FIRST RESULTS OF A CHALLENGE TEST STUDY ON PSEUDOMONAS SPP. IN VACUUM-PACKED PLAICE FILLETS

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I. INTRODUCTION

Pseudomonas spp. is one of the main specific spoiling bacteria of fresh fishery products [1,2]. Knowing its acceptable load at the time of packaging and its growth potential during the shelf life is essential to ensure the freshness of fish fillets. Till now the growth potential of *Pseudomonas spp.* in fresh fish fillets has never been calculated because it is difficult to design a challenge test with a non-pathogenic bacterium consistently present in the product and using the food matrix itself. In this study, on vacuum-packed plaice fillets we overcame the problem inoculating *Pseudomonas aeruginosa* because it is a species not often present in foods so far [3,4] but for which there are selective and differential media.

II. MATERIALS AND METHODS

The ISO 20976-1:2019 standard was used as a guide for the design of the study. The analyses were carried out on a batch of vacuum-packed plaice fillets with a shelf life of 6 days. The inoculations took place on the morning of production on 24 test units and with a suspension created by mixing 3 reference strains of *Pseudomonas aeruginosa* (ATCC 9027, 27853,15442) at a known concentration. 8 control units were inoculated with sterile physiological solution. All the samples were initially stored at + 4°C but, starting from day 2 (T2), 12 samples were subjected to the simulation of thermal abuse in the warehousing/retail and home conservation phases, so they were transferred from +4°C to +6°C and, at T7, 4 samples were additionally moved to +8°C. The analysis were carried out at T0, T2, T4, T7, T9 in 3 test units and 1 control unit for each time and for both kind of storage. Petri dishes with Pseudomonas Isolation Agar medium (Liofilchem®) were used and the standard ISO 7218: 2013 was followed for the count. Control units were also used to measure pH, water activity (a_w) and to quantify the Total Viable Count (TVC) on Plate Count Agar medium (Merck®) following ISO 4833-1:2022. From a statistical point of view, a logarithm to base 10 transformation, the arithmetic mean and the standard deviation (s.d.) were performed when possible. The growth potential (Δ) of *Pseudomonas aeruginosa* was calculated subtracting the initial (T0) average load from the highest average load (among the analysis times within the shelf life); this was done for both storage conditions.

III. RESULTS AND DISCUSSION

Both *Pseudomonas aeruginosa* (Figure 1) and TVC (Figure 2) loads were higher in samples stored under thermal abuse than in those kept under refrigeration temperature. The growth potentials of *Pseudomonas aeruginosa* calculated were: 0.34 Log_{10}(CFU/g) at 4°C and 0.32 Log_{10}(CFU/g) at +6/8°C. In general pH and a_w decreased (Figure 3); the decrease in a_w was predictable for the progressive accumulation of liquid in the package towards the end of the shelf life and in the following period.

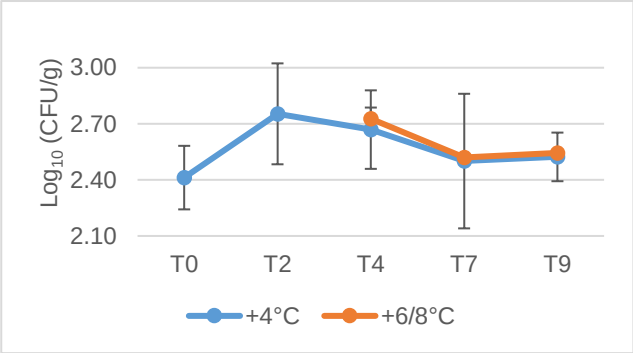

Figure 1. Average loads ± s.d. of *Pseudomonas aeruginosa* in test units during the days of vacuum storage.

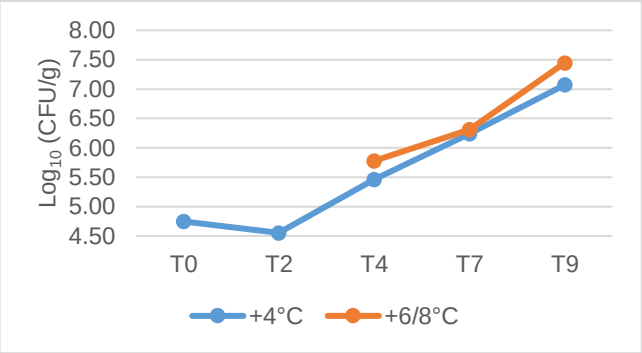

Figure 2. Total Viable Counts in control units during the days of vacuum storage.

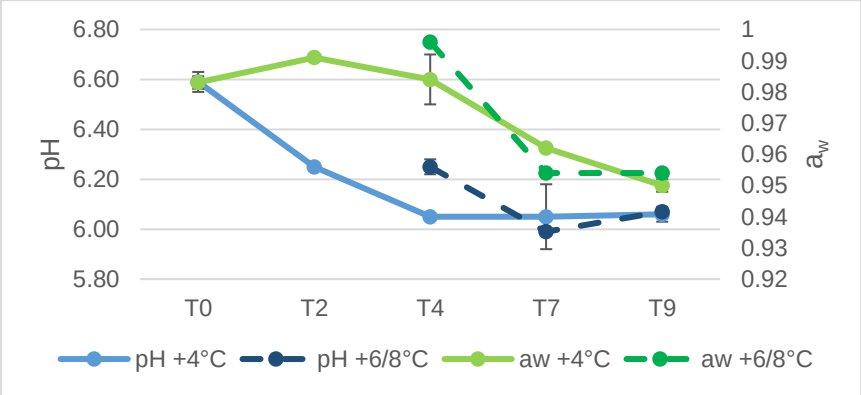

Figure 3. pH and a_w average ± s.d. in control units during the days of vacuum storage.

IV. CONCLUSION

Our study provides a basis and a strategy for developing challenge tests for *Pseudomonas* on food. By induction, the results allow us to state that *Pseudomonas spp.* growth potential in vacuum-packed plaice fillets is less than 0.5 Log_{10}(CFU/g) and, according to ISO 20976-1:2019, this enough to state that the bacterium did not show significant growth. Current data seems to confirm that even in vacuum-packaging it is crucial to have loads of *Pseudomonas spp.* between 10^4-10^5 CFU/g (or lower) at the beginning of the shelf life; 10^5 -10^6 CFU/g represents the average value found in plaice fillets of the same manufacturer [4] using EU hygiene standards in the fish industry. However, further analysis on a greater number of samples belonging to different batches is necessary to consolidate the results and to carry out a more in-depth statistical analysis of the data.

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