's that the number of papers published around the chemistry of polyaniline grew to an accelerated degree. Polyaniline may be easily made and is usually fabricated by the oxidation of aniline via either electrochemical or chemical oxidation – normally under acidic conditions (Feast et al., 1996). Polyaniline has potential to be used as a sensing material for gaseous molecules which has been demonstrated by the growth of research in this area as seen above in Figure 1.1. Another aspect of this project is studying the deposition of polyaniline thin films and optimising film thickness and responsiveness to certain gas molecules.

The main body of the research in this thesis will be focusing on the changing characteristics of polyanilines conductive properties for dc and ac circuits whilst exposed to degrading food stuffs. It is known that polyaniline exists in one of its four oxidation states (Lindfors and Ivaska, 2005). The change in resistance and impedance of polyaniline that has been measured is attributed to the change in oxidation state of the material. Other work will concentrate on the biology of the degradation of food stuffs and correlating volatile emissions to bacterial growth. These results will then be linked back to the previous study of changes in conductance of polyaniline when exposed to the food stuff.

Timestrip plc is an enterprise with a UK head office in Hitchin, Bedfordshire. They currently have a market capitalisation of over £26 million and trade on the AIM section of the London Stock Exchange. Timestrip currently produce indicator labels that can measure a period of elapsed time from activation. This time period ranges from fourteen days to periods of six months. The labels are activated when a sealed dye reservoir has been broken which allows the dye to flow down a capillary tube via capillary action. Figure 1.2 shows the current format of the dye based indicators. These labels are used for applications in food and medicine storage to allow users to know when the items have perished and should be discarded.



Figure 1.2 The product portfolio of Timestrip at present.

A ‘Timestrip’ attached to a product would inform the user of a given time before the product would have to be consumed by, rather than relying on the sell by date. This novel product has created a new food safety market and has allowed Timestrip to gain an impressive client list, including Nestlé, Henkel, and Whirlpool among others (Hill 2007).

Timestrip are also aiming to enter novel markets for newly emerging applications of sensors in supply chains. At the moment there are a number of examples of RFID non intelligent sensors being deployed into supply chains for minimising shrinkage and wastage. Sensors such as these take the form of very small tags that are attached to goods in a supply chain. These labels allow easy identification and location of products in the supply chain. Timestrip propose to capitalise on the many obvious benefits from developing a sensor product that can also analyse the product that it is attached to. The main differentiation is that their sensor will give an indication of its freshness and more importantly for the retailer how many shelf days a product has before it should be withdrawn from sale. This technology could, moreover,

having been developed be applied to a variety of further applications. This may include the development of intelligent wound dressings that would inform clinicians of when the wound needs to be re-dressed.

The issue of food wastage from consumers, suppliers and retailers has recently been highlighted by several organisation and governmental departments, including the Waste and Resources Action Programme (WRAP) and the Department for Environment, Food and Rural Affairs (DEFRA) respectively. Food Wastage is seen as an unnecessary burden for the environment since the majority of uneaten and spoiled food and discarded food packaging is ultimately sent to land fill. With the introduction of government targets to reduce volumes being sent to landfill, a technology that could monitor and suggest improvement to food manufacturers and suppliers would be of great importance. The types of intelligent packaging, the market structure and other significant research will be discussed in the literature review.

1.1 Research objectives

The overall aim of the project was to produce a sensor that can act as an early warning for unsafe food for consumption. The overall synopsis is to produce a sensing element that does not require contact with the foodstuff being analysed and can be integrated easily into existing food packaging. Further detailed objectives that were completed within this EngD are detailed below.

The first objective of this project was to gain an understanding of the chemistry occurring between the polyaniline strips and the gas within the head space of the degrading food stuff-salmon or herring. This was necessary so that the results were then used to understand the processes occurring on polyaniline, such as for example, a reduction/oxidation of the polymer.

The second objective was to analyse the headspace of the gas generated using a range of complimentary gas chromatography techniques. This has included SIFT-MS (selected ion flow tube – mass spectroscopy) and GCMS-TD (thermal desorption gas chromatography mass spectroscopy). An understanding of the change in the gaseous environment within the pseudo packaging overlapped with studies on the changing populations of bacteria on the

foodstuff. For this project the focus has been on changes in total populations and changes in specific spoilage organisms.

A further objective of this research was to improve and optimise the method of manufacturing the polyaniline strips that are to be used in this project. The main objective has been to make the process more reproducible as well as maintaining the low cost of manufacture for the sensor. Originally, the method for making the strips was to some extent irreproducible and gave inconsistent thickness of coating of polyaniline, which affected the resistivity of the material and hence the resistance/impedance signal changes.

Further objectives are to produce a fully working prototype that can be used in the supply chain and be incorporated into packaging. This must be durable, inexpensive and easy to manufacture. The prototype will also have to suit the other components and specifications that are being developed by Timestrip and are outside the scope of this project; examples could include here the insertion of a power source for the sensor.

The final objective is to develop the management and commercial aspect of producing these labels from an end consumer (marketing) and retailer (supply chain management) perspective. This involved a study that ran alongside the technological development of the sensor so that considerations could be made for the design of the sensors. A study into the amount of wastage that occurs in the food supply chain and a study of supply chain best practices, provided indications for the optimal deployment of the sensors. A market summary of intelligent labels is also included.

The results presented within this thesis are in the process of being transformed into research papers that aim to be published as soon as awaiting patent applications have been filed.

Chapter 2

Introduction and Literature Review

2 Introduction

The aim of this chapter is to provide background and an insight into the scientific principles and theory surrounding the experimental work that is presented in this thesis. A short overview of polymer chemistry is followed by a specific review of the nature of conducting polymers. The research carried out for this project involved polyaniline and there is a detailed review of recent literature into the use of this polymer as a sensor for volatile gases. The next section of this chapter provides a summary on the aspects of food spoilage and safety, followed by a review of food spoilage technologies and research.

2.1 Polymers: A background

Polymer chemistry relates to large sized molecules which are often referred to as macromolecules. They are defined as substances consisting of large molecules that are made from repeated units (monomers) and held together by covalent bonds (Daintith and Martin, 2005) and can be categorised into two main groups. These are condensation polymers and addition polymers. The grouping determines the mechanism of synthesis. Polymers exist in nature such as DNA, natural rubber and cellulose but it is in the last 100 years that mankind has harnessed the understanding and ability to synthesise polymers in the lab. The uses of polymers range from fibres, plastics to surface coatings and resins.

Polymers can exist as two units (dimer), three units (trimers) or several units (oligomers). The amount of repeating units is referred to as the degree of polymerisation and is given the symbol \bar{X}_n which can be used in determining the relative molar mass of a polymer by multiplying this factor with the molecular mass of the monomer. The degree of polymerisation is usually represented schematically by n as can be seen in below in Figure 2.1 which represents the addition polymerisation of ethene to polyethene.

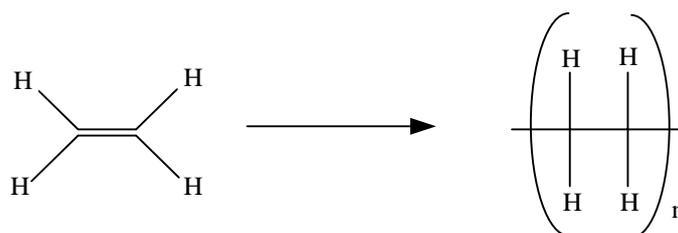


Figure 2.1 Polymerisation of ethene to polyethene

Polymers are of huge economic and social importance in the modern age from the production of large volume bulk materials such as packaging, synthetic fabrics and fibres like Nylon and polyesters to highly specialised polymers, for example Kevlar™ and catalysts (Nicholson, 1991).

2.1.1 Polymerisation

As mentioned previously, there are two main methods of polymer synthesis, namely, addition and condensation. Addition polymerisation requires the monomer to be unsaturated (i.e. have a carbon-carbon double bond) as in the example in Figure 2.1. Condensation polymerisation requires two different starting materials, such as a dicarboxylic acid and a diol, to produce a polymer and a small side product; in this case a polyester and water are produced. A schematic of this reaction can be seen below in Figure 2.2 where X and Y represent an alcohol group and a carboxylic acid group respectively. The rest of the molecule is omitted for clarity. The combination of the two functional groups has produced XY as a waste product (i.e. water). The mechanism of polymer chain length increase for condensation reaction is via a step-growth polymerisation. Figure 2.2 shows the result of two steps of polymerisation. The chain length increases by a repeating unit every time an X and Y monomer terminal react.

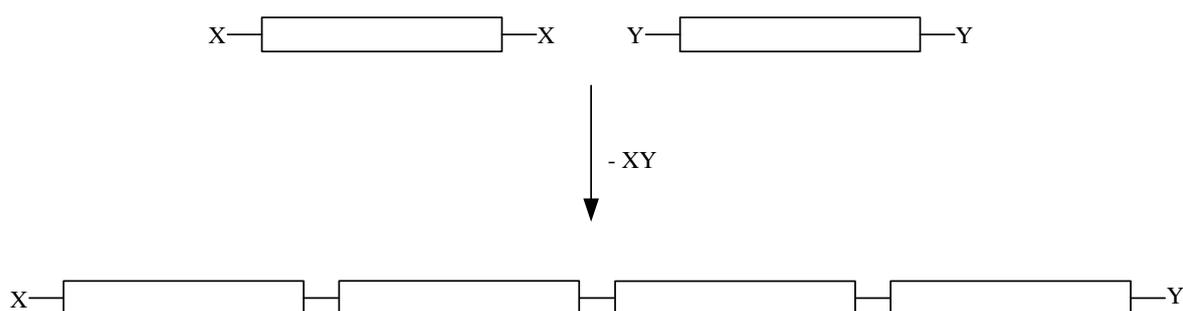


Figure 2.2 Simplification of the condensation polymerisation

Other groups such as amides can act as the X or Y group and produce amide and peptide bonds that are found correspondingly in synthetic fibres and proteins.

The mechanism involved in addition polymerisation is known as chain polymerisation. Chain reactions occur after the formation of either a free radical or ionic species in solution. Once initiated, the reaction is propagated by the continuous replenishment of free radical species until the concentration of unreacted substance is extremely low or zero. The reaction terminates when two free radicals or charge species combine.

2.2 Conducting polymers

This section draws on previous research in the field of a specific branch of organic polymers that are able to transfer an electric current through the polymer chain. For the main part of their history, polymers were deemed to be excellent electrical insulators and were applied to uses such as cable coatings and coatings to prevent corrosion. Over the last forty years, research has shown that conducting polymers can be produced to rival the conductance of metals such as copper as well as having all of the other attributes that are expected of an organic macromolecule (Heeger, 2001).

2.2.1 Background

A conducting polymer is an organic based polymer that can act as a semiconductor or a conductor. Examples include polyacetylene, polypyrrole, polythiophene and polyaniline. The first instance of polymer conduction was observed in 1963 by Bolto, McNeill and Weiss (Bolto et al., 1963). The research showed that some polypyrroles showed signs of conductivity. Polypyrrole is a derivative from polyacetylene and can be seen in Figure 2.9. The conducting polymers discussed within this thesis all have spatially extended π -bonding systems that allow the transfer of electrons or other charge carrying species. These are often referred to as conjugated polymers in the literature (Pron and Rannou, 2002; Kertesz, 1997).

Conjugated polymers can act as semiconductors or conductors. The terms refer to the formation of bands of electrons and shells within compounds and elements (Pron et al., 1988). Band theory is used to explain the mechanisms of conduction in solids. The theory stems from the formation of energy bands in solid materials from discrete orbital energy levels found in single atom systems. Bands form because of the affect of neighbouring nuclei in a 3d lattice on the electronic energy levels. Once these bands have formed they can facilitate electrons passing from one quantum state to the next if there overlap between bands

or sufficient energy to overcome any band gaps. The level of electrons in a system which is reached at absolute zero is called the Fermi level. The Fermi level in a given system represents the energy in a solid at which the average number of particles per quantum state is half, i.e. one half of the quantum states are occupied. In a conductor, the Fermi level sits within the conduction band, whereas in an insulator the Fermi level rests within the valence band. For semiconductors, the Fermi level lies within the band gap, i.e. the space between the conduction and valence band (Daintith and Martin, 2005).

In terms of electronic configuration, the valence band has the outer most electrons. If this band is full, there is nowhere for electrons to move to which means that the material is an insulator. For electrons to be able to move requires the Fermi level to be within a partly filled band, known as a conduction band, which allows electrons to transfer through these different quantum states. Most metals are good conductors because the conduction band is not completely filled or there is sufficient overlap with the valence band, allowing the conduction band to provide different electronic states, as seen in Figure 2.3. The schematic gives the 3p and 3s electron shells for a single metallic atom in the third period of the periodic table that overlap to become bands that overlap in energy.

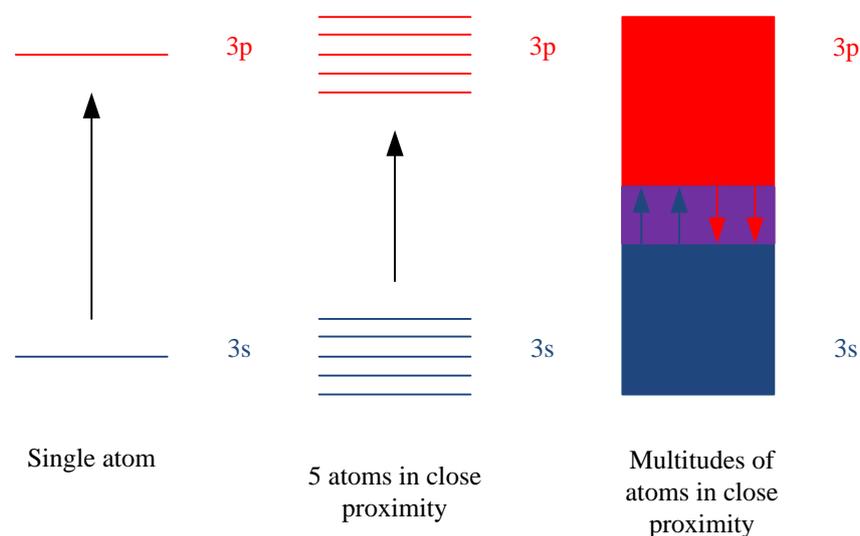


Figure 2.3 The formation of bands in a conducting solid in the 3rd period and overlap between the valence and conduction bands (Source: Atkins, 2001)

In an insulator, there is no overlap of the valence and conduction bands and there is a sufficient gap of energy between the two bands. Electrons cannot easily move between the

two bands and therefore the flow of electrons is prohibited by this gap in energy. The energy gap can be made up of bands of forbidden electronic states or represent the gap between the next available energy level for electrons. In an intrinsic semiconductor, this band gap is much smaller than an insulator and providing that enough energy is given to the solid via a thermal change or disturbance in electronic field then electrons can jump this gap and move from the valence band into the conduction band, leaving behind a hole in the valence band. At absolute zero the semiconductor would act like an insulator but at a given temperature there is enough energy to allow electrons to transfer into the higher band. Both of these situations can be seen in below in Figure 2.4.

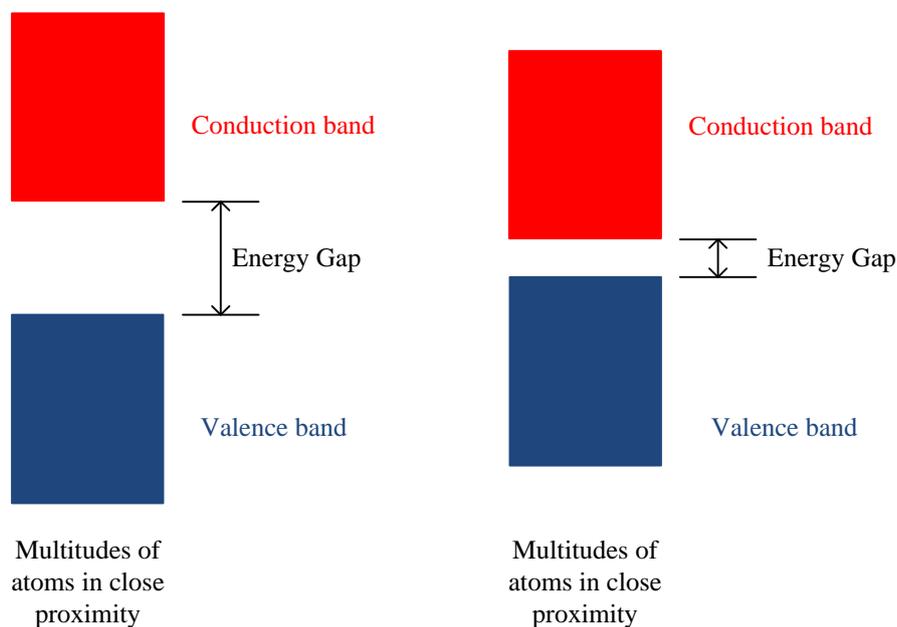


Figure 2.4 The energy gaps between bands in an insulator and a semiconductor (Source: Atkins, 2001)

There is also a way to make semiconductors out of material that would normally act as an insulator. There are two types of semiconductors which arise from the placing of impurities into the original material. These are known as extrinsic semiconductor and the band gap in these materials have foreign orbitals for electrons to use. In an extrinsic semiconductor there are two types of mechanisms for conduction that occur. Silicon for example, has a valence of four; that if doped with something of a higher valence (for example arsenic) this creates an extra electron per atom. This allows partial filling of the conduction band with these unpaired electrons. This type of conduction is called n-type semiconduction and uses electrons as the main charge carrier. The other example to consider is if a material is doped

with a material with a lower valence, for instance, silicon with boron (valence of three). Here, the valence band is now depleted of some electrons creating a hole for each electron missing from a bonding pair. The main charge carrier here is the holes that are formed when the electrons move out of the valence shell. These are known as p-type semiconductors. Figure 2.5 shows the methods of conduction for both of these types of semiconduction.

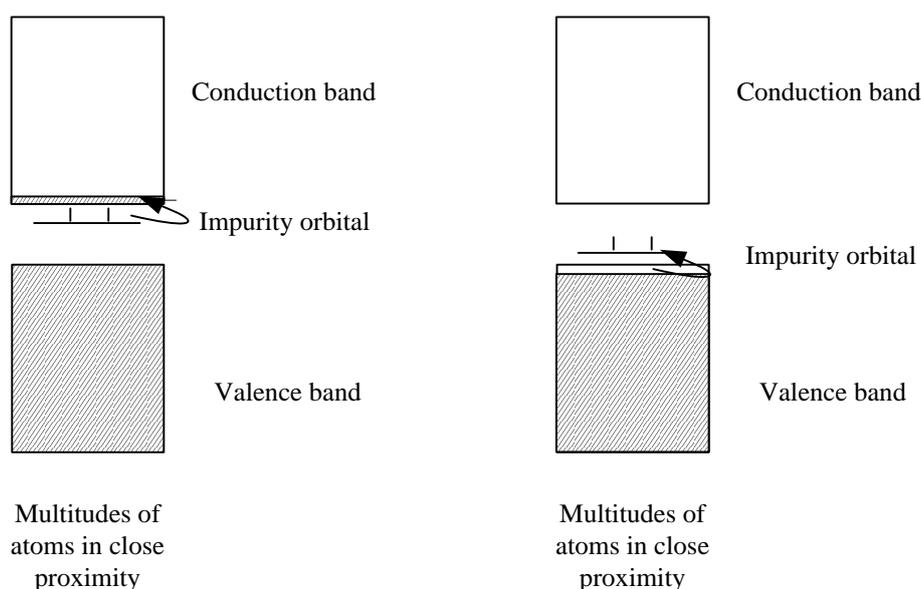


Figure 2.5 The different types of semiconductor, extrinsic and intrinsic (Source: Atkins, 2001)

Other materials other than inorganic solids and metals can behave as semiconductors and conductors. Material once thought to be insulating or at best semiconducting was proven to be conducting under the right conditions. In 1977, work by Shirakawa, MacDiarmid and Heeger was published on the conductivity of polyacetylene (Shirakawa et al., 1977). This work contributed to the authors receiving a shared Nobel Prize in 2000 and focused on the effect of doping the polyacetylene semiconductor films with halogen derivatives to create a conducting polymer. Polyacetylene does not behave as a normal semiconductor such as silicon (Przyluski, 1991). The band gap created by undoped polyacetylene (Figure 2.6) is expected to be large and to act like an insulator. This was not the case.

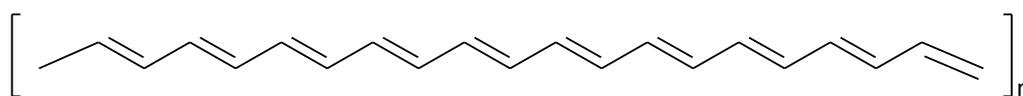


Figure 2.6 Undoped polyacetylene

This is derived from the fact that we consider polyacetylene to be a 1 dimension lattice. It is composed of sp^2 hybridised carbon atoms and can be viewed as a simplified representation of graphite. The carbon-carbon bonds in graphite are of similar length, however this is not the case in polyacetylene. There are 3 electrons in each sp^2 hybrid which results in a half filled orbital on some of the carbons in the chain.

This produces a Peierls distortion (Kertesz, 1997; Atkins, 2001) as the carbon atoms change the length of the covalent bonds to get to a lower energy level. The change in bond distance is seen to decrease by 0.06 Å (comparing to 1.54 Å for C-C and 1.34 Å for C=C) (Przyluski, 1991). This distortion allows the polymer to act as semiconductor by moving the highest occupied molecular orbit (HOMO) and the lowest unoccupied molecular orbital to within 1.5 eV. This can be seen in Figure 2.7 where the HOMO level is a π bonding and the LUMO level is a π^* antibonding. The band gap is described as “soft”. This means that the gap is changeable by adding electrons (doping) or by heating (Doblhofer and Zhong, 1991).

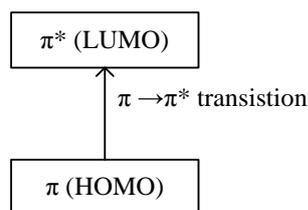


Figure 2.7 The transition of electrons in polyacetylene that allows it to behave as a semiconductor (Source: Pron et al., 1988)

Polyacetylene conducts when mistakes or defects occur in the bond alternation. This creates single electrons that occupy a level of energy in the middle of the band gap. Note that this is the reverse of a normal semiconductor (as temperature increases, conductance increases) because as temperature increases, the number of defects increases and therefore the number of unpaired electrons increases.

The doping of polyacetylene is more of a redox process than the doping of the inorganic semiconductor counterparts (Levi and Aurbach, 2008). The electron addition or removal via an oxidation or reduction causes the polymer to conduct like a metal. For most conjugated polymers, n-type doping refers to a reduction where as p-type doping refers to an oxidation (Heeger, 2001). The hybridised orbitals lose or gain electrons which change the band from being half full and vary the Fermi level. This corrupts the Peierls distortion that occurs when

the band is half full and creates a more delocalised structure of bonding as seen in Figure 2.8. The attraction of an electron in one repeat unit to the nuclei of a neighbouring atom leads to this structure.

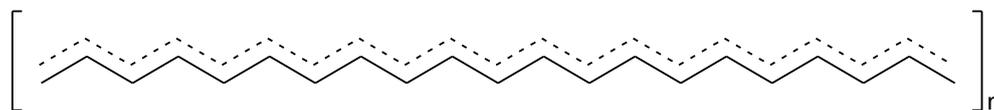


Figure 2.8 Doped polyacetylene with delocalised electrons, which acts as a metal

The material is then free to conduct electrons or transfer positive holes at a much higher conductivity, closer to that of metallic copper (Heeger, 2001). This is the mechanism for conduction that most conducting polymers follow. This is obtained by the overlapping p_z orbitals of the carbon atoms in the chain (Pron and Rannou, 2002; Pron et al., 1988).

The other conjugated polymers follow a similar method of conduction with the overlap of bands formed by distortion. Figure 2.9 below shows the structures of majority of conjugated polymers that can conduct electricity. Most of the examples utilise the electron rich benzene rings in their mechanism of charge transfer. The aromatic ring also offers delocalised π electrons into the polymer chain. Polyaniline has a range of different properties due to the overlap of p_z orbitals from the nitrogen atoms in the chain (Pron and Rannou, 2002).

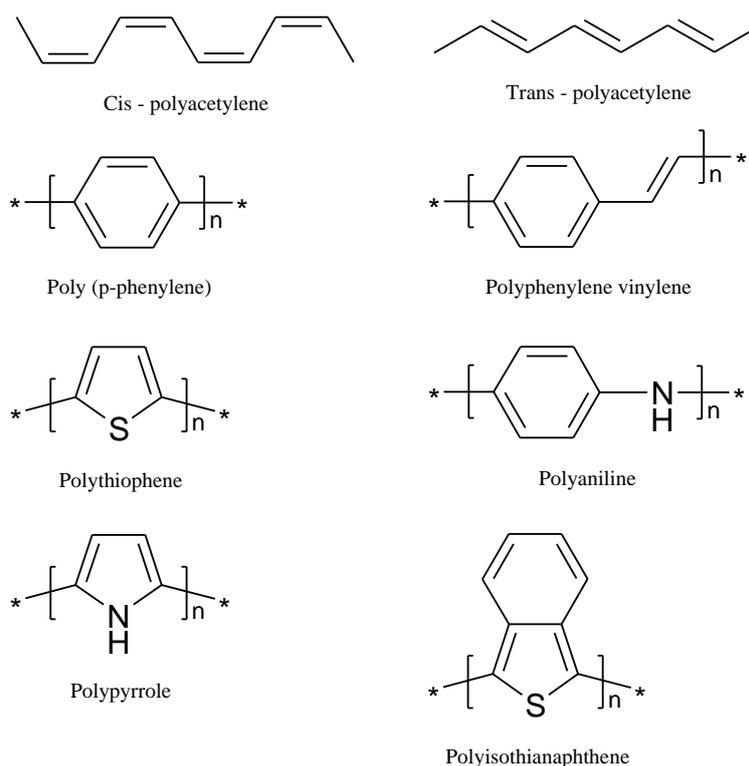


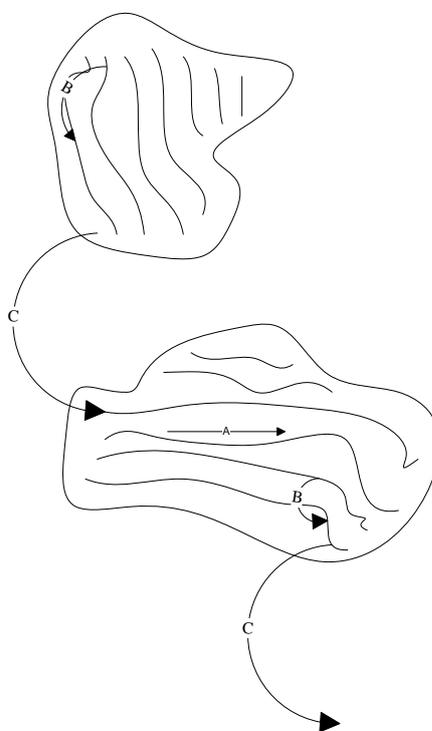
Figure 2.9 Typical (shown uncharged) structures of conducting polymers (Source: Walton and Lorimer, 2000)

The different characteristics of some of the conjugated polymer are given below in Table 2.1. Polyaniline has excellent thermal stability and provides a reasonable conductivity measurement (Chiang and MacDiarmid, 1986). In terms of processability, polyaniline is rated as good and can therefore be used in a wide variety of applications. In terms of cost, aniline is far cheaper than any of the other monomers used to synthesise conjugated polymers making the use of this polymer much more economic. The synthesis of polyaniline is also much easier compared to methods required to produce the other conjugated polymers. The properties of polyaniline can be tuned more easily than other conducting polymers. These reasons contribute to why polyaniline is popular among research communities in this field (Feast et al., 1996; Bhadra et al., 2009; Gospodinova and Terlemezyan, 1998).

Table 2.1 the conductivity, stability and processability of a number of doped conjugated polymers (Source: Bhadra et al., 2009)

Polymer	Conductivity (S cm ⁻¹)	Stability	Processability
Polyacetylene	10 ³ - 10 ⁵	Poor	Limited
Polyphenylene	1000	Poor	Limited
Poly(phenylene vinylene)	1000	Poor	Limited
Poly(phenylene sulphide)	100	Poor	Excellent
Polypyrroles	100	Good	Good
Polythiophenes	100	Good	Excellent
Polyaniline	10	Good	Good

The transfer of charge (positive hole or electron) through the backbone chain of a polymer is just one method in which conduction can occur. Polymer chains are often held together by intramolecular covalent bonds or intermolecular dipole-dipole bonds. In a large piece of polymer that is synthesised in the lab, charge transfer can take place between the intermolecular bonded chains. Often in a polymer mix there are large colloids of polymer particles that exist. Therefore charge transfer can also occur between these large particles (Li et al., 2009). Figure 2.10 below shows a schematic of these three systems of charge transfer between particles of conjugated polymer.

**Figure 2.10** Schematic of conduction pathway in a conducting polymer showing A = Intrachain, B = Interchain and C = Interparticle (Source: Walton and Lorimer, 2000)

2.2.2 Uses of conducting polymers

Heeger summarised in 2001 that the field of conducting polymers was an exciting and growing field due to four opportunities it had created. The overlap of chemistry and physics meant that the research carried out on conducting polymers was multidisciplinary. The four main opportunities were that:

1. Research on conjugated polymers increased the understanding of the underlying chemistry and physics of π -bonded macromolecules.
2. Research on conjugated polymers allowed studies to be carried out on the bond length alternation in long chained polymers and the importance of the interaction of electrons throughout the polymer lattice.
3. Research into the metal-insulator transition and the effect of Peierls distortions as well as instability of on one dimensional metal lattices.
4. Research into the use of these polymers which share similar optical and electronic properties with metals and semiconductors while still retaining the mechanical and chemical properties of polymers.

This final point is suggested as the most important and is where the bulk of contemporary research is carried out on conducting polymers (Heeger, 2001). Following from the successful synthesis and doping of polyacetylene, the area of electronic conducting polymers has received great interest. This is due to the growing amount of applications of this relatively new scientific field. These include polymer/oxide batteries (Kerr et al., 1996), redox super capacitors (Hashmi and Upadhyaya, 2002), molecular recognisers (Teasdale and Wallace, 1993) and as light emitting diodes (Kim et al., 2000).

Conducting polymers can be used in a wide variety of other applications. These vary depending on the methods used in doping the polymer. The uniqueness of these materials stems from the ability to conduct electricity and have the same inherent properties of normal polymeric materials. The controlling of the Fermi level over the energy gap allows

conjugated polymers to be used as light emitting diodes/capacitors and polymer photodiodes (Stenger-Smith, 1998). Figure 2.11 shows the various applications of conducting polymers that can be achieved through different methods of doping.

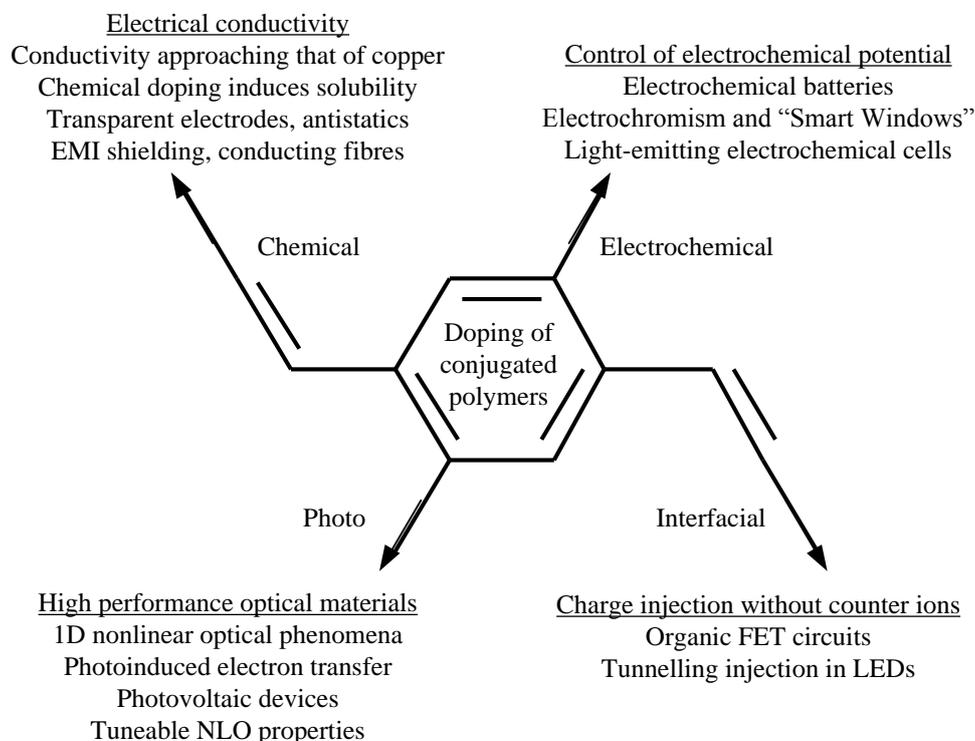


Figure 2.11 Summary of the uses of doped conjugated polymers (Source: Heeger, 2001)

Conjugated polymers have received a great deal of interest as chemical sensing materials due to the relationship between conductivity and chemical doping (Adhikari and Majumdar, 2004). The change in conductance can be registered in various ways and differs from polymer to polymer. The full potential of uses of conducting polymers is understood but to yet be fully harnessed (Li et al., 2009).

In addition, Conducting polymers usually possess an inherent ability to change colour due to the nature of electrons within the compounds. A π - π^* transition in the benzene rings within polyaniline is seen with the naked eye and by using UV-Vis spectrometry. Different amounts of chemical doping with H^+ ions causes polyaniline to change to different oxidation states. These states appear as different colours, allowing the polymer to act as a visible pH meter (Zagórska et al., 1997).

2.3 Polyaniline

Polyaniline (often referred to in the literature as PANI, PAn or PAni) is a conducting polymer. Polyaniline is formed either using a chemical or electrochemical method. Recently, research interest has been growing around using this polymer as a sensor. This is due to the ability of the polymer to react to various gas or liquid species which in turn affects the conductivity or the colour of polyaniline.

2.3.1 Polyaniline

Polyaniline has been the subject of broad research since the discovery of the material to become a conductor from an insulator under certain conditions (Epstein et al., 1987). The material was first synthesised and recorded many years before it was realised that the material could conduct electrical current. The first discovery of the polymer was by Runge in 1834 and initially research was carried out to analyse the material by Letheby in 1862 (Letheby H., 1862). The various oxidation states of polyaniline were determined at the beginning of the twentieth century by Green et al (Green and Woodhead, 1912). It is over the last twenty years, however, that the majority of research on this polymer has taken place (Li et al., 2009). Interest has been shown due to the polymers ability to conduct electricity as well as the redox capabilities that the polymer possesses (Epstein et al., 1987).

2.3.2 Chemical Synthesis

There are two methods of producing polyaniline as a bulk macromolecular polymer (Abbas et al., 2007) either as a chemically deposited thin film (Stejskal et al., 1999) or as an electrochemically deposited film (Trivedi, 1997). There are also various methods of inducing the chemical polymerisation reaction other than by using a chemical oxidant. These methods include gas phase plasma polymerisation (Cruz et al., 1997), autocatalytic polymerisation (Liao and Gu, 2002) and inverse emulsion polymerisation (Rao et al., 2002). The oxidation of aniline is completed with an oxidising agent, usually a persulphate (Syed and Dinesan, 1991), under acidic condition. The general reaction scheme is given below in Figure 2.12.

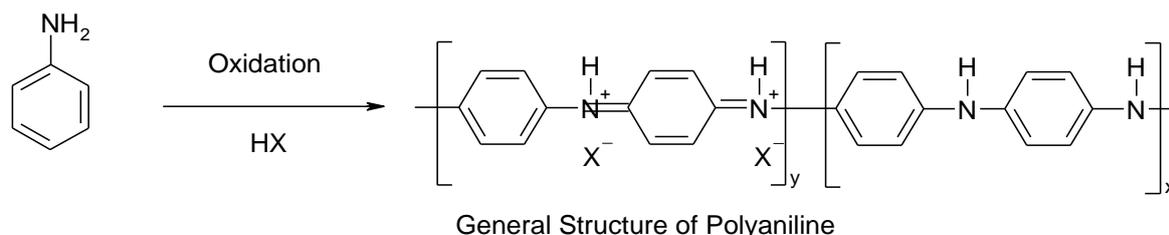


Figure 2.12 The general reaction scheme and structure of polyaniline (Source: Nicolas-Debarnot and Poncin-Epaillard, 2003)

The chain of polyaniline can be formed by various different combinations of the two repeating units. In the above schematic, these are described as x and y. Varying the conditions and the method of synthesis can lead to the formation of four different oxidation states of polyaniline with varying percentages of these x and y moieties. These are summarised below in Table 2.2 along with the names, colour and the conductivity.

Table 2.2 Accompaniment to above figure showing the ratio of parts x and y for each variation of the structure of polyaniline (Source: Nicolas-Debarnot and Poncin-Epaillard, 2003)

y-value	x-value	Name	Colour	Conductivity (S cm ⁻¹)
0	1	Polyeucoemeraldine base	Transparent	<10 ⁻⁵
		Polyprotoemeraldine base		<10 ⁻⁵
0.5	0.5	Polyemeraldine base	Blue	<10 ⁻⁵
(Without X ⁻ species)		Polynigraniline base		<10 ⁻⁵
1	0	Polypernigraniline base	Purple	<10 ⁻⁵
(Without X ⁻ species)				
0.5	0.5	Polyemeraldine salt	Green	~15

The conducting polyemeraldine salt is produced via a process originally known as ‘protonic acid doping’ (Chiang and MacDiarmid, 1986). This occurs when the moderately oxidised states, particularly the polyemeraldine base, are protonated. This creates the doping effect necessary to promote conductivity and provides the charge carriers (H⁺ ions). This explains why it is essential to complete the reaction under acidic conditions to synthesise the conducting salt.

The mechanism for the formation of polyaniline is still not fully understood (Gospodinova and Terlemezyan, 1998). The literature suggests that the routes of chemical and electrochemical synthesis of polyaniline are similar, with the latter being more controlled and clean due to the ability to regulate the amount of redox occurring in the reaction (Ayad and Shenashin, 2004; Abrantes et al., 2001).

Chemical synthesis requires three reactants, aniline, an acidic medium and an oxidant. More commonly hydrochloric acid and sulphuric acid are used for the acid. There are various oxidants that can be used (Syed and Dinesan, 1991; Malinauskas, 2001) but usually ammonium persulphate ((NH₄)₂S₂O₈) is used (Syed and Dinesan, 1991). The most popular synthesis is with a 1M aqueous hydrochloric acid solution (pH=0-2) with ammonium persulphate/aniline molar ratio ≤ 1.15 to create a high yield and high conduction. The procedure requires a slow, drop by drop, addition of the oxidant to the aniline/HCl solution after cooling both to 0°C.

Electrochemical synthesis can be carried out via three routes. Electrical synthesis can be carried out via three routes. These are galvanostatic (constant current), potentiostatic (constant voltage) or potentiodynamic (variable current and voltage). All of these methods require a three electrode assembly comprising a working electrode, a counter electrode and a reference electrode (in most cases, a saturated calomel electrode, SCE, or Ag/AgCl electrode (Nicolas-Debarnot and Poncin-Epaillard, 2003)). The deposition of polyaniline is onto the working electrode which can range from platinum (Mohilner et al., 1962), iron (Mengoli et al., 1981a), copper (Mengoli et al., 1981b) and gold (Paul et al., 1985). The polyaniline can then be removed by immersion in an acidic solution.

Polymerisation is believed to follow a condensation route with the step growth of the polymer chain via the production of free radical species (Nicolas-Debarnot and Poncin-Epaillard, 2003). The first stage of the polymerisation requires the formation of a radical of aniline which is induced by acidic oxidative conditions. The radical is formed on the nitrogen as these electrons are lost more readily than those delocalised in the benzene ring. This reaction can be seen below in Figure 2.13.

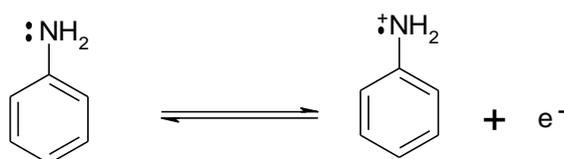


Figure 2.13 Formation of the aniline radical (Source: Geniès et al., 1990)

The radical that is formed has three resonance states. These are displayed below in Figure 2.14. Due to the stability provided by sterics and the increased reactivity given by the inductive effect, structure 2 is favoured for further reaction.

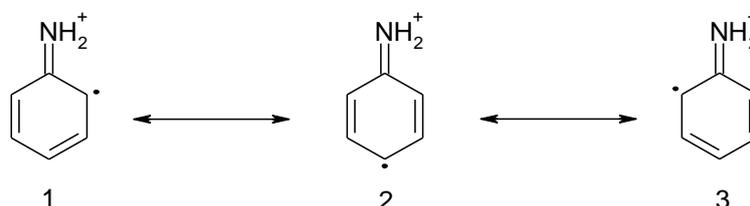


Figure 2.14 The three resonance forms of the aniline radical (Source: Geniès et al., 1990)

An aniline dimer is formed when an aniline radical reacts with the (2) resonance form from above. This dimer then undergoes rearrangement and loss of H^+ forming a stable dimer (Koval'chuk et al., 2001). This can be seen below in Figure 2.15. This dimer is then able to form a radical and proceed through another step of rearrangement to form a longer chain until the reaction is complete.

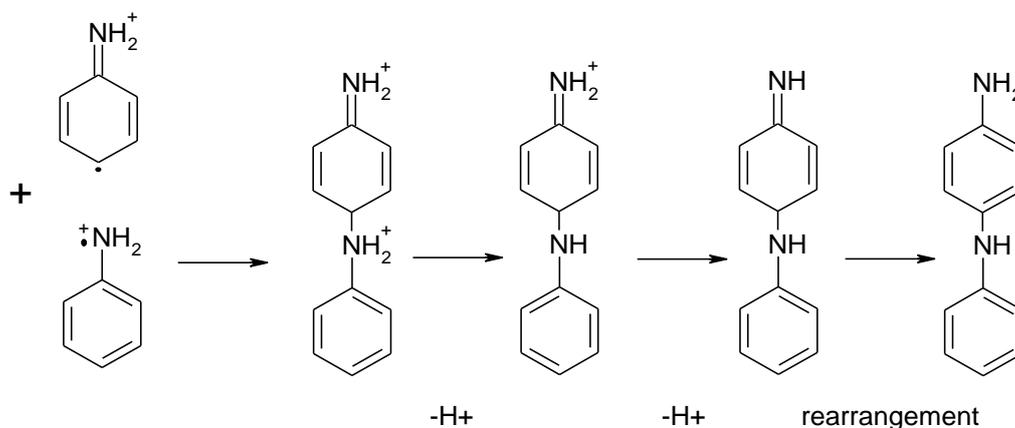


Figure 2.15 The formation of the aniline dimer in the chain polymerisation reaction (Source: Geniès et al., 1990)

The ability of polyaniline to exist in different oxidation states is also of interest due to the differing colours produced by the π - π^* aromatic electron transitions. The colours produced are shown in Table 2.2 and correspond to the oxidation states that can be seen below in Figure 2.1.

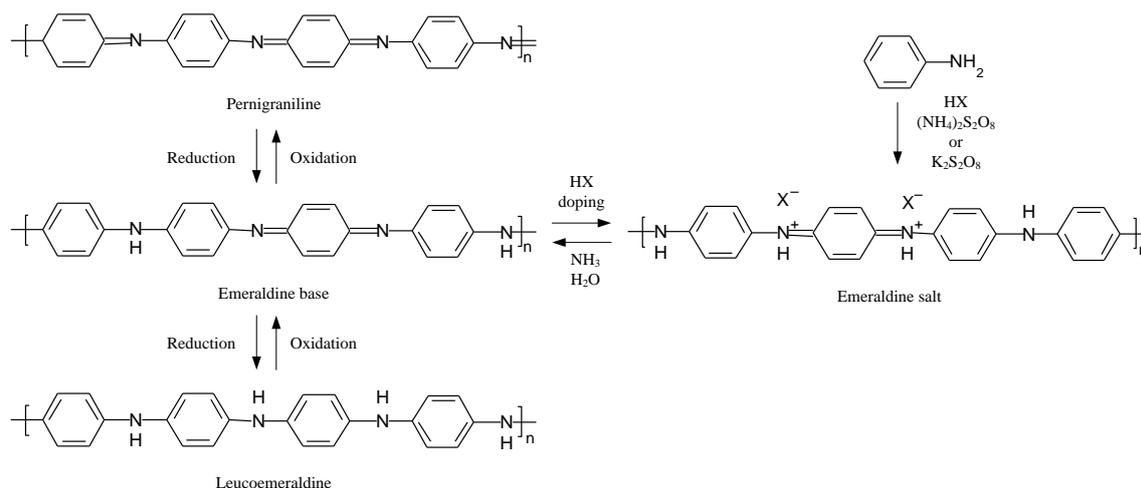


Figure 2.16 Schematic diagram showing the chemical structure, synthesis, reversible acid/base doping/dedoping and redox chemistry of polyaniline (source: Li et al., 2009)

Once formed, the conductive form of the polymer can be converted to the non-conducting polyemeraldine base under basic conditions. As previously mentioned, this is also seen as a visible colour change. Polyemeraldine salt is strongly green in colour where as polyemeraldine base is dark blue. This has been quantified in the literature by using techniques such as UV-Vis spectrometry due to the change in molecular dipole moments and the aromatic π - π^* shift (Ram et al., 1997). The approximate values of these transitions are given below in Table 2.3 together with an example of the spectrums in Figure 2.17.

Table 2.3 The approximate values of UV-Vis absorption for the different redox states of polyaniline (Source: Kang et al., 1998)

Transition responsible	Polyaniline redox state		
	Polyemeraldine salt	Polyemeraldine base	Leucoemeraldine
π - π^*	330 nm (2-3 eV)	283 nm and 327 nm (3.8-4.5 eV)	343 nm (3.6-4.0 eV)
Charge-transfer transition between Nitrogen and quinoid	635 nm (1.5 eV)	530 nm (2-2.3eV)	Weak 637 nm (1.5-1.9eV)

The weak absorption for the Leucoemeraldine state can be explained by the depletion of the quinoid species within the polymer structure that usually gives rise to this band. The shift in the broad absorption peaks observed in polyemeraldine base and salt can be easily distinguished in Figure 2.17 below.

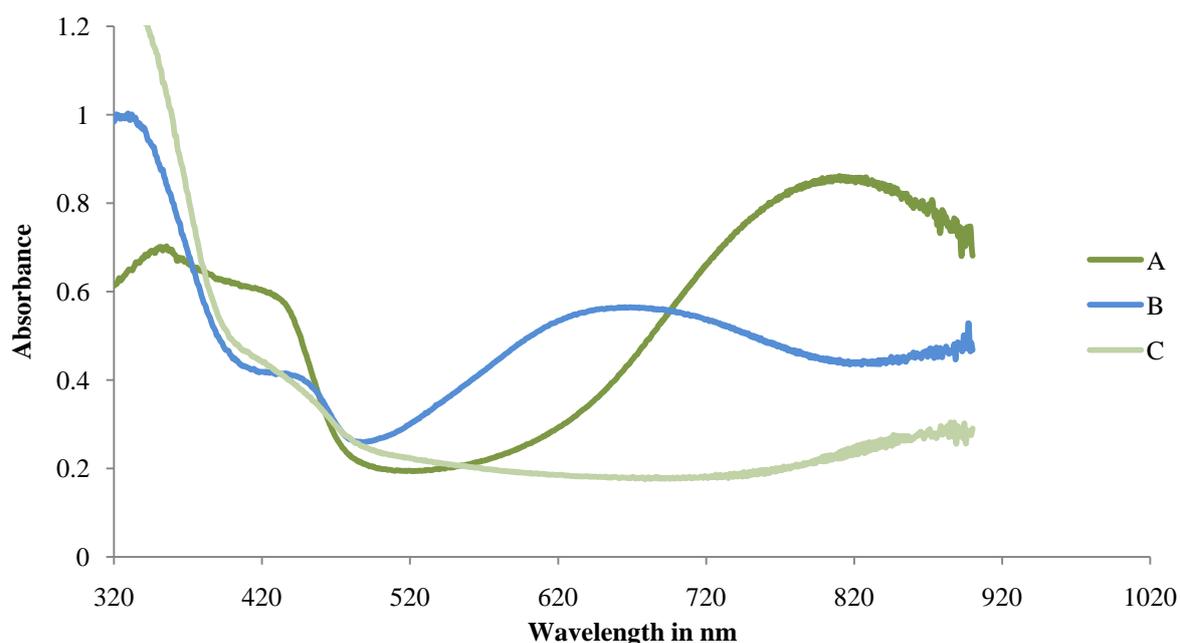


Figure 2.17 UV-Vis Spectrum of polyaniline thin films on a Melinex® substrate. (A=polyemeraldine salt, B= polyemeraldine base and C= polyleucoemeraldine)

From the above table and figure, the change in the π - π^* transition can be seen. This corresponds to the change in the band gap of the material (Ram et al., 1997) and explains the change in conductivity as the band gap increases between the redox states. The different energy of these band gaps can be explained below in Figure 2.18.

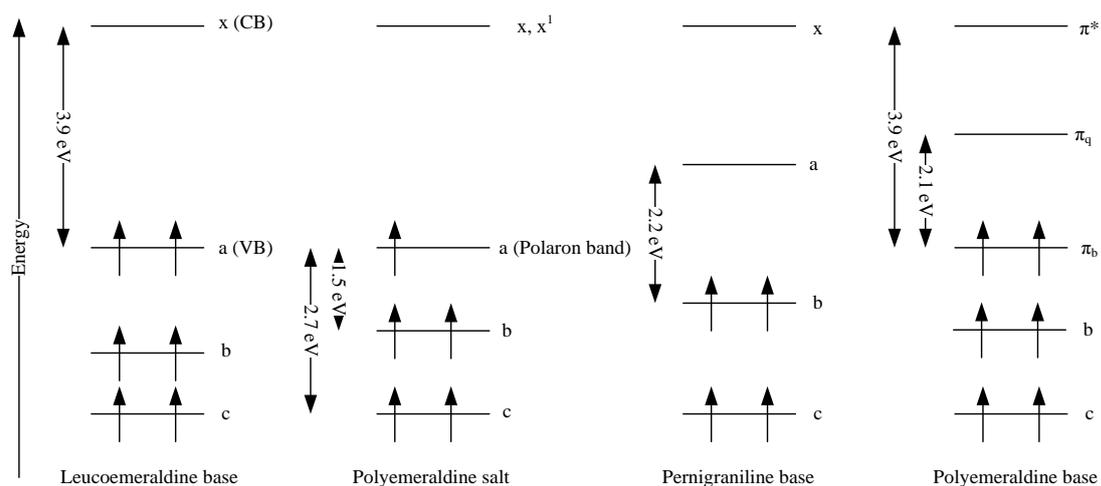


Figure 2.18 The different band gaps present in polyaniline redox states (Source: Huang and MacDiarmid, 1993)

In the above schematic, the electron transitions responsible for the corresponding UV-Vis spectrum can be observed. The small band gap and the half filled polaron defect band (formed when the material is oxidised) in polyemeraldine base provide the materials' ability to conduct electricity. Note that the diagram is simplified to show schematically the different properties of polyaniline. Band b in polyemeraldine is shifted slightly higher in energy due to the destabilisation caused by the polaron band. The upper polaron defect band (x^1) is nearly degenerate with band x (Huang and MacDiarmid, 1993). The formation of a bipolaron can occur with charges on two adjacent nitrogen atoms in the chain with quinoid rings or on two subsequent nitrogen atoms and benzene rings (polar) as seen below in Figure 2.19. The accepted structure that permits better conductivity is the former due to the relative proximity of the charged species and the quinoid functional group (Mažeikienė et al., 2007).

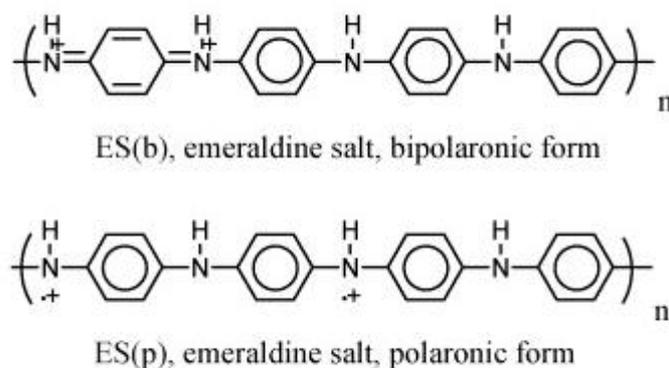


Figure 2.19 The forms of polyemeraldine salt (Source: Huang and MacDiarmid, 1993)

When the polymer is further oxidised to polynigraniline another electron is removed changing the HOMO and LUMO to band a and c respectively. The change from polyemeraldine salt to base removes the polaron band introduced by chemical doping. This changes the HOMO and LUMO to π electron systems within the benzene ring (π_b) and the quinoid-imine system (π_q) former being similar to Leucoemeraldine and the latter being present only in this redox state (Chiang and MacDiarmid, 1986; Anand et al., 1998). Other research in this area investigated the effect of introducing nanofilms of polyaniline to silicon semiconductors (de Vasconcelos et al., 2005). The investigation was able to show that the material could act as a hetero-junction using the silicon energy levels and the upper and lower polaron bands. There has also been recent research into the effect on nitro-substituents on the benzene rings in polyaniline with regards to the UV-Vis spectra that is produced (Ando et al., 2008). The electron withdrawing effect of the nitro (NO_2) groups on the benzene rings was

able to show that the π - π^* transitions in polyemeraldine salt are due to nitrogen and quinoid electrons.

Other techniques used to identify the structures of the redox states of polyaniline include direct absolute weight methods via measurements of scattered light from lasers (Kolla et al., 2005) and Raman spectroscopy (Lindfors and Ivaska, 2005). The first method mentioned above used a laser light source with a wavelength of 785 nm and a differential refractor to estimate the different structures. Raman spectroscopy has been used to demonstrate the change of electrochemically deposited polyaniline from the polyemeraldine salt to the polyemeraldine base (Lindfors and Ivaska, 2005). The change in Raman spectra was observed from a pH change of 2 to 9 at interrogating wavelengths of 514 nm, 633 nm and 780 nm. The UV-Vis spectra can be seen below in Figure 2.20 to demonstrate the change of the polymer from one state to another. The Raman spectroscopy showed that there was an increase in Raman intensity and absorption energy of the carbon-nitrogen double bonds and carbon-carbon aromatic bonds using a laser to interrogate at 633 nm. The Raman data has been tabulated within the paper released by Lindfors et al (2005).

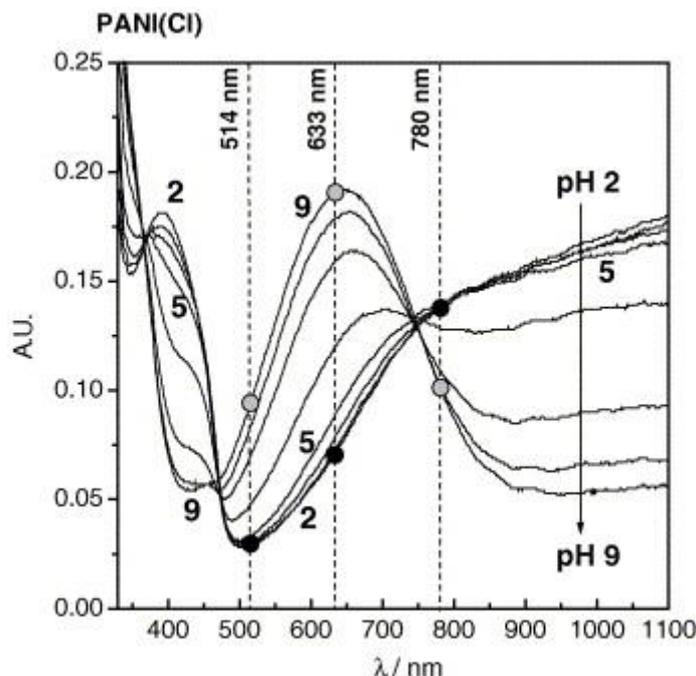


Figure 2.20 The UV-Vis Spectra of electrochemically deposited polyaniline exposed to different pH (Source: Lindfors and Ivaska, 2005)

Similar work has used Raman spectroscopy to characterise polyaniline films via the polaron formation in polyemeraldine salt from the polyemeraldine base (Bernard and Hugot-Le Goff,

2006a). Other research has used Raman spectroscopy to view differences in structure when sulphate (SO_3) groups are substituted onto the benzene rings within the polymer (Mažeikienė et al., 2007; Bernard and Hugot-Le Goff, 2006b). The research by the former showed that the substituted groups were able to self-dope polyaniline and extend the range of pH that the polymer was able to conduct in.

2.3.3 Methods of manufacture of polyaniline films

As mentioned previously, there are two main branches of polyaniline synthesis. These are electrochemical synthesis and chemical synthesis. The focus of this EngD is on deposition of films that have been manufactured via the chemical process. Polyaniline can be deposited as a film on numerous substrates in various ways (Malinauskas, 2001). There are numerous substrates such as glass (Stejskal et al., 1999), polymer (Yang et al., 1995) and fibres (Li et al., 1997). Chemical oxidation of aniline has been widely covered in the literature. In terms of bulk polymerisation, the optimum conditions for producing the highest yield with the best conductivity were found by Cao et al (1989). The suggested oxidising reagent was ammonium persulphate in a high ratio to aniline. Using concentrated hydrochloric acid also increased the conductivity of the bulk polymer as protons were available to form the polyemeraldine salt. It was also suggested that the temperature of reaction should be between 0 and $-5\text{ }^\circ\text{C}$ over a twenty minute period (Cao et al., 1989).

There has been further research into optimisation of this bulk polymerisation technique yielding similar conditions (Pron et al., 1988; Stejskal and Gilbert, 2002). The former work importantly discussed the competition between the formation of the polymer and the degradation of the polymer. Polyaniline can decompose if it is over-oxidised. The latter study focused on the repeatability of the polymerisation technique and found that using these optimised conditions that the technique was highly reproducible even within labs where the synthesis had not been tried before.

The method known as in-situ film formation refers to the creation of thin films of deposited polyaniline. These films are formed on substrates within the reaction vessel or on the walls of the reaction vessel itself. This technique has also been referred to in the literature as chemical bath deposition (Hu et al., 1999). This method has been widely reported as giving

thick films with high conductivity ideal for gas sensing (Nicolas-Debarnot and Poncin-Epaillard, 2003; Lange et al., 2008).

The first instance of this method being reported as a method of thin film formation showed that polyaniline could be deposited as a strong adhering layer onto a range of different substrates (MacDiarmid and Epstein, 1989) including fibres, textiles and gold (Gregory et al., 1989). The method involves dipping or submersing the selected substrate into a solution of aniline and hydrochloric acid and then adding an oxidising reagent. The initial process once the addition of the oxidant is complete is the formation of radical cations that are adsorbed onto the surface of the substrate. These cations can be singular aniline ions or small chained polymers in the Polypyrrograniline form (Gregory et al., 1989). The oligomers in the Polypyrrograniline that form rapidly polymerise leading to the growth of polymer chains (Trivedi and Dhawan, 1993).

More recent studies into this technique have tried to grasp a better understanding of the kinetics of this deposition (Mazeikienė and Malinauskas, 2000) and have shown that preformed polyaniline films that are used on a substrate have an autocatalytic effect on new layers of polyaniline depositions (Liao and Gu, 2002). Research carried out by Stejskal et al over the last decade has been focused on explaining the complete mechanism of this deposition as well as using different techniques such as FTIR (Trchová et al., 2005), X-ray photoelectron spectroscopy and UV-Vis spectrometry (Stejskal et al., 1999). Initial studies showed that a thicker film formed on a glass substrate at lower temperatures and a linear relationship between film thickness and UV-Vis absorbance at 400 nm was also shown (Stejskal et al., 1999). These are shown in Figure 2.21 and Figure 2.22 respectively.

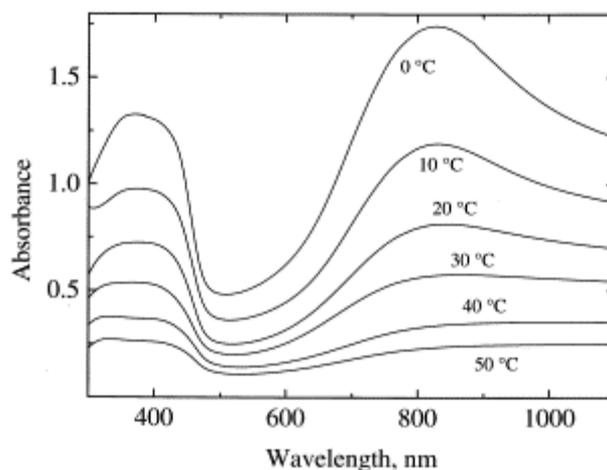


Figure 2.21 The increase of absorption over the spectrum of polyaniline synthesised at different temperatures (Source: Stejskal et al., 1999)

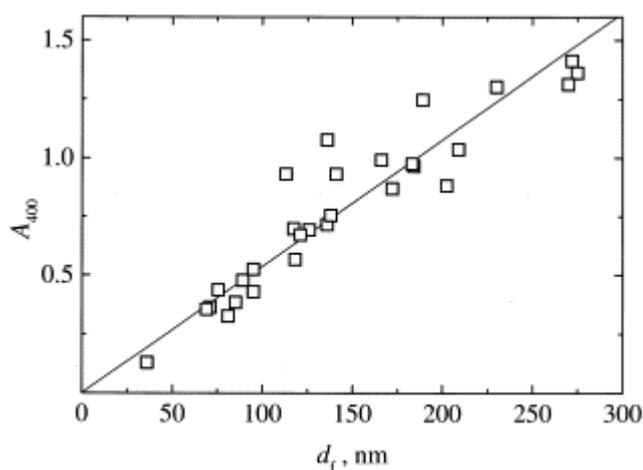


Figure 2.22 The relationship between film thickness of polyaniline film and the absorbance at 400 nm (Source: Stejskal et al., 1999)

Film thickness was measured indirectly by interferometry using the displacement of an interference pattern produce from a monochromatic light source reflecting from a groove of known depth. The method was reported to work well on films of thickness between 50-200 nm and was not precise on films with rougher surfaces (which occur on thicker films) and on thin films. The conductivity of the films in this study did not have the same relationship. The conductivity of the films produced was independent of both the temperature applied to the polymerisation mix and the concentration of the hydrochloric acid used. There was a link, however, to the ratio of oxidant to aniline used and the film conductivity. A ratio of 1.5 was deemed to produce the most conductive films with an acidic concentration between 1 and 2 M (Stejskal et al., 1999). There was not a lot of variation between the conductivity of films produced in different concentrations of reagent solutions within the range used. The reproducibility of the films was also reported to be poor due to various conditions including

differences in film washing and drying, variations of temperature in the reaction vessel due to the exothermic nature of the polymerisation reaction and the order and speed in which the reagents are added into the reaction vessel (Stejskal et al., 1999).

Further work has suggested a mechanism for the formation of these thin films (Sapurina et al., 2001). This study indicated that there were three stages of film deposition that occur; induction, oxidative polymerisation and then a period after polymerisation when no further reaction takes place. These periods were demonstrated by measuring the temperature of the reaction mix (0.2 M aniline with 0.25 ammonium peroxydisulphate) at room temperature. This can be seen below in Figure 2.23.

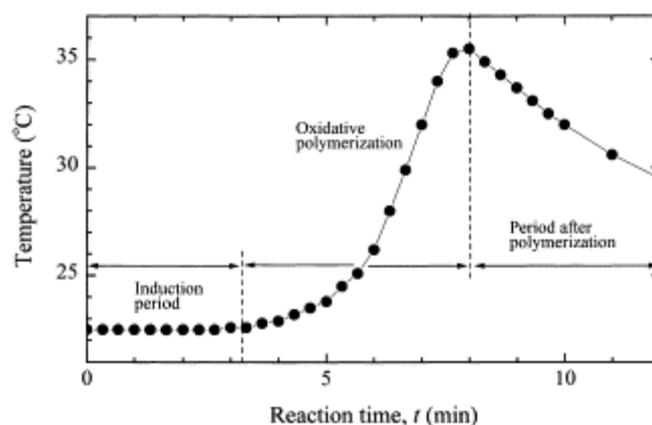


Figure 2.23 Reaction periods of in-situ film formation of polyaniline (Source: Sapurina et al., 2001)

Evidence supplied by film thickness measurements using the relationship of UV-Vis absorbance at 400 nm and surface morphology via a scanning electron microscope supported the purposed reaction mechanism. This is shown below in Figure 2.25. In the first part of the mechanism, aniline cations produced from oxidation are absorbed onto the substrate surface (a). These ions react and interact with other polymer chains forming long brush like chains. Polymerisation can occur here even faster than the rate in bulk solution due to the creation of reaction sites on the substrates. This partially ordered brush like state gives rise to a very smooth surface film (b). Once this surface has got to a certain point more nucleation of charge particles and polymer chains become absorbed onto the polymer surface rather than the glass (c). This complex surface morphology can now encapsulate whole colloids of unattached polyaniline and other crystalline matter (d). This increases the surface roughness and can be seen below in Figure 2.24.

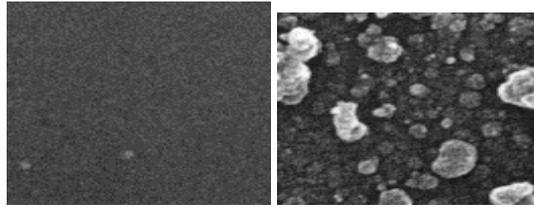


Figure 2.24 Surface morphologies at stage a and d of film formation (Source: Sapurina et al., 2001)

This mechanism also helps in the explanation of why these in-situ films form better on surfaces that have been pre-coated with a polyaniline layer (Sapurina et al., 2001).

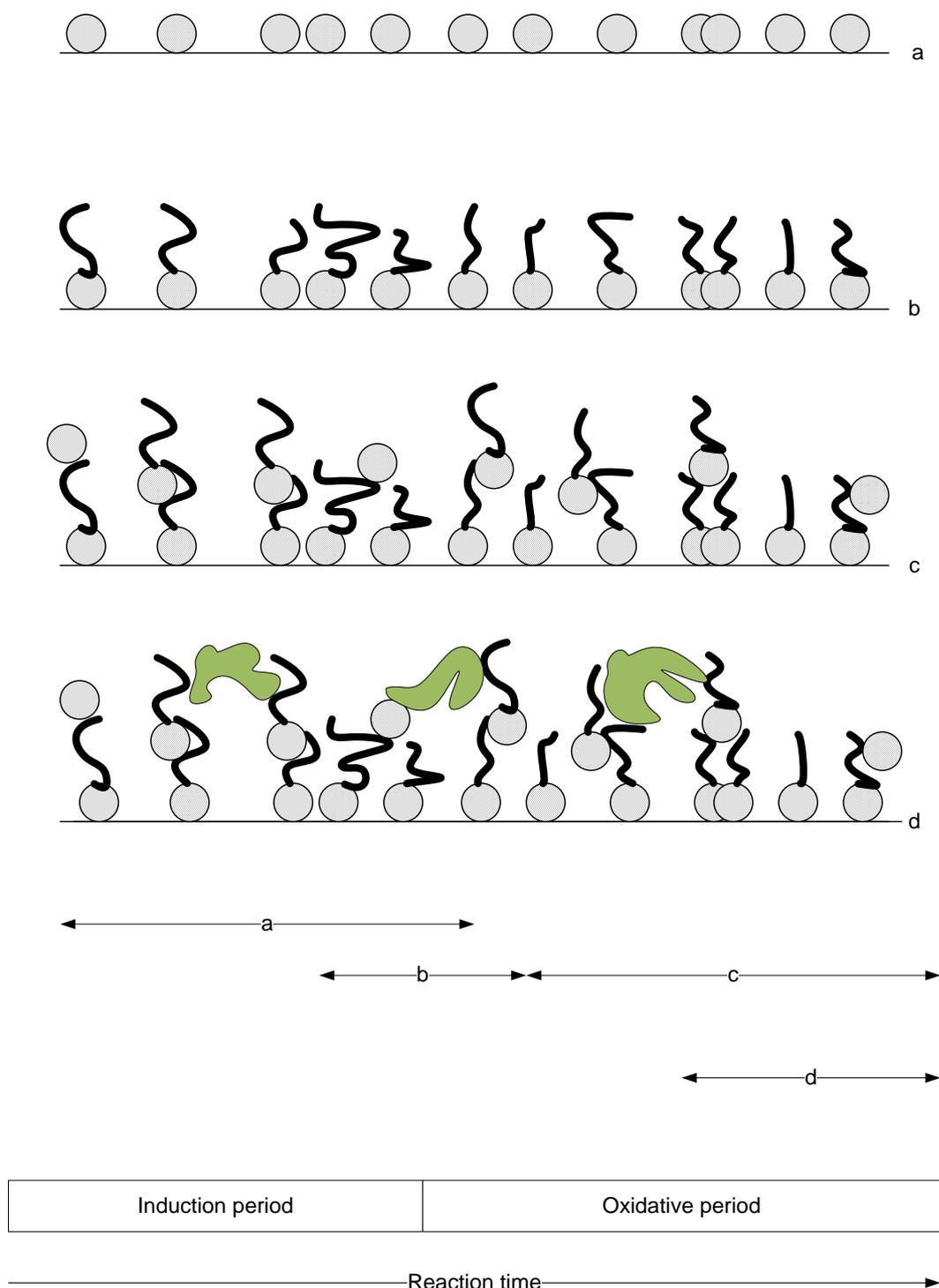


Figure 2.25 The model for in situ film formation of polyaniline (Source: Sapurina et al., 2001)

The films produced are often so thin that the deposited layer can be measured using a quartz crystal micro-balance system (Ayad and Shenashin, 2004). Work by Ayad et al has shown that film thickness can be related linearly to UV-Vis absorption at 345 nm (Ayad and Shenashin, 2003). Ayad et al also showed that a pre-deposited layer of polyaniline increased

the growth kinetics of polyaniline films (Ayad et al., 2003). The optimum condition found by this research was a ratio of 1.5 between oxidant and aniline concentrations (Ayad et al., 2004; Ayad et al., 2003). A large range of conductivity was also reported.

Research carried out by Travain et al (2007) showed that they were able to model the growth of polyaniline films to a Johnson-Mehl-Avrami model under certain conditions and concentrations as given below in Equation 2.1.

Equation 2.1 The relationship between film absorbance at 815 nm and time the substrate is left in reaction mix (Source: Travain et al., 2007)

$$A = k \left\{ 1 - \exp \left[- \left(\frac{1}{\tau} \right)^n \right] \right\}$$

Where A is the absorbance at 815 nm, k and n are constants and τ is the characteristic time (Travain et al., 2007).

This model was only deemed to be well fitting to concentrations below 0.112 moles of aniline in 1 litre of 1 M hydrochloric acid. Atomic force microscopy has also been used to determine film thickness but has been noted to give poor results on glass due to the weak adhesion of the polyaniline film to glass (Avlyanov et al., 1995).

The knowledge of polymerisation kinetics occurring more steadily on a surface pre-coated with polyaniline lead to an investigation by Madathil (2005) in using separate containers for the oxidant and aniline solutions. The introduction of the coated substrate into each of these solutions, followed by washing stages showed an increase in layer thickness via UV-Vis spectroscopy (Madathil, 2005). Each cycle of this scheme increased the thickness and conductivity by a controlled and reproducible amount. The study carried out up to five repeats of this cycle to compare the growth of the films in this manner. The synthesis route and UV-Vis spectra can be seen below in Figure 2.26.

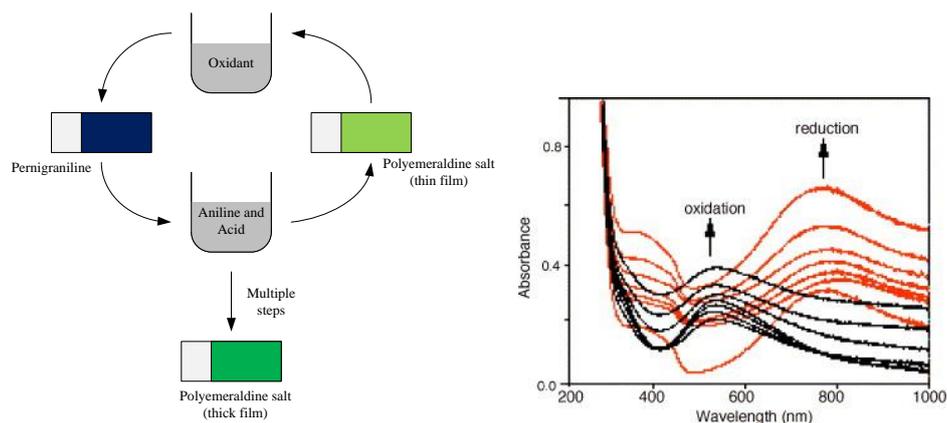
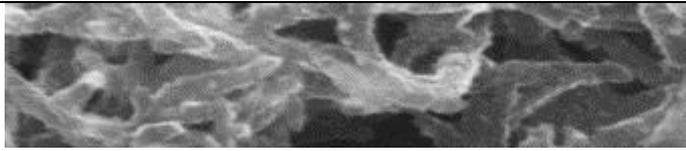
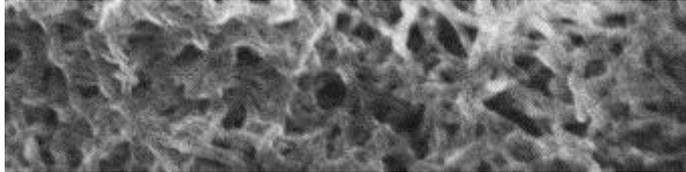
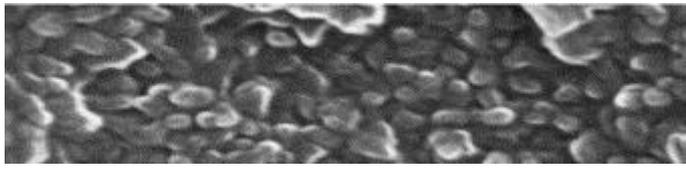
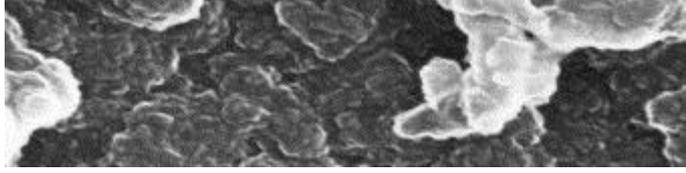
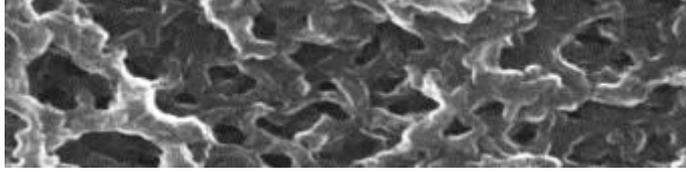


Figure 2.26 The process involved in two pot synthesis of polyaniline films (Source: Madathil, 2005)

This method was able to show that the formation of polypernigraniline is the initial step in the polymerisation film growth process. The observation of the film thickening and changing colour between dipping in the different solutions has been shown by the UV-Vis spectra. The results were able to show that better conducting films were manufactured using hydrochloric acid as the dopant. The film thickness and surface morphology also altered depending on the acidified doping component (Madathil, 2005). Hydrochloric acid and nitric acid produced nano-fibres with a diameter of 50 nm and 40 nm respectively. This was compared to the porous structures from methane sulphonic acid. Hydrogen bromide and sulphuric acid showed much denser structures based on the nano-fibre structure shown by the other strong acids. The change in the thickness of the fibres is possibly explained by the radius of the ions used in doping. Thickness was measured using white-light surface profiler. This has been summarised below in Table 2.4.

Table 2.4 Conductivity and thickness of polyaniline films synthesised with different acids Source: (Madathil, 2005)

Dopant	Thickness (μm)	Conductivity (S cm^{-1})	Morphology (scanning electron microscope scale ^a 3 cm = 500 nm, scale ^b 5 cm = 1 μm)
HCl ^a	10.	25-32	
HNO ₃ ^b	0.7	15-20	
HBr ^a	0.9	11-15	
H ₂ SO ₃ ^a	0.9	6-11	
MeSO ₃ H ^b	0.8	2-3	

Other mechanical methods of film production include plasma polymerisation (Cruz et al., 1997), Inverse emulsion polymerisation (Rao et al., 2002), vapour-phase polymerisation (Kim et al., 2007), Langmuir-Blodgett films (Xie et al., 2002) and self-assembly (similar to in-situ) (Li et al., 2000). There have also been attempts made to produce polyaniline blends and composites using other polymers in the reaction mix such as rubber and polyphenylacetylene (Cataldo and Maltese, 2001).

2.3.4 Polyaniline films as gas sensors

There has been a large amount of research on polyaniline as a sensor for many different gases. Research has also been carried out into whether polyaniline could act as a molecular recognisor (Teasdale and Wallace, 1993) for an array of different chemical species (including NO_3^- , CH_3COO^- , PO_4^{3-} and CO_3^{2-}). It was found that polyaniline produced a different change

in colour, resistance and impedance to different reagents - and also that the selectivity could be modified by changing the frequency of the exciting ac potential.

There has also been recent research into polyaniline as a sensor for hydrogen (Grzeszczuk, 1994), ammonia (Hong et al., 2004) carbon dioxide (Irimia-Vladu and Fergus, 2006) and carbon monoxide (Dixit et al., 2005). Other work includes using conjugated polymers to detect sodium dodecyl sulphate via a change in resistance (Binas and Sevilla, 2005).

There have been numerous studies for using polyaniline as a sensor for volatile compounds (Kim et al., 2005; Lee et al., 2003; Hwang et al., 2003). A polyaniline sensor was able to detect methanol, ethanol, benzene and toluene vapour via resistance change down to 100 ppm (Kim et al., 2005). An array of polyaniline and polypyrrole sensors was setup to test the recognition of chloroform, toluene, ethanol and benzene. The sensor array was constructed of eight conducting polymers that varied in thickness on an alumina substrate. The sensors made with the higher amount of oxidant performed the best and the signals produced were transferred via a computer program into actual volatile recognition (Lee et al., 2003). A similar study determined sensitivity via UV-Vis absorbance changes of a conducting polymer array sensor (Hwang et al., 2003).

This project is focused on the production of a polyaniline sensor that is able to detect changes in volatiles produced by degrading fish. This detection would either be observed as a resistance change, an impedance change or a colour change. When dealing with a sensor of this nature, selective doping is necessary to obtain monitoring of your chosen molecule (Huh et al., 2003). For instance, in the case of degrading foodstuffs, the tracer volatile will most likely be an amine, and so doping of polyaniline to prevent its reaction with water vapour and alcohols would be needed to prevent interference. Consideration of doping would also depend on whether the sensitivity of the polymer to amines is changed or lowered.

The construction of the sensing element is also proven to be of importance (Agbor et al., 1995; Zhang et al., 2006; Liu et al., 2004). The correct accessibility of the polyaniline surface to the selected volatile reactant is required. The use of nano materials in sensor fabrication has been proven to be utilised to act as a filter for larger molecules that could interfere with measurements of the change in impedance (Bekyarova et al., 2004; Virji et al.,

2009; Virji et al., 2005). The inclusion of a polyaniline coated fabric into a mesh for use of toxic vapour detection (Collins and Buckley, 1996) has proven successful in determining low concentrations of ammonia, nitrogen dioxide and DMMP (a chemical warfare stimulant). For this project this practice is unnecessary due to the expected high concentration of spoilage volatiles from degrading fish.

There has also been research into biogenic amine vapour detection using polyaniline as an optical and chemiresistor sensor (English et al., 2006). This study found that by plotting the change in resistance over a time period, rapid reversible responses to small (10ppb) injections of analyte (butyl amine) were observed. It was also commented that these detection responses draw close parallels to olfaction in biological systems, a factor that has also been discussed in other work (Riul et al., 2003). This has led to the potential application of polyaniline films as pH sensor. Optical changes of polyaniline over a range of pH caused by the exposure of the film to ammonia and acetic acid has been recorded (Asijati et al., 2005). The films were made using the in-situ method and the substrate was polystyrene sheet. Film thickness was measured at 100 nm and iron (III) chloride solution was used as the oxidant. The response time of these films to volatile from 0.8 M ammonia solution was approximately 20 minutes at a wavelength of 610 nm. A similar study using ammonium persulphate as the oxidant provided a polyaniline sensor that was able to detect gaseous ammonia at 40 ppm via absorbance at 600 nm (Quinto and Sevilla, 2005). Other methods of deposition have a poorer sensitivity of ammonia. Layers of polyaniline deposited from bulk polymerisation by centrifugal force showed a sensitivity of 1000 ppm via optical changes at 655 nm and resistance changes of the polymer.

Other methods of manufacture of polyaniline based ammonia sensors include emulsion polymerisation (Wu et al., 2000), nylon coated fibres (Hong et al., 2004) and Langmuir-Blodgett films (Kukla et al., 1996). The sensing ability of polyaniline has led to the introduction of thin films of the polymer to be introduced into systems like electronic noses and tongues (Riul et al., 2003; Barisci et al., 2002).

The reaction mechanism of polyaniline with simple gases and other volatiles has been covered widely in the literature (Bai and Shi, 2007). The majority of this research focused on the reaction of polyaniline with ammonia. These studies were designed to determine the

optical effects of ammonia gas with polyaniline. The detection limit of ammonia has been tested as low as 10 ppm (Nicho et al., 2001) for undoped polyaniline. This work employed an absorbance wavelength of 632 nm and detected change via an optical-transmittance bridge. This method may prove to be more sensitive to volatile gases than the chemiresistor approach; however, it is more costly and not practical for the design of a remote sensor since absorbance techniques are used.

Further studies of the effects of ammonia and acetic acid on polyaniline (Asijati et al., 2005) showed optical changes in the visible range, allowing the polymer to be used as a visible pH meter. There have also been several publications relating to the detection of gases using near IR (Christie et al., 2003), IR (Ohsaka et al., 1984) and Raman spectroscopy (Lindfors and Ivaska, 2005) on polyaniline.

In terms of this project, the main area of interest is the reaction of polyaniline with the volatiles produced from meat as it degrades due to bacterial activity. There is evidence that the majority of these volatiles are small chain alcohols and amines for salmon (Chantarachoti et al., 2006; Wierda et al., 2006) and herring (Chung et al., 2007; Mjøs and Solvang, 2006). An understanding of the mechanism of polyaniline reacting with these molecules is therefore necessary so that dopants can be added to make polyaniline more efficient and susceptible to these gases.

Figure 2.27 below shows the mechanism for the reaction of polyaniline with gaseous ammonia. The reaction results in an oxidation of the conducting polyaniline salt and a production of ammonium ions. The volatiles produced by salmon and herring include amines, which tend to be more stable when positively charged in comparison to ammonium ions due to the inductive effect (English et al., 2006). Therefore the mechanism for the reaction of polyaniline producing amides will be similar to the one below but more favourable towards the oxidation of polyaniline due to the stability of the ions produced (English et al., 2006).

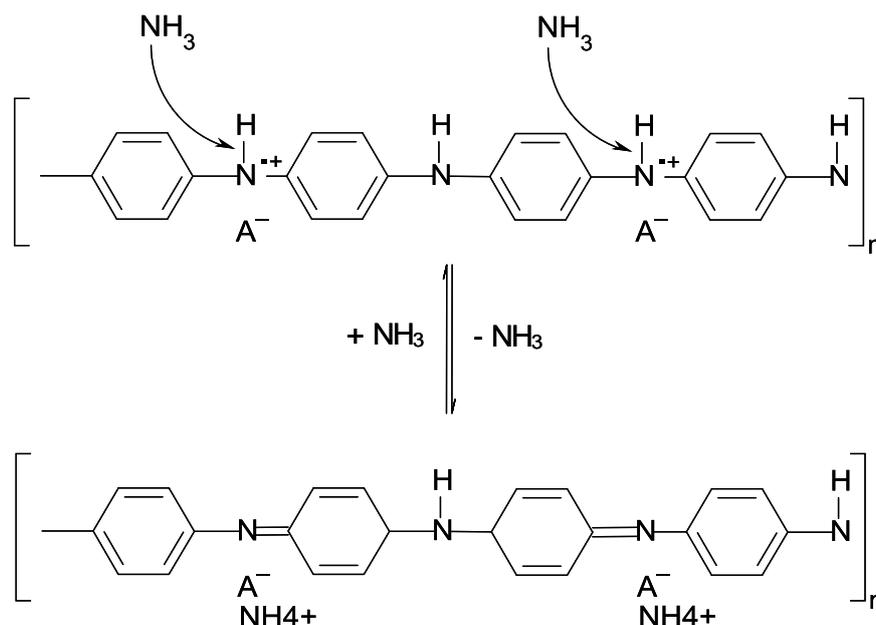


Figure 2.27 The reversible reaction of polyaniline with ammonia (Source: Hong et al., 2004; Zhang et al., 2006; Bai and Shi, 2007)

Polyaniline-Pd nanocomposite films exposed to several alcohols (methanol, ethanol and isopropanol) showed high selectivity when interacting with methanol down to 10 ppm (Athawale et al., 2006). The resistance change of the polyaniline was proportional to the mixture of methanol with other alcohols. Since the interactions are only physical in nature, desorption of the methanol molecules was extremely quick and so the materials proved reusable and stable. Figure 2.28 below shows the reaction scheme of polyaniline-Pd with methanol, which was determined by using FT-IR. The spectra show that the imine nitrogen is reduced by the methanol molecules to an amine in the presence of palladium nano-particles. In air, methanol molecules are desorbed due to moisture.

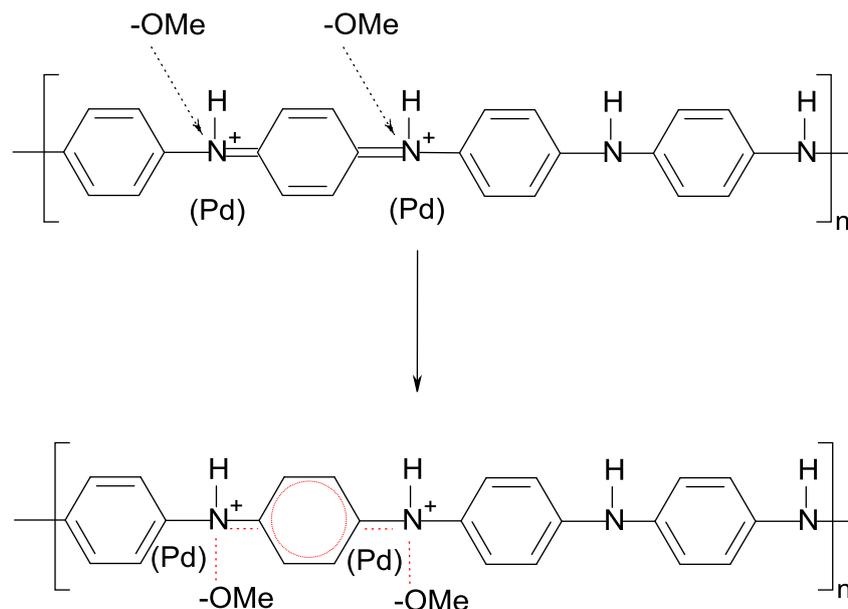


Figure 2.28 The interaction of polyaniline (doped with Pd) with Methanol (Source: Athawale et al., 2006)

The kinetics of adsorption has also been studied for films of polyaniline. Research has been carried out on using Langmuir adsorption isotherms of volatiles onto films of polyaniline (Bai and Shi, 2007). The Langmuir isotherm is designed to model the equilibrium process between surface and gas phases. There are a number of simple assumptions that are often criticised since they are not truly valid, especially at high surface coverage (Pillings and Seakins, 2003). The model suggests that only a monolayer of gas can be adsorbed, however, further layers of gas in most situations can be physisorbed. In this case we are only interested in the surface adsorbate interactions which result in an oxidation/reduction reaction. These can only occur in a monolayer due to polyaniline having specifically charged sites for interaction with volatiles so the assumptions for this model can be taken as being reasonable.

The equilibrium reactions for adsorption and desorption are represented below in Figure 2.29. X represents the adsorbate chemical species and the site to which it adsorbs onto the surface at a relevant site.

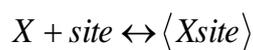


Figure 2.29 The Equilibrium process of chemical species X adsorbing/desorbing to a site.

The rate of surface desorption, ρ_{-1} , is assumed to be proportional to the fraction, θ , of the surface covered. It follows also that the rate of adsorption, ρ_1 , is proportional to the fraction of vacant sites, $(1-\theta)$, and to the pressure, p , of the gas X. Equation 2.2 shows this relationship at equilibrium.

Equation 2.2 The equilibrium of adsorption and desorption on a surface.

$$k_1 p(1 - \theta) = k_{-1} \theta$$

If b is allowed to be substituted into Equation 2.2 as $b = k_1/k_{-1}$ then Equation 2.3, the Langmuir adsorption isotherm is derived.

Equation 2.3 The Langmuir adsorption isotherm.

$$\theta = \frac{bp}{(1 + bp)}$$

The principal is as p increases from zero, θ rises, linearly at first and then when at full coverage, θ tends to unity since bp becomes much larger than unity. Experimentally ΔH_{ad} can be determined. The assumption here is that the heat of adsorption is independent of θ , which then allows k_1/k_{-1} to be determined by a study of the volume of gas taken up versus pressure. The experiment is then repeated over a range of temperatures.

The interaction of ammonia with polyaniline has been studied (Hu et al., 2002). In this report, the changes in the optical properties of polyaniline were used as a sensor for ammonia adsorption. The research proved that the polyaniline surface has two energetically different adsorption sites for ammonia molecules and is inhomogeneous. The shape of the adsorption/desorption curves also suggested a monolayer reversible chemisorption process. It was also concluded from the experimental evidence that the Freundlich isotherm was followed by this material rather than the Langmuir type isotherm. This conclusion was reached via the decreasing logarithmic dependence of the heat of adsorption of ammonia against the concentration of ammonia and the increasing logarithmic dependence of the activation energy of adsorption on the concentration of ammonia.

The Freundlich isotherm suggests that the rate of absorption is more suited to logarithmic scales. Equation 2.4 below shows the Freundlich isotherm, where c_1 and c_2 are constants determined experimentally.

Equation 2.4 The Freundlich Isotherm (Source: Atkins, 2001)

$$\theta = c_1 p^{\frac{1}{c_2}}$$

The mechanics of adsorption allow the material to be used as highly responsive sensor. The ability of a small amount of volatile vapour (10 ppm) to change the structure of the polymer so that it cannot conduct as efficiently allows polyaniline to act as such a responsive gas sensor. A review of the above mathematically approach to determining the actual amount of substance present can be found in the literature (Hu et al., 2002).

2.3.5 Other uses of polyaniline

Other uses of polyaniline are being researched and novel applications are routinely being discovered. This is due to the robustness of the polymer and the ease of processability (Pron and Rannou, 2002). Figure 2.30 below summarises some of the current uses and future applications of polyaniline.

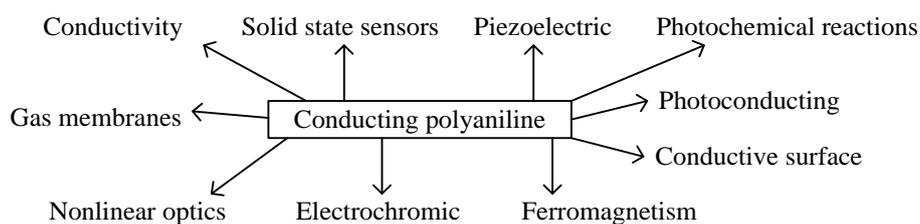


Figure 2.30 A schematic overview of the uses of polyaniline (Source: Li et al., 2009; Trivedi, 1997)

Conjugated polymers provide a material that has the normal properties of organic solids and also the ability to act as a conductor or semiconductor. The various methods of synthesis allow these polymers to be used in a wide variety of uses. Polyaniline is one of the most

researched conjugated polymers due to the conductivity and colour changing ability that the polymer possesses.

2.4 Food spoilage and Safety

This section will give a detailed overview of relevant literature in the field of food microbiology. The focus of this EngD project is specifically on fish and seafood produce, especially salmon and this is reflected in this part of the literature review. The legislation of food safety is stringent and this section will give an overview of the emerging concepts in food quality analysis that aim to be in accord with the legislation.

2.4.1 Bacterial growth

The amount of bacteria in food offers key information about the hygiene and quality of the produce. Bacteria are often the cause of spoilage of seafood products and the growth of many bacteria and their metabolites pose a health risk towards consumers. There is therefore a requirement to be able to accurately predict the growth of specific spoilage bacteria as well as detection of the bacteria before the food stuff is visibly spoiled and can be detected by the human nose or naked eye.

Population studies of bacteria over a set time allow for the determination of bacterial growth curves. Due to the large numbers involved when counting and numerating bacterial population counts are usually expressed in logarithms. Bacterial growth occurs in four stages as can be seen in Figure 2.31. The details of these phases are given below:

1. Lag phase – once microorganisms are introduced into fresh culture no instant growth is visible. This phase can vary in length due to a number of factors which include if the cells were damaged or injured, if the medium was chilled or if the inoculum was from a previous culture.
2. Exponential phase – in this stage the microorganisms are growing at their fastest possible rate. The maximal rate depends on the media used, the conditions (such as temperature, pH or available nutrients) and their genetic potential. The phase is called

the exponential phase as each organism divides and doubles at regular intervals. The curve is smoothed due each microorganism producing an equivalent at slightly different moments to each other.

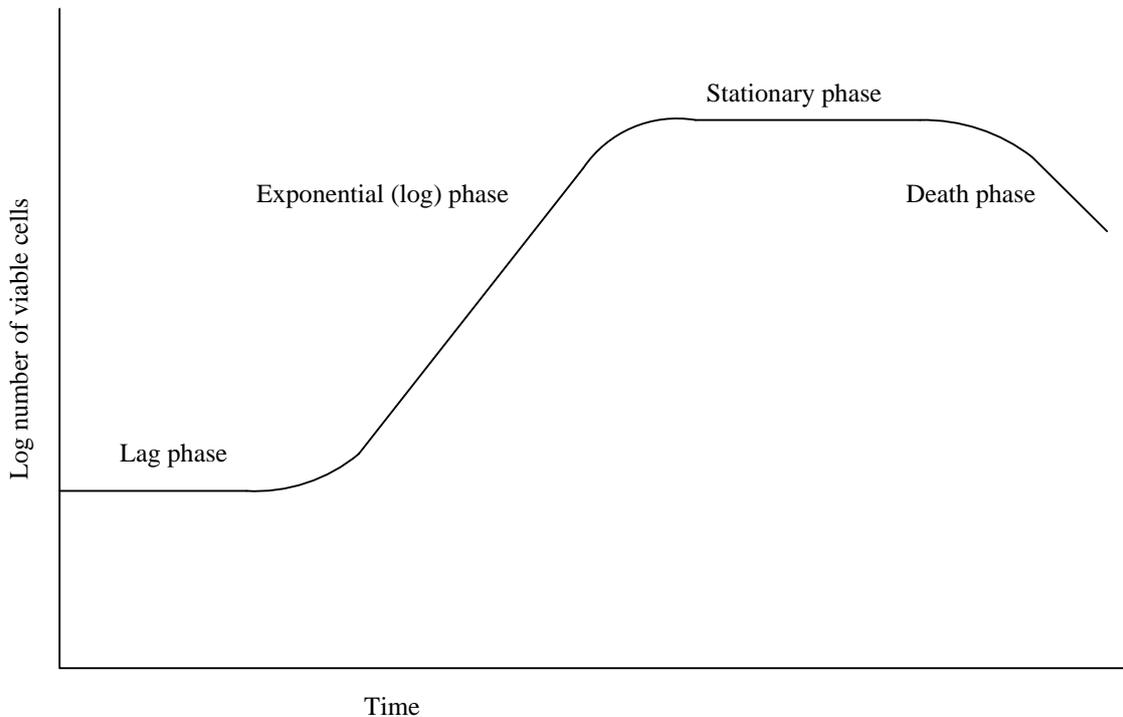


Figure 2.31 The bacterial growth curve including labels of the four stages (Source: McKellar and Lu, 2009)

3. Stationary phase – at this point the population growth is halted which flattens the growth curve to a horizontal position. The level of this stationary point again depends on several factors, again including available nutrients, microorganism type or the accumulation of waste products.
4. Death phase – the build-up of toxic waste products plus the lack of availability of key nutrients causes the decline of population numbers of bacteria. The death rate of cells is said to diminish at a logarithmic pace due to large proportions of cell numbers dying at the same time.

The rate of growth of bacteria during the exponential phase is of high importance to people studying in the field. This data gives evidence of the behaviour of the cells to the environment, temperature or nutrients they have been exposed to. Generation time is defined as the time taken for the population of bacteria to double. To simplify this, the increase in population is always given as 2^n where n is the number of generations in a time period, t . The initial population number can be expressed as N_0 , the population at time t as N_t so that using Equation 2.5 below, the population size at given time during the exponential phase can be represented as:

Equation 2.5 The population at a given number of generations during the exponential phase

$$N_t = N_0 \times 2^n$$

This equation can be rearranged to find the number of generations as:

Equation 2.6 Rearrangement to find number of generations

$$n = \frac{\log N_t - \log N_0}{0.301}$$

Where 0.301 is the \log_2 . The mean growth rate constant, k , is a measurement of the rate of growth within a culture and is the number of generation in a unit of time. This is usually given in the units of generations per hour and is expressed below.

Equation 2.7 The mean growth rate expressed as a function of time

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

To calculate the mean time taken for a bacteria population to double in size, i.e. $N_t = 2N_0$, requires substituting in g , which is defined as the mean doubling time, so that:

Equation 2.8 The mean growth rate constant as a product of population doubling over a time period

$$k = \frac{\log(2N_0) - \log N_0}{0.301g}$$

Equation 2.9 Simplification of the above equation

$$= \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

This can be simplified to show that k is a reciprocal of g and is given below.

Equation 2.10 The dependence of mean generation time on the mean growth rate constant.

$$k = \frac{1}{g} \text{ or } g = \frac{1}{k}$$

These equations form the basic tools of microorganism growth modelling which is used as a means for determining population growth rates, population sizes and generation times. A review of bacterial modelling will be given later in this thesis.

2.4.2 Bacterial and Chemical spoilage of food

Food stuffs provide bacteria with a nutrient rich environment for growth. Food spoilage is a major problem that has a detrimental effect on the economy, health and the environment. Food spoilage is defined as any change which renders an item of food unacceptable for human consumption (Hayes, 1985). Evidence of food spoilage is often obvious and is detected by the human eye. Spoilage can take the guise of visible growth of microorganisms (e.g. fungi and moulds), slime or physical damage. Food spoilage is also a very complex system of chemical and biological reactions occurring at variable rates depending on temperature, pH, food type and the way in which the food item is processed (Koutsoumanis et al., 2005). Figure 2.32 shows the nature of food product deterioration with the external conditions. There is an overlap and interaction between chemical spoilage and microbial spoilage that gives rise to the difficulty in modelling food spoilage and growth of spoilage organisms (Huis in't Veld, 1996).

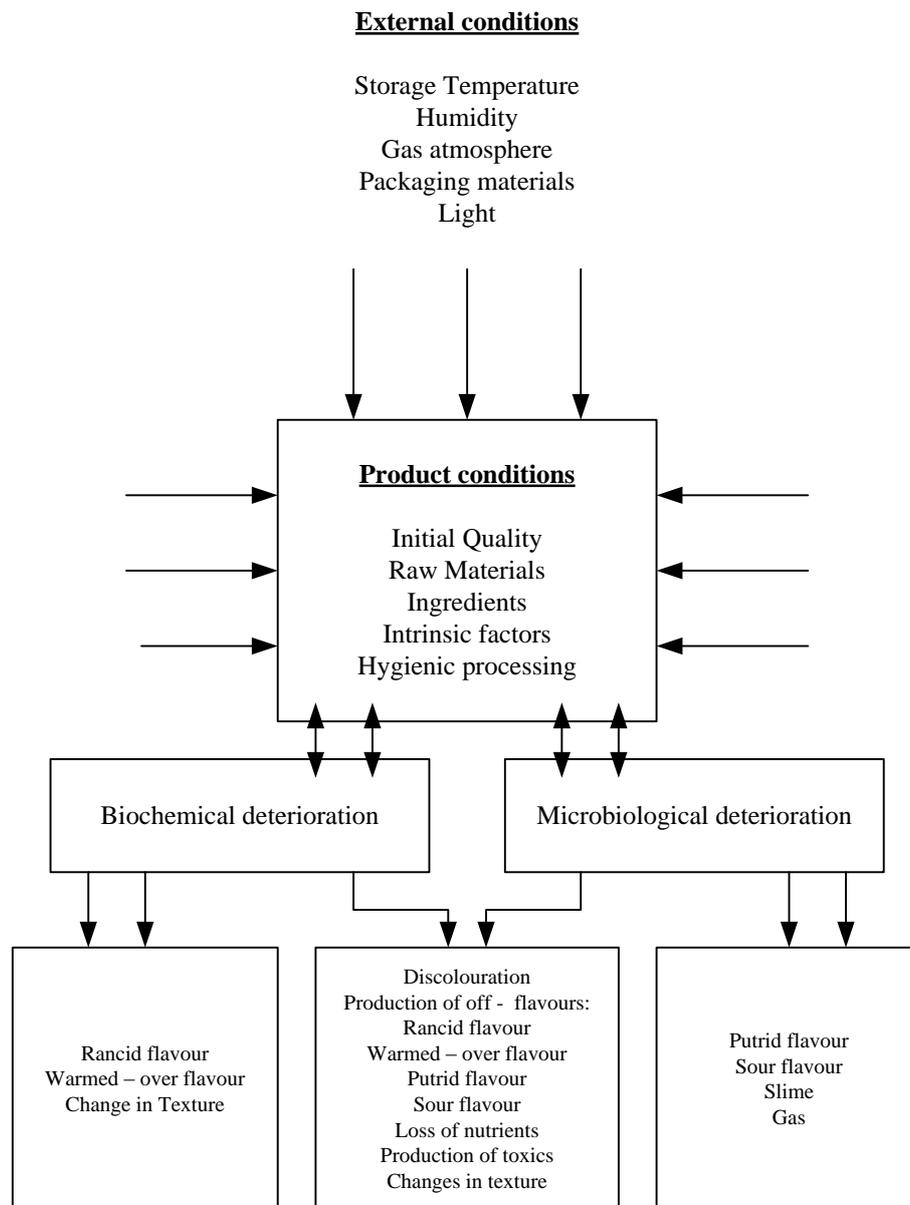


Figure 2.32 The quality deterioration of foods during storage (Source: Huis in't Veld, 1996)

The issue of quality of produce and measurement of spoilage is argued to be mainly subjective (Nychas et al., 2008), whereas food safety concerns - and definitions are very concise and in the developed world depend on legislation (Koutsoumanis et al., 2005; Dalgaard, 1995a). There is an increase in research focus around the role of supply chain and logistics of food stuffs due to today's global food production and lengthening distances between source and consumption (Ross, 1 November 1996; Rönnow, 2006; Smith and Sparks, 2004). There are many different ways to prevent or delay food spoilage such as

refrigeration, packaging food in a modified atmosphere or by using antimicrobial agents (Hogan and Kerry, 2008). These are all concepts that are deemed to be preventative measures. There is also a requirement to provide data on actual food spoilage and condition. For this to occur there needs to be an understanding of the factors that contribute to food spoilage.

Microorganism growth is the main cause of most food spoilage. There are numerous invasive methods for the determination of food quality measuring bacterial populations or levels of spoilage metabolites (Olafsdóttir et al., 1997; Rehbein, 1997; Rodriguez-Perez et al., 2003). The importance of certain organisms and their effect on food spoilage has been long understood (Huis in't Veld, 1996). It is now considered that monitoring of these organisms could lead to a technology that would be able to determine shelf-life of food. Specific spoilage organisms (SSO) are microorganisms that can act as indicators of food spoilage (Hozbor et al., 2006). As the name suggests, they are specific to certain food types and conditions (Nychas et al., 2008). Research has been carried out into the interaction of these organisms with the food stuff, temperature (Olafsdottir et al., 2006) and other spoilage organisms (Gram et al., 2002).

There are modelling techniques that can predict the growth of these SSOs which require a wealth of data to yield adequate accuracy (Huis in't Veld, 1996). Currently there are other ways of determining food quality; Nychas et al (2008) suggest the common defects in meat products and the bacteria that cause these. A summary of their findings can be found below in Table 2.2.

Table 2.5 Problems of meat spoilage and the causal bacteria (Source: Nychas et al., 2008)

Defect	Meat Product	Bacteria responsible
Slime	Meats	<i>Pseudomonas</i> , <i>Lactobacillus</i> , <i>Enterococcus</i> , <i>Weissella</i> , <i>Brochothrix</i>
H ₂ O ₂ greening	Meats	<i>Lactobacillus</i> , <i>Enterococcus</i> , <i>Weissella</i> , <i>Leuconostoc</i>
H ₂ S greening	Vacuum packed meats	<i>Shewanella</i>
H ₂ S production	Cured meats	<i>Vibrio</i> , <i>Enterobacteriaceae</i>
Sulphide odour	Vacuum packed meats	<i>Clostridium</i> , <i>Hafnia</i>
Cabbage odour	Bacon	<i>Providencia</i>
Putrefaction	Ham	<i>Enterobacteriaceae</i> , <i>Protus</i>
Bone Taint	Whole meats	<i>Clostridium</i> , <i>Enterococcus</i>
Souring	Ham	Lactic acid bacteria, <i>Enterococcus</i> , <i>Micrococcus</i> , <i>Bacillus</i> , <i>Clostridium</i>

These defects are visible once spoilage has occurred. There are two current methods of food spoilage analysis that are used to determine quality and safety; microbiological and sensory analysis (European Commission, 2005). Both of these methods have limitations and drawbacks. Microbiological analysis of food spoilage often requires high cost tools for analysis and uses destructive procedures that give results corresponding to past events (Nychas et al., 2007). It is almost impossible to use microbiological techniques for evaluation of food quality in real-time as there is a required incubation time for plates and media. Sensory evaluation of food quality is entirely subjective and requires a panel of highly trained experts to analyse the foodstuff on pre determined quality indices. This method is also costly, largely subjective and proves difficult to carry out routinely. For most meat products the preferred method of analysis is via the sensory analysis (Nychas et al., 2008).

There has been considerable effort recently to provide new methods of quality analysis in the food industry (Dalgaard et al., 2007; Dainty, 1996; McMeekin et al., 2006). These new methods are aimed at linking decrease in food quality to biochemical changes occurring within the food. From one perspective, there is the use of the emerging field of bioinformatics in combination with microbiological modelling to determine the shelf-life of foodstuffs with the main focus on meat products (Olafsdóttir et al., 1997; McMeekin et al., 2006; Koutsoumanis and Nychas, 2000; Gospavic et al., 2008). Most of these proposed systems have not been received well within the food industry and as a result acts more as a research tool (Nychas et al., 2008). The main reason for this is the lack of real information

required to input into the initial models, such as, SSO present, variations in lag phase of SSO, food type and unpredicted fluctuations in temperature (Dalgaard et al., 2007). Table 2.6 includes a summary of the present software used to model SSO growth and hence product shelf life.

Table 2.6 Various software available to evaluate food spoilage (Source: Nychas et al., 2008)

Websites or software for food spoilage
Kinetics characteristics of certain spoilage bacteria are available from the growth predictor (UK) – www.ifr.ac.uk/safety/GrowthPredictor/ (Based on data previously used in the FoodMicromodel software).
The French approach ‘Sym’previus – www.symprevius.org is under development and also will provide kinetic data on this website.
Seafood Spoilage (Shelf-life of seafood and growth of SSO) (Dalgaard et al., 2007)
Safety monitoring and assurance system (Greek predictive microbiology application software under development)
Pathogen Modelling Program (USA) – www.arserrc.gov/mfs/pathogen.htm 37 models of growth, survival and inactivation. This is frequently updated and most used (~5000 downloads per year)

The other method that has been suggested as a replacement to the two incumbent quality determining techniques is via the detection of volatile microbial metabolites. As bacteria grow on food they produce by-products from their digestion of available nutrients. Quantification of the change in these metabolites could give an indication of microbial populations. There has been a considerable amount of research into the field of matching metabolites to bacteria (Korkeala et al., 1987; Aquilanti et al., 2007; FU et al., 1992; Ntzimani et al., 2008; Chen et al., 2010). Table 2.7 below gives an overview of the current metabolite compounds and their respective tests used to determine bacterial spoilage of food.

Table 2.7 Compounds potentially useful for the assessment of shelf-life of raw meat and fish under different packaging conditions (Source: Nychas et al., 2007)

Compound	Test	Packaging conditions	Red meat and poultry
Glucose	Enzymatic kit	air, VP, MAP	Y
Acetate	Enzymatic kit, HPLC	VP, MAP	Y
Gluconate	Enzymatic kit	air, VP, MAP	Y
Total lactate	HPLC	VP, MAP	Y
D-lactate	Enzymatic kit	VP, MAP	Y
Ethanol	Enzymatic kit, GLC	VP, MAP	Y
Free amino acids	Chromatometric	Air	Y
Ammonia	Enzymatic, colorimetric	Air	Y
Acetone, methyl ethyl, ketone, dimethyl sulphide, dimethyl disulfide hydrogen sulphide	GLC, GC/MS, sulphur selective detector	VP, MAP	Y
Diacetyl, acetoin	Colorimetric	VP, MAP	Y
Biogenic amines	HPLC, sensors, enzymic test, GLC, enzyme electrodes, test strips	air, VP, MAP	Y
Diamines	Amperometric electrodes (enzymatic systems)	Air	Y
Microbial activity	Enzymic/resazurin	Air	Y
Volatile odours	Electronic noses, PTR-MS (chemical sensors)	air, VP, MAP	Y
Proteolysis (amides, amines, etc)	FT – IR, NIR, MIR	air, VP, MAP	Y

^aVP = vacuum packed, MAP = modified atmosphere packed

Over the last two decades efforts have been made to relate bacterial growth to specific metabolite (Nychas et al., 2008; Ellis and Goodacre, 2001). Considering the correct metabolite can be very difficult. The initial approach in this field was to specifically correlate certain metabolites to growth of bacteria. Using this approach means that if a change in the food ecology occurs which does not favour the production of the specific metabolite, then other spoilage may occur undetected. The first stage of this research also requires expensive equipment and highly trained personnel in the detection and measurement of the chosen metabolite.

Several criteria have been suggested in the selection of an effective metabolite to indicate food safety (Jay et al., 2005), these are as follows:

1. Be easily and rapidly detectable.
2. Be easily distinguished from other members/species/molecules present.
3. Have a close relationship to a pathogen or spoilage bacteria.
4. Always be present when the pathogen or spoilage bacteria are present.
5. Have a production rate that is greater than any pathogen or growth rate of spoilage bacteria.
6. Be absent from foods that do not have any spoilage bacteria present or at a certain lower threshold of population.

The relationship between a potential indicator and the levels of pathogenic specie(s) created should be that the former is in higher concentration than the latter and that the indicator should out live the lifetime of the pathogenic species present. This can be seen in Figure 2.33 below.

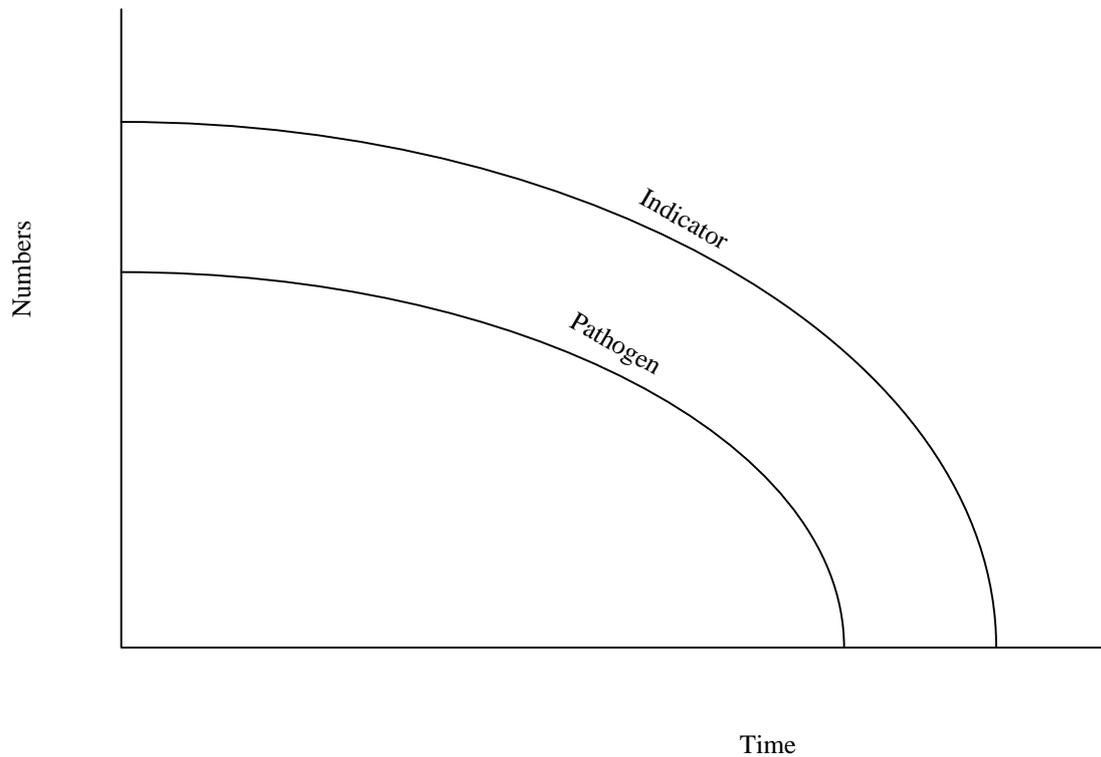


Figure 2.33 Idealised relationship between an indicator organism and the relevant pathogen(s). The indicator should exist in higher numbers than the pathogen during the existence of the latter (Source: Jay et al., 2005)

The selection, measurement and analysis of these marker metabolites requires expensive and specialist equipment (Borch et al., 1996). Research carried out by Plutowska et al (2007) suggested that the combination of several gas analysis techniques should be used to produce aroma profiles, or aromagrams, for the detection and monitoring of spoilage metabolites (Plutowska and Wardencki, 2007). Aromas arise from food products at all stages of the production process. The research also contains numerous examples in the literature of techniques used to determine the quality indices of many food items. These include techniques such as electronic nose (El Barbri et al., 2007; Haugen et al., 2006; Rajamäki et al., 2006) and various forms of headspace and gas chromatography analysis (Mjøs and Solvang, 2006; Eyles and Adams, 1986; Chinivasagam et al., 1998).

2.4.2.1 Amines as food spoilage markers

Several groups have researched the potential of using amines and biogenic amines as potential marker of spoilage for meat and fish (Ntzimani et al., 2008; Edwards et al., 1987; Balamatsia et al., 2007; Rokka et al., 2004; Yano et al., 1995b). These studies all conclude that an increase in biological spoilage is related to the increase in production of nitrogen containing compounds. Biogenic amines are reported to be produced through bacterial enzyme activity and the levels depend on the micro-ecology of the food sample (Halász et al., 1994). Other amines such as trimethylamine (TMA) occur due to break down of specific amino acids due to microbial growth and activity (Halász et al., 1994; Bardócz, 1995; Ruiz-Capillas and Moral, 2005). Biogenic amines have been used to determine quality of food products such as chocolate, meat, wines and fish (Önal, 2007). According to the literature there is a gap in the knowledge of the chemical variations of volatile amines occurring in meat, fish and poultry (Balamatsia et al., 2007; Al-Bachir and Mehio, 2001; Byun et al., 2003; Mitchell et al., 2002). Ion mobility spectroscopy has been used to determine the quality of chicken meat via the detection of volatile amines such as TMA (Bota and Harrington, 2006).

2.4.2.2 Seafood spoilage

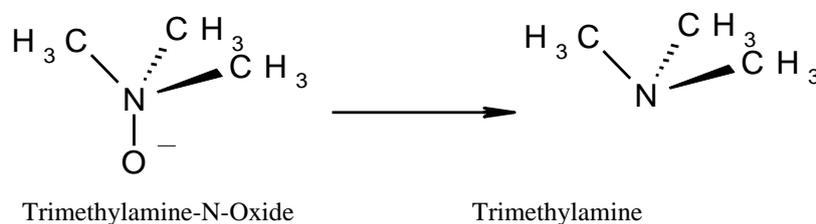
Recent research in this field has led to an increase in understanding of the mechanisms of seafood spoilage. Contributing factors to seafood spoilage include the contamination of the product from the original environment/habitat, processing (ice storage or sterile canning) and the growth conditions that are derived from storage temperature, pH and microbial interactions (Gram and Huss, 1996). The relatively high pH of post-mortem fish tissue (>6.0) allows pH sensitive microorganisms to grow. There is also an abundance of non-protein-nitrogen (NPN) present which provides low molecular weight and water soluble nitrogen for bacterial growth. The majority of this is found in compounds such as trimethylamine oxide (TMAO), unchained amino acids and nucleotides (Gram and Huss, 1996). Other than bacterial growth; there are other parameters of fish spoilage, these are summarised below in Table 2.8

Table 2.8 Review of damage caused by microbial spoilage of food (Source: Gram and Huss, 1996)

Microbiological activity	Sensory manifestation
Breakdown of food components	Production of off-odour and flavour
Production of extracellular polysaccharide	Slime formation
Growth per se of moulds, bacteria or yeasts	Large visible pigmented or non-pigmented colonies
CO ₂ from carbohydrate or amino acids	Production of gas
Production of diffusible pigments	Discolouration

Current methods to evaluate seafood and fish shelf-life include the sensory analysis in terms of taste, odour and texture. The current systems of labelling and freshness definitions are defined by current EU regulations (European Commission, 2005; European Commission, 1996). This section will now discuss other emerging methods of quality and freshness determination in fish products.

TMAO is present in all marine based fish. Many spoilage bacteria are able to use TMAO as an electron acceptor in anaerobic respiration which results in the formation of TMA (Gram and Huss, 1996; Gram, 1992; Dalgaard, 1995b). TMA is responsible for the fishy off odour that is often present on spoiled food products. This can be seen below in Figure 2.34.

**Figure 2.34 Trimethylamine-N-oxide reduction to trimethylamine (Source: Jay et al., 2005)**

TMA, ammonia and dimethylamine (DMA) are excellent metabolites for use as spoilage markers as the levels of these chemicals on freshly caught fish are extremely low and rise with bacterial activity (Oehlenschläger, 1997). Determining levels of total volatile basic nitrogen (TVB-N) is also suggested as a method of freshness indication. TVB-N consists of ammonia, DMA and TMA and is good for use as a spoilage indicator but is not as good as a freshness indicator as the levels remain low and unchanged during the first 8-14 days of fish stored at low temperatures (Oehlenschläger, 1997).

As mentioned previously, the current methods of assessment of food quality are microbial and sensory analysis. In terms of fish and seafood, there are numerous examples of research in the literature of bacterial detection (Gram et al., 1987), microbial modelling (Dalgaard et al., 1997) and microbial/sensory assessment (Ruiz-Capillas and Moral, 2005; Leroi et al., 1998; Chytiri et al., 2004) for a variety of fish. In terms of odour analysis, there have been numerous studies using a variety of techniques to determine changes in concentration of volatile metabolites. Fish odour comprises a variety of different compounds which has been simplified in Figure 2.35.

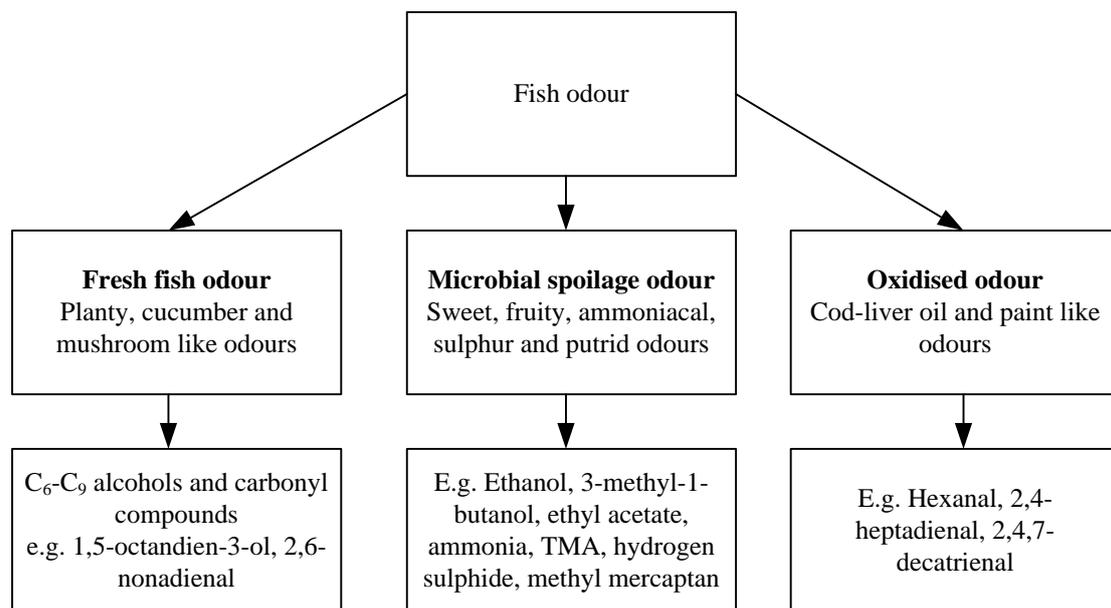


Figure 2.35 Categorisation of fish odours and some of the compounds responsible (Source: Olafsdóttir et al., 1997)

From the findings in the literature, there are a variety of compounds that make up this complex odour. From these many compounds there are a few that are suggested as possible quality indices. These have been summarised below in Table 2.9. The majority of the cases report TMA as a good quality marker; however other product specific markers (such as smoke flavourings for smoked salmon), organic acids and carbonyl compounds have been suggested. Gram et al (2002) suggested that ammonia, biogenic amines, organic acids and sulphur containing compound from amino acids as by products from SSOs (Gram and Dalgaard, 2002).

Table 2.9 An overview of research into the volatiles produced by spoilage bacteria on fish

Method of detection	Fish	Major spoilage indicator	Other volatiles
Gas chromatography	Hake (<i>Merluccius merluccius</i> or <i>Merluccius capensis</i>)	TMA (Veciana-Nogues et al., 1996)	DMA (Veciana-Nogues et al., 1996)
	Salmon (various)	Unknown markers (Nakai et al., 1999)	NA
	Capelin (<i>Mallotus villosus</i>)	Sulphur containing compounds e.g. SO ₂ (Olafsdottir et al., 1997b; Olafsdottir et al., 1997a)	NH ₃ (Olafsdottir et al., 1997b; Olafsdottir et al., 1997a)
Gas chromatography mass spectrometry	Cod (<i>Gadus morhua</i>)	TMA (Olafsdottir et al., 2005)	H ₂ S and SO ₂ (Olafsdottir et al., 2005)
	Herring and blue whiting	TMA and Sulphur containing compounds (Mjøs and Solvang, 2006)	Other amines and amides (Mjøs and Solvang, 2006)
	smoked salmon (<i>Salmo salar</i>)	Biogenic amines (Jorgensen et al., 2001) organic acids and carbonyls (Jónsdóttir et al., 2008)	H ₂ S and amines from isolated spoilage bacteria (Joffraud et al., 2001) smoke based compounds (Jónsdóttir et al., 2008)
Solid phase micro extraction gas chromatography mass spectrometry	Tropical prawns (<i>Penaeus</i> spp.)	Amines and sulphides (Chinivasagam et al., 1998)	Carbonyls and esters (Chinivasagam et al., 1998)
	Whiting (<i>Merlangius merlangus</i>)	TMA and DMA (Béné et al., 2001; Duflos et al., 2006)	Alcohols, sulphur containing compounds and carbonyls (Duflos et al., 2006)
	Cod (<i>Gadus morhua</i>)	TMA (Duflos et al., 2006)	Alcohols, sulphur containing compounds and carbonyls (Duflos et al., 2006)
	Mackerel (<i>Scomber scombrus</i>)	TMA (Duflos et al., 2006)	Alcohols, sulphur containing compounds and carbonyls (Duflos et al., 2006)
	Salmon (<i>Salmo salar</i>)	TMA and DMA (Béné et al., 2001)	NA
	King-Salmon (<i>Oncorhynchus</i>)	Organic acids (Wierda et al., 2006)	Aromatic hydrocarbons (Wierda et al., 2006)

Method of detection	Fish	Major spoilage indicator	Other volatiles
Static headspace analysis-olfactometry	<i>tshawytscha</i>) White herring (<i>Ilisha elongate</i>)	TMA (Chung et al., 2007)	H ₂ S (Chung et al., 2007)
	Smoked salmon (<i>Salmo salar</i>)	organic acids and carbonyls (Jónsdóttir et al., 2008)	smoke based compounds (Jónsdóttir et al., 2008)
Electronic nose	Atlantic salmon (<i>Salmo salar</i>)	Alcohols, aldehydes, carbonyl (Haugen et al., 2006)	Smoke related compounds (Haugen et al., 2006)
	Alaska pink salmon (<i>Oncorhynchus gorbuscha</i>)	Volatile mix produced by spoilage (Chantarachoti et al., 2006)	NA

There are many other different factors in the determination of quality of seafood for the end consumer, which include value, availability and freshness. Freshness is a function of several factors such as volatile freshness indicators. Figure 2.36 shows the dependence of freshness on these factors and the relationship between freshness and consumer quality. Included within this study are the measurement of adenosine triphosphate (ATP) degradation to hypoxanthine and measurement of k . Post-mortem changes in fish flesh include the degradation to ATP to inosine monophosphate (IMP) by enzymes originating from the fish itself. IMP is then changed to hypoxanthine and inosine at a much slower rate. This latter reaction requires an input from bacterial enzymes as the time from death increases (Olafsdóttir et al., 1997). Studies have shown that the breakdown of ATP closely parallels the loss of freshness and in the literature the measurement of ATP degradation is given as a k value (Hattula, 1997; Hattula and Kiesvaara, 1996; Gill, 1995; Hamada-Sato et al., 2005). This technique requires details of handling and storage conditions to have any meaning and it should be noted that K values vary for each species of fish.

Lipid oxidation of unsaturated fats in fish can be attributed to the change in odour, texture and colour. There is also a detrimental effect on the nutritional value of the food. Oxidation occurs immediately after catch and becomes significant once the fish is stored above 0 °C, where oxidation spoilage is more prevalent than microbial growth (Harris and Tall, 1989). Oxidation is initiated by several factors including the increase in free iron and the decrease of available antioxidants (Hultin, 1994). There are many invasive methods used to determine

the extent of lipid oxidation, such as for example, gas chromatography, high-performance liquid chromatography (Eriksson and Svensson, 1970) and spectrophotometry (Gray, 1978). For accurate determination a combination of these methods is required which can be time consuming and expensive (Olafsdóttir et al., 1997).

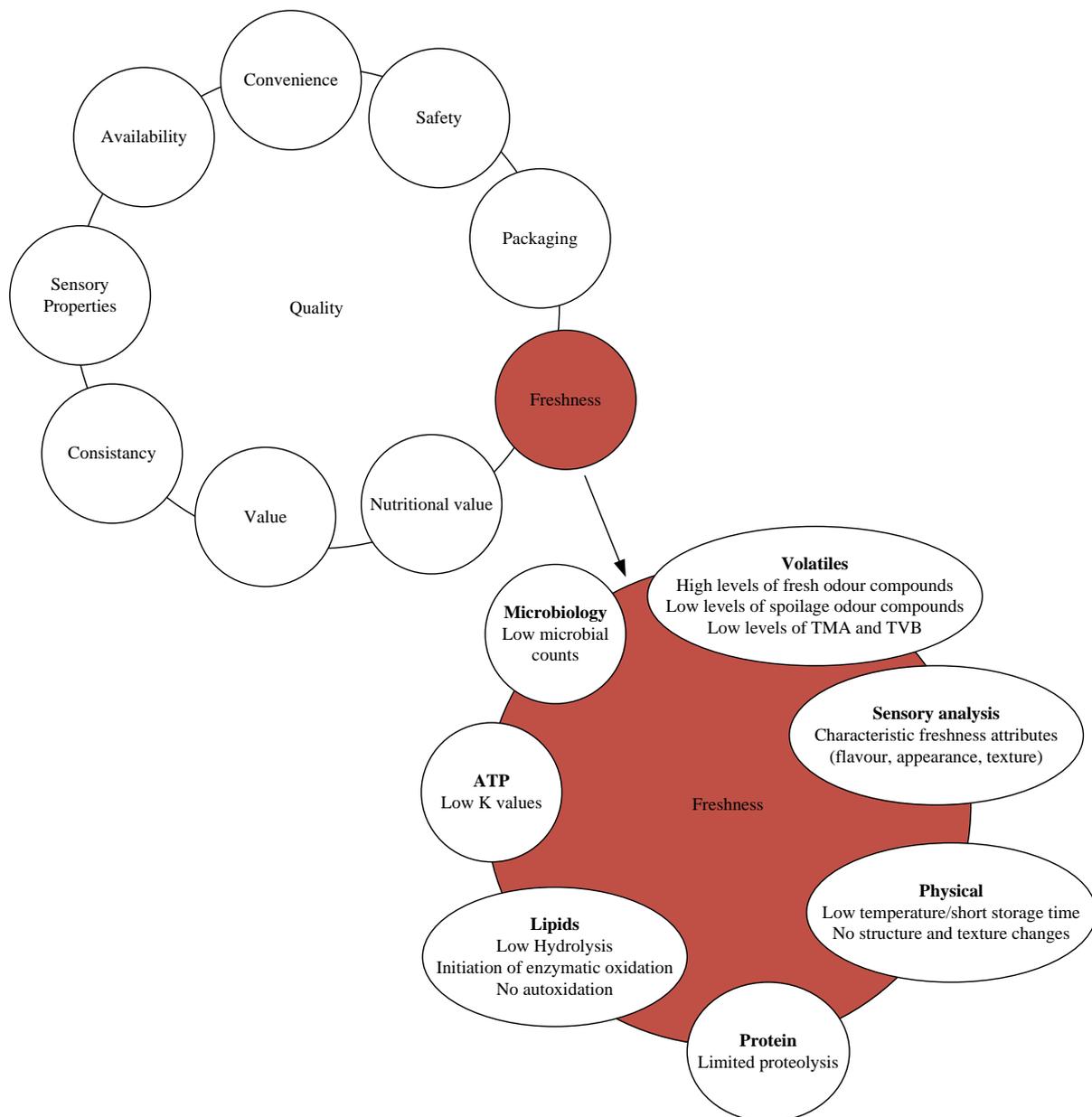


Figure 2.36 Relationship between quality and freshness and functions and determinates of freshness
(Source: Olafsdóttir et al., 1997)

Sensory evaluation of fish is expected to be used within industry to evaluate freshness and spoilage. The method is used to indicate measure, analyse and interpret characteristics of food. It is a very complex manner in which to evaluate fish freshness and quality and uses a

panel of experts, usually with about 8-10 people involved. Panels are selected by examining potential judges' ability to complete existing tests and once selected there are continual and rigorous training to make sure that the standard. As the name implies, the main tools used to perceive the quality of the food is through the senses of sight, smell, taste and touch of humans (Olafsdóttir et al., 1997). The panel rates the fish samples on certain criteria, which involve three types of tests used either on their own or in combination. The first stage of testing is used to determine differences between samples (discriminative), such as the triangle test, used as a method to compare two similar and one different sample of fish. Ranking tests are used by panels to rank samples in terms of a characteristic for example, levels of salt by taste or difference in colour of samples. Conversely, both of the tests can also be used to rate the strength of a panel.

Descriptive tests include rating the appearance of skin, gills and eyes, smell of the fish and the stiffness of the flesh. A score is then given to the sample based on these factors and this is called the quality index method (QIM). Structured scaling provides the assessor with a set of fixed number ratings and descriptive terms are used selectively to produce more precise ratings. Profiling is used to compare the fish sample to a reference sample. Attributes such as flavour, texture and toughness are assessed by a scoring system which is used to rank the quality of the fish sample.

The final method of testing is a measurement of the influences of the local preferences against the testing measures. Many local preferences exist for the taste and general state of food products and fish is no exception. The affective test is used to determine if the results found from any of the discriminative or descriptive test have any significance to the overall market in which the fish product is to be sold.

Quality control of the panel is assessed by the inspection of the final product. Figure 2.37 displays the different types of tests that can be used to determine fish quality and freshness by using the sensory analysis method. A thorough review of this method can be found in the literature (Olafsdóttir et al., 1997; Gill, 1995).

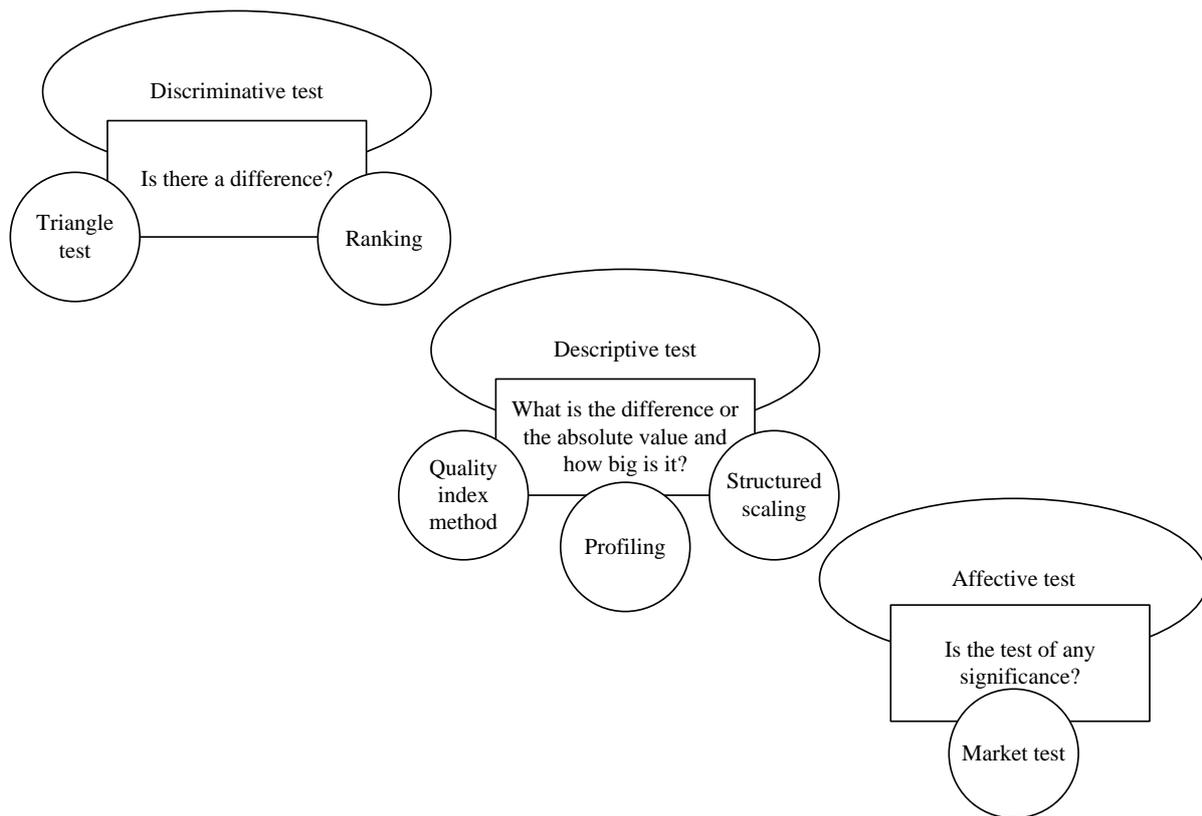


Figure 2.37 The sensory tests used industry to qualify fish freshness (Adapted from: Gill, 1995)

Some of the measurements for sensory analysis are now being taken on recently introduced machines capable of making better judgement than a panel of human judges. Equipment and techniques such as the electronic nose and VIS spectroscopy are being introduced to combine with the human test results (Olafsdottir et al., 2004). The combination of these new techniques has given rise to an artificial quality index (AQI) and has proven successful in providing an accurate replacement technique for analysing total fish quality. The drawbacks of using AQIs to measure fish quality and freshness is that it requires at least five pieces of expensive and technical equipment to be in the same lab or factory to perform a thorough analysis. This can often be more expensive and invasive than allowing a human panel to analyse the fish samples (Olafsdottir et al., 2004).

Microbial analysis is used in fish quality determination as much as any other food. Microbial analysis techniques use plate counting, testing and isolation techniques to verify bacterial populations and bacteria species present (Topic-Popovic et al., 2007). The majority of research is used to determine spoilage bacteria of different fish (Gram, 1992; Gram et al., 1987). Fish meat is a complicated medium and can produce a variety of conditions regarding pH and nutrient availability. Research has also shown that the SSOs of a fish not only

depends on the species but also from where the fish was sourced and the storage atmosphere (Gram and Huss, 1996; Dalgaard, 1995b). Table 2.10 below shows a summary of the research towards determining SSO for different packaging atmospheres and locations.

Table 2.10 SSO of fresh and packed fish stored below 4°C or in ice (Source: Gram and Huss, 1996)

Atmosphere	Specific spoilage organisms of fresh chilled fish			
	Temperate waters		Tropical waters	
	Marine	Fresh	Marine	Fresh
Aerobic	<i>S. putrefaciens</i> <i>Pseudomonas</i> spp.	<i>Pseudomonas</i> spp.	<i>S. putrefaciens</i> <i>Pseudomonas</i> spp.	<i>Pseudomonas</i> spp.
Vacuum	<i>S. putrefaciens</i> <i>P. phosphoreum</i>	Gram-Positive bacteria Lactic acid bacteria	Lactic acid bacteria and others	Expected to be Lactic acid bacteria
CO ₂	<i>P. phosphoreum</i>	Lactic Acid Bacteria	Lactic acid bacteria TMAO reducing bacteria	Expected to be lactic acid bacteria TMAO reducing bacteria

There are examples in the literature of modified atmosphere packaging affecting the evolution of TMA and total volatile basic nitrogen producing bacteria (Debevere and Boskou, 1996). The research suggested that varying the amount of oxygen and keeping carbon dioxide levels constant in the atmosphere of the packaging reduces the oxidation of TMAO. A 60% 40% 0% mix of carbon dioxide, oxygen and nitrogen respectively produced the least amount of TMA and had an inhibiting effect on *Shewanella putrefaciens*. Another study conducted by Drosinos et al (1997) showed the effect of modified atmospheres on the production of lactate and acetate from fish flesh. The research showed that under a modified atmosphere designed to inhibit growth of TMA producing bacteria that lactate and acetate could be used as quality marking metabolites (Drosinos and Nychas, 1997).

The literature also provides data on the numbers of quality rejection for these SSOs used in fish quality determination and the typical volatile compounds that are manufactured by these bacteria. Table 2.11 presents the finding of this research.

Table 2.11 Specific spoilage bacteria and typical spoilage compounds of some fish products (Source: Huss et al., 1997)

Product	Typical SSO	Number at rejection ¹	Typical volatile compounds
Fresh fish, aerobic chill store	<i>S. putrefaciens</i> ²	10 ⁸ - 10 ⁹	TMA, H ₂ S, CH ₃ SH,
	<i>Pseudomonas</i> spp. ³	10 ⁸ - 10 ⁹	ketones, aldehydes, esters, non-H ₂ S sulphides
Fresh fish, chilled, MA packed	<i>P. Phosphoreum</i> ²	>10 ⁷	TMA, Hx
	Lactic acid bacteria ³	>10 ⁸	NH ₃
Fresh fish, >10°C	Vibrionaceae	10 ⁷ -10 ⁸	TMA, volatile sulphides
Sous vide cooked fish	<i>Clostridium</i> spp.	>10 ⁶	strong fecal, sulphhydryl odours
Sugar-salted Herring	Halophilic, anaerobes ⁴	10 ⁷ -10 ⁸	Indole, H ₂ S, acids
	Osmotolerant yeasts	10 ⁴ -10 ⁵	fruity

¹ number in cfu g⁻¹, ² typical of marine, temperate water fish, ³ typical of freshwater fish and fish from warmer waters, ⁴ not identified

Microbial analysis has shown that SSO fractions have a major role in fish and seafood spoilage (Huss et al., 1997). The total viable count (TVC) of bacterial populations increases as normal following the stages of bacterial growth. SSO growth also follows the expected bacterial growth model but lags slightly behind the TVC population. A product is rejected at a sensory level usually once the SSO have become the dominate bacteria in the TVC and have reached a certain population. The overall shelf life in microbial analysis is said to be from the time of death until this population is reached. Figure 2.38 below shows the overall model that is expected for fish spoilage. For clarity and comparison, a pseudo-chemical spoilage indicator has been added to the figure to show the ideal relationship between the SSO and spoilage indicators.

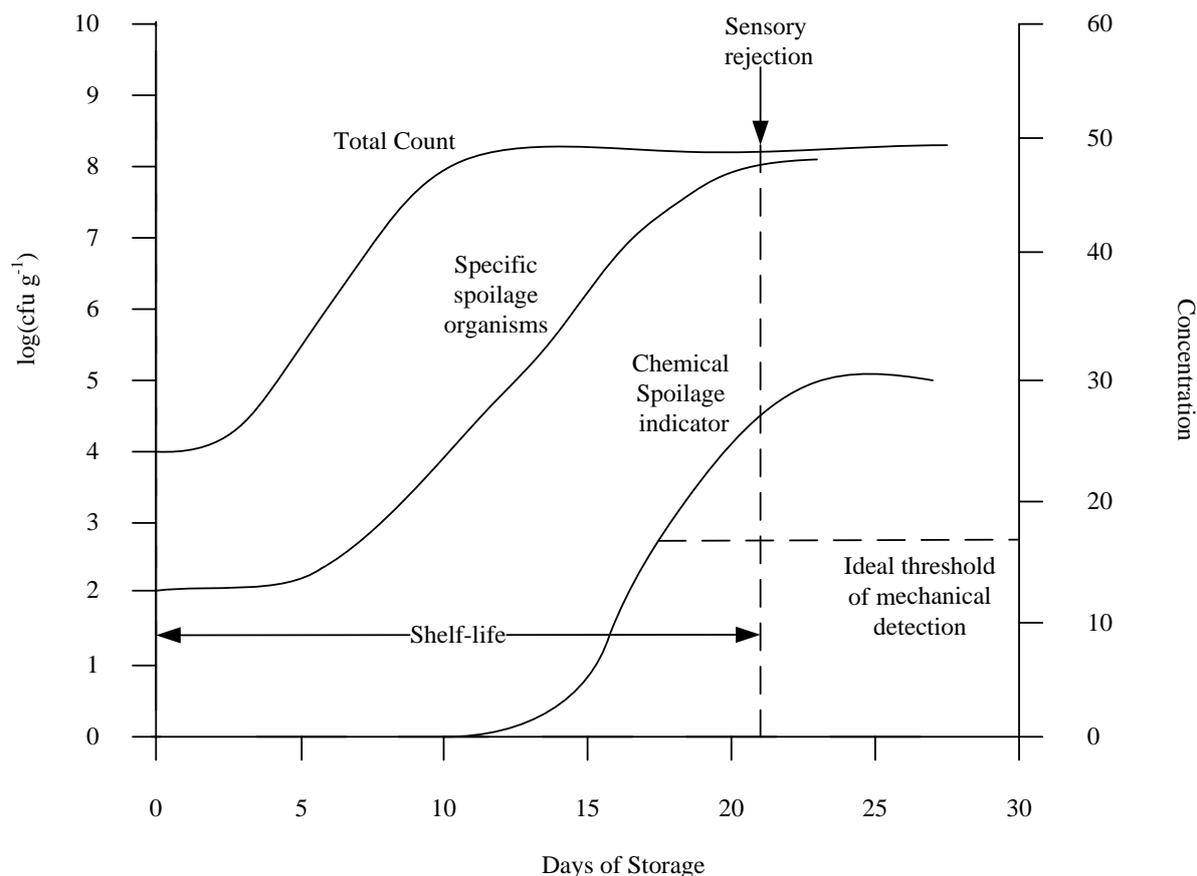


Figure 2.38 Model of changes in total viable count, specific spoilage organisms and chemical spoilage during chill storage of a fish product (Source: Huis in't Veld, 1996; Gram and Huss, 1996)

There have been several studies into comparing sensory and chemical changes in fish and seafood to microbial analysis. Research into the correlated changes of these factors in squid mantle (Paarup et al., 2002) gave evidence towards the effect of pressure on bacterial growth. The work also showed that TMA, ammonia and other biogenic amines increased at different rates over time of storage. Bacterial populations of *Pseudomonas* spp. and other spoilage increased in relation to loss of sensory quality and their respective chemical spoilage indicators.

Other research in this area has shown that there is a strong correlation between the numbers of SSO and the shelf-life of sea salmon stored on ice (Hozbor et al., 2006). *Shewanella putrefaciens* was identified as the major producer of TMA and hydrogen sulphide. Other SSOs that were identified included *Pseudomonas* spp., *Vibrionaceae*, *Enterobacteriaceae* and *S. Alga*. In this case and the shelf-life under these conditions was reported to be ten days.

Another study considered the effect of storage temperature on microbial spoilage and measured various freshness parameters such as odour, microbial growth and QIM to build a multivariate quality prediction model (Olafsdottir et al., 2006). The research gave evidence that the model produced was able to predict with a good degree of accuracy the freshness and sensory quality of haddock fillets. The model was eventually constructed to take into account the levels of ammonia, hydrogen sulphide, *Pseudomonas* counts and the time-temperature profile as these were suggested to be the most important factors to determine freshness.

Another group researched the sensory, physical, chemical and microbiological changes in European sea bass fillets (Poli et al., 2006). Various bacterial populations were ascertained over a time period of 8 days of storage on ice. These techniques were compared to the EU standards and legislations derive for determining fish quality (European Commission, 2005; European Commission, 1996) and found that the sensory analysis and microbial analysis techniques were concurrent with the regulations. The report mainly looked at the effect of modified atmosphere packaging and found that the overall effect of this was greater at delaying the production of hydrogen sulphide and TMA. This was because of the decrease in the growth rate of the bacteria that produce these gases.

The aim of this section was to compare and comment on the emerging methods for analysing fish quality and freshness. These included measurement of ATP degradation, TMA production, protein decomposition and bacterial changes. An overview of the majority of post-mortem changes in fish are represented below in Figure 2.39. The figure shows a simplified explanation of all of the post-mortem changes that occur simultaneously. The previously mentioned techniques have been used in combination or separately to determine fish freshness to provide evidence of an overlap between the different mechanics and measurement of fish spoilage.

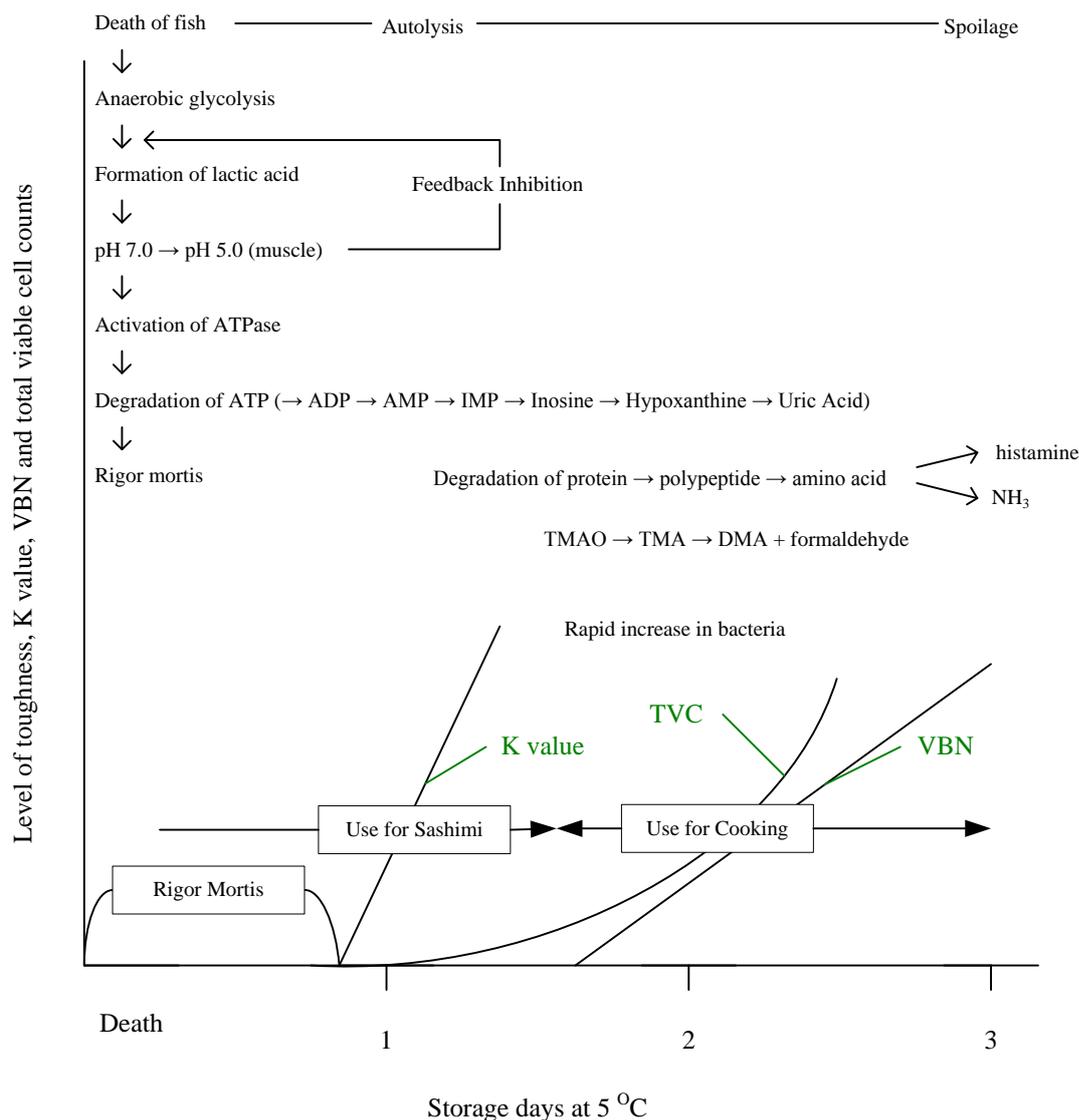


Figure 2.39 Post-mortem changes in fish meat where k is the rate of spoilage, TVC is total viable count and VBN is the amount of volatile base nitrogen (Source: Hamada et al., 2002)

Evaluating fish freshness and spoilage requires the amalgamation of several areas of science. Bacterial analysis, biochemistry and analytical chemistry skills are just some of the expertise required to provide an accurate description of the changes and processes involved in developing complete models for fish and seafood spoilage. Much of this research should be carried out in collaborations between research groups to provide these models (Olafsdóttir et al., 1997; Gram et al., 2002). Consumers demanding more knowledge about their purchases and expecting better food quality will act as major drivers for this to occur.

2.5 A review of food spoilage technologies

Modern-day food packaging is expected to provide containment and protection for food produce whilst being informative, attractive, easy to use and environmentally friendly. Consumer demands are primarily focussed towards food products that are fresh and unprocessed. Retailer demands are by contrast focused towards cost-effective measures that meet consumer demands whilst extending product shelf-lives. These requirements have led to a rapid development of innovations within the packaging industry. There are two key emergent branches that aim to meet these demands, namely, active packaging and intelligent packaging. Although there are products and technologies that are already available and in use, the focus of this section will be on future developments and the potential convergence of these two branches.

2.5.1 Overview of current technologies

Figure 2.40 presents an overview of active and intelligent packaging and the aspects that they can potentially improve. The diagram provides the previously mentioned four key functions of packaging. The terms active and intelligent packaging are situated above the roles that they aim to enhance. For example, active packaging provides a means to improve the protection of the contents, where intelligent packaging enhances the communication of data to the user. It is important to note that in some cases these categories overlap since some active packaging systems also include improvements based on user-convenience, such as microwave susceptors (Yam et al., 2005) which are, for example, used to allow better cooking performance in microwave food stuffs. In the schematic of Figure 2.40, overlaps between packaging features are represented by the areas in grey.



Figure 2.40 The model of packaging functions (Adapted: Yam et al., 2005)

Innovation and advances in the fields of intelligent packaging are aimed towards packaging improvements that facilitate improved communication to the user, whether the user is a warehouse computer system or a food consumer. The information that is to be communicated will be much more than just the sell-by date, the product brand or the ingredients. With future developments in packaging technology, it is hoped that the user will be able to determine quantitatively to what extent degradation of the food has occurred, the quality of the food stuff and how much time is left before the food becomes spoiled.

2.5.1.1 Active packaging

Active packaging is defined as ‘packaging in which subsidiary constituents have been deliberately included in or on either the packaging material or the packaging headspace to enhance the performance of the packaging system’ (Robertson, 2006). The interaction of the

active features can be through a chemical (modified atmosphere) or biological (antimicrobial agents) interface to provide an extended shelf-life or an addition to the packaging that enhances its performance.

Examples of active technologies used in packaging are shown below in Table 2.12. As previously mentioned, the underlying function of these technologies is to protect and increase the longevity of the product within the packaging. The examples given in the table act to preserve and protect the food product so that it is able to maintain the desired flavour and a customary appearance through delaying or hindering bacterial spoilage. This is achieved by modifying the atmospheric conditions of the packaging or by changing the surface of the packaging.

Table 2.12 Examples of active packaging applications for use within the food industry (Adapted: Kerry et al., 2006)

Active packaging	Application
Absorbing/scavenging properties	Oxygen, carbon dioxide, moisture, ethylene, flavours, taints, UV light
Releasing/emitting properties	Ethanol, carbon dioxide, antioxidants, preservatives, sulphur dioxide, flavours, pesticides
Removing properties	Catalysing food components removal: Lactose, cholesterol
Temperature control	Insulating materials, self-heating and self-cooling packaging, microwave susceptors and modifiers, temperature-sensitive packaging
Microbial and quality control	UV and surface treated packaging materials

Although these technologies are not the focus of this thesis, it is important to acknowledge their significance as they are often used in conjunction with intelligent packaging.

2.5.1.2 Intelligent packaging

Smart or intelligent packaging is a widely used term that often covers many different branches of technology and packaging design. Although there is no formal academic definition for the terminology “*smart/intelligent packaging*”, many agree that it can be defined as any packaging that goes beyond the use of simple materials in conjunction with printed barcodes or labels (Kerry and Butler, 2008). The term intelligent packaging is often used to describe improvements in existing materials or methods to extend shelf-life by preventing microbial growth (Coma, 2008; Sivertsvik et al., 2002). Intelligent packaging is

also used to illustrate additional design features to packaging that are convenient and that may enhance the usability of a product.

The less stringent definition of intelligent packaging allows for a greater scope of technologies and products. Table 2.13 below summarises the main ideas around this topic of research and proposes potential or available technologies that could be used.

Table 2.13 Examples of intelligent packaging applications for use within the food industry (Adapted: Kerry et al., 2006)

Intelligent packaging	Application
Tamper evidence and pack integrity	Breach of pack containment
Indicators of product safety/quality	Time Temperature indicators (TTI), gas sensing devices, microbial growth, pathogen detectors
Traceability/anti-theft devices	Radio frequency identification (RFID) labels, tags, chips
Product authenticity	Holographic images, logos, hidden design print elements, RFID

The focus for this thesis will be towards the development of intelligent packaging systems that can be used alongside food products to facilitate better stock management and safety within the food industry. The key driver for this technology is for an improvement for food safety and food quality assurance (Ahvenainen, 2003b). The improvement for better food safety directly benefits the end-consumer, however, with this technology there may also be direct benefits for food retailers and suppliers. Sustainability of food supply chains is a topic which is generating considerable political interest and these acts as another driving force for the development of this technology. High levels of waste can occur within the food industry, most of which is sent to landfill (C-Tech Innovation Ltd, 2004). Agreements from government and environmental departments suggest that there needs to be long term alternatives to landfill through the reduction of waste production (Defra, 2007b). This potentially could help drive the use of optimal technologies in supply chains to determine the cause of high wastage levels. Consumers are adding to this pressure as they become more environmentally aware in and more curious to a range of issues when it comes to selecting products based on traceability and health benefits. The ultimate aim is to produce technologies that could communicate and assure consumers around the issues of traceability and safety as well as reducing waste occurrence in supply chains.

For sealed packed produce, the only source of quality assurance at present is the packaging material and its integrity. This is communicated via the branding and the information

available on the label, including a sell-by date, the source of contents and location of production. With the consumer trend for fresher, less preserved foods of high quality, a potential market is emerging for an intelligent packaging that can identify food spoilage or deterioration of quality without being invasive, destructive or expensive. For the purposes of this chapter, the focus will be on the interactivity of the packaging with the consumer and the retailer as well as how reliable the information provided is in terms of food quality and safety - in other words, producing packaging that conveys data to the user that is both easily and comprehensive and accurate.

2.5.2 Time temperature indicators and freshness quality indicators (TTI and FQI)

Indicators can be defined as devices or substances that inform the user about the presence, absence or concentration of another substance. They can also indicate the extent of a reaction between substances by means of a distinctive change, such as colour. They are said to differ from sensors as they do not require a receptor or transducer and can impart information through a direct change or reaction (Kerry and Butler, 2008). The subtle difference between TTIs and FQIs is what each one interacts with. A thorough overview of TTIs is presented later in this section and can be found elsewhere in the literature (Taoukis and Labuza, 2003). Several authors have discussed their reliability and function (Selman, 1995; Taoukis et al., 1991; Singh and Wells, 1985). There are also several examples of FQIs in the literature (Kerry et al., 2006; Kerry and Butler, 2008; Hurme et al., 2002; Pacquit et al., 2008; Smolander, 2003). Consideration will be given later in this review to the principles and the operation of these two different types of indicators. Figure 2.41 below shows the distinction between TTIs and FQIs when used as intelligent labels. TTIs rely on modelling and predictive behaviour of microbial growth when temperature abuse occurs. FQIs on the other hand, interact with the metabolites caused by microbial growth and do not require microbial growth models.

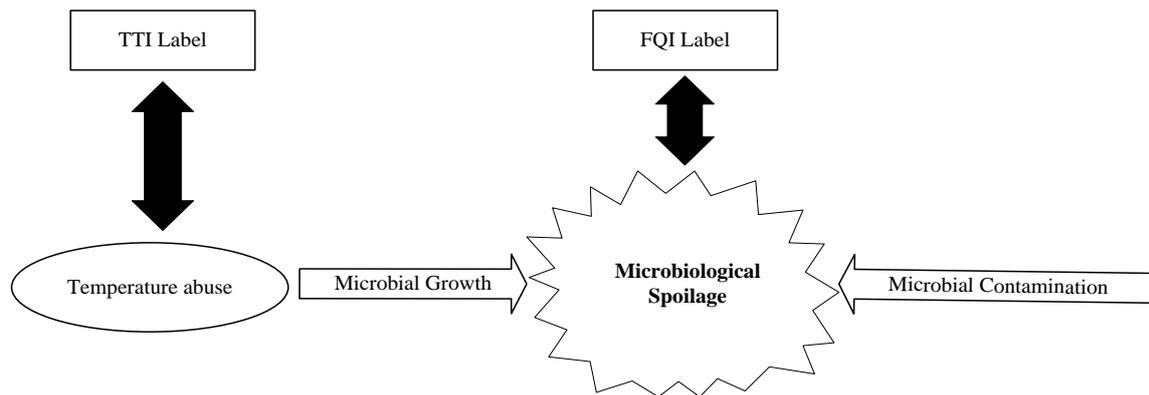


Figure 2.41 Comparison of the information obtained by FQI and TTI labels (Adapted from: Smolander, 2003)

Here, the focus is on freshness quality indicators (FQI) and time temperature indicators (TTI) as methods for determining food spoilage and estimating shelf-lives of differing products. As mentioned previously FQIs provide direct food quality information by reacting to changes taking place within the foodstuff. These changes can be due to either the external or the internal environment. Internally, these changes are caused by chemical degradation or microbiological activity and metabolism as the food perishes. TTIs on the other hand monitor the temperature and time lapse over a predetermined amount of time so that they are able to estimate how degraded food items changed due to an external environment variation, which in this case would be temperature. For example, if a sensor is set to measure an item that is to be stored for 5 days at 7°C , then it would indicate when 5 days had elapsed. If this item had been subjected to a higher temperature then the indicator would behave differently and would react quicker to inform the user that the food has degraded sooner.

Temperature is deemed to be the most important external factor controlling food spoilage. Storage temperature has a direct influence on the kinetics of the chemical and biological changes that occur in food products. Currently there are three types of commercially available TTIs, namely (i) critical temperature indicators, (ii) partial history indicators and (iii) full history indicators (Singh, 2000). There has been extensive review of these indicators throughout the literature (Taoukis and Labuza, 2003; Selman, 1995; Taoukis et al., 1991; Singh and Wells, 1985; Claeys et al., 2002; Smolander et al., 2004). Although the technology has been available for over 20 years, the rate of adoption or implementation has been very slow. This has been a result of the high cost of the technology and the lack of supportive information technology to allow appropriate utilisation (Yam et al., 2005). The

information that is obtained by these sensors is also deemed to be limited, especially in the context of cheaper FQI technologies becoming available (Kerry and Butler, 2008).

Most FQIs respond to changes of the gaseous headspace of the packaging. Several techniques exist which correlate changes in certain gases to microbial growth or chemical spoilage. This technology is usually concerned with items such as meat and fish that give rise to distinctive aromas once they spoil or become unsafe to eat. There are also examples of this technology in use with other items such as fruit and vegetables in addition to materials that are sensitive to changes in concentrations of oxygen and carbon dioxide (Ahvenainen, 2003a). Mostly they are concerned with food that has extremely short shelf-life. Table 2.14 summarises some of the points that are raised from previous research (Hurme and Ahvenainen, 1996).

Table 2.14 Comparisons of the advantages and disadvantages of TTI and FQI technology (Adapted: Pacquit et al., 2008)

Time Temperature Indicators		Food Quality indicators	
Advantages	Disadvantage	Advantages	Disadvantage
Easily Read	No Relation to Actual Food Spoilage	Easily Read	Not Yet Commercially Available
Accepted QA and Adaptable	No Concern for Food Safety	Direct Indication of Quality	Affixed Inside Packs
Commercially Available	Storage and Activation Issues	Potential Cheap Unit Cost	Potential Limitation of Materials i.e. Toxicity
Cheap Unit Cost	Not Suitable for Rapid Spoilage Products	Low Developmental Costs	No Concern for Food Safety
Science Understood			Difficulty in Applying to all Food Products
Maturing Technology			

The main issue with both technologies is the limitation of concern over food safety. In both cases the indicators are there to measure food spoilage which is followed as the food degrades and spoilage bacteria populations' increase to unsafe levels. This does not take into account the situation where a small number of pathogenic bacteria are sourced onto the food stuffs (e.g. Salmonella or E. coli (Hurme et al., 2002)). The presence of very small count of

these bacteria makes the food unsafe. A TTI is concerned only with the external environment and so it has no method of detecting the bacteria, whereas a FQI may not have the sensitivity or ability to detect such small populations especially if produced commercially on a small budget. Another potential pitfall of these technologies is their sensitivity to spoilage. If the lower limit of detection of spoilage is too high, then users may already be able to determine if the food is spoiled by visually checking it. On the other hand if the limit is too low, then food that is edible would be deemed by the sensor to be unfit for consumption. An advantage is that TTIs and FQIs allow certain levels of tailoring for the levels of detection since there are different standards and regulations for spoiled food throughout the world that are adhered to (Singh, 2000).

Another important aspect is the contrast of what kind of produce the user is labelling. The technology in a TTI label functions primarily with food stuffs that have to be kept at a low and constant temperature. It is only when there are deviations from this temperature that the indicator behaves differently to one that has been attached to a correctly stored item. There is also the issue that the reliability of the indicator increases with the amount of time it is set to measure. This limits the use of TTIs to mainly chilled or frozen long-life goods (Singh and Wells, 1985; Riva et al., 2001).

FQIs solely rely upon the change in headspace gas of the food. The data that they collect relies heavily on the bacterial growth and the metabolites that are produced. The gas has to be of the correct type and concentration to react effectively with the sensing element. This limits the deployment of this kind of food monitoring technology to aromatic and perishable food stuffs. This is the main reason that research in this area focuses on meat, fish and other short shelf-life food (Kerry et al., 2006; Pacquit et al., 2008). Table 2.15 gives an overview to the pockets of research devoted to the area of freshness indicators. There are numerous examples in the literature of different metabolites being used as potential indicators of quality as suggested below.

Table 2.15 Summary of the freshness indicating metabolites and indicator concepts available for their detection (Source: Smolander, 2008)

Quality indicating metabolite	Corresponding food types	Potential indicator and sensor principle	Commercial freshness indicating product
Ethanol	Seafood (Rehbein, 1993; RANDELL et al., 1995), fresh produce (González-Aguilar et al., 2004)	Enzymatic determination for package headspace (Smyth et al., 1999; Cameron and Talasila, 1995)	
Organic acids	Fresh fish (Drosinos and Nychas, 1997; Kakouri et al., 1997), Meat (Nychas et al., 1998), Poultry (Smolander, unpublished)	Published methods applicable as package integrated concepts not available	
Glucose	Meat (Dainty, 1996)	Determination by knife type probe (inside the products) (Kress-Rogers and Brimelow, 2001)	
Volatile Nitrogen compounds (e.g. ammonia, dimethylamine, trimethylamine)	Seafood (depending on season and species) (e.g. (Dainty, 1996))	Determination of volatile sulphur compounds from the package headspace, reaction based on pH sensitive dyes as a visual colour change (e.g. (Williams and Myers 2005; Williams et al. 2006; Miller et al. 1999)) or with an optical sensor(e.g. (Oberg et al., 2006; Pacquit et al., 2006; Byrne et al., 2002))	FreshTag (Cox Recorders, USA) (previously available) FreshQ (Food Quality Sensor International, Inc., USA)
Biogenic amines (e.g. tyramine, cadaverine, putrescine, histamine)	Poultry (Rokka et al., 2004; Schmitt and Schmidt-Lorenz, 1993), Beef (Edwards et al., 1987; Yano et al., 1995b; SMITH et al., 1993; Yano et al., 1995a), Pork (Ordonez et al., 1991)	Electrochemical biosensors or spectrophotometric assay based on enzymatic determination (Yano et al., 1995a; Okuma et al., 2000; Niculescu et al., 2000; Frébort et al., 2000; Punakivi et al., 2006) , direct contact with food required (e.g. (Yano et al., 1995a; Okuma et al., 2000; Niculescu et al., 2000; Frébort et al., 2000; Punakivi et al., 2006))	
Carbon dioxide	Indication of microbial growth in several food types ((FU et al., 1992; Mattila et al., 1990)) Indication of product ripeness (Korean kimchi) ((Hong and Park, 2000))	Determination of CO ₂ from packaging headspace, reaction based on pH sensitive dyes (e.g. (Hong and Park, 2000; Morris 2006; Horan 2000))	For appropriate level of CO ₂ in MA-packages (Sealed Air, USA) (Previously available)
ATP degradation products	Meat, especially seafood (Hattula, 1997)	Test Strips and electrochemical biosensors based on enzymatic determination, direct contact with food required (e.g. (Yano et al., 1995b; Mulchandani et al., 1990))	Transia GmbH (Germany) test strips

Quality indicating metabolite	Corresponding food types	Potential indicator and sensor principle	Commercial freshness indicating product
Sulphuric compounds	Poultry meat (Rajamäki et al., 2006; Lea et al., 1969), Seafood (Olafsdóttir et al., 1997)	Determination of volatile sulphur compounds from the package headspace, reaction based on colour change of myoglobin ((Smolander et al., 2002)), colour change of nano-scale silver layer (Smolander et al. 2004), colour change of package integrated, RF readable silver layer based tag ((Smolander, 2003))	Freshness Guard Indicator (UPM Raflatac, Finland)
Undefined Volatiles	Meat (deli, cooked, raw)	Determination of volatile compounds from the headspace of a storage bag or container, reaction based on visual colour change of a dye	It's Fresh™ (It's Fresh! Inc.) (concept for consumers use only)
Microbial Enzymes	Not specifically defined, several	Determination of substrate conversion due to the enzymatic activity, reaction requiring a direct contact with the food ((Van Veen 2004))	
Pathogenic Bacteria	Not specifically defined, several	Immunochemical determination requiring a direct contact with the food product ((Bodenhamer 2000; Goldsmith 1994; Woodman 2002))	Toxin Guard™ (toxin Laert Inc, Canada)

2.5.2.1 Overview of technology

The role of the intelligent label is to respond to changes in the external environment. The change could be a simple fluctuation in temperature along the cold chain or an increase in volume of a product due to seasonality. Considering the example of temperature abuse then a TTI label could be attached to a temperature sensitive product, where a change in temperature affects the state of the TTI label (Taoukis and Labuza, 2003; Selman, 1995). The change displayed by the TTI could then be fed into a data processing unit that could model and estimate the best business recommendations on how to manage the packaged food through the supply chain (Yam et al., 2005). Figure 2.42 shows the potential feedback loop of a TTI where once a decision has been made from the data gathered from the labels and an action has been taken in real time so that the data can be matched to an appropriate model.

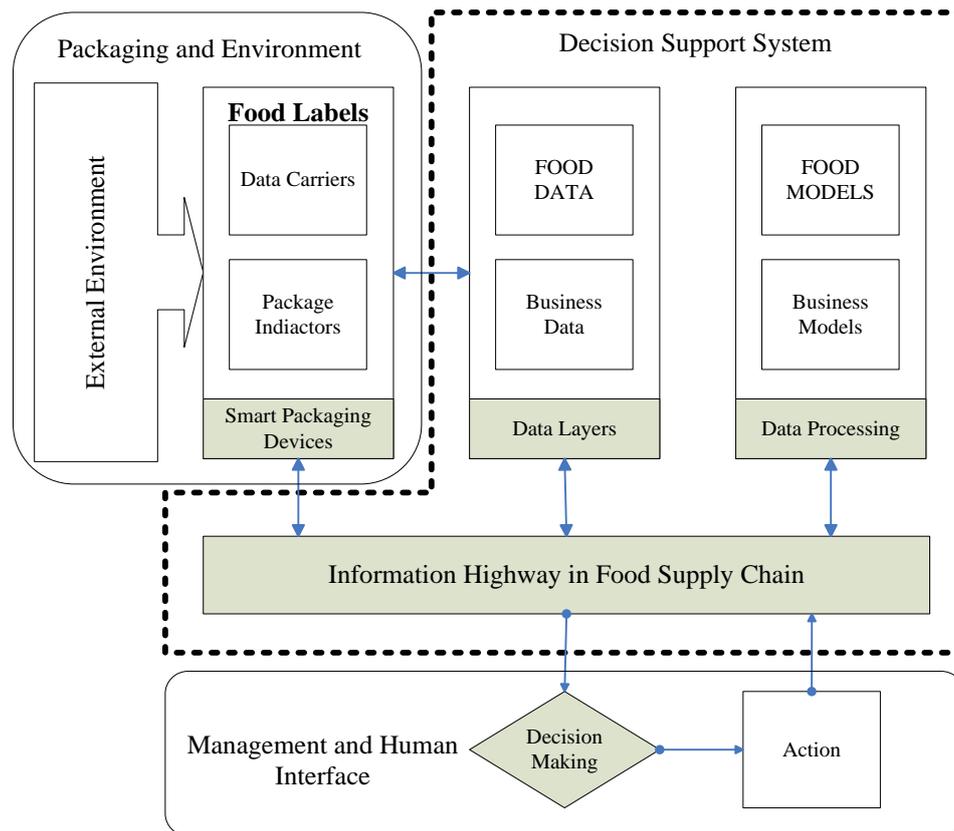


Figure 2.42 The possible information flow diagram of a supply chain implemented with a TTI intelligent label (Adapted: Yam et al., 2005)

Although the model is simplistic, it shows the data flow from the external environment, through the labelling technology and into a management decision. In this situation an indicator, such as a TTI, could highlight areas in supply chains which are not maintained or are inefficient at keeping food items chilled. As the data is recorded in real-time, food suppliers would get warning of failure or problems and be able to cope better with managing such a problem. Another use for TTIs has been to provide evidence in insurance claims due to logistical negligence (Tsironi et al., 2008). Transit vehicles that have failed to provide adequate temperature control for chilled products have been shown to be at fault from the data from these sensors.

As previously stated, one of the main barriers preventing the integration of TTIs into supply chains over the last 20 years has been the cost and also the requirement of a data and information handling system. Over recent years, production costs of TTIs and FQIs have been lowered and now it is almost unheard of to not have a data base or computing network setup for supply chain management purposes. These two factors have enabled a route to

commercialisation for these products. There are still however intrinsic problems that exist with introducing and producing new products and services such as these plus attributing costs and reliance on the present system. What may be required is a dramatic shift from one labelling type to another to provide a pathway for either technology.

An improvement on this model is not to rely solely on measuring changes to the external environment. If we were able to measure directly within the packaging the food and the impact from a change in external environment, then a more robust food model could be generated and a more reliable business recommendation could be made on what decision to be taken. A food quality indicator (FQI) could be used in this case to measure direct food changes and spoilage to ascertain actual bacterial changes in the food. Figure 2.43 shows the extra input of information into the food label. A change in temperature could be recorded (external factor) and then a monitoring of the spoilage on the food could also be determined (internal factor).

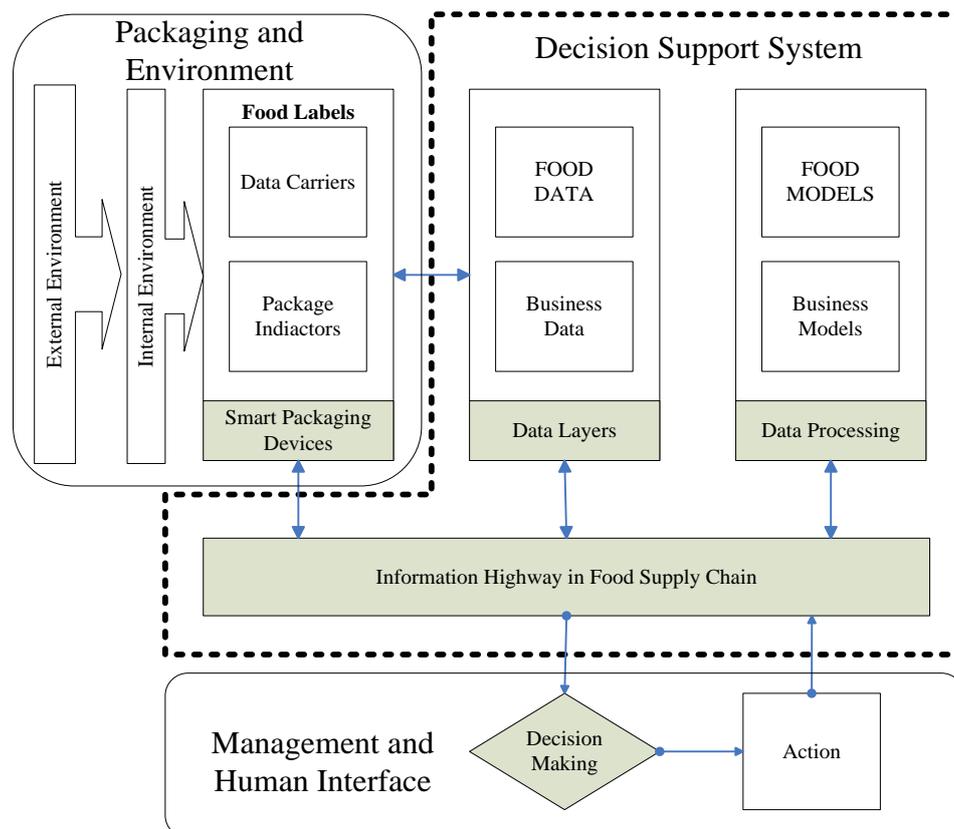


Figure 2.43 The possible information flow diagram of a supply chain implemented with intelligent packaging (Adapted: Yam et al., 2005)

The potential application for this technology, if used and harnessed correctly, is to provide a real time flow of information to food producers, retailers and consumers (Kerry et al., 2006). There are a lot of examples of these kinds of technologies that are in use currently (Pacquit et al., 2008; Hurme and Ahvenainen, 1996; Han et al., 2005). In the literature there are also countless examples of sensors and indicators that are being developed (Adhikari and Majumdar, 2004; Butler, 2001; Connolly, 2007). The next section will examine both the impact of TTIs and FQIs as intelligent food labels as well as comparing the two technologies.

2.5.3 Review of companies and their products

This section will give a complete overview of the current market of pseudo competitors. As this is an emerging market there are many branches of similar products. The main two technologies are TTIs and FQIs. There are more commercially available TTIs at present as the concept for a time temperature monitoring device has been around for over 20 years (Taoukis and Labuza, 1989). These intelligent labels have not been as successful as was predicted due to many factors. FQIs on the other hand are seen as a modern alternative to TTI technology and aim to supersede the incumbent even before TTIs have become a mainstay technology.

2.5.3.1 TTI technology

The main principle of TTI technology is that a label reacts to display to the user if a certain time has elapsed at a given temperature. If the temperature has been raised then that is accounted for in the kinetics of the reaction within the label and so the remaining life of the TTI is diminished accordingly.

TTI technology requires an initial model for determining the shelf-life and quality of the perishable product that it is to be attached to. The overall objective is to determine quality indices, A , which correlates with time, t . The indices can be a chemical change, a biological change or a physical change. Usually these are off-odours or colours from oxidation, measurements of microbial growth or texture loss. We can express the change of indices A in terms of time and temperature, T , as:

Equation 2.11 Relationship of quality indices to time

$$f(A) = k(T)t$$

Where $f(A)$ is the quality function of the product and k is the reaction constant. This is then related an Arrhenius expression, where the rate constant, k , is given as an exponential function of the inverse absolute temperature which is shown below:

Equation 2.12 The rate constant expressed as an Arrhenius equation

$$k = k_{ref} \exp \left[\frac{-E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]$$

In Equation 2.12, k_{ref} refers to the reaction rate constant at a reference temperature T_{ref} . E_a is the activation energy of the reaction of the quality indices and R is the universal gas constant (Taoukis, 2008). The activation energy of food spoilage chemical reactions and microbial growth is within the range of 30-140 kJ mol⁻¹ (Taoukis et al., 1997).

As with all reaction kinetics, the order of reaction has an overall effect on the equation being used. The appearance of the quality function for zero, first and nth order reactions are shown below in Table 2.16.

Table 2.16 The form of the quality functions for different reaction orders. (Source: Taoukis and Labuza, 1989)

Reaction Order	Quality Function $f(A)_t$
0	$A_0 - A_t$
1	$\ln \left(\frac{A_0}{A_t} \right)$
n (n≠1)	$\frac{1}{n-1} (A_t^{1-n} - A_0^{1-n})$

Integrating Equation 2.11 over a time period of time zero to time t gives the change in the quality indices during a known varied temperature exposure. T_{eff} is defined as the effective temperature, which is a constant temperature at which the same quality changes are observed over a time period compared to the exposure to a variable temperature distribution.

The kinetics of $f(A)$ are matched to a designed response function of $F(X)$, (given below in Equation 2.13), where X is a measurable change on a TTI label being either a colour or similar change.

Equation 2.13 The response function of a TTI

$$F(X) = kt$$

With k being an Arrhenius function of T as before, then the effective temperature notion, as mentioned above, can be used here also. Therefore for a TTI label that shares the same effective temperature and the same temperature abuse as a food item it is attached to in the cold-chain the response function can be expressed as:

Equation 2.14 The response function of a TTI

$$F(X)_t = k_{I_{ref}} \exp \left[\frac{-E_{AI}}{R} \left(\frac{1}{T_{eff(TTI)}} - \frac{1}{T_{ref}} \right) \right] t$$

In Equation 2.14, $k_{I_{ref}}$ and E_{AI} are the selected Arrhenius parameters for the TTI that is in use, and are determined from the intercept and the slope of an Arrhenius plot. These vary depending on the sensor that is in use and can be matched to fit the food product that they are assigned to. Table 2.17 provides some data on the type of reaction and activation energy of the sensors.

Table 2.17 Characteristics of some of the commercially available TTI labels (Source: Pocas et al., 2008)

TTI	Working principle	E_a (kJ/mol)
CheckPoint®	Diffusion	33-50
eO®	Enzymatic reaction	50-113
TT Sensor™	Solid state polymerisation	84-100
Fresh-Check®	Solid state reaction	Not available
OnVu™	pH change due to microbial growth	Not available
MonitorMark®	Diffusion reaction	Not available

Data has been gathered around what are considered to be the main TTI products that are available. These are in comparison to the current technology and products available from Time Strip Plc. The usability and scientific details of each TTI will be considered.

2.5.3.1.1 Vitsab, Sweden: Checkpoint® I and III products

Vitsab are a Swedish company that produce colour changing enzymatic TTIs commercially known as Checkpoint®. Currently these labels are designed to be used for meat, seafood and vegetable products. In the USA the labels have received acceptance for use on seafood from the Food and Drug Administration (FDA). These indicators are based on a colour change that is controlled by a fall in pH induced by a controlled enzymatic hydrolysis of a lipid substrate. The label consists of two parts, which are separate compartments, one holds an aqueous solution of a lipolytic enzymes and the other contains the lipid substrate suspended in a mix of aqueous medium and a pH indicator (Hogan and Kerry, 2008). Figure 2.44 shows the CheckPoint® III type EH label which is used for seafood HACCP applications. Activation of this label requires a pressure to be applied to the label so that a seal between the two components can be broken. This can be done either manually or by an online automation system.

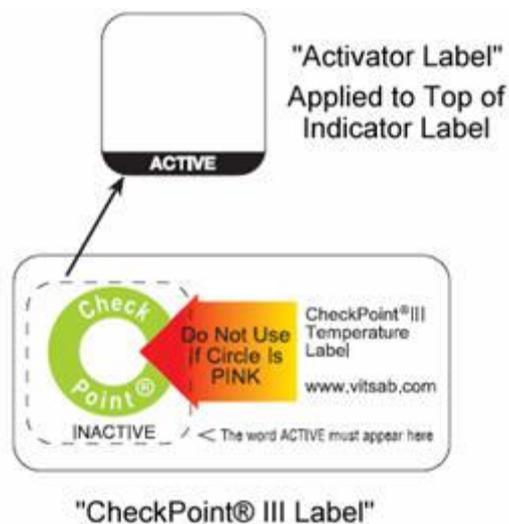


Figure 2.44 Vistab CheckPoint label III (source: www.vitsab.com)

The combination of different enzyme-substrate concentrations enable the sensor's response to vary according to the lifetime and the storage temperature of the item it is attached to. The drop in pH, which is caused by the hydrolysis of the substrate, is observed by the user as a

visual change in colour via the pH indicator. This means that no technical equipment is required to evaluate the results from the TTI which still allows a degree of subjectivity into any results given by this label. Vitsab produce a five point colour scale which can be used to check the results of the sensor and help to determine the freshness of the item it is attached to. The label also requires accurate information concerning the starting bacterial concentration to act effectively.

2.5.3.1.2 Cryolog, France: (eO)[®], echo[®] and TRACEO[®] systems

(eO)[®] is a label which can indicate levels of freshness through changes in colour. The label is in the form of a flower and comprises a small gel pad that transforms from green (fresh) to red (spoilt). Figure 2.45 below shows the label along with a possible use on packaged sandwiches.



Figure 2.45 Cryolog (eO) (Source: www.cryolog.com/en/)

The change in colour occurs as food microorganisms within the gel grow after activation to produce a change in pH. The label produces a clear and sudden change which enables the user to easily determine that the food item has perished.

Cryolog are also developing Echo, a system of sensors that will be able to remotely monitor temperature and relay this information back into a central system. This data will then be translated and interpreted to deliver a microbiology forecast to estimate the remaining freshness of the item the sensor are attached to.

TRACEO[®] is a label that is used for cold-chain products and is placed over the barcode of the item as seen below in Figure 2.46.

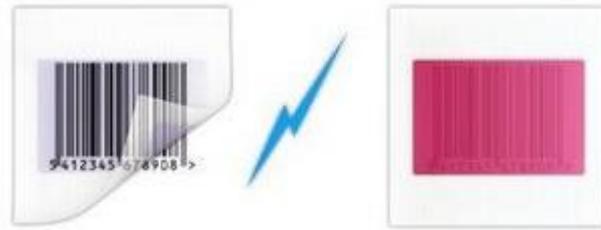


Figure 2.46 Cryolog TRACEO® label system (source: www.Cryolog.com/en/)

The label changes from clear to red to inform the user if the item has perished or if there has been temperature abuse along the cold-chain. This prevents the barcode from being scanned at the checkout. This technology has been superseded by the advent of the Echo® and e(O)® systems.

2.5.3.1.3 Avery Dennison Corp., USA: TT Sensor®

The TT Sensor® is based on the principles of diffusion. A change in concentration of a polar compound via diffusion causes a distinct colour change of the indicator from yellow to fluorescent pink as seen in Figure 2.47.

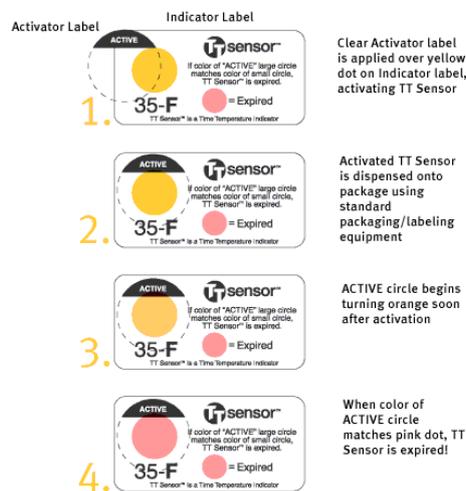


Figure 2.47 Avery Dennison Corp. TTsensor (source: www.averydennison.com)

The sensor is designed to be incorporated within the cold-chain and would display to the user whether the food has perished or the temperature in the supply chain had been abused. This is determined via the time and temperature dependence of the diffusion of the substrate and the concentration of the polar compound required for the colour change. Currently the labels

conform to the FDA criteria and HACCP plans for fresh seafood. The labels are available in three standard time-temperature profiles although customisation is possible to match specific requirements.

2.5.3.1.4 Temp-Time Technology, USA: Fresh-Check® indicator and Lifelines Freshness Monitor®

Temp-Time Technology produced two labels that can monitor the time and temperature after a point of activation. The Lifeline Freshness Monitor® is designed to be used in the food retail and supply industries and requires a laser optic wand to read the label. The Fresh-Check® indicator, however, is for consumer use only and can be interpreted using the naked eye. Figure 2.48 shows the stages of the label as the middle spot changes to indicate spoiled produce.



Figure 2.48 Temp-Time Technology Fresh-Check indicator (source: www.temptimecorp.com)

The Fresh-Check indicator is a TTI based on a simple, temperature dependant polymerisation reaction. Once activated, diacetylene crystals polymerise via a 1,4-addition polymerisation to produce a dark coloured polymer in the middle spot. The colour of the outer ring is designed to provide consumers with a comparison. The indicator is designed to be used in conjunction with the 'use-by date of the produce it is attached to. The kinetics of the indicator can be correlated to main pathogenic bacteria found in food such as salmonella and listeria.

2.5.3.1.5 Ciba Specialty Chemicals, Switzerland: OnVu™

OnVu™ TTI use colour changing organic pigments to display elapsed time and temperature fluctuations. As with all TTIs, the kinetics of the reaction is dependent on temperature. The label consists of an apple shaped icon with a middle heart which has the pigment readily printed on it. Figure 2.49 shows a sample label before activation.



Figure 2.49 An OnVu TTI label (source: www.onvu.com)

The label is in a stable condition until it is activated by UV light. At this point the heart becomes a deep blue colour and a filter is placed over the top to prevent the pigment from receiving further UV light. The pigment then fades to a shade of grey similar to the surrounding reference colour to show that the food has perished. Again as the same with previously mentioned labels, these labels can be tailored to different situations and food types.

2.5.3.1.6 SIRA Technologies, USA: Food Sentinel System™

The TTI produced by SIRA Technologies is incorporated into the barcode label of the item it is attached to. The modified barcode incorporates a thermochromic ink that is transparent once printed and activated. After temperature fluctuations the ink changes via an irreversible reaction to a deep magenta and prevents the bar code from being scanned. Figure 2.50 below shows the label on activation (left) and after a temperature abuse elapse (right).



Figure 2.50 the Food Sentinel System (source: www.siratechnologies.com)

The added benefits of this labelling system are the incorporation of the indicator into the existing barcode so that nothing else is required to be adhered to the packaging and the high stability of the ink which means the label could be used throughout the entire manufacture, supply and retail chain. There is not remark on time elapse and instead this TTI works in conjunction with the “use-by” date already printed on the food item.

2.5.3.1.7 3M, USA: Monitor Mark

3M are renowned for their production of innovative products and in turn they have produced a TTI that is based on temperature dependant diffusion of polymer materials that can be tuned to certain time and temperature conditions. Figure 2.51 shows the product below where each window shows the user the time elapsed since activation.



Figure 2.51 3M Monitor Mark (source: www.3m.com)

The irreversible colour change occurs when migration of a polymer blend flows onto a light reflective porous glass matrix. This occurs at a rate that is temperature dependant so as to detect temperature abuses. The label can be tuned to the desired time period and transition temperature with simple modifications of the polymer concentration and the glass matrix.

2.5.3.2 FQIs products currently available

FQIs are used to directly measure changes in food items that directly link to the quality of the product. Many of the products that exist in this area register gas changes and inform the user of actual microbial growth. This compares to TTIs label systems notifying the user when the TTI has predicted that the food item has gone beyond the shelf-life. Obviously, the added benefits of having actual data on food spoilage means that the user has better information on the quality and safety of the food item. As previously discussed, the difficulty of producing an effective FQI label is finding a metabolite or gas that directly correlates to food quality. Many FQIs are similar in their functioning to TTIs in that they rely on a colour change to report bacterial spoilage.

2.5.3.2.1 UPM Raflatac, Finland: Freshness Guard

UPM are a producer of a variety of paper and pulp materials, with the Raflatac division specialising in labels and labelling systems. The Freshness Guard is displayed below in Figure 2.52 and has been developed to be used as a detector of change in head space gases. The company also produces RFID labels and has aims to combine both technologies to produce the “Shelf-Life Guard”. Both of these labels systems conform to the EU regulations and UPM have shown interest in producing a similar system to be used for fish and seafood.



Figure 2.52 The freshness Guard attached to turkey portions (Source: Smolander et al. 2004)

The label is manufactured to react with hydrogen sulphide with nano – scale silver particles dispersed over the label surface (Smolander et al. 2004). The original colour of the thin silver layer is light brown and is segmented into a surround of similar colour. Once silver sulphide is formed the layer becomes opaque and a warning cross becomes visible on the layer. The label was produced to be used in cold chains especially during high seasonal demand for poultry produce (Christmas and Easter).

2.5.3.2.2 Cox Recorders, USA: Fresh-Tag

The Fresh-Tag is an FQI used for the determination of seafood quality. The label is designed to react to increased levels of amines as seafood spoils. A wick was inserted into the packaging without piercing the integrity. The wick would respond to amines by changing colour. The technology present in the Fresh-Tag was developed by the National Centre for Toxicological Research in the USA and then licensed out to Cox Recorders. Unfortunately, data regarding the product and the progress of research has been available as Cox Recorders was purchased by Sensitech in 2004, which were then more recently acquired by Carrier Corp. These companies do not have any records of the device (Pacquit et al., 2007).

2.5.3.2.3 Food Quality Sensor International (FQSI) Inc, USA: FreshQ

The FreshQ is designed by FQSI and incorporates research into using pH sensitive dyes for the detection of volatile amines produced from food spoilage (Williams and Myers 2005; Williams et al. 2006). Figure 2.53 gives the design of the label which could be incorporated into the packaging by adhering it to the inside of the packaging.

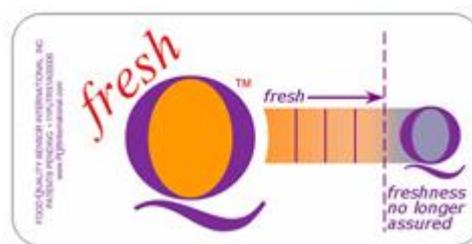


Figure 2.53 FreshQ FQI label (Source: www.fqsi.com)

The literature and patent describe a device that changes colour to indicate spoilage starting at an orange colour and changing to a grey shade once bacteria have reached a critical level.

The sensitivity of the label can be tuned by adjusting the original pH of the dye when it is deposited on the label. Thus, the device can also be used for detecting acidic compounds from spoilage if required. The sensor is to be marketed towards the supply chain and consumer markets by collaborating with supermarket chains, meat and poultry suppliers - and food science research institutions to produce a label costing less than US\$0.01.

2.5.3.2.4 It's Fresh Inc, USA: It's Fresh®

It's fresh have a portfolio of products aimed at the food spoilage detection market, as can be seen in Figure 2.54. Included within this collection they have developed a sensor that is only to be used by end consumers, the It's Fresh® indicator. The portfolio also contains an active packaging ethylene remover and a TTI for cold chain purposes.



Figure 2.54 It's Fresh Inc product portfolio (Source: www.itsfresh.com)

The It's Fresh® indicator is designed to be incorporated into either specially designed food containers or sealable bags using them as the substrate. The sensor detects extracellular enzyme activity and is detected by a colour change of the label. The drawbacks of this system are the requirement of a sterile environment to function properly as well as a need for the substrate to be in direct contact with the food stuff.

2.5.3.2.5 Toxin Alert Inc, Canada: Toxin Guard

This product is under development for commercial and military purposes. The sensor comprises a polyethylene film with immobilised labelled antibodies used to detect certain pathogenic bacteria. Further applications of this technology include the capture and detection of pesticide residues or genetically modified proteins (Bodenhamer 2000).

2.5.3.3 Current research and new products

This section will give an insight into the growing area of research that is being conducted towards the production of a viable food safety or spoilage indicators. This section covers new developments in the field of FQIs being produced by current research.

Over the last decade the Adaptive Sensor Group comprising Diamond et al, in collaboration with Unilever, have been developing an amine sensitive sensor to determine bacterial populations (Pacquit et al., 2006; Byrne et al., 2002; Pacquit et al., 2007; Loughran and Diamond, 2000). The original work describes the potential use of a calixarene as an acid-chromic dye sensor as a sensor for food spoilage. Calixarenes are macrocyclic molecules that are built up from aromatic compounds that often produce hydrophobic chambers that attract small molecules or ions. In the case of this work, the cavity is pre-arranged for the complexation of Li^+ ions chelating to the carbonyl oxygen atoms and the four phenoxy oxygen atoms is depicted in Figure 2.55. The labile proton present on the phenoxy group produces the change in colour when lost to volatile amines (i.e. NH_3 , DMA and TMA). The use of Li^+ ions gave the most sensitive response and the research has shown that the sensitivity and linear response of the dye to could be tuned by adjusting the ratio of ion to dye.

Initially the results showed that volatile amines could be detected by the visible change of colour of the dye by the naked eye (McCarrick et al., 1993), however, more accurate measurements were taken by using reflectance of UV-Vis spectroscopy (Loughran and Diamond, 2000).

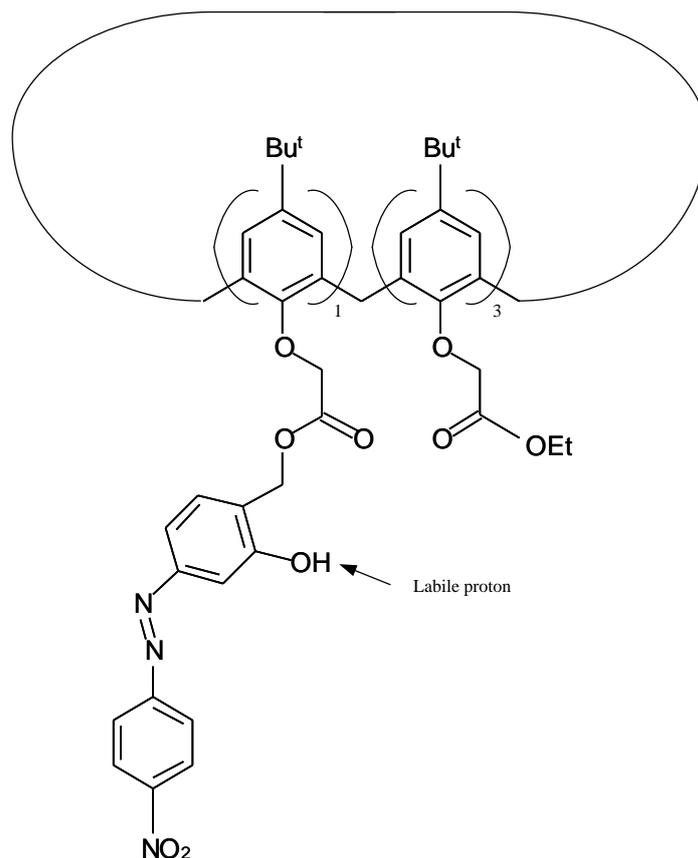


Figure 2.55 Structure of the mononitrophenolazophenol calix[4]arene tetraester derivative used as the acidochromic dye in the study completed by Diamond et al (Source: Loughran and Diamond, 2000)

The original experiments were conducted on ice and at room temperature ($22^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and concluded that an increase in volatile amines correlated to an increase in 515 nm absorbance from reflectance UV-Vis spectrum Figure 2.56. The results showed better sensitivity at higher temperatures and this is not explained as to whether this is because of the increased rate of production of volatile amines, the increased rate of evaporation of amines, the rate or kinetics of the deprotonation reaction - or the change in the headspace concentration of another indicating gas. The paper also commented on the increase of other organic compounds changing the spectrum of the dye but not at 515 nm as this correlated to the deprotonation of the phenoxy group.

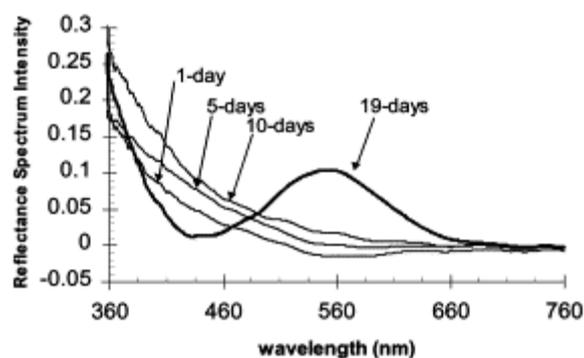


Figure 2.56 The long term study of cod samples on ice showing an increase at ~ 500 nm as the dye is deprotonated (Source: Loughran and Diamond, 2000)

The next two papers that follow from the research group use Bromocresol Green (BCG) dye immobilised onto a cellulose polymer based substrate as a sensor for food spoilage (Pacquit et al., 2006; Byrne et al., 2002; Pacquit et al., 2007). BCG is a dye that also has a labile proton and changes colour from brown to green if this proton is removed. The dye has a pK_a of 4.72 (Pacquit et al., 2006) when in the sodium salt form, making the dye comparable to a weak acid. Figure 2.57 shows the change and resonance forms for the dye.

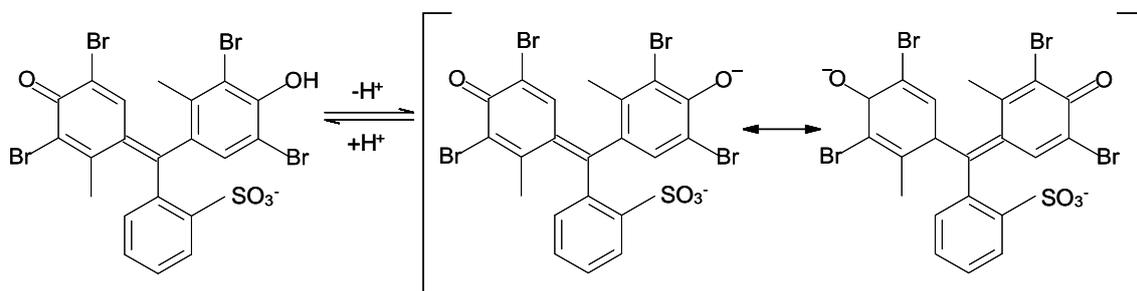


Figure 2.57 The BCG dye change from brown to green with addition or removal of a proton (Source: Pacquit et al., 2006)

The study aimed to correlate colour changes in the colour of the dye to increases in populations of *Pseudomonas* spoilage bacteria in cod and whiting fish samples. An LED was used to monitor the colour change that occurred at 483 nm (acid dye) to 615 nm (basic dye) using a reflectance colorimeter and a photodetector. The sensor required a hydrophobic membrane to prevent water from interfering with the dye. The experimental setup can be seen below in Figure 2.58.

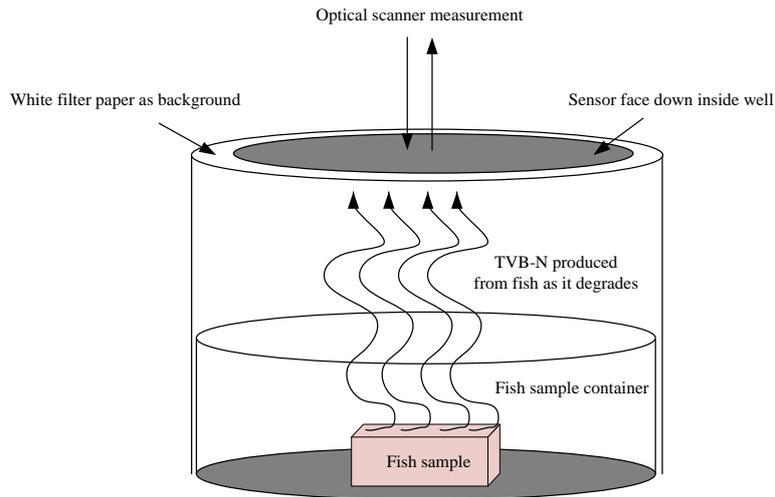


Figure 2.58 experimental design for fish spoilage monitoring (Source: Byrne et al., 2002)

The sensor was able to signal when the level of *Pseudomonas* and total viable count (TVC) was 10^7 CFU g^{-1} , which was reached after 18 hours at 20 °C (Pacquit et al., 2006). There is no evidence of the sensor being able to conform to these bacteria levels at lower temperatures and the dye has to be protected from water as condensing water can affect the signal. Figure 2.59 shows the normalised response of the sensor to bacterial growth on fresh cod. Further studies using BCG dye with whiting and cod at higher temperatures gave similar results with the sensor responding once the level of *Pseudomonas* had reached 10^7 CFU g^{-1} (Pacquit et al., 2007). The authors suggest that further work is required for a sensor to give a similar response at lower temperatures.

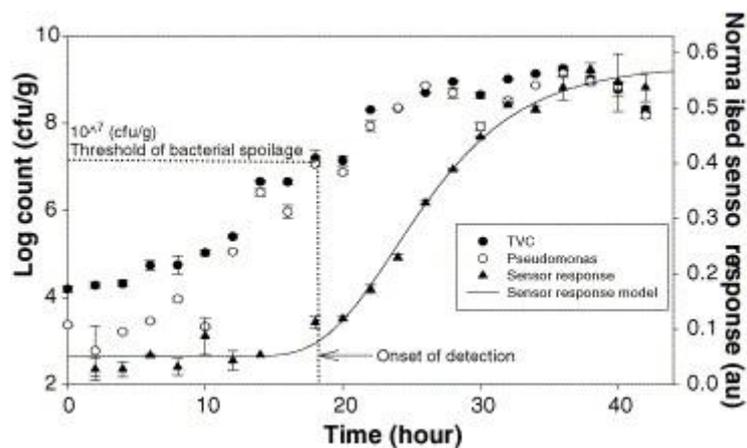


Figure 2.59 BCG dye normalised response to amine produced by bacterial growth in fresh cod samples at 20°C (Source: Pacquit et al., 2006)

Other work in this field is not as commercially focused as previous examples but is included in this review to give a complete overview of other techniques being used to monitor food

freshness. There are many examples of using semiconducting metal oxide (SMO) sensor arrays (El Barbri et al., 2007; Hammond et al., 2002; Schweizer-Berberich et al., 1994). The sensors work in a similar way to the polyaniline sensor being developed for this project. The sensor acts as a chemiresistor, changes in the headspace gas produce a change in electrical conductivity. The sensor is oxidised or reduced depending on the food product. Research has been carried out to show the correlation between bacterial growth on different species of fish to conductivity changes of some commercially available SMO films (Hammond et al., 2002). This paper also suggests a neural network that could determine what type of fish and at the state of degradation. The sensor would require specialist software if it were to be used in industry and does not integrate into existing packaging very well.

Research has also been conducted into the use of potentiometric sensors to determine freshness of sea bream (Barat et al., 2008, Barratt and Oliveira, 2001). The study used gold and silver electrodes and directly inserted them into fish tissue to determine freshness. The technique discussed was invasive and produced poor correlations between changes in conductivity to several spoilage factors, such as pH change, bacterial population increase and ATP decrease.

2.5.4 Prospects of intelligent packaging

Several authors have commented on the positive future for intelligent packaging and cited reasons for their beliefs throughout the literature (Yam et al., 2005; Kerry and Butler, 2008; Smolander, 2003; Byrne 1997). The main underlying factors to the expected success are:

- Consumers are driven by freshness and safety.
- Consumer demands will always increase.
- Government's demand for decrease in food waste and increase in food standards.
- As more exotic foods become common place and supply chains become global there will be a need for heightened traceability and logistic chains will become longer.

- The ability for intelligent packaging to offer control and data for entire industrial and retail supply chains. The information gathered will give users points for improvement and facilitate stock management.

It is expected that intelligent packaging of the future will be as much as a mainstay to consumers and retailers as the barcode is today (Byrne 1997). The labels of the future are expected to act as information providers as well as analytical tools. Underlying trends will affect the uptake of the technology, such as the rise in cost of food, the decrease in size and price of electronic components and innovations like printable conductive inks (Smolander, 2003). All of the technologies mentioned so far require an understanding of the microbial environment of food stuffs. For the field of intelligent packaging to progress there needs to be overlap in the fields of food science, bacterial modelling and the formation of quality indicating compounds.

Active packaging is expected to be adopted in the near future as it compliments many of the consumer trends present in today's food markets (Hurme et al., 2002). Intelligent packaging may not come to fruition until much later due to the factors mentioned above. Hurme suggests that research required to produce a FQI is so multidimensional that development of the technology represents an opportunity for collaboration between specialist companies and universities (Hurme et al., 2002).

2.5.4.1 Importance of technology

This section will highlight the key areas in which this technology could reduce costs in the future and will discuss the problems that it can help to overcome. It is anticipated that intelligent packaging will play an important role in the following areas:

- Food production and supply industry
- Food retailing
- Consumers' food awareness

These areas share similar problems that intelligent packaging can help to solve or minimise (Hurme et al., 2002). The first of these problems is the wastage of food. Intelligent packaging can aid the reduction of food waste via improved communication of food degradation to consumers - and could potentially reduce the amount of food waste from poor food production and supply chain systems. In a similar vein, there is the issue of stock management, traceability and information flows within the food industry. Above all, there are consumer concerns of safety and quality to consider, as well as overcoming confusion caused by over reliance on date codes (i.e. use-by and best-before dates).

In recent times, food wastage has become an important environmental, economical and political focal point for research and debate (BBC, 2008; Hogg et al., 2007; WRAP, 2007). WRAP (Waste and Resources Action Programme) of the UK recently reported that as much as a third of food bought by consumers is thrown away and placed into land fill (WRAP, 2007). The food drink and tobacco industry is responsible for 11% (7.5 mega tonnes) of the total industrial and commercial waste in the UK with the retail and wholesale sector being responsible for a further 19% (12.9 mega tonnes) (Defra, 2007b). There are three key stages throughout the food supply chain which can be envisaged as a supply pipeline. Figure 2.60 below shows the simplified three stages of food production, supply and consumption together with the waste produced at each stage. It is important to note that an amount of waste will be unavoidable. There are many examples in this context such as, for example, banana skins from a food production facility manufacturing banana containing goods.

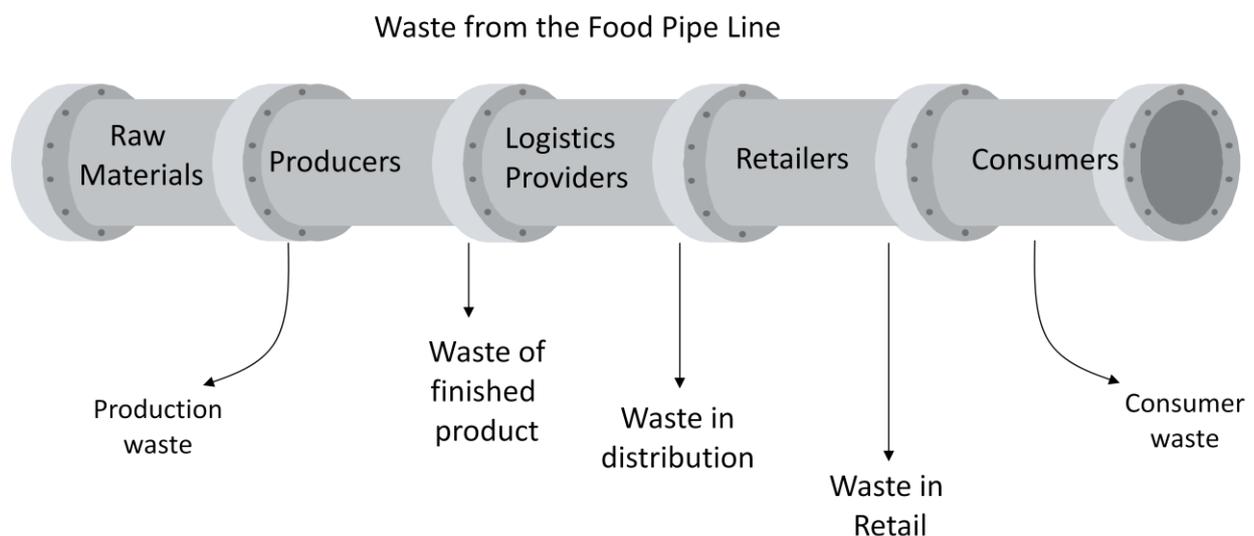


Figure 2.60 The three areas of waste production (Source: Mena, 2008)

A significant driver for the food industry to uptake this technology is the increase in production efficiency which in turn would aid in the reduction of production cost. As mentioned previously, governmental pressures on issues surrounding waste production and disposal are growing. Some types of wastage could be better understood if there was a more accurate means of determining the source or sources from a food supply chain. In terms of food traceability, packaging with sensors that follow a product from ‘farm to fork’ would provide key information about the producer and the route through the supply chain to the eventual sale to a consumer (Yam et al., 2005). In a situation where a product needs to be recalled then traceability would mean that less drastic measures would need to be taken to purge the supply chain. For example, if there was an emergency food recall due to a contaminant from one factory, then the information obtained from an intelligent label would prevent the waste of a whole product line and only the product which had passed through that factory. Another advantage as mentioned previously would be an enhanced consumer confidence in knowing where the item had come from if the label was able to display such information (Müller-Vieira et al., 2005).

Another important driver that needs to be considered for the introduction of intelligent packaging is the requirement of the consumer. A packaging technology that could prevent consumers buying and eating unsafe or poor quality produce would show obvious benefits. Research has shown, however, that there are several potential barriers to overcome when introducing new packaging technology (Hurme and Ahvenainen, 1996).

Table 2.18 shows the potential barriers and challenges to be overcome when introducing packaging technology to be used by the general public.

Table 2.18 Problems and solutions encountered with introducing new products using active and/or intelligent packaging techniques (Adapted: Hurme and Ahvenainen, 1996)

Problems	Solutions
Consumer attitude	Consumer research: education and information
Doubts over the performance	Storage tests before launching; consumer education and information
Increased packaging cost	Use in selected, high quality products; marketing tool for increased quality and QA
False sense of security, ignorance of date markings	Consumer education and information

Mishandling and abuse	Active compound/sensor incorporated into label or packaging film; consumer education and information
False complaints and returns of packs with indicators	indicator automatically readable at the point of purchase
Difficulty of checking every indicator at point of purchase	Bar code labels: intended for QA for retailers only; RFID system within stores

These solutions would require substantial investment before any real returning benefit is seen from the consumer, retailer or food producer (Han et al., 2005). The function of this technology for industrial use provides more opportunity and benefits in the short term. Using an indicator or sensor for monitoring food spoilage would require a design that integrates easily into existing supply chains as well as interpreting changes and potential hazards that the foodstuff is exposed to. The information that is obtained from the indicators has to be simple and accurate so that the data can be used to make key decisions. A sudden change in temperature in a cold chain would require a decision to be made around the safety of the food as well as an assessment on the scale of the problem.

2.5.5 Potential collaborating technologies

RFID has long been heralded as a potential breakthrough in supply chain management and as the solution to a lot of inventory management problems. In this section, a consideration of a system that has benefited from RFID (radio frequency identification tags) technology will be discussed. Comparisons between this case study and the food industry will then be drawn to demonstrate the potential impacts of RFID technology in collaboration with smart packaging technology. This thesis will not go into detail on the working of or technical aspects of RFID. An excellent overview has been written elsewhere which explains the workings and limitations of the technology (Clarke, 2008) and the reader is also referred to work by these other authors (McMeekin et al., 2006; Kerry et al., 2006; Trappey et al., 2009).

RFID permits the transfer of electronic data and therefore classifies as a separate intelligent device and does not fall into either the sensor or indicator categories. The concept is that tags are attached to items (ranging from cattle, containers, pallets, individual packets etc.) to give the user a real-time collection of data. This data is transmitted to an information system and allows analysis and tracking of the object the tag is attached to. For some, RFID technology

is seen as the natural evolution of the barcode in that it gives objects identification as well as a potential array of other information.

The case study of Marks and Spencer (Stafford, 2008) shows how RFID technology can be transferred to the clothing department. The original technology was used in the food supply chain as a measure of volume and inventory management. In the case study, intelligent labels were attached to individual items of clothing that were then tracked and registered during their journey through the supply chain. When transferring the technology from food to clothing, some of the key benefits were deemed to be:

- Improved store service
- Product visibility
- Inventory accuracy
- Improved processes

In this case the tags were only used as a stock control and were unable to emit signals without the correct interrogating signal. This meant that they were able to avoid the issue of breaching customer privacy. The intelligent label that would be used as a food monitoring device would need to be able to communicate with the user at any point to highlight any problems. This is a potential hurdle in light of recent bad press that RFID has received for being an intrusive technology into consumer privacy. Another problem is the cost of an integrated label that would require adequate power to monitor food and signal any problems if necessary. This would require a reliable power source and could place the cost of the technology out of the range of potential users.

It is often said that retail is detail. RFID technology in the first instance acts as a descriptor of what it is attached to. Consider a plastic tray in a food depot containing packaged portions of chicken breast; a written tag on the side of the tray would be able to confirm what the contents were and a few more key pieces of information (weight, date and place of origin etc.) which would then be manually entered onto a stock management system. A barcode could provide a method of relaying this information, and maybe more, to a stock management system. If a real time device, such as an RFID tag with an attached FQI, were to be attached

to the tray then much more information could be ascertained. This information could include more data on the source of the meat, the route so far taken by the tray through the supply chain pipeline, the predicted shelf-life remaining of the meat and so forth.

Figure 2.61 below shows a simple model of a supply chain using RFID technology in conjunction with a FQI tag. Upon entry and exit of every stage of the diagram, the RFID chip is updated with where it has been; a remote reading could then be taken of the state of the food stuff that is attached.

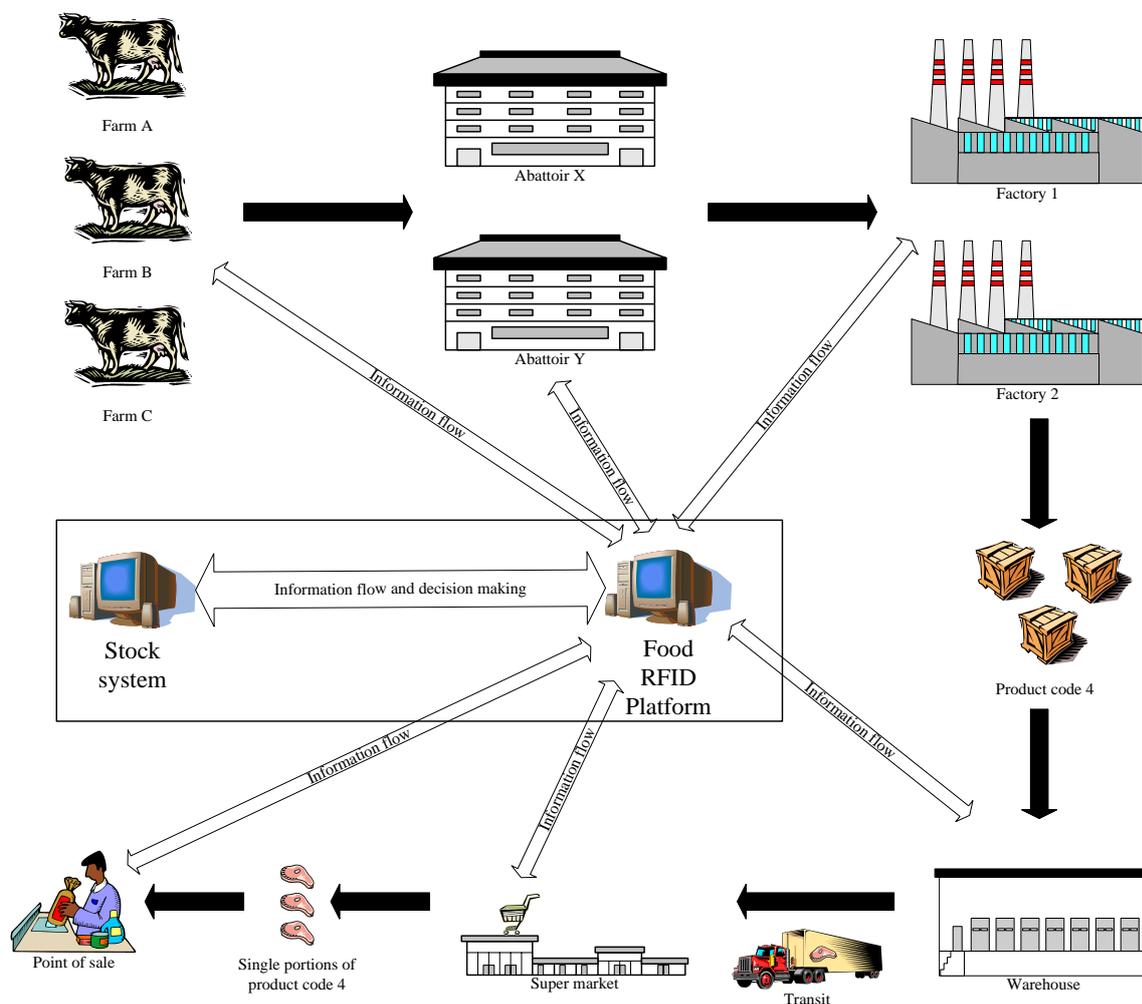


Figure 2.61 a simplified map of a supply chain and how an intelligent tag could be used for stock control and product monitoring (Adapted from: Stafford, 2008)

Following this simplified diagram, a generic meat product (here named *product code 4*) is followed from producer to point of sale. The emphasis here is on the different routes the product could take through a supply chain and also the breakdown of original produce to the

final finished product. The wealth of information that could be obtained from a system like this would help to find weakness in efficiency throughout the chain as well as helping manage inventory levels and supply. In the case of Marks and Spencer, there is already an RFID framework that is set up to work with food inventory management. Adding to this existing framework, a sensor to estimate product safety and condition would enhance the ability for managers to make stock management decisions.

For many, the idea of an RFID tag attached to an individual item, such as a single piece of chicken, for the purposes of stock management in the food industry would be complex and expensive. If the tag were to provide more information about the remaining shelf-life and possible contamination, then the cost and information trade off would be better balanced. With the falling cost of RFID tags and the pressure from governments for food retailers to hit waste targets, it will not be long until a major retailer invests in a system such as this.

Chapter 3

Materials and Methods

3 Introduction

This chapter provides details of the materials and methods employed throughout this EngD project. This includes the preparation and characterisation of polyaniline film coatings, the modification of the sensors and the preparation of samples for optical and spectroscopic analysis. The techniques used for bacterial analysis will also be discussed.

3.1 The synthesis of polyaniline films

3.1.1 Reagents

All reagents used were of analytical grade unless specified. Aniline Hydrochloride (99% purity), potassium persulphate, ammonium persulphate, hydrochloric acid, acetic acid, sodium acetate and sodium chloride were all purchased from Sigma Aldrich (Dorset, UK).

3.1.2 Materials

A4 250 μm thick polyester Melinex® sheets were used to deposit the polymer to closely mimic food packaging film. These were purchased from Cadillac plastics (Swindon, UK). Glass microscope slides and test tubes were acquired from Fisher Scientific Ltd. (Loughborough, UK).

3.1.3 Equipment

An Analytical Electrochemical Workstation 2-10 (Sycopel Scientific Instruments, UK) was used in conjunction with ECProg3 user interface software (version 3.6) for experiments involving the electrochemical deposition of polyaniline. Screen printed electrodes with a carbon working electrode interface and silver/silver chloride reference electrode (Microarray Limited, UK) were used as the substrate for the electrochemical deposition.

3.1.4 Method

The method used for production of 1 coated Melinex® A4 sheet used the following method which was adapted from previous research into polyaniline film production (Kukla et al., 1996; Kukla et al., 2009). A glass test-tube, 24 mm in diameter and 150 mm in length, was cleaned and then a cut piece of Melinex® sheet was rolled and pressed against the inside of the glass. Aqueous solutions of 2 M aniline hydrochloride in 0.1 M hydrochloric acid and 0.6 M potassium persulphate were prepared. Due to the low solubility of potassium persulphate, the solution required heating to allow the substance to be fully dissolved. Aniline hydrochloride was then poured into the tube so that it was half filled. The rest of the tube was then filled with potassium persulphate.

It was observed that the mixture turned blue as soon as the potassium persulphate solution was added and then proceeded to become increasingly cloudy as the polymerisation reaction took place and solid polymer precipitated. This mixture was then left for 20 minutes. The residual polymer sludge was removed and washed from the reaction vessel using 0.1 M hydrochloric acid. The Melinex® sheet was then removed from the tube and then rinsed three times with 0.1 M hydrochloric acid to remove any solid debris and to ensure that the polymer remained green in colour. It was then left to dry overnight. This was repeated until the desired amounts of sheets were coated. The sheets were then cut into the desired area.

3.1.5 Variations on this method

This method was also repeated using 0.6 M ammonium persulphate to compare the oxidation strength of both reagents and the effect of deposition of the polymer film. As the solubility of this reagent is much higher than potassium persulphate no heat and stirring was required for complete dissolution in deionised water. A range of concentrations of oxidising reagent were also used to compare any effect of this variation. The reaction vessel was also heated and cooled to investigate any effect of deposition on the surface of the Melinex® films.

Other substrates that were used included solid plastic such as disposable Petri dish and microscope glass slides, both from Fisher Scientific Ltd. (Loughborough, UK). These were used to compare the thickness and grafting ability of the polymer film. These were placed inside the reaction vessel.

3.1.6 Electrochemical deposition of polyaniline films

The mechanical method of deposition was compared to the electrochemical method used to deposit polyaniline. A buffered solution of pH 4 100ml 0.2 M aniline hydrochloride was made containing 7.5×10^3 moles of acetic acid, 2×10^3 moles of sodium acetate and 7.5×10^2 moles of sodium chloride. Individual screen-printed carbon electrodes were immersed into 5 ml aliquots of this solution and connected to the Sycopel potentiostat. The equipment was set up to scan between -200 mV to +800 mV and then sweep back down from +800 mV to -200 mV. The sweep rate was set at 50 mV s^{-1} and was repeated over various scan repeats for analysis in film thickness.

3.2 Sensor manufacture

The coated sheets produced from the previous method were used to develop the sensors described within this project. The method detailed here gives the optimal manufacturing process for the sensors used in the later experiments in section 5 and build upon previous work by Madathil (2005) for the deposition of thin and uniform layers of polyaniline (Madathil, 2005). The reader is referred to section 5 for the detailed explanation and development of the method of manufacture and testing.

3.2.1 Reagents

Aniline Hydrochloride (99% purity), potassium persulphate, ammonium persulphate, hydrochloric acid were all purchased from Sigma Aldrich (Dorset, UK).

3.2.2 Materials

Plastic centrifuge tubes (50ml) from Fisher Scientific Ltd. (Loughborough, UK) were used as vessels to contain the two solutions. Clamp and boss along with stands can be used to hold these vessels whilst the reaction is taking place. Gold coated crocodile clips and wire from Maplin (Rotherham UK) were used as connectors.

3.2.3 Equipment

An Agar B7341 Automatic Sputter Coater (Agar Scientific Ltd., Essex, UK), in conjunction with a Pfeiffer Rotary Vane Vacuum Pump (Pfeiffer Vacuum Ltd., Newport Pagnell, UK)-Figure 3.1 was used for the sputter coating of the sensors edges with a thin and conductive gold surface applied to provide a good connection to the crocodile clips.



Figure 3.1 Sputter coater used in sensor fabrication

A Philips XL30 FEG SEM was used for all scanning electron microscopy. Electrochemical analysis was carried using INCA “Point and ID” system onboard the electron microscope. A DEKTAK ³ST surface profiler (Veeco, Cambridge, UK) was used to measure the surface profile of the deposited films and estimate the thickness.

3.2.4 Method

Sheets of polyaniline coated Melinex ® were cut into desired size and shape with an area of 2.5 cm² (\pm 0.5 cm²). These sensors were then tested to determine whether the conductivity was suitable for manufacture. This was established by measuring the resistance across the surface of the sensor. The DC resistance of sensors was checked using a Metrix MX54C multi-meter. The acceptable upper limit of measured resistance across the sensor was 30 k Ω , anything above this threshold was rejected (> 10% of the coated sensors manufactured).

The following synthesis followed a two pot dipping process. Using the centrifuge tubes, an equal amount of the polyaniline solution and the oxidising reagent were placed into two separate tubes. A quantity of 40 ml was adequate so that the entire length of the coated film could be submerged into the solutions. The coated strip was first placed in aniline 2 M solution and then washed in 0.1 M hydrochloric acid solution. The strip was then dipped into a 0.6 M solution containing the oxidant (either potassium or ammonium persulphate) and then washed in deionised water followed by 0.1 M hydrochloric acid. If required, the strip would then be dipped again in the aniline hydrochloric acid solution, washed and then dipped

in the oxidant. These steps could be repeated any number of times to get the desired film thickness and conductivity.

A colour change of green to blue was observed when the film was dipped in the oxidant solution signifying a change from polyemeraldine salt to base. A further colour change of blue to green was observed when the film was washed and dipped in the aniline acid solution. This process can be seen below in Figure 3.2. The cycle was repeated a variety of times and film thickness, conductivity and reproducibility were analysed.

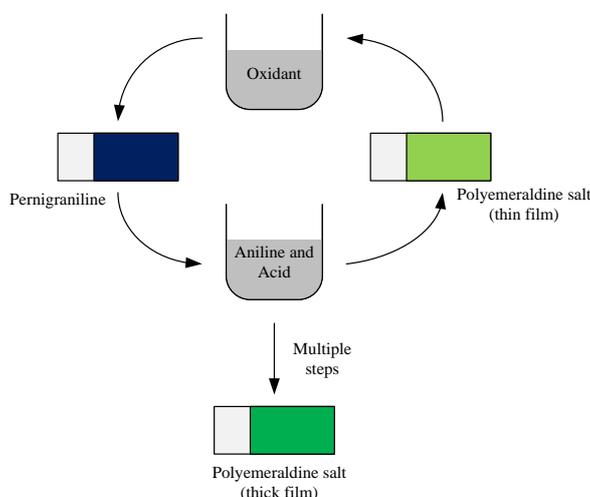


Figure 3.2 A two pot strategy of polyaniline film synthesis (Source: (Madathil, 2005))

Scanning electron microscope and other surface analysing techniques were conducted to compare the morphology of the films to those in the literature. Surface roughness and thickness studies were performed via DEKTAK surface analysis.

These thick films were then cut to the desired length and covered whilst the ends were gold sputter coated to give gold connections at either end. A current of 30 mA was passed under 0.1 mbar of pressure for 60 seconds to produce a thick enough gold coating.

3.3 SIFT sample preparation

The SIFT-MS technique was used to analyse gas head space of fish and meat samples. The same methodology was followed for all analysis.

3.3.1 Materials

The materials required to make a head space bag were as follows. An inflatable Nalophan bag (KALLE, UK) was attached via a polypropylene tube from Fisher Scientific (Loughborough, UK) and fitted with a Swagelok fitting (Swagelok, England) to a Luer counterpart, Figure 3.3. The Luer fitting could be directly connected to the SIFT-MS intake device. Nalophan is available in sleeves of different diameters and its length may be varied according to sample volume required. The material is inert and does not emit large amounts of volatiles itself making the material ideal for this use.



Figure 3.3 completed nozzle fittings for headspace bags including a Swaglock and Luer fitting

Salmon and herring fish were sourced from Tesco supermarket (Kingston, Milton Keynes, UK) and were gutted, prepared and frozen into appropriate sizes before storage at $-40\text{ }^{\circ}\text{C}$ within one hour of purchase. All salmon and herring samples were sourced from the Atlantic.

3.3.2 Equipment

The analysis of samples was carried out on a Profile 3 SIFT-MS system (Instrument Science Limited, Cheshire, UK) Figure 3.4, using Trans Spectra profile 3 Beta software (Instrument Science Limited, Cheshire, UK).



Figure 3.4 the Profile 3 SIFT-MS system used for headspace analysis

3.3.3 Method

Head space bags were manufactured by first cutting a desired length of sheeted tubing. For all these experiments, bags were cut from tubing of 15 cm in width and to a length of 40 cm. The bottom of the tubing was then concertinaed and then double folded over itself. Cable ties were then used to seal the bottom of the bag by constricting the concertinaed fold. The sample was inserted into the bag at this point. The plastic nozzle with cap was then inserted and rested at the top of the bag which was then folded around the shaft of the nozzle. Two cable ties were used to hold this in place and to ensure the bag was air tight. The bag was then inflated with clean and hydrocarbon free air. An example of a headspace bag can be seen below in Figure 3.5.



Figure 3.5 A bag used for headspace analysis

Each sample of salmon and herring (1 g) was introduced into the constructed bag and then sealed. Samples that had been taken from lower or higher temperatures than room temperature were given a period of twenty minutes to adjust before analysis. Once the samples had adjusted to room temperature they were then each applied in turn to the sample intake of the SIFT-MS. An average scan between a mass to charge ratio of 16 atomic mass units to 200 atomic mass units was carried out 10 times. This was repeated for each of the precursor ions (H_3O^+ , NO^+ and O_2^+). The mass viewing software was then used to interpret the result in terms of counts being converted into parts per billion (ppb) concentrations.

3.4 GCMS-HS sample preparation

Gas chromatography mass spectroscopy studies were carried out in parallel to the SIFT-MS experiments for comparison of the results and the performance of the techniques. The system available at Cranfield Health uses thermal desorption (TD) for loading samples onto the chromatogram column.

3.4.1 Reagents

Fully deuterated toluene (Supelco) in AR grade methanol (Sigma) was used to produce a 100 $\mu\text{g ml}^{-1}$ solution for the standard added to each analysis. Salmon and herring fish were sourced from Tesco supermarket (Kingston, Milton Keynes, UK) and were gutted, prepared and frozen into appropriate sizes before storage at $-40\text{ }^{\circ}\text{C}$ within one hour of purchase. All salmon and herring samples were sourced from the Atlantic.

The bags used for collecting headspace volatiles were made using the same method, materials and equipment as for the SIFT-MS headspace bags.

3.4.2 Materials

Thermal desorption stainless steel tubes, Figure 3.6, were used to load and store samples of headspace volatiles which were sourced from Markes International (Markes International Limited, UK). The tubes were packed with tenax and carbotrap sorbent and sealed using brass fittings.



Figure 3.6 A thermal desorption tube

3.4.3 Equipment

A Perkin Elmer AutoSystem XL gas chromatograph equipped with an ATD 400 thermal desorption system and TurboMass mass spectrometer (Perkin Elmer, Wellesley, MA) was used to analyse gas samples. The components of this machine can be seen below in Figure 3.7.



Figure 3.7 Perkin Elmer instrument setup. On the right is the thermal desorption unit which feeds samples onto the chromatograph (middle) with the mass spectrum unit on the left

An Agilent ADM 2000 gas flowmeter (Agilent Technologies Limited, UK) pump was used to load samples and standards onto the TD tubes.

3.4.4 Method

TD tubes were loaded with the sample head space gas from the bags used in the SIFT-MS experiments. 1g of fish sample was placed into a separate bag for each sample. The tubes were connected to the flow pump and the sample bag via gas tight fitting. A flow rate of 50 ml min^{-1} was then applied to the system for two minutes to allow the tube to adsorb headspace volatiles. Brass fittings of the tubes were replaced with PTFE caps. The tubes were then loaded with glass wool and 50 ng of the standard solution (d8-toluene) and attached to a helium flow line at 500 ml min^{-1} for 20 seconds. The glass wool was then removed and the tubes were placed in the loading carousel of the ATD 400 thermal desorption system.

Adsorbed volatiles were analysed via the following method. TD tubes were desorbed by purging for 2 minutes at ambient temperature then for 5 minutes at $300 \text{ }^\circ\text{C}$. Volatiles purged from the tubes were captured on a cold trap which was initially maintained at $30 \text{ }^\circ\text{C}$. The trap was then heated to $320 \text{ }^\circ\text{C}$ and maintained at that temperature for 5 minutes whilst the exhaust effluent was transferred to the gas chromatograph via a heated ($180 \text{ }^\circ\text{C}$) transfer line coupled directly to a Zebron ZB624 wall-coated open tubular column (dimensions $30 \text{ m} \times 0.4 \text{ mm} \times 0.25 \text{ mm ID}$) (Phenomenex, Torrance, CA). The gas chromatograph oven was

programmed to maintain 50 °C for 4 minutes following injection. The temperature was then raised at a rate of 10 °C min⁻¹ to an upper limit of 220 °C and held at this temperature for 9 minutes. The mass spectrometer was operated using the full scan mode (range m/z 33 to 200). The resulting mass spectra were combined to form a total ion chromatogram (TIC) by the GCMS integral software (TuboMass version 4.1).

Results from the chromatograph and spectrometer were analysed using the National Institute Standard and Technology library database via the Automated Mass Spectral Deconvolution and Identification System software (U.S. Department of Commerce, Gaithsburg, MD, USA).

3.5 Bacterial analysis

Bacteria populations and growth data were required to correlate and compare to the analysis of the head space composition and the sensor response. For this experiment, three temperatures were chosen to compare the growth of bacteria from salmon and herring.

3.5.1 Reagents and media

Tryptone soya broth (comprising pancreatic digest of casein (17 g l⁻¹), papaic digest of soybean meal (3 g l⁻¹), sodium chloride (5 g l⁻¹), dibasic potassium phosphate (2.5 g l⁻¹) and glucose (2.5 g l⁻¹)) (Oxoid), tryptone soya agar (comprising pancreatic digest of casein (15 g l⁻¹), enzymatic digest of soya bean (5 g l⁻¹), sodium chloride (5 g l⁻¹) and agar (15 g l⁻¹)) (Oxoid) and bacteriological peptone (LABM) were all sourced from Fisher Scientific Ltd. (Loughborough, UK). Pseudomonas selective agar (comprising peptic digest of animal tissue (20 g l⁻¹), magnesium chloride (1.4 g l⁻¹), potassium sulphate (10 g l⁻¹), Triclosan (Irgason) (0.025 g l⁻¹) and agar (13.6 g l⁻¹)) (Fluka), Sodium Chloride (Sigma) and glycerol (Sigma) was sourced from Sigma Aldrich (Dorset, UK). Salmon and herring fish were sourced from Tesco supermarket (Kingston, Milton Keynes, UK) and were gutted, prepared and frozen into appropriate sizes before storage at -40 °C within one hour of purchase. All salmon and herring samples were sourced from the Atlantic.

3.5.2 Materials

Sterile Petri plates (Fisherbrand), sterile 10 ml pipettes (Costar), sterile 25 ml and 50 ml universals (Nunc) were all sourced from Fisher Scientific Ltd. (Loughborough, UK). Sterile bags for homogenisation of samples (Plastiques Gosselin) were sourced from Fisher Scientific Ltd. (Loughborough, UK). All other aliquots were measured using standard Gilson pipettes and sterilised tips sourced from Fisher Scientific Ltd. (Loughborough, UK).

3.5.3 Equipment

A Lab Blender 400 stomacher (Seward Medical), Figure 3.8, sourced from Seward Medical (London, UK) was used to homogenise fish samples. A temperature controlled incubator (TISS) sourced from TISS (Hampshire, UK) was used to store fish samples at set temperatures and incubate bacterial plates.



Figure 3.8 Lab Blender 400 Stomacher unit

All work was carried out in a sterile environment provided by either a class I or class II laminar flow cabinet provided by MDH International Figure 3.9. Sterilisation was carried out using Astell AMA 240 BT autoclave system (Astell Scientific Limited, Kent, UK). A glass rod (Fisher Scientific Limited, Loughborough, UK) was used to produce a plate spreader which was stored in 70 % isopropanol solution and flamed before and after use.



Figure 3.9 Laminar flow cabinets used for experiments carried out under sterile conditions.

A Gallenkamp colony counter (Gallenkamp Company Limited, UK) was used to tally plate counts for both the total viable and pseudomonas plates.

3.5.4 Method

In preparation of the media, agar plates and broth were made up in accordance to the instruction indicated by the producer. This involved adding a certain mass of powdered media into a 1 litre volume. For Pseudomonas isolation agar, 10 ml of glycerol was also added as instructed. Tryptone soya broth media was prepared and stored in 9 ml aliquots for one in ten serial dilutions. Bacteriological peptone solutions were made up into 40 ml aliquots consisting of 1 % peptone and 0.85 % sodium chloride per volume of water. All solutions were sterilised using the autoclave system.

An appropriate number of 4g samples of salmon or herring were cut from whole fish samples. These were then sealed into sterile stomacher bags and stored at the desired temperature in a constant temperature incubator. The rate at which samples were analysed depended on the storage temperature. The experimental plan between temperatures can be seen below in Table 3.1.

Table 3.1 Sample size and sampling rate for bacterial study at different temperatures

Temperature (°C)	Time between samples (hour)	Number of samples
24	1	24
14	3.6	20
4	6	20

For each experimental measurement, a sample was taken from the incubator and then a 40 ml aliquot of peptone solution was warmed slightly and then added. The liquid and fish were then homogenised by using the stomacher for a period of 60 seconds. The homogenous mix was now a 0.1 g ml⁻¹ solution and was then serial diluted by taking 1 ml and diluting in the pre-prepared 9 ml tryptone soy broth solutions. The serial dilutions were carried out to an appropriate level of dilution. Both sets of agar plates were given 0.1 ml of this dilute solution which was then spread over the plate using a sterile glass spreader. Tryptone soy agar was used to measure the total viable counts from the fish samples, with pseudomonas agar being used to selectively grow only these types of bacteria. Plates were produced in triplicates for each dilution step analysed. The plates were then incubated at 30 °C and for a period of 48

hours. Plate counts were taken manually after this period using the plate counter. This raw data was then analysed and modelled using Microsoft Excel and JMP stats package.

3.6 Polyaniline film as a food spoilage sensor

The films produced from the previous methods were investigated for the possible use as a food spoilage sensor. The majority of experiments were carried out at room temperature.

3.6.1 Reagents

Salmon and herring fish were sourced from Tesco supermarket (Kingston, Milton Keynes, UK) and were gutted, prepared and frozen into appropriate sizes before storage at $-40\text{ }^{\circ}\text{C}$ within one hour of purchase. All salmon and herring samples were sourced from the Atlantic.

3.6.2 Materials

Various containers were used and modified to act as the chamber holding the sensor and food stuffs. These included 30 ml universals and 50 ml centrifuge tubes from Fisher Scientific Ltd. (Loughborough, UK). A modified glass jar with a volume of 2.5 l (loaned from Dr. Leon Terry) was used in test of the reaction of polyaniline to different volatiles, see Figure 3.10.



Figure 3.10 A 2.5 litre gas jar used for testing different volatiles with gold pin connectors.

Gold coated crocodile clips from Maplin (Rotherham UK) were used as connectors. Gold plated socket and pins, electrical resistors and platinum wires sourced from RS components (RS Components Limited, UK) were used to create quick changeable connections as can be seen in Figure 3.10.

3.6.3 Equipment

A Shimadzu UV 2100 UV-Vis spectrometer (Shimadzu, Milton Keynes, UK) was used for the characterisation of polyaniline films. The UV-Vis machine can be seen below in Figure 3.11. An Analytical Electrochemical Workstation 2-10 (Sycopel Scientific Instruments, UK) was used in conjunction with ECProg3 user interface software (version 3.6) for continuous DC experiments at room temperature. The AC impedance was measured using a GillAC potentiostat (ACM Instruments Limited, Cumbria, UK). The DC resistance of sensors was pre-checked using Metrix MX54C multi-meter.



Figure 3.11 The Shimadzu UV 2100 UV-Vis Spectrometer used for film analysis

For the experiments involving a variable temperature, a specialised piece of equipment loaned from the Food Security and Environmental Health group managed by Dr. Leon Terry within Cranfield Health. The equipment can be seen below in Figure 3.12 and consists of a block of aluminium with two reservoirs and heat exchangers at either end.

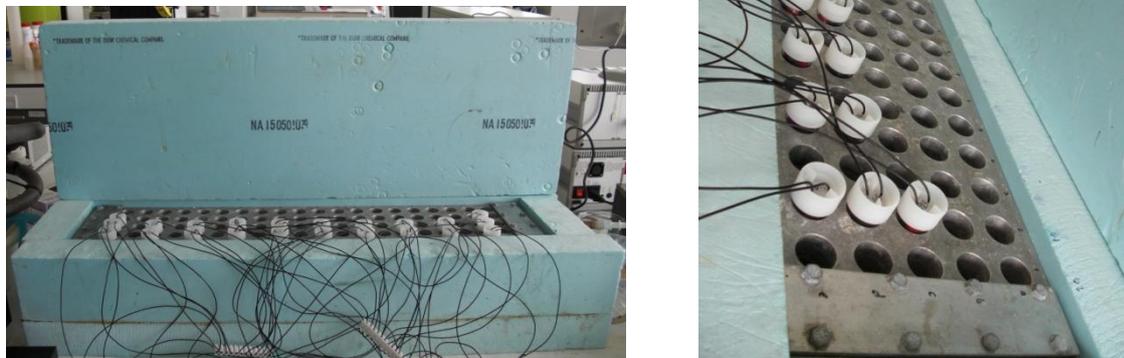


Figure 3.12 Specialist aluminium block used to create temperature gradient loaned from Dr. Leon Terry

Throughout the block, holes are drilled a set distance apart 5 rows across so that if the difference between the two heat exchangers is 20 °C, the increase in temperature from one row of holes to the next is 1 °C. The block is heavily insulated so that thermal stability can be kept over a long period. To keep one heat exchange below room temperature, a recirculation cooler was required (modified Churchill chiller thermal circulator loaned from Dr. Leon Terry). The cooler, Figure 3.13, was filled with CarPlan Blue star anti freeze (Screw Fix Direct Limited, UK) in a ratio of 20 % per litre.



Figure 3.13 Modified cooler used to create a cold heat exchange at one end of the block

A Techne water bath coupled with a Fisher Brand water pump and heater (both sourced from fisher scientific), Figure 3.14, were used as the heated thermal exchange at the other end of the aluminium block.

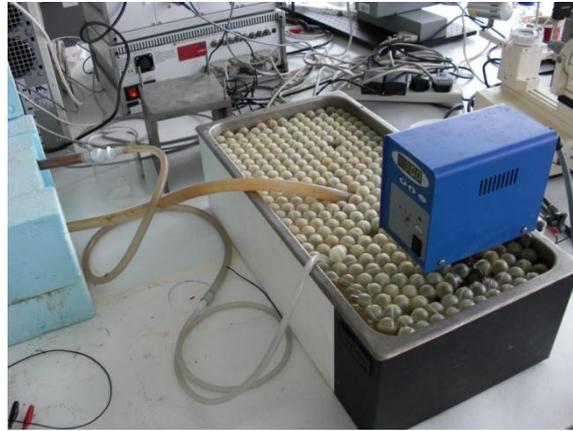


Figure 3.14 Warm water bath used to create hot heat exchange at other end of the block

A complete setup of this equipment is shown below in Figure 3.15 with the cooler and the water bath placed at either end of the aluminium block. The wires seen in the figure are connected to samples and the blue foam on top of the block usually rests over to provide heat insulation.



Figure 3.15 Entire setup of variable temperature experiment

Data storage and analytics were carried out using a CDL field computer (Cranfield Diagnostics Limited, UK.) in conjunction with a specialist software package. This was a custom made data storage unit and had 14 channels of input.

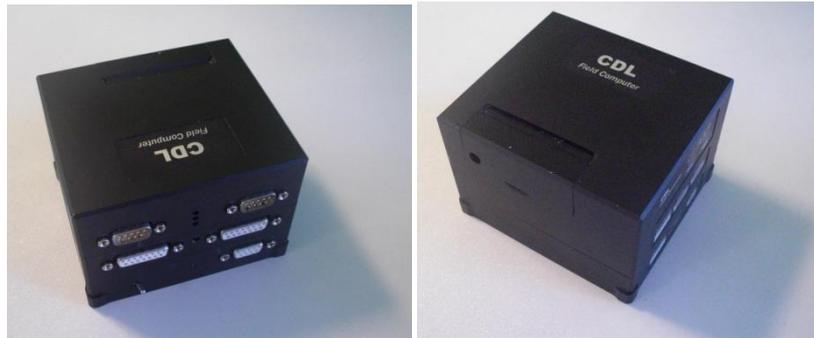


Figure 3.16 The CDL field computer used in the variable temperature experiments

3.6.4 Method

Polyaniline sensors were placed in a 25 ml or 50 ml sterile centrifuge tube or 2.5 l gas jar and then connected to the equipment the sensor was to be interrogated by. The polyaniline sensor was placed above the meat sample in the sterile tube and held in place with crocodile connectors. The wires were threaded through small holes made in the lid of the tubes which was then screwed into place. Multiple layers of Polyfilm seal were used over the lid and the wires to make sure that the unit was gas tight once the unit was ready to make measurements. The layout of this setup can be seen below in Figure 3.17.

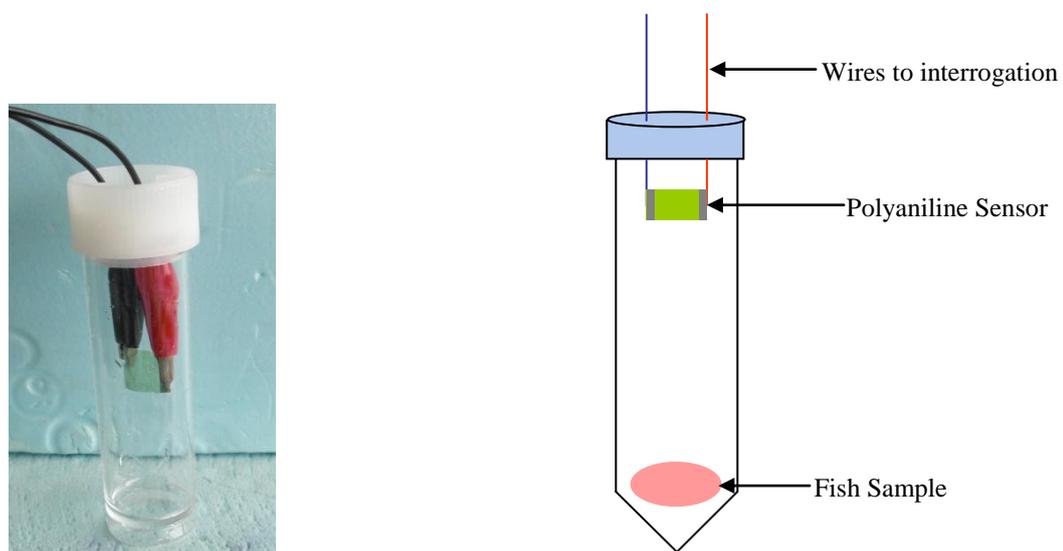


Figure 3.17 Sensor placed within proximity of fish sample.

Initial experiments were carried out at room temperature using single channel instruments. For DC measurements using the Sycopel instrument, a static voltage of 1 V was applied across the sensor and the change in resistance was measured by a change in current. For AC impedance measurement, the ACM instrument was used and set to scan between 1-10000Hz

and ac signal amplitude of 5mV RMS. These machines could be modified to take a reading every hour over the course of a number of days.

For the variable temperature experiments using the aluminium block, the cooler was set to -8°C and gave antifreeze at a temperature of 1°C at the heat exchange. The water heater was set to 30°C and this temperature was observed at the heat exchanger. For the measurement of change in DC resistance of polyaniline the 14 channel CDL unit was used in conjunction with a potential divider. A supply of five volts was given to all channels which then passed through a known value of resistance (R_1). A signal of the voltage share for this resistor was sent to the field computer. The circuit was completed with a polyaniline sensor held within a reaction chamber at a set temperature. The change in resistance of this chemiresistor (R_2) was measured by the change in voltage out signal and monitored by the field computer. The circuit diagram can be seen below.

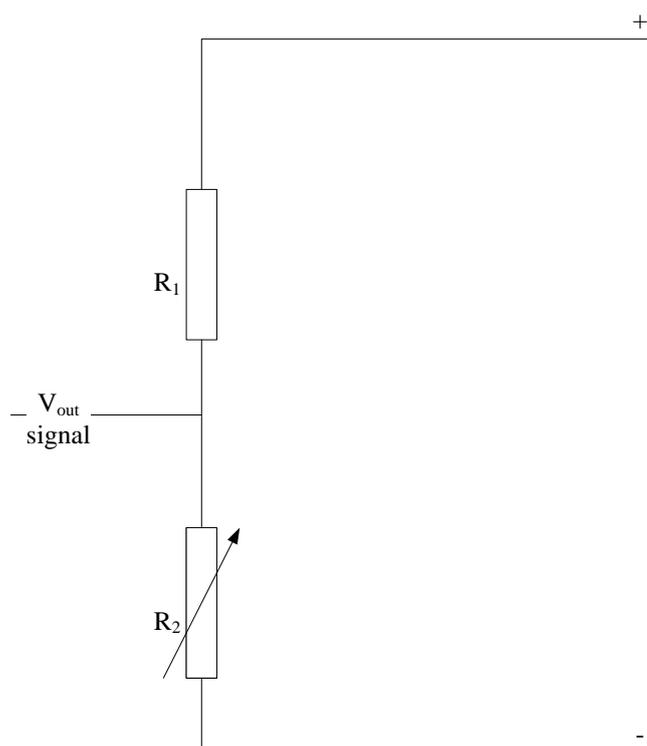


Figure 3.18 Electronic circuit setup for detecting resistance change in the sensor

In all cases of using the CDL computer, a blank channel was left to analyse the output 5 V signal to ensure it was steady. For a control experiment was also repeated with deionised water instead of fish samples.

Chapter 4
Waste Generation in the Food Chain and its Possible Reduction
with the Use of Intelligent Packaging

4 Introduction, scope and objectives

The work presented in this chapter represents the findings from a study carried out over a 12 month period (July 2008 June 2009) focussed towards investigating waste from the food and drink industry. The research provided a platform for collaboration between a number of schools within Cranfield University as well as the Institute of Grocery Distribution (IGD). The results from this study have been published as a separate scientific report for the Department of Environment Food and Rural Affairs (DEFRA) (Mena et al., 2009). Along with the scientific studies undertaken within the project, the evidence presented in this chapter provides vital strategic information to producers of intelligent packaging aimed at the food retail market. The role of the researcher in this project was as a part time research assistant for the entire duration of the project and was involved in all stages of the project. This included data collection, report writing, presenting and data analysis.

The study followed on from numerous other pieces of research in the field of waste volumes produced by either by food consumers (such as the study conducted by the Waste Resources Action Programme (WRAP)), food manufacturers and food retailers. The aim of the project was to establish the root causes of waste and identify key best practices within the industry arising between food manufacturer and retailers in the UK. The results given in this chapter are qualitative in nature and arose from interviews with selected food companies.

The data gathered has been presented as to protect the provider. All interviews conducted were in confidence and the results have been coded to protect the identification of the participants. Project partners included the majority of large food retailers as well as a number of sizeable food manufacturers and suppliers. Since the project was completed, a number of other studies have been commissioned in similar fields to be completed by the same project team.

4.1 Background to the problem

Waste is a major issue facing the retail, food and packaging industries in the UK. In 2005 the majority of food retailers and producers signed an agreement known as the Courtauld

Commitment aimed to raise awareness of the problems of food waste and reduce overall levels of food and packaging waste (WRAP, 2008). A year later in 2006, the Food Industry Sustainability Strategy (FISS) presented the industry with the challenge of reducing the volume of food and packaging wastage and to recycle or otherwise gain value from waste that does arise, without compromises to food quality and safety (Defra, 2006). Currently there are over 30 major retailers, brands and suppliers and 92% of grocery supermarket that have signed up to these two Agreements.

There has been recent investment into encouraging best practice programmes by the UK government which include WRAP and Envirowise initiatives to help the industry reduce waste and meet the targets set by the FISS. The target for these programmes has been waste minimisation for packaging, manufacturing and home waste and there have been signs of success. An area that has received less scrutiny is the waste generated between the production and retail stages of food manufacture. Waste occurring at these stages has both financial and environmental effects as produce at these stages has had a large amount of value adding activity already expended on it which equates to an accumulating cost and energy to the producer. Therefore a reduction of waste at this stage would dramatically cut costs by making processes more efficient and also reduce emissions from unnecessary energy usage.

From previous studies it has been estimated that the food and retail sector produces about 30% of all industrial and commercial waste in the UK (Defra, 2007a). Absolute volume figures estimate this at between 18-22 million tonnes per annum (Defra, 2007a; Cabinet Office, 2008). Although waste is generated along the chain, studies have shown that the consumer waste figures are comparatively lower at around 6.7 million tonnes for food waste (WRAP, 2007) and an additional 5.2 million tonnes for food related packaging waste (Hogg et al., 2007). According to the latter study, this relates to generation of 15 million tonnes of CO₂ and other green house gases from wasted energy and emissions from landfill (Hogg et al., 2007). For the retail sector there are a wide range of figures available which estimate waste at 0.4 million tonnes (Cabinet Office, 2008) whilst WRAP and Envirowise reported 1.5 million tonnes (WRAP, 2007) and 12 million tonnes respectively (Envirowise (2002)). Food manufacturing waste estimates also present a wide range in figures from 3.5 million tonnes (WRAP, 2007) to 6.6 million tonnes (Cabinet Office, 2008). The spread in these figures suggest that the waste

data for these industries are based on very rough estimates which contain a high degree of error.

4.2 Supply chain concepts and structure

This section will give the reader an understanding of supply chain structure, composition and operation. An overview of the UK food and drink industry will also be given to provide the necessary statistics for a discussion of the issues facing this commercial sector.

4.2.1 Overview of supply chain management

Supply chain management is a concept that has been used in a variety of unique events throughout history (for example, the building of the pyramids) and has evolved into the modern doctrine that it is today. The principles involved are the efficient flow of materials to meet customer requirements and an effective information flow in reverse parallel to support and enhance this. The idea of supply chain management as a standalone discipline was conceived in the late 1950s and increase in study in the area began in the 1980s. Eventually there was a significant increase in publications in these areas from the 1990s to present day (Huan et al., 2004).

Supply chain management is often cited as logistics and simple movement of goods. This, however, is not the case; modern day, successful supply chain management involves the entire length of the buyer, supplier and producer network and the supervision of relationships in between. Martin Christopher defines supply chain management as “*The management of upstream and downstream relationships with suppliers and customers to deliver superior customer value at less cost to the supply chain as a whole.*” (Christopher, 2005). This puts more emphasis on the management of relationship and expectations rather than the simple movement of goods to produce a more profitable outcome for everyone involved in the chain.

Figure 4.1 below shows what is often incorrectly referred to as the supply chain. This is in fact the logistics management process within supply chain management. It does, however give an introduction to the role of operations of supply chains. It should be noted that the roles that occur within the logistics process can be used for the foundations of modern supply chain

management. The usual role of the firm is to produce goods by flowing materials from left to right whilst adding sufficient value so as to compensate the overall cost of the supply chain.

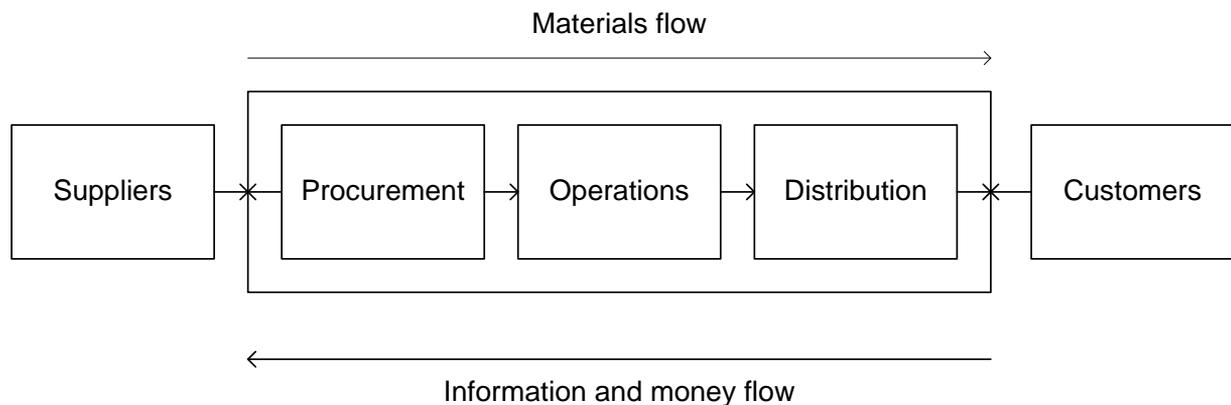


Figure 4.1 the logistics management process (Source: Christopher, 2005)

Often whole industries can be represented as supply chains within supply chains, since individual firms can act as a division in the main function of trading goods from raw materials. In the modern understanding of supply chain management, a firm's customer can be another firm's supplier further along the chain rather than being a point of end consumption (Power, 2005). A good example of this is the computer industry, where firms are often integrated into producing complex goods from raw materials, such as silicon. A firm at the start of the chain, purifying raw materials, will be a supplier for firms producing components which in turn will supply these to computer manufacturers and retailers which will eventually be sold to end consumers. There is also an added confusion when the firm is involved in the service industry rather than in the manufacturing industry. For this reason there is a need to reduce the complexity of supply chains down to a simplified model that would allow complete understanding of the supply chain process.

Modern business competition is said to be through the understanding and control of supply chains rather than of management of individual firms (Christopher, 2005). There is a need to reduce the complexity of supply chains so that all of the processes and operations can be understood and modelled. The universal model that is used for supply chain management known as the supply chain operations reference (SCOR) model that has been developed by the Supply Chains Council (Supply Chain Council, 01/10/2009). This model is routinely used as a

strategic planning tool which then allows senior management to reduce the complexity levels of supply chains as shown within Figure 4.2.

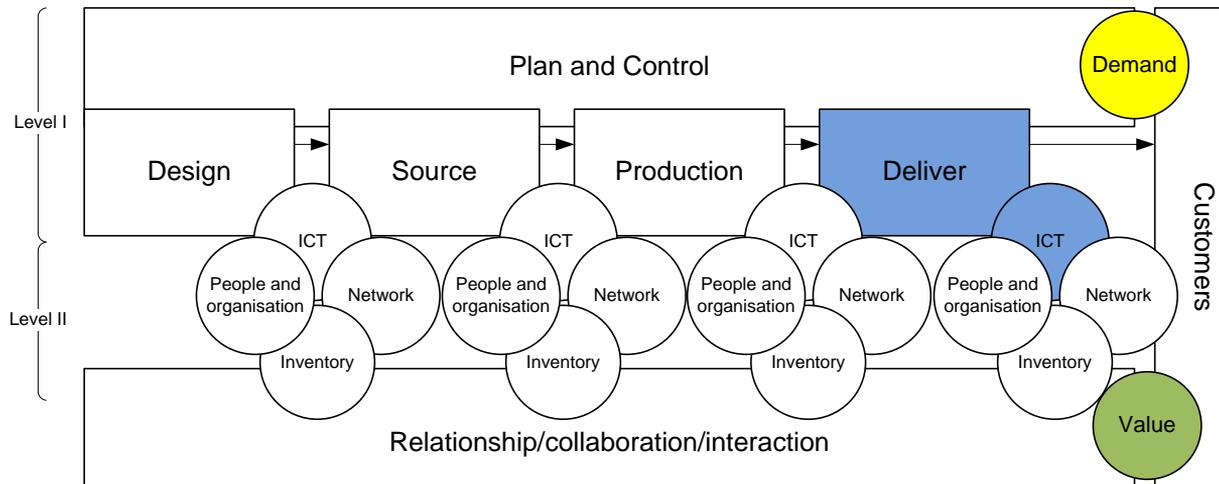


Figure 4.2 the SCOR template for supply chain management (Source: Supply Chain Council, 01/10/2009)

Figure 4.2 outlines the model with the five key stages of the supply chain where material is flowing left to right (with arrows) and information is returning right to left (absent from the diagram for clarity). These five functions are:

1. Design - considers the initial design of the supply chain and the product that will eventually flow through it, including optimisation, delivering to market segments and coping with expected demand. Thorough design procedures can lead to facilitated control of a supply chain and allow process evolution to occur within the supply chain without causing major disruption.
2. Source – relates to the sourcing of people, raw materials and goods. This is a vital stage to consider for management as strategic sourcing can provide excellent quality material and personnel as well as strong relationships between the firms' suppliers.

3. Production – This stage is the most visible part of the supply chain and encompasses the benefits from the previous stages. This is often the stage where value is added to the materials that are used by the firm.
4. Deliver – this is the final part of the supply chain model and represents the delivery of goods or services to customers.
5. Plan and control – this sits above all of the other stages to show that it the link used in managing and controlling the entire supply chain. There needs to be considerable links and overlap between this stage and all others to equal customer demand. Supply chains are there to provide demand fulfilment from the demand creation provided by marketing functions (Christopher, 2005).

These are seen as level I functionalities and are achieved by the careful management of the level II core components. These include controls of information and communications technology (ICT), Networks within these stages, inventory level management and people management. The focus for this project is the deliver function of the supply chain and specifically the role of ICT in improving this part of the process (these are highlighted in blue on the diagram). ICT includes technologies such as the one being studied for this project or similar devices such as TTIs.

Overall, the supply chain is setup to deliver value for end consumers as well as providing an integrated network of trust. This is represented by the box at the bottom of the diagram and is the important part of delivering value to a firm's customers. The definition of value is important to clarify. Value is defined as follows by Professor Michael Porter, "*A business is profitable if the value it creates exceeds the cost of performing the value activities. To gain competitive advantage over its rivals a company must either perform these activities at a lower cost or perform them in a way that leads to differentiation and a premium price (more value)*"

(Porter, 1998). In terms of competitive advantage, there are two dimensions that are used to calculate it. These are the product excellence and process excellence used in creating the product. Christopher argues that there is equal weighting of these two dimensions of competitive advantage which is defined below in Equation 4.1.

Equation 4.1 Definition of competitive advantage of a firm in terms of supply chain systems (source: Christopher, 2005)

$$\textit{Competitive advantage} = \textit{product excellence} \times \textit{process excellence}$$

This project is created to highlight the importance of process excellence. A key indicator of process performance is the amount of waste produced by the overall process. The current emphasis of many companies is on product performance to provide a competitive advantage. Christopher argues that competitive advantage can be more easily achieved by sharing awareness of process excellence. In the case of food supply chains, this could be in the minimisation of waste or investment in supply chain technology. Figure 4.3 below shows where the emphasis should be for competitive supply chains.

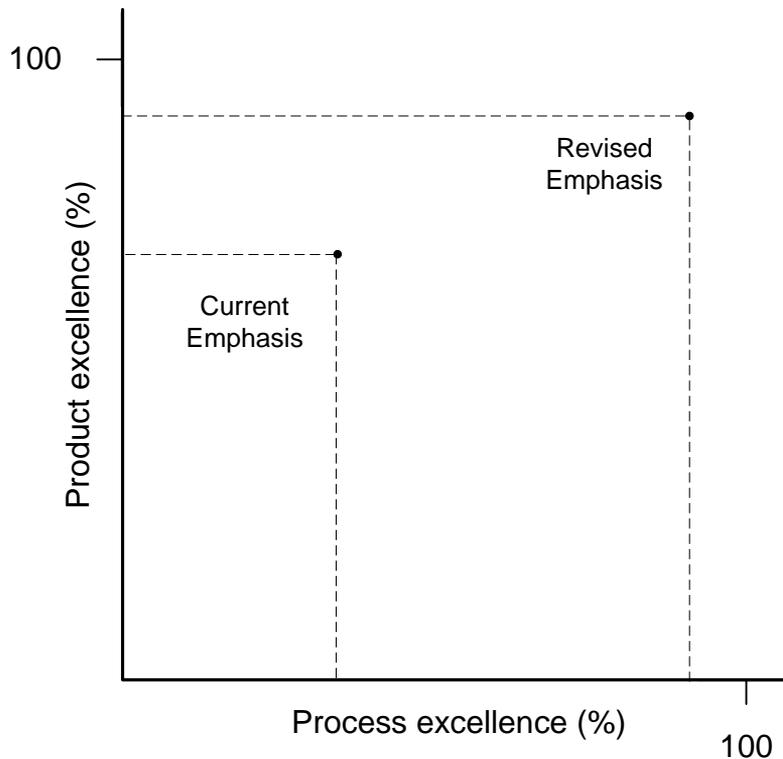


Figure 4.3 investing in process excellence can yield greater benefits (Source: Christopher, 2005)

One way in which process excellence can be improved is by focusing on the relationship between suppliers and retailers (Power, 2005). This can be achieved by integration of firms within a supply chain. There are several methods of integration of firms which include partnerships, alliances, cooperation and complete mergers. Most business see integration as relinquishing control and potentially harmful for competition. There are also issues of trust and information sharing between firms which can be seen as a barrier to collaboration (Kotzab, 1999). The time between the start of integration and the reaping of these benefits also deters many firms from entering collaborative agreements. Many sectors, including the food and drink industry, are becoming more integrated as research has shown the benefits of collaboration and integration amongst firms in a supply chain (Sheu et al., 2006). Overall supply chain performance and satisfaction increases with the amount of retailer – supplier collaborations. Table 4.1 below shows the expected change in views from firms that view themselves as individuals in a large supply chain compared to those that are deemed to be part of the bigger picture.

Table 4.1 the extended enterprise change in viewpoint (source: Kearney, 2000)

Single company view	Extended enterprise view
Focus on own customer	Focus on the ultimate consumer
Increase own profits, push waste through supply chain	Increase profits for all
Consider own costs only	Consider total cost
“spread the business around” philosophy	Team with the best
Guard information, ideas and resources	Share information, ideas and resources

Allowing a more integrated approach to supply chain management means giving the suppliers and customers total visibility to your processes, sales data and strategies. The argument pro integration states that the customers at the end of the chain are ultimately the supplier’s customers. Any strategy, policy or market segment that a retailer chooses should be aligned with their suppliers.

Table 4.2 shows the effect of collaboration between a retailer and supplier. In this case it was a product produced and supplied via Birds Eye Walls group to Tesco. The strategic collaboration meant that complete visibility of the final consumer was given to the supplier, Birds EYE Walls. This involved Tesco providing point of sales data, stock handling procedures and promotional management control to the supplier.

Table 4.2 Benefits if supply chain integration from Tesco - Birds Eye Walls case (Source: Walker, 1994)

	Before	After
Product availability	85%	99.5%
Lead time	7 days	2 days
Tesco stock	3.4 weeks	1 week
Birds Eye Walls Stock	7 weeks	3.5 weeks

It is evident that the effect of collaboration is largely positive on the normal Key Performance Indicators (KPI) in supply chains. This report hope to find evidence to suggest that collaboration and supply chain integration can also have a positive effect on waste levels.

4.2.2 The food and drink industry structure

The UK food and industry is an essential part of the economy, providing the 60.9 million inhabitants (Office for National Statistics, 2008) with food, drink and tobacco. The amount spent on food, drink and tobacco equates to over £106 billion per year (including VAT) which is approximately £1,750 per capita, with an additional £81 billion spent on catering services (Office for National Statistics, 2008). The sector equates to 7% of the Gross Value Added (GVA) and supplies jobs to 14% of the UK workforce. The industry also has negative environmental effects, such as pollution, waste and CO₂ emissions.

The structure of the supply chain for the food and drinks industry comprises six main components:

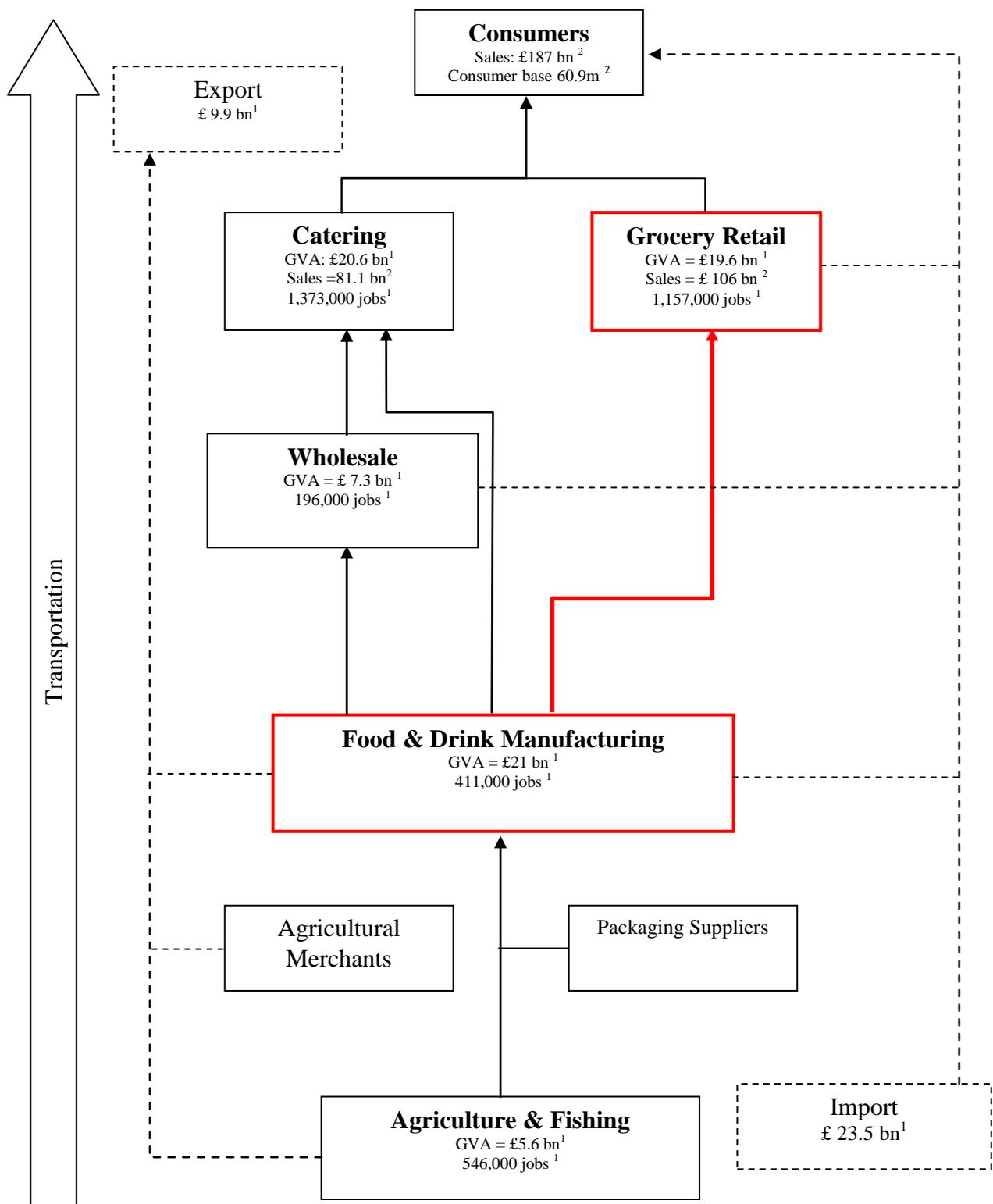
- Consumers
- Retailers
- Caterers
- Wholesalers
- Manufacturers
- Primary Producers (including farming and fishing)

Other additional contributors to the industry include packaging suppliers, agricultural merchants, logistic service suppliers and waste managers. An overview of this structure can be seen in Figure 4.4 and includes figures on GVA, total sales and number of employed people for each stage. For this study it is important to note that the focus will be retail and manufacturing, the two largest stages in terms of employment and GVA.

4.2.2.1 Consumers

The UK food and drink supply Chain is a complex network of firms and organisations. The network is continuously evolving to satisfy changing consumer demands and governmental policies. Customer demand trends change continuously, with the current prevalent drift of emphasis towards healthy eating as well as locally and ethically sourced foods. This has

pressurised many retailers to either improve existing products or introduce new ranges of products such as organic foods (Taylor, 2006). In this way, consumers provide a key role in shaping the structure of food supply change and control a certain aspect of product demand.



Sources: 1. Defra (2007) Food Statistics Pocketbook
2. ONS (2008)

Note: GVA = Gross Value Added

Note: Some values differ between sources

Figure 4.4 the UK Food and Drinks Supply Chain (Adapted: IGD 2007))
D. Hobday

Figure 4.5 Consumer Expenditure on food by type in 2007 (£m at rsp) (Source: National Statistics Online, 2008) Figure 4.5 and Table 4.3 give the current data on consumer expenditure on food, drink and tobacco for the UK. The largest categories in terms of expenditure are tobacco, meat and alcoholic beverages. Table 4.3 shows that these categories have been increasing at below the average rate of growth of 22%. These trends are balanced by the increase in expenditure on fish (39%), fruits (47%) and vegetables (23%). This is evidence of consumer change in food markets as health and fitness campaigns are changing the contents of the average shopping baskets (Intel, 2007). The change in consumer's demand for these short shelf-life products has implications on food supply chains. Fish products and many fresh fruits and vegetables require temperature controlled supply chains to counter their relatively brief shelf-life. The increased stress on these supply chains created by growing demand of these products is expected to create waste.

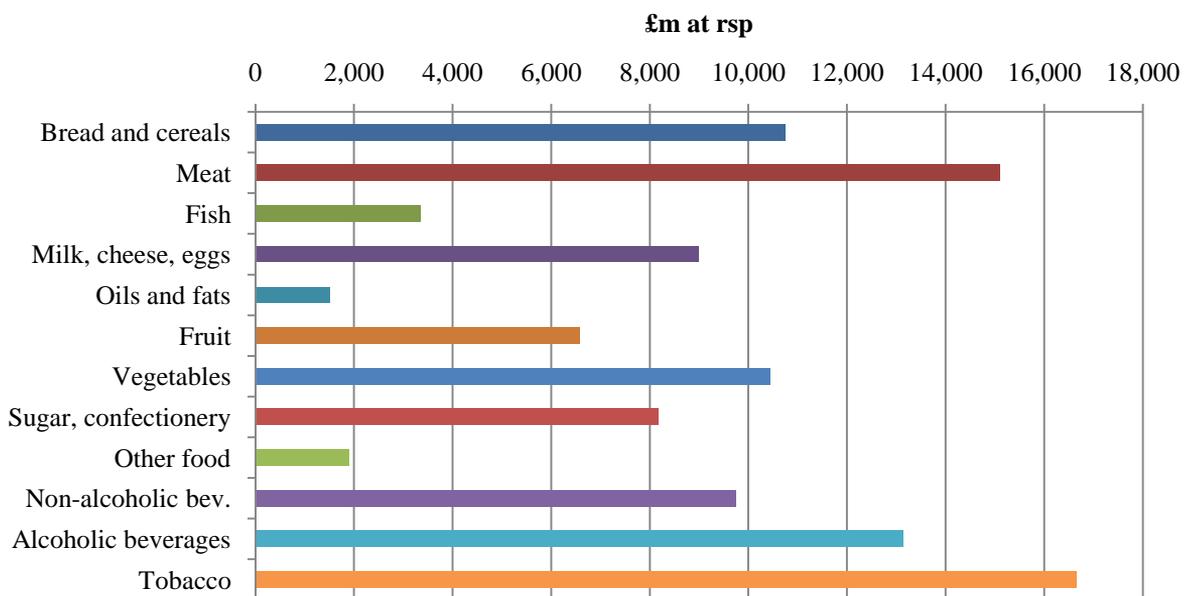


Figure 4.5 Consumer Expenditure on food by type in 2007 (£m at rsp) (Source: National Statistics Online, 2008)

Table 4.3 UK consumer expenditure on food by type at current prices (£m at rsp) 2002 - 2007 Source: (National Statistics Online, 2008)

	2002	2003	2004	2005	2006	2007	Change 2002-2007
Bread and cereals	9,016	9,309	9,642	9,889	10,306	10,750	19%
Meat	12,579	13,128	13,689	13,795	14,303	15,105	20%
Fish	2,405	2,397	2,447	2,661	2,985	3,341	39%
Milk, cheese, eggs	7,522	7,658	7,791	8,219	8,444	8,998	20%
Oils and fats	1,207	1,208	1,212	1,248	1,402	1,499	24%
Fruit	4,489	4,752	5,109	5,579	5,974	6,578	47%
Vegetables	8,473	8,595	8,739	9,170	9,490	10,428	23%
Sugar, confectionery	6,830	6,946	7,125	7,196	7,241	8,174	20%
Other food	1,463	1,514	1,603	1,609	1,672	1,891	29%
Non-alcoholic bev.	7,326	7,667	8,164	8,173	9,112	9,739	33%
Alcoholic beverages	11,344	12,027	12,213	12,344	12,417	13,140	16%
Tobacco	14,622	15,270	15,500	15,729	16,105	16,642	14%
TOTAL	87,276	90,471	93,234	95,612	99,451	106,285	22%

rsp — retail selling prices

There are a number of social economic trends that are affecting the consumer. These trends in turn affect the industry. A dramatic change in the global economic climate occurred in 2006 which has had several affects. Prices of many agricultural commodities have increased substantially as a result of growing demand from Asia, higher energy costs, poor harvests and governmental policies, such as the growing support for bio-fuels (Cabinet Office, 2008). The decrease in consumer confidence and global recession has also had an effect on the demand of food (Defra, 2007a). Consumers are being advised to avoid throwing food away unnecessarily and retailers have expanded their own brand ranges.

The UK population is growing slowly and is expected to rise by approximately 1 million from 2007 to 2012 (Mintel, 2007). The composition of the population is expected to change more considerably with the number of children set to decrease and rapid increases in the age groups over 45 and over 65. These trends are set to compliment the decrease in household size and increase in the number of people living alone. These changes are likely to see a pattern of growth in demand of fresh produce, local produce and premium products as well as a decrease in larger pack sizes (Mintel, 2007).

Lifestyle changes are expected to impact upon the food and drink market. Consumers are expected to become more health conscious and the growth in demand of fresh fruit and vegetables is a key indicator that this is already happening (Cabinet Office, 2008). Healthy produce often has a short shelf-life and an increase in demand for these products could potentially increase waste levels.

Consumers are increasingly concerned with ethical issues. Figure 4.6 shows the results of a recent study to find the prevalent attitudes of grocery shoppers. Waste related issues such as packaging, carrier bags and recycling are considered important by many consumers.

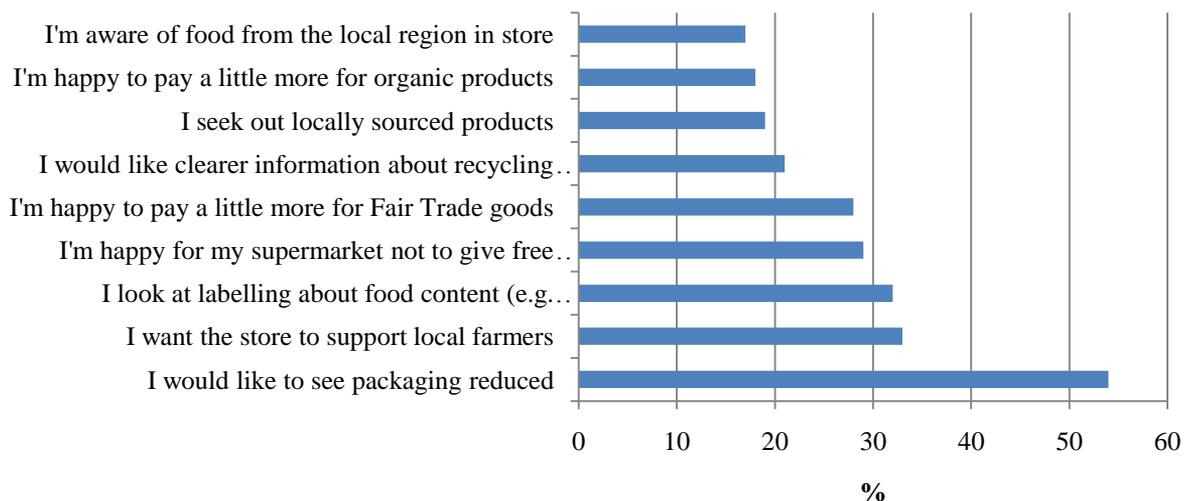


Figure 4.6 Attitudes towards grocery shopping (Source: Mintel, 2006)

Other issues that concern shoppers include climate change, waste, pollution pesticides and food miles (Cabinet Office, 2008). Further market research has also shown the importance of Fair Trade, local farmer support and environmental impacts of consumer choices (Mintel, 2007). There is a reported gap, however, between what consumers say and what consumers do in terms of ethical issues around food (Cabinet Office, 2008).

4.2.2.2 Retail

Total household expenditure on food, beverages and tobacco through UK retailers for 2007 was estimated around £106 billion, which relates to 46% of total retail sales (Office for

National Statistics, 2008; Mintel, 2007). Food and non-alcoholic beverages sales alone represent £76 billion. The retail sector comprises 55,000 enterprises with over 100,000 outlets employing over 1.2 million people (Defra, 2007a; Defra, 2008). The larger multiples of food retail (with turnover greater than £1 billion) dominate the market and in turn this is lead by the big four names (Tesco, Sainsbury's, Asda and Morrisons) which together account for 75% of grocery sales (Cabinet Office, 2008).

Figure 4.7 and Table 4.4 give the market share data for the leading retailers over the period January to June 2008 and January to June 2007.

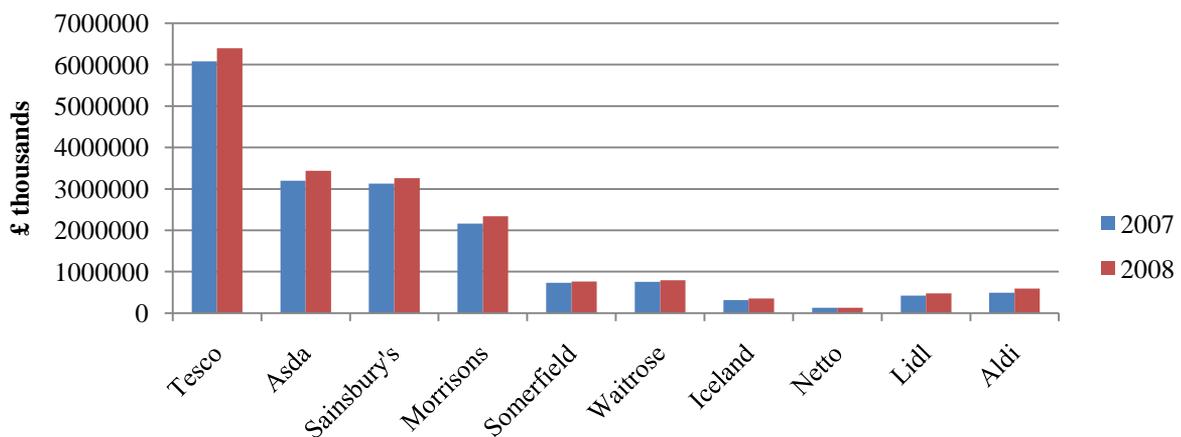


Figure 4.7 UK supermarket sales 2007 - 2008 (Source: TNS Global UK, 19/08/08)

These figures show that Tesco dominate the market with over 31% of the market share. Tesco market growth has only been 5.1% in the last year and is weak compared to the rising market share of some of the discounters such as Aldi (20.7%) and Lidl (12.8%). This has been referred to in the media as the “Aldi effect” and is evidence of the effect of the global economic strain on consumers.

Table 4.4 UK Supermarket sales 2007 - 2008 (Source: TNS Global UK, 19/08/08)

	12 Weeks to 17 June 2007		12 Weeks to 15 June 2008		Change
	£000s	%	£000s	%	%
Total Till Roll	27,453,255		28,256,150		2.9
Total Grocers	19,272,003	100.0%	20,460,948	100.0%	6.2
Total Multiples	17,887,744	92.8%	19,050,222	93.1%	6.5
Tesco	6,080,382	31.6%	6,392,222	31.2%	5.1
Asda	3,198,374	16.6%	3,438,175	16.8%	7.5
Sainsbury's	3,126,682	16.2%	3,262,042	15.9%	4.3
Morrisons	2,162,141	11.2%	2,337,697	11.4%	8.1
Somerfield	736,923	3.8%	767,119	3.7%	4.1
Kwik Save	27,608	0.1%	-	0.0%	-100
Waitrose	754,776	3.9%	794,343	3.9%	5.2
Iceland	315,059	1.6%	354,107	1.7%	12.4
Netto	128,041	0.7%	130,242	0.6%	1.7
Lidl	425,370	2.2%	479,644	2.3%	12.8
Aldi	494,563	2.6%	597,011	2.9%	20.7
Farm Foods	91,106	0.5%	103,332	0.5%	13.4
Other Freezer Centres	44,150	0.2%	48,236	0.2%	9.3
Other Multiples	302,569	1.6%	346,051	1.7%	14.4
Total Coops	840,713	4.4%	863,994	4.2%	2.8
Total Independents	543,547	2.8%	546,732	2.7%	0.6
Total Symbols	190,939	1.0%	195,337	1.0%	2.3
Other Independents	352,608	1.8%	351,396	1.7%	-0.3

A wide variety of retail formats are available in the UK and Table 4.5 gives an overview of these. Included is the potential waste causing factors for each format.

Table 4.5 Retail formats

Convenience	<p>Small stores with sales area up to 3,000 square feet.</p> <p>Usually located in busy city centres, residential areas, small towns, petrol stations forecourts.</p> <p>Stocking mainly food (higher margin products such as ready meals), everyday essentials, newspaper, magazines, tobacco products and a limited range of alcoholic beverages.</p> <p>Product selection is limited when compared to other formats.</p> <p>Waste as a percentage of sales is likely to be higher than in other formats due to the higher proportion of short shelf-life products such as sandwiches and chilled foods.</p>
Supermarket	<p>Sales area between 20,000 and 50,000 square feet.</p> <p>Larger than convenience stores and offers a wider selection of products.</p> <p>Usually located close to residential areas to be convenient for consumers.</p> <p>Offer a wide variety of food and household merchandise and some offer a limited range of non-food products.</p> <p>Convenient shopping hours (some are open 24 hours)</p> <p>Wider product range and ample shopping hours could result in lower volumes of food waste compared to convenience stores.</p>
Hypermarket	<p>Sales area range above 60,000 sq ft.</p> <p>Usually located in suburban or out-of-town locations that are accessible by automobile.</p> <p>Large retail facility which carries an enormous range of products. Full lines of groceries and general merchandise including electronics, clothing, furniture, etc.</p> <p>Provide additional services such as photo processing, opticians, café, restaurant, cash machines, etc.</p> <p>Convenient shopping hours (some 24 hours)</p> <p>Wider product range and ample shopping hours could result in lower levels of food waste as a proportion of sales. However, waste non-food products due to damage could be substantial in terms of value.</p>

4.2.2.3 Manufacturing

The food manufacturing industry comprises a variety of sectors and processes such as, meat processing, brewing, dairy, confectionary, frozen ready meals etc. The majority of food manufacture is performed by large organisations, which operate across a range of food markets (Fenn, 2007). The largest firms in the market make up 3.8% of the total market but generate over 75% of all food manufactured in the UK (Cabinet Office, 2008). The industry had estimated sales of £114 billion for 2006 (Fenn, 2007) and accounted for 17% of all manufacturing (Cabinet Office, 2008). This made the food and drink manufacturing industry the largest manufacturing sector in the UK. The industry comprises 6,500 firms with a total of around 411,000 employees (Defra, 2007a; Fenn, 2007).

In terms of industry output, approximately 75% of sales go to retailers, 10% to caterers and 15% are exported (Cabinet Office, 2008). This is why large retailers are often stated to have strong bargaining power over manufacturers who in turn increase pressure to reduce costs and is frequently cited as reasons for the increase in consolidation within the industry (Fenn, 2007). The larger firms often have higher margins than smaller firms and are better structured to cope with pressures from food retailers. Table 4.6 presents a list of the UK's leading food producers and gives data on profit, turnover and employment; this table briefly outlines company activities and products.

Waste has been a long-standing concern of the food manufacturing industry and many companies have addressed this issue through quality systems and continuous improvement processes. However, food manufacturing processes also cause some waste which is inevitable due, for example to skins, carcasses and other trimmings. Specific issues related to food manufacturing waste will be addressed in the following section.

Table 4.6 UK' Largest food Manufactures (sources: Fenn, 2007, FAME database (Bureau Van Dijk) and company websites)

Company		Brief Description	Turnover/Profit 2007	Employees 2007
Associated Foods PLC	British	Diversified multinational. Main sectors include sugar, bread, tea and oil.	£6.8bn / 508m	750,000 (84,636 UK)
Grampian Food Group Ltd	Country	Fresh, frozen and added-value chicken, pork, beef, lamb and turkey products	£1.81bn / £40.5m §	20,000 §
Dairy PLC	Crest Group	Chilled dairy foods (milk, cheese, yoghurt, desserts and ice cream	£1.57bn / 64.6m †	8,342 †
Nestle UK Ltd.		Part of Nestle, the world's largest food company. Provides a wide range of products including cereals, chilled dairy products, chilled meats, milk and cream, confectionery, and coffee among others.	£1.29bn / (£62.4) §	5,418 §
Premier Ltd	Foods Group	Wide range of products including bread, cakes, preserves, beans, soup, noodles, vinegar, sauces, salt, chilled ready meals and desserts.	£1.269bn / £65m	13,593 §
Unilever		Unilever, the Anglo-Dutch giant, is one of the leading producers of FMCG, including food. Some of their leading food brands include PG Tips, Pot Noodle, Flora, Bertolli, Knorr, Ragu, Marmite, Hellman's, Coleman's and Slim Fast	UK: £ 1.01 bn / 128 m World: £29.65 bn / £3.82 bn	175,000 (2,886 UK)
United Biscuits		Leading European manufacturer of biscuits and snacks. Main brands include Hula Hoops, McCoys, KP Nuts, Jaffa Cakes, and McVitie's.	£897m / £69m §	6,863 §
Northern Foods PLC		Produces a wide range of products in added-value convenience foods. Main markets include pizza, biscuits, ready meals, sandwiches, salads and puddings.	£ 888.5m / £34.4m §	10,700 §
HJ Company	Heinz	Subsidiary of HJ Heinz Company (USA). Main	£570.7m /	2,259 §

Company	Brief Description	Turnover/Profit	Employees
		2007	2007
Ltd	products include canned food (e.g. beans, pasta, and fish), baby foods, biscuits, cakes, cereals and snacks, chilled desserts, dairy products, frozen desserts, ready meals, salads, salad dressing, sandwiches, sauces, soups and soya.	£123.3m) §	
Uniq PLC	Supplies sandwiches, salads, desserts, salmon and seafood products, cheese, dips, party foods and ready meals to major supermarkets.	£541.2m / (£58.5m) §	7,600 § (2,887 UK)
Bernard Matthews Ltd	Frozen products, cooked sliced meats, pastries and sandwiches.	£422m/ (£26m) §	6,650 §

§ = results from 2006

† = results from 2008

4.2.2.4 Logistics

Logistics has become an important differentiator in the food supply chain and retailers have used it as a mechanism to control, organise and manage end to end supply chains. This has occurred due to the competitiveness of the market and the diversity of the products that are on offer. The complexity of food supply chain has also made it necessary to require a logistic system that is both efficient and adaptable (Bourlakis and Weightman, 2004).

Due to the wide range of products managed and sold by the large retailers there is a need for the use of distribution centres. The majority of products traded by retailers are held in distribution centres before reaching the front of store. Most tend to use their own transportation fleets to replenish stock levels while others rely on third party providers such as Eddie Stobart and Wincanton.

Figure 4.8 shows a simplified generic logistic network. For food retailers, the greater the control over the secondary distribution phase equates to more efficient transportation and replenishment systems. Control is afforded by heavy investment in IT systems and

infrastructure to manage this phase. The overall aim is to have complete control over reducing inventory levels and also decrease product lead times (Cachon and Fisher, 2000).

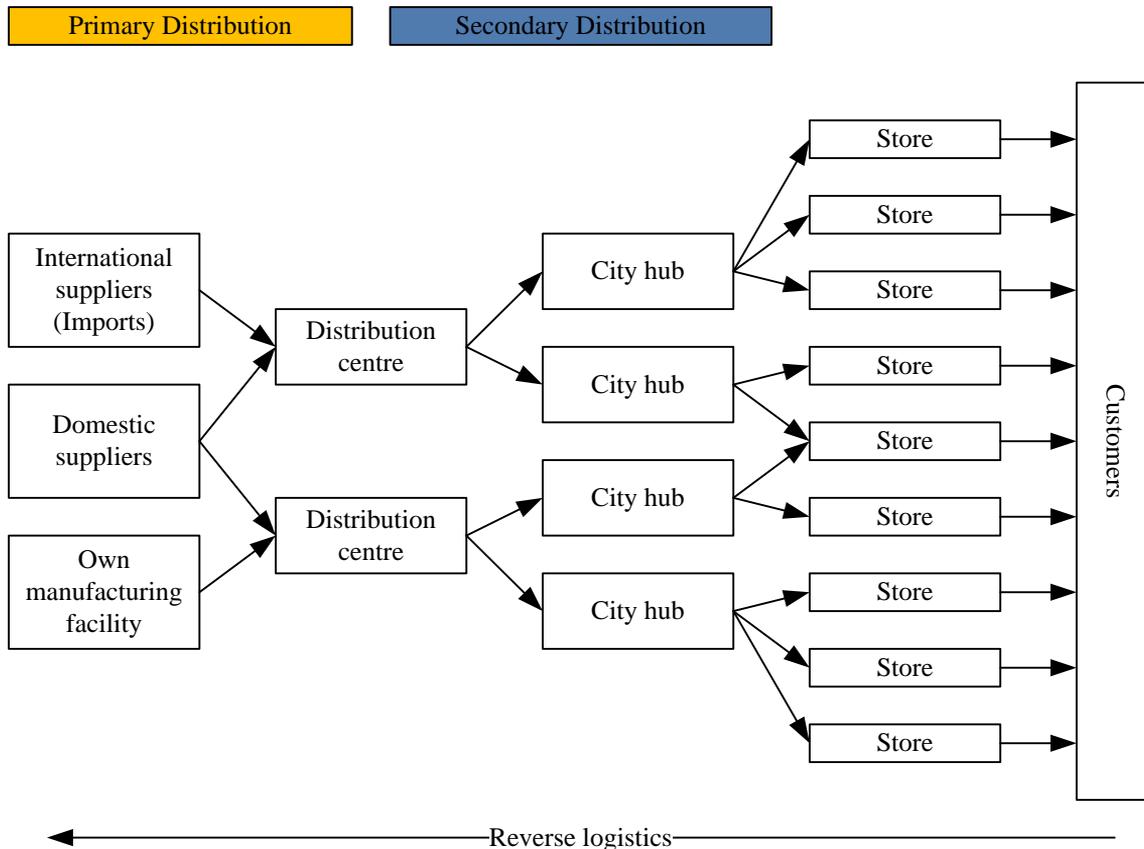


Figure 4.8 retail logistics (Adapted from: Agarwal, 2007)

Waste is influenced by a variety of management practices and factors. One of these relates to the way inventories are managed. High inventories are likely to lead towards products being damaged or exceeding the shelf-life of the product. Figure 4.9 shows data of inventory levels across a range of products. Foods with short shelf-lives are reported to have much lower levels of inventory compared to items such as frozen foods or alcoholic beverages, which have relatively longer shelf-lives.

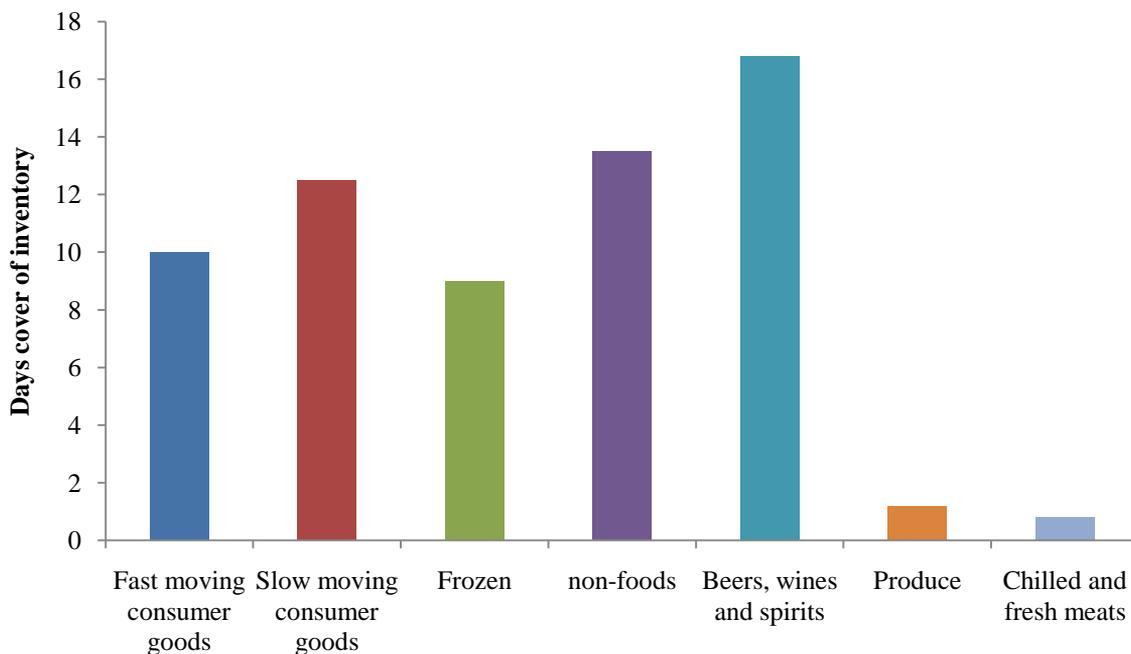


Figure 4.9 Inventory levels by product category (Source: IGD, 2007)

4.2.2.5 Wholesalers and primary producers

Wholesalers provide an essential link between supply activities (agriculture and Manufacturing) and the market activities (retail and catering). 2007 saw the total number of food wholesalers estimated at 14,096 with sales of £17.8 billion (Defra, 2007a; IGD, 2007). The market place is highly concentrated with the top 30% (with sales over £1million) secure almost 94% of sales (Mintel, 1999).

There are two main types of food and grocery wholesalers. These are cash and carry and delivery wholesalers. Cash and carry wholesalers have customers that purchase and collect goods from the store and cater for general groceries, tobacco and confectionery etc. These stores account for 53% of the wholesale market (IGD, 2007). Delivery wholesalers offer a delivery service of goods to the customers' location for a fee and often offer a wider range of products including frozen and chilled goods, household goods, health and beauty products etc and account for 47% of the market (IGD, 2007).

Primary production in the food and drinks industry comprises a wide variety of activities. The two main categories are farming and fishing. Farming contributes £6.6 billion a year to the UK economy, representing around 0.8% of the economy. The sector also provides employment to over half a million people and uses 18.6 million hectares of land [around three quarters of the UK's land area] (Defra, 2001). Fishing, the smaller of the two categories, contributes £660 million to the UK economy and provides 17,000 jobs (Defra, 2001).

4.3 Waste in UK supply Chains

4.3.1 Defining waste

For this project the EU guidelines which define waste as “any substance or object the holder discards, intends to discard or is required to discard” were used (WRAP, 2007). This will include all facets of physical waste including wasted produce and wasted packaging. Not included in this project was the study of wasted time, energy and resources.

The management of food waste is a significant and prevailing political issue with strong economical and environmental concerns. Recent news and reports highlight the growing concerns of food and packaging wastage volumes that are being sent to landfill (BBC, 2008; Vidal 15th April 2005). The majority of the data presented, however, only relates to information collected from consumers (WRAP, 2007). It was expected that this study would give a qualitative indication of the practices and performances of the food supply chain.

It has been acknowledged that the European food system produces an enormous amount of waste from both packaging and food (Ethical Corporation, 2006) and it has been estimated that approximately 25% of material that is introduced into the supply chain is wasted (C-Tech Innovation Ltd, 2004; Green and Johnston, 2004). Charities such as FareShare have estimated that up to 25% of the food sent to landfill by the food manufacturing and retail industries is either edible or could be turned into compost or energy (Green and Johnston, 2004). It is thought that this could feed more than 250,000 people which has provided a *raison d'être* and growth for charities such as FareShare.

The overall cost of waste is often undervalued (Binyon, 2007) and it is estimated that the cost of waste can be applied to the firms' turnover due to hidden costs, shown in Figure 4.10. It follows that an item wasted at a later stage in the supply chain has had more production, transportation, energy use and additional costs attributed to it, therefore the higher the waste value of that item.

Waste can also be divided into avoidable and unavoidable streams when items of food cannot be processed further into by-products or co-products. Unavoidable waste mostly comprises inedible parts of raw food, for example, fruit and vegetable produce with inedible skin/peel will cause waste if it is to be prepared into a 'ready to eat product'. There is scope for further study into the causes, limitations and usage of the unavoidable waste created by production and manufacture of certain foodstuffs. For example, the UK poultry industry produces 150,000 tonnes of feathers every year, which costs the industry around £3 million in landfill charges per annum (C-Tech Innovation Ltd, 2004).



Figure 4.10 The true cost of waste from hidden costs (Source: Binyon, 2007)

4.3.2 Areas of waste

The area of focus for this project is the post-production/pre-consumer phase of the food supply chain as shown in Figure 4.11 (Mena, 2008). As mentioned previously, reports from WRAP have shown the volumes and values of waste produced at the consumer end of the supply chain (WRAP, 2007) to be one third of all food bought by consumers. Data gathered by C-Tech Innovation Ltd (Chabukswar et al., 2001) showed that the food sector accounted for over a third of all the waste products in the UK in 2004 which equated to a total of 17 million tonnes.

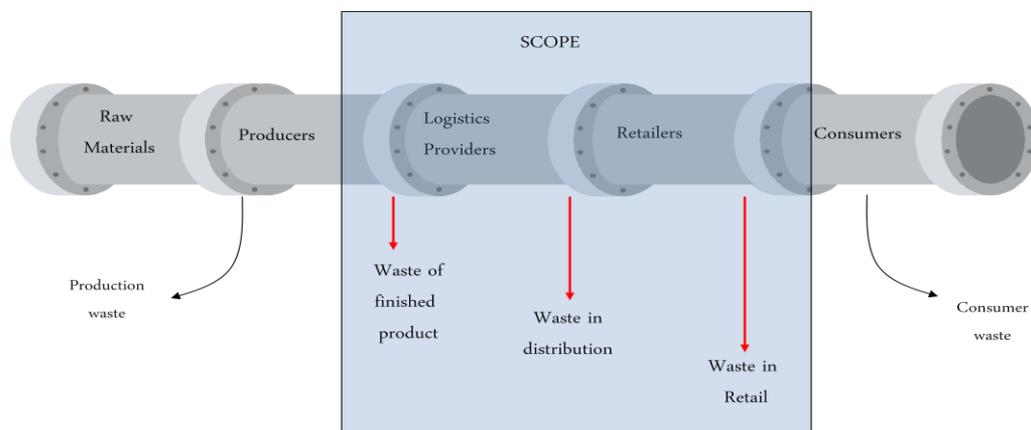


Figure 4.11 Focus of the research project (Source: Mena, 2008)

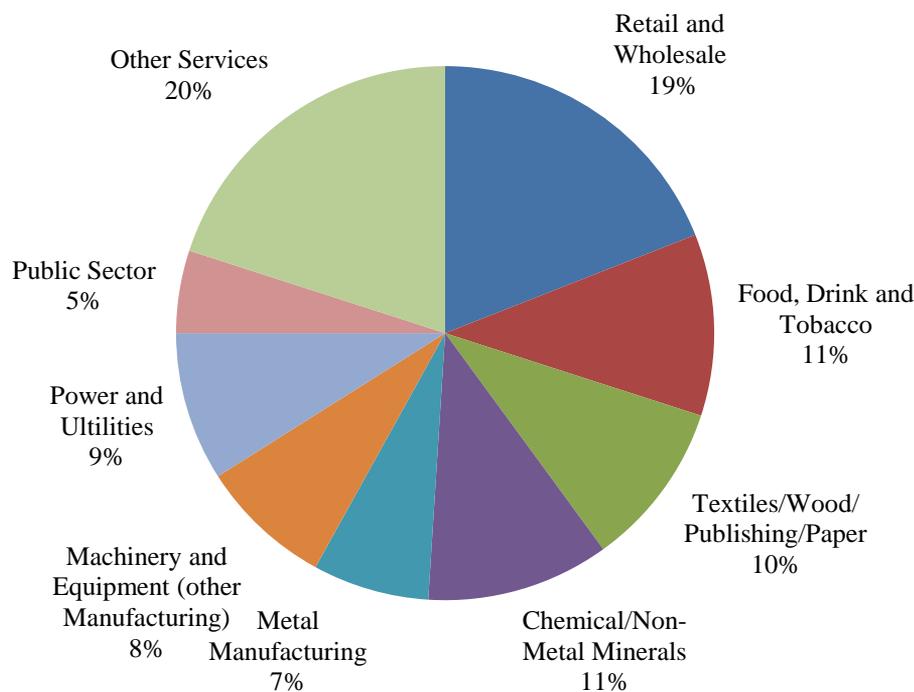
The research also showed that approximately 15% of this 17 million tonnes arose from food manufacture and a further 21% from distribution, retail and consumption (C-Tech Innovation Ltd, 2004). Separate Government figures suggest that roughly 17 million tonnes of food are being put into landfill at a cost of over £175 million a year (Defra, 2007a). For this project, the three key areas of study include:

- Retail
- Distribution
- Production

It is anticipated that there will be significant findings of waste of materials in these areas as current statistics show this trend. From Figure 4.12, it can be seen that the main contributors to

waste in the commercial and industrial sectors are the retail and wholesale, and food, drink and tobacco industries (Defra, 2007a). The majority of waste in the years of this study (2002/03) came from retail and wholesale (19%) and the food, drink and tobacco sector (11%). This equates to over 20 million tonnes of waste from these sectors combined.

Commercial and Industrial Waste by Sector- Total 67.9 Million Tonnes



Source: Environment Agency Commercial and Industrial Waste Survey 2002/03

Figure 4.12 Wastage figures from industry and commerce by sector (Source: Defra, 2007a)

The food industry has worked together with government agencies and other organisations such as WRAP to deliver actions on this amount of waste which has led to agreements, such as the Courtauld Commitment, for the reduction of packaging waste and identifying areas of food waste. Of the 7 million tonnes of waste produced each year by the food, drink and tobacco industry it is estimated that 4.1 million tonnes are food or food processing by-products, which constitutes nearly two thirds of the total commercial and industrial food waste. The majority

of this food waste often returns to the supply chain for use in food production or food processing but about 1.9 million tonnes ($\approx 46\%$) of this is land filled directly (Defra, 2007a). The Food Industry Sustainability Strategy has given a target of a 15-20% reduction in the food manufacturing industry's own waste by 2010. The implementation of this target is now being discussed (Defra, 2006).

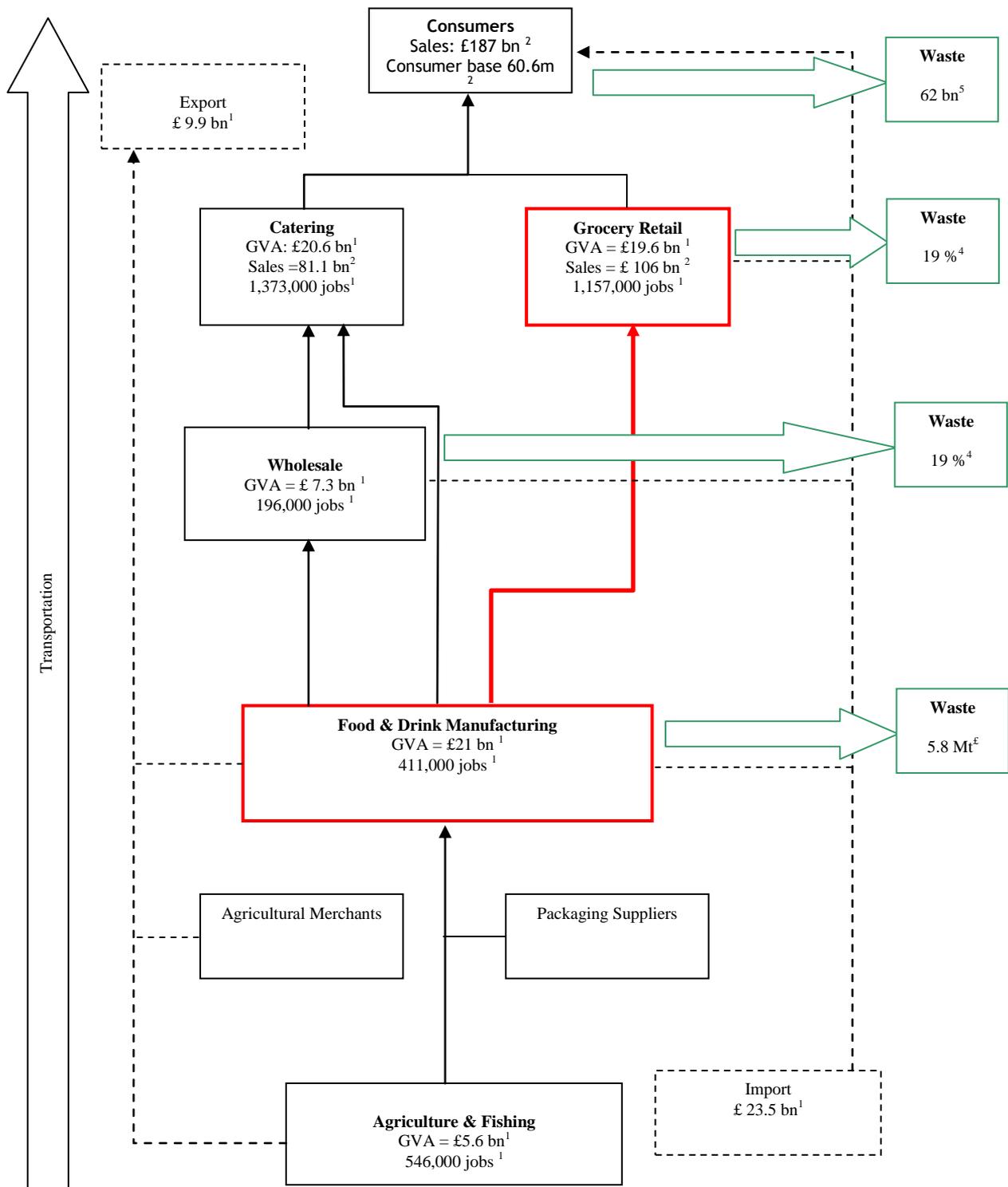
The supply chain layout for the UK is shown in Figure 4.13 This diagram shows the flow of materials through the industry from raw materials to end consumers. It also identifies income from sales as well as the value adding to the food from these sales. This highlights another problem in the cost of waste, in that a food item could have several different prices or values attributed to it throughout the supply chain. This draws attention to a need for a standard of measuring for waste, including packaging and material waste to be used in this project. It is expected that throughout the course of this investigation, a best practice of estimating values of waste will arise from communications with several of the project partners.

The waste that has been described in Figure 4.12 shows only the gross value of wastage occurring at that stage of the supply chain. This project will look to identify the root causes of these wastes in terms of production as well as evaluating the price and value of the waste – to be discussed later in this section. This will result in a stratum of waste values or problems from different operations which will be the makeup of the gross, top line figure of waste, (see Figure 4.13). It will also help identify wasteful processes and inefficient operational techniques as well as possible solutions to waste reduction. This will correlate to round table discussions already occurring as a result of the Courtauld agreement (Defra, 2007a) (see Figure 4.14).

Although out of the scope of this project, it is worth noting the waste recorded from agriculture and raw food manufacture. The amounts of waste in this diverse industry run at about 30-40% of production (C-Tech Innovation Ltd, 2004) which is extremely high. This is due to the rigid contracts with processors and retailers who usually specify the size and shape of fruits and vegetables with exact numbers per order.

The shrinking size of the food wholesale market means that any surplus or “rejected” food items have nowhere to move up the supply chain. The management of this waste is well practised and often involves re-ploughing or composting for crops and specialist retail streams for livestock waste. An example of waste becoming a resource can be found in the meat and livestock sector. The hides and skins from the animals can be used as valuable resources for the leather industry and prevents increases in landfill volumes and therefore costs.

Figure 4.13: The economic summary of the UK food chain (Source: (IGD, 2007)).



- Sources: 1. Defra (2007) Food Statistics Pocketbook
 2. ONS (2008)
 3. Biffaward (2006) The Mass Balance Movement
 4. Defra (2007) Waste Strategy for England 2007
 5. Wrap (2007) Understanding Food Waste

Note: Some values differ between sources

4.3.2.1 Retail

From Figure 4.12 it is certain that there are large volumes of waste found in the Retail segment. This is a result of food items, especially chilled, being high in complexity and in a compound form (an example being sandwiches, which contain varied, processed ingredients that are not able to be reversed into raw ingredients). There is a lack of independent, robust research into the waste produced by retailers. This is mainly because of the perceived value of this information and the difficulty in ensuring confidentiality.

In terms of the value-added chain, the most valuable items can be found at the highest point of Figure 4.13. It would seem that the highest 'costs' of waste arise from this commercial segment. As expected, the larger supermarkets are responding to these costs and the Courtauld Commitment to fall in line with legislation and the Food Industry Sustainability Strategy (FISS) as well as to recoup any financial reward from minimising potential waste. A summary of this agreement is given in Figure 4.14.

Courtauld Commitment:

- Design out packaging waste growth by 2008
- Deliver absolute reductions in packaging waste by 2010
- Identify ways to reduce food waste

Figure 4.14 Summary of the Courtauld Commitment (Source: Defra 2007)

The agreement represents 92% of UK grocery supermarkets and over 30 major retailers, brands and suppliers which include names like Heinz, Sainsbury's and Procter and Gamble. The agreement has delivered on its first target by zeroing growth in packaging waste, despite increase in sales and population (WRAP, 2008). This was accomplished using innovative packaging formats, reducing the weight of packaging and increasing the use of refill/self-dispensing systems with collaboration on packaging design guidance.

Taking a large retailer like Tesco, a retailer also involved in the Courtauld Agreement, it is possible to see the effects such agreements are having on their management and treatment of waste. According to their website they are tackling issues such as recycling:

“At the moment we recycle 71% of waste from our stores and we aim to increase this to 80% by 2008.” (Source: TESCO, 2007)

There is also evidence of managing waste by using reusable green packaging trays and cutting down on the use of cardboard (estimated saving of 132, 000 tonnes of cardboard saved so far since the introduction of 2006) as well as being involved with FareShare to distribute surplus food to avoid landfill (TESCO, 2007).

4.3.2.2 Manufacturing and Distribution

This sector inevitably causes waste as discussed previously, this waste might be from an unavoidable source, such as vegetable skins and animal carcasses, or from food trimmings and processing. Figure 4.15 presents the example of tea biscuits resulting from a mass balance analysis. This analysis shows that the process generates 3.5 tonnes of visible waste per 75 tonnes of production, this equates to 4.6% of output.

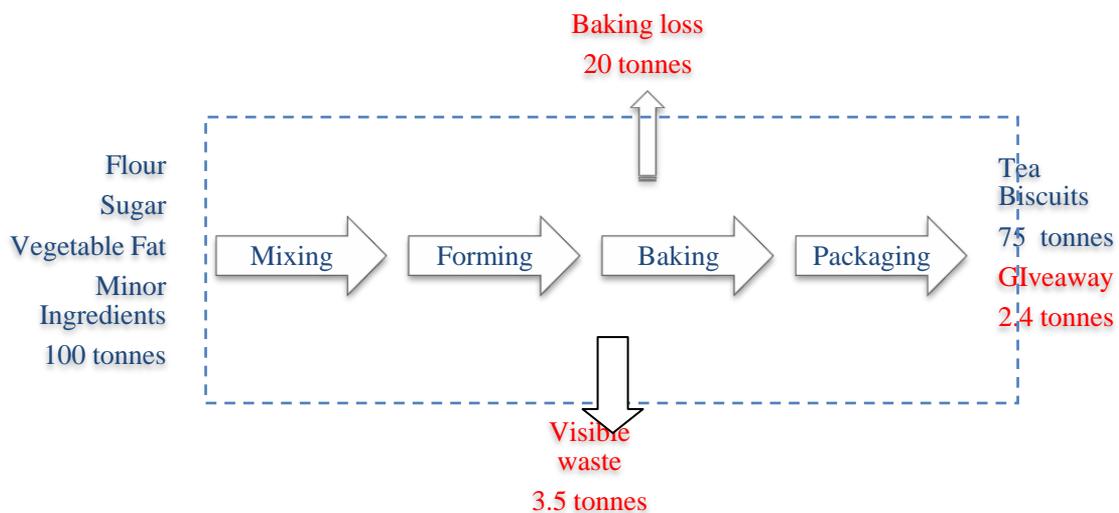


Figure 4.15 the Mass Balance process for United Biscuits McVities Tea Biscuits production (Source: C-Tech Innovation Ltd, 2004)

Some of this is lost as visible quantifiable waste; other losses of mass might be caused by water vapour escaping during the baking process. This method of mass balance is used in the industry to benchmark waste efficiencies of food production (C-Tech Innovation Ltd, 2004). In other convoluted food processes that require several ingredient streams and cooking steps, such as ready - made meals, it can be much more difficult to estimate manufacture waste. Using the above method for each step simplifies the end calculation as long as mass measurements are reliable.

Distribution of food post-manufacture also poses a challenge to food waste. The segments at highest risk at this stage are the chilled and frozen food segments which depend on a constant temperature to avoid food spoilage. Other challenges include damage caused by transit and errors emerging from forecast discrepancies which are not passed onto retailers (Food and Drink Federation, 2007; Fellows, 2000).

Table 4.7 shows some of the key points identified by research into increase in waste in production and distribution (Fellows, 2000). This project will investigate reasons why such waste matter occurs, as well as relating best practices for avoiding and dealing with such items of waste.

Table 4.7: Food processing unit operations and associated wastes (Source: Fellows, 2000)

Category	Operations	Example Wastes produced
Ambient Temperature Processing		
Raw Materials Preparation	Cleaning, Sorting, Grading and Peeling.	Cleaning water effluent (BOD or COD), peelings, hair, feathers, grit, blood, contaminated foodstuffs.
Size Reduction	Chopping, Cutting, Slicing, Dicing, Milling of Solid Foods, Pulping, Emulsification and Homogenisation of Liquids.	Poor Quality (too course/fine) products with loss of nutritional/sensory characteristics. Dust agglomerates. Waste off-cuts. Fat bearing effluents from colloidal products (e.g. Dairy). Risk of pathogenic contamination in emulsification (e.g. Dairy)
Mixing and Forming	Mixing, Forming malformed pieces.	Wrongly proportioned batches, poorly mixed ingredients.
Separation and Concentration	Centrifugation, Filtration, Expression, Solvent Extraction, Membrane Concentration.	Separated solids (e.g. after clarification of liquids), press residues (e.g. fruit juice extraction).
Fermentation and use of Enzymes	Fermentation, Enzyme Technology.	Spent biomass.
Other	Irradiation Pulsed Electronic Field, High Pressure, Pulsed Light and Ultrasound.	
Processing with Heat Application		
Preservation/stabilisation	Blanching, Pasteurisation and Sterilisation.	Under blanched food wastage, effluent, thermophilic bacterial contamination, heat spoilage, heat transfer surface film build-up and product loss due to unsuccessful treatment.
Evaporation and Distillation Extrusion	Evaporation and Distillation Extrusion.	Heat transfer surface film build-up, distillation residues, strip down residues.
Dehydration	Hot air driers, Heated surface driers, Rehydration.	Heat transfer surface film build-up.
Baking and Roasting	Direct and indirect heating ovens, Batch and Continuous ovens.	Write off of oven contents if process interruption exceeds products buffering capacity.
Frying	Shallow Frying, Deep-fat frying.	Contaminated fats and particulates
Direct and Radiant Heating	Dielectric Heating, Ohmic Heating Infrared heating.	
Processing with Heat Removal		
Chilling	Fresh foods, processed foods, Mechanical refrigerators, Cryogenic chilling, Chill Storage, Modified and controlled atmosphere storage and packaging.	Spoiled food in equipment failure
Freezing	Freezing.	Spoiled food in equipment failure and thawed water.
Freeze Drying and Concentration	Freeze drying and Freeze Concentration.	

Category	Operations	Example Wastes produced
Pre & Post-Processing Operations		
Coating and Enrobing	Battering, Coating and Dusting.	Over/under coated product and appearance failures.
Packaging Filling and Sealing	Packaging, Filling and Sealing.	Packaging waste, product give away and spoiled goods if seal fails.
Handling, Storage and Distribution	Raw Materials and ingredients, Waste management and disposal, Storage and Distribution.	Losses due to transit damages and mismatch of forecast to actual demand.

4.3.2.3 Packaging

It has been estimated that 10.5 million tonnes of packaging entered the UK waste stream in 2006 (Defra, 2007a) and according to Envirowise, the food and drink industry is responsible for over 50% of this (Binyon, 2007). Over 5 million tonnes of food industry packaging enters the waste stream every year and in terms of raw materials alone costs £4 billion. Adding on other expenses such as disposal and recovery payments would give a truer and much larger figure of the cost. There are also environmental costs involved in land filling packaging, such as greenhouse gas emissions as well as low bio-degradation rates.

There is a trade-off between excessive packaging and protection of the product. The main aim of packaging is to protect foodstuffs and extend shelf-lives of products. However, if the package become more protective, it will be inevitable to produce more waste. Packaging has proven itself necessary for the modern production of food. The majority of waste created from packaging materials comprises glass, cardboard and plastics. Most of these are able to be reused and recycled so disposal to landfill is not an effective use of these resources.

4.3.3 Policies and Practices (UK and EU)

This section provides an overview of the current policies and legislation relating to waste and the food and drink industry. The majority of the information has been collated from two sources (C-Tech Innovation Ltd, 2004; NetRegs, 2008). The right legislation aims to prevent waste from occurring as well as increasing recycling, energy recovery and other waste

minimisation methods by making them more attractive and necessary as waste management approaches (Food and Drink Federation, 2007).

Table 4.8 shows a summary of the main EU and UK legislation relevant to the food and drink industry. In terms of the EU directives, they were all introduced to set up adequate waste control and waste management. The Framework directive on Waste, introduced in 1975 and revised in 1991, deals with the regulatory framework for the implementation of the European Commission's Waste Management Strategy of 1989, covering waste avoidance, disposal and management. The hazardous waste directive was introduced in 1991 to align management of these materials across Member States. The UK uses special waste regulations to conform to this directive and includes lists and definitions of special wastes as well as provisions for their treatment, storage and disposal. The food industry treats items unfit for human consumption, such as preservatives or peeling sludge as special waste.

The Integrated Pollution Prevention and Control (IPPC) directive introduced methods such as the "polluter pays" to attempt to reduce pollution at source by using BAT (best available techniques). The polluter pays for the environmental damage they are deemed to have caused to air, soil or water. In the UK, the Pollution Prevention and Control Act, 1999, is used for this purpose against large food and milk processing operations including intensive poultry and pig farming.

Table 4.8: Selected European and UK waste related legislation (Source: C-Tech Innovation Ltd, 2004)

European	Main Features	UK	Main Features
Framework Directive on Waste 75/442/EEC (91/156/EEC)	Waste control regimes and waste management plans. Regulatory framework for 1989 waste management strategy.	Environmental Protection Act 1990	Waste management licensing. Integrated Pollution Control (IPC). Waste producers duty of care. BATNEEC
Hazardous Waste Directive 91/689/EEC	Definitions of hazardous wastes. Wastes requiring special disposal.	Special Waste Regulations 1996	Implementation of Hazardous Waste Directive. Storage, treatment and disposal of hazardous controlled wastes. Definitions and lists of special wastes, including several food related categories.
Integrated Pollution Prevention and Control 96/61/EC	Prevention of waste at source, "polluter pays" principle, Best Available Techniques (BAT).	Environmental act 1995	Environment Agency (SEPA in Scotland) as a primary enforcement body. Producer responsibility for waste.
		Pollution Prevention and Control Act 1999	Phased in enactment of IPPC Directive including major food processing and agri-operations
		The Pollution Prevention and Control (England and Wales) Regulation 2000	
		Pollution Prevention and Control (Scotland) Regulations 2000	
Landfill Directive 1999/31/EC	Proscription of landfilling certain hazardous wastes. Reduction targets for biodegradable municipal waste.	Landfill (England and Wales) Regulation 2002	Designation of Landfill Directive in England and Wales
		Pollution Prevention and Control (designation of Landfill Directive) (Scotland) Order 2003	Designation of Landfill Directive in Scotland
Packaging and Packaging Waste Directive 94/62/EC	Reuse, Recycling and Recovery targets for packaging and packaging waste.	The Producers Responsibility Obligations (Packaging Waste) Regulations 1997 and subsequent amendments.	Implementation of Packaging Directives and amended (increased) targets for recovery and recycle levels.

European	Main Features	UK	Main Features
Animal By-Products Regulation	Categorises animal by-products in three categories with stringent disposal requirements.	Animal By-Products Order 1999 (Amended 2001) Water Resources Act 1991 Water Industry Act 1991	Limitation on use of material of animal origin to prevent disease entering the food chain. Water and effluent management, consents and charges.

The landfill directive's aim is to reduce landfill as a disposal route by prohibiting some hazardous and liquid wastes and by setting ambitious reduction targets for others such as biodegradable municipal waste (35% of 1995 figures by 2018 for the UK, with the 2004 figure at over 80% of biodegradable waste going to landfill). The directive was established to be used as a lever to force major change in the handling of some wastes and to promote more sustainable treatment and applications of new technologies. The UK has landfill regulations which are subject to ongoing amendments but with the targets of the EU directives in mind.

The directive on packaging was introduced with the intention of minimising the environmental impact of packaging and packaging waste. Other aims were to promote reuse, recycling and recovery of different articles of waste. Five-year targets were set including a 50-60% recovery of packaging waste and a 25-45% target of packaging to be recycled. The UK enforces this with its own regulations and further amendments. The regulations cover manufacturers, packers and fillers, importers and retailers of food products.

The EU regulation of animal by-products introduced in 2003 categorises waste into three sections:

- Category 1: High risk to be incinerated.
- Category 2: Materials unfit for human consumption. Most types of this material must be incinerated or rendered.
- Category 3: Material which is fit for but not destined for human consumption.

The UK has its own order introduced in 1999 and amended in 2001 and again in 2003 (UK Government 1999; UK Government 2001). This aims to minimise disease transmission such as BSE. The current legislation requires the prevention of feeding livestock catering waste which have come into contact with animal carcasses or material presenting similar hazards.

Further relevant UK legislation includes the Environmental Protection Act, 1990, (UK Government 1990) which licenses companies handling controlled wastes and allocates the monitoring and enforcement of pollution control, usually by local authorities. The waste producer is expected to deal with their wastage by BATNEEC (best available technology not at excessive cost). The Environment Act, 1995 is used to promote producer responsibility for recycling, recovery and re-usage of resources. Wastewater legislation is complex and is an amalgamation of the Water Resources Act, 1991 and the Water Industries Act, 1991. The primary concerns of these legislations are the release of water that is hazardous to the environment and responsible water resource management.

4.3.4 Existing Practices

Throughout the UK retail industry there are efforts to match the targets set by UK and EU directives mentioned previously. Many of these targets are focused on reducing the amount of waste that is sent to landfill. The range of these targets is broad when considering the 5 of the largest food retailers. Many of the targets are available on the company's own websites. Table 4.9 overleaf gives an overview of the existing practices in waste management and environmental targets from the top five food retailers/super markets in the UK.

Table 4.9 Existing practices summary table in the main retails companies in UK

Practices	Tesco		Asda		Sainsbury's ¹		Morrison's		Co-operative Group ²	
	Targets achievements	Future Targets	Targets achievements	Future Targets	Targets achievements	Future Targets	Targets achievements	Future Targets	Targets achievements	Future Targets
Reduce waste created in the operations	Reduction of 9 % of waste created by UK stores operations in 2007 ³ . The amount of waste recycled has been of 70 % ⁴ . Currently projects to use one-way packaging with clearly and readily labels by suppliers in the distribution operations. Reusable transit trays has saved over 130 000 tonnes of cardboard.	Recycle 80 % of the waste by 2009. Reduction in packaging by 25 % by 2010.	It launches initiatives to spread the utilisation of plastic bags made of recycling materials.	Target zero waste to landfill by 2010.	A decrease of 6 % in absolute terms during 2008. Technical Management training to suppliers.	Reduction the waste to landfill by 50 % relative to sales by 2012, against a 2005/06 baseline. Reduce suppliers travel by 5 million Km.	Achieved 18 % of long term target.	Reduce volume of waste to landfill by 50 % by 2010.	Consider the merger with United Co-operatives the amount of waste re-used and recycled increased in 4771 tonnes in 2007 against 2006 baseline.	Ensure that less than 50 % of total waste arising are land filled by 2013
Food waste control	Delivery of surplus fresh food to homeless shelters in partnership with FareShare.	Use food waste for producing energy.	It launches initiatives to spread the utilisation of plastic bags made of recycling materials.	Target zero waste to landfill by 2010.	A decrease of 6 % in absolute terms during 2008. Technical Management training to suppliers.	Reduction the waste to landfill by 50 % relative to sales by 2012, against a 2005/06 baseline. Reduce suppliers travel by 5 million Km.	Achieved 18 % of long term target.	Reduce volume of waste to landfill by 50 % by 2010.	Consider the merger with United Co-operatives the amount of waste re-used and recycled increased in 4771 tonnes in 2007 against 2006 baseline.	Ensure that less than 50 % of total waste arising are land filled by 2013
Recycling facilities	Installation of 45 "reverse vending machines", which separate different kinds of materials and compact them ready to recycle, increasing the average of recycle materials from 4 to 8,3 tonnes each week.	It is planned install 100 machines by March 2009.	Using a backhauling process is recycling to some of 5 Asda Service Centres 65 % of the stores waste.	Goal, eliminate the remaining 35 % of waste which is no currently reprocessed.	Increase to 50 % of recycle material in the standard carrier from June 2008.	Drive down carrier bag usage by 50 % by April 2009, against April 2008 baseline.	Recycled 72 % of available store waste.	Increasing the proportion of recycled available store waste to 80 % by 2010.	In 2007, it was recycled or reused almost 40000 tonnes of waste. 1249 tonnes were through own recycling centre in Manchester	Commit to increase recycling facilities.
Packaging	Launch a system for registering suppliers' data online, which support packaging improvements. In 2007 own-brand packaging in electrical and clothing lines has reduced by as much as 40 %.	Reduction in the packaging by 25 % by 2010.	Reduction of 10 % own label food packaging. Sourcing paper from sustainable forest, certified by Forestry Steward Council.	Reduction by 25 % own label food packaging.	90 % SO's (Sainsbury's Organic products) packaging will be recyclable, reusable or compostable. 75 % of wood-based products have certified by Forestry Steward Council.	Reduction the amount of packaging by 25 % by May 2010, and 50 % from these is hoped will be from recycled material on fruit and vegetables.	Achieved 44 % of long term target.	Increase recycled content of standard plastic carrier bags to 50 % by 2010.	In late 2007 was restated the target of 15 % of reduction packaging, include transit packaging.	Initiate projects to support reduction in primary packaging on own-brand food products by 15 % by 2010.
External factor	Progress with product carbon and labelling footprints.	Reduction a 50 % CO ₂ emissions ⁵ by 2020.	Commit to achieve Government's Courtauld Commitment.	Commit to use 18000 tonnes each year less packaging.	New stores use green technology to recycle waste which provided energy. Reduce CO ₂ emissions per case transported by 5 % by March 2009 against a 2005/06 baseline.	Reduction CO ₂ emissions per square metre by 25 % by 2020.	Reduction of 10818 tonnes of CO ₂ emissions due to transportation efficiencies. And 58 % road miles travelled per pallet of stock and 35 % empty road miles travelled of target by 2010.	Save 8 % haulage CO ₂ emissions by 2010, 2005 baseline.	Commit to reduce the carbon footprint.	It called an independent lifecycle analysis of common packaging options for a given application.

¹ <http://www.j-sainsbury.co.uk/cr/index.asp>² <http://www.co-operative.coop/food/ethics/Environmental-impact/>³ Total waste in UK was 487 000 tonnes.⁴ Most of the recycle wasted was cardboard and plastic. The target in 2007 to recycle was 75%.⁵ It is considered to worldwide operations. In UK the commitment is reduce by 5, 5 % in their existing stores and distribution centres.

4.3.5 What happens to waste

The waste management hierarchy (Defra, 2007a) gives industry a structure for the management options for waste. The options are as follows:

- Waste prevention
- Re-use
- Recycle
- Energy recover
- Disposal.

Figure 4.16 shows the relationship between the steps in terms of priority and ideal quantities, i.e. more waste prevention than waste re-use. Waste prevention aims to avoid producing waste in the first place and should be carried out ideally before any of the other solutions in the hierarchy. The main aim is to cut down waste going to landfill and to obtain the full potential from materials and foodstuffs rather than produce waste for the sake of (Defra, 2007b).

For the food industry, general waste minimisation activities include improving operational practices, increasing control of existing waste operations and introducing innovative process technology (C-Tech Innovation Ltd, 2004). Implementing this step presents the challenge of investing money into operations. However, this investment will be recouped as other disposal methods, such as landfill and incineration, become more costly and deemed more environmentally damaging.

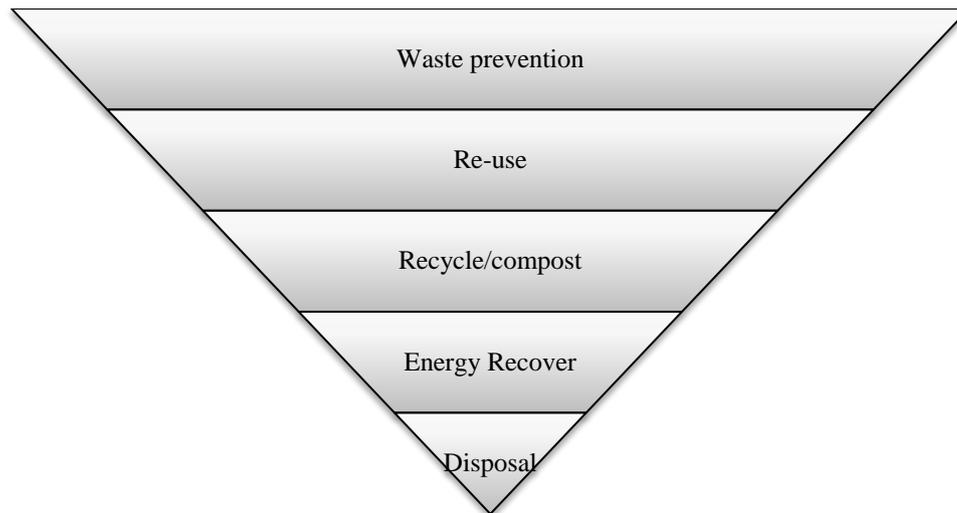


Figure 4.16 the waste hierarchy (Source: Defra, 2007b)

Re-use of materials where possible is the next step. However in the food industry there are barriers to applying this proposal. The limiting factor is generally hygiene requirements; for example, any re-use of packaging would require a high standard of cleaning before it can be re-used which is often not cost effective (C-Tech Innovation Ltd, 2004).

In terms of recycling and composting, the food production industry has a wealth of options available. These include composting, land spreading, aerobic digestion etc. These can often lead to further income streams because the by-products can either be resold to food producers or re-used by the manufacturer in other operational procedures (Fellows, 2000).

Other options of waste disposal that would lie at the bottom of the hierarchy include incineration, rendering and landfill. Incineration is generally perceived by the public to be environmentally unfriendly and a health damaging method of disposing of waste which is surrounded by high levels of legislation and regulation. In other countries where technology for converting incinerated waste into energy has progressed substantially (where conversion efficiency is approximately 75%) (C-Tech Innovation Ltd, 2004)) it is deemed to be a tolerable diversion route for waste from landfill. It should be noted that ultimately the resulting ashes are often land filled.

The rendering of animal by-products from the meat production chain is estimated to be a cost-effective means of disposal at least in the medium term due to the legislation mentioned

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previously in this report (UK Government 1999)(Yu and Huh, 2006). It is estimated that 1.75 million tonnes of this waste is to be dealt with annually which produces 0.25 million tonnes of fat and 0.4 million tonnes of protein meal through rendering (C-Tech Innovation Ltd, 2004).

Landfill is the UK's prevalent waste disposal route and handles 50% of industrial waste. There have recently been several drivers for change for the implementation of the waste hierarchy and to reduce this figure. These include categorising waste (into hazardous, non-hazardous or inert), reduction of active landfill sites and a ban on tyres going into landfill. Also to be imposed are the banning of liquids, the requirement of pre-treatment for non-hazardous waste (both from October 2007) and the planned closure of some landfills by 2009 (Defra, 2007b).

4.3.6 Known causes of waste

Waste is an undesirable effect resulting from the complex interaction of management practices, product characteristics, consumer trends and environmental factors. Incidents leading to food waste are seldom the result of a single cause but rather from a combination of factors occurring simultaneously. For instance, poor information sharing combined with a short shelf-life chilled product and a spell of cold weather could lead to substantial amounts of waste due to the fluctuation in demand. Hence it is difficult to attribute specific amounts of waste to each cause. It is possible, however, to identify those causes that appear to be having the most influence on waste.

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Table 4.10 presents a short description of the leading causes of waste in the food and drinks industry gathered from the literature.

Table 4.10: Causes of Waste

	Cause	Description
Management Factors	Forecasting	Estimating the demand for a product is a complex and inherently inaccurate activity which can be affected by many factors such as weather, seasonality, marketing campaigns, product launches, promotions and special occasions such as Christmas and Easter. Forecasting error has a direct impact on waste, particularly for products with short shelf-lives. Hence the forecasting approaches and methods used by both retailers and manufacturers are key to reducing waste.
	Information sharing	Accurate and timely information is essential for good planning and forecasting. When information is scarce there tend to be large variations between forecast and orders which often result in waste. Furthermore variations caused by poor information sharing can amplify across the supply chain (i.e. bullwhip effect).
	Promotions	Demand during promotional periods is notoriously difficult to forecast and the increased forecasting error is likely to lead to increased waste. Furthermore, promotions can also increase household waste as customers might buy unusually large quantities of product.
	Shelf-life policies	Most mainstream retailers have policies of only accepting product with a high proportion of shelf-life remaining (usually over 70%). This is particularly problematic for own label producers who are unable to sell the product through other channels, such as discount retailers.
	Inventory Management	Inventory management policies, particularly around safety stock levels are likely to have an impact on waste.
	Stacking and shelving	Stocking and shelving can have an impact on product damage but also on product selection by customers which will prefer those products with the longest shelf-life available.
	Penalties and availability targets	Penalties are a mechanism used to ensure that deliveries are made on time and in full. However, they can encourage manufacturers to over-produce to cover themselves against the risk of penalties or de-listings.
Product Factors	Product characteristics	Some characteristics inherent to the product such as shelf-life and temperature regime tend to generate waste. However, technology can be used to alter some of these characteristics.
	Packaging	Packaging plays a dual role in terms of waste; on the one hand it protects the product from damage and can help to extend its shelf life, having a positive effect on waste. On the other, the amount of packaging on a product has a direct impact on household waste and to some degree on waste generated at other stages in the chain.
	Product damage	Poor practices in product storage and handling coupled with packaging and palletising practices can result in damaged products which are discounted or discarded.
	Product recalls	Product recalls are relatively rare events. However, when they occur they are likely to generate large amounts of waste, particularly for products with long shelf-lives since they are likely to have more stock in the pipeline.

	Cause	Description
Environmental and Consumer factors	Customer trends	Trends in customer demand can have a substantial impact on waste levels. Environmental trends, for instance, are already having a positive influence on the reduction of packaging waste. Other trends such as the increase in fresh foods and some convenience foods with short shelf lives can have a detrimental effect on waste.
	Weather	Weather patterns have a strong effect on demand for some products, particularly fresh produce and beverages which are likely to affect waste levels. Although these events are beyond the control of the companies involved, actions taken to monitor and react to changing weather patterns could help to minimise waste.
	Catastrophic failures	Temperature controlled supply chains suffer the risk of a potential catastrophic failure in warehousing or transportation equipment. Although these events are rare, their impact is bound to be substantial in terms of the volume of waste generated.
	Seasonality	Seasonality of both supply and demand affects forecast accuracy, production levels and inventory levels. All of these factors could lead to waste.

4.4 Methodology

The food supply chain encompasses large variations in product specifications and characteristics. These include shelf-life, storage temperature and seasonal/demand variability. The project also required sets of quantitative data to estimate the size of the problem and also qualitative data to determine root causes and best practices. Therefore the methodology required needed to allow for different product types and an interface that allowed the gathering of volumes and numbers as well as concepts and ideas.

The decision was made to segment and collect data around a multiple case-study research design. Food types were segmented into either cold chain or ambient chain categories and were then further divided based on their shelf-life. The product would then be analysed in the two stages of manufacture and retail within the supply chain. This would facilitate the understanding of the issues emerging between the two parties and potentially provide solutions that could be applied to both.

The original research design incorporated 16 cases which would cover a range of product types. These were divided into the following categories as seen below in Table 4.11. Once the project was underway it was clear that the main product category for waste was chilled produce and hence the shift in balance of cases towards this group. Ultimately 20 cases were

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undertaken and the majority of these were centred on products with short shelf-lives in the cold chain. The names of companies and products are kept anonymous for confidentiality reasons.

Table 4.11 case studies conducted (planned vs. Actual)

	Cold Chain		Ambient	
	Frozen (long shelf-life)	Chilled (short and medium shelf-life)	Short shelf-life	Long shelf-life
Planned	3	5	5	3
Actual	2	10	5	3
Example	Ice-Cream, Frozen Veg.	Meats, dairy, drinks, sandwiches	Fruit and Vegetables	Confectionary, canned and bottled product

4.4.1 Data Collection

The case-study research involved three forms of data collection

1. Semi structured interview (a blank form can be found in the appendix) to allow issues and causes of waste to be discussed and understood.
2. Review of company records to estimate the size of waste.
3. Process observation, to see the physical and information process active within the supply chain.

The interviews and observations were used to provide quantitative and qualitative data and supply information on the magnitude of the problem and identify root causes. Although it was not possible to conduct all three forms of data collection for all cases, in 17 cases it was possible to use at least two methods. The data for the remaining three cases was collected via a workshop in which three different suppliers of fruits and vegetables completed a questionnaire based on the interview questionnaire and then discussed the issues collectively.

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In each interview case the interview lasted approximately 1 hour and conducting face to face for the majority of interviews. At least two members of the research team were present. With one member conducting the interview and at least one other taking notes. These notes would then be discussed and agreed by the interview team and then sent to the interviewee for validation.

Interviews were coordinated with managers who had a direct responsibility for waste within their organisation. If there were no specific waste management personnel then a manager with supply chain responsibilities was selected.

The interview protocol was developed over three stages. A first draft was prepared and then reviewed by all members of the research team and adjusted. This next draft was then piloted over the first two cases and then modified for a final time with an additional question. The interview was designed to collect data from four key areas:

- Contact details and demographic, to cover details on the company and the product
- Quantitative waste data, if available, specific data concerning waste volumes and percentages
- Causes of waste and good practices, to provide discussion on the main areas of waste
- Destination of waste, to provide a discussion on how waste is managed

Waste management information was considered by many participants to be sensitive and relating to certain parts of corporate strategy. Therefore confidentiality was of high importance and for this reason a detailed confidentiality agreement was offered to all participating companies. The sensitivity of data occasionally prevented the collection of the numerical data on waste.

4.4.2 Data analysis

Due to the aims of the project being causes of waste and best practices from waste management, the majority of the data collected was in qualitative format. The data from each individual case was initially coded and placed into a standard case-study template and then

key information was extracted and placed into a table to enable a cross case comparison. The data from these tables was analysed in the following ways:

- Analysis of waste by product type, products were grouped by temperature control in order to analyse the outstanding issues for each product grouping
- Analysis of root causes, a method using current reality trees (CRTs) was used to analyse the complexity of casual connections and observations that somehow correlate to waste
- Analysis of good practices, by focusing on the practices used in each of the cases and seeking any similarity or patterns across the cases in that category.

This project did not produce any data that required any statistical analysis and so no statistical tolls were applied to the data.

4.5 Analysis

4.5.1 Case studies

The case studies are presented in the table formats below so that cross case analysis can be carried out without having to use the original data collect for each individual case. This method makes it easier to investigate the similarities and differences across the category and helps to indentify key information from each interview. The interviews have also been coded for ease of reference with F for frozen products (Table 4.12), C for chilled (Table 4.13 and Table 4.14) and A for ambient (Table 4.15 and Table 4.16).

Table 4.12 Frozen products case data

	F1	F2
Product category	Vegetables	Ice cream
Temperature regime	Frozen	Frozen
Focus	Retail	Retailer – supplier
Shelf-life	6 months +	18 months
Lead-time	Day 1 for day 3	Day 1 for day 3 in RDC, day 4 in store
Demand variability	Stable with some seasonality	Highly seasonal / Weather dependent
Stock	7 days depot 7 days store	7 days in store Stockless depot
Waste	0.12% Very Low	Very low
Main causes of waste	- Damage (packaging) - Failure in refrigeration (rare but high impact)	- Inaccurate promotional forecast - Human error (inventory) - Failure in refrigeration equipment (rare but high impact) - Recalls (rare but high impact)
Good practices	- Clarity of responsibilities - Performance measurement - Forecasting and replenishment software - Orders place automatically by adjusted re-order point system - Sales visibility to all suppliers - Reducing waste to landfill	- Clear responsibilities for waste management - Clear promotional planning process (not always followed) - Clear process for shelf management (not always followed) - Continuous replenishment related to till sales. - Product with short shelf-life left sold through other channels
Destination of waste	Landfill (aim to reduce to zero)	- Landfill - Retailer has trial with FareShare

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Table 4.13 chilled products case data

	C1	C2	C3	C4	C5
Product category	Milk	Vegetables (bagged salad)	Red meat	Margarine	Sandwiches (pre-packed)
Temperature regime	Chilled	Chilled	Chilled	Chilled	Chilled
Focus	Retail	Supplier – Retail	Retail – producer	Retailer – supplier	Retailer
Shelf-life	3 days (average in retail)	3 days	7 days	8 weeks	2 days
Lead-time	Day 1 for day 3	Variable (seasonality)	Day 1 for day 2 in store	D1 for D3 in RDC, D4 in store	Day 1 for day 3 (store)
Demand variability	Stable / predictable	Seasonal (summer uplift)	Irregular	Stable	Irregular (seasonal and weather a)
Stock	1.5 days at depot 1.5 days at store		1 day	7 days in store - stockless depot 3 days at supplier	1 day
Waste	0.1 % (Very Low)	High – Very high	Low	Low	7% (Very high)
Main causes of waste	- Damage (poor handling)	- Unpredictable demand - Inaccurate forecasting - Retailers service level requirements (over stock) - Promotions - Rejected deliveries (quality) - Packaging design changes - Seasonality of supply	- Product out of shelf-life	- Inaccurate promotional forecasts - Poor stock rotation (shelf) - Failure in refrigeration equipment (rare but high impact) - Recalls (rare but high impact)	- Irregular demand - Inflated orders to make shelves look full - Poor stock rotation (shelf) - Failure in refrigeration equipment

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	C1	C2	C3	C4	C5
		(longer transport in winter)			
Good practices	<ul style="list-style-type: none"> - Clarity of responsibilities - Performance measurement - Forecasting and replenishment software - Orders place automatically by adjusted re-order point system - Sales visibility to all suppliers - Reducing waste to landfill 		<ul style="list-style-type: none"> - Butcher in store helps to preserve the product for longer - Shorten lead times (vertical integration) - Visibility of promotions (vertical integration) - Availability sacrificed in promotions - Forecasting influenced by historic data, seasonal events and weather. - Ordering managed centrally but store managers can flex - Culture of waste reduction 	<ul style="list-style-type: none"> - Product with short shelf-life left sold through other channels - Clear responsibilities for waste management - Clear promotional planning process (not always followed) - Clear process for shelf management (not always followed) - Continuous replenishment related to till sales. - Product with short shelf-life sold through other channels that mainstream retailers 	<ul style="list-style-type: none"> - Forecasts are manually adjusted to account for regional variations - Promotions run constantly so don't affect waste - Change packaging from plastic to cardboard to increase recycling (although cardboard is less durable) - Clear responsibility for waste
Destination of waste	Landfill (aim to reduce to zero)	Landfill	<ul style="list-style-type: none"> - Landfill - Trials on anaerobic digestion 	<ul style="list-style-type: none"> - Recycle secondary packaging - Landfill - Retailer has trial with FareShare 	<ul style="list-style-type: none"> - Local arrangements with charities to collect unsold product - Mainly goes to landfill

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Table 4.14 Chilled products case data continued

	C6	C7	C8	C9	C10
Product category	Cooked poultry (organic)	Cooked poultry	Fruit drinks	Milk (own brand)	Fresh meat
Temperature regime	Chilled	Chilled	Chilled	Chilled	Chilled
Focus	Producer	Producer	Producer	Producer (single retailer)	Producer
Shelf-life	10 days	26 days	20-40 days	12 days	8-9 days
Lead-time	Same day for orders before 2.00; else day 1 for day 2	Same day for orders before 2.00; else day 1 for day 2	12 to 36 hrs	2 days from farm to depot 2 days to store	Day 1 for delivery to store on day 2
Demand variability	Irregular (low volume; weather)	Irregular (Promotion; weather)	High	Stable (some seasonality)	Variable (seasonal and weather)
Stock	1.5 days	2.5 days	8-10 days	0.5 days	1.5 days
Waste	1.85 % (producer) Very low	0.38% (producer) Very low	3% in store; 1% in manufacture Intermediate	0.02% Very low	6-10% main retailers; 15-30% in convenience (High – Very High)
Main causes of waste	<ul style="list-style-type: none"> - Combination of poor forecast accuracy and short shelf-life - Low volume - Packaging changes and price changes can cause waste 	<ul style="list-style-type: none"> - Combination of poor forecast accuracy and short shelf-life - Promotions planning with retailers (base demand is stable but promotions cause variability) - Packaging changes and price changes can cause 	<ul style="list-style-type: none"> - Forecasting error - Promotional forecasting - Cannibalisation during promotions - Product damage 	<ul style="list-style-type: none"> - Rejected deliveries - Poor stock rotation - Wrong date coding - Planning errors - Cannibalization (brand promotions slow down demand, but not major impact) 	<ul style="list-style-type: none"> - Bad weather - Achieving a balance between OSA and waste - Differences in appearance (customer pick) - Volatility in small retailers - Occasional damage

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	C6	C7	C8	C9	C10
		waste			
Good practices	<ul style="list-style-type: none"> - Collaborative forecasting delivers more accurate forecast but is resource intensive (particularly for seasonal products) - Use of alternative routes to market, such as discounters and pet food producers for product with short remaining shelf-life 	<ul style="list-style-type: none"> - Collaborative forecasting delivers more accurate forecast but is resource intensive (particularly for seasonal products) - Use of alternative routes to market, such as discounters and pet food producers for product with short remaining shelf-life 	<ul style="list-style-type: none"> - Sharing of data and close collaboration with retailers - Pasteurisation technologies to increase product life. - Use of packaging to increase shelf-life. - Developing new forecasting model to reduce forecast error. 	<ul style="list-style-type: none"> - No waste to landfill - Effective system leading to very low levels of waste. 	<ul style="list-style-type: none"> - Clear responsibilities for waste - Partnership with retailers - Regular meetings with retailers - Use of implants with large retailers to reduce forecast error - Some promotions create less unpredictability (e.g. link deals) - Some retailers treat product better (e.g. handling, ergonomics, lighting, stock , temperature) - Looking at approaches to extend shelf-life
Destination of waste	<ul style="list-style-type: none"> - Packaging waste is recycled - General waste goes to landfill. - Products can be stripped to separate waste. 	<ul style="list-style-type: none"> - Packaging waste is recycled - General waste goes to landfill. - Products can be stripped to separate waste. 	<ul style="list-style-type: none"> - Damaged product goes to landfill - Planning to give away product with short shelf-life left. 	<ul style="list-style-type: none"> - Animal feed, bio-gas and composting - Milk cannot be sent to landfill - Packaging is recycled - Durable tertiary packaging 	

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Table 4.15 Ambient product case data

	A1	A2	A3	A4
Product category	Fruits and Vegetables (potatoes)	Fruits and Vegetables (raspberries)	Fruit and vegetables (general)	Pasta sauce
Temperature regime	Ambient	Ambient	Ambient	Ambient
Focus	Retail	Supplier – Retailer	Retail - producer	Retailer – supplier
Shelf-life	3-5 days	3 day	Product dependent (short)	9 months
Lead-time	Day 1 for day 2 in RDC day 3 in store	N/A	Day 1 for day 2 in store	Day 1 for day 3 in RDC, day 4 in store
Demand variability	Seasonal (winter uplift)	Seasonal supply and demand (summer uplift)	Irregular	Stable Seasonal variations
Stock	1.5 days in depot 1.5 days in store	N/A	1 day in store (average)	7 days in store 4-7 days in dept 14-28 days at supplier
Waste	Low	High – Very High	Low	Low
Main causes of waste	- Inaccurate forecasting - Insufficient shelf space available - Exceptionally poor handling	- Quality expectations (reject) - Weather effects on demand - Difficult to predict demand - Poor store handling	- Product damage - Difficulties in predicting demand accurately	- Inaccurate promotional forecast - Damage - Product handling - Recalls (rare but high impact)
Good practices	- Efficient handling (reduce order – delivery time) - Clarity of responsibilities - Performance measurement - forecasting and replenishment	- Train packers to select appropriate fruit. - Promotions used to manage waste (cope with seasonal fluctuations)	- Shorten lead times (through vertical integration) - Visibility of promotions (vertical integration) - Availability sacrificed in	- Product with short shelf-life left sold through other channels - Clear responsibilities for waste management - Clear promotional planning

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	A1	A2	A3	A4
	software - Orders place automatically by adjusted re-order point system - Sales visibility to all suppliers		promotions - Forecasting influenced by historical data, seasonal events and weather. - Ordering managed centrally but store managers can flex - Experimenting with packaging to protect products - Culture of waste reduction	process (not always followed) - Clear process for shelf management (not always followed) - Continuous replenishment related to till sales. - Product with short shelf-life sold through other channels that mainstream retailers
Destination of waste	Landfill (aim to reduce to zero)	Landfill	- Recycling or landfill - Trials on anaerobic digestion	- Landfill - Retailer has trial with FareShare

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Table 4.16 Ambient product case data continued

	A5	A6	A7	A8
Product category	Oils	Drinks	Fruits and Vegetables (peppers)	Fruits and Vegetables (citrus)
Temperature regime	Ambient	Ambient	Ambient	Ambient
Focus	Producer	Producer	Supplier - Retailer	Supplier
Shelf-life	12-18 months	75 days – 24 months	2 weeks	10 days (more dependent on cold chain)
Lead-time	3-4 days	From 1 to 3 days depending on customer	N/A	Same day
Demand variability	Stable (some seasonality and weather impacts)	Moderately stable (seasonality, marketing initiatives and weather)	Seasonal and weather dependent	Stable with 3 fold increase in Christmas
Stock	17 – 21 days	11 days	N/A	10 days southern hemisphere; 2 -3 days northern hemisphere
Waste	5-10%:-High – Very high	Very Low (most waste is packaging)	Intermediate	Around 5% Moderate - High
Main causes of waste	<ul style="list-style-type: none"> - Forecasting error; out of shelf-life - Damage (broken glass) - Quality issues (rejects) 	<ul style="list-style-type: none"> - Some promotions can cause waste - Product can get damaged but not very significant 	<ul style="list-style-type: none"> - Depot rejections: low quality product - Damage: handling and in-store damage by customer - Waste occurs when weather is poor and there is over availability of the product. 	<ul style="list-style-type: none"> - Product quality issues like mould and disease - Incorrect temperature / humidity storage - Packaging / labelling errors
Good practices	- Visibility of retailer information	- Product with short life left is	- Use of forecasting software; use	- Promotions agreed with retailer

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	A5	A6	A7	A8
	<p>(POS) and forecast but need to pay for POS data!</p> <ul style="list-style-type: none"> - Storage and in store displays can impact waste - Product with short shelf-life left can be sold to discounters, but not for own brand product. - The threat of penalties for not delivering OTIF motivates to reduce forecast error. 	<p>discounted</p> <ul style="list-style-type: none"> - Seasonality and weather fluctuations don't affect waste due to long shelf-life 	<p>forecast from retailer</p> <ul style="list-style-type: none"> - Demand management approach to reduce gaps in supply chain and inconsistencies in stock levels 	<p>help to reduce waste</p>
Destination of waste	<ul style="list-style-type: none"> - Can be destroyed or reworked - Some returnable packaging 	<ul style="list-style-type: none"> - Mainly recycle - 4.5% of all waste goes to landfill 	<ul style="list-style-type: none"> - Landfill 	<p>28 % is land-filled</p> <p>Packaging is recycled</p>

4.5.2 Causes of waste

A complete summary of the cases is provided below in Table 4.17 and gives a brief description of the product characteristics, levels of waste (very low (<1%), low (1-3%), medium (3-5%), high (5-7%) and very high (>7%) and the main causes of waste.

Table 4.17 Main causes of waste

Products	Product Characteristics	Waste Levels	Main Causes of Waste
Fresh red meat (pre-packed)	Chilled, Short shelf-life	V High	Bad weather, shelf-life, balance between availability and waste, differences in appearance, volatility in small retailers, occasional damage
Sandwiches	Chilled; very short shelf-life	High – V High	Planning and forecasting, variability of demand and shelf-life
Vegetables (bagged salads)	Chilled, very short shelf-life	High – V High	Unpredictable demand / forecasting, shelf-life, service level requirements, promotions, rejected deliveries (quality), packaging design changes, seasonality of supply (longer transport in winter)
Fresh fruit (raspberries)	Chilled + Ambient; short shelf-life	High – V high	Quality expectations (reject), weather effects on demand, demand predictability, handling
Oils	Ambient, long shelf-life	High – V high	Forecasting error, damage (broken glass), quality issues (rejects)
Fresh fruits (citrus)	Ambient, short shelf-life	High	Poor quality issues, incorrect storage, packaging and labelling issues
Fresh veg (peppers)	Ambient, short shelf-life	Med	Poor quality, handling and storage, weather fluctuations
Fruit drinks	Chilled; short-long shelf-life	Med	Forecasting, promotions (cannibalization) shelf-life, product damage
Fresh Veg. (general)	Ambient; short-medium shelf-life	Low	Product damage, difficulties in predicting demand accurately
Potatoes	Ambient; short shelf-life, seasonality	Low	Handling, shelf-life, inaccurate forecasting
Margarine	Chilled; medium shelf-life	Low	Temperature control, shelf-life, promotions, stock rotation
Cooked poultry	Chilled; short shelf-life; promotional, low volume	Low	Promotions planning, temperature control, shelf-life, demand variability
Cooked poultry (organic)	Chilled; short shelf-life, high value	V Low	Promotions planning, temperature control, shelf-life,

Products	Product Characteristics	Waste Levels	Main Causes of Waste
Milk	Chilled; short shelf life; low demand variability	V Low	Temperature abuse
Milk (own brand)	Chilled; short shelf life; low demand variability	V Low	Poor stock rotation, wrong date coding, planning errors, promo cannibalization
Beverages	Ambient; long shelf-life	V Low	Poor inventory management, promotions
Past Sauce	Long life; fragile packaging	V. Low	Handling
Ice Cream	Frozen; long life, variable demand	V Low	Temperature control, handling
Frozen vegetable	Frozen; long shelf-life	V Low	Temperature control, handling
Fresh Veg. (general)	Ambient; short-medium shelf-life	Low	Product damage, difficulties in predicting demand accurately

Red meat was the only product to be classified as high waste which was attributed to a variety of factors. These included the product's short shelf-life, weather and product damage. The products categorised in the high waste range were all susceptible to product damage, forecasting errors and promotions. However, only one product was classified as long shelf-life (oils). The high waste levels of this product can be attributed to packaging waste and quality rejection; the case study also highlighted poor inventory management and storage conditions as a cause for waste. The products with medium to very low levels of waste are mixed between both ends of the shelf-life spectrum. These include canned beverages and chilled milk. Wastage levels tend to be low for the chilled goods due to the high volume of sales and throughput. Wastage levels for frozen products tend to be lower because forecasting and seasonal variations are much easier to predict. Table 4.17 shows that there are common waste causes for the majority of products, such as, forecasting, promotions and shelf life.

Figure 4.17 shows this data represented as a matrix of waste levels vs. shelf-life. There is clear pattern with shelf-life and wastage, that low level wastage items are usually long shelf-life items.

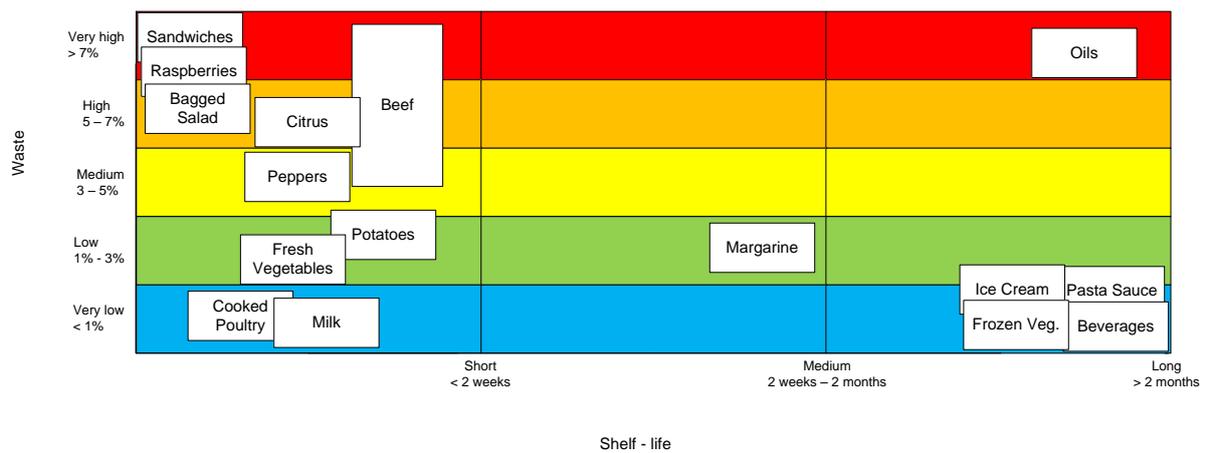


Figure 4.17 A waste Vs shelf-life matrix (Source: Mena et al., 2009)

Again, it is also important to note that not all goods with short shelf-lives have high levels of wastage. Milk, potatoes and cooked poultry have low to very low levels of waste. This is attributed to the high, predictable and stable demand for these products. These products are also not substantially affected by seasonality, weather and promotions. This is a key finding since it shows that these causes are not independent.

Shelf-life is the main cause of waste production, which is not surprising as short shelf-life limits the available time for the product to be sold to consumers. There are few management practices that can be put into effect to limit the impact of shelf-life on waste as the life is inherent within the product itself. Life extending technologies and correct cold chain maintenance were the identified as the key for shelf-life extension from this research.

Figure 4.17 also shows the range of waste levels found for the two beef cases. The cause of this range is due to the different supply chains from the two cases. The company with the case that reported low waste the company was found to have high levels of vertical integration in the supply chain and that the meat was butchered in store. In the case with very high reported waste, the supplier reported that larger retailers were better at managing waste, where as smaller, convenience style stores had very high levels of waste. This is evidence of the impact that management decisions have on wastage levels.

4.5.2.1 Root causes of waste

The previous analysis highlighted three key points about the causes of waste. They were that many causes are shared across products, that causes are independent of each other and that some causes are out of the control of management (shelf-life and weather). These issues presented further questions and so analysis was carried out to understand and identify the root causes of waste. The tool used for this technique was a current reality tree (CRT). This tool maps the logic between an effect and its cause creating a tree where the top shows the symptoms and the bottom shows the root causes. This is shown in Figure 4.18 and shows at the top the creation of waste by suppliers and retailers and the root causes at the bottom. The root-cause map is segmented into three, colour coded groups:

- a) **Mega Trends:** These are industry wide trends that affect the problems of waste. Examples are “increasing demand for fresh products” (71) and “products out of season” (83). These are important social-economical factors affecting the waste problem but company strategy can have little or no impact on.
- b) **Natural constraints:** These are factors that have an influence on waste but are associated with the product or process. Examples include “fresh Produce” (41) and “longer lead times for imported products” (80).
- c) **Management root causes:** These are factors affecting waste where management practice has a direct impact. These are the root causes that are identified as potential targets for waste reducing strategies. By eliminating these issues organisation will be able to reduce their amount of waste. Each of these causes is discussed following Figure 4.18.

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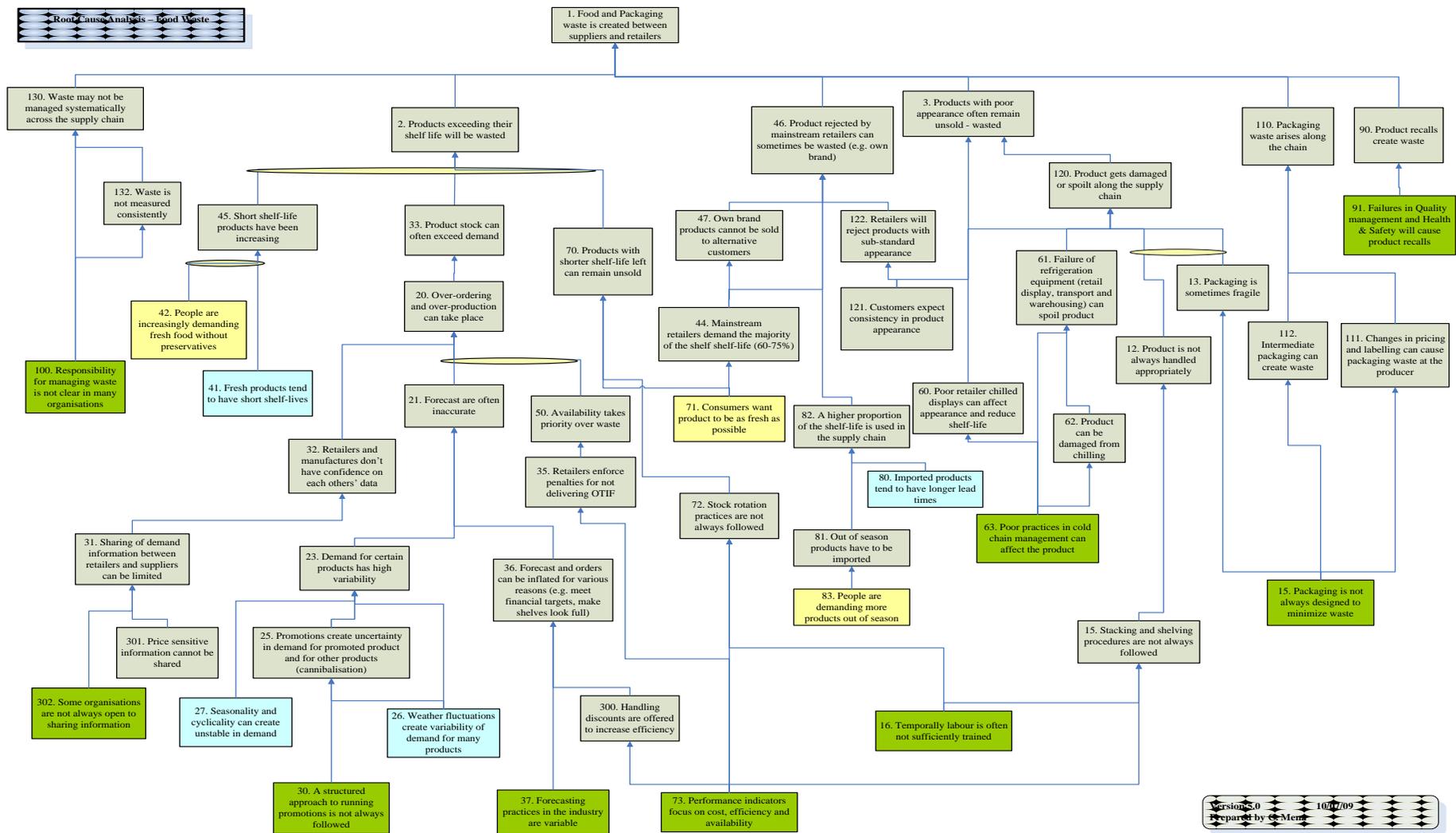


Figure 4.18 Root cause map from data collected (Source: Mena et al., 2009)

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The following discussion is centred on the findings from the CRT for the underlying causes of waste. Working from left to right the first root cause is in box 100 which concerns responsibility of waste management. Some of the companies that were studied had clear roles and responsibility for waste volumes where as others did not have a specific, waste focused role. Waste should be managed and measured to prevent future increases in waste volumes. Measuring wastage can also lead to a better understanding of process failings and highlight areas of improvement.

The next cause that is raised is information sharing and is found within box 302. As explained previously in this chapter, information is the key for excellent performance in modern supply chains (Lee et al., 2000). In supply chains that deal with short shelf-life products information is essential for efficiency. Accurate and timely information is required for good planning and forecasting. If this flow of information is constricted then variations between forecast and orders can increase and in turn lead to waste. This error can carry down a supply chain and amplify in what is known as the “bullwhip effect” (Lee et al., 1997). This can leave suppliers or producers at the end of supply chains with wildly exaggerated orders and cause waste throughout the chain. Some companies that were questioned already understood the value of information sharing within supply chains, although, others did not. In some instances retailers would charge suppliers for point of sale data, while others would give it for free. The withholding of information also can undermine confidence in this information once shared or purchased by suppliers.

Following from this point, the next box contained the point of forecasting as a cause of waste. This was one of the most common raised issues from the interview process as a source of waste production. Estimating the demand for a product can be extremely complicated and can be compounded by a product’s shelf-life and demand. The methods used are often found to be regularly inaccurate due to uncontrollable factors such as the weather but also because of seasonality, marketing campaigns, product launches, promotions and seasonal events such as Christmas and Easter. The error in forecasting can never be eliminated due to the uncertainty involved, however many of the companies interview showed more scientific approaches to forecasting. Companies using more informal measures or no forecasting at all had products with high waste. An example of this was the case study of sandwiches, where

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the short shelf-life item was used as a footfall product but no regard was given to estimating demand and therefore high waste was a result.

Waste is not currently considered a key performance metric and all of the companies who took part in this scheme used measures such as cost, efficiency and stock availability to determine success. Box 73 has highlighted this as a potential root cause of waste within supply chains. This report argues that waste has an effect on all of these measures but is currently sacrificed for the benefit for these other performance indicators. This report found that retailers often have a policy of rejecting products with less than 70% of shelf-life remaining. If this product is complex in nature (ready meals or cooked sliced meat) and has already been packaged and produced for an own brand product, then the channels of getting the product to market are dramatically reduced to discount retailers or waste.

The research also revealed that poorly trained labour (box 16) can lead to improper stacking and stock rotation procedures leading to product damage. This issue was deemed to be more prevalent over the Christmas period due to high demand leading to increased temporary labour being required.

The next root cause is shown in box 63 and relates to cold chain management. Cold chains are utilised to prevent spoilage of certain products requiring temperatures below ambient temperatures. Cold chain abuse which can be caused by equipment failure or improper storage of goods increases volumes of waste. From this investigation it was found that situations involving total disruption to the cold chain, such as equipment failure, were rare but catastrophic in terms of waste when they did occur.

The penultimate root cause of waste is the issue of poorly designed packaging or the over use of packaging materials. As discussed previously in this thesis, packaging has to be used in order to protect and preserve the contents so as to prevent waste. This packaging has to go to waste at some point either in the supply chain or at the end point with the consumer. This project found that excessive packaging should be avoided and that specific labelling and packaging that is used in promotions can lead to large volumes of avoidable waste. Packaging is often purchased in large quantities so a retailer acting on the sudden removal of

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a promotion or change in product type can leave suppliers with large quantities of redundant packaging and labels.

The final root cause to be discussed is Quality management from box 91. Issues with product quality can lead to rejections and product recalls. Rejection of fruit and vegetables is a very common problem and is variable and seasonal. The loss of quality of a product is often deemed more important to the companies than the waste created by the disposal of the product. Product recalls are relatively rare events and compare with cold chain equipment failure. When they do occur there are normally large quantities of waste that arise. Interesting to note is that the shorter the shelf-life, the more waste that is created due to the necessity to have sufficient product within the product supply pipeline.

4.5.3 Good practices

This research has shown that there is a wide range in abilities within the industry for companies managing waste. This section is used to highlight some good practices that arose from the interviews of certain companies used to reduce waste.

The data from the interviews suggest that creating and maintaining clear roles within an organisation to manage waste generates a culture of accountability and reduces waste volumes. These roles need to be set throughout the corporate structure (corporate, facility and process) to have maximum effect with clear waste reduction targets and incentives. This would also mean using waste as a key performance measure so that processes are viewed from a waste perspective. This facilitates the observation of high waste areas and eases implementation of investment, training and process improvement.

The research also supported evidence that procedures such as Collaborative Planning Forecasting and Replenishment (CPFR), Vendor Managed or Co-Managed Inventory (VMI/CMI), the sharing of sales data and the use of “implants” at the customer’s premises can have a positive impact on reducing forecasting error and consequently on waste volumes. There are many potential benefits to these systems and there was evidence to show waste levels were lower in products that were from information integrated supply chains. The cost of implementation and the time required for setup means that many smaller companies cannot afford to invest in such systems and practices.

As discussed previously, forecasting techniques within the industry ranges from complex computer systems and software to having a relatively casual approach. The companies with lower wastage levels used very sophisticated statistical methods, usually based on factors, such as weather predictions and point of sale data. Good practice in this arena would be the use of dedicated information technologies with the associated statistical techniques to help reduce forecasting error and waste. This again comes at a cost to the implementer but this would show returns on investment by reducing waste and improving product availability.

In agreement with the literature, the data from the interviews showed that promotions cause large variations in demand on products and lead to waste. Interestingly this was intensified for products with irregular demand and short shelf-life. Promotions can be used effectively in the fresh fruit and vegetables produce to remove gluts of over production as is seen in the summer months with soft berries. Most companies used very clear maps and processes for managing promotions, however, problems arose when these plans were not followed or ignored. Good practice in promotion management requires effective planning, implementation and analysis of performance through the lifecycle of the promotion as well as an understanding of the impact of promotions on waste, availability, cannibalisation of other products and waste.

Improvements of process efficiency and effectiveness would lead to reduced lead times and increase the available lifetime of the product for retailers. Measures such as direct delivery to retailers would not only improve on this but also help in the reduction of carried inventory by suppliers. Retailers can also implement improved training for their staff to reduce wastage from poor stock rotating and information management. The majority of retailers suggested that issues such as poor product handling of delicate or short shelf-life products have been improved by these methods. Cold chain maintenance is also imperative for waste reduction and from the interviews there was evidence that companies that maintain and replacement of refrigeration equipment were better at preventing this sort of waste. Some companies already make use of temperature monitoring equipment to check the integrity of their cold chain.

Good practise involving packaging require the careful equilibrium of using packaging to protect and preserve contents by extending the shelf-life of the produce balanced with

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volumes of packaging used. The best organisations with low levels of primary packaging waste focused on this point of trade off. The research also suggested that the implementation of novel packaging technologies, such as modified atmospheres, lead to the complete rearrangement of supply chain strategy. The reusability of secondary and tertiary packaging also lead to reduced levels of this waste. An example of this is using plastic palates for movement once in store.

The companies that have implemented the waste management hierarchy, as seen in Figure 4.16, were the best at minimising waste levels. The first approach by these companies is to lower waste levels by implementation of the measures mentioned so far. In order to avoid waste of those products with short shelf-lives which were perishing, there was a strategy to “re-use” by using alternative routes to market, such as discounters, wholesalers, street traders and even charities. Other uses of spoiled products included animal feed and bio fuel/composting for energy generation. The recycling of products and packaging, however was proven to be more difficult and implemented less amongst the suppliers questioned. This was because of the required level of effort, equipment and personnel to separate out products, packaging and spoilt food. The majority of cardboard and some plastics were recycled by most of the companies involved in the study.

4.6 Potential strategies for novel packaging technologies

This section will compliment the literature review with evidence for the use of intelligent packaging, such as TTIs and the technology designed in this research, within food supply chains. The evidence from the research for this project suggests that any intelligent packaging design should be able to integrate with the products original packaging and not cause any additional packaging waste. Where possible it should also be re-usable or recyclable. In terms of preventing or lowering product waste then the label should also be geared towards products that have short shelf-life and variable consumer demand.

The benefits of using intelligent packaging can be divided into three categories; general benefits, benefits from having real time information on products and benefits from the extension of the shelf – life of the products they are attached to. These are represented in Table 4.18.

Table 4.18 benefits of using TTI and other such technology in supply chains (Adapted from: Sahin et al., 2007)

Category	Benefits
General Benefits	Conformity with norms and regulations
Benefits of having real time information on products	Improvements in the reputation and competitiveness of companies Simplification of product freshness control procedures Improvement in product rotation Reduction in cost of pertaining product quality inspections Reduction in the cost related to product recall procedures Raise Awareness of operators concerning time and temperature conditions Less litigations between actors of the cold chain Prevention of frauds in use by date labels Reduction in the cost associated with discarded products Decision making in case of equipment failure
Benefits from extension of product's shelf-life.	Efficiency in the production process Better organisation of replenishment and transportation process Reduction in shrinkage Reduction of store stock outs Extension of the potential geographic coverage of companies
Benefits from having information on a product's remaining shelf-life.	Better control and trust between supply chain actors Better inventory management based on remaining shelf-life Pricing based on product remaining shelf-life Improvements in quality for the end consumer

To determine a quantifiable benefit from using this technology, studies have been completed into the potential reduction of stock inspection that could be fully automated by using an RFID component integrated into intelligent label (Sahin et al., 2007). Figure 4.20 shows this as a step by step calculation and this example estimates a cost reduction of 4500 Euros per year.

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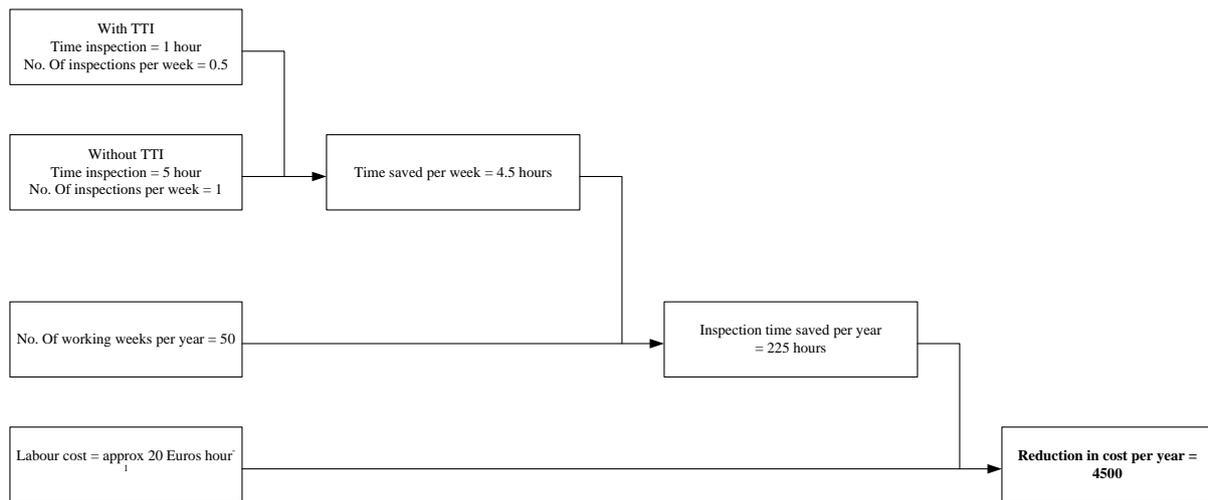


Figure 4.19 Simplified evaluation of the annual reduction of inspection costs using TTI technology (Source: Sahin et al., 2007)

Using the model of economic production quality (EPQ), developed to be a basic inventory model (Silver and Peterson, 1985), to consider one item in a pseudo single – stage system then we can see another beneficial effect of this technology. To simplify the model we define demand as a constant and known value and production rate of the product is also at a constant rate. At the beginning of a production period of length T , a fixed quantity of is produced, Q , and depleted at a constant rate, D . A fixed ordering cost is incurred for each order of size Q . These cost are to cover production equipment setup cost, production release cost etc. A proportional inventory holding cost is incurred for each unit of product in inventory during a unit of time. The model is used to determine the EPQ of Q^* i.e. the optimal production time period $T^* = Q^*/D$ which minimises the total average cost that is the sum of the holding cost and can be seen in Figure 4.20.

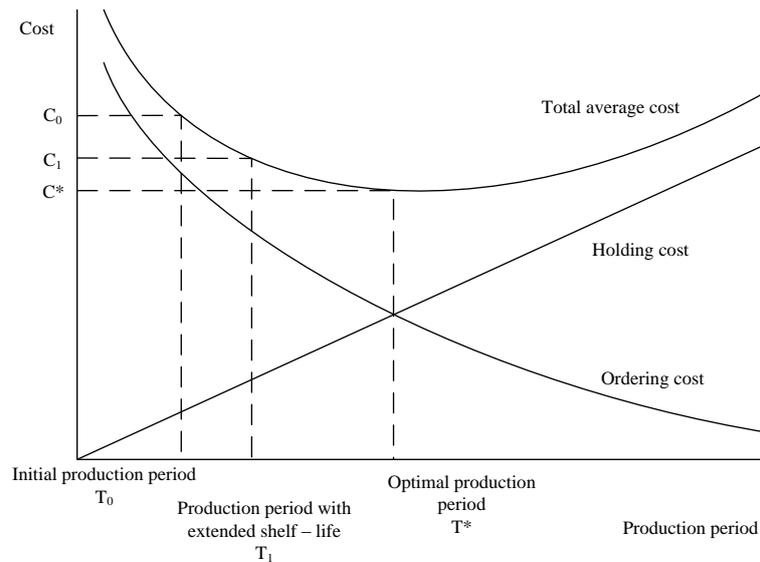


Figure 4.20 Average costs incurred with and without using TTIs (Source: Sahin et al., 2007)

If we now consider a product with a shelf-life shorter than time T^* , the optimum total average cost cannot be used and will increase. Without information of shelf-life and to be on the safer side, the firm is expected to reduce the time to T_0 . With the information available from intelligent packaging and the potential lengthening of shelf-life, T_0 could be increase to T_1 and therefore decrease overall total average cost and move closer to the optimum production time (Sahin et al., 2007).

The benefits that have been highlighted from this report mainly centre on wastage and include the potential for better stock management, decreased shrinkage and better management of equipment failures and product recalls.

4.7 Conclusions

The research carried out for this project showed that waste levels generated between suppliers and retailers ranges between 0.1% and 10% and is dependent on the variation of a products sales volume and a product shelf-life. This is considerably lower than those levels of waste reported for food consumers, which accounted to a third of all purchases (WRAP, 2007).

The majority of products with higher levels of waste are fresh meats, fruits and vegetables. Those products that have a short shelf-life but lower levels of waste, such as milk, can be explained by their relatively stable demand patterns. Therefore it is reasonable to conclude

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that these factors are independent, and that the combination of both a short shelf-life with variable consumer demand leads to product with high waste.

The root cause analysis found that there were three groups of issues affecting waste; these were mega trends, natural constraints and management root causes. The identification of these root causes along with discussions around industry good practices was able to produce a series of recommendations to help organisations to improve the way they manage waste as can be seen in Table 4.19.

Table 4.19 the recommendations for best practice from the project (Source: Mena et al., 2009)

Recommendation	Description
Ensure there is accountability for waste	Clear accountability is a prerequisite for managing waste. Organizations that have a person responsible for waste management tend to have a much better understanding of the scale and causes of the waste problem. This understanding is a first step for reducing waste.
Promote a culture of waste reduction	The case studies revealed that some organisations promoted a culture of waste reduction and this culture was driving all other activities in the organisation, such as training, performance measurement and incentives.
Embark in collaborative activities:	Poor information sharing and lack of trust among supply chain partners can lead to waste. The case studies showed that some retailers are open to sharing information with their suppliers and in some cases they can even have employees from the supplier (implant) working on site, so that they can be in close communications. This kind of practices have proved to be effective in reducing forecasting error and hence waste, however, they can also expensive since they demand considerable resources from both suppliers and retailers.
Analyse promotions more closely and consider the impact on waste	Poor promotional practices can create waste when sales do not achieve the expected demand, particularly in the case of products with short shelf-life. Understanding the impact of different promotion

Recommendation	Description
	<p>mechanics and working together using collaborative approaches can help to minimise the negative impact of promotions. In some cases, promotions can even help to reduce waste by helping to move product that otherwise would not reach the consumer.</p>
<p>Be more analytical about forecasting</p>	<p>Although forecasts will never be perfectly accurate it is possible to reduce forecast error by using statistical techniques supported by information systems. From the case studies it appears that some retailers have an analytic approach to forecasting while others rely on more informal approaches. Given the impact that accurate forecasting can have on availability and waste, investing in forecasting methods appears to be a fruitful strategy.</p>
<p>Manage process efficiently and effectively</p>	<p>The way processes are managed can affect waste at all stages in the chain, including the home. This involves efforts to reduce lead-times to increase product home life and discipline in to ensure products are not damaged along the chain and that stock rotation is managed appropriately. Furthermore it can also include efforts to extend shelf-life through improvements in technology and understanding of the biochemical and physical changes that occur to a product through the supply chain and how these can be mitigated against or at least minimised/delayed.</p>
<p>Maintain the cold chain</p>	<p>Interruptions to the cold chain can be caused by a failure in refrigeration equipment at any stage of the chain or by poor process management. Investments in both equipment maintenance and process management to reduce cold chain abuse can be paid back through reductions in waste, although this needs to be quantified.</p>
<p>Consider the natural characteristics of the product</p>	<p>Some products, particularly fresh fruits and vegetables, are subject to natural variability and retailers and producers should to make allowances for variations during the season in order to reduce waste. For example, the use flexible data code management to reflect seasons and state of product would have a</p>

Recommendation	Description
Use packaging effectively	<p>direct impact on waste.</p> <p>Packaging plays a dual role in terms of waste; on the one hand it protects the product from damage and can help to extend its shelf-life, having a positive effect on waste. On the other, the amount of packaging on a product has a direct impact on household waste and to some degree on waste generated at other stages in the chain. Organisations need to look closely at packaging and decide what the right balance for each product is.</p>
Reduce, Reuse, Recycle	<p>A number of alternatives exist to divert waste to landfill. Many organisations look for alternative markets, such as discounters, wholesalers, charities, animal feed, composting and energy generation. Recycling, particularly for packaging materials, is now a common practice for many. Using these alternatives not only compensates for some of the losses of not selling the product at full price, but also reduces waste to landfill.</p>

The use of intelligent packaging technology could be used to realise some of the good practices and policy recommendations. The main strategic barriers to entry for these labels are the cost of implementation and the trust required for organisations to collaborate and share information. With the industry structured as it is, one route for entry would be to partner a small food manufacturer and supply with a larger food retailer. This is so that there is sufficient data relating to consumer behaviour and retailing trends to compliment the relative small cost of implementing the IT and electronic infrastructure required. The direction of the Grocery sector seems to be towards more integrated landscape, with competition between complete supply chains rather than small firms within them. Intelligent packaging would be an ideal way to improve process excellence and forge a stronger competitive advantage.

Chapter 5

Novel Synthesis of Polyaniline Films and Their Characteristics

5 Introduction

This chapter will detail on the development of robust and reproducible polyaniline films produced via an in situ deposition approach. The sensor that is to be developed for use as a food sensor is required to integrate with existing packaging as much as possible. As already stated in the previous chapter, a large part of food waste is packaging-and consumers are demanding that less packing be used on food products. A very brief comparison of electrochemical deposition will be given to compare the method of in situ film deposition. The method of electrochemical deposition has not been studied further since it could not be deposited easily onto existing packaging used in the fish industry and the cost per sensor would be higher.

The reproducibility and reliability of in situ films will be described along with the possible areas for improvement. A variety of techniques have been used to study these films including scanning electron microscope images, surface profiling and UV-Vis spectroscopy.

5.1 In situ deposition of polyaniline films

Deposition of polyaniline films initially took place on varying substrates including glass, acetate sheets and gold sputter coated glass slides. These initial layers were then subjected to growth via the two pot synthesis method described in the literature review (Madathil, 2005). This method was chosen as the deposited amount of polyaniline in each film layer is more regulated and the chain reaction of polyaniline can be controlled by keeping both reagents separate. The films were analysed by their colour, morphology, their initial level of resistance and their ability to adhere to the substrate.

This study aimed to produce a commercially viable sensor that could be incorporated into packaging of certain food products which would indicate food spoilage either by a change in colour, resistance or both.

The first study was to improve the reproducibility of manufacturing thin films of polyaniline within reasonable tolerances. Sheets of polyaniline coated Melinex were cut into 2 cm² sensors which were then gold sputter coated at either end of sensor. These were then

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connected to a multi-meter via gold crocodile clips to measure the resistance. The initial reaction of polyaniline with an oxidant created a batch of sensors that had a wide range of resistance. This can be seen below in Figure 5.1.

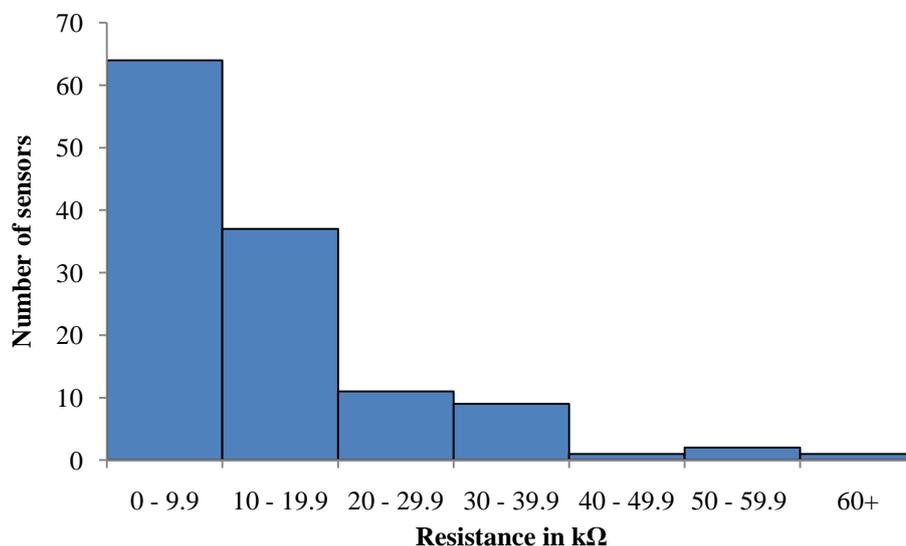


Figure 5.1 The distribution of resistance from initial coating of polyaniline on acetate

The majority of sensors produced were below 30 k ohms in resistance; however, a large spread of data was found. Using the two pot synthesis technique the overall resistance was decreased with every cycle of synthesis. The number of cycles of this synthesis that were studied were 1, 5 10, 15, 20 and 50. It was observed that the distribution of resistance in sensors produced after 5 cycles was dramatically decreased. These sensors were reliable and robust enough for use in experiments with degrading food stuffs. The effect on repeated cycles of dipping can be seen below in Figure 5.2.

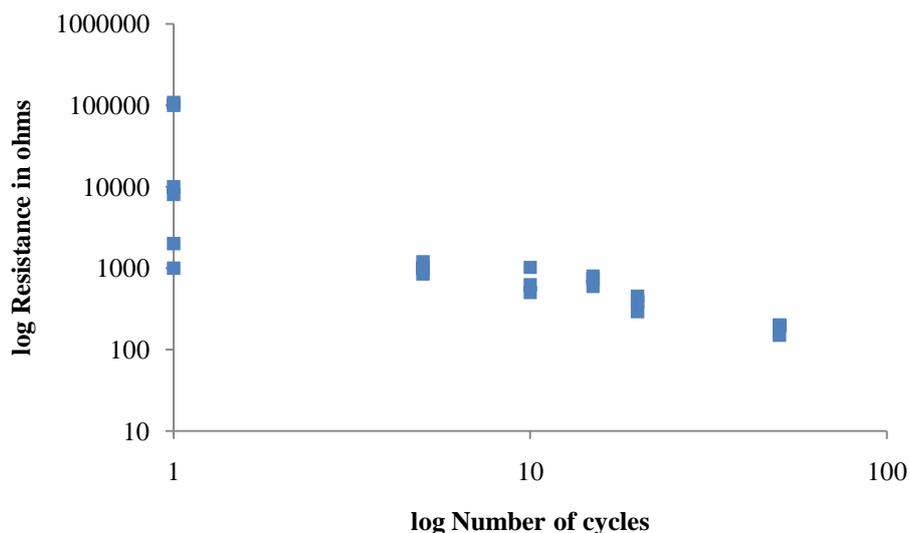


Figure 5.2 The effect of repeated dipping on deposited layers of polyaniline with upper and lower range

The decrease in the range of resistance can be seen in the figure above along with a decrease in average resistance by a magnitude of 10 after only 5 dips. After a further 50 dips, the films become visibly thicker and much more reproducible, however, the amount of reproducibility is negligible.

The original choice of substrate was glass following from the studies of in situ films in the literature. Other assumptions were that adhesion would be reasonable on this surface and that the sensor would be easier to be used in further experiments. This was incorrect however and after a 5 cycles, the deposited layers of polyaniline started to lose adhesion and were removed. This observation is shown below in Figure 5.3. This is important to note as it shows that this method of film manufacture could benefit from optimisation experiments. On rougher organic surfaces, such as those from a Melinex or poly acetate substrate, the surface adhesion is much improved and the loss of coating was not observed.



Figure 5.3 Loss of adhesion of the polyaniline multilayer when a glass substrate is used (left) compared to a plastic substrate (right)

This is most likely to be explained due to the poor original adhesion of the original layer of polyaniline formed from the in situ reaction whilst the polymerisation reaction took place. Additional layers of polyaniline add to the overall weight of the layer which is removed after excess layering.

Poly acetate Melinex films were used instead for the remainder of this study. The film was chosen as it could best replicate the films used in most packaging films used in the fish industry. For the experiments carried out on the scanning field emission gun (S-FEG) electron microscope, a gold sputter coated surface was created on a glass slide to provide a conducting substrate.

5.1.1 Imaging techniques

This section reviews the experiments completed to study the surface morphologies and investigate coating thickness using several imaging techniques. These will be described in turn showing the resulting data from performing the two pot synthesis technique.

5.1.1.1 SEM and elemental analysis

An environmental scanning electron microscope (Phillips XL30) was used in conjunction with elemental analysis (Inca point and ID) to characterise in situ deposited polyaniline thin films on polyacetate. An SEM of the original layer formed after the substrate is submersed in the polymerisation reaction-can be seen below in Figure 5.4

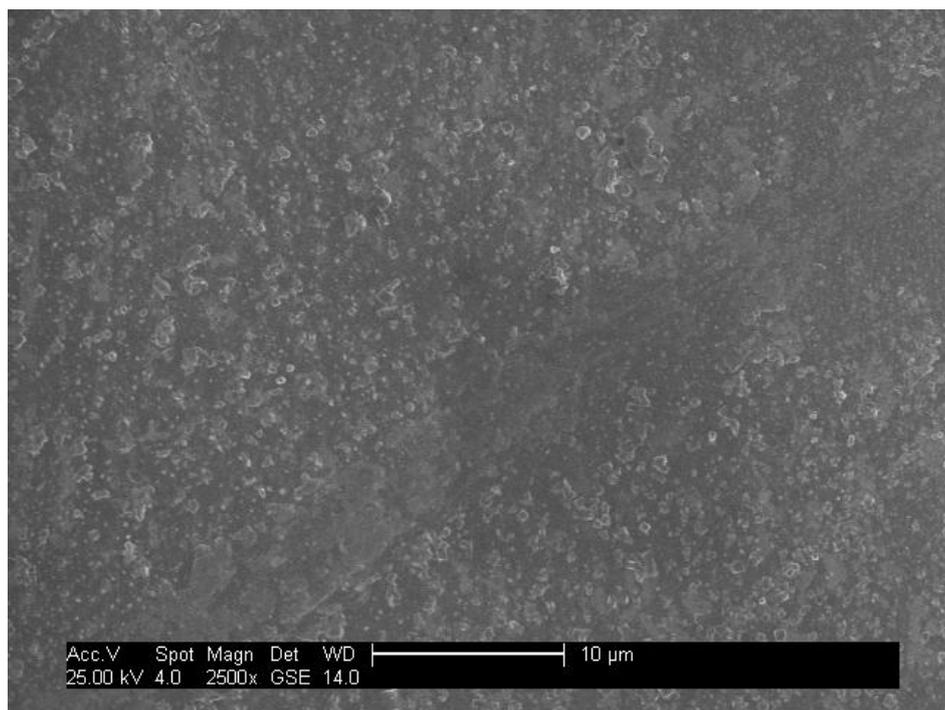


Figure 5.4 In situ deposited layer of polyaniline on a polyacetate substrate

The image shows a mixture of deposited polyaniline and debris left from the polymerisation reaction. The debris is most likely to be residual crystals of oxidant that have not dissolved and large oligomer particles that have become imbedded in the layer as shown in Figure 2.25. Closer inspection of the debris can be seen in Figure 5.5.

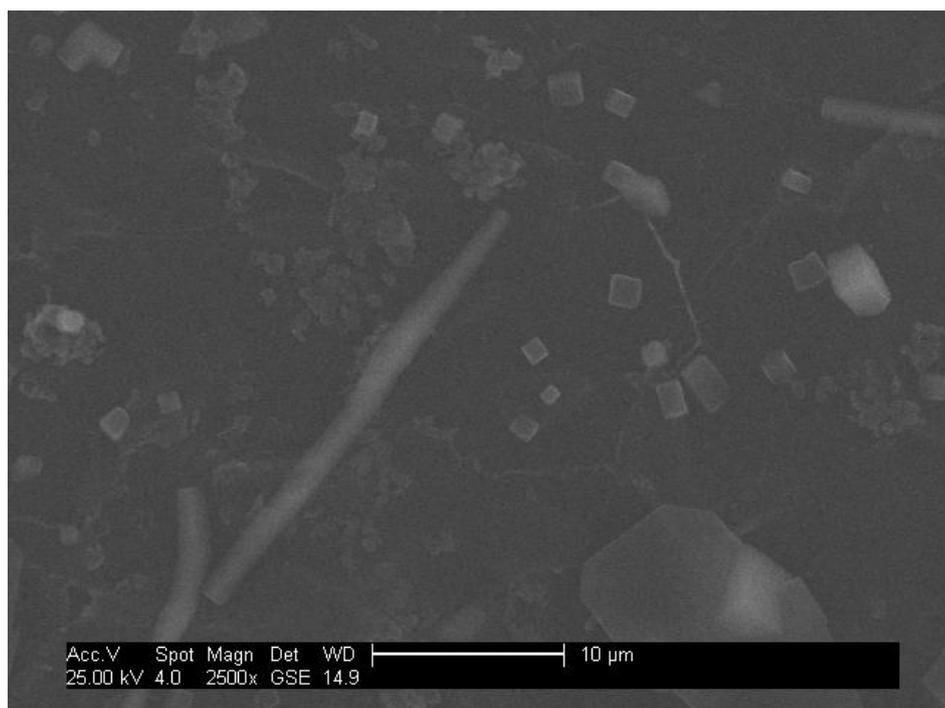


Figure 5.5 Polyaniline after 1 cycle and unwashed showing remaining crystals of oxidant and other debris

Elemental analysis was carried out on the larger crystals to determine their composition. In this case (in Figure 5.6) potassium persulphate was used as the oxidant; the elemental composition is shown in Table 5.1.

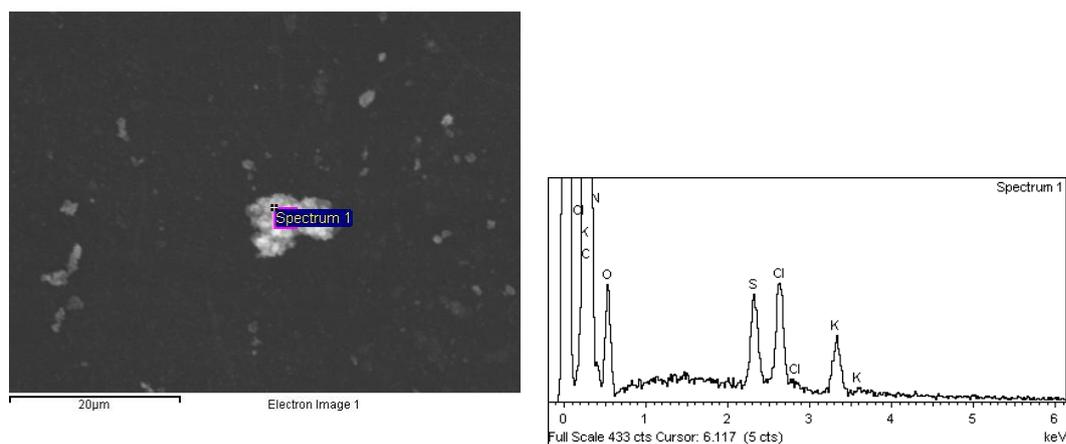


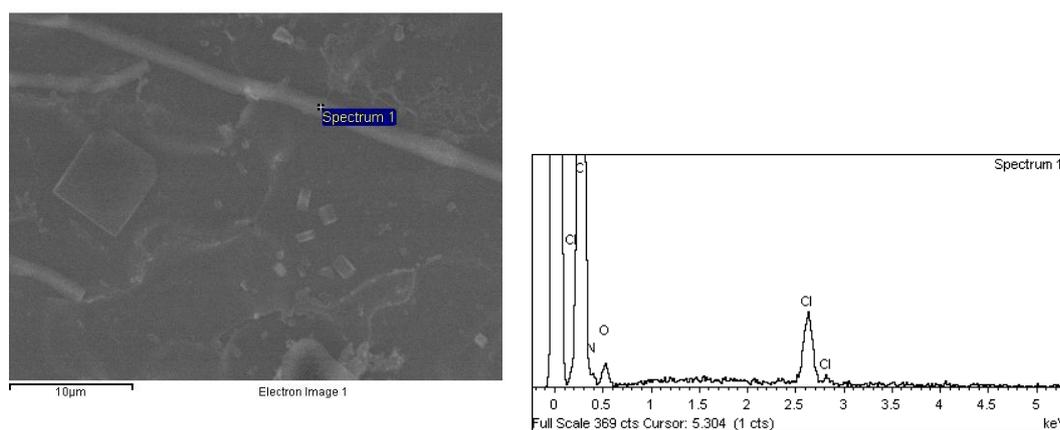
Figure 5.6 Elemental analysis of surface debris on polyaniline layer

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Table 5.1 Data from elemental analysis of surface debris of polyaniline layer

Element	Weight%	Atomic%
C	72.65	77.65
N	13.97	12.80
O	10.68	8.57
S	0.84	0.33
Cl	1.19	0.43
K	0.68	0.22
Totals	100.00	

The results show that the debris in this case is a crystal of potassium oxidant that has not dissolved due to its low solubility in water. Further analysis of the surface was conducted on the bulk of the surfaces produced. The results of elemental analysis for these surfaces can be seen in Table 5.2 and Figure 5.7.

**Figure 5.7 Elemental analysis of polyaniline surface layers****Table 5.2 Data from elemental analysis of polyaniline surface without debris**

Element	Weight%	Atomic%
C	89.04	92.74
N	0.00	0.00
O	7.90	6.17
Cl	3.07	1.08
Totals	100.00	

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The results show that the majority of the surface comprises the constituent elements of polyaniline. Due to the thin surface and the substrate, the high percentage for carbon is expected as the substrate material comprises carbon and some oxygen.

The morphology of growth of the polyaniline layers on this plastic film substrate concurred with the findings in the literature. A coral like structure is grown from the original in situ film deposition after each cycle of dipping. The SEM microscope was used to study the morphologies of films up of up to 50 cycles of the two pot process. The results can be seen below in Figure 5.8. The deposition of each film increases the thickness visibly. Using the electron microscope the morphologies of these films is comparable to those seen in various papers from the literature (Stejskal et al., 1999; Stejskal and Gilbert, 2002).

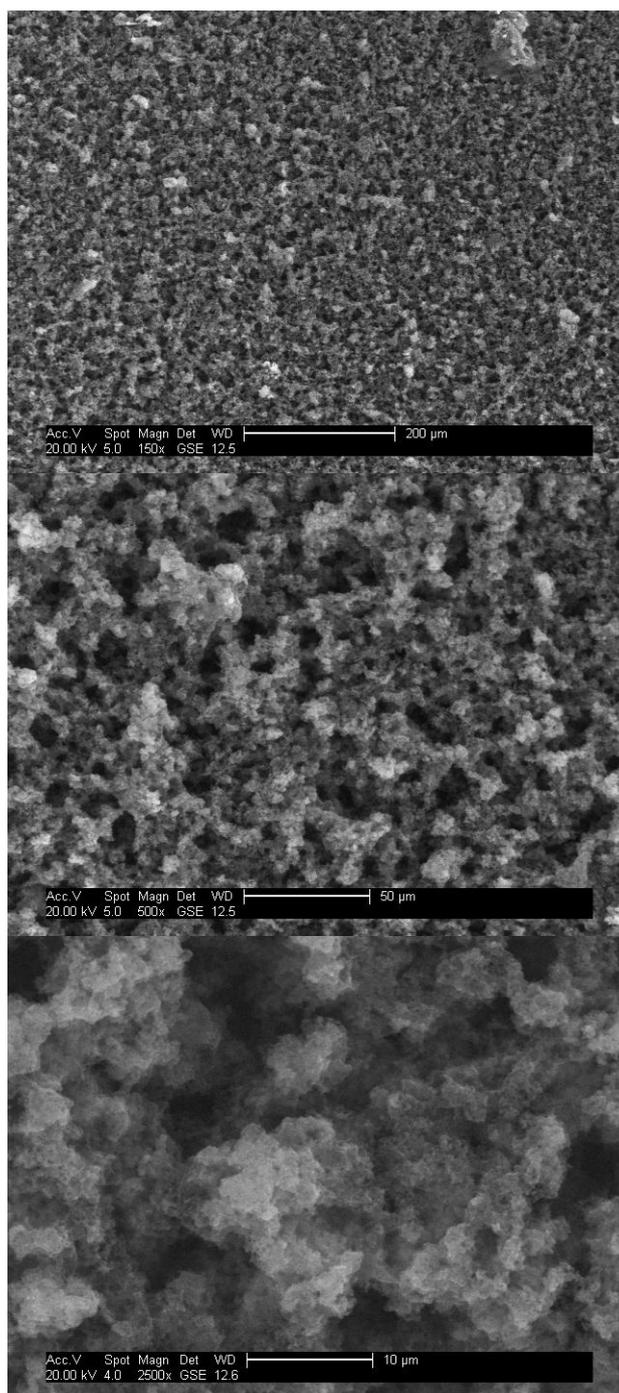


Figure 5.8 a, b+c SEM image of the morphology of a polyaniline surface on Melinex after 50 cycles at varying functions of magnification (a = 150 time magnification, b = 500 times magnification and c = 2500 time magnification)

After noticing that the glass slide produced poor adhesion, a flake of polyaniline that had been deposited was used to view the thickness of the film. The flake was positioned onto the positioning block used in the SEM chamber and the flake was attached using silver paint. This can be seen in Figure 5.9 below.

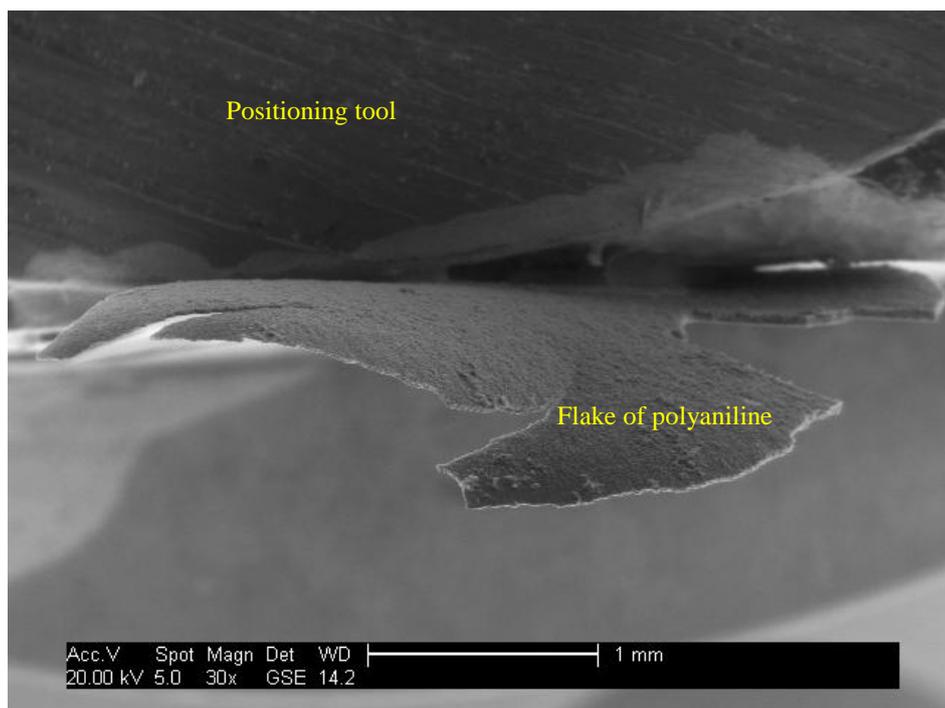


Figure 5.9 SEM image of a flake of polyaniline layer deposited on glass after 50 cycles.

After this initial experiment it was suggested that film thickness could be measured on a conducting substrate using a scanning field emission gun electron microscope. The data from this could be used to study the build up of polyaniline over the course of the several cycles of the two pot synthetic process. Although the results would not directly correspond to film thickness on the preferred substrate of plastic polyacetate sheets, they would give images on film morphologies using this method compared to cleaner techniques such as screen printing using conducting inks and electrochemical deposition.

5.1.1.2 S-FEG study of deposition on gold sputter coated glass

Gold sputter coated slides were immersed in a vessel used for the aniline polymerisation reaction as before, then removed and finally washed. These slides were then used in the two pot synthesis method and then snapped cleanly using a diamond cutter on the back of the slide to produce a coated edge. This edge was then positioned in the electron microscope to view the deposited film. A schematic showing the production of the gold coated slide can be seen below in Figure 5.10.

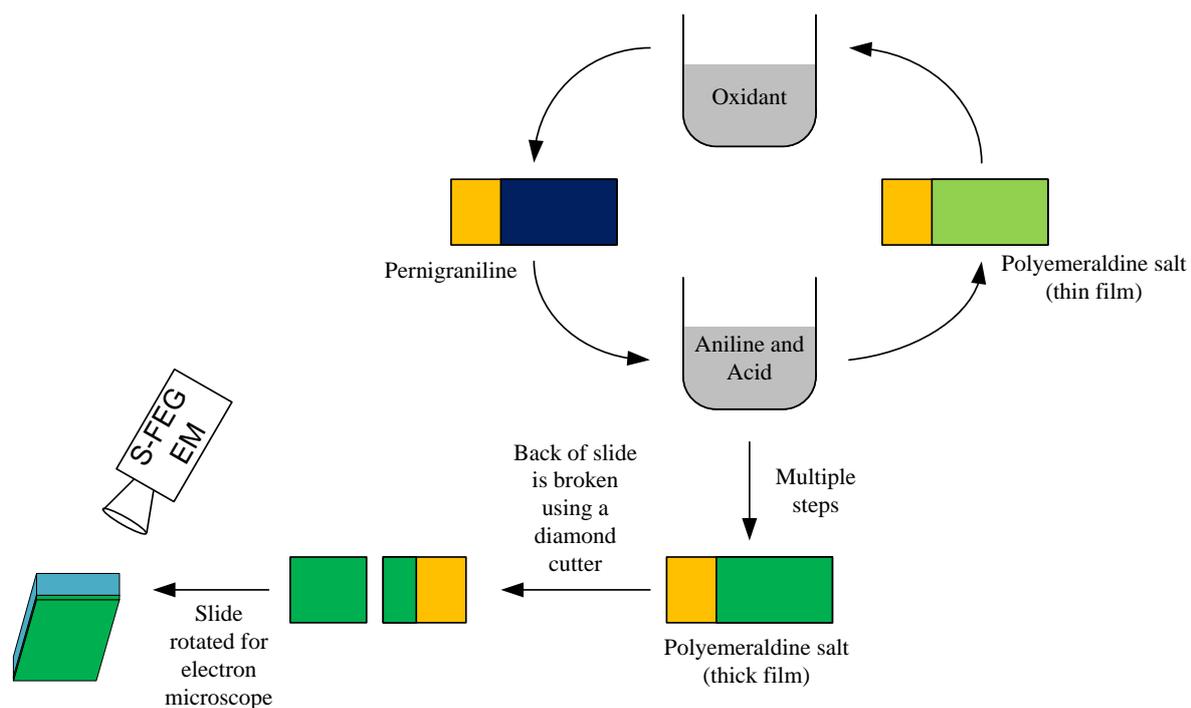
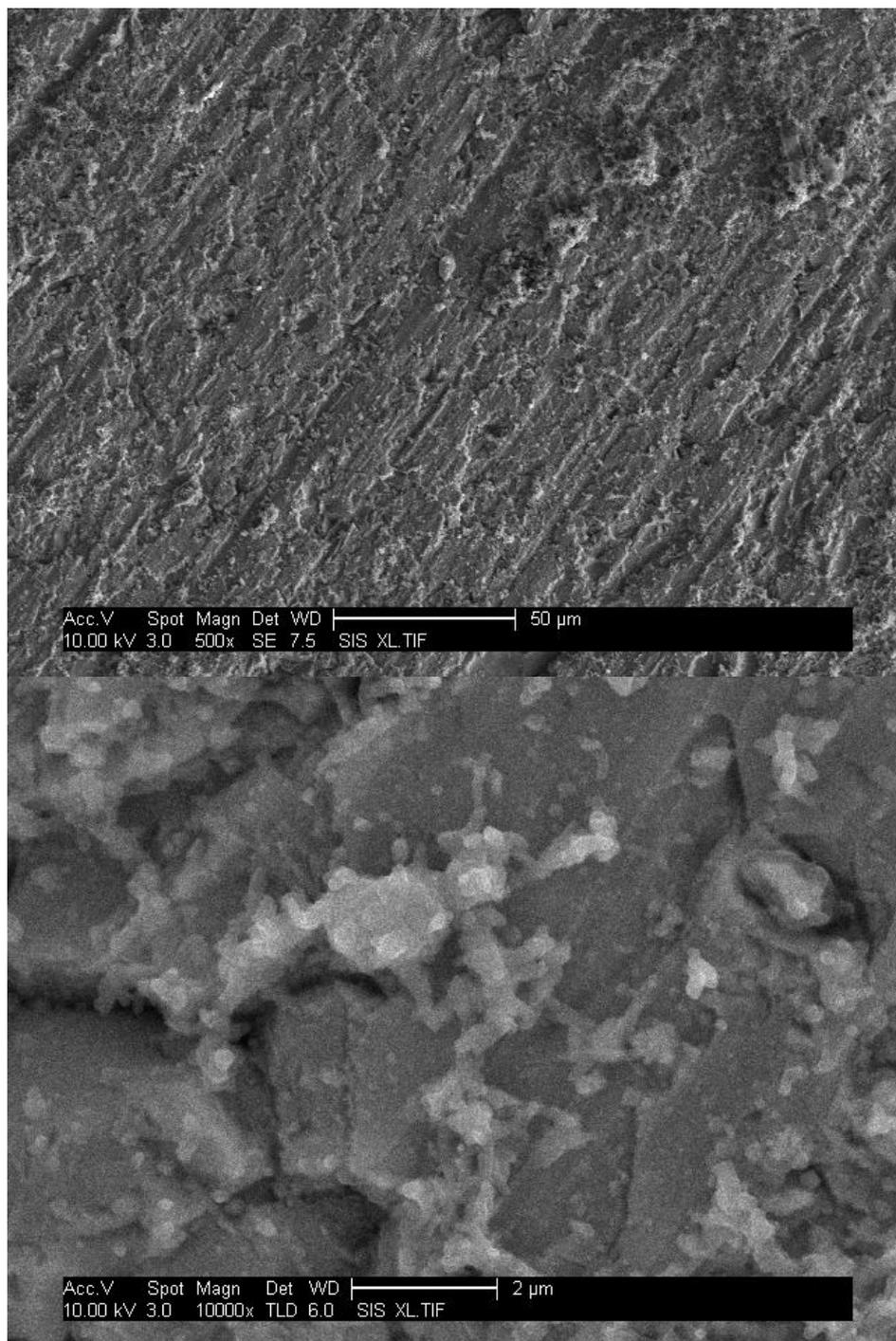


Figure 5.10 Modified method for estimation of film thickness using scanning field emission gun electron microscopy

The images produced from this experiment show detailed morphology of the films produced by the two pot synthesis of thick polyaniline films. The first sets of images are from a top down view onto the slide of an in situ layer of polyaniline without any additional layering from the two pot process. These images can be seen below in Figure 5.11.



The morphology of the layer is similar to that which is seen in the literature. The growth of these coral like structures of polyaniline from a solution of hydrochloric acid is expected. The surface has been randomly scattered with large blocks of the deposited polymer as can be seen in the top image. A side on view of the slide (presented in Figure 5.12) shows that the

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coverage of these oligomer particles is over the majority of the surface and that the deposited layer is extremely thin.

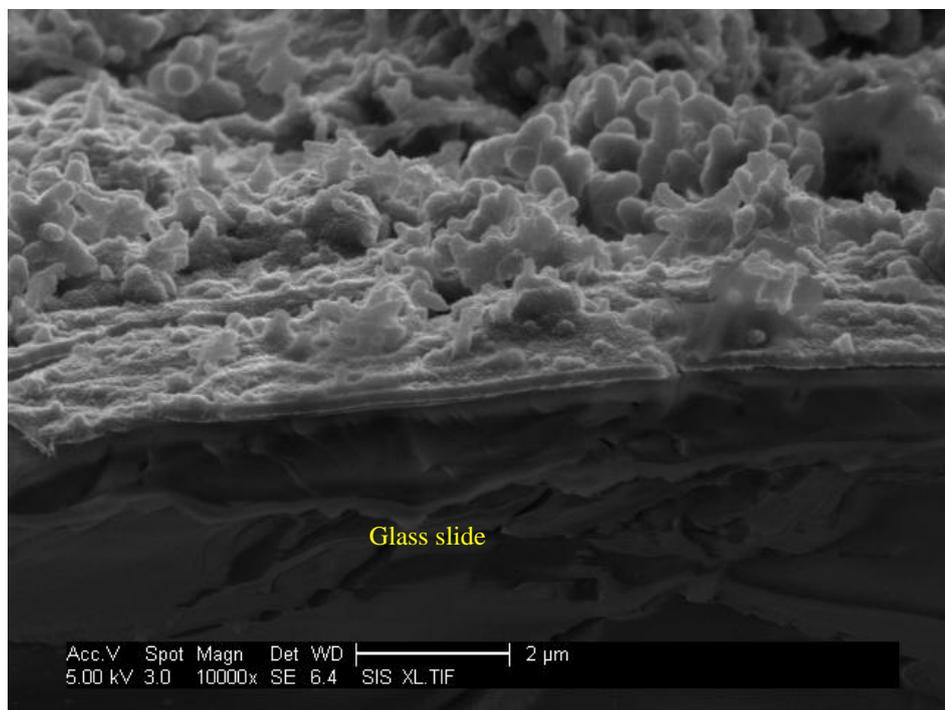
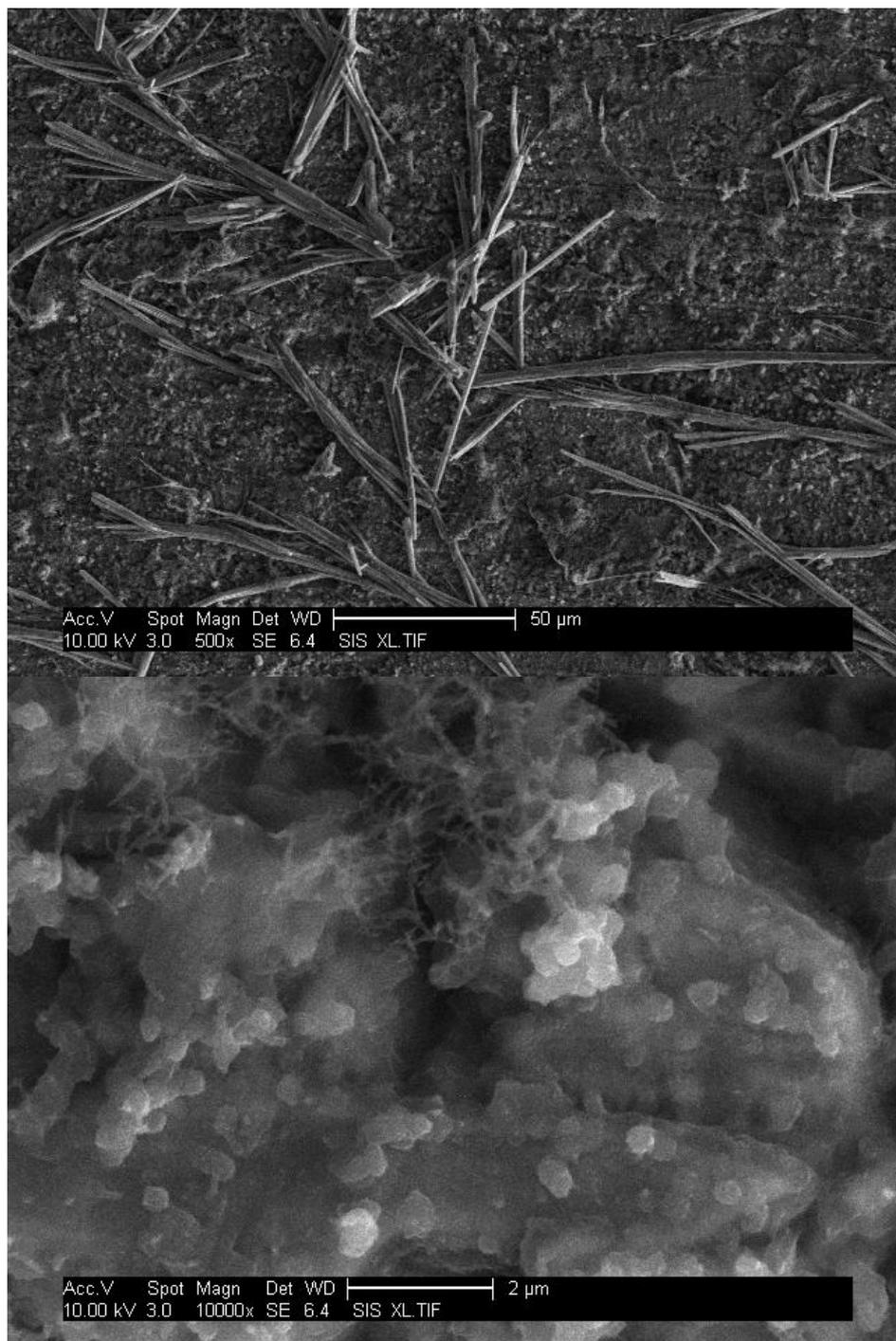


Figure 5.12 Film thickness of the polyaniline after in situ deposition

Using the microscope the best estimate of film thickness was approximately 120 nm. It should also be noted that the film thickness varies due to the build up of deposited polymer around random sites as seen in the above image.

The second set of images is taken from an in situ film after 1 cycle of the two pot layer synthesis method. These images can be seen in Figure 5.13.



The images show that there is a build up of non-coral like strands of polymer which are encapsulated by further growth of the polymer around them. A close up image of the films shows that the overall morphology of the deposited polymer is similar to previous images (bottom picture). The strand-like particles are expected to be polymer chains that have

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formed in solution and have become deposited onto the surface. The intertwining of these stands with the existing coral like structure can be seen below in Figure 5.14.

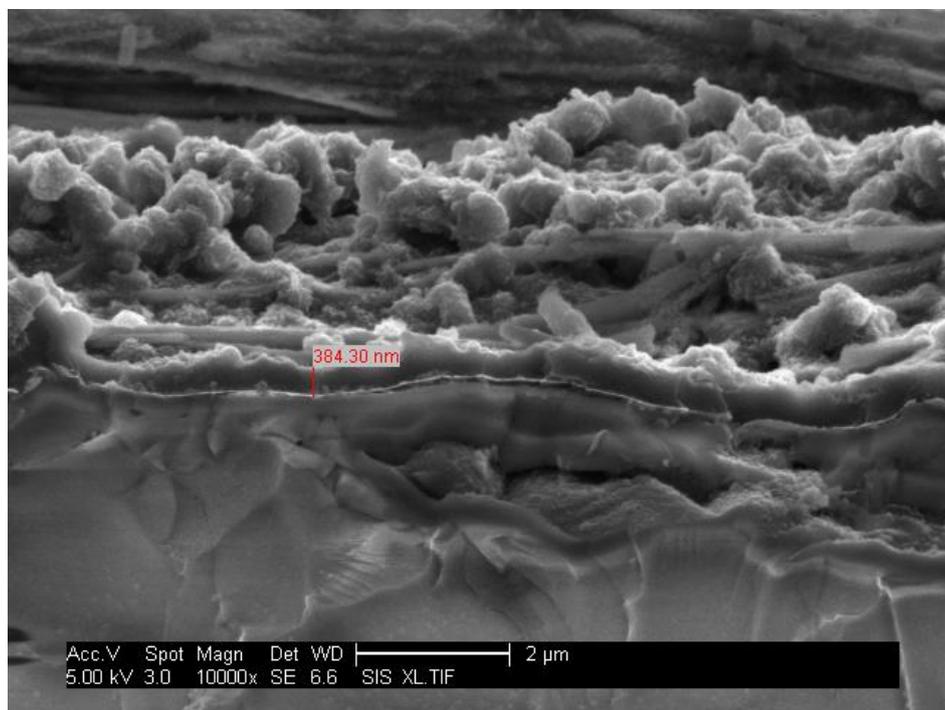


Figure 5.14 Estimated film thickness from a side on view of the glass slide

The overall profile of the surface has become more consistent compared to the in situ film. The film thickness has also increased to approximately 384 nm which was measured using a series of images at this angle. The films do not visually appear any different to those formed using just the in situ method. The difference in structure morphology at this point can only be seen using an electron microscope.

After 5 cycles of the two pot synthesis on pre-deposited in situ films the difference in film thickness can be seen with the naked eye. The film still appears to be translucent on the substrate and there is no physical sign of deposition other than the green colour of the film itself. An image of the morphology of the deposited polymer layer can be seen below in Figure 5.15.

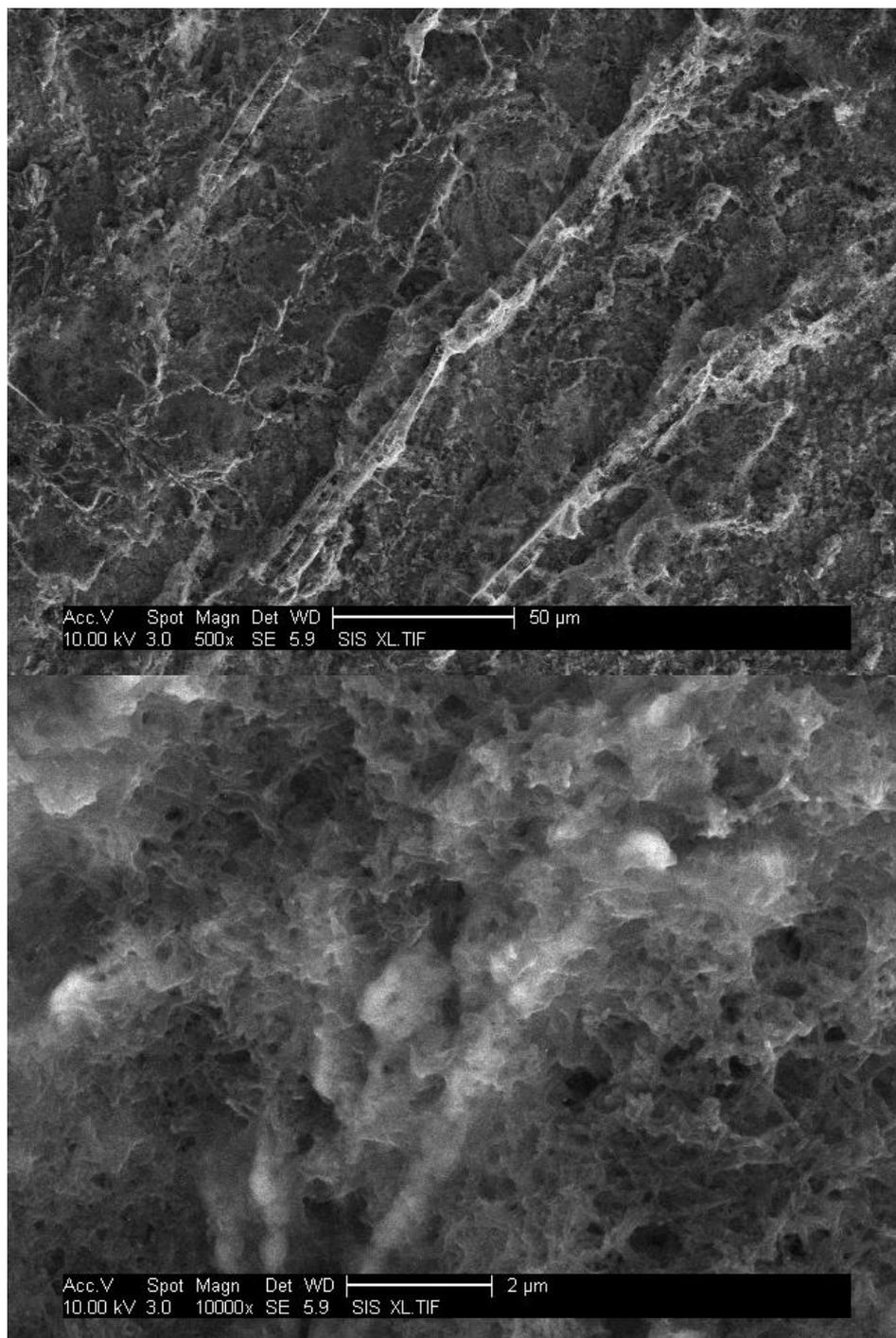


Figure 5.15a+b Polyaniline layer morphology after 5 cycles of the two pot process (a = 500 times magnification, b = 10000 times magnification)

There is no sign of the long strand like features on the surface and the entire surface appears to be more uniform in terms of the composition. The surface has more features on it and has ridges and other characters that could possibly have formed randomly in areas where originally more polymer was deposited in the in situ reaction. The morphology of the polymer after this amount of cycles appears to be much finer and more interweaved. A

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possible explanation of this could be related with the time allowed for reaction in the two pot process. The substrate is submerged for a fraction of time compared to the in situ reaction so the morphology is expected to be smaller and more concentrated. After 5 cycles the original in situ layer has been completely coated. A cross section of this coating can be seen below in Figure 5.16.

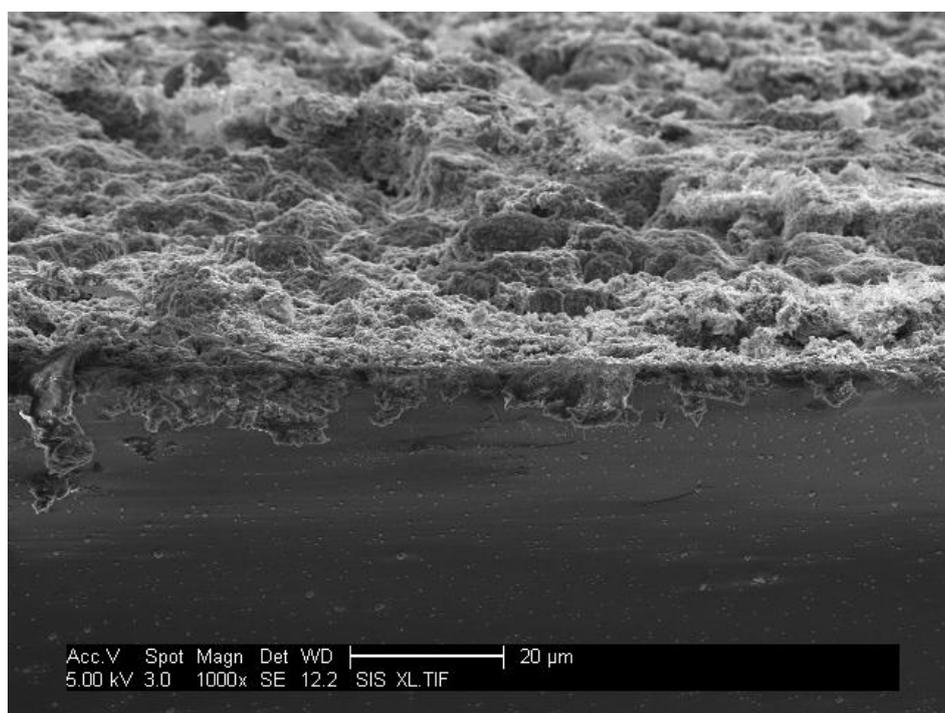
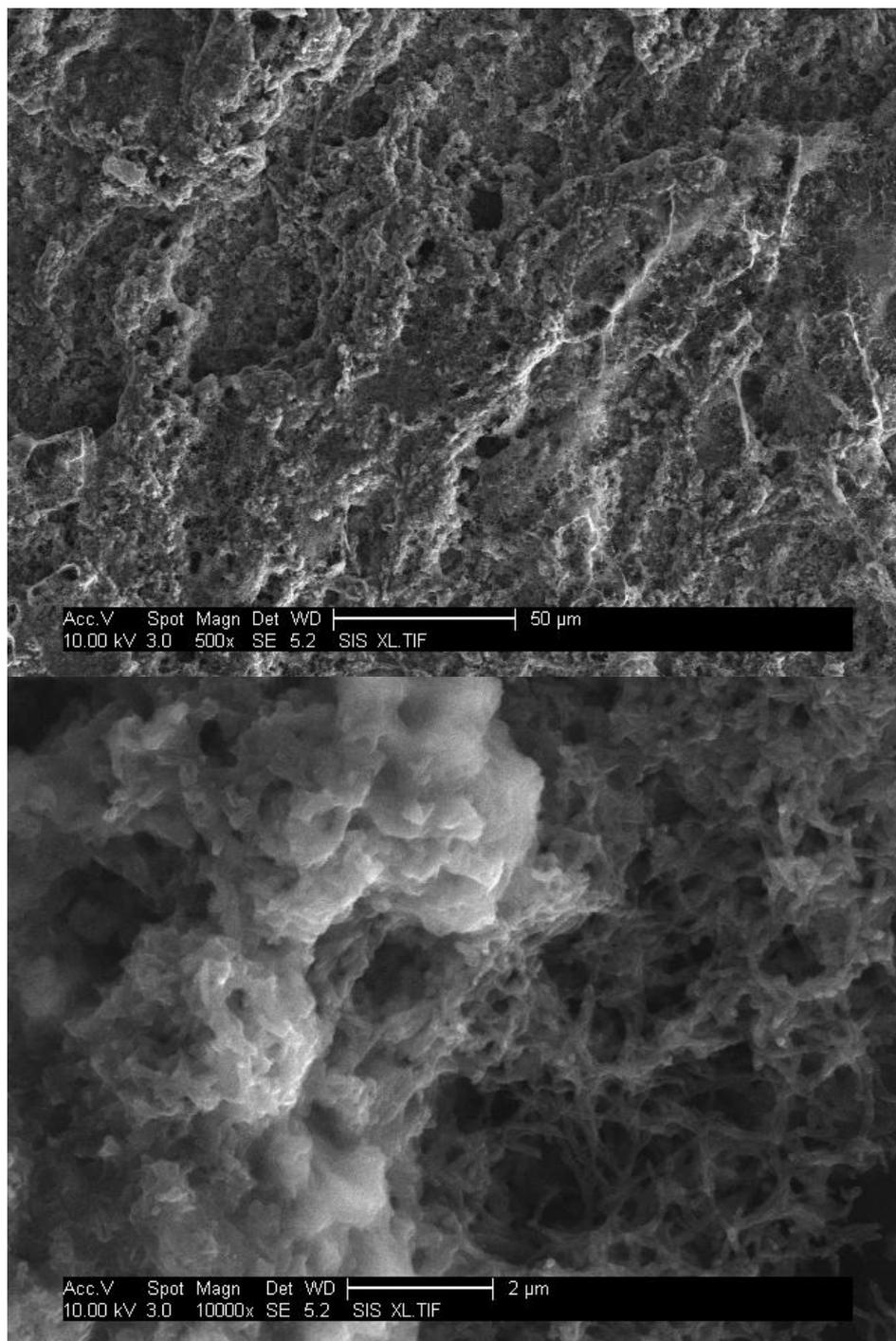


Figure 5.16 Image used to estimate film thickness from a side on view of the glass slide

The images collected along this cross section estimated the film thickness to be approximately 680 nm. Using the decreased scale it is possible to see the coverage of the surface with random features present. The overall smoothness of the surface is comparably more uniform than previous two images with fewer cycles.

After 10 cycles, the surface appears black rather than green and the surface has the consistency of a fine powder adsorbed onto the substrate rather than a thin film coating. The morphology of the polymer coatings can be seen below in Figure 5.17.



In terms of features and uniformity, the surface of this film is comparable to that observed after 5 cycles. The surface features again appear random although they are not sufficiently raised above the plane of the surface to cause considerable change in thickness. The image of Figure 5.17(b) shows the morphology of the deposited polymer which again has a fine and interwoven appearance. This time there are also thicker clumps of polyaniline depositions

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where more polymer has been deposited. These appear to exist in random places and are explained by the nature of this polymerisation technique compared to more controllable methods. Figure 5.18 shows the cross section of the cut in the glass slide.

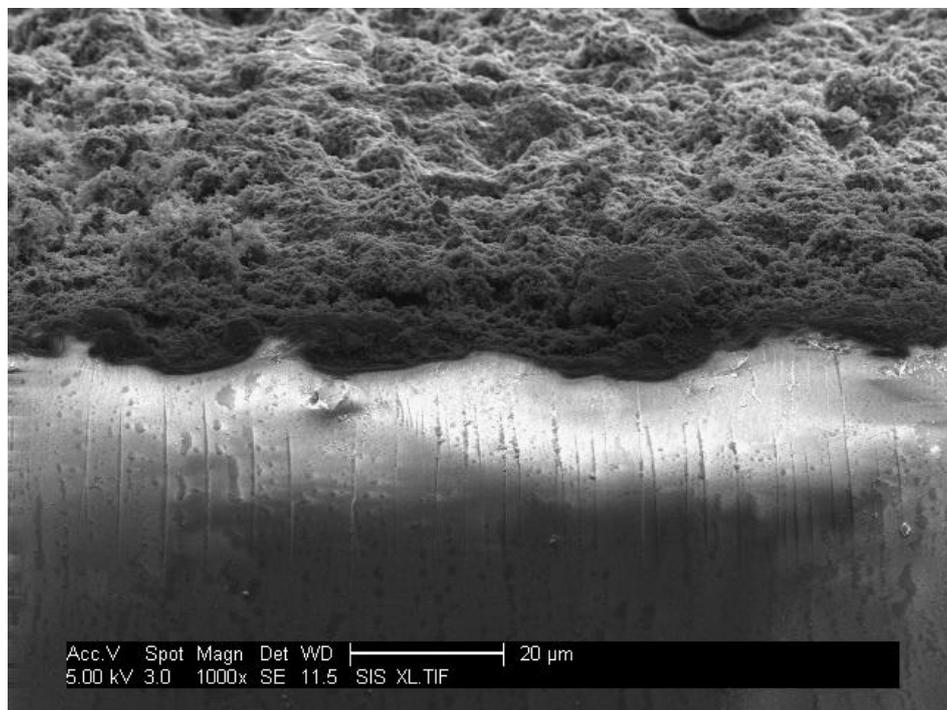


Figure 5.18 Image used to estimate film thickness from a side on view of the glass slide

The images collected along this cross section estimated the film thickness to be approximately 1560 nm. The topography of the surface of this image shows the film consistency to be comparable to that of the 5 cycles.

After 15 cycles using this substrate there is no visible trace of the original layer deposited by the in situ method. The film that has grown is more powdery in appearance than previous films. Images of the film surface and the morphology can be seen in Figure 5.19.

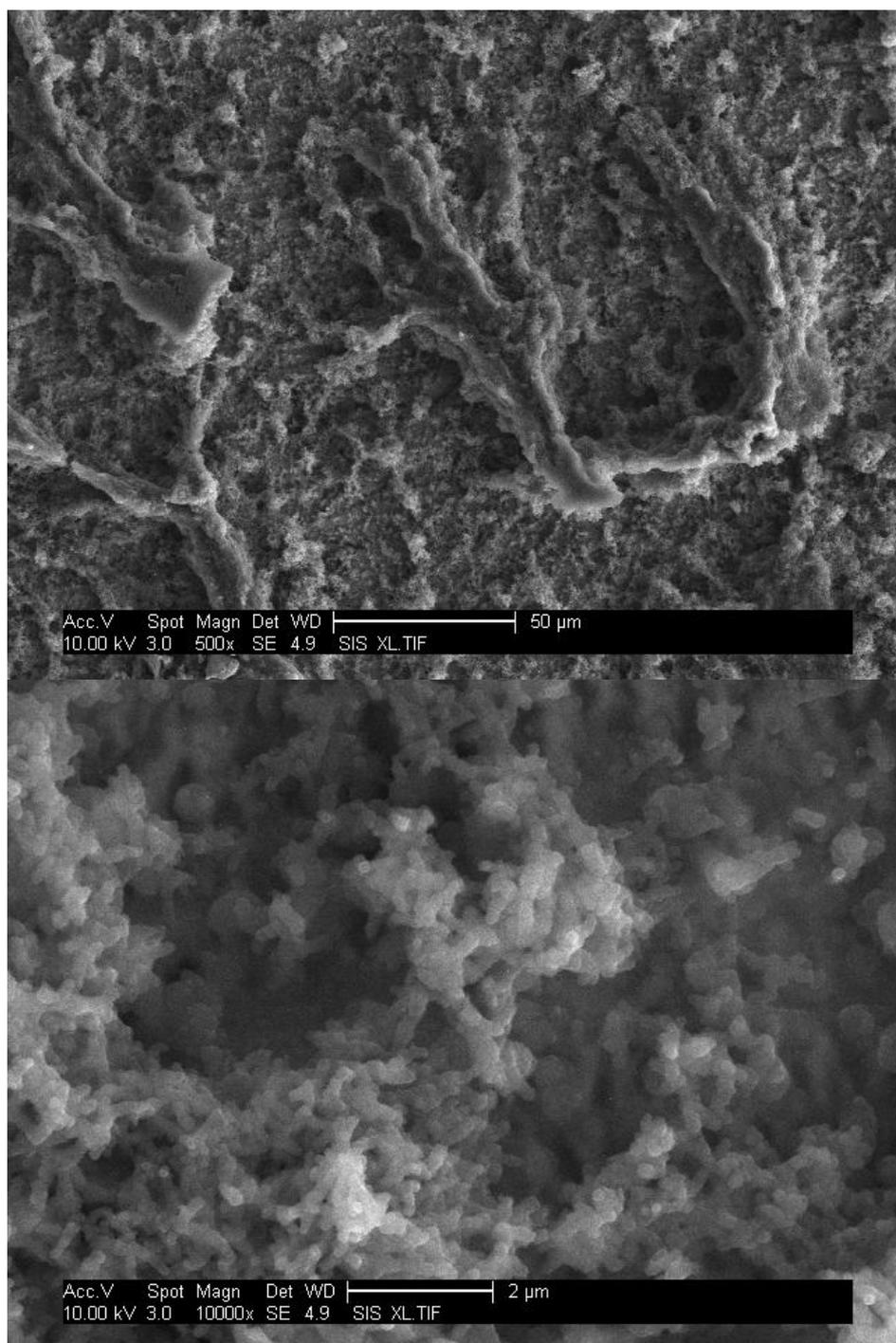


Figure 5.19a+b Polyaniline layer morphology after 15 cycles of the two pot process (a = 500 times magnification, b = 10000 times magnification)

The surface has now built up around any features that have randomly grown over each cycle and the surface appears more distinct with large clusters of deposits. The morphology of the deposited aggregates appears much thicker due to the increased amount of exposure of the surface to the two reactants. The polyaniline appears to be more linked and explains the

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observed increase in conductivity. Film thickness has been estimated from images such as Figure 5.20.

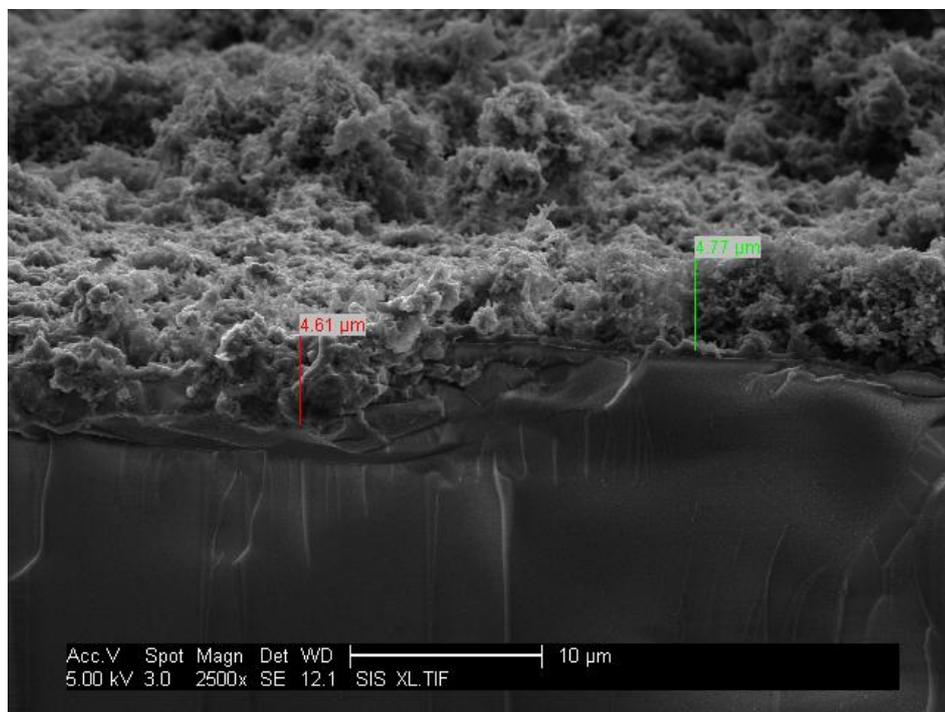
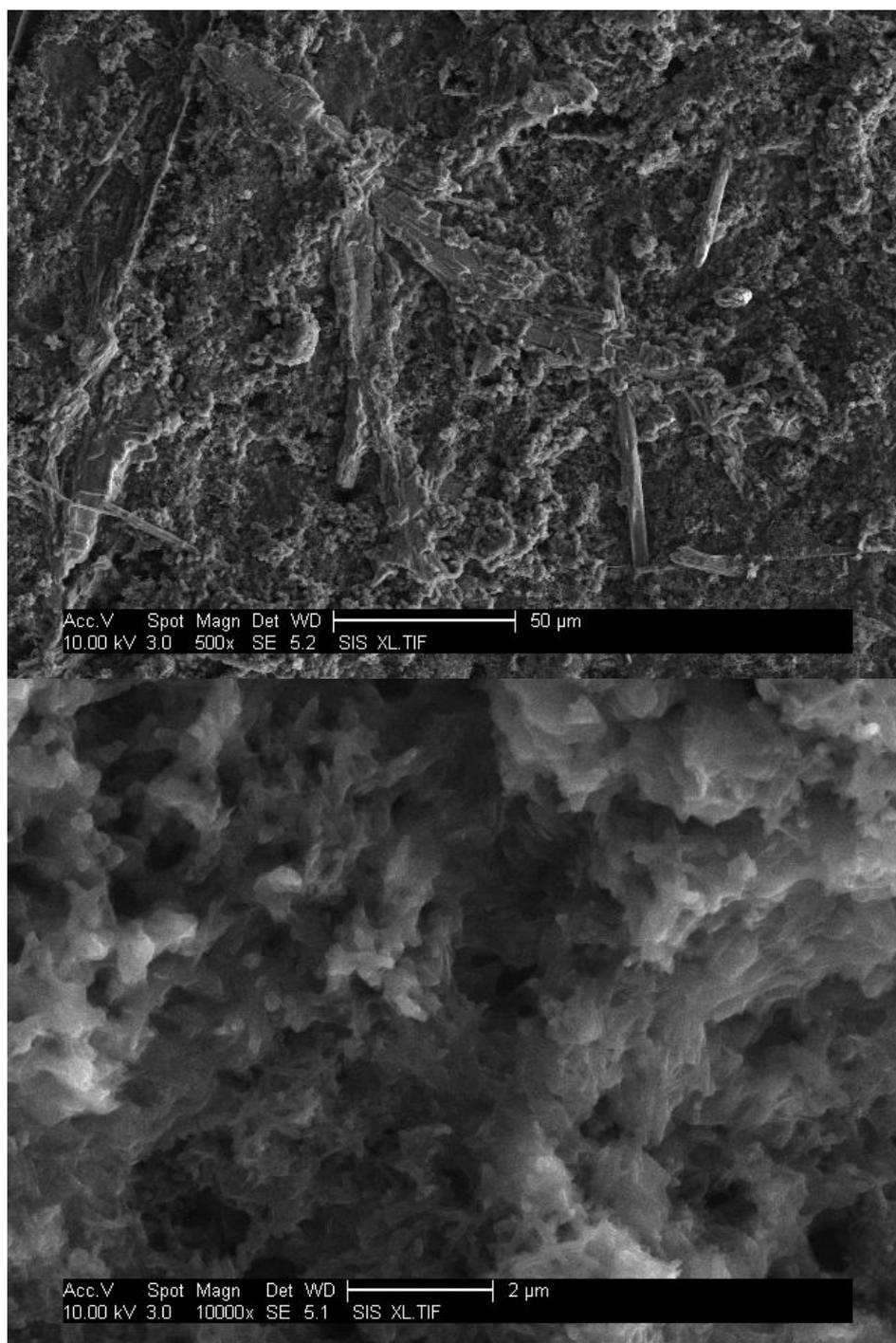


Figure 5.20 Estimated film thickness from a side on view of the glass slide

The film thickness after 15 cycles has been approximated to 4770 nm using this method. The surface topology is described as featured much like the surface seen after 10 dips. The features again include clusters of mainly the coral like particles of polyaniline with less of the longer strands visible.

After 20 dips the surface coating appeared black to the human eye and more powdery with poor adhesion of the powder like particles. Contact with any surface or and mechanical strain removed a large amount of black dust. An image of the surface can be seen in Figure 5.21.



The surface now appears to have thicker stands of polyaniline particles within the layer. These have formed whilst the cycle of dipping and washing has taken place and have been encapsulated by the growing layer of deposited polyaniline. These loosely intertwined fibres are responsible for the powdery like appearance of the surface and explain the weakness of the top layer of the surface. The morphology of the top layer of polymer is consistent with

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previous examples with the stands becoming thicker and more linkage between the structures. A cross section of the layer can be seen below in Figure 5.22.

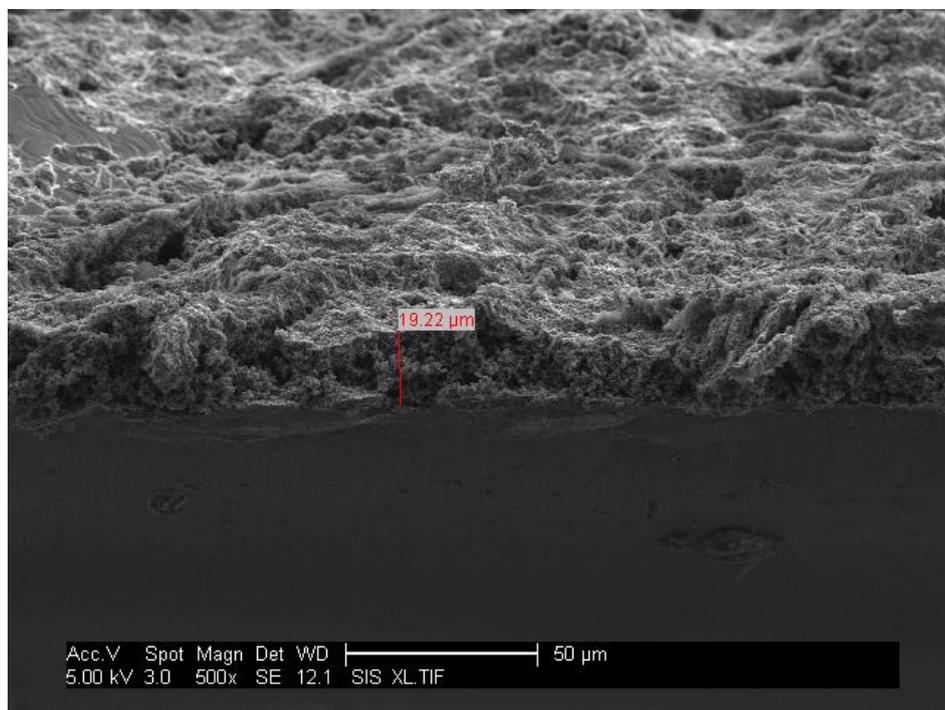


Figure 5.22 Estimated film thickness from a side on view of the glass slide

Film thickness has been estimated at 19740 nm. The surface has more features with a relatively uniform thickness. The surface is now becoming more of a thick layer coating than a thin film which fits in with the observations seen with the naked eye.

Figure 5.23 shows the layer after 50 cycles using the two pot process. The surface is now crumbly and powdery in appearance with the slightest touch removing large amounts of black powder. The addition of the gold coating has improved adhesion of the original in situ layer so that the substrate is able to support a layer of this thickness.

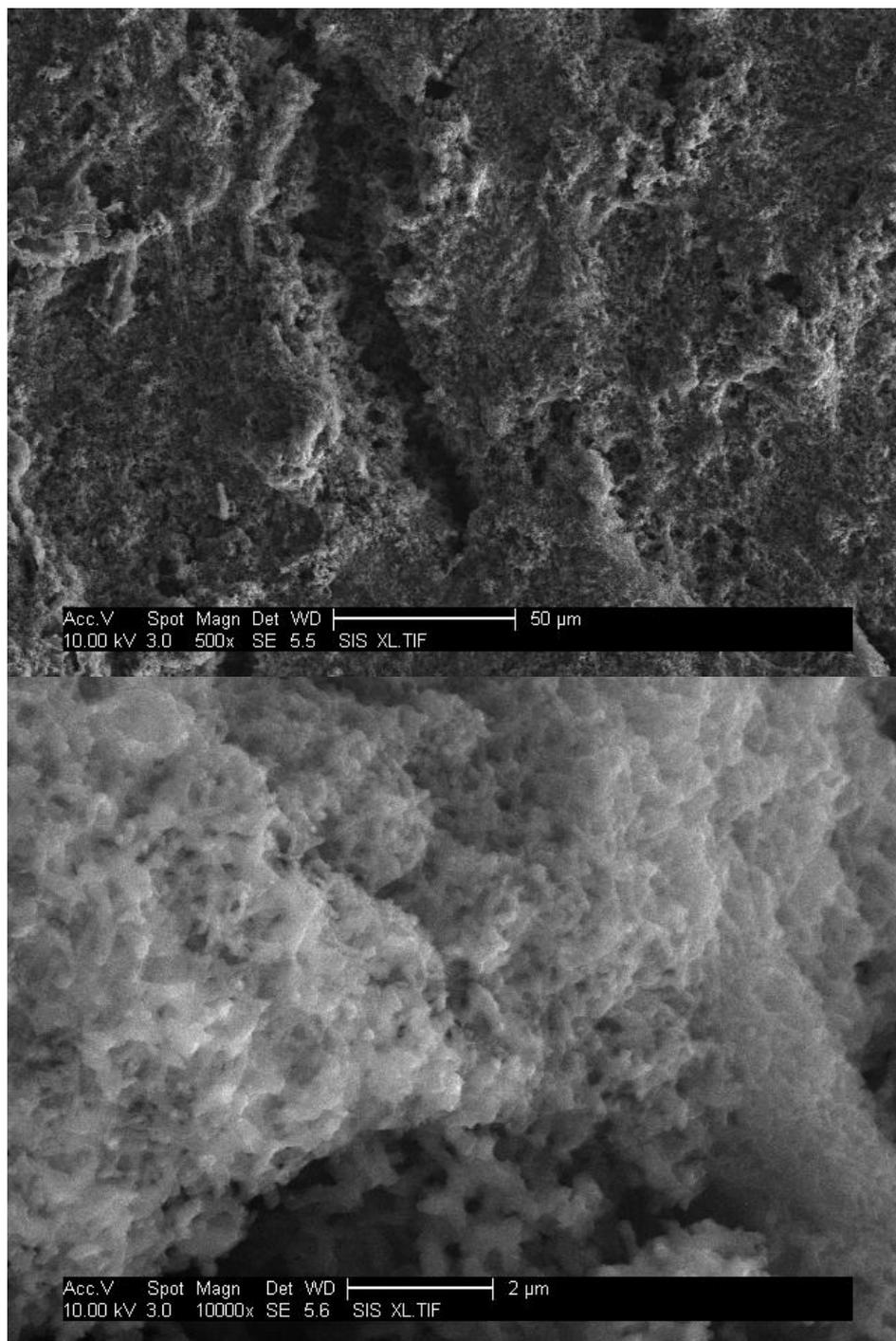


Figure 5.23a+b Polyaniline layer morphology after 50 cycles of the two pot process (a = 500 times magnification, b = 10000 times magnification)

Due to the poor consistency of the layer, there is unavoidable and visible contamination of both reagent pots after approximately 30 cycles. The polymerisation reaction is observable in both the reagents so much so that each cycle produces a visibly thicker layer. The surface of appears to be featured randomly like previous examples. The morphology of the polymer overall has changed dramatically as seen in the previous experiment using the SEM and

Synthesis and Characterisation of Polyaniline Films

Melinex films. The coral like polyaniline growths are much thicker and intertwined almost into a completely covered layer with no visible gaps. Figure 5.24 shows the cross section of the layer.

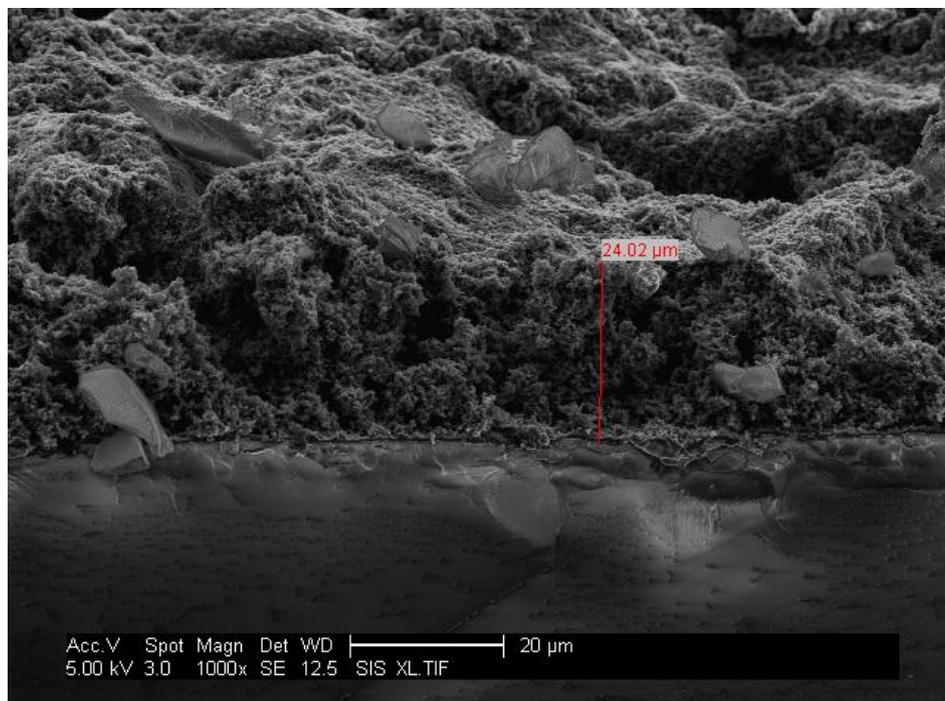


Figure 5.24 Film thickness estimated after 50 cycles of the two pot process

Film thickness after 50 cycles has been estimated at 24020 nm using this technique and substrate. The layer consists of relatively large features and debris due to aggregates that have been gathered from the reagent mixture once polymerisation has started. The surface appeared to be relatively more uniform than lower cycles.

The data of film/layer thickness from these images has been summarised below in Table 5.3 using mean values from all the images gathered.

Table 5.3 Estimated mean film thickness of polyaniline deposited onto gold coated glass substrate after a number of cycles of two pot synthesis after an original in situ film deposition.

Number of cycles	Estimated film thickness in micrometers
0	0.12
1	0.38
5	0.68
10	1.56
15	4.77
20	19.74
50	24.02

The trend for film thickness has been plotted below in Figure 5.25 for cycle reaction using this substrate.

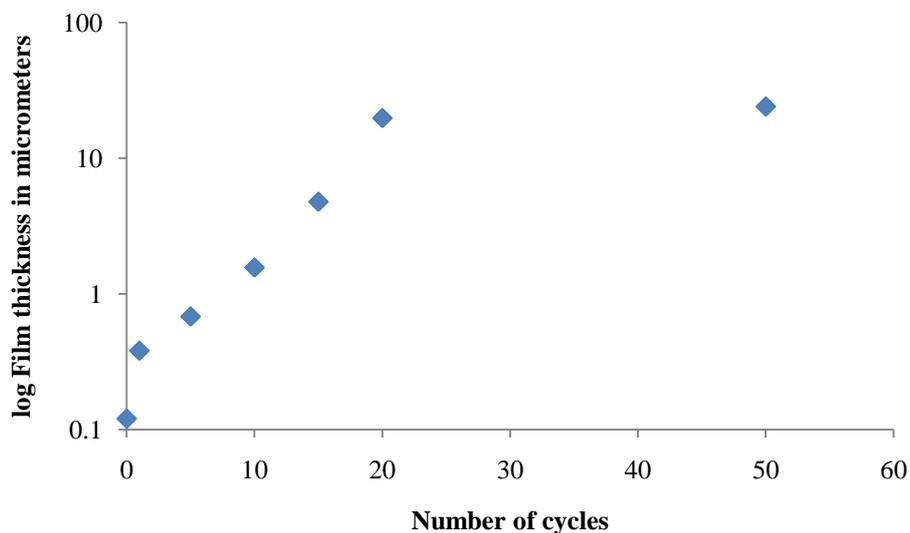


Figure 5.25 Graph showing log of film thickness versus the number of cycles through the two pot process

The relationship between the log of film thickness and number of cycles appears to be linear with an R squared value of 0.982 for values 1 to 20 cycles. The equation that describes this relationship is given below in Equation 5.1.

Equation 5.1 Relationship between film thickness and number of cycles where N = the number of cycles

$$thickness = 0.2531 \times e^{(0.206 \times N)}$$

There is a large increase from the original in situ deposited film to the first layer created from using one cycle of this method. There is also a cut of point where the number of cycles

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reaches a maximum in terms of affectively increasing the thickness of the film. Observations suggest that this is approximately 30 cycles due to the consistency of the layer becoming too powdery to adhere to the surface. Once this occurs then deposits in the reagent pots cause the polymerisation reaction to commence and each layer is deposited as a thin sludge.

For the purposes of this project, a commercial sensor would need to integrate with existing packaging to be commercially successful. Following on from this experiment a study of film thickness on a plastic film substrate was performed. The technique used previously to estimate film thickness could not be used due to the nature of the substrate so a new strategy was adopted.

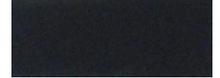
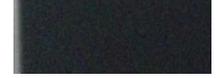
5.1.1.3 UV – Vis Spectroscopy

UV-Vis spectroscopy has been used in previous experiments to estimate film thickness of in situ on various transparent substrates including glass and plastics. Following on from the previous experiment, the thickness of films deposited on a Melinex substrate was experimental measured using UV-Vis spectroscopy.

Polyaniline is renowned for its various oxidation states and the colours that are displayed. This project has investigated polyaniline as a food spoilage sensor not only as a variable electrical resistor but also as a colour changing indicator. For the purposes of this chapter, the technique will be used to estimate film thickness.

As described in the previous experiment, the more cycles that are completed using the two pot method, the thicker the film of deposited polyaniline becomes. This has also been observed whilst using the Melinex substrate. This affect can be visibly observed and is summarised below in Table 5.4.

Table 5.4 The appearance of films deposited onto Melinex sheets after various number of cycles

Number of cycles	Description of film	Appearance of film
0	Very light green in colour No visible deposition	
1	Slightly darker in colour No visible deposition	
5	Dark green in colour No visible deposition	
10	Very dark green Slight signs of deposition	
15	Almost black in colour Powdery deposition on film	
20	Black in colour Appears to be powder	
50	Black in colour Appears to be powder	

The thicker films lose their translucent appearance and make them ineligible for this kind of analysis. Films after 5 cycles of dipping have been selected for this analysis. In terms of a food sensor, a product that can crumble or contaminate food stuffs would not be suitable for safety reasons.

The literature has already suggested a model for estimating film thickness using absorbance measurements at 400 nm (Stejskal et al., 1999). The relationship is linear up until the upper limits of absorbance. This can be seen below in Figure 5.26.

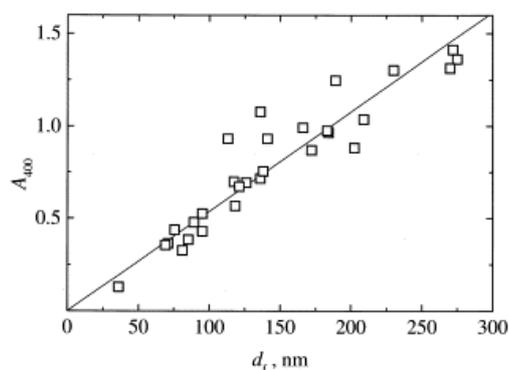


Figure 5.26 Model used for estimating film thickness (Source: (Stejskal et al., 1999))

Using this model and the data gathered from the UV-Vis spectrometer estimations of film thickness can be made. Using the spectrometer gave meaningful readings up until 10 cycled

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coating. At this point the film was too thick and blocked out all radiation that was attempted to be passed through it. Table 5.5 shows the data that was calculated from this experiment.

Table 5.5 estimated film thickness from the above model at 400 nm

Number of cycles	Estimated film thickness in micrometers
0	0.096
1	0.141
5	0.426
10	Too thick for model

For estimating the film coatings above 5 cycles of dipping the substrate in the two pots the films became too thick to measure using UV-Vis spectroscopy and this model could not be used. Therefore a different technique was required due to the thickness of the deposited layer.

5.1.1.4 DEK – TAK

This surface profiling technique was used to determine film thickness on the thicker layers found after 5 cycles of synthesis. The machine uses a diamond stylus to register the change in a given surface. The limits of the vertical range are 50 Å to 2620 kÅ however for rough surfaces it can become difficult to register changes in substrate and feature changes on a surface. An overview of the instrument is given below in Figure 5.27.

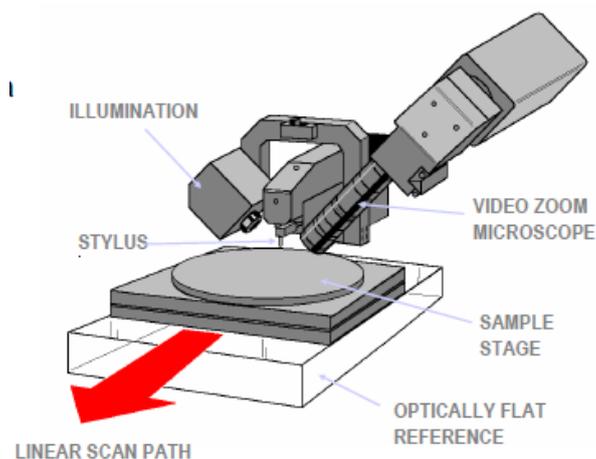


Figure 5.27 Layout of equipment used in the surface profiling of polyaniline (Source: www.Veeco.com)

For this experiment, an area was marked off the substrate using insulating tape. The stylus was then moved along the coated surface until it met the step change from coated to non-coated surface. Figure 5.28 shows the machine in operation using this step measurement method. The blue solid represents the uncoated substrate and the green area corresponds to a thick layer of deposited polymer.

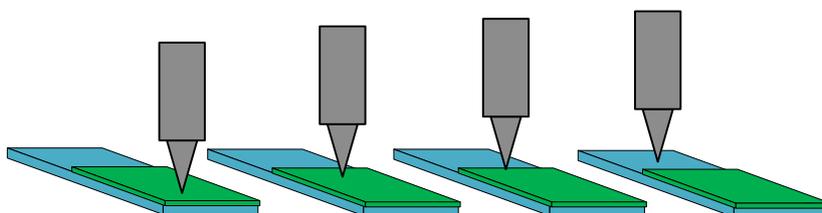


Figure 5.28 The recording of a surface profile using the Dek-Tak stylus

The machine was adjusted to measure this step change and Table 5.6 below gives the setup for the parameters used in this experiment.

Table 5.6 The machine settings for each scan

Machine parameter	Setting
Scan length	2000 μm
Time for scan	Low (50 s)
Resolution	High
Data points	8000
Scan resolution	0.250 μm
Range of measurement	1310 k \AA
Force of Stylus	30 mg

Table 5.7 shows the data collected from using this technique and these have been plotted along with the results from the UV-Vis spectroscopy experiment in Figure 5.29.

Table 5.7 Data collected from Dek-Tak experiment

Number of cycles	Estimated film thickness in micrometers
10	5.25
15	16.82
20	19.44
50	28.96

The results show that on this substrate there is also the same trend in terms of increase in the log of layer thickness and the number of cycles of synthesis performed. The upper and lower confidence limits have also been placed on the graph; these show that the more cycles that are completed the more reproducible the technique becomes.

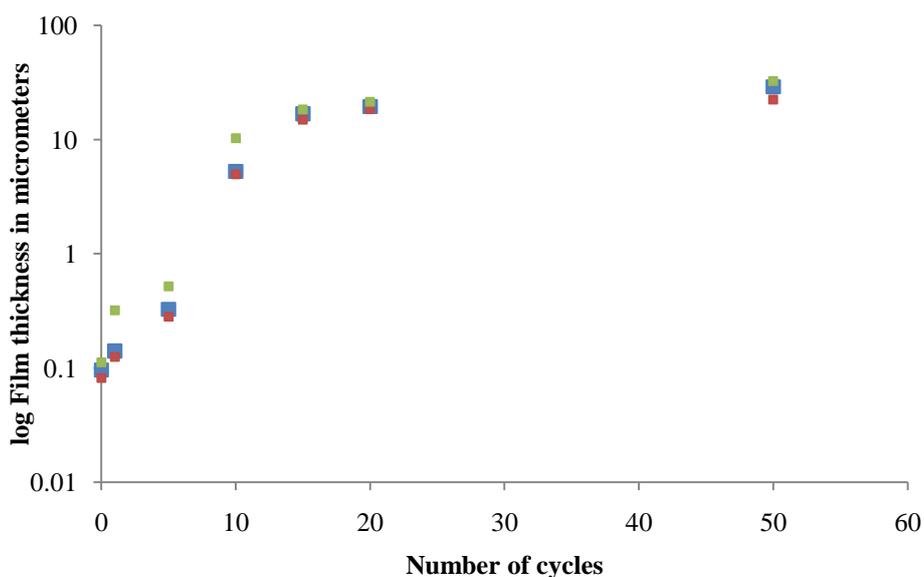


Figure 5.29 Film thickness of polyaniline deposited on Melinex using UV-Vis spectroscopy and Dek-Tak surface profiling (■ = upper bound. ■ = mean value and ■ = lower bound)

The results also show that the choice of substrate makes a difference in the first 10 cycles of layer production. Films produced on the gold coated substrates were thicker up until 10 coats. The same effect of polymerisation in the reagent solutions was also observed using this substrate and lead to the diminishing effect of this technique. The equation for film thickness on this substrate can be seen below in Equation 5.2 which has an R^2 value of 0.9113.

Equation 5.2 Thickness of polyaniline deposited on Melinex substrate where N = the number of cycles

$$Thickness = 0.1288 \times e^{(0.286 \times N)}$$

The data point at 50 cycles does not conform to the relationship seen for the rest of the data set which is caused by the uncontrollable polymerisation reaction occurring after pieces of polyaniline are dropped into the reagent solutions.

5.2 Other techniques of deposition

This thesis has mainly been concerned with the improvement of the technique used to produce in situ films of polyaniline. As mentioned in the literature review, there are other techniques that can be used to deposit polyaniline films such as electrochemical and gas vapour techniques. This method has been chosen to be investigated because of the comparative low cost and the quality and reproducibility of the layers that are produced. The method of two pot synthesis could potentially be scaled up industrially and used on existing packaging films. SEM images of polyaniline films formed through electrochemical polymerisation can be seen below in Figure 5.30.

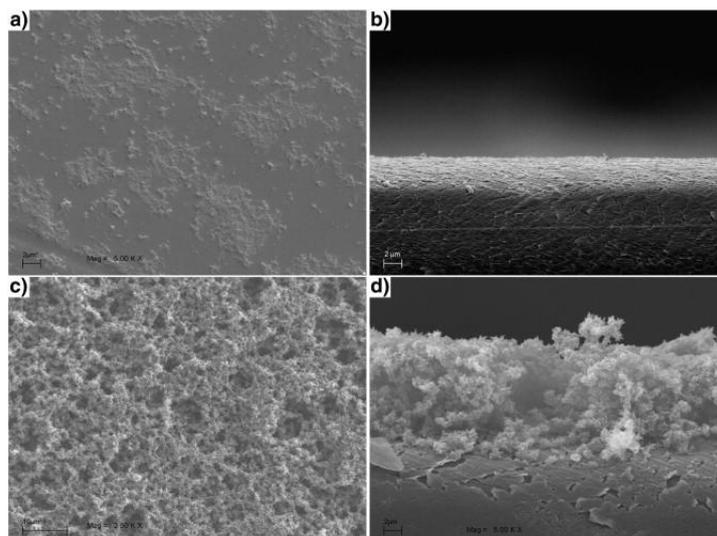


Figure 5.30 A selection of SEM images for polyaniline formed at 1.2 V showing morphology and cross sections (a and b are thin films, c and d are thick films) (Source: (Golczak et al., 2008))

The films produced are of a much higher purity and show similar morphologies to the films formed using the in situ two pot method. The film thickness of the samples shown above is dependent on time and applied voltage. A film with a thickness of 200 nm can be achieved using 1.2 V for 2 seconds and if left for a minute the film thickness can increase up to 12,000 nm (Golczak et al., 2008).

5.3 Conclusions

The techniques used in this study have been employed to determine the characterisation of polyaniline films that are grown using a two pot synthesis process. Original layers were deposited using an in situ method. This film was then subjected to various numbers of cycles of the two pot process.

Previous results have shown that in situ films have a limit in terms of maximum thickness and conductivity that can be controlled with temperature. In situ films are also inconsistent in terms of conductivity and thickness and the method of controlling the polymerisation reaction has proven difficult. The results in this chapter have used this novel method of synthesis to controllably improve in situ films. It has been shown that the more cycles that are performed on an original layer, the better the conductivity will be with improved reproducibility.

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Further investigations into the development of these films over the course of 50 cycles on various substrates have been undertaken. The data from the various experiments show that there is an exponential trend of increasing film thickness for every cycle that is completed. The maximum number of times that the reagent solutions can be used is estimated at 30 times. After this the solution starts to polymerise due to debris that has been left in by the decomposing thick layers.

This method of sensor development has been used to optimally design the sensors that will be used in the final part of this thesis. Films that have been cycled 5 times will be used to make the sensors that are used as the food quality indicators for further study in this thesis. This is because the layers show sufficient conductivity with a high enough standard of reliability and reproducibility to be used as commercial sensors. The layers are also robust while being and thin enough to not decompose or disintegrate which has been seen on layers after 10 cycles.

Chapter 6
Monitoring and Modelling Bacterial Changes in Salmon and Herring

6 Introduction

This chapter will deliver results on bacterial growth for salmon and herring fish samples. The overall aim of the experiments was to quantify bacterial growth and spoilage rates for these two fish species so that comparisons could be made to the results gathered from the polyaniline sensor being produced. The models were used to test the effect of external storage temperature on growth rate of the total bacterial population and specific spoilage organisms (SSOs). In this case, the selected SSO was *Pseudomonas* species which were isolated using selective media techniques. The growth model curves were fitted using primary and secondary methods and a comparison of the observed results to the literature followed.

6.1 Bacterial modelling

Bacterial modelling is the practice of fitting observed results of bacterial growth into a format such as a curve so that variables such as lag time and growth rate can be estimated. This field has seen constant improvement in data collection and understanding over the last twenty years. In terms of data collection, there are a number of ways that bacterial populations can be enumerated. The standard technique that is used either as a standalone method or reference is to use a viable colony plate counting approach. Other techniques in this field are used to provide process automation or to be more informative. Examples of a more automated population technique include turbidity measurements and impedance measurements of an electrolytic media. These techniques can be used for studies involving large numbers of variables and experiments. Flow cytometry and microscopy are techniques that are used to interrogate sizes of colony and specific cell growth rates. The experiments in this chapter used plate counting methods for data collection.

Bacterial growth curves have a distinct shape that can be segmented into four stages, as discussed previously in section 2.4. These are lag, exponential, stationary and death. These stages can be seen schematically in Figure 6.1 in the order in which they occur.

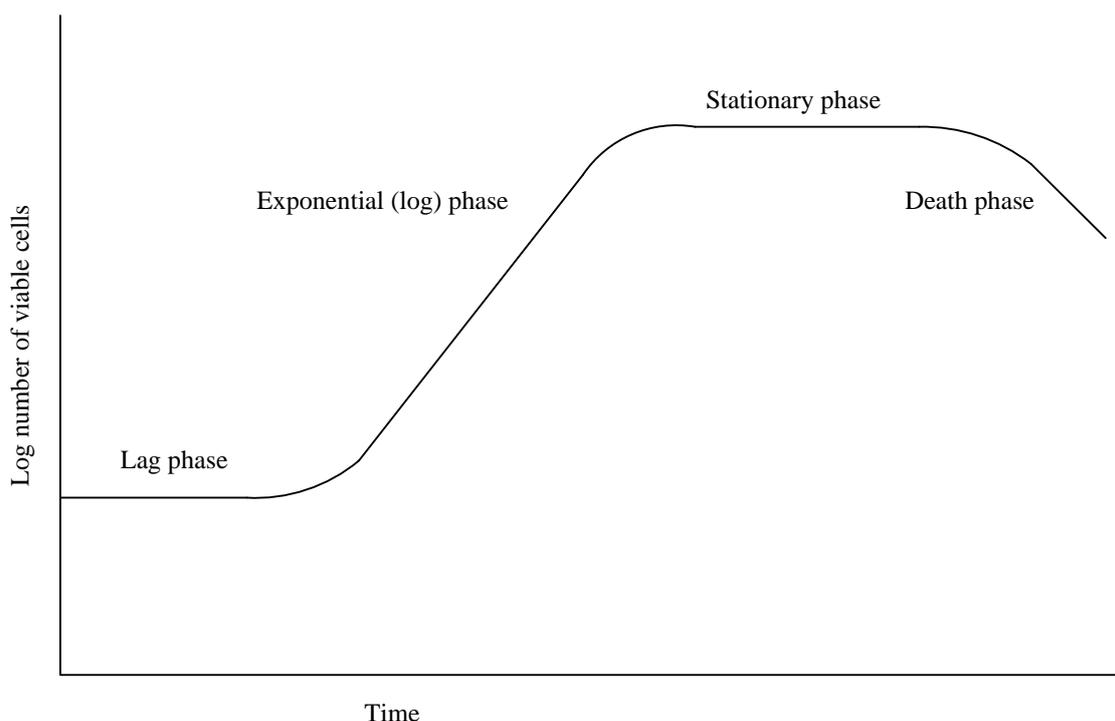


Figure 6.1 Schematic of bacterial growth Source: McKellar and Lu, 2009)

Initial reviews of predictive microbiology explain that the original concept was to provide information on the behaviour and response of microorganisms to a variety of different environmental conditions such as pH changes, temperature changes and a_w changes (Ross and McMeekin, 1994). In the food industry, models of this kind can be used in conjunction with other knowledge, such as chemical degradation, to predict spoilage times. There are two approaches to modelling, empirical and mechanistic (Ross and Dalgaard, 2009). Empirical models use basic mathematical terms and relationships to provide parameters that have no real meaning. Mechanistic models provide parameters and terms that relate directly to bacteriological functions of a system.

The simplest modelling of bacterial growth uses a logistic equation to fit to the sigmoidal shape of the bacterial growth curve (Gibson et al., 1988). The shape of this curve also has four stages so is comparable as a model. Gibson et al (1987) produced the model with the parameters A, B, C and M which have no biological meaning.

Equation 6.1 Gibson model for bacterial growth (Source: (Gibson et al., 1987))

$$L(t) = A + \frac{C}{1 + e^{(-B(t-M))}}$$

In this equation, $L(t)$ is the number of cells at time t , A is the asymptotic count as t decrease to zero i.e. the original count of bacteria, C is the difference between the final count after growth and A , B is the relative growth rate at M where M is the time at which absolute growth rate is at a maximum (Gibson et al., 1988). These parameters can be seen below in Figure 6.1.

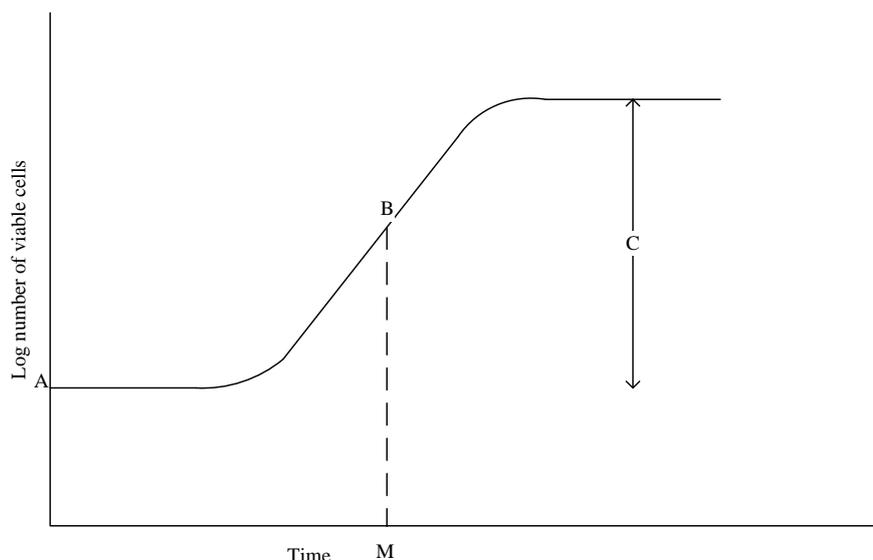


Figure 6.2 Parameters used in Gibson and Gompertz models

Following on from this, a simple rearrangement and modification led to the Gompertz model. This can be seen below in Equation 6.2.

Equation 6.2 Modified Gompertz equation for bacterial growth (Source: Gibson et al., 1988)

$$L(t) = A + C \exp\{-\exp[-B(t - M)]\}$$

The Gompertz equation above is used as an empirical model but the parameters can be used to give mechanistic measurements. The parameters of the above equations are used in the equations below in Equation 6.3 to give measurements of lag time (λ), exponential growth rate (μ) and generation time. The symbol e is used to express the exponential growth constant.

Equation 6.3 The equations used to give bacterial parameters form the Gompertz equations (Source: Gibson et al., 1988)

$$\mu = \frac{BC}{e}$$

$$\lambda = M - \frac{1}{B} \quad \text{or} \quad \lambda = M - \frac{1}{B} + \frac{\log_{10}N(0) - A}{BC/e}$$

$$\text{Generation time} = \frac{\log_{10}(2)e}{BC}$$

The expression of lag time has been corrected to the expression on the right to give a better model fit to the data (McMeekin and Ross, 1996). This is because the coefficient A is not used to represent the population at $t=0$ and instead requires this correction factor to determine the initial population. One drawback of empirical formulations is that they do not facilitate the calculations of statistics and standard errors around parameters of the growth rate.

Using the Gompertz equation can lead to errors and underestimation in the calculated generation time (Miles et al., 1997) and can give a negative value for λ in some cases (Juneja and Marks, 1999). Further research into the possibility of using bacteriological functions in place of the empirical mathematical functions of the previous equations was introduced using terms such as μ and λ within the model (Zwietering et al., 1991; Zwietering et al., 1990). This modified equation can be seen below in Equation 6.4.

Equation 6.4 Modified Gompertz equation with bacteriological terms included (Source: Zwietering et al., 1990)

$$L(t) = A \exp \left\{ -\exp \left[\frac{\mu_m e}{A} (\lambda - t) + 1 \right] \right\}$$

There has been a study in comparing the use of different primary models using sigmoidal curves (Logistic, Gompertz, Richards, Schnute and Stanard) and the best model of fit in most cases was the modified Gompertz equations in terms of ease of use and statistical accuracy (Zwietering et al., 1991). Other primary models include the Baranyi model which uses a

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single variable to describe the physiological state of the cells. This model is cited as being at least as good as the modified Gompertz model but has to be carried out in standardised laboratory conditions (Baranyi and Roberts, 1994).

The changing of the parameters from empirical to mechanical also allows for the use of actual bacterial parameters to be used in further analysis and secondary models. Secondary models can be used to characterise foods and environments under certain extrinsic conditions (Ross and Dalgaard, 2009). This can be the effect of temperature changes or pH changes on the growth rate predicted from primary modelling. In Figure 6.3 below, the effect of temperature and pH on the growth of *Escherichia coli* O157:H7 which shows the possible range of μ and an optimal μ_{\max} (Rosso et al., 1995).

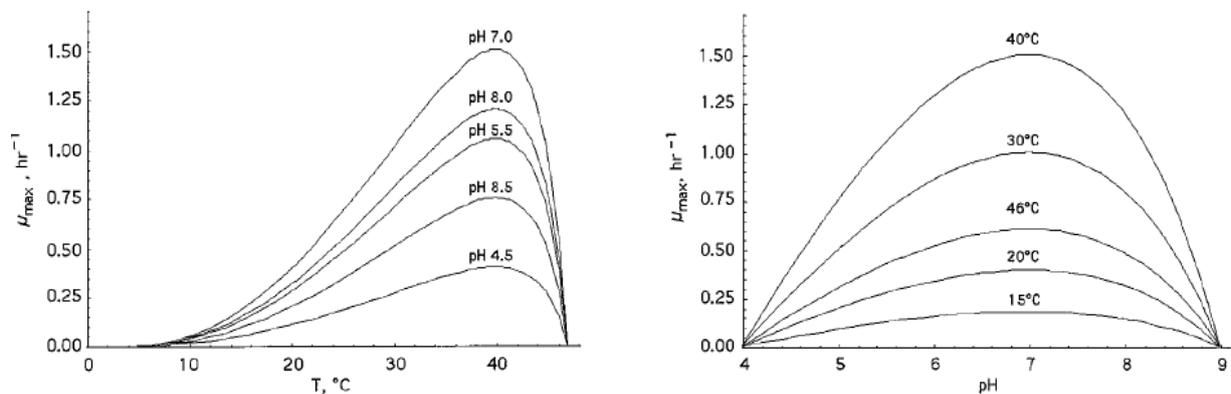


Figure 6.3 The modelling of pH and temperature to the growth rate of bacteria (Source: Rosso et al., 1995)

The model shown above used previous models to produce secondary models for predicting growth rates (Zwietering et al., 1991; Zwietering et al., 1990). Due to the shapes of the variable temperature curves it has been suggested that a linear relationship is present between the square root of μ and a region of a T_{\min} and a T_{\max} (the peak of the curve i.e. optimal μ) (McMeekin and Ross, 1996).

This chapter will provide results from bacterial growth studies on salmon and herring at three constant temperatures chosen to simulate refrigeration (4 $^{\circ}\text{C}$), cold chain abuse (14 $^{\circ}\text{C}$) and consumer abuse of product (24 $^{\circ}\text{C}$) – as well as the effect this has on the product. Primary models will be created and the critiqued followed by a simple secondary model that will be used in later chapters. The models that are created in this chapter will be used in the analysis

of data from subsequent chapters into volatile emissions from salmon and herring at different temperatures as well as correlating sensor responses to the growth of bacteria.

6.2 Bacterial models for fish and seafood

There are a number of examples of these models being used in the seafood industry. These models are often used in conjunction with existing tests for fish quality (Larsen et al., 1997). Due to the short shelf-life of these products and the increased global demand (National Statistics Online, 2008) there is a growing requirement for better understanding of the mechanics of spoilage to minimise waste and shrinkage. Improving shelf-life prediction has always been a main target for research in the field of predictive microbiology (McMeekin and Ross, 1996). The importance of being able to associate the effects of chemical detection to actual data on remaining shelf-life of a food product or early detection of spoilage has been understood for over a decade (McMeekin and Ross, 1996). Traditional methods are often time consuming and can require counts of spoilage bacteria close to the spoilage limit for detection of trace spoilage metabolites. Predictive microbiology also has inherent limitations itself which include the difficulty in measuring an initial level of population of bacteria, variability in bacterial lag times, the dependency of growth on a variable medium (i.e. food) and the non-microbial factors that contribute to food quality deterioration (McMeekin and Ross, 1996). There is also the effect of extrinsic factors on biological growth that have to be accounted for in a model that measures growth over a varying temperature.

A systematic experimental approach to shelf-life prediction requires several experimental stages to acquire the correct amount of data necessary to build an effective model (Koutsoumanis and Nychas, 2000). The layout of the planned experiments for this section can be seen below in Figure 6.4. Stages 2 and 4 will be discussed within this chapter.

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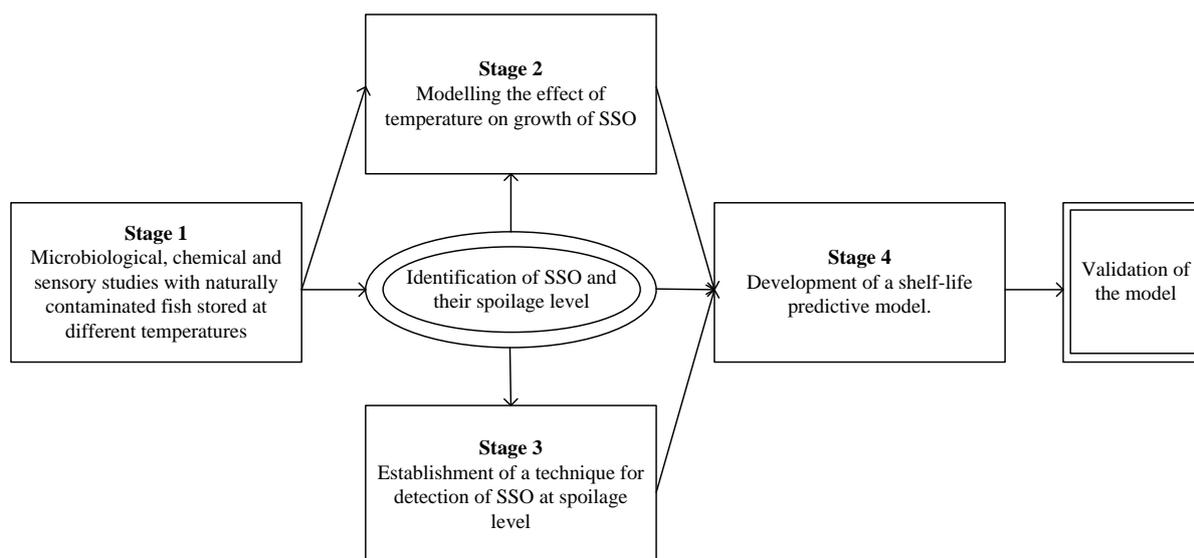


Figure 6.4 Systematic approach for creating a valid model for bacterial growth (adapted: Koutsoumanis and Nychas, 2000)

Studies in this area have used populations of $10^{6.5} - 10^{7.0}$ colony forming units as level of spoilage that can be identified by the naked eye and conforms to the end of a products shelf life (Hozbor et al., 2006; Koutsoumanis and Nychas, 2000). Lone Gram et al (2002) were able to show the interactions between the spoilage bacteria *Shewanella putrafaciens* (hydrogen sulphide and trimethyl amine producing) and *Pseudomonas* spp. (dimethyl sulphide and slime producing). The research was able to show that both SSOs prefer low aerobic conditions and a high pH. Both bacteria interacted to spoil the fish media at 0 °C with both having comparable growth rates and similar lag times (Gram and Melchiorson, 1996).

Using sensory analysis with chemical and biological data provides enough information to build a suitable model. This has been shown in various studies in the literature using a variety of techniques to validate growth models including multivariate statistics (Olafsdottir et al., 2006) and stochastic process risk models (Rasmussen et al., 2002).

The data from bacterial modelling for food products is often used in applications such as TTI. Initial studies took used predictive microbiological and took into account growth measurements to predict shelf-life as well as previous storage temperatures (Avery et al., 1996). Using predictive modelling techniques and TTIs, Taoukis et al (1999) were able to provide a shelf-life control system for chilled fish under simulated dynamic conditions. Using various TTIs, the research showed that the indicators were able to give reliable data of

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spoilage by *Pseudomonas* spp. and *Shewanella putrafaciens*. The time of spoilage given by the TTIs were in agreement with the sensory panels used to assess the quality of fish (Taoukis et al., 1999).

Other studies over the last few years have shown that TTIs can be deployed into real life situations to give accurate shelf-life and spoilage measurements (Tsironi et al., 2008; Taoukis, 2008; Giannakourou et al., 2005). The use of TTIs has been shown to be of more benefit against the use of long standing retailer best practice such as “first in first out” (Tsironi et al., 2008). There has also been evidence of TTIs being more accurate than declared shelf-life labels, such as sell-before dates, and issues such as shelf-life variance on large packages and containers of food items (Giannakourou et al., 2005).

Further applications of predictive microbiology in the fish industry include using tertiary models such as application software (Dalgaard et al., 2007; Dalgaard, 1997). The possibility of being able to calculate shelf-life of fish products was explored via modifications of a secondary model to produce growth rates over variable temperatures (Dalgaard, 1995a) and have been developed into the Seafood Spoilage and Safety Predictor (SSSP) (Dalgaard et al., 2007).

The main issue with predictive microbiology and subsequent technologies is the handling of data and information in the supply chain. At present, systems like hazard analysis and critical control point (HACCP) management are used in supply chains to control the safety of food products. This system has removed the end of chain product testing to testing and hazard identification along all points of food supply chains. Real-time data of food spoilage on chilled products has been suggested as the natural progression of the HACCP system (McMeekin et al., 2006) and with emerging information technologies such as RFID, the possibility of integrating TTIs or FQIs with an sufficient information technology system will potentially be a root to market for these intelligent labels (McMeekin et al., 2006).

6.3 Bacterial modelling for salmon

The study of bacterial growth on salmon commenced with an experiment into the change in bacterial populations over a 24 hour period when stored at 24 °C. These studies were carried out in a comparison to previous work which examined the changes of total bacterial and

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Pseudomonas spp. populations on samples of whole cod (*Gadus morhua*) and whole whiting (*Merlangius merlangus*) (Pacquit et al., 2007). The research presented in this work suggested a spoilage population limit from $10^{6.3}$ up to 10^7 colony forming unit counts per gram of *Pseudomonas* spp. in the fish samples which concurs with the rest of the literature. The salmon was sold with an expected shelf life of 72 hours if kept below 5 °C. The raw data can be seen below in Figure 6.5 with blue representing the total viable population and red the *Pseudomonas* spp. populations at a given time.

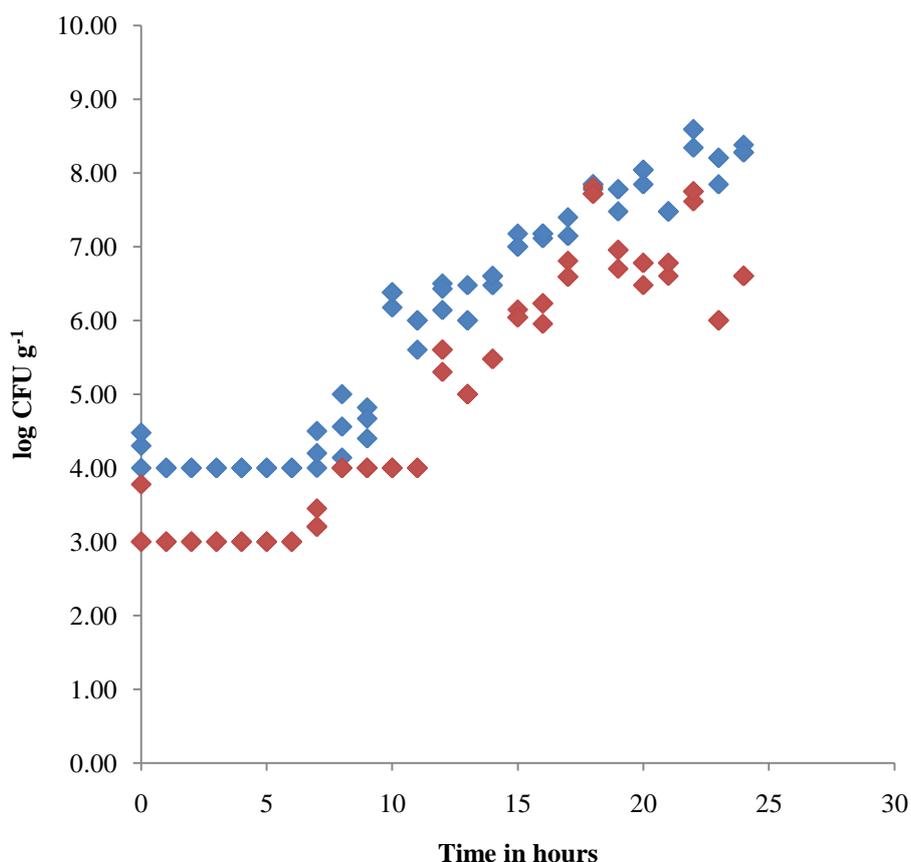


Figure 6.5 Raw data from plate counts from salmon at 24 °C (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Initially the Gompertz model was fitted to the data, as can be seen in Figure 6.6 with a sum of squares value of 7.419 and 13.906 for total populations and spoilage populations respectively. Using this model, the growth of both the total viable population and the *Pseudomonas* spp. population can be seen. The growth curves are very similar as expected with relatively short lag times due to the high temperature. The coefficients that have been calculated for the model can be seen below in Table 6.1 and Table 6.2 with the calculated standard error. As

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mentioned previously, these coefficients have no actual biological meaning but can be used to calculate lag times and growth rates.

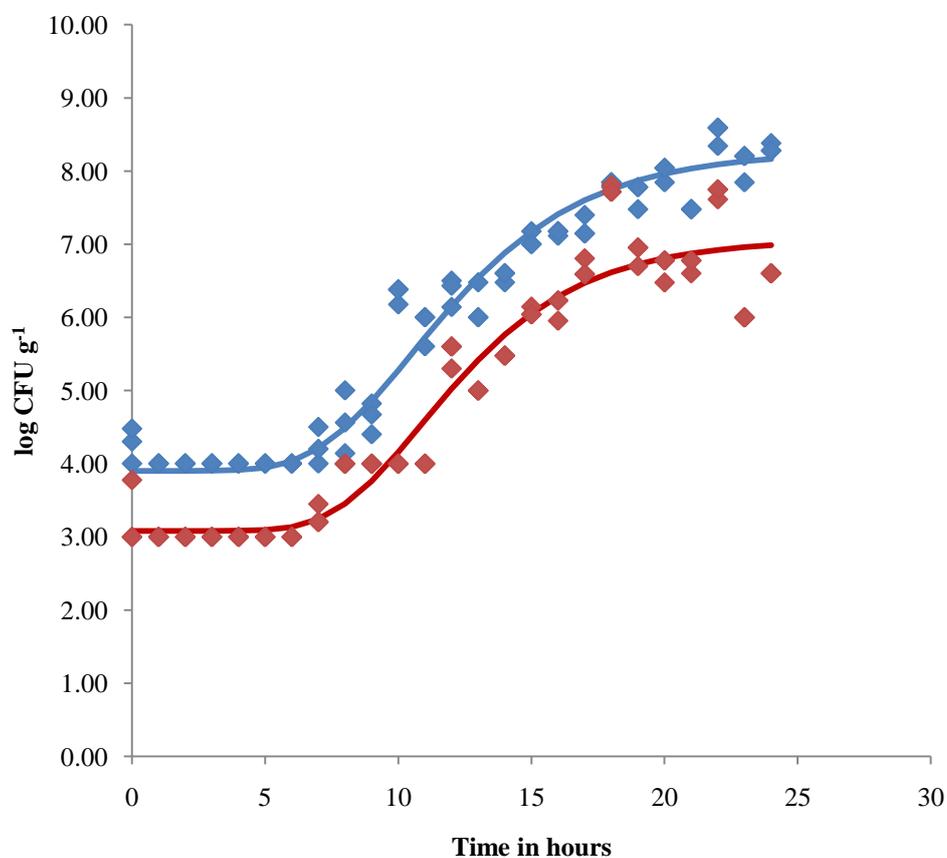


Figure 6.6 TVC and *Pseudomonas* growth curves with Gompertz model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.1 Growth coefficients predicted from Gompertz model for total viable count

Coefficient	Predicted value	Standard error
A	3.98	0.085
B	0.23	0.025
C	4.37	0.19
M	10.53	0.30

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Table 6.2 Growth coefficients predicted from Gompertz model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.08	0.010
B	0.299	0.043
C	3.98	0.20
M	10.91	0.34

Overlaid onto this plot is the Zwietering model in darker blue and red in Figure 6.7 with a sum of squares of 7.419 and 13.907 for total viable populations and *Pseudomonas* spp. populations respectively. Using this model the actual growth rate and lag time are estimated from the best fit model. These can be found below in Table 6.3 and Table 6.4.

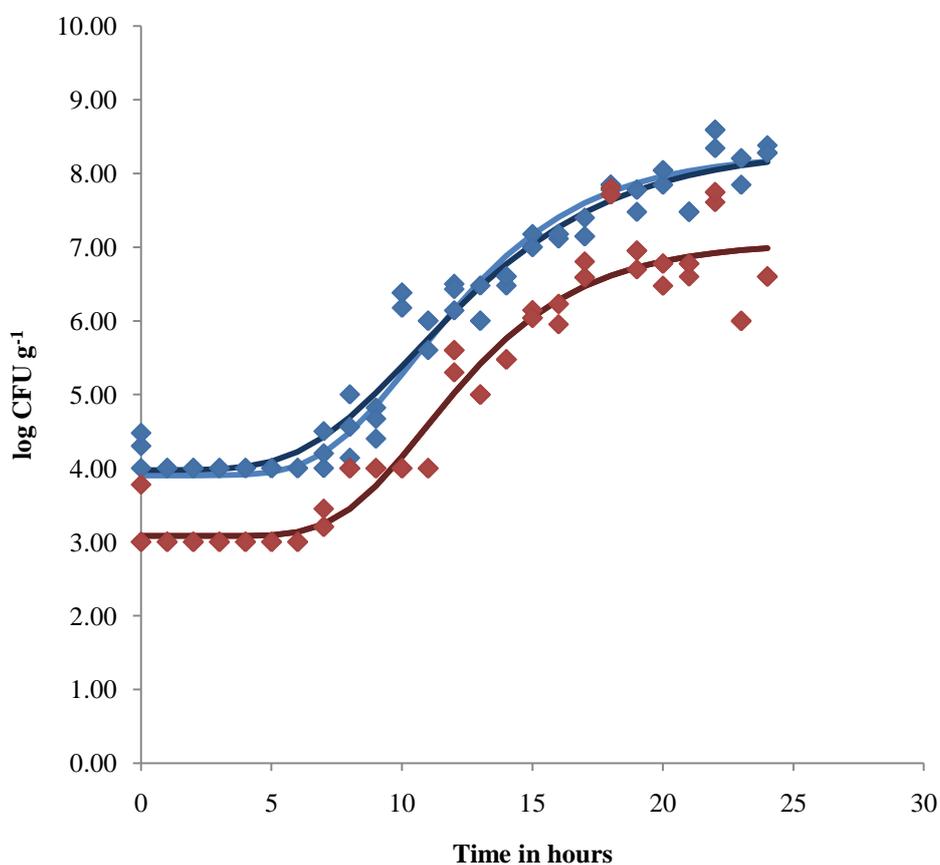


Figure 6.7 TVC and *Pseudomonas* growth curves with Zwietering model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

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Table 6.3 Growth coefficients predicted from Zwietering model for total viable count

Coefficient	Predicted value	Standard error
A	3.98	0.085
μ	0.37	0.028
C	4.37	0.19
λ	6.23	0.51

Table 6.4 Growth coefficients predicted from Zwietering model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.08	0.099
μ	0.44	0.049
C	3.98	0.20
λ	7.56	0.58

The Zwietering model appears to fit the raw data of the total viable count by shortening the lag time compared to the Gompertz model. The results also show that the high temperature favours the growth of *Pseudomonas* spp. in salmon with a slightly longer lag time. This is potentially due to *Pseudomonas* spp. being a specific spoilage organism for fish such as salmon and that it is the most favoured organism for growth under these conditions.

The lag time for both population counts is below 8 hours and the time it takes the total viable count to reach 10^7 cfu g⁻¹ is below 15 hours and the *Pseudomonas* spp. population reaches the lower spoilage limit of $10^{6.3}$ cfu g⁻¹ within 17 hours. Other reasons for the rapid growth time could be down to sampling error although this was minimised by doing replicate experiments with plate counts in triplicate. This also may be due to cell damage that was caused by storing samples at temperatures below -30 °C before the modelling.

The next temperature set was conducted at 14 °C and the raw data can be seen below in Figure 6.8. The experiment was carried out over a longer time period of 72 hours in anticipation of a longer lag period and expected slower growth rate. A population study was carried out every 3.6 hours rather than every hour.

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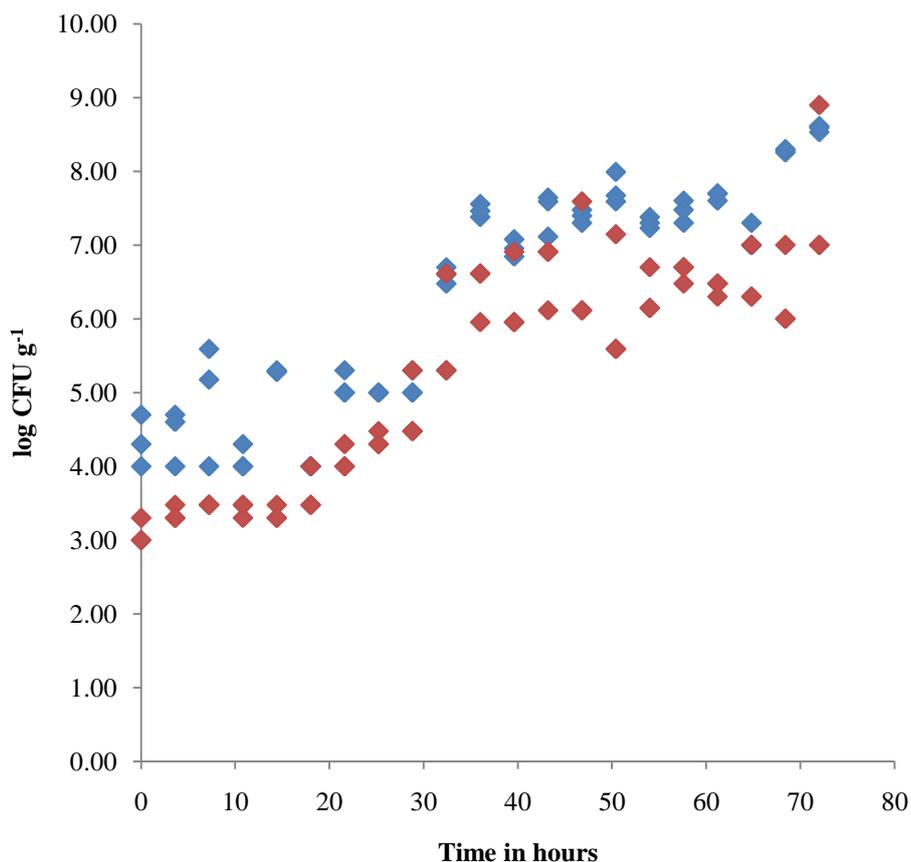


Figure 6.8 raw data from plate counts from salmon at 14 °C (■ = total volatile count and ■ = *Pseudomonas* spp. count)

The Gompertz equation was initially fitted to the data and is shown in Figure 6.9 with a sum of squares of 14.576 and 15.824 for total viable populations and *Pseudomonas* spp. populations respectively. The model shows a longer lag time and slower growth rates. The final populations are also reached after approximately 45 hours at this temperature. The coefficients for this equation can be seen below in Table 6.5 and Table 6.6 for total populations and *Pseudomonas* spp. respectively.

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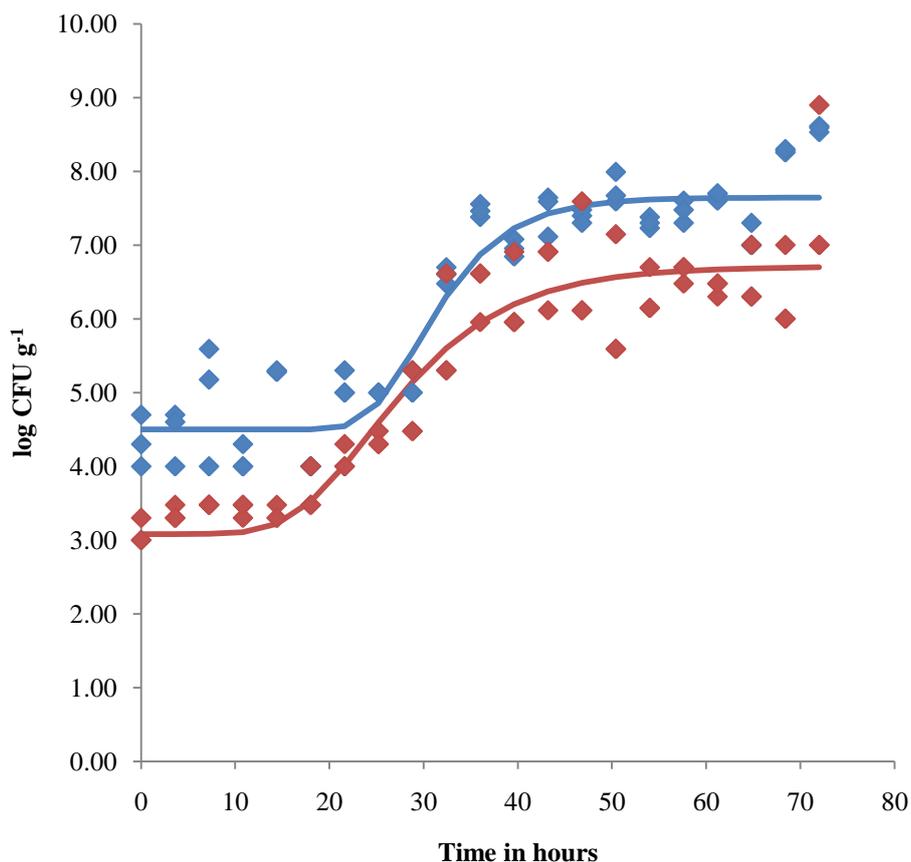


Figure 6.9 TVC and *Pseudomonas* growth curves with Gompertz model (■ = total viable count and ■ = *Pseudomonas* spp. count)

Table 6.5 Growth coefficients predicted from Gompertz model for total viable count

Coefficient	Predicted value	Standard error
A	4.58	0.11
B	0.19	0.043
C	3.15	0.16
M	29.33	0.96

Table 6.6 Growth coefficients predicted from Gompertz model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.37	0.13
B	0.14	0.031
C	3.29	0.18
M	25.72	1.13

Using the Zwietering model as described previously, the lag time and growth rate can be estimated. This can be seen in Figure 6.10 with a sum of squares of 14.576 and 15.824 for total viable populations and *Pseudomonas* spp. populations respectively. These have been

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overlaid on top of the Gompertz model in darker colours. In this case, the model conforms to the previously used Gompertz model with no modifications on observed lag time or growth rate. These are shown below in Table 6.7 and Table 6.8.

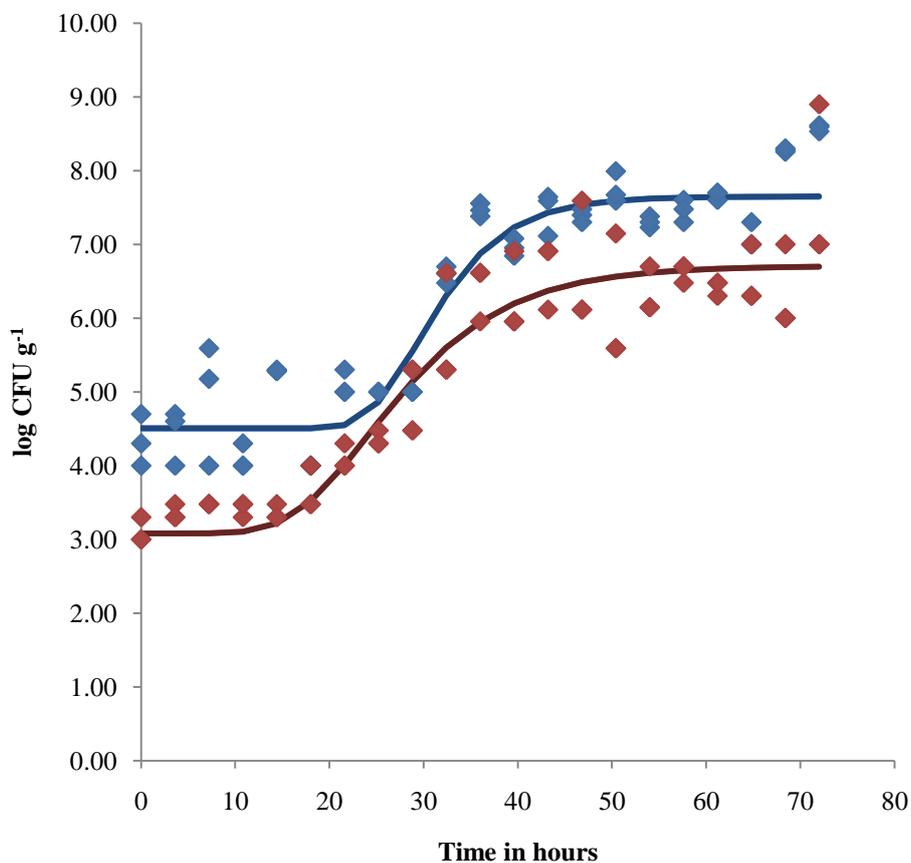


Figure 6.10 TVC and *Pseudomonas* growth curves with Zwietering model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.7 Growth coefficients predicted from Zwietering model for total viable count

Coefficient	Predicted value	Standard error
A	4.58	0.11
μ	0.22	0.045
C	3.15	0.16
λ	24.05	1.69

Table 6.8 Growth coefficients predicted from Zwietering model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.37	0.13
μ	0.18	0.033
C	3.29	0.19
λ	19.05	1.97

At this temperature both of the models fit the data set on the exact same curve. There would be no need to modify the Gompertz equation to fit the data set apart from to get the actual figures for μ and λ . Both sets of results show a lower growth rate than at the higher temperature. The growth rate of the total population is comparable to the growth of the SSO population. The lag time of the total populations is roughly 4 hours shorter compared to the total population with the spoilage population reaching levels above $10^{6.3}$ cfu g⁻¹ after 40 hours. The total population reaches a level of 10^7 cfu g⁻¹ in approximately 37 hours at this temperature.

There is quite a degree of variance around the *Pseudomonas* plate counts after 35 hours which is most likely caused by initial population variance in replicate samples from different portions of salmon as can be seen at the start of experiment. The coefficient C in this experiment is also lower than the value recorded in the higher temperature experiment. This is could be due to the change of rate of necessary chemical degradation to maximise bacterial growth at this temperature.

The final data set for salmon was recorded at a temperature of 4 °C. The raw data for this data set can be seen in Figure 6.11 with a sum of squares of 13.655 and 20.017 for total viable populations and *Pseudomonas* spp. populations respectively. For this experiment, a sample for population analysis was taken at 6 hour intervals. The length of the experiment in this case was 120 hours in total. Final populations were much higher in this experiment were much higher than at the other two temperatures which is likely to be caused by the extended length of time allowed for the experiment and the conditions being advantageous for the growth of favoured SSO. Also the longer length of the experiment allows for further growth into the stationary phase.

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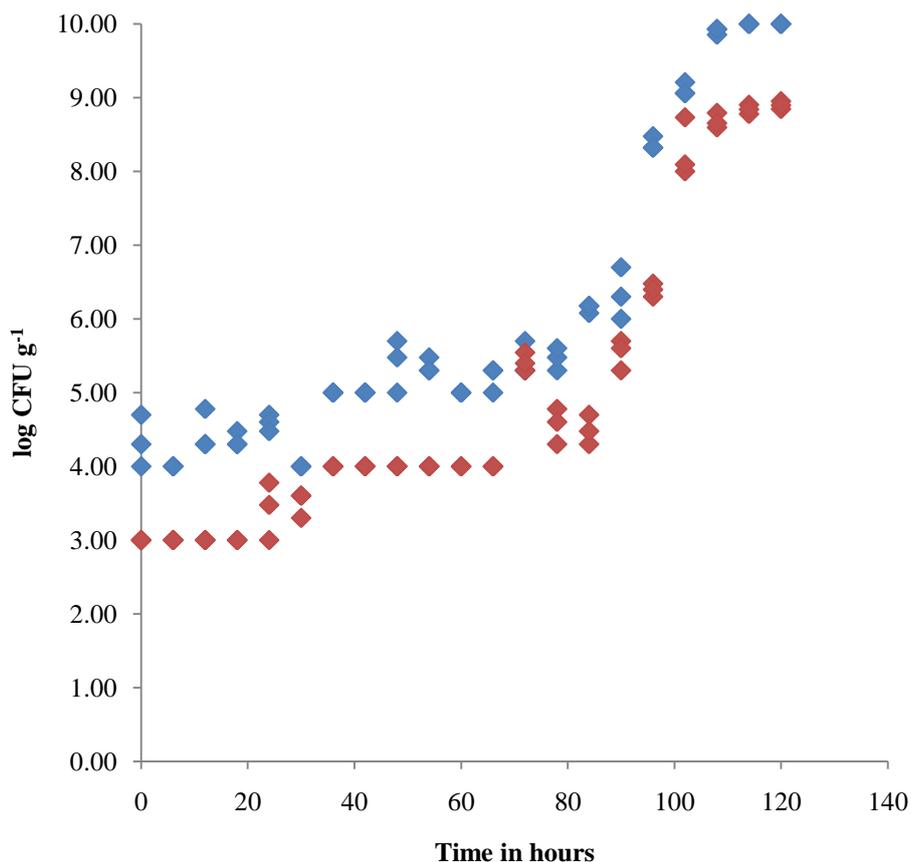


Figure 6.11 raw data from plate counts from salmon at 4 °C (■ = total volatile count and ■ = *Pseudomonas* spp. count)

The domination of SSOs would mean more growth nutrients available to them and with a slower growth rate these nutrients would be used more efficiently. These reasons could explain the higher overall final populations at this lower temperature.

The Gompertz model was fitted to this curve and can be seen below in Figure 6.12 with a sum of squares of 13.655 and 20.107 for total viable populations and *Pseudomonas* spp. populations respectively. At this temperature sample variance is much lower as is expected at a lower growth rate with the standard errors for the Gompertz coefficients reflecting this in Table 6.9 and Table 6.10.

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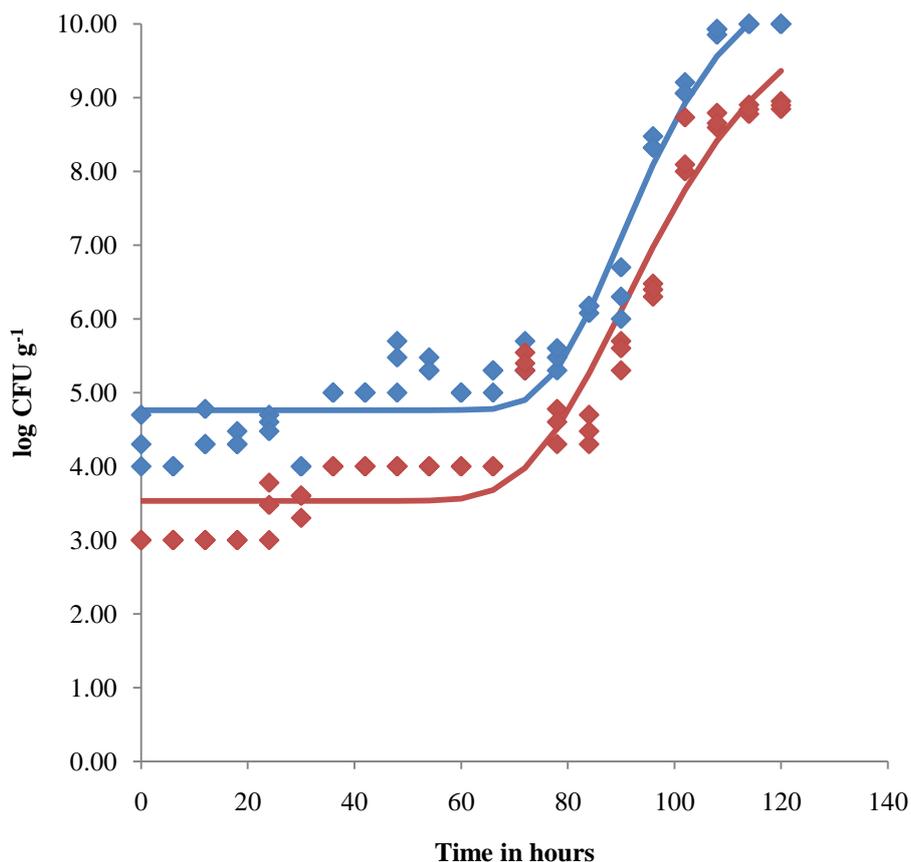


Figure 6.12 TVC and *Pseudomonas* growth curves with Gompertz model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.9 Growth coefficients predicted from Gompertz model for total viable count

Coefficient	Predicted value	Standard error
A	4.78	0.074
B	0.094	0.013
C	5.56	0.23
M	88.63	0.98

Table 6.10 Growth coefficients predicted from Gompertz model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.53	0.10
B	0.044	0.0087
C	7.00	0.81
M	89.97	3.26

Using the Zwietering model on this data set shows a slightly shorter lag time expected for the *Pseudomonas* spp. populations and no change in the total viable populations. This can be

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seen below in Figure 6.13. The growth rate and other biological measurements from this model can be seen in Table 6.11 and Table 6.12.

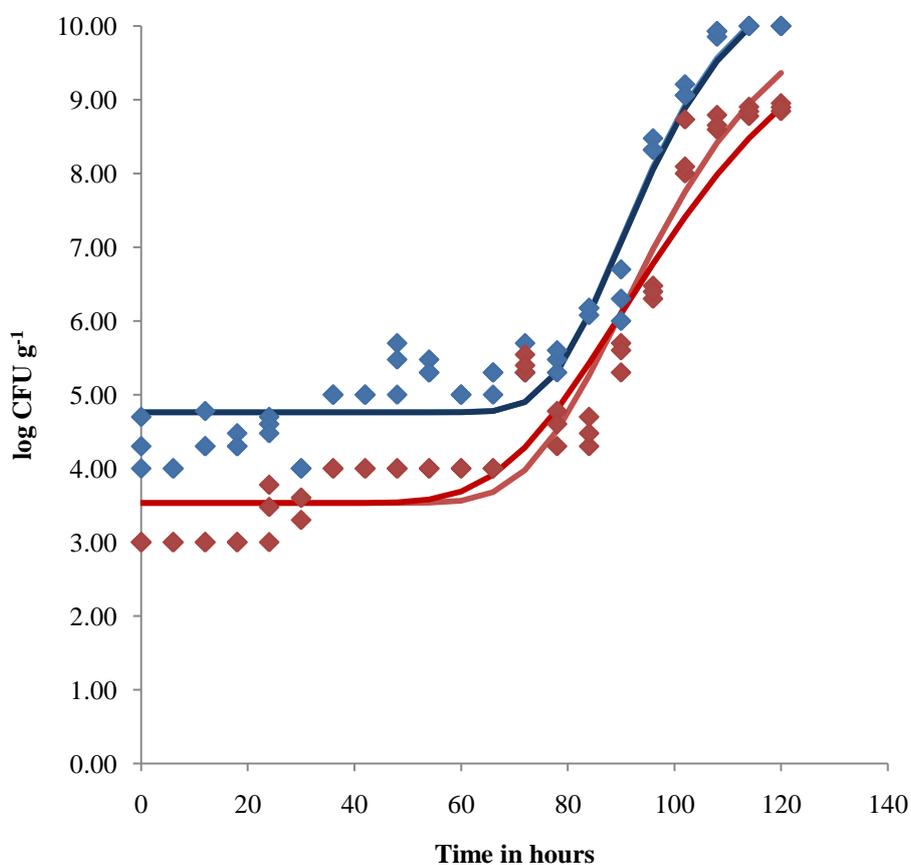


Figure 6.13 TVC and *Pseudomonas* growth curves with Zwietering model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.11 Growth coefficients predicted from Zwietering model for total viable count

Coefficient	Predicted value	Standard error
A	4.79	0.074
μ	0.19	0.022
C	5.56	0.23
λ	78.04	1.64

Table 6.12 Growth coefficients predicted from Zwietering model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.53	0.10
μ	0.11	0.011
C	7.00	0.81
λ	67.41	2.72

The data from this experimental model shows that the lag time for *Pseudomonas* spp. is less than that of the total viable count by more than 10 hours which has not been seen in the other two higher data sets. The growth rate however is slower than the total viable count so that the population of *Pseudomonas* spp. never exceeds more than 30 % of the total population.

In terms of shelf-life, the spoilage criteria of population of SSO at $10^{6.3}$ cfu g⁻¹ is reached after 93 hours which exceeds the stated 72 hour shelf-life given by the use-by date label. The time taken for the total population to reach 10^7 cfu g⁻¹ was approximately 92 hours. The production of slime at this temperature was also more visibly noticeable after 80 hours but no strong fishy odour was present at the end of the experiment which has been noticed at the higher temperatures.

6.4 Bacterial modelling for herring

Following on from the models of salmon, herring fish were used to compare growth rates and lag times. Salmon and herring have similar composition in terms of water, lipid and protein content (Jay et al., 2005) but differ in terms of climate. The fish was sourced from the Atlantic Ocean and had a given use-by date of 48 hours after purchase if stored below 5 °C. This was lower than that of salmon because of the increased distance from source to store and because the product was sold as a whole fish.

For these experiments the suggested limits of spoilage will again be from $10^{6.3}$ up to 10^7 colony forming unit counts per gram of *Pseudomonas* spp. These experiments commenced with a study of growth of bacteria on herring at 24 °C. The raw data from this experiment is presented below in Figure 6.14 . The variance of the plate count for this data set is greater after 16 hours where the growth rate is at its maximum value.

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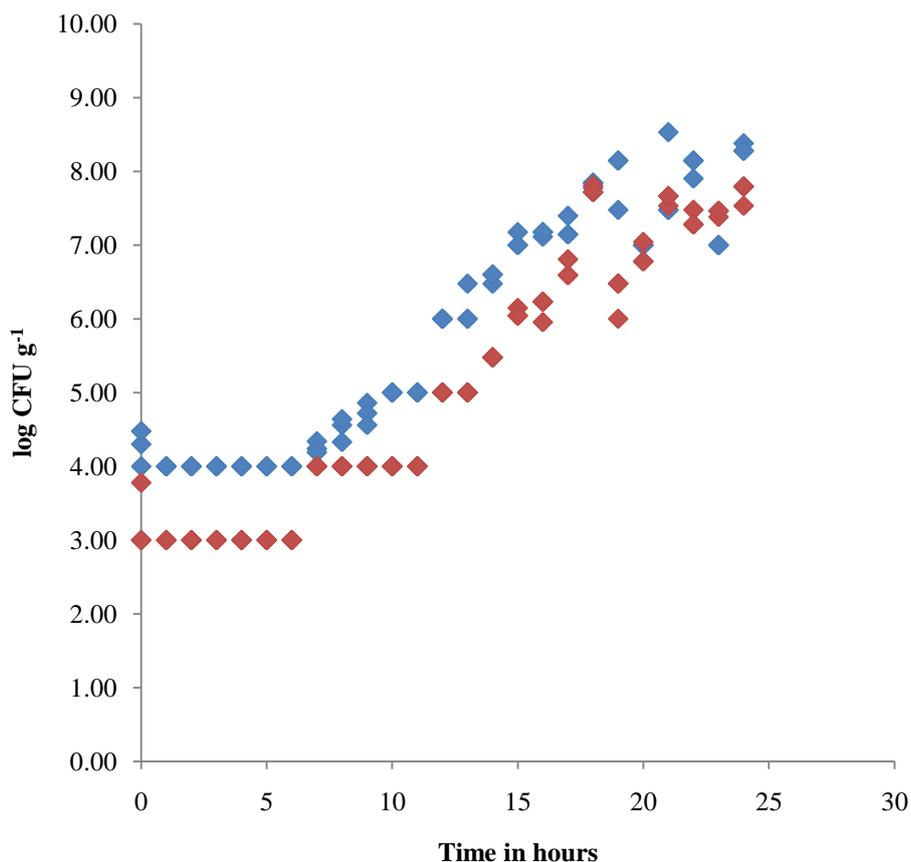


Figure 6.14 raw data from plate counts from herring at 24 °C (■ = total volatile count and ■ = *Pseudomonas* spp. count)

The Gompertz model was again fitted to the data set and the curve can be seen below in Figure 6.15 with a sum of squares of 7.607 and 9.027 for total viable populations and *Pseudomonas* spp. populations respectively. The low values for C in the total population study are due to low observed populations at times 23 hours and 20 hours. This is why the model meets the population of the *Pseudomonas* spp. towards the end of the study. The coefficients for the curve model can be seen in Table 6.13 and Table 6.14.

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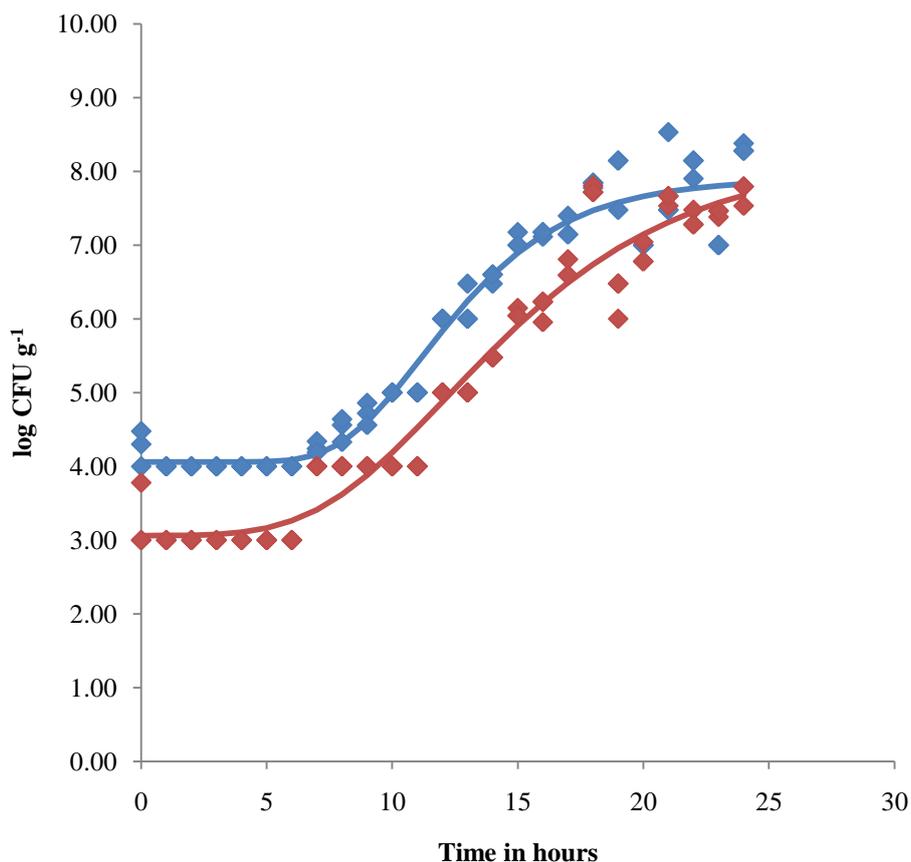


Figure 6.15 TVC and *Pseudomonas* growth curves with Gompertz model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.13 Growth coefficients predicted from Gompertz model for total viable count

Coefficient	Predicted value	Standard error
A	4.06	0.071
B	0.31	0.035
C	3.84	0.15
M	11.16	0.25

Table 6.14 Growth coefficients predicted from Gompertz model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.06	0.093
B	0.19	0.024
C	5.14	0.33
M	12.21	0.42

These data points were then fitted to the Zwietering model to estimate values of μ and λ and can be seen below in Figure 6.16 with a sum of squares of 7.027 and 9.027 for total viable populations and *Pseudomonas* spp. populations respectively. The fit of the curve is

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essentially the same as the Gompertz model with the same error of the final total populations of total viable count effecting the value of C. The values of lag time and growth rate are given in Table 6.15 and Table 6.16.

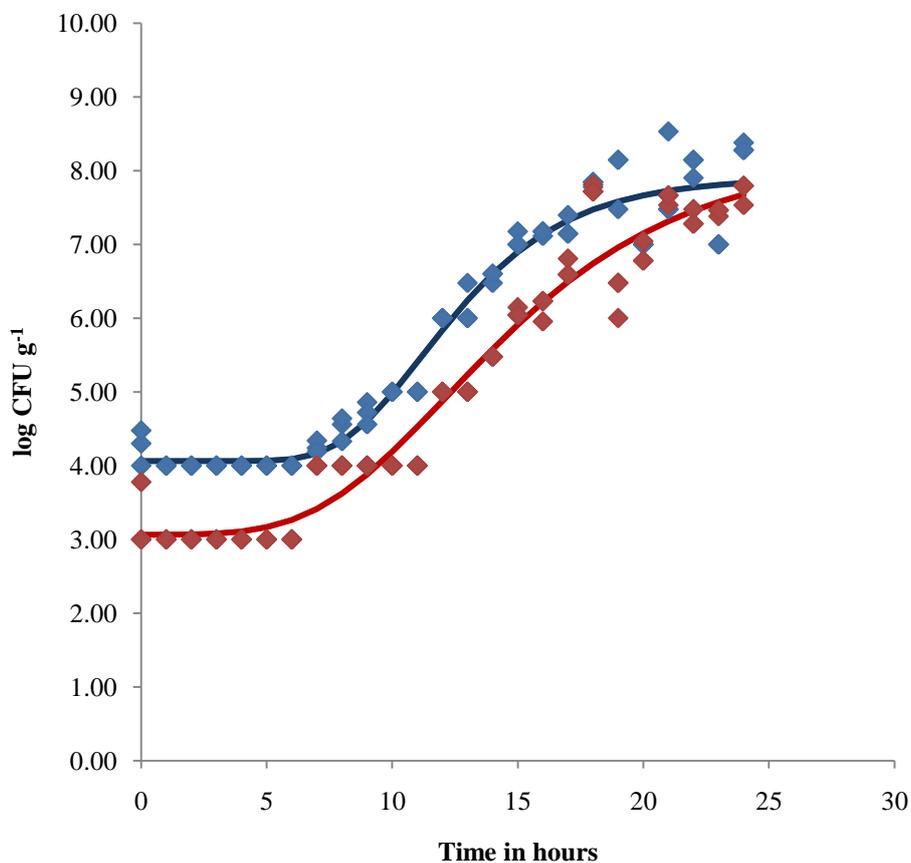


Figure 6.16 TVC and *Pseudomonas* growth curves with Zwietering model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.15 Growth coefficients predicted from Zwietering model for total viable count

Coefficient	Predicted value	Standard error
A	4.06	0.071
μ	0.44	0.038
C	3.84	0.15
λ	7.96	0.43

Table 6.16 Growth coefficients predicted from Zwietering model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.06	0.093
μ	0.36	0.025
C	5.14	0.33
λ	6.93	0.58

The growth rate of both the total population and the population of *Pseudomonas* spp. is high as might be expected due to the temperature. The total population growth rate is faster than that of the *Pseudomonas* spp. which is not similar to the findings of salmon. The lag times for both of the data sets are also quite similar with the total population having a shorter lead time of one hour compared to the *Pseudomonas* spp. which is similar to that of salmon.

The spoilage limitations for this set of data of $10^{6.3}$ cfu g⁻¹ for *Pseudomonas* spp. is reached at 16 hours where as the limit of 10^7 cfu g⁻¹ is reached at 18 hours. Total population count exceeds 10^7 cfu g⁻¹ after 15 hours which is also comparable to the values that were found for salmon.

The next data set was conducted at 14 °C and the sampling interval was the same for that of the salmon experiment. The raw data is presented below in Figure 6.17.

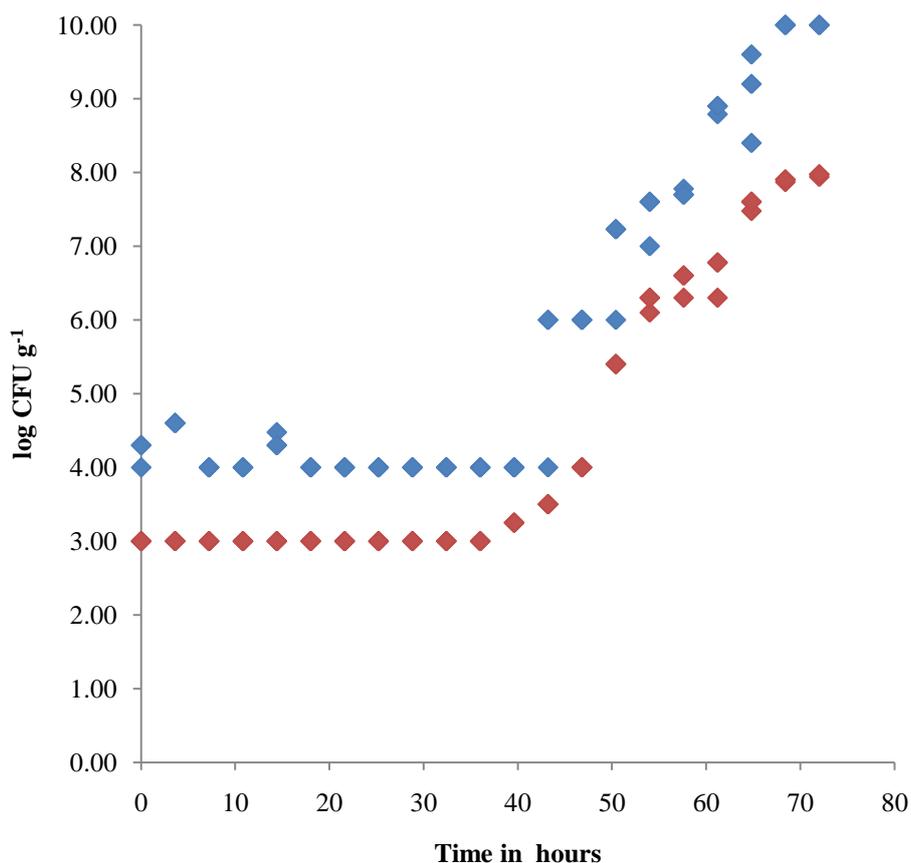


Figure 6.17 raw data from plate counts from herring at 14 °C (■ = total volatile count and ■ = *Pseudomonas* spp. count)

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The data set was used in creating a Gompertz model which is presented below in Figure 6.18 with a sum of squares of 9.211 and 2.150 for total viable populations and *Pseudomonas* spp. populations respectively. The curve has a different shape to that of salmon at this temperature which is due to the different level of nutrients present on the fish media as well as potential sampling errors from the experimental procedure. The coefficients for this Gompertz model can be found in Table 6.17 and Table 6.18.

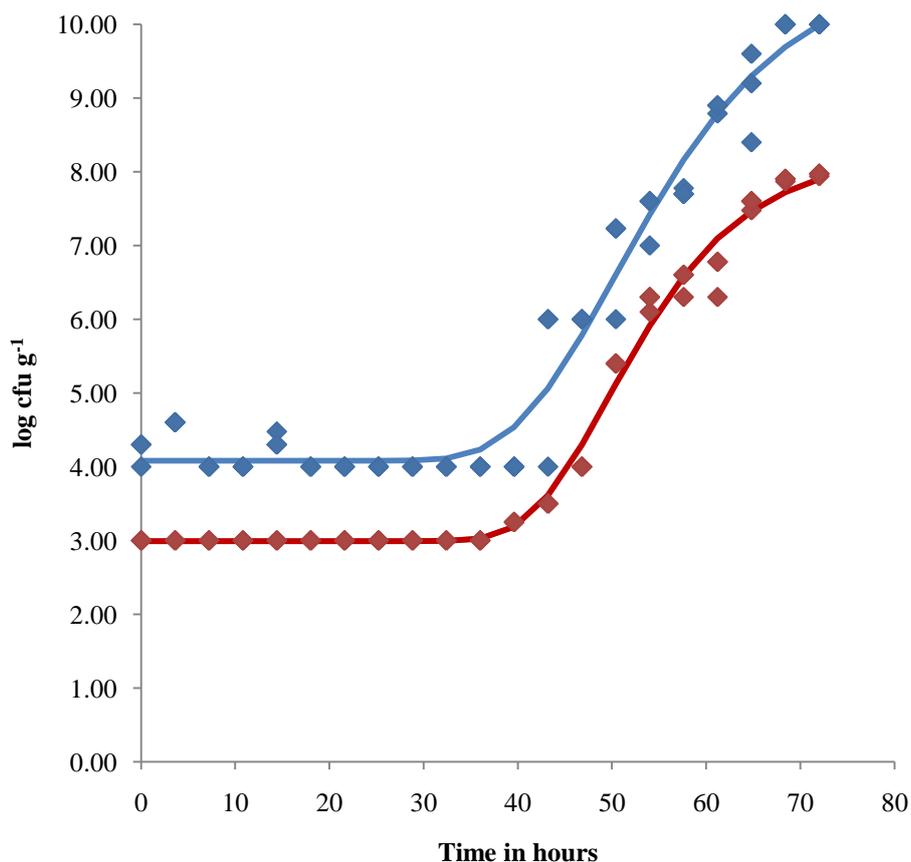


Figure 6.18 TVC and *Pseudomonas* growth curves with Gompertz model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.17 Growth coefficients predicted from Gompertz model for total viable count

Coefficient	Predicted value	Standard error
A	4.08	0.072
B	0.093	0.013
C	6.74	0.48
M	50.21	1.02

Table 6.18 Growth coefficients predicted from Gompertz model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	2.99	0.033
B	0.12	0.0087
C	5.25	0.15
M	49.54	0.38

The Gompertz model was modified as before and the Zwietering model was fitted and overlaid to estimate actual values of the growth rate and the lag time for both the total and *Pseudomonas* spp. populations. This can be seen below in Figure 6.19 with a sum of squares of 9.211 and 2.150 for total viable populations and *Pseudomonas* spp. populations respectively. The estimated values for growth rate and lag time can be seen below in Table 6.19 and Table 6.20.

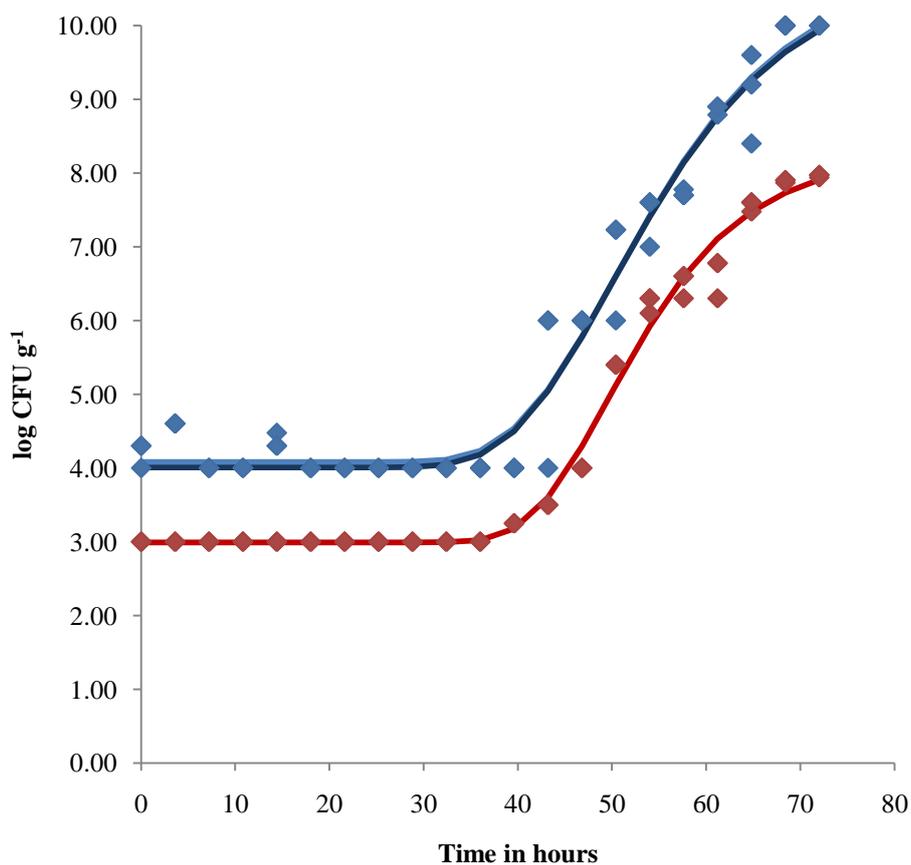


Figure 6.19 TVC and *Pseudomonas* growth curves with Zwietering model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

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Table 6.19 Growth coefficients predicted from Zwietering model for total viable count

Coefficient	Predicted value	Standard error
A	4.08	0.072
μ	0.23	0.017
C	6.74	0.48
λ	39.51	1.03

Table 6.20 Growth coefficients predicted from Zwietering model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	2.99	0.033
μ	0.23	0.011
C	5.25	0.16
λ	41.30	0.53

At this lower temperature the growth rate and the lag time for both the total populations and the SSO has changed as expected. The lag times have lengthened considerably when compared to the values for salmon at this temperature and herring at 24 °C. The growth rates for both population studies are the same value and are notably higher compared to those of salmon.

In terms of the time taken until spoilage, the samples took 56 and 60 hours to reach $10^{6.3}$ cfu g^{-1} and 10^7 cfu g^{-1} levels of population respectively. This is much slower than that of the model predicted for salmon and shows that the samples of herring potentially should have a longer shelf life at a lower temperature. The total population reaches a level of 10^7 cfu g^{-1} organisms after 48 hours.

The final experiment took place at 4 °C with a sampling interval of 6 hours. The raw data for this experiment can be seen below in Figure 6.20. The final populations for both the total and SSO count were higher than those seen in previous higher temperatures and comparable to those observed in salmon at this temperature. The reasons for this are expected to be the same as those given for salmon.

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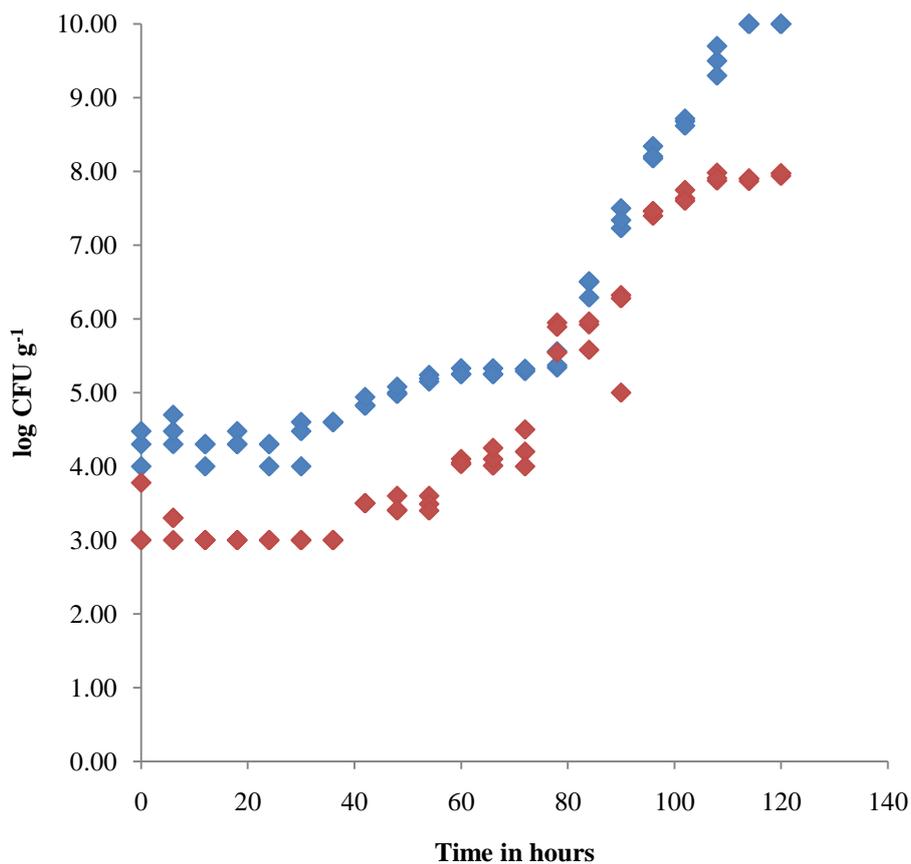


Figure 6.20 raw data from plate counts from herring at 4 °C (■ = total volatile count and ■ = *Pseudomonas* spp. count)

The Gompertz model was fitted to the data using the same technique as previous and the resulting curve can be seen below in Figure 6.21 with a sum of squares of 8.340 and 7.456 for total viable populations and *Pseudomonas* spp. populations respectively. The values for the coefficients for these equations can be seen in Table 6.21 and Table 6.22.

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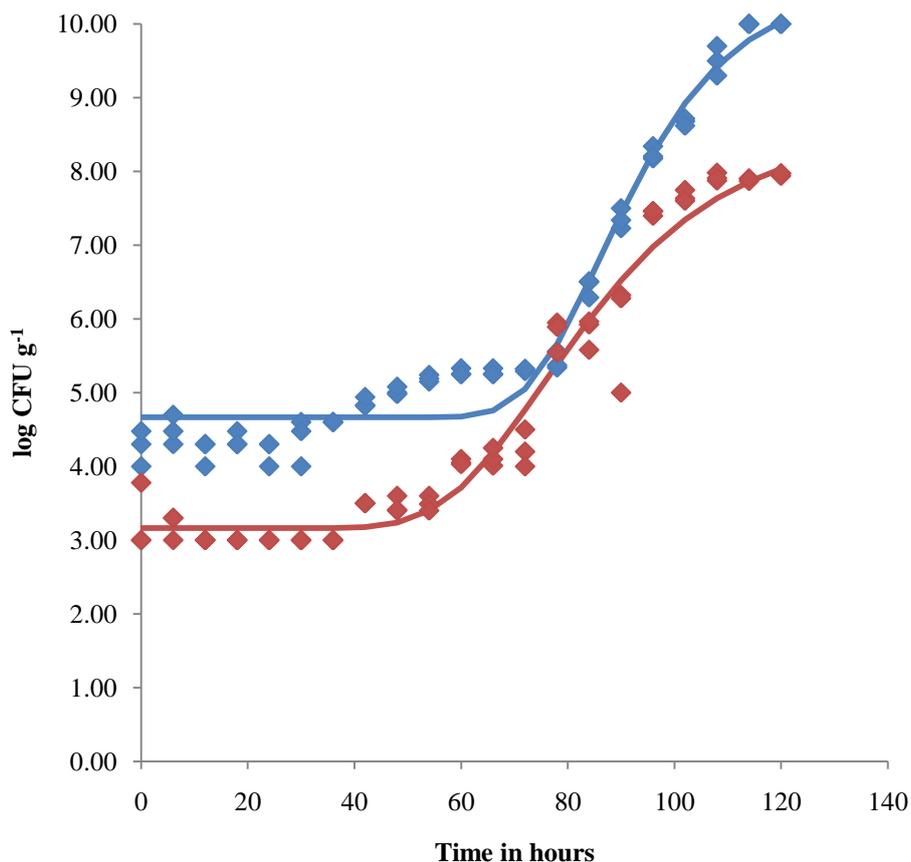


Figure 6.21 TVC and *Pseudomonas* growth curves with Gompertz model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.21 Growth coefficients predicted from Gompertz model for total viable count

Coefficient	Predicted value	Standard error
A	4.67	0.061
b	0.072	0.0078
C	5.85	0.23
M	86.02	0.96

Table 6.22 Growth coefficients predicted from Gompertz model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.16	0.067
b	0.053	0.0056
C	5.36	0.23
M	75.59	1.29

The curves show a shorter lag time for the SSO compared to the total population and this can also be seen on the Zwietering curve overlaid in Figure 6.22 with a sum of squares of 8.340

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and 7.456 for total viable populations and *Pseudomonas* spp. populations respectively. The data estimated from this model is presented in Table 6.23 and Table 6.24.

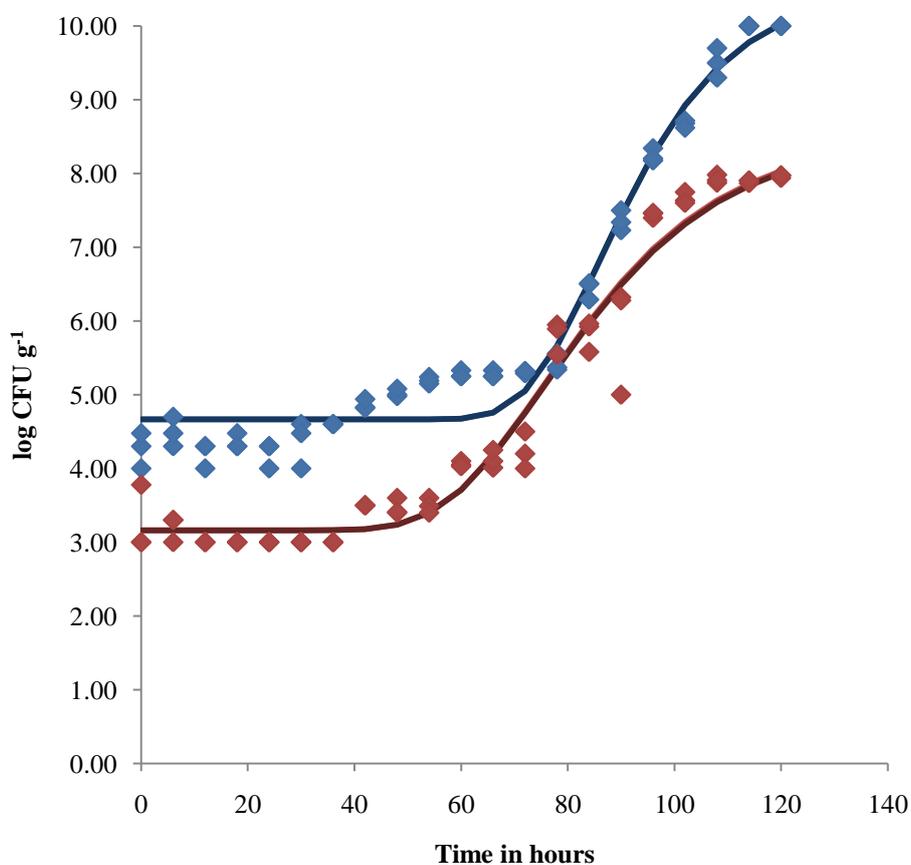


Figure 6.22 TVC and *Pseudomonas* growth curves with Zwietering model (■ = total volatile count and ■ = *Pseudomonas* spp. count)

Table 6.23 Growth coefficients predicted from Zwietering model for total viable count

Coefficient	Predicted value	Standard error
A	4.67	0.061
μ	0.15	0.012
C	5.85	0.23
λ	72.07	1.48

Table 6.24 Growth coefficients predicted from Zwietering model for *Pseudomonas*

Coefficient	Predicted value	Standard error
A	3.16	0.067
μ	0.10	0.0075
C	5.36	0.23
λ	56.62	1.92

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The growth rates for both of these curves are low as might be expected for this temperature. The lag time for the SSO is much shorter than that of the total population. This could be due to favourable conditions in the fish media environment for *Pseudomonas* spp. or potentially higher initial populations. This has also been seen in the salmon data sets for this temperature which indicates that the temperature and conditions are favourable for the growth of SSOs.

The growth rate for the SSO was marginally slower than that observed for the total population which explains why the growth curve does not overtake the total viable count. The stationary phase population is much lower for the SSO than the total population and the SSO never exceeds more than 35 % of total population.

The spoilage levels of $10^{6.3}$ cfu g⁻¹ and 10^7 cfu g⁻¹ were reached after 81 and 96 hour respectively which indicates again that the information supplied on the use-by label was incorrect by a significant margin. A level of 10^7 cfu g⁻¹ was reached after 84 hour for the total population.

6.5 Comparisons of temperature and fish on growth

The three temperatures have yielded the growth rates for both salmon and herring. These will now be used to create a secondary model that can be used in correlating the food sensor response and shelf-life. The models will be made using the fitted growth rates compared against changing temperatures for both the total count and the SSO count (*Pseudomonas* spp.). The model for the total viable count on salmon is presented below in Figure 6.23 with the upper and lower limits for the square root of growth rates displayed in Figure 6.24.

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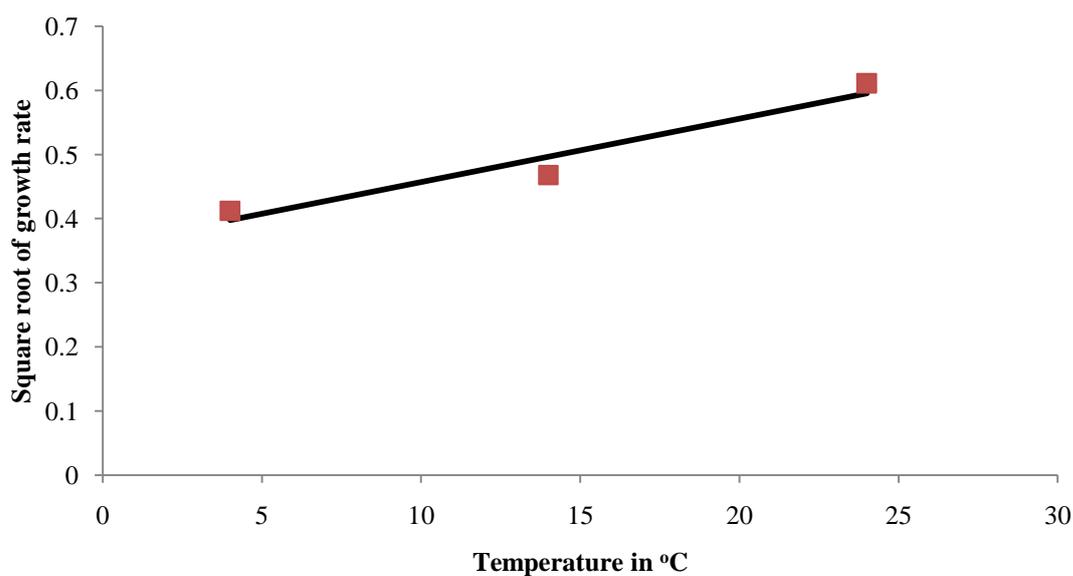


Figure 6.23 Relationship between growth rate and temperature for total population count on salmon

The line has an R squared value of 0.939 and the details of the model are given below in Table 6.25.

Table 6.25 The terms used in the model of total population growth for salmon under a variable temperature

Term	Estimate	Std error
Intercept	0.357	0.0409
Gradient	0.00994	0.002526

The upper and lower limits from the estimates predicted from statistical analysis of this model are presented below in Figure 6.24.

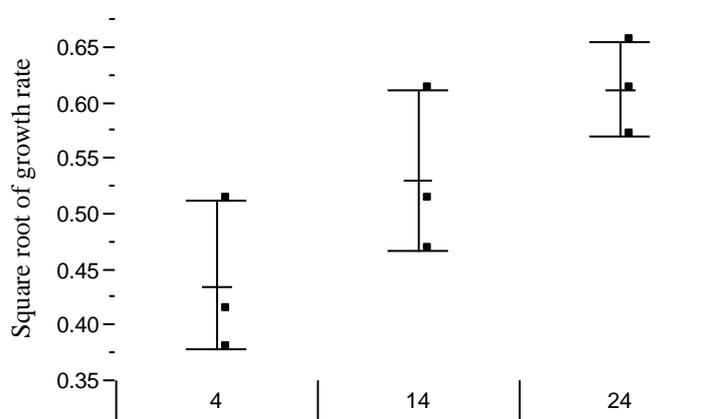


Figure 6.24 Upper and lower limits of the growth rate over the three given temperatures

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The change in the standard deviation of the estimated points on the model is shown below in Figure 6.25. The relatively low values of standard deviation show that the predicted value for all three temperatures are good estimates for growth rate.

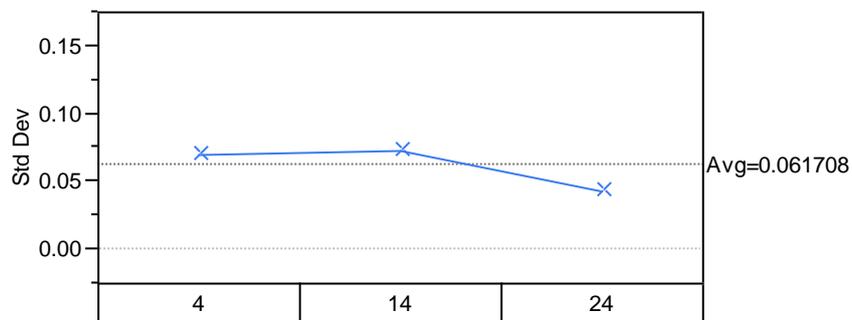


Figure 6.25 Spread of the standard deviation from the models presented

The model for temperature effect on growth of the *Pseudomonas* spp. on salmon can be seen in Figure 6.26. The terms for this model are presented in Table 6.26. The graph has an R square value of 0.895.

Table 6.26 The terms used in the model of total population growth for salmon under a variable temperature

Term	Estimate	Std error
Intercept	0.241	0.0899
Gradient	0.0162	0.00555

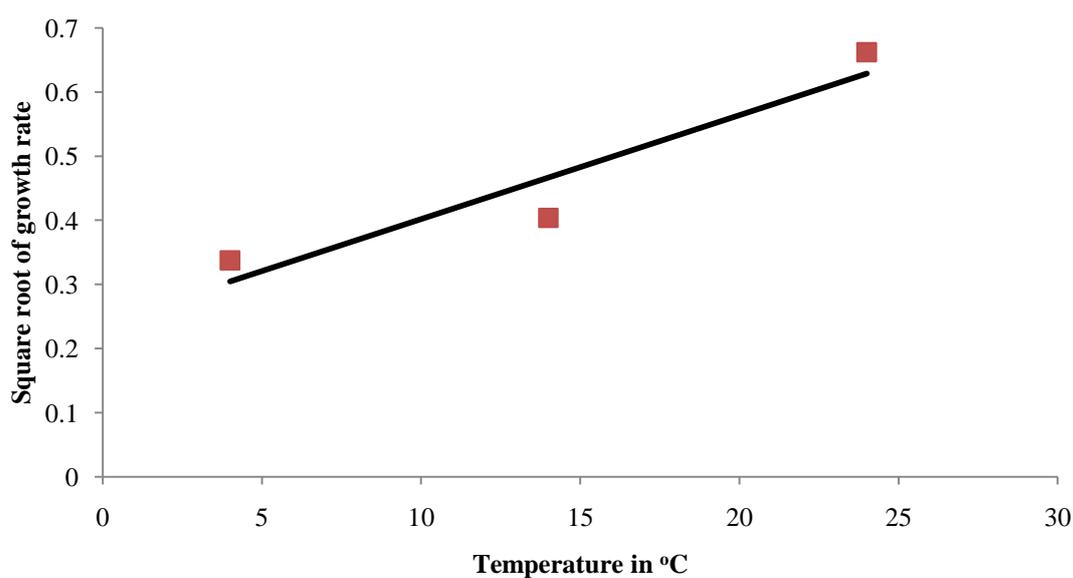


Figure 6.26 Relationship between growth rate and temperature for SSO population count on salmon

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The graph shows that there is poor linear correlation between these data which is possibly caused by the value estimated at 14 °C.

The upper and lower limits from the estimates predicted from statistical analysis of this model are presented below in Figure 6.27. The model presented for 14 °C has wider boundaries for upper and lower confidence limits and therefore a larger range of possible values. In contrast, the model presented for the lower temperature appears to be a much better fit.

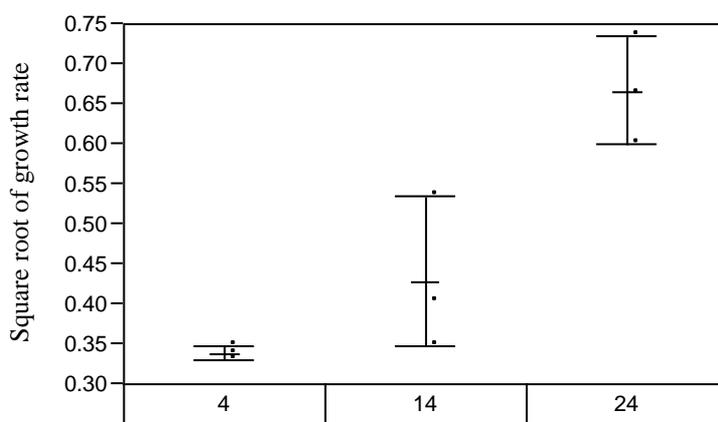


Figure 6.27 Upper and lower limits of the growth rate over the three given temperatures

In Figure 6.28 the range of these deviations can be seen. The large increase in spread for the value at 14 °C can again be seen here and is why the secondary model produced is poor.

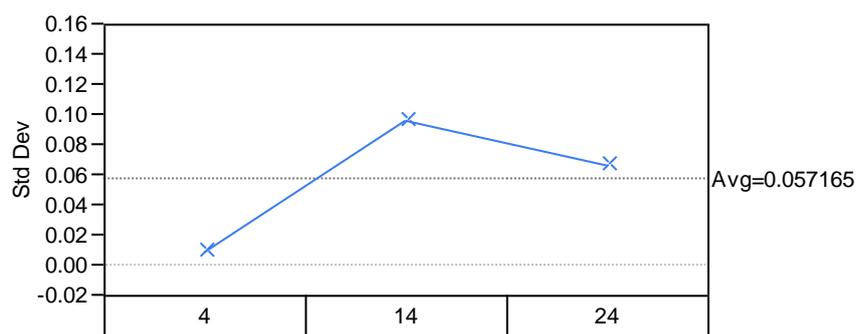


Figure 6.28 Spread of the standard deviation from the models presented

A secondary model for the total population growth rate on herring is presented below in Figure 6.29 along with the coefficients being displayed in Table 6.27. The model has an R squared value of 0.961.

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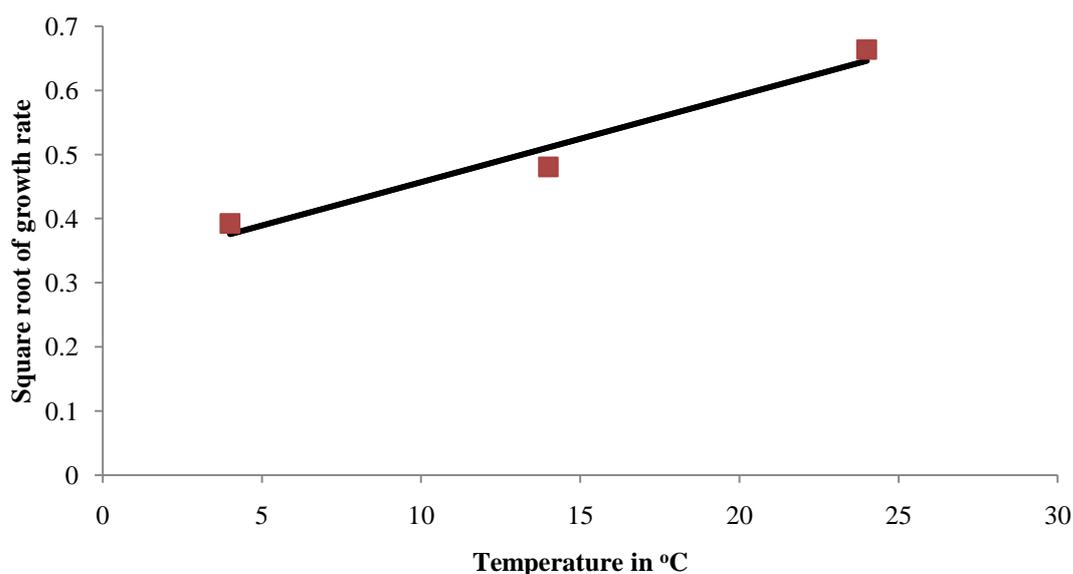


Figure 6.29 Relationship between growth rate and temperature for total population count on herring

Table 6.27 The terms used in the model of total population growth for salmon under a variable temperature

Term	Estimate	Std error
Intercept	0.322	0.0443
Gradient	0.0136	0.00274

The model also shows an improved linear correlation with much tighter upper and lower confidence limits for all values of temperature. This can be seen below in Figure 6.30.

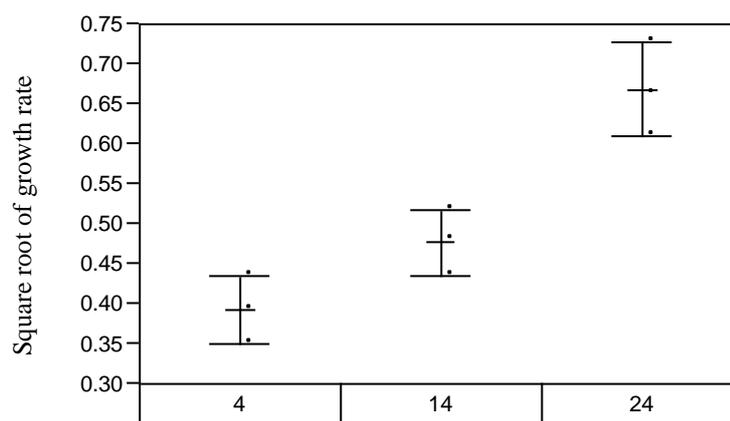


Figure 6.30 Upper and lower limits of the growth rate over the three given temperatures

Figure 6.31 shows that there are fairly similar deviations in growth rates at all temperatures.

Monitoring and Modelling Bacterial Changes in Salmon and Herring

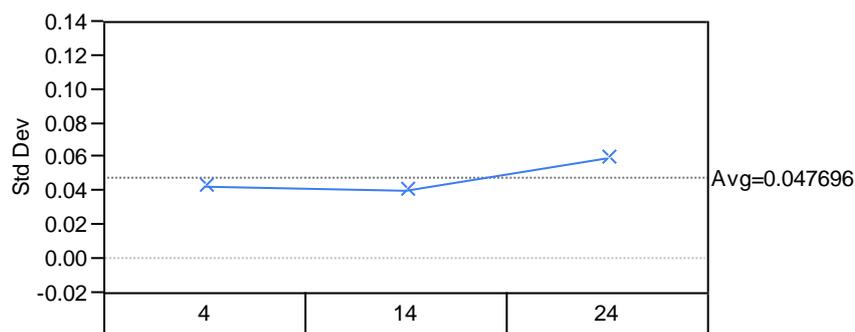


Figure 6.31 Spread of the standard deviation from the models presented

Figure 6.32 shows the model fit of temperature to growth rate of *Pseudomonas* spp. on herring. The model fits a linear correlation well with an R squared value of 0.990.

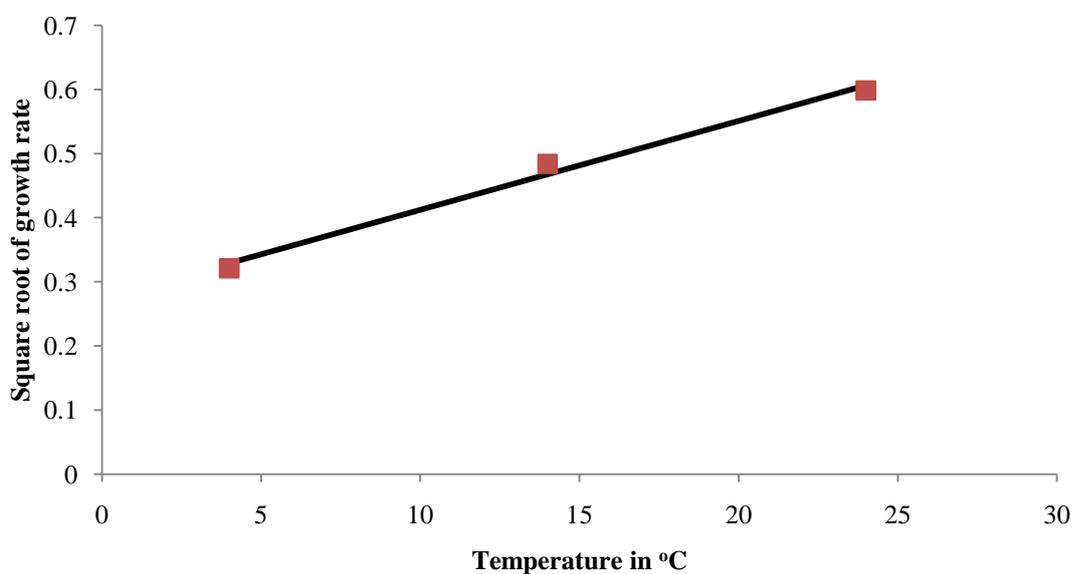


Figure 6.32 Relationship between growth rate and temperature for SSO population count on herring

Table 6.28 shows the values of intercept and gradient for this model.

Table 6.28 The terms used in the model of total population growth for salmon under a variable temperature

Term	Estimate	Std error
Intercept	0.273	0.0226
Gradient	0.00139	0.00139

The fit for this model is much better than the other presented within this chapter, however, the range of growth rate can be seen Figure 6.33. This shows that the upper and lower

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confidence limits for growth rate values are very small in this case and that the primary models fit the data.

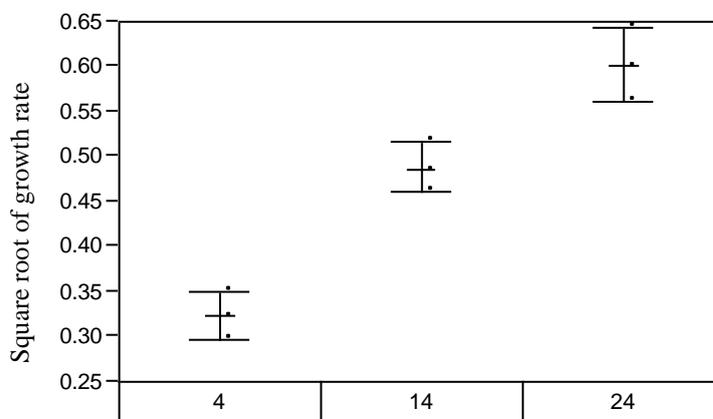


Figure 6.33 Upper and lower limits of the growth rate over the three given temperatures

Figure 6.34 shows the standard deviation for all values of μ over the three temperatures.

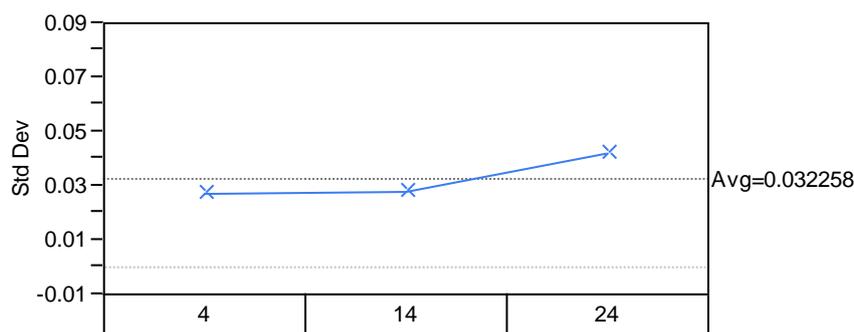


Figure 6.34 Spread of the standard deviation from the models presented

The spread in the standard deviation is again low and is not comparable of the deviation that was seen in the model created for *Pseudomonas* spp. in salmon.

6.6 Conclusions

For the purposes of this thesis, the SSO that has been chosen to study are *Pseudomonas* spp. in reaction to spoilage of salmon and herring. This chapter used the results gained from experiments in determining both total and spoilage populations of bacteria in forming models to estimate growth rates at three temperatures so that secondary models could be used to correlate growth rate to storage temperature. An analysis of the models has also been

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completed with statistical analysis for fit of curve and also the assessment of the upper and lower confidence limits of the value of μ predicted by the models.

This work presented a study on the modelling of bacterial growth on samples of salmon and herring using the Gompertz model as a primary model. This was modified into the Zwietering model so that the growth rate and lag time could be found directly from the simulations used to fit the curves to the raw data. Simulations and the presented models reflect the quality of data that is used as an input. Therefore poor or inherently inconclusive data on bacterial growth will produce an inadequate model. The primary models produced for this experiment show that these techniques can be used to quantify bacterial growth either indirectly (Gompertz model) or directly (Zwietering model).

The poorest models came from the data set with the worst shape for growth (salmon at 14 °C). The modelling software was unable to produce a model with a large range in confidence limits and a relatively high sum of squares value. The experiments were able to show that the growth rates of both the SSO and the total population increase with temperature with a much shorter lag time and that the increase of the square root of growth rate increases linearly with temperature.

The growth rates of SSO and total population for both fish are similar over the range of temperatures with the majority of total population growth rates being greater than that of the *Pseudomonas* spp. growth rate. The lag times for both sets are also comparable apart from the models from the data of salmon at 14 °C which is likely to be caused by poor data from either infection of sample fish or by poor regulation of incubator temperature.

Limitations of this experimental technique include the dependence of good models on robust initial data. The modification of the equation should allow for an easier estimation of growth rates and lag times which have been seen in this chapter. If this technique is to be used in the creation and validation of food sensor in industry then the scale of work would potential require a large amount of resources. This could be resolved using other techniques for population enumeration that were highlighted at the start of this chapter. Other potential hazards include the variability of food as a media for bacterial growth and the fluctuations of temperatures in industrial cold chains. These techniques often gather data at one storage

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temperature to build models that are used to work over a given time and a fluctuating temperature (Dalgaard, 1995a).

Limitations of the experiment include the number of temperatures used in observing the change in growth rate. A more robust model could have been produced if more temperatures had been logged. This would have been a lot more time and resource consuming and may not have improved the model sufficiently to justify this. The models that have been created can be used in conjunction with other analysis carried out in the thesis to show whether thin films of chemically deposited polyaniline can be used as food spoilage sensors.

Chapter 7
Gas Chromatography Mass Spectroscopy and Selective Ion Flow
Tube Mass Spectroscopy Studies of Degrading Salmon and
Herring

7 Introduction

This chapter will investigate the volatile emissions from degrading samples of salmon and herring via two analytical techniques. The results from this section will be used in comparison with the bacterial growth data from the previous chapter and the sensor response to these volatiles in the subsequent chapter.

The first technique will be gas chromatography mass spectroscopy used to qualitatively analyse the change in headspace constitution as the food samples degrade. The second technique, selected ion flow tube mass spectroscopy, will be used to quantitatively estimate the changes in the headspace. These techniques have been chosen for comparison as they both use different methods to analyse gas samples.

7.1 Gas chromatography and mass spectroscopy

Gas chromatography mass spectroscopy (GCMS) has become a mainstay of modern analytical chemistry. Chromatography refers to the separation of components in a mixture via a moving phase such as a gas or liquid. The modern market for GC instruments sales annually is estimated to be over \$1 billion (McNair, H.M, 1998). The technique uses two principle components to analyse volatile and gaseous compounds within a given sample. These are the gas chromatography column and the mass spectrometer used to identify species that have been separated. The technique first requires separation of the gas constituents followed by individual analysis of each separated component via a mass spectrometer unit.

For the purposes of this thesis, this technique has been used to qualitatively select marker gases that can be used as spoilage indicators. The literature review has already suggested that for samples of fish-amines and ammonia are the most favoured gases that could be used as spoilage indicators and that polyaniline is a suitable material for the detection of these species.

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7.1.1 Overview of concepts of gas chromatography and mass spectroscopy

Gas chromatography mass spectrometry requires an inert carrier gas to flow gaseous samples through a heated column and into a mass spectrum unit. The principles of gas chromatography are similar to those of all chromatography techniques, in that the aim is to separate a complicated mixture of gases so that they can be identified. Figure 7.1 shows a schematic overview of a generalised GCMS system with the key component labelled.

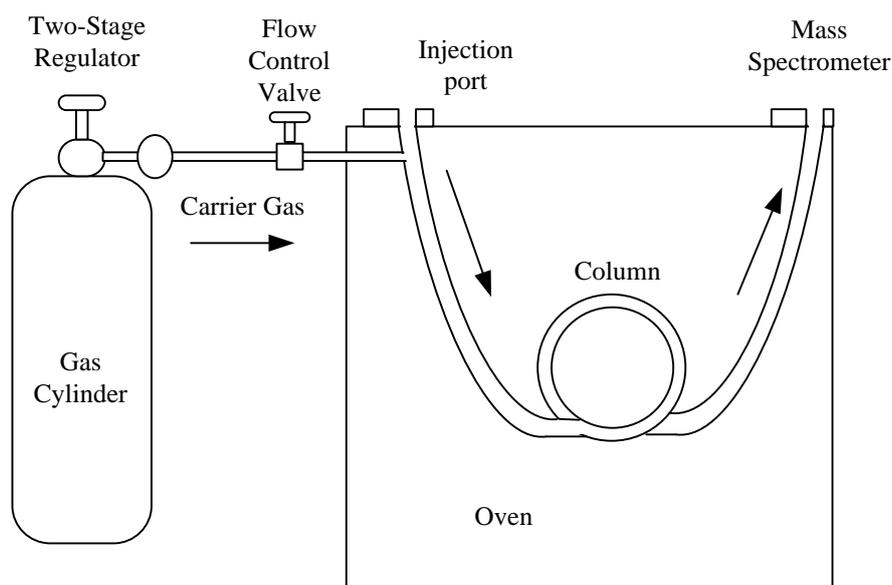
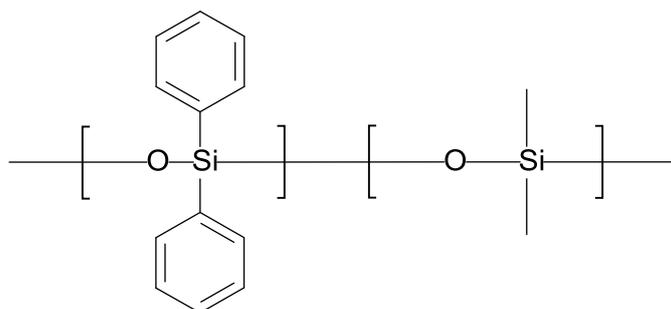


Figure 7.1 Simplified overview of a GCMS

A constant flow rate of carrier gas is streamed through the system and samples are injected into the injection port to pass through the column for separation. The separated gases emerge at the other end of the column and flow directly into a mass spectrometer. The mass spectrum is constantly completing a scan at a set frequency so that the output at any time on a chromatogram can be viewed. Separation of gases occurs in the column which can be up to 60 metres in length. The flow rate and length of column determine the amount of time it takes for the carrier gas to flow through the column. Similar to liquid chromatography, the column is packed with an adsorbing solute or lining so that the gaseous species can separate. For these experiments, a capillary column was used with a fused silica lining with a polysiloxane 6% cyanopropylphenyl and 94% dimethylpolysiloxane (Figure 7.2) which acted as the porous stationary phase. Table 7.1 shows a comparison of column length against experimental factors such as speed and resolution.

Table 7.1 Comparison of column length and its effect on experimental method

Column length	Resolution	Speed of analysis
Long (60-100 m)	High	Slow
Short (5-10 m)	Moderate	Fast
Medium (25 30 m)	Good compromise/starting point	Good compromise/starting point

**Figure 7.2 Chemical structure of the polysiloxane coating of the column**

The polysiloxane allows for weak and strong molecular interaction between gas samples and the lining of the wall of the column. These include Van der Waal and Debye permanent induction forces. The lack of hydroxyl groups on the polysiloxane explains why this column is not an optimum choice for the species of interest in these experiments (amines and sulphides) as there are limited sites for absorbance. Experiments using this technique and equipment will still be able to supply information on the qualitative increase of potential spoilage indicator compounds.

In this case, software by AMDIS was used in the deconvolution of the chromatograms that were produced from the raw data. An example of one of these chromatograms can be seen in Figure 7.3. The blue cursor indicates the time after the start of analysis which corresponds to a mass spectrum that was taken at that time as gas was leaving the column.

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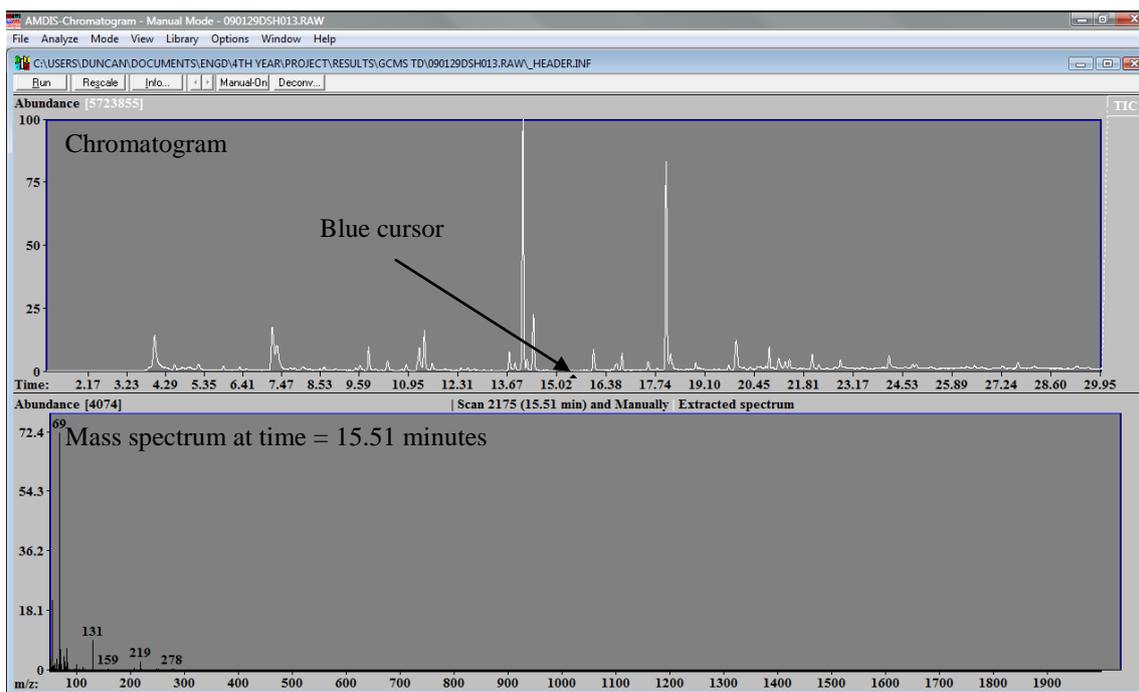


Figure 7.3 Screen print of the AMDIS software showing a chromatogram (top) and mass spectrum for selected time (below)

The view shows the complete chromatogram with the mass spectra that was taken at 15.51 minutes. The spectra can then be used to identify what gas caused the observed peak by observing specific fragment patterns or by whole mass. This can often be complicated due to high numbers of fragments caused by larger molecules. This difficulty is overcome by using a compound search library to determine matching patterns and fragments.

7.1.2 Overview of GCMS TD instrument

The instrument that was used in these sets of experiments uses a thermal desorption unit for gas sample injection. The brass tubes that were used to collect and store samples from the headspace of the degrading fish are filled with an adsorbing material. As the gas from the salmon and herring samples is passed through the tube, molecules of the volatile gas are adsorbed onto the sorbent and can be stored at low temperature (below 5 °C) for a week. Before analysis takes place, the tubes are loaded with a known amount of standard which is inert and unusual enough as to not be confused with any gaseous species already on the tubes. In this case the standard is fully deuterated toluene as can be seen below in Figure 7.4.

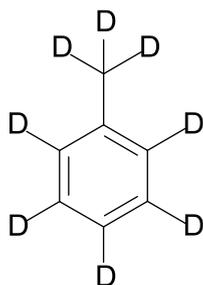


Figure 7.4 Structure of the standard used in the experiments involving GCMS

To remove the gaseous species from the sorbent, the tubes are purged at high temperature onto a cold trap onboard the sample injector ion the GCMS system. The cold trap is then heated to temperatures above 300 °C and then injected into the GC column. The column is then heated over a set time period with either a steady temperature increase or a changing rate of temperature increase. Whilst this is happening, the gases collected from the experiment are passing through the column and become separated as discussed previously. The exhaust from the column is continually registered by the mass spectrometer and several spectra are produced per second which are then used in identifying the separate gas species.

7.2 Selected Ion Flow Tube Spectroscopy

Selected ion flow tube mass spectroscopy (SIFT-MS) is a relatively new technique for the simultaneous real time quantification of trace gases. SIFT-MS has been used in a variety of different situations to quantitatively measure several trace gases. Like most mass spectroscopy based techniques SIFT-MS relies on the creation of ionic species which are then detected and counted. The significance difference with this technique is the manner in which the sample is ionised. SIFT-MS compares the chemical ionisation of the sample with three precursor ions (H_3O^+ , NO^+ and $\text{O}_2^{+\bullet}$) which react with the trace gases for a defined space of time. Flow tubes coupled with mass spectrometers are often used in physical chemistry to analyse kinetic data for certain reactions. With the addition of selected ions into the flow tube as well as knowing the reaction constants- k , for the volatiles that are being analysed then concentrations can be calculated. This will be covered later in this chapter.

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7.2.1 Overview of SIFT MS

Mass spectroscopy has become a vital and popular means of analysing substances for molecular weights, structure and composition. All techniques of mass spectroscopy require charged particles for the apparatus to work successfully. Rather than using 'hard' ionisation techniques, such as electrospray ionisation or fast atom bombardment, SIFT-MS uses chemical ionisation. Chemical ionisation requires proton transfer from cations produced from an ion source. The proton transfer takes place in a flow tube of set length whilst in a stream of inert helium carrier gas.

SIFT-MS is also a technique that can be used for a wide range of trace gas detection and quantification in real-time. Compared to other electron ionising mass spectroscopy techniques, SIFT-MS does not require selective membranes to lower the amount of gas loading at the ion source from water vapour, O_2 and N_2 . These membranes are expensive and sometimes can inhibit the transfer of trace gasses of interest (Smith and Sparks, 2004). Figure 7.5 below shows the layout of the instrument.

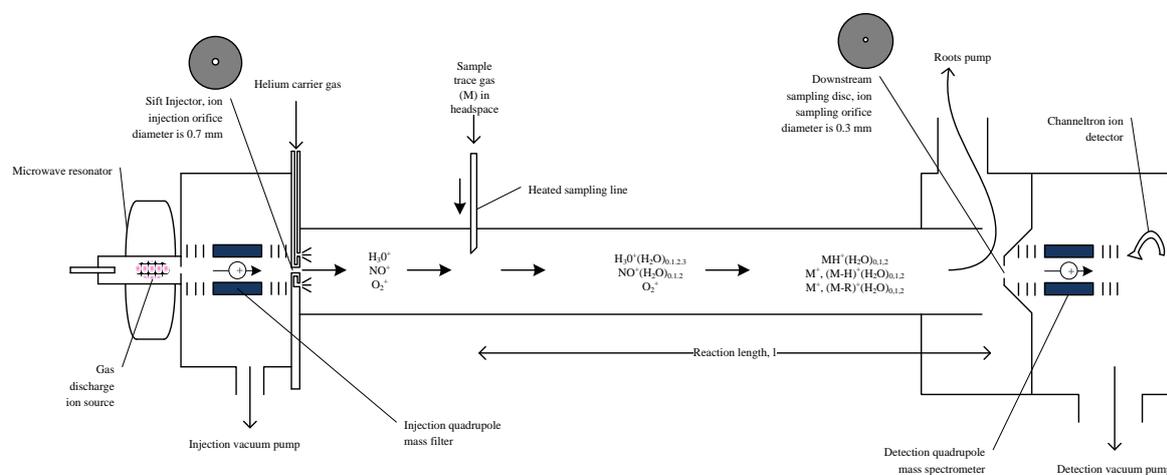


Figure 7.5 Layout of a SIFT-MS (adapted: Smith and Sparks, 2004)

As described previously, the analyte reacts with one of the three precursor ions (H_3O^+ , NO^+ and O_2^+) once it has entered the SIFT-MS. The ionisation reaction is allowed to proceed for the amount of time it takes from the length of the flow tube at a given flow rate. At the end of the chamber is an ion detector which can only distinguish charged species. A comparison between the two techniques used in this chapter can be seen below in Table 7.2.

Table 7.2 Comparisons of the two techniques for the analysis of headspace volatiles (Source: Amann et al., 2007)

Characteristic	GCMS	SIFT-MS
Ionisation method	Electron impact ionisation or chemical ionisation	Chemical ionisation using precursors
Used to detect	Molecular fragments and identification using fragmentation patterns	A variety of ionic species characteristic of the precursor ions and the reactant molecules
Fragmentation	Unavoidable and desirable as substances can be identified	Does not occur but is well characterised for many reactions by SIFT studies
Substances which cannot be detected	Depends on pre-concentration; ethane can't be detected at ppb levels using SPME	Lower order hydrocarbons and species of low proton affinity and high ionisation energies
Advantages	Different substances in gas sample can be identified	On-line, real time absolute quantification of several compounds simultaneously to good accuracy. Water vapour used as internal calibration
Potential problems	On-line measurement is only possible in chemical ionisation mode, different protocols for different classes of substances; water in samples may cause problems	Identification of isomers is sometimes difficult
Limit of detection	Depends on pre-concentration method and detector (ppb and even ppt)	Currently at 0.1 ppb s ⁻¹
Calibration	The whole chain of sample preparation and measurement has to be calibrated for each compound separately	Once the reaction time and mass discrimination are determined for a given instrument, universal kinetic library files can be used for absolute quantification

The counts of ions that are collected by the ion detector then have to be converted into meaningful approximations of concentrations of expected analyte. There are several

instrumental factors that need to be considered before these calculations can be made. These are as follows:

- The flow rate of the sample into the reaction chamber. The sample flow tube is a heated capillary which has a limited surface area to prevent loss of trace gasses by surface adsorption. Therefore there is also a minimised effect of species from previous samples appearing again in further experiments. The flow rate of samples in the capillary can also change as the tube is heated. In order to overcome this sampling is delayed until the temperature is stabilised.
- Flow rate of the carrier gas (helium). This is a straight forward measurement and is regularly checked on the instrument used in these experiments.
- Flow tube pressure. The SIFT-MS instrument used in these experiments has a gauge to monitor the pressure in the flow tube. In other systems there may be a need for a calibration factor to account for any pressure drops between the gauge position and the midpoint pressure in the flow tube.
- Flow tube temperature. The flow tube temperature has a direct effect on the quantification of volatile gases and should be checked and monitored frequently.
- Precursor and product ion intensities. As previously stated, the data that is collected from the MS unit of the SIFT is interpreted as count rates. This data represents the ion swarm that is being delivered to the end chamber through the sampling orifice. The physical measurement of counts relies on the number densities of ions with different mass to charge ratios (m/z) similar to all over practices of mass spectroscopy. In the equipment that is being used in these experiments there are two effects that are accounted for in calculations. The first of these is the effective dead time of the combination of the electron multiplier, the pulse amplifier and the discriminator and is labelled τ_d . If this effect is not accounted for it can distort the count rates if they are high. The τ_d is characterised by using the known abundance ratio of two isomers which include two isotopes. An example of this is the H_3O^+ molecule with an $\text{O}^{18}/\text{O}^{16}$ ratio of 4×10^{-3}). The other factor is the mass

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discrimination factor M_r which is any mass discrimination that is found in the analytical spectrometer or ion detection system. This can be characterised by comparing the measured ion count rate to ion current collected by the downstream sampling disc (which has an inbuilt ammeter) for species of known m/z values.

The mass discrimination factor can be calculated using the H_3O^+ precursor m/z value of 19 and the two constants of f_{m1} and f_{m2} which are calculated by the Sift-MS equipment for a given resolution setting of the analytical quadrupole mass spectrometer. The equation is entirely empirical and should be used with caution when using other SIFT-MS systems. The equation can be seen below in Equation 7.1.

Equation 7.1 Calculation of the mass discrimination factor (Source: (Španěl et al., 2006))

$$M_r(m/z) = 1 + f_{m1}(m/z - 19) + f_{m2}(m/z - 19)^2$$

The calculated value is then used in calculating the corrected ion signal, I , with the dead time and the multiplier count rate N/t_s . The symbol t_s corresponds to the time used to acquire the number of counts, N . The equation for the dead time corrected signal intensity can be seen below in Equation 7.2.

Equation 7.2 Calculation of the dead time corrected ion signal (Source: (Španěl et al., 2006))

$$I = M_r(m/z) \frac{N/t_s}{1 - \tau_d N/t_s}$$

The reaction time of the volatiles entering the tube is related to the length of the flow tube, l , and the mean velocity of the ions along the flow tube v_i , this is expressed below in Equation 7.3.

Equation 7.3 The calculation of the reaction time (Source: Španěl et al., 2006)

$$t_r = \frac{l}{v_i}$$

The length is considered to be the geometric distance between the sample injection point and the sampling orifice. The mean velocity flow rate is calculated from the flow rate of the carrier gas and the sample of volatiles injected into the system (Španěl et al., 2006). The reaction of specific volatile species with the ion precursors is estimated from two principle components. These are the reaction constants, k , and the diffusion coefficients, D . Both of these constants can be found either from data tables or studies in the literature. The SIFT-MS instrument can also be used to determine these values if pure volatiles are injected into the equipment (Španěl et al., 2006; Spanel and Smith, 1998a; Spanel and Smith, 1998b).

An example of the reaction that occurs for ammonia using the H_3O^+ precursor can be seen below in Figure 7.6.

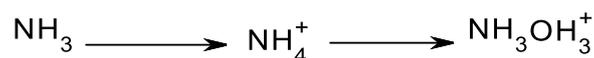


Figure 7.6 Reaction scheme for the first two ionisations of ammonia

Here, the reactions are both dependant on the diffusion of ammonia in the flow tube as well as the reaction constants for the ionisation of ammonia and the addition of a precursor ion to create an ionic species. These will be seen as peaks on the spectrum at 18 and 36 respectively. From knowing the value of the reaction rate and the diffusion coefficient, actual concentrations can be calculated using the following model in Equation 7.4 which is calculated via the SIFT-MS software.

Equation 7.4 Equation used in within the SIFT-MS software for calculations of absolute concentrations of trace gases

$$[M] = \frac{1}{k_1 t_r} \ln \left(1 + k_1 \frac{f_{p1} I_{p1} / D_{ep1} + f_{p2} I_{p2} / D_{ep2} + \dots}{f_{i1} I_{i1} k_1 + f_{i2} I_{i2} (k_1 + k_2) / 2 / D_{ei2} + \dots} \right)$$

The above equation uses f_p coefficients which correspond to the products formed from reaction with the precursors and are usually equal to 1. The symbol I_p corresponds to the ion signals for all the products ions after being corrected for the dead time and mass discrimination. The coefficients on the bottom row correspond to the reactions that occur for

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the precursor ion species and are used as a relative comparison to the increase in signal of the interrogated volatile. The values for the constants are common for all injected samples and values for corrected signals are used from the collected data. The expression is given in the expanded logarithmic form to correct for the decrease in the precursor signal which is observed when large concentrations of the interrogated analyte are present.

7.2.2 Usage of SIFT MS instruments

The SIFT MS technique has been used in a variety of situations where on-line trace gas analysis is required. These applications include breath analysis (Španěl et al., 2006; Smith and Španěl, 2005), food flavouring compounds (Španěl and Smith, 1999) and specific compounds such as amines (Spanel and Smith, 1998a) as well as sulphur containing organic molecules (Spanel and Smith, 1998b).

The technique has had relative success in these areas and the above studies have been used in the collection of data (k values, ionic precursor interactions and reactions). The SIFT-MS can be used to directly measure the components of gas samples including breath. The technique requires no sample storage or preparation. The interfacing software can also be setup to show either a full scan array of the injected sample or a graphical representation of actual concentration increase for target molecules. The latter interface has been suggested for use in patient screening for diseases that produce atypical trace compounds in breath. The equipment would be able to provide quick and reliable results without invasive sampling methods being used (Amann et al., 2007; Wang et al., 2008).

Other research of interest in this area includes a study into the volatile compounds produced by *Pseudomonas* spp. and related bacteria (Wang et al., 2004). The species that were considered in this paper were highly substituted alcohols, alkenes and esters such as 2-methyl-1-butanol and 4-methyl-pentadiene. The authors also commented that volatiles such as trimethyl amine, ammonia, and some organosulphides could also be used in detecting growth of *Pseudomonas* spp. (Spanel and Smith, 1998a; Spanel and Smith, 1998b; Wang et al., 2004).

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7.2.3 Sample analysis of SIFT MS spectra

An example of the spectra produced from the SIFT-MS can be seen below in Figure 7.7.

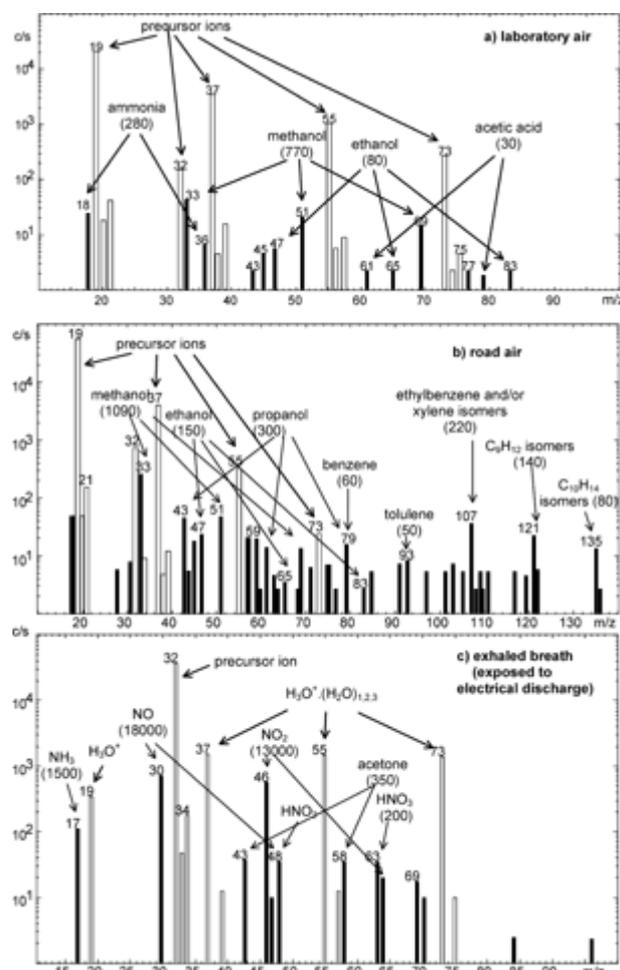


Figure 7.7 a, b+c An example of three SIFT-MS spectra showing the precursor peaks (H_3O^+) in white (Source: Smith and Španěl, 2005)

The spectrum produced is a complete analysis of (m/z) of the whole injected sample with count rates displayed on the y axis. In comparison to the GCMS method, no previous separation of gases is required as the sample is injected in bulk. The white bars indicate precursor and precursor complexes and the black bars indicate volatile species present in the injected sample that have either become charged by chemical ionisation by reacting with the precursor or have become encapsulated in a complex with the precursor ions. The spectra in Figure 7.7 show the difference in air collected from the laboratory (a), the road (b), and exhaled from breath (c).

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The obvious limitations of this technique compared with GCMS is that it requires the interrogated species to be able to be chemically ionised which is overcome by using several ionic precursors that have a low activation energy of reaction with the majority of volatile gases of interest.

7.3 Results from TD-GCMS study

As previously stated, the use of GCMS in this thesis is for qualitative analysis of the species present in degrading samples of salmon and herring. GCMS can be used as an accurate quantitative method; in this case however, the choice of column and the method of sample loading are not appropriate for analysis of the type of volatiles that are expected to be produced. In this case the SIFT MS is able to offer relatively faster and accurate approximations of concentrations of trace volatiles. The pathways of these techniques are shown below in Figure 7.8 which shows that the SIFT MS technique is a more direct approach to measuring sample headspace.

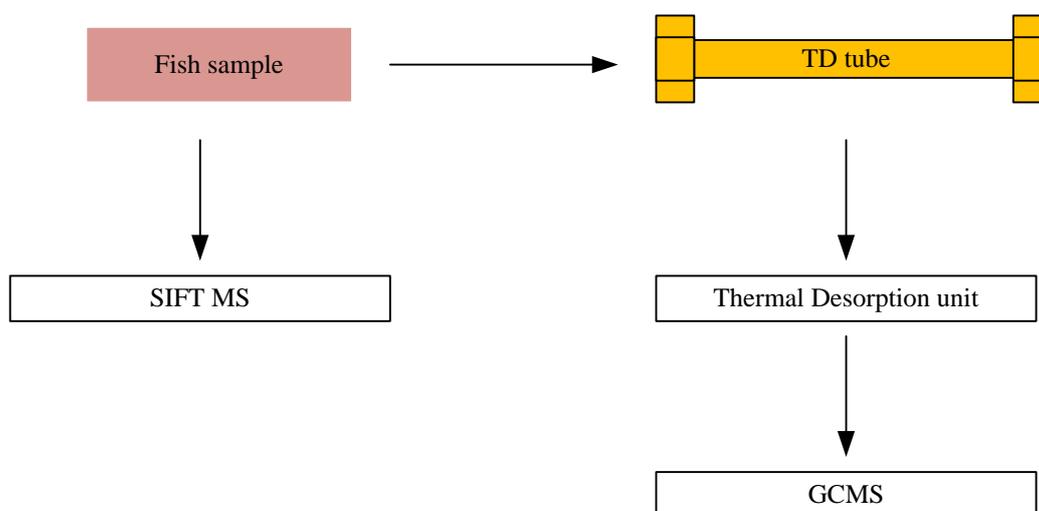


Figure 7.8 Pathways of analysis using both sets of equipment

The results from these experiments will correspond to reports in the literature for increases of spoilage markers such as trimethyl amine and other nitrogen containing compounds as well as sulphur containing compounds and other organic volatile organics (esters, carbonyls and alcohols etc). Ammonia has been omitted from this study as the relative atomic mass of the molecule was below the lower limit set by the equipment.

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7.3.1 Salmon

During the process of decay it was observed that the change in odour from fresh samples to sour and putrid samples followed after a certain number of days at room temperature. The initial experiments were used to find species of interest for the SIFT-MS experiments as well as for comparison with existing data already published in the literature. As already stated, the main compound of interest as a marker for fish spoilage is the production of trimethyl amine. Due to the setup of the machine, the detection of ammonia was below the lower limit of m/z ratio of the mass spectrometer to cut out readings of lower mass species such as water, helium carrier gas and molecular nitrogen found in air.

Table 7.4 gives the main compounds of interest discovered from degrading samples of salmon at room temperature.

Table 7.3 The major compounds found from degrading salmon at room temperature

Nitrogen	Sulphur	alcohols	Carbonyl	Miscellaneous
Trimethyl amine, pyrrole, acetonitrile	Dimethyl trisulphide, dimethyl disulphide, Cystine, sulphur dioxide	Phenol, Ethanol, isopropyl alcohol, pentanol	Butanone, butandione, pentanal	Acetic acid, carbon dioxide, sulphuric acid, hexamethyl cyclotrisiloxane, acetic anhydride

The main species that were seen to increase over the spoilage period were sulphur containing compounds such as dimethyl disulphide and hydrogen sulphide as well as alcohols and trimethyl amine. The combination of these volatile and strongly scented gases with other scented species such as esters and carbonyl compounds. The production of the siloxane species is a by product of the column being used in these experiments.

7.3.2 Herring

The above experiment was repeated with herring samples, Table 7.4 gives the major volatile compounds present in the headspace analysis using the GCMS TD technique.

Table 7.4 The major compounds found from degrading herring at room temperature

Nitrogen	Sulphur	alcohols	Carbonyl	Miscellaneous
Trimethyl amine, pyrrole, acetonitrile, ethylenimine	Dimethyl trisulphide, dimethyl disulphide, Cystine, sulphur dioxide, methyl sulphinyl ethene	Phenol, Ethanol, isopropyl alcohol, pentanol	Butanone, pentanal, hexanal, acetone	Acetic acid, carbon dioxide, sulphuric acid, hexamethyl cyclotrisiloxane, acetic anhydride

Again, the major nitrogen containing species was trimethyl amine due to the inability of the technique to register molecules of ammonia. Sulphide species were relatively lower compared to samples of salmon although this may have been caused by the presence of more amines and other gases. The production of the siloxane species is a by product of the column being used in these experiments.

There was also a complicated mixture of oxygen containing alkanes and alkenes which added to the aroma produced by the rotting sample. It was observed that the smell from herring was much more noticeable at an earlier time frame when compared to the time taken to register spoilage odours from salmon. After approximately 1 day of storage at room temperature the aroma of rotting fish was observed from herring; in salmon it took an extra day for a similar strength of aroma to be produced. The smell from both fish was also slightly different as can be expected due to the condition of both fish at point of purchase and the different biological composition. Herring samples were noted for their stronger and more “fishy” aromas and salmon was noted for its more pungent, egg like odour.

Further work was required to study the change in concentration of spoilage marker volatiles and so SIFT-MS was used to quantifiably study the change in selected species over a time period of seven days and a variety of storage temperatures. The selected spoilage indicators were trimethyl amine, ammonia, dimethyl amine, hydrogen sulphide and disulphide dimethyl.

7.4 Results from SIFT-MS study

The results that are presented within this section are quantitative values of the selected spoilage markers found in degrading salmon and herring. These have been selected using the GCMS technique from the above experiments. Absolute concentrations of the headspace gases are shown here in either parts per million or parts per billion. The SIFT-MS equipment is noted as having accuracy down to 0.1 parts per billion and can measure with relative accuracy up to 20 ppm (Španěl et al., 2006). The selected species are expected to react in the following way as seen below in Table 7.5 with complex ions from precursors also formed.

Table 7.5 The chosen markers for spoilage indication for salmon and herring with their corresponding reaction with each SIFT-MS precursor (Source: (Spanel and Smith, 1998a; Spanel and Smith, 1998b; Scaronpanel and Smith, 2000))

Volatile	Atomic mass	Reaction with H_3O^+	Reaction with NO^+	Reaction with O_2^+
Ammonia	17	NH_4^+ (100)	No reaction	NH_3^+ (100)
Trimethyl amine	59	$(\text{CH}_3)_3\text{N.H}^+$ (90) $\text{C}_3\text{H}_8\text{N}^+$ (10)	$(\text{CH}_3)_3\text{N.H}^+$ (100)	$\text{C}_3\text{H}_8\text{N}^+$ (65) $(\text{CH}_3)_3\text{N}^+$ (35)
Dimethyl amine	45	$(\text{CH}_3)_2\text{NH.H}^+$ (100)	$(\text{CH}_3)_2\text{NH.H}^+$ (95) $\text{C}_2\text{H}_6\text{N}^+$ (5)	$(\text{CH}_3)_2\text{NH.H}^+$ (70) $\text{C}_2\text{H}_6\text{N}^+$ (30)
Hydrogen sulphide	34	H_3S^+ (100)	No reaction	H_2S^+ (100)
Dimethyl disulphide	62	$(\text{CH}_3)_2\text{S}_2\text{H}^+$ (100)	$(\text{CH}_3)_2\text{S}_2^+$ (100)	$(\text{CH}_3)_2\text{S}_2^+$ (80) $\text{CH}_3\text{CH}_2\text{S}^+$ (10) CH_3S_2^+ (5) CH_2S^+ (5)

The following gases were chosen for separate reasons. In the literature, fish quality is often linked to volatile amine and ammonia (Oehlenschläger, 1997). Combined with sulphurous compounds they are often responsible for the recognisable fishy off-odour related to fish spoilage. Trimethyl amine was specifically chosen due to the nature of its production in spoiling fish with the breakdown of trimethyl amine oxide (Jay et al., 2005). Dimethyl amine was selected to be a comparison gas for these reactions. Although the concentration of dimethyl amine does increase with spoilage, it does so at a much slower rate. Ammonia is produced from the breakdown of amino acids and proteins by bacteria and is used as an overall indicator of bacterial growth. Total volatile basic nitrogen (TVB-N) is also approximated by totalling the concentrations of the most abundant nitrogen containing compounds that can act as a proton donor. This is important as the sensor produced for this thesis is likely to react with any available basic species.

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The sulphur compounds were chosen to compare to the concentration of the nitrogen compounds and also to compare to the observations of increased odour.

All the kinetic data and diffusion coefficients were taken from previous papers and loaded into the SIFT-MS library so that absolute concentrations could be approximated using the automation mode on the SIFT-MS interface software.

7.4.1 Salmon

The first set of experiments used 5 separate samples of salmon to monitor the change in nitrogen containing compounds and was recorded at 24°C. The results from this can be seen below in Figure 7.9.

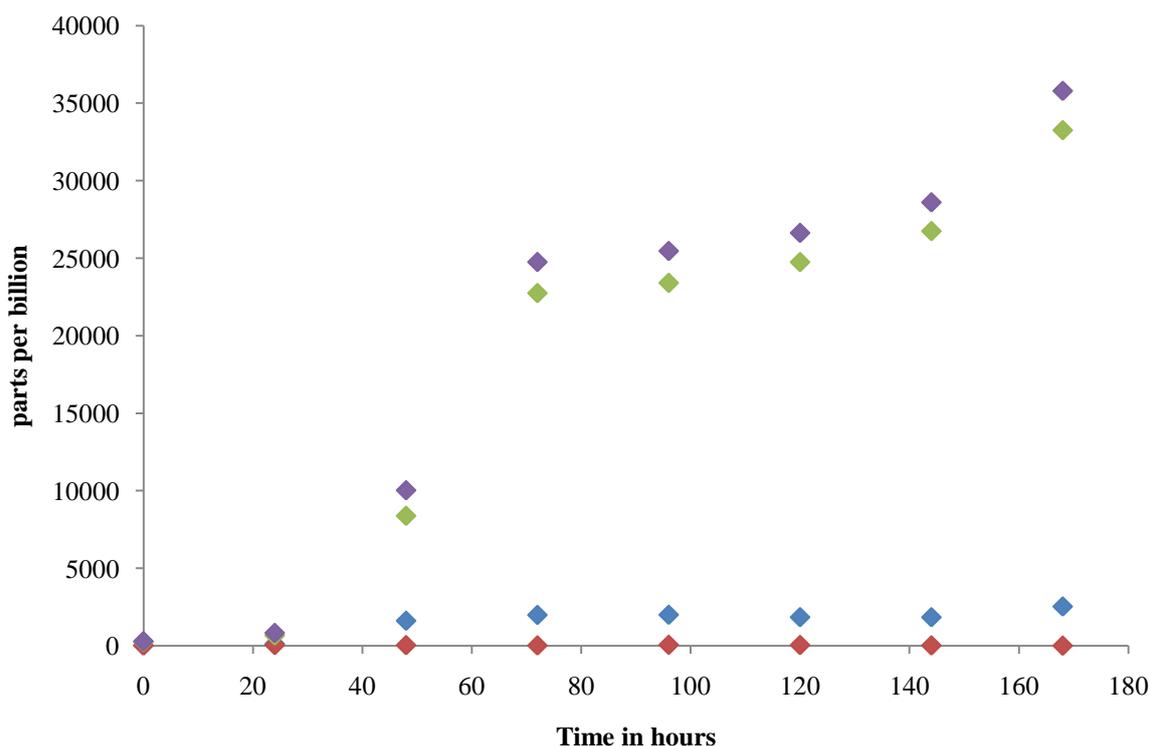


Figure 7.9 SIFT-MS analysis of degrading salmon at 24 °C (■ = total basic nitrogen, ■ = ammonia, ■ = trimethyl amine and ■ = dimethyl amine)

At this high temperature the spoilage rate of the fish sample is expected to be high and the recorded values for the bacterial growth shows that growth rate is near to the highest optimum value (Huss et al., 1997). Therefore the breakdown of nutrients on the fish flesh is

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occurring at the fastest rate. The TVB-N rises very sharply after 24 hours and then flattens out after 72 hours. This expected to be an exponential increase but possible due to sampling error or standard error within the samples the flatness is not observed. Instead, after 6 days there is apparent further production of TVB-N.

The majority of this TVB-N is ammonia which mirrors the pattern of rise in concentration of the TVB-N. The increase of ammonia is again an exponential growth after 24 hours and flattens after approximately 72 hours. Starting concentration of ammonia in the headspace of fish was quite high at around 500 ppb and rose to nearly a 100 fold increase of 40000.

The remaining compounds of trimethyl amine and dimethyl amine (TMA and DMA) behaved similarly but with the expected changes as expressed in the literature (Pacquit et al., 2008). Although the levels do not reach the same magnitude, the rise of TMA concentration is similar to that of ammonia and in the case of TVB-N, the original concentration was lower than 20 ppb and rose to 2537 ppb.

On the other hand, the levels of DMA stayed fairly constant at around the 40 ppb levels with no exponential rise until day 7 where the level was 169 ppb. This is as expected as volatile DMA is known to evolve from fish where degradation is reliant on the TMAOase enzyme similar to TMA.

The next set of experimental data was taken at a lower temperature of 14°C which was also previously used in the bacterial growth experiments. The result from this can be seen in Figure 7.10.

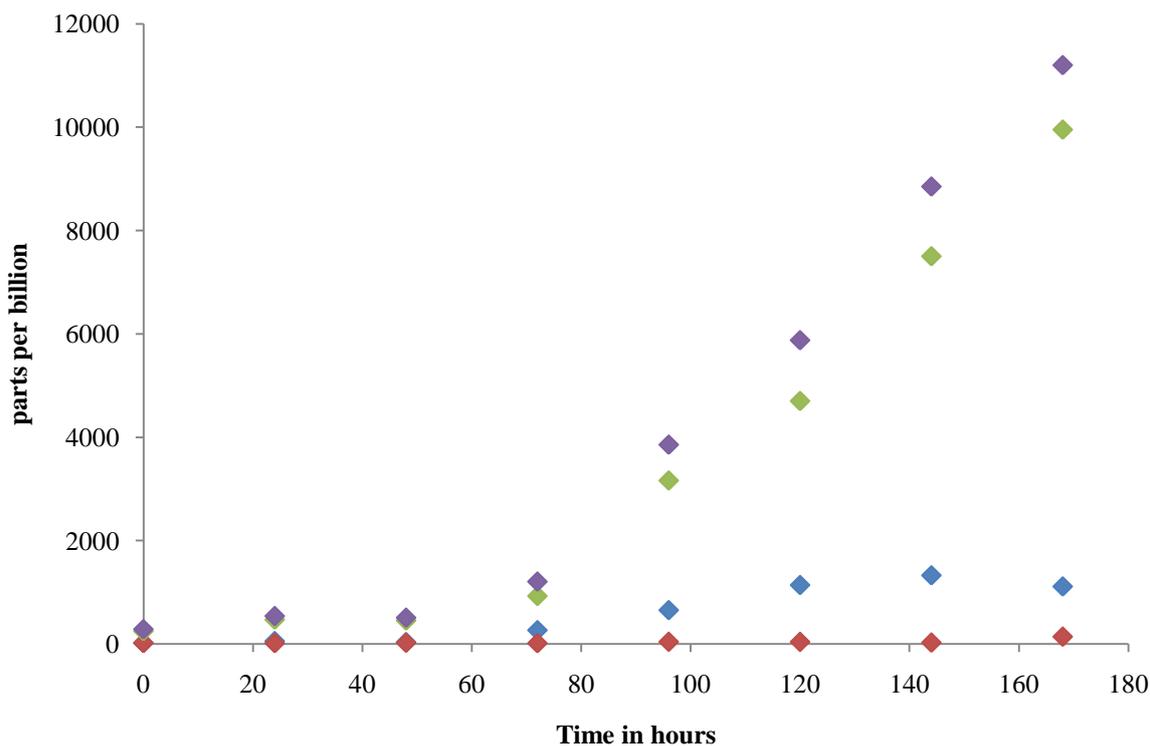


Figure 7.10 SIFT-MS analysis of degrading salmon at 14 °C (■ = total basic nitrogen, ■ = ammonia, ■ = trimethyl amine and ■ = dimethyl amine)

At this lower temperature the breakdown of amino acids and proteins by bacteria and autolysis is slowed down compared to the previous higher temperature experiment. This is reflected in the results where the concentration of TVB-N does not start to increase until after 48 hours, where the increase rises steadily after this time from an initial concentration of around 500 ppb to over 10000 ppb. Again the major component of this gas is ammonia which makes up nearly 90 % of all TVB-N in the headspace.

Ammonia rises from initial levels close to 400 ppb and rises to nearly 10000 ppb following the same pattern for TVB-N. The amount is much less than that observed at a higher temperature and it is expected that that after 7 days the amount would still rise as the graph shows no signs of levelling out.

TMA levels also show a similar pattern of increase but on a much smaller scale. At this temperature observed levels of TMA rose from approximately 18 ppb to 1100 ppb. The rise in concentration started after approximately 48 hours and rose much faster relative to the change seen in TVB-N with ammonia and the graph appearing to level out after 6 days. This

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is possibly due to the growth of bacteria reaching the stationary phase or the breakdown of the TMAOase enzyme under these conditions.

DMA levels follow a very similar pattern to those observed at 24 °C with the initial level of concentration not changing until day 7 when there is a slight rise from approximately 40 ppb to 130 ppb.

The next set of experimental data concerning changes in concentration of nitrogen containing compounds in the headspace was conducted at 4°C. A graph showing these results can be seen below in Figure 7.11.

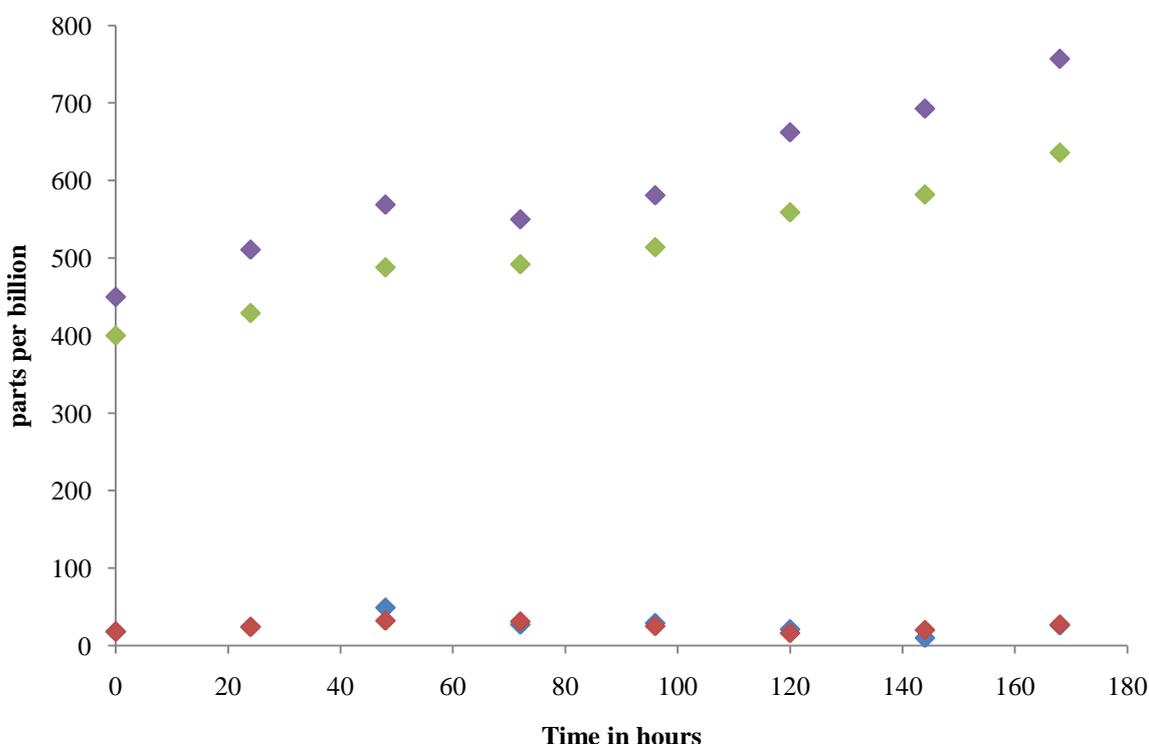


Figure 7.11 SIFT-MS analysis of degrading salmon at 4 °C (■ = total basic nitrogen, ■ = ammonia, ■ = trimethyl amine and ■ = dimethyl amine)

Here the initial levels of TVB-N concentrations can be seen quite clearly at around 550 ppb, and the increase in concentration is much slower than the rate seen at higher temperatures. After 7 days the concentration is around 700 ppb. As before, the major component of this collection of nitrogen containing gases is ammonia which rises from levels of 400 ppb to 636 ppb. Both of these components rise in concentration levels after 96 hours and the rate of increase is retarded compared to higher temperatures.

The levels of both TMA and DMA stay fairly constant around the 20 ppb concentrations. This is because bacterial activity and enzymatic breakdown of TMAO is slower at this temperature as expected from the previous chapter.

A further study of the increase in TMA and TVB-N was carried out using the aluminium temperature block so that the effect of temperature on TVB-N and TMA concentrations could be monitored as well as time. This data is represented below in Figure 7.12.

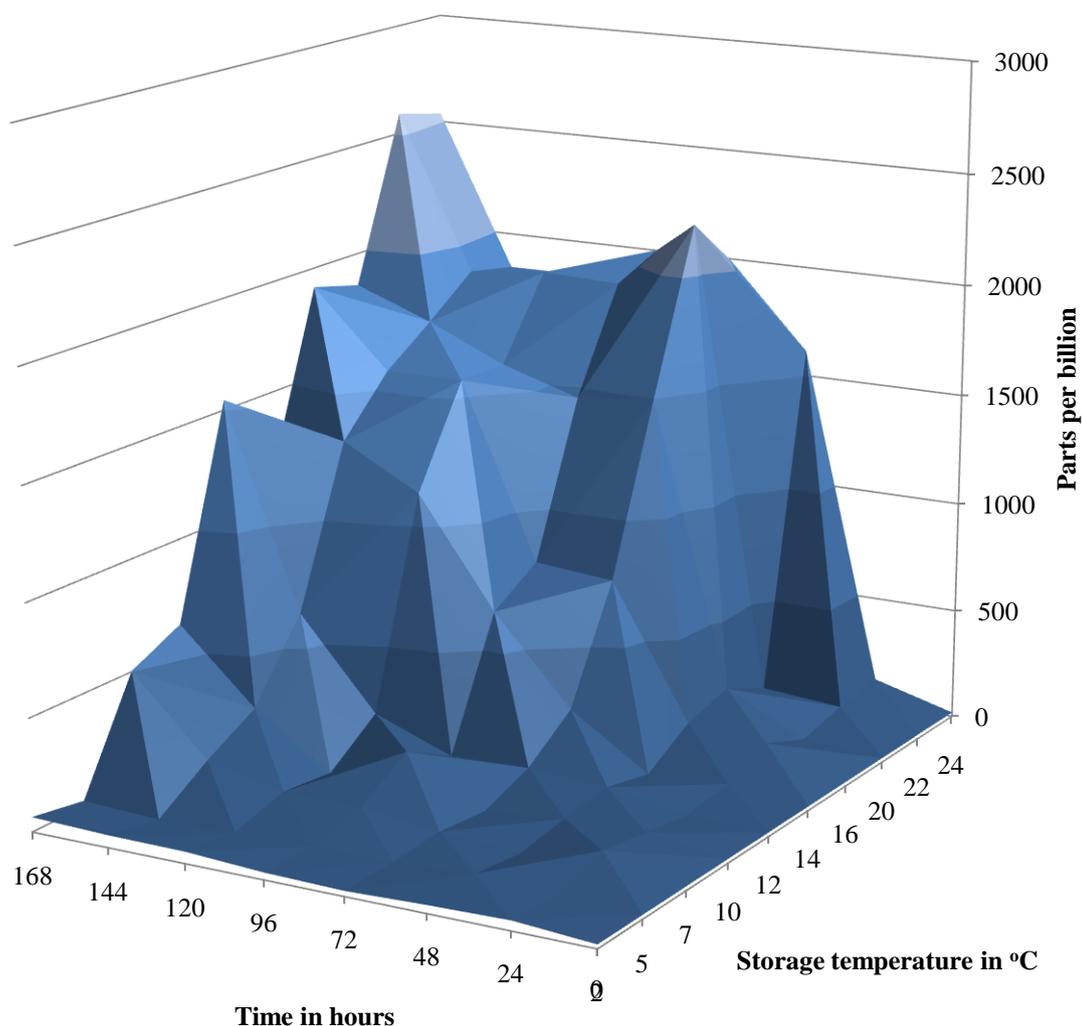


Figure 7.12 Concentration of TMA from degrading salmon with time and temperature

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As expected the increase is much more dramatic when the temperature is higher. A general trend is that the higher the temperature the less time is required for the rise in gaseous concentrations of TMA to occur. A similar graphical representation of TVB-N levels over changing time and temperature can be seen below in Figure 7.13.

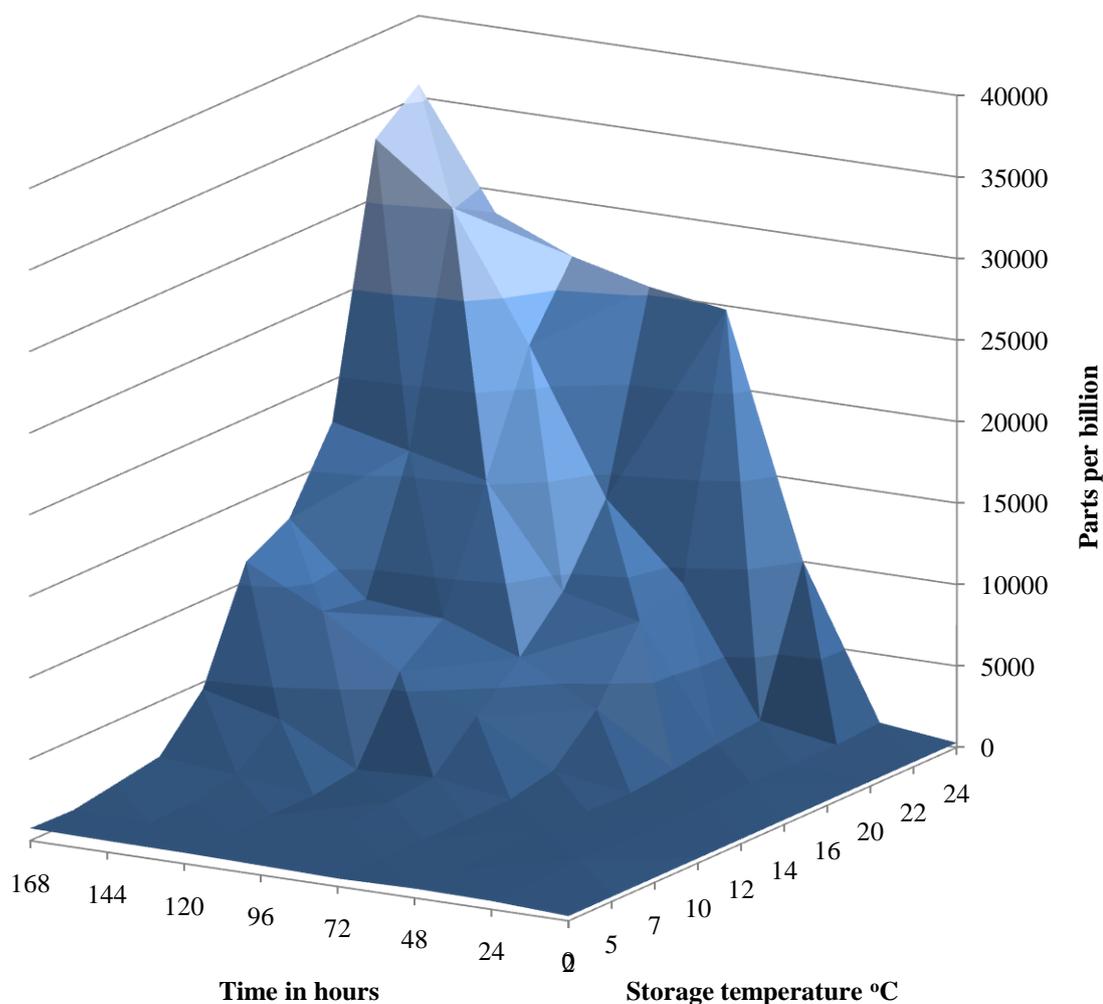


Figure 7.13 Concentration of total basic nitrogen from degrading salmon with time and temperature

The shape of the graph is similar to the shape of the previous chart for TMA. The overall concentrations of TVB-N are much greater than those of TMA which is expected from the previous results. The rise in TVB-N occurs at a much faster rate and climbs to an overall concentration of approximately 35 ppm. The effect of storage temperature has a greater effect on the evolution of TVB-N compared to time.

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The final two experiments investigated the change in concentration of two sulphur containing compounds that are produced from degrading fish samples. The first of these experiments involving the change of dimethyl disulphide can be seen below in Figure 7.14.

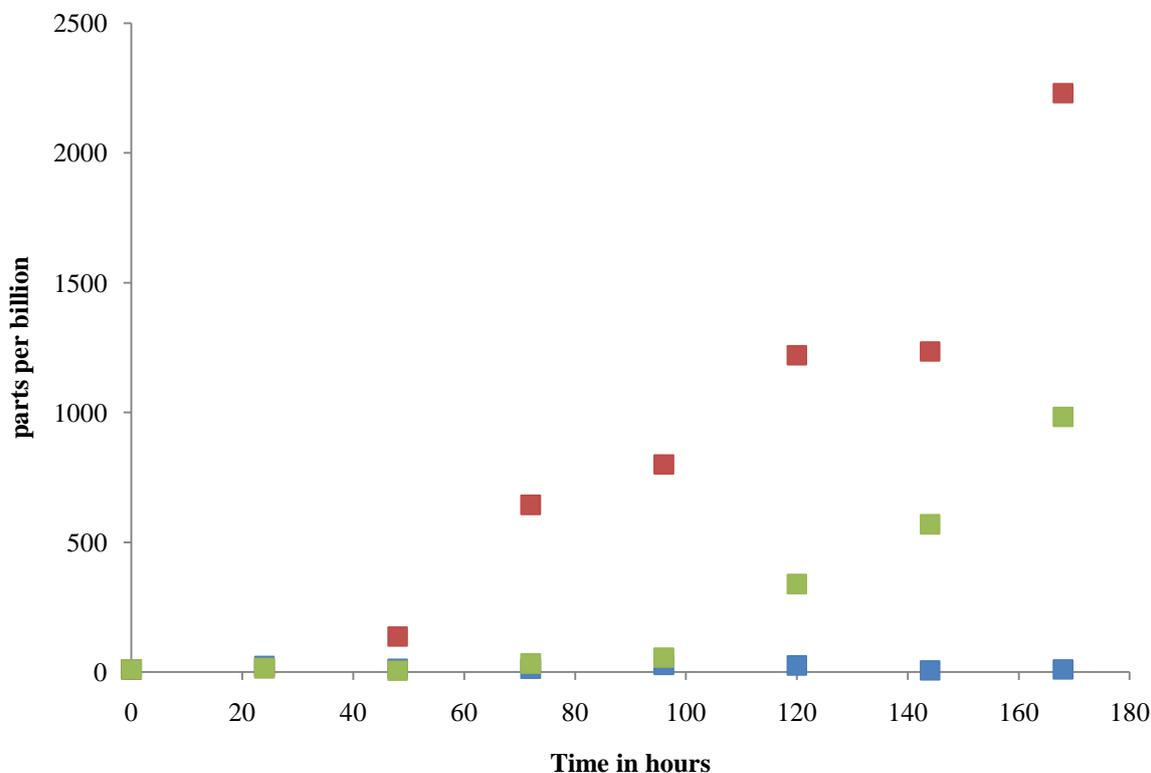


Figure 7.14 SIFT-MS analysis of dimethyl disulphide in head space of degrading salmon (■ = 24 °C, ■ = 14 °C and ■ = 4 °C)

In the above figure the rise of dimethyl disulphide can be seen over a range of three temperatures. This molecule has already been recognised as a spoilage indicator produced by certain bacteria such as *Pseudomonas* spp. (Wang et al., 2004). As expected, the concentration of dimethyl disulphide increases at a greater rate and to a higher amount at higher temperatures. This is due to the higher rate of breakdown of proteins and amino acids in the flesh either by autolysis or by bacteria. The increase in sulphur volatiles also explains the rise in putrid aromas released by the degrading fish samples. The rise of this spoilage product occurs after 24 hours at high temperatures and after 96 hours at medium temperatures.

Initial levels of this compound are approximately 10 ppb and at 24 °C this rises to a level of over 2200 ppb. At 14 °C the amount after 7 days is almost 1000 ppb and at the lowest temperature, 4 °C, the concentration rises to only 30 ppb.

The next spoilage volatile to be investigated was hydrogen sulphide. The same study as above was conducted at three different temperature and the results can be seen below in Figure 7.15.

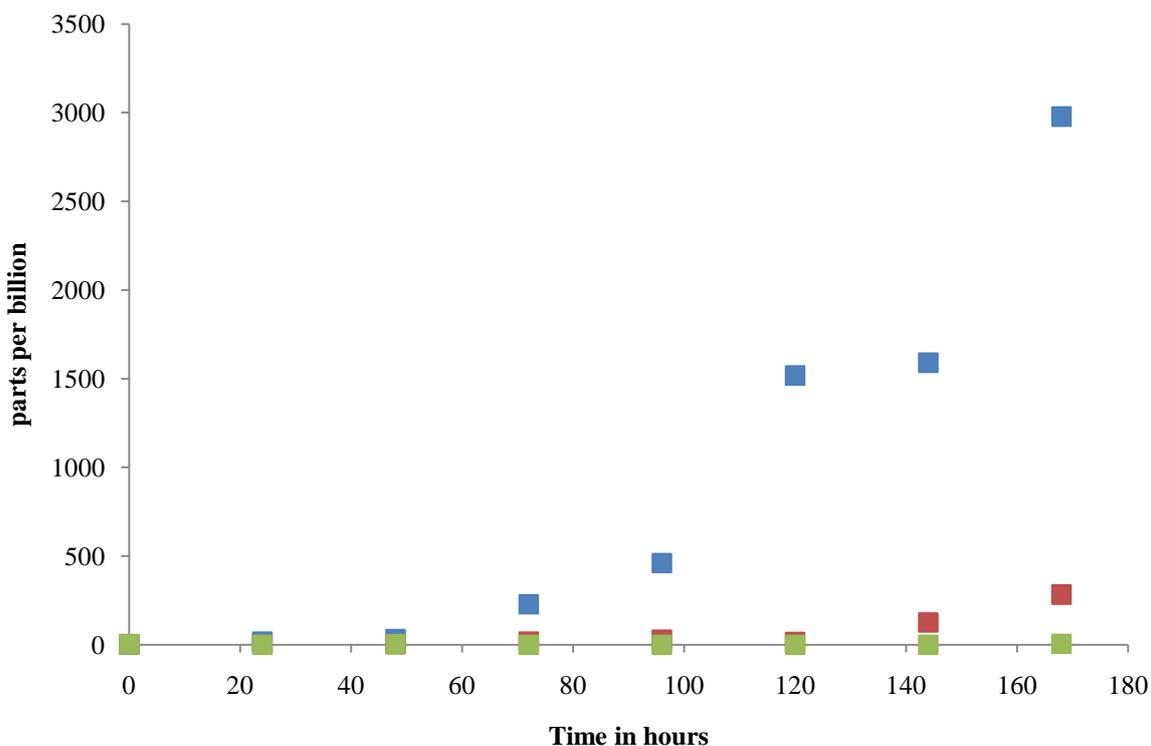


Figure 7.15 SIFT-MS analysis of hydrogen sulphide in head space of degrading salmon (■ = 24 °C, ■ = 14 °C and ■ = 4 °C)

Compared to the previous experiment, the levels of this spoilage marker follow the same patterns of increase with a sharper rise at higher temperatures after 72 hours. At high temperatures the concentration rises from near 0 ppb to almost 3000 ppb. There is a very sharp increase after the sixth day of storage at 24 °C which might be due error within the previous samples taken at either 148 or 120 hours.

At 14 °C the increase of this molecule is modest and rises to a final level of 283 ppb. The lower temperature shows no increase in this marker for spoilage. This is likely to be down to the slower rate of bacterial growth and autolysis of salmon at these temperatures.

7.4.2 Herring

The previous experiments were repeated using samples of herring rather than salmon for comparison of the gases from the fish as it degrades. Figure 7.16 shows the nitrogen containing spoilage species that are evolved as herring degrades.

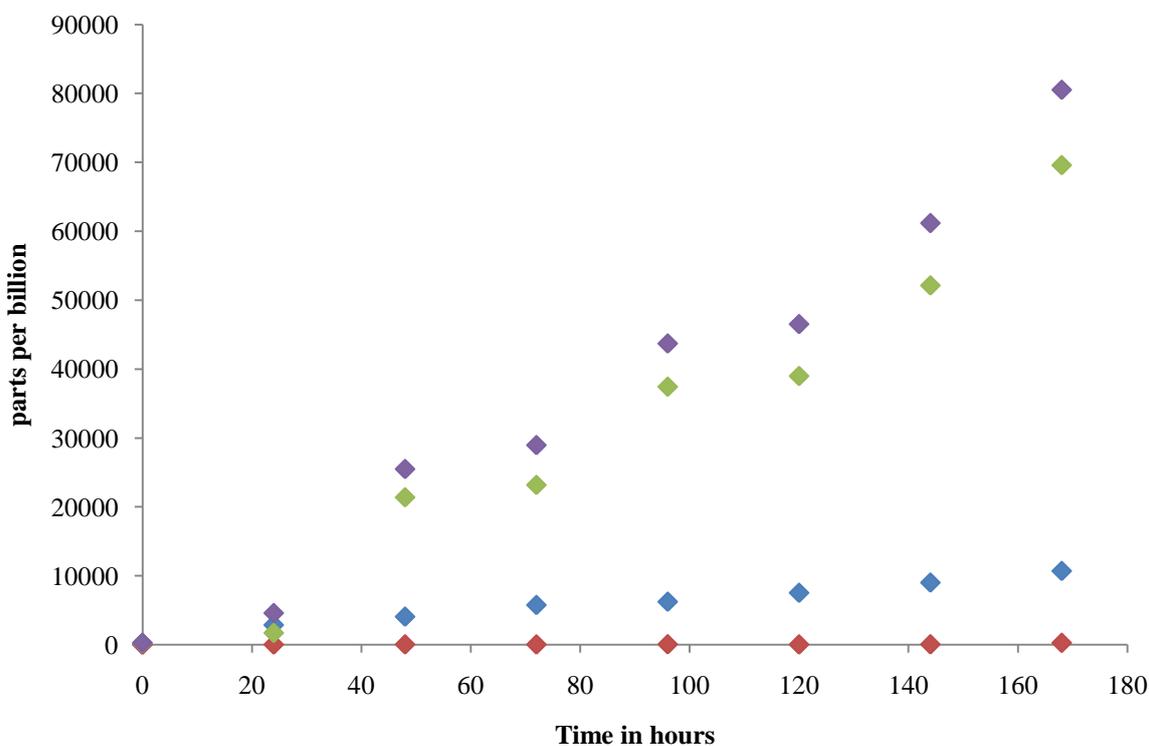


Figure 7.16 SIFT-MS analysis of degrading herring at 24 °C (■ = total basic nitrogen, ■ = ammonia, ■ = trimethyl amine and ■ = dimethyl amine)

At this high temperature the rise of TVB-N occurs after 24 hours. The initial level of TVB-N concentration is at a similar level to that of salmon at around 500 ppb. The rise occurs much earlier than compared to salmon and the final level is over 80000 ppb which is over twice the level seen from degrading salmon.

The major component of this gas is again ammonia. During this experiment, initial concentrations of ammonia in the headspace are 400 ppb. The major increase is also after 24 hours and rises in a linear fashion until a maximum of nearly 70000 ppb is reached. This again is much higher than the observed value seen for salmon but in proportion to the ratio of TVB-N observed for both fish.

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Levels of TMA also rise after the 24 hour period from low levels of 25 ppb to a level of over 13000 ppb. The concentration increases at a steady rate after 24 hours and does not appear to level out. In comparison to salmon, the final concentration and rate of rise of TMA is much higher and faster respectively. The proportion of TMA is also greater in terms of TVB-N in herring than that seen in salmon. This can possibly be explained by the whole fish having much higher levels of the TMAOase enzyme and other nutrients compared to the washed and prepared samples of salmon that were purchased. The shelf life of the herring was also much shorter than that of the salmon which would explain the rise in all nitrogen containing compounds occurring much earlier in herring.

Levels of DMA remained fairly constant below 50 ppb until day 7 when concentrations of over 250 ppb were observed. This is similar to the results found in the case of salmon. Again this is expected as DMA is formed as a by product of autolysis rather than bacterial growth which is occurring rapidly at this temperature.

The next storage temperature for investigation was 14 °C and the results from this experiment can be seen below in Figure 7.17.

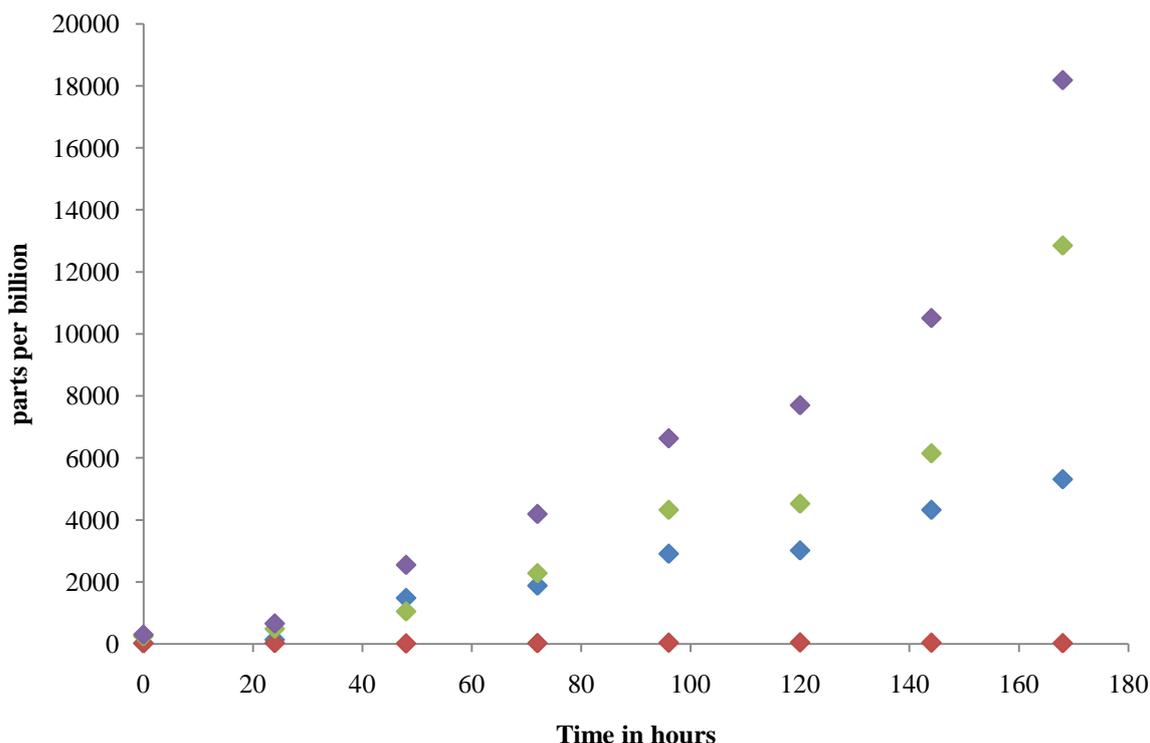


Figure 7.17 SIFT-MS analysis of degrading herring at 14 °C (■ = total basic nitrogen, ■ = ammonia, ■ = trimethyl amine and ■ = dimethyl amine)

The rise in TVB-N rises very steadily after a period of 24 hours. The rate of increase in concentration is less than for the temperature of 24 °C and comparable to that observed for salmon at this temperature. The concentration rises from a background of 500 ppb up to 18000 ppb. The change is linear from 24 hours up to around 120 hours and then there is large increase in concentration. This is expected to be down to standard error within the samples.

The major component of the TVB-N is not always ammonia. Up until day 7, the level of ammonia is relatively similar to the level of TMA and is sometimes exceeded by TMA (48 hours). This has not been seen in any of the other experiments so far. Ammonia concentration rose from an initial concentration of 400 ppb to a level of 12850 ppb and followed the same linear relationship as TVB-N with a large increase observed on the final day. TMA concentration rose from 25 ppb up to levels of 5309 ppb over the course of the seven days with a rise occurring after 24 hours.

DMA levels again stayed constant at around 20-30 ppb with no increase shown over the course of the 7 day experiment. This is comparable to all experiments so far and is expected for reasons presented previously.

The final experiment monitoring the 4 nitrogen containing compounds can be seen below in Figure 7.18. This was conducted using herring stored at 4 °C.

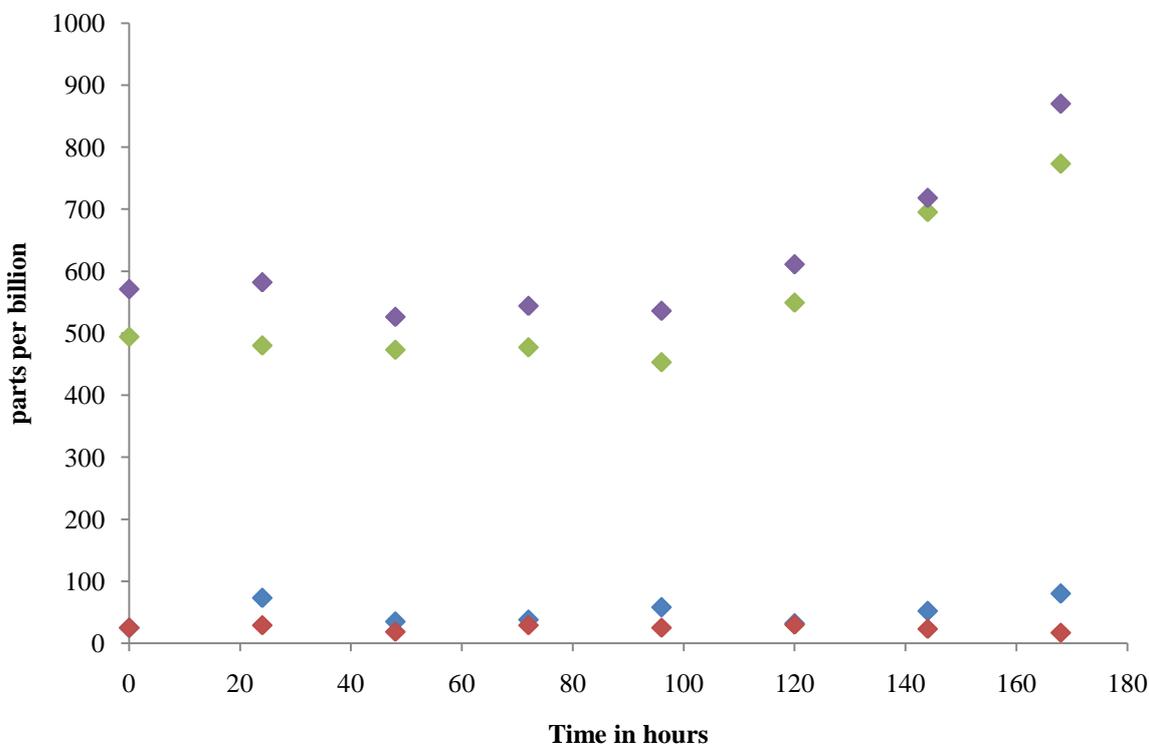


Figure 7.18 SIFT-MS analysis of degrading herring at 4 °C (■ = total basic nitrogen, ■ = ammonia, ■ = trimethyl amine and ■ = dimethyl amine)

TVB-N and ammonia concentrations are very similar at this temperature and both start from initial levels of 500 ppb and 400 ppb respectively. The rise occurs after 96 hours and is at a much slower rate than that observed at lower temperatures and reaches levels of around 750 ppb for ammonia and nearly 900 ppb for TVB-N. These are both similar levels and rates to those observed for salmon although fractionally higher.

Levels of TMA do not change until the sixth day of storage when a small increase is noted from initial levels of 25 ppb up to a final level of 80 ppb. There is no change in the levels of TMA for salmon over the 7 day period and this may reflect the lower shelf-life of the herring. This may also be an indication of the effect of having whole fish samples with a higher variation of nutrients and TMAO available for decomposition.

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DMA levels stay constant at around the 30 ppb level and do not increase over the storage time. This directly compares with the observations from salmon and also shows the limited breakdown of proteins and amino acids in autolysis at this temperature.

The effect of storage temperature and time was investigated using the aluminium block variable temperature equipment and the SIFT-MS. As with salmon, the first volatile spoilage marker to be investigated was TMA. The results of this can be seen below in Figure 7.19.

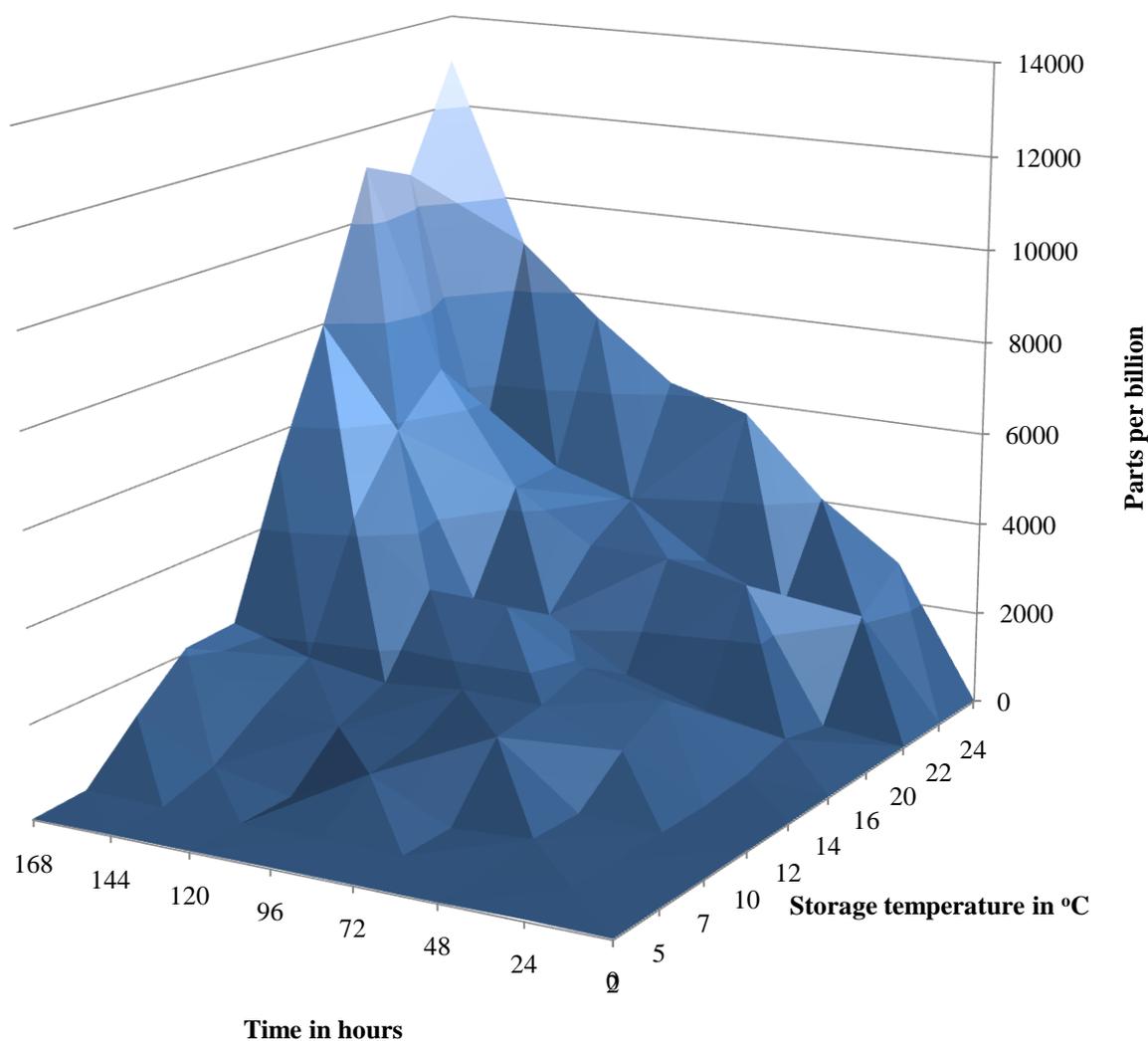


Figure 7.19 Concentration of TMA from degrading herring with time and temperature

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The rise in TMA can be seen to be time and temperature dependant. The overall observable trend is that as temperature and time increase, the concentration of TMA increases. This graph also shows that the level of TMA observed from degrading herring is a lot higher than those values for salmon. The length of storage does not have as dramatic effect of TMA production as the temperature. The surface of the graph is much smoother for herring than that observed for salmon which is probably a reflection of the greater levels of TMA that are produced by the former.

Figure 7.20 below shows the effect of storage time and temperature on the production of all nitrogen containing species in the headspace of degrading herring.

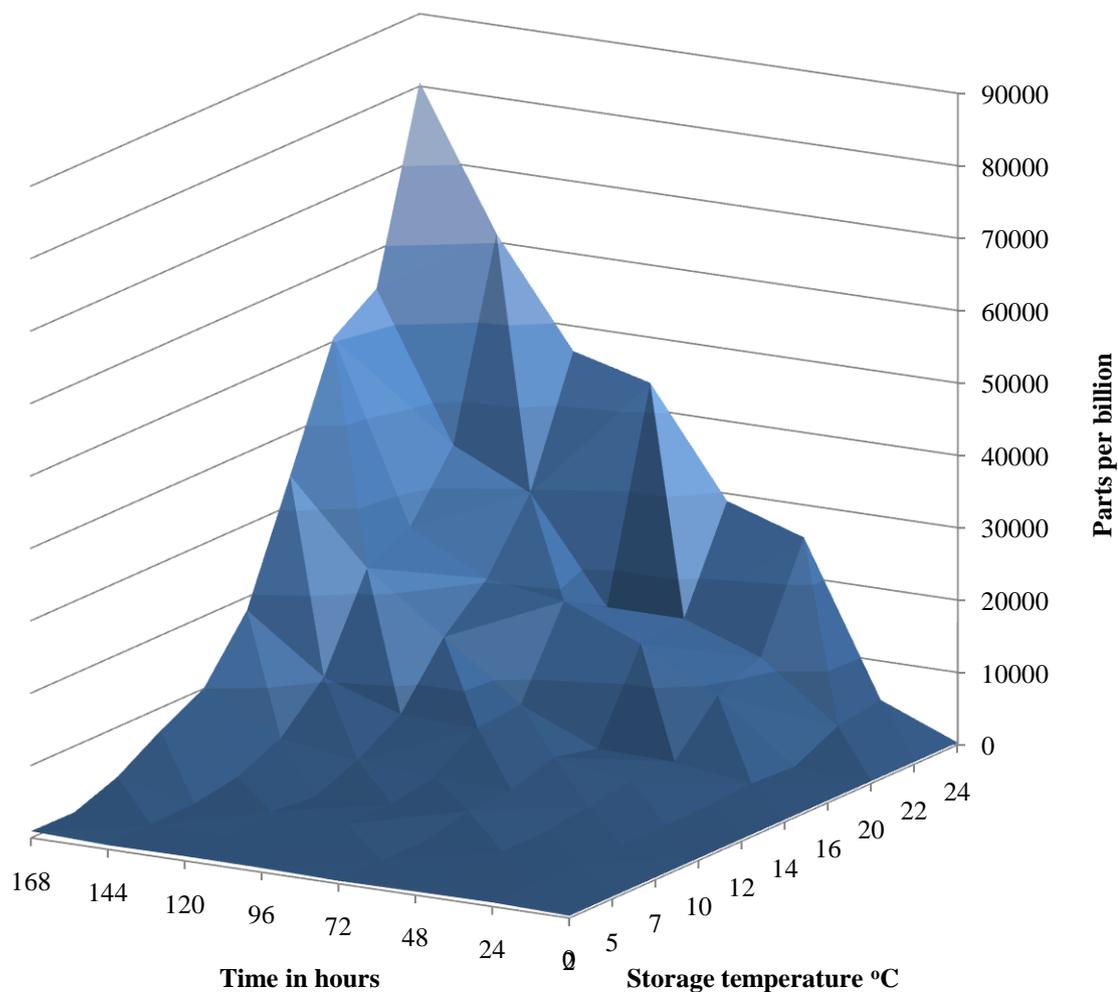


Figure 7.20 Concentration of total basic nitrogen from degrading herring with time and temperature

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The graph shows that there is a strong correlation between storage time and temperature and the production of nitrogen containing compounds. In herring, observed levels of TVB-N change at a faster rate than the levels observed for TMA which is expected from the results shown previously.

The shape of this surface is much steeper than that observed for salmon. This is mainly due to the higher amount of TVB-N in these samples. The levels represent averages of several experimental replicates as the higher concentrations were at the upper limits of feasible detection of the SIFT-MS instrument. Although this is the case, the amounts can be taken as relative concentrations and not exact levels.

The experiments for sulphur containing compounds were repeated for samples of herring. The storage temperatures and times that were investigated for these sulphur compounds were the same as before. The comparison of the concentration of dimethyl disulphide is given below in Figure 7.21.

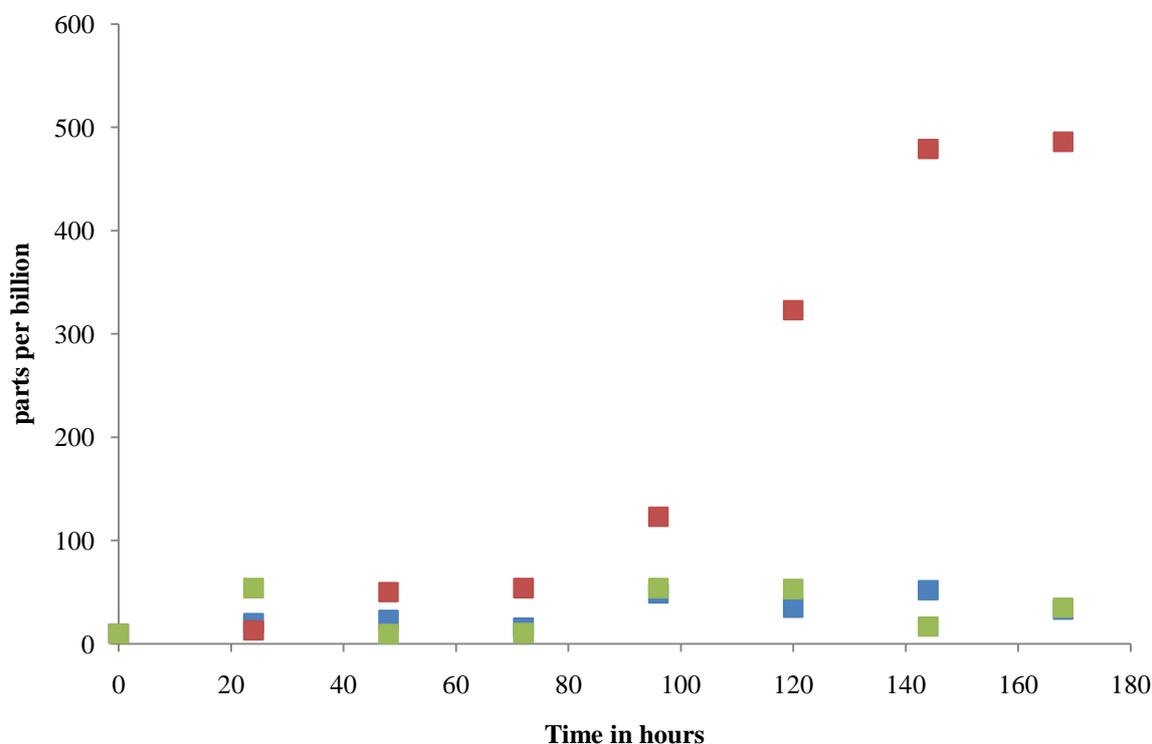


Figure 7.21 SIFT-MS analysis of dimethyl disulphide in head space of degrading herring (■ = 24 °C, ■ = 14 °C and ■ = 4 °C)

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The results show a definite increase in dimethyl disulphide that occurs after 72 hours at the higher temperature. The initial level of this compound was approximately 10 ppb in the headspace of herring and at 24 °C this level rose to nearly 500 ppb. The rise is fairly steady and appears to plateau at this higher value. At the two lower temperatures there is no change in the observable level of this volatile.

Compared to salmon, the resulting concentration is much lower. This may be due to the different available nutrients, the growth environment or the micro fauna. The increase starts at the same time for herring as it does for salmon. The lower amounts of this compound could explain the observed difference in odour of the rotting salmon and rotting herring.

The final set of data using the SIFT-MS instrument investigated the change of hydrogen sulphide with storage time and temperature. The results of this experiment are displayed below in Figure 7.22.

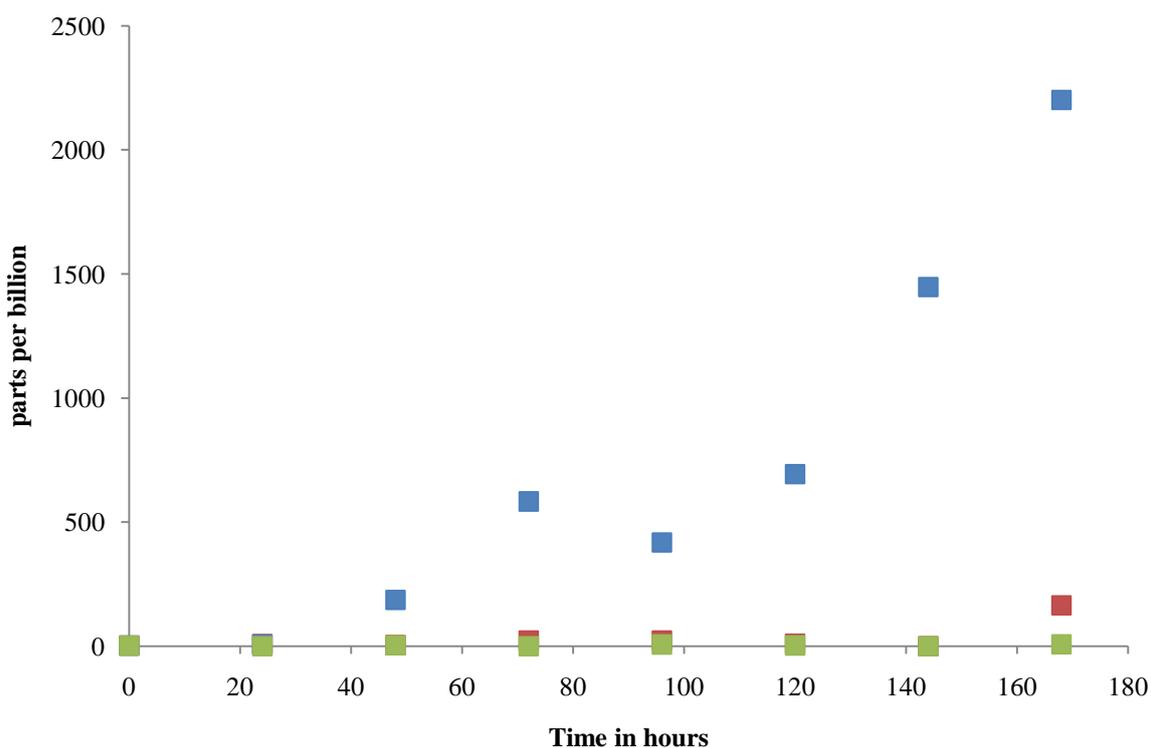


Figure 7.22 SIFT-MS analysis of hydrogen sulphide in head space of degrading herring (■ = 24 °C, ■ = 14 °C and ■ = 4 °C)

The results show an increase in at the highest temperature after 24 hours. The level of hydrogen sulphide in herring is initially near 0 ppb and rises to over 200 ppb over the 7 day

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period. The rise is much faster towards the end of the week. When herring is stored at 14 °C there is a slight increase after 6 days of storage. The concentration after 7 days at this temperature is 283 ppb. At 4 °C there is no change observed over the 7 days.

Compares to salmon the overall concentration is fairly similar but slightly lower and the observed change and rate of change occurs at approximately the same time.

7.5 Conclusions

This chapter has shown the change in headspace of degrading samples of salmon and herring. This is required so that the results from the previous experiment can be related to bacterial growth and ultimately to the sensor response that will be monitored by either a colour change or change in resistance.

The study aimed to find trends in changes if headspace gases of salmon and herring at various temperatures. Previous studies had shown that degrading fish samples create pungent odours that are a complex mixture of volatile compounds. These include nitrogen, oxygen and sulphur containing organic molecules. The molecules of interest for this thesis were nitrogen containing amines and ammonia which were studied over a range of temperatures. These were shown to increase over time and the rate of increase was heavily dependent on the storage temperature.

Also investigated for comparison was the change in concentration of sulphur containing compounds which also contribute to the characteristic putrid smells attributed to rotting fish. These were also shown to increase over time at a faster rate at higher temperature. They were overall at a much lower concentration of amines and ammonia and relatively similar to the levels observed for TMA.

In terms of the two fish, it appeared that salmon produced less volatile nitrogen containing compounds than herring other all of the temperatures that were investigated. The salmon samples, however, were found to produce more of the sulphur containing compounds. As a general trend, the two fish produced more volatiles when left at a higher temperature which is expected.

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The experiment itself was limited to only two fish which were prepared slightly differently and sourced from different methods of farming. This may have had lead to the observed changes of concentrations and the higher levels that were present in the un-gutted herring. Further work should be done on portions of flesh that were taken from whole salmon prepared in the lab and also across a wider range of fish to see if biological composition has a larger effect.

The two techniques used in this chapter have given two sets of complementing results. The first technique was used as a qualitative indicator of the composition of the complex mixture of headspace gas from salmon and herring. TD-GCMS could not be relied upon to give a good estimate of actual concentrations as the column and sorbent that was inside the TD tubes was not entirely suitable for this kind of analysis. The study was successful in determining which of the volatiles produced should be used as potential markers for spoilage and which volatiles should be investigated by the SIFT-MS.

The SIFT-MS study was able to gauge concentrations of the volatile species and the precision of the technique was good below the maximum level of detection of 20 ppm. After this limit had been reached, the ion swarm in the detector becomes too complex and a wider standard deviation from the recorded mean is observed. These have been omitted from the graphs for clarity but at higher concentrations the reliability of the accuracy of the results is of more concern. The results given over this limit are given to show the relative increase and should not be taken as actual concentrations.

Other studies in this area use other techniques for headspace sampling and analysis of the composition of fish as it degrades. These techniques could be used in conjunction with the SIFT-MS and TD-GCMS to provide further evidence of volatile emissions that would aid the commercialisation of the sensor being produced in this thesis.

Chapter 8
Polyaniline as a Food Quality Indicator for Salmon and Herring

8 Introduction

This final result section will use all of the previous results in conjunction with results in this chapter to assess the use of polyaniline films as food spoilage sensors. The first part of this chapter will identify which volatile produced from degrading fish that polyaniline is most susceptible to in terms of changing from polyemeraldine salt to base. DC response of polyaniline to degrading samples of salmon and herring are presented along with a comparison of the data gathered for bacterial populations and headspace changes.

There will also be a comparison of the change in colour of the thin films produced as another method of monitoring fish spoilage using UV-Vis spectroscopy at certain wavelengths. Other techniques such as AC impedance interrogation over a range of frequencies will be used to characterise the change of the films on exposure to degrading fish samples.

The sensors that were used throughout these experiments was manufactured in the same way as discussed in chapter 5, with an in situ thin film being layered with 5 cycles of the two pot synthesis process. All chemicals used in this chapter for testing the sensor detection were sourced from Sigma Aldrich (Dorset, UK) and were above 99 % purity unless otherwise stated. The experiments were carried out under standard laboratory conditions unless otherwise stated.

8.1 Polyaniline as a sensor for food volatiles

Using the results from the previous chapter on headspace analysis, it is clear that the sensor for spoilage detection for salmon and herring will require the ability to detect low levels of amines and ammonia. As shown in the literature, the reaction of polyemeraldine salt with ammonia is well reported and it was expected that this and any other amines will form the major reactants that produce change in the manufactured sensors.

An initial experiment was conducted to determine the effect of common molecules found in air and the headspace of degrading salmon and herring with regard to their effect on the conductivity of polyaniline. Several containers of 50 ml volume were setup and 150 μ l of each volatile was added. The containers were closed and air tight and then after 7 days they

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were opened and the films resistance was measured and compared to the previous value. The results can be seen below in Table 8.1.

Table 8.1 The change in resistance of polyaniline when exposed to various volatile (150 μ l) over a 5 day period

Reactant	Initial resistance (k Ohms)	Resistance after 120 hours (k Ohms)
Ethanol	5	11
Benzene	5	9
Propanol	5	32
Formaldehyde	5	52
Butanol	5	38.5
Acetic acid	5	16
Water	5	11
Hexane	5	9.7
Cyclo hexanone	5	9.8

The highly substituted alcohols and the aldehyde show a better ability to increase the resistance of the polyaniline films. No colour change in the films was observed and the film remained green, indicating that the polyaniline was in the conducting polyemeraldine base form. The change in resistance for the larger alcohol molecules is due to the increased stability of the alkoxide ions that react with the film as seen in Figure 2.28. The concentration of these in the headspace under standard conditions would be very low and suggest why the resistance change observed is very small.

A further investigation into the change of resistance of the polyaniline films was completed using 150 μ l of ethanol in a 50 ml sealed vessel. The polyaniline film was exposed for 5 days and an interrogation of the films resistance was taken every hour. The results of this study can be seen below in Figure 8.1.

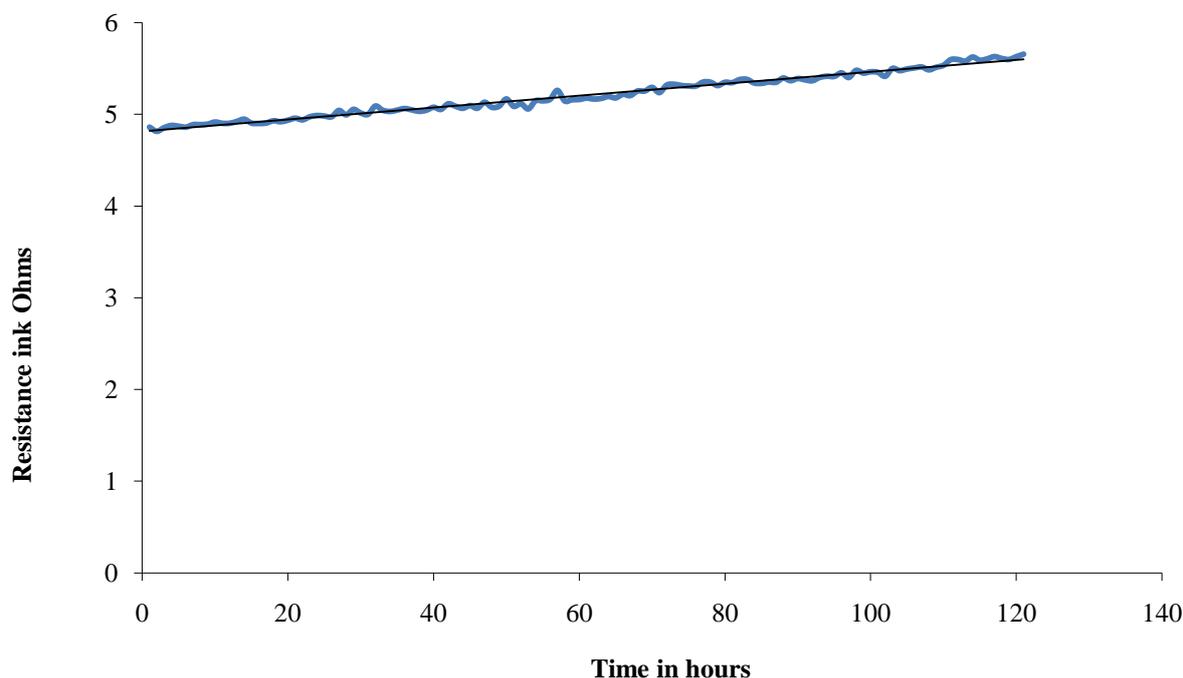


Figure 8.1 Resistance change of in situ film when exposed to 150 μ l of ethanol

The change in resistance of the film after exposure to ethanol is low and over time there is a linear increase in resistance over time. From these results, the effects of the compounds studied so far show no major effect on the colour or resistance of the sensor.

The next study was the effect of ammonia and trimethyl amine on the resistance of the sensor. A previous study using 150 μ l of both compounds in a sealed 50 ml vessel has shown that there was an increase in resistance in the order of magnitude of 10^3 . An amount of 150 μ l of both ammonia and TMA solutions were added separately to 2.5 l gas jars with a polyaniline sensor after 1.5 minutes of stabilisation. The experiment continued for a further 30 minutes recording the change in resistance of the polyaniline film. The results from this experiment can be seen below in Figure 8.2.

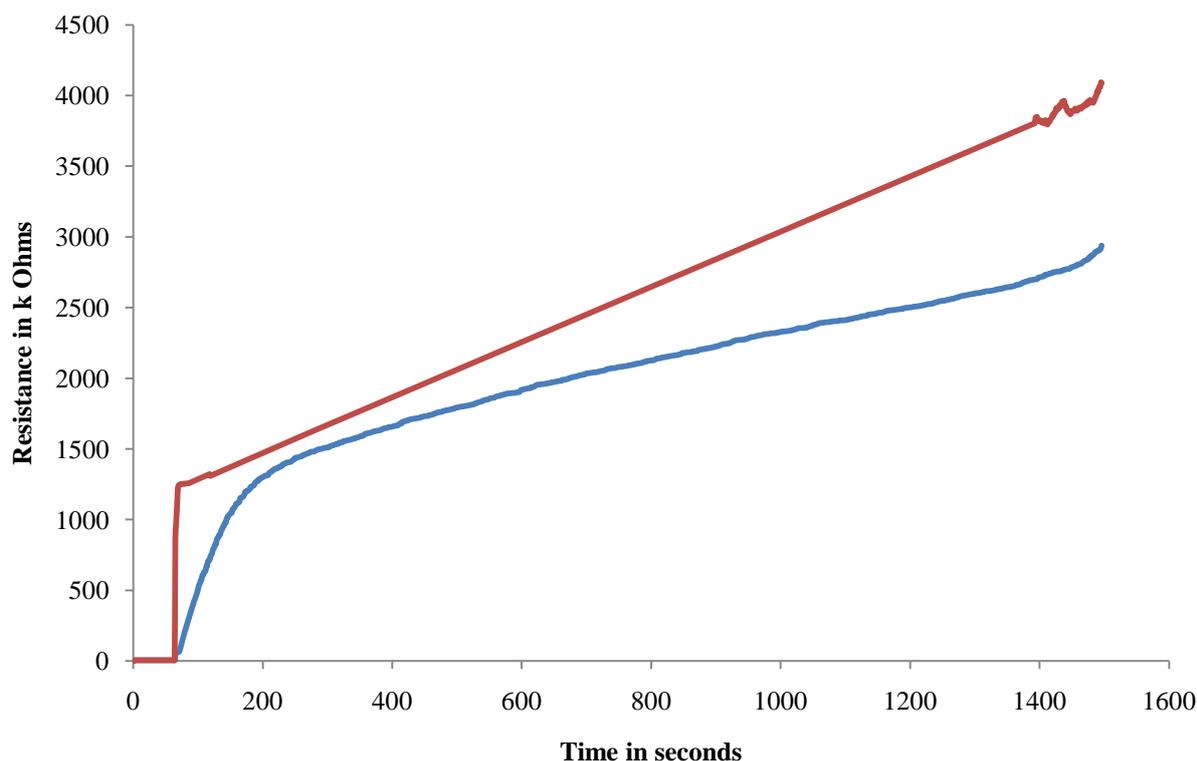


Figure 8.2 Response of polyaniline sensor to 150 μ l of 2.0 M ammonia in ethanol (■) and TMA 25 % wt. in water (■)

After the injection of the nitrogen containing compound there is an exponential increase in the resistance of the polyaniline sensor. This effect is much greater for ammonia than TMA which is possibly due to the higher volatility of this species and the increased concentration. The graph shows that the effect of nitrogen containing species on polyaniline films is greater than that on any of the other molecules tested. An instant colour change of green to blue was also observed when the films were exposed to these two compounds. This correlates with the findings in the literature in terms of the effect of these compounds on polyaniline formed in other ways, such as electrochemically polymerised films and nano fibres. The proposed reaction mechanism is reported in Figure 2.27 which shows the transformation of polyemeraldine salt in to polyemeraldine base.

Further testing was completed on the sensors response to water vapour over a period of 1 day at 30 °C. The sensor was placed in a 50 ml vessel with 10 ml of water sealed inside. The results from this experiment are shown in Figure 8.3.

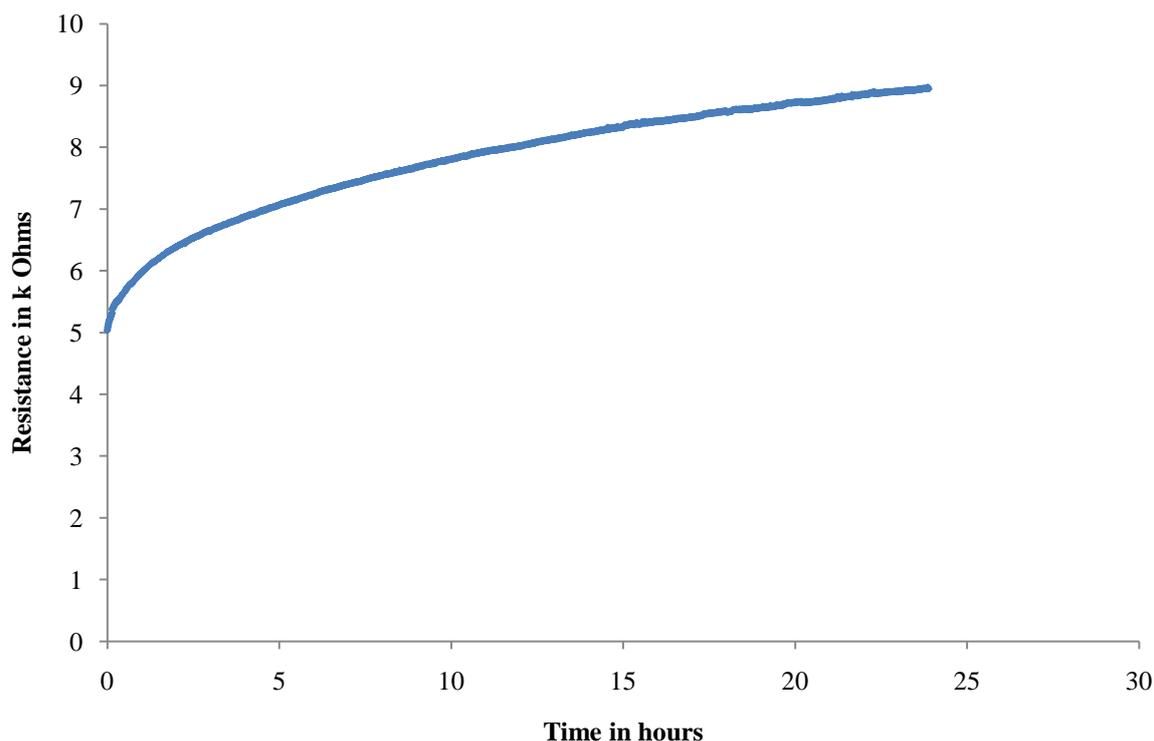


Figure 8.3 The response of polyaniline to excess water at room temperature

The response of the sensor to water vapour is similar to that observed for nitrogen containing compounds. The scale of change in resistance is considerable less – as expected. These results indicated that a sensor developed for low level amine or ammonia detection may pose problems with interaction with water vapour which has been already indicated in the literature (Nicho, 2001). For the purposes of this research, the effect of water on the sensor can be considered negligible due to the high concentrations of nitrogen containing species in the headspace of degrading fish samples.

Finally an experiment was conducted to determine the sensors stability in laboratory air whilst being interrogated for change in resistance. The results from this experiment can be seen below in Figure 8.4.

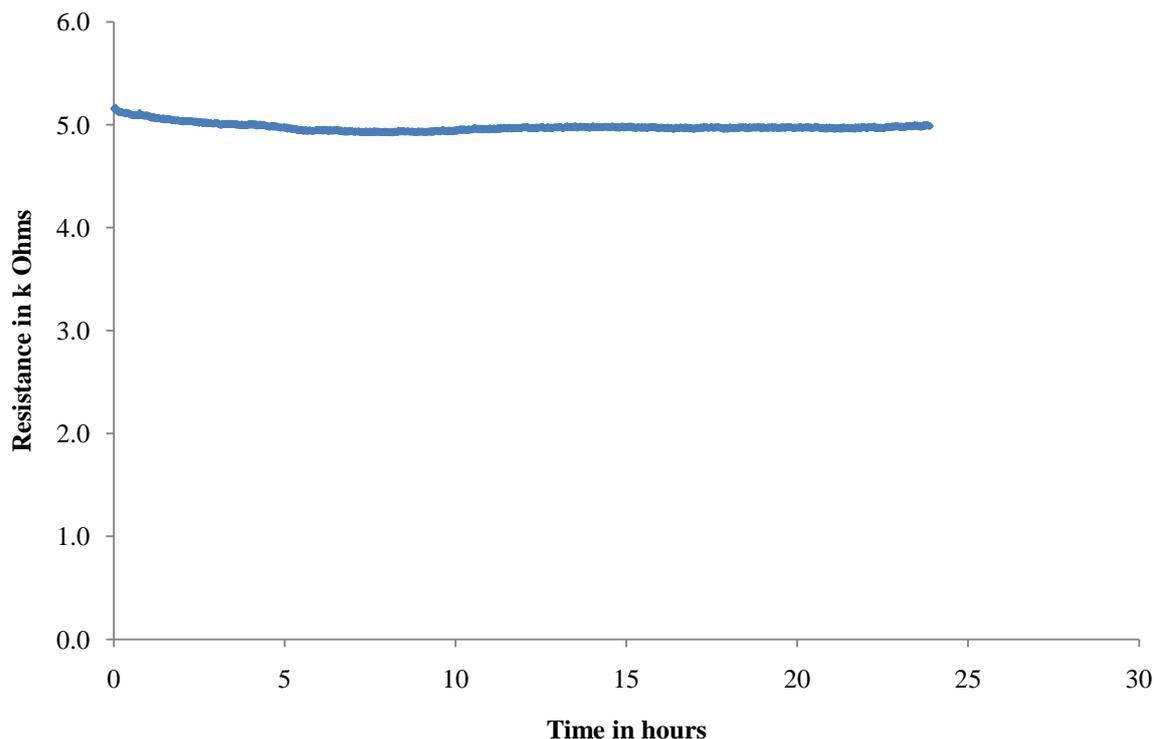


Figure 8.4 The effect on exposing polyaniline to air

The results show that the sensor is stable in laboratory air over a period of 1 day. Further testing showed that the sensor was able to stay stable over longer periods of up to 2 years if stored in correctly.

These initial investigations were able to show that the polyaniline sensors produced for this project have a different response to different volatiles. The most effective change of colour and resistance was seen when polyaniline was exposed to nitrogen containing compounds such as trimethyl amine and ammonia.

8.2 Polyaniline as a sensor for food spoilage – chemiresistor

After assessing the sensor response to a variety of volatiles, the next stage was to assess polyaniline response to food stuffs and determine if the detection limit of the sensor would correlate to the growth of bacteria within the degrading salmon and herring samples. The sensor response was first investigated as a chemiresistor sensor with the change in resistance being monitored.

Polyaniline as an FQI for Salmon and Herring

8.2.1 Across a range of temperatures

The first experiment investigated the effect of temperature on the sensing ability of polyaniline films of salmon and herring spoilage. The samples of salmon and herring were placed in 25 ml sterile vessels and sealed, so that no headspace gas could escape. The experimental process attempted to simulate conditions of packaging and storage for fish products. The change in resistance of films exposed to fish samples over a range of temperatures (27-2 °C) was recorded. The temperatures of interest were the same temperatures used in previous experiments (24, 14 and 4 °C). The results from this experiment can be seen below in Figure 8.5.

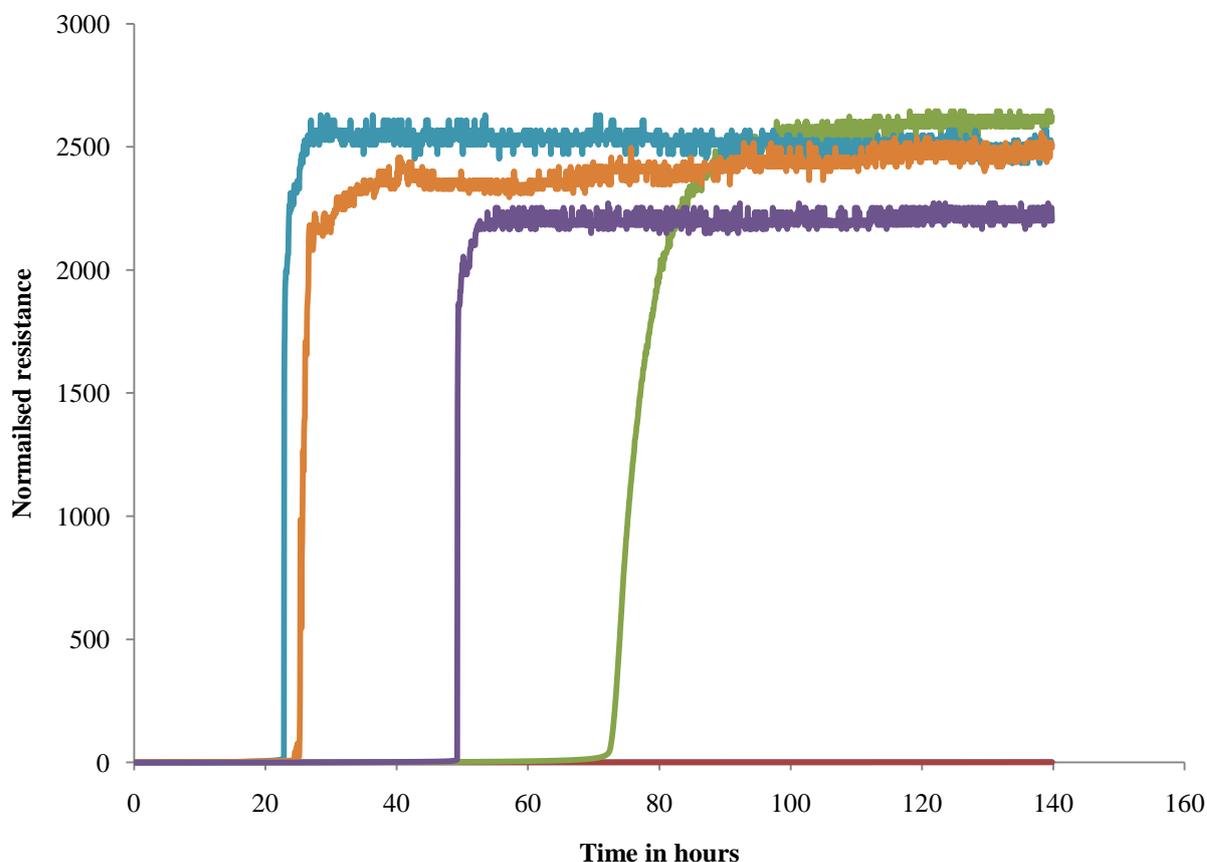


Figure 8.5 Normalised resistance change when polyaniline sensor was exposed to salmon and herring at various temperatures (■ = salmon at 24°C, ■ = herring at 24°C, ■ = salmon at 14°C, ■ = herring at 14°C, ■ = salmon at 4°C and ■ = herring at 4°C)

The increase in resistance was normalised by dividing the original resistance by the observed resistance after time t . The resistance of the sensor was calculated from the potential divider equation. The resistance change that was observed was similar to that seen in the previous study with ammonia and TMA. The increase in resistance of the film occurs at different

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times for different temperatures with the highest temperatures showing a change in resistance first proceeded by lower temperatures. Salmon and herring also exhibited different reaction times of the sensor in terms of the large resistance increase, especially at mid range temperatures as seen in the above graph. This is possibly due to the higher concentrations of TVB-N that were observed in the previous chapters at these temperatures.

Table 8.2 summarises the findings from these experiments with the mean time taken for the sensor to respond to salmon and herring.

Table 8.2 Time taken for polyaniline sensor to reach critical point in salmon and herring over various temperatures

Temperature	Time taken for herring (hours)	Time taken for salmon (hours)
27	17	18
24	22	24
22	23	26
20	27	30
16.5	32	43
14	49	72
12	74	89
10	99	103
7	128	124
4	Not reached	Not reached
2	Not reached	Not reached

The time taken for the sensor to respond to the degrading fish increases as storage temperature is lowered. At the lowest temperatures (below 7 °C) no change in the sensor was recorded. This is an issue which was seen in the previous chapter where at 4 °C a low concentration of TVB-N was observed. Although spoilage has occurred before the end of the 5 days of this experiment according to the bacterial study, no response is given. This shows a possible limitation of polyaniline as a food spoilage detector at lower temperatures. The lower temperature slows the rate of TVB-N that is vaporised into the headspace either because the mechanisms of production of TVB-N have slowed or that the rate of vaporisation is too low. This effect may be due to both of these problems.

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The populations of bacteria and the levels of TVB-N have been estimated in the previous chapter and are presented below in Table 8.3.

Table 8.3 The level of TVB-N and bacterial populations of salmon and herring at the time of sensor response

Temperature (°C) and fish		Approximate TVB-N (ppb)	Bacterial population (logCFU g ⁻¹)
Salmon	24	1000	TVC = 8.9 SSO = 6.9
	14	1200	TVC = 8.1 SSO = 7.0
	4 (after 100 hrs)	500	TVC = 8.4 SSO = 7.0
Herring	24	1800	TVC = 7.9 SSO = 7.1
	14	2000	TVC = 6.5 SSO = 5.0
	4 (after 100 hrs)	500	TVC = 8.5 SSO = 7.1

The table shows that the sensitivity of the polyaniline sensor as a food spoilage indicator is between 1 and 2 ppm in terms of TVB-N created in the headspace of degrading salmon and herring. The reaction timing of the sensor correlates well with the spoilage of salmon at 14 and 24 °C. As previously discussed, the sensitivity of the polyaniline film is not enough to monitor the small changes of TVB-N occurring at lower temperatures for both salmon and herring. The sensor does not correlate well with the spoilage of herring below 24 °C. At this high temperature, the sensor responds to a change of spoilage organisms at the acceptable level of 7 log CFU g⁻¹. At 14 °C the sensor reacts too early at 50 hours to an increasing concentration of TVB-N. The sensor reaction should be delayed to 62 hours. This gap is approximately 12 hours and could act as an early warning signal for users, i.e. a forewarning of impending spoilage if storage conditions are not improved.

A further set of experiments were conducted to estimate the effect of water on the sensor in this environment in case there was any alteration in the sensor behaviour. Figure 8.6 shows the change in actual resistance of the films when exposed to 0.5 ml of water in 25 ml sterile sealed containers. These results compare to the original experiment in Figure 8.3 where an excess of water was exposed to the sensor at a higher temperature.

Polyaniline as an FQI for Salmon and Herring

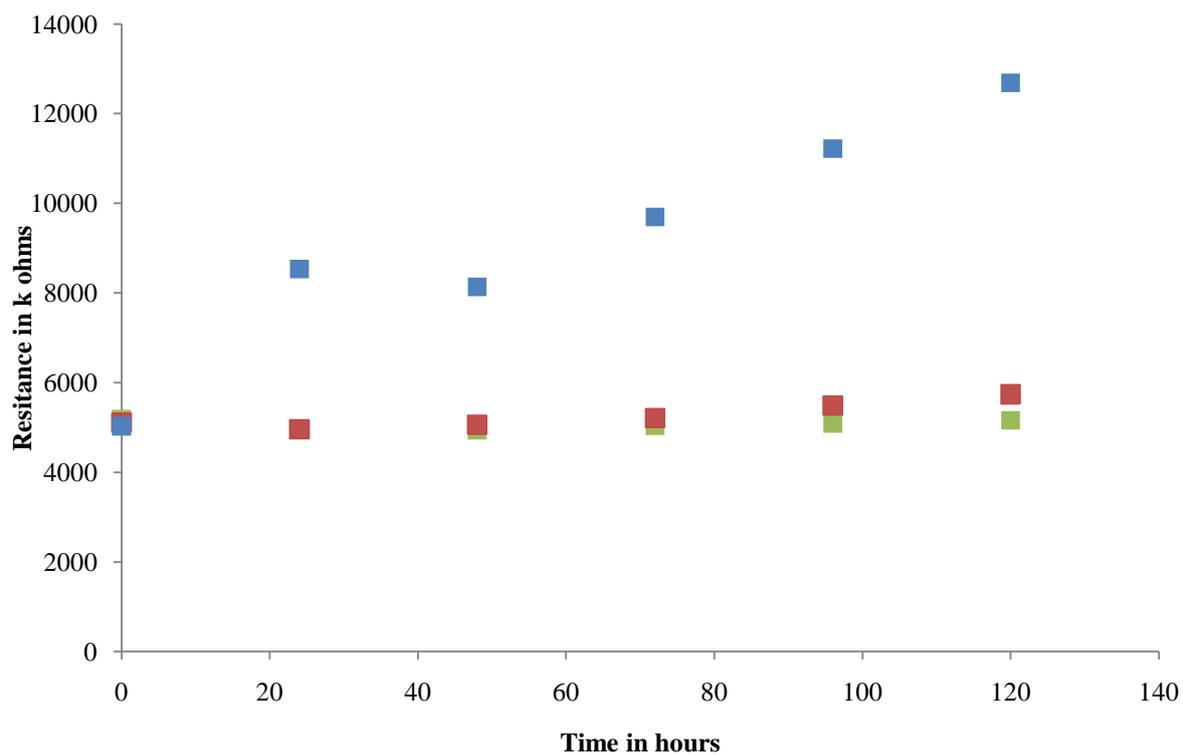


Figure 8.6 Change in resistance of polyaniline film when exposed to 0.5 ml of water over a range of temperatures (■ = at 24°C, ■ = at 14°C and ■ = at 4°C)

The slight increase in resistance of the films is not comparable to that observed when the sensor is placed in the headspace of salmon and herring samples. The effect of water vapour on the films can be considered negligible in terms of polyaniline as a food spoilage sensor.

8.3 Polyaniline as a sensor for food spoilage – Chromatic sensor

As previously stated, other than changing the conductivity of polyaniline films, ammonia and amines can also change the appearance of the film in terms of colour. Until now these have been observed, however, in this section an attempt is made to characterise this change using UV-Vis spectroscopy. Figure 8.7 shows the spectra of the various forms polyaniline using this technique of spectroscopy.

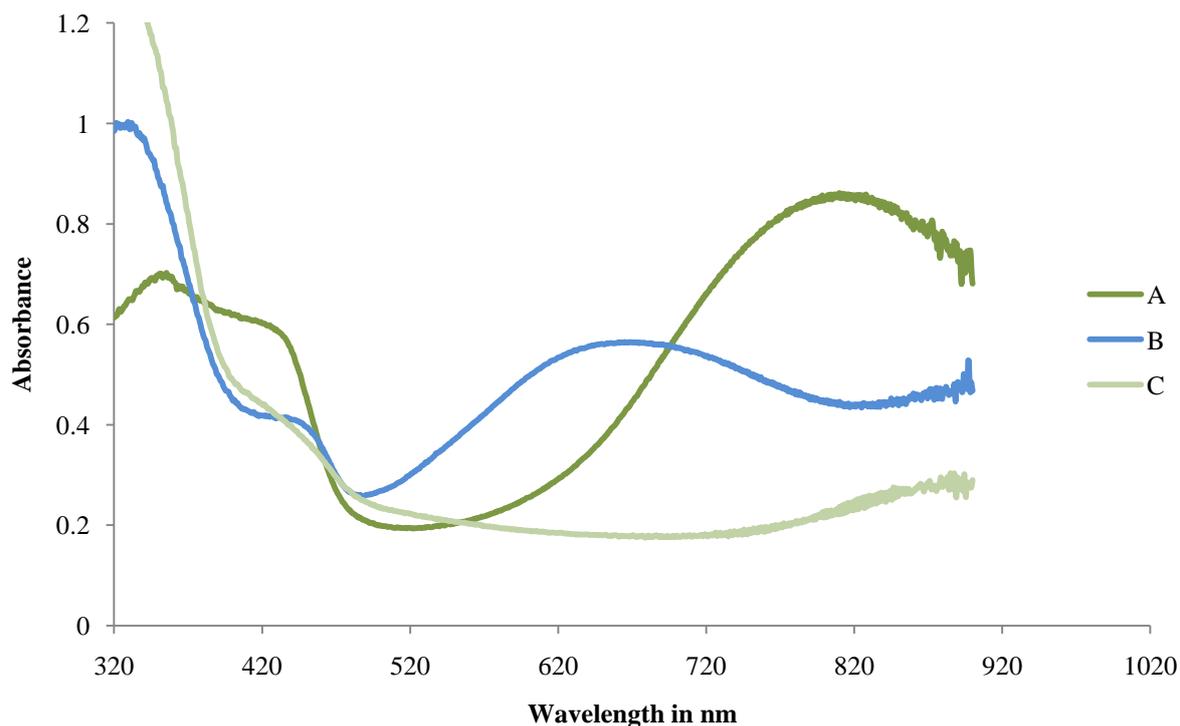


Figure 8.7 UV-Vis Spectrum of polyaniline thin films on a Melinex® substrate. (A=polyemeraldine salt, B= polyemeraldine base and C= polyleucoemeraldine)

It was observed that during experiments using salmon and herring that the colour of the polyaniline sensor changed from green to blue over the course of spoilage. Once the vessels were placed in experimental conditions they were not disturbed until reading had been completed. After the experiment had finished the films were taken and studied for the variation of the colour change over the range of storage temperatures after 5 days exposure to salmon or herring.

This colour change was also visible to the naked eye and can be seen in Figure 8.8.



Figure 8.8 Range of colours displayed by polyaniline on exposure to fish (herring in the above figure)

The colour change was dependant on storage temperature and was of higher visible magnitude when the fish was stored at higher temperature, the sensor below in Figure 8.9 was stored with herring at 4 °C.



Figure 8.9 Colour of sensor after exposure to herring after 7 days at 4°C

The colour of the sensor does not appear to have changed on inspection with the naked eye. A sensor stored at 14 °C with herring can be seen below in Figure 8.10.



Figure 8.10 Colour of sensor after exposure to herring after 3 days at 14°C

There is a visible colour change in the above sensors as there is change between states of polyemeraldine. This is occurring due to the increase in concentration of TVB-N in the headspace. The change in colour appeared all over the sensor and did not appear to favour any specific site on the sensor.

The final change is observed when storage temperatures exceed 17 °C. After 5 days the colour change of the sensor is complete and has changed from green to a dark blue. An example of this is given below in Figure 8.11.



Figure 8.11 Colour of sensor after exposure to herring after 1 days at 24°C

Due to the observable change of the sensor and because the substrate is transparent in the regions of interest, UV-Vis spectroscopy could be used to further characterise this colour change. For the first experiment as a control, 500 µl of water was placed in the vessel containing the sensor and was stored at various temperatures. The sensor was exposed to

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these conditions for a period of 5 days after which UV-Vis spectra were recorded at certain wavelengths which correspond to the changes seen for the transformation of polyemeraldine salt into polyemeraldine base. These wavelengths were 800 nm, 650 nm and 550 nm. Polyemeraldine salt was expected to have high absorbance at 800 nm and weaker absorbance at 550 nm. Polyemeraldine base was expected to have weak absorbance at 800 nm and high absorbance at 550 nm. The graphs are shown with a mean value and also the upper and lower confidence intervals at 95 % for each data set.

The first experiment investigated the change of absorbance at 800 nm of polyaniline sensors when exposed to 500 μ l of water and the results of this can be seen below in Figure 8.12.

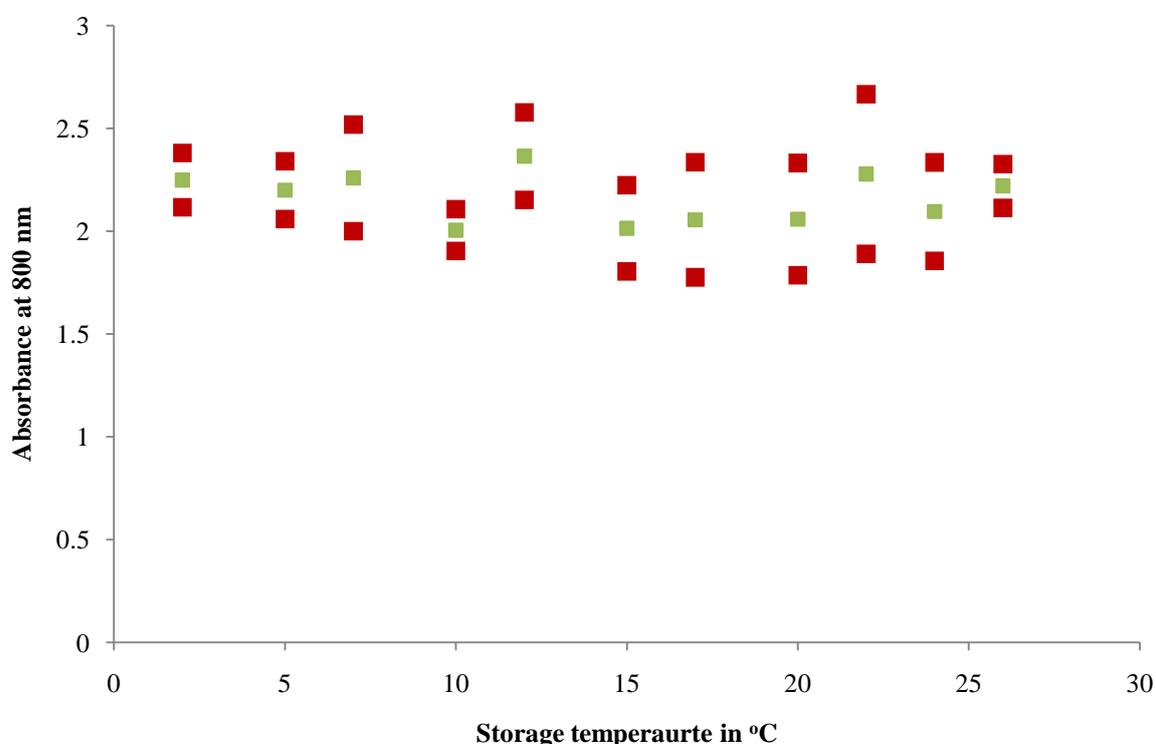


Figure 8.12 Absorbance of polyaniline film at 800 nm after exposure to water for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The above graph shows that there is no change in absorbance at this wavelength when the sensor is exposed to water. The upper and lower bounds of error in the replicate sets show the variance of absorbance of the manufactured sensor which is fairly consistent across the range of temperatures. It can be assumed that water vapour has no effect on the absorbance at this wavelength.

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The next experiment Figure 8.13 shows the effect of absorbance at 800 nm on the polyaniline films when they are exposed to salmon for 5 days at varying temperature.

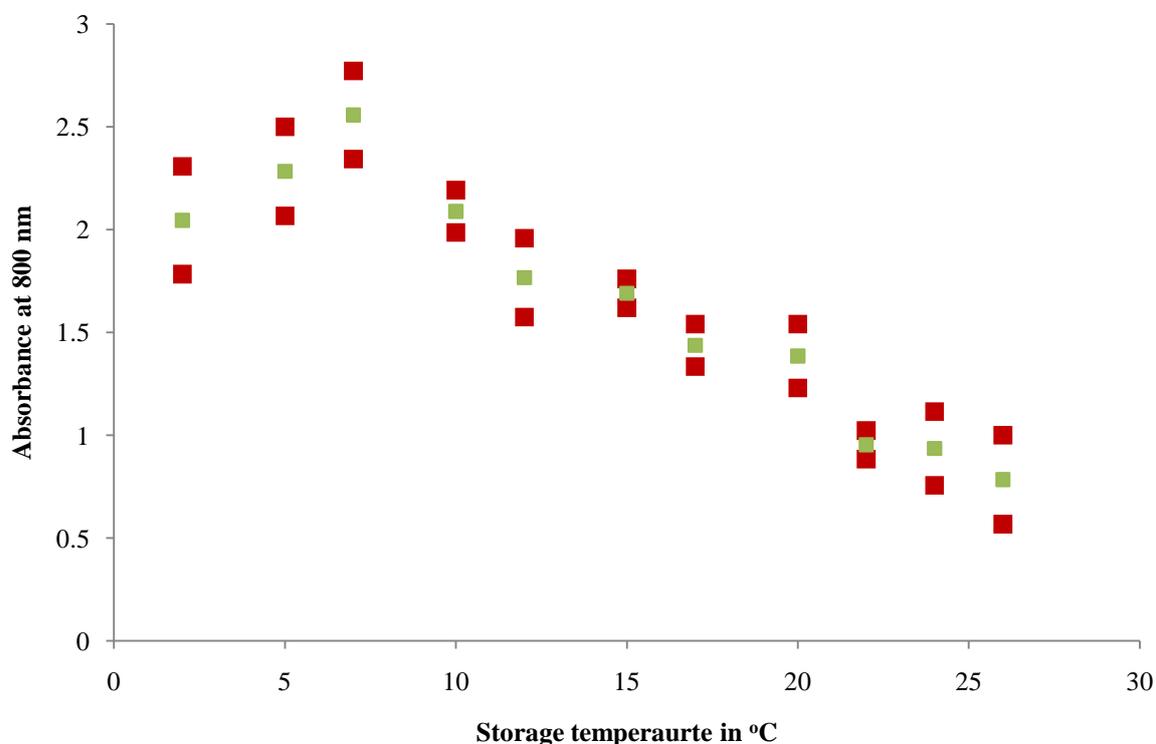


Figure 8.13 Absorbance of polyaniline film at 800 nm after exposure to salmon for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The results show that at lower temperatures there is a slight increase in absorbance which might be due to complex interactions that are occurring in the headspace at these temperatures as the results from the SIFT-MS experiment showed that TVB-N was too low for detection at these temperatures. The film must be interacting with another gaseous species that is enhancing the polyemeraldine salt phase.

Once above these lower temperatures, the absorbance at this wavelength starts to decrease in a linear fashion owing to the decrease in the green coloured polyemeraldine form of polyaniline.

The upper and lower error points displayed on the graph show again that there is a consistent variance in absorbance across the data set which is due to the standard error of film deposition and the random nature of the technique used to grow the layers of polyaniline.

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The next experiment investigated the changes of absorbance at 800 nm for polyaniline films exposed to herring for 5 days at varying temperatures. The results are displayed below in Figure 8.14.

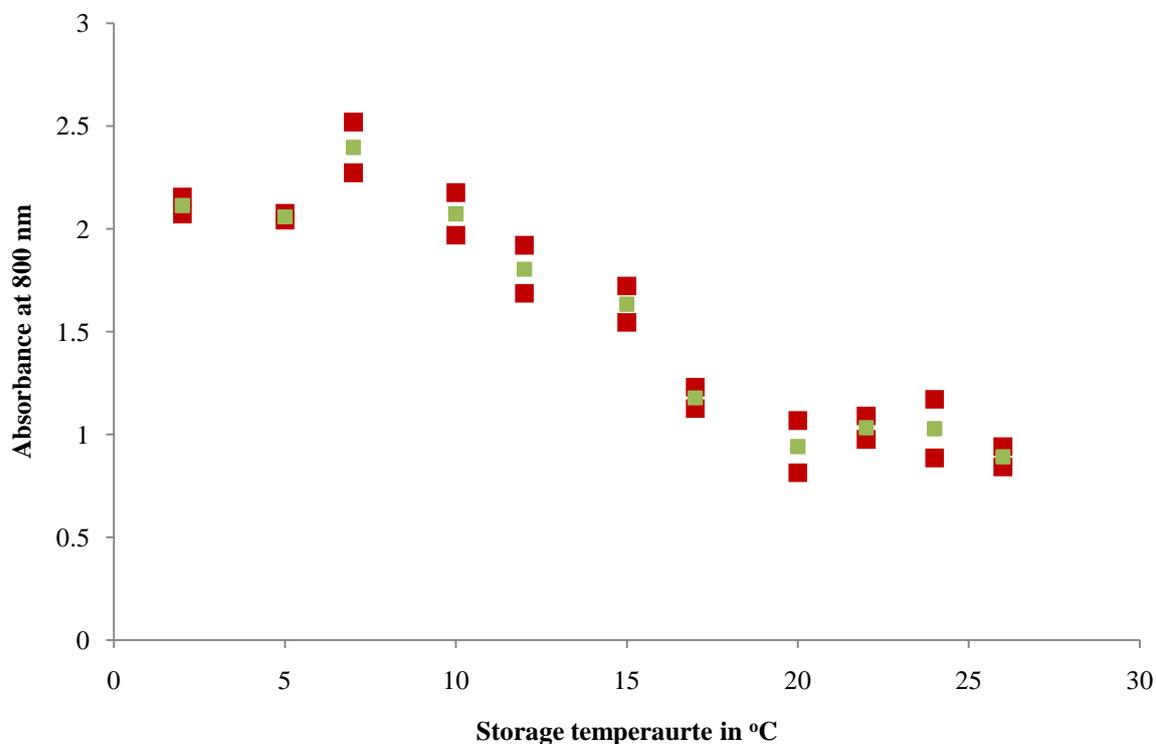


Figure 8.14 Absorbance of polyaniline film at 800 nm after exposure to herring for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The decrease in absorbance is similar to that of salmon but occurs faster and levels out at a lower temperature than that observed for salmon. This can be attributed to the higher concentrations of TVB-N seen in the SIFT-MS experiments.

Again, the upper and lower error points displayed on the graph show that there is a consistent variance in absorbance across the data set which is due to the standard error of film deposition and the random nature of the technique used to grow the layers of polyaniline.

The next set of experiments observed changes in the absorbance of the films at 650 nm. The experiment was repeated for water, salmon and herring. The results for the experiments with 500 μ l of water can be seen below in Figure 8.15.

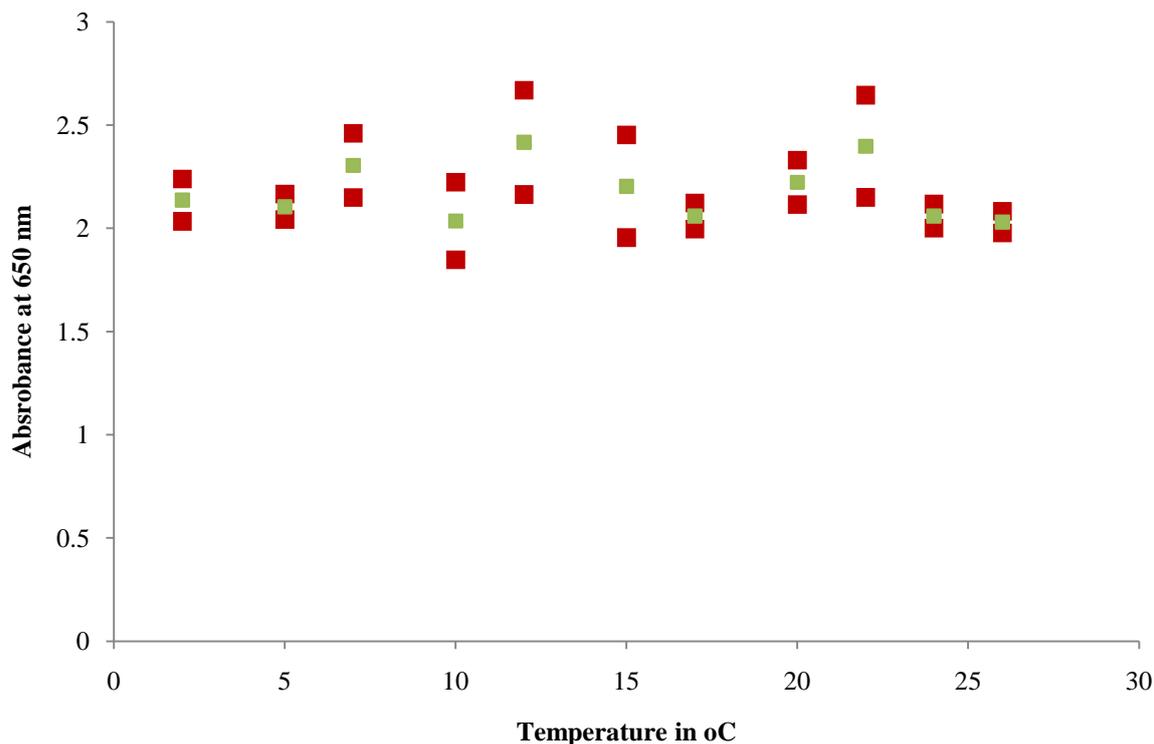


Figure 8.15 Absorbance of polyaniline film at 650 nm after exposure to water for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The sensor shows stability at this wavelength when exposed to water across the range of temperatures investigated. The upper and lower error bounds again show that the reproducibility of the sensor is high but with some expected error attributed to the method of formation of the films.

The next experiment exposed the polyaniline sensors to samples of salmon and after 5 days with the absorbance at 650 nm being recorded for storage of the fish at a range of temperatures. Figure 8.16 shows the results of this experiment.

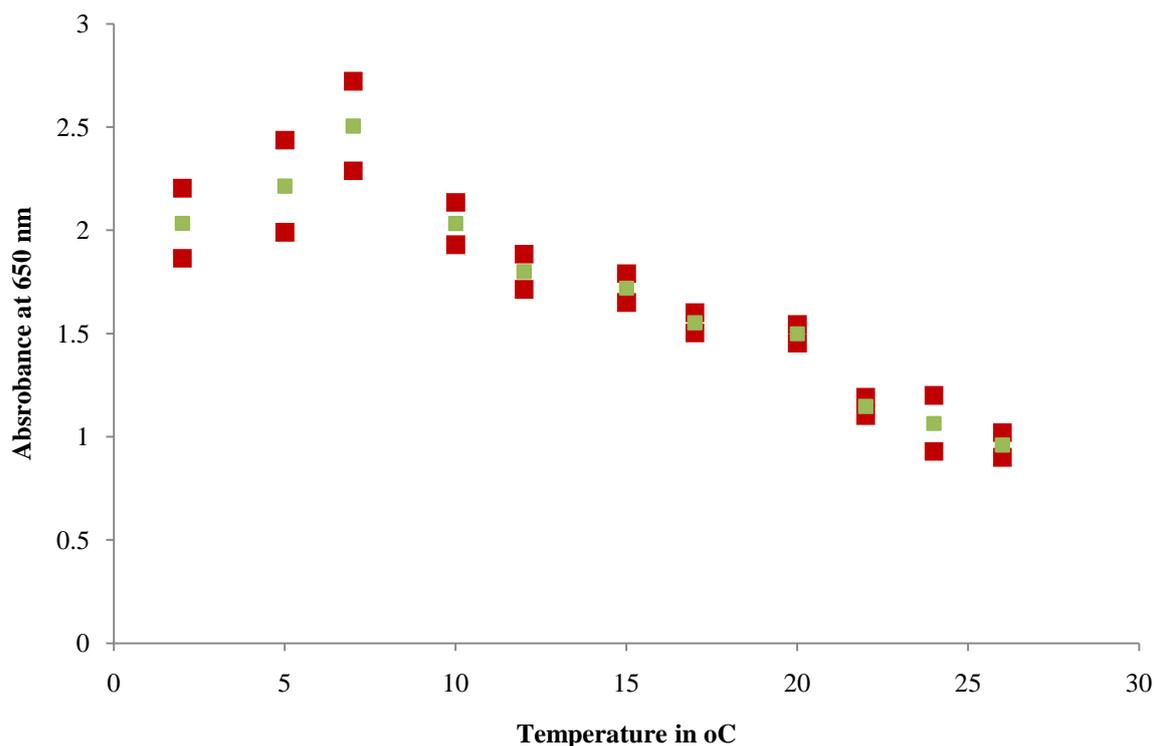


Figure 8.16 Absorbance of polyaniline film at 650 nm after exposure to salmon for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

Similar to the results seen in the previous experiment, there is an increase in absorbance at lower temperatures. This is possibly explained by reasons mentioned previously to do with the complex mixture of gases present in the headspace at these lower temperatures and the low value of TVB-N concentration. The absorbance is then seen to decrease linearly until a minimum is reached. This minimum is slightly higher than that observed for the higher wavelength due to the cross over between the green and blue forms of the polymer.

The upper and lower error bounds become closer as the absorbance decreases at the higher temperatures. In general these bounds are close and reflect the accuracy of the data.

The experiment was repeated using herring at this wavelength. Figure 8.17 shows the response of the sensor to a variety of storage temperature after 5 days storage.

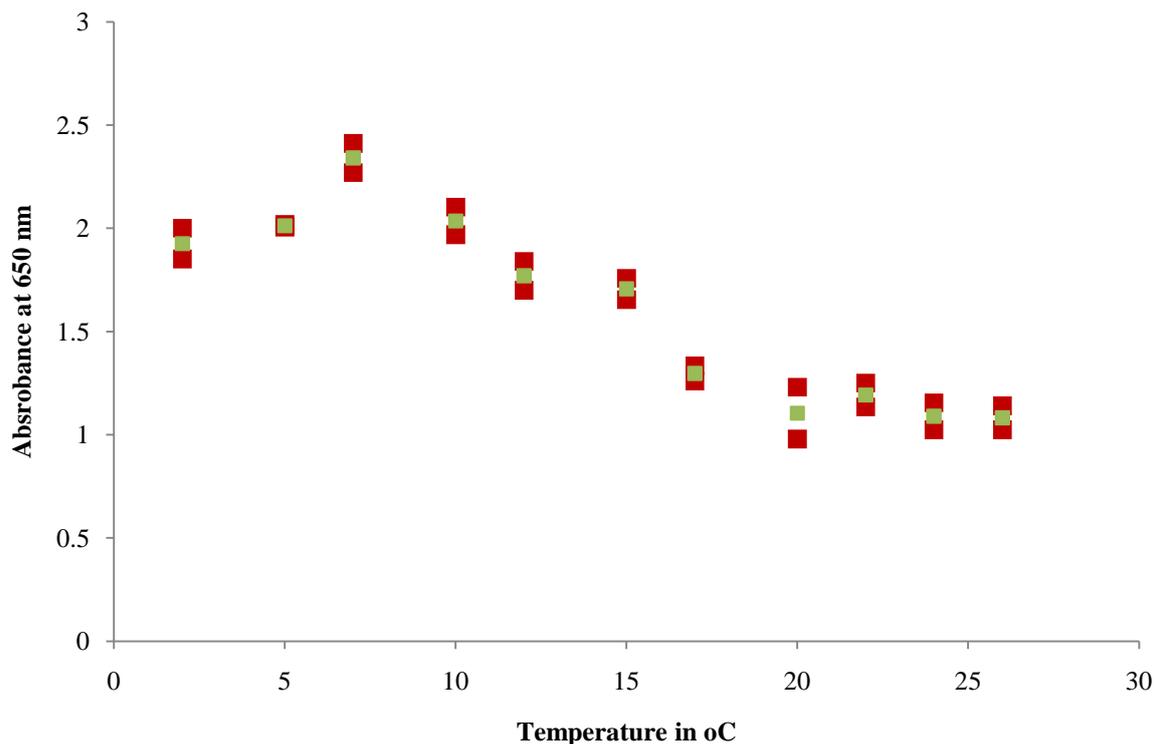


Figure 8.17 Absorbance of polyaniline film at 650 nm after exposure to herring for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The results again compliment those observed at the higher wavelength with a steeper linear decrease of absorbance than salmon. This again is expected to be attributed to the higher concentration of TVB-N. At this wavelength the increase at lower temperatures is also visible and is possibly down to error in the samples or more probably down to the complex nature of the composition of the headspace gas.

Absorbance decreases to approximately the same level as seen at the higher wavelength and the upper and lower bounds show the reliability of the data recorded for this experiment.

The final set of these experiments investigated the change in absorbance at 550nm for polyaniline films exposed to salmon, herring and water. The first set of results are found below in Figure 8.18 and show the change in absorbance at this wavelength of films exposed to 500 μ l of water.

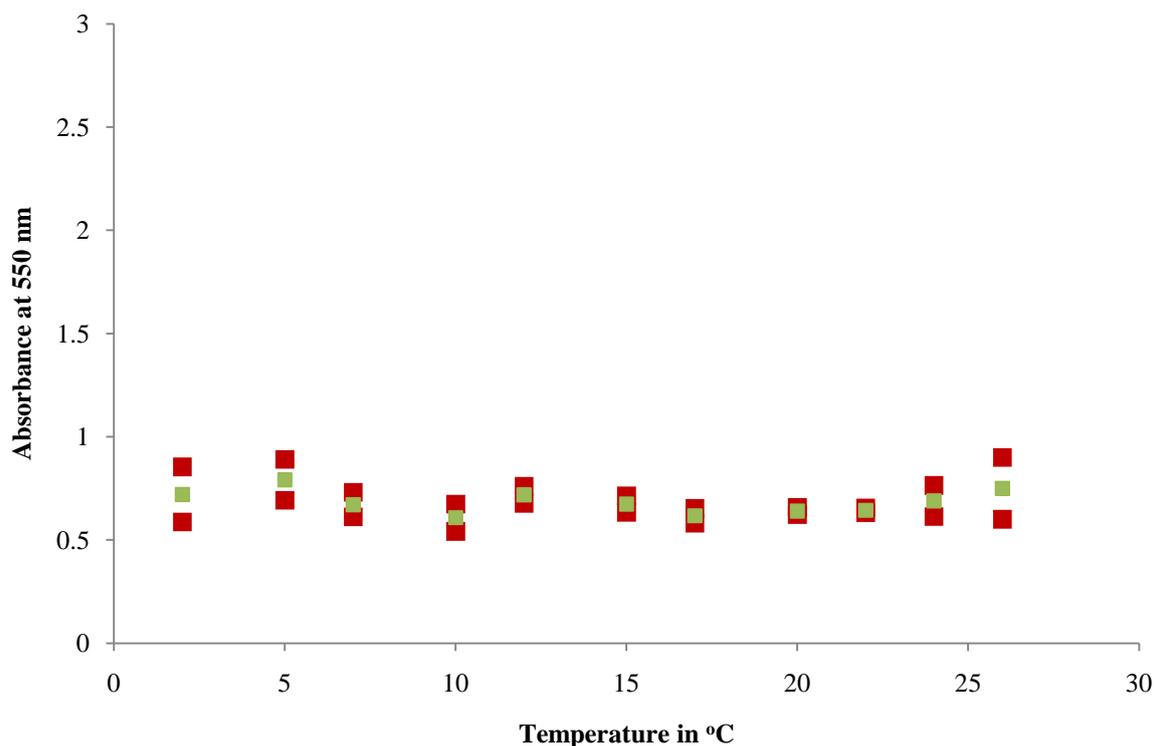


Figure 8.18 Absorbance of polyaniline film at 550 nm after exposure to water for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The data set shows that there is low initial absorbance for this wavelength and that there is no noticeable change when the polymer is exposed to water over a range of temperatures.

The reliability of the measurements at this wavelength is shown by the upper and lower bounds of confidence on the graph.

The next set of data can be seen below in Figure 8.19; this shows the effect of storage temperature of salmon on the change in absorbance of polyaniline sensors placed in the headspace over a 5 day period.

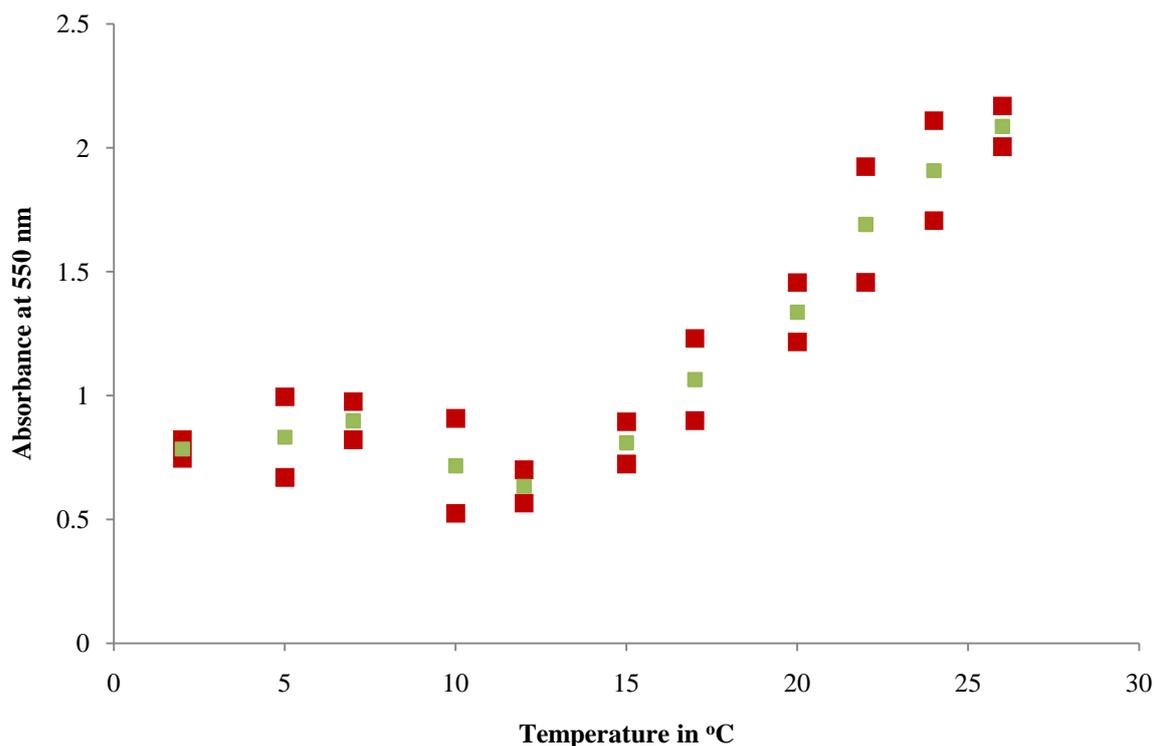


Figure 8.19 Absorbance of polyaniline film at 550 nm after exposure to salmon for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The change in absorbance at this wavelength is a linear increase and this is only observed at temperatures above 17 °C. This shows that this wavelength is a poorer describer of shelf-life and spoilage than the other two that have been described previously. After 5 days, all of the samples have past acceptable standards for foods spoilage but only some of the sensors give an indication of this.

The errors again are robust enough for each sample for the model to be accepted. In general, there is a bigger spread of error at the higher temperatures which is due to either fluctuations in rates of spoilage or the layers of the sensor or both.

The final graph in this data set show the same experiment with samples of herring instead of salmon. The results can be seen below in Figure 8.20.

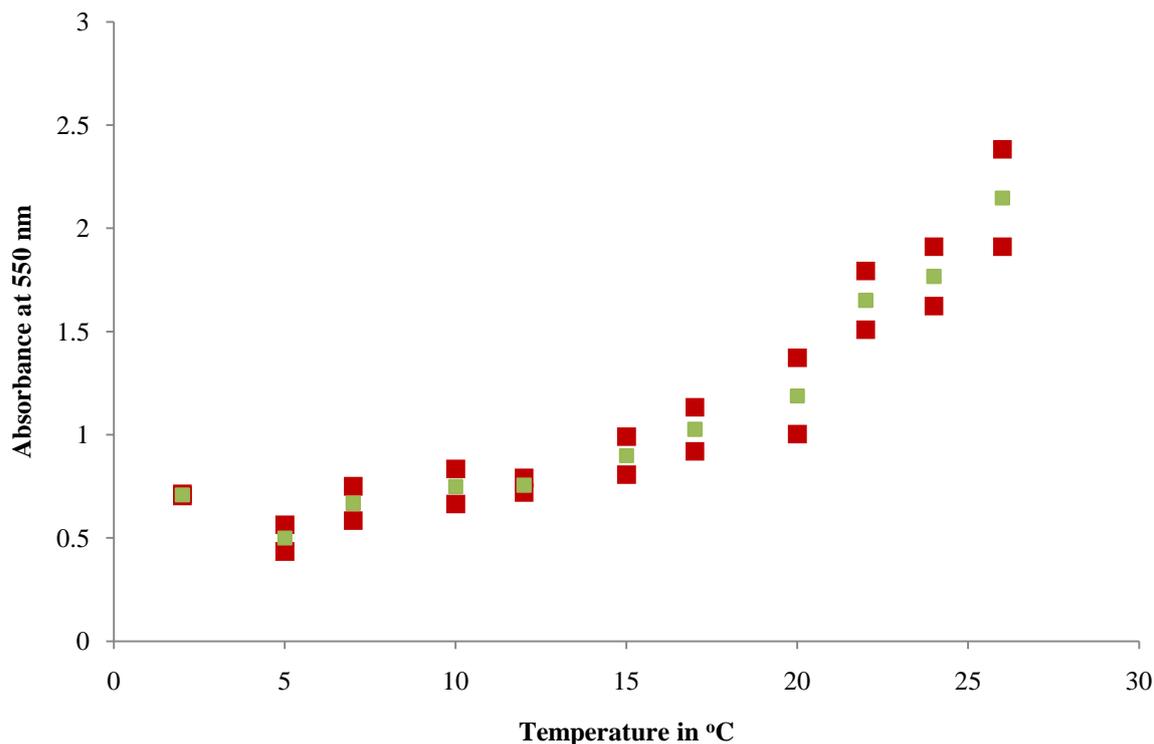


Figure 8.20 Absorbance of polyaniline film at 550 nm after exposure to herring for 5 days at various temperatures (■ = mean value, ■ = upper and lower values)

The increase of the absorbance here is also linearly upwards as expected and is similar to that observed for salmon. This is mainly due to the increase in concentration of TVB-N in the headspace at higher temperatures. The sensor does not respond effectively until storage temperatures over 17 °C even though all of the samples will have failed quality inspections in industry and harbour dangerous levels of spoilage bacteria and metabolites.

The errors recorded for this experiment again show that there are slight variations in the sensor response. Reasons for this have been previously discussed and include the method of formation of the sensor and variations in spoilage kinetics of each herring sample.

8.4 Other techniques of detecting change in polyaniline films

After the completion of the above studies further research was completed in order to determine any other methods of observing physical change in polyaniline after exposure to degrading food stuffs. Following on from measurements of resistance, an investigation into the effect of using an alternating current on the film was investigated. A brief summary of

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AC currents and impedance is given below, followed by the results found from this investigation.

8.4.1 AC circuits

The definition of impedance is:

“Impedance, Z, is the measure of the opposition of a circuit to the passage of a current and therefore determines the amplitude of a current.”

In a dc circuit this is R alone, however, in an ac circuit the reactance, X, also has to be accounted for. Equation 8.1 shows impedance being expressed as:

Equation 8.1 Mathematical expression for impedance

$$Z^2 = R^2 + X^2$$

The reactance of a material is its capacitance reactance, X_C , and inductance reactance, X_L . To understand this better, first we must consider a pure resistor with no capacitance, when ohms law is obeyed as in Equation 8.1. In the reality the relationship is not as simple due to the sinusoidal behaviour of the current and voltage. A purely sinusoidal voltage is expressed below in Equation 8.2 as:

Equation 8.2 Mathematical expression of sinusoidal voltage

$$e = E \sin \omega t$$

Where ω is the angular frequency, which is 2π times the conventional frequency in Hz. The amplitude is E with a frequency of rotation of ω . The observed voltage at any given time is given by e. For current, the signal is not in phase with the voltage. The signals are separated by a phase angle, ϕ . Usually E is taken as a reference signal for ϕ to be measured relative to it. In the demonstration figure, the current lags the voltage. Equation 8.3 below expresses this as:

Equation 8.3 Mathematical expression of sinusoidal current

$$i = I \sin(\omega t + \varphi)$$

Where φ is assigned a quantity, in this case it is negative and is 90° . This relationship can be seen below in Figure 8.21.

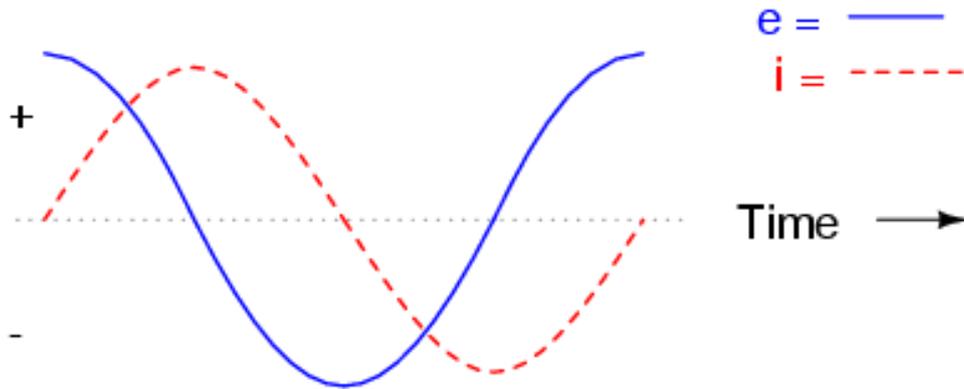


Figure 8.21 Relationship between e and i

The impedance of a pure resistor as stated before is simply the ratio of e and i. The reactance of a material is also a function of frequency. The reactance depends on the materials ability to act as a capacitor or inductor when the current is alternating. These effects contribute to the overall observed impedance of the material along with the resistance.

The reactance of capacitors and inductors is calculated by the equations as below. Note that these equations are only correct for circuits of pure capacitance or pure inductance. The overall effect of reactance is calculated by vectors. This is expressed below in Figure 8.3.

Equation 8.4 Mathematical expression of the reactance of a capacitor and an inductor

$$X_C = -\frac{1}{\omega C} \quad X_L = \omega L$$

Figure 8.22 shows the current changes due to the properties of inductance. The changes include phase shift of the current and amplitude changes. The combination of the resistance

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and the inductive/capacitance reactance, in this case inductive only, equals the total impedance.

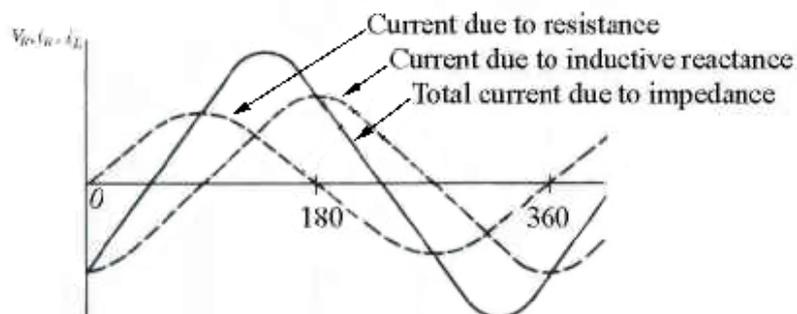


Figure 8.22 A visual explanation of the impedance effect on current (Source: Atkins 2001)

The total impedance is calculated by vectors. Figure 8.23 shows the vectors from the above graph. The phase angle is the change in phase of the current due to the reactance relative to the resistance. The impedance is then calculated to be the vector between the two quantities.

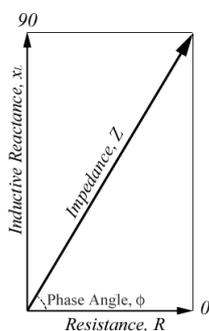


Figure 8.23 The vectors of inductance and resistance being added to equal the impedance

The impedance measurements for the experiments presented in the next section were scanned over a range of frequencies to determine if the sensor could show enhanced functionality.

8.4.2 Impedance changes

A brief study was conducted into the change in impedance signal of the polyaniline films to degrading food stuffs. The studies for salmon can be seen below in Figure 8.24.

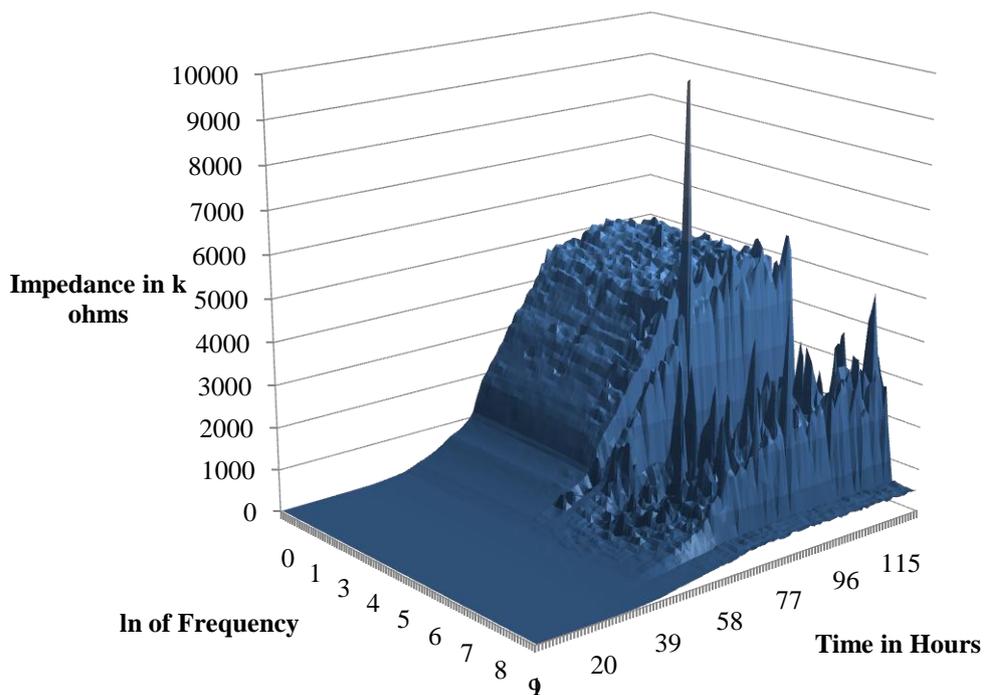


Figure 8.24 AC impedance of polyaniline exposed to salmon at 20 °C interrogated over a range of frequency (1-10000 Hz)

The results show a poorer response to degrading salmon as the sensor shows the larger increase after 40 hours of storage at this temperature which is approximately 10 hours behind the time at which the change is monitored using the sensor in a dc circuit. The change in impedance is not as sudden as the increase in dc resistance seen in previous experiments.

The sensor also does not behave as expected above 100 Hz frequency of ac. This is possibly due to the conduction mechanism of conducting polymers, in that the transfer of charge between two clusters of polyaniline may be limited to a frequency range below 100 Hz. This region was different for every sensor used which is most likely caused by subtle differences in the layers of the polyaniline deposited. The method of manufacture is not reproducible in terms of the microscopic details of the films produced, which is probably reflected in this observation. This was also noticed in the range of absorbance in the previous experiment determining the change in colour of the films exposed to degrading fish. The method of conduction in conducting polymers can be seen in Figure 2.10.

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The above experiment was repeated for herring and the results can be seen below in Figure 8.25.

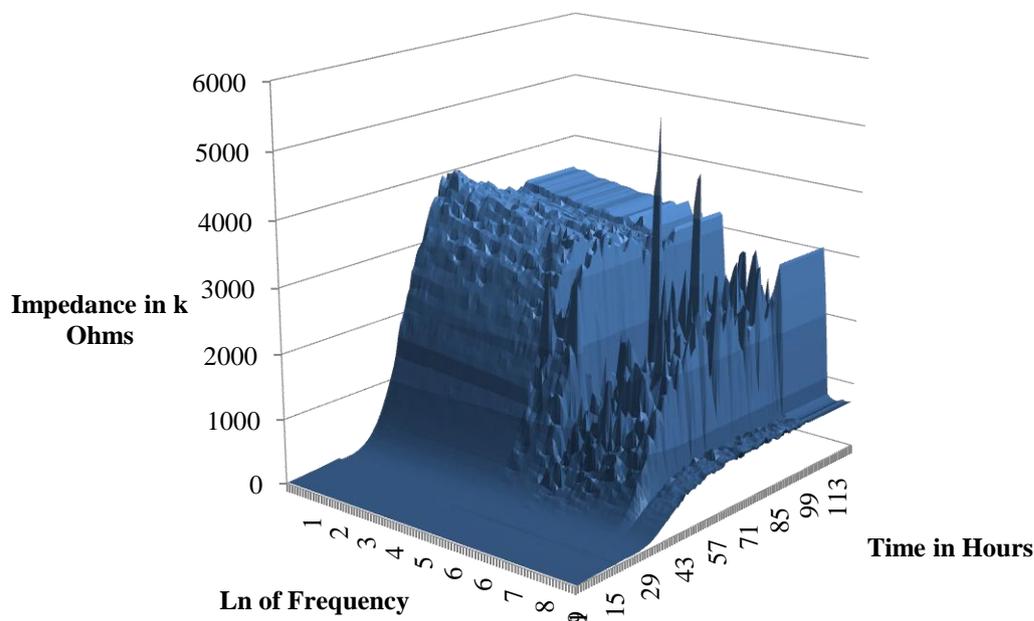


Figure 8.25 AC impedance of polyaniline exposed to herring at 20 °C interrogated over a range of frequency (1-10000 Hz)

The signal response to herring shows that the sensor is again slightly behind to the dc response but by a much smaller margin. In dc circuits, the resistance change of the sensor was observed at 27 hours and in ac circuits the change occurs after 33 hours.

Again the quality of the signal is poorer after 100 Hz which again was different for each sensor that was used. This is probably down to the reproducibility and conduction method of the polyaniline sensor.

8.5 Conclusions

The polyaniline sensors that have been developed during this project have been tested for their response to degrading samples of salmon and herring. The results have shown that there is measureable and observable change when the films are exposed to certain vapours. These include amine and ammonia. The changes that have been measured in this chapter are the colour, resistance and impedance of the polyaniline sensor when exposed to fish at a variety

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of temperatures. The range of temperatures studied in this chapter was consistent with previous chapters. The majority of experimentation took place at 24, 14 and 4 °C.

The sensors showed a definite response to nitrogen containing compounds when compared to the response observed for other molecules such as alcohols and carbonyls. This is to be expected as it has been widely reported in the literature that this is the case. Therefore it was a reasonable assumption that polyaniline sensors could be applied as FQI for degrading salmon and herring if one of the major spoilage volatiles in the headspace was ammonia.

The results have shown that polyaniline does respond to increased concentration of TVB-N in the headspace but this does not always correlate to when spoilage of the fish product has occurred. The results show that occasionally if the concentration of TVB-N is too high then the sensor reacts to display a false positive. The implications of this are also unfortunate as it would mean that edible food would be sent to land-fill. This was observed in the case of herring at 14 °C.

The three methods of sensor interrogation include measurements of change in dc resistance, colour from green to blue and ac impedance changes. All of these methods correlate to changes in the headspace of TVB-N and are in agreement with each other in terms of variation in the storage temperature of the fish samples. If a wavelength was to be measured to indicate quality then the results suggest that either 650 nm or 800 nm would give a more reliable indication of spoilage.

The sensor is limited for use above temperatures of 7 °C as it fails to respond to increasing bacterial populations below this threshold. The suggested reason behind this is because of the low concentration of TVB-N in the headspace at these temperatures due to low volatility of the compounds and also the slowed mechanisms of spoilage within the fish sample.

There may be further ways of improving the sensor including using another method of film deposition or a combination of conducting polymers in an array sensor. The problem with this approach is that the polymer could not be able to be deposited on existing material used for fish packaging and may also increase the cost of implementation of each sensor.

Chapter 9

General Conclusions

9 General Conclusions

The main aim of this thesis was to investigate the development of a polymer sensor for food spoilage detection for possible future commercialisation. The polymer that was chosen to be investigated for this purpose was polyaniline. This polymer is known to conduct in certain oxidation states. The conductivity of polyemeraldine salt is known to change physical properties such as colour and conductivity on exposure to certain compounds. These compounds are usually basic in nature and the effect of ammonia on polyaniline is well documented in the literature.

The degradation of fish is known to produce several amines and ammonia as well as other compounds which add to the unpleasant and distinctive aroma of degrading fish. These compounds are produced by autolysis and bacterial growth as compounds such as amino acids and proteins are broken down. This made fish a suitable starting point for the application of polyaniline as a food sensor. Comparisons of two fish species were chosen due to the different composition in terms of biology and industrial handling. Herring, which is a fish with higher fat content than salmon was sold as a whole fish and was sourced from open waters. Salmon on the other hand was bought as a pre-gutted and washed fillet from a specialised farm.

As shown in the commercial research in chapter 4, high value chilled food with a short shelf-life presents the greatest challenge to the food and drink industry in terms of volumes and value of waste sent to land-fill. Although fish was not directly investigated in this study the model that was presented in Figure 4.17 would position fish in the top left corner of the shelf-life versus waste matrix. A reasonable assumption is that the conclusions from chapter 4 carry across to the fish industry and fresh fish products. Vertical integration or information sharing at the supplier-retailer interface is of great importance for products that cause large volumes of waste. The minimisation of wastage is one of the benefits that come from these practices.

The sensor being developed for this project has been expected to integrate with existing packaging and not cause any excess waste since this is an important aspect to consider in

General Conclusions

terms of consumer attitudes as well as from an environmental perspective. The commercial sensor that will eventually be used in supply chains to replace printed dates will have to integrate with information systems as well as being easily understood by end consumers. The sensor presented in this thesis could be used in conjunction with an RFID tag to present data upon changes in resistance of the polyaniline film. The colour change that has been shown could also be used and easily comprehended by end consumers to show that the food has perished. The combination of both of these factors has not been observed on any existing products in the market sector for intelligent packaging.

The increase in growth rate of bacterial populations on salmon and herring can be correlated with a rise in temperature. The experiments in chapter 6 showed that the effect of temperature increase on samples of salmon and herring - provided a linear increase in the square root of the growth rate providing an initial secondary model. The results from this could also be correlated with the emissions of volatile compounds and the decrease in the quality of the fish samples. The appearance of slime and changes in colour of the flesh were visible indicators of the state of the food product and that the sample had passed an acceptable level of spoilage.

The data from the studies into the headspace of both the fish studied showed that there is an increase of TVB-N once spoilage starts to occur. The rate and amount of these nitrogen compounds in the headspace relates to an increase of storage temperature. An increase in storage temperature allows the increase rate of production of these compounds. At lower storage temperatures, the delay in this increase is longer, much like the lag time observed for bacterial growth, and the rate of increase is much slower. Other volatiles measured that relate to spoilage (sulphides) also showed a similar pattern. The results of this study from the GCMS and SIFT-MS correlate with observed bacterial growth of the total count and also of the spoilage organism populations.

The sensors in this project could be further developed into a viable commercial product. The findings in this thesis have shown early indications that the polyaniline films could be developed into a sensor for fish spoilage, early warning of spoilage or storage temperature abuse. The sensor has the benefit of showing two physical changes when exposed to degrading fish; these are colour and conductivity. This could allow a sensor to be developed

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based around the conductivity of the films using RFID technology for integration into supply chain systems. The colour change that is observed furthermore does not require spectroscopic equipment for interrogation and could be interpreted by consumers and end users without the need for them to invest in any costly technology. FQI products already available use similar methods to communicate remaining shelf-life to users. The sensor from this project could have two methods of effective communication.

The reliability of the sensor is an issue that will need to be addressed in future work. A sensitivity analysis will need to be completed to determine the lower limits of detection for the sensor. At present, the lowest recorded limit using the method of manufacture in this thesis is between 1 and 2 ppm of TVB-N in fish headspace. This is comparatively better than the majority of polyaniline based ammonia sensors reported in the literature. The results have shown that the polyaniline films show larger changes in resistance to nitrogen containing compounds when compared to other compounds such as alcohols and carbonyl compounds.

Ultimately the issue of food waste is one of the largest contemporary problems facing the food industry and governments. Addressing this will call in to question levels of food waste sent to land-fill by consumers, food retailers and food suppliers. A technology that can help tackle the issue of food waste is expected to be of significant importance in minimising levels of waste by better stock control and product management.

Chapter 10

Suggestions for Further Work

10 Suggestions for Further Work

There are several parts of this thesis that have been identified for expansion by carrying out further research. With respect to chapter 4, further research similar to the work presented in this thesis is already being conducted into causes and volumes of waste created by the fish industry by the same team to compliment the data gathered on other food stuffs. Other methods of deposition of thin films could also be investigated and compared against those formed in chapter 5 as well exploring the deposition of other conducting polymers using the two pot synthesis method.

Other methods of film manufacture could also be indentified and compared to the results gather in chapter 5. Other techniques for thin film deposition, such as spin coating and gas vapour methods, are expected to be poorer in terms of their conductivity. The ease of deposition, film conductivity and film morphology could be investigated. The commercial prospects of the sensor would mean that a comparison of cost-effectiveness would also have to be taken into account when comparing these methods.

In chapter 6, the application and comparison of other growth models such as the Baranyi model could be used to compare the data gathered from these simulations. The experiment could also be repeated over a wider range of times and temperatures building on the secondary models initially produced by the three temperatures. There is also scope for simulating storage temperature abuse and monitoring the sensor robustness under a fluctuating temperature. Another possible area to study is into the growth of other SSOs and the comparisons of other food stuffs such as chicken, beef and lamb. Further bacterial techniques could also be involved in the scaling up and automation of bacterial population enumeration. The quality of the models produced in this thesis could be improved with the collection of more data on populations while the resolution of the models could also be improved.

Further methods of gas analysis could be carried out on salmon and herring as well as using techniques such as high performance liquid chromatography. In conjunction with this, SIFT-MS analysis could also be carried out on other meats and food stuffs to analyse the

Suggestions for Further Work

spoilage markers that could be used to quantify food spoilage. The sensor also requires the exact detection limits to be determined.

The food sensor developed in chapter 8 could be used for other purposes, such as wound monitoring in an intelligent medical dressing. The use of other conducting polymers should also be investigated and compared along with an array such those as an electronic nose. The use of sterile fish samples would also be of interest to compare whether the sensor is detecting bacterial spoilage - or autolysis and natural breakdown of the food stuff.

Chapter 11

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Chapter 12

Appendix

12 Appendix

12.1 Blank questionnaire from DEFRA waste project

Company (optional)		Date	Time	Interviewer
Name (optional)	E-mail (optional)		Telephone (optional)	
Title and responsibilities (optional)				
1.0 Who is responsible for waste in the organisation?				
1.1 Product (s) Reviewed				
1.2 Brief description of Product				
1.3 Scope: Manufacture <input type="checkbox"/> Logistics <input type="checkbox"/> Retail				
1.4 Product Type				
<input type="checkbox"/> Fast Moving Goods (FMG)		<input type="checkbox"/> Slow Moving Goods (SMG)		
<input type="checkbox"/> Chilled / Fresh Meats		<input type="checkbox"/> Produce		
<input type="checkbox"/> Frozen		<input type="checkbox"/> Beer, Wine and Spirits		
1.5 Product shelf-life (days)				
1.6 Lead time (hours from order to delivery)				
1.7 Demand Variability (comments on seasonality, cyclicity and promotions)				
1.8 Average stock cover (days):				
1.9 Total production volume (per year):				
1.10 Percentage of wasted product (over a year)				
1.11 Tonnage of wasted product (per year):				
2. CAUSES OF WASTE				
2.1 What are the main causes of waste for this product?				
2.2 What is the impact of forecasting practices on waste?				
2.3 What is the impact of information sharing on waste?				

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2.4 What is the impact of promotions on waste for this product?
2.5 What is the impact of lead-times on waste?
2.6 What is the impact of shelf-life policies on waste? (i.e. proportion of shelf-life accepted)
2.7 What is the target stock level for this product? 2.7.1 What is the impact of safety stock level policies on waste?
2.8 Are there any specific stacking and shelving policies for this product? 2.8.1 What is the impact of these policies on waste?
2.9 What are the penalties for not delivering on-time in-full (OTIF)? 2.9.1 What is the impact of such policies on waste?
2.10 Are there any specific characteristics of this product that make it more susceptible to creating waste?
2.11 What is the impact of product damage on waste for this product?
2.12 What is the impact of packaging design on waste? 2.12.1 What kind of intermediate packaging is used for this product? 2.12.2 What is the impact of “ready for shelf” packaging on waste?
2.13 What would be the impact of a product recall and emergency product withdrawals (EPWs) on wasted product?
2.14 What is the impact of weather changes on waste for this product?
2.15.1 What would be the impact on waste of a catastrophic failure on warehousing equipment? 2.15.2 What would be the impact on waste of a catastrophic failure on transportation equipment?
2.16 What is the impact of seasonality on waste for this product?
2.17 Have we missed any other important cause of waste for this product?

3. Destination of waste

3.1 What happens to waste of damaged product?
3.2 What happens to waste of product that exceeds its shelf life?
3.3 What happens to product that exceeds the proportion of shelf-life demanded by retailers but it is still safe to eat?
3.4 What happens to packaging waste (i.e. intermediate packaging)?