SYNTHESIS, CHARACTERIZATION AND APPLICATION OF PH-SENSITIVE INDICATORS FOR FRESHNESS MONITORING OF GILTHEAD SEABREAM Sparus aurata FILLETS DURING REFRIGERATED STORAGE

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Introduction

Significant amounts of fish and seafood are wasted worldwide due to spoilage and degradation during various stages of the cold chain (EUMOFA, 2022). An essential aspect of fish and seafood quality assurance is the rapid and accurate identification of the quality level and remaining shelf life (Tsironi et al., 2018). The development of novel smart packaging solutions to monitor fish spoilage could contribute to accurately inform consumers about the freshness of fish products and, consequently, reduce fish waste (Mohebi et al., 2015; Li et al., 2022).

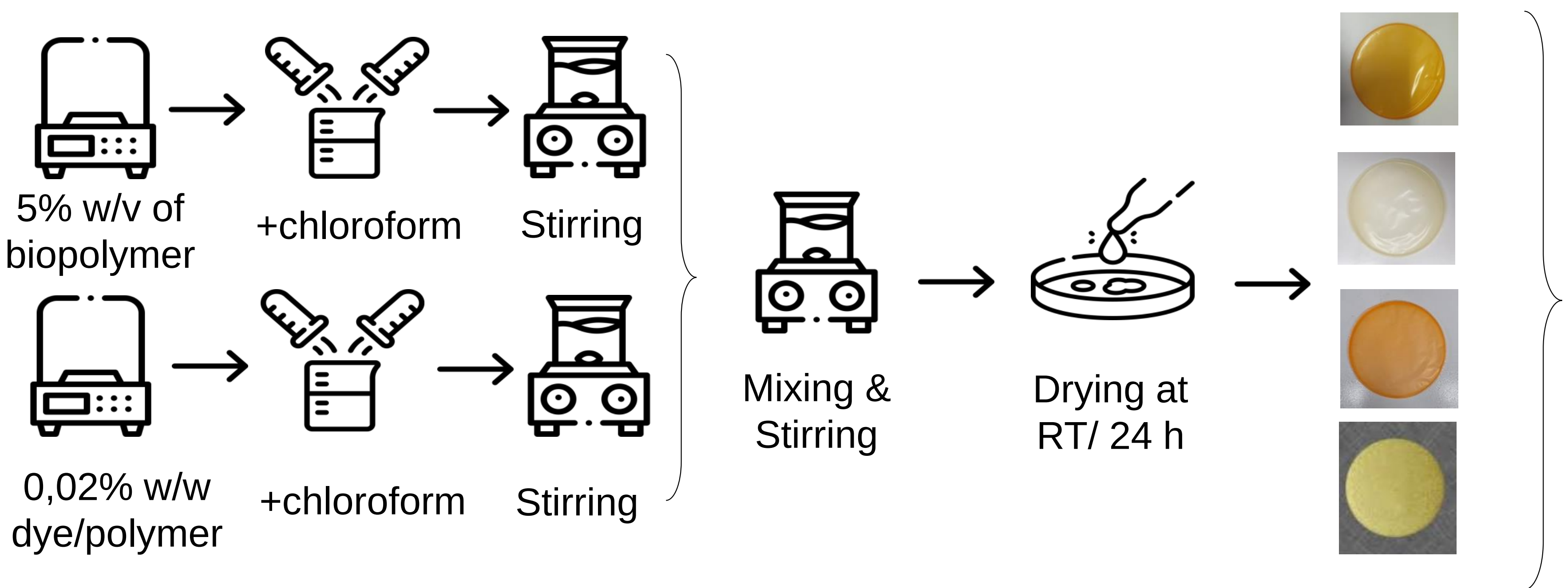
Aim

The aim of the study was to design and characterize novel pH-sensitive indicators which are based on biodegradable materials and ultimately test their ability to inform about food spoilage of perishable gilthead seabream fillets stored under refrigerated and abused temperature conditions.

Experimental design

Polymers
- ❖ Polyhydroxybutyrate (PHB)
- ❖ Polylactic acid (PLA)

pH-sensitive dyes
- ❖ Methyl Red (MR)
- ❖ Bromothymol Blue(BB)

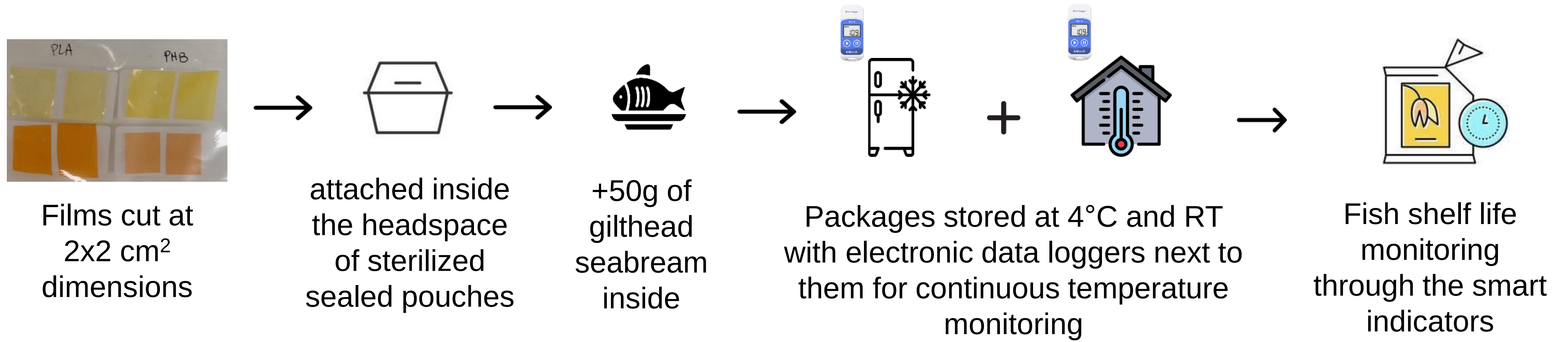


5% w/v of biopolymer → +chloroform → Stirring

0,02% w/w dye/polymer → +chloroform → Stirring

→ Mixing & Stirring → Drying at RT/ 24 h →

Smart films were characterized for:
- ✓ optical and mechanical properties
- ✓ UV–Vis barriers
- ✓ Wettability
- ✓ Microstructure
- ✓ Infrared spectra
- ✓ Sensitivity to volatile amines commonly produced during fish spoilage:
  - ❖ NH_3
  - ❖ Trimethylamine (TMA)
  - ❖ Dimethylamine (DMA)



Films cut at 2x2 cm^2 dimensions → attached inside the headspace of sterilized sealed pouches → +50g of gilthead seabream inside → Packages stored at 4°C and RT with electronic data loggers next to them for continuous temperature monitoring → Fish shelf life monitoring through the smart indicators

During the shelf life test, the following parameters were monitored:
- ✓ Color response of the indicators (L, a, b measurement)
- ✓ In-package gas composition
- ✓ Microbial growth (total viable counts)
- ✓ TVB-N content
- ✓ pH
- ✓ Wettability of the enclosed indicators

Results

Table 1. Mechanical properties of the PLA based smart films.

Film sample	Thickness (μm)	Tensile Strength (MPa)	Elasticity (%)	Young Modulus (MPa)
Control (PLA)	96.2 ± 6.8	18.6 ± 2.7	25.3 ± 10.2	1030 ± 218
Methyl Red (PLA)	56.9 ± 1.7	29.5 ± 4.9	18.0 ± 8.2	2022 ± 164
Bromothymol Blue (PLA)	68.8 ± 6.2	32.1 ± 2.3	41.3 ± 25.7	1816 ± 680

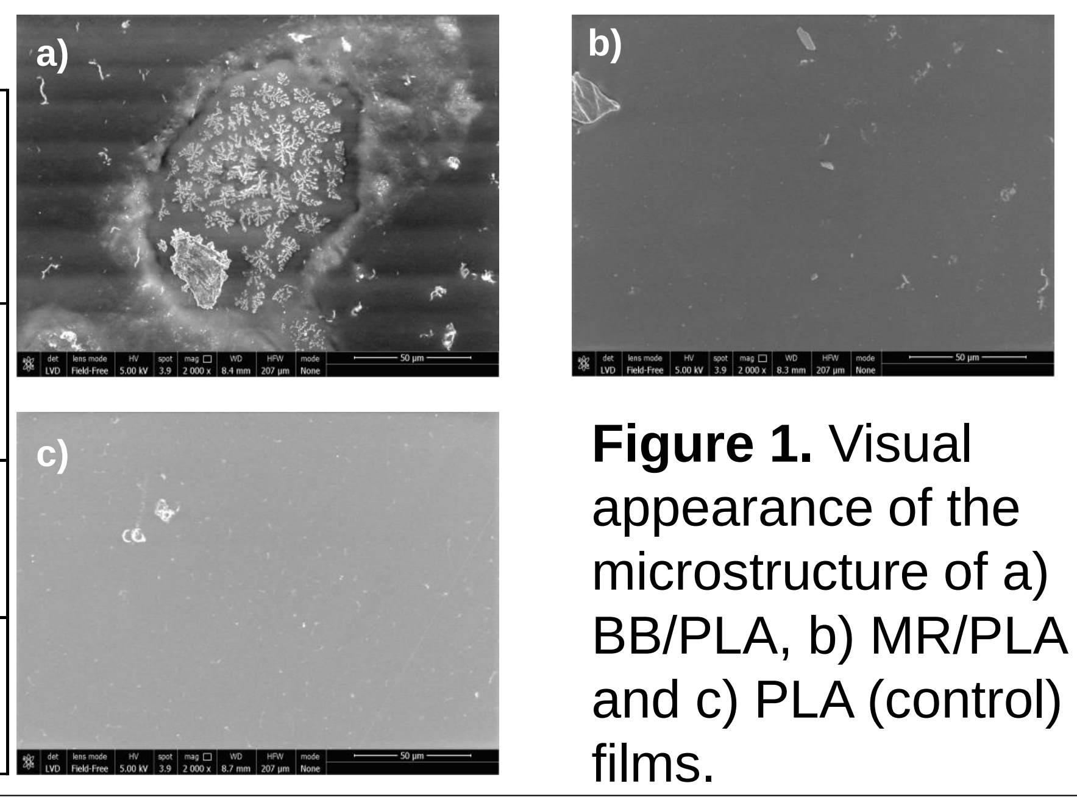


Figure 1. Visual appearance of the microstructure of a) BB/PLA, b) MR/PLA and c) PLA (control) films.

Table 2. UV–Vis light transmittance (200–800 nm) of PLA based smart films.

Film Sample	200.0 nm	300.0 nm	350.0 nm	400.0 nm	500.0 nm	600.0 nm	700.0 nm	800.0 nm	Opacity
Control (PLA)	1E-08	34.5	42.7	46.5	51.7	54.4	55.9	57.0	2.7
Methyl Red (PLA)	1E-08	33.3	47.2	42.7	18.2	60.7	62.2	63.1	3.8
Bromothymol Blue (PLA)	1E-08	31.4	39.5	41.6	49.7	52.7	54.3	55.2	4.0

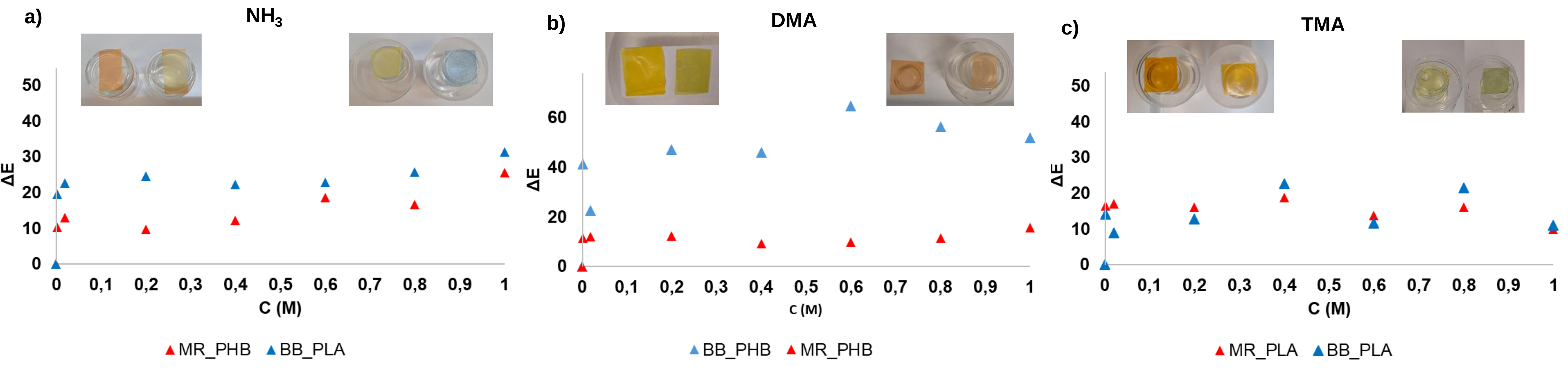


Figure 2. Color change (ΔE) after exposure to a) NH_3 of the MR/PHB and BB/PLA films, b) DMA of the BB/PHB and MR/PHB films and c) TMA of the MR/PLA and BB/PLA films.

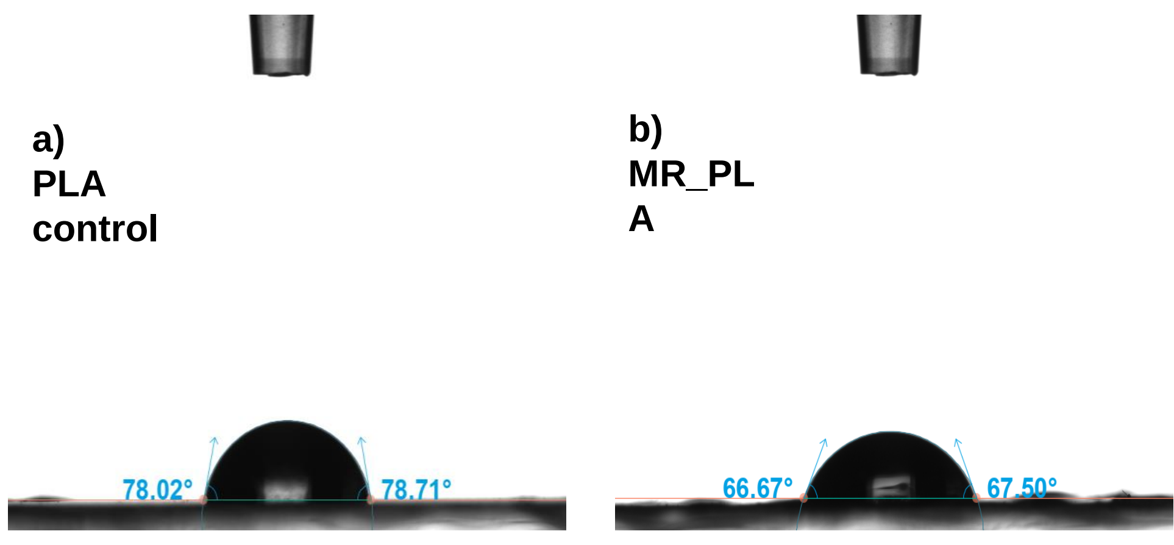


Figure 3. Water contact angle of a) PLA films (control) and b) MR_PLA films.

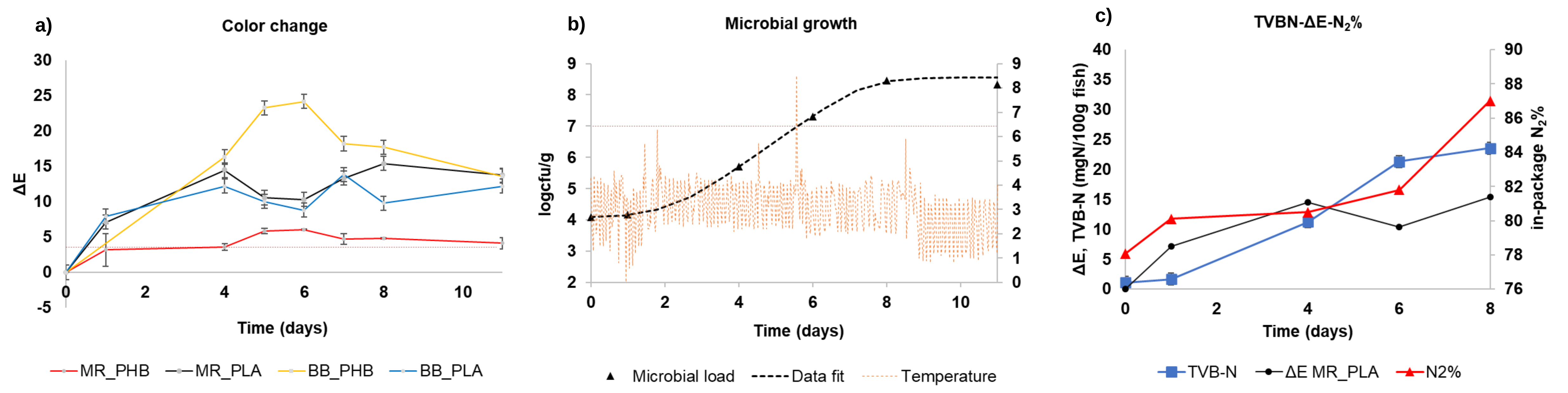


Figure 4. Visual appearance of the results obtained during the isothermal storage of gilthead sea bream fillets a) color change (ΔE) of the 4 smart indicators during fish shelf life, b) TVC growth and c) correlation of TVB-N and in-package N_2% concentration with the color change of the MR/PLA indicator.

Figure 5. Gilthead seabream fillets stored in RT after 30 hours with noticable color change of the MR/PHB smart indicator.

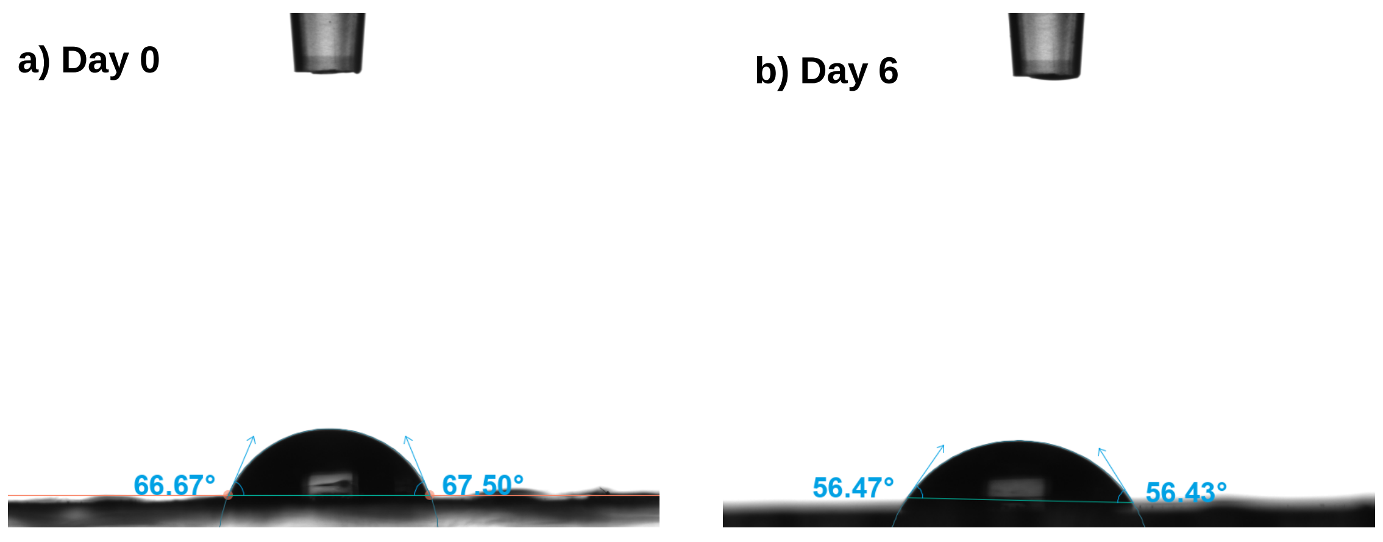


Figure 6. Water contact angle of MR/PLA films at a) day 0 and b) day 6 of storage.

Conclusions

- ➢ All smart indicators provided a color change visible to the human eye (ΔE>3) in both isothermal (4°C) and abused (RT) conditions which correlated with fish spoilage process.
- ➢ Gilthead seabream in RT spoiled after 30h (6,54 logcfu/g TVC at 24h)
- ➢ MR/PLA provided the most accurate representation of the microbial growth, TVB-N and in-package N_2% progress (isothermal storage)
- ➢ Hydrophilicity of the indicators increased (drop of contact angle) during their enclosure to the package headspace, creating a potential barrier to the indicators' best performance

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