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# Preservation practices and safety of fresh shrimp (*Penaeus notialis*) sold in Beninese markets

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**Abstract**
The microbiological characteristics of fresh shrimps during storage in ice (FSPI) (1–4.5 °C) and at ambient temperature (FSKAT) (27.5–29.5 °C) was evaluated in Beninese selling market conditions to assess hygiene and shrimp safety in artisanal preservation practices. Furthermore, samples of FSPI and FSKAT sold at the retail markets were collected and analyzed using bacteriological and physicochemical methods. The acceptable limits for aerobic mesophilic bacteria (AMB) [7.0 $Log_{10}$ (CFU/g)] and trimethylamine (TMA) (5 mg/100 g) were exceeded after 12 days (FSPI) and 9 h (FSKAT). Most market samples (75% FSPI, 92% FSKAT) were non-compliant with the acceptable limit for AMB. The maximal limits specified were exceeded regarding *Enterobacteriaceae*, *E. coli,* and *Salmonella*, up to 75%, 92%, and 42%, respectively (FSKAT) and 33%, 67%, and 75% (FSPI). About 33% (FSPI) and 58% (FSKAT) samples were non-compliant with the TMA limit. All the samples were within the acceptable limits of histamine and tyramine. However, training stakeholders in good handling and hygienic practices is necessary.

**Keywords** Ice · Hygiene · Spoilage · Biogenic amines · Quality

## 1 Introduction

Shrimp is a perishable product. Enzymatic and microbiological changes greatly influence its quality and safety during post-capture storage. According to Aitken et al. [1] and Shamshad et al. [2], shellfish spoil more rapidly than fish for some reasons. First, shellfish are smaller, and small fish spoil more quickly than larger ones. Second (and more critical), the gut is usually not removed immediately after capture; however, post-mortem autolytic changes occur fast. A third reason is that the chemical composition of shellfish tissue is different, and it contains a lot of non-protein nitrogenous compounds that contribute to more rapid spoilage. Finally, black spots, or melanosis, a discoloration indicative of spoilage, always

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occur in shrimp [3]. Therefore, the shrimp processing industry must develop a storage method to maintain shrimp's high quality and freshness.

In Benin, shrimp fishing plays a significant socio-economical role. This activity occupies about 21000 fishermen living in more than 200 villages in the country's south, who catch wild shrimps in the brackish waters of lake Ahémé, lake Nokoué, and Lagoon of Porto-Novo [4]. The annual production of fresh shrimp in Benin, estimated to be approximately 3000 tons, concerns mainly the *Penaeidae,* of which *Penaeus notialis and P. monodon* constitute the major species [5]. More than 75% of this production is intended for the artisanal sector [6]. In 2005, the government self-banned shrimp export to Europe in reaction to the shortcomings noted by the Food and Veterinary Office (FVO) of the European Commission in the legislative and sanitary arrangements for the export-oriented shrimp sector in Benin [7]. Since then, all the national shrimp collection has been directed towards the artisanal sector. In artisanal conditions known as unclean, fresh shrimps are preserved in ice for 3 to 7 days or kept at ambient temperature (about 30 °C) to be sold in local markets [8]. This study aims at evaluating hygiene in preservation practices and shrimp safety in selling conditions in Beninese markets.

## 2 Materials and methods

### 2.1 Fresh shrimp storage experiment as practiced by stakeholders

Wild shrimps (*Penaeus notialis*) with a size of 55 to 60 shrimp/kg, fished from lake Nokoué, were purchased from fishermen at wharves in Cotonou Township, Benin. Shrimps have washed trices in tap water to remove slime and other extraneous material and divided into two batches. One batch was kept in ice in a plastic basket with a shrimp/ice ratio of 1:2 (w/w). The basket was heightened on PVC pipes and enclosed in an icebox. As sellers used to, flake ice was added twice daily at 8 a.m. and 5 p.m. (experiment 1). The second batch was kept in another plastic basket without ice at ambient temperature (experiment 2). These two experiments were performed, each in 3 trials, with a fresh shrimp seller in markets of Saint Michel (ambient temperature) and Ganhi (preservation in ice), following their customary practices. The temperature was monitored in the mass of the product undergoing the storage conditions using a thermocouple (TSTEMP 10 K, Oakton, Singapore). Samples were withdrawn for analysis at 2-day intervals for experiment 1 and 3-h intervals for experiment 2.

### 2.2 Sampling of fresh shrimp as sold in retail markets

Twenty-four (24) samples comprising 12 samples of fresh shrimp preserved in ice and 12 other samples of fresh shrimp kept at ambient temperature were randomly purchased from retail markets of Ganhi and Saint Michel (Cotonou), Comè (Comè city center) and Ahouangbo (Porto-Novo) (Table 1). Samples were collected in sterile stomacher bags, kept in ice, and transported to the laboratory within 2 h for immediate microbiological analysis and pH determination or stored at − 20 °C until other chemical analyses.

### 2.3 Microbiological analyses

Ten (10) shrimps (random selection) of each sample were ground in a sterile stomacher bag using a laboratory blender (Stomacher Lab-Blender 400, model N BA 6021, Seward, London, UK). Twenty-five grams (25 g) of ground samples were suspended in 225 ml of buffer peptone water (Oxoid CM0509B, Basingstoke, Hampshire, England) and homogenized for 2 min in the laboratory blender. Serial decimal dilutions were prepared in buffer peptone water as described by ISO 6887–3 [9] and inoculated in different media to enumerate aerobic mesophilic bacteria (Plate count agar, Oxoid

**Table 1** Fresh shrimp collected from retail markets for analysis

| Retail markets | FSIS | FSATS | Total |
|---|---|---|---|
| Ganhi (Cotonou) | 3 | 3 | 6 |
| Saint Michel (Cotonou) | 3 | 3 | 6 |
| Comè (Comè city center) | 3 | 3 | 6 |
| Ahouangbo (Porto-Novo) | 3 | 3 | 6 |
| Total | 12 | 12 | 24 |

*FSIS* Fresh shrimps preserved in ice for selling; *FSATS* Fresh shrimp kept at ambient temperature for selling

CM0463B), *Staphylococcus aureus* (Baird-Parker agar, Oxoid CM0275B), *Enterobacteriaceae* (Violet Red Bile Glucose Agar, Oxoid CM0485B), *Escherichia coli* (TBX, Oxoid CM0945B), *Pseudomonas* spp. (Pseudomonas agar, CM0559 and *Salmonella* (Rappaport–Vassiliadis broth, Oxoid CM0669B; Muller Koffman broth, Oxoid CM0343B; X.L.D, Oxoid CM0469B; Salmonella, Shigella Agar (Oxoid CM0099B), as described by Kpoclou et al. [10].

## 2.4 Physicochemical analyses

### 2.4.1 Moisture content and pH determination

The pH of the samples was determined as described by Goulas and Kontomina [11] using a digital pH meter (Inolab pH 730 WTW 82362 Wellheim, Germany). The dry matter content was determined by oven drying 5 g of ground samples at 105 °C until a constant weight was reached [12].

### 2.4.2 Total volatile basic nitrogen (TVBN) and trimethylamine (TMA) determination

Total volatile bases of nitrogen (mg N/100 g) were determined in trichloroacetic acid (TCA) extracts of whole peeled shrimp, as described by Malle and Poumeyrol [13] using steam distiller (Büchi distillation Unit K-350, Switzerland). Briefly, 100 g of whole peeled shrimp sample was weighed and blended with 200 ml of 7,5% aqueous TCA (Tricholoroacetic acid). The blend was then centrifuged at 400 × g for 5 min and the supernatant liquid was filtered (Whatman N 3 filter paper). Twenty-five milliliters (25 ml) of filtrate were loaded into the distillation tube followed by 5 ml of 10% NaOH. Steam distillation was carried out using Kjeldahl-type distillatory (VELP mark, model UDK-6, Milan, Italy). A beaker containing 10 ml of a 4% boric acid solution to which three to five drops of the indicator solution, Tashiro Mixed Indicator (2 g Methyl–red and 1 g Methylene–blue are dissolved in 1000 ml 95% ethanol) have been added, was placed at the end of the condenser. Distillation was continued until a final volume of 50 ml was obtained in the beaker). The contents of the collection beaker were titrated from green to a pale pink end point. A procedural blank was done using 200 ml Trichloroacetic acid with no sample and titrated as before. The concentration of trimethylamine (TMA) was determine by modification of the TVBN method adding 35 ml of formaldehyde to 25 ml of filtrate. Steam distillation was then performed as for TVBN determination. The concentrations of TVBN and TMA were determined as follows:

$$\mathrm{TVB-N\ (mg/100g)\ =\ 14\ mg/mol\ x\ a\ x\ b\ x\ 2\ x\ 300\ /\ 25\ ml}$$

$$\mathrm{TMA\ (mg/100g)\ =\ 14\ mg/mol\ x\ c\ x\ b\ x\ 2\ x\ 300\ /\ 25\ ml}$$

where: a and c = ml of sulphuric acid. b = molarity of sulphuric acid.

### 2.4.3 Biogenic amines

**2.4.3.1 Standard solutions** Putrescine, cadaverine, histamine, spermine, spermidine, tyramine, tryptamine, 2-phenylethylamine, and 1,7-diaminoheptane were obtained from Sigma (St. Louis, MO, USA). Individual stock solutions (10 mg/mL) were prepared by dissolving each biogenic amine standard in a 5% trichloroacetic acid (TCA) solution. A working solution of 1,7-diaminoheptane (1.5 mg/ml) obtained by diluting 10 mg/ml stock solution with TCA 5% was used as an internal standard. A pool of the 8 other biogenic amines was obtained by mixing 100–1000 µL of each stock solution with TCA 5% to 10 ml. All the standard solutions were kept for 6 months at 4 °C.

**2.4.3.2 Sample preparation** Five grams of fresh shrimps were mixed with 100 µL internal standard working solution into a 50 ml Falcon tube (Becton Dickinson Labware (NJ, USA)). Then, 20 ml of trichloroacetic acid (TCA) 5% was added; the mixture was vortexed and let stand for 10 min. Homogenization was realized with an UltraTurrax mixer (T25 basic) (IKA®-Werke, Staufen, Germany) for 30 secs. Next, the solution was filtered through a paper filter (MN 640 M ¼, diameter 150 mm, Macherey–Nagel, Düren, Germany) into another 50 ml Falcon tube, and TCA 5% was added to 50 ml. One milliliter of the extract was poured into a 15 ml Falcon tube. Three hundred µL $NaHCO_3$ saturated, and 250 µL NaOH 2N was added. The dansylation was realized by adding 1 ml dansyl chloride (20 mg/ml in acetone) and incubating the tubes at 70 °C for 20 min. After dansylation, 1 ml glycine (30 mg/ml in water) was added to bind to the dansyl chloride in excess, and the tubes were incubated at 70 °C for 20 min. Then acetone was evaporated under a nitrogen stream, and 2 ml

water was added. Next, biogenic amine derivatives were extracted with 4 × 1 ml of diethyl ether followed by evaporation of the solvent to dryness in a Savant™ Universal SpeedVac™ Vacuum System (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the addition of 500 µL of acetonitrile/water (50:50, v:v). Finally, the solution was filtered through an Acrodisc® filter (GHP Acrodisc® 13 mm syringe filters with 0.2 µm GHP membrane, Pall Life Sciences, MI, USA) and transferred into an injection vial.

**2.4.3.3 UPLC-Fluorescence analysis** Amines were analyzed on a UPLC Acquity system integrated autosampler (Acquity Sample Manager FTN), solvent delivery system (Acquity QSM H Class), and column heater coupled to an Acquity Fluorescence detector, all from Waters Corporation (Milford, MA, USA). The column used was an Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 µm), with a UPLC BEH C18 VanGuard pre-column (2.1 × 5 mm, 1.7 µm), both from Waters Corporation. The mobile phase was acetonitrile (solvent A) and water (solvent B). The gradient elution conditions were: 50% of solvent A maintained for 1.75 min, from 50 to 60% of solvent A within 6.75 min, from 60 to 85% within 1.80 min, and from 85 to 100% within 2.40 min; then, conditions were held for 0.30 min, and the contribution of solvent A was decreased to 50% over 0.30 min and maintained for 2.40 min. The oven temperature was set at 40 °C, and the injection volume was 5 µL. The flow rate was 0.6 ml/min.

The peaks were identified by comparing their retention times with the corresponding standards. The fluorescence detection was applied at 300 nm for excitation and 480 nm for emission. Results were calculated using Empower 3 software (Waters). The limits of quantification (LOQ) of the method were 5.9 mg $kg^{-1}$ for tryptamine and 2-phenylethylamine and 5.9, 4.5, 2.5, 14.9, 10.2, 2.0, and 0.8 mg $kg^{-1}$ for putrescine, cadaverine, histamine, tyramine, spermidine, and spermine, respectively. The recovery of extraction was ranging between 95.4 and 104.8% for all biogenic amines.

### 2.5 Data analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, version 16). Data are presented as mean with standard deviation; the significant differences were set at $p < 0.05$. One-way ANOVA and SNK (Student, Newman, and Keuls) range tests were used. In addition, correlation analyses between physicochemical and microbiological parameters were studied.

## 3 Results and discussion

### 3.1 Microbiological characteristics of shrimps

#### 3.1.1 Fresh shrimps during storage in ice and at ambient temperature

The microbial loads of fresh shrimp during storage in ice and at ambient temperature are shown in Fig. 1. It appears that during storage in ice, the temperature in the mass of shrimps oscillates between 1 and 4.5 °C. During 14 days, the aerobic mesophilic bacteria (AMB) load and *Pseudomonas* load increase progressively from 3.8 $Log_{10}$ (CFU/g) to 8.0 $Log_{10}$ (CFU/g) and from 3.4 $Log_{10}$ (CFU/g) to 6.8 $Log_{10}$ (CFU/g), respectively. The load of *Enterobacteriaceae* wavered between 2.8 $Log_{10}$ (CFU/g) and 3.4 $Log_{10}$ (CFU/g), while *S. aureus* was absent throughout the experimentation. When shrimps are stored at ambient temperature (27.5–29.5 °C), AMB, *Pseudomonas* and *Enterobacteriaceae* increased from 3.7 $Log_{10}$ (CFU/g), 3.5 $Log_{10}$ (CFU/g), and 2.7 $Log_{10}$ (CFU/g) to 9.1 $Log_{10}$ (CFU/g), 8.4 $Log_{10}$ (CFU/g) and 4.8 $Log_{10}$ (CFU/g), respectively within 15 h. Therefore, keeping at an ambient temperature (27.5–29.5 °C) is favorable for microbial development in fresh shrimp. This testifies to the preservative effect of ice. AMB load superposes *Pseudomonas* load either during storage in ice or keeping at ambient temperature; this indexes *Pseudomonas* as dominant microorganisms of the aerobic mesophilic flora. The same tendency was observed for similar storage conditions (0 °C and 28 °C) in a previous study on fresh shrimp (*Penaeus notialis*) collected from the same lake (Nokoue) in Benin by Dabade et al. [14]. These authors showed the progressive growth of total viable count (TVC), *Pseudomonas* spp., and *Enterobacteriaceae*, with *Pseudomonas* spp. as the dominant microorganism group in shrimp storage at 0 °C. At 28 °C storage, they identified *Pseudomonas* spp. among the dominant bacteria groups ($H_2S$-producing bacteria, *Pseudomonas* spp., and Lactic acid bacteria). In our study, only *Pseudomonas* spp. have been enumerated in these groups. Ray and Bhunia [15] reported that Gram-negative aerobic rods, such as *Pseudomonas* spp. and several Gram-negative rods are the major shrimp spoilage bacteria. However, because of the relatively shorter generation time, spoilage by psychrotrophic *Pseudomonas* spp. predominates under aerobic storage at

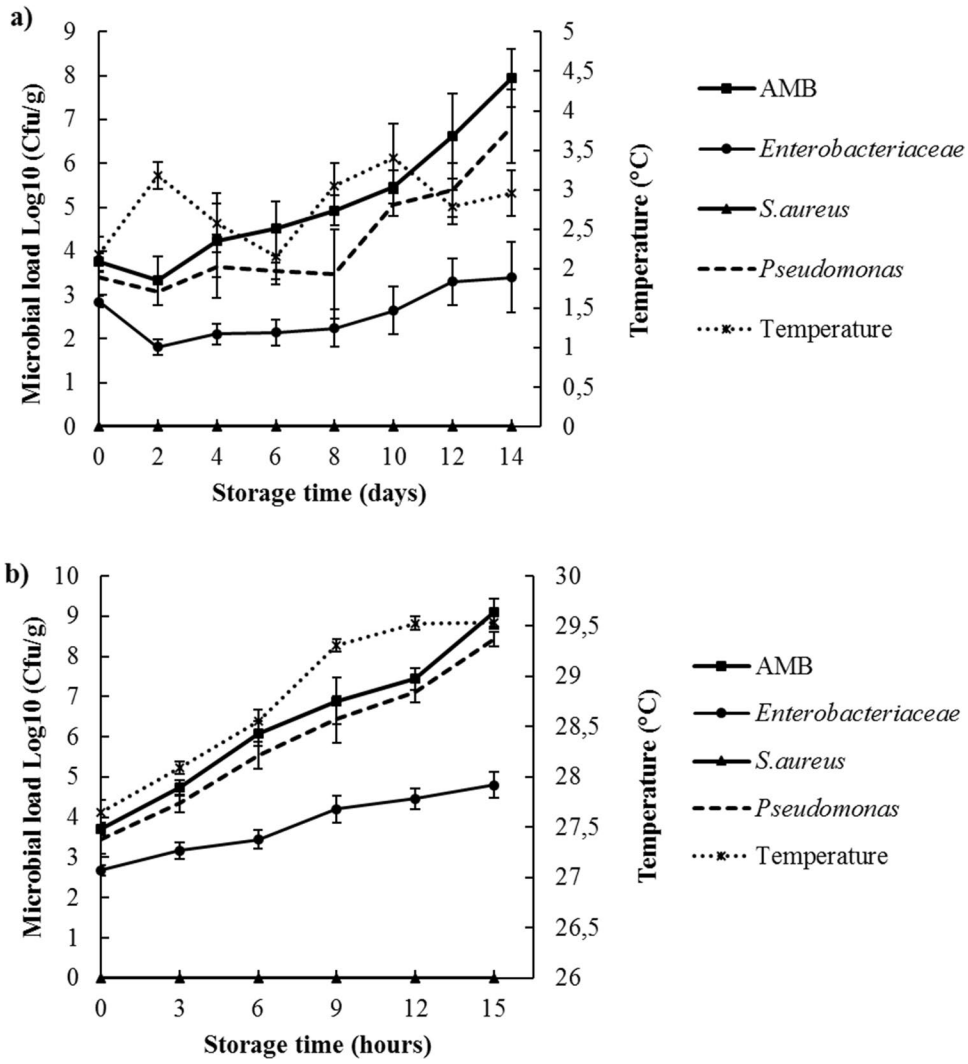


**Fig. 1** Changes in microbial loads during fresh shrimps storage in ice (**a**) and at ambient temperature (**b**)

both refrigerated and slightly higher temperatures. Other previous studies have identified *Pseudomonas* as the main flora in the deterioration of shrimps from tropical waters [16–19]. The International Commission on Microbiological Specifications for Foods [20] set up maximum limits of 7.0 $Log_{10}$ (CFU/g), 4.0 $Log_{10}$ (CFU/g), 2.0 $Log_{10}$ (CFU/g), and absence in 25 g for AMB, *S. aureus*, *E. coli,* and *Salmonella* respectively in raw crustaceans. Furthermore, the German Society for Hygiene and Microbiology [21] recommended 5.0 $Log_{10}$ (CFU/g) for *Enterobacteriaceae* for raw shrimp. In this study, the maximal limit of AMB was exceeded after 12 days and 9 h of storage in ice and at ambient temperature, respectively (Fig. 1). This agrees with data reported by Dabade et al. [14], in which TVC attained the limit of 7.0 $Log_{10}$ (CFU/g) after 12 days of storage at 0 °C and 28 °C respectively., Based on a sensory analysis, these authors identified the rejection times of 8–10 h and 10–12 days at 28 °C and 0 °C.

### 3.1.2 Fresh shrimp collected from retail markets

AMB count was up to 9.0 $Log_{10}$ (CFU/g) and 8.5 $Log_{10}$ (CFU/g) in FSPI and FSKAT, respectively (Table 2). Most of the FSKAT samples (75%) were contaminated with S. aureus, and none of them complied with the maximal limit set by the ICMSF [20]. Except for *Salmonella* tests, FSKAT was less safe than FSPI (Table 2) for all microbiological criteria. This could be due to the preservative role of ice, as reported by Dabade et al. [14] in a comparative study of shrimp storage at 28 °C, 7 °C and 0 °C.

Many FSPI and FSKAT were non-compliant with *Enterobacteriaceae*, *Salmonella,* and *E. coli*. This points out the possibility of human or animal fecal sources of contamination (often correlated with contamination by digestive pathogens) of shrimp, even in the environment, they are caught from or during post-harvest handling. Indeed, raw shrimps used for

**Table 2** Microbial loads ($Log_{10}$ (CFU/g)) and status of fresh shrimps collected from retail markets

| Samples | Tests | Mean | SD[a] | Minimum | Maximum | Standards | No-compliant samples (%) |
|---|---|---|---|---|---|---|---|
| FSPI (n = 12) | AMB | 6.8 | 1.6 | 5.2 | 9.0 | 7.0 | 42 |
| | *S. aureus*[b] | 3.8 | – | 3.8 | 3.8 | 4.0 | 0 |
| | *E. coli* | 4.1 | 0.7 | 3.3 | 4.9 | 2.0 | 67 |
| | *Enterobacteriaceae* | 4.7 | 1.30 | 3.3 | 6.8 | 5.0 | 33 |
| | *Pseudomonas* | 6.6 | 1.3 | 4.7 | 8.3 | – | – |
| | *Salmonella*[c] | – | – | – | – | Absence | 75 |
| FSKAT (n = 12) | AMB | 7.5 | 0.5 | 6.7 | 8.5 | 7.0 | 83 |
| | *S. aureus* | 4.4 | 2.0 | 4.0 | 5.6 | 4.0 | 75 |
| | *E. coli* | 4.3 | 1.1 | 1.8 | 5.7 | 2.0 | 92 |
| | *Enterobacteriaceae* | 5.2 | 1.0 | 3.3 | 6.2 | 5.0 | 75 |
| | *Pseudomonas* | 6.8 | 0.5 | 6.1 | 7.4 | – | – |
| | *Salmonella* | – | – | – | – | Absence | 42 |

*FSPI* fresh shrimps stored in ice; *FSKAT* fresh shrimps stored at ambient temperature; *AMB* Aerobic Mesophilic Bacteria; *CFU* colony forming unit; *n* number of samples; *Standard* ICMSF [20]

[a]*SD* standard deviation

[b]coagulase-positive staphylococci

[c]Presence/absence test in 25 g of sample

experiments with sellers were initially contaminated with *Enterobacteriaceae,* a group of microorganisms including *E. coli and Salmonella*, but were not infected with *S. aureus* (Fig. 1). The non-compliance of FSPI and FSKAT for *Enterobacteriaceae*, *Salmonella,* and *E. coli*. Could be associated with microorganisms growth during the shelf-life at market before they were collected. Indeed, Dabade et al. [14] reported growth of shrimp during sotorage at 28 °C, 7 °C and 0 °C. FSKAT exceeded the microbiological specifications in storage experiments with sellers after 9 h. Furthermore, all the samples of this shrimp collected in retail markets for analysis were non-compliant at more than 75% for all microbiological criteria, except for *Salmonella* (42%). Fresh shrimps stored at ambient temperature at the sale points present more risk for consumers' health.

### 3.2 Physico-chemical characteristics of shrimps

The results showed that pH, TMA, and TVBN increased significantly ($p < 0.05$) during storage both in ice (1–4.5 °C) and at ambient temperature (27.5–29.5 °C) (Table 3). The initial value of TVBN and TMA was 17.34 mg/100 g and 1.39 mg/100 g, respectively (FSPI) and 17.64 mg/100 g and 1.39 mg/100 g, respectively (FSKAT). These values are lower than those (30.1 mg/100 g and 2.5 mg/100 g, respectively) reported by Dabade et al. [14]. As argued by these authors, the difference would be due to the shrimp's non-protein nitrogen content, which depends on feeding; catching season; size, age, sex, microbial activity, and the methods used for determination. In this study, TVBN and TMA determination were achieved on an extract of ground tissue of peeled shrimp by several authors [22–26], while Dabade et al. [14] measured these data on whole fresh shrimp. Heu et al. [27]. reported TVBN values up to 9.8 mg/100 g and 5.6 mg/100 g in processing by-products (heads, shells, and tails) of Northern pink shrimp (*Pandalus borealis*) and spotted shrimp (*Trachypenaeus curvirostris*) respectively while 12.6 mgN/100 g and 11.9 mg/100 g were reported in muscles. Limam et al. [28] wrote for caramote prawns (*Penaeus kerathurus*), 10.3 mg/100 g (muscle) and 12.4 mg/100 g (by-product).

Changes in pH, TMA, TVBN, and biogenic amines could be due to post-mortem microbial and biochemical reactions of shrimps' spoilage. Indeed, previous studies [22, 29–31] have shown that shrimp spoilage started due to endogenous enzymes and microorganism reactions after death. They result in basic nitrogen such as urea, ammonia, and trimethylamine inducing pH change [22, 29–31]. Our study's pH increases significantly from 6.86 to 7.52 (FSPI) and from 6.89 to 7.62 (FSKAT). Furthermore, in this study, data showed that pH is positively correlated with TMA, TVBN, and AMB: 0.92, 0.86, and 0.87, respectively, for storage in ice and 0.96, 0.93, and 0.86 for storage at ambient temperature (Table 4).

Gram-negative bacteria dominate microbial flora in shrimp. These microorganisms initially metabolize the non-proteinic nitrogen (NPN) compounds by decay (oxidation), followed by putrefaction to produce different types of volatile

**Table 3** Physicochemical characteristics of fresh shrimp during storage in ice and at ambient temperature

| Time | | pH | TMA (mg/100 g) | TVBN (mg/100 g) | Dry matter (%) |
|---|---|---|---|---|---|
| Shrimp storage in ice (1–4.5 °C; n = 3) | | | | | |
| Time (days) | 0 | 6.86 ± 0.05[a] | 1.39 ± 0.16[a] | 17.43 ± 0.73[a] | 20.23 ± 0.12[a] |
| | 2 | 7.01 ± 0.03[b] | 1.64 ± 0.16[a] | 20.58 ± 0.84[ab] | 20.08 ± 0.09[a] |
| | 4 | 7.15 ± 0.03[c] | 2.35 ± 0.31[ab] | 22.68 ± 2.17[b] | 19.27 ± 0.36[a] |
| | 6 | 7.28 ± 0.05[d] | 2.94 ± 0.48[b] | 27.72 ± 2.15[c] | 18.79 ± 0.07[ab] |
| | 8 | 7.33 ± 0.01[e] | 3.07 ± 0.44[b] | 32.76 ± 1.97[d] | 17.87 ± 0.05[bc] |
| | 10 | 7.37 ± 0.02[f] | 4.33 ± 0.85[c] | 39.48 ± 2.17[e] | 17.18 ± 0.02 [cd] |
| | 12 | 7.42 ± 0.01 [g] | 4.70 ± 0.90[c] | 56.28 ± 4.00[f] | 17.11 ± 0.03 [cd] |
| | 14 | 7.52 ± 0.04 [h] | 6.43 ± 1.33[d] | 70.98 ± 3.73 [g] | 16.27 ± 0.06[d] |
| Shrimp storage at ambient temperature (27.5–29.5 °C; n = 3) | | | | | |
| Time (hours) | 0 | 6.89 ± 0.05a | 1.39 ± 0.16[a] | 17.64 ± 0.97[a] | 22.87 ± 0.52[a] |
| | 3 | 7.05 ± 0.04[b] | 2.73 ± 0.69[b] | 19.61 ± 0.84[a] | 22.75 ± 0.41[a] |
| | 6 | 7.20 ± 0.02[c] | 3.61 ± 0.52[c] | 22.09 ± 0.89[a] | 22.62 ± 0.28[a] |
| | 9 | 7.39 ± 0.02[d] | 4.58 ± 4.58[d] | 40.19 ± 0.16[b] | 22.45 ± 0.19[a] |
| | 12 | 7.51 ± 0.01[e] | 6.97 ± 0.40[e] | 65.39 ± 5.77[c] | 22.36 ± 0.52[a] |
| | 15 | 7.62 ± 0.01[f] | 9.16 ± 0.81[f] | 83.87 ± 3.83[d] | 22.17 ± 0.31[a] |

Data expressed as mean ± standard deviation (n = 3)

*n* number of trials

[a, b, c, d, e, f, g, h]values in the same column followed by the same letter are not significantly different (p > 0.05)

**Table 4** Linear correlation between physicochemical and microbiological parameters for quality assessment during shrimp storage in ice (1–4.5 °C)

| | pH | TMA | TVBN | Dry matter | AMB | *Enterobacteriaceae* | *S. aureus* |
|---|---|---|---|---|---|---|---|
| Storage in ice (1–4.5 °C) | | | | | | | |
| TMA | 0.92 | | | | | | |
| TVBN | 0.86 | 0.98 | | | | | |
| Dry matter | – 0.96 | -0.96 | – 0.92 | | | | |
| AMB | 0.87 | 0.98 | 0.99 | – 0.94 | | | |
| *Enterobacteriaceae* | 0.48 | 0.73 | 0.80 | – 0.67 | 0.82 | | |
| *S. aureus* | – | – | – | – | – | – | |
| *Pseudomonas* | 0.77 | 0.96 | 0.96 | – 0.88 | 0.96 | 0.84 | – |
| Storage at ambient temperature (27.5–29.5 °C) | | | | | | | |
| TMA | 0.96 | | | | | | |
| TVBN | 0.93 | 0.90 | | | | | |
| Dry matter | – 0.99 | – 0.98 | – 0.95 | | | | |
| AMB | 0.86 | 0.97 | 0.92 | – 0.99 | | | |
| *Enterobacteriaceae* | 0.98 | 0.96 | 0.93 | – 0.99 | 0.98 | | |
| *S. aureus* | – | – | – | – | – | – | |
| *Pseudomonas* | 0.99 | 0.98 | 0.93 | – 0.99 | 0.99 | 0.99 | – |

*TMA* trimethylamine, *TVBN* Total volatile basic nitrogen, *AMB* Aerobic mesophilic bacteria

compounds such as $NH_3$, trimethylamine, histamine, putrescine, and cadaverine [15]. In this study, AMB and Gram-negative bacteria such as *Pseudomonas* and *Enterobacteriaceae* are positively correlated with TMA (0.96 and 0.48 respectively for FSPI; 0.98 and 0.96 for FSKAT) and TVBN (0.96 and 0.80 respectively for FSPI; 0.93 and 0.93 for FSKAT) (Table 4). Dry matter decreasing during storage in ice could be due to meltwater absorption or protein degradation in shrimp (Table 3). In this study, dry matter is negatively correlated with pH, TMA, TVBN, and AMB during the two storage trials (– 0.96; – 0.96; – 0.92 and – 0.94, respectively, for FSPI and – 0.99; – 0.98; – 0.95 and – 0.99 for FSKAT) (Table 4).

Nitrogen basic compounds such as TMA and TVBN are indexed as indicators for shrimp spoilage [24, 32, 33]. Chinivasagam et al. [34] suggested 30 mgTVBN/100 g, while Cobb et al. [22] reported 5 mg TMA/100 g and 30 mg TVBN/100 g as limits of acceptability used for raw shrimp in some regions of the world. However, Zeng et al. [25] indicated that the TVBN limit might be questionable for shrimp because higher levels have been found even in fresh shrimp. Indeed, several authors reported TVBN values above 30 mg/100 g in freshly caught shrimp. The initial TVBN values of 38.2 mg/100 g, 34 mg/100 g, 33.5 mg/100 g, and30 mg/100 g have been reported respectively by Cob et al. [23] in white shrimps (*Penaeus setiferus* and *P. aztecus*), Chinivasagam et al. [34] in white shrimp (*Penaeus merguiensis*), Zeng et al. [25] in northern shrimp (*Pandalus borealis*) and Lopez et al. [35] in pink shrimp (*Parapenaeus longirostris*). Dabade et al. [14] reported 30.1 mg/100 g in freshly caught shrimp (*Penaeus notialis*) from the origin (lake Nokoué) as those used in the present study. Regarding the TMA as a freshness indicator, the acceptability limit was exceeded after 12 days and 9 h, respectively, in FSPI and FSKAT, as shown in the AMB count (Fig. 1). Gram et al. [36] and Gram and Huss [17] indexed TMA as a product of bacterial spoilage. Regarding the marketed shrimps, data show that the mean value of TMA was lower than the acceptability limit (5 mg TMA/100 g) (Table 5). However, considering samples individually, 33% and 58% of FSPI and FSKAT collected from retail markets were non-compliant with the TMA acceptability limit (Table 5). The odds are that a consumer buys non-compliant shrimp. Therefore, fresh shrimp, sold in Beninese markets (open-air, 1–4.5 °C or 27.5 °C–29.5 °C), is not of the required quality. Guidance documents must be developed to help stakeholders with good handling and hygienic practices.

### 3.3 Biogenic amines in shrimps

During both storage in ice (1–4.5 °C) and at ambient temperature (27.5–29.5 °C), tryptamine, 2-phenylethylamine, histamine, and tyramine were not detected beyond their limit of quantification value of 5.9, 5.9, 14.9 and 10.2 mg $kg^{-1}$ respectively, except that tyramine appeared after 15 h of storage at ambient temperature, up to 530.4 and 272.2 mg $kg^{-1}$ for both trials (Tables 6 and 7). However, until the 12th day and the 9th hour for which FSPI and FSKAT, respectively, exceeded the TMA acceptable limit, putrescine, cadaverine spermidine, and spermine were detected with maximal values of 117.9, 2.6, 2.2, and < 0.8 mg $kg^{-1}$respectively for storage in ice and 31.5; 74.4; 4.3 and 7.1 mg $kg^{-1}$ for storage at ambient temperature.

Biogenic amine formation results in amino acid decarboxylation from protein degradation by the metabolism of alternative microorganisms [37]. They are non-volatile low-molecular weight nitrogenous organic bases that may represent a health threat for humans if ingested at certain levels. Among them, histamine is the most frequently implied in seafood poisoning, whose symptoms include skin flushing, hypotension, and diarrhea [38]. Nout [39] suggested Toxic levels of 50–100 mg of histamine/kg and 100–800 mg of tyramine/kg of food. Likewise, according to the scientific opinion published by the European Food Safety Authority [40], histamine concentrations between 50 and 200 mg/kg may cause adverse health effects, and levels above 200 mg/kg histamine are reported to cause toxic effects in humans. In this study, histamine and tyramine were not detected beyond respective LOQ values of 14.9, and 10.2 mg $kg^{-1}$ before the maximal limits for TMA were attained during storage trials (Tables 6 and 7). Likewise, both FSPI and FSKAT collected from retail markets agreed with this recommendation on biogenic amines (Table 8). Putrescine and cadaverine are known to enhance the toxicity of histamine and tyramine [41]. In this study, these amines were detected, but no maximal limit value was recommended.

**Table 5** Physicochemical characteristics of fresh shrimp collected from sellers in retail markets

| Parameters | FSPI (n = 12) | | | FSKAT (n = 12) | | | Status (No-compliant samples) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Minimum | Maximum | Mean | Minimum | Maximum | FSPI | FSKAT | Limit value | Reference |
| pH | 7.32 | 7.12 | 7.50 | 7.39 | 7.21 | 7.54 | – | – | – | – |
| TMA (mg/100 g) | 3.96 | 2.23 | 6.12 | 4.12 | 3.01 | 6.02 | 33% | 58% | 5 | Cobb et al. [22] |
| TVBN (mg/100 g) | 26.99 | 19.24 | 33.42 | 29.84 | 23.02 | 33.24 | – | – | – | |

*FSPI* fresh shrimp preserved in ice, *FSKAT* fresh shrimp, kept at ambient temperature, *TMA* trimethylamine, *TVBN* Total volatile basic nitrogen; *n* number of samples; *Limit value* limit of acceptability

**Table 6** Change in biogenic amines (mg $kg^{-1}$) during shrimp storage in ice (1–4.5 °C) (n = 2)

| | Trial | LOQ | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 2 | 4 | 6 | 10 | 12 | 14 |
| Tryptamine | 1 | 5.9 | nd | nd | nd | nd | nd | nd | nd |
| | 2 | | nd | nd | nd | nd | nd | nd | nd |
| 2-Phenylethylamine | 1 | 5.9 | nd | nd | nd | nd | nd | nd | nd |
| | 2 | | nd | nd | nd | nd | nd | nd | nd |
| Putrescine | 1 | 4.5 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| | 2 | | 4.8 | 7.0 | 10.5 | 6.5 | 109.6 | 117.9 | 99.5 |
| Cadaverine | 1 | 2.5 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| | 2 | | 9.9 | 27.6 | 15.9 | 4.2 | 3.0 | 2.6 | < LOQ |
| Histamine | 1 | 14.9 | nd | nd | nd | nd | nd | nd | nd |
| | 2 | | nd | nd | nd | nd | < LOQ | nd | nd |
| Tyramine | 1 | 10.2 | nd | nd | nd | nd | nd | nd | nd |
| | 2 | | < LOQ | nd | < LOQ | < LOQ | < LOQ | < LOQ | 11.9 |
| Spermidine | 1 | 2.0 | < LOQ | 2.8 | 3.4 | 2.3 | 2.6 | 2.2 | < LOQ |
| | 2 | | 6.0 | 2.9 | 3.1 | < LOQ | < LOQ | nd | nd |
| Spermine | 1 | 0.8 | nd | 4.3 | 4.6 | < LOQ | 2.8 | < LOQ | 1.5 |
| | 2 | | 2.0 | 1.3 | < LOQ | 9.3 | 8.4 | nd | 10.8 |

*LOQ* limit of quantification; *n* number of trials; *nd* not detected

**Table 7** Change in biogenic amines (mg $kg^{-1}$) during shrimp storage at ambient temperature (27.5–29.5 °C) (n = 2)

| | Trial | LOQ | Storage time (hours) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 3 | 6 | 9 | 12 | 15 |
| Tryptamine | 1 | 5.9 | nd | nd | nd | nd | nd | nd |
| | 2 | | nd | nd | nd | nd | nd | nd |
| 2-Phenylethylamine | 1 | 5.9 | nd | nd | nd | nd | nd | nd |
| | 2 | | nd | nd | nd | nd | nd | < LOQ |
| Putrescine | 1 | 4.5 | < LOQ | 4.9 | 6.5 | 31.5 | 119.4 | 165.5 |
| | 2 | | < LOQ | < LOQ | 6.3 | 30.4 | 87.2 | 134.7 |
| Cadaverine | 1 | 2.5 | < LOQ | < LOQ | 16.9 | 74.4 | 124.3 | 373.7 |
| | 2 | | < LOQ | < LOQ | 14.7 | 62.2 | 124.4 | 353.6 |
| Histamine | 1 | 14.9 | < LOQ | nd | < LOQ | < LOQ | nd | nd |
| | 2 | | < LOQ | < LOQ | < LOQ | < LOQ | nd | < LOQ |
| Tyramine | 1 | 10.2 | nd | nd | nd | nd | nd | 530.4 |
| | 2 | | nd | nd | nd | nd | < LOQ | 272.2 |
| Spermidine | 1 | 2.0 | 3.0 | 3.6 | 3.3 | 4.3 | 2.7 | 7.1 |
| | 2 | | 2.3 | 3.4 | 3.9 | 2.8 | 2.1 | 2.7 |
| Spermine | 1 | 0.8 | 2.8 | 4.3 | 6.0 | 4.3 | 7.2 | 1.0 |
| | 2 | | 6.5 | 6.2 | 6.8 | 7.1 | 7.4 | 1.1 |

*LOQ* limit of quantification; *n* number of trials; *nd* not detected

## 4 Conclusion

This study revealed that, as preserved by sellers in markets, fresh shrimp becomes stale after 12 days of preservation in ice (1–4,5 °C) (shrimp/ice ratio, 1:2) and 9 h of storage at ambient temperature (27.5–29.5 °C). As sold in retail markets, fresh shrimps are safe from toxic biogenic amines (histamine and tyramine). However, they are of poor hygienic quality from the physicochemical and microbiological points of view. In addition, most samples collected from markets were non-compliant with the TMA limit specified and limits specified for microorganisms of fecal

**Table 8** Biogenic amines content (mg $kg^{-1}$) of fresh shrimp collected from retail markets

| Biogenic amines | LOQ | FSPI (n = 12) | | | FSKAT (n = 12) | | |
|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | Median | Minimum | Maximum | Median |
| Tryptamine | 5.9 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| 2-Phenylethylamine | 5.9 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| Putrescine | 4.5 | < LOQ | 146.6 | 6.4 | < LOQ | 9.9 | < LOQ |
| Cadaverine | 2.5 | < LOQ | 287.1 | 27.9 | 5.4 | 56.4 | 15.0 |
| Histamine | 14.9 | < LOQ | < LOQ | < LOQ | < LOQ | 27.6 | < LOQ |
| Tyramine | 10.2 | < LOQ | 35.4 | < LOQ | < LOQ | < LOQ | < LOQ |
| Spermidine | 2.0 | < LOQ | 4.7 | < LOQ | < LOQ | 7.3 | 4.1 |
| Spermine | 0.8 | < LOQ | 17.2 | 8.9 | 3.5 | 21.5 | 9.0 |

*FSPI* fresh shrimp stored in ice; *FSKAT* fresh shrimp stored at ambient temperature; *LOQ* Limit of quantification; *n* number of samples

contamination, such as *E. coli*, *Enterobacteriaceae* and *Salmonella*. Therefore, developing guidance documents and training stakeholders for good handling and hygienic practices is necessary.

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**Data availability** The dataset generated for this study are available on request to the corresponding author.

**Declarations**

**Competing interests** The author declares no competing interests.



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