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Arrhenius equation modeling for the shelf-life prediction of vacuum packed sea bass incorporated with bioactive preservative

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Abstract
This study aimed to study the effect of inoculation of *Lactococcus lactis* and *Carnobacterium piscicola* and/or *Citrus* essential oil (*CEO*) addition on vacuum-packed sea bass (*Dicentrarchus labrax*) fillets quality during 21 days of storage at 4°C. Throughout storage, both *LAB* and *CEO* preserved a high content of polyunsaturated w-3 fatty acids, prevented my of ibrillar proteins proteolysis and improved the textural properties of fish. Then, using an Accelerated Shelf Life Test (ASLT) the shelf life of fish fillets was estimated based on total viable count (TVC) and total volatile basic nitrogen (TVB-N). For these parameters, developed kinetic models served to determine the quality loss. Overall, the combined adjunction of *LAB* and *CEO* was an efficient tool resulting in sea bass fillets quality preservation and significant shelf life extension by approximately 3-5 days (control *CEO*), 4 days (control *LAB*) and 5 days (*LAB+CEO*).

Keywords: *Citrus* essential oil, lactic acid bacteria, sea bass quality, shelf life, vacuum packaging

Introduction

Fish is the favored food in many countries due to its richness in proteins, vitamins, minerals, and omega-3 polyunsaturated fatty acids (Durmus, 2020) [7]. Although its broader consumption, the high perishable fish products (Sofra *et al*., 2018) [24] are characterized by short shelf-life brought about both biological and chemical reactions such as lipid oxidation and microbial activities (Kachele *et al*., 2017) [12] originating from natural flora or from contamination of fish (Lopez de Lacey *et al*., 2014) [14]. Therefore, spoilage is a complicated process (Kachele *et al*., 2017; Guirattana *et al*., 2016) [12] linked to the formation of ammonia, biogenic amines, responsible of unpleasant and unacceptable off-flavours due to the production of trimethylamine (Lopez de Lacey *et al*., 2014) [14]. So that, the freshness quality related to safety and nutritional quality of fish can be affected by handling, processing and storage conditions from the catch to the consumers (Boulares *et al*., 2017) [3].

For these reasons, the improvement of efficient preservation techniques for extending shelf-life and improving quality of fresh fish fillets is crucial (Guirattana *et al*., 2016). In this context, the application of several microorganisms, their actives substances, plant extracts such as essential oils (EOs) and their pure components is interesting, due to their relatively safe status (Guirattana *et al*., 2016). Lactic acid bacteria (LAB) could be used for the improvement of the microbiological quality and nutritional value of fish muscle without changing its sensory characteristics (Boulares *et al*., 2017) [3]. Also, more attention has been paid to the biopreservative effects of natural compounds of the herbal medicine (Bouzenna *et al*., 2016) [2] known to present biological activities (Lou *et al*., 2017) [15]. In this context, *Citrus limon* is a medicinal plant of the family *Rutaceae* found in Tunisia and other Mediterranean countries (Ben Hsouna *et al*., 2017) [1] and having many important natural chemical components such as ascorbic acid, citric acid, minerals, flavonoids and essential oils (Bouzenna *et al*., 2016) [2].

Recently, Citrus EO is an economic and natural antioxidant, antifungal and antibacterial component widely used in food industries (Mahato *et al*., 2019) [16] due to its active compound mixture (Ben Hsouna *et al*., 2017) [1]. Also, the use of *Citrus* EO was based on the resulted sensory attributes in food like the pleasant smell and like-lemon taste (Lou *et al*., 2017) [15].

Hence, this study is aimed to investigate first, the protective effect of lactic acid bacteria strains combined with *Citrus limon* essential oil and vacuum packaging on biochemical and textural properties of sea bass fillets during 21 days of storage at 4°C. Then, the effects of this combined treatment on sea bass fillets shelf life extension were evaluated using an accelerated shelf life test.

Materials and methods

Fish preparation, treatment and storage

Fresh farmed sea bass (*Dicentrarchus labrax*) (fish with an average weight of 400 g) were purchased and kept in ice throughout transportation to the laboratory. The same day of their capture, fish were immediately gutted, headed, washed and aseptically hand filleted.

Then, two LAB strains (*Lactococcus lactis* KF147 and *Carnobacterium piscicola* AT 71101238000999) were prepared (appropriately diluted in 0.9% NaCl solution) to inoculate the fillets at a cell density of 8 log CFU/ml according to the study of Boulares *et al*. (2017) [3]. Thus, five batches were prepared:

- Fresh untreated sea bass fillets were used as control,
- Sea bass fillets inoculated with two LAB strains in association named control *LAB*,
- Sea bass fillets treated with pure *Citrus* essential oil at a concentration of 0.5% (v/w), named Control *CEO*. Pure Citrus essential oil was purchased from the Laboratory of medicinal plants CERINA, Tunisia.
- Fresh sea bass inoculated with LAB strains and treated with CEO, named *LAB+CEO*.

Bacterial suspension and essential oil were sprayed on the surface and into the muscle of sea bass fillets before being well distributed with sterilized gloved fingers (Boulares *et al*., 2017) [3]. Finally, sea bass fillets were immediately sampled (day 0), packed under vacuum in polyethylene keeping boxes and cold stored at 4 °C for up to 21 days. Sampling was evaluated on days 0, 7, 14 and 21 with three replicates.

Quality assessment during storage

pH measurement

pH measurement was determined as described by Boulares *et al*. (2018) [2], at room temperature, on different homogenized control and treated filleted.

Fatty acid composition

The extraction of lipid fraction and fatty acids content were determined according to Boulares *et al*. (2017) [3]. Chloroform/methanol (2:1 v/v) solution was used for the extraction of lipids. Then, fatty acids content was performed based on gas chromatography injection of methyl esters of fatty acids stored at – 40 °C (Boulares *et al*., 2017) [3]. The proportion of each fatty acid was expressed as a percentage of the total fatty acids.

Muscle proteins extraction

Extraction of sarcoplasmic proteins (SP) and myofibrillar proteins (MP) of fresh farmed sea bass muscle was performed as previously described by Boulares *et al*. (2017) [3]. The obtained protein fractions stored at −80 °C were analyzed by Sodium dodecyl sulphate–polyacrylamide gel electrophoresis SDS-PAGE (Mauriello *et al*., 2002) [17] using 4% poly-acrylamide stacking gels and 15% separating gels.

Texture measurement

Fillets texture was carried out using a TA-XT2 Texture Analyzer (Stable Microsystems Haslemere, UK). Fish fillets were subjected to a penetration test using a cylindrical plunger (16 mm in diameter) at a constant speed of 0.1 mm/s and at a deformation level of 20%. Then, the parameter hardness (N) was recorded and three measurements were performed on each fillet for averaging the valid values. Hardness is defined as the maximum force (N) that occurs at the compression cycle (Kaewprachu *et al*., 2017; Periago *et al*., 2005) [13, 18].

Shelf life kinetic study

Estimation

The shelf life estimation of vacuum-packed sea bass fillets inoculated with LAB strains and/or treated with Citrus EO was evaluated using an Accelerated Shelf Life Test (ASLT) carried out as a function of total viable count (TVC) and total volatile basic nitrogen (TVB-N). Sea bass fillets used in this study were stored at controlled isothermal conditions of 4°C, 14°C and 24°C (10 fillets per temperature) in high-precision (±0.2 °C) incubators. Sea bass fillets were taken in appropriate time intervals to allow an effective kinetic analysis of quality deterioration based on variation of storage temperature (4 °C: 0, 3, 6, 9, 12, 15, 18 and 21 days; 14 °C: 0, 3, 6, 9, 12, 15 days and 24 °C: 0, 3, 6 and 9 days). Quality deterioration assessment was performed for two replicated storage experiments.

Kinetic data analysis

To determine the reaction order of TVC and TVB-N, zero and first-order kinetics were hypothesized by applying the general reaction rate expression:

$$\frac{d[A]}{dt} = K[A]^n \qquad \textbf{(Eq 1)}$$

Where [A] is the total viable count (TVC) and total volatile basic nitrogen (TVB-N), k is the reaction rate constant ($days^{-1}$), n is the reaction order and t is the reaction time (days). The order which gave the best regression (R^2) among the experimental values and the theoretical half-life ($t_{1/2}$) of the two tested parameters was chosen as representative of the present study.
Pseudo first-order rate constants of TVC and TVB-N were determined by linear regression of at least eight points from the initial part of the curves. The effect of temperature ranging from 4 to 24 °C on the rate of TVC and TVB-N was determined by means of the Arrhenius equation:

$$k = k_0 \exp(-Ea/RT) \quad \textbf{(Eq 2)}$$

Where k is the reaction rate constant, R is the universal gas constant (8.31 J K^{-1} mol^{-1}), T is the absolute temperature (°K), Ea is the activation energy (J mol^{-1}) of the studied action and k_0 is the pre-exponential factor of the frequency factor (Sofra *et al*., 2018) [24].
This equation allows the define the TVC and TVB-N indicating the end of the shelf life based on the risk level. While the TVC and TVB-N follows a pseudo-first order kinetics, the shelf life of sea bass fillets can be finally predicted accordingly:

$$SL\ (Days) = \frac{Ln\ [A_L] - Ln\ [A_0]}{K_{4°C}} \quad \textbf{(Eq 3)}$$

SL is the shelf life expressed as days, A_L corresponded to the limit values of TVC and TVB-N of sea bass fillets, A_0 is the TVC and TVB-N values at initial storage time. K and T represents the pseudo zero rate constants at the selected temperature.

Total viable count (TVC) enumeration
TVC were enumerated on Plate Count Agar (PCA, Oxoïd, Ltd., Basingstoke England) after incubation at 30°C for 72 h (Boulares *et al*., 2017) [3].

Total volatile basic nitrogen (TVB-N) determination
TVB-N content in sea bass fillets was measured using the FIA technique performed as reported by Ruiz-Capillas *et al*. (2000). Briefly, 1 g of the ground sample were weighed in a container and homogenized with 2 mL distilled water and 250 mL 6% perchloric acid solution for 2 min. The obtained blend was centrifuged at 12000 rpm for 10 min. The supernatant was used for Flow Injection Analysis (FIA) and standards of ammonium chloride were prepared by taking appropriate dilutions (Boulares *et al*., 2017) [3].

Statistical analysis
The results were performed in two replicates and presented as mean and standard deviation (SD). Analysis of variance (ANOVA) in SPSS software version 20 (IBM Corp 2011) and Duncan's test were used at a significance level of 5%.

Results and Discussion
pH variation
In the present study, according to the results displayed in Figure 1, the starting pH value of fresh farmed sea bass fillets was about 6.54±0.06 which was in line with other studies (Boulares *et al*., 2018) [2]. In fact, in live fish muscle, the pH value is close to 7.0. However, postmortem pH vary from 6.0 to 7.1 depending on several factor such as season, species, and feed composition (Boulares *et al*., 2017, Durmus, 2020) [3, 7]. This value increased significantly ($p < 0.05$) in control fillets and reached 6.99±0.07 after 14 days of refrigerated storage. This finding was due to fish spoilage bacteria proliferation and the breakup of nitrogen compounds producing basic compounds like ammonia, trimethylamine and other biogenic amines (Durmus, 2020) [7]. Following this period, pH values decreased toward the end of storage. However, initial pH values had undergone a preliminary rise until the 7th day in fillets inoculated with LAB alone or combined with CEO. Then, pH decreased at end of storage period. Otherwise, the pH of fillets treated with CEO alone remained unchanged, during all storage period, due to the antagonistic effect of citrus EO against spoilage bacteria delaying the formation of volatile compounds as reported by Durmus (2020) [7].
Besides, the lowest final pH (6.49 ± 0.01) was noted in fish fillets inoculated with selected LAB strains showing their capacity to produce lactic acid and bacteriocins which inhibited food poisoning bacteria as reported by Boulares *et al*. (2017) [3].

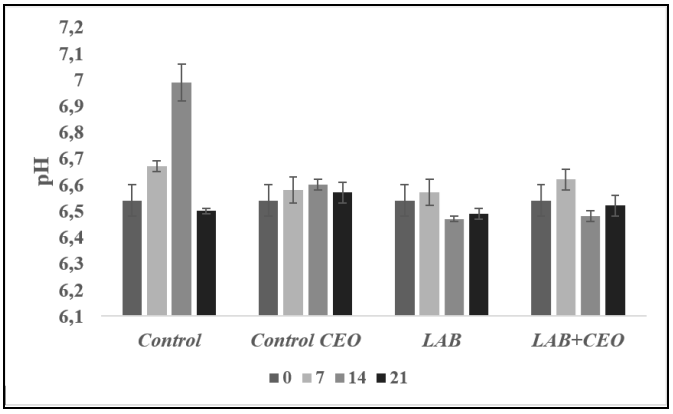

Fig 1: pH changes in fresh farmed sea bass fillets during 21 days of vacuum-packaging storage at 4°C (0 d (■) 7 d (■) 14 d (■) and 21 d (■)

Fatty acids variation

The fatty acid (FA) changes of total lipids extracted from sea bass analyzed fillets during 21 days of refrigerated storage are presented in Table 1. Saturated (SAFA) and monounsaturated (MUFA) fatty acids can be made in the human body, while, linoleic acid (C18:2 w-6) and linolenic acid (C18:3 w-3) belonging to polyunsaturated fatty acids (PUFAs) must be provided by diet (Ozden and Erkan, 2008) [19]. In this study, the prominent fatty acids were oleic (C18:1 w-9), followed by palmitic (C16:0), palmitoleic (C16:1 w-7), docosahexaenoic (DHA C22:6 w-3), linoleic (C18:2 w-6) and eicosapentaenoic (EPA C20:5 w-3) acids, which have beneficial effects for human health while they are considered as essential fatty acids (Boulares *et al*., 2017; Ozden and Erkan, 2008) [3, 19]. During all the storage period, FAs content increased with storage and the highest content was registered for MUFA (ranging from 37.77 to 44.64), followed by PUFA (28.49 to 34.47) and SAFA (23.74 to 26.93) as found by Boulares *et al*. (2017) [3] and Ozogul and Ozogul (2007). MUFA and SAFA contents increased, while PUFA content decreased at the end of refrigerated storage to reach 28.49, 30.11, 30.16 and 30.29 % for untreated control, *CEO* control, control *LAB* and *LAB+CEO*, respectively. In this connection, lipid oxidation is the main reaction in relation to lipase activity, fish species and storage conditions that indicate the freshness of fish (Cheng *et al*., 2015) [6]. Besides, light and high temperature lead to the oxidation of FA and their break down into low molecular weight substances (aldehydes, ketones and carboxylic acid groups) which result in nutritional and organoleptic (smell, texture and color) alterations of fish (Cheng *et al*., 2015) [6]. Moreover, the initial content of linoleic acid (12.25%) decreased significantly ($p<0.05$), during storage. After 21 days at 4°C, the lowest content (7.46%) was observed at untreated control and very close values (10.68 and 10.67%) were noted at control *LAB* and *LAB+CEO*. Equally, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents decreased slowly during storage or remained constant. In fact, into the body linolenic acid can be converted to arachidonic acid, or EPA and DHA as described before by Ozden and Erkan (2008) [19]. These results were confirmed by the stability of the ratios w-6/w-3, PUFA/SAFA and IDO during storage period, which was in line with the results found by Periago *et al*. (2005) [18]. Therefore, both LAB association and *Citrus EO* alone or in combination with vacuum packaging preserved a good content of PUFA by inhibiting their oxidation and enhancing the nutritional quality of sea bass fillets. These findings confirmed those highlighting the antioxidant and antimicrobial activities of *Citrus* EO against a huge range of fish spoilage bacteria (Iturriaga *et al*., 2012) [11] and its synergistic effect with probiotics and their actives substances such as bacteriocins (Shipradeep *et al*., 2016) [23].

Table 1: Fatty acids changes (%) in vacuum packed sea bass fillets during 21 days of storage at 4°C

	Sea bass fillets								
	Control			***Control CEO***		***Control LAB***		***LAB+CEO***	
Storage (D)	0	14	21	14	21	14	21	14	21
Fatty acids									
C14:0	2,80	4,42	4,21	3,02	2,64	4,10	2,81	3,69	2,93
C15:0	1,07	0,70	0,00	0,24	2,04	0,28	0,57	0,90	0,17
C16:0	16,36	18,04	18,74	17,36	18,24	16,86	18,65	17,57	18,80
C16:1 w7	10,62	12,75	16,58	8,61	11,22	9,20	12,25	12,14	12,22
C16:2 w4	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C16:3 w4	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C18:0	3,51	3,77	3,74	3,84	3,50	2,56	3,80	2,00	3,78
C18:1 w9	24,68	27,05	26,02	28,26	28,67	28,37	29,75	27,42	29,17
C18:1 w7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C18:2 w6	12,25	7,18	7,46	10,39	9,88	11,57	10,68	10,07	10,67
C18:3 w3	1,64	1,36	1,70	2,90	2,14	2,16	1,61	2,05	1,46
C18:4 w3	1,91	1,44	1,77	2,04	0,73	2,06	0,97	1,40	0,58

C20:1 w9	2,47	2,38	2,04	3,00	3,35	3,02	1,73	2,85	2,06
C20:4 w6	0,50	0,91	0,70	1,46	0,76	1,28	0,74	0,55	0,71
C20:4 w3	0,40	0,35	0,38	0,47	0,40	0,89	0,39	0,68	0,49
C20:5 w3	6,57	6,69	6,32	6,90	6,50	6,71	6,57	6,99	6,72
C22:5 w3	0,70	0,81	0,70	0,67	0,59	0,78	0,55	0,74	0,69
C22:6 w3	9,38	9,85	9,46	9,00	8,73	9,02	8,65	9,82	8,97
SAFA	23.74	26.93	26.69	24.46	26.42	23,80	25,83	24.16	25.68
MUFA	37.77	42.18	44.64	39.87	43.24	40,59	43,73	42.41	43.45
PUFA	33.35	28.59	28.49	33.83	30.11	34,47	30,16	32.30	30.29
w-3	20.60	20.50	20.33	21.98	19.47	21,62	18,74	21.68	18.91
w-6	12.75	8.09	8.16	11.85	10.64	12,85	11,42	10.62	11.38
Total FA	93.86 [a]	97.70 [b]	99.82 [e]	98.16 [c]	99.77 [e]	98,86 [d]	99,72 [e]	98.87 [d]	99.42 [e]
∑w-6/∑w-3	0.62	0.39	0.40	0.54	0.55	0.59	0.61	0.49	0.60
IDO	0.91	0.92	0.84	0.92	0.83	0,93	0,82	0.96	0.83
PUFA/SAFA	1.36	1.06	1.07	1.38	1.14	1,45	1,17	1.34	1.18

Values are means of three replicates, standard deviation are in the range [±0.00 to ±2.54]. Means with different superscripts are significantly different ($p < 0.05$).

Evolution of proteolysis of muscle proteins

Proteins are nutritional component of fish muscle that mainly comprise sarcoplasmic protein, myofibrillar protein and insoluble matrix protein (Cheng *et al*., 2015) [6]. Based on SDS-PAGE technique, the present study explains the qualitative changes affecting different muscular proteins of fresh sea bass fish fillets after their inoculation with LAB strains and/or treatment with *Citrus* EO, during 21 days of refrigerated vacuum packaging. The electrophoretic patterns of sarcoplasmic proteins (SP), showed the presence of 13 major protein bands (Figure 2-a). The major observed protein bands are as follows in average molecular weights: 100 kD, 60 kDa, 51 kDa, 41–39 kDa. For myofibrillar proteins (MP), 14 bands were observed as shown in Figure 2-b. These results were similar to those described elsewhere (Boulares *et al*. 2017) [3]. Moreover, the SP profile of untreated control fillets did not represent important proteolytic variation at the end of refrigerated storage, while an alteration of the MP profile arose in the untreated fillets. These findings were partially in accordance with those reported by Mauriello *et al*. (2002) [17] suggesting the possible endogenous proteolytic activity resulting from the co-operation between endogenous enzymes and proteases of microbial origin. In addition, no important proteolytic changes were observed, at the end of storage, on the SP profiles resulting from the combined effect of LAB combinations and *Citrus* EO. However, MP proteins were sensitive to both the treatment with CEO and the storage. In fact, at the end of storage, a decrease in the intensity bands of myosin heavy chain (MCL, 200 kDa), α-actinin (α-AC, 108 kDa), desmin (50 kDa), actin (AC, 42 kDa), tropomyosin (TMP, 35 kDa), troponine (T, 32 kDa) and myosin light chains (MLC, 18 kDa) was noted. Similarly, it was clear the appearance of bands in the range of 60 ± 150 kDa, between tropomyosins (35 and 32 kDa) and myosin light chains (MLC1 and MLC2, 24 and 21 kDa) as a result of the breakdown of MHC and high molecular weight MP proteins fragments as observed by Boulares *et al*. (2017) [3] and Mauriello *et al*. (2002) [17]. It can be concluded thus, that SP were more stable then MP during fish *post-mortem* period and during vacuum packaging storage (Boulares *et al*., 2017) [3]. On the other hand, the inoculated LAB mixture alone or combined with CEO retard the proteolysis of MP by maintaining the intensity of protein bands when comparing with untreated control and *CEO* control, at the end of storage period. This result was attributed to the wide antibacterial activity of inoculated LAB strains (*Lactococcus lactis* and *Carnobacterium piscicola*) against undesired psychrotrophic and proteolytic bacteria in fish products.

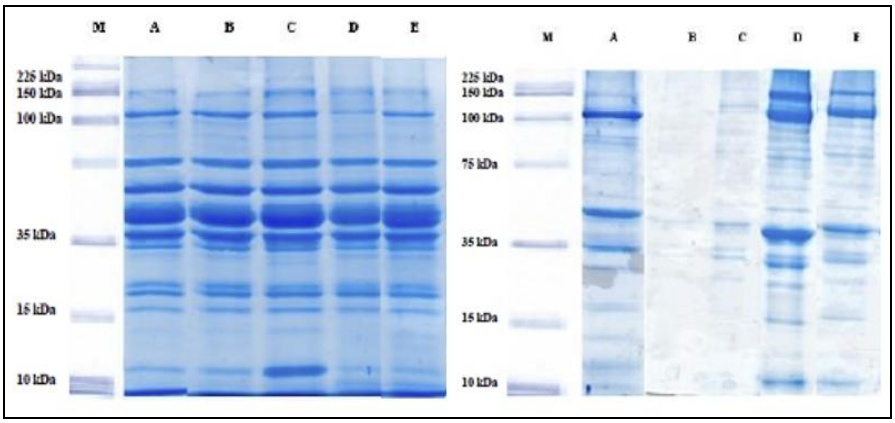

Fig 2: SDS-PAGE of sarcoplasmic (a) and myofibrillar (b) proteins hydrolysis Lane M: perfect protein marker; lane A: uninoculated control at 0 h of storage; lane B: untreated control at 21 days of storage; lane C to E: treated fillets at 21 days of storage; lane C: fillets treated by *Citrus essential oil (Control CEO)*; lane D: fillets inoculated with LAB combination (*Control LAB*) lane E: fillets inoculated with LAB combination and treated with CEO (*LAB+CEO*)

Hardness variation

Texture is the main important quality criterion for fish muscle (Kaewprachu *et al*., 2017) [13]. This measurement evaluate fish freshness and serve as a control method for fish quality in the seafood industry (Cheng *et al*., 2015) [6]. Thus, a soft muscle texture is considered as negative for consumers which demand an appropriate fish hardness (Kaewprachu *et al*., 2017) [13].

In the present study, as shown in Figure 3, fresh farmed sea bass fillets muscles were initially firm and springy with an initial hardness value of 5.52 ± 0.2 N. With longer storage time, this value decreased significantly ($p<0.05$) to reach 2.3 ± 0.9 N, 2.03 ± 0.45 N and 2.67 ± 0.57 N respectively, at untreated control, *CEO* control and *LAB* control, at the end of storage. Also, during the whole storage, the combined treatment of *Citrus* EO and LAB combinations led to a better texture characteristics with a final hardness value of 5.25 ± 1.06 N indicating that this treatment delayed the softening of the protein network fish muscle. Besides, this softening of fish muscle, initially having a resilient and firm flesh, was highly related to many factors mainly the duration of the rigor mortis, pH, microbial activity and *post-mortem* degradation of structural proteins during refrigeration by endogenous enzymes (Mi *et al*., 2017; Periago *et al*., 2005) [18, 21]. Besides, Fuentes *et al*. (2010) [9] reported that farmed fish were softer than wild fish due to their low activity. In addition, lipid content and humidity of muscle had an important role on texture parameters by decreasing the hardness of muscle. Besides, lean fish was much stiffer than fatty fish (Cheng *et al*., 2015) [6]. This finding was inconsistent with that observed by Mi *et al*. (2017) [18] showing that the hardness of fish muscle increased during storage probably due to several reasons especially the polymerization of lipids and/or proteins, denaturation of the myofibrillar proteins leading to water loss and dryness at the surface of the fish muscle. In this context, this result contributes to confirm all the obtained results about the preservative effect of *LAB+CEO* on fatty acids and muscle proteins changes.

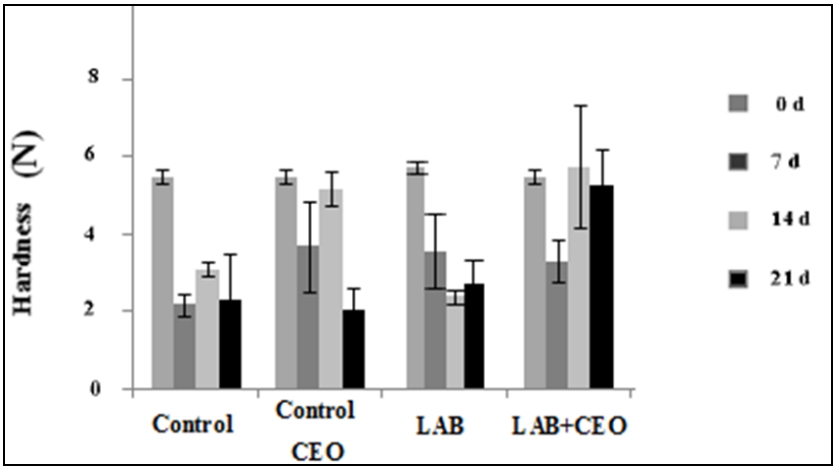

Fig 3: Hardness variation in vacuum packed sea bass fillets during 21 days of refrigerated storage at 4°C (0 d (■) 7 d (■) 14 d (■) and 21 d (■)

Shelf life Assessment

Total viable count (TVC) Variation

Mesophilic bacteria count is a useful parameter for qual ty evaluation of the aquatic products because post-processing contamination and microbial deterioration are the most common causes of fish spoilage (Mi *et al*., 2017; Kachele *et al*., 2017) [18, 12].

In this study, the logarithmic evolution of TVC counts of treated and untreated vacuum packed sea bass fillets, during storage at different temperatures during 21 days followed an apparent first order kinetic. The initial total viable count (TVC) was about 4.2 log CFU/g which suggest that the fish fillets were of good quality. This result was in line with those of Boulares *et al*. (2017) [3] and Cheng *et al*. (2015) [6]. In fact, according to Kachele *et al*. (2017) [12], several factors affect the initial microbial load in fish essentially fish species, water temperature, good manufacturing practices and transportation conditions. However, at the end of storage period, TVC in control fillets reached 7.2 log CFU/g exceeding the maximum acceptable limit of 7.0 log CFU/g for fresh fish (Kachele *et al*., 2017) [12]. This finding indicated that the microbiological shelf-life of control sea bass fillets was about 19 to 21. In comparison, a shelf-life of more than 21 days has been registered for vacuum packed sea bass fillets after biopreservative treatment combining LAB inoculation and or *Citrus EO* addition and refrigerated storage since they not reach this level over the entire storage period with value not exceeding 6.7 log CFU/g. In addition, the maximum acceptable level was exceeded in fillets on the 12th day of storage at 14°C for untreated control while this count was reached after 15 days for the three remained treated fillets. However, sea bass fillets stored at 24 °C became spoiled and unhealthy for human consumption after 12 and 10 days respectively for untreated control and all the LAB and/or CEO treated fillets.

These results showed that microbiological growth in sea bass fillets stored at 4 °C was significantly ($p < 0.05$) decreased due to LAB inoculation and/or CEO addition under vacuum packaging conditions, especially in fillets treated with both LAB strains and CEO. This finding correlated with those reported by Kachele *et al*. (2017) [3]

suggesting that the vacuum packaging combined with cold storage reduce significantly the proliferation of TVC due to the reduction of oxygen concentration within the package. On the other hand, under such conditions growth of more adaptive microorganisms such as LAB take place and predominantly influence the spoilage process (Sofra *et al*., 2018) [24]. Therefore, in the present study, inoculation of the combination of *Lc. lactis* and *C. piscicola* and incorporation of *Citrus* EO extended the shelf life of sea bass fillets due to the production of bacteriocins and the active components particularly limonene of the EO as previous concluded in the studies of Ben Hsouna *et al*. (2017) [1], Mahato *et al*. (2017) and El Bassi *et al*. (2009) [8].

Total volatile basic nitrogen (TVB-N) variation

The TVB-N is the main vital indicator of freshness or spoilage of fish and fish products. It is composed of trimethylamine, dimethylamine and ammonia resulting from the action of endogenous enzymes on proteins and non-protein nitrogenous compounds (Kachele *et al*., 2017, Durmus, 2020) [17, 2]. The initial TVB-N content in fresh sea bass fish was 12.4 mg N/100 g indicating the good quality of sea bass fillets. This value was in agreement with those reported in other studies reported that this content varied between 5 and 20 mg N/100 g (Kachele *et al*., 2017; Durmus, 2020) [12, 7].

In this study, logarithmic TVB-N values of control and different treated fillets stored at different temperatures increased with storage following apparent first order kinetic. In fact, TVB-N values increased with storage time in all treated fillets. This enhancement was attributed to spoilage accompanied with the production of ammonia and the desamination of amino acids. According to Kachele *et al*. (2017) [12], the products are considered of high quality when TVB-N values were lower to 25 mg N/100 g, while, they are considered to be of acceptable quality with TVB-N values between 25 and 30 mg N/100 g. However, the products with TVB-N values of 35 mg N/100 g are considered to be spoilt. In our study, TVB-N values in control fillets rose up to 35.07 mg N/100 g on day 18 and a higher level of 40.54 mg N/100 g on day 21. However, for control *LAB*, control *CEO* and *LAB+CEO* vacuum packed fillets, the TVB-N values were significantly lower compared with untreated vacuum packed fillets and distinctly less than the upper acceptability limit value of 35 mg N/100 g during the whole storage period ($p < 0.05$) indicating that this combined treatment retarded fish spoilage. In fact, untreated control fillets stored at 4°C were spoiled, after 18 days, since his TVB-N value exceeded the upper limit. However, vacuum packed fillets stored at 4°C and inoculated with LAB strains and/or treated with CEO fillet had significantly ($p < 0.05$) lower values than that of the control and didn't exceed this limit until the end of storage period.

Moreover, there was significant difference ($p < 0.05$) in TVB-N values between temperature storage. In fact, untreated fillets stored at 14°C and 24°C spoiled after 5 days and 4 days, respectively. However, the shelf life of treated fillets stored at 14°C was about 7 or 8 days, while after 6 days all fillets were spoiled at 24°C. Besides, we concluded that storage at refrigerated condition (4 °C) under vacuum packaging conditions better preserved the freshness of fillets. Equally, fillets inoculated by LAB strains and treated with CEO showed higher capacity to reduce the TVB-N content and were expected to have the best shelf life. This fact confirms our microbiological results reporting that lower oxygen concentration and antimicrobial activities of both LAB and CEO inhibited the growth of various flora and resulted in low volatile compounds production (Sofra *et al*., 2018; Boulares *et al*., 2017) [24, 3].

Determination of shelf life

Plots of natural logarithm of TVC and TVB-N contents in sea bass fillets versus storage period for each temperature are presented in Figures 5 and 6, respectively. The kinetic parameters obtained in this study were listed in Table 2. The end of shelf-life was correlated with a TVC level and TVB-N contents of 7 log (CFU/g) and 35 mg TVB-N/100 g, respectively. The results showed that linear correlation coefficients (R^2) ranged from 0.876 to 0.998 and 0.945 to 0.995, respectively for TVC and TVB-N, indicating that the corresponding kinetics followed a first-order reaction according this equation:

$$\mathbf{Ln\ [A] = ln\ [A_0] + k.t} \qquad \textbf{(Eq 4)}$$

Where $[A_0]$: is the initial TVC count or the initial TVB-N value.

It is clear that the reaction rate values (k) of TVC and TVB-N increased with the increase of storage temperature from 4°C to 24 °C. In addition, the data showed that the rate constant of TVC and TVB-N varied as function of treatment type. Moreover, significant differences ($p<0.05$) were observed between control and treated samples (*CEO+LAB*, control *LAB* and control *CEO*). However, the results revealed that no significant variation ($p>0.05$) was noted between control *LAB* and control *CEO* in term of rate constant. In this regard, the higher value of rate constant were found in untreated control while the lowest values were equally registered in *LAB+CEO* and control *LAB* fillets showing a longer shelf life. In the other hand, the half time ($t_{1/2}$) is the time required for the TVC and TVB-N in sea bass to decay up to 50 % of its initial count or concentration, and it was determined according to the following equation:

$$\mathbf{t_{1/2}\ (Days) = \frac{-\ Ln\ (0.5)}{k}} \qquad \textbf{(Eq 5)}$$

At 4 °C, the half-time values of all samples varied significantly ($p<0.05$) from 2.11 to 2.65 days for TVC and from 12.83 to 15.40 days for TVB-N, respectively. Similarly, the results showed that there was no significant ($p>0.05$) variation of half-time between control *LAB* and control *CEO*. The results indicated that the lower formation rate found in treated sea bass resulted in a longer shelf life than that for control sea bass fillets. The statistical results showed that there is a significant differences ($p<0.05$) between treated and untreated sea bass fillets in term in term of shelf life. Moreover, the shelf life (SL) of all sea bass fillets stored at 4 °C in terms of TVC count and TVB-N value were found between 19.83 to 24.93 days and 19.04 to 24.12 days, respectively. Finally, the inoculation of *LAB* strains and *Citrus* EO separately or in combination was efficient to improve the biochemical and microbiological quality of sea bass fillets and to extend the shelf life by about 3-5 days (control *CEO*), 4 days (control *LAB*) and 5 days (*LAB+CEO*), respectively for TVC and TVB-N. These findings were in line with those of Durmus (2020) [7]. Showing that nanoemulsions of lemon EO increased the shelf life of rainbow trout, sea bass and mackerel.

Table 2: Kinetic parameters and shelf life of vacuum-packed sea bass fillets at different storage temperatures

Sea bass Fillets	Parameters	Storage temperature (°C)	Constant rate k ($Days^{-1}$)	Half-time $t_{1/2}$ (Days)	R^2	Shelf life (Days)
		4	0.332 ± 0.08^{a}	2.11 ± 0.04^{a}	0.998	
Control	TVC	14	0.627 ± 0.10^{b}	1.10 ± 0.08^{b}	0.962	19.83 ± 0.09^{a}
		24	0.765 ± 0.13^{c}	0.91 ± 0.09^{b}	0.963	
		4	0.054 ± 0.02^{a}	12.83 ± 0.13^{a}	0.995	
	TVB-N	14	0.223 ± 0.19^{b}	3.11 ± 0.10^{b}	0.955	19.04 ± 0.08^{A}
		24	0.256 ± 0.15^{c}	2.71 ± 0.08^{c}	0.968	
Control CEO	TVC	4	0.281 ± 0.10^{a}	2.46 ± 0.10^{a}	0.996	
		14	0.458 ± 0.15^{b}	1.51 ± 0.10^{b}	0.995	23.16 ± 0.12^{b}
		24	0.571 ± 0.13^{c}	1.21 ± 0.11^{c}	0.988	
		4	0.045 ± 0.01^{a}	15.40 ± 0.14^{a}	0.954	
	TVB-N	14	0.176 ± 0.12^{b}	3.93 ± 0.13^{b}	0.970	24.12 ± 0.16^{B}
		24	0.243 ± 0.20^{c}	2.85 ± 0.09^{c}	0.980	
Control LAB		4	0.271 ± 0.11^{a}	2.55 ± 0.10^{a}	0.982	
	TVC	14	0.472 ± 0.18^{b}	1.46 ± 0.07^{b}	0.991	24.01 ± 0.20^{d}
		24	0.587 ± 0.20^{c}	1.18 ± 0.09^{c}	0.993	
		4	0.047 ± 0.07^{a}	14.74 ± 0.14^{a}	0.970	
	TVB-N	14	0.180 ± 0.13^{b}	3.85 ± 0.11^{b}	0.952	23.09 ± 0.13^{C}
		24	0.279 ± 0.09^{c}	2.84 ± 0.19^{c}	0.970	
LAB+CEO		4	0.261 ± 0.18^{a}	2.65 ± 0.06^{a}	0.876	
	TVC	14	0.428 ± 0.12^{b}	1.61 ± 0.09^{b}	0.980	24.93 ± 0.16^{c}
		24	0.535 ± 0.17^{c}	1.29 ± 0.10^{c}	0.899	
		4	0.045 ± 0.11^{a}	15.40 ± 0.24^{a}	0.970	
	TVB-N	14	0.183 ± 0.07^{b}	3.78 ± 0.16^{b}	0.945	24.12 ± 0.20^{B}
		24	0.261 ± 0.14^{c}	2.66 ± 0.09^{c}	0.965	

TVC: Total Viable Count; TVB-N: Total Volatile Basic Nitrogen.
Means with different letters in the same column, at different temperature, are significantly different at the 5 % level.

Conclusion

This study developed a preservation treatment for vacuum packed sea bass fillets by the adjonction of two lactic acid bacteria and/or *Citrus* essential oil. LAB and *Citrus* EO used separately or in combination resulted in significant improvement in microbiological, biochemical and rheological quality under refrigerated vacuum storage of sea bass fillets when compared to untreated control. This combined treatment gave promising results by preserving a good content of PUFA w-3 fatty acids in sea bass fillets and delaying the softening of fresh fish muscle. Finally, the accelerated shelf life test used in order to set expiration date of vacuum packed and refrigerated sea bass fillets approved the efficiency of the use of *LAB* in combination with *CEO* by slowing down the microbial growth and increasing the shelf life of this product by about approximately 5 days.

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