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Development of a smart packaging for the monitoring of fish spoilage

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Abstract

There is much interest from the fisheries industry in developing rapid methods to evaluate real-time freshness of fish and seafood products. Emphasis is on the ones that would reflect and account for the products history and their storage conditions from "harvest-to-home". The development of a "smart packaging" that monitors the microbial breakdown products in the headspace of packaged fish is described. When fish spoils it releases a variety of basic volatile amines which are detectable with appropriate pH indicating sensors. These are prepared by entrapping within a polymer matrix a pH sensitive dye that responds, through visible color changes to the spoilage volatile compounds that contribute to a quantity known as total volatile basic nitrogen (TVB-N). Laboratory trials on fresh fish filets showed that the sensor accurately tracks the increase in amines concentration in the package headspace. The response was also found to correlate to changing microbial populations (total viable count or TVC and *Pseudomonas* spp.). In addition, leaching of the dye was assessed over time to assess the suitability of the sensor formulation for food packaging application.

Keywords: Smart packaging; TVBN; Volatile amines; Fish spoilage indicator

1. Introduction

The potential of smart packaging technology is far reaching, from food safety and drug's use monitoring to postal delivery tracking and embedded security tags (Butler, 2004). For the customer, a safer, easier, more interactive and more enjoyable life. For the manufacturer, it can identify supply chain inefficiencies, reduce costs and errors, improve product performance and ultimately increase profit. But it also has the potential to reclaim lost consumer confidence in the food supply given the recent food crisis. Furthermore, within the European Union (EU), food "traceability" is now a legal requirement since the new General Food Law (European Parliament & of the Council, 2002) came in effect on 1st January 2006. This will establish a chain of responsibility throughout the whole food chain and there is consequently great interest from the fisheries industry, retailing industry, consumer's right watchdogs and food safety controlling bodies in developing accurate, cost effective, rapid, reliable, non-invasive and non-destructive methods or devices to evaluate real-time freshness of fish and seafood products. Emphasis is on the ones that would reflect and account for the products history and their storage conditions from "harvest-to-home". An excellent review of existing multi-sensor techniques has been provided by Nesvadba (2003).

One concept to meet this requirement is that of the development of a simple freshness color indicator in the form of in-package sensor spots that monitors spoilage in fish and seafood products. Volatiles amines, such as trimethylamine (TMA), ammonia (NH_3) and dimethylamine (DMA) contribute to a quantity known as total volatile basic nitrogen (TVB-N) and are the characteristic substances responsible for the fishy odour and flavour encoun-

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tered in fish after having past the initial phase of freshness. Their contents in freshly caught marine fish investigated immediately after hauling is normally found to be on a very constant level (Oehlenschlager, 1997) Over time and depending on the fish species, feeding habits, temperature and general storage conditions (atmosphere, aw, microbial cross-contamination etc.) TVB-N levels increase as a result of bacterial metabolism. Fresh fish and seafood harbours a quite heterogeneous micro-flora. Often during storage, a specific bacteria group, known as specific spoilage organisms (SSO), will outgrow the others and cause the most chemical changes often. In the case of marine, temperate water fresh fish stored aerobically in chilled conditions (0–4 °C), *Pseudomonas* spp. and *Shiwanella putrefaciens* are the typical SSO involved (Huss, Dalgaard, & Gram, 1997). Using the proposed pH sensor in correlation with continuous temperature logging may help identify patterns of abusive fish storage conditions (Crowley, 2005).

An inexpensive and simple chemical sensor that allows the real-time and non-destructive determination of fish freshness was described in a previous study (Pacquit et al., 2006). In an enclosed food package, as the fish product spoils, a pH increase occurs over time within the headspace which can be detected with an appropriate pH indicating sensor. The fundamental characteristic of pH indicator dyes that change color when placed in an acidic or basic environment is the key element of this sensor. The sensor response is independently monitored with a simple, inexpensive reflectance colorimeter that we have developed based on LEDs and a photodiode. This apparatus was also extensively described in our previous work (Irish Patent, 2004; Pacquit et al., 2006). It clearly demonstrated the correlation of the sensor response and the change in bacterial population over time. The fish used was Cod and was caught at the end of spring.

In this paper, we present further results from the ongoing assessment of this sensor formulation. In particular, the occurrence of dye leaching was investigated as well as the correlation between sensor response and microbial growth over time in fish caught at the beginning of the autumn. Amines levels change with fish species but also feeding habits, fishing ground and depth of living (Oehlenschlager, 1997). Fish are available virtually all year round and not two harvests are the same. If incorporated within a smart packaging, this sensor must be consistent with all batches of fish from a particular species. This study attempted to investigate variation in sensor response with a different batch of Cod caught at a different time in the year and probably in a different fishing ground (details of fishing ground not available) as well as with a new fish species, Whiting.

2. Experimental

2.1. Materials

Bromocresol green or BCG (sodium salt), cellulose acetate (M_w approximately 30,000 g mol^{-1}) and all ammonium bromide salts used in this study, such as octadecyl trimethyl ammonium bromide, tetrahexyl ammonium bromide, tetraoctyl ammonium bromide or cetyl trimethyl ammonium bromide, were obtained from Sigma–Aldrich (Dublin, Ireland); dibutyl sebacate (DBP) and nitrophenyl octyl ether (NPOE) were obtained from Fluka Chemicals (Dublin, Ireland) and optically clear polyethylene teraphthalate (PET, 175 microns grade) sheets were obtained from HIFI Industrial Film Ltd. (Dublin, Ireland).

2.2. Sensor fabrication

Sensor spots are prepared by entrapping within a polymer matrix solution a selected pH sensitive dye (i.e. BCG) which is then spin-coated at 1000, 2000 and 3000 rpm onto optically clear PET substrate discs as described previously (Pacquit et al., 2006). Alternatively, the sensors can be made by using an automatic pipette and simply depositing 3–4 μl of the same solution within a series of self-adhesive paper ring reinforcements on the same PET substrate (Byrne, Lau, & Diamond, 2002). The PET discs were left overnight in the dark to dry after which individual 5 mm diameter discs were punched out with a common office desk hole puncher. The method was found very reproducible. A hydrophobic gas permeable membrane was added to protect the sensor coated surface from excess humidity while allowing gaseous compounds to pass through. The optically clear PET allowed diffuse reflectance measurements to be made from the sensor rear (i.e. from the side not exposed to the sample).

2.3. Leaching studies

A very simple way to prevent the leaching of a water soluble dye from a polymer matrix is the addition of a quaternary ammonium salt to form an ion pair and create a lipophilic film as described by Grady, Butler, MacCraith, Diamond, and McKervey (1997), Weigl and Wolfbeis (1995) and Werner, Klimant, and Wolfbeis (1995). Consequently, a series of ammonium salts (cetyl trimethyl ammonium bromide, Octadecyl ammonium bromide, Tetrahexyl ammonium bromide and Tetraoctyl ammonium bromide) were tested. Formulation coated PET strips were soaked in a small water container for approximately 48 h after which a wavelength spectrum of the water (400–700 nm) was carried out to detect the presence of BCG, if any.

2.4. Film thickness: differences in signal steady-state changes

In the same experiment described previously (Pacquit et al., 2006) where nine replicate tissue samples of 1.05 ± 0.05 g were removed from the cod filets and allowed to spoil at 20 °C, response differences among sensors of various thicknesses (1000, 2000 and 3000 rpm) were investigated. Responses were monitored every 2 h with the reflectance colorimeter. At each time interval, five measure-

ments were performed per sensor so that a total of 15 measurements were carried out for sensors of each thickness.

2.5. Fish spoilage trial

2 set of sensors (2×5) spin-coated at 1000 rpm, were placed at the bottom of wells in two separate standard 24-well plates. Freshly caught whole cods (*Gadus morhua*) and whole whiting (*Merlangius merlangus*) were filleted at a local harbour market (Howth, Ireland) and placed in a sealed ice container. Aseptic techniques such as the use of disposable gloves, bactericide built-in cutting board and flame sterilized scalpel were used to avoid sample contamination. Ten replicate tissue samples of $1.05\ g \pm 0.05$ were removed from the filets. $5\times \sim 1$ g cod and $5\times \sim 1$ g whiting filet samples were placed in separate individual polypropylene caps and inserted in the inverted (wells opening facing down) 24-well plate (Fig. 1). Each well cap was sealed with fast cure epoxy to create a permanent gas-tight seal and prevent leakage of amines. Samples were at no time in direct contact with the sensors (i.e. only headspace was sampled). Reference wells did not contain any fish sample and the whole plate-well was left at room temperature (19–21.5 °C) over a period of 55 h. The sensors responses were monitored with the optical scanner described previously (Pacquit et al., 2006). In this paper, BCG sensors displayed a color change from yellow to blue, easily visible to the naked eye (Fig. 2).

2.6. Microbial analysis of cod samples

Simultaneously to the above spoilage trial, 19 samples of approximately 25 g were removed from the same cod filets, under the same aseptic conditions, and placed in zip lock freezer bags. They were also allowed to spoil at room temperature under the same conditions and every 2 h, along with every sensor response measurement, a sample was transferred into a freezer. Samples were in a frozen state at core in less than 1 h. TVC counts were determined, using the pour plate method, on plate count agar (Oxoid CM463) while the spread plate method was used on agar base (Oxoid CM733) with CFC (cetrimide fucidin cephalosporin) selective supplement (Oxoid SR103) to give Pseudomonad counts. Plates were counted after 48 h incubation at 30 °C and results were correlated with the sensor response.

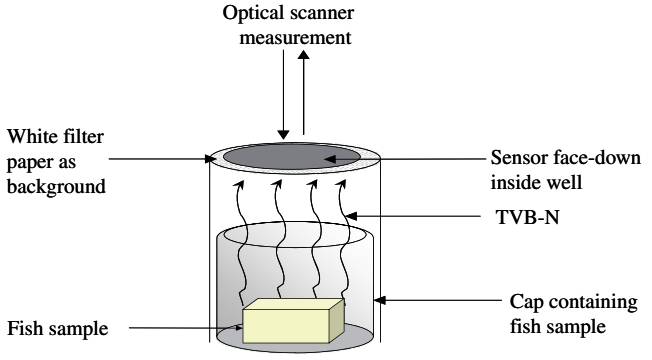

Fig. 1. Experimental design for fish spoilage monitoring (based on Byrne et al., 2002).

3. Results and discussion

3.1. Leaching studies

Fig. 3 shows the effect of ammonium bromide salts on BCG leaching. Tetraoctyl ammonium bromide reduced leaching by 82% and was selected for the fish spoilage trial. It is expected that leaching amounts would significantly decrease considering the actual sensor size (5 mm diameter) and the presence of a gas permeable membrane that will act as a barrier against condensation mist and prevent the sensor spot to come in direct contact with the food product or food exudate within the packaging. A pre-wash step may also purge the sensing membrane of all excess unbound-dye on the surface. No evidence of leaching was visible throughout the various fish spoilage analysis carried out.

3.2. Film thickness: differences in signal steady-state changes

Previous results (Pacquit et al., 2006) had indicated that the higher the spin-coating speed, the thinner the sensor

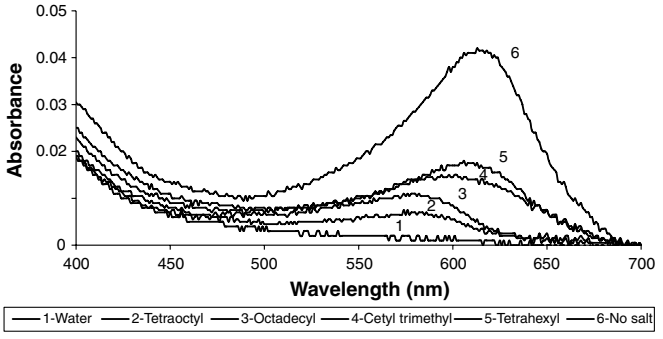

Fig. 3. Effect of ammonium bromide salt on bromocresol green leaching.

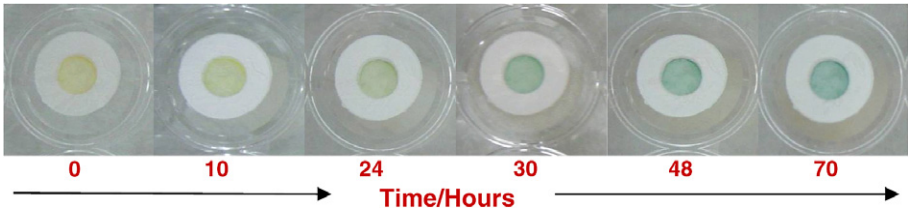

Fig. 2. Typical BCG sensor response to a spoiling whiting sample.

with average thickness results of 2.57, 1.36 and 1.01 μm at a spin rate of 1000, 2000 and 3000 rpm, respectively. Furthermore, sensors with the greatest thickness (1000 rpm) showed the biggest dynamic range when exposed to standard ammonia.

Fig. 4 shows the rate of change in color of the sensors of various thickness exposed to spoiling cod at room temperature. Responses in all three thicknesses were similar in time. Prior to the first 18 h, no color change was detected by the reflectance colorimeter. Thereafter, the sensors steadily changed color from yellow to blue (≈20–38 h). However, the thickest sensors, coated at 1000 rpm, showed a higher signal than those coated at 2000 or 3000 rpm and a more intense color change was visible to the naked eye allowing a better visualisation of the occurrence of spoilage. This thickness was selected for the following fish spoilage trial.

3.3. Fish spoilage trial

Fig. 5 shows the change in TVB-N level monitored by the color sensor in spoiling cod at room temperature. For the first 14 h, no color change was detected by the reflectance colorimeter but at 16–18 h, a definite increase in reflectance was recorded. The sensor gradually changed color from yellow to green then to blue in approximately 43h where no further color change was observed. During this entire period, the sensors fitted in the reference wells which did not contain any fish samples remained in their yellow form.

Similar results were obtained with the whiting samples (Fig. 6) where the sensor gradually changed color from the 14th hour up to the 45th hour after which no further color change was observed. The intensity of the response was equivalent in both cod and whiting, indicating that these species released a comparable amount of volatile amines.

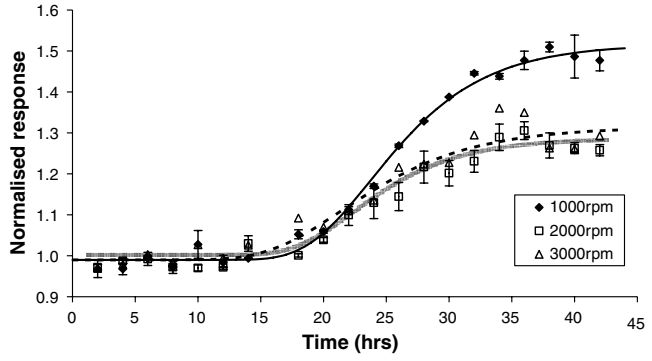

Fig. 4. Differences in signal steady-state changes between sensors of various thicknesses. The error bars are SEM (standard error of the mean) values.

3.4. Microbial analysis of cod and whiting samples

It is difficult to use reported bacterial counts in the literature to define exact spoilage thresholds as they can vary depending on the catch season, catch maturity, geographical location and above all fish species. However, Gram and Huss (1996) and Huss et al. (1997) suggested that when stored aerobically, spoilage of iced fish is reached at levels of 10^8–10^9 cfu/g of specific spoilage organism while Koutsoumanis (2001) and Olafsdottir et al. (1997, 2004) both reported TVC and *Pseudomonas* values of 10^7 cfu/g for fresh fish samples to reach end of shelf life.

Figs. 5 (cod) and 6 (whiting) show ranges of microbial population commonly associated with spoilage in white fish and the specific level of 10^7 cfu/g was reached after about 18 h in cod and 14 h in whiting. In Fig. 5, the TVC counts were found slowly increasing from approximately 10^4 cfu/g during the initial 10 h but sharply rising from then on, reaching values of 10^7 cfu/g at approximately 18 h before stabilising at 26 h just above 10^7 cfu/g. Clearly, this coincides with the onset of color change in the sensors (14–24 h) suggesting that the concentration of spoilage compounds in the headspace has reached the sensor lower detection limit.

Thus, the sensors accurately track this increase in volatile base concentration in the package headspace. The region of change in sensor color coincides with higher levels of *Pseudomonas* spp. in the fish tissue. Therefore, the scanner measurements of on-package sensor color are useful

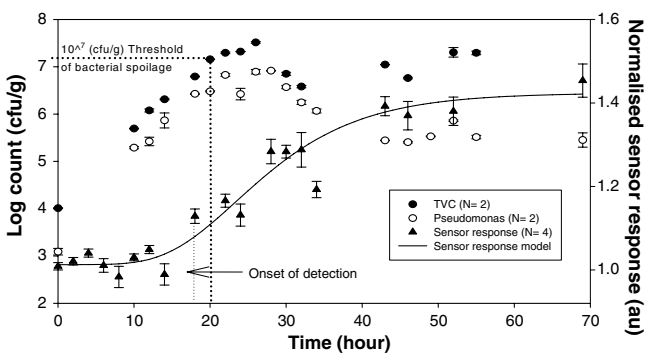

Fig. 5. Normalised data showing the correlation between sensor response and bacterial population (TVC and *Pseudomonas* spp.) in cod filet samples at 21 °C. The error bars are SEM (standard error of the mean) values.

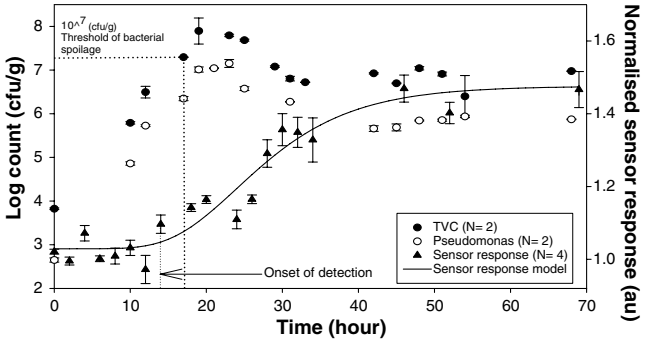

Fig. 6. Normalised data showing the correlation between sensor response and bacterial population (TVC and *Pseudomonas* spp.) in whiting filet samples at 21 °C. The error bars are SEM (standard error of the mean) values.

indicators of *Pseudomonas* spp. population and therefore spoilage of the fish samples. This is consistent with our earlier findings (Pacquit et al., 2006). However, contrary to the latter in which the *Pseudomonas* counts rose from about 65% to approximately 100% of the TVC counts in just 18 h, in this study the *Pseudomonas* counts did not exceed 80% of the TVC counts from a 50% initial load.

4. Conclusions

The results presented in this study indicated that a fast and sensitive detection of spoilage compounds in fish can be achieved by a non-invasive colorimetric method. The sensor response was found to correlate with bacterial growth patterns in cod and whiting fish samples thus enabling the "real-time" monitoring of spoilage. Ongoing research is currently assessing the sensor response at refrigerated temperature (0–4 °C) as bacterial population and bacterial activity are both temperature dependants.

These colorimetric sensors offers the potential of developing dynamic "best-before" dates that may lead to important and exciting improvements in the quality assurance sector. Future development includes the study of the system response in various scenarios of temperature abuse, chilled storage disruption and packaging puncture. These should provide a clear insight into the full potential of this sensor as an on-package food quality indicator.

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