Development of a biopolymer-based freshness indicator for fish freshness monitoring during storage

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ABSTRACT

Fish and seafood, increasingly recognized for their key role in food security and nutrition, have reached an all-time record of 214 million tonnes of production. However, their high perishability and short shelf-life calls for novel processing and packaging methods for quality retention and shelf-life extension. As freshness is considered a priority for fish production and consumer acceptability, the development of novel technologies and systems intended to continuously monitor quality and shelf-life is necessary. Smart packaging refers to systems capable of providing consumers and stakeholders with information regarding food quality from production up to the consumption, as freshness is monitored during transportation, retail display, and domestic storage. The development of smart materials would also contribute to the fish and seafood waste reduction which accounts for 30-35% of total fish production. The aim of the study was to design a biopolymer-based smart packaging system for the evaluation of fish freshness via a pH-sensitive colour response. The application of the developed smart system on fish fillets during storage at constant conditions was evaluated. pH-sensitive freshness indicators were prepared by the immobilization of extracted anthocyanins (from grape peels) into a solid matrix of PVA (polyvinyl alcohol)/starch using the solvent casting method. After fabrication, the smart films were evaluated for colour, thickness, ammonia sensitivity and colour responsivity at constant and variable conditions. Application of the indicator into the fish packaging was performed by attaching the indicator inside the headspace of sterilized sealed packaging pouches containing 50 g of fish fillets. Samples were stored at constant and variable temperature conditions for shelf-life evaluation, based on microbiological (spoilage bacteria) and chemical (pH, TVB-N) physical quality indices determination, in parallel with sensory evaluation. Preliminary results indicate that the fabricated smart indicator based on anthocyanins incorporated into PVA/starch adequately monitored ammonia vapor concentrations and the spoilage process of fresh fish during storage (ΔE>3), by providing shelf-life prediction of fish fillets ranging from 5 days at 5°C to 16 hours at 25°C (corresponding to E_a=70kJ/mol). These values were validated by experimental determination of quality level fish based on microbiological and chemical analyses.

KEY-WORDS: freshness indicator, pH-sensitivity, anthocyanins, biopolymers, fish

INTRODUCTION

According to data derived from FAO (2022) the demand for aquatic foods has shown remarkable increase (average annual rate of 3.0 percent since 1961), in comparison to the 1.6 percent of population growth rate. However, its intrinsic characteristics (neutral pH, high water activity and increased number of non-protein nitrogen molecules) make it a food product with limited shelf life [1]. Especially during fish transportation, implications such as uncontrolled storage conditions and transportation temperature could lead to microbial growth and consequently fish quality degradation and reduced shelf-life [2]. Cold chain management is an important element in ensuring standards of quality and safety of fish and seafood [3].

Up to date, numerous technologies have been developed and are currently applied in order to minimize the perishability of fish and extend their shelf life, mainly focusing on processing and preservation technologies (e.g., smoking, superchilling, high hydrostatic pressure) [4]. Although prolonging fish shelf life via the aforementioned methods is effective against food waste, limiting the unnecessary fish waste is also an important goal. Packaging plays a crucial role for the preservation of perishable food at any stage of the cold supply chain. Accurate information of end users about the exact expiry point and remaining shelf life of fish products can contribute to waste reduction, which accounts for 30-35% of total fish production [1, 5].

Freshness indicators are a recently developed smart packaging technology that aims to monitor food alterations and communicate them to the end user through a colorimetric response [6]. pH freshness indicators provide information about changes that occur in the environment in which the food is conditioned, since they are not in actual contact with the food item itself. For instance, anthocyanins are red when found in acidic environment and as the pH increases turn to colorless-purple and finally dark green-blue. The changes in the internal environment of a food package are caused by the food deterioration process [7]. Therefore, it is very important to be able to properly interpret and link the corresponding path of changes from the food matrix to the in-package conditions and finally to the color change of the pH freshness indicator. This study aims to develop a smart biopolymer-based pH sensitive indicator which would monitor the in-package changes during fish spoilage at both constant and variable storage conditions to simulate potential temperature fluctuations in the actual cold chain.

METHODS

Anthocyanin extraction and characterization

Anthocyanins were extracted from dried grape peels which were blended with 80/20 v/v distilled water/ethanol at a plant material-to-solvent ratio of 1:20 (w/v). Mild stirring followed (24h at 25 ± 2°C, in the dark) and then mixtures were filtered and centrifuged at 5000×g for 10 min at 4°C. Anthocyanins were concentrated through a rotary evaporator with vacuum pump (37°C and 25 mmHg).
The total anthocyanin content was evaluated through spectrophotometry (520 and 700nm) using a pH differential technique [8]. The pH-dependent colour-changing ability of anthocyanins was measured by dissolving anthocyanins within pH 2–12 buffers and recording their colour with a colourimeter (Eye-one Pro, X-Rite, Grand Rapids, MI, USA).

Smart indicator preparation and characterization

Freshness monitoring indicators were prepared by immobilizing anthocyanins-rich plant extracts (0,37% w/w) in starch:PVA matrices with a 3:1 ratio using the solvent casting method. After complete dissolution of the dry mass, 15 mL of the film making solution were cast into clean Petri dished and placed in an incubator at 40°C for 24 h to dry. After that, the films were peeled from the Petri dishes and stored at 50% RH for further use.

Film thickness was measured with a digital micrometer (IP65, SAMA Tools, Viareggio, Lucca, Italy) at ten different randomly chosen positions. The average and standard deviation of ten measurements were recorded.

The colour parameters of the films were measured according to the CIELAB system with a colourimeter (Eye-one Pro, X-Rite, Grand Rapids, MI, USA). Also, colour responsivity of the pH-sensitive indicator was evaluated at different pH values at a range of pH 2-12. The parameters that were measured were L- (lightness), a- (greenness/redness) and b- (yellowness/blueness) values and ΔE (total colour difference) was calculated according to Equation 1. With ΔE > 3, human eyes could identify color changes [9].

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

To evaluate the sensitivity of the fabricated indicator to the ammonia vapor, ammonia sensitivity test was performed according to Zwang et al. (2019) using NH_3 solutions with concentrations varying from 0.002 -1 M [10]. This test was acquired to show the potential of color response toward the volatile nitrogen components such as total volatile basic nitrogen (TVB-N) which are produced in different amounts during fish spoilage.

Application of the smart indicators for monitoring fish freshness

The colorimetric films were attached inside the headspace of sterilized packaging sealed bags containing 50 g of meagre (*Argyrosomus regius*) slices and the bags were then stored isothermally at 0-25°C and at variable conditions for shelf-life monitoring. Miniature electronic dataloggers (Elitech RC-5) were used to constantly monitor fish storage temperature. Measurements were carried out in appropriate time intervals (day 0, 1,2,5,7,9 and 12) which allow for efficient kinetic analysis of the quality degradation of the tested samples.

At each sampling point, the in-package gas composition was measured with a gas analyzer (Danseonsor, CheckPoint 3). Afterwards, the colour of the smart indicators was measured to determine the colour change (ΔE), as described before as well as the change in a-value from red to green (Δa). Chemical indices, such as pH and total volatile basic nitrogen (TVB-N) and microbiological growth (total viable counts, TVC) of fish were determined during storage at all tested storage conditions.

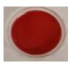
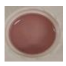
The quality level of fish was determined using the Arrhenius-based kinetics developed from the data obtained from the isothermal experiments. To demonstrate the integrated effect of temperature variability on product quality, the term of effective temperature T_{eff}, i.e. the constant temperature that results in the same quality value as the variable temperature distribution over the same time period), was introduced [11].

RESULTS AND DISCUSSION

Characterization of anthocyanin extract

The grape peels anthocyanin content was ~339 mg/L cyanidin-3-glucoside equivalents. The color parameters of the original anthocyanin extract (control) as well as the ones after the extract's exposure to various pH buffer solutions are presented in Table 1.

Table 1. *Color parameters of the anthocyanin extract (control and after exposure to pH buffers).*

pH	L	a	b	image
control	26.9634	43.8668	30.8424	
2	27.9036	45.5312	34.9325	
5	41.8575	19.3663	11.9644	
6	31.5819	17.7074	5.7203	
7	25.854	12.5077	8.2835	
8	15.6392	6.397	2.429	
12	35.0038	20.779	43.576	

Characterisation of smart films

The average film thickness was 0.11±0.02 mm. The L, a and b colour parameters of the fabricated smart films were 73.94 and 35.95 and -6.39, respectively. The results of the colour responsivity and ammonia sensitivity tests that were carried out at the smart films are both presented in Figure 1.

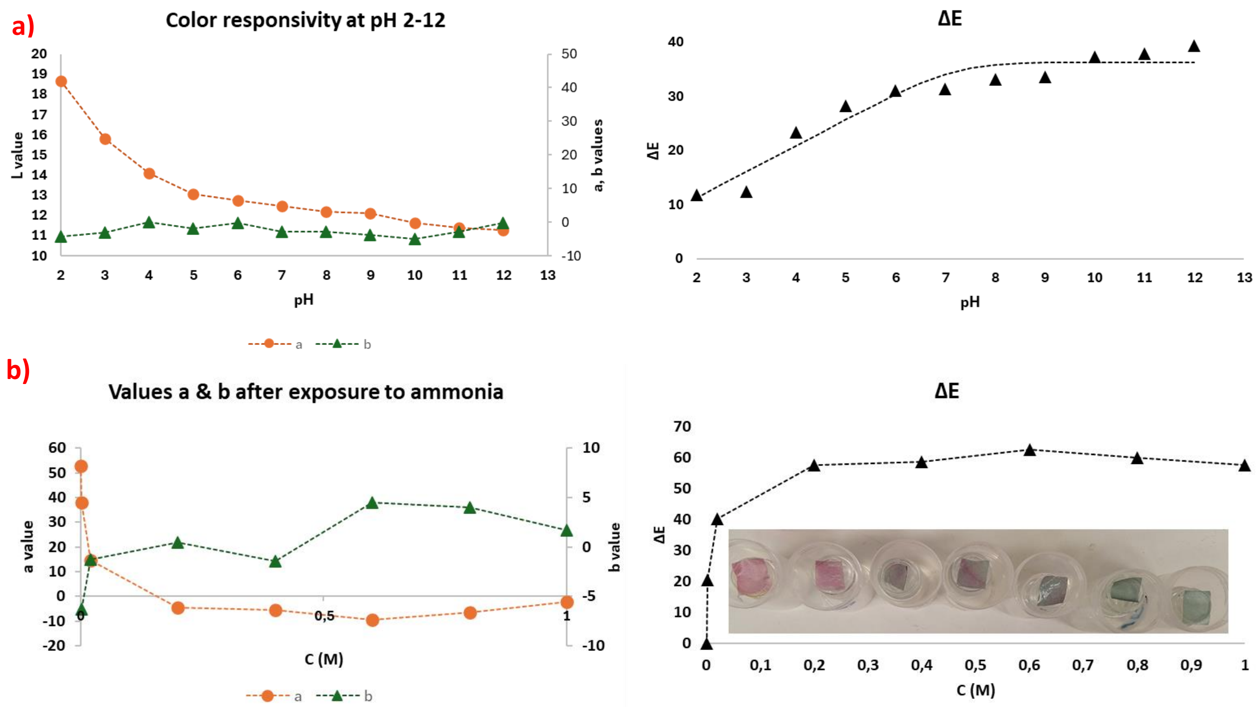


Figure 1. *L (lightness), a (greenness/redness) and b (yellowness/blueness) values and ΔE (total color difference) after exposure of smart films to a) pH buffer solutions 2-12, and b) 0.002M-1M ammonia vapor concentrations.*

Fish freshness monitoring

The fresh fish slices were stored for 12 days at constant and variable temperature conditions. The initial TVC of fish fillets was 4.4 logCFU/g (day 0) and the threshold TVC value of 7 logCFU/g was exceeded at day 7 of constant storage, while for variable storage conditions it was exceeded at day 5, as shown in Figure 2. *Pseudomonas* spp. population growth was observed during the initial storage days at both conditions but begun to decrease after the oxygen depletion inside the package (day 5). Enterobacteriaceae increased significantly and reached 5.9 logCFU/g at day 7 and 5 of constant and variable storage conditions, respectively. The T_{eff} determined by Arrhenius kinetics for variable storage conditions of fish was 6,15°C whereas for constant 2,53°C.

Fish flesh pH of both storage cases increased from 6.58 to 6.67 (day 5) and dropped again to 6.56 (day 12) possibly due to lactic acid bacteria accumulation in the last days of storage.

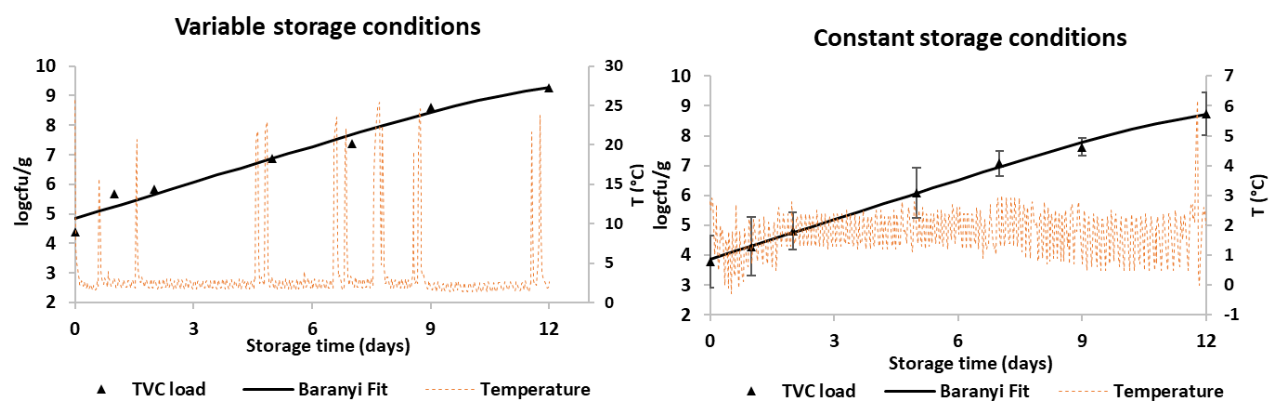


***Figure 2**. Microbial growth of TVC under variable and constant storage conditions.*

Total volatile basic nitrogen was found higher at the fish stored under variable conditions compared to constant, as expected. The same trend was observed in the in-package nitrogen (N_2%) concentration as well as the smart indicator color change (ΔE), indicating the adequate ability of the fabricated starch/PVA based smart indicator to monitor fish freshness and highlight the occurrence of variable storage conditions during the cold management chain (Figure 3).

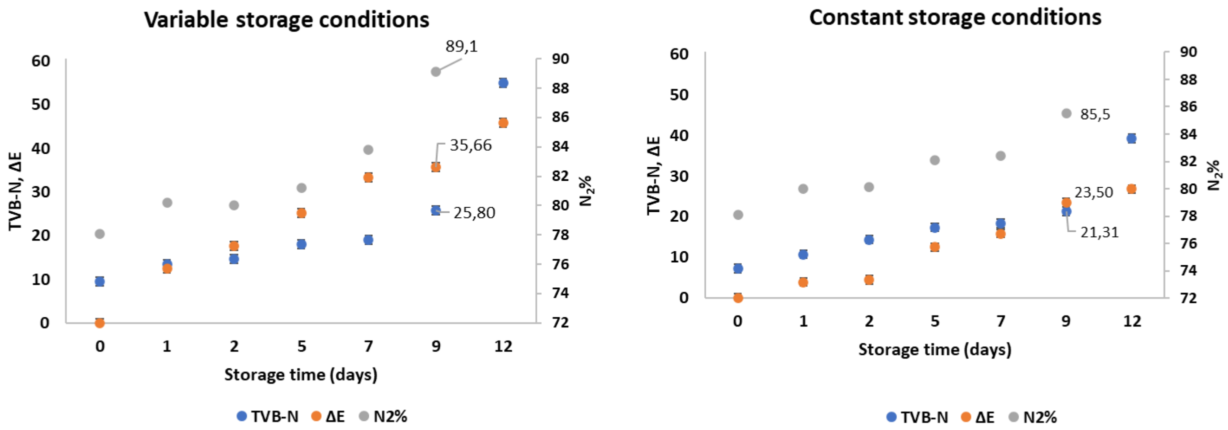


***Figure 3.** Schematic presentation of TVB-N, color change of the smart indicator and in-package nitrogen concentration during variable and constant storage of fish.*

As observed by the color measurements of the smart indicators, a value (indicating red hue) was constantly decreasing during the storage experiment, with a more intense drop being noticed in the variable storage conditions, where fish spoilt faster (Figure 4). This finding is also depicted in the measurements of ΔE and Δa ($a_{initial}$-a_{final}) which are progressively rising with the increasing spoilage rate of fish and are constantly higher in the variable scenario than the constant one (Figure 5).

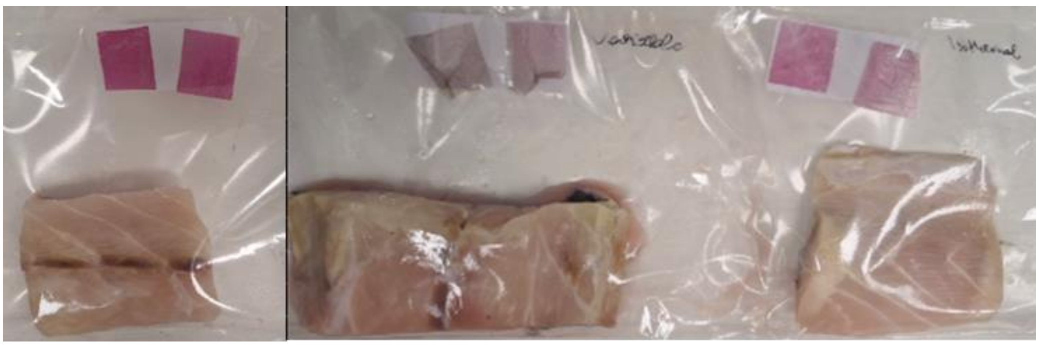


***Figure 4.** Day 0 and end of storage experiment (variable and constant conditions).*

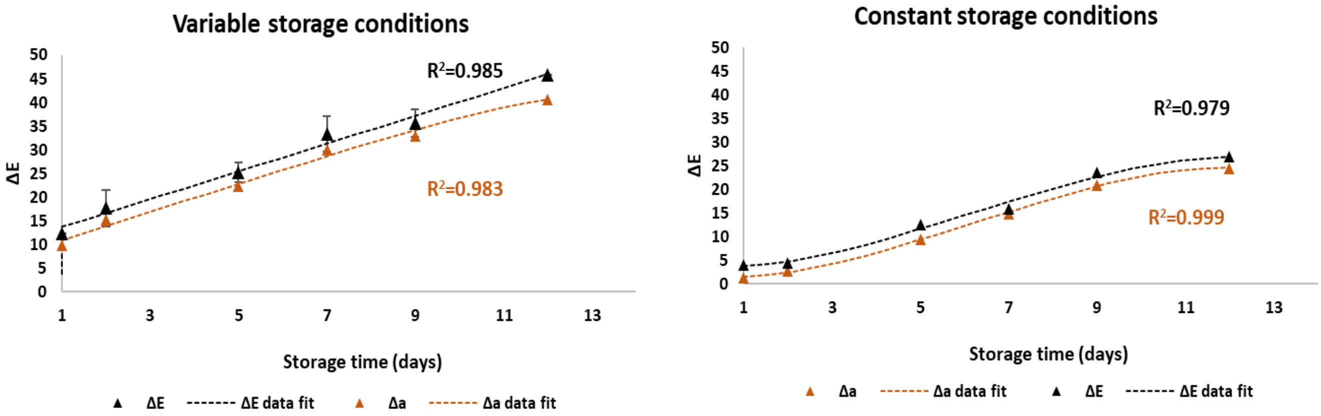


Figure 5. *ΔE and Δa progress during the shelf life evaluation of fish in constant and variable conditions.*

Further experimental procedures (e.g., smart film stability, morphology analyses etc.) would lead to the development of an integrated smart packaging system for continuous monitoring of fish freshness in the actual cold supply chain.

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