ELSEVIER

Food Chemistry 69 (2000) 97–103

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Section

Monitoring of volatile bases in fish sample headspace using an acidochromic dye

Michael Loughran, Dermot Diamond*

Biomedical and Environmental Sensor Technology Centre, Dublin City University, Dublin 9, Ireland

Received 26 July 1999; received in revised form 19 September 1999; accepted 19 September 1999

Abstract

The release of volatile amines such as trimethylamine (TMA), dimethylamine (DMA) and ammonia from fish samples has been detected and continuously monitored through changes in the colour of a sensitive acidochromic dye using UV–Vis reflectance spectroscopy. Changes in the colour of the calix[4]arene-based dye immobilised on test paper disks in contact with the headspace of cod and whiting samples could be determined by an increase in absorbance centred on ca. 500–510 nm. Fresh whiting produces larger, and more rapid colour changes than fresh cod, suggesting that the rate of release of volatile bases from whiting is faster. The sensitivity of the dye response can be tuned by varying the ratio of $LiClO_4$ to dye in the dye solution due to the formation of a more acidic Li^+–dye complex. Volatile components absorbing at lower wavelengths (below 410 nm) were detected in cod samples but not whiting.

Keywords: Fish freshness; Cod; Whiting; Calixarene; Acidochromic dye; Reflectance spectroscopy

1. Introduction

There is increasing interest in the development of rapid methods for the determination of food freshness and general quality indicators. The availability of such indicators would enable freshness to be certified at various stages along the distribution chain, and ultimately, could be incorporated into 'intelligent' packaging that conveys information on the freshness of the packaged food to the consumer. The importance of fish and fish-based products in the global food market market has risen sharply in recent years. For example, the Food and Agriculture Organization of the United Nations (FAO, 1998a) estimated that in 1998, the world fish market was worth more than $250 bn, and predicted the annual demand would increase by ca. 30% to around 120 million tonnes by 2010 (FAO, 1998b). Given this increasing demand, and calls for better management of global fish stocks, a key element in the overall strategy must be to reduce wastage and generate routes to more accurate estimations of shelf-life, and means to extend shelf life (e.g. through various modified atmosphere packaging techniques; Phillips, 1996).

Furthermore, with the increasing trends towards mass central processing of large amounts of food, producers have been increasingly adopting the philosophies of zero defect manufacture (ZDM) via good manufacturing practice (GMP) procedures developed by the electronics and semiconductor industries. Critical in the implementation of ZDM is the concept of hazard analysis — critical control point technique (HACCP) (Sutherland, Varnam & Evans, 1986). Under this regime, critical control points along the production line should be monitored using rapid analytical methods so that immediate action can be taken if a problem is detected. However, conventional measurements of bacterial populations based on plate count procedures typically require several days incubation before results are available.

An alternative approach is to measure some aspect or effect of the metabolic activity of the microorganisms, which can then be related to the level of microbial contamination in the food sample.

One approach is to measure the ATP generated by bacteria in the sample. This has been achieved using bioluminescence, but the endogenous extra-cellular

* Corresponding author. Tel.: +353-1704-5404; fax: +353-1704-8021.

E-mail address: dermot.diamond@dcu.ie (D. Diamond).

0308-8146/00/$ - see front matter
PII: S0308-8146(99)00224-1

ATP must be destroyed prior to releasing the bacterial ATP. Results can be obtained in about 1 h (Chappelle, Piccolo & Deming, 1978; Stannard & Wood, 1984). Another approach is to monitor the electrical impedance of the tissue, as changes in impedance can be related to the concentration of micro-organisms. However, for quantitative results, assays can take up to 24 h (Hardy, Kraeger, Dufour & Cady, 1977). Zhang and Lee have developed a rapid and simple near-infrared (NIR) spectroscopic method, for direct determination of free fatty acids (FFA) in fish oil. In their research, they claimed that the FFA change in mackerel had the same trend as hypoxanthine (Hx) change, and that this could therefore be used as a rapid determination of fish freshness index. Generally, it is recognized that Hx content in fish is probably the most accurate indicator of fish freshness. Cheng, Peng and Yang (1998) reported a bilayer coated-wire electrode incorporating xanthine oxidase to estimate fish freshness. The biosensor was based on detecting hydrogen peroxide released from the enzymatic reaction. This method is supposed to be relatively simple with rapid determination. An excellent agreement with standard methods (e.g. total volatile basic nitrogen, TVB-N) was demonstrated. Matsumoto, Asada and Murai, (1998) have also used biosensors to demonstrate the simultaneous determination of inosine monophosphate (IMP) and L-glutamate (GL) using two enzyme reactors in parallel by flow injection analysis. Other biosensors aimed at determining fish freshness include a multienzyme approach (Hu & Liu, 1997) based on a 5′-nucleotidease (NT) membrane and a nucleoside phosphorylase (NP)-xanthine oxidase (XO) membrane co-immobilised on a Clark type oxygen electrode, and amperometric electrodes modified with xanthine oxidase, nucleoside phosphorylase and nucleotidase that could detect ATP degradation products (Ghosh, Sarker & Misra, 1998). However, in general, enzyme-based biosensors tend to be difficult to make reproducibly and suffer from stability and lifetime problems. Hence they tend to require a high degree of skill and experience to obtain reliable results (Diamond, 1998).

1.1. Volatiles in packaged fish headspace

A large number of volatile components have been identified in fish samples using steam distillation followed by analysis by GC–MS. For example, 3-pentanone, isopentyl alcohol and 2-heptanone have been identified as possible volatile indicators of the freshness of mackerel (Zhang & Lee, 1997). Alasalvar, Quantich and Grigor (1997) combined static headspace analysis (SHA)/GC/MS and dynamic headspace analysis (DHA)/GC/MS to identify aldehydes, ketones, alcohols and esters in fresh mackerel aroma whereas acid and sulphur-containing compounds gave spoiled-odours. However, while this techique is very sensitive and can identify many components in a single run, the equipment is expensive and can only be operated by dedicated, skilled personnel.

1.2. Volatile amines in fish tissue

Volatile amines such as trimethylamine (TMA), dimethylamine (DMA) and ammonia are generated by the enzyme catalysed decomposition of trimethylamine oxide (TMAO), which is present in significant quantities in most marine species. A recent detailed study (Olafsdóttir et al., 1997) of concentration-time profiles of these volatile bases in ice-stored cod demonstrated that:

- TMA is produced in the greatest quantities, rising rapidly to >40 mg TMA-N/100 g sample after 23 days on ice. However, this increase begins after around 12 days and coincides with the onset of microbiological spoilage.
- Ammonia follows a similar profile, but the amounts generated are lower, reaching ca. 14 mg ammonia-N/100 g sample after 23 days.
- In contrast, although DMA levels are relatively low, they rise steadily to around 1.0 mg DMA-N/100 g sample after ca. 12 days, and thereafter, increase more rapidly to ca. 3.0 mg DMA-N/100 g sample.
- The total volatile base content (TVB-N), as one might expect, is a combination of these three, and shows a gradual initial rise over the first 12 days or so on ice, and thereafter rises rapidly.

This suggests that TVB-N measurement would be difficult to use as a precise measurement of freshness, but could be used to indicate that microbiological spoilage had occurred if the levels were high (i.e. fish has been on ice for more than 10–12 days). In fact, TVB-N levels have been recommended as markers in the fishery industry for produce that no longer fit for human consumption. Since March 1995, a decision of the European Commission has fixed TVB-N limits for certain categories of fishery products (95/149/EEC). This is based on the European directive on fish hygiene (91/493/EEC).

1.3. Rapid detection of volatile bases in fish headspace

The most commonly used method of determining volatile bases in fish samples is GC and, increasingly, GC–MS. However, as previously mentioned, this equipment, while very sensitive, is expensive and requires a dedicated skilled operator, and is therefore only suitable for use at central locations in the distribution chain that have access to analytical laboratories. Consequently, attention has been focused on alternative sensor-based approaches as these are very rapid and relatively inexpensive, and could potentially be used on-line at many points in the distribution network. Gupta

and Misra (1997) demonstrated a manganese phthalocyanine based thin-film gas microsensor for TMA. The sensor response was found to increase with fish spoilage as TMA emission from tissue increased. Li, Endo, Hayashi et al. (1994) described a system consisting of an immobilised mould, *Penicillium decumbens*, an oxygen electrode and a flow cell. However, while the equipment was inexpensive, the TMA assay took ca. 30 min to perform. Like other biosensors, this device would be difficult to adapt to mass production due to the difficulties in stabilising biomaterials in sensor configurations.

Our interest in TMA and other volatile bases emitted during food spoilage arises from our development of a very sensitive chromogenic dye based on a calix[4]arene macrocyclic receptor. Calixarenes have been studied extensively as active agents for a wide variety of sensor applications (Diamond & McKervey, 1996). The derivative used in this research contains a cavity pre-arranged for efficient complexation of metal cations defined by four carbonyl oxygen atoms and four phenoxy oxygen atoms (Fig. 1), with a single azophenolnitrophenol dye moiety substituted at one of the four pendent ester groups. Originally, our goal was to develop systems capable of providing visible transduction of metal ion binding (McCarrick, Harris, Diamond, Barrett & McKervey, 1993). However, we have also demonstrated that the very similar tetra-azophenolnitrophenol derivative can be used to provide sensitive detection of ammonia when immobilised on fibre optic probes, and that the sensitivity can be tuned according to whether the free ligand or the metal ion complex is used as the dye (Grady, Butler, MacCraith, McKervey & Diamond, 1997). The Li^+-complex was found to produce to most sensitive response, probably due to the electrostatic effect of the metal ion which is thought to be in close proximity to the labile acidic proton (Fig. 1). Furthermore, it was shown that the sensitivity and linear range of the dye response could be tuned as the Li^+-dye ratio was varied. Initial studies also showed that TMA could be detected very sensitively, and that paper disks impregnated with the dye could provide visual indication of spoilage of fish samples through reaction of the volatile bases released during spoilage (McCarrick, Harris & Diamond, 1994). In this paper, we demonstrate that sensitive measurements of increasing TVB-N levels in fish headspace are possible by monitoring changes in the colour of paper disks impregnated with the Li^+-dye complex using reflectance UV–Vis spectroscopy. Furthermore, the rapid increase in the levels of TMA associated with the onset of microbial spoilage of cod on ice can be easily noticed by eye as a distinct change in the colour of the test disks from pale yellow to dark brown.

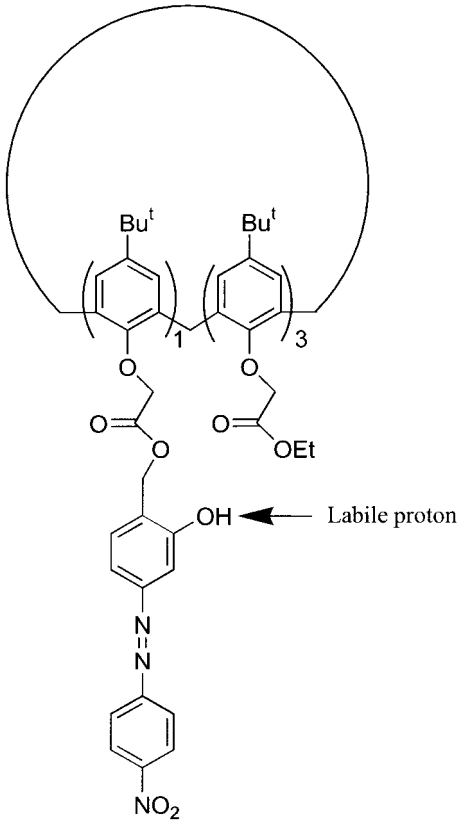

Fig. 1. Structure of the mononitrophenolazophenol calix[41arene tetraester derivative used as the acidochromic dye in this study: $C_{93}H_{88}N_3O_9$ FW 1483.

2. Experimental

2.1. Laboratory instrumentation

UV–Vis spectra were recorded with an Ocean Optics PS1000 miniature photodiode array spectrometer (Ocean Optics Inc., Dunedin, FL) which included: Ocean Optics Spectrascope Software Version 2.5 93/95; Ocean Optics Fibre Optic Reflectance Probe R200-7; light source: Ocean Optics LS-1 tungsten halogen lamp.

2.2. Chemicals and reagents

The monoazophenolnitrophenol calix[4]arene dye was synthesized as reported previously (McCarrick, Harris & Diamond, 1996). Spectroscopic grade methanol for preparation of 5mM calixarene solutions was obtained from Fluka Chemie AG (Buchs, Switzerland). Lithium was used as the perchlorate salt (A.C.S. reagent grade), and purchased from Aldrich Chemical Co. (Gillingham, Dorset, UK).

2.3. Preparation of dye impregnated indicator disks

Filter paper disks (3 mm diameter) were immersed in 200 μl of a 5 mM calixarene-dye solution for 5 min. The disks were prepared in batches of six to prevent the aggregation of too many filter paper disks within the

calixarene solution. Such aggregation would create uneven adsorption of calixarene dye on both surfaces of the filter paper. The more sensitive Li^+-calixarene complex was simply produced by adding lithium perchlorate to the calixarene solution (e.g. 10:1 or 3:1 w/w lithium perchlorate–calixarene) and test disks produced in the same manner. After soaking in the dye solution, the disks were removed and left to dry for 20 mins in a laminar flow cabinet at room temperature. The indicator disks were then attached to a polypropylene sample vial with Pritt-stick™ adhesive over a 1.0 mm hole drilled in the vial cap.

2.4. Preparation of fish samples

Freshly caught cod and whiting were bled, gutted, iced into 90 l boxes and stored at 0–1°C. Both species of fish were skinned and filleted within 24 h from time of catch. The fresh cod and whiting fillets were stored on ice, in separate polystyrene ice boxes, in a refrigerator at 0 ± 1°C. During storage, melting ice was replenished in order to maintain a constant storage temperature. Samples were removed at various intervals, and UV–Vis spectra of indicator disks exposed to a number of different fish samples were recorded at room temperature except for the last experiment (extended duration–19 days) in which the cod samples were kept on ice for the entire duration of the experiment. The samples were:

(i) fresh whiting (1-day on ice since catch)
(ii) fresh cod (1-day on ice since catch)
(iii) cod stored for 3 days on ice since catch
(iv) fresh cod kept on ice during spectral monitoring for 19 days.

In the above experiments (i)–(iii), the spectral measurements were obtained at room temperature (i.e. samples removed from fillets stored on ice and placed into sample vial at room temperature). In the case of (iv), samples were removed from a fillet that was 1-day on ice since catch and monitored for 19 further days on ice.

2.5. Experimental set-up

A schematic illustration of the experimental set-up used to record continuous UV–Vis spectra of the indicator disks exposed to various fish samples is shown in Fig. 2. The UV–Vis reflectance probe which formed part of the Ocean optics PS1000 was inserted into a pre-drilled hole in an aluminium cylinder that contained the sample vial so that the probe was held at a fixed distance and angle from the indicator disk surface. Reflectance spectra of the disk colour were recorded continuously at fixed intervals during the various timescales of the experiments. Spectral data were imported into EXCEL 97 for post run data processing.

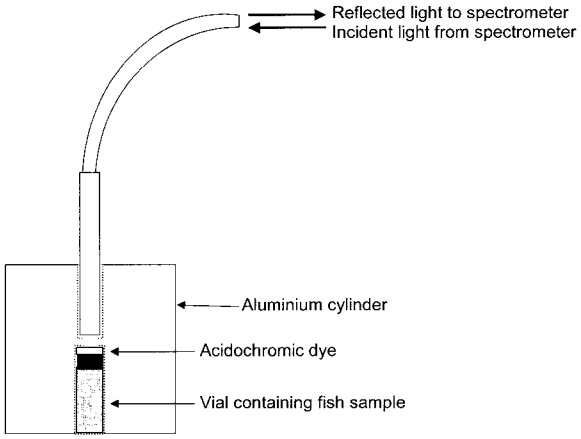

Fig. 2. A schematic illustration of the experimental set-up used to record continuous UV–Vis spectra of the indicator disks exposed to various fish samples. The fibre optic probe is held in position by insertion into a pre-drilled aluminium cylinder. The vial containing the fish tissue sample is also held in position in the cylinder in a second pre-drilled chamber. This means that the probe can be positioned very reproducibly close to the indicator disk surface.

3. Results

3.1. Fresh whiting and cod (24 h on ice from time of catch)

Indicator disks were prepared from a solution that was 3:1 w/w lithium perchlorate:calixarene dye. The fish samples were obtained approximately 24 h after time of catch, and were stored on ice during this period.

There was no distinct smell of amine from either fish sample nor was there any visible change in the colour of the indicator disks exposed to either the whiting or cod samples during the experiment.

The differential reflectance spectra recorded of the indicator disk colour during the 30 h after exposure to the fresh whiting and cod are shown in Fig. 3a and b, respectively. These spectra were obtained by subtracting the first spectrum (at $t = 0$) from subsequent spectra recorded at regular intervals over 30 h. They show a very clear change in absorbance with a broad band occurring over the range 450–550 nm, which is indicative of deprotonation of the dye (McCarrick et al., 1993). A number of features are worth noting about these results:

- Volatile bases released from the fish samples are deprotonating the dye immobilised on the indicator disks.
- The rate of colour change is greater for the whiting samples than the cod samples. This is clearly demonstrated in Fig. 3c which shows the change in absorbance at 515 nm as a function of time for the whiting and cod samples. This suggests that volatile bases are generated more rapidly in whiting than cod.

- On deprotonation, the conventional liquid-phase UV–Vis absorbance spectrum of the dye typically has an increasing absorbance centred on ca 510 nm, and decreasing absorbance centred on about 380 nm, with an isosbestic point at about 425 nm. This is more or less obtained with the whiting sample (see Fig. 3a) but with the cod sample the region below 410 nm appears to be distorted, and an increase in the reflectance spectrum absorbance seems to occur in this region (Fig. 3b).

3.2. Cod samples 3-days on ice

While the spectral changes obtained with the cod samples above were small, a distinct increase in absorbance centred at 510 nm occurred (see Fig. 3b and c).

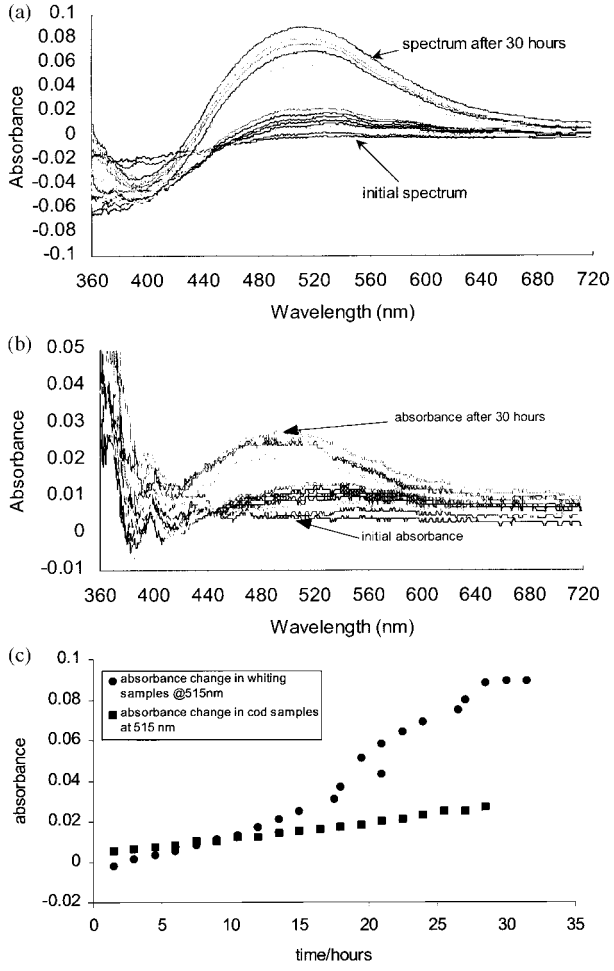

Fig. 3. (a) Differential reflectance spectra of 3:1 w/w $LiC1O_4$-dye disk in contact with fresh whiting (24 h on ice since catch), 2.0 g of fish at room temperature (22 ± 1°C), for 30 h. (b) Differential reflectance spectra obtained with 3:1 w/w $LiC1O_4$-dye indicator disk in contact with fresh cod samples (24 h on ice since catch): 2.0 g fish monitored at room temperature (22 ± 1°C), for 30 h. (c) Change in absorbance observed over a period of 30 h at room temperature obtained from (a) and (b) at 515 nm.

However, the signals obtained were low level, and the noise on the differential spectra in Fig. 3b is at the bit resolution of the instrument, so in subsequent experiments, a lithium perchlorate:calixarene ratio of 10:1 w/w was employed in order to enhance the dye sensitivity. In this experiment, a sample of cod was removed from a fillet that had been stored for 3 days on ice from time of catch, and transferred to a vial. The colour of an indicator disk exposed to this sample was then monitored via reflectance spectra over a period of 12 h at room temperature. The differential reflectance spectra obtained are illustrated in Fig. 4. Once again, a number of points are worth commenting on:

- The broad increasing band centred around 500–510 nm is once again obtained, which is convincing evidence of deprotonation of the indicator by volatile bases released from the cod sample.
- The spectral changes obtained at the absorbance maximum are much larger than before at ca. 0.08 after 12 h compared to less than 0.03 after 30 h. This is due to the more sensitive response of the dye generated by the increased amount of lithium perchlorate relative to the calixarene in the dye mixture (10:1 w/w compared to 3:1 w/w) and not due to the increased number of days on ice for the sample, as the concentration of TVB-N in cod samples has been shown to increase only slightly during this period of storage on ice (Olafsdóttir et al., 1997).
- A large *increase* in the absorbance is observed below about 410 nm. This would appear to be a feature of cod samples and not whiting.

3.3. Reproducibility of absorbance increase below 410 nm in cod samples

The increase in absorbance observed in cod samples below 410 nm is not explained by deprotonation of the

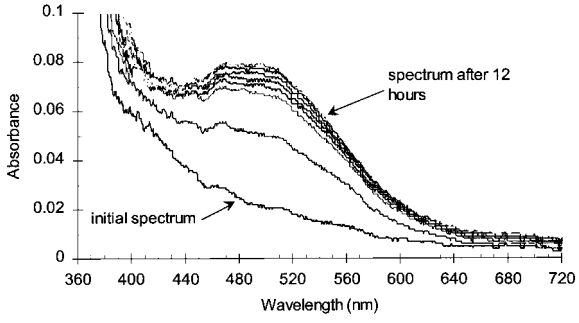

Fig. 4. Differential reflectance spectra of the colour of 10:1 w/w $LiClO_4$-dye indicator disk in contact with cod sample. Monitoring commenced after storing the cod for 3 days on ice from catch: 2.0 g fish exposed to disk at room temperature (22 ± 10°C), for 12 h during monitoring.

dye by volatile bases, but is more likely due to the release of volatile organics that inherently absorb below 410 nm. In order to verify this response feature, samples were taken from the same cod fillet stored on ice at 24 hours [Fig. 5, spectrum (a)] and 48 h [Fig. 5, spectrum (b)] after catch and the spectra obtained. This procedure was repeated in triplicate. The differential spectrum [Fig. 5, spectrum (c)] confirms that the absorbance increases markedly below 410 nm in all three cases. Good reproducibility is evident by the rather small standard deviation [satellite lines above and below spectrum (c)]. These results are important for several reasons;

- The increase in absorbance below 410 nm is clearly not due to deprotonation of the dye, as there is no characteristic increase in absorbance around 500–510 nm.
- This feature is real and reproducible, and is associated with cod and not whiting.
- It is most likely due to the presence of volatile organic compounds in the headspace of the cod samples that have a strong absorbance in this region of the spectrum.
- It occurs in fresh cod samples and increases rapidly during the first few days of storage on ice.

3.4. Long-term studies of cod on ice

In this experiment, a sample was taken from a fresh cod fillet (24 h on ice from catch) and the spectrum of the calixarene dye (10:1 w/w $LiClO_4$:dye) monitored over 19 days at 0–2°C. Fig. 6 shows differential spectra obtained by subtracting the initial spectrum from subsequent days. Once again, the increase in absorbance is observed at lower wavelengths after 1, 5, 10 and 14 days, but no indication of volatile base release is apparent. However, by 19 days, a strong absorbance at 550 nm is is clearly present indicating that the concentration of volatile base in the headspace is now significant, and that bacterial spoilage has occurred.

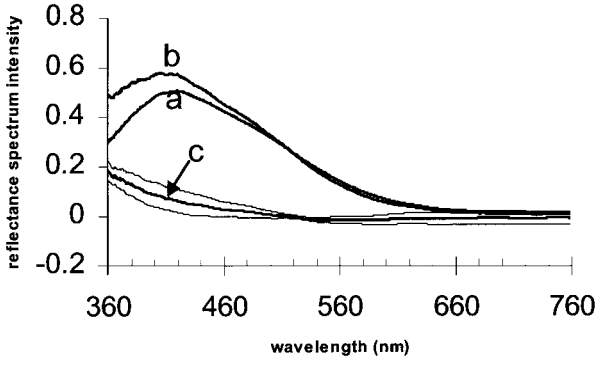

Fig. 5. Differential reflectance spectra obtained for cod samples that had been stored for 24 h from time of catch (a) and the same samples after a further 24 h stored on ice (b). Spectra are the mean of three obtained with different samples taken from the same fillet. The difference spectrum (c) is shown with lines at ± the first standard deviation to give an indication of the reproducibility of the measurements.

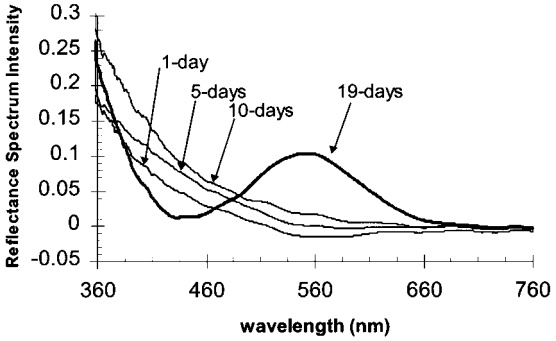

Fig. 6. Long-term study of cod samples on ice. Changes in the reflectance spectrum after 1, 5, 10, 14 and 19 days are shown. There is little indication of deprotonation of the indicator dye (10:1 lithium perchlorate–calixarene w/w) even after 14 days. However, by 19 days, there is a large increase in the spectral intensity above 500 nm. Once again, a large increase in the intensity is obtained at lower wavelengths, even after 1 day.

These results agree with those discussed earlier, in that there is no evidence of volatile base emission from cod on ice for up to 12 days, but as microbial spoilage begins to take effect, the levels of volatile bases, and in particular TMA increase rapidly.

4. Conclusions

The calixarene dye, like other acidochromic dyes, provides a simple means for detecting the build up of volatile bases in the confined headspace associated with packaged fish. The dye does not distinguish between the various bases (NH_3, DMA, TMA) but rather responds to any volatile base capable of deprotonating the dye. However, unlike conventional acidic dyes, the calixarene dye sensitivity can be greatly enhanced through the formation of a Li^+-dye complex, which enables it to detect lower levels of volatile bases.

Reflectance spectroscopy enables relatively slight changes in the colour of the indicator disks to be detected (before they are visible to the eye). Furthermore, the responses obtained in this study are fairly reproducible, despite the very simple fabrication technique employed. Clearly, it would be possible to greatly improve the reproducibility of the dye response if a more automated approach was adopted (e.g. screen printing, spin coating, etc.) to deposit very precise amounts of the dye on the substrate surface.

While it may be difficult to distinguish the relatively small changes in colour by eye, it might be possible to define a characteristic 'response spectrum' for fish samples at various stages before the onset of gross microbial spoilage, and hence predict the shelf-life of the fish. A

visible change in the colour of the disk indicates that there is a relatively high level of volatile base in the sample headspace, and the food is therefore probably unfit for consumption. Such materials may therefore play a role in the development of 'intelligent packaging' that conveys some idea of the freshness of the packaged food to the consumer. Alternatively, they may be employed in more specialised configurations that facilitate more sensitive instrument-based measurements of the total volatile base levels in packaged food.

Acknowledgements

The authors wish to thank Unilever Research Centre Colworth, Bedfordshire, UK for useful discussions and for providing support for this project.

References

FAO (1998). *http://www.fao.org/WAICENT/FAOINFO/FISHERY/FISHERY.HTM* (press release 98/38).

FAO (1998). *http://www.fao.org/WAICENT/FAOINFO/FISHERY/FISHERY.HTM* (press release 98/31).

Phillips, C. A. (1996). *Int. J. Food Sci. Technol., 31*(6), 463–479.

Sutherland, J. P., Varnam, A. H., & Evans, M. G. (1986). A colour atlas of food quality control. Wolfe Publishing Ltd.

Chappelle, E. W., Piccolo, G. L., & Deming, J. W. (1978). *Methods in Enzymology, 57,* 65–72.

Stannard, C. J., & Wood, J. M. (1984). *Journal of Applied Bacteriology, 55*(3), 429–439.

Hardy, D., Kraeger, S. J., Dufour, S. W., & Cady, P. (1977). *Applied Environmental Microbiology, 84*(1), 14–17.

Zhang, H. Z., & Lee, T. C. (1997). *Journal of Agricultural and Food Chemisty, 45*(9), 3515–3521.

Cheng, Q., Peng, T. Z., & Yang, L. J. (1998). *Analytica Chimica Acta, 369*(3), 245–251.

Matsumoto, K., Asada, W., & Murai, R. (1998). *Analytica Chimica Acta, 358*(2), 127–136.

Hu, S. S., & Liu, C. C. (1997). *Electroanalysis, 9*(16), 1229–1233.

Ghosh, S., Sarker, D., & Misra, T. N. (1998). *Sensors and Actuators: B-Chemicals, 53*(1–2), 58–62.

Diamond, D. (1998). *Principles of chemical and biological sensors*. New York: Wiley Interscience.

Zhang, H. Z., & Lee, T.-C. (1997). GC–MS analysis of volatile flavour compounds in mackerel for assessment of fish quality. In *Flavor and lipid chemistry of seafoods* (pp. 55–63) Washington, DC: American Chemical Society.

Alasalvar, C., Quantick, P. C., & Grigor, J. M. (1997). Aroma compounds of fresh and stored mackerel (*Scomber scombrus*). In *Flavor and lipid chemistry of seafoods* (pp. 39–54). Washington DC: American Chemical Society.

Olafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E., & Heia, K. (1997). *Methods to determine the freshness of fish in research and industry*. Fair Programme of the EU, AIR3CT94–2283.

Gupta, S., & Misra, T. N. (1997). *Sensors and Actuators: B-Chemical, 41*(1–3), 199–202.

Li, N. J., Endo, H., Hayashi, T., Fujii, T., Takai, R., & Watanabe, E. (1994). *Biosensors and Bioelectronics, 9*(8), 593–599.

Diamond, D., & McKervey, M. A. (1996). *Chemical Society Reviews*, 15–24.

McCarrick, M., Harris, S. J., Diamond, D., Barrett, G., & McKervey, A. M. (1993). *Analyst, 118,* 1127–1130.

Grady, T., Butler, T., MacCraith, B. D., McKervey, M. A., & Diamond, D. (1997). *Analyst, 122,* 803–806.

McCarrick, M., Harris, S. J., & Diamond, D. (1994). *Journal of Materials Chemistry, 4*(2), 217–221.