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Use of oxygen sensors for the non destructive measurement of oxygen in packaged food and beverage products and its impact on product quality and shelf life

A Thesis submitted in the Fulfilment of the Requirements for the Degree of Doctor of Philosophy

Presented by

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This Thesis is dedicated to my grandfather

Walter 'Opi' Hempel,

Who opened me to the world of food and I miss him everyday

Abstract

The principal objective of this thesis was to investigate the ability of reversible optical O_2 sensors to be incorporated into food/beverage packaging systems to continuously monitor O_2 levels in a non-destructive manner immediately post-packaging and over time. Residual levels of O_2 present in packs can negatively affect product quality and subsequently, product shelf-life, especially for O_2-sensitive foods/beverages. Therefore, the ability of O_2 sensors to continuously monitor O_2 levels present within food/beverage packages was considered commercially relevant in terms of identifying the consequences of residual O_2 on product safety and quality over time. Research commenced with the development of a novel range of O_2 sensors based on phosphorescent platinum and palladium octaethylporphyrin-ketones (OEPk) in nano-porous high density polyethylene (HDPE), polypropylene (PP) polytetrafluoroethylene (PTFE) polymer supports. Sensors were calibrated over a temperature range of -10°C to +40°C and deemed suitable for food and beverage packaging applications.

This sensor technology was used and demonstrated itself effective in determining failures in packaging containment. This was clearly demonstrated in the packaging of cheese string products. The sensor technology was also assessed across a wide range of packaged products; beer, ready-to-eat salad products, bread and convenience-style, muscle-based processed food products. The O_2 sensor technology performed extremely well within all packaging systems. The sensor technology adequately detected O_2 levels in; beer bottles prior to and following pasteurisation, modified atmosphere (MA) packs of ready-to-eat salad packs as respiration progressed during product storage and MA packs of bread and convenience-style

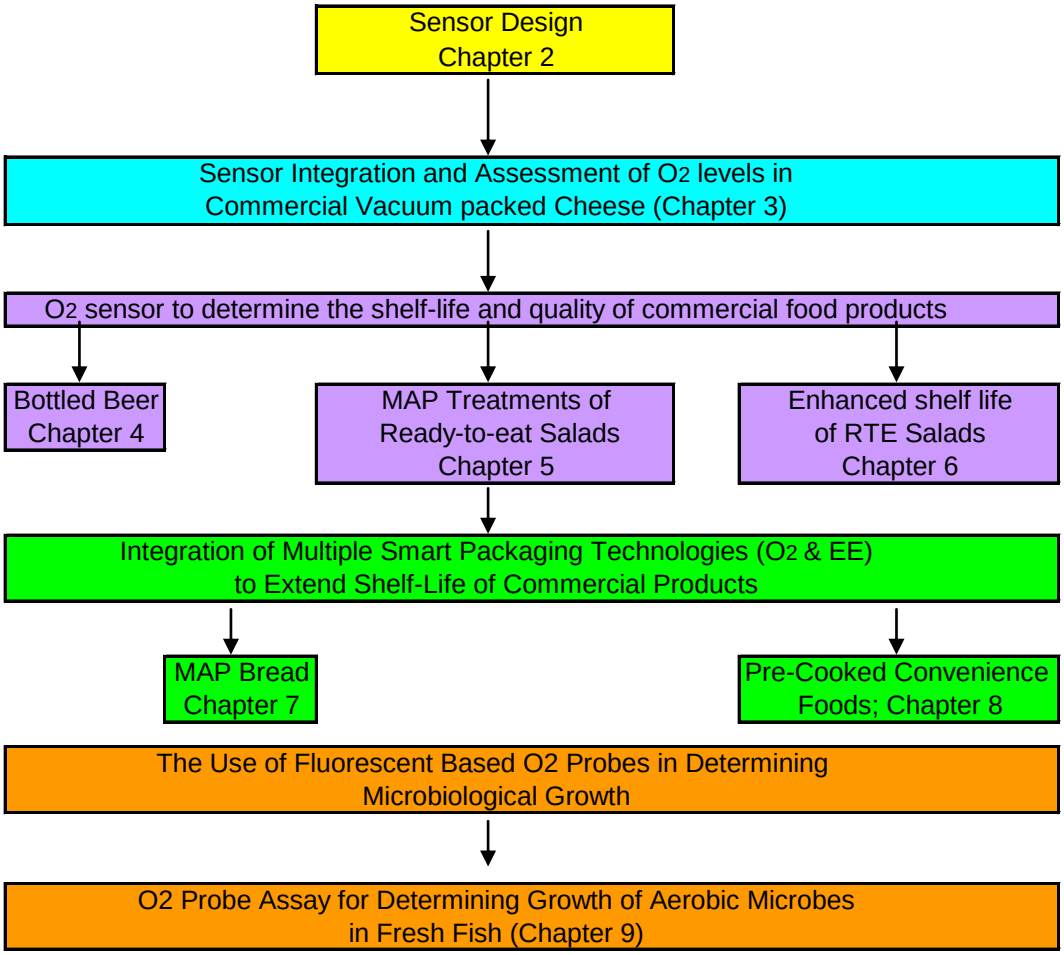
muscle-based products as mycological growth occurred in food packs over time in the presence and absence of ethanol emitters. The use of the technology, in conjunction with standard food quality assessment techniques, showed remarkable usefulness in determining the impact of actual levels of O_2 on specific quality attributes.

The O_2 sensing probe was modified, miniaturised and automated to screen for the determination of total aerobic viable counts (TVC) in several fish species samples. The test showed good correlation with conventional TVC test (ISO:4833:2003), analytical performance and ruggedness with respect to variation of key assay parameters (probe concentration and pipetting volume). Overall, the respirometric fish TVC test was simple to use, possessed a dynamic microbial range (10^4-10^7 cfu/g sample), had an accuracy of +/- one log(cfu/g sample) and was rapid. Its ability to assess highly perishable products such as fish for total microbial growth in <12 hr demonstrates commercial potential.

Keywords: Optical O_2 Sensors, Food Packaging, Sensory Evaluation, Shelf-Life, Storage, Modified Atmosphere Packaging, Vacuum Packaging, Ethanol Emitters

Thesis Overview Schematic

Use of oxygen sensors for the non destructive measurement of oxygen in packaged food and beverage products and its impact on product quality and shelf life



Thesis Objective

The objective of this thesis was to develop and optimize a reversible O_2 sensor system for assessment in food and beverage products using conventional packaging systems and materials. Integrate an optical O_2 sensor suitable for industrial food packaging applications. Establish the universal O_2 sensor as a quality control ‘smart packaging’ device in a range of commercial foods, withstanding all types of packaging systems (Modified atmosphere, bottling, vacuum, etc.). Demonstrate the use of multiple smart packaging technologies (Active - ethanol emitters & intelligent - O_2 sensors) in commercial food products to extend shelf life. And finally, to modify the O_2-sensitive dye in a rapid Respirometric microbiological assay

CHAPTER I

Literature Review

1.1 Role of Food Packaging

The role of food packaging is essential and pervasive; essential because without packaging the safety and quality of food would be compromised, and pervasive because almost all food is packaged in some way (Robertson, 2010). Food packaging performs a number of fundamental functions: to contain, protect and preserve the food or beverage from contamination, physical damage and spoilage, respectively, to facilitate the handling, distribution, transport and storage of goods and to provide information and promote the sales of retail-ready products in an economic, environmental and legally acceptable manner. A distinction is usually made between various "levels" of packaging; primary (technical functions and sales promotion), secondary (product collation and handling) and tertiary packaging (collation and transport). A primary package is one that is in direct contact with the contained product, and provides containment, protection and preservation (technical functions) as well as promoting the product through the provision of information in as cost-effective, convenient, legal and environmentally-friendly manner as possible. The packaging sector represents about 2% of Gross National Product (GNP) in developed countries and about half of all packaging is used to package food (Robertson, 2010). The quality of most foods and beverages decreases during storage and over time. These products allow for a finite length of time before the product becomes unacceptable for sale and use; described ultimately as shelf-life. Manufacturers generally attempt to provide the longest practical shelf-life consistent with costs, the pattern of handling, use and demand by distributors, retailers and consumers (Robertson, 2006).

1.2 Conventional packaging materials used in the packaging of oxygen-sensitive products

In the manufacturing of foods, packaging materials and techniques play a major role in the preservation of foods, enabling a longer product shelf-life to be achieved. Each single material used in food packaging imparts a specific function that aids in the containment, protection, design and barrier properties. Throughout the history of food packaging, many materials have been used. Each material has its own unique advantages which will determine its use in the role of packaging food and beverages. Some of the most extensively used materials for the industrial packaging of O_2-sensitive foods and beverages today are presented subsequently.

1.2.1 Glass

Glass is one of the oldest packaging materials and still maintains an important place today. Glass has many advantages as a packaging material. It is solid, transparent, inert, impermeable, odorless, and available in a wide range of shapes, sizes and colours to provide customer appeal (Brody & Marsh, 1997). Glass is primarily made from silica sand and other raw materials that are naturally abundant and inexpensive. It can form containers that are rigid and easily transported, filled and stored. Products look better, taste purer and are secure when packaged in glass (Grayhurst, 2012). Glass is 100% recyclable and is environmentally friendly when viewed in a number of ways; however, glass is heavy, thereby adding to shipping costs (Yam, 2009) through the consumption of non-renewable fossil fuels. There are two main types of glass containers used in food packaging; bottles which have narrow openings and jars and pots which have wide openings. Glass bottles are heavily utilized in the beverage industry. The single greatest use of glass in the food

industry is in the packaging of thermally processed and high-end carbonated beverages (Duncan and Hannah, 2012).

1.2.2 Plastics/Polymers

Plastics are widely used as food packaging materials as they are flow-able and moldable under certain processing conditions, they are generally inert, but not necessarily 100% impermeable, are cost-effective in meeting market needs, are lightweight and provide choices in respect of transparency, colour, heat-sealing and barrier function (Kirwan & Strawbridge, 2003). Plastics have primary properties of strength and toughness. Specific plastics can meet the needs of a wide range of temperatures, from frozen food processing (-20ºC) and chilled storage (4ºC) to high temperatures of hot filling (100ºC), microwavable reheating (>100ºC) ultra high temperature processing (120 ºC) and retort sterilization (>120ºC). Plastics are utilized in the food industry because they offer a wide range of appearance and performance properties which are derived from the inherent features of the individual plastic material and how it is processed and used (Kirwan & Strawbridge, 2003). Gases such as O_2, CO_2, N_2 together with water vapour and organic solvents permeate through plastics, however the rate of permeation depends on the type of plastic, thickness, method of processing, storage temperature and the concentration or partial pressure of the permeate molecule (Lagarón, 2011). There are a large number of polymers used in food packaging applications, the predominant forms consisting of; polyesters, polyethylenes (PE), polypropylene (PP), polystyrene (PS), polyamides (PA), polyvinyl chloride (PVC), polyvinyl alcohol (PVOH) and ionomers. The selection of a plastic material for food packaging will depend on the characteristics of the food or beverage product in question. In the case of O_2 sensitive foods a high O_2 barrier plastic will be selected. Good performing O_2 barrier plastics commonly used around food and beverage products today include;

polyethylene terephthalate (PET), PVOH, ethylene vinyl alcohol (EVOH), PA and polyvinyldene chloride (PVDC). The extent of their application depends on cost and the importance of an O_2 barrier layer to the food product. In the case of laminates (1.2.4) which combine multiple material layers, a plastic may be chosen as the barrier material in the laminate for the product of choice.

1.2.3 Metal

Metals are used for a various range of food packaging applications. Metal packages for food products must perform the following basic functions if the contents are to be delivered to the ultimate consumer in a safe and wholesome manner; preserve and protect the product, resist chemical attack by product components, withstand the handling and processing conditions (excess heat processing), withstand the external environment conditions, have the required shelf-life display properties at the point of sale, provide easy opening and simple/safe product removal and be constructed from recyclable raw materials (Page, Edwards & May, 2003). In addition, these functions must be performed satisfactorily until well after the end of the stated shelf-life. For most metal food and drink containers the cost of the metal itself is 50-70% of the total container cost. Metal-based food packaging containers are manufactured using steel or aluminum, the choice being application-dependent (Page, 2012). The use of metal in retort pooches or laminates is very common. The integration of a metalized layer imparts specific barrier properties to extend shelf-life and quality of the packaged food. An aluminium foil is most commonly found in retort pouches, it should be inert, heat stable, dimensionally stable and heat resistant to 121^{o}C. Laminates containing metal should demonstrate adequate performance against O_2, other gases and moisture vapour.

1.2.4 Laminates / Multilayer packaging materials

Laminates or multilayer flexible packaging offers packaged goods manufacturers the opportunity to match the functional needs of their products precisely to the materials used to package them. Multilayer flexible packaging consists of combinations of metal, plastic, or cellulosic substrates, individually ranging in thickness of 2.5 to 250um. These materials are then fashioned into various container formats that hold the product. Laminate materials provide documented levels of barrier to environmental factors; light, moisture and O_2 (Yam, 2009; Robertson, 2012). Oxidation of oils, fats and vitamins in foods and other products can be retarded by barrier-based multilayer materials if the O_2 rich atmosphere within the package is replaced with an inert gas. Multilayer flexible packaging is successfully used for retort processing of foods. A barrier layer can be added to plastic materials through a number of methods including adhesive lamination, extrusion coating, co-extrusion, co-injection, and vacuum deposition. Coating and lamination are often employed to improve O_2 barrier properties of packages, but can also be effective at reducing light transmission depending on the materials used and manufacturing methods employed (Duncan & Hannah, 2012). Aluminum is the most commonly used material for metallization of films. Flexible polymer or paper packages containing metal layers can be generated using laminate technology or by vacuum deposition onto a substrate. Initially used for decoration, metallization is now widely used in flexible and multilayer packaging to improve gas and moisture properties, heat resistance, light reflectance and electrical conductivity (Kerry, 2012). Aluminum foil used in a laminate construction is typically 5-17um in thickness, whereas a vacuum deposited aluminum coating is typically between 10-40nm. Metallization can be applied to a variety of polymer materials as well as paper, provided the paper can be treated to enable adhesion of the metal particles. Laminates or multilayer packaging materials that incorporate a metal foil account for approximately 17%

of all produced packaging films (Tartakowski, 2010). Paper is commonly used in laminates as a decorative layer for design, colour and printability. The paper layer does not actively participate in barrier properties.

1.3 Conventional packaging systems for oxygen-sensitive food and beverage applications

Each packaging system or format is carefully selected to provide the functions of food packaging as well as integration with food processing and practicality of use (Emblem, 2012). A range of packaging systems that are commonly employed by the manufacturers of O_2-sensitive food and beverage products are described in the following sections.

1.3.1 Aseptic packaging

Aseptic processing is the process by which a sterile (aseptic) product is packaged in a sterile container. Sterility is achieved with a flash heating process usually in excess of 90ºC, retaining more nutrients and using less energy than conventional sterilization such as retort or hot-fill canning. Aseptic food preservation methods allow processed food to keep for long periods of time without the need of preservatives, as long as food packages remain sealed. Most aseptic packages are laminations of paperboard, plastic and aluminum foil. In aseptic packaging, the products are sterilized with high heat for a short time followed by rapid cooling. The package is sterilized separately, and then both product and package are combined in a sterile chamber and sealed therein. In almost all methods of aseptic packaging, aluminum foil is used as a barrier to light and O_2 (Yam, 2009). Bag-in-box technology is commonly used because it provides strong containers that are lightweight and easy to handle prior to being filled. A bag-in-box package is an example of an aseptic package which is flexible, collapsible, and a fully sealed bag made from one or more plies

of synthetic films, a closure and tubular sprout through which contents are filled and dispensed, and an outer rigid box or container (Robertson, 2006). The physical strength of the bag is of prime importance and it must remain intact during distribution and subsequent storage. The bag-in-box system can be an alternative to heavier packaging materials such as metal and glass and results in a less weight of waste. The materials incorporated into bag-in-box systems are generally fully recyclable.

1.3.2 Retort (long-life) packaging

The standard definition relating to retortable flexible barrier materials is that they are food packaging materials capable of withstanding specific thermal processing in a closed retort at temperatures above 100ºC (Brody & Marsh, 1997). Retortable packages must maintain material integrity as well as barrier properties for desired end-product usage during product-to-package handling, thermal processing and subsequent shipping abuse. For shelf-stable food packages, the packaging materials used must be retortable and still maintain extended barrier characteristics against such effects as light, O_2, moisture and environmental exposure. The primary reason for specifying a package to be retortable is for thermal sterilization, or at least, minimal destruction of microbial content, which could cause illness or spoilage of contained products. The most positive effects on foods packaged in retortable packages are the resultant shortened process times for commercial sterilization and improved product quality (Brody & Marsh, 1997). Pasteurization (>70ºC x 2 mins) used in retort is to effectively destroy all spore-forming microorganisms and to significantly reduce the number of natural spoilage microorganisms, thereby extending the shelf-life of the product. Sterilization refers to the complete destruction of microorganisms including spores. Products are heated to temperatures as high as 120ºC for several minutes (Thippareddi & Sanchez, 2006). Thermal sterilization is usually associated with the canning

process. A typical three layer *retortable pouch* structure would consist of PET for strength, aluminium foil as moisture, light and gas barrier, and an inner layer of CPP for heat sealing, strength and compatibility with all foods. Retortable trays usually have a rigid or semirigid structural supporting body and a sealable flexible lid. They provide all the advantages of retortable pouches but are selected due to their ease of flat filling or top loading of products and potential to increase production-line speeds (Yam, 2009). Metal cans perform very well in a retort environment reported earlier (1.2.3). Glass jars and lightweight aluminum rigid containers are also well established packaging materials which can be used for retorting purposes. Glass as a retortable medium has the advantages of very low interaction with the contents and offers visibility of the product. It is essential to use the correct overpressure during the retorting to prevent the lid being distorted, and also the jars prior to processing to prevent shock breakage (Holdsworth, 1997). Special handling is required to prevent closure lids on jars from releasing their vacuum seal, easy-open scored lids on aluminum cans from fracturing, and aluminum can bodies from paneling owing to excessive internal or external pressure.

1.3.3 Hot-fill packaging

A wide range of foods can be preserved by filling at hot temperatures (>85ºC), sealing of the package under a steamed vacuum and holding there for a short period of time before cooling. The heat from the hot fill inactivates microorganisms which are sensitive to heat. This method is employed routinely in the filling of foods into glass jars. Polymers like PP, PET, high-density polyethylene (HDPE) and polycarbonate (PC) are used when plastics are chosen for food packaging; these polymers are heat stable for hot fill products (Lee, Yam & Piergiovanni, 2008).

1.3.4 Bottling

The use of glass and plastic bottles has been used commercially for liquids for greater than 50 years. Carbonated beverages represent the biggest single packaging market with over 100 billion beer and soft drink containers filled each year (Yam, 2009). Performance requirements are quite severe, as the contents are under pressure, they must hold their carbonation, and must withstand summer storage and pasteurization temperatures for beer (Grayhurst, 2012). There are three primary types of bottles used for beverages;

Plastic bottles – Method of manufacture is stretch blown PET, where the stretching of the polymer is needed to maximize tensile strength and gas barrier, which in turn enables bottle weight to be low enough to be industrially economical. The use of plastic bottles allows for recycling and also prevents the dangers associated with glass breakages.

Non-returnable glass bottles - Glass bottles have a cost advantage which carries through to retail level. The use of glass allows manufacturers great choice in bottle design and colour, and the glass is seen as environmentally-friendly as the material called cullet can be easily recycled. Non-returnable glass bottles are lightweight, ranging from 130g to 180g for 10oz and 16oz bottles, respectively (Brody & Marsh, 1997). These bottles are employed in single use products before discarding/recycling.

Refillable glass bottles – Thicker and heavier glass, weighing about 300g for 16oz bottle. Accounts for about 1/3 of all soft drinks and 1/5 of beer market (Yam, 2009). The bottles are reusable and refillable and primarily used where collection is feasible in what is known as tied systems. Major product brands can have labels permanently silk-screened on to the glass surface and are promoted for their environmental benefits. Refillable or returnable bottles are collected by supplier and reused for packaging where they undergo vigorous cleaning steps and are repeatedly subjected to elevated temperatures. The thicker grade of glass provides protection during these heat treatments.

1.3.5 Modified Atmosphere packaging

Modified atmosphere (MA) and vacuum packaging are two packaging methods used widely in the manufacture of O_2-sensitive food products. Some of the primary deteriorative reactions that occur in food are as a result of the excess presence of O_2 include; enzymatic and chemical reactions as well as physical and biological changes. Food packaging techniques can help combat these detrimental changes by preventing such reactions from taking place or delaying them, thereby resulting in an extended shelf-life (Robertson, 2006). Oxygen-related reactions resulting in food deterioration has been well documented. The role of O_2 in oxidative reactions negatively affects polyunsaturated fats and pigment stability, as well as promoting the growth of aerobic spoilage microorganisms. The importance of MA packaging to the food industry cannot be understated. The ability to provide an atmosphere within food packs to alter the natural progression of its state over time can result in an extended shelf-life. The ability to extend short shelf-life products which are typically O_2-sensitive foods is vital to manufacturers dealing with large markets.

MA packaging relies on mixtures of atmospheric gases like O_2, CO_2 and N_2 in concentrations that differ to air. The atmospheres created by manipulating these gases can sometimes be augmented through the addition of small amounts of other gases, such as; carbon monoxide, ethanol, sulphur dioxide or argon to maintain foods in a 'fresh' state during transport, distribution and retailing (Yam, 2009). The typical gases used in commercial food MA packaging applications consist of the following (Lee, *et al.*, 2008; Gontard & Guillaume, 2012; Coles, *et al*., 2003; Han, 2005; Brody & Marsh, 1997);

Oxygen (O_2): Most reactions with food constituents involving O_2 are degradation reactions resulting in the oxidative breakdown of foods into their constitutive parts. O_2

combines readily with fats and oils and cause rancidity. Most spoilage organisms require O_2 to grow and will cause off odours in the presence of sufficient O_2. O_2 will however be required in fresh red meat MA packaging applications, where the promotion of myoglobin to oxymyoglobin produces a rich cherry-red colour, which is found to be desirable by consumers.

Carbon Dioxide (CO_2): This gas dissolves in water and in fat and solubility increases with decreasing temperature. It has an acidifying effect consequently. CO_2 has selective bacteriostatic effects, which means that it acts as an antimicrobial substance against obligate and rapid food spoilage microorganisms like *Psedudomonas* sp. *CO_2* levels used in foods must be carefully calculated to prevent the formation of abnormal flavours and can also impact on product colour (Artes Calero, 2003; O'Sullivan, Cruz-Romero & Kerry, 2011).

Nitrogen (N_2): Is the most abundant component in air (~78%) and can be used in either gaseous or liquid forms. It is physiologically inert in its liquid and gaseous forms and is used in packaging primarily as a filler gas to prevent pack collapse following *CO_2* absorption by products and to exclude other gases (by flushing), especially *O_2*, from products at the point of pack sealing.

The combination of gases used for applications depends on many factors, such as the type of product being packed, packaging materials used, storage temperature, fat and moisture content, bacterial population, colour requirements (meat) etc. The packaging system should have sufficient headspace to provide enough gas to interact with the entire product (Cruz-Romero & Kerry, 2011). Package integrity is an essential requirement for maintaining the high quality of, for example, sterilised foods and MA packaged foods (Hurme, 2003). MA packaging can be created in two ways; passive MA packaging, where

in the case of fresh produce, respiration rate are matched with films of appropriate permeability to generate an atmosphere that passively evolves as a result of consumption of O_2 and evolution of CO_2 due to respiration, and active MA packaging, where a package is evacuated and then flushed with the desired gas mixture. The use of absorbers can be used in active MA packaging to scavenge O_2, CO_2 and ethylene to help maintain an atmosphere that promotes extended shelf-life (Vakkalanka *et al*., 2012). MA packaging offers many advantages to consumers and food products. To the consumer, it offers convenient, high quality food products with an extended shelf-life, it also reduces and sometimes eliminates the need for chemical preservatives, leading to a more 'natural' and 'healthy' product. At the same time, producers also enjoy the benefits of increased shelf-life (Floros & Matos, 2005).

1.3.6 Vacuum Packaging

Vacuum packaging involves the placing, either manually or automatically, of a perishable food inside a polymer-based packaging and then, by physical or mechanical means, removing air from inside the package so that the package material remains in close contact with the product surfaces after sealing under vacuum (Brody & Marsh, 1997). Factors such as the product being packaged, the barrier properties of the package material being employed, the level of air removal and the storage temperature used, can substantially delay chemical and/or microbial deterioration of the food product in question, and will in many cases, dramatically extend the period of eating quality. The effectiveness of vacuum packaging for shelf-life extension is mainly due to the low or very low amount of residual O_2, left in packs after sealing, as a consequence of the air extraction; oxidative reactions and aerobic respiration are reduced (Lee, *et al*., 2008). The use of manipulative packaging materials can be used in the practice of vacuum packaging. A food manufacturing process

that utilizes vacuum packaging will describe foods that are in a limited O_2 environment. The reduction of O_2 can vary due to packaging materials and pressures used during processing. An O_2 free packaging application can include food items that have been 'shrink-wrapped', where a plastic or laminate has been subjected to great pressure, forming a second skin around the food product (Yam, 2009). Vacuum skin packaging (VSP) is a packaging approach that uses a film that fits very tightly to the food surface, leaving little space for the accumulation of any fluid produced by drip loss (O' Sullivan & Kerry, 2012).

1.4 Importance of O_2 control in the packaging of oxygen-sensitive food products

The presence of O_2 in the environment immediately surrounding an O_2-sensitive food product can be responsible for a number of effects that can be negative to food quality. Oxygen found in the headspace of food packaging can result in the reduction of food quality by processes that include oxidation of fats and proteins as well as promoting aerobic microbial spoilage. Oxidative deterioration in foods involves oxidation on both the aqueous phase (proteins) and the lipid phase (fats) resulting in major sensory changes to flavour, aroma and overall perception to the eventual consumer. Formation of free radicals is an early event that occurs prior to the progression of oxidation and is most often associated with the aqueous phase (Skibsted, 2010). The elimination of O_2 in food packaging has been exploited to safely package foods and extend shelf-life. Also the addition of CO_2 to combat fast spoiling aerobic microorganisms has been employed. The availability of O_2 in abundance in food packaging is responsible for a range of degradative processes.

1.4.1 Oxidation of essential food components

-Lipids

Lipid oxidation is recognised as an important cause of quality deterioration of many foods like edible oils, muscle-based foods like fish and meat, and milk and dairy products. The spontaneous reaction of atmospheric O_2 with organic compounds leads to a number of degradative changes that reduce the lifetime of many products of interest to the chemical industry, especially polymers, as well as causing the deterioration of lipids in foods (Hudson, 1990). When foods are in contact with air, food components are oxidized directly or indirectly. The most frequent occurring reaction is lipid oxidation and this is one of the most important factors limiting shelf-life in many food products, resulting in the formation of unhealthy compounds such as free radicals and reactive aldehydes (Jacobsen, 2010). This results in the reduction or destruction of essential fatty acids, formation of off-flavours and aromas. Therefore control of lipid oxidation reactions is necessary for the preservation of foods. Oxidation of lipids is initiated by irradiation including exposure to visible light, by enzymes and metal catalysts. Heat and pressure accelerate lipid oxidation when initiated (Skibsted, 2010). The primary event in lipid oxidation is formation of free radicals followed by appearance of lipid hydroperoxides as primary lipid oxidation products further leading to secondary lipid oxidation products. Lipid oxidation will also lead to significant changes in the sensory properties of the food where, odour, flavour, colour and texture, which are detected by the consumer and ultimately may determine the shelf-life of the product. The individual effects of lipid oxidation on specific foods is well documented (Velasco, 2010; Skibsted, 2010). The limitation of O_2 in food sensitive products is achieved with the use of antioxidants as well as processing steps that alter the atmosphere in contact with the foods (i.e. vacuum and MA packaging).

-Pigments

Oxidative deterioration of pigments will also affect the appearance and colour of foods (Jacobsen, 2010). The interplay between pH, O_2, light, metals, and other factors promote *pigment oxidation*. The presence of light and O_2 cause heme pigments to become oxidized resulting in brown pigments (Skibsted, 2010; Richards, 2010). Novel packaging strategies using various gas mixtures in the headspace can be utilized to decrease pigment and lipid oxidation in muscle-based food products.

-Proteins

Oxidation of food proteins is currently of great interest. The fact that proteins are targets for reactive O_2 species was ignored for several decades while lipid oxidation was studied in depth (Lund *et al*., 2011). Protein oxidation can be defined as the covalent modification of a protein induced either directly by reactive O_2 species or indirectly by reaction with secondary by-products of oxidative stress (Shacter, 2000). In products containing both lipids and proteins such as muscle foods, dairy products etc., lipid oxidation will accompany the oxidation of proteins and this may have a significant impact on the textural changes of the product (Jacobsen, 2010). These processes are commonly linked to a decrease in muscle protein functionality, leading to increased water losses, weakened protein gels or destabilised emulsions (Xiong, 2000). Packaging under high O_2 concentrations may cause an increase in the oxidation of lipids and proteins in meat. These reactions affect the functional, sensory and nutritional quality of meat and meat products (Zakrys *et al*., 2012). Protein oxidation can also result in the loss of enzyme activity and protein solubility, as well as the formation of protein complexes and non-enzymatic browning reactions and products.

-*Vitamins*

Vitamins by their definition are essential to health and have to be obtained from the diet on a regular basis. Factors that affect the stability of vitamins include; O_2, light, moisture and pH. The deterioration of vitamins can take place naturally during storage and losses can occur during the processing and preparation of foods and their ingredients, particularly those subjected to heat treatment. Each individual vitamin has varying sensitivities to O_2, where an example of an O_2 sensitive vitamin is Vitamin A. It is sensitive to atmospheric O_2 where decomposition is catalysed by the presence of trace minerals. Vitamin A is commercially available as a preparation that includes an antioxidant and often a protective coating (Ottaway & Ottaway, 2010). Vitamins sensitive to O_2 include; Vitamin A, C, D and K. The type of packaging can have a significant effect on vitamin stability and the quality of the barriers to O_2 moisture and light is very important.

1.4.2 Promotion of aerobic microbial spoilage

Microbial growth is affected by a range of intrinsic and extrinsic factors. Such extrinsic factors include; O_2, CO_2, temperature, humidity and competition with other micro-organisms. The use of a specific packaging system can address one or a number of these issues. Specific microbes need O_2 to propagate and are known as aerobes, while others cannot propagate in the presence of O_2 (anaerobes). In general, moulds and yeasts need O_2 to propagate (Emblem, 2012). Obligate aerobes require O_2 in order to survive and multiply in a process known as cellular respiration; these organisms use O_2 to oxidize substrates in order to obtain energy. Facultative anaerobes may use O_2, but also have anaerobic methods of energy production. Microaerophiles are facultative anaerobes that may use O_2 only at low concentrations. Foods that have a short shelf-life often employ O_2 reducing packaging

technologies to limit the degradative processes exerted by O_2 on food quality. Aerobic microbes that can spoil food include; bacteria, yeasts and moulds. Some of these aerobic microorganisms can result in food poisoning and some are so dangerous that they can be fatal when consumed (Jay, Loessner & Golden, 2005). The range of food spoilage bacteria is broad, where each specific food can be associated with a specific spoilage microorganism. Research in this field is well documented, detailing the common bacteria's, yeasts and moulds responsible for spoiling food. CO_2 is commonly used to prevent the growth of aerobic microorganisms. Moulds and Gram-negative bacteria are most sensitive to CO_2. The application of CO_2 in the packaging of O_2 sensitive foods is very broad. Some microorganisms are killed by prolonged exposure to CO_2 but usually its effects are bacteriostatic (Adams & Moss, 1995). Vacuum packaging is an example of another packaging technology that removes O_2 from the package to enhance the shelf-life of foods susceptible to aerobic spoilage.

Table 1.1 A list of common and well documented aerobic food spoilage organisms (Adams & Moss, 2008; Jay *et al*., 2005)

Aerobic Bacteria	Aerobic Yeast and Moulds
Staphylococcus species	Aspergillus species
Streptococcus species	Cladosporium species
Enterobacteria species	Penicillium species
Bacillus	Rhizopus species
Pseudomonas aeruginosa	
Nocardia	
Mycobacterium tuberculosis	

1.4.3 Effect of O_2 on sensory perception of foods

Most food and beverage products have a defined shelf-life that is governed by firstly microbiological safety and then product sensory quality. At the end of shelf-life, undesirable characteristics develop which can be microbiological, chemical or physical. Sensory analysis is generally performed in parallel with the monitoring of specific sensory attributes that are used as indices of sensory quality. These attributes may include the loss of particular fresh sensory notes and the development of oxidised or stale sensory notes in food (O' Sullivan, 2012; O'Sullivan, Kerry & Byrne, 2011). Sensory evaluation of food stuffs is the most reliable method to determine whether the product is acceptable for consumption, at least when it concerns evaluation of off-flavour formation due to lipid oxidation. Lipid oxidation products can give rise to different sensory impressions in different food products where the term 'rancid' is often used to describe the off odours and flavours produced by such reactions. Only the secondary oxidation products are responsible for the undesirable changes in the aroma and flavour properties of foods caused by lipid oxidation (Jacobsen, 2010). There is a number of sensory testing methods available all using a panel of consumers, trained or untrained to ascertain the quality of foods (Rodgers, 2010). The detection of off flavours and aromas can be easily detected by these methods, and manufacturers utilize this form of analysis to ascertain further shelf-life information.

1.5. Smart packaging technologies

As outlined above, conventional food and beverage packaging systems and applications have evolved and developed over the years to provide the technical and sales functions necessary to stabilise and promote this segment of fast moving consumer goods.

However, this primary supporting conventional packaging structure is struggling to keep pace with greater challenges being placed upon it through a range of global developments; movement of goods over longer distances, requirement for longer shelf-lives with products, greater demands for product safety, product branding and authenticity etc.

A more recent packaging approach to providing conventional packaging systems with secondary support structures in the form of smart packaging devices has taken place in recent years. These smart packaging technologies can help maintain product quality or safety, extend product shelf-life, provide information on product quality or safety, or simply greater consumer interaction with the product. Smart packaging is generally defined as packaging that provides additional levels of useful functionality beyond protecting, containing and providing information about the product (Yam, 2009) and is generally comprised of active and intelligent systems. The active and intelligent packaging market accounts for <2% of the total global food market. Value for the global active and intelligent packaging market for food and drinks is $4.2 and $1.1 billion dollars (estimate – Data Monitor 2010).

1.5.1 Active Packaging

Active packaging refers to the incorporation of certain additives into packaging systems (whether loose within the pack, attached to the inside of packaging materials or incorporates within the packaging materials themselves) with the aim of maintaining or extending product quality and shelf-life (Kerry, O'Grady & Hogan, 2006). Active packaging has been classified as a subset of smart packaging and referred to as the incorporation of certain additives to food containers with the aim to extend shelf-life (Day, 2008). The concept of active packaging started with a shift in the protection function of

packaging from passive to active. Active packaging has been defined as a system in which the product, the package and the environment interact in a positive way to extend shelf-life or to achieve some characteristics that cannot be obtained otherwise (Lee *et al*, 2008). Another definition of active packaging states that packaging that performs some desired role in food preservation other than providing an inert barrier to external conditions (Rooney, 1995). All active packaging technologies involve some physical, chemical or biological action for altering the interactions between the package, the product, and the package headspace to achieve certain desired outcomes. The two primary forms of active packaging include; absorbing/scavenging systems and emitters/releasing systems.

1.5.2 Absorbing Systems/Scavengers

Active scavengers are utilised in food packaging to absorb components that have detrimental effects in food. Foods that are susceptible to O_2 and which lead to the propagation of many degradative processes, can integrate scavengers within food packaging systems. *O_2 scavengers* can therefore help maintain food product quality by decreasing food metabolism, reducing oxidative rancidity, inhibiting undesired oxidation of pigments and vitamins, control enzymatic discolouration and inhibiting the growth of aerobic microorganisms (Rooney, 1995).

O_2 scavengers are the most commercially important sub-category of active packaging for food products and the most well known take the form of small sachets containing various iron-based powders containing an assortment of catalysts. These chemical systems often react with water supplied by the food to produce a reactive hydrated metallic reducing agent that scavenges O_2 within the food package and irreversibly converts it to a stable oxide. These scavengers are capable of reducing O_2 to levels of 0.01% which is much lower than the typical 0.3-3.0% residual O_2 levels achievable by MA packaging (Day, 2008).

Non-metallic scavengers include those that use organic reducing agents such as ascorbic acid, ascorbate salts or catechol. They also include enzymatic O_2 scavenger systems using either glucose oxidase or ethanol oxidase, which could be incorporated into sachets, adhesive labels or immobilised onto packaging film surfaces (Day, 2008). Oxygen scavengers were first marketed in Japan in 1976 by the Mitsubishi Gas Chemical Co. Ltd under the trade name Ageless™. This sparked the development of a wide range of similar scavengers by Japanese companies including Toppan printing Co. Ltd and Toyo Seikan Kaisha Ltd (Rooney, 1995). The acceptance by consumers of such innovated packaging is most likely reason why O_2 scavengers have been such a commercial success in Japan (Robertson, 2006). In recent years the development of O_2 scavenging adhesive labels that can be adhered to the inside of packages and the incorporation of O_2 scavenging materials into laminated trays and plastic films have enhanced and will help the commercial acceptance of this technology (Day, 2008). Other forms of scavengers are available in the form of CO_2, moisture, ethylene, flavours and UV light scavengers.

Carbon dioxide absorbers or scavengers consist of either calcium hydroxide and sodium hydroxide, or potassium hydroxide, calcium oxide and silica gel, may be used to remove CO_2 during storage in order to prevent the bursting of packs (Hogan & Kerry, 2008). Carbon dioxide scavenger applications include use in packs of dehydrated poultry products and beef jerky (Ahvenainen, 2003). A detailed example of the use of an active packaging system is the use of CO_2 scavengers for fresh roasted or ground coffees, which produce significant volumes of CO_2 when packaged hot. This would lead to the eventual bursting of packs due to the build up of CO_2 in sealed packs. This problem is overcome in two ways, the use of one way valves to allow CO_2 to escape packs or the use of CO_2 scavengers. These CO_2 scavenger sachets and labels are more common and are commercially used for canned and foil pouched coffees in Japan and USA (Rooney, 1995).

In recent time, coffee producers are moving to the use of one way valve system to release excess gas from the packs. The use of CO_2 scavengers is incorporated to food packaging where respiration occurs throughout the storage of the food, where O_2 is converted to CO_2. This action results in the bloated appearance of the pack. Common food stuffs that use CO_2 include fresh meat and fish, nuts and coffee.

Another form of important commercial scavengers is ethylene scavengers. Ethylene is a plant hormone that accelerates the respiration rate and subsequent senescence of horticultural products such as fruit, vegetables and flowers (Day, 2003 & 2008). Effective systems utilise potassium permanganate immobilised on an inert mineral substrate such as alumina or silica gel. Potassium permanganate is used as an active ethylene scavenger and is used in food applications such as the packaging of fruit and vegetables.

Moisture absorbers are added to food packaging to absorb moisture being released by foods resulting in a visually unacceptable product. PVA blankets are commonly placed on the under side of fresh meat products. Silica gel and activated clays and minerals are also used in moisture absorbers (Day, 2008). Commercial moisture absorbers include Toppan Sheet™ (Toppan Printing Co. Ltd, Japan), Thermarite™ (Thermarite Pty Ltd, Austraila) and Fresh-R-Pax™ (Maxwell Chase, Inc., Douglasville, GA, USA).

Flavour and Odour absorbers have potential in active packaging technology (Hogan & Kerry, 2008). They are incorporated to food packaging to remove the generation of off flavours throughout the course of the storage period. Flavour and odour absorbers have a number of mechanisms which include acetylated paper, citric acid and activated carbon (Lopez-Rubio, Lagaron & Ocio, 2008).

Table 1.2 A history of commercial *O2* scavenger and *CO2* emitting systems

Manufacturer	Country	Trade name	Mechanism	Packaging form
Mitsubishi Gas Chemical Co. Ltd.	Japan	Ageless	Iron based	Sachets/Labels
Toppan Printing Co. Ltd	Japan	Freshilizer	Iron based	Sachets
Toyo Seikan Kaisha Ltd.	Japan	Oxyguard	Iron based	Sachets
Ciba Speciality Chemicals	Switzerland	Shelfplus 02	PET copolyester	Films, bottles/containers
W.R. Grace Co. Ltd	USA	PureSeal	Ascorbate/Metallic Salts	Bottle crowns
Multisorb Technologies, Inc.	USA	FreshMax	Iron based	Labels
		Fresh Pax	Iron based	Labels
		Fresh Pack	Iron based	Labels
Mitsubishi Gas Chemical Co. Ltd.	Japan	Ageless type E	Iron based	Sachets/Labels
		Fresh Lock	Iron based	Sachets/Labels
Toppan Printing Co. Ltd	Japan	Freshilizer type CV	Iron based	Sachets
Mitsubishi Gas Chemical Co. Ltd.	Japan	Ageless type G	Ferrous carbonate/ Metal halide catalyst	Sachets/Labels
Emco Packaging Systems	UK	ATCO	Iron based	Sachets/Labels/Caps
Pillsbury Co.	USA	Oxysorb	Ascorbatic acid/Copper	Sachets
Cryovac, Sealed Air Corporation	USA	Cryovac OS2000	Polymer based	Film

1.5.3. Releasing systems/Emitters

The principle of a releasing system is for a gas to be released over time in an atmosphere causing a positive effect to food and thus extend shelf-life. Commercial types of emitters include; CO_2, ethanol, antioxidant and preservative releasers. Carbon dioxide emitters can be produced in sachet or label forms. These emitters provide a number of functions in food packaging. Primarily, they provide active CO_2 as an antimicrobial to aerobic microbes to extend shelf-life, as well as provide abundant gas for in-pack integrity (TemaNord, 2000). Such emitters can also be used in a dual form where the emitter is combined with an O_2 scavenger. In order to prevent package collapse, the labels have been developed to absorb O_2 and generate an equal volume of CO_2. Ageless® G (Mitsubishi Gas Company, Japan) and Freshpax® M (Multisorb Technologies Inc, USA) are two commercial examples of dual action combined CO_2 generators and O_2 scavengers. CO_2® technologies have commercialized CO_2 emitter pads for use with fresh produce such as

strawberries (CO_2 Technologies, 2013). Ethanol emitters are a subset of preservative-releasing technologies that are available in sachet form. The use of ethanol as an antimicrobial agent is well documented (Matz, 1989 & Brody, 2001). It is particularly effective against mould but can also inhibit the growth of yeasts and bacteria. Several reports have demonstrated that the mould-free shelf-life of bakery products can be significantly extended after spraying with 95% ethanol to give concentrations of 0.5-1.5% (w/w) in the products. However a safer and more practical method of generating ethanol is through the use of ethanol emitting sachets (Day, 2008; Rooney, 1995). Active packaging materials like ethanol vapour generators consist of ethanol absorbed or encapsulated in carrier materials and enclosed in polymer packets. The ethanol permeates the selective barrier and is released into the headspace within the package (Appendini and Hotchkiss, 2002). Many applications of ethanol emitting sachets have been patented, primarily by Japanese manufacturers. These include Ethicap™, Antimold 102™ and Negamold™ (Freund Industrial Co. Ltd), Oitech™ (Nippon Kayuka Co. Ltd), ET Pack™ (Ueno Seiyaku Co. Ltd), Oytech L (Ohe Chemicals Co. Ltd) and Ageless™ type SE (Mitsubishi Gas Chemical Co. Ltd). All of these films and sachets contain absorbed or encapsulated ethanol in a carrier material that allows controlled release of ethanol vapour. Ethanol emitters are relatively expensive compared with other active packaging technologies and hence their use tends to focus on premium food items. Nevertheless, ethanol emitters represent a relatively small but growing area of the food active packaging market (Day, 2008). An example of the positive effect of ethanol emitters in the shelf-life of bread has been seen by Latou, *et al.* (2010), and Salminen, Latva-Kala, Randell, Hurme, Linko and Ahvenainen (1996) to extend the shelf-life of sliced wheat bread. Antioxidant emitters are of similar design to ethanol and CO_2 emitters. An active antioxidant compound is incorporated to a sachet (i.e. BHA, BHT and tocopherol) and added to food packaging. Foods that are susceptible to fat

or protein oxidation can utilise such emitters to increase shelf-life of the product (TemaNord, 2000).

1.5.4 Intelligent Packaging in the Food Industry

The term intelligent packaging has also been used with ambiguous and vague meanings, and sometimes used interchangeably with smart packaging. According to the Wiley encyclopaedia of packaging technology, a package is intelligent if it has the ability to sense the environment and communicates with humans: for example an intelligent package is one that can monitor the safety/quality condition of a food product and provide early warning to the consumer or food manufacturer (Yam, 2009). Lee, *et al*. (2008) defined intelligent packaging as a packaging system that is capable of carrying out intelligent functions (such as detecting, sensing, recording, tracing, communicating and applying scientific logic) to facilitate decision making in order to extend shelf-life, enhance safety, improve quality, provide information, and warn about possible problems. Such packaging systems contain devices that are capable of sensing and providing information about the functions and properties of the packaged foods. These types of devices can be categorised into three groups; external indicators (attached to outside of packages), internal indicators (inside the packs) and the third type are indicators that increase the efficiency of information flow and effective communication between product and the consumer (Han, Ho & Rodrigues, 2005). Intelligent packaging can play an important role in facilitating the flow of both materials and information in the food supply chain cycle. In order for these packaging systems to be practical they should be easy to use, cost effective and capable of handling multiple tasks.

Table 1.3 Types of Intelligent packaging applications

Indicators of product safety and quality	Gas sensing devices, microbial growth, pathogen detection and Time-temperature indicators
Package integrity and Tamper evidence	Pack containment failures
Anti-theft and Traceability devices	RFID labels and tags
Product authentication	Logos, holographic images and RFID

1.5.5. Product Authenticity / Tamper Evidence / Anti-theft devices

Although theft and counter fitting are not too common in the food industry, they do pose a huge economic burden in other industries. Electronic article surveillance (EAS) is used to deter the theft of high priced goods. Such devices are commonly found in food retailers, where expensive items such as alcohol can be seen fitted with EAS devices. Tampering is another global issue, and therefore more sophisticated anti-tampering devices or packages with responsive technology are necessary to control and minimize these problems (Han, Ho & Rodrigues, 2005). The use of thermochromic materials can provide a closure which 'bruises' during any attempt to tamper with product thus alerting the consumer before the goods are purchased (Ahvenainen, 2003). The use of Holograms, tear labels and tapes, micro-tags and diffraction devices are also used to deter counterfeiting.

1.5.6. Traceability

RFID (Radio Frequency Identification Devices) technology does not quite fall into either sensor or indicator classification but rather represents a separate electronic information based form of intelligent packaging (Kerry *et al.,* 2006). (RFID) is an electronic, information-based form of intelligent packaging (Hogan & Kerry, 2008). RFID are non-contact, wireless data communication system, where programmed with unique

information and attached to objects for identification and tracking purposes. The tags can contain a variety of information, such as location, product name, product code and expiration dates (Han, *et al.,* 2005). RFID systems contain a chip and an antenna, and an external host system that can power the device allowing information to be transferred to the reader (Järvi-Kääriäinen, 2003). RFID technology has been available for approximately 40 years although its broad application in packaging is a relative recent development. *Rafsec* (UPM, Finland) produce RFID transducers, antennas and the fixings for chip to tag mounting. Tags can be added to labels, packages and products. RFID tags can be of great use when products are to be recalled. Traceability of products can be addressed when using RFID tags. Incidents such as food poisoning that cause product recalls can be made more efficiently when using this technology. RFID can create the ability to trace and recall if necessary not only the products but the also its ingredients and packaging materials (Järvi-Kääriäinen, 2003).

1.5.7. Safety and Quality indicators

A wide range of safety and quality indicators are available for the food industry. These can vary by design to accumulate information throughout the shelf-life and storage of the product. Food safety and quality indicators include gas sensing devices, microbial growth indicators, pathogen detection devices and time-temperature indicators (Kerry, *et al.,* 2006). Some safety and quality sensors fall under the heading of biosensors which will be discussed in greater detail later.

-Freshness indicators

There are at present a large number of "Freshness" indicators that are used to indicate if the product quality has been impaired due to the exposure of unfavourable conditions

during storage and transportation. Most freshness indicators are based on a colour change of a biosensor indicator tag due to the presence of microbial metabolites produced during spoilage (Smolander, 2003). Freshness indicators can function on the principle of pH change, sensitivity to volatile N_2 compounds and hydrogen sulphide, and miscellaneous microbial metabolites. The freshness indicators are mainly attached to packaging materials and can indicate a visual result to be read by manufacturers and consumers. Below is an example as to the varying forms of commercial Freshness indicators;

FreshTag® (COX Technologies) is an example of a freshness biosensor indicator label where a colour indicating tag is attached to the outside of packs. Typical use for such an indicator is in fresh seafood, where volatile amines produced by spoilt fish contact the reagent turning tag colour to pink (Han, Ho & Rodrigues, 2005) (Meat and Poultry online, 2012).

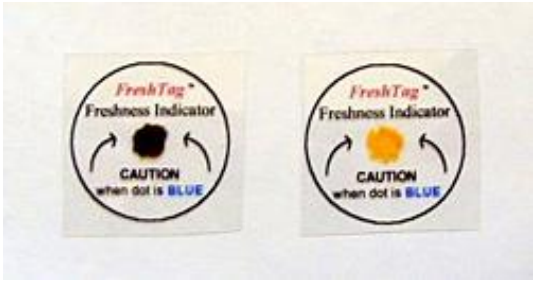


Fig 1.1 *FreshTag*® (COX Technologies)

Toxin Guard (Toxin Alert Inc.) is a polyethylene based packaging material containing immobilised antibodies which detect the presence of pathogenic bacteria like *Salmonella*, *E.Coli 0157* and *Listeria* (Han, Ho & Rodrigues, 2005; Hogan & Kerry, 2008). pH dye indicators are used to monitor the formation of CO_2 due to microbial growth and is one of the most frequent applications in food packaging industry.

The UWI Label (UWI Technology Limited) is a label that is utilised in food packaging to provide the consumer with information on the freshness of the product. The label will detail information that allows the consumer to know when the container (i.e. jar) was first opened and its relationship to the use by date once opened.

Fig 1.2 The *UWI* Label

(UWI Technology Limited; http://www.uwitechnology.com/uwilabelsolution.html)

-*Thermochromic indicators*

Thermochromic inks are dyes that react in reference to temperature and not in a chemical nature (Yam, 2012). The uses of such thermochromic inks have become synonymous with the beverage industry utilising the technology to display a readiness for consumption. An example of such is the Coors Light® branded bottle, where a thermo chromic ink is used to symbolize that the beverage has reached the desired temperature for consumption.

Fig 1.3 *Coors Light*® 330ml amber bottle (Coors Brewing Company, CO, USA)

The presence of a 'blue mountain' represents that the sample has reached the targeted consumption temperature. Above (4ºC) the desired consumption temperatures, the ink remains at natural state where it is found to be transparent, matching the colour of the label material, in this case silver.

-Time-Temperature Indicators (TTI)

The temperature variations in a food product can lead to changes in product safety and quality. There are two types of temperature indicators; simple temperature indicators and time-temperature indicators (TTI's). Temperature indicators show whether products have been heated above or cooled below a reference or critical temperature, warning consumers of about the potential survival of pathogenic microorganisms and protein denaturation, for example, freezing or defrosting processes (Han & Rodrigues, 2005). A time temperature integrator or indicator can be defined as a simple, inexpensive device that can show an easily measureable time temperature dependant change that reflects the full or partial temperature history of a food product to which it is attached (Taoukis & Labuza, 2003). TTI indicators display a continuous temperature dependant response of the food product.

Response is made to chemical, enzymatic or microbiological changes that should be visable and irreversible, and is temperature dependant. TTI's provide an overall temperature history of the product during distribution. A number of commercially available TTI's are listed below;

Check-Point® SmartLabel™ is a simple adhesive attached to food cartons to check for temperature abuse. Is has the ability to monitor food packs from the processor to the retailer and stays with the package until the point of retail sale (Vitsab International, Sweden). Check-Point® tracks the time-temperature relationship and makes it visible in a colour dot, starting white to green on activation, where a yellow to red colour will appear toward end of shelf-life. The use of such labels are used by meat, seafood and vegetable processors to monitor for temperature abuse during the distribution and storage of their products up to the point of retail sale (Vitsab International, 2012).

Fig 1.4 *Check-Point® SmartLabel™* (Vitsab International, Malmo, Sweden)

Table 1.4 List of commercially available TTI's

Sensor	Company
CheckPoint®	Vitsab International, Sweden
Fresh-Check®	Temptime Corp., NJ, USA
OnVu™ TTI	Ciba Speciality Chemicals & Freshpoint, Switzerland
TT Sensor™ TTI	Avery Dennison Corp., USA
eO® TTI	Cryolog, France.
3M Monitor Mark®	3M Co., MN, USA

Other commercial forms of TTI's include; 3M Monitor Mark (diffusion based indicator label) (3M, USA), I Point™ (I-Point Biotechnologies, Sweden) labels are enzymatic TTI's whose colour changes as a result of pH variations due to enzymatic hydrolysis of lipid substrates. Lifelines' Freshness Monitor (Lifelines Technologies, NJ, USA) and Fresh-Check™ (TempTime Corporation, USA) are solid state polymerisation reactions to give a colour change (Singh, 2000).

-Gas indicators

Other safety and quality indicators can include the use of Gas indicators (Kerry, *et al*., 2006; Hogan & Kerry, 2008). Gas sensors are devices that respond to the presence of a gaseous analyte by changing the physical parameters of the sensor and are monitored by an external device and these can include O_2 and CO_2 sensors (Kress-Rodgers, 1998b). The uses of gas indicators are available in commercial forms. The principle of using an in-pack device to detect gases in food packaging can provide quality information to manufacturers and consumers. Ageless Eye® O_2 indicator is an in-package monitor which indicates the presence of O_2 at a glance (Mitsubishi Gas Company, Japan). When O_2 is depleted within packs, a colour change from pink to blue occurs. The Oxy_2Dot® (Oxy Sense Inc.) is a non invasive, light sensitive O_2 sensor. Oxy_2Dot® is placed in packs prior to filling and sealing

and read by a fibre optic reader. Other commercial O_2 indicators include Vitalon® and Samso-Checker®.

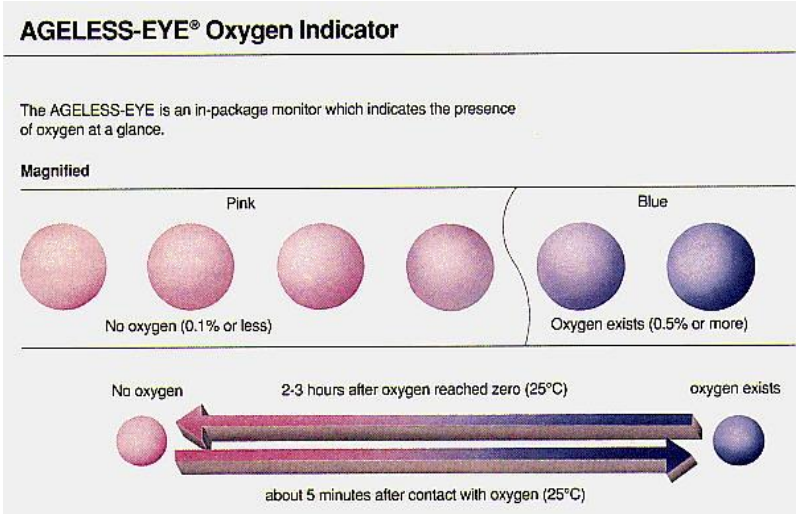


Fig 1.5 *Ageless Eye*® (Mitsubishi Gas Chemical Company, Japan)

Carbon dioxide indicators are also available in commercial form. The uses of such indicators are valuable in MA packaging where high levels of CO_2 are desired. The indicator displays the desired concentration of CO_2 inside the pack. This allows incorrectly packaged products to be immediately repacked and eliminates the need for destructive, labour intensive and time consuming quality control procedures (Han & Rodrigues, 2005). Cryovac Sealed Air Ltd. (SC, USA), produce labels containing a visible CO_2 indicator, used to identify machine faults and gas flushing problems (Cryovac® CO_2 indicator).

Fig 1.6 *Cryovac® CO_2* indicator (Sealed Air Ltd., SC, USA)

Inks have been developed for detecting O_2. These inks can be activated using UV light where the colour changes from blue to colourless. If no O_2 is present the colour of indicator remains colourless. The blue colour activation will occur when O_2 is present above 0.1% (Mills, 2005).

UV activated technology forms the basis of UPM Shelf Life Guard (UPM, Finland). This technology is a smart packaging solution that aids consumers and the food industry to monitor and shelf assess their produce's shelf-life. The colour change of the packaging label allows the consumer to make a personal assessment of the product. The label turns from transparent to blue, informing the consumer that air has replaced the modified atmosphere gases within the package.

Fig 1.7 *Shelf Life Guard* (UPM, Finland)

RipeSense® (Jenkins Group, NZ) is the worlds first intelligent sensor label that changes colour to indicate the ripeness of fruit (RipeSense.com). It functions be reacting to the aromas released by the fruit as it ripens. Initial state is a red colour that changes to orange and then yellow that consumers can match with their eating preference for future selection.

Fig 1.8 *RipeSense*® indicator (Jenkins Group, NZ)

1.5.8. Biosensors

Food quality control is essential in the food industry. Food producers are increasingly demanding the efficient control methods, particularly through on-line or at-line quality sensors to satisfy consumers and regulatory requirements and to improve the feasibility of automated food processing and quality of sorting (Yam, 2009; Kerry, *et al*., 2006). Novel sensing technologies using biomaterials or nanomaterials can be used to detect quality and safety attributes in packaged foods. These sensing technologies range from rapid non destructive and non contact to highly specialized micro-sensing and nano-sensing structures. Today, the most important quality parameters and concepts in food production control are; sensory, nutritional, compostion, detection of foreign bodies, microbial safety, shelf-life, production hygiene, HACCP and packaging (Yam, 2009). Biosensors are a subgroup of chemical sensors where the detection of a chemical component with a bio-recognition molecule (Atalay, Verboven, Vermeir & Lammertyn, 2008). These are compact analytical devices that detect, record and transmit information pertaining to biological reactions (Kerry, et al., 2006; Yam, Takhistov & Miltz, 2005). These devices consist of a bioreceptor (such as enzymes, antigens, microbes, hormones and nucleic acids) specific to a target analyte and a transducer (optical, electrochemical etc.) to convert biological signals to a quantifiable electrical response (Hogan & Kerry, 2008). The transducer can measure the weight, electrical charge, potential, temperature, or optical activity of the substance. The potential advantage of using biosensors for food analysis is a rapid, specific quantification without the need for extensive sample preparation. The specificity of biological components for particular analytes reduces the need for extensive sample extraction and analyte isolation before measurement can take place (Scott, 1998). Intelligent packaging systems incorporating biosensors have the potential for extreme specificity and reliability. A commercial example of a biosensor is ToxinGuard™.

ToxinGuard™ (Toxin Alert, Ontorio, Canada) is a visual diagnostic system that incorporates antibodies in polyethylene-based plastic packaging and is capable of detecting *Salmonell*a sp., *Campylobactor* sp., *E.coli* 0157 and *Listeria* sp. ToxinGuard™ can be targeted to detect freshness degradation, as well as the presence of specific food hazard such as pesticide, or indicators of genetic modification.

The Food Sentinel System™ (SIRA Technologies, CA, USA) is another biosensor system that is capable of continuous detection of contamination through immunological reactions occurring in part of a barcode. The barcode becomes unreadable due to the presence of contaminating bacteria.

1.6. Oxygen Sensors

The use of intelligent packaging, specifically in the form of O_2 sensors will be discussed in greater detail. The use of a smart packaging technology that can non-destructively monitor the level of O_2 within food packs is of great benefit to manufactures and retailers (Kerry & Papkovsky, 2002; Smiddy, *et al.*, 2002b & O'Mahoney, et al., 2004). The ability to measure O_2 immediately post-packaging can provide valuable information on the shelf-life of the product, especially where, O_2 limiting packaging techniques are used such as MA and vacuum packaging.

1.6.1. Optical Oxygen Indicators for Sensing in Food Packaging

For optimum quality assurance, cost-effective methods for rapidly assessing, and preferably measuring on-line, of the chemical and physical properties and the microbial status of raw materials, process streams and end products is necessary (Kress-Rodgers, 1998). The role of O_2 in food degradative processes is well documented. The development

of improved methods to assess food conditions such as freshness, or changes due to microbial spoilage, oxidative rancidity or degradation induced by O_2 and/or heat has been important to manufacturers in recent years (Kress-Rodgers, 1986). Food products must be tested and their shelf-life predicted correctly to ensure that they reach the consumers in good condition (Kress-Rodgers, 2001). The marker approach for the development of novel instrumentation for the rapid assessment of complex food conditions has great potential, particularly in the determination of food freshness. It can offer unique solutions to the problems of determining the food status with respect to microbial or oxidative spoilage (Kress-Rodgers, 2001). The use of certain O_2 sensitive dyes in sensors would prove useful in the monitoring of foods that are susceptible to O_2 degradative processes like oxidation and aerobic spoilage.

The effectiveness of phosphorescent dyes, consisting of platinum (II) and Palladium (II) complexes of porphyrins and some related structures, for practical O_2 sensing, due to their long lifetimes and suitable spectral characteristics (Papkovsky *et al.,* 1991 & 1995). Phosphorescent complexes of porphyrin-ketones were designed for use as O_2 probes by Papkovsky *et al.,* 1995, where their favourable properties included high stability, water insoluble, high melting points, biogenic origin and low toxicity. Advantages of optical O_2 sensing include it being a non-invasive technique for measuring O_2 through translucent material, the solid state sensor being inert and not consuming O_2 or participating in other chemical reactions (Wolfbeis, 1991).

The ability of optical sensors to monitor the levels of O_2 in raw and cooked beef was investigated using O_2 sensors that were attached to the inside of the lidding material in MA packs or inserted into vac-packs, and were capable of monitoring changes in O_2 levels in all packaged samples (Smiddy, 2002a). Also, another application would be to assess the effectiveness of O_2 free or O_2 limited packages, where Smiddy *et al*, 2002b, showed that

sensors used in applications involving MA packaging of cooked and processed meats, where O_2 was eliminated by using 70% N and 30% CO_2, where in reality a large percentage of packs did contain O_2, even just after packaging. Clearly if such high levels of O_2 are present in packs where measures were put in place to exclude its presence, it is imperative that a non destructive method to continuously assess O_2 levels in packs be developed. The overall use of O_2 sensors showed potential for commercial use and detected levels of O_2 in the ranges previously reported for packaged meat products. The continuous and non destructive means of analysing O_2 in meat packs provides a more realistic means of assessing actual levels of O_2 in retail meat packs (Smiddy, et al., 2002b & 2002c). Such O_2 sensors can be produced cheaply, are disposable and when used in conjunction with accurate instrumentation provide rapid determination of O_2 concentration (Kerry & Papkovsky, 2002).

O_2 sensors and intelligent inks that are used for O_2 sensing in food packages should have the following criteria (Mills, 2005);

1. They should be very inexpensive, for the use in every single food package,
2. Should not require expensive analytical instrumentation,
3. Un-trained personal should be able to monitor sensors,
4. Should have non-toxic, non-water soluble components,
5. Have very long shelf lives (at ambient conditions),
6. Be only activated once the packaged is sealed,

7. Made to respond to changes of 0.1% level, or 0.5 – 2 % level for non- scavenged MA packs.
8. Exhibit an irreversible response toward O_2,
9. And should be easily incorporated into the food package.

1.6.2. Optical O_2 Sensing, The Mechanism:

Most optical dyes for O_2 sensors are luminescence based. Luminescence is the general term given to describe processes leading to the emission of light. It is always in a response to an input of energy of some type, where different types of luminescence are distinguished by the addition of a prefix to denote the type of energy involved. (Rendell, 1987). Fluorescence is distinguished from other types of photoluminescence by the fact that the excited molecule returns to the ground state immediately after excitation. This lifetime in the excited state, although very short, is in fact very long compared with other events on the molecular scale (Rendell, 1987). Compounds exhibiting fluorescence are called fluorophores or fluorochromes. These absorb light where energy is taken up for the excitation of electrons to higher states. The process of absorption is rapid and is immediately followed by a return to lower energy states, which can be accompanied by emission of light. Phosphorescence is the term used if the delay is greater than fluorescence (Mason, 1993). Quenching reactions are easy to perform, require only a small sample, usually are non-destructive, and can be applied to almost any system that has an intrinsic or extrinsic fluorescent probe. The most important characteristic is the value of information that these reactions can provide. Solute quenching reactions using quenchers such as

molecular O_2, acryl amide or iodide ion provides information about the location of fluorescent groups in a molecular structure (Eftink, 1992).

In such systems the luminescence is associated with an electronically excited lumophore and is quenched by molecular O_2, which is irreversible (Bacon & Demas, 1987). Luminescent probe molecules are encapsulated in gas permeable material (ex. Silicone rubber or organic polymers) to create a thin film O_2 indicator, (Mills, 2005). Optical O_2 sensors work by the luminescence quenching effect of O_2 (Papkovsky, et al, 2000). A long decay fluorescent or phosphorescent dye embedded into a solid state polymer matrix normally comprising the active element of the sensor (Papkovsky, 1995 & 2004). O_2 penetrates the sensitive dye polymer coating by simple diffusion and quenches luminescence of the dye by a dynamic collisional mechanism. Quantitation of O_2 is allowed by measuring changes in luminescent parameters from the O_2 sensor element contacting the sample (using a predetermined calibration). Sensors used in experimentation include a Platinum (II)-octaethylporphine-ketone dye (PtOEPK) embedded on Durapore filter paper, forming the actual sensor (Smiddy, 2002 (c)). The O_2 sensor is based on the quenching effect of solid-state phosphorescent sensors by molecular O_2, which diffuses into the polymer and quenches dye molecules. Such sensors should allow compatibility with simple and inexpensive optoelectonic measuring devices (LED's, photodiodes, etc), minimize interference by scattering and sample fluorescence and allow long term operation without calibration (Papkovsky, 1995). The sensor membrane is excited by the intensity-modulated light source (LED), and the phase shift of phosphorescence signal with respect to the excitation is monitored, which is related to O_2 concentration (Papkovsky, 1995). The efficiency of quenching correlates with the O_2 concentration, causing a decrease of the phosphorescence intensity and lifetime of the sensor. The mechanism of sensing is physical,

O_2 is not consumed, no chemical reaction takes place and quenching is reversible (Papkovsky, 1995 & Smiddy, *et al.*, 2002).

1.6.3 Commercial forms of O_2 sensors

Currently there are a large number of intelligent indicators that can give information about food packages as described above. Depending on the type of indicator, information can be obtained from the food package without the need to destroy the actual package. Such indicators are applied to the inside of a food package, to give information on the storage conditions and package effectiveness, and are utilized in applications where foods are stored in packages with reduced O_2 concentrations (Ahvenainen, 2003).

There are many forms of O_2 sensors available for commercial use. A detailed example of one of the most commercially available O_2 sensors that utilize an O_2 sensitive fluorescent dye is the ***O2xyDOT®*** (OxySense, TX, USA). *O2xyDOT®* is the O_2 sensor that is placed inside a bottle or package prior to filling. The measurement is achieved by placing the fibre optic reader/pen on the outside of the package immediately above the *O2xyDOT*. The dot is addressed from the outside of the package via the optic pen to make O_2 measurements without penetrating the package itself. (*OxySense*, TX, USA)

The *O2xyDOT*® features include:

- A 5mm diameter with a thickness of 0.2mm,
- Allows multiple measurements on the same package,
- Has detection limits of 15 ppb in water and 0.03% in gas,
- Is totally passive, therefore does not interfere with contents of the package and does not consume O_2,
- Is suitable for measuring O_2 both in gases and liquids,
- Is a robust low cost sensor that can be used on a disposable basis,
- Is mechanically stable and can be sterilized and go through pasteurisation process without losing calibration,
- Can be used in oil, water or gas,
- Independent of pH (2-10) and salt concentrations,
- And is not affected by other gases.

The *O2xyDOT* works on the principle of fluorescent O_2 sensing. The typical indicators used are Ruthenium complexes and porphyrins, both of which are compatible with low cost light emitting diodes. (*OxySense.com*). Other commercial O_2 indicators include Vitalon® and Samso-Checker®.

The use of fluorescent dyes can be used to construct 'laboratory made' O_2 sensors. Research that incorporates the use of O_2 sensors in the monitoring of O_2 levels in packaged foods has been carried out. Smiddy et al., 2002 has reported a number of positive experiments, showing the non-destructive monitoring of O_2 in packaged foods. The uses of modified atmosphere and vacuum packaging to limit the levels of O2 in O_2 sensitive foods

have been investigated. Using O_2 sensors, Smiddy, *et al*., 2002a, states it is necessary to monitor O_2 levels within packs of O_2 sensitive foods. Due to analysis of modified atmosphere packaged samples with O_2 sensors, where packages were prepared to eliminate O_2 by using a mixture of gases (70% N_2 and 30% CO_2), but in reality, a large percentage of these packs contained O_2 even just after packaging (Smiddy, *et al.*, 2002a & Smiddy, *et al.*, 2002c). The ability to continually assess O_2 levels within packs through continual, non-destructive assessment using O_2 sensors provides a far more realistic shelf-life stabilities of foods held in both vacuum or modified atmosphere food packaging systems (Smiddy, *et al.*, 2002c).

CHAPTER II

Phosphorescent Oxygen Sensors Based on Nano-Structured Polymeric Matrices

Contents of this chapter was submitted and accepted as part of the manuscript entitled - Phosphorescent Oxygen Sensors Based on Nanostructured Polyolefin Substrates; R.N. Gillanders, A. Hempel, J.P. Kerry and D.B. Papkovsky, 2010. *Analytical Chemistry*, 82, 466-468.

Abstract

Novel oxygen (O_2) sensors based on phosphorescent platinum octaethylporphyrin-ketones (OEPk) in nano-porous high density polyethylene (HDPE), polypropylene (PP) and polytetrafluoroethylene (PTFE) polymer supports were evaluated. The fabrication methods of the sensors are described, with subsequent optimisation techniques and sensing characteristics investigated. The sensors were heat-treated at elevated temperatures to enhance their stability, and compared with established sensors based on Pt-OEPk in polystyrene on Durapore films. The HDPE- and PP-based sensors showed good sensitivity to O_2, with good reproducibility and stability. The sensors were calibrated over a temperature range of -10°C to +40°C, and are found to be suitable for food packaging applications. The sensors were easily fabricated and provided a new material for O_2 sensor applications.

2.1. Introduction

Quenched fluorescence O_2 sensors have received considerable interest in recent years chiefly due to their low-cost, stability and reliability (Ogurtsov, Papkovsky & papkovskaia, 2001; Amao, Asai, Miyashita, & Okura 2000; O' Mahoney, O'Riordan, Papkovskaia, Kerry & Papkovsky, 2006; Gillanders, Tedford, Crilly & Bailey, 2005; Papkovsky, 1995; Papkovsky, Papkovskaia, Smyth, Kerry & Ogurtsov, 2000). In these systems, the phase shift of an emitted luminescent signal relative to the excitation signal is measured, and the shift is converted to O_2 concentration. The optically interrogated component of a sensor normally consists of sol-gel or polymer-based thin film coatings, drop- or dip-coated onto a solid substrate, which can give rise to heterogeneous dye/matrix environments (Mills, 1998; Ogurtsov & Papkovsky, 2003; Ogurtsov & Papkovsky, 2006). These heterogeneous environments often cause differences in quenching behaviour due to differing quenching sites within the film, resulting in errors outside the parameters of the sensor calibration. Many sol-gel based systems can be complicated to fabricate, with several chemistry steps involved with lengthy drying times (Maccraith, McDonagh, O'Keeffe, McEvoy, Butler, & Sheridan, 1995). An ideal O_2 sensor should be easy to fabricate in large batches, be reproducible, and cost-effective. A significant amount of research has been conducted on the type of O_2 sensor consisting of a dye immobilised or impregnated into a porous support. A large variety of support matrices have been used for O_2-sensitive dyes. Nano-structured polymers (Volynskii, Yarysheva & Bakeev, 2006) have not yet to date been utilised as an O_2 sensor support. These polymers were considered due to the attractive properties of nano-structured polymers: a highly-controllable degree of porosity on the polymer surface can be achieved with treatment of the polymer under particular conditions; no further

chemistry is required; it can be produced in large batches, and is relatively inexpensive. To fabricate these polymers, a standard polymer film is deformed under an adsorptionally active liquid medium, resulting in a polymer dispersion of oriented macromolecules, separated by microvoids. By controlling the temperature and tensile stress of the deformation, it is possible to accurately control the structure of these materials. It is proposed that a dye solution can be coated directly onto the polymer, and subsequently treated either thermally or mechanically to entrap the dye, resulting in the final sensor. Therefore, it is possible to remove a step from the standard sensor fabrication process: the dye can be coated directly onto the substrate matrix without any pre-mixing stages, and a large number of sensors can be fabricated quickly. Currently, optical O_2 sensing is receiving widespread interest in industrial applications, particularly within the food packaging industry due to its non-destructive nature, the rapidity in which measurements can be made, and the possibility of large-scale samples (O'Mahoney, et al., 2006; Smiddy, Fitzgerald, Kerry, Papkovsky, O'Sullivan & Guilbault, 2002a; Smiddy, Papkovsky & Kerry, 2002b; Smiddy, Papkovskaia, Papkovsky & Kerry, 2002c). The objective of this study was to produce an optical O_2 sensor which satisfies the above conditions, while being economical and having advantageous properties such as reproducibility, repeatability, compatibility with standard packaging materials, ease of integration and O_2 sensitivity, would be a significant contribution to this field. The sensors reported in this work are easily fabricated and are based on nano-structured polymer films impregnated with well-known O_2-sensitive dyes Pt-OEPk (Papkovsky, 1995; Mills & Lepre, 1997; Papkovsky, 2004).

2.2. Materials and Methods

2.2.1 Materials

Platinum-octaethylporphyrin ketone (Pt-OEPk) was obtained (Luxcel Biosciences, Ireland**)**. Hexane was supplied by Sigma-Aldrich (Ireland) and used as received. Durapore sheets (type GV) were supplied by Whatman (England), and gas cylinders of 100% nitrogen, 0.5%, 1%, 2%, 5%, 8% and 21% O_2, balanced with nitrogen, were supplied by Irish Oxygen, Cork.

2.2.2 Sensor preparation

To prepare the nano-structured polymer, the starting polymer underwent tensile strain under heptane, a plasticizing liquid environment. Several different polymers were prepared in this fashion: high-density polyethylene (HDPE), polypropylene (PP) and polytetrafluoroethylene (PTFE/Teflon) (Luxcel Biosciences, Ireland). All polymers were stored between cardboard sheets, and secured along the tensile strain to ensure minimal wrinkling. Sensors based on the nano-structured polymers were prepared in the same way: a piece of polymer of approximately 12 x 20 mm was cut along the tensile strain. The strip was then dip-coated in a Pt-OEPk solution in hexane, both at 1 mg/ml. The untreated sensor was allowed to dry, while the heat-treated sensors were placed between two microscope slides, to ensure uniformity in the film, and treated at 90°C or 120°C for 1 hour. After drying, both films were subsequently rinsed in hexane to remove any unbound dye from the surface. Sensors were stored in sealed bags in the dark when not in use. As a reference, sensors based on Pt-OEPk and PS (Polystyrene) were prepared on Durapore by reconstituting a PTK-1 kit (Luxcel Biosciences, Ireland). A volume of

1.0 ml of ethylacetate was added to the pre-mixed components and incubated at room temperature. The resulting solution was then spotted in 4μL aliquots on the Durapore sheets and allowed to dry before the sheet was cut into individual sensors. The sensors were stored in the dark until required for further use.

2.2.3 Sensor calibration

The sensor strip was placed in an in-house designed aluminium flow cell and held in a water bath to provide temperature stability. The gas was introduced to the flowcell inlet, passed over the sensor strip continuously for several minutes, and exited via an outlet. Optical measurements were performed with a bench-top phase-fluorometric detector (Luxcel Biosciences, Ireland; Fig.2.6), with a variable modulation frequency: 2.614 kHz was used for Pt-OEPk-based sensors.

[Fig.2.6]

2.3 Results and Discussion

2.3.1 Sensor design

Initially, calibrations were made on untreated sensors, before incubation at elevated temperatures and recalibration. It was expected that by treating the sensors close to or at the glass transition temperature, the dye molecules would become secured in the nanovoids, providing a more reproducible sensor. The glass transition temperature (Tg) of HDPE, PP and PTFE is typically around 130°C, 160°C and 327°C, respectively, so treatment at 90°C and 120°C would facilitate partial

deformation, allowing dye to be securely embedded in support materials. Qualitative evidence of this was shown when untreated and treated sensors were rinsed in hexane. The untreated sensors lost most of their dye, turning the hexane a pink colour, while the treated sensors lost very little dye, with support material retaining their colour. All treated sensors were subsequently rinsed in hexane prior to use to enhance reproducibility, since it was believed that any unbound dye could cause error, particularly in the intensity domain. To determine the effect of heat-treating the sensors, comparison between the heat- and non-heat-treated sensors was made. A clear benefit of heat-treating the sensor is shown in Figure 2.1, where the treated sensor yields higher intensity values at 0 and 21% O_2 than the untreated sensor. This result points to an improvement in dye homogeneity across the matrix with heat-treatment.

[Fig 2.1]

As can be observed, both phase and intensity increase after heat-treatment in the case of the PE-based film. The proposed mechanism for craze formation (openings for dye embedding) and sensor fabrication is presented in Figure 2.2.

[Fig 2.2]

This mechanism suggests that with initial tensile drawing of the polymer, craze nucleation begins. As tensile drawing continues, the crazes propagate in the direction perpendicular to the strain, and the dimensions increase leading to craze collapse resulting in a well defined 3-D network of nanopores (<15nm). Subsequent to this,

the dye is coated heterogeneously on the surface of the film pre-treatment. On the application of heat, the polymer deforms close to the glass transition temperature, aligning the dye molecules within the nanopores, creating a more homogeneous environment.

2.3.2 Detailed characteristics

Six sensors of each polymer were fabricated as described above, with one batch of sensors untreated, and a batch each of PE and PP treated at 90°C and 120°C. PTFE was not heat-treated at these temperatures, since the high glass transition temperature of this polymer (~327°C) would disallow any significant change in the physical properties of the material. The following calibration results presented in Table 2.1 were performed at 25°C and are the mean values for six films (st.dev <0.5):

[Table 2.1]

An increase in $\Delta\phi$ (change in phase) can be observed in (both PE and PP-based) sensors treated at 120°C over those treated at 90°C; this may be attributed to a greater degree of dye homogeneity within the support matrix, due to a higher level of polymer deformation during heat-treatment. If the molecules are evenly distributed within the matrix, then aggregation is less likely to occur, giving rise to fuller quenching site accessibility. Thus treatment temperatures closer to the glass transition temperature of the polymer are more desirable for these sensors. To establish whether treatment at 120°C provided the optimal treatment temperature, and to determine if any further treatment affects the calibration further, six PE-based sensors were treated for one hour, and calibrated in both phase and intensity domains. The sensors were

then re-treated at 120°C, re-calibrated, and compared with the initial calibration values.

[Table 2.2]

The results are expressed in Table 2.2, with corresponding standard deviations for the initial calibration. As can be seen, the second set of calibrations decreased slightly. This may be due to a higher degree of deformation rendering the dye molecules within the bulk polymer rather than the pores. Calibration curves, shown in Figure 2.3, compare the mean phase (top) and intensity (bottom) response of the solvent-crazed polymer-based sensors with the established Durapore/PS sensor. All sensors were measured six times for statistical integrity. PP-based sensors showed slightly higher sensitivity to O_2 than Durapore/PS-based sensors, with $\Delta\phi$ values of 30.8° and 29.31°, respectively, while PTFE-based sensors (Teflon) have a $\Delta\phi$ value of 10.53°. The PE-based sensors also have a comparable $\Delta\phi$ to O_2 as PP- and Durapore/PS-based sensors, with a value of 30.15°. These figures indicate that the solvent-crazed support matrices have high solubility for O_2. The Durapore/PS-based sensor exhibits a higher intensity signal, which is due to a higher surface concentration of Pt-OEPk.

[Figure 2.3]

2.3.3 Temperature effects

Since these sensors are to be used in packaging applications, they are expected to operate over a large temperature range, in this case -10°C (i.e. frozen foods) to

+40°C (e.g. hot deli counters). It is well-known that temperature has a significant effect on the emission of luminescent dyes, so a full calibration over the temperature range is necessary for a more complete calibration set. In Figure 2.4, a PE-based sensor (treated at 90°C) was calibrated over the above mentioned range in 10°C steps. The high dependency on temperature can be noted at 5% O_2 for example, where the phase angle can be seen to drop from approximately 21° to 7°. Thus with this calibration matrix, a measured phase angle in conjunction with a measured temperature can provide a more accurate O_2 concentration.

[Figure 2.4]

2.3.4 Dissolved oxygen sensing

The physical properties of the PP-based sensors also showed promise for use as a dissolved O_2 (DO) sensor. The sensor is quite pliant but robust: DO sensors are often used in aggressive environments [37], and can be submerged for prolonged periods of time. The "locked-in" nature of the dye within the nano-porous polymer should prevent any leaching of the dye over time. An initial experiment was conducted to test the viability of the PP-based sensor, with nitrogen and air alternately introduced to approximately 1.5 ml of deionised water, and the phase angle measured as described above. The response is illustrated in Figure 2.5, where the reversibility of the sensor in the dissolved phase is clearly demonstrated.

[Figure 2.5]

2.4. Conclusions

Novel O_2 sensors were developed using the dye Pt-OEPk in a variety of solvent-crazed polymer supports – polyethylene, polypropylene and PTFE. The PE and PP polymers were subject to heat-treatment at 90°C and 120°C, and their performance compared with untreated sensors; it was found that heat-treating the sensors increased the stability, sensor-to-sensor reproducibility, and signal repeatability. However, treating the films close to the glass transition temperature slightly decreased sensitivity, probably due to molecules becoming inaccessible within the bulk polymer. The physical properties of the PTFE film were not conducive to the same heat-treatment effects. The PE- and PP-based films were compared favourably with established Durapore-based sensors. The sensors were characterised over an O_2 range of 0-21 kPa and a temperature range of -10°C - + 40°C. The sensors have been found to be highly suitable for use in the food packaging industry, while the PP-based sensors have also shown promise as DO sensors. Future work on these sensors will model the response mathematically, and investigate the physical parameters.

2.5. Acknowledgements

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2.6. TABLES AND FIGURES

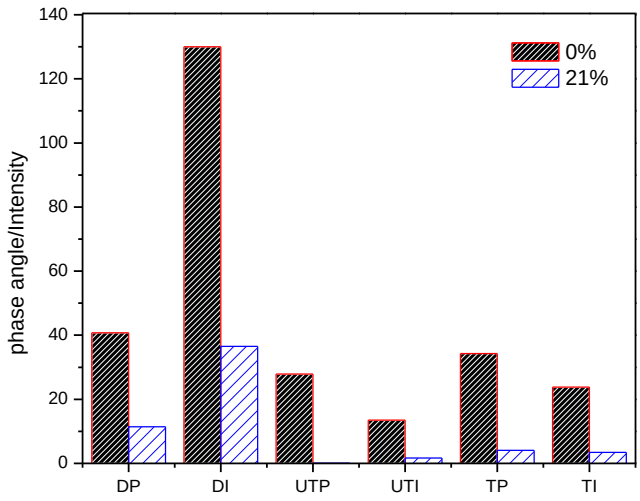

Figure 2.1 Comparison of untreated PE and PE treated at 90°C in the phase and intensity domains at 0 and 21% O_2, compared with Durapore, where DP – Durapore (phase); DI – Durapore (intensity); UTP – untreated PE (phase); UTI – untreated PE (intensity); TP – treated PE (phase); TI – treated (intensity)

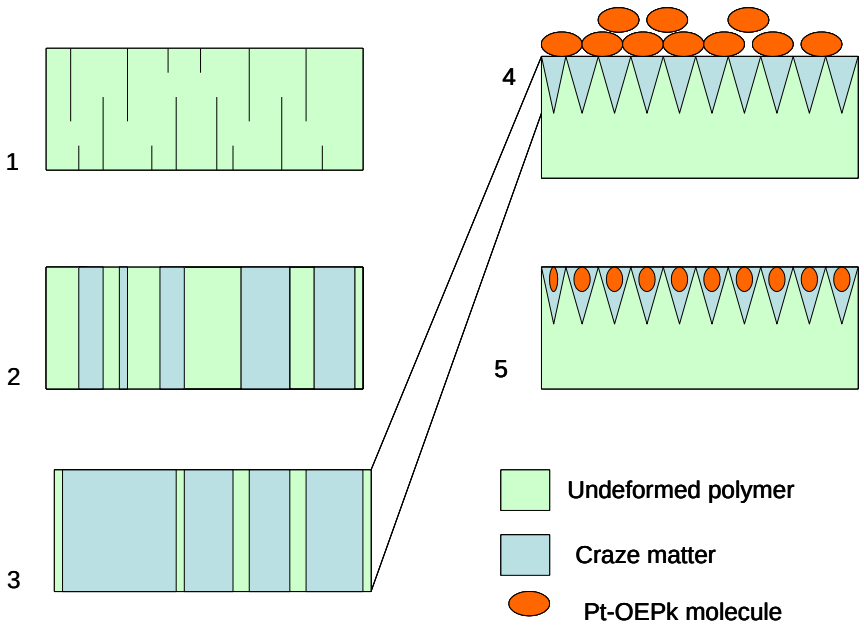

Figure 2.2 Proposed stages of craze formation and sensor fabrication, where 1: craze nucleation; 2: craze widening; 3: craze collapse; 4: dye coating in solution, pre-treatment; 5: impregnated dye, post-treatment

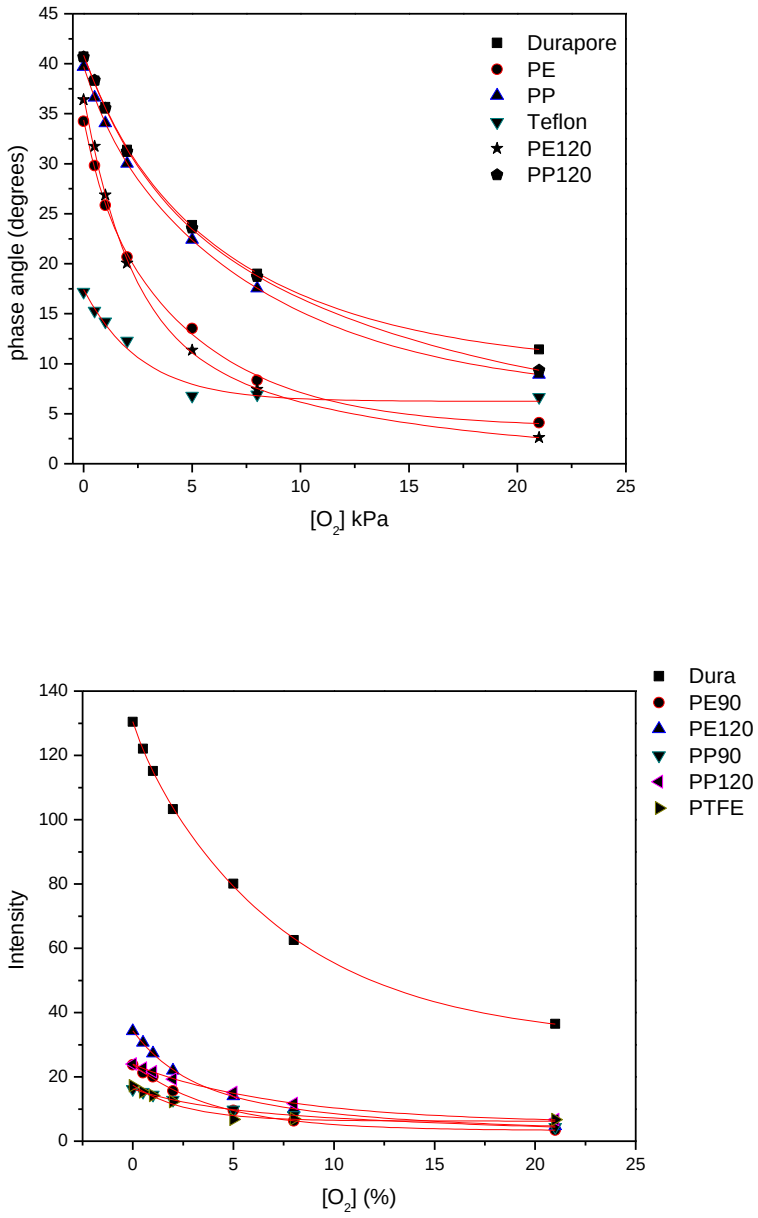

Figure 2.3 Phase shift (top) and Intensity response (bottom) as a function of oxygen concentration for Pt-OEPk in Durapore/PS (■), 90°C-treated PE (●), 90°C-treated PP (▲), PTFE (▼), 120°C-treated PE (★), and 120°C-treated PP (⬟)

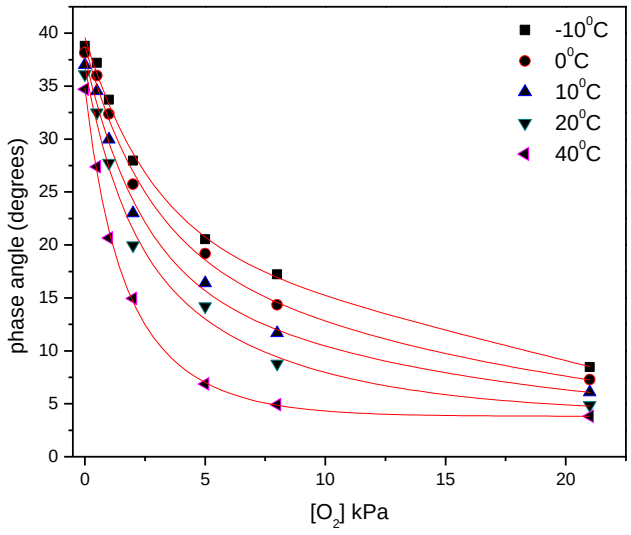

Figure 2.4 Effect of temperature on response of PE film treated at 90°C

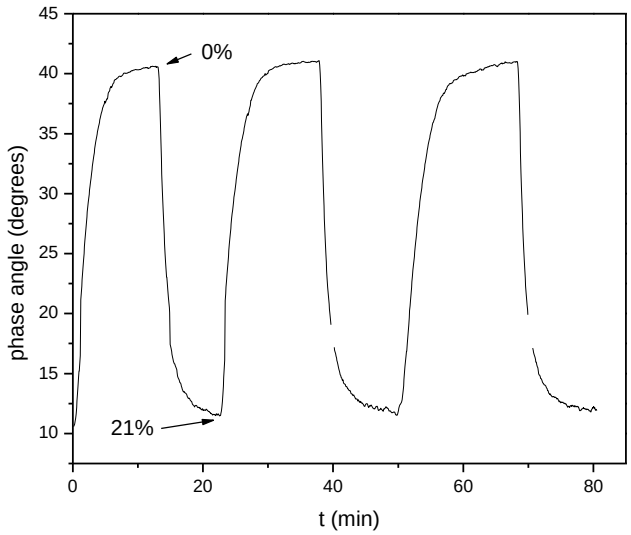

Figure 2.5 – Response of PP-based sensor to dissolved oxygen (DO) between nitrogen and air

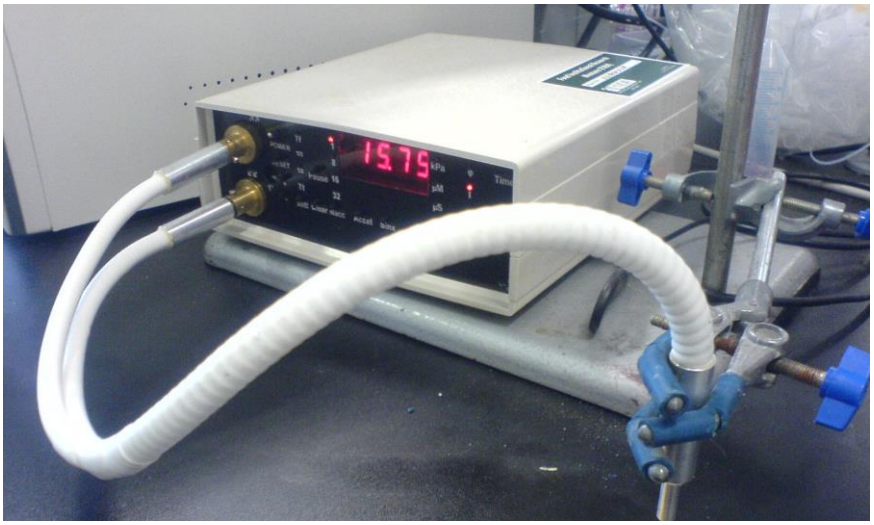

Figure 2.6 Luxcel Phase Detector (Luxcell Biosciences, Cork, Irl)

Table 2.1 Mean Phase-domain calibration results (st.dev < 0.5) for untreated PE, 90°C PE, 120°C-treated PE, 90°C-treated PP, 120°C-treated PP, PTFE, and Durapore-based sensors at varying O_2 concentrations

Host Matrix	0%	0.5%	1%	2%	5%	8%	21%	ΔΦ
Untreated PE	27.92	23.83	19.77	14.66	7.18	4.75	0.11	27.81
90 PE	34.25	29.82	25.84	20.67	13.53	8.36	4.10	30.15
120 PE	36.41	31.73	26.86	20.05	11.35	7.42	2.61	33.80
90 PP	39.71	36.63	34.06	30.01	22.42	17.53	8.93	30.78
120 PP	40.68	38.34	35.54	31.23	23.57	18.73	9.34	31.34
PTFE	17.19	15.30	14.22	12.29	6.78	6.91	6.66	10.53
Durapore	40.73	18.32	35.67	31.40	23.88	19.01	11.42	29.31

ΔΦ represents the change in phase from 0 to 21% O_2

Table 2.2 Effects of re-treating PE-based sensors at 120°C, compared with Durapore-based sensors (± st.dev).

120°C	1st Treatment	2nd Treatment		1st Treatment	2nd Treatment		Durapore	
O_2%	Phase angle (Φ)	Phase angle (Φ)	ΔΦ	Intensity (*I*)	Intensity (*I*)	ΔI	Phase angle (Φ)	Intensity (*I*)
0	36.4 ± 0.5	35.9 ± 0.4	0.50	39.91 ± 8.03	34.24 ± 5.94	5.67	40.73 ± 0.31	130.45 ± 9.55
0.5	31.72 ± 0.46	31.13 ± 0.66	0.61	36.01 ± 7.18	30.65 ± 6.24	5.36	38.32 ± .035	122.1 ± 6.18
1	26.86 ± 0.38	26.73 ± 0.42	0.47	30.05 ± 6.49	27.33 ± 5.98	2.72	35.67 ± 0.27	115.15 ± 6.6
2	20.04 ± 0.46	20.44 ± 0.73	0.25	23.76 ± 5.95	21.97 ± 5.21	1.78	31.4 ± 0.28	103.25 ± 6.22
5	11.35 ± 0.39	11.72 ± 0.44	0.08	14.91 ± 3.44	14.01 ± 2.89	0.90	23.9 ± 0.18	80.05 ± 5.8
8	7.41 ± 0.32	7.31 ± 0.21	0.21	10.13 ± 1.91	10.04 ± 1.55	0.01	19 ± 0.49	62.56 ± 5.74
21	2.6 ± 0.21	2.78 ± 0.18	0.08	5.01 ± 1.16	1.68 ± 1.11	0.33	11.42 ± 0.32	36.49 ± 2.55

CHAPTER III

Detection of Cheese Packaging Containment Failures using Reversible Optical Oxygen Sensors

This Chapter is in the form of an accepted manuscript and published in the *International Journal of Dairy Technology*;

Hempel, A.W., Gillanders, R.N. Papkovsky, D.B. and Kerry, J.P. 2012. International Journal of Dairy Technology, 63, (3), 456-460.

Abstract

This study investigated the capacity of optical O_2 sensors to non-destructively detect containment packaging failures in commercial vacuum packed cheddar cheese. Unacceptable O_2 levels in packs lead to product deterioration and can significantly reduce shelf life. Vacuum packed cheddar cheese was monitored for O_2 levels over time at 4ºC with optical sensors pre fitted to packaging laminates (PET/PP). Oxygen levels present in packs immediately after packaging were 3% on average. Further ingress of O_2 into all packs occurred over storage time and levels ranged from 3.6% to 8.2% after 6 days. Information obtained using O_2 sensors led to further assessments and identification of the causes of packaging containment failure.

Keywords: Vacuum packaging, Cheese, O_2 sensors, shelf life, storage

3.1 INTRODUCTION

Packaging is increasingly recognised as an important factor in protecting and controlling the safety and quality of cheese. In the case of hard varieties, a complete barrier package (i.e. vacuum package) is preferred to eliminate O_2 related deterioration (McSweeney, 2007). Hard variety cheeses (<39% moisture content) are typically packaged in laminates, predominantly consisting of polyamide (both orientated and non-orientated) and polyethylene and applied as pre-formed pouches or as flow-wrapping in horizontal form, fill and seal operations where gas flushing, vacuum packaging or a combination of both are used prior to the sealing. Vacuum packaging involves the placing of a perishable food inside a plastic package and then by physical or mechanical means, removing air from inside the package so that the packaging material remains in close contact with the product surfaces after sealing (Brody and Marsh, 1997). A combination of both vacuum packaging and storage temperature dramatically extends quality and shelf life of food products.

Essentially, the overall packaging objective is to prevent or significantly delay the growth of aerobic spoilage bacteria, yeasts and moulds, reduce oxidation reactions and prevent physical contamination of the cheese from external physical entities such as dirt, moisture, insects, grease/oil etc. Vacuum packaging of food products allows for the almost complete removal of O_2 to oxygen-sensitive products, therefore extending shelf life. It is an effective means of eliminating possible biological and chemical contaminants from the space surrounding the food (Lee, 2008). Cheese in general is a highly perishable product and can be found to mould quickly when in contact with O_2. Moulding and oxidation are the primary causes for spoilage of cheese and cheese products (Fox and McSweeney, 2006).

Packaging films for cheddar cheese must be sufficiently impermeable to O_2 to prevent fat oxidation and mould growth. Oxygen that is present in the space between the package material and cheese (produced either as a product of enzymatic action in the cheese, left in the package after sealing or diffusing through the package material) determines whether or not microbial growth will occur on the surface of the cheese (Robertson, 2006). It is likely that problems of mould growth on hard cheeses packaged in commonly used films are a function of hygienic conditions in the package room, the degree of vacuum inside the package and the integrity of the heat seal, rather than O_2 permeability of the package material used (Robertson, 2006).

The use of optical sensors in the monitoring of O_2 has attracted great interest in recent years (Ogurtsov and Papkovsky 2003, Papkovsky 1995 & 2004 and O' Riordan et al., 2005). Optical O_2 sensors which use a fluorescent-based dye which react with O_2 can be used within food packaging to assess the levels of O_2 present. Oxygen-sensitive probe platinum octaethylporphyrin-ketone (Pt-OEPK) in polystyrene, spotted on Durapore membranes have been utilised in such applications (Papkovsky, 2004). The use of optical sensors in the non-destructive monitoring of O_2 in food applications such as cooked meats (Smiddy et al., 2002a), vacuum packaged beef (Smiddy et al., 2002b), cooked chicken patties (Smiddy et al., 2002c), MAP cheddar cheese (O'Mahoney et al., 2006) and *sous vide* products (O'Mahoney et al., 2004) have been investigated. These assessments were carried out, not alone to determine O_2 levels in food packs, but to assess the consequences of different levels of O_2 on the shelf life stability of various O_2-sensitive food types. The successful packaging of hard cheese under vacuum is essential in order to meet commercial storage requirements and retailing shelf life and this will only be achieved by removing the O_2 from the food product.

While the O_2 sensor technology described above has been used in numerous lab-based trials to assess the impact that oxygen levels have had on the safety and quality of numerous oxygen-sensitive foods during simulated retail storage, the technology had more limited exposure to addressing industrial packaging problems. Consequently, when the group was approached by a cheese company to help solve a packaging containment problem during storage and up to the point of consumption, a study plan was initiated with the objective of determining how the oxygen sensor technology could be used to determine what was causing a loss of containment, where this problem was occurring and how the problem might be addressed and solved. This was the focus of this study.

3.2. MATERIALS AND METHODS

3.2.1. Sensor preparation and calibration

Oxygen sensors were prepared by spotting 40µl Pt-OEPK (Platinum octaethylporphyrin-ketone) from ready to use 2ml glass vials (Luxcel Biosciences, Ireland) on Durapore paper using a Gilson P100 micropipette and allowed to dry. The sensors were cut to a size of 1cm² and a total of 40 sensors were produced. Six sensors were then screened, where they were placed into a customised flow cell for calibration using seven O_2 concentrations ranging from 0%-21% O_2 (0.0, 0.5, 1.0, 2.0 , 5.0, 8.0, 21.0%, respectively) using bottled gases provided by BOC gases, Ireland. A phase value was obtained at each O_2 concentration using a phase detector (Luxcel Biosciences, Ireland) and these data points were used to construct a calibration curve (Fig 3.1). Phase results are converted to O_2 using a calibration function of $y = -2.6728x + 38.854$. To simulate the conditions of refrigerated storage, this calibration step was conducted using a water bath at 4ºC, in which the flow cell was half submerged.

[Fig 3.1]

3.2.2. Sample preparation

Commercially prepared cheddar cheese was made available in 20 g strips. The sensors were placed in positions of visibility within the thermoformed well in the bottom plastic-based laminate web on the vacuum packaging line. The cheese sample was placed over the sensors and covered by another plastic-based laminate top web, then held under vacuum and heat sealed. The plastic packaging material used was a

laminate consisting of PET12/PP50 (μm) with an O_2 permeability of 110 ml/m^2/24h. Analysing the samples using sensors allowed for the non-destructive measurement of O_2 in cheese packs over time, thereby maintaining initial package containment. A total of two sensors were placed in each pack, one on each plastic layer. A total of 40 samples of cheddar cheese were packaged individually and sealed. Each pack was then numbered. Packaging was carried out using a MultiVac C500 vacuum packer (MultiVac Ireland Ltd, Dublin, Ireland). All packs were visually checked for failures following vacuum packaging. The packs were then stored in a cold room (4°C) and tested twice daily (morning and evening), with the first measurement coming four hours after packaging.

3.2.3 Leak Detection Using Water Submersion

Water submersion tests were carried out using a 5 litre water bath, where pack samples, identified as having high levels of O_2 present via the presence of O_2 sensors, were individually submerged in water for 5 minutes, and samples visually assessed for leaks, which were inconsistently demonstrated by the appearance of gas bubbles. Leak locations were marked and further studied using microscopy.

3.2.4 Light Microscopy

Using a light microscope (Olympus, BX51), all samples were assessed for visual defects at various magnifications (4x, 10x, 20x). Heat seals were monitored for air channelling due to possible heat seal failure. Further assessment, using microscopy,

analysed pack areas for over stretching at ridged points along the pack/product interface. Images were obtained using an integrated Pixelink PL-A662 megapixel firewire digital camera (PixeLINK, Ottawa, Canada).

3.3 RESULTS AND DISCUSSION

3.3.1 Sensor readings

Each sample was measured twice daily; the first measurement was taken 4 hrs after packaging and the final measurement taken at 148 h. Using the calibration graph in Fig 3.1, the corresponding O_2 concentration in the pack was determined. All 40 samples were tested in this manner and recorded over 148 h. In the first measurement, an average of 2.63 ± 0.92 % O_2 was measured across the 40 packs, where the lowest and highest O_2 present in samples were 0.84%, and 5.2%, respectively. The presence of these O_2 levels in vacuum packed cheese is unacceptable as the process of vacuum packaging would be expected to remove O_2 to extremely low levels. Clearly, the failure to remove O_2 would leave cheese products susceptible to quality deterioration. Fig 3.2 represents the amount of O_2 present within all samples, at the commencement of storage (4 h after packaging) and at the end of storage (148 h after packaging). Across all vacuum packaged samples it was observed that there was a notable increase in O_2 within all packs over time.

[Fig 3.2]

On final assessment of the cheese packs, the average O_2 content was determined to be in excess of 5.5 ± 1.19 % O_2. Samples with as high as 8.2% O_2 was observed.

As outlined previously, sensors had been placed on both sides and at both ends of the cheese samples, essentially monitoring each plastic layer or sealing area for possible packaging faults. Differences in O_2 readings between sensors were found to be negligible, where differences in percentage O_2 readings varied between sensors on average by less than ~0.1%. These levels are represented in Table 3.1.

[Table 3.1]

This consistency in O_2 determination suggested that O_2 was entering the pack at various points and was being dispersed evenly throughout the cheese packs. An average profile was constructed to show the level of O_2 ingress into a pack over time. This is represented by Fig 3.3

[Fig 3.3]

Table 3.2 depicts the number of packs that can be categorised on the basis of O_2 level at specific analysis times throughout storage. After 4 hours the majority of packs contained between 2.00-3.99% O_2. This situation persisted up to 120 hours storage when a significant shift in O_2 within packs increased so that the majority of packs contained between 4.00-5.99%. This remained the case up to 144 h and 148 h of storage, but only narrowly, as there were almost as many packs (18 versus 14 packs and 21 versus 16 packs) within the 4.00-5.99% and 6.00-9.00% O_2 categories. The steady increase in O_2 levels across all packaged samples was alarming and clearly indicated that further assessment of the packaging materials was necessary in order to determine the reasoning behind this rapid ingress of O_2 within cheese packs.

Consequently, physical tests were carried out on the packaging materials to determine the cause of excessive O_2 permeation.

[Table 3.2]

3.3.2. Light Microscopy

Cheese pack samples which were determined to have the highest levels of O_2 were submerged in a water tank. The objective of carrying out this step was to determine if air bubbles could be observed emanating from the packs or if water was entering the packaging. On inspection, samples were inconsistently seen to have air bubbles leaking from the packs. The leaking areas on the packs were marked and observed under a light microscope. It was noted that the air escaping from the packs were all located at points in the packaging which lay adjacent to the main body of the cheese and not from the surrounding heat seal. At first it was thought that the heat seal was failing. However, using microscopy at 4x, 10x and 20x magnification, the seal appeared to be intact in all cases. Fig 3.4 shows a typical view at 10x magnification of the heat seal area with no visible break or channelling in the network.

[Fig 3.4]

The marked locations on the cheese packs where air was visibly seen escaping was also observed under microscope. The top web of plastic which is pulled onto the product when under vacuum was the packaging layer that was found to have holing in all cases, located at the edges of each cheese sample. Fig 3.5 shows the typical pinhole found. It is thought that with a combination of excessive vacuum pressure and

over-stretching of the plastic material at rigid points along the cheese pack samples was causing the pinhole formation within the laminate used in all packs. This information was passed directly to cheese packaging company from which changes were undertaken to reduce the future likelihood of holes being formed. The reduction in vacuum pressure as well as the placement of plastic 2 (top layer) onto the samples resulted in the immediate elimination of such pinholes being produced.

[Fig 3.5]

Following industrial adjustment of the packaging process another trial was conducted to see whether or not pin-holing was still occurring in cheese packs. This trial, involving the sensor assessment of 25 cheese pack samples, contained O_2 levels of <1% O_2 over a 7 day storage period. Repeats of the submersion test showed no leaks present and locations at which holes were normally found were monitored again using microscopy and no pinholes were detected.

The use of such optical sensors in food packaging systems show great application potential for the monitoring of O_2 in low O_2 packaging conditions (Fig 3.6). The suitability of such a technology to vacuum packaging is apparent from this study and can help ascertain the levels of O_2 present in packs immediately after packaging. In this case the sensors allow for the level of O_2 present post packaging to be determined and lead to the discovery of faults with operational packaging procedures.

3.4. Conclusion

With the addition of further testing, the O_2 sensor showed adequate qualities in the assessment of a vacuum packaged food product. The reversible properties of the sensor allowed for the monitoring of O_2 over time, to indicate the O_2 permeability in the pack and the level at which ingress was occurring. With such a high level of O_2 ingress, further assessment of the packs was carried out to ascertain the cause of such a high rate and resulted in the visualisation of holing caused by excessive vacuum pressure. This pressure has been altered by the manufacturers resulting in more stable packaging and O_2 ingress. The sensors show good applications in assessing the competence of O_2 free or low O_2 packaged foods. This novel method of testing adds to the range of food and packaging types carried out by this research group.

3.5. Acknowledgements

Financial support of this work by the Irish Department of Agriculture, Food International Research Measure (FIRM) grants 06RDC468 is gratefully acknowledged

3.6. Tables and Figures

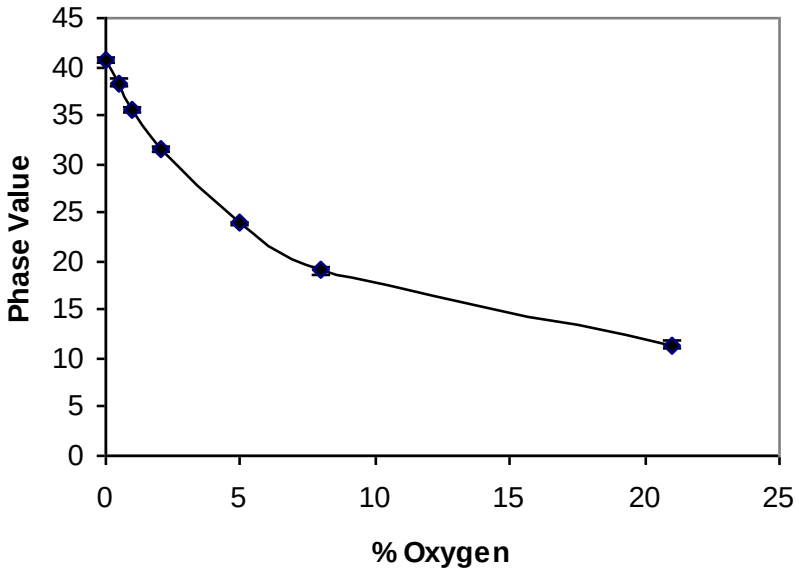

Fig 3.1 Calibration Curve for O_2 sensors. Results shown are means and ± standard deviation of six sensors.

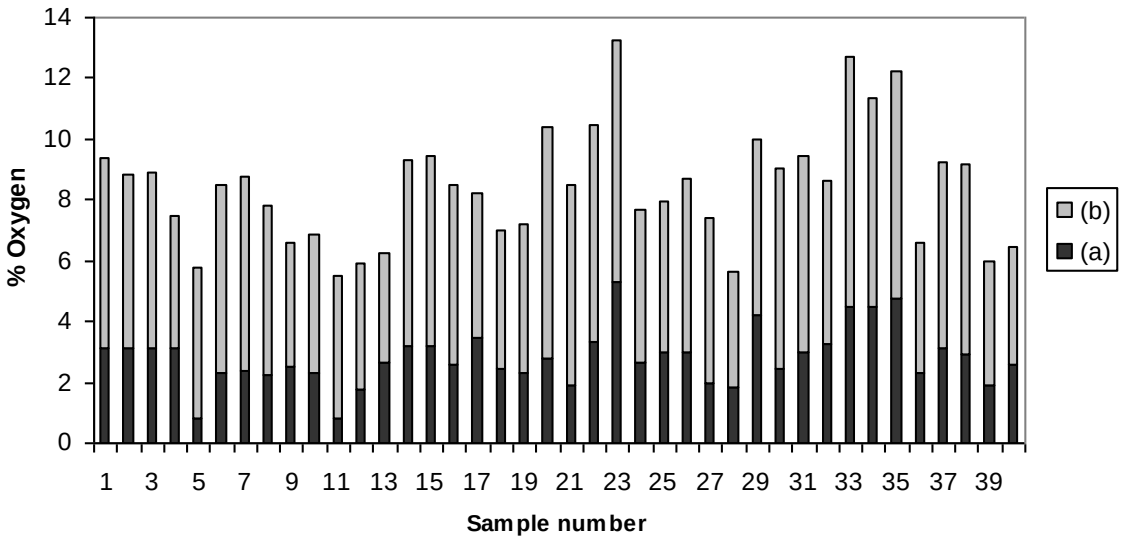

Fig 3.2 Oxygen concentration detected in vacuum packed cheddar cheese after 4 (a) and 148 (b) h of storage.

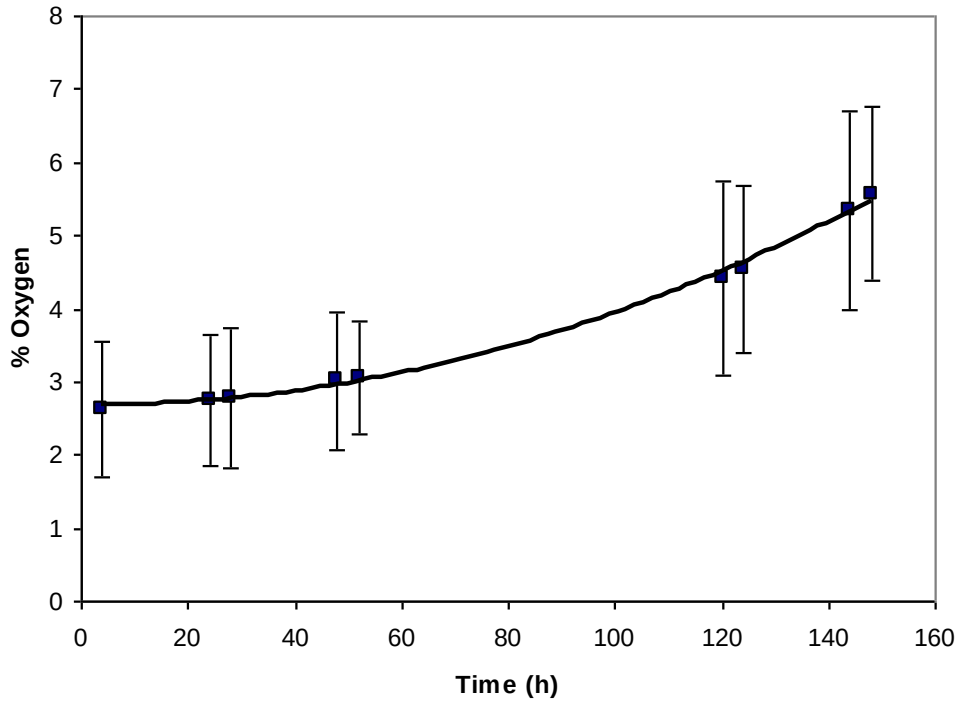

Fig 3.3 Mean increase in O_2 content in cheddar cheese packs over time. Results shown are means ± standard deviation.

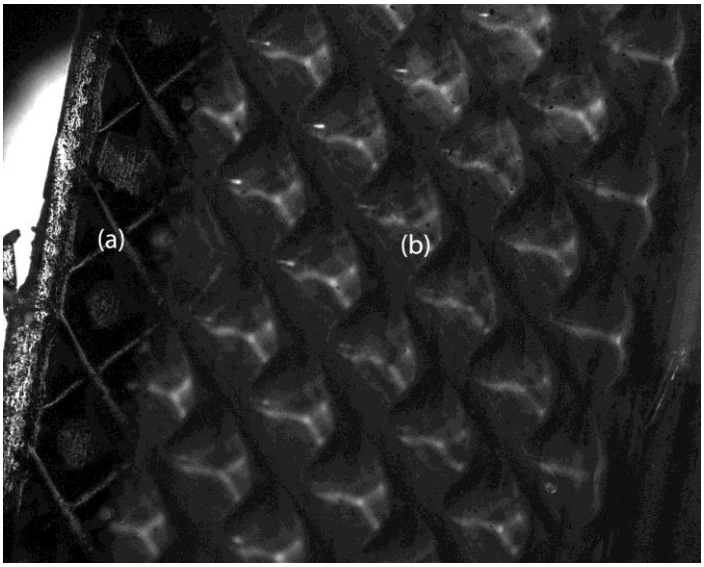

Fig 3.4 10x Magnification of heat seal area on cheese pack. The outer edge represents the heat seal area (a) clearly showing no break or channelling within the seal area and (b) represents unfused pack interior.

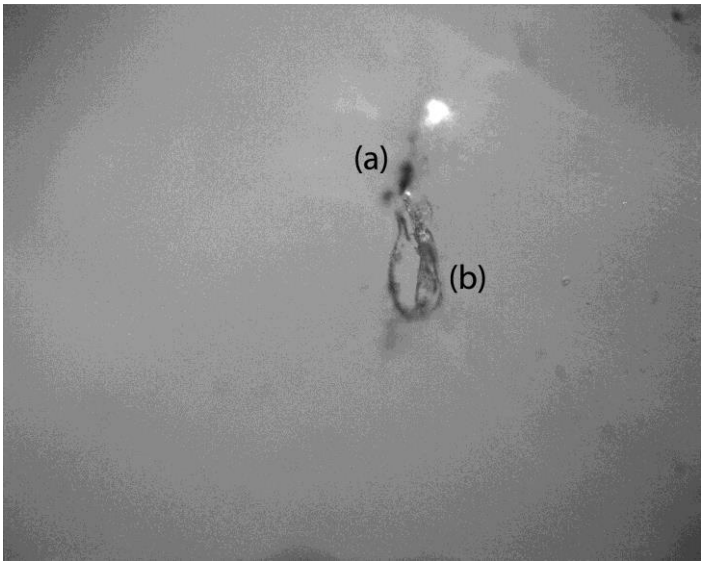

Fig 3.5 10x Magnification of pinhole formed in cheese packaging, (a) denotes the position of a pin hole formed during packaging, (b) represents a water drop present inside the pack.

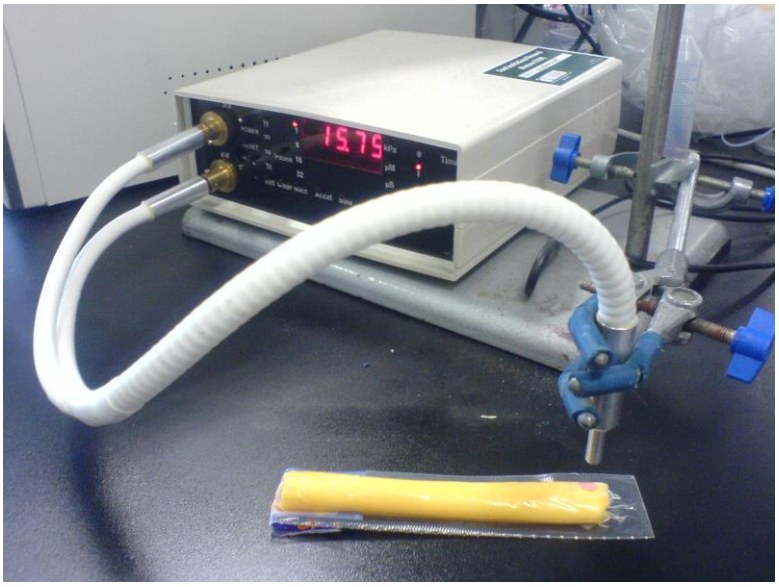

Fig 3.6 Cheese sample and Luxcell Phase detector

Table 3.1 Percent O_2 found with sensors placed on underside and topside of samples

	Mean O2%		
Time (hrs)	Underside	Topside	Difference
4	2.65	2.61	0.04
24	2.77	2.72	0.05
28	2.81	2.75	0.06
48	3.05	2.99	0.06
52	3.11	3.02	0.09
120	4.46	4.37	0.09
124	4.58	4.49	0.09
144	5.37	5.33	0.04
148	5.61	5.53	0.08

Table 3.2 Number (%) of cheese packs containing ranged O_2 levels throughout storage.

	% O2			
Time (h)	0-1.99%	2-3.99%	4-5.99%	6-9%
4	20	67.5	12.5	0
24	20	77.5	2.5	0
28	22.5	62.5	15	0
48	15	72.5	12.5	0
52	15	80	5	0
120	2.5	35	47.5	15
124	0	37.5	50	12.5
144	0	20	45	35
148	0	7.5	52.5	40

CHAPTER IV

Use of Optical Oxygen Sensors to Monitor Residual Oxygen in Pre- and Post-Pasteurised Bottled Beer and its Effect on Sensory Attributes and Product Acceptability during Simulated Commercial Storage

This Chapter is in the form of an accepted manuscript and published in *LWT – Food Science and Technology*;

Hempel, A., O'Sullivan, M.G. Papkovsky, D.B. and Kerry, J.P. 2013. *LWT – Food Science and Technology*, 50, (3), 226-231.

Abstract

Disposable optical O_2 sensors were used to non-destructively assess the levels of residual oxygen in the head space of commercially produced Lager beer in 330ml clear glass bottles. Monitored before and after pasteurisation, O_2 concentration was observed to drop as O_2 diffused into the beer. Oxygen levels between 0% and 5% were present in the bottle headspace pre-pasteurisation. Oxygen diffusion into the beer was monitored over time under refrigeration (~ 4ºC) and a sensory panel was used to determine the effect of residual O_2 on the sensory quality of the beer. The headspace in the bottles was also monitored over time where O_2 increased to a level of ~0.5% on average over the course of the shelf life. Interestingly, results showed that beer samples possessing higher levels of O_2 present prior to pasteurisation also possessed the most negative sensory attributes associated with the beer, particularly those consistent with beer staling. The developed optical sensor was shown to act as a predictor of sensory quality and may have on-line applications in the beer packaging sector.

Keywords: Optical, Oxygen sensor, Beer, Packaging, Sensory, Storage

4.1 Introduction

Alcohol-based beverages contribute €6.6 billion (euro) to the Irish national economy and provide over 100,000 jobs, which makes this industry a vital part of Irish social and economic life. Beer constitutes the biggest alcohol-based product category, accounting for about 50% of the market, and an average of 40% of all beer brewed in Ireland is exported (Irish Business and Employers Confederation, 2010). Pale lager is the most widely consumed and commercially available style of beer in the world. The flavour of these products is usually mild and the producers often recommend that the beers be served refrigerated. In general, lagers display less fruitiness and spiciness than ales, simply because the lower fermentation temperatures associated with lager brewing causes the yeast to produce fewer of the esters and phenols associated with those flavours. The typical brewing process is finalised by filling, packaging and pasteurisation steps. Depending on the packaging selected, pasteurisation can take place prior to, or post filling. The only successful way to prevent beer spoilage by microorganisms is to thermally destroy organisms via pasteurisation (Wainwright, 1999a). In most popular cases, tunnel pasteurisation is carried out when bottling. Tunnel pasteurisers allow for the destruction of microorganisms and residual yeasts at 65-68ºC over a period of 20 minutes. The tunnels are usually divided into a number of heating zones where atomized water sprays heat the filled containers as they pass on a conveyor giving incremental rises in temperature until pasteurisation is achieved (Fellows, 2000).

The packaging of beer using glass bottles is still a preferred packaging approach. Despite negative features such as packaging fragility and final package weight, glass bottles used for beer packaging still possesses features that still make it the most suitable form of packaging for use on high speed filling lines, namely, it is inert and

so does not chemically interact with the beer itself, provided that the correct closure system is used, glass is completely hermetic and consequently prolongs product shelf-life, it offers greatest container rigidity and consequently, it is easily, filled, stored and transported and is available in a variety of sizes, shapes and colours. Products look better, taste purer and are secure when packaged in glass (Yam, 2009). When used as a beer package, the premium image and end-use market make glass very acceptable to the consumer, especially traditional consumers who perceive bottled beer as being of a superior quality (Giles, 1999). The final processing of larger-style beer generally results in the production of a clear beer which should possess a commercial shelf-life, as a minimum, of six months.

However, beer products, if inappropriately processed or packaged can oxidise, may spoil due to microbial growth or develop hazing (which can produce turbid-like appearances in the beer) during storage. Flavour stability in beer depends to a great extent on the lack of oxidation of the beer. If there is a relatively large volume of air in the final container, oxidation inevitably occurs (Wainwright, 1999b). At the end of the fermentation stage, beer is completely free of O_2. At this point, beer is highly susceptible to oxidation, which has the following affects on the end product; undesirable taste, cloudy/hazy beer, increased beer astringency and darkened beer colour.

During the filling process, beer bottles are lifted to an individual filling point with a tube which projects into the bottle. The bottles are filled based on height, weight or volume. At the end of filling process, there should be less than 2ml of gas space in the bottle and the O_2 content in the beer should be below 0.1mg/L (Giles, 1999). Prior to capping, the bottles are usually at full fill point with foam. This level of carbonation resulting in foaming helps expel O_2 from the bottle and usually keeps

O_2 from entering the bottle. Production of flavour-active compounds like carbonyls are associated with extended storage. Oxygen plays a key role in bottled beer, where, beer of high O_2 contains significantly higher levels of carbonyls after storage. Although a degree of protection is obtained by minimising the O_2 content of beer after packaging, a high level of oxidation during earlier stages of brewing also results in flavour instability in the final beer (Varnam and Sutherland, 1994). Almost all beers taste best immediately after they have been produced and usually start to deteriorate/age/stale over time; this is where best before dates are set. The primary quality issue associated with bottled beer is the change of its chemical composition during storage, which subsequently alters its sensory properties (Vanderhaegen, Neven, Verachtert & Derdelinckx, 2006 and Vanderhaegen, Delvaux, Daenen, Verachtert & Delvaux, 2007).

Optical O_2 sensors have been used as a non-destructive method of O_2 detection in recent years (Papkovsky, 1995 & 2004 and O' Riordan, Voraberger, Kerry & Papkovsky, 2005). Optical O_2 sensors use a phosphorescent dye embedded in a polymeric film which is quenched by O_2 and can be used within food packaging to assess the levels of O_2 present. Oxygen-sensitive platinum octaethylporphyrin-ketone (Pt-OEPK) in polystyrene is used as a typical optical sensing probe. The use of such sensors have been well documented and used in food packaging applications, such as modified atmosphere packaging and vacuum packaging systems, where foods such as beef (Smiddy, Fitzgerald, Kerry, Papkovsky, O'Sullivan & Guilbault, 2002a) processed meats (Smiddy, Papkovsky & Kerry, 2002b) chicken patties (Smiddy, Papkovskaia, Papkovsky & Kerry, 2002b), sous vide products (O'Mahoney, O'Riordan, Papkovskaia, Orgurtsov, Kerry & Papkovsky, 2006) and cheese (O'Mahoney, O'Riordan, Papkovskaia, Kerry & Papkovsky, 2002), have all been

assessed. The reversibility of the sensor response allows for continuous, non-destructive measurement of O_2 while contained within a food package up to the expiration date and beyond. The use of optical O_2 sensors provide the perfect tool to measure the level of O_2 present in bottled beer during processing, transport, storage and sale. The principle objective of this experiment was to determine the effectiveness of incorporating an O_2 sensor into lager beer bottles and predicting the sensory quality of the beer with respect to oxidation and staling. The level of O_2 in bottled beer pre- and post-pasteurisation was measured and its affect on sensory attributes was assessed during shelf life stability testing conducted over a storage period of seven months.

4.2 MATERIALS AND METHODS

4.2.1 Sensor Preparation

Optical O_2 Sensors were prepared using (Platinum octaethylporphyrin-ketone) (Pt-OEPK) (Luxcel Biosciences, Cork, Ireland) spotted on Durapore (Millipore Inc, Bedford, USA) paper using Gilson P100 pipette (Gilson, WI, USA) and allowed to dry and cut to a size of 5mm diameter. The sensors were batch-calibrated using a phase detector (Luxcel Biosciences, Cork, Ireland) and a customised load cell, where one sensor was flushed with O_2 of varying concentrations (0, 0.5, 1, 2, 5, 8, 21%, respectively) and their phase determined. A calibration curve was constructed representing phase versus O_2, to convert phase readings to percent O_2. These were then attached to 3cm stickers for adhesion to the interior neck of the bottles. In order to withstand the bottling process (pre-wash, wash, drying, filling and capping) initial trials were carried out within the bottling plant to establish the adequate adhesion required for stickers to remain attached to the bottles throughout the various steps

involved in the filling process. Three forms of stickers were used; pricing stickers (Avery price marking), labelling stickers (Avery 35mm laser labels) (Avery Dennison, California, USA) and masking tape (3M Scotch tape, 3M, Minnesota, USA). Using all three types of adhesive-coated stickers available, six bottles were pre-fitted with each type of potential sensor supporting adhesive-coated matrix in the bottle neck area and passed through the commercial bottling process. All three forms of stickers were assessed for their ability to; adhere to the applied internal surface of the bottle, resist peeling, resist flaking and resist curling. These were assessed over a 30-day period to determine which sticker was optimally suited for a larger and more long-term beer storage study. Once adhesive label selection was made, sensor materials were spotted to a 5mm diameter in the centre of the stickers. These constituted the sensors for further application and have a shelf life of over one year. Sensors were incorporated to beer bottles and exposed to the complete packaging process including pasteurisation. Immediate post packaging sensor checks and recalibration were carried out to insure sensors withstood the temperature shock inflicted by the pasteurisation process. Results yield no change in performance to sensors and re-confirm initial calibration checks.

4.2.2 Sample Preparation

A local brewery (Beamish and Crawford Ltd., Cork, Ireland) provided access to their production facilities and provided all of the samples necessary for this study. Empty clear coloured glass 330ml bottles were collected and fitted with sensors in the upper neck of the bottle. Once prepared these bottles were transported to the bottling facility and added on to the filling line. Once washing, drying, filling and capping were complete, the bottles were taken off-line where sensors were visually assessed

and instrumentally read for the first time. Following this procedure, the bottles were again returned to the bottling line so that the bottles could go through the pasteurisation process. Pasteurisation was carried out in a tunnel pasteuriser for 25 minutes where samples were subjected to 68ºC heat and then cooled back to refrigeration temperature (4ºC). A batch of 48 samples were produced and regularly monitored for O_2 over time (bottles were read in triplicate, where standard deviation is <0.1% O_2). Samples were categorised according to their pre-pasteurisation O_2 content and were grouped into levels that represent 0, 0.5, 1.0, 2.0 and 5.0mls of O_2 per 100mls of air and these were designated as 0.0%, 0.5%, 1.0%, 2.0% and 5.0%, respectively. These groups were subsequently used for sensory analysis. The level of O_2 present prior to pasteurization was assessed in an attempt to ascertain if this O_2 level would have an impact on shelf life and product sensory attributes. All samples were continuously measured on a monthly basis, for seven months, while maintained at 4ºC. Equipment was blanked each test day before readings were taken, using sensors maintained at 0% and 21% O_2.

4.2.3 Sensory Analysis-Flash Profiling

A 26 member sensory panel was recruited in University College Cork, Ireland. Age range of panellists was 21-40 years old and 50:50 ratio of men: women. Selection criteria for panellists were availability and motivation to participate on all days of the experiments and that panellists were regular beer consumers. All panellists had previous experience in carrying out sensory analysis and thus were not considered completely näive (O'Sullivan et al., 2002) as the flash profile involved brief training. Panellists were trained in a short session by being presented with fresh (1 month old) and aged beer samples (8 months old) and the relevant sensory descriptors in Table

4.1 described (O'Sullivan, Cruz-Romero and Kerry, 2011). This assisted panellists in identifying the sensory attributes that describe fresh and stale beer attributes and allowed the evaluation of the products on a monthly basis. The descriptors used include those for oxidized flavour, stale flavour, liking of flavour, carbonation and overall acceptability etc. Hedonic descriptors were included as intensive sensory training was not undertaken and thus bias was not introduced in to the panel. Flash profiling was carried out in panel booths conforming to international standards (ISO 8589: 2007). Beer samples were categorised by the level of O_2 present in the product pre-pasteurization and were assigned to the nearest O_2 category outlined; 0%, 0.5%, 1 %, 2% and 5%. The level of O_2 present was assessed by panellists to ascertain if beer containing the highest O_2 levels also possessed the most perceived negative attributes. Five samples were taken to represent varying O_2 contents present in the beer samples pre pasteurisation and assessed monthly over the course of the labelled shelf life. Samples were held at refrigeration temperatures (4ºC) up to the moment of sampling. One bottle representing each category was relinquished for every test day. Each panellist was presented with five samples (0%, 0.5%, 1% 2% and 5% O_2 respectively) and asked to assess the attributes, according to a ten-point scale. A list of descriptors that were used is presented in Table 4.1. Each consumer was presented with coded (three digit) samples which were assessed blind. The order of the presentation of all test samples was randomized to prevent first order and carryover effects (MacFie, Bratchell, Greenhoff and Vallis 1989).

4.2.4 Statistical analyses

ANOVA-Partial Least Squares Regression (ASLPR) was used to process the mean data accumulated from the 26 panellists during the sensory evaluation. Principal component (PC) 1 versus PC 2 is presented; other PC's did not yield additional information. To derive significance indications for the relationships determined in the quantitative APLSR; regression coefficients were analysed by jack-knifing (Table 4.3).

4.3. RESULTS AND DISCUSSION

4.3.1 Sensor selection

An initial trial was carried out to enable the selection of a sensor sticker stable enough to withstand the cleaning and filling rigors associated with a typical commercial beer bottling process. This process was essential to select a strong adhesive sticker combined with the sensor to specifically withstand the pre-wash, wash, fill, capping and pasteurisation stages of bottling. The use of Avery pricing stickers were selected for further trials as no adhesive failures were observed, whereas bottles fitted with labelling stickers and masking tape were found to have multiple failures within 48 hours of processing (Table 4.2). Failures were assessed post-filling and over time (for 30 days). The selection and application of optical O_2 sensors to commercial beer bottles using adhesive stickers, was successful and again demonstrated the potential applicability of the O_2 sensor technology to practical industrial settings. Previous and successful industrial applications of the O_2 sensors have been shown by this research group for; cheese (O' Mahoney, 2006), processed meats (Smiddy, 2002b), beef (Smiddy, 2002a) and chicken (Smiddy, 2002c).

4.3.2 Sensor Readings

Sensors were pre-fitted in the neck of clear glass bottles, and added to the bottling line. All 48 sensors were observed to have withstood the bottling process with no visible failures recorded; all showing good adhesion. When all bottles were capped and labelled during packaging process, they were taken off the line for the first optical reading. All 48 samples were then read by phase detector, showing a relatively wide range of 0-5% O_2 present within the headspace of the bottles. Once recorded, bottles were placed back on the line for final stage – pasteurization. After pasteurization, the bottles were refrigerated to 4°C and sensors were monitored again for phase. The level of O_2 present at this stage appeared to have reduced dramatically, as across all samples, only traces of O_2 remained. This suggests that the O_2 levels present in the headspace diffused into the beer during pasteurization. Fig 4.1 represents the categories used to describe the O_2 levels found in pre-pasteurised samples. Only trace amounts of O_2 were present in bottles after pasteurization, with 0.24% O_2 being the highest level recorded.

Over the shelf life period, it was observed that only a minor increase in O_2 was apparent in bottled beers (Fig 4.2). From initial to final readings an average increase from 0.01% to 0.49% was observed. This increase is believed to be as a result of O_2 not being utilised in aging and deterioration processes within beer and excess O_2 being released into the headspace once more. This is confirmed when noting that bottles with the highest O_2 present in sample headspace at month 7, match those samples with highest O_2 present pre-pasteurisation. Samples categorized as 5% were found to have the highest level of O_2 present in headspace throughout storage. By final month 7, 5% O_2 samples were found to be above the average O_2 level (0.49%),

where an average between 0.5-0.6% O_2 (SD <0.1%) was found. Conversely, 0% and 0.5% grouped samples were found to have a lower than average O_2 level in headspace throughout the shelf life period, averaging 0.4% O_2 (SD <0.1%). The level of O_2 present prior to pasteurization was assessed in an attempt to ascertain if this O_2 level would have an impact on shelf life and product sensory attributes.

4.3.3 Sensory Analysis

Sensory analysis involved flash profiling which was carried out on samples with varying pre-pasteurization O_2 contents for the categories 0-5% O_2, to assess the changes in descriptors over time. Flash profiling of beer, which had been bottled and stored for one month, showed that little difference was determined by panellists between all product categories. However, as time progressed; deteriorative quality attributes became more apparent in some beer samples and it was determined that samples possessing the highest levels of O_2 pre-pasteurization were also associated with the most negative sensory attributes. Sensory evaluation on a progressive month to month basis showed a continued decline in positive beer attributes in samples that were deemed to possess high O_2 pre-pasteurisation.

From the data in Table 4.3, the level of significance for each descriptor can be seen. Samples found to have 0% O_2 present pre-pasteurisation were seen to be positively associated with beer colour ($P \leq 0.05$), liking of flavour ($P \leq 0.01$), fresh flavour ($P \leq 0.05$), hop flavour ($P \leq 0.05$), carbonation ($P \leq 0.05$) and overall acceptability ($P \leq 0.001$). An O_2 level of 0.5% was also found to be positively correlated to overall acceptability ($P \leq 0.05$). 0% O_2 samples confirmed significant negative correlation to oxidised and stale flavours ($P \leq 0.001$). Levels of 2% and 5% became associated with oxidised and stale flavours and became negatively correlated

with liking of flavour ($P \leq 0.01$), fresh flavour ($P \leq 0.05$) and overall acceptability ($P \leq 0.01$). The positive correlation of 5% O_2 samples with oxidised and stale flavours was extremely significant ($P \leq 0.001$).

Therefore increases in pre-pasteurisation headspace O_2 levels reduced positive fresh beer sensory descriptors, increased negative stale and oxidised descriptors and also reduced the overall acceptability of the beer. In terms of shelf life, it can be seen that from months 1 to 7, a remarkable decline in product quality occurred. A positive correlation can be seen in month 1 with fresh flavour ($P \leq 0.05$) and month 2 with liking of flavour, fresh flavour and overall acceptability ($P \leq 0.001$). Mid way through the trial, month 3 showed overall acceptability to still be positively correlated ($P \leq 0.05$). However, from month 5 and onwards, panellists noted a significant and negative change in beer quality. Month 5 samples became negatively correlated with liking of flavour, fresh flavour and overall acceptability ($P \leq 0.01$). In the case of commercial products, this level of unacceptability at month 5 suggests that in cases of high O_2 present pre-pasteurisation (>2%) do not meet the suggested best before dates of six to seven months. On final assessment in month 7, the reduced quality of the samples was confirmed as sulphur aroma ($P \leq 0.05$), light struck ($P \leq 0.01$), oxidized flavour ($P \leq 0.001$) and stale flavour ($P \leq 0.001$) were all positively correlated and the overall acceptability descriptor was negatively and significantly correlated ($P \leq 0.001$). The results confirm that the increased level of O_2 present pre-pasteurisation has a negative affect on sensory attributes in beer over time. Levels of O_2 in excess of 1% were determined to be unacceptable to consumers. Figure 4.3 represents an overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 5 O_2 level groups.

In this study a notable number of samples (20%) had O_2 levels in excess of 2% O_2 pre-pasteurisation. This level of O_2 had detrimental effects on the sensory quality of the beer during storage. With this in mind, the ability to exert quality control at the filling and bottle capping step during beer production would enable bottlers identify those containers in possession of unacceptable levels of O_2. Such information would allow beer producers to identify unacceptable beer products at source and remove them prior to entering the distribution chain. However, more importantly, the presence of unacceptably high O_2 levels in bottled beer would allow beer bottlers to make adjustments on the filling line in order to optimise the process, particularly at the point of filling. Generally, the shelf life of beer is mostly determined by its microbial, colloidal, foam, colour and flavour stabilities. Beer contact with O_2 causes a rapid deterioration in product flavour and the type of flavour changes depends on the O_2 content of the beer (Vanderhaegen *et al*., 2006). Generally, there is a need to optimize production processes towards extending the shelf life of beer (Vanderhaegen, Neven, Coghe, Verstrepen, Verachtert & Derdelinckx, 2003). In bottled beer especially, excessive amounts of O_2 may cause a rapid change in aroma and taste. It has become evident that levels of O_2 throughout the brewing process can also affect the shelf life of beer downstream. Minimizing the formation of reactive O_2 species in beer must be the first step for improving beer flavour stability (Vanderhaegen *et al*., 2006). Oxygen triggers the release of free radicals, which can easily react with many constituents, leading to rapid changes in the flavour profile of beer. The reactivity of the O_2 species increases with their reduction status. The concentration of free radicals during the aging of beer increases with increasing levels of iron or copper present in the beer, with increasing O_2 concentrations or with higher storage temperatures (Kaneda, Kano, Koshino & Ohya-Nishiguchi, 1992). Since O_2 is recognised to cause such detrimental

effects on beer flavour, brewers have tried to minimize the O_2 pick up in finished beer prior to capping and modern filling equipment are reported to achieve total O_2 levels in the bottle of less than 0.1 mg/l beer (Vanderhaegen *et al*., 2006). However, this study clearly demonstrated that even when using such equipment, O_2 levels in bottled beer prior to capping and pasteurisation were significantly higher and resulted in subsequent sensory quality deterioration.

4.4 Conclusion

The use of optical O_2 sensors shows a potential application to the beer bottling industry. This type of application adds to further work carried out by this research group in applying optical O_2 sensors to different food packaging systems. This novel and unique method in using O_2 sensors to monitor the level of O_2 present pre-pasteurisation and post packaging provides important information on the shelf life and sensory quality of the product during storage. Acceptable limits could be set on the packaging line for the removal of samples with high O_2 rates which would result in unacceptable units for retail supply. Therefore, a volume of O_2 present before pasteurisation could be selected, in this case 1%, and monitored to ensure sample sensory quality to consumers during the conventional six/seven month shelf life associated with beer. Furthermore, O_2 sensors could be employed on line for long or short product runs to determine filling efficiencies. Such usage of the sensor technology would allow for greater quality control and process optimisation, thereby enhancing the quality assurance of bottled beer production and supply.

4.5 Acknowledgments

Financial support of this work by the Irish Department of Agriculture, Food International Research Measure (FIRM) grants 06RDC468 is gratefully acknowledged.

4.6 TABLES AND FIGURES

Table 4.1 Sensory terms for the naive assessor evaluation of Lager beer

Attribute	Description
Colour	0 = light amber, 10 = dark amber
Fruity Ester Aroma	0 = none, 10 = extreme
Stale Aroma	0 = none, 10 = extreme
Sulphur Aroma	0 = none, 10 = extreme
Light Struck Aroma	0 = none, 10 = extreme
Liking of Flavour	0 = extremely dislike, 10 = extremely like
Papery Flavour	0 = none, 10 = extreme
Fresh Flavour	0 = none, 10 = extreme
Hop Flavour	0 = none, 10 = extreme
Burnt Flavour	0 = none, 10 = extreme
Fruity Ester Flavour	0 = none, 10 = extreme
Oxidised Flavour	0 = none, 10 = extreme
Stale Flavour	0 = none, 10 = extreme
Carbonation	0 = none, 10 = extreme
Overall acceptability	0 = extremely unacceptable, 10 = extremely acceptable

Table 4.2 Suitability of adhesives to withstand bottling process (30 days)

Adhesive Type	Sample No.	Process Failure	Time Failure	Failure Rate
Avery Pricing Sticker	6	0	0	0%
Avery Laser Stickers	6	2	0	33%
3M masking Tape	6	2	1	50%

Table 4.3 Significance of estimated regression coefficients (ANOVA values) for the relationship of sensory terms as derived by Jack-Knife uncertainty testing for oxygen present in headspace of beer bottles. One sample per category was used per test day.

	Oxygen (%) present Pre-Pasteurization					Month Storage				
	0% O_2	0.5% O_2	1% O_2	2% O_2	5% O_2	1	2	3	5	7
Beer Colour	0.04*	0.29 ns	-0.23 ns	-0.26 ns	-0.16 ns	0.51 ns	0.90 ns	0.07 ns	-0.65 ns	-0.19 ns
Fruirt/Ester Aroma	0.07 ns	0.12 ns	-0.60 ns	-0.02*	-0.16 ns	0.45 ns	0.15 ns	0.92 ns	-0.05*	-0.18 ns
Stale Aroma	-0.15 ns	-0.53 ns	0.29 ns	0.81 ns	0.07 ns	-0.19 ns	-0.21 ns	-0.01**	0.61 ns	0.16 ns
Sulpher Aroma	-0.009**	-0.34 ns	0.23 ns	0.46 ns	0.009**	-0.24 ns	-0.06 ns	-0.001***	0.88 ns	0.04*
Light Struck	-0.07 ns	-0.31 ns	0.30 ns	0.42 ns	0.007**	-0.46 ns	-0.65 ns	-0.05*	0.83 ns	0.01**
Liking of Flavour	0.004**	0.14 ns	-0.81 ns	-0.01*	-0.008**	0.08 ns	0.001***	0.29 ns	-0.004**	-0.07 ns
Papery Flavour	-0.22 ns	-0.52 ns	0.30 ns	0.80 ns	0.22 ns	-0.15 ns	-0.34 ns	-0.02*	0.73 ns	0.33 ns
Fresh Flavour	0.02*	0.24 ns	-0.91 ns	-0.02*	-0.04*	0.03*	0.00***	0.02*	-0.004**	-0.19 ns
Hop Flavour	0.03*	0.22 ns	-0.98 ns	-0.02*	-0.10 ns	0.10 ns	0.001***	0.19 ns	-0.003**	-0.20 ns
Burnt Flavour	-0.54 ns	-0.89 ns	0.32 ns	0.44 ns	0.24 ns	-0.04 ns	-0.004**	-0.001***	0.09 ns	0.45 ns
Fruity Ester Flavour	0.72 ns	0.73 ns	-0.54 ns	-0.18 ns	-0.99 ns	0.14 ns	0.03*	0.09 ns	-0.03*	-0.98 ns
Oxidised Flavour	-0.001***	-0.08 ns	0.26 ns	0.04*	0.001***	-0.93 ns	-0.08 ns	-0.02*	0.09 ns	0.001***
Stale Flavour	-0.001***	-0.12 ns	0.24 ns	0.08 ns	0.001***	-0.77 ns	-0.37 ns	-0.006**	0.23 ns	0.001***
Carbonation	0.04*	0.24 ns	-0.92 ns	-0.08 ns	-0.07 ns	0.08 ns	0.002**	0.37 ns	-0.06 ns	-0.12 ns
Overall Acceptability	0.001***	0.05*	-0.33 ns	-0.007**	-0.001***	0.49 ns	0.001***	0.03*	-0.01**	-0.001***

ns = not significant
* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$

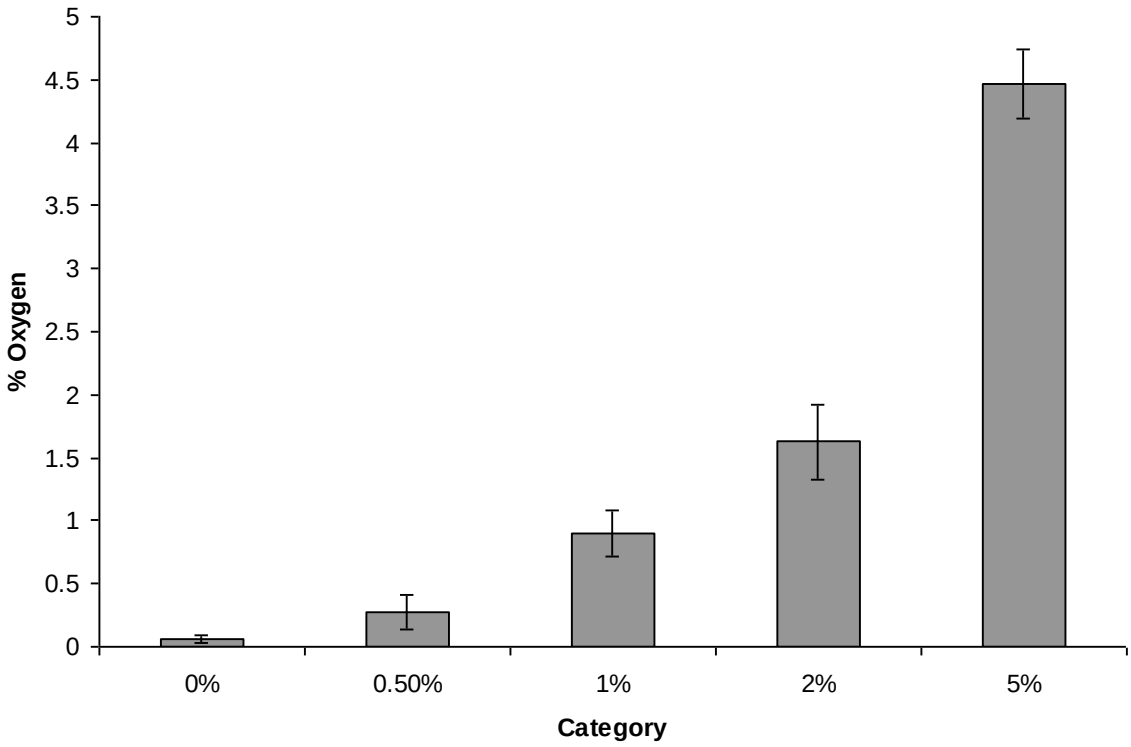

Fig 4.1 Sample categories assigned by Pre-pasteurisation oxygen content, yielding groups of 0%, 0.5%, 1%, 2% and 5% oxygen, consisting of 11, 10, 9, 13 and 5 bottles respectively.

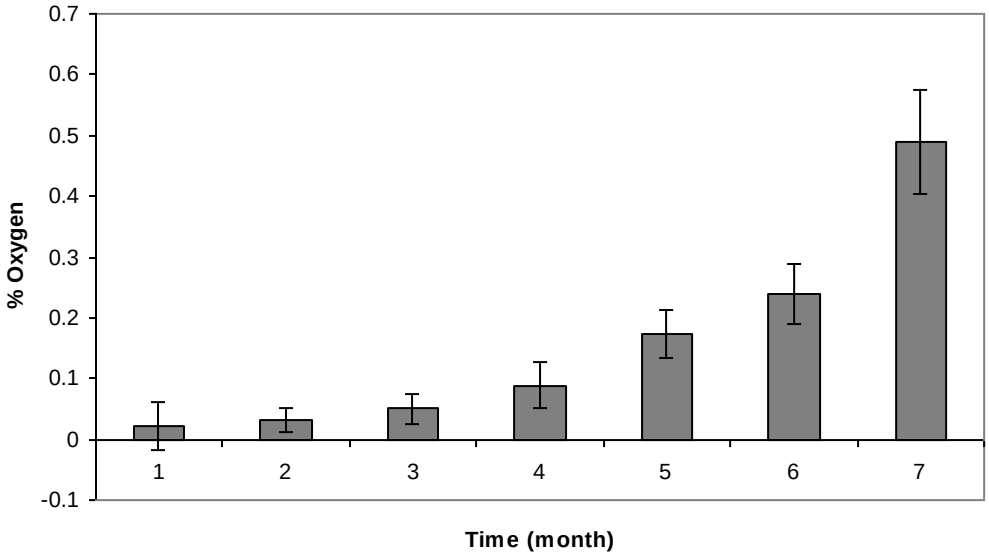

Fig 4.2 Represents the mean level of oxygen found in the headspace (post-pasteurisation) of all 48 bottles during storage including standard deviation.

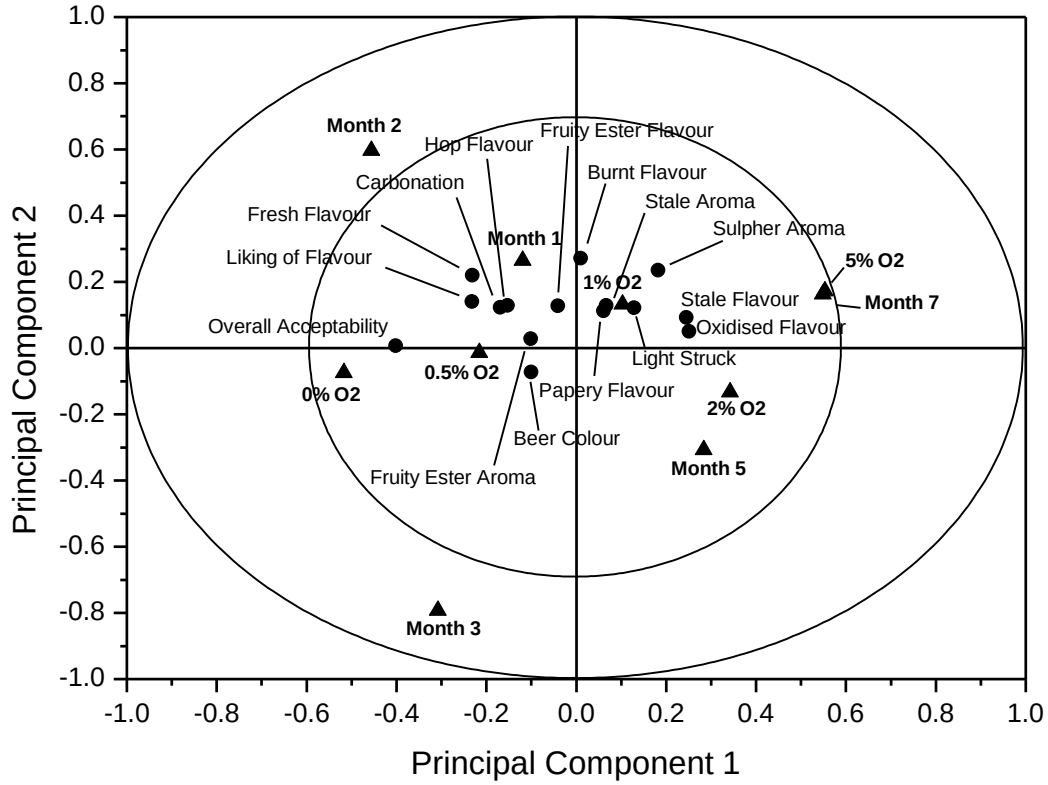

Fig 4.3 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 5 Oxygen level groups.

CHAPTER V

Non-Destructive and Continuous Monitoring of Oxygen Levels in Modified Atmosphere Packaged Ready-to-Eat Mixed Salad Products Using Optical Oxygen Sensors, and its effects on sensory and microbiological counts during storage

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Abstract

The objective of this study was to determine the percentage O_2 consumption of fresh, respiring ready-to-eat (RTE) mixed leaf salad products (Iceberg salad leaf, Caesar salad leaf and Italian salad leaf). These were held under different modified atmosphere packaging (MAP) conditions (5% O_2, 5% CO_2, 90% N_2 (MAPC - Commercial Control), 21% O_2, 5% CO_2, 74% N_2 (MAP 1), 45% O_2, 5% CO_2, 50% N_2 (MAP 2) and 60% O_2, 5% CO_2, 35% N_2 (MAP 3) and 4°C for up to 10 days. The quality and shelf-life stability of all packaged salad products were evaluated using sensory, physio-chemical and microbial assessment. Oxygen levels in all MAP packs were measured on each day of analysis using optical O_2 sensors allowing for non-destructive assessment of packs. Analysis showed that, with the exception of control packs, O_2 levels for all MAP treatments decreased by approximately 10% after 7 days of storage. Oxygen levels in control packs were depleted after 7 days of storage. This appears to have had no detrimental effect on either the sensory quality or shelf-life stability of any of the salad products investigated. Additionally, the presence of higher levels of O_2 in modified atmosphere packs did not significantly improve product quality or shelf-life stability; however, these additional levels of O_2 were freely available to fresh respiring produce if required. This study shows that the application of optical sensors in MAP packs were successful in non-destructively monitoring O_2 level, or changes in O_2 level, during refrigerated storage of RTE salad products.

Keywords: Modified atmosphere packaging, ready-to-eat (RTE), packaging, sensory analysis, shelf life

5.1 Introduction

Consumption of packaged, convenience-style, ready-to-eat (RTE) horticultural produce has increased in recent years. RTE salads are perceived as being healthy, nutritious and convenient meals or snacks which have undergone minimal processing. The minimal processing of fresh RTE produce has two purposes; firstly, it ensures that the produce is maintained effectively and conveniently in its fresh, nutritious state and secondly, to enable the product to have a sufficient shelf life to allow for distribution and consumption within a region (Alzamora, Tapia & Lopez-Malo, 2000). Watada & Qi, 1999, state that fresh cut processing of vegetables increase respiration rates and cause major tissue disruption. Physiological changes occurring during processing can be accompanied by shrinkage, softening of texture, flavour loss, decay and an increased rate of vitamin loss resulting in a shorter shelf life (Sandya, 2010). Modified atmosphere (MA) technology is popularly used in the minimal processing of fruits and vegetables including fresh RTE products. One of the most essential functions of a modified atmosphere (MA) pack is to maintain integrity. If the pack leaks, the optimized atmosphere within the food pack will become compromised as the protective MA gas mixes with normal atmosphere, consequently resulting in the loss of the beneficial effect of the MA used (Smolander, Hurme & Ahvenainen, 1997). In MAP applications, reduced O_2 and high CO_2 levels are used to extend product quality by controlling firmness, enzymatic browning and decay of fresh vegetables. According to Rogas-Grau et al. (2009), in pack O_2 concentration must be sufficient to limit respiration but also prevent anaerobic respiration. Not enough O_2 or too much CO_2 could result in the formation of undesirable metabolites. Using low levels of O_2 and high concentrations of CO_2 in combination with a low storage

temperature (<7ºC), has been proposed by researchers as the optimal conditions for storing fresh-cut vegetables, maintaining sensorial and microbial quality (Jacxsens, et al., 2000). Virtually all fruits and vegetables are susceptible to spoilage by microorganisms at a rate that depends on a range various extrinsic and intrinsic factors. Spoilage of vegetables usually occurs during storage, transport and while waiting to be processed (Alzamora *et al.,* 2000). Mechanical damage of vegetables during processing may increase the likelihood of decay and growth of microorganisms (Alzamora *et al.,* 2000). Aerobic microbial counts in lettuce have been reported to range from 10^3 to 10^9 colony forming units (cfu) g^{-1}, with most containing between 10^5 and 10^7 cfu g^{-1} (Szabo, Scurrah & Burrows, 2000).

Recently, the application of super-atmospheric O2 concentrations (>70% O2) have been used as an alternative to low O_2 MA applications to inhibit microbial growth, as well as preventing undesired anoxic respiration, thus maintaining the fresh like sensory qualities of RTE produce (Amanatidou *et al*., 1999; Rojas-Grau *et al*., 2009).

Jacxsens, *et al.,* 2001, found that high O_2 atmospheres were found to inhibit enzymatic browning in vegetables. Additionally, there was a reduction in yeast growth observed. The high O_2 atmosphere can be applied as an alternative for low O_2 MA for some specific types of RTE vegetables that are vulnerable to spoilage by yeasts and enzymatic browning.

Smolander, *et al*., 1997, states that in addition to leak detection, it is very important to establish that the correct gas mixture has been delivered within an MA package. This can be determined at the point of manufacture instrumentally, by employing a detection devise such as an O_2 analyser as part of the packaging machine (Smolander *et al*., 1997). A number of different gas analysers are available for

commercial applications however; these methods are destructive in nature. A non-destructive method for O_2 monitoring during storage is present in the form of optical O_2 sensors (Ogurtsov and Papkovsky, 2003; Papkovsky, 1995, 2004; O'Riordan, Voraberger, Kerry & Papkovsky, 2005). Optical O_2 sensors use a phosphorescent dye embedded in a polymeric film which is quenched by molecular O_2. Oxygen-sensitive platinum octaethylporphyrin-ketone (Pt-OEPK) in polystyrene is used as a typical optical sensing probe. This technology has been used previously in a number of food packaging (MAP and vacuum packaging) applications which include; fresh beef (Smiddy *et al*., 2002(a)), cooked meats (Smiddy *et al*., 2002(b)), chicken patties (Smiddy *et al*., 2002(c)), cheddar cheese (O'Mahoney, O'Riordan, Papkovskaia, Kerry & Papkovsky, 2006 & Hempel, Gillanders, Papkovsky & Kerry, 2012), bottled beer (Hempel, O'Sullivan, Papkovsky & Kerry, 2012) and sous-vide products (O'Mahoney, O'Riordan, Papkovskaia, Ogurtsov, Kerry & Papkovsky, 2004). The use of optical O_2 sensors allows for the accurate monitoring of O_2 in packs over time and the reversible reactions of the sensors have proven themselves to be useful in detecting defects in packaging, if present. While O_2 sensors have been used successfully in food packaging applications previously, the products investigated were not living, respiring products which might significantly impact on pack gas composition over time. Consequently, the objectives of this study were to assess the ability of O_2 sensors to monitor O_2 levels, and changes in O_2 levels, within packs of fresh, respiring RTE salad-based products packaged under different MAP conditions over time and to ascertain if the alterations in O_2 levels had an impact on quality and shelf-life stability of these products.

5.2 Materials and methods

5.2.1 Sample preparation

Commercially produced MA pre-packed, RTE salad leaf products were produced commercially using industrial standard packaging conditions (5% O_2, 5%-CO_2, 90% N_2, experimental control) along with three other experimental MAP gas mixes which varied in O_2 levels (21%, 45% and 60%) from the control with corresponding decreases in N_2 content (Table 5.1). A total of 90 g of Caesar salad consisting of Cos leaves; Iceberg Lettuce and Italian mix salad consisting of Cos, Frisee, Radicchio and Lollo Rosso were packed in low O_2-permeable (<1cm^3/m^2/24 hr at standard temperature and pressure (STP)) polystyrene/ethylvinylalcohol/polyethylene trays and flushed with modified atmosphere using a vacuum-sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) with a gas mixer (Witt-Gasetechnik GmbH & Co. KG, Witten, Germany). Trays were heat-sealed with a low O_2 permeable ((<1cm^3/m^2/24 hr at STP) laminate film, Cryovac Satina (Cryovac Inc, Sealed air Corporation, New Jersey, USA) and stored at 4ºC. All samples were provided by a local fresh fruit and vegetable processing company.

[Table 5.1]

5.2.2 Optical sensor readings

All sample packs were pre-fitted with an optical sensor for the non-destructive and continuous monitoring of O_2 in MA packs. Optical O_2 sensors were prepared using Pt-OEPK (Platinum octaethylporphyrin-ketone) (Luxcel Biosciences, Ireland)

spotted on Durapore (Millipore Inc, Bedford, USA) paper using Gilson P100 pipette (Gilson, WI, USA) and allowed to dry and cut to a size of 5mm diameter. These were then attached to 3cm Avery price marking stickers (Avery Dennison, USA) for adhesion to the underside of the Cryovac films used. Sensors were calibrated prior to use by placing in a customized flow cell and subjected to O_2 levels ranging from 0-100% O_2 (BOC gases, Ireland). The sensors were utilised in conjunction with a phase detector (Luxcel Biosciences, Cork, Ireland) allowing for regular non-destructive monitoring of O_2 within packs. Samples were read daily.

5.2.3 Sensory Analysis

A 26-member sensory panel was recruited in University College Cork, Ireland. Panellists were chosen based on their availability and motivation to participate on all days of the experiments and that panellists were regular salad consumers. All panellists had previous experience in carrying out sensory analysis. Sensory references were provided to help panellists identify certain sensory descriptors, i.e. freshness in samples. Sensory analysis was carried out in panel booths conforming to international standards (ISO 8589: 2007). A list of descriptors for salad sensory analysis was selected and can be seen in Table 5.2. Samples were held at refrigeration temperatures (4ºC) up to the moment of sampling. Each panellist was presented with 12 samples in two servings. Samples were assigned a random three digit code to allow for a blind assessment. Three RTE salads were made available comprising of Caesar salad, Iceberg salad and an Italian mix salad, packaged in four different atmospheres (Control, MAP1, MAP2 and MAP3) and asked to assess quality, according to a 10-point scale.

[Table 5.2]

5.2.4 Microbial Testing

Total viable counts (TVC) for all RTE salad-based products were carried out on sampling days 1, 3, 7 and 10 to determine the microbial counts present in all samples. TVC's were carried out in accordance with ISO standards, method 4833:2003 utilising agar plates, incubation at 30ºC and counting the microbial colonies after 48 hr. Results are expressed as Log_{10} values.

5.2.5 Statistical analyses

ANOVA-Partial Least Squares Regression (ASLPR) was used to process the mean data accumulated from the 26-panellists during the sensory evaluation. Principal component (PC) 1 versus PC 2 is presented; no additional information was produced from other PC's. The validated explained variance for the model constructed was 11.03% and the calibrated variance was 14.07%. Regression coefficients were analysed by jack-knifing (Table 5.4) to derive significance indications for the relationships determined in the quantitative APLSR.

5.3 Results and discussion

5.3.1 Oxygen readings

The average O_2 levels found in RTE salad packs for all packaging treatments are shown in Table 5.3. Control MA packs consisting of 5% O_2 showed that by days three and seven of storage, O_2 levels had decreased to 1.4-1.7% and 0.1%, respectively; the latter level of O_2 remaining static until day 10 of refrigerated storage.

MAP1 salads packed in 21% O_2 showed an initial decline in O_2 after three days of storage to 14%. Oxygen levels continued to decrease over time and by day 10 of refrigerated storage MAP1 salad packs contained between 8.45-9.25% O_2. MAP2 salads packs containing 45% O_2 showed the most dramatic decrease in O_2 levels over time. By Day 3, MAP2 samples contained 35-37% O_2. Over the storage period to Day 10, MAP2 readings showed that product packs contained between 24.2-27.5% O_2. This represents an 18.3% decrease in O_2 level during storage.

[Table 5.3]

Salads were also packaged in high O_2 atmospheres (MAP3) to determine if any changes were notable in respiration levels and product quality. Packaging fresh produce at high MAP atmospheres as an alternative to low O_2 (<5%) has been investigated in fruit (Van der Steen, Jacxsens, Devlieghere and Debevere, 2001) and vegetables (Jacxsens *et al*, 2001). By days three, seven and ten of storage MAP3 samples showed a reduction of approximately 3%, 3% and 2% O_2, respectively, with a total reduction in packs of 8-9% O_2 by Day 10. Interestingly, MAP3 salad leaf samples behaved very differently to equivalent materials packed under MAP1 and MAP2 conditions in terms of O_2 consumption over time in that less O_2 was consumed by salads packed under MAP3 conditions. The data obtained has demonstrated the beneficial application of optical sensors in non-destructively determining the amount of O_2 being consumed by respiring salad leaves in MAP. The typical MA selected for packaging of RTE salad leaves commercially incorporates an O_2 level of less than 5% (Sandhya 2010, Jacxsens, 2001 and Ahvenainen 1996). Results indicate that a product

that continues to respire, even after processing, may requires a greater amount of O_2 than typical commercial MAP (<5% O_2) formats provide.

5.3.2 Sensory and visual analysis

Sensory analysis was carried out to see if differences in salads packaged using varying O_2 levels in MA packs had an effect on sensory quality throughout storage. The 26-panellists were asked to assess all three salad types packaged using four different MAP treatments described previously and rate descriptors on a ten-point scale. The lists of descriptors used are presented in Table 5.2. Sensory results generated in this study represent the level of acceptability perceived by panellists for sample types, difference in packaging treatment and storage time (Table 5.4). Panellists showed a significant liking ($P \leq 0.001$) for Iceberg-based salads, whereas a significant disliking ($P \leq 0.001$) for the Italian mixed salad was also expressed. No strong opinion in terms of liking or disliking for Caesar salad was expressed by panellists. No significant preference for any salad type was found to be influenced by MAP O_2 levels in terms of taste or appearance. This indicates that the panellists found no perceivable difference in taste caused by the packaging gases. Storage time affected the sensorial quality of salad products. Samples on Day 1, as expected, were found to be perceived in a 'fresh' state, where product quality was found to be positively correlated to Overall Appearance ($P \leq 0.001$), Flavour and Acceptability ($P \leq 0.001$), and negatively correlated to Wilting, Off-flavour and Browning ($P \leq 0.001$). Day 7, showed changing characteristics in the quality of the samples transitioning to a state of unacceptability where overall appearance and acceptability is found to be negatively correlated ($P<0.05$) and the onset of off flavour and aromas becoming apparent ($P<0.05$). By Day 10 of storage, data generated showed that RTE salad

products were no longer suitable for retail sale, as each product sample was positively correlated ($P \leq 0.001$) to browning, wilting, off-flavour, off-aroma and loss of texture. This in turn resulted in a significant negative correlation to Overall Acceptability and Appearance ($P \leq 0.001$). Fig 5.1 represents the APLSR plot for sensory results.

[Table 5.4]

[Fig 5.1]

In-pack visual analysis was also carried out by panellists on the visual (in-package) acceptability of all salads held over the storage time. The objective was to see if panellists could perceive differences in salads packaged under different atmospheres, and its affect on appearance and overall visual quality. Table 5.5 shows the results of visual assessment using Jack-Knifing uncertainty testing. Results suggest that panellists preferred Italian-style salads in terms of Overall Appearance ($P \leq 0.01$) (positively correlated). Conversely, panellists least preferred iceberg salads ($P<0.05$) in terms of Overall Appearance (negative correlation). This could be attributed to the richer, more vibrant colours of the Italian mixed leaves, compared to the singular colour associated with Iceberg lettuce. No significance in liking or disliking was found for Caesar salad. Storage assessment showed panellists expressing significant liking for Overall Appearance, Colour, Package quality and Purchase appeal ($P \leq 0.001$) on Day 1, as expected. Day 7 continues to be significantly acceptable in terms of overall appearance ($P \leq 0.01$) and colour ($P \leq 0.001$). A significant negative correlation to Appearance, Colour, Package Quality and Purchase Appeal ($P \leq 0.001$) was determined by Day 10 of storage. It is apparent that

panellists found the quality attributes of Browning and Bloated Appearance ($P \leq 0.001$) significantly correlated to Day 10. Of the four packaging treatments assessed within this study, panellists found salads packed in 5% O_2 to be significantly correlated with Package Quality ($P \leq 0.05$), Purchase Appeal ($P \leq 0.05$) and Overall Appearance ($P \leq 0.05$). These were the only significantly positive affects that were determined in this study using the Control, which represented the commercial packaging norm for RTE salad products.

[Table 5.5]

5.3.3 Microbial assessment of RTE MAP salad products

Data generated for microbial assessment of RTE MAP salad treatments over a 10-day storage period is presented (Table 5.6). After 1 day of storage, results showed relative uniformity in TVC counts for all treatments. Iceberg lettuce had the lowest initial microbial load of the three salads assessed and continued to have the lowest TVC counts on each testing day thereafter. On Day 1 of storage, all three salad products presented similar TVC results. Even after three, seven and 10 days of product storage, no clear 'best' or 'worst' treatment could be determined and consequently, no significant differences were determined.

[Table 5.6]

Interestingly, for all three salad types, both Control (<5% O_2) and MAP3 (>60% O_2) treatments had the lowest Log_{10} TVC results up to Day 10. The TVC obtained in this study were comparable to figures published by Szabo et al. (2000), where aerobic plate counts were found in fresh produce to range from 10^3 to 10^9 cfu g^{-1} (Approx. Log_{10} 3-9). Microbial limits were arrived at by day seven in this study for a number of treatments and concur with data presented by Harrigan (1998). From the data generated in this study, the use of high O_2 levels in MAP formats does not appear to accelerate the growth of aerobic microorganisms in a way that drastically reduces product quality. In fact, it appears that as O_2 level increased in packs, the smaller the differences that occurred in microbial counts when compared to the control. Rojas-Grau, et al. (2009) stated that the concept of packaging respiring products in a higher O_2 atmosphere may be beneficial in inhibiting the growth of naturally occurring spoilage organisms, prevent undesired anoxic respirative processes and maintain the fresh like quality of fresh cut produce. Jacxsens et al. (2001) reported that high O_2 packaging was particularly effective in inhibiting enzymatic discolouration, preventing anaerobic fermentation reactions and inhibiting microbial growth in MAP fruit and vegetable products. The purpose of choosing minimally processed foods is to deliver a fresh-like product to consumers ensuring food safety and sensory qualities while extending the shelf-life (Alzamora et al., 2000).

Minimally processed vegetables should preserve sensory, nutritional and microbiological shelf life for 4-7 days, and this should be even longer (to 21days) depending on the market requirements.Therefore, further research is required to investigate packaging process and product optimisation for RTE fresh produce and in systems that allow for continuous, rapid and non-destructive assessment of product quality and package integrity.

5.4 Conclusions

The ability to monitor O_2 in a non-destructive manner can provide valuable information regarding O_2 utilisation by fresh vegetative produce within MA packaging. Information gathered by optical means provides a clear understanding of the levels of O_2 being utilised within packs over time. Packaging salads in elevated O_2 atmospheres showed an O_2 decline in the range of 8-20% over a 10 day period, indicating the level of respiration taking place within packs. Of all three elevated oxygen treatments, MAP3 (60% O_2) samples behaved differently to samples packed in MAP1 and MAP2, where less O_2 was consumed over a 10 day shelf life study. It is apparent that the level of O_2 commonly used in commercial MA packaging of <5% is insufficient for respiring salad leaves that expect a shelf life greater than seven days. In terms of sensory analysis, no significant preference was perceived in any of the treatment atmospheres in terms of taste or acceptability. In general, it would appear that consumers did not find the packaging atmosphere as having any affect on product quality. In terms of microbial quality, MAPC and MAP3 have the lowest Log10 TVC values, where low (MAPC) and high (MAP3) O_2 atmospheres appear to have slowed the growth of microbes in comparison to MAP1 and MAP2 samples. The general findings of this study provide useful information about the level of O_2 utilised within MA packs, and the significance of this in terms of product quality and stability. This clearly demonstrates the potential that exists for commercial exploitation of this smart packaging technology within the fresh produce packaging industry.

5.5 Acknowledgement

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5.6 Tables and Figures

Table 5.1 Gas composition of MAP salad samples

Sample	O_2	CO_2	N_2
Control	5%	5%	90%
MAP1	21%	5%	74%
MAP2	45%	5%	50%
MAP3	60%	5%	35%

Table 5.2 List of descriptors for Sensory and Visual Analysis

Attribute - Sensory	Descriptor	Scale
Overall appearance liking	*The liking of appearance*	0 = extremely dislike, 10 = extremely like
Wilting Appearance	*Appearance of leaf wilting*	0 = none, 10 = extreme
Leaves Superficial Browning (LSB)	*Appearance of surface leaf browning*	0 = none, 10 = extreme
Leaves Edge Browning (LEB)	*Appearance of leaf edge browning*	0 = none, 10 = extreme
Texture/Non-Crispy	*Crispy to non-crispy leaf texture*	0 = none, 10 = extreme
Off Aroma	*Off Aromas*	0 = none, 10 = extreme
Off Flavour	*Off Flavours*	0 = none, 10 = extreme
Overall Flavour liking	*The Overall liking of Flavour*	0 = extremely dislike, 10 = extremely like
Overall Acceptability	*The Overall Acceptability of the product*	0 = extremely dislike, 10 = extremely like
Attribute - Visual	Descriptor	Scale
Overall appearance	*The liking of appearance*	0 = extremely dislike, 10 = extremely like
Colour	Light – dark Green	0 = light, 10 = dark
Browning	Degree of Browning	0 = none, 10 = extreme
Bloated Appearance	Degree of pack bloating	0 = none, 10 = extreme
Package Quality	Package and pack content quality	0 = none, 10 = extreme
Purchase Appeal	Rate the purchase appeal of packs	0 = extremely dislike, 10 = extremely like

Table 5.3 Mean percentage Optical O_2 sensor readings for Control, MAP1, MAP2 and MAP3 salad formats (+/- Standard deviation)

	Day Storage				
Sample	Day 0	Day 3	Day 7	Day 10	Differential
Italian Mix Control	4.7 ± 0.31	1.5 ± 0.32	0.1 ± 0.11	0.1 ± 0.10	4.60
Caesar Control	4.6 ± 0.23	1.4 ± 0.26	0.1 ± 0.09	0.1 ± 0.12	4.50
Iceberg Control	4.8 ± 0.19	1.7 ± 0.17	0.1 ± 0.07	0.1 ± 0.11	4.70
Italian Mix MAP1	20.2 ± 2.42	14.6 ± 1.51	11.1 ± 1.44	9.2 ± 1.26	11.02
Caesar MAP1	20.5 ± 1.30	13.9 ± 0.64	10.9 ± 0.62	8.4 ± 0.89	12.10
Iceberg MAP1	20.4 ± 1.41	14.4 ± 1.20	11.5 ± 1.82	8.9 ± 2.14	11.50
Italian MAP2	44.8 ± 3.04	37.3 ± 3.10	32.0 ± 4.90	27.2 ± 1.77	17.58
Caesar MAP2	44.9 ± 3.88	35.6 ± 3.12	29.5 ± 2.23	24.2 ± 1.36	20.68
Iceberg MAP2	44.3 ± 5.59	37.4 ± 4.54	30.6 ± 3.10	27.5 ± 3.51	16.67
Italian MAP3	57.8 ± 0.17	55.2 ± 0.48	50.8 ± 0.13	48.7 ± 0.32	9.14
Caesar MAP3	56.6 ± 0.53	53.9 ± 0.19	50.7 ± 0.39	47.7 ± 0.30	8.93
Iceberg MAP3	57.8 ± 0.33	55.8 ± 0.26	51.7 ± 0.36	49.7 ± 0.16	8.04

Table 5.4 Significance of estimated regression coefficients (ANOVA values) for the relationship of sensory terms as derived by Jack-Knife uncertainty testing for O_2 levels in MAP (P values)

	Salad Type			Oxygen in MAP				Day Storage		
	Italian Mix	Ceasar	Iceberg	MAPC	MAP1	MAP2	MAP3	Day 1	Day 7	Day 10
Overall Appearance	-0.001***	0.27	0.001***	0.37	0.61	0.81	0.12	0.001***	-0.04*	-0.001***
Wilting Appearance	0.001***	0.28	-0.001***	-0.30	-0.67	-0.77	-0.19	-0.001***	0.07	0.001***
Leaves Superficial Browning (LSB)	0.001***	0.27	-0.001***	-0.26	-0.61	-0.83	-0.13	-0.001***	0.05*	0.001***
Leaves Edges Browning (LEB)	0.001***	0.27	-0.001***	-0.31	-0.64	-0.82	-0.11	-0.001***	0.06	0.001***
Texture/Non-Crispy	0.003**	0.26	-0.01**	-0.37	-0.60	-0.87	-0.15	-0.001***	0.13	0.001***
Overall Flavour	-0.001***	0.27	0.001***	0.31	0.61	0.89	0.12	0.001***	-0.03*	-0.001***
Off Aroma	0.001***	0.26	-0.001***	-0.39	-0.53	0.65	-0.16	-0.001***	0.03*	0.001***
Off Flavour	0.001***	0.26	-0.001***	-0.35	-0.67	-0.82	-0.11	-0.001***	0.03*	0.001***
Overall Accepatability	-0.001***	0.27	0.001***	0.22	0.61	0.82	0.12	0.001***	-0.04*	-0.001***

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Table 5.5 Significance of estimated regression coefficients (ANOVA values) for the relationship of sensory terms as derived by Jack-Knife uncertainty testing for O_2 levels in MAP by In-pack Visual Analysis (P values)

	Salad Type			% Oxygen in MAP				Day Storage		
	Italian	Ceasar	Iceberg	MAPC	MAP1	MAP2	MAP3	Day 1	Day 7	Day 10
Overall appearance	0.003**	-0.56	-0.04*	0.03*	-0.95	-0.78	-0.11	0.001***	0.004**	-0.001***
Colour	0.001***	-0.78	-0.001***	0.70	-0.83	-0.52	-0.43	0.001***	0.001***	-0.001***
Browning	-0.73	0.29	0.40	-0.008**	0.91	0.29	0.13	-0.001***	-0.07	0.001***
Bloated Appearance	-0.25	0.46	0.72	-0.03*	0.97	0.56	0.08	-0.001***	-0.87	0.001***
Package Quality	0.03*	-0.49	-0.17	0.03*	-0.97	-0.70	-0.11	0.001***	0.20	-0.001***
Purchase Appeal	0.03*	0.46	-0.19	0.02*	-0.99	-0.62	-0.12	0.001***	0.18	-0.001***

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Table 5.6 Growth of total viable counts (log_{10} CFU/g ± standard deviation) on salads leaves over time under various packaging atmospheres and stored at 4 °C

	Log10 Values			
Sample	Day 1	Day 3	Day 7	Day10
Italian Mix MAPC	4.96 ± 0.09	5.02 ± 0.14	^6.00 ± 0.25	^6.32 ± 0.12
Italian Mix MAP1	4.87 ± 0.08	5.13 ± 0.06	^6.29 ± 0.32	^6.98 ± 0.21
Italian Mix MAP2	4.83 ± 0.03	5.04 ± 0.19	^6.03 ± 0.15	^6.42 ± 0.19
Italian Mix MAP3	4.90 ± 0.45	5.34 ± 0.19	5.75 ± 0.21	^6.37 ± 0.08
Caesar MAPC	4.69 ± 0.14	5.07 ± 0.21	5.70 ± 0.03	^6.01 ± 0.06
Caesar MAP1	5.35 ± 0.53	5.70 ± 0.22	5.89 ± 0.09	^6.35 ± 0.02
Caesar MAP2	4.97 ± 0.18	5.09 ± 0.04	^6.11 ± 0.14	^6.42 ± 0.25
Caesar MAP3	4.81 ± 0.20	5.17 ± 0.15	5.84 ± 0.17	^6.30 ± 0.15
Iceberg MAPC	3.55 ± 0.21	4.09 ± 0.25	5.00 ± 0.12	5.23 ± 0.09
Iceberg MAP1	3.56 ± 0.05	4.66 ± 0.14	5.70 ± 0.20	5.87 ± 0.15
Iceberg MAP2	3.76 ± 0.05	4.75 ± 0.09	5.63 ± 0.13	5.98 ± 0.30
Iceberg MAP3	3.63 ± 0.12	4.57 ± 0.23	5.12 ± 0.33	5.73 ± 0.10

^ = exceeds microbial limits of 6 log_{10} CFU/g

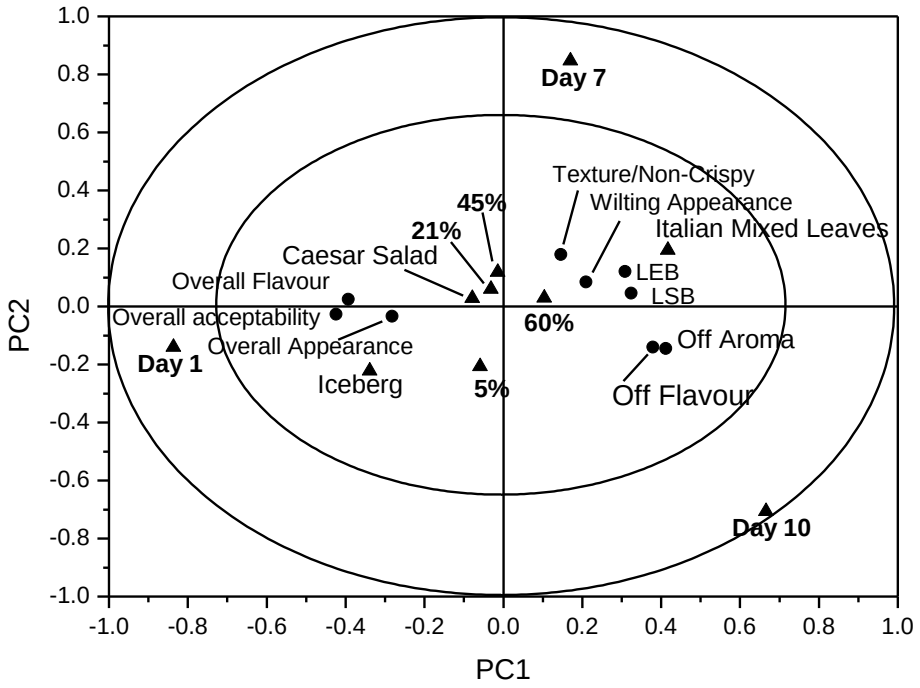

Fig 5.1 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 4 O_2 level groups.

CHAPTER VI

Assessment and Use of Optical Oxygen Sensors as Tools to Assist in Optimal Product Component Selection for the Development of Packs of Ready-to-Eat Mixed Salads and for the Non-Destructive Monitoring of in-Pack Oxygen Levels Using Chilled Storage

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Hempel, A.W., O'Sullivan, M.G., Papkovsky, D.B. and Kerry, J.P. 2013. Assessment and use of optical oxygen sensors as tools to assist in optimal product component selection for the development of packs of ready-to-eat mixed salads and for the non-destructive monitoring of in-pack oxygen levels using chilled storage

Abstract

Optical O_2 sensors were used to ascertain the level of O_2 consumed by individual salad leaves for optimised packaging of ready-to-eat (RTE) Italian salad mixes during refrigerated storage. Seven commonly found leaves in Italian salad mixes were individually assessed for O_2 utilisation in packs. Each leaf showed varying levels of respiration throughout storage. Using the information obtained, an experimental salad mix was formulated (termed Mix 3) which consisted of the four slowest respiring salad leaves—Escarole, Frisee, Red Batavia, Lollo Rosso. Mix 3 was then compared against two commercially available Italian salads; Mix 1 (Escarole, Frisee, Radicchio, Lollo Rosso) and Mix 2 (Cos, Frisee, Radicchio, Lollo Rosso). Optical sensors were used to non-destructively monitor O_2 usage in all mixes throughout storage. In addition to O_2 consumption, all three salad mixes were quality assessed in terms of microbial load and sensorial acceptability. In conclusion, Mix 3 was found to consume the least amount of O_2 over time, had the lowest microbial load and was most sensorially preferred ($p < 0.05$) in terms of overall appearance and acceptability. This study clearly shows the potential that O_2 sensors possess in terms of assisting in the optimised development of commercial RTE salad products.

Keywords: modified atmosphere packaging; ready-to-eat salads; packaging; storage; sensory; O_2 sensors

6.1 Introduction

The growth in the ready-to-use vegetable market (~10% p.a) has been largely due to increasing demand by consumers for fresh, healthy and convenient foods (Rico, Martin-Diana, Barat & Barry-Ryan, 2007). The most important motivation for purchasing minimally-processed vegetables relates to convenience and speed, especially for consumers who buy these products during their weekend shopping (Ragaert, Verbeke, Devlieghere & Debevere, 2003). Consumer demand for freshness and convenience has led to the evolution and increased production of numerous varieties of minimally-processed vegetables presented in a wide range of packaging formats. Vegetables are, in general, highly perishable products that require controlled handling conditions throughout the distribution chain, from producer to consumer, in order to maintain quality and safety and to increase product shelf life (Pocas, Delgado & Oliveira, 2008). Mixed prepared vegetables deteriorate rapidly and typically possess short shelf lives. Prepared salads comprised of several different components can present unique challenges through widely varying requirements and respiration rates (Sandhya, 2010). The shelf-life of ready-to-eat (RTE) vegetable products or salads established by manufacturers is usually 7–14 days depending on the type of fresh produce selected, and is determined by loss in organoleptic qualities (Garcia-Ginemo and Zurera-Cosano, 1997). As a result of peeling, grating and shredding, produce will change from a relatively stable product with a shelf-life of several weeks or months to a perishable entity that possesses a very short shelf life, even as short as 1–3 days at chill temperatures. It is possible to achieve a shelf-life of 7–8 days at refrigeration temperatures (5 °C), but for some markets this is not enough and a shelf-

life of 2–3 weeks is sometimes necessary (Ahvenainen, 1997). Consequently, modified atmosphere packaging (MAP) technology is largely used for the extended storage of minimally processed fruit and vegetables, including fresh RTE salad products. Oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) are the gases typically implemented in MAP of fresh produce, with O_2 in packs generally being employed between 1% and 5% in order to support product respiration but used at low enough concentration to discourage the proliferation of microbial spoilage in the form of bacteria and fungi. More recently, the use of high O_2 atmospheres (*i.e.*, >70% O_2) have been used as an alternative technique to low O_2 equilibrium modified atmosphere (3% O_2) (Jacxsens, Devlieghere, van der Steen & Debevere, 2001). The concentration of O_2, as well as CO_2, within the pack relates to the metabolic state of the produce (Rico, Martin-Diana, Barat & Barry-Ryan, 2007) and indicative of product quality and potential shelf-life.

Optical O_2 sensors have been shown to provide valuable information in food packaging applications (Papkovsky, 1995; Papkovsky, 2004; O'Riordan, Voraberger, Kerry & Papkovsky, 2005). The use of a non-destructive method of O_2 detection to monitor O_2 levels in different forms of food packaging provides ample information as to the package environment conditions of a food product throughout storage and shelf life. Problems associated with packaging and containment failures can be instantly observed post packaging using this technology (Hempel, O'Sullivan, Papkovsky & Kerry, 2013). Research has been carried out using O_2 sensors in specific food applications, such as; MAP cheese (O'Mahoney, O'Riordan, Papkovskaia, Kerry & Papkovsky, 2006), vacuum packed cheese (Hempel, Gillanders, Papkovsky & Kerry, 2012), MAP and vacuum packed beef (Smiddy, Fitzgerald, Kerry, Papkovsky, O'Sullivan & Guilbault, 2002), cooked meats (Smiddy, Papkovsky & Kerry, 2002),

MAP and vacuum packed chicken (Smiddy, Papkovskaia, Papkovsky & Kerry, 2002), as well as *sous vide* products (O'Mahoney, O'Riordan, Papkovskaia, Ogurtsov, Kerry & Papkovsky, 2004).

To date, the use of O_2 sensors have not been employed to monitor O_2 levels in retail packs of fresh produce and have never been evaluated as a technology to assist in the optimised development of commercial RTE salad products. Therefore, the objective of this study was to employ the use of non-destructive O_2 sensing technology to ascertain the level of O_2 consumed by individual salad leaves so that the optimised packaging of RTE Italian salad mixes might be determined. An increase in the O_2 gas fill of such MAP salads is also used as an alternative to traditional 3%–5% O_2.

6.2 Experimental Section

6.2.1 Sample Preparation

Salad leaves used in this study were grown in Ireland (and in close proximity to the processing facility where product packaging was undertaken) and consisted of the following salad leaf varieties: Escarole, Frisee, Radicchio, Lollo Rosso, Cos, Iceberg and Red Batavia. Salad leaves undergo treatments such as washing and shredding by manufacturer prior to packaging. Approximately 170 g of each salad leaf was packaged individually for assessment, using an Ishida SE multi head weigher (Ishida Europe, Birmingham, UK) and stored at 4 °C. A Sandiacre Novus 350 (HayssenSandiacre, USA) was used to form, fill and seal samples in orientated polypropylene/low density polyethylene laminate films (220 × 290 mm) of 45 micron thickness with modified atmospheres. Control RTE salad mixes were produced

commercially with normal packaging conditions (5% O_2, 5%–10% CO_2 and 85%–90% N_2) and monitored for O_2 levels over time. In order to appropriate the correct level of O_2 to a mixed salad product, it was necessary to understand the individual O_2 requirement for each salad leaf within the product mix. Individual salad leaves were packaged using a higher O_2 level (21% O_2, 5%–10% CO_2 and 69%–74% N_2) to assess the true O_2 levels utilised by each leaf and subsequent salad mixes during the shelf life period. Mixes of salad leaves were also prepared as described; totalling 170 g, with approximately 25% of each leaf and contents are described in Table 6.1. Preliminary trials were carried out packaging typical commercial gas (5% O_2, 5%–10% CO_2 and 85%–90% N_2) without salad fill and monitored for O_2 over time. This was undertaken to assess the level of permeation of gases using these commercial packaging materials over a 14 day period. The level of permeation was negligible as sample free packs were seen to hold initial gas fill within <0.5% O2, showing low permeability.

[Table 6.1]

6.2.2. Optical Sensor Preparation and Readings

Oxygen sensors were prepared using Pt-OEPK (Luxcel Biosciences, Cork, Ireland) spotted on Durapore (Millipore Inc., Bedford, MA, USA) paper using Gilson P100 pipette (Gilson, WI, USA) and cut to a size of 5mm in diameter. These sensor materials were then placed on 3 cm Avery price marking stickers (Avery Dennison, Pasadena, CA, USA) for adhesion to packaging films. A Mocon OpTech-O_2 Platinum reader (Mocon Inc., Minneapolis, MN, USA) was used in conjunction with O_2

sensors allowing for regular non-destructive monitoring of O_2 within packs. The Mocon OpTech-O_2 device allows for reliable and accurate O_2 readings from 0.001% to 25% O_2 in 0.5 s. The device causes excitation of the phosphorescent dye embedded in the optical O_2 sensor and is quenched by molecular O_2. Calibration of instrumentation was carried out using a Platinum CalCard allowing for instant calibration before each set of measurements were taken. O_2 readings were taken at Day 0, 3, 5, 7 and 10 for commercially prepared MAP RTE salads, individual salad leaves and modified salad mixes. The final measurement was taken on Day 10 of storage as no further O_2 drop was observed and samples had exceeded microbial limits.

6.2.3. Microbial Testing

Microbial testing was carried out during refrigerated shelf-life assessment of individual salad leaves and RTE Italian salad Mixes 1, 2 and 3 on sampling days 1, 3, 5, 7 and 10. Total viable counts (TVC) were carried out to determine microbial numbers (colony forming units (cfu)/g samples) in accordance with ISO standard method (ISO 4833:2003) using total plate count agar and an incubation temperature of 30 °C. The counting of microbial colonies was carried out after 48 h incubation and results were expressed as log values. Results reported up to day seven before exceeding acceptable limits.

6.2.4. Sensory Analysis

A sensory panel made up of 26 members was recruited in University College Cork, Ireland. Panellists were selected based on their availability on test days and were regular consumers of RTE salad products. All panellists had previous experience in carrying out sensory analysis. A list of descriptors for sensory analysis was selected and these are presented in Table 6.2. Each panellist was presented with six samples at room temperature (three salad mixes and tested in duplicate) and asked to assess the attributes, according to a 10-point scale. Sensory analysis was carried out during shelf-life evaluation of RTE Italian-style salad products on refrigerated storage days 1, 3 and 7. Sensory analysis was carried out in panel booths conforming to international standards (ISO 8589:2007).

[Table 6.2]

6.2.5 Statistical analyses

ANOVA-Partial Least Squares Regression (APLSR) was used to process the mean data accumulated from the 26 panellists during the sensory evaluation. Principal component (PC) 1 *versus* PC 2 is presented; other PC's did not yield additional information. To derive significance indications for the relationships determined in the quantitative APLSR; regression coefficients were analysed by jack-knifing (Table 6.3).

[Table 6.3]

6.3. Results and Discussion

6.3.1. Oxygen Readings

In commercial Irish applications, Italian mixed RTE salad leaf products are packaged in low O_2 modified atmosphere conditions consisting of <5% O_2. This practice concurs with similar reported practices (Rojas-Grau, Oms-Oliu, Soliva-Fortuny & Martin-Belloso, 2009). In these studies sensors were placed in commercial packs of RTE Italian mixed salads and O_2 levels were monitored over time. From Figure 6.1 it is clear that the level of O_2 provided in such packs may be insufficient for optimised product respiration, as by day 3 of storage, less than 0.5% O_2 remained within packs. This move towards an anoxic environment leads to quality-linked deteriorative processes within the pack (Soliva-Fortuny & Martin-Belloso, 2003; Harrigan, 1998).

[Figure 6.1]

It was necessary to understand the individual O_2 requirement for each salad leaf within the product mix. Consequently, individual salad leaves typically used within Italian-styled RTE mixed salad leaf products (Escarole, Frisee, Radicchio, Lollo Rosso, Cos, Iceberg and Red Batavia) were packaged individually within MAP formats (21% O_2, 5%–10% CO_2 and 69%–74% N_2) to ensure adequate O_2 was available for respiration) containing a pre-fitted optical O_2 sensor. The individual

salad leaf packs were monitored over seven days for O_2 levels to ascertain O_2 consumption by each salad leaf type. Table 6.4 shows the amount of O_2 consumed by each salad leaf type during storage (up to Day 7, exceeding microbial limits). The O_2 differential between leaves showed that after seven days, samples ranged in O_2 utilization by up 3.2% O_2. For example, the Radicchio salad leaf was found to consume the greatest amount of O_2 over the seven days of storage (15.79%), whereas the Lollo Rosso leaf was determined to consume the least amount of O_2 (12.47%) over the same period of time. The consumption of O_2 in packs appears to slow after Day 7, at which the product is spoilt.

[Table 6.4]

The data generated in this study showed that the amount of O_2 required by individual salad leaves to respire adequately over a seven day storage period was far greater than the typical <5% O_2 provided in typical commercial MA packaging applications. This insufficient level of O_2 utilised in MAP could lead to anaerobic respiration with the production of undesirable metabolites and associated physiological disorders (Soliva-Fortuny & Martin-Belloso, 2003).

It is apparent that each leaf respires at different rates under the same conditions. The level of O_2 utilisation can be categorised in a table representing each salad leaf in the order of increasing respiratory levels. Table 6.5 outlines O_2 consumption by individual salad leaves in order of increasing O_2 utilisation on storage days 3, 5 and 7. Escarole, Lollo Rosso and Frisee were consistently the slowest respiring salad leaves while Cos, Radicchio and Iceberg were consistently found to utilise the greatest amount of in-pack O_2. Individually packed leaves underwent microbial (TVC) testing.

[Table 6.5]

6.3.2. Microbial Testing

Each individually packed salad leaf was measured by total viable count (TVC) assay. Testing was carried out on Day 1, 3, 5, 7 and 10 to establish the microbial load in colony forming units per gram of sample. Table 6.6 represents the log cfu/g of each salad leaf on each testing day. With the exception of Cos and Iceberg, all other total viable counts appear to be quite similar between log 5 and log 6 by day five. With the exception of Iceberg, all salads were deemed unfit for consumption by Day 7 as all counts had exceeded log 6. Cos had the lowest counts on Day 1 of storage, but by Day 7 were found to be quite similar to all other leaves. Iceberg was the only leaf that had lower microbial numbers than the rest, with its final count being log 6.1 at Day 10, exceeding acceptable limits. Microbial counts determined in this study over time concur with that reported in the scientific literature (Harrigan, 1998; Szabo, Scurrah & Burrows, 2002).

[Table 6.6]

In the case of Radicchio, a correlation between highest level of O_2 utilisation of all leafs and microbial counts at Day 7 are observed. The inverse of this can be seen with Lollo Rosso, where it was found to consume the lowest level of O_2 over 10 days and had the second lowest log TVC value. Results can also be ranked in terms of which salad leaf has the lowest microbial load in order to understand the difference in

microbial quality across all samples. This can be seen in Table 6.7, where salad leaves are listed in order of lowest microbial counts (1) (log cfu/g sample) to highest (10). From this information it can be seen that at Day 1, Cos and Frisee are the lowest and highest microbial load, respectively. By day 7 Iceberg and Radicchio has the lowest and highest microbial load, respectively.

[Table 6.7]

6.3.3. Shelf-Life Comparison of an Experimentally Formulated MAP Italian Salad Leaf Mix with Two Commercial Forms

Based on the results obtained by monitoring the level of O_2 utilised by each salad leaf (from seven leaf types typically used to construct Italian style mixed leaf RTE salads), an experimental salad mix was formulated using the four lowest respiring leaves determined by the O_2 sensor previously. This Italian-styled formulation (Mix 3) and two similar commercially available formulations (Mixes 1 and 2) were modified atmosphere packaged using the same packaging conditions, in the same manufacturing plant, using salad materials grown and processed in the same manner. All three salad mixes were produced in volume and a shelf-life evaluation study undertaken. Oxygen measurements were taken throughout product storage and optical O_2 sensor readings showed that Italian salad Mix 3 had the lowest O_2 consumption of all three salad mixes (Table 6.8). After seven days of storage, Salad Mix 3 had a residual O_2 level of 9.25% remaining in packs compared to 8.12% in Mix 1 and 7.61% in Mix 2. As outlined previously, salad Mix 3 was formulated using the four slowest respiring leaves and so it makes logical sense that packs containing this

product mix had the highest remaining O_2 found in packs over the four days of sampling. The concentration of O_2 inside the headspace is related to the metabolic state of the samples (Rico, et al., 2007). Commercial salad Mixes 1 and 2 both use the Radicchio leaf which was found to be the highest O_2 consumer (15.79%) of all seven leaf types assessed previously and commonly found in Italian-style mix leaf salads. Salad Mix 2 was also comprised of the Cos leaf, which was also found to be amongst the highest O_2 consumers (13.33%) of all leaves assessed. Consequently, the use of Radicchio and other high respiring leaves in mixes ultimately results in a final product that consumes greater amounts of O_2. Therefore, it was essential to provide a modified atmosphere with enough O_2 to allow sufficient O_2 for respiration irrespective of what leaves selected. It is important to recognize that extremely low O_2 levels or excessively high CO_2 levels can result in the generation of off-flavours or visible tissue damage (Beaudry, 1998).

[Table 6.8]

All three salad leaf mixes were assessed for microbial quality during refrigerated storage (Table 6.8). Microbial counts for salad Mix 1 and Mix 2 were found to yield similar results on all days of analysis throughout storage and have exceeded acceptable limits by Day 7. Results showed that salad Mix 3 consistently had lower microbial counts on each sampling day throughout storage when compared to commercial salad Mixes 1 and 2. It was clear that the initial microbial counts at Day 0 were much lower in Mix 3, where the selection of slower respiring leaves had resulted in a product requiring less O_2 over time. By day 7, Mix 3 was found to still maintain acceptable limits of <log 6 (Harrigan, 1998; Szabo, Scurrah & Burrows,

2002), where log 5.79 was recorded. The use of salad leaf mixes which are found to use less O_2 over time appears to result in a product that has a lower microbial count than that of one which consumes more.

6.3.4. Sensory and Statistical Analysis

Salad Mixes 1, 2 and 3 were assessed for sensory acceptability over the seven day storage period (days 1, 3 and 7). Panellists were made aware of relevant descriptors (Table 6.2) and rating scale. The shelf-life of all salad mixes was perceived in a predictable manner, where Day 1 showed all salads to be in a "Fresh" state, where overall appearance ($p \leq 0.01$), flavour ($p \leq 0.05$) and acceptability ($p \leq 0.01$) were significantly and positively correlated. Day 7 data contrasted significantly with salad samples found to be negatively correlated with overall appearance, flavour and acceptability, along with a positive correlation for wilting appearance ($p \leq 0.01$) and non-crispy texture ($p \leq 0.01$). As for the individual salads, it was apparent that salad Mix 3 was favoured amongst panellists over salad Mixes 1 and 2, where overall appearance and acceptability were significantly correlated ($p \leq 0.01$) and descriptors such as wilting appearance and non-crispy texture were found to be negatively correlated ($p \leq 0.05$) showing a true preference for salad Mix 3. This selection appears to be attained by the use of Red Batavia instead of Radicchio. Panellists were asked if any individual leaf was found to be particularly undesirable and in this case 16 of the 26 panellists (61.5%) found the Radicchio leaf to be undesirable within the salad mix. The Radicchio leaf is understood to be a spicier and a more bitter intense leaf (Tordoff & Sandell, 2009) which most panellists found undesirable when compared to the Red Batavia alternative. Both salad Mixes 1 and 2 had no

significantly positive or negative attributes associated when compared with each other. The use of higher levels of O_2 (21%) in packaging did not appear to have an adverse affect on browning of the salad leaves. Data derived by Jack-Knifing uncertainty testing for individual salad leaves find no significance in leaf superficial browning (LSB) or leaf edge browning (LEB). Figure 6.2 represents an overview plot of the mean data from the ANOVA correlation values for all three mixes.

[Figure 6.2]

With the understanding that Mix 3 was found to be the most desirable in terms of sensory analysis, the formulation of a mix with a lower microbial load and slower respiration rate compared well to common commercially produced salad mixes. The use of an optical O_2 sensor in determining the O_2 respiration levels shows a novel and effective tool in understanding the O_2 levels used in respiring salads leaves packaged in modified atmosphere conditions. This valuable information let the development of a product with a longer, more stable shelf life and a product more acceptable to consumers (fig 6.3).

6.4 Conclusion

The ability to non-destructively assess the level of O_2 utilized by individual salad leaves was successfully achieved using optical O_2 sensors. Information gathered using O_2 sensors showed that an O_2 level in excess of 10% is required to provide salad leaves with enough O_2 to comfortably respire within a typical shelf-life period of seven days. The apparent lack of O_2 within such packs has been previously shown to cause many deleterious quality processes. The use of optical O_2 sensors showed that a salad mix can be optimised by understanding specific product requirements, like that for O_2, and subsequently, careful product selection; thereby assisting in extending product shelf-life and promoting quality.

6.5 Acknowledgements

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6.6 Tables and Figures

Table 6.1 Leaf content in salad mixes

Sample	Leaf content
Mix 1*	Escarole, Frisee, Radicchio, Lollo Rosso
Mix 2*	Cos, Frisee, Radicchio, Lollo Rosso
Mix 3*	Escarole, Frisee, Red Batavia, Lollo Rosso

Table 6.2 List of descriptors for Sensory Analysis

Attribute - Sensory	Descriptor	Scale
Overall appearance liking	*The liking of appearance*	0 = extremely dislike, 10 = extremely like
Wilting Appearance	*Appearance of leaf wilting*	0 = none, 10 = extreme
Leaves Superficial Browning (LSB)	*Appearance of surface leaf browning*	0 = none, 10 = extreme
Leaves Edge Browning (LEB)	*Appearance of leaf edge browning*	0 = none, 10 = extreme
Texture/Non-Crispy	*Crispy to non-crispy leaf texture*	0 = none, 10 = extreme
Off Aroma	*Off Aromas*	0 = none, 10 = extreme
Off Flavour	*Off Flavours*	0 = none, 10 = extreme
Overall Flavour liking	*The Overall liking of Flavour*	0 = extremely dislike, 10 = extremely like
Overall Acceptability	*The Overall Acceptability of the product*	0 = extremely dislike, 10 = extremely like

Table 6.3 Significance of estimated coefficients (ANOVA values) for sensory terms as derived by Jack-knife uncertainty testing for Italian-style salad leaf Mixes 1, 2 and 3

	Salad Type			Day Storage		
	Mix 1	Mix 2	Mix 3	Day 1	Day 3	Day 7
Overall Appearance	-0.38	-0.36	0.005**	0.007**	0.47	-0.0003***
Wilting Appearance	0.41	0.34	-0.009**	-0.018*	-0.46	0.0014**
Leaves Superficial Browning (LSB)	0.53	0.25	-0.21	-0.19	-0.51	0.16
Leaves Edges Browning (LEB)	0.51	0.21	-0.12	-0.14	-0.48	0.093
Texture/Non-Crispy	0.34	0.41	-0.012*	-0.016*	-0.50	0.01**
Overall Flavour	-0.38	-0.47	0.13	0.018*	0.52	-0.03*
Off Aroma	-0.49	-0.64	-0.47	0.51	0.62	-0.51
Off Flavour	-0.53	-0.57	-0.44	0.42	0.68	-0.47
Overall Accepatability	-0.33	-0.44	0.033*	0.0046**	0.51	-0.0041**

ns = not significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Table 6.4 Mean consumption (+/- standard deviation) of O_2 by seven individual salad leaf types typically used in Italian-style RTE mixed salad leaf products

	Storage (Day)					Differential (%)
Salad Leaf	Day 0	Day 3	Day 5	Day 7	Day 10	Day 0-7
Escarole	20.82 ± 0.12	13.31 ± 0.32	9.42 ± 0.33	8.20 ± 0.28	8.08 ± 0.17	12.62
Cos	20.63 ± 0.14	10.52 ± 0.51	8.21 ± 1.21	7.34 ± 1.21	7.17 ± 0.92	13.29
Lollo Rosso	20.91 ± 0.11	12.01 ± 0.47	9.80 ± 0.87	8.54 ± 0.82	8.36 ± 0.76	12.37
Frisee	20.52 ± 0.10	12.10 ± 1.58	9.39 ± 2.77	8.01 ± 3.13	7.93 ± 2.14	12.51
Radicchio	20.44 ± 0.08	10.04 ± 0.73	5.78 ± 0.91	4.57 ± 0.90	4.57 ± 0.63	15.87
Iceberg	20.70 ± 0.12	9.09 ± 0.74	6.11 ± 1.12	5.26 ± 1.01	4.88 ± 0.45	15.44
Red Batavia	20.72 ± 0.09	12.31 ± 1.97	8.90 ± 0.84	7.50 ± 0.88	7.29 ± 0.37	13.22

Table 6.5 Ranking of individual salad leaf types in terms of O_2 consumption within refrigerated MA packs; lowest O_2 consumption (1) to highest O_2 consumption (7)

Ranking	Day 3	Day 5	Day 7	Day 10
1	Escarole	Lollo Rosso	Lollo Rosso	Lollo Rosso
2	Red Batavia	Escarole	Escarole	Escarole
3	Frisee	Frisee	Frisee	Frisee
4	Lollo Rosso	Red Batavia	Red Batavia	Red Batavia
5	Cos	Cos	Cos	Cos
6	Radicchio	Iceberg	Iceberg	Iceberg
7	Iceberg	Radicchio	Radicchio	Radicchio

Table 6.6 Table represents the mean log cfu/g of each salad leaf (± standard deviation), ^Exceeds limits of 10^6 or 6 Log_{10} cfu/g; n/d - not determined.

	Storage (Day)				
Salad Leaf	Day 1	Day 3	Day 5	Day 7	Day 10
Cos 4ºC	2.2 ± 0.27	3.1 ± 0.47	4.3 ± 0.16	^6.3 ± 0.09	n/d
Escarole 4ºC	4.5 ± 0.14	4.9 ± 0.09	5.3 ± 0.28	^6.5 ± 0.04	n/d
Frisee 4ºC	4.8 ± 0.09	5.1 ± 0.17	5.5 ± 0.19	^6.5 ± 0.08	n/d
Lollo Rosso 4ºC	4.3 ± 0.22	4.8 ± 0.31	5.4 ± 0.14	^6.0 ± 0.13	n/d
Radicchio 4ºC	4.6 ± 0.34	5.0 ± 0.07	5.8 ± 0.21	^6.5 ± 0.12	n/d
Red Batavia 4ºC	4.5 ± 0.29	5.1 ± 0.19	5.6 ± 0.17	^6.5 ± 0.09	n/d
Iceberg 4ºC	3.0 ± 0.08	3.8 ± 0.70	4.3 ± 0.05	5.6 ± 0.04	^6.1 ± 0.07

Table 6.7 Salad leaves ranked in terms of lowest $\log_{10}$ cfu/g (1 to 7) on days 1, 3, 5 and 7

Rank	Day 1	Day 3	Day 5	Day 7
1	Cos	Cos	Cos	Iceberg
2	Iceberg	Iceberg	Iceberg	Lollo Rosso
3	Lollo Rosso	Escarole	Escarole	Cos
4	Red Batavia	Lollo Rosso	Lollo Rosso	Red Batavia
5	Escarole	Frisee	Frisee	Frisee
6	Radicchio	Red Batavia	Red Batavia	Escarole
7	Frisee	Radicchio	Radicchio	Radicchio

Table 6.8 Represents the mean % O_2 by optical sensor (*) and Log cfu/g incl. standard deviation (^) found in each mix on Day 0, 3, 5 and 7; n/d - not determined.

		Day Storage				
Sample	Leaves	Day 0	Day 3	Day 5	Day 7	Day 10
Mix 1*	Escarole, Frisee, Radicchio, Lollo Rosso	20.33 ± 0.11	13.45 ± 1.54	13.45 ± 1.54	8.13 ± 1.45	7.93 ± 1.22
Mix 2*	Cos, Frisee, Radicchio, Lollo Rosso	20.18 ± 0.10	12.98 ± 1.19	12.98 ± 1.19	7.61 ± 1.80	7.11 ± 1.46
Mix 3*	Escarole, Frisee, Red Batavia, Lollo Rosso	20.64 ± 0.05	14.69 ± 0.59	14.69 ± 0.59	9.25 ± 0.59	8.15 ± 0.37
Mix 1^	Escarole, Frisee, Radicchio, Lollo Rosso	3.24 ± 0.34	4.84 ± 0.24	5.52 ± 0.30	6.81 ± 0.28	n/d
Mix 2^	Cos, Frisee, Radicchio, Lollo Rosso	3.12 ± 0.15	4.00 ± 0.21	5.31 ± 0.23	6.21 ± 0.31	n/d
Mix 3^	Escarole, Frisee, Red Batavia, Lollo Rosso	2.67 ± 0.17	3.86 ± 0.12	4.97 ± 0.09	5.79 ± 0.20	6.21 ± 0.27

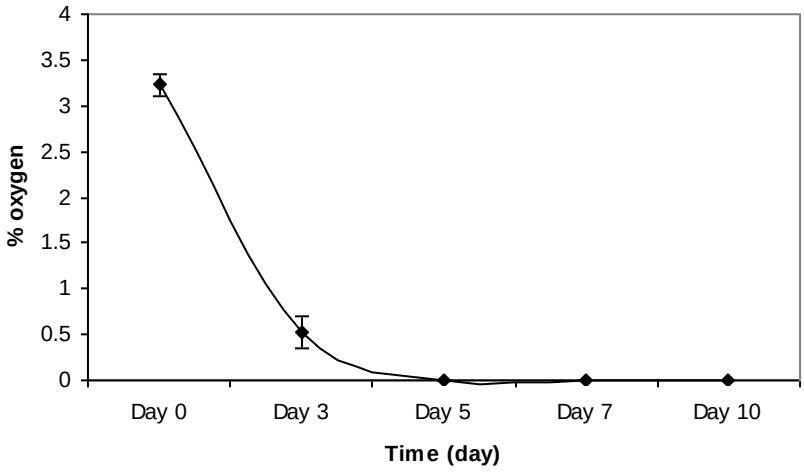

Fig 6.1 Represents the mean (n=12) O_2 levels in packs of commercially available Italian mix leaves salad (± standard deviation)

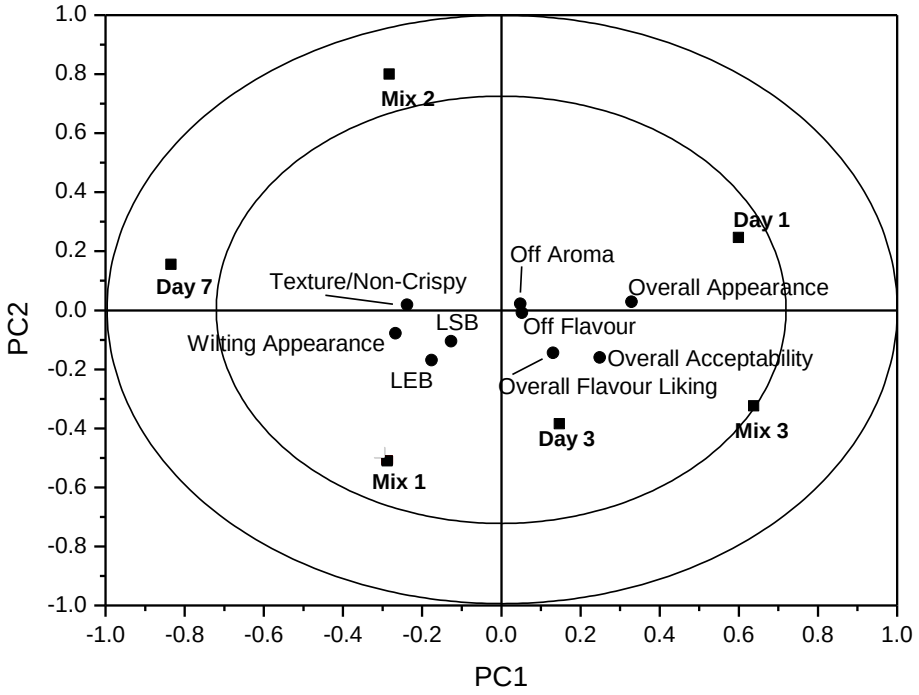

Fig 6.2 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the three salad mixes

Fig 6.3 Sample pack of mixed salad leaf salad

CHAPTER VII

Use of smart packaging technologies for monitoring and extending the shelf-life quality of modified atmosphere packaged (MAP) bread: application of intelligent oxygen sensors and active ethanol emitters

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Abstract

Following commercial assessment of the packaging materials and systems used for MAP speciality breads, a study was carried out to assess bread quality and shelf-life following the implementation of an improved MAP (10% CO_2, 90% N_2) process, and compared to controls (held in air). O_2 sensors permitted the continuous and non-destructive monitoring of in-pack O_2 levels over time. Ethanol emitters (EE) or product surface sprays (ES) were used for MAP and control treatments to establish their effects on product shelf-life. MAP samples had a continuous O_2 level of <0.1% throughout storage. Samples packed in air showed contrasting O_2 profiles, where control samples showed that O_2 levels were depleted by day 6, whereas controls utilising EE and ES demonstrated a reduced O_2 decline over time. ES in control packs slowed O_2 decline over time, with complete O_2 depletion occurring by day 14, whereas the use of EE in control packs showed an even slower decline with complete O_2 depletion occurring by day 35 of storage. The use of ES, but particularly EE, reduced mycological counts in bread samples, reflecting the reduced O_2 utilisation in control packs. This was mirrored, but not to the same extent, for bread held under MAP conditions. Sensory evaluation of breads demonstrated that the use of ethanol in packs produced no negative organoleptic issues. In conclusion, O_2 sensors clearly demonstrate their ability to assess pack containment and O_2 utilisation within packs over time by yeasts/moulds while the use of EE controlled mycological growth, thereby extending product shelf-life.

Keywords**:** Oxygen Sensing, MAP, Shelf life, Packaging, Sensory

7.1 Introduction

In 2008, the bakery products market in Western Europe was estimated to be worth €98.4 billion, accounting for 8.9% of the entire food and drinks market (FFT, 2009). In the UK alone, the bread market is worth £3.4 billion and is one of the largest markets within the British retail sector (The Federation of Bakers, 2012). Therefore, with such lucrative product markets, any packaging technology that can impact positively on product quality will be seriously considered. Fresh baked bread is naturally a highly perishable product, with a typical shelf-life of less than seven days when stored under optimum conditions. This degree of perishability in bread-based products is hard to delay without causing negative sensory attributes. Bread is typically spoiled by many yeasts and moulds, of which the *Penicillium* species are by far the most common spoilage moulds (Legan, 1993). Critical factors influencing the successful storage and shelf-life of bread include pH and water activity of the product, the presence of anti-microbial additives and preservatives.

Typically and traditionally, fresh bread products are packaged using waxed paper or polyolefin-based plastic packaging. In more recent times, higher value, speciality-type breads have been packaged using laminate constructions and packaged under modified atmospheres (MA). Modified atmosphere packaging (MAP) is a non-invasive and additive free way to extend the quality and shelf-life of fresh food products. MAP is the enclosure of food products in packages comprised of gas barrier materials that rely on mixtures of gases in concentrations different to those of air, to retard deterioration processes in foods extending product shelf life (Yam, 2009). MAP offers many advantages to consumers and food products. To the consumer, it offers convenient, high-quality food products with an extended shelf life. It also reduces and

sometimes eliminates the need for chemical preservatives, leading to more 'natural' and 'healthy' products, while producers enjoy the benefits of increased shelf life (Floros & Matos, 2005). A combination of various gases can be utilised in the MAP of breads, thereby creating an atmosphere capable of extending shelf life. Carbon dioxide has the ability to selectively inhibit the growth of micro-organisms, specifically yeasts and moulds and so is the most popular gas used for bakery goods (Cauvain & Young, 2000). Nitrogen acts as an inert filler gas to displace O_2 during gas flushing (if drawing a vacuum to remove air from packs is not practiced), and to prevent pack collapse. Commercially produced speciality breads are frequently packaged under MAP in order to reduce aerobic conditions, thereby preventing mould growth. However, it is difficult to reduce O_2 content to a very low level in bakery product packs due to the porous interior of many of these products which tend to trap O_2 in such a way that it does not readily interchange with the gas which is flushed and flowing through the package (Matz, 1989) at the point of air evacuation or displacement. The capacity to monitor O_2 levels within packs in a continuous and non-destructive manner would assist in determining the effectiveness of MAP systems for bakery product application.

Optical O_2 sensors have proven to be a novel and effective way of non-destructively detecting and monitoring O_2 (Papkovsky, 1995 & 2004). The use of a reversible optical sensor incorporating a phosphorescent dye that is quenched by molecular O_2 has been well documented in food packaging applications. These include; MAP cheese (O'Mahoney, O'Riordan, Papkovskaia, Kerry & Papkovsky, 2006), vacuum packed cheese (Hempel, Gillanders, Papkovsky & Kerry, 2012), MAP and vacuum packed beef (Smiddy, Fitzgerald, Kerry, Papkovsky, O'Sullivan & Guilbault, 2002a), cooked meats (Smiddy, Papkovsky & Kerry, 2002b), MAP and

vacuum packed chicken (Smiddy, Papkovskaia, Papkovsky & Kerry, 2002c), as well as *sous vide* products (O'Mahoney, O' Riordan, Papkovskaia, Ogurtsov, Kerry & Papkovsky, 2004) and bottled beer (Hempel, O'Sullivan, Papkovsky & Kerry, 2013). Common food packaging systems which incorporate O_2 limiting techniques (i.e. Vacuum packaging or MAP) can be assessed for efficiency immediately post-packaging, and subsequently during storage, using optical O_2 sensors. While the use of MAP can undoubtedly extend the shelf-life of bakery goods, shelf-life can be further extended through the use of smart and active packaging systems, for example the use of ethanol emitters to lower or control the growth of mycological entities.

It has been suggested that the addition of ethanol to modify the atmosphere in MAP bakery foods could increase shelf life (Matz, 1989). Ethanol has been demonstrated to extend the shelf life of packaged bread and other baked products where ethanol vapours have been shown to be effective in controlling *Asperigillus* and *Penicillium* species and various spoilage yeasts (Brody, 2001). 'Smart' packaging comprise of active and intelligent forms of packaging. Smart packaging devices are defined as small inexpensive labels or tags that are attached onto packaging to facilitate communication throughout the supply chain so that appropriate actions may be taken to achieve desired benefits in food quality and safety enhancements (Lee, Yam & Piergiovanni, 2008). Active packaging materials like ethanol vapour generators consist of ethanol absorbed or encapsulated in carrier materials and enclosed in polymer packets. The ethanol permeates the selective barrier and is released into the headspace within the package (Appendini and Hotchkiss, 2002). There are several ways of adding ethanol for this purpose; the simplest would be the injecting or depositing of a small amount of the material into packets immediately before it is sealed. Another approach is to use ethanol sachets, containing

encapsulating ethanol, which release ethanol vapour, thereby imparting a preservative effect in the packaging headspace. Different commercial sources of alcohol (whisky, brandy) are used in many premium bakery products, not only to inhibit mould growth but to impart unique flavours associated with such alcohol-based products (Cauvain & Young, 2000). Ethanol appears to have sufficient promise as an in package preservative to warrant further investigation in bakery goods (Brody, 2001).

The objective of this study was to assess the combined application of smart packaging technologies (one intelligent form and one active form) with MA packaged speciality bread to assess pack integrity, packaging performance and shelf-life stability. Problems associated with elevated mould counts resulting in a reduced shelf life were assessed. The ability of ethanol to impede mycological growth is well documented (as described above), however published literature in packaging ethanol based smart technologies are few and far between. Ethanol is used as a preservative in speciality bread both solely and in addition to MAP to ascertain if extension in shelf life can be obtained.

7.2 Materials and methods

7.2.1 Initial assessment of commercial MA packaged Ciabatta bread using oxygen sensors

Commercially produced MA packaged (10% CO_2 : 90% N_2) bread was assessed for O_2 content and pack ingress during shelf-life using non-destructive optical O_2 sensors which were placed within packs on-line prior to gassing and sealing. The use of an optical O_2 sensor was used to determine O_2 levels to understand the level of O_2 present in packs immediately post packaging and throughout storage period to ascertain why moulds counts were resulting in a product of reduced shelf life. The monitoring of O_2 levels in these commercial packs showed that O_2 levels were in excess of 13% immediately after packaging and this subsequently led to a reduction in product shelf-life as evidenced by excessive mould growth. A sealing fault was determined to be responsible for elevated levels of O_2 in packs and this was subsequently amended. Following these initial studies, it was decided to investigate if the corrected commercial packs of bread could be monitored again and further enhanced in terms of shelf-life using a combination of smart packaging technologies.

7.2.2 Sensor preparation and calibration

Optical O_2 Sensors were prepared using Platinum octaethylporphyrin-ketone (Pt-OEPK, Luxcel Biosciences, Cork) in polystyrene and 4μL was spotted on Durapore paper (Millipore Inc, Bedford, USA) using a Gilson P100 pipette (Gilson, WI, USA) and allowed to dry and cut to a size of 5mm diameter. Avery price marking stickers (Avery, California, USA) were used to attach sensors to packaging materials prior to packaging process. Six sensors were then taken at random for calibration

where each placed into a customised flow cell (Luxcel Biosciences, Cork) for calibration using seven O_2 concentrations ranging from 0% O_2 to 21% O_2 (BOC gases, Ireland). A phase value was obtained at each O_2 concentration using a phase detector (Luxcel Biosciences, Cork, Ireland) and these data points were used to construct a calibration curve (Fig 7.1), to convert phase readings to percentage O_2.

[Figure 7.1]

7.2.3 Ethanol Emitter/Spray preparation

Ethanol emitting (EE) LDPE-based sachets containing 3ml of alcohol gel (Selden, Derbyshire, UK) were used in this study. Prior to use, sachets were pierced prior to being placed and fixed within packs. The use of gel-based alcohol provided for the slow release of ethanol vapour into the packaging headspace over time compared to the spray technique which would result in rapid vaporisation within the sample headspace immediately after sealing the package. Ethanol sprayed (ES) samples were carried out by rotation of each sample 360 degrees whilst a 3ml (Ethanol 70%, Carbon Group, Ringaskiddy, Cork) direct spray was administered, using a manual 1 litre SprayMist liquid sprayer (B&Q, Eastleigh, UK).

7.2.4 Sample preparation

Commercial Ciabatta bread (consisting of wheat flour, yeast, milk, water, sugar, salt and butter) samples were packaged in MAP gas levels consisting of 10% CO_2 and 90% N_2 (BOC gases, Ireland). Each bread pack contained two Ciabatta loafs (2 x 100g) and were packaged in high barrier (1 cm^3/m^2, 24h, bar) OPA/PE laminate films of 38 micron thickness and stored at room temperature (21ºC). An optical O_2 sensor

was attached to the inside of all bread packs pack prior to gas flushing and sealing. Each pack was numbered and best before dates labelled (6 days). Further samples were packaged with air (~21% O_2) to serve as control. The use of ethanol was also incorporated into a number of packs and used in 'sachet' and 'spray' forms. Samples using ethanol were prepared as described previously using EE and ES formats and labelled. Oxygen measurements were taken daily during shelf-life evaluation. All experimental treatments are presented in Table 7.1.

[Table 7.1]

7.2.5 Microbiological Testing

For the microbiological analysis of bread, 10g of sample was diluted with 90mls of ringer solution (Merck, Germany) and blended for 1.5 minutes in a stomacher (Colworth Stomacher® 400).With this solution; dilutions from 10^{-1} to 10^{-6} were prepared. Dilutions were plated onto total viable count agar supplied by Sigma-Aldrich, Ireland. Total viable counts (TVCs) were carried out in accordance with ISO standards, method 4833:2003 utilising agar plates, incubation at 30ºC and enumeration of colonies after 48 hours. Results were expressed in Log_{10} colony forming units (CFU)/g sample. Yeast and mould detection was carried out daily using the same dilutions described above and plated on Compact Dry YM medium (Hyserve, Germany), incubated at 25 ºC for 7 days. Samples exceeding microbial limits are tabled, where Day 1, 5, 10 and 17 are listed. A limit for mould/yeast growth was set at log_{10} 5 cfu/g sample (ICMSF, 1986). Yeast counts were within limits at each test day.

7.2.6 Sensory Analysis design

Twenty-six panellists were chosen from University College Cork, Ireland. Panellists were chosen on their availability and motivation to be present on all days of experiment as well as regular consumers of speciality bread products. All selected panellists had previous experience in carrying out sensory analysis and consisted of 50:50 male and female, with an age range from 18-40 years old. In conjunction with ISO standards (ISO 8589: 2007) sensory analysis was carried out in individual booths. It was necessary to assess the quality changes with the use of ethanol to see if flavour and aroma characteristics were affected. Each panellist was presented with 6 samples all assigned with a random three digit identification code to allow for blind assessment and asked to rate descriptors according to a ten point scale. A list of descriptors can be seen in Table 7.2. Samples provided for assessment included Control (WOE - Without Ethanol, air + EE and air + ES) and MAP (WOE, EE and ES) and were assessed on storage Days 1, 5 and 10.

[Table 7.2]

7.2.7 Statistical Analysis

ANOVA-Partial Least Squares Regression (APLSR) was used to process the mean data accumulated from the 26 panellists during the sensory evaluation. Principal component (PC) 1 versus PC 2 is presented (Figure 7.3); other PC's did not yield additional information. The validated explained variance for the model constructed was 51.13% and the calibrated variance was 53.19%. To derive significance indications for the relationships determined in the quantitative APLSR; regression

coefficients were analysed by jack-knifing (Table 7.6). All analyses were performed using the Unscrambler Software, version 9.7 (CAMO ASA, Trondheim, Norway).

7.3 Results and discussion

7.3.1 Oxygen measurements within commercial packs of MAP Ciabatta bread products

In order to understand the O_2 levels typically found in commercially produced packs, sensors were added to commercial bread samples packed for retail distribution, in factory. Using optical sensors the level of O_2 could be determined non-destructively and immediately after packaging and daily throughout 10 days of product storage. Ten commercial bread samples were packaged using MAP (10% CO_2 and 90% N_2) thereby providing an atmosphere free of O_2. However, during evaluation of product quality and packing system performance it was observed that O_2 was present in excess quantities (>13.5%) within packs immediately after packaging (Figure 7.2). By day 6 of product storage, a steady increase in O_2 levels within packs was observed to 20.1%. From days 6 to 9, O_2 levels decreased dramatically to 3.1%. Interestingly, this large decrease in O_2 content also coincided with the visual presence of mould observed in packs on day 8 of storage. The utilisation of O_2 appears to have been linked to this rapid onset of mould growth. By day 10, a rapid rise in O_2 to atmospheric levels (21%) occurred once more, indicating that mycological demand for O_2 had diminished. On examination of the packaging process, it was determined that the reason for the high levels of O_2 present in packs was due to faulty heat sealing equipment which the commercial company were unaware of. Once rectified, the packaging process was brought back within a state of control and ideal MAP

conditions delivered subsequently, the level of in-pack O_2 was repeatedly found to be < 0.5% in bread packs post-servicing. Further samples were produced to ensure this level was maintained over time for quality control purposes. In terms of MAP, it is obvious that the intended atmosphere required to extend shelf-life of speciality breads could not have been delivered due to faulty packaging equipment and a subsequent inability to hold containment, thereby culminating in a shorter product shelf-life than that originally targeted. The use of optical sensors have been previously shown to be a valuable and novel tool in determining excess levels of O_2 in low O_2 cheese packaging formats due to packaging containment failures brought about predominantly by the physical damage to packaging materials during the pack forming process (Hempel *et al*. 2012). The reversible nature of the optical sensors demonstrates a novel and effective way of continuously monitoring fluctuations in O_2 levels within packs, from O_2 ingress within packs to O_2 consumption by microbial, biochemical and chemical processes (Smiddy *et al.*, 2002(a); O'Mahoney *et al.*, 2004; Hempel *et al.*, 2013).

[Figure 7.2]

7.3.2 Oxygen measurements within packs of MAP Ciabatta bread products containing O_2 sensors and ethanol-based active packaging treatments

In order to produce a longer and more stable shelf-life, Ciabatta samples were produced with the use of Ethanol emitters (EE) and Ethanol Spray (ES) and compared to control (in the absence of ethanol -WOE) breads over time. Control samples (Air) and MAP (10% CO_2 and 90% N_2) samples were prepared using EE, ES and WOE. The optical sensor was incorporated into all packaging formats so as to continuously

monitor O_2 levels over time. Non-destructive O_2 assessment was carried out until visual mould growth was observed. Mean O_2 levels found for each treatment are presented in Table 7.3.

[Table 7.3]

Control (Ciabatta held in air) samples possessed O_2 levels of between 20-21% on day 0 while MAP (Ciabatta held in 10% CO_2 and 90% N_2 following commercial packaging improvements) possessed O_2 levels of <0.5% O_2 at the same time point. All samples were monitored over time (Table 7.3) until mould was visually observed, a point at which the samples are clearly unacceptable to consumers. All MAP samples showed a continuous O_2 free environment within packs up to 35 days. Control samples showed varying results depending on what treatment was used. Control WOE samples showed a complete utilisation of O_2 by day 6 of storage (manufacturer's expiry date). It was noted that visual mould growth was observed by day 5 in Control WOE samples.

The reduction of O_2 over time can be correlated with the onset and increase in mould growth. The use of ES on samples packed in air reduced the onset of O_2 decline to day 6 of storage, where O_2 levels decreased by only 4%, compared to an O_2 free environment being produced in control samples over the same period of time. The depletion of O_2 for the Air + ES treatment occurred by day 14 of storage, where visual mould growth appeared by day 11 of storage. The packaging treatment of Air + EE samples appears to have had a continuous effect on O_2 levels over time, where initial O_2 levels decreased by 4% on day 14 of storage. However, by day 35 O_2 levels were still in excess of 16.0%. The reduced level of O_2 decline can be correlated to the

propagation of lower mould counts as no visual mould growth was observed for Air + EE samples up to day 24 of storage. The appearance of visual mould growth is listed in Table 7.4. The use of an ES on MAP samples appears to have only prevented visual mould growth by a single day in MAP treatments.

[Table 7.4]

The delivery of intended MAP (10% CO_2 : 90% N_2) significantly improved the shelf-life extension of bread samples. Packaging in MAP WOE had a continuous O_2 level of <0.5% over the course of the shelf-life study, providing a near O_2-free environment for up to 35 Days. Samples were visually mould-free for 12 days. The use of MAP + ES extended the mould free appearance of bread products by one further day, whereas MAP + EE samples had the greatest mould free status for the duration of 30 days. Although the appearance of mould does affect consumer acceptability, it does not adequately address product safety in terms of true microbial counts. Consequently, microbial enumeration was carried out to establish the true shelf-life associated with bread products from each experimental treatment.

7.3.3 Microbiological Assessment

The Log 10 cfu/g sample for mould growth in all bread treatments is shown in Table 7.5. By day 5 of storage, Control WOE samples exceeded microbiological limits of $>10^5$ or Log 5 cfu/g (Forsythe, 2000) and displayed visual mould growth. Determined mould counts were in line with those reported by Latou, Mexis, Badeka & Kontominas (2010). By day 10 of storage, samples packed in Air + ES exceeded limits, without visual mould being determined (Day 11). Interestingly, this extends the

six day shelf-life originally targeted by the commercial manufacturer of MAP speciality breads previously outlined in this study. The use of EE in both Air and MAP bread samples extensively increased shelf life. By day 10 of storage, all EE bread samples were within acceptable limits. By day 17 of storage, microbial limits were exceeded for the first time for Air + EE and MAP + EE packaging treatments, where Day 16 is found to have recorded the last day EE samples were within acceptable limits. Consequently, the bread samples held under these treatment conditions had an extended shelf-life of 10 days from the commercial standard of six days. No visual mould growth was observed for either treatment on day 17 of storage. Although the levels of O_2 in all MAP samples post-packaging were found to be extremely low (<0.5%), the eventual propagation of mould in samples containing EE could possibly occur due to the limited availability of O_2 within packs and through the possible ingress of small amounts of O_2 through the packaging materials over time. It is clear that the use of EE dramatically reduced the occurrence of mould growth in bread. The ability of EE to extend the shelf-life of bread packaged in normal atmospheres showcases the effectiveness of active packaging in providing bread manufacturers with an alternative means of prolonging product shelf-life. The use of EE could be of a great benefit for commercial manufacturers who would prefer to use such active packaging sachets instead of employing MAP at all. The results generated here echo the suggestion made by Brody (2001) that ethanol appears to have sufficient promise as an in-package preservative in bakery goods (Brody, 2001).

[Table 7.5]

7.3.4 Sensory Analysis

With the use of ethanol in packaging applications, it was necessary to understand if the ethanol imparted a flavour and aroma to the product. Table 7.6 represents the results derived by Jack-knifing uncertainty testing of panellist's scores. In terms of ethanol treatments, Air + EE and MAP + EE samples were found to be acceptable to consumers, where fresh aroma, overall flavour and overall acceptability were all found to be significantly correlated ($P \leq 0.001$). Ethanol aroma and flavour was found to be negatively correlated with the use of EE ($P \leq 0.01$), indicating that consumers did not appear to perceive ethanol in samples resulting in an overall acceptable product ($P \leq 0.001$). It has been observed that the use of EE was shown not to affect initial odour, taste and texture in sliced bread (Latou *et al*. 2010). The use of an ethanol spray (ES) however, results in a product that is perceived to have ethanol present in abundance and where Air + ES and MAP + ES are found to be significantly correlated to ethanol flavour ($P \leq 0.001$) and ethanol aroma ($P \leq 0.001$) resulting in a product that was found to be unacceptable ($P \leq 0.01$). It is apparent that by day 5 panellists perceived samples to be unacceptable ($P \leq 0.001$) as flavour ($P \leq 0.001$) and appearance ($P \leq 0.001$) became negatively correlated. By day 10 of storage, Control samples were not assessed due to microbial limits being exceeded, resulting in samples being perceived with more positive scores. Consequently, aroma was found to be significantly acceptable ($P \leq 0.05$). Of the six treatments assessed, only MAP WOE, Air + EE and MAP + EE samples scored high for overall acceptability ($P \leq 0.001$) to panellists. It is clear that the technique employing an ethanol spray (ES) to samples produced a product that was negatively impacted upon in terms of flavour and aroma, thereby reducing its acceptability and overall general appeal ($P \leq 0.01$).

[Table 7.6]

[Figure 7.3]

It is clear that utilizing EE to extend shelf life is successful in yielding a product that is found to be acceptable to consumers in terms of sensory quality ($P \leq 0.001$). Therefore, the option to employ such emitters could be of great benefit to bread manufactures that have an option or preference in packaging highly perishable bread products without the need of modified atmosphere gases which present technical challenges and elevate equipment and production costs.

7.4 Conclusion

Packaging faults and containment failure were detected by O_2 sensor in commercial bread products. Intended modified atmosphere fill to eliminate O_2 in packaging was accurately determined and monitored over time for commercial products. The reduction in O_2 during shelf life trial is shown to be related to the microbial quality of samples by propagation and respiration of moulds. The use of ethanol emitters is shown to extend shelf life without the need of additional modified atmosphere gas. Acceptable limits for microbial quality were maintained for 16 days when packaged in air using ethanol emitters. Sensory analysis shows that the use of ethanol emitters has no negative effect on product quality compared to ethanol spray technique and controls. The results of this study provide valuable information in terms of understanding what levels of O_2 are present in MA packaging and its resulting cause in quality. The addition of ethanol to extend shelf life shows great promise in

delaying the onset of inevitable mould growth. The potential for such smart packaging technology like O_2 sensors and ethanol emitters in the bread industry cannot be understated.

7.5 Acknowledgements

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7.6. TABLES AND FIGURES

Table 7.1 Sample preparation and abbreviation

Sample	Gas	Abbreviation
Control (without Ethanol)	Air	Control WOE
Air + Ethanol Emitter	Air	Air + EE
Air + Ethanol Spray	Air	Air + ES
MAP (without Ethanol)	10% CO_2, 90% N_2	MAP WOE
MAP + Ethanol Emitter	10% CO_2, 90% N_2	MAP + EE
MAP + Ethanol Spray	10% CO_2, 90% N_2	MAP + ES

Table 7.2 Sensory terms for the naive assessor evaluation of Ciabatta bread

Attribute	**Description**
Overall Appearance	0 = Extremely Dislike, 10 = Extremely Like
Off Aroma	0 = None, 10 = Extreme
Ethanol Aroma	0 = None, 10 = Extreme
Fresh Aroma	0 = None, 10 = Extreme
Overall Flavour Liking	0 = Extremely Dislike, 10 = Extremely Like
Ethanol Flavour	0 = None, 10 = Extreme
Astringent Flavour	0 = None, 10 = Extreme
Overall acceptability	0 = Extremely unacceptable, 10 = Extremely acceptable

Table 7.3 Mean O_2% levels in Ciabatta bread packaging treatments during storage (35 days) including standard deviation

	Day					
Sample	1	6	14	21	28	35
Control WOE	20.60 ± 0.56	<0.1	<0.1	<0.1	<0.1	<0.1
Control + EE	21.17 ± 0.40	18.35 ± 0.35	17.08 ± 0.12	16.77 ± 0.25	16.4 ± 0.31	16.38 ± 0.10
Control + ES	20.43 ± 0.64	16.70 ± 1.70	<0.1	<0.1	<0.1	<0.1
MAP WOE	0.4 ± 0.08	<0.1	<0.1	<0.1	<0.1	<0.1
MAP + EE	0.4 ± 0.09	<0.1	<0.1	<0.1	<0.1	<0.1
MAP + ES	0.4 ± 0.10	<0.1	<0.1	<0.1	<0.1	<0.1

Table 7.4 Visual mould occurrence

Treatment	Day
Control WOE	5
Air + EE	25
Air + ES	11
MAP WOE	12
MAP +EE	30
MAP +ES	13

Table 7.5 Log10 cfu/g sample for yeasts and moulds in bread samples held under different packaging treatments

	Log10 cfu/g			
Sample	Day 1	Day 5	Day 10	Day 17
Control WOE	2.39 ±	[1]4.57 ± 0.52	ND	ND
Air + EE	<1	2.40 ± 0.21	3.23 ± 0.18	^[1]5.14 ± 0.08
Air + ES	<1	3.49 ± 0.15	^[1]5.35 ± 0.44	ND
MAP WOE	<1	2.72 ± 0.42	^[1]5.07 ± 0.15	ND
MAP + EE	<1	2.34 ± 0.31	3.11 ± 0.09	^5.03 ± 0.05
MAP + ES	<1	2.69 ± 0.27	4.50 ± 0.17	^[1]6.49 ± 0.52

^Exceeds limits >$Log_{10}5$

[1]Yeast and mould appearance

ND not determined as mould visually observed on bread

Table 7.6 Significance of estimated coefficients (ANOVA values) for sensory terms as derived by Jack-knife uncertainty testing for packaging treatments for bread

	Treatment						Time		
	Control WOE	Air + EE	Air + Spray	MAP WOE	MAP + EE	MAPS	Day 1	Day 5	Day 10
Overall Appearance	0.25	0.14	0.33	0.097	0.21	-0.54	0.001***	-0.001***	0.30
Off Aroma	0.28	-0.12	0.006**	-0.001***	-0.001***	0.02*	-0.01**	0.01**	-0.05*
Ethanol Aroma	-0.001***	-0.01**	0.001***	-0.001***	-0.001***	0.001***	-0.45	0.63	-0.83
Fresh Aroma	-0.33	0.001***	-0.007**	0.001***	0.004***	-0.04*	0.001***	-0.001***	0.61
Overall Flavour	-0.11	0.001***	-0.001***	0.001***	0.001***	-0.001***	0.001***	-0.001***	0.35
Astringent Taste	0.48	-0.001***	0.01**	-0.001***	-0.001***	0.03*	-0.10	0.01**	-0.82
Ethanol Flavour	-0.001***	-0.01**	0.001***	-0.001***	-0.001***	0.001***	-0.25	0.11	-0.81
Overall Acceptablility	-0.11	0.001***	-0.008**	0.001***	0.001***	-0.001***	0.001***	-0.001***	0.49

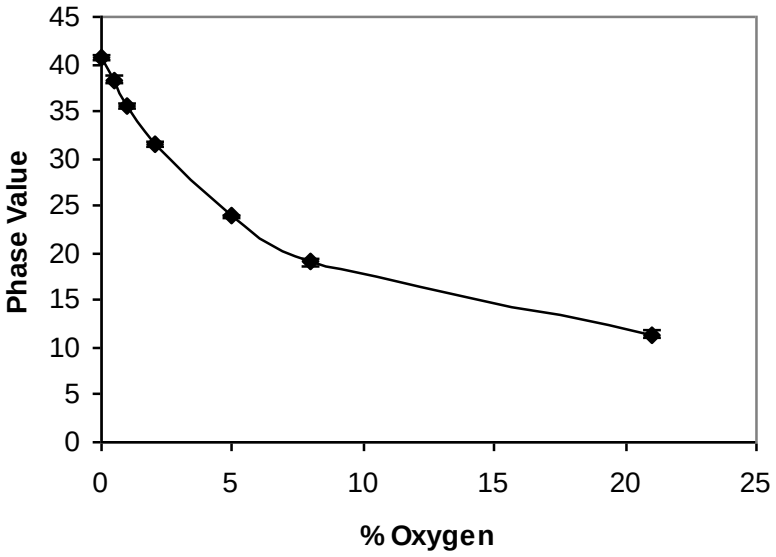

Fig 7.1 Calibration curve for optical O_2 sensors

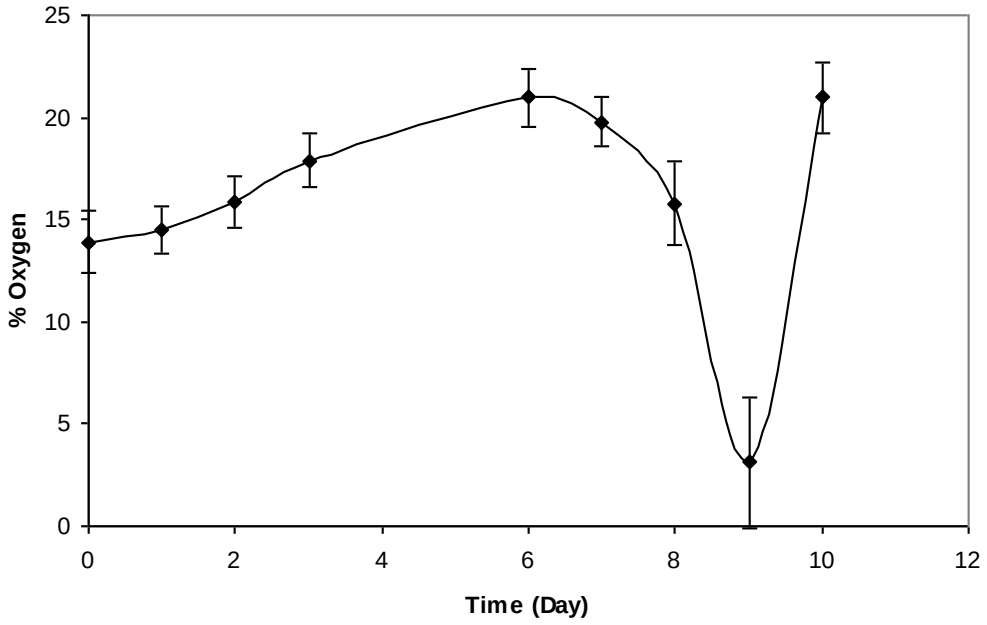

Fig 7.2 Mean O_2 profile of in factory commercially packaged Ciabatta samples held under MAP conditions.

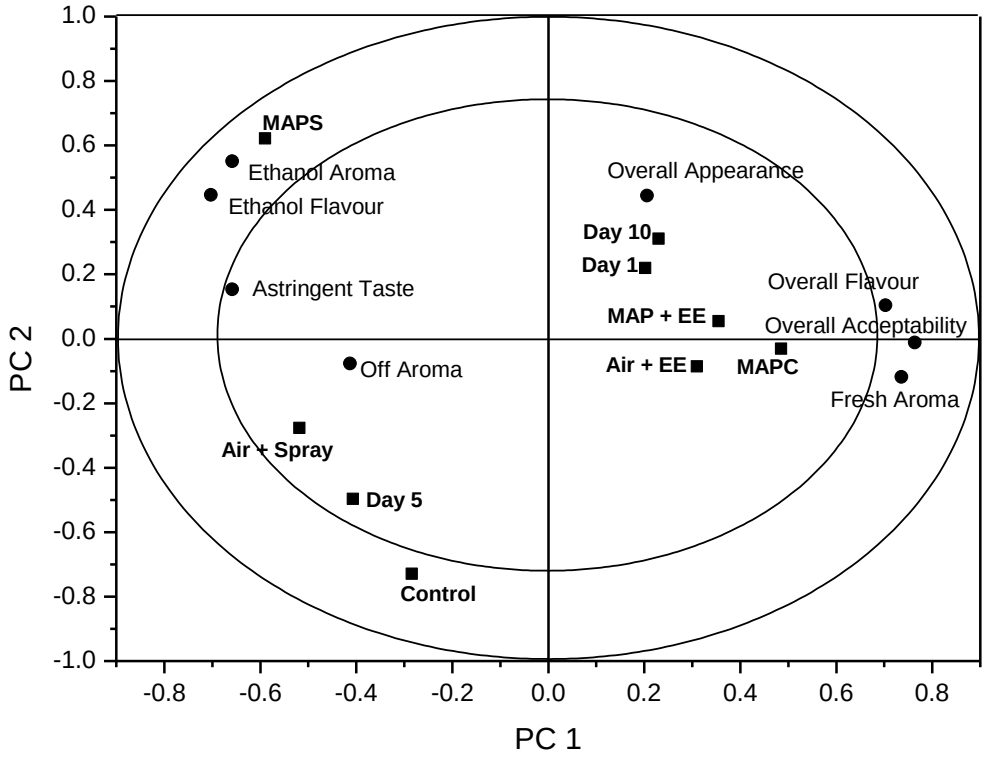

Fig 7.3 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each bread packaging treatment

Chapter VIII

Use of Optical Oxygen Sensors in Non-Destructively Determining the Levels of Oxygen Present in Combined Vacuum and Modified Atmosphere Packaged Pre-Cooked Convenience-Style Foods and the Use of Ethanol Emitters to Extend Product Shelf-Life

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Abstract

O_2 sensors were used to non-destructively monitored O_2 levels in commercially packed pre-cooked, convenience MAP foods. A substantial level of O_2 (>15%) was present in packs resulting in a shorter than expected shelf-life, where the primary spoilage mechanism was found to be mould. Various combinations of vacuum (0-0.6 MPa) and gas flush (0.02-0.03 MPa) (30% C O_2/70% N2) settings were assessed as treatments that result in the desired shelf-life (28 days). This was achieved using the combined treatment of vacuum 0.35 MPa and gas flush 0.02 MPa which resulted in a reduction of 6-9% O2 in all three samples (battered sausages (BS), bacon slices (BA), and meat and potato pies (PP)). Reduced O_2 levels reflect the microbial quality of products, which has been successfully reduced. Duplicate samples of all product packs were produced using ethanol emitters (EE) to see if shelf-life could be further extended. Results showed a further improvement in shelf-life to 35 days. Sensory analysis showed that ethanol flavour and aroma was not perceived by panellists in two of the three products assessed. This study demonstrates how smart packaging technologies, both intelligent and active, can be used to assist in the modification of conventional packaging systems in order to enhance product quality and safety and through the extension of product shelf-life.

Keywords: modified atmosphere packaging; convenience foods; packaging; storage; sensory; oxygen sensors, ethanol emitter

8.1 Introduction

Convenience foods can be defined as commercially prepared foods designed for ease of purchase, preparation and consumption (De Barcellos, Grunert & Scholderer, 2011). Such food items can be single elements of a meal or can be complete food items. It is widely believed that the importance of convenience in food is still on the increase, at least in many countries, and that changing demographics are a major driver in this process (Brunner, van der Horst & Siegrist, 2010; Scholderer & Grunert, 2005). Retailer focus and greater consumer demand for quality maintenance and shelf-life extension of retailed convenience-style food products continues to challenge the development of these food forms. The shelf-life of heat and serve or ready-to-eat foods is usually limited by two factors; microbial growth and the O_2 sensitivity of the product. Therefore, the two main requirements when packaging convenience-style products under modified atmosphere (MA) is that O_2 should be excluded and a fungistatic or bacteriostatic agent be present (Subramaniam, 1993). The uses of advanced technologies have been researched in an attempt to exert greater control over the production and stabilisation of convenience-style food products. Technologies such as radiation treatment (Kanatt, Shobita Rao, Chawla & Sharma, 2010) and high pressure processing (Jofre, Aymerich, Grebol & Garriga, 2009; Vercammen, Vanoirbeek, Lurquin, Steen, Goemaere, Szczepaniak, Paelinck, Hendrickx & Michiels, 2011) have been used to increase the shelf-life of convenience-style foods, but are associated with high costs and utilisation issues. Simple and more commonly used technologies such as packaging may prove to be cheaper and more practical in terms of exerting greater process control during the manufacture of convenience-style food products. Technologies like modified

atmosphere (MA) packaging and vacuum packaging are utilised across the food industry to create packaging solutions capable of providing a sufficient shelf-life for the chilled chain distribution of numerous food types The use and manipulation of such packaging systems have not been exploited sufficiently and in combination with new packaging materials offer opportunities to enhance greater control around food production, especially in the area of convenience-style food production. The use of MAP involves the use of O_2, CO_2 and N_2 in ratios that differ to normal atmospheric air. These altered concentrations retard deterioration processes and maintain foods in a 'fresh' state for extended periods of time (Yam, 2009). Reduced O_2 levels, to that found in air is commonly applied to O_2-sensitive food packaging in order to reduce or delay oxidation reactions in foods. Aerobic microbial growth and oxidation reactions are the principal mechanisms responsible for food deterioration. Elevated levels of CO_2 are utilised for selective antimicrobial effects, primarily targeted towards food spoilage microorganisms (Floros & Matos, 2005). Vacuum packaging is also utilised in extending shelf-life in foods applications, where the atmosphere that normally surrounds the food is removed. Vacuum packaging of food products can be seen as an effective means of eliminating possible biological and chemical contaminants from the space surrounding the food (Lee, Yam & Piergiovanni, 2008).

Smart packaging is generally defined as packaging that provides additional levels of useful functionality beyond protecting, containing and providing information about the product (Hogan & Kerry, 2008; Yam, 2009). Smart packaging encompasses and incorporates intelligent and active packaging formats. A smart packaging component can be described as intelligent if it has the ability to sense the environment and communicate its findings with the buyer or consumer; for example an intelligent package is one that can monitor the safety and/or quality condition of a food product

and provide early warning to the consumer or food manufacturer (Kerry, O'Grady & Hogan, 2006). A form of intelligent packaging that has received much interest is that of optical O_2 sensors (Papkovsky, 1995; Papkovsky, 2004; Kerry & Papkovsky, 2002). Fluorescent-based O_2 sensors represent the most promising systems to date for remote measurement of headspace gases in packaged products. A number of disposable O_2 sensing prototypes has been developed that can be produced at low costs and provide rapid determination of O_2 concentration (Kerry & Hogan, 2002; Papkovsky, Papkovskaia, Smyth, Kerry & Ogurtsov, 2000). Sensors normally consist of a fluorescent or phosphorescent dye encapsulated in a solid polymer matrix and added to a suitable support material. If present, molecular O_2 quenches the luminescent dye and can be quantified against predetermined calibrations. The process is reversible and yields no by-products (Hogan & Kerry, 2008). Research utilising optical O_2 sensors across a wide range of foods has been extensively published. Foods ranging from MAP cheese (O'Mahoney, O'Riordan, Papkovskaia, Kerry & Papkovsky, 2006), vacuum packed cheese (Hempel, Gillanders, Papkovsky & Kerry, 2012), MAP and vacuum packed beef (Smiddy, Fitzgerald, Kerry, Papkovsky, O'Sullivan & Guilbault, 2002a), cooked meats (Smiddy, Papkovsky, Kerry, 2002b), MAP and vacuum packed chicken (Smiddy, Papkovskaia, Papkovsky & Kerry, 2002c), as well as sous vide products (O'Mahoney, O'Riordan, Papkovskaia, Ogurtsov, Kerry & Papkovsky, 2004) have been monitored for O_2 levels using non-destructive, reversible, optical O_2 sensors. Further research has been carried out in the bottled beverage sector, where O_2 levels were determined in pre-pasteurised beer (Hempel, O'Sullivan, Papkovsky & Kerry, 2013). The ability to non-destructively assess the levels of O_2 present immediately after packaging can provide valuable information into the shelf-life and overall quality of the packaged food at any

time point during the life-time of the product. Post packaging assessment could lead to the development of acceptable limits that could ensure product quality throughout shelf life and during storage periods (Hempel, Gillanders, Papkovsky & Kerry, 2012). Another form of smart packaging is that of active packaging. This is defined as packaging in which subsidiary constituents have been deliberately included in, or on, either the packaging material or the package headspace to enhance the performance of the package system (Robertson, 2006). Scavengers, emitters, absorbers and releasers are commonly utilised active packaging materials incorporated to food packaging applications to extend shelf-life. The use of ethanol is particularly effective against mould but can also inhibit the growth of yeasts and bacteria (Day, 2008). Ethanol sachets containing ethanol-release vapour imparts a preservative effect in the packaging headspace (Brody, Strupinsky & Kline, 2001). Many forms of ethanol emitting sachets have been patented and available for purchase including; Ethicap™, Antimould 102™ and Negamold™ (Freund Industrial Co. Ltd) and Ageless™ type SE (Mitsubishi Gas Chemical Co. Ltd). However, limited applications of such technologies have been reported in the scientific literature.

The objective of this study was to assess various packaging technologies and assess their capacity to extend the shelf-life of a range of commercial convenience-style products that were found to have a reduced shelf-life primarily influenced by mould spoilage. The integration of O_2 sensors in food packs to monitor the levels of O_2 remaining in packs post packaging and the application of ethanol emitters in extending the shelf-life of three convenience-style food products were investigated.

8.2 Experimental Section

8.2.1 Optical O_2 Sensor & Analysis

Optical O_2 sensors were prepared by using well known Platinum octaethylporphyrin-ketone (Pt-OEPK) (Luxcel Bioscience, Cork, Ireland), spotted 4uL on Durapore paper (Millipore Inc, Bedford, USA), allowed to dry and cut to size of 5mm. Sensors were then attached to stickers (Avery price marking stickers, California, USA) for adhesion to the underside of packaging laminates. Sensors were read using a Mocon Op-Tech O_2 Platinum (Mocon Inc, MN, USA) measurement device, which allows for instant O_2 readings ranging from 0.001 – 25% O_2 in 0.5 seconds. This system allows for the handheld instrument to be transportable with the use of a portable computer with Mocon Op-Tech software and complies with standards ASTM F2714. Instrumentation underwent calibration using a Cal-Card, where a simple gas-free method of calibration was carried out using two scan zones of 0% O_2 and air. All food packs described in this study contained O_2 sensors and all packaging samples were read daily using this non-destructive method.

8.2.2 Ethanol Emitter Preparation

Ethanol emitters (EE) were used as an in pack antimicrobial sachet. They were prepared in house, by using 3ml of alcohol gel (Selden, Derbyshire, UK) and placed in pouches formed by using Excell LDPE polymer films (supplied by Fispak, Dublin, Ireland) and heat-sealed using a Henkelman Polar 80 vacuum packer and sealer (Henkelman BV, Hertogenbosch, NL). The use of gel-based alcohol provided for the slow release of ethanol vapour into the packaging headspace. Pouches were micro perforated before being placed into food packs to allow for the release of ethanol over time in the headspace of packaging.

8.2.3 Sample preparation

Pre-cooked convenience-style foods were made available from an Irish food manufacturer. Products, including; pre-cooked bacon slices (BA), battered sausages (BS) and beef & potato pies (PP) were selected to monitor the efficiency of the packaging process through the shelf-life evaluation of these products as the company in question had highlighted these products as being problematic in terms of reduced shelf-lives due to mycological growth. All samples were packed in thermoformed retail-ready 2mm thick PS/EVOH/PE trays (250mm x 170mm) (<1 cm3/m2/24h O_2 permeability at 20ºC) and contained through the application of a high barrier lidding laminate Cryovac ULM491 (<1 cm3/m2/24h O_2 permeability at 4ºC) at 43µm thickness which was heat-sealed to the tray after product filling. Each product varied in unit pack content. Table 8.1 highlights the number of product units present in each pack, for each commercial product-type. Repeat samples were also prepared with ethanol emitters (EE) placed in packs and compared. Packaging was carried out using an FP Speedy 2 (ILPRA, Italy) packaging station, sealing two trays per cycle. A combined process of vacuum (1-2 seconds) application followed by gas flushing and sealing (2.5 seconds) using a gas mix of 30% CO_2 and 70% N2 (BOC gases, Ireland), process designed to exclude in-pack O_2. The level of vacuum pressure and gas fill was carried out to manufacturers packaging settings listed in Table 8.1. Unit specifications showed that the equipment had a pressure capacity which ranged from 0 to 0.60 MPa (Max.). Table 8.1 also presents the new packaging settings selected for the shelf-life optimisation study. Samples packaged under normal conditions were prepared with the use of a sensor which was pre-attached to laminate materials prior to entering the packaging process. All samples were monitored over time to determine the level of O_2

present immediately following pack sealing and following its removal from the processing line. Subsequently, repeat samples were produced to compare the effects of applying a range of different pressure settings for both the vacuum and gas flushing processes on line and determining the impact of these process modifications on O_2 levels in product packs. All samples were refrigerated at 4ºC immediately after packaging.

[Table 8.1]

8.2.4 Microbial Analysis

Microbial testing was carried on samples for Total Viable Counts (TVC) and yeasts and moulds. Samples were tested on a weekly basis for 5 weeks (35 days). TVC was determined using total viable count agar (Sigma-Aldrich, MO, USA), with dilutions of 101 - 106 and incubated at 30ºC for 48 hr. Limits for Total viable count (TVC) were Log10 6 (EC:2073/2005). Yeast and mould counts were assessed using dilutions described above and plated on compact dry yeast and mould plates (Hyserve, Uffling, Germany) and incubated at 25ºC for 7 days. Colonies were counted and presented in Log10 cfu/g sample and limits were exceeded when mould counts reached 10^5/g or 5 log10 (cfu/g) (EC:2073/2005).

8.2.5 Sensory Analysis Design

Sensory analysis was carried out to determine if the use of EE had an effect on product quality perception. Twenty-six panellists were chosen form University College Cork, Ireland to partake in the study. The selection criteria for panellists included; availability to attend on each day of the study, motivation and were regular

consumers of ready-cooked products, especially product types similar to those being assessed in this study. Panellists were all aged between 21 and 40 and consisted of a 50:50 male and female balance. Testing was carried out in accordance with ISO standards (ISO: 8589: 2007), where individual booths were provided and samples were assigned random three digit codes for blind assessment. Panellists were asked to rate descriptors on a ten point scale. A list of descriptors used for products can be seen in Table 8.2. Sensory analysis was assessed on day 14 and 35, to allow maximum exposure of foods to ethanol over a 35-day shelf-life. Panellists were provided with six samples, consisting of three products packaged at new packaging settings and replicates packaged with ethanol emitters. Samples were presented in a cooked state as instructed by pack guidelines.

[Table 8.2]

8.2.6 Statistical Analysis

Raw data was accumulated from sensory evaluation and processed using ANOVA-partial least squares regression (APLSR). The optimal number of components in the APLSR models presented was determined to be two principle components (Fig 8.2). Principle component (PC) 1 versus PC 2 is presented, as other PC's did not yield any additional information. The validated explained variance for the model constructed was 18.52% and the calibrated variance was 25.43%. To derive the significance indictors for the relationships determined in the quantitative APLSR, regression coefficients were analysed by Jack-Knifing (Table 8.6). All analyses were performed using the Unscrambler Software, version 7.6 (Camo ASA, Trondheim, Norway).

8.3 Results and Discussion

8.3.1 Packaging assessment and Optical O_2 readings

The research undertaken in this study was conducted, in conjunction with industrial involvement, to ascertain packaging performance for a range of convenience-style food products which were processed and packaged to meet a 28-day retail-required shelf-life. The company partner involved in this research employed two basic packaging approaches to pack all manufactured products; one which pulled a vacuum (0.01 MPa) around trayed-products prior to heat-sealing and, the second which pulled a vacuum (0.20 MPa), followed by gas flushing (0.06 MPa) prior to heat-sealing. These two packaging approaches were chosen for initial study to monitor O_2 presence within packs. To this end, BS were chosen as a test product for assessment by both packaging approaches, i.e. BS using vacuum tray packaging only (0.01 MPa) and, BS using vacuum (0.20 MPa) and gas flush tray packaging (0.06 MP). Continuous non-destructive monitoring of O_2 by optical sensors throughout product storage showed that both commercial packaging methods employed were quite poor in terms of removing O_2 from food packs to achieve the commercially-desired and expected O_2-less state. The mean O_2 profiles of BS packaged using the two packaging approaches described are presented in Figure 8.1. The vacuum method (0.01 MPa) only reduced the mean O_2 level within packs to 17.5% (just slightly lower than normalised levels found in atmospheric air – 21%). This level of O_2 in food packs is undesirable as it can lead to elevated microbial growth and oxidation reactions resulting in a product with a shorter than expected or required shelf-life (Hempel, O'Sullivan, Papkovsky & Kerry, 2013a; Hempel, O'Sullivan, Papkovsky & Kerry, 2013b). During product storage, it was observed that O_2 declined by 9% by day

24 and this coincided with the appearance of visual mould growth on BS. This resulted in a shorter shelf-life than required by the manufacturer. The combined use of vacuum and MAP produced similar results to that determined for the vacuum-only packaging treatment, with O_2 levels in packs immediately post packaging determined as 16%. Again, the gradual decline in O_2 for the vacuum and MAP combination over time closely matched that of the vacuum packaging only profile. Consequently, the overall finding from this preliminary product storage trial was that the commercial packaging approaches used to package BS products in no way came near to achieving targeted in-pack O_2 levels (Hempel, Gillanders, Papkovsky & Kerry, 2012).

[Figure 8.1]

Equipped with the determined knowledge that O_2 levels in BS packs were much greater than expected or desired, a range of packaging treatments were chosen to ascertain if altering packaging regimen had any impact on the O_2 levels remaining in packs post packaging. This was undertaken by altering the packaging pressures applied when using various vacuum/MAP combinations for use again with BS products. The mean O_2 levels present in product packs under these varying packaging treatments, immediately after packaging and throughout product storage, are shown in Table 8.3.

[Table.8.3]

The combination of vacuum/MAP levels and their effect on O_2 levels were shown to vary greatly over the 35 day storage trial. In general, the application of high

vacuum levels lowered initial O_2 concentrations in packs on day 0. Vacuum pressures applied at 0.35 and 0.60 MPa removed the greatest volume of O_2 from packs, averaging 8.3% O_2 post-packaging. The increase in MAP fill pressure from 0.02 to 0.03 MPa appears to have had a negligible effect on O_2 levels. From this study, it was determined that the application of the packaging treatment V 0.35/G 0.02 was optimal for the packaging of rigid tray formats of BS. Additionally, the reduced O_2 levels in packs also eliminated the presence of visual mould on products up to 35 days of product storage. Therefore, it was concluded that this packaging treatment should be applied to a number of food products in order to see whether or not the packaging modifications applied could lower O_2 levels in other product packs and more importantly, extend the shelf-life of these products to greater than 28 days. The use of a lower gas flush setting (0.20) was selected for further studies as it appeared that no further improvement in O_2 levels was achieved by increasing this level further, thereby saving on gas utilization and cycle time per tray. The 0.35 MPa vacuum treatment was also chosen for further studies as it requires less time per packaging cycle and produced equivalent results when compared against higher vacuum pressures.

8.3.2 Revised packaging treatment

A total of three convenience-style ready-cooked food products were chosen for shelf-life studies to ascertain if the new packaging treatment (V 0.35/G 0.02) was suitable for maintaining an acceptable product with a shelf-life of 28 days. As previously described retail products consisting of battered sausages (BS), beef and potato pie (PP) and bacon slices (BA) were chosen for assessment. All samples were prepared as described previously, using an optical O_2 sensor to monitor O_2 levels

throughout storage. The new packaging treatment (V 0.35/G 0.02) was also compared against the original commercial packaging treatments used at the start of these studies (vacuum application (0.01 MPa) and vacuum application (0.20 MPa), followed by gas flushing (0.06 MPa). The use of ethanol emitters (EE) was added to duplicate treatments of the above to determine if further shelf-life extension could be achieved for all products (beyond 28 days) using these active packaging components. The mean levels of O_2 recorded for all experimental treatments over time are shown in Table 8.4. Packaging treatment V 0.35/G 0.02 had reduced levels of O_2 immediately post packaging and throughout storage when compared to the original commercially-used packaging procedures. In the case of BS the O_2 differential was reduced from 15.7% to 2.5% O_2 using the new settings. This trend continued for the other product types examined, where the initial concentration of in-pack O_2 was lowered following the application of the newly modified packaging settings. Product packs containing EE provided an antimicrobial effect which was signalled by a lack of O_2 utilisation in packs over time.

[Table 8.4]

8.3.3 Microbial analysis

Microbial analysis was carried out on a weekly basis to establish the mould counts present in all sample treatments. It was identified that mould was the primary microbial spoilage mechanism across the product range. Table 8.5 presents the log10 mould counts from days 0 to 35. On days 0, 7 and 14, near identical counts were observed across all product treatments, yielding no trends or significant differences

between treatments. Day 21 shows the first notable difference across all treatments. It is clear that all samples are still within acceptable limits at day 21. The normal commercial packaging format employed at the start of this study for each of the three products examined exceeded yeast/mould limits by day 28. However, the application of the new packaging settings was seen to extend shelf-life for all products up to day 35 of storage. It can be noted that the use of EE in all samples further extended shelf-life by an additional 7 days (Day 42) before exceeding limits, compared to samples packed under the new packaging conditions (V 0.35/G 0.02). The ability to extend shelf-life further could benefit product manufacturers, distributors and retailers who require longer shelf-lives, as transportation distances to market increase and waste minimisation measures become more demanding.

[Table 8.5]

The utilisation of EE showed great potential in extending product shelf-life without the need for more advanced processing or packaging equipment or materials. It has been observed that EE have the ability to dramatically extend shelf-life in food products (Latou, Mexis, Badeka & Kontominas, 2010). Total viable count (TVC) limits of Log10 6 (EC: 2073/2005) were not reached in any product sample packaged using the new experimental settings, either with or without EE by day 35.

8.3.4 Sensory and Statistical Analyses

EE were used in selected product packs in order to ascertain if they could potentially extend shelf-life. The continued presence of ethanol in the headspace of the packs was assessed to see if product taste or aroma was affected during sensory assessment of these products. A total of 26 panellists were provided with 6 samples, three products that were packaged both with and without EE. Questionnaires designed with a range of descriptors were rated by panellists to best describe the taste and aroma profile of each sample. Table 8.6 represents the significance of sensory relationship terms. Findings from this study show that only one of the six products assessed were found to be unacceptable. BS + EE were found to impart negative effects on flavour and aroma, where ethanol aroma and flavour were negatively and significantly correlated ($P < 0.001$). Other samples continued to be acceptable to panellists where PP + EE and BA + EE were found to have an overall significant ($P < 0.01$) liking for flavour and overall acceptability ($P < 0.001$). This would lead to the belief that the continued presence of ethanol in samples had no effect in two of the three EE-containing samples. The negative results associated to BS + EE could be due to the increased level of fat associated with this particular product, causing an off flavour ($P < 0.001$) and imparting an ethanol aroma ($P < 0.001$). In the case of BS the sample prepared without EE was found to be significantly acceptable compared to sample BS + EE. The use of EE in food products has been seen to have no effect on taste and/or aroma in bread products (Latou, Mexis, Badeka & Kontominas, 2010; Hempel, O'Sullivan, Papkovsky & Kerry, 2013b). Figure 8.2 represents an overview plot of the mean data from the ANOVA correlation values for all six samples. The ability to extend shelf-life using EE without reducing the sensory quality of certain

food products shows great potential of active packaging technologies. These technologies can allow foods to be stable for long periods of time without the need for further processing and the addition of food preservatives and additives during manufacture.

[Table 8.6]

[Figure 8.2]

Results showed that the use of EE in a variety of ready-cooked, convenience-style foods have a positive effect in extending shelf-life without being accompanied by a decline in perceived product quality. The use of such antimicrobial sachets could provide industry to evaluate what packaging techniques are adopted. The use of EE is without a doubt one of the most exciting interactive packaging technologies available to the food industry (Smith, Hoshino & Abe, 1995), however, the technology is grossly underutilised in commercial retail packs of food today.

8.4 Conclusion

Optical O_2 sensors were shown to readily integrate with commercial packaging of ready-cooked, convenience-style food products. The ability to non-destructively measure O_2 immediately after packaging and throughout shelf life was reported. O_2 levels in excess of that intended was clearly recorded. Vacuum and MA packaging methods were shown to have O_2 levels in excess of 15%. Alteration of packaging settings were monitored for changes in these O_2 levels and resulted in a decrease of O_2

levels to 8%. Best performance settings were selected by results obtained by O_2 sensor readings resulting in a new packaging setting for improved product containment. Although O_2 is clearly available for degradative processes within packs, the lowered O_2 levels obtained maintained an acceptable product for longer than the required shelf-life. The use of EE further extended product shelf-life through, antimicrobial control without adversely affecting overall acceptance of product quality.

8.5 Acknowledgements

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8.6 Tables and Figure

Table 8.1 Sample list with packaging settings (Vacuum – V/ Gas – G) expressed in MPa.

Sample	Abbreviation	No. of Units per pack	Original Packaging Settings (MPa)	New Packaging Settings (MPa)
Battered Sausages	BS	6	V 0.20 / G 0.06 or V 0.01	V 0.35 / G 0.02
Bacon Slices	BA	8	V 0.01	V 0.35 / G 0.02
Beef & Potato Pie	PP	4	V 0.01	V 0.35 / G 0.02

Table 8.2 List of descriptors for sensory analysis

Attribute	Description
Overall Appearance	0 = Extremely Dislike, 10 = Extremely Like
Off Aroma	0 = None, 10 = Extreme
Ethanol Aroma	0 = None, 10 = Extreme
Acid Aroma	0 = None, 10 = Extreme
Overall Flavour Liking	0 = Extremely Dislike, 10 = Extremely Like
Off Flavour	0 = None, 10 = Extreme
Sour Flavour	0 = None, 10 = Extreme
Astringent Taste	0 = None, 10 = Extreme
Ethanol Flavour	0 = None, 10 = Extreme
Overall acceptability	0 = Extremely unacceptable, 10 = Extremely unacceptable

Table 8.3 Mean O_2 levels (%) found in BS packs held under varying packaging treatments (STDEV ≤ 0.50%). V – Represents the level of vacuum pressure applied (MPa), G – represents the level of gas flushing pressure applied (MPa).

							Differential
Sample	D0	D7	D14	D21	D28	D35	D0-D35
V (0.01) / G (0.02)	14.7	13.5	13.3	12.5	10.4	4.1	10.7
V (0.04) / G (0.02)	15.4	13.7	13.5	13.5	12.6	5.0	10.4
V (0.08) / G (0.02)	14.7	13.5	13.2	12.4	11.8	7.0	7.7
V (0.20) / G (0.02)	12.2	11.5	10.4	9.8	8.1	6.8	5.4
V (0.35) / G (0.02)	8.2	6.9	6.7	6.3	6.0	4.5	3.7
V (0.60) / G (0.02)	8.1	6.5	6.3	6.1	6.0	4.4	3.7
V (0.01) / G (0.03)	14.3	13.5	13.1	12.2	10.1	4.0	10.3
V (0.04) / G (0.03)	15.1	13.7	13.5	13.5	12.6	5.0	10.1
V (0.08) / G (0.03)	15.0	13.5	13.0	12.2	11.5	7.1	7.9
V (0.20) / G (0.03)	11.4	11.4	10.1	9.5	7.8	6.8	4.6
V (0.35) / G (0.03)	8.4	6.7	6.5	6.3	6.1	4.6	3.8
V (0.60) / G (0.03)	8.2	6.4	6.3	6.0	5.8	4.5	3.7

Table 8.4 Mean O_2 levels found in all samples over shelf life study (inc. Standard deviation). V – Represents the level of vacuum pressure (MPa), G – represents the level of gas flush (MPa).

	Time (day)						Differential %
Sample	D0	D7	D14	D21	D28	D35	D0-D35
BS Normal 1 (V 0.01)	16.5 ± 0.81	13.2 ± 0.74	13.8 ± 0.75	12.6 ± 0.57	8.6 ± 0.62	0.9 ± 0.44	15.7
BS Normal 1 (V 0.01) + EE	16.6 ± 0.70	15.3 ± 0.83	14.6 ± 0.52	13.9 ± 0.66	10.4 ± 0.87	5.7 ± 1.20	10.9
BS Normal 2 (V 0.20/G 0.06)	12.9 ± 1.00	11.1 ± 0.91	10.2 ± 0.61	9.7 ± 0.83	6.4 ± 0.76	4.1 ± 0.86	8.8
BS Normal 2 (V 0.20/G 0.06) + EE	12.8 ± 0.90	11.3 ± 0.40	10.4 ± 0.64	9.9 ± 0.85	8.7 ± 0.57	6.1 ± 0.77	6.7
BS (V 0.35/G 0.02)	7.5 ± 0.39	6.7 ± 0.48	6.8 ± 0.32	6.8 ± 0.36	7.0 ± 0.20	5.0 ± 0.27	2.5
BS (V 0.35/G 0.02) + EE	8.4 ± 0.41	6.8 ± 0.37	6.7 ± 0.38	6.5 ± 0.44	6.7 ± 0.22	7.4 ± 0.11	1.0
PP Normal (V 0.01)	14.1 ± 1.10	13.1 ± 0.83	12.5 ± 0.64	8.4 ± 0.76	2.7 ± 0.46	0.9 ± 0.62	13.2
PP Normal (V 0.01) + EE	14.4 ± 0.94	13.8 ± 0.73	12.9 ± 0.88	9.4 ± 0.72	6.4 ± 0.65	4.7 ± 0.80	10.7
PP (V 0.35/G 0.02)	7.3 ± 0.51	6.5 ± 0.47	6.4 ± 0.47	6.3 ± 0.56	5.5 ± 0.32	2.2 ± 0.43	5.1
PP (V 0.35/G 0.02) + EE	6.9 ± 0.33	6.6 ± 0.38	6.8 ± 0.13	5.9 ± 0.23	6.0 ± 0.27	4.3 ± 0.11	2.7
BA Normal (0.01)	16.9 ± 1.31	16.5 ± 0.73	16.4 ± 0.33	15.3 ± 0.37	14.4 ± 0.64	8.2 ± 0.89	10.7
BA Normal (0.01) + EE	16.8 ± 1.00	16.5 ± 0.84	16.2 ± 0.76	15.8 ± 0.53	15.1 ± 0.45	11.7 ± 0.44	5.1
BA (V 0.35/G 0.02)	9.5 ± 0.45	7.9 ± 0.53	7.7 ± 0.45	7.7 ± 0.27	7.7 ± 0.36	7.9 ± 0.40	1.6
BA (V 0.35/G 0.02) + EE	7.1 ± 0.22	6.3 ± 0.46	6.1 ± 0.32	6.1 ± 0.13	6.8 ± 0.12	6.3 ± 0.18	0.9

Table 8.5 Mean Log10 mould counts for varying treatments (MPa) across three products, n/d – not determined due to ^exceeding limits (10^5/g or 5 log10 (cfu/g); EC: 2073/2005) or presence of visual mould.

	Time					
Sample	D0	D7	D14	D21	D28	D35
BS Normal 1 (V 0.01)	2.8 ± 0.14	3.2 ± 0.21	3.9 ± 0.32	4.5 ± 0.17	^6.1 ± 0.10	n/d
BS Normal 1 (V 0.01) + EE	2.9 ± 0.09	3.1 ± 0.15	3.7 ± 0.35	4.0 ± 0.22	^5.1 ± 0.09	n/d
BS Normal 2 (V 0.2/G 0.06)	2.6 ± 0.25	3.0 ± 0.07	3.5 ± 0.23	4.3 ± 0.27	^5.8 ± 0.10	n/d
BS Normal 2 (V 0.2/G 0.06) + EE	2.8 ± 0.18	3.2 ± 0.21	3.4 ± 0.32	3.8 ± 0.19	^5.0 ± 0.28	n/d
BS (V 0.35/G 0.02)	1.8 ± 0.29	2.2 ± 0.45	2.7 ± 0.49	2.9 ± 0.34	3.6 ± 0.37	4.8 ± 0.07
BS (V 0.35/G 0.02) + EE	1.7 ± 0.27	1.8 ± 0.29	2.0 ± 0.49	2.3 ± 0.27	3.1 ± 0.32	4.1 ± 0.29
PP Normal (V 0.01)	2.8 ± 0.20	3.2 ± 0.26	3.7 ± 0.15	4.2 ± 0.18	^6.8 ± 0.11	n/d
PP Normal (V 0.01) + EE	2.7 ± 0.28	3.2 ± 0.37	3.6 ± 0.41	3.9 ± 0.50	^5.2 ± 0.07	n/d
PP (V 0.35/G 0.02)	1.7 ± 0.17	2.1 ± 0.12	2.4 ± 0.07	2.8 ± 0.04	3.4 ± 0.16	4.5 ± 0.21
PP (V 0.35/G 0.02) + EE	1.5 ± 0.04	2.0 ± 0.13	2.2 ± 0.09	2.5 ± 0.11	3.0 ± 0.27	4.4 ± 0.31
BA Normal (V 0.01)	2.9 ± 0.10	3.1 ± 0.16	3.6 ± 0.19	4.2 ± 0.14	^6.8 ± 0.08	n/d
BA Normal (V 0.01) + EE	2.8 ± 0.06	3.0 ± 0.12	3.2 ± 0.22	3.8 ± 0.15	^5.6 ± 0.12	n/d
BA (V 0.35/G 0.02)	1.4 ± 0.07	2.0 ± 0.09	2.4 ± 0.17	2.9 ± 0.21	3.7 ± 0.04	4.8 ± 0.05
BA (V 0.35/G 0.02) + EE	1.2 ± 0.05	1.3 ± 0.08	1.6 ± 0.14	1.9 ± 0.18	2.4 ± 0.10	3.9 ± 0.07

Table 8.6 Significance of estimated regression coefficients (ANOVA p-values) for the relationships of sensory terms as derived by jack-knifing uncertainty testing of ready cooked foods.

	Sample						Time	
	BS	BS + EE	PP	PP + EE	BA	BA + EE	Day 14	Day 35
Overall Appearance	0.93 ns	-0.33 ns	0.27 ns	0.23 ns	0.47 ns	0.78 ns	0.64 ns	0.58 ns
Off Aroma	-0.003**	0.001***	-0.001***	-0.004**	-0.85 ns	-0.001***	-0.40 ns	0.42 ns
Ethanol Aroma	-0.001***	0.001***	-0.001***	-0.003**	-0.90 ns	-0.001***	-0.61 ns	0.64 ns
Acid Aroma	-0.04*	0.02*	-0.19 ns	-0.17 ns	-0.53 ns	-0.28 ns	-0.59 ns	0.63 ns
Overall Flavour Liking	0.002**	-0.001***	0.002**	0.004**	0.99 ns	0.001***	0.67 ns	-0.61 ns
Off Flavour	-0.002**	0.001***	-0.003**	-0.005**	-0.74 ns	-0.001***	-0.56 ns	0.38 ns
Sour Flavour	-0.003**	0.001***	-0.002**	-0.007**	-0.83 ns	-0.001***	-0.48 ns	0.46 ns
Astringent Taste	-0.002**	0.001***	-0.015*	-0.02*	-0.94 ns	-0.001***	-0.81 ns	0.77 ns
Ethanol Flavour	-0.001***	0.001***	-0.001***	-0.002**	-0.65 ns	-0.001***	-0.48 ns	0.37 ns
Overall Acceptablility	0.003**	-0.001***	0.001***	0.002**	0.75 ns	0.001***	0.45 ns	-0.42 ns

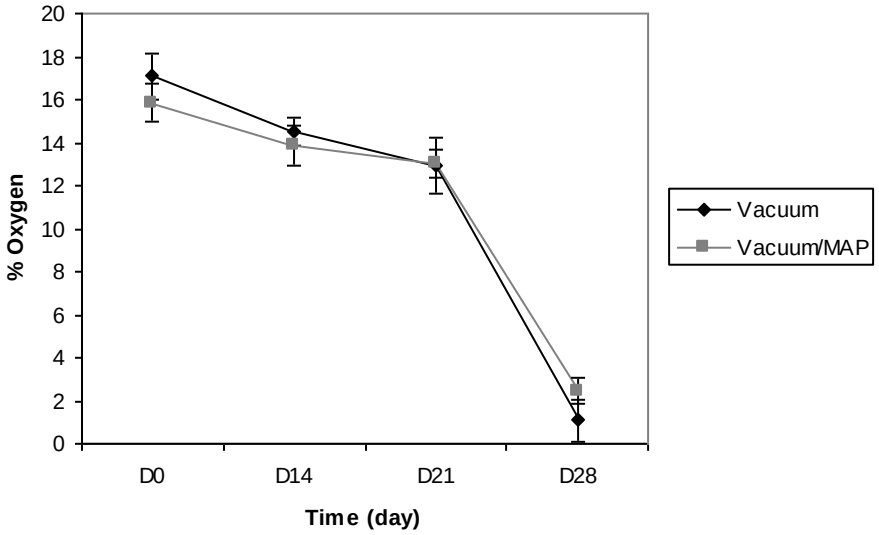

Figure 8.1 Existing packaging assessment; Mean O_2 profiles(+/- standard deviation) for BS packaged under vacuum or packaged under a combination of vacuum and MAP; vacuum (0.01 MPa) and vacuum/MAP (0.20/0.06 MPa)

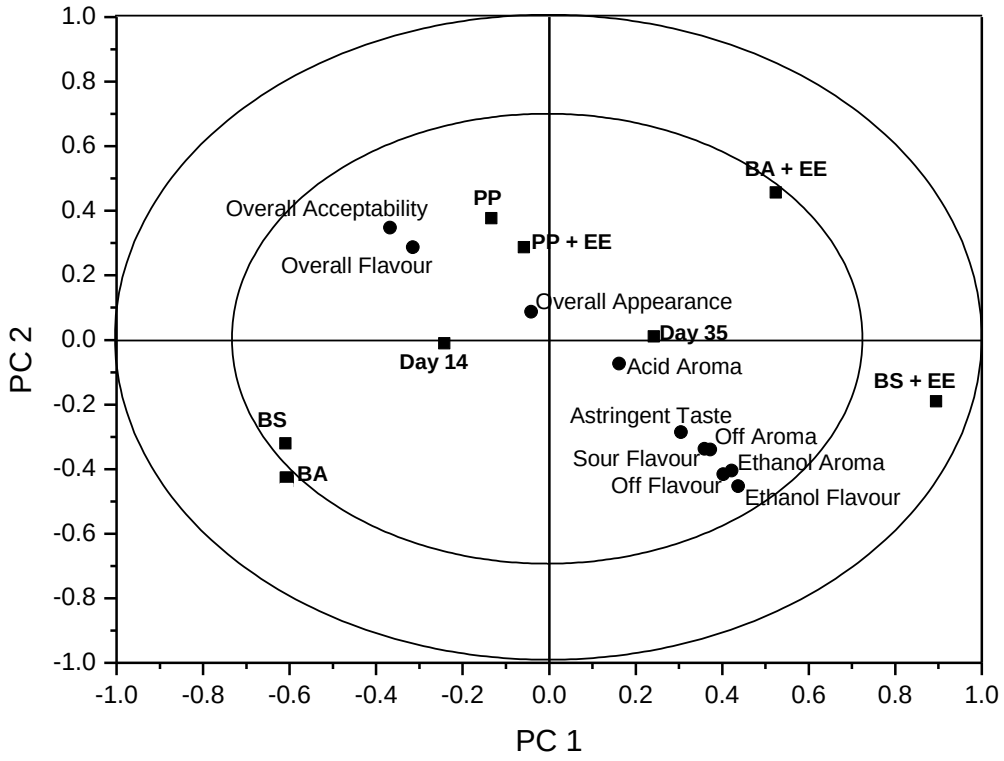

Figure 8.2 An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the eight treatment groups assessed by a trained sensory group. Shown are the loadings of the X- and Y- variables for the first two PC's for averaged data validated over replicates. ■ = Sample and days of analysis. ● = Sensory descriptor.

CHAPTER IX

Analysis of total aerobic viable counts in raw fish by high throughput optical oxygen respirometry

This Chapter is in the form of an accepted manuscript and published in *Journal of Food Protection*;

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Abstract

A simple, miniaturised and automated screening assay for the determination of total aerobic viable counts (TVC) in fish samples is presented. Fish tissue homogenates are prepared in peptone buffered water medium (PBW) according to standard method, and aliquots are dispensed into wells of a 96-well plate together with the phosphorescent O_2 sensing probe GreenLight^{TM}. Sample wells are covered with mineral oil (barrier for ambient O_2) and the plate was monitored on a standard fluorescent reader at 30ºC. The samples produce characteristic profiles with a sharp increase in fluorescence above the baseline level at a certain threshold time (TT) which can be correlated with their initial microbial load. Five different fish species: salmon, cod, plaice, mackerel and whiting are analyzed. Using the conventional agar plating method, the relationship between the TT and TVC load (cfu/g) was established, calibration curve generated and the test was validated with 169 unknown fish samples. It shows a dynamic range of 10^4 – 10^7 cfu/g, accuracy of +/- one log(cfu/g), assay time of 2-12 h (depending on the level of contamination), ruggedness with respect to the key assay parameters, simplicity (three pipetting steps, no serial dilutions), real-time data output, high sample throughput and automation. With this test, quality of fish samples, CFU-per-gram levels and their respective time profiles were determined.

9.1. Introduction

Food in general is a highly perishable product because of high water activity (Abbas, Saleh, Mohamed & Lasekan, 2009), relatively high pH and the presence of autolytic enzymes (Robertson, 2006). Commercially, this high rate of perish ability often makes the transport and marketing of fish a challenge (Otwell, Kristinsson & Balaban, 2006). Fresh fish by its nature has a low microbial load both internally and externally. The muscle tissues are usually sterile in healthy fish, while large populations of bacteria are present on the external surfaces, gills and intestines. There may be as much as 10^2-10^6 bacteria per cm^2 on skin surfaces (Gram & Dalgaard, 2002; Robinson, 2000). As soon as fish are caught and processed, a series of bacteriological, chemical, physical, and histological changes develop in the muscle tissue (Jeremiah, 1996). Significant microbial spoilage and chemical changes in fish cause sensory changes to a degree that it becomes unacceptable to the consumer. Autolytic, chemical and microbiological processes produce undesirable sensory changes in fish, which include discoloration, changes in texture, odour and flavour as well as slime and gas formation. Microbial growth is the main reason for the development of off flavours and odours rendering fish products unacceptable or spoiled (Gram & Huss, 1996; Robinson, 2000). The high degree of perish ability of fish has limited its consumption in a fresh state to areas close to capture.

To preserve the freshness of fish products, especially during prolonged transportation and storage, and extend their shelf life, various packaging and holding temperature techniques are used, including freezing, cooling, refrigeration (Davies, Davies & Abowei, 2009), vacuum and modified atmosphere packaging (Ibrahim, Nassar & El-Badry, 2008; Sivertsvik, Jeksrud & Rosnes, 2002). At the same time, prolonged storage and transportation requires efficient control measures, to ensure

high quality and safety of fish products (including individual fish and pieces). In particular, their microbial load has to be carefully controlled and maintained below the acceptable threshold levels.

The conventional microbiological method called Total aerobic Viable Counts (TVC) provides quantification of viable microorganisms in a sample. Traditionally, TVC in fish and other samples has been done by agar plating which takes 24-48 h to generate results (ISO, 2003). This macro-method involves multiple dilutions of each sample and manual or semi-automated readout (counting of grown colonies). When dealing which such a perishable product as fish, a more rapid, simple and automated micro-method which provides determination of TVC in large number of samples would be highly desirable.

A number of alternative tests and systems suitable for TVC determination in fish have been described, which utilize different chemistries, detection principles and instrumentation, including Petrifilm APC by bioMerieux (Blackburn, Baylis & Petitt, 2008), Simplate by BioControl (Townsend & Naqui, 1998), impedance based Maltus, Bactometer, Rabit and Bactec systems (Duran & Marshall, 2002; Russell, 2000), optical VITEK (Odumeru, Steel, Fruhner, Larkin, Jiang, Mann & McNab, 1999), MicroFoss (Odumeru & Belvedere, 2002) and respirometry (O'Mahoney, et al., 2005; Papkovsky, 2004).

In particular, optical micro-respirometry uses fluorescence based O_2 sensing probes, standard 96-well plates and fluorescent reader detection to monitor growth of aerobic cells and micro-organisms via their respiration. Its initial food safety application has been described for raw meat (O'Mahoney, O'Donovan, Hynes, Moore, Davenport & Papkovsky, 2005) where TVC are determined directly in crude homogenates in peptone buffered water (PBW) medium. This screening assay has

been successfully validated and certified by the American Organization of Analytical Chemists (AOAC). It has now been adopted by a number of meat producing companies and food safety laboratories.

In this study we applied the optical O_2 micro-respirometry assay methodology (Papkovsky, Hynes & Will, 2006) using commercial GreenLight^{TM} probe to develop a similar TVC test for fresh fish samples. In such a test, the probe produces a large increase in fluorescence upon the depletion of dissolved O_2 by growing microorganisms, which occurs when a certain level of respiration is reached (threshold). For different samples fluorescent profiles are expected to be similar in shape, but shifted with respect to each other according to their initial TVC load: samples with higher TVC values ascend earlier, with low TVC - later. The samples are sealed with mineral oil to reduce back diffusion of atmospheric O_2. Using five different species of fish, we investigated matrix effects on assay performance, performed optimisation of assay parameters (dilutions, volumes, timing), generated calibrations for each species and combined calibration. The assay was validated with a panel of real (unknown) samples and benchmarked against conventional agar plating TVC test.

9.2. MATERIALS AND METHODS

9.2.1. Materials

Samples of salmon, cod, whiting, plaice and mackerel filets were purchased from local retailers in Cork. Refrigerated storage room was set at 4ºC, 14ºC or room temperature (24ºC) for fish storage trials. Sterile peptone buffered water (PBW) was prepared fresh using the ingredients from Sigma-Aldrich (Ireland) and Milli-Q water. Stomacher machine and sterile stomacher bags were from Seward (Ireland). Sterile 96-well flat-bottom microplates with lid made of clear polystyrene were from Sarstedt (Ireland). GreenLight^{TM} O_2 probe and mineral oil were from Luxcel Biosciences (Ireland). Plate Agar was from Merck (Germany).

9.2.2 Respirometric assay

Fluorescent reader Safire (Tecan, Switzerland) was set up as follows: measurement mode - fluorescence; excitation filter - 380nm; emission filter - 650nm; gain - 60. Temperature of the microplate compartment was set at 30ºC. Measurement of the microplate was carried out in kinetic mode, with each test well measured every 10 min over 2-12 h. The assay was performed as follows; GreenLight^{TM} probe was reconstituted in 10 mL of sterile PBW to produce stock solution. Fish samples (stored at 4˚C, 14˚C or 24˚C), were taken by cutting 10g squares from the edges of each fillet containing skin on the outside, placed in a stomacher bag together with 90 mL of PBW and homogenized for 1 min. After this, 100 µl aliquots of the homogenate were transferred to the wells of a 96 well plate. Subsequently, 100µl aliquots of probe stock and 100µl of mineral oil (seal from ambient O_2) were dispensed in each well. One fillet per type of fish represents a fish sample (one 10g replicate) and was used per

testing day, analysed in triplicate by the respirometric method and in duplicate by conventional TVC. Negative controls (PBW with probe) and blanks (PBW without probe) were also included. The plate was then placed in the fluorescent reader and monitored at 30ºC using the above settings to determine threshold time (TT) for each sample. TT was taken as the time to reach the threshold of fluorescence intensity set to be 400 FU (fluorescence units). At this threshold the sample containing rapidly proliferating micro-organisms starts to rapidly deplete the dissolved O_2. Initially (at relatively low cell numbers) the sample remains oxygenated and probe fluorescent signal stays flat and low being quenched by dissolved O_2. When the threshold is reached, the sample undergoes rapid deoxygenation (seen as steep increase of probe fluorescence) followed by levelling off when dissolved O_2 is depleted (unquenched probe).

The procedure of fish TVC assay includes six simple steps according to the flow chart shown in Scheme 9.1. Compared to the previously described assay for raw meat (O'Mahoney, O'Donovan, Hynes, Moore, Davenport & Papkovsky, 2005), the procedure has been rationalised to three 100 µl pipettings requiring just one micropipette. The homogenisation step and medium used are the same as in conventional agar plating TVC method.

1. Take 10g of each **Fish Sample** and 90mls of sterile PBW, homogenize in stomacher bag for 1 min

↓

2. Dispense 100µl aliquots of **homogenates** into wells of a sterile 96WP

↓

3. Reconstitute a vial of **GreenLight™ probe** in 10 ml PBW and dispense 100µl into sample wells (negative control is usually included)

↓

4. Dispense 100µl of **mineral oil** to each well (seal from air oxygen)

↓

5. Read the plate on **Fluorescent reader** at 30ºC for 2-12 h

↓

6. **Analyze** measured fluorescence profiles, determine (using software) the TT values and cfu/g load for each sample

Scheme 9.1: Flow chart of the Respirometric TVC Assay for fresh fish

During the initial set-up of the assay, positive controls (medium spiked with *E.coli)* and blanks (medium without probe) were also included to ensure sufficient sensitivity and proper operation of the instrument, whereas at later stages these controls are not necessary. Plate preparation time should be kept to a minimum (<20 min). Where required, the same homogenates were also used in agar plating TVC test (see below). The respirometric assay can also be run on other fluorescent readers, for example Victor^3 (Perkin Elmer) and Omega (BMG) readers which are spectrally

compatible with the probe and allow temperature control and measurements in kinetic mode in 96 well plates.

To determine possible matrix effects, measurements were conducted at several different dilutions of fish homogenates: the standard 1:10 dilution, 1:20, 1:40 and 1:80 dilutions. Spiking with *E.coli* was also used to assess matrix effects on microbial growth and calibration. In this case, frozen cod filet with low TVC (tested by agar plating) was thawed for 3 h at room temperature, homogenized in PBW, then spiked with *E.coli* stock to produce concentrations between $5*10^1$ and $5*10^7$ cfu/g, and measured as above.

To validate the new TVC assay, a panel of fresh fish samples with unknown levels of microbial contamination (salmon, cod, mackerel, whiting, N=169) was obtained from local retailers on different days, several samples each day. Each sample was tested by the new respirometric test and their cfu/g values were determined by applying the combined calibration. In parallel, the samples were analyzed by the conventional TVC test (ISO:4833:2003 method) and the results were compared and plotted against each other.

To test the ruggedness of the respirometric assay, two different errors were introduced: pipetting volume and probe concentration (Table 9.1). Since standard protocol involves 3 consecutive additions of 100µl volumes (Probe + Sample + Oil), an error in each was introduced applying a lower (70µl) and higher (120µl) pipetting volume. An error in probe concentration was introduced using a lower (50%) and higher (150%) probe dilution compared to the standard conditions. The effects of these errors were tested at two different contamination levels: 10^4-10^5 CFU/g (low) and 10^6 CFU/g (high), with negative controls (media only, $<10^3$ CFU/g) included in

each test. Samples were taken from cod filets which were stored at 24ºC for 2 days (high CFU/g) and at 4ºC (low CFU/g), and analysed in 5 repeats (N=5).

[Table 9.1]

In the storage trials, fish samples were kept at 4ºC, 14ºC and 24ºC, and analyzed periodically by the respirometric and conventional TVC test: daily for 4ºC and 14ºC tests and hourly for 24ºC (due to fast deterioration).

9.2.3 Conventional TVC test

TVC on agar plates was performed by the standard ISO: 4833:2003 method, using PBW medium, incubation at 30ºC and counting the colonies of bacteria after 48 h (ISO, 2003).

9.2.4 Statistical analysis

The possibility of the calibration relation between TVC (cfu/g) and TT (h) being modulated by other factors like species of fish or trial effects was investigated by fitting a general linear model of the form:

$$\begin{aligned} th_{ijk} = \mu + b\log\left(TVC_{ijk}\right) + \alpha_i + \beta_j + \gamma_i \log\left(TVC_{ijk}\right) \\ + \delta_j \log\left(TVC_{ijk}\right) + \phi_{ij} + \varphi_{ij} \log\left(TVC_{ijk}\right) + \varepsilon_{ijk} \end{aligned} \quad (1)$$

Where th_{ijk} stands for the threshold recorded on the *k*-th sample on the *j*-th trial for the *i*-th species and similarly for TVC_{ijk}. Here μ stands for overall mean of TT values, *b* (TVC) for the overall slope of the regression of TT on log(*TVC*), a_i (Species) and b_j (Trial) for the main effect on the mean of the *i*-th species and *j*-th trial respectively, γ_i (TVC:Species) and δ_j (TVC:Trial) for the effect on the slope of the *i*-th species and *j*-

th trial respectively, ϕ_{ij} (Species: Trial) for the interaction (combined) effect on the mean of the *i*-th species and *j*-th trial, φ_{ij} (TVC:Species:Trial) for the interaction (combined) effect on the slope of the *i*-th species and *j*-th trial and ε_{ijk} (Error) for measurement error. For estimation and hypothesis testing, measurement errors were assumed to have a Gaussian distribution with identical variance and be mutually independent. The significance of effects in model (1) was measured using *F*-tests computed by a three factor analysis of variance (ANOVA) (Zar, 2000). The acronyms in parentheses are used to represent each effect in the ANOVA table. Based on the significant terms identified by the ANOVA procedure, a reduced model of the form:

$$th_{ijk} = \mu + b\log\left(TVC_{ijk}\right) + \varepsilon_{ijk} \qquad (2)$$

was fit to the data by least squares. The fitted calibration model was examined for adequacy by examining the residuals (estimated errors) for outliers and constancy of variability. Outliers identified by this process were removed for estimating the final calibration model. The assumption of Gaussianity of measurement error was checked using a quantile quantile plot (Venables & Ripley, 2002). The quality of fit was quantified by the R^2 statistic.

The respirometric TVC assay (TVC_R) was computed using the relation:

$$TVC_R = \frac{th - \mu}{b} \qquad (3)$$

Where *th* is the observed TT. The quantities μ and *b* are obtained from the final calibration model in (2). For validation, we compare TVC_R values against standard *TVC* values using agar plating across a range of validation samples i = 1,..., 169, by linear regression:

$$TVC_{Ri} = c + mTVC_i + \varepsilon \qquad (4)$$

For a perfect validation, we would expect $c = 0$ and $m = 1$ (the line y = x), but the actual values are likely to be different due to sampling variability. However, we can check for adequacy of the validation by checking if 95% of the data values are within ±1.96 standard deviation (SD) of the ideal line, where SD due sampling variability is estimated from the residual error of the fitted regression model in (Duran & Marshall, 2002; Zar, 2000).

Analysis of ruggedness testing was performed using a two factor ANOVA (Zar, 2000) where the factors were: 1) the level of sample contamination (high and low cfu/g), and 2) either the assay volume (70, 100 and 120 μl) or the probe concentration (50, 100 and 150ml). Significance of effects was measured by standard ANOVA F-tests (Zar, 2000). Statistical analysis was done using the R package (cran.r-project.org).

9.3. RESULTS AND DISCUSSION

9.3.1 Assay set-up

The probe is added to crude homogenates of fish tissue in growth promoting medium (PBW) during plate preparation, and then changes in fluorescence are monitored at 30^{o}C. Prominent changes in probe fluorescence due to microbial growth allow unambiguous identification of positive samples and quantification of their TVC load on the basis of measured TT. The TTs are determined for all the samples on the plate and converted to cfu/g using pre-determined calibration. Theoretical sensitivity of the respirometric assay is 1 cfu/well (O'Mahoney & Papkovsky, 2006), however, sample volume (0.1 ml) and dilution during the homogenisation (1:10) should be factored in for food samples. Statistical variability at low cell numbers (1-10 cells), possible matrix effects and data scattering reduces the sensitivity further, down to 10^3

-10^4 cfu/g (limit of detection, LOD). Samples producing flat profiles with low fluorescent signal are defined as negative (below the LOD). Up to 96 samples can be analyzed on a plate in one run.

[Fig 9.1]

9.3.2 Analysis of Fish Matrix Effects and Optimisation of Assay Conditions

To assess matrix effects in the respirometric assay, fish samples with relatively low level of contamination (10^5-10^4 cfu/g range, verified by conventional TVC) were initially measured at different dilutions of the homogenates: 1:10, 1:20, 1:40 and 1:80. Representative respiration profiles for one such salmon sample are shown in Fig 9.1. As with pure microbial cultures (O'Mahoney, Green, Baylis, Fernandes & Papkovsky, 2009) and raw meat homogenates (O'Mahoney, et al., 2005), the samples showed characteristic sigmoidal profiles. In contrast, negative samples produce flat profiles, as their O_2 concentration is not changing.

From these profiles a good linearity between measured TT values and sample dilution is seen (Fig 9.1 processed data). The threshold is the point at which the fluorescence signal shows a sharp increase above the basal level. Corresponding TT is compared with the results of conventional TVC cfu/g results which show that sample matrix has no significant effect on assay performance and that at different sample dilutions the microorganisms proliferate at about the same rate (exponential growth).
To mimic the responses at different initial microbial load, homogenate of cod sample with low level of microbial contamination (<10^2 cfu/g, 1:20 homogenate dilution) was spiked with increasing concentrations of *E.coli* and measured. Fig 9.2 shows that spiked homogenates produce consistent profiles in the assay and give a linear

relationship between TT and *E.coli* concentration (cfu/ml). In this matrix, doubling time of *E.coli*, the limit of detection and maximal monitoring time were determined: 25.6 min, 50 cfu/g, and 10-12 h, respectively.

[Fig 9.2]

From these experiments, 1:20 dilutions of fish homogenates was selected as standard for further work, as it provides convenience with pipetting (standard 100 µl aliquots throughout) and no undesirable matrix effects.

9.3.3 Establishment of Calibration

To establish the relationships between the TVC (cfu/g) and TT (h) and generate calibrations which can be used for the analysis of samples with unknown microbial load, we analyzed panels of samples of different fish types (fresh salmon, cod, whiting, plaice and mackerel). The selection of fish was made to cover the spectrum of different types of tissue, i.e. white and red tissue, fresh and seawater fish, flat and thick body, low-fat and oily fish. Each sample homogenate underwent parallel analysis by conventional agar plating TVC method and by the respirometric assay. Accounting for potentially slower growth rates of microorganisms present in fish samples, plate monitoring time was extended to 12-16 h.

From the ANOVA analysis (results not shown) it was apparent that the only significant source of variation in the calibration is in relation is TVC level. More specifically, the calibration relation is not significantly different across species or trial or any combination of factors. This justifies a simple regression model of the form (2), where other factors are not included.

Fig 9.3 shows the combined calibration for the four fish species (salmon, cod, whiting and mackerel), after exclusion of the top two outliers. We see that the majority of points lie within the ± 1.39SD band, as expected. However, the R^2 value is moderate, indicating the presence of substantial variability in the data. The relationship obtained from the fitting and analytical equation for conversion of measured TT values into cfu/g is given in Fig 9.3. It is worth noting that inclusion of the two outliers significantly changes the calibration relation (it becomes TT =– 2.40(cfu/g) + 18.69). Treating fish types individually, the R^2 and the parameters of equation are as follows, for salmon (TT = -2.94(cfu/g) + 21.35, R^2 = 0.72) and whiting (TT = -2.36(cfu/g) + 18.73, R^2 = 0.70) is a higher R^2 obtained and for cod (TT = -2.41(cfu/g) + 19.01, R^2 = 0.53) and mackerel (TT = -1.49(cfu/g) + 13.00, R^2 = 0.39) a lower one.

[Fig 9.3]

As already mentioned fish is a quite difficult product to work with as it is less known the chemical and physical changes that fish is developing within muscle tissue post-mortem and how this is influencing the respirometric method. In an earlier work performed on different types of raw meat (beef, pork, lamb and poultry) by the same method (O'Mahoney, et al., 2005), a combined R^2=0.86 was obtained, as compared to R^2=0.56 for the fish.

9.3.4 Problematic Samples

At the same time, certain fish samples, particularly fresh plaice, were seen to produce high scattering of results of the respirometric assay and worse correlation with conventional TVC test (Fig 9.4). We explain this by plaice being a flat fish with a low ratio of muscle tissue volume to skin surface, resulting in a less predictable sampling of surface bacteria than for the other fish species tested. Likewise, the scattering of results from frozen fish samples was significantly greater than for fresh fish, although the calibration equation was similar. This suggests that freezing impacts the bacteria in fish tissue and affects their normal growth during the assay. Particular reasons may include freeze damage to microorganisms by the crushing and spearing action of ice crystals as well as lethality resulting from cell dehydration effects. The rate of freezing, storage temperature and temperature fluctuations during storage influence the extent of sub lethal injury and death of micro organisms. Thawing is more injurious to micro organisms than freezing, and the effects vary according to species. Even simple thawing of a frozen microbial population without intervening storage causes slight to moderate reduction in number of live organisms (Robinson, 2000). Due to the large variance of results, plaice and frozen fish samples were excluded from further testing in the respirometric TVC assay.

[Fig 9.4]

9.3.5 Assessment of Ruggedness of Respirometric TVC Assay

The results of assay ruggedness test with respect to pipetting volume are summarised in Table 9.2. Using this data, ruggedness was tested against two factors: 1) the level of sample contamination (high and low cfu/g), and 2) the pipetting volume

(70, 100 and 120 μl). To examine the relative contributions of these factors to measurement variation, we modelled log (Response) as a function of them, yielding the analysis of variance (ANOVA) results shown in Table 9.3.

[Table 9.2]

[Table 9.3]

As expected, Table 9.3 shows that the main source of variability is sample microbial load (*cfu/g)*, whereas variability due to pipetting volume and residual error appear to be negligible by comparison (relative means square of 0.1% and 0.02% respectively). Further analysis showed a marginally significant trend (p-value = 0.04) in measurements due to change in assay volume. We note that the significance occurs due to the very small value of residual error (due to replication).

Probe concentration ruggedness test produced similar results (not shown). The main source of variability was again sample microbial load (*cfu/g)*, whereas variability due to probe concentration and residual error appear to be negligible by comparison (relative means square of 0.2% and 0.01% respectively). Further analysis showed a significant trend (p-value = 0.003) in measurements due to change in probe concentration. We note that the significance occurs due to the very small value of residual error (due to replication).

9.3.6 Assay validation

Fig 9.5 shows correlation between the two methods. A diagonal line shows the ideal correlation between the two methods (predicted=observed line). Although the

validation trend line produced by linear regression fit of all the data points does not match this ideal line, one can see that 93.5% of data points (158/169) lie within ±1.96SD of the ideal line. This is close to the expected 95%. One can see that respirometric assay provides the accuracy of TVC determination in fish samples of approximately +/- 1 log (cfu/g). For a simple, fast, high throughput screening test, this analytical performance is considered to be quite good (though not as good for raw meat samples).

[Fig 9.5]

In addition, storage trials were carried out to ascertain the natural spoilage rates of fish at different temperatures. It is known that bacteria grow faster at high temperatures. The Q10-rule implies that for every 10ºC increase in temperature the growth doubles, i.e. Q10=2 (Dworkin, Falkow, Rosenberg, Schleifer & Stackebrandt, 2006; Tjoelker, Oleksyn & Reich, 2008). Of course this can vary depending on bacteria and sample used. Representative data for salmon are shown in Fig 9.6.

[Fig 9.6]

After linearization of the three curves slopes were observed which increase from 4ºC to 24ºC with a factor of Q10=2.5, particularly 4ºC=0.31, 14ºC=0.78 and 24ºC=1.97.

9.4 Conclusions

A simple, rapid and robust screening test for TVC in raw fish sample was developed which relies on fluorescence based micro-respirometry in standard 96-well plates. Assay conditions including pipetting volumes, sample dilution, matrix effects were optimised to streamline the procedure and produce reliable results. The test was applied to five different fish types: fresh cod, salmon, whiting, mackerel and plaice as well as frozen fish (all used as crude homogenates in PBW) for which individual calibrations and combined calibration were generated. The test showed good correlation with conventional TVC test (ISO:4833:2003), analytical performance and ruggedness with respect to variation of key assay parameters (probe concentration and pipetting volume). Although linear regression fit was not perfect (R^2=0.56), vast majority of data points lay within 1.39SD. At the same time, plaice and frozen fish showed lower correlation with conventional TVC method which can be explained by generic structure of these fish samples resulting in a less predictable sampling and higher scattering of data. The respirometric test was then validated with a panel of unknown fish samples (N=169), where it correlated well with conventional TVC test. Although correlation trend line produced by linear regression does not match the ideal line, 93.5% of points lie within ±1.96SD, i.e. very close to the anticipated 95%.

Overall, the respirometric fish TVC test provides general simplicity (homogenisation and pipetting) and miniaturisation, a dynamic range (10^4-10^7 cfu/g), accuracy of +/- one log (cfu/g), high speed and automation. Highly contaminated samples can be identified quickly (2-12 h depending on the level of contamination – see Fig 9.3), positive samples can be seen as the measurement progresses (real-time data output). Its ability to assess highly perishable products such as fish in <12 h shows good application usage for industry in testing samples far quicker and reliably

and making safety and quality assessments in large number of samples. The test offers simple set-up (conventional microplates and fluorescent reader), significant savings on labor, lab space and waste requirements, and it overcomes many drawbacks of conventional TVC testing.

9.5 Acknowledgement

Financial support of this work by the Irish Department of Agriculture, grants 06RDC468 and 08RDC642, is gratefully acknowledged.

9.6. TABLES AND FIGURES

Table 9.1 Ruggedness test parameters

No.	Assay variable	Standard protocol	Test parameters	
1	Pipetting volume (µl)	100 + 100 + 100	70 + 70 + 70	120 + 120 + 120
2	Probe concentration (%)	100	50	150

Table 9.2 Experimental data for pipetting volume ruggedness test

		Pipetting vol		
Pipetting vol error	Replicate no.	70µl	100µl	120µl
Log sample (high CFU)	1	6.79	6.96	6.98
	2	6.79	6.96	6.97
	3	6.79	6.95	6.97
	4	6.79	6.94	6.96
	5	6.79	6.94	6.96
	Avg	6.79	6.95	6.97
	SD	0	0.01	0.01
Log sample (low CFU)	1	3.75	3.88	3.99
	2	3.66	3.77	3.93
	3	3.57	3.66	3.84
	4	3.48	3.57	3.66
	5	3.11	3.38	3.66
	Avg	3.51	3.65	3.82
	SD	0.25	0.19	0.15
Log sample (negative control)	1	<3	<3	<3
	2	<3	<3	<3
	3	<3	<3	<3
	4	<3	<3	<3
	5	<3	<3	<3
	Avg	NA	NA	NA
	SD	NA	NA	NA

NA, not applicable, based on data.

Table 9.3 Two-factor ANOVA for sample volume ruggedness experiment

Factor	Degrees of freedom	Sum of Squares	Mean Squares	F-statistic	P-value
Microbial load (cfu/g)	1	78.83	78.83	3976	<0.001
Assay volume	2	0.29	0.147	7.4	0.002
Error	26	0.52	0.02	-	-

- NA, not applicable, based on data.

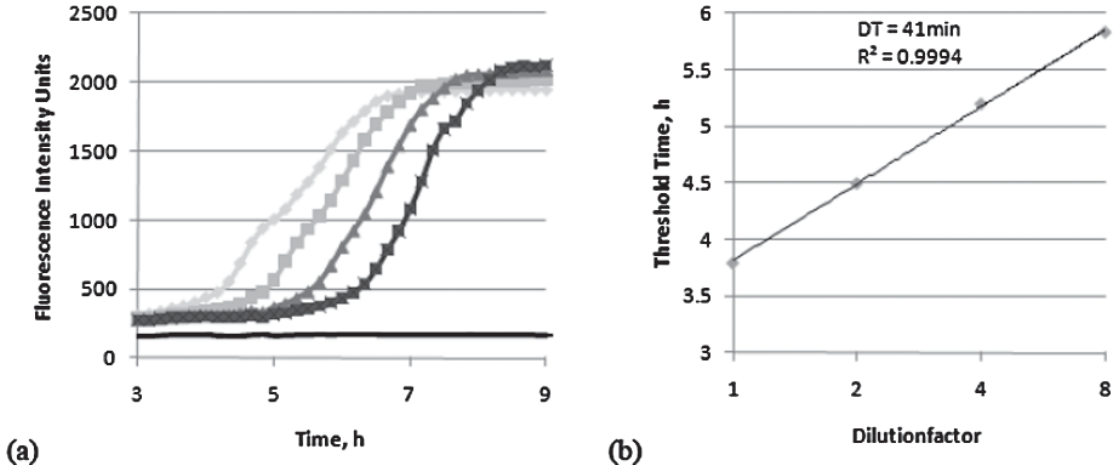

Fig 9.1 Typical profiles (a) of salmon homogenate measured at different dilutions: 1:10, 1:20, 1:40 and 1:80 (from left to right) and negative control (flat line), and the relationship (b) between TT (at 400 FU) and sample dilution. Doubling time (DT) calculated from the slope is shown on the graph.

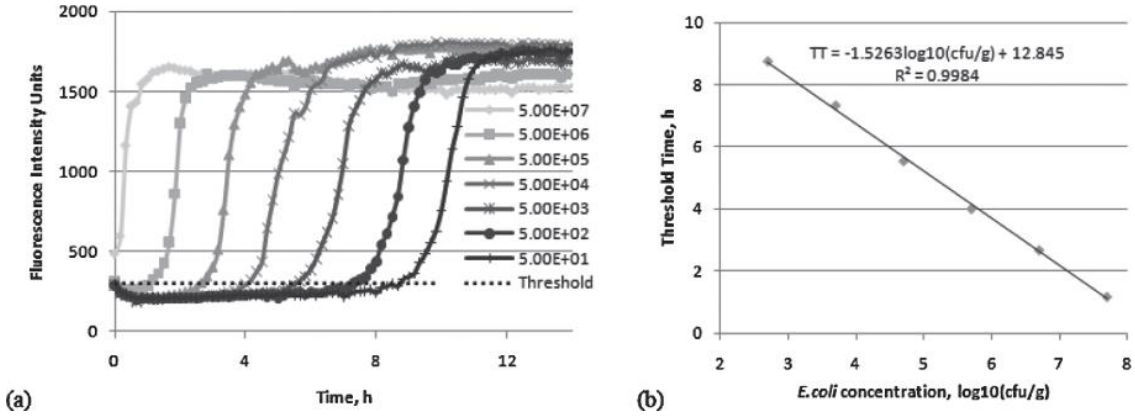

Figure 9.2 Respiration profiles (a) of cod homogenate samples (~10^2 CFU/g) spiked with concentrations of E.coli (threshold), and the resulting relationships (b) between TT and E.coli concentration.

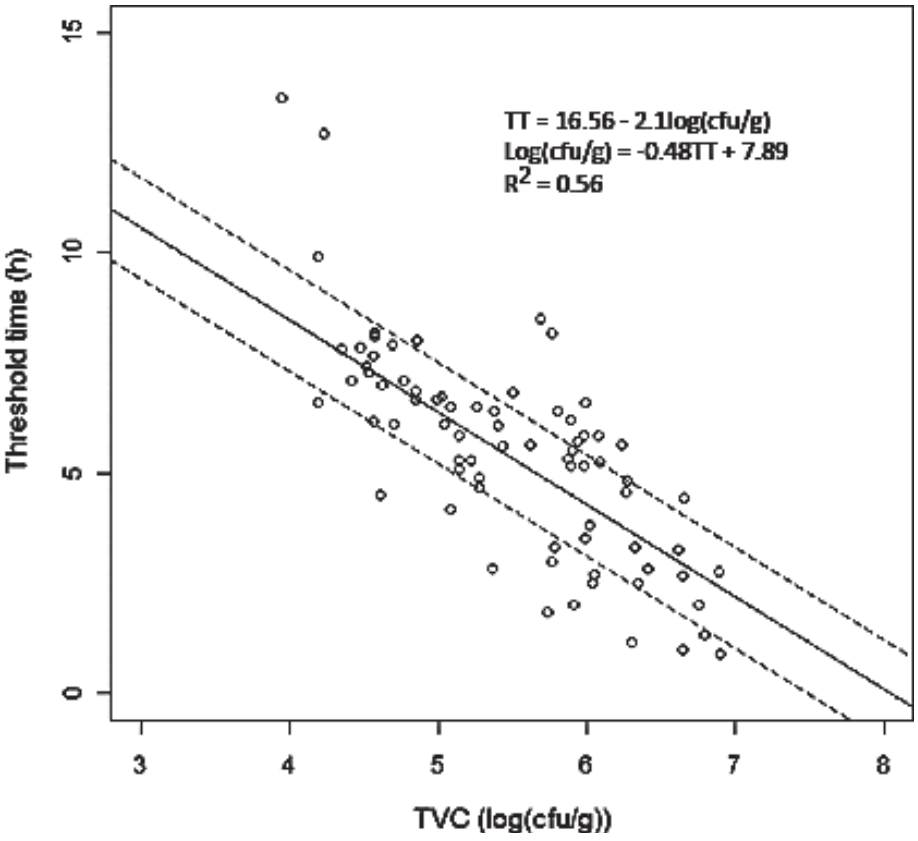

Figure 9.3 Combined calibration curve (solid line) for the fish samples (n = 75; Cod, 23: mackerel, 8; Salmon, 23; whiting, 21), TT (hours) versus log (CFU per gram). Dotted line denotes 1 SD (SD = 1.39) band around the calibration curve.

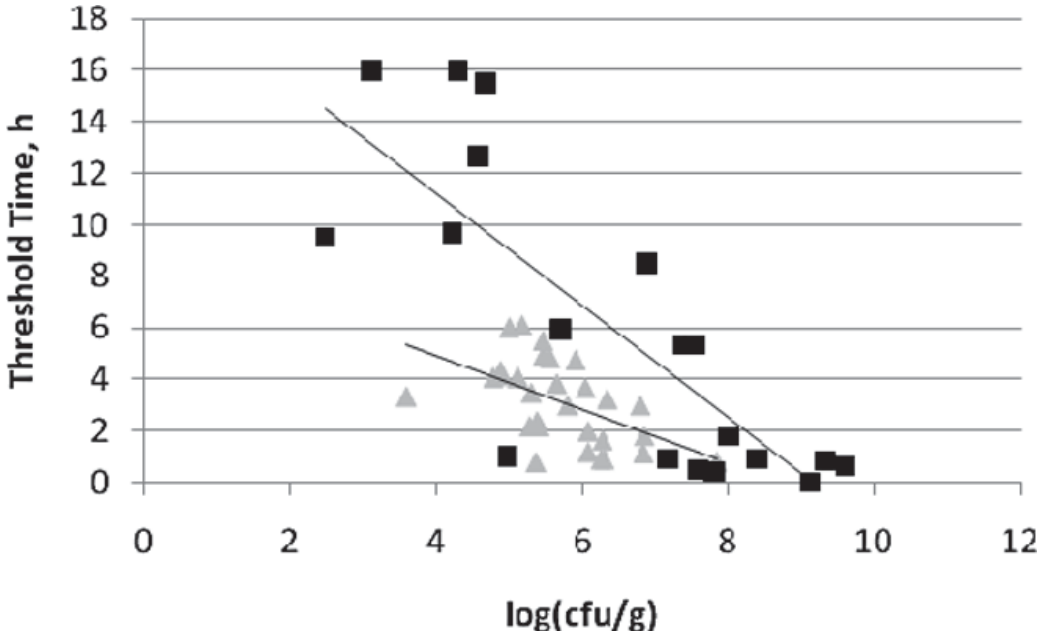

Figure 9.4 The relationship between the Respirometric TT values and CFU per gram in the standard TVC test for plaice (▲; $y = -1.0597x + 9.1813$, $R^2 = 0.295$) and frozen fish (■; $y = -2.1725x + 19.92$, $R^2 = 0.639$) samples.

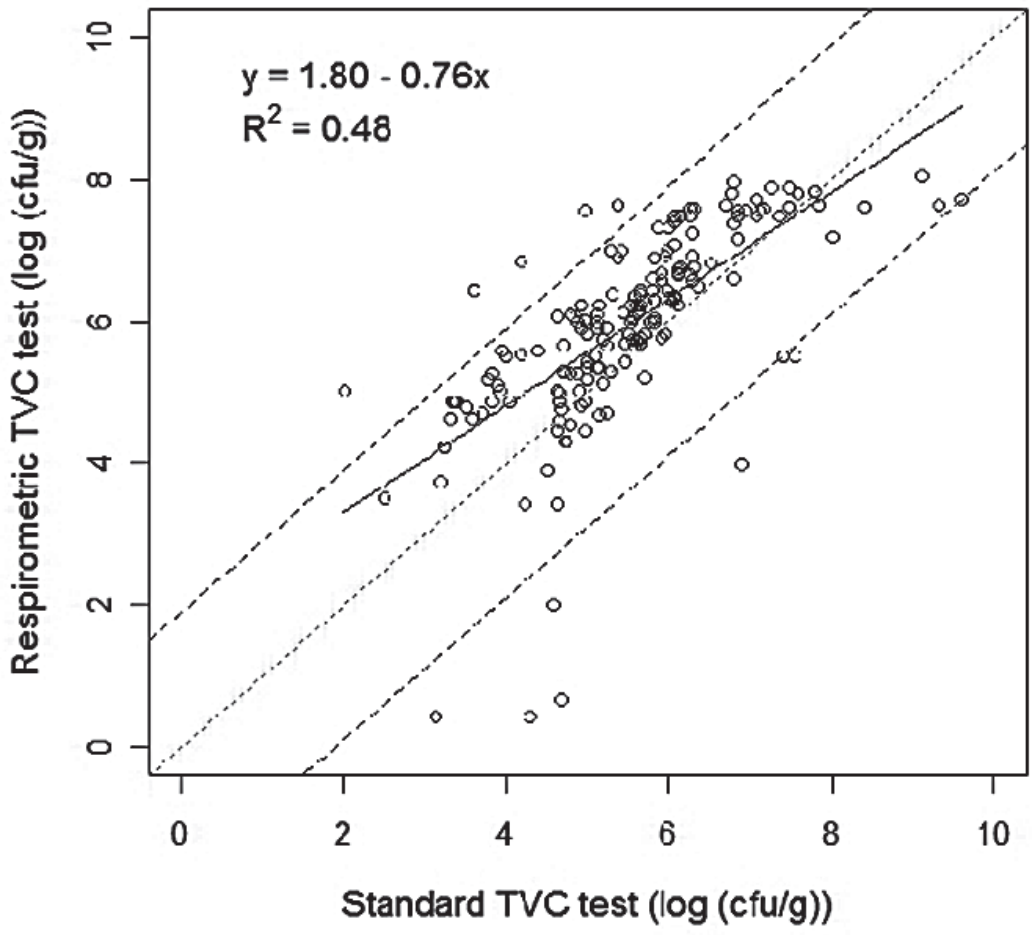

Figure 9.5 Assay validation with unknown fish samples form different retailers. Solid line was produced with linear regression fit of the Respirometric and standard TVC test values. Dotted line shows the ideal case: y = x. From regression, SD = 0.97 CFU/g. Dashed lines indicate sampling variability range (ideal is ± 1.96 SD).

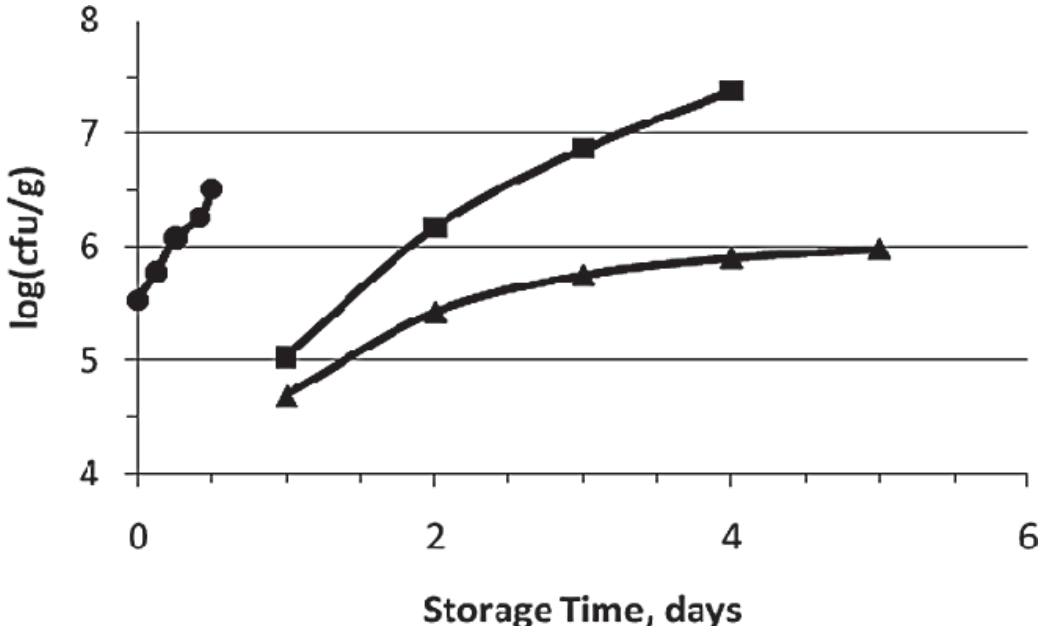

Figure 9.6 Time profiles of microbial load (log cfu/g) for salmon fillets stored at different temperatures: 24ºC (●), 14 ºC (■), and 4 ºC (▲)

Overall Discussion

Sensors are a novel and valuable addition to the field of smart packaging technologies. Sensing devices, such as the sensor system utilised in this thesis, allows for communication to manufacturers and retailers of physical and chemical modifications taking place within their food products immediately post-packaging and throughout the products shelf-life. Information collected from these devices can allow a user to non-destructively assess their products before being released into the supply chain, along the supply chain and at the point of sale. This leads to continuous faulty product detection and assurances that the intended processing steps and atmospheres to ensure a stable and targeted product quality and shelf- life is ultimately delivered to the consumer.

The first phase of research for this thesis began with the selection of an optical O_2 sensor that could be successfully integrated into food/beverage packaging systems. Consequently, Chapter 2 describes the selection of dyes and support materials that were suitable for producing optical O_2 sensor systems for food trials. Experimentation began with fluorescent dyes capable of monitoring O_2 in a non-destructive and reversible manner, with no permanent chemical reactions having taking place. Optical O_2 sensors based on phosphorescent platinum and palladium octaethylporphyrin-ketones (OEPk) in nano-porous high density polyethylene (HDPE), polypropylene (PP) and polytetrafluoroethylene (PTFE) polymer supports were evaluated. The sensors were heat-treated at elevated temperatures to enhance their stability, and compared with established sensors based on Pt-OEPk in polystyrene on Durapore films. The HDPE- and PP-based sensors showed good sensitivity to O_2, reproducibility and stability. Sensors used were calibrated against 0-21% O_2 over a temperature range of -10°C to +40°C, and were found to be suitable for food packaging applications. The sensors were easily fabricated and provided for a new

material for O_2 sensor applications. The PE- and PP-based films compared favourably with established Durapore-based sensors. Durapore-based sensors are highly suitable for use in the food packaging industry.

Using the knowledge gathered from the research conducted in Chapter 2, the next stage was to assess the performance of the sensor technology in non-destructively monitoring O_2 levels in O_2-sensitive commercial food packaging systems. Consequently, the objective of Chapter 3 was to investigate the effectiveness of the O_2 sensor technology in a vacuum packaged cheese string product. A local manufacturer of vacuum packed cheddar cheese had detected shelf-life issues pertaining to their product. The optical sensor developed in this thesis was applied to the commercial cheese packaging materials and detected elevated levels of O_2 (3%) immediately post packaging. Continued O_2 monitoring by the sensors showed increasing levels of O_2 over time (up to 8.2% after 6 days). Due to the information obtained by optical sensor it was clear that a packaging fault was present. In order to ascertain what fault was being continuously produced in all samples, a series of simple tests were carried out. Visual checks on packs yielded no apparent faults, however submerging the packs in water clearly showed O_2 leaking from within the packs. With the origin of the leaks marked, they were assessed under a light microscope to show the appearance of holes being formed in the corners of the cheese packs. It was believed that the use of excess pressures caused an over-stretching of the plastic films at these rigid points of the product packs. The manufacturer was informed and the problem rectified by minimising the pressures used during the stretching of the plastic around the cheese products. Therefore, the sensors proved themselves to be a valuable tool in evaluating the performance of low O_2 barrier

packaging around O_2-sensitive foods as indicated by the presence of a fault, thereby indicating containment failure.

Chapter 4 describes the use of the sensor in another O_2 sensitive product; in this case larger beer. There were two objectives to this study; firstly to design a 'universal' sensor that could withstand even the most harsh food packaging processes and secondly, the detection of O_2 in headspace of the beer bottles and its effect on shelf-life quality. The development of a 'universal sensor' that incorporated an adhesive support material was selected for adhesion to the inside of bottles and strong enough to withstand rigorous pre-processing and food processing steps. An O_2 sensor was prepared with an adhesive strong enough to withstand pre-processing steps like washing and processing steps like pressure filling, inversion and pasteurisation. The in-bottle O_2 sensor showed great application in determining the levels of O_2 present immediately post-packaging. The levels of O_2 present in sealed beer bottles at the pre-pasteurisation stage were as high as 5%. As no vacuum or modified atmosphere is applied during the capping process and an elimination of air is dependent upon the frothing of beer brought about by carbonation prior to sealing, it was investigated if the levels of O_2 observed had an effect on product quality and shelf life. The levels of O_2 reported pre-pasteurisation had all dissipated after the heat treatment stage (pasteurisation). This quantity of O_2 present in bottles prior to pasteurisation has no means of being removed from the sealed bottle, so it was believed that the O_2 had 'transferred' into the liquid and chemically reacted there. This theory was tested over the course of the shelf-life, where samples with varying O_2 levels present pre-pasteurisation were assessed by sensory analysis to determine product quality and acceptability. Beer samples containing 2% O_2 or greater were found to be significantly unacceptable before a six month storage period was reached, whereas

liking of flavour ($P \leq 0.01$), fresh flavour ($P \leq 0.05$) and overall acceptability ($P \leq 0.01$) were all negatively correlated compared to beer samples containing 1% O_2 or less. The level of O_2 detected by the O_2 sensor was found to be capable of being used to pre-determine the shelf-life quality of the beer immediately after packaging. This information could provide manufacturers with valuable acceptable limits ($O_2 \leq 1\%$) at pre-pasteurisation stage to ensure optimal product shelf-life quality before samples leave the manufacturing facility. Such uses for sensor technologies would allow for greater quality control and process optimisation, thereby enhancing the quality assurance for bottled beer.

Chapters 5 and 6 employ the O_2 sensor in modified atmosphere (MA) packs of respiring food products and where the atmosphere in direct contact with food is continuously changing. Fresh, ready-to-eat salads were assessed for their O_2 utilisation in packs over time. Chapter 5 describes how commercially produced ready-to-eat salads packed in MAP (5% O2, 5% CO2 and 90% N2) were depleted of O_2 by day 7 of storage, demonstrating that the typical employment of 5% O_2 in packs was insufficient in providing adequate O_2 for continuous and typical aerobic respiration. It was investigated if higher levels of O_2 use allowed for continued respiration throughout storage, maintained product quality, yet circumvented anaerobic spoilage.

Elevated O_2 levels of 21, 40 and 60% were chosen to ascertain O_2 affects on MAP product quality and shelf-life. Of the three packaging treatments; MAP1 (21% O_2, 5% CO2, 74% N2), MAP2 (45% O_2, 5% CO2, 50% N2) and MAP 3 (60% O_2, 5% CO2, 35% N2), the highest O_2 level in MAP 3 showed the least O_2 consumption by all salads over time whilst providing no detrimental effects on either sensory quality or shelf-life stability of the salads. The elevated levels of O_2 in MAP did not

significantly improve product quality; however, these levels provided abundant levels of O_2 freely available for fresh respiring produce if required.

The research conducted in Chapter 5 was continued in Chapter 6. In this study, individual salad leaf varieties were packaged separately under MAP conditions and assessed in terms of O_2 consumption over time. Seven leaf varieties (typically used together to form a commercial 'Italian salad mix') were packed in 21% O_2, 5% CO_2 and 74% N_2 to provide adequate O_2 for respiration as determined in Chapter 5. Each leaf variety showed a varying demand for O_2 as demonstrated by the O_2 sensor. Microbial and sensory analysis was also carried out for all packaged products. It was proposed that a mix of the four slowest respiring leaves could result in a product with extended shelf-life. The four slowest respiring leaves were prepared and tested against two commercially produced alternatives. The new salad formulation termed mix 3 was found to consume the least amount of O_2 over time, resulting in a lower microbial load and a higher sensory acceptance ($p < 0.05$) in terms of overall appearance and acceptability compared to the commercial mixes used in this study. The identification of lower O_2 dependant leaves resulted in a product that could be used by manufacturers to extend the shelf-life of a highly perishable product. In this study, O_2 sensors showed their potential in assisting product optimisation.

Chapters 7 and 8 focused on the use of multiple smart packaging technologies to assess the levels of O_2 in MAP commercial food products and to further extend the shelf-life of these products. Chapter 7 describes the assessment of existing packaging practice for commercial MAP bread samples. Information obtained by the O_2 sensors determined the presence of excess levels of O_2 in bread packs. Following the accurate delivery of target gas (10% C O_2, 90% N_2) resulting in negligible levels of O_2 (<0.1%), the shelf life of MAP bread samples were restored to the expected 6 days.

Bread samples were then packaged, using the target MAP atmosphere (10% C O_2, 90% N_2) in combination with ethanol, to determine if product shelf-life could be extended through the control of spoilage microorganisms. Ethanol was administered in two ways; a direct ethanol surface spray (ES) and by the use of an ethanol emitter (EE). Control samples packed in air packaged both with and without EE and ES treatments were used for comparative purposes. The use of EE was most effective treatment in MAP breads and this was demonstrated through the degree of mycological control delivered. The use of EE in bread packs showed that the expected shelf-life of commercial MAP products could be extended by a further 11 days. MAP control samples (packaged in air) without ethanol showed excessive mould growth within 5 days of storage and this rapid growth was evidenced through the consumption of O_2 in packs as demonstrated by the O_2 sensor. Sensory evaluation of breads demonstrated that the use of ethanol in packs produced no negative organoleptic issues.

Chapter 8 describes how faulty vacuum and gas delivery systems in commercial packaging plants can be responsible for shorter than expected shelf-life in pre-cooked convenience foods. O_2 sensors determined that excess levels of O_2 were present in a range of commercial pre-cooked convenience foods (>15%) after vacuum packaging and modified gas filling. A range of combinations of vacuum and gas fill were carried out to ascertain the greatest O_2 reduction in food packs. The new levels of 0.35 MPa vacuum and 0.02 MPa gas flush reduced O_2 by 6-9%. The reduced levels of O_2 reflect the microbial quality of the products; microbial counts being reduced as a consequence. Duplicate samples of these products were also packaged but contained EE as described in Chapter 7. The use of EE extended the shelf-life of all products to

35 days. These studies clearly demonstrate how smart packaging technologies can be used to enhance product quality and safety, as well as extending product shelf-life.

The final section of this thesis investigated the possibility of modifying the O_2 sensor technology described in order to produce a probe which might be used to rapidly determine the total aerobic viable counts (TVC) associated with food products. Therefore, Chapter 9 describes a simple, rapid and robust screening test for TVC in raw fish which was developed using fluorescence based micro-respirometry. Using 96 well plates, fish homogenates were prepared and mixed with a phosphorescent O_2 sensing probe. Samples were covered in mineral oil to prevent contact with atmopshereic air and monitored on a fluorescent reader at 30ºC. Characteristic profiles were produced with a sharp increase in fluorescence referred to as the threshold time (TT) as O_2 in samples was being utilised in relation to microbial growth. Five species of fish were chosen for assessment; Salmon, Cod, Plaice, Mackerel and Whiting. Conventional TVC testing was used to determine the relationship of initial microbial load and threshold time (TT), resulting in a calibration curve being generated and the test validate for 169 samples. A dynamic range of 10^4-10^7 cfu/g samples was established and the assay completed within 12 hours. This test determined the microbiological quality of fish, cfu/g sample levels and deteriorative spoilage time profile for each sample. The ability to analyse a highly perishable food product for microbiological quality was achieved simply, rapidly and in a more cost-effective manner when compared to more conventional means of microbial enumeration.

The research carried out in this thesis has demonstrated the ability of optical O_2 sensors to non-destructively monitor O_2 in commercial food products. This has resulted in establishing a universal O_2 sensor, which before this thesis had been

limited to a laboratory environment and introduced the intelligent device into industrial and commercial applications. The accuracy, ease and versatile nature of the sensor show great application in food packaging where the quality of the food can be monitored throughout storage and shelf-life.

Overall Conclusions

The objective of this thesis was to extensively assess the use of optical O_2 sensors within a range of commercial food packaging systems. In summary, the research findings from this thesis indicate the potential of O_2 sensors in non-destructively monitoring the O_2 levels in a range of packaged commercial foods. The ability to monitor O_2 in packaging systems that range from modified atmosphere to vacuum is thoroughly described. The commercial value of such a technology is apparent, where foods susceptible to O_2 in terms of spoilage and sensory quality are described. The potential uses for such O_2 sensors can be of great importance to food manufactures, where products can be assessed immediately post-packaging and throughout the shelf-life period. The unique versatility of the sensor would be of benefit to a wide range of food manufacturers. The ability to determine O_2 levels in products at manufacturing stage provided an insight into the quality and shelf-life stability of various products and demonstrated the effectiveness, or otherwise, of the packaging materials and systems employed for product packaging. Successful modification of the O_2 sensor technology to produce a probe which could be used to rapidly determine the total aerobic viable counts (TVCs) associated with food products was achieved. A simple, rapid and robust screening test for TVCs in raw fish was developed using fluorescence-based micro-respirometry.

Future Work

The ability of sensors to monitor O_2 in industrial food applications has now been thoroughly described. Future work will involve further research into materials to help reduce the cost of the sensors, to be more industrially attractive, as well as an attempt in reducing the size of the sensor for discrete placement in packs. Implementation of the sensor within packaging materials can also be researched.

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