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Study and application of Time-Temperature Integrator smart labels for monitoring the cold chain of active modified atmosphere packaged sea bass

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ABSTRACT

Time Temperature Integrators (TTIs), inexpensive smart labels that indicate the time-temperature history per product unit, can support effective management of fish products supply chain. Shelf life of sea bass (*Dicentrarchus labrax*) in modified atmosphere packaging (MAP) with or without CO_2 emitters (50% CO_2) was modeled via microbial indices in the temperature range of 0-10 °C. Response of lipase/fatty acid ester based TTIs (VITSAB AB) was modeled for different enzyme concentrations/substrates. Temperature dependence was expressed through the activation energy (E_a) of the Arrhenius equation. Shelf life of MAP sea bass based on microbial growth was 18 days at 0°C (E_a=67 kJ/mol). TTIs E_a ranged from 95 to 140 kJ/mol. Matching TTI and product requires appropriate response time and E_a within 40 kJ/mol. TTI with methyl myristate substrate and 12 units enzyme concentration was selected for shelf life monitoring and its applicability in the MAP fish cold chain.

Keywords: Smart packaging, Time temperature integrators, Active modified atmosphere, Packaging, Cold chain monitoring.

1. INTRODUCTION

Refrigerated food products are complex systems that undergo changes which lead to quality deterioration, especially due to handling during distribution in the cold chain. Temperature is one of the main factors of quality's maintenance and any deviation from acceptable limits increases rates of quality deterioration and decreases the remaining shelf life.

In order to monitor the time-temperature history of foods, the application of Time-temperature integrators (TTIs) has been proposed. TTIs are small and inexpensive smart labels that can be placed on the food packaging and can show an easily measurable, time and temperature dependent colour change. This change cumulatively indicates the time-temperature history of the product from manufacture to consumption. The TTI can be used auxiliary with the expiry date, providing information on possible temperature deviation during storage or more generally for product mismanagement. For an effective TTI based cold chain management system to be developed mathematical models describing the effect of temperature on the evolution of food spoilage under dynamic storage conditions are prerequisite. Additionally, a full mathematical kinetic study of the TTI response as a function of temperature is required (Taoukis and Labuza, 1989).

Fish are highly perishable foods mainly due to the rapid growth of microorganisms that causes quality deterioration in a short period of time unless preservation techniques are used. Modified atmosphere packaging (MAP) can effectively prevent contamination and extend the shelf life of refrigerated fish. The application of such hurdles reduces the rates of fish products spoilage caused by microbial growth. MAP is a system where the air within a package is replaced by a

mixture of different gases. The most commonly used gases in MAP for fish are carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2). The concentration of CO_2 has been reported to play a major role in the inhibition of spoilage and pathogenic microbial growth and thus may delay the growth of respiratory organisms (Stammen et al. 1990, Tsironi et al. 2019).

Despite the preservative effect of MAP on packed fish products, it may be space-demanding due to the need for a high gas volume/product volume ratio. On the other hand, low ratios reduce the inhibitive effect of CO_2 on microbial growth. In addition, high concentrations of CO_2 in the package headspace may result in significant dissolution of CO_2 in the fish tissue. Carbon dioxide emitters utilize the O_2 in the package headspace in order to form CO_2 and enhance the concentration of a CO_2/N_2 headspace in the package without requiring an additional insertion of gas (Hansen et al. 2009, Tsironi and Taoukis 2018).

2. MATERIALS AND METHODS

2.1 Kinetic study of microbial growth on MAP sea bass

Fresh gutted sea bass (*Dicentrarchus labrax*) (weight: 300g/fish) was obtained in ice by a leading Greek aquaculture company (Selonda S.A.). Samples were transported to the Laboratory of Food Chemistry and Technology within 1 day after harvesting and packed in high-density polyethylene (HDPE) pouches in modified atmosphere consisted of 50% CO_2, 40% N_2 and 10% O_2 (Boss NT42N, Bad Homburg, Germany). One fish was placed in each package and the gas/sample volume ratio was 3:1. In half number of samples, selected CO_2 emitters (dimensions 300mm × 130 mm × 40 mm, McAirlaid's Inc., Steinfurt, Germany) were used in order to evaluate the effect of active MAP on shelf life extension.

MAP and active MAP (coded as ACT-MAP) samples were stored at controlled isothermal conditions (0, 5 and 10 ^{o}C) in high precision incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) for shelf life analysis. Temperature in the incubators was constantly monitored with electronic, programmable dataloggers (COX TRACER, Belmont, NC). Quality assessment of the sea bass was based on microbial load and specifically on total viable count (TVC), *Pseudomonas* spp., *Enterobacteriaceae* spp. and lactic acid bacteria (LAB). Their growth was enumerated by appropriate plate count method as described by Tsironi and Taoukis (2017). Gas headspace was evaluated using the CheckMate 9900 O_2/CO_2 device (PBI Dansensor, Ringsted, Denmark). The microbial growth was modelled using the Baranyi Growth model and for curve fitting, DMFit software (IFR, Institute of Food Research, Reading, UK) was used. The microbial growth rate (k) at each temperature was estimated and the temperature-dependence of rates was modeled via the activation energy (E_a) of the Arrhenius equation (Eq. (1)):

$$\ln(k) = \ln(k_{ref}) - \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \quad \text{Eq. (1)}$$

where k_{ref} is the microbial growth rate in d^{-1} at a reference temperature T_{ref} (T_{ref}=4 °C=277.15 K), T is the temperature in K, E_a is the activation energy in J mol^{-1} that indicates the temperature dependence of the selected microorganism growth rate, and R is the universal gas constant (Taoukis et al., 1997).

2.2 Time Temperature Integrators application and modelling

In order to match the quality degradation of MAP and ACT-MAP gutted sea bass with appropriate TTI, different enzymatic TTI were kinetically studied, provided by VITSAB AB (Malmo, Sweden). VITSAB enzymatic TTIs are based on a controlled enzymatic hydrolysis of a lipid substrate which decreases the pH and thus the TTI colour changes progressively from green to yellow/orange and finally to red. More precisely, M-type enzymatic TTI contains methyl myristate as lipid substrate and a *Rhizopus orizae* enzyme, while LP-type contains a mixture of trilaurine/tripalmitine and the same enzyme. Different enzyme concentrations were studied for M-type (5U, 10U, 15U, 20U, 25U, 50U, 75U and 100U) and LP-type enzymatic TTI (100U, 150U, 300U and 500U). Following inactivation, TTIs were stored at 0, 2.5, 5, 10 and 15 °C. Colour change was measured instrumentally using the Eye-One PRO (x-rite, Michigan, USA) and was expressed via CIELab scale (L, a, b).The enzymatic TTI response change was described by the normalized value (a + b) of the CIELab scale as shown in Eq. (2).

$$norm(a+b) = \frac{(a+b)-(a+b)_{min}}{(a+b)_{max}-(a+b)_{min}} \quad \text{Eq. (2)}$$

where a and b are parameters of the CIELab colour scale.

Plotting Eq. (2) as a function of storage time resulted in a sigmoidal function described by a logistic type equation (Eq. (3)).

$$F(x) = norm(a+b) = \frac{1}{1+exp\left(\frac{k_1-t}{k_2}\right)} \quad \text{Eq. (3)}$$

where k_1 and k_2 are the response rate constants which are functions of enzyme concentration and storage temperature and t is the storage time in days. At each case, these constants were determined by non-linear regression (SigmaPlot 12.0, Systat Software Inc.). In addition, the values k_1 and k_2 can be expressed as a function of enzyme concentration and temperature (Eq. (4)), so a composite model was developed for each type of TTI (M-type and LP-type) which predicts the TTI response of a known enzyme concentration at selected temperature and storage time.

$$F(x) = norm(a+b) = \frac{1}{1+exp\left(\frac{k_{1ref}*C^{-A}*exp\left[\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]-t}{k_{2ref}*C^{-B}*exp\left[\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}\right)} \quad \text{Eq. (4)}$$

where k_{1ref} and k_{2ref} are the response rate constants in d^{-1} at a reference temperature (T_{ref}=4 °C=273.15 K), T is the temperature in K, E_a is the activation energy in J mol^{-1}, R the universal gas constant and A, B are constants.

2.3 Matching the appropriate enzymatic TTI for shelf life monitoring of gutted sea bass

In order to select the appropriate enzymatic TTI for packaged gutted sea bass the growth curves of the shelf life determining microbial indicator and the response of the TTI were combined and compared in pursue of an adequate match in the range of 0–10 °C. Practically, this required the TTI response kinetics to be similar to the growth kinetics of the target microorganism. Considering that this close match is not always achievable, a suitable TTI could be selected if it showed satisfactory correlation with the target microorganism at high (abuse) temperatures. The selected quality index of MAP and ACT-MAP sea bass was total viable counts (TVC) growth and the shelf life estimation was determined as the time required to reach the acceptability limit of 7.0 log CFU g^{-1} (ICMSF 1986).

3. RESULTS AND DISCUSSION

3.1 Gas changes

CO_2 concentration in the MAP gutted sea bass package headspace decreased due to its dissolution in the fish tissue and reached the minimum value of 40% after 15, 11 and 4 days of storage at 0, 5, and 10 °C, respectively. Afterwards, CO_2 concentration increased (up to 50% approximately) due to the metabolic activity of the spoilage bacteria. On the other hand, CO_2 concentration in the ACT-MAP gutted sea bass package headspace initially decreased up to 46% and then increased, indicating that the CO_2 emitters started to produce CO_2 due to the contact of the emitter ingredients in the liquid absorber pad and the liquid loss. The highest CO_2 values (60.5–63.5%) were observed at the end of the storage time at all the examined temperatures.

O_2 concentration showed a descending trend with time while at the end of the storage period the concentration exhibited zero levels. This trend was related to the increased microbial population of the total viable counts, for both MAP and ACT-MAP gutted sea bass samples.

3.2 Microbial growth and shelf life estimation

The microbial growth rates of TVC, *Pseudomonas* spp., *Enterobacteriaceae* spp. and LAB in MAP and ACT-MAP gutted sea bass stored at each temperature are presented in Table 1. The initial TVC for the fresh gutted sea bass was approximately 4 log CFU g^{-1}. The MAP gutted sea bass samples reached the limit value of 7 log CFU g^{-1} (ICMSF 1986) on days 18, 10, and 6 at 0, 5, and 10 °C, respectively.

The ACT-MAP samples reached the TVC limit value on days 22, 17, and 10, respectively, indicating significant inhibitory effect of CO_2 emitters on the TVC microbial growth due to a lag phase increase. In addition, the growth rates of TVC were lower for the ACT-MAP samples compared to the MAP samples, as shown in Table 1.

The initial *Pseudomonas* spp. counts were approximately 3.0 log CFU g^{-1}, reaching final values of 5.0-6.0 log CFU g^{-1}. The *Pseudomonas* spp. growth rates were significantly lower for ACT-MAP samples compared to MAP samples at all tested temperatures.

Enterobacteriaceae spp. growth rates increased under MAP and ACT-MAP with increasing storage temperature from 0 to 10 °C. In addition, the CO_2 emitter use, decreased the growth of *Enterobacteriaceae* spp. at all tested temperatures. The final load reached the value of 6.0 log CFU g^{-1} at 5 and 10 °C and the value of 4.0 log CFU g^{-1} at 0 °C. In case of LAB, the load remained below the spoilage level of 6.0 log CFU g^{-1}. It was concluded that the CO_2 concentration in the package headspace did not present a statistically significant effect on LAB in MAP and ACT-MAP samples.

Table 1. Growth rate (k in d^{-1}), for the total viable count (TVC), *Pseudomonas* spp. and *Enterobacteriaceae* spp. in gutted sea bass during the isothermal storage at 0–10 °C.

Storage Temperature	0 °C	5 °C	10 °C
Total viable count			
MAP	0.175 ± 0.010	0.337 ± 0.028	0.496 ± 0.027
ACT-MAP	0.138 ± 0.017	0.277 ± 0.016	0.308 ± 0.041
Pseudomonas spp.			
MAP	0.165 ± 0.005	0.347 ± 0.029	0.345 ± 0.142
ACT-MAP	0.108 ± 0.027	0.129 ± 0.006	0.191 ± 0.012
Enterobacteriaceae spp.			
MAP	0.245 ± 0.093	0.339 ± 0.103	0.625 ± 0.069
ACT-MAP	0.204 ± 0.017	0.292 ± 0.019	0.378 ± 0.063

The temperature effect on the microbial growth rates for all the examined microorganisms (TVC, *Pseudomonas* spp., and *Enterobacteriaceae* spp.) was described by the activation energy (E_a) which is summarized in Table 2. According to the results, E_a values for the TVC, *Pseudomonas* spp. and *Enterobacteriaceae* spp. growth rates were in the range of 37–52 kJ mol^{-1} and 48–67 kJ mol^{-1} for ACT-MAP and MAP samples, respectively, not showing statistically significant differences between the two group of samples.

Table 2. Kinetic parameters of the Arrhenius model for the microbial growth rate for total viable count (TVC), *Pseudomonas* spp. and *Enterobacteriaceae* spp. in gutted sea bass during the isothermal storage at 0–10 °C.

Total viable count	MAP	ACT-MAP
E_a (kJ mol^{-1})	67.2	51.8
k_{ref} (d^{-1})	0.279	0.211
***Pseudomonas* spp.**	**MAP**	**ACT-MAP**
E_a (kJ mol^{-1})	47.8	36.7
k_{ref} (d^{-1})	0.252	0.131
***Enterobacteriaceae* spp.**	**MAP**	**ACT-MAP**
E_a (kJ mol^{-1})	60.0	39.7
k_{ref} (d^{-1})	0.342	0.266

3.3 TTI modelling and application

The TTI response was adequately described by sigmoidal equation (Eq. (2)) as a function of storage time and the parameters k_1 and k_2 were determined. In both TTI types (M-type and LP-type) increasing enzyme concentration and storage temperature, led to accelerated colour change. The response rate constants were plotted as a function of temperature in Arrhenius plots. Increasing enzyme concentration did not have a significant effect on observed activation energy values for both k_1 and k_2, ranging from 88 to 101 kJ mol^{-1} for k_1 and from 78 to 95 kJ mol^{-1} for k_2. The E_a values for LP-type ranged from 118 to 167 kJ mol^{-1} for k_1 value and from 117 to 164 kJ mol^{-1} for k_2.

The response data for M-type and LP-type TTI were used to develop a composite model (Eq. (4)) and the calculated constants are shown in Table 3. Based on these models, the response times of the enzymatic TTIs at each temperature were calculated. The experimental and predicted response times were found to be well correlated ($R^2_{M\text{-}type}$=0.97 and $R^2_{LP\text{-}type}$=0.84), showing wider variation at higher

response time values. The predicted response times for all tested TTIs are presented in Fig. 1.

Table 3. Parameters of the composite model of Eq. (4) for M-type and LP-type enzymatic TTI

Enzymatic TTI	E_a (kJ mol^{-1})	$k_{1,ref}$ (d^{-1})	$k_{2,ref}$ (d^{-1})	A	B
M-type	96.9	127.7	24.9	0.86	0.85
LP-type	137.4	1247.0	177.2	1.04	0.89

3.4 Matching appropriate TTI with shelf life of MAP and ACT-MAP sea bass

To select the appropriate enzyme concentration for monitoring the shelf life of MAP and ACT-MAP sea bass during distribution in the cold chain, the shelf life curves of the studied TTIs and the products were combined to obtain an adequate match as described by Tsironi et al. (2015) and the results are depicted in Fig. 1. According to the results, M-type of TTI matched better with both products due to similar response duration and E_a values (within ±40 kJ mol^{-1}), while match was not satisfactory between the response times examined for LP-type TTI and both sea bass products. Comparing the developed predictive models, the required enzyme concentration for the TTI labels was identified and finally M-21U and M-12U were selected as the most suitable TTIs for monitoring the shelf of MAP and ACT-MAP sea bass, respectively (Fig. 2). In both cases, if products are stored at low temperatures (0-2.5 °C), the end of shelf life will be determined by the nominal expiration date, while in cases of higher temperatures (in case of temperature abuse within the cold chain) the TTI will conservatively signal poor quality before the end of shelf life. The application of the proposed TTIs was evaluated in validation trials performed by attaching TTI tags on packaged gutted sea bass samples and storing at dynamic temperature conditions (expected in the real cold chain). At different time intervals TTIs response was measured and used to predict the remaining shelf life and was compared to the experimentally determined (based on microbiological analysis) remaining shelf life, the error in predicting the remaining shelf life was within acceptable limits of error (<20%).

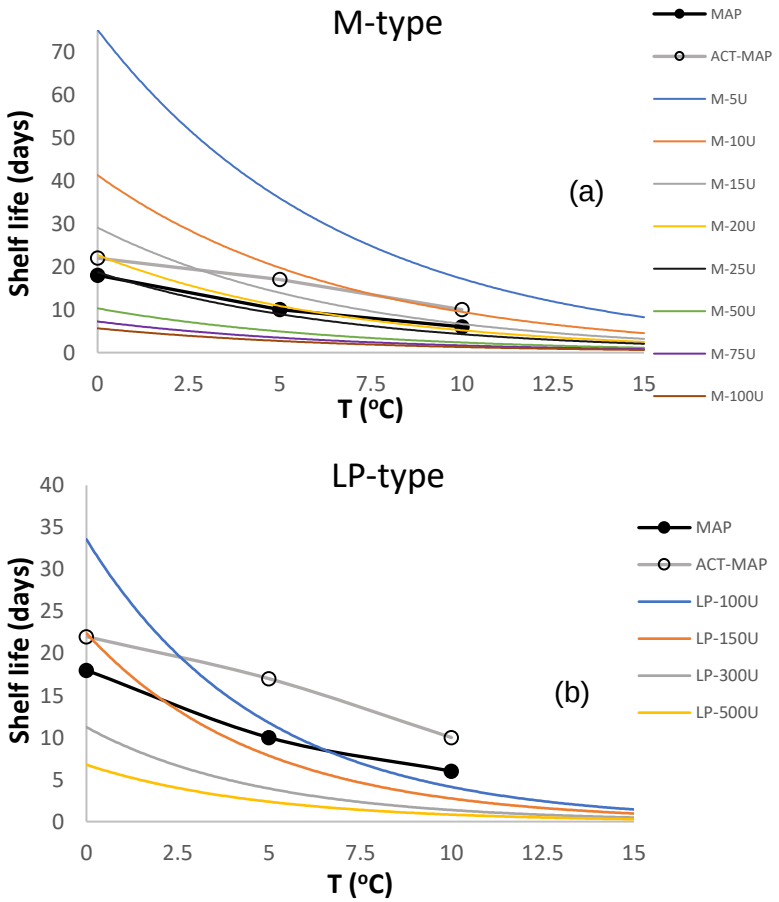

Figure 1: Total response time of (a) M-type and (b) LP-type enzymatic TTI as a function of temperature and enzyme concentration calculated by the composite model (Eq. (4)) and shelf life of MAP (●) and ACT-MAP (○) sea bass

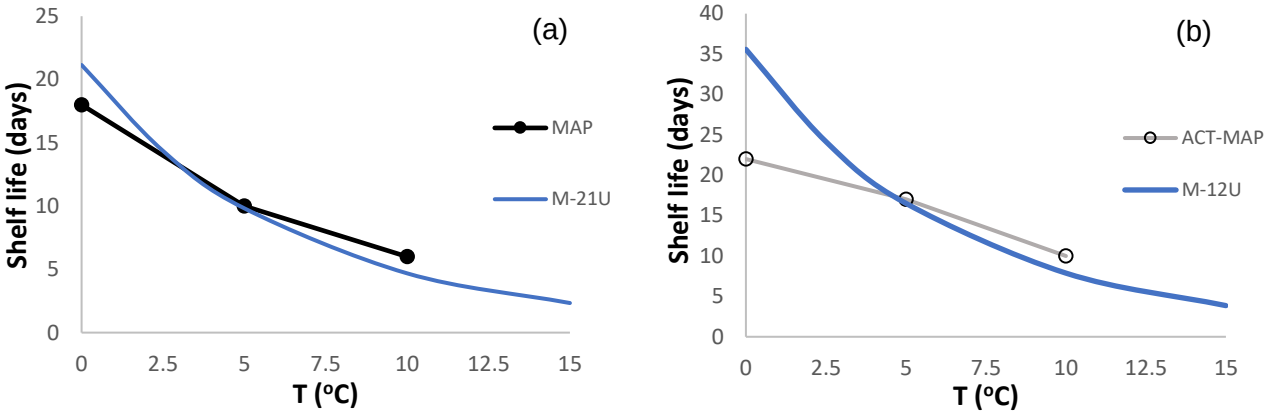

Figure 2: Suitable M-type enzymatic TTI for monitoring the shelf life of (a) MAP and (b) ACT-MAP sea bass

4 CONCLUSIONS

MAP is an important food packaging technology for extending the shelf life and maintaining the quality of perishable refrigerated food products such as fresh fish. MAP with CO_2 emitters, which produce CO_2 in contact with water from liquid leaking of fish tissues, extended the shelf life of gutted sea bass. MAP samples were acceptable up to 18, 10 and 6 days of storage at 0, 5 and 10 °C respectively while the use of CO_2 emitters led to a 4-7 days shelf life extension. CO_2 concentration initially decreased and then increased due to microbial growth in both types of packaging and relevant CO_2 production from emitters in case of ACT-MAP fish. The kinetic study of different types of TTI (M and LP-type) and different enzyme concentrations (5-100 units for M-type and 100-500 units for LP-type) at 0-15 °C allowed the development of a mathematical kinetic model where TTI response time is expressed as a function of concentration, storage temperature and time. Finally, M-21 U and M-12 U enzymatic TTI labels were found to be well correlated with the shelf life of MAP and ACT-MAP sea bass making them good candidates to monitor shelf life at any stage of the cold chain.

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