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Procedia Engineering 47 (2012) 1362 – 1365

Procedia Engineering

www.elsevier.com/locate/procedia

Proc. Eurosensors XXVI, September 9-12, 2012, Kraków, Poland

Fish freshness decay measurement with a colorimetric array

Patricia Zaragozá[a], Susana Ribes[b], Ana Fuentes[b], José-Luis Vivancos[a,c,e] *, Isabel Fernández-Segovia[b], José Vicente Ros-Lis[a], José Manuel Barat[b], Ramón Martínez-Máñez[a,d,e]

[a]*Centro de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universitat Politècnica de València – Universitat de València, C° Vera, S/N, Valencia 46022, Spain*
[b]*Departamento de Tecnología de Alimentos. Universitat Politècnica de València. C° Vera, S/N, Valencia 46022, Spain*
[c]*Departamento de Proyectos de Ingeniería. Universitat Politècnica de València, C° Vera, S/N, Valencia 46022, Spain*
[d]*Departamento de Química. Universitat Politècnica de València, C° Vera, S/N, Valencia 46022, Spain*
[e]*CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)*

Abstract

The development of rapid non-destructive techniques to determine fish quality and shelf-life is vital to guarantee food safety. The aim of this study was to evaluate a new optoelectronic nose composed by eight sensing materials prepared by the incorporation of pH indicators and chromogenic reagents selective to metabolites into inorganic materials (aluminium oxide and silica gel) in the shelf-life assessment of fresh sea bream in cold storage. Physico-chemical and microbial analyses were carried out periodically throughout the cold storage, as well as colour measurements on the colorimetric array. The results obtained using the chromogenic array were in accordance with those obtained from physico-chemical and microbial analyses, which showed a clear loss of freshness from day 4 to day 7. This confirms the potential usefulness of colorimetric reagents for detection of sea bream spoilage.

Keywords: chromogenic array; sea bream; spoilage; freshness

* Corresponding author. José L. Vivancos, Universitat Politècnica de València, Cº de Vera s/n, 46022 Valencia, Spain. Tel.: +34-963-877-000; fax: +34-963-879-869.
E-mail address: jvivanco@dpi.upv.es.

1877-7058
doi:10.1016/j.proeng.2012.09.409

1. Introduction

Fish is a highly perishable food product whose freshness and quality rapidly decline post-mortem. Loss of freshness and spoilage of fish are complicated processes and various factors such as species and different storage conditions influence the spoilage pattern. The current methods used in the food industry to determine fish shelf life are based on physical, chemical, microbiological and sensory measurements [1]. However, some of these methods are tedious, expensive, time-consuming and they require skilled personnel, so that it is of interest to develop rapid non-destructive quality control techniques, which can be applied at any stage of the supply chain [2].

The development of reliable methods to assess the freshness of fish as well as the evaluation of quality criteria has been the goal of fish research for many years [3]. The use of chromogenic chemosensors is a promising technique, since they are usually cheap, versatile, can be printed on the package, colour changes can be easily measured using cameras or other image capturing systems, and in certain circumstances, they may allow the naked eye detection of colour changes through transparent films [4]. Although some chromogenic indicators have been described, they are generally based on a single compound and have some limitations such as lack of specificity (offering false positives or false negatives). Additionally, the presence of certain target metabolites is not necessarily an indication of poor quality. More exact correlations seem necessary among target metabolites, product type and organoleptic quality and safety. The possibility of false negatives is likely to dissuade producers from adopting indicators unless specific indications of actual spoilage can be guaranteed. Thus, although some attempts have been made in single analyte indicators, the most promising, potent and versatile approach to be applied in complex matrixes is the use of optoelectronic noses, built by an array of dyes able to offer information through suitable colour changes [5]. Indeed in the last few years, the use of arrays of non-specific sensors has proved a suitable approach to analyse complex systems, and a number of examples of electronic noses and tongues to monitor fish freshness can be found in the literature [6], unlike examples of chromogenic arrays that are scarcer [4].

The aim of this study was to evaluate a rapid and easy-to-use system, using an array of chromogenic indicators with different chemical recognition properties, in the shelf-life assessment of fresh sea bream in cold storage.

2. Materials and Methods

2.1. Chromogenic array preparation

The five dyes used in the study were bromocresol purple, resorufin, bromophenol blue, phenol red (acquired from Sigma Aldrich (St. Louis, MO, USA)), and a dinuclear complex of rhodium (synthesized according to known procedures [7,8]. The selection of these dyes was based on previously reported optoelectronic noses and on our own experience in designing colorimetric probes [9]. These include pH indicators, (phenol red, bromocresol purple, bromophenol blue), Lewis acids (dinuclear rhodium complex), and oxidation-reduction indicators (Resorufin). The supports (aluminium oxide and silica gel) and the analytical-grade solvents (dichloromethane and ethanol) were purchased from Scharlau Chemie, S.A. (Barcelona, Spain).

Solutions were prepared by dissolving a certain amount of dye and the inorganic support (aluminium oxide or silica gel) in the appropriate solvent (dichloromethane or ethanol), obtaining a total of 8 chromogenic materials. The solutions were stirred for 8 h to give maximum dye absorption in the material. After that, the solvent was evaporated in a rotary evaporator to obtain the materials.

2.2. Sample preparation

Sea bream from a Spanish farm (Frescamar Alimentación, S.L., Burriana, Castellón, Spain) of commercial size 400-600 g was used as raw material. Fish were transported to the laboratory in polyspan boxes with ice. Eighteen fish were weighed and fifteen were individually placed in aluminum trays of 2.7 L. The five dyes in different supports (a total of eight different sensing systems) described in Table 1 were placed inside the tray close to the fish samples (Figure 2). In addition, three trays containing the colorimetric array were also packaged in the absence of fish and used as controls. Petri dishes filled with water were placed in the control trays in order to reproduce the moisture conditions. Studies with the samples were repeated three times. All trays were wrapped with plastic film and stored at 4 ºC for 11 days.

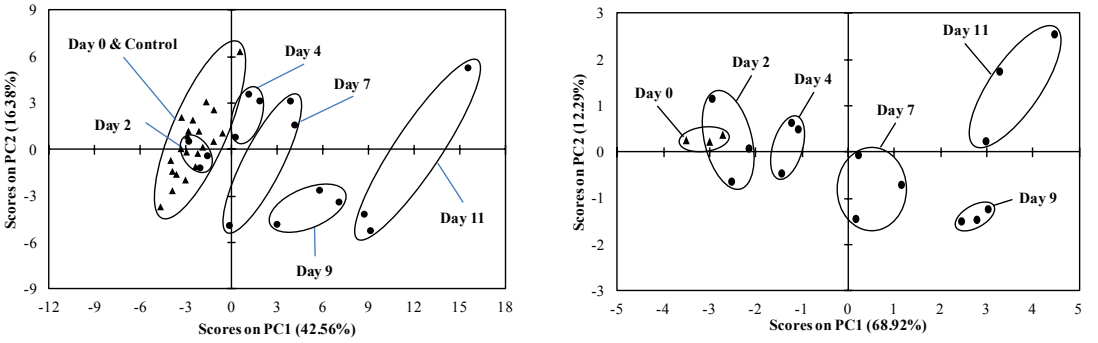
Physicochemical and microbiological analyses of samples were performed at days 0, 2, 4, 7, 9 and 11 of storage. Before analyses, samples were headed, gutted and filleted, obtaining 2 fillets from each fish.

Photographs of the trays were obtained at each day of analysis in all the trays stored.

3. Results and Discussion

The evolution of pH, drip loss, ATP related compounds, K_1-value and microbial counts showed a loss of freshness that took place mainly from day 7 up to the end of the storage period. All these parameters exhibited values typical of fresh fish from day 0 to day 4; however, the levels of these physico-chemical parameters and the counts of mesophilic and *Enterobacteriaceae* at day 7 of storage corresponded to spoiled fish.

Colour data were analysed using Principal Component Analysis (PCA) a linear, supervised, nonparametric pattern recognition method. The original variables, in this case, were the responses of the 8 sensing materials used in the sensor array. All 48 dimensions (i.e., 8 R, G and B coordinates and 8 L, a and b coordinates) would take one of the 256 possible values. Different groups can be observed (Fig 1.a.); data of days 0 and 2, as well as the controls (colorimetric array without fish) are mixed in a unique group, being day 4 next to this group. The other three sampling days (7, 9 and 11) are separated in three different groups, being the distance from the first days higher as the storage time increased.



(a) (b)

Fig. 1. (a) PCA performed using RGB and L*a*b* coordinated of the colorimetric array; (b) PCA performed with data from the physico-chemical and microbial analyses.

These results agree with the values obtained in the physico-chemical and microbial analyses, which registered a significant loss of freshness from day 0 to 7. In order to confirm this correlation a PCA was

performed with data from the physico-chemical and microbial analyses (Fig 1.b). The mean centrering pre-processing technique was applied to a dataset of 18 measurements (i.e., 6 sampling day x 3 replicates) and 10 features (moisture content, pH, TVB-N, drip loss values, mesophilic bacteria *Enterobacteriaceae* counts, ATP related compounds and K_1-value). In general, the groups identified were the same as those from the data of the colorimetric measurements.

4. Conclusions

An array of chromogenic indicators with different chemical recognition properties has been developed for the shelf-life assessment of fresh sea bream in cold storage. Results suggest that the colour measurements can be used effectively to discriminate the samples into four groups; the first one grouped data of days 0, 2 and 4, in the second, third and fourth groups were data of days 7, 9 and 11, respectively. Moreover these results are in accordance with those obtained in the physico-chemical and microbial analyses.

Acknowledgements

The financial support from the Spanish Government (project MAT2009-14564-C04-01), the Generalitat Valenciana (project PROMETEO/2009/016) and Universitat Politècnica de València (project ref. 2711) is gratefully acknowledged.

References

[1] Ólafsdóttir G, Nesvadba P, Natale CD, Careche M, Oehlenschlager J, Tryggvadottir SV, et al. Multisensor for fish quality determination. *Trends in Food Science &Technology* 2004;**15**:86–93.

[2] Pons-Sánchez-Cascado S, Vidal-Carou MC, Nunes ML, Veciana-Nogués MT. Sensory analysis to assess the freshness of Mediterranean anchovies (Engraulis encrasicholus) stored in ice. *Food Control* 2006;**17**:564–9.

[3] Natale CD, Ólafsdóttir G, Einarsson S, Martinelli E, Paolesse R, D'Amico A. Comparison and Integration of Different Electronic Noses for Freshness Evaluation of Cod-Fish Fillets. *Sensors Actuators B: Chem.* 2001;**77**:572–8.

[4] Huang X, Xin J, Zhao JA. Novel Technique for Rapid Evaluation of Fish Freshness using Colorimetric Sensor Array. *J.Food Eng.* 2011;**105**:632–37.

[5] Pacquit A, Lau KT, McLaughlin H, Frisby J, Quilty B, Diamond D. Development of a Volatile Amine Sensor for the Monitoring of Fish Spoilage. *Talanta* 2006;**69**:515–20.

[6] Kuswandi B, Jayus, Restyana A, Abdullah A, Heng LY, Ahmad M. A novel colorimetric food package label for fish spoilage based on polyaniline film. *Food Control* 2012;**25**:184–9.

[7] Esteban J, Ros-Lis JV, Martínez-Máñez R, Marcos MD, Moragues M, Soto J, et al. Sensitive and selective chromogenic sensing of carbon monoxide by using binuclear rhodium complexes. *Angew. Chem. Int. Ed*. 2010;**49**:4934–7.

[8] Moragues M.E., Esteban J, Ros-Lis JV, Martínez-Máñez R, Marcos MD, Martínez M, et al. Sensitive and selective chromogenic sensing of carbon monoxide via reversible axial CO coordination in binuclear rhodium complexes. *J. Am. Chem. Soc.* 2011;**133**:15762–72.

[9] Salinas Y, Ros-Lis JV, Vivancos JL, Martinez-Manez R, Marcos MD, Aucejo S, et al. Monitoring of chicken meat freshness by means of a colorimetric sensor array. *Analyst* 2012;**DOI:10.1039/c2an35211g**