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Quality changes, potential spoilage organisms, and shelf-life prediction of brackish river prawn (*Macrobrachium macrobrachion*) at different storage temperatures

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ABSTRACT

The brackish river prawn (*Macrobrachium macrobrachion*) is a species of commercial importance in West Africa. However, like other fishery products, it is prone to deterioration due mainly to microbial activities. The present study aimed at evaluating the spoilage characteristics of *M. macrobrachion* and predicting the growth of the main spoilage bacteria as well as the shelf-life of the product as a function of storage temperature. Freshly caught brackish river prawn samples from Lake Aheme were aerobically stored at 0, 7, 15, and 28 °C and, at predetermined times during storage, they were taken for microbiological, chemical, and sensory analysis. At sensory rejection times, the spoilage potential of 185 isolates from specific groups of organisms enumerated was assessed in prawn of which the endogenous microbiota was heat inactivated. Isolates capable of producing strong off-odor were identified using 16S rRNA sequencing. Models predicting the maximum growth rate of *Pseudomonas* spp. and H_2S-producing bacteria in the brackish river prawn as well as the shelf-life of the product were developed. These models were validated using an independent experiment during which prawn was stored at 0, 4, 10, and 25 °C. Results showed that *Pseudomonas* spp. at 0 °C, *Pseudomonas* spp. and H_2S-producing bacteria at 7 °C, and H_2S-producing bacteria at 15 °C and 28 °C were the dominant groups of microorganisms during storage. As expected, total volatile basic nitrogen, trimethylamine, and pH with initial values of 21.2 ± 3.0 mg-N/100 g, 4.1 ± 0.8 mg-N/100 g, and 7.46 ± 0.15 increased during storage reaching approximately 35 mg-N/100 g, 10 mg/ 100 g and 8, respectively at sensory rejection times which were 7 h at 28 °C, 1.2 d at 15 °C, 4.6 d at 7 °C, and 11.7 d at 0 °C. The main spoilage organisms were *Citrobacter braakii* at 28 °C, *Citrobacter braakii, Pseudomonas kurunegalensis,* and *Shewanella bicestrii* at 15 °C, *Shewanella putrefaciens, Shewanella baltica,* and *Pseudomonas bubulae* at 7 °C, and *Pseudomonas versuta* at 0 °C. The validation of the developed models showed an adequate agreement between the predicted and observed values. This study highlights the specific spoilage characteristics of the brackish river prawn and reveals that Gram-negative rod bacteria are the main spoilage organisms even at high storage temperatures, contrary to many earlier reports on the spoilage of tropical fishery products.

1. Introduction

Macrobrachium macrobrachion, known as brackish river prawn, is an important component of the ecology of rivers and estuaries along the West coast of Africa (Akinwunmi and Moruf, 2021; Koussovi et al., 2023). The species was found to be the most dominant prawn species in some West African countries. For example, in the Lagos Lagoon (Nigeria), *M. macrobrachion* constitutes about 60 % of all prawn landings and up to 83 % of all *Macrobrachium* fishery catches during the rainy season (Enin, 1998; Onunkwor et al., 2022). In Benin Republic, the species is widely distributed in waterbodies and streams of the country and its demand is high for local consumption as well as exportation mainly to neighboring countries (Koussovi et al., 2023). However, it is challenging for stakeholders to maintain the freshness of the product. In fact, like other crustaceans, *M. macrobrachion* is highly prone to deterioration due to its high water activity, neutral pH, and relatively high content of free amino acids (Odeyemi et al., 2021, 2023; Zeng et al., 2005). Microorganisms are the major cause of this deterioration and it is well established that only a fraction of microorganisms associated with fishery products, called "specific spoilage organism(s) (SSOs)", really

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contribute to spoilage (Gram and Dalgaard, 2002). Knowledge of SSOs and their metabolites, which can be used as chemical indicators of spoilage, is of importance to develop strategies and tools for better fishery products quality and safety management (Dabadé, 2015). For example, mathematical models can be used to predict the progression of spoilage processes in foods; thus they can help in decision-making regarding food quality management (Bruckner et al., 2013; Dalgaard and Huss, 2020). Spoilage characteristics, however, even for the same fishery product, may depend on geographical origin and other unknown factors interacting with microbial growth (Gram and Huss, 1996). For example, Lalitha and Surendran (2006) identified *Pseudomonas*, *Aeromonas hydrophila*, *A. veronii* boivar sobria and *Shewanella putrefaciens* as the dominant spoilage organisms of the giant river prawn (*Macrobrachium rosenbergii*) stored in ice in India. At room temperature (28 °C ± 2), Jeyasekaran and Ayyappan (2002) found that in the same species of giant river prawn, *Lactobacillus* sp. was dominant followed by *Staphylococcus* sp. and *Bacillus* sp. In the white prawn *Exopalaemon modestus* during ice storage, lactic acid bacteria especially, *Lactococcus garvieae* was the dominant spoilage organism (Du et al., 2017). *Pseudomonas* species were found to be the dominant spoilage organisms in *Macrobrachium vollenhovenii* under ice storage (Akintola and Bakare, 2011). This dynamic nature of the microbial spoilage process indicates that the SSOs and their spoilage domains must be determined before kinetic models can be developed (Dalgaard and Huss, 2020). To date, to our knowledge, there is no report on specific spoilage organisms of brackish river prawn (*M. macrobrachion*) nor on their spoilage domain and spoilage level. This study aimed at (i) evaluating the quality changes of *M. macrobrachion* stored at different temperatures, (ii) identifying the major spoilage bacteria of *M. macrobrachion*, and (iii) developing mathematical models predicting the growth of spoilage bacteria of *M. macrobrachion* as well as the shelf-life of the product as a function of storage temperatures.

2. Materials and methods

2.1. Prawn collection and preparation

Four independent samplings were carried out to collect the brackish water prawn (*Macrobrachium macrobrachion*) from Lake Aheme, one of the most important fishing areas in the Benin Republic (Dabadé et al., 2014). Three independent samplings were used for mathematical model development and the last sampling was for the validation of the developed models. At each sampling date, freshly caught prawn samples were immediately cooled with ice and transferred to Laboratory (road transportation) within approximately 2 h. At the Laboratory, samples were aseptically packed loose in sealed sterile polyethylene bags (Twirl'em sample bags, Labplas Inc., dimensions: 305 × 178 mm; thickness: 89 μm) and stored at 0, 7, 15, and 28 °C, for the development of the models. As for their validation, the storage temperatures were 0, 4, 10, and 25 °C. Thermochron iButton (DS 1921G) devices were placed in some prawn samples to record temperatures during storage. For storage at temperatures >0 °C, Sanyo MIR-154 refrigerated incubators were used. For 0 °C, prawn samples were stored in ice as previously described (Dabadé et al., 2015a). Prawn samples were taken for microbiological, chemical, and sensory analysis at pre-determined times for each storage temperature (Table S1).

2.2. Microbiological analysis

A 25 g prawn sample was transferred aseptically to a stomacher bag, diluted 10 times in physiological saline peptone solution (0.85 % NaCl (SIGMA, Germany), 0.1 % peptone (OXOID, England) and mixed using a stomacher (Seward Laboratory Stomacher 400, England). From this primary dilution, appropriate successive decimal dilutions were prepared.

Total Viable Counts (TVC), and H_2S-producing bacteria were enumerated on double-layered plates of iron agar supplemented with 0.04 % L-cysteine (SIGMA) and incubated at 25 °C for 72 h as described by Gram et al. (1987). From the same iron agar, black colonies were counted as H_2S-producing bacteria and TVC as the sum of black and white colonies (Gram et al., 1987). Enterobacteriaceae were enumerated on double-layered plates of violet red bile glucose (VRBG) medium (OXOID) and incubated at 37 °C for 24 h. *Pseudomonas* spp. were enumerated on spread plates of *Pseudomonas* agar base (OXOID) supplemented with cetrimide, fucidin, and cephaloridine (CFC) (OXOID) at 25 °C for 48 h. Lactic acid bacteria (LAB) were enumerated on double-layered plates of de Man, Rogosa, and Sharp agar (MRSA) (OXOID) incubated at 30 °C for 72 h.

2.3. Chemical analysis

Total volatile basic nitrogen (TVBN), trimethylamine (TMA), and pH were determined in prawn samples during storage. To determine the pH, distilled water (20 mL) was added to 10 g of ground prawn. The mixture was homogenized and the pH was measured in duplicate using a pH meter (InoLab 730, Germany). TVBN and TMA were determined using the method recommended by the European Commission (EC, 2005), as previously described (Dabadé et al., 2015a).

2.4. Sensory analysis

The overall acceptance of the prawns based on their odor, color, and texture was assessed using a scale with three categories: 1 = prawn with good quality, 2 = prawn with marginal quality, but still acceptable, and 3 = spoiled prawn (Argyri et al., 2010; Dabadé et al., 2020; Dalgaard et al., 1993) by 10 panelists experienced in prawn freshness evaluation. Sensory rejection time was defined as the moment when 50 % of the panelists evaluated samples to be in category 3.

2.5. Identification of the main spoilage bacteria

2.5.1. Prawn samples inoculation and spoilage potential assessment

During the microbiological analysis of the prawn samples, at least 15 colonies of each of the following microorganisms: H_2S-producing bacteria, Enterobacteriaceae, *Pseudomonas* spp., and lactic acid bacteria capable of growing during storage at each of the tested temperatures were randomly selected from the media used to enumerate them at sensory rejection times. These colonies (185 in total) were checked for purity on MRSA for lactic acid bacteria and on tryptone soya agar (TSA) for H_2S-producing bacteria, Enterobacteriaceae, and *Pseudomonas* spp.. Pure cultures of isolated colonies were inoculated individually to freshly caught prawns of which the endogenous microorganisms were inactivated as previously described (Dabadé et al., 2015a). The inoculated prawn samples were stored at the temperature of the initial isolation of the colony. Panelists assessed the ability of the inoculated culture to spoil the prawn (off-odor production) using the three-point scale described in 2.4. Strains having received a score of three by at least 50 % of the panelists were considered as the main spoilage organisms of the product and were identified following the procedure described below.

2.5.2. DNA extraction, PCR conditions, and sequencing

DNA of the isolates having shown strong spoilage activity was extracted using the protocol described in the genomic DNA purification kit (Promega Corporation). The DNA extracts were used to amplify the 16S rRNA gene with polymerase chain reaction (PCR) using the Labcycler Basic (SensoQuest). The PCR was performed using 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1540R (5′-TACGGYTACCTTGTTACGACT-3′) primers. The 16S rRNA gene was amplified using the PCR conditions previously described (Houngbédji et al., 2018). The PCR products were sequenced at Microsynth Seqlab (Germany). The DNA Baser Sequence Assembler v5.15 (2020) (http://www.DnaBaser.com) was used to assemble the forward and reverse sequences. The

sequences were compared against the GenBank database using the basic local alignment search tool (BLAST) (Altschul et al., 1990). The sequences were deposited in the GenBank database under the accession numbers OP222972-OP223017.

2.6. Modeling the shelf-life of brackish river prawn as a function of storage temperature

Three empirical models: exponential model (1), Ratkowsky model (2) (Ratkowsky et al., 1982), and Arrhenius model (3) were fitted to the rates of spoilage determined as the reciprocal of shelf-life (RS, day^{-1})

$$RS = b_1 \times exp(a \times T) \quad (1)$$

$$RS = b_2^2 \times (T - T_{\min})^2 \quad (2)$$

$$RS = b_3 \times exp\left(\frac{-E_a}{R \times K}\right) \quad (3)$$

With T, the storage temperature (°C); R, the gas constant 8.314 (J mol^{-1} K^{-1}); K, temperature in Kelvin. b_i, a, T_{min} and E_a are coefficients to be estimated.

To stabilize the variance over the temperature range, both the models and the RS-data were ln-transformed before the fitting. The performance of the three models was assessed by fitting the models to the ln-transformed data and comparing the root mean square error of the model ($RMSE_{\text{model}}$) as described by Dabadé et al. (2015a).

$$RMSE_{\text{model}} = \sqrt{\frac{\sum (\text{observed}_i - \text{fitted}_i)^2}{n - s}} \quad (4)$$

With observed$_i$, observed values; fitted$_i$, described values; n, number of data points; s, number of parameters of the model.

2.7. Modeling the growth of the spoilage organisms as function of storage temperature

2.7.1. Primary modeling

The reparameterized Gompertz model (Zwietering et al., 1990) (Eq. (5)) was fitted to the microbial growth data obtained during prawn storage at 0, 7, 15, and 28 °C using the Excel's solver function.

$$log_{10}(N_t) = log_{10}(N_0) + [log_{10}(N_{\max}) - log_{10}(N_0)]exp\left\{-exp\left[\frac{\frac{\mu_{\max}}{ln(10)} \cdot exp(1)}{[log_{10}(N_{\max}) - log_{10}(N_0)]}(\lambda - t) + 1\right]\right\} \quad (5)$$

where t is time (h), N_t is the number of microorganisms at time t (CFU/g), N_0 is the number of microorganisms at time zero (CFU/g), $N_{\max}$ is the maximum number of microorganisms (CFU/g), $\mu_{\max}$ is maximum specific growth rate (h^{-1}), and λ is the lag time (h).

2.7.2. Secondary modeling

The Ratkowsky equation (Eq. (6)) (Ratkowsky et al., 1982) was used to model the effect of temperature on microbial growth.

$$\mu_{\max} = [b_1(T - T_{\min})]^2 \quad (6)$$

where $\mu_{\max}$ is maximum specific growth rate (h^{-1}), T is temperature (in degrees Celsius), b_i, $T_{\min}$, are coefficients to be estimated. $T_{\min}$ (in degrees Celsius) is the minimum theoretical temperature for growth. Eq. (6) was square-root transformed prior to the fitting.

2.7.3. Model validation

An independent experiment was performed to validate the models developed in this study that predict the growth rate of dominant groups of microorganisms during storage at 0, 7, 15 and 28 °C. Prawn samples were collected and stored at 0, 4, 10 and 25 °C. At pre-determined times (Table S1), samples were taken for microbiological analysis and sensory analysis.

The performance of the developed models was assessed by a graphical comparison of predicted and observed values. In addition, the acceptable prediction zone method developed by Oscar (2005) was applied. The relative errors (RE) for maximum growth were calculated as follows:

$$\text{RE for shelf-life} = (\text{predicted} - \text{observed})/\text{predicted} \quad (7)$$

$$\text{RE for } \mu_{\max} = (\text{observed-predicted})/\text{predicted} \quad (8)$$

RE less than zero represents fail-safe predictions and RE above zero represents fail-dangerous predictions (Oscar, 2005). The boundaries of the acceptable prediction zone proposed by Oscar (2005), which were −0.3 (fail-safe) and 0.15 (fail-dangerous) were used.

For shelf-life data, the bias (Eq. (9)) and the accuracy (Eq. (10)) factors according to Ross (1996) were calculated as follows:

$$B_f = 10^{\left(\sum log\left(t_{\text{predicted}}/t_{\text{observed}}\right)/n\right)} \quad (9)$$

$$A_f = 10^{\left(\sum |log\left(t_{\text{predicted}}/t_{\text{observed}}\right)|/n\right)} \quad (10)$$

$t_{\text{predicted}}$ are the predicted shelf-life values (in days), t_{observed} are the observed shelf-life values (in days). n is the number of observations. B_f values higher than 1 show that the predicted shelf-life is on average longer than the observed shelf-life. $B_f = 1$ suggests, on average, a perfect agreement between predicted and observed values. The accuracy factor assesses the absolute deviation between the observed values and the predicted values. $A_f = 1$ shows a perfect agreement between predicted and observed values. The larger the accuracy factor, the less accurate is the average estimate (Ross, 1996).

2.8. Shelf-life model comparison and benchmarking

The model developed in this study predicting the shelf-life of *M. macrobrachium* was compared to existing models predicting the shelf-life of fishery products (Eqs. (11)–(13)).

$$\text{Shelf-life } (T) = \frac{\text{Shelf-life } (T_0)}{(1 + 0.1 \times T)^2} \quad (11)$$

$$\text{Shelf-life (T)} = \text{Shelf-life}(T_0) \times exp(-0.12 \times T) \quad (12)$$

$$\text{Shelf-life (days)} = 4.5 \times 10^{-15} \times exp\left(\frac{9650}{\theta}\right) \quad (13)$$

In all models, T and θ are storage temperatures in °C and K, respectively.

Eq. (11) used as a common shelf-life prediction model for seafood (Dalgaard and Huss, 2020), derived from the root square model (Eq. (6)) suggested for the growth of microorganisms with a $T_{\min}$-value of −10 °C. Eq. (12) is the model developed typically for tropical fresh seafood shelf-

life prediction Dalgaard and Huss (2020). Eq. (13) is the model developed for tropical brackish water shrimp (*Penaeus notialis*) shelf-life prediction (Dabadé et al., 2015a).

The comparison was based on the differences between the predicted shelf-lives by the models and the observed shelf-lives during the model validation experiment conducted in this study.

In addition, data from literature on shelf-life of various fresh prawn or shrimp species harvested or caught mainly from tropical regions and stored aerobically at temperatures ranging from 0 to 30 °C were benchmarked to compare graphically the outcomes of the model developed in this study for the prediction of *M. macrobrachium* shelf-life. The performance of the model with these data from literature on shelf-life was also assessed using the bias factor (B_f) (Eq. (9)) and the accuracy factor (A_f) (Eq. (10)).

2.9. Statistical analysis

A Student's two-tailed *t*-test or one-way ANOVA (IMB SPSS Statistics 19.0) followed by Tukey's test as post hoc comparison of means was used to compare the means of bacterial concentrations, TVBN, TMA, and maximum specific growth rate from prawn stored at various temperatures. The significance level was set at $P < 0.05$.

3. Results

3.1. Microbial growth during brackish water prawn storage

The microbial growth data of the prawn samples with the fitted reparameterized Gompertz model are shown in Fig. 1. The calculated kinetic parameters (Eq. (5)) are shown in Table S2. The initial total viable count (TVC) was 5.8 ± 0.5 log CFU/g. The initial concentrations of H_2S-producing bacteria, Enterobacteriaceae, *Pseudomonas* spp., and lactic acid bacteria varied from 4.0 ± 0.5 log CFU/g (Enterobacteriaceae) to 4.4 ± 0.6 log CFU/g (H_2S-producing bacteria). During storage, TVC increased to reach the maximum concentration (ca. 9.4 log CFU/g) after 21 h, 4 days, 9 days, and 18 days, at 28 °C, 15 °C, 7 °C, and 0 °C, respectively. The dominant group of microorganisms during prawn storage was temperature-dependent. At 28 °C and 15 °C, H_2S-producing bacteria were the dominant group of microorganisms with a maximum concentration of approximately 9.0 log CFU/g while at 0 °C, the average

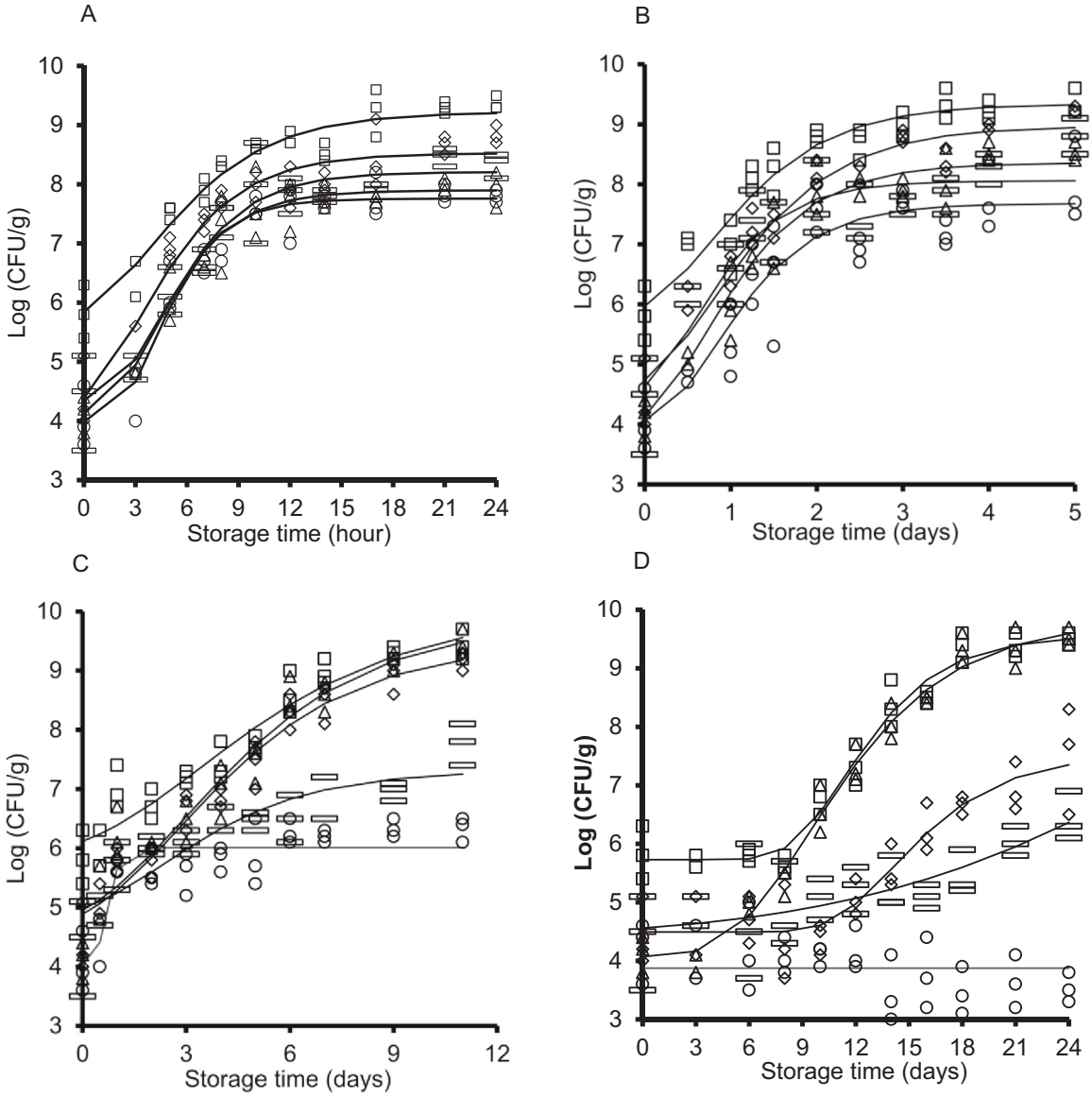

Fig. 1. Evolution of total viable counts (□), H_2S-producing bacteria (◇), *Pseudomonas* spp. (△), Enterobacteriaceae (○), and lactic acid bacteria (═) of brackish water prawn stored at 28 °C (A), 15 °C (B), 7 °C (C), and 0 °C (D). The reparameterized Gompertz model (solid line) was fitted to the growth data.

maximum concentration of this group of microorganisms was below 8.0 log CFU/g, far behind that of *Pseudomonas* spp. which were the dominant group of microorganisms at this temperature with a maximum concentration of 9.4 log CFU/g. At 7 °C, *Pseudomonas* spp. and H_2S-producing bacteria co-dominated reaching a maximum concentration of approximately 9.2 log CFU/g. Enterobacteriaceae and lactic acid bacteria (LAB) grew well in prawn stored at 28 °C and 15 °C, reaching a maximum concentration of approximately 8.0 log CFU/g. No Enterobacteriaceae growth was observed at 0 °C while at 7 °C, slight growth was noticed with a maximum concentration of 6.0 log CFU/g. LAB reached a maximum concentration of approximately, 6.0 log CFU/g and 7.0 log CFU/g at 0 °C and 7 °C, respectively.

3.2. Chemical changes during brackish water prawn storage

Chemical changes occurring in brackish water prawn during storage at various temperatures as revealed by TVBN, TMA and pH values are shown in Fig. 2. The average initial TVBN value was 21.2 ± 3.0 mg-N/100 g. This value increased throughout the storage at all tested temperatures but, as expected, with higher rate at high storage temperatures reaching concentrations ranging from 65.8 ± 7.7 mg-N/100 g (0 °C) to 221.5 ± 9.2 mg-N/100 g (28 °C) at the end of the storage (Fig. 2A). With an average initial value of 4.1 ± 0.8 mg/100, TMA showed similar trend like TVBN during prawn storage (Fig. 2B). The initial pH value was 7.46 ± 0.15. It also increased during storage reaching a value of approximately 8.3 at the end of storage for all the tested temperatures.

3.3. Sensory changes during brackish water prawn storage

The results of the prawn sensory evaluation are presented in Table 1. Prawn samples were deemed to be of good quality by all panelists at the beginning of storage time. As expected, the percentage of the panelists evaluating the prawn samples to be of marginal or defective quality increased with storage time. The quality of the prawn was judged defective when there was off-odor production and/or noticeable changes in prawn color or texture. The sensory rejection time defined as the time when at least 50 % of the panelists evaluate prawn to be spoiled was 7 h at 28 °C, 1.2 d at 15 °C, 4.6 d at 7 °C, and 11.7 d at 0 °C.

3.4. Main spoilage organisms of brackish water prawn

The spoilage assessment results and the identity of isolates with high spoilage activity (strong off-odor production) are shown in Table 2. At 28 °C, 16/60 strains tested were capable of producing strong off-odor according to at least 50 % of the panelists. They belong mainly to the family of Enterobacteriaceae (n = 7), Pseudomonaceae (n = 4) and Aeromonaceae (n = 4). *Citrobracter braakii* was the species having the strongest spoilage activity based on the percentage of panelists identifying it as having strong spoilage activity (90–100 %). All isolates from *Pseudomonas* selective agar were identified as *Pseudomonas kurunegalensis,* except for one strain identified as *Aeromonas enteropelogenes*. No Lactic acid bacteria isolates tested were found to show strong spoilage activity in prawn during storage. At 15 °C, similar spoilage organisms were identified with again *Citrobacter* species namely *C. braakii* and *C. freundii,* and *Pseudomonas kurunegalensis* representing >60 % of the potential spoilage organisms identified at this temperature. However, almost 20 % of the isolates showing strong spoilage activity were identified as *Shewanella bicestrii.* At 7 °C *Shewanella putrefaciens, Shewanella baltica,* and *Pseudomonas bubulae* were found to be the main spoilage organisms while at 0 °C *Pseudomonas versuta* was the main isolate having shown strong spoilage activity.

3.5. Modeling the maximum growth rate as a function of temperature

Of the different specific groups of microorganisms enumerated on brackish water prawn stored at 0, 7, 15 and 28 °C, only *Pseudomonas* spp.

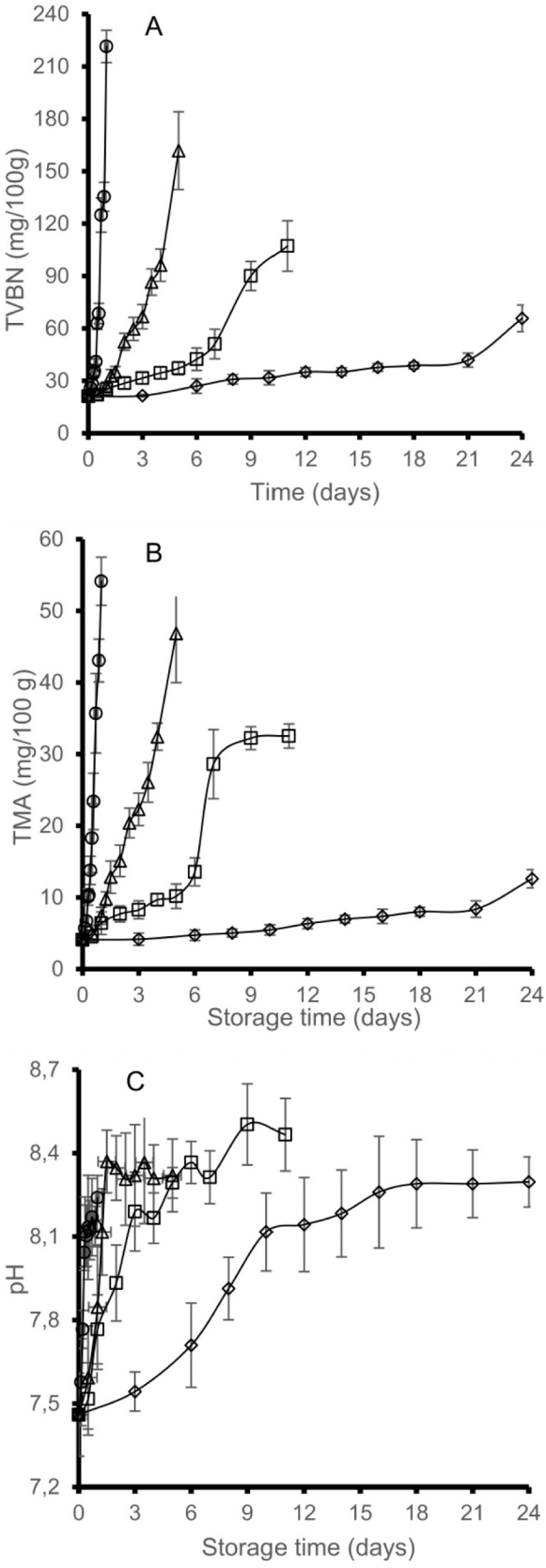

Fig. 2. Evolution of total volatile basic nitrogen (TVBN) (A), trimethylamine (TMA) (B), pH (C) of prawn stored at 28 °C (○), 15 °C (△), 7 °C (□), and 0 °C (◇). Bars represent the standard deviation of three independent samples.

Table 1
Prawn overall quality evaluation (% of panelists) during storage at 28 °C (A), 15 °C (B), 7 °C (C), and 0 °C (D).

A

Score	Storage time (hours)						
	0	3	5	7	8	10	12
1 = Prawn of good quality (no defect)	100	73.3	3.3	0	0	0	0
2 = Prawn of marginal quality, but still acceptable	0	26.7	80	50	40	20	0
3 = Spoiled prawn (rejected)	0	0	16.7	**50**	60	80	100

B

Score	Storage time (days)						
	0	0.5	1	1.25	1.5	2	2.5
1 = Prawn of good quality (no defect)	100	90	6.7	0	0	0	0
2 = Prawn of marginal quality, but still acceptable	0	10	83.3	46.7	33.3	16.7	0
3 = Spoiled prawn (rejected)	0	0	10	**53.3**	66.7	83.3	100

C

Score	Storage time (days)							
	0	0.5	1	2	3	4	5	6
1 = Prawn of good quality (no defect)	100	96.7	90	66.7	6.7	0	0	0
2 = Prawn of marginal quality, but still acceptable	0	3.3	10	33.3	93.3	63.3	40	0
3 = Spoiled prawn (rejected)	0	0	0	0	0	36.7	**60**	100

D

Score	Storage time (days)							
	0	3	6	8	10	12	14	16
1 = Prawn of good quality (no defect)	100	93.3	63.3	3.3	0	0	0	0
2 = Prawn of marginal quality, but still acceptable	0	6.7	36.7	93.4	70	46.7	13.3	0
3 = Spoiled prawn (rejected)	0	0	0	3.3	30	**53.3**	86.7	100

Boldface indicates sensory rejection times.

and H_2S-producing bacteria were able to grow at all these temperatures and showed significant spoilage activities. The effect of temperature on the maximum growth rates of these groups of microorganisms is shown in Fig. 3. The estimated parameters of the Ratkowsky model (Eq. (6)) used were presented in Table 3. The maximum growth rate of *Pseudomonas* spp. was significantly higher than that of H_2S-producing bacteria at 0 and 7 °C, but at 15 and 28 °C, no significant differences were found.

3.6. Modeling the shelf-life of brackish water prawn as a function of temperature

Of the three models used, the exponential model was the best fitting model. Indeed, its root mean square error of the residuals ($RMSE_{model}$) was 0.11 while those of Ratkowsky and Arrhenius models were 0.28, and 0.18, respectively. The fitted parameters from the exponential model a and b_1 were 0.135 ± 0.008 (95 % CI) and 0.089 ± 0.13 (95 % CI), respectively. With these estimated parameters the shelf-life (the reciprocal of the rate of spoilage) of tropical brackish water prawn *Macrobrachium macrobrachion* could be predicted at a given storage temperature using Eq. (14)

$$\text{Shelf-life (days)} = 11.2 \times exp(-0.135T) \quad (14)$$

With T, storage temperature in degree Celsius.

The predicted brackish water prawn shelf-life as a function of temperature is shown in Fig. 4.

3.7. Validation of the developed models

3.7.1. Validation of the model predicting prawn shelf-life at constant storage temperatures

The observed shelf-lives from another independent experimentation using different storage temperatures were plotted to the Figure showing the predicted model (Fig. 4) for graphical comparison purpose. The calculated bias factors and accuracy factors of the model were 1.05 and 1.12, respectively. Moreover, the calculated relative errors for shelf-life Eq. (7)) were almost all within the boundaries of the acceptable prediction zone proposed by Oscar (2005) (Fig. 5). These results showed that there was an adequate agreement between the predicted and observed shelf-life values of prawn as depicted in Table 4.

3.7.2. Validation of the model predicting the maximum growth rates of *Pseudomonas spp. and H_2S-producing bacteria as a function of temperature*

For graphical comparison purposes, the observed growth rates from another independent experimentation using different storage temperatures were plotted to the Figure showing the models predicting the maximum growth rate as function of temperature (Fig. 3). In addition, like with the model predicting the shelf-life, the calculated relative errors for maximum growth rates (Eq. (8)) were in majority, within the boundaries of the acceptable prediction zone proposed by Oscar (2005) at all storage temperatures tested except for the highest temperature (25 °C) where most of calculated relative errors were the above the upper limit of the acceptable zones (Fig. 5).

3.8. Comparison of the model predicting the shelf-life of *M. macrobrachion* to other models and its benchmarking with shelf-life data of prawn or shrimp from literature

With a shelf-life of 11.7 d at 0 °C (the average found in our study), the common model (Eq. (11) (Table 4) used for fresh seafood shelf-life prediction gives satisfactory predictions (<20 % differences between predicted and observed values) at low storage temperatures (≤10 °C) but unrealistic predictions at high storage temperatures, with >180 % difference between predicted and observed values at 25 °C. The tropical fresh seafood shelf-life prediction model developed by Dalgaard and Huss (2020) (Eq. (12)) (Table 4) gives a better prediction than the common model at 25 °C, since the difference was 75 % instead of 187.5 %, but this difference is still high. Even at 10 °C, the difference between the predicted and observed shelf-life was >40 % with this tropical fresh seafood shelf-life prediction model. The model developed for the prediction of shelf-life of tropical brackish water penaeid shrimp (*Penaeus notialis*) (Eq. (13)) (Dabadé et al., 2015a) gives satisfactory predictions with the palaemonid shrimp (*Macrobrachium macrobrachion*) at temperatures ≤10 °C, but up to 50 % difference between the predicted and the observed shelf-life was recorded at 25 °C, while only 12.5 % difference was obtained with the new developed model in this study (Table 4).

As shown in Fig. 6, the model predicting the shelf-life of *Macrobrachium macrobrachion* gives, overall, a satisfactory prediction of shrimp or prawn based on the reported shelf-lives in the literature. The B_f and A_f values obtained were 0.81 and 1.35, respectively.

Table 2
Spoilage potential assessment and identities of isolates capable of producing strong off-odor at 28 °C, 15 °C, 7 °C and 0 °C.

T[a] (°C)	Culture media	Number of isolates tested	Number of isolates with strong spoilage activity	% Similarity and GeneBank closest relatives	GenBank given accession number	% of panelists judging the isolates to have strong spoilage activity ($n = 10$)
28	VRBGA[b]	15	5	99.6 % *Citrobacter braakii*	OP222977	100
				99.7 % *Citrobacter arsenatis*	OP222978	70
				99.8 % *Klebsiella variicola*	OP222979	50
				99.2 % *Escherichia coli*	OP222980	80
				99.9 % *Enterobacter ludwigii*	OP222981	80
	Iron Agar[c]	15	6	98.5 % *Aeromonas veronii*	OP222994	50
				99.8 % *Citrobacter braakii*	OP222995	100
				99.6 % *Citrobacter braakii*	OP222996	90
				99.8 % *Aeromonas veronii*	OP222997	50
				99.9 % *Aeromonas dhakensis*	OP222998	50
				98.7 % *Shewanella bicestrii*	OP222999	50
	PSA[d]	15	5	99.7 % *Aeromonas enteropelogenes*	OP223013	60
				99.8 % *Pseudomonas kurunegalensis*	OP223014	50
				99.8 % *Pseudomonas kurunegalensis*	OP223015	70
				99.8 % *Pseudomonas kurunegalensis*	OP223016	70
				99.7 % *Pseudomonas kurunegalensis*	OP223017	80
	MRSA[e]	15	0	–	–	–
15	VRBGA	15	5	99.4 % *Citrobacter braakii*	OP222972	80
				99.4 % *Citrobacter freundii*	OP222973	70
				99.2 % *Citrobacter braakii*	OP222974	80
				99.6 % *Citrobacter pasteurii*	OP222975	90
				99.7 % *Escherichia coli*	OP222976	60
	Iron Agar	15	7	99.7 % *Aeromonas caviae*	OP222987	60
				99.7 % *Aeromonas caviae*	OP222988	50
				99.5 % *Citrobacter braakii*	OP222989	80
				98.8 % *Shewanella bicestrii*	OP222990	50
				99.9 % *Shewanella bicestrii*	OP222991	70
				99.5 % *Citrobacter freundii*	OP222992	50
				99.2 % *Shewanella bicestrii*	OP222993	50
	PSA	15	4	99.9 % *Pseudomonas kurunegalensis*	OP223009	80
				99.9 % *Pseudomonas kurunegalensis*	OP223010	50
				99.1 % *Pseudomonas kurunegalensis*	OP223011	60
				99.8 % *Pseudomonas kurunegalensis*	OP223012	70
	MRSA	15	0	–	–	–
7	Iron Agar	15	5	99,5 % *Shewanella putrefaciens*	OP222982	60
				99,5 % *Shewanella baltica*	OP222983	50
				99,5 % *Aeromonas veronii*	OP222984	60
				99,9 % *Shewanella baltica*	OP222985	50
				99,5 % *Shewanella putrefaciens*	OP222986	60
	PSA	15	5	99.7 % *Shewanella baltica*	OP223004	60
				99.8 % *Pseudomonas bubulae*	OP223005	50
				99.8 % *Pseudomonas bubulae*	OP223006	60
				99.9 % *Pseudomonas bubulae*	OP223007	50
				99.4 % *Shewanella baltica*	OP223008	60
0	PSA	20	4	99.9 % *Pseudomonas versuta*	OP223000	100
				99.8 % *Pseudomonas bubulae*	OP223001	80
				99.9 % *Pseudomonas versuta*	OP223002	90
				99.9 % *Pseudomonas versuta*	OP223003	90
	Iron Agar	**15**	**0**			
	Total	**185**	**46**	–	–	–

[a] Temperature, the strains were isolated from naturally contaminated prawns and assessed for spoilage potential at the same storage temperature.
[b] Violet red bile glucose agar
[c] Only black colonies so H_2S-producing bacteria were tested.
[d] *Pseudomonas* selective agar.
[e] de Man, Rogosa and Sharp agar.

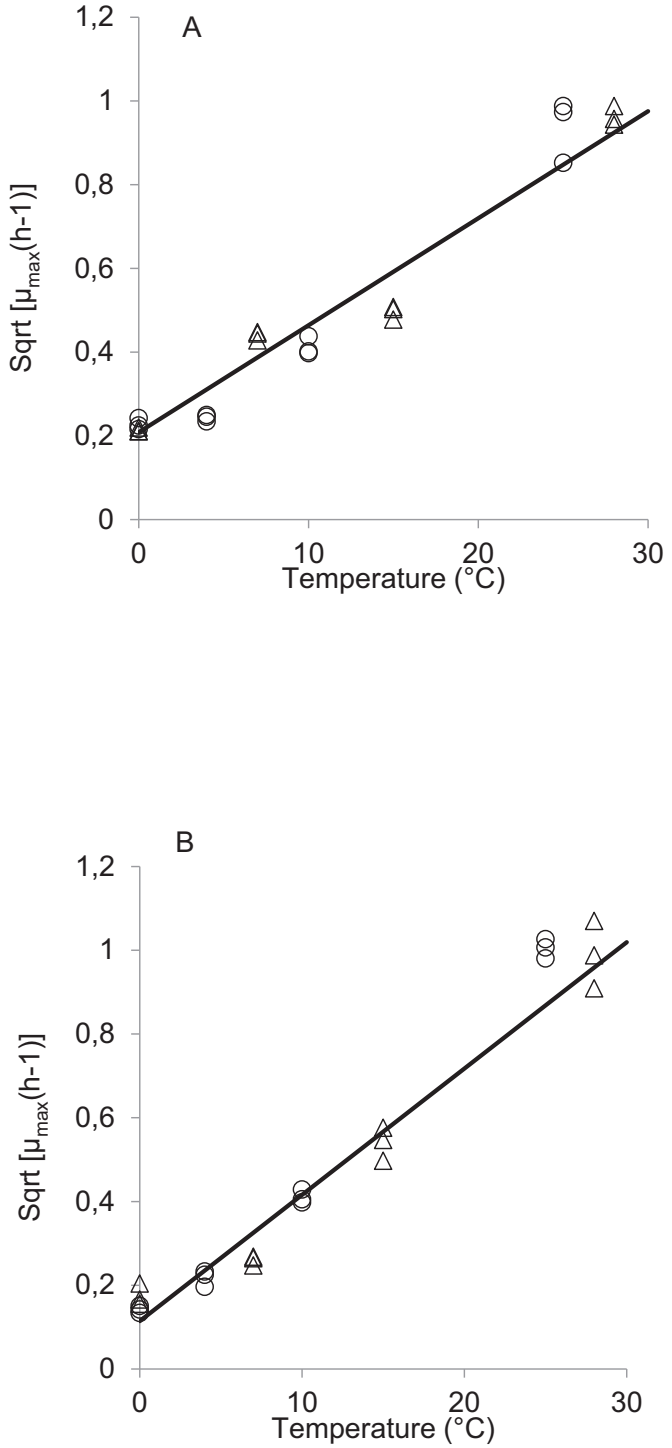

Fig. 3. Maximum specific growth rate of *Pseudomonas* spp. (A) and H_2S-producing bacteria (B) in brackish water prawn as function of temperature modelled by the Ratkowsky equation (on square root scale) (solid line) with model-dependent data (△) and data from an independent experimentation for the validation of the model (○).

Table 3
Parameters (with 95 % C.I.) of the temperature growth rate dependency estimated with the Ratkowsky equation (Eq. (6)).

Microorganisms	T_{min}	b_1
Pseudomonas spp.	−8.2 (−10.7, −5.6)	0.026 (0.022, 0.030)
H_2S-producing bacteria	−3.8 (−6.1, −1.5)	0.030 (0.026, 0.034)

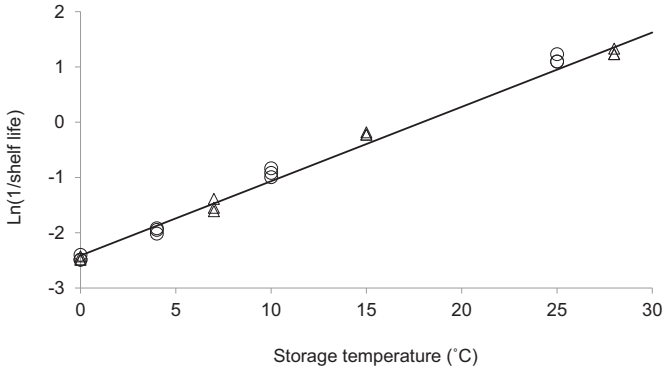

Fig. 4. Predicted brackish water prawn shelf-life (solid line) with model-dependent data (△) and observed shelf-lives from an independent experimentation for the validation of the model (○)
Legend: time is in days.

4. Discussion

This study aimed to evaluate the quality changes of *M. macrobrachion* stored at different temperatures, to identify the major spoilage bacteria of *M. macrobrachion*, and to develop mathematical models predicting the growth of spoilage bacteria of *M. macrobrachion* as well as the shelf-life of the product as a function of storage temperatures, and to validate the models.

Although *Macrobrachium macrobrachion* is called "prawn" in this study like in many other previous studies (Akinwunmi and Moruf, 2021; Koussovi et al., 2023; Lawal-Are and Owolabi, 2012) it is also called "shrimp" by other authors (Ekanem et al., 2011; Eume et al., 2022; Ezemonye et al., 2019).

In Benin, *Macrobrachium* spp. (family of Palaemonidae) are known as fresh water "shrimp" in contrast to *Penaeus* spp. (family of Penaeidae) known as marine or brackish water shrimp. Although both crustaceans can be found in brackish waters in Benin (lakes and lagoons), it is important to note that *Macrobrachium* spp. are found in these brackish waters only when their salinity is nearly 0 ‰ due to the flood of the rivers that brings freshwater to these brackish waters, usually from September to December each year. During this period, Penaeid shrimps are absent from the brackish waters (Dabadé, 2015). The spoilage characteristics of Penaeid shrimp, namely *Penaeus notialis* from brackish waters in Benin have been previously reported from various studies (Dabadé, 2015; Dabadé et al., 2020; Dabadé et al., 2022). For the first time, insight has been given into the spoilage characteristics of Palaemonid "shrimp", namely *M. macrobrachion* from the same brackish waters.

The initial total viable counts in *M. macrobrachion* (5.8 log CFU/g) are similar to that reported in tropical Penaeid shrimp (Dabadé et al., 2015a; Shamshad et al., 1990) and in tropical Palaemonid shrimp such as *Macrobrachium vollenhovenii* in Nigeria (Jimoh et al., 2017). Although these initial total viable counts are high, it seems to be the reality of tropical shrimp microbiology as pointed out by ICMSF (2005) stating that tropical shrimps carry higher initial numbers of bacteria, 5–6 log CFU/g, than cold-water species, 2–4 log CFU/g.

During the storage of the brackish river prawn, *Pseudomonas* spp. and H_2S-producing bacteria were the dominant microorganisms at low (≤7 °C) and high storage temperatures, respectively as it was the case with the tropical brackish water shrimp *Penaeus notialis* caught from the same Lake (Dabadé et al., 2015b). However, the conceptual minimal temperature T_{min} for pseudomonad growth in *M. macrobrachion* (Table 3) is higher than that of pseudomonad growth in *Penaeus notialis* -12.0 ± 1.1 (95 % CI), but are similar to some previously values (−8 to −5 °C) reported for the same group of microorganisms (Li et al., 2020a; Manthou et al., 2019). Strains variability and the nutritional composition of the products could explain the diffences in T_{min} values obtained

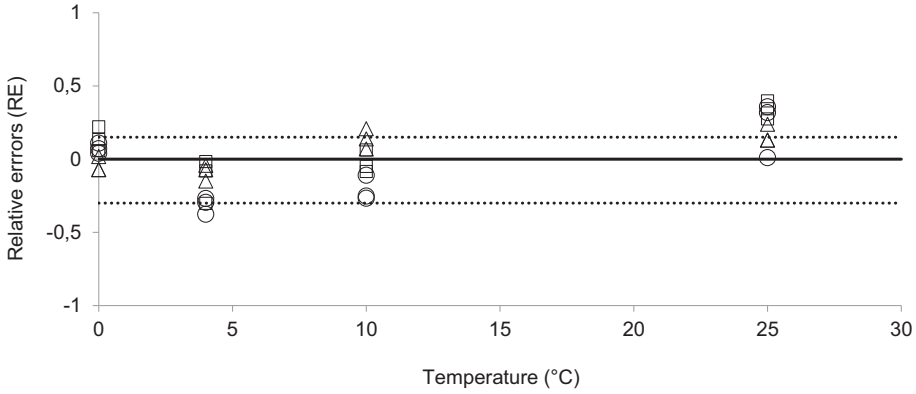

Fig. 5. Relative errors for brackish water prawn shelf-life (△), maximum specific growth rate of *Pseudomonas* spp. (○), and maximum specific growth rate of H_2S-producing bacteria (□) plot against storage temperature with an acceptable prediction zone from an RE of −0.30 to 0.15. The dotted lines indicate the boundaries of acceptable prediction zone.

Table 4
Comparison between the predicted shelf-lives using various models and the observed ones of brackish river prawn during storage at constant temperatures.

Storage temperature (°C)	Types of model[a]	Observed shelf-life (h)	Predicted shelf-life (h)	Difference[b](%)
0	**Eq. (14)**	**280**	**269**	**+3.9**
	Eq. (13)		238	+15
4	**Eq. (14)**	**170**	**157**	**+7.6**
	Eq. (11)		143	+15.9
	Eq. (12)		174	−2.4
	Eq. (13)		143	+15.9
10	**Eq. (14)**	**60**	**70**	**−16.7**
	Eq. (11)		70	−16.7
	Eq. (12)		85	−41.7
	Eq. (13)		68	−13.3
25	**Eq. (14)**	**8**	**9**	**−12.5**
	Eq. (11)		23	−187.5
	Eq. (12)		14	−75.0
	Eq. (13)		12	−50

Shelf-life (T_0) was considered to be 11.7 days (the average found in this study). Predictions from the model developed in this study, observed values from the validation experiment carried out in this study and the differences between these predicted and observed values are in bold.

a

$$\text{Shelf-life (days)} = 11.2 \times \exp(-0.135T) \quad (14)$$

Model developed in this study.

$$\text{Shelf-life } (T) = \frac{\text{Shelf-life } (T_0)}{(1+0.1\times T)^2} \quad (11)$$

Common shelf-life prediction model for seafood Dalgaard and Huss (2020)

$$\text{Shelf-life (T)} = \text{Shelf-life}(T_0) \times \exp(-0.12 \times T) \quad (12)$$

Model developed typically for tropical fresh seafood shelf-life prediction Dalgaard and Huss (2020)

$$\text{Shelf-life (days)} = 4.5\times10^{-15} \times \exp\left(\frac{9650}{\theta}\right) \quad (13)$$

Model developed for tropical brackish water shrimp (*Penaeus notialis*) shelf-life prediction Dabadé et al. (2015a)

In all models T and θ are storage temperaturein (ºC and K, respectively

[b] Difference between observed and predicted shelf-life values.

(De Filippis et al., 2019; Koutsoumanis, 2001). The conceptual minimal temperature T_{min} for H_2S-producing bacteria growth in *M. macrobrachion* obtained in this study is within the same range of that reported by Hoel et al. (2017) in sushi (a dish containing raw fish).

Pseudomonas isolates were detected as active spoilers of *M. macrobrachion* during storage at all tested temperatures but the prevailing *Pseudomonas* species were temperature-dependent: *P. versuta* during iced storage, *P. bubulae* at chilling temperatures and *P. kurunegalensis* at high storage temperatures (≥15 °C). *P. versuta* has also been found to be an active of blunt snout bream (*Megalobrama amblycephala*) a freshwater fish species, during storage at 4 °C (Li et al., 2020b). So far, no reports on the other species as active spoilers of fishery products have been documented.

Although H_2S-producing bacteria were able to grow at 0 °C, their maximum growth rate was significantly lower than that of *Pseudomonas* spp., but no significant differences were obtained between both groups of microorganisms at 7 °C, 15 °C or 28 °C (data not shown). At the sensory rejection times of the prawn at 0 °C (11–12 days), H_2S-producing bacteria were still on lag phase (Fig. 1D) that could be the reason why isolates from this group of microorganisms did not show any strong spoilage activity. Therefore, our findings were not in agreement with the reports by Gram and Dalgaard (2002) and Vogel et al. (2005) who showed that H_2S-producing bacteria of the genus *Shewanella* especially *S. baltica* and *S. putrefaciens*, were the main spoilage bacteria of iced marine fish. In line with our finding, Gram et al. (1990) reported that *S. putrefaciens* played no significant role in the spoilage of tropical fish (Nile perch) during ice storage. However, *S. baltica* and *S. putrefaciens* were identified at 7 °C as active spoilers of *M. macrobrachion.* They can be considered as psychrotrophic *Shewanella* as classified by Vogel et al. (2005). *Shewanella bicestrii* is another species of the genus identified in this study only during storage at 15 °C and 28 °C. This is the first time this organism was identified as spoilage organism of a fishery product and might be classified as mesophilic *Shewanella.* At high storage temperatures, other members of H_2S-producing bacteria isolated as active spoilers of *M. macrobrachion* were *Aeromonas* spp. This is in agreement with the experiment by Gram et al. (1990) in which *Aeromonas* spp. were able to produce hydrogen sulfide (H_2S) and were the dominant spoilage organisms in tropical fish (Nile perch) stored at ambient temperature. In farm reared freshwater prawn *Macrobrachium rosenbergii* in India, Lalitha and Surendran (2006) also reported the presence of *Aeromonas* spp. producing H_2S and dominant at 20 °C. *Citrobacter braakii* and *C. freundii* were two species of Enterobacteriaeae isolated in this study among H_2S-producing bacteria at 15 °C and 28 °C. To our knowledge, this is the first report on *Citrobacter braakii* as an active spoiler of a fishery product. The organism has been isolated however in raw milk and possessed protease activity (Pukančíková et al., 2016).

It is well documented that at high storage temperatures, Enterobacteriaceae species are actively involved in fishery products spoilage producing metabolites such as indole and putrescine, which cause strong off-odor (Dabadé et al., 2015a; Mendes et al., 2002). The spoilage characteristics of *M. macrobrachion* are in agreement with these reports.

What is surprising regarding the spoilage characteristics of the

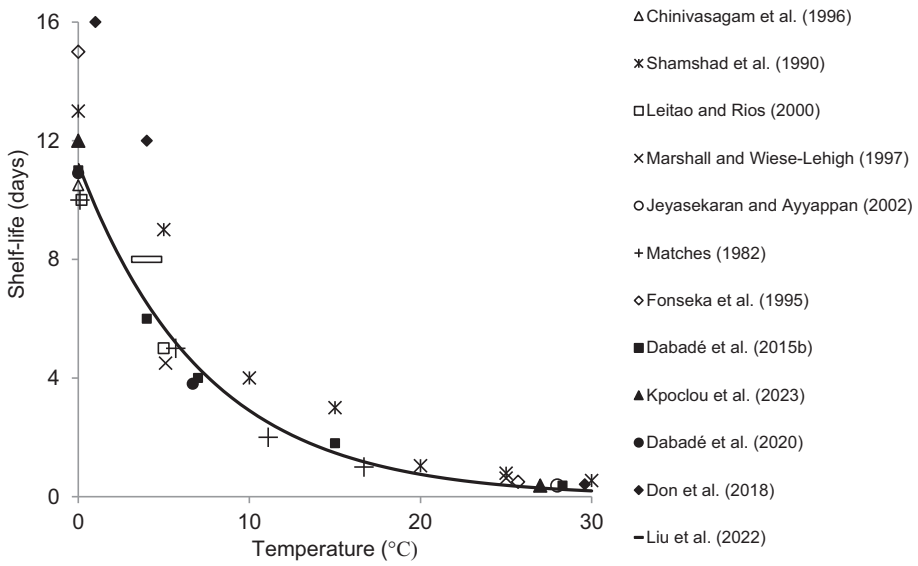

Fig. 6. Comparison of predicted brackish river prawn shelf-life (solid line, Eq. (14)) and observed prawn or shrimp shelf-life in literature (Don et al., 2018; Fonseka et al., 1995; Kpoclou et al., 2023; Leitao and Rios, 2000; Liu et al., 2022; Marshall and Wiese-Lehigh, 1997; Matches, 1982).

brackish river prawn is the total absence of lactic acid bacteria isolates on the list of its potential spoilage microorganisms. In fact, in tropical brackish water shrimp *Penaeus notialis* caught from the same Lake Aheme, lactic acid bacteria, namely *Vagococcus* spp., *Lactococcus garvieae*, *Carnobacterium maltaromaticum* were found to part of the main spoilage organisms during storage of the product at temperatures varying between 0 and 28 °C (Dabadé, 2015). Also, in other prawn of the same family (Palaemonidae) of that of *Macrobrachium macrobrachion,* Du et al. (2017) reported that *Lactococcus garvieae* was the dominant spoilage organism in white prawn (*Exopalaemon modestus*) during ice storage. Even, in the same genus *Macrobrachium,* Jeyasekaran and Ayyappan (2002) found that *Lactobacillus* sp. was dominant in tropical freshwater prawn *Macrobrachium rosenbergii* during storage at room temperature (28 °C ± 2). These results support the assertion that even for the same seafood product, spoilage may develop differently (Gram and Huss, 1996). A controversial role of LAB in fresh meat spoilage was also documented as sometimes they can cause perceivable spoilage signs and sometimes fail to do so (Pothakos et al., 2015). It is known that there is an intra-species variation in the ability of LAB strains to cause spoilage (Björkroth et al., 1998; Pothakos et al., 2014). The absence of lactic acid bacteria isolates on the list of potential spoilage microorganisms of *Macrobrachium macrobrachion* in this study might be justified by the types of LAB strains associated with the product.

The initial value of TVBN (21.2 mg-N/100 g) in fresh brackish river prawn *Macrobrachium macrobrachion* is similar to that of fresh *Macrobrachium rosenbergii* reported by Azam et al. (2013) (20.9 mg-N/100 g) and Haque et al. (2020) (19.3 mg-N/100 g). In contrast, this initial value is lower than that of fresh brackish water shrimp (*Penaeus notialis*) caught from the same Lake, which was 30 mg-N/100 g (Dabadé, 2015). At sensory rejection times, TVBN values obtained in this study in *Macrobrachium macrobrachion* (32–35 mg-N/100 g) were less than half of *Penaeus notialis* caught from the same Lake (Dabadé et al., 2015b). Various factors, namely fish species, catching season and region, fish size, age, sex, and microbial activity can affect TVBN values (García et al., 2022; Goulas and Kontominas, 2007) and as a result, standard rejection values cannot be applied to all species of shrimp (Chinivasagam et al., 1996; Dabadé, 2015).

The initial value of TMA (4.1 mg-N/100 g) found in the present study was similar to that reported by Haque et al. (2020) in *M. rosenbergii* and by Dabadé et al. (2015a) in *Penaeus notialis.* The TMA values at sensory rejections times were 6 mg-N/100 g at 0 °C and approximately 10 mg-N/100 g at all other storage temperatures tested. These values are similar to the limit of TMA for acceptable pink shrimp (11.4 mg-N/100 g) (Mendes et al., 2005). It is well documented that trimethylamine oxide (TMAO) present in many fishery products can be reduced to TMA during spoilage (Gram and Dalgaard, 2002). Although, to our knowledge, the concentration of TMAO in the brackish water prawn (*Macrobrachium macrobrachion*) has not yet been reported in the literature, Lalitha and Surendran (2006) found that several spoilage organisms including *Aeromonas* spp., *Shewanella putrefaciens*, *Enterobacter,* and *Citrobacter* also isolated in this study, were able to reduce TMAO to TMA in sterile *Macrobrachium rosenbergii* broth.

The initial value of pH (7.5) is comparable to the initial values reported in other shrimp species by Dabadé et al. (2015a) (7.4), Zeng et al. (2005) (7.41), López-Caballero et al. (2007) (7.3), but higher than reported by Sriket et al. (2012) (6.5) in *M. rosenbergii.* Basic compounds production during storage as a result of microbial or enzymatic activity could explain the increase in pH value observed.

In the present work, good correlations were found between TVBN and TMA values and sensory scores ($R^2 > 0.90$) (data not shown) as it was observed with tropical brackish water shrimp (Dabadé et al., 2015a).

Although some empirical models have been developed to satisfactory predict the shelf-life of fishery products, they may fail to give adequate predictions in certain cases depending on the types of the fishery products (Dalgaard and Jorgensen, 2000). The types of spoilage organisms associated with the fishery products and the shift in the dominant spoilage organisms according to the storage temperatures could explain the differences in the predictions by a given model for a given fishery product. In *M. macrobrachion*, we found that even at high storage temperatures, Gram-negative rod bacteria (Aeromonads, *Shewanella, Pseudomonas,* Enterobacteriaceae) were dominant and contributed actively to the spoilage of the product, while the active spoilers at high storage temperatures in *Penaeus notialis* were lactic acid bacteria and Enterobacteriaceae. This difference in spoilage organisms' composition may explain why a shorter shelf-life is obtained at high storage temperatures for *M. macrobrachion.*

The validation of the developed model predicting the shelf-life of *M. macrobrachion* by comparison of predictions and shelf-life observed in other studies with various tropical prawn or shrimp species from different regions in the world, leads to less precision than that obtained using the observed shelf-life from our validation experiment with the same prawn species (the bias factors and accuracy factors of the model were 1.05 and 1.12 with our own validation data with same prawn

species, while they were 0.81 and 1.35, respectively with shelf-life data in other studies). This finding is not surprising since spoilage characteristics can vary from one species to another. However, the B_f value of 0.81 obtained shows that the model gives acceptable predictions even with prawns or shrimp from various species since this value ranged between 0.75 and 1.25 (Dalgaard, 2000). Therefore, the model developed in this study for the prediction of shelf-life of fresh *M. macrobrachion* stored aerobically at constant temperatures can be used as a tool in decision-making regarding the management of the quality of tropical brackish water mainly but also other fresh shrimp or prawn caught or harvested from tropical regions worldwide and stored aerobically at constant temperatures.

In conclusion, the present work shows that during storage of the brackish river prawn *M. macrobrachion* at low or high temperatures, Gram-negative rod bacteria are the main spoilage organisms. The active spoilers were identified as *Pseudomonas versuta* at 0 °C, *P. bubulae*, *Shewanella baltica* and *S. putrefaciens* at 7 °C, *P. kurunegalensis*, *S. bicestrii, Citrobacter braakii*, *C. freundii, C. pasteurii*, *Aeromonas veronii* at 15 °C, and *P. kurunegalensis, Citrobacter braakii, C. arsenatis, Escherichia coli,* and *Aeromonas* spp. at 28 °C. As expected, Total volatile basic nitrogen (TVBN) trimethylamine (TMA) and pH increased during storage but with a high rate at higher storage temperatures. TVBN value of 35 mg-N/100 g can be set as the limit for *Macrobrachion macrobrachium.* A model predicting the shelf-life of *M. macrobrachium* during storage at constant temperatures was developed and its validation shows that it can be used to appropriately predict the shelf-life of the product.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2023.110344.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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