Western Indian Ocean

JOURNAL OF

Marine Science

Chief Editor José Paula

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Marine Science

Chief Editor **José Paula** | Faculty of Sciences of University of Lisbon, Portugal

Copy Editor **Timothy Andrew**

Published biannually

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Effect of initial handling by artisanal fishermen on the quality of penaeid shrimps in Kurawa on the north coast of Kenya

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Abstract

This study was conducted to determine the effect of initial handling on the quality of shrimps during and after catch by the artisanal fishermen in Kurawa on the north coast of Kenya. Fishermen collected shrimps from salt ponds and carried them using both insulated and bucket containers. A portion of samples was preserved with ice immediately after catch (control), while the rest were carried in buckets to the landing site and a portion preserved immediately upon arrival (0-hr). The remaining samples were sub-sampled and preserved with ice after 2 hours (2-hr), 4 hours (4-hr) and 6 hours (6-hr), respectively. Biochemical parameters (Total Volatile Bases - Nitrogen, Trimethylamine-Nitrogen, Peroxide Value, p-Anisidine Value) and proximate composition parameters (dry matter, protein, fat and ash content) were used to monitor quality changes of samples with delayed icing. Results showed a significant increase ($p < 0.05$) in biochemical parameter values over time. This indicated progressive deterioration in quality of shrimp with increased delay in icing. Samples preserved immediately after catch (control) exhibited best quality, followed by 0-hr, 2-hr, 4-hr and 6-hr samples, respectively. In general, increased delay in icing caused significant deterioration of shrimp quality although most samples remained within acceptable standard quality limits for the 6-hr delayed icing period.

Keywords: Shrimps, artisanal, handling, quality, icing, Kurawa

Introduction

Decapod crustaceans including penaeid shrimps are economically important marine resources in the world with increasing demand in both domestic and export markets (Sawant *et al.*, 2012). Shrimps are highly susceptible to post harvest spoilage and therefore require handling at low temperatures (Chatzikyriakidou and Katsanidis, 2011). It has been established that initial handling activities such as delayed icing, temperature variation during handling and mode of packaging have contributed majorly to quality loss of captured shrimps (Tsironi *et al.*, 2009; Ali *et al.*, 2013; Imran *et al.*, 2013). Proper handling to ensure maintenance of shrimp quality is therefore essential from an economic point of view as well as for human health

considerations. In most cases, artisanal fishers fail to adhere to proper handling practices due to a lack of proper fish handling facilities at the landing sites. This, coupled with negligence, results in serious shrimp quality loss. In Bangladesh, Nowsad (2010) reported serious post-harvest losses in the fisheries sector on an annual basis due to ignorance and negligence in handling and processing of fish products at different stages of the supply chain.

In Kenya, shrimp fishing takes place in Malindi-Ungwana Bay where artisanal and commercial/semi industrial bottom trawl fisheries are conducted (Fulanda *et al.*, 2011; Munga *et al.*, 2013). The artisanal fishers mostly utilize the shallow region of the bay

while the commercial fishers fish the deeper parts (>3 nautical miles offshore). The penaied shrimp species exploited in Malindi-Ungwana Bay are mainly *Fenneropenaeus indicus*, *Penaeus semisulcatus*, *Penaeus monodon*, *Penaeus japonicus* and *Metapenaeus monoceros* (Munga *et al.*, 2013). A preliminary study on the status of the artisanal shrimp fishery in the Malindi-Ungwana Bay (KMFRI, 2014) reported the existence of poor handling by artisanal fishermen and lack of adequate handling facilities. However, the extent of quality loss was not yet established quantitatively.

Biochemical quality parameters have been used to quantify the extent of quality losses in seafood products, and standard acceptable limits have been established (Lannelongue *et al.* 1982; Bono and Badaluco, 2012; Okpala *et al.*, 2014; Codex Alimentarius Commission, 2015). This includes the use of Total Volatile Bases-Nitrogen, Trimethylamine-Nitrogen, Peroxide values, *para*-Anisidine values and Thiobarbituric acid parameters as indicators of quality loss. Total Volatile Bases Nitrogen (TVB-N) in particular, has been reported (Malle and Poumeyrol, 1989) to be one of the most widely used methods to estimate the degree of quality deterioration in fish, cephalopods such as squid, and crustaceans. TVB-N includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile nitrogenous compounds associated with seafood spoilage. Trimethylamine (TMA-N) provides an accurate indication of bacterial spoilage in some species with its presence being due to the bacterial reduction of trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species (Malle and Poumeyrol, 1989). Peroxide (PV) and p-anisidine value (p-AV) on the other hand are standard oxidative quality parameters based on measurements of oil primary oxidation products (peroxides) and secondary oxidation products (aldehydes), respectively (Kasmiran *et al.*, 2016). Oil oxidation is an undesirable series of chemical reactions in which oxygen is added, or hydrogen/electrons are withdrawn, that degrades the quality of an oil giving rise to flavour quality loss, often referred to as rancidity (Kasmiran *et al.,* 2016). According to the FAO/WHO Codex Alimentarius Commission (2015), quality standard acceptability limits for PV is set at 5 meqO$_2$/kg while that of p-AV is set at ≤ 20 meqO$_2$/kg. Any value above the set standard is considered undesirable. Proximate composition determination is also required as it is also affected as quality deteriorates, especially due to deamination of amino-acids and nucleotide catabolites. Poor handling (lack of immediate icing) in the study area was likely to enhance the processes of quality deterioration and compromise the quality of shrimps. This study was undertaken to quantify the effect of delayed icing on shrimp quality after catch to establish the extent of quality loss for purposes of effective management during initial handling.

Materials and Methods

This study was conducted at Kurawa landing site on the north coast of Kenya in Malindi-Ungwana Bay. The study site lies between latitudes 2°30′–3°30′S and longitudes 40°00′– 41°00′E. Kurawa is located in the vicinity of salt ponds that were constructed for salt production, and fishermen harvest shrimp from the ponds. Water is introduced to the ponds using water pumps. Fishermen utilize the ponds situated closest to the water inlet where the brine is less concentrated. Since the ponds restrict the movement of shrimps to the sea, shrimp fishing in this area continues throughout the year with variations in catches experienced during the northeast and southeast monsoon periods. Seine nets are the dominant gear used for shrimps harvesting. However, some fishermen deploy their nets in the pump inlet area to trap the shrimps that are sucked through the pump together with water during pumping periods (twice a day during high tides). According to fishermen in Kurawa, spring tides provide better shrimp catches than neap tides. Use of ice is rarely practiced here, except for by the shrimp dealers who bring small quantities in non-insulated containers and gunny bags. Motorcycles are the primary means of transport for the shrimp dealers who mainly transport the product to Malindi town for sale.

Sampling design

Completely randomised design was adopted for this sampling. Six identified fishermen collected samples from the various fishing grounds within Kurawa salt ponds, using both insulated containers (for immediate preservation at the point of catch using ice) and bucket containers (for carrying the remaining sample to the landing site for preservation). The samples from the six fishermen were used as replicates. The sample immediately preserved in ice in an insulated container was considered as the control. The samples carried in buckets to the landing site by each fisherman were placed on separate mats and each immediately sub-sampled and preserved in ice using an insulated container (0-hr sample). The rest

of the samples were sub sampled every 2 hours for a period of six hours (2-hr, 4-hr and 6-hr samples), respectively. Samples that were iced immediately at the point of catch before landing were labelled as Control, while those sub-sampled immediately after landing were labelled as 0-hr. Those sampled and iced 2, 4, and 6 hours after landing were labelled as 2-hr, 4-hr and 6-hr samples, respectively. All the preserved samples were thereafter transported using insulated containers to Kenya Marine and Fisheries Research Institute (KMFRI) in Mombasa for laboratory analysis.

Sample treatment

In the laboratory, a 250 gm sub-sample was taken from each of the 0-hr, 2-hr, 4-hr, and control samples for quality evaluation. The samples were homogenised using a mincer and subjected to laboratory biochemical (TVB-N, TMA-N, PV) and proximate composition (% Dry matter, % protein, % fat, and % ash) analysis. The analyses were independently conducted for samples of each fisherman (six replicates), and results recorded appropriately. A total of 36 samples were analysed in triplicates.

Laboratory analysis of biochemical parameters
Total volatile basic-nitrogen (TVB-N)

A sample of approximately 10 g of ground shrimps was homogenized with 10 ml of 20% Trichloroacetic acid (TCA) (w/v) using a magnetic stirrer. The homogenized sample was then cooled for 5 minutes and the TVB-N determined according to the standard methods of analysis adopted from Siang and Kim (1992) using a Conway's Micro diffusion Unit. The TVB-N was calculated using the following formula:

$$\text{TVB-N (mg/100 g samples)} = (14 \times N \times (X-y) \times 100)/ \text{(weight of sample (g))}$$

Where:
N = Normality of the H_2SO_3
X = Volume (ml) of H_2SO_4 required for titration
y = Volume (ml) of H_2SO_4 required for blank

Trimethylamine-Nitrogen (TMA-N) analysis

Trimethylamine in shrimp samples were determined by the Conway technique (Siang and Kim, 1992), which is the same as the TVB-N determination except that prior to addition of potassium carbonate (K_2CO_3), 1 ml of 10% neutralized formalin was pipetted into the extract to react with ammonia, and thus allow only the TMA-N to diffuse over the unit. The TMA-N was calculated using the following formula:

$$\text{TMA-N (mg/100 g samples)} = (14 \times N \times (X-y) \times 100 \times 100)/ \text{(weight of sample (g))}$$

Where:
N = Normality of the H_2SO_3
X = Volume (ml) of H_2SO_4 required for titration
y = Volume (ml) of H_2SO_4 required for blank

Peroxide Value (PV) analysis

Crude fat was extracted according to the Bligh and Dyer (1959) method and determination of PV was done according to Kirk and Sawyer (1991), with some modifications. Approximately 0.3 g of whole shrimp oil was dissolved in 10 ml chloroform and 15 ml acetic acid. The homogenate was shaken vigorously for 30 seconds and drops (1 ml) of fresh saturated aqueous potassium iodide solution added to the mixture and allowed to stand in the dark for 5 minutes. Approximately 75 ml of distilled water was added to the mixture to release the iodine from solution and titrated with 0.01 M sodium thiosulfate solution against a blank. The PV was then calculated as follows;

$$\text{Peroxide Value (meq } O_2/\text{kg)} = (10 \times \text{volume of titrant (ml)})/\text{(weight of oil in kg)}$$

p-Anisidine Value (p-AV) analysis

The Anisidine value was determined according to the American Oil Chemists Society (AOCS) official methods (1997). The reaction produces a solution that is measured through spectrometric methods at 350 nm. A 0.3 g of extracted oil sample in a 10 ml volumetric flask was diluted to volume with isooctane. The absorbance (As1) of the solution was measured at 350 nm wavelength using a spectrophotometer with solvent as reference (Ab2). Approximately 2.5 ml of the oil solution was pipetted into the first test tube and another 2.5 ml of solvent into the second test tube. Approximately 0.5 ml of p-Anisidine reagent was added to each and shaken. After 10 minutes, the absorbance of the sample (As2) was taken at 350 nm using the solution of the second test tube as reference (Ab2). The value was expressed as;

$$\text{p-AV meq O2/kg} = (10 \times (1.2 \times As_2 - Ab_2) - (As_1 - Ab_1))/M$$

Where:
As_1 and As_2 = first and second measure of absorbance of samples
(As_1 = samples with no p-Anidine reagent, As_2 = samples solution + p-Anisidine reagent)
Ab_1 and Ab_2 = first and second measure of absorbance of the blank

(Ab_1= solvent with no p-Anisidine reagent, Ab_2= solvent +p-Anisidine)
M = weight of the sample (g)

Determination of Proximate Composition

Crude protein analysis

Crude protein content in shrimp meat was determined based on the Kjeldahl method (AOAC, 1990). A sample of 5 g was digested in sulphuric acid in the presence of copper sulphate as a catalyst. Thereafter, the sample was placed in the distillation unit (2400 Kjeltec Auto Sample System). The acid solution was made alkaline by adding sodium hydroxide solution. The ammonia was steam distilled into boric acid with indicators. The boric acid was then simultaneously titrated with 0.02 M H_2SO_4.

Calculation of % N = ((A-B) x N x 1.4007)/ (w)

Where:
A and B are volume (ml) of sample titrant and Blank titrant respectively
N is the normality of the titrant
W is the weight of sample (g)

Crude fat content analysis

Crude fat content in shrimp flesh was determined by the AOCS (1997) official method of analysis. The sample was extracted with petroleum ether, with a boiling range of between 40-60° C. The extract was recovered using a rotary evaporator. The extract was dried, weighed and the fat content calculated as follows:

% crude fat content = ((weight of fat + container) - (weight of container)/ (weight of sample (g)) x100

Dry Matter analysis

Dry matter was calculated by analysing moisture content according to AOCS (1997) official method of analysis, after which the moisture (%) content was subtracted from 100%. 5 g of crushed whole shrimps was dried for 24 hours on a pre-weighed aluminium foil in an oven at 105° C and cooled in a desiccator to room temperature. The weight was then taken and recorded accordingly. Calculation of the moisture content was carried out as follows:

Moisture content (%wet basis) = (Initial weight-final weight)/(initial weight) x 100
Dry matter was calculated as follows:
Dry matter (%) = 100% - % moisture content

Ash content analysis

Ash content was determined according to the AOCS (1997) official method of analysis. A shrimp sample weighing 5 g was dried for 24 hours in an oven at 105° C and cooled in a desiccator to room temperature, weighed and recorded accordingly. The samples were put in a micro furnace at 450° C for six hours on pre-weighed aluminium foil to ash. The ash content was calculated as follows:

% Ash content = (initial weight before ash-final weight after ash)/(initial weight before ash) x100

Data Analysis

Data were analysed using MINITAB® 14 statistical software. All data was tested for normality using the method of Shapiro Wilk (1965) before being subjected to one way analysis of variance (ANOVA). Where differences were noted, tests for significance differences in means were conducted using Turkey's pairwise comparison analysis. All tests were considered significant at a confidence level of 95% (α = 0.05).

Results

Changes in proximate composition of shrimps during initial handling

The results of proximate composition were as indicated in Table 1 below.

The results show an increase in percentage of dry matter as delay in icing increased. However, only the 6-hr delayed icing samples were significantly different from the rest. The Control sample (shrimp iced immediately after catch) gave the lowest value (20.10 ± 0.70%) while the 6-hr sample gave the highest value (27.90 ± 1.07%). A pairwise comparison test did not show any significance differences between the control, 0-hr, 2-hr and 4-hr samples for dry matter content. For protein content (%), the 4-hr and 6−hr samples differed significantly (p < 0.05) from the control, 0-hr and 2-hr samples. Samples for both fat (%) and ash (%) content did not show significance difference (p > 0.05) for the 6-hr delayed icing period. The increase in dry matter with time during delayed icing was mainly attributed to decrease in moisture content as a result of drying through evaporation when preservation was delayed. However, even though an increase in the dry matter (%) was detected, a significant difference was only apparent in the sample with a delayed icing period of 6 hours.

Table 1. Turkey's pairwise comparison on mean shrimp proximate composition during delayed icing at Kurawa sampling station.

	Control	0-hr	2-hr	4-hr	6-hr
Dry matter	20.10±0.70[a]	22.86±0.83[a]	23.33±0.90[a]	23.99±1.06[a]	27.90±1.07[b]
Protein	12.04±0.28[a]	12.04±0.28[a]	12.95±0.12[a]	11.55±0.24[b]	14.59±0.25[c]
Fat	1.22±0.05[a]	1.28±0.16[a]	1.43±0.07[a]	1.43±0.10[a]	1.42±0.12[a]
Ash	2.85±0.29[a]	3.02±0.16[a]	3.41±0.35[a]	3.43±0.23[a]	3.85±0.20[a]

N = 6. Different letters (superscripts) in the same row indicates significance difference ($p < 0.05$). Values are given as mean ± standard error (SE). Units of the parameters are given in percentages.

Changes in biochemical quality parameters during initial handling

During the delayed icing period, there were significant changes in TVB-N, TMA, PV and the p-AV with all the biochemical parameters increasing with time (Table 2).

Changes in TVB-N during the 6 hours delayed icing period ranged from 1.84 ± 0.30 mg to a maximum of 16.04 ± 0.78 mg N/100 g. The results showed a consistent increase in the level of TVB-N values as delay in icing period increased. However, the values were still within the edible acceptability limit (≤ 20 mg N/100 g) despite the significant loss in quality as icing was delayed.

Changes in TMA-N followed the same pattern as those of TVB-N since TMA-N forms part of the TVB-N. The TMA-N results were 1.58 ± 0.25 mg N/100 g for the control and 4.38 ± 0.25 mg N/100 g for the 6 hours delayed icing samples respectively. Taking into consideration that the limit of acceptability for TMA-N

was considered at 5 mg N/100 g (Bono and Badaluco, 2012), the quality loss within the 6 hours delay in icing did not surpass the limit of acceptability. However, there was appreciable loss in the quality of shrimps due to delay in icing.

PV increased significantly with delayed icing period. Values ranging from 4.03 ± 0.30 meq O2/kg for the control to a maximum of 16.25 ± 0.90 meq O2/kg for the 6-hr sample were observed. Turkey's pairwise analysis showed that the differences were mainly attributed by the control and 0-hr samples only. Otherwise, the 2-hr, 4-hr and 6-hr samples were not significantly different. Consumer acceptability of PV value in fish was given as 0-2 mmol/kg as very good, 2-5 mmol/kg as good, 5-8 mmol/kg as acceptable and 8-10 mmol/kg as spoilt (Okpala *et al.*, 2014) Conversion of these values to meq O2/kg gives values of 0-4 meq O2/kg as very good, 4-10 meq O2/kg as good, 10-16 meq O2/kg as acceptable and 16 - 20 meq O2/kg as spoilt. Going by the results obtained in this study,

Table 2. Turkey's pairwise comparison on mean biochemical quality parameters during delayed icing at Kurawa sampling station.

	Control	0-hr	2-hr	4-hr	6-hr	Standard limits of acceptability
TVB-N	1.84±0.30[a]	5.34±0.55[b]	8.60±0.43[c]	13.16±0.83[d]	16.04±0.78[e]	< 25 mg N/100g (Bono and Badaluco, 2012)
TMA-N	1.58±0.25[a]	2.00±0.28[a]	3.02±0.56[b]	3.35±0.28[b]	4.38±0.25[b]	5 mg N/100 g (Bono and Badaluco, 2012)
PV	4.03±0.30[a]	8.85±0.59[b]	13.00±0.78[c]	14.04±1.20[c]	16.25±0.90[c]	10-16 meq O_2/Kg (Okpala *et al.*, 2014)
p-AV	4.32±0.44[a]	2.88±0.10[a]	11.62±0.35[b]	28.37±0.56[c]	50.85±0.83[d]	≤ 20 meq O_2/Kg (Codex Alimentarius commission, 2015)

N = 6. Different letters in the same row (superscripts) indicates significance difference ($p < 0.05$). Values are given as means ± standard error (SE). TVB-N and TMA-N are given in mg%. PV and p-AV are given in meq O_2/kg.

all sample were still within the acceptable range, though delayed icing for six hours pushed the shrimp quality almost to the spoilt end of the spectrum.

Para-anisidine values showed a significant increase with delayed icing period. There was significant differences in the 2-hr, 4-hr and 6-hr samples. However, the control and 0-hr samples did not exhibit any significant difference. The Codex Allimentarius Commission (2001) sets the limit of acceptability for p-Anisidine values at < 20 meq O2/kg. Results showed that the 4 hours delayed icing period pushed the quality indicator value beyond this limit of acceptability.

Discussion
Changes in proximate composition of shrimps during initial handling
Proximate composition of the samples showed insignificant changes in fat and ash content throughout the delayed icing period. Dry matter and protein changed significantly after four and six hours delayed icing respectively. Shrimp protein content (%) of between 12.04 and 14.59 indicates a good protein source similar to those reported by Sephan and Benjakul (2012). The deamination of amino-acids and nucleotide catabolites by bacterial activity did not affect protein composition significantly as it was seen to remain stable throughout the six hour delayed icing period. Equally, no major effect was noted in the fat and ash content indicating no or little effect on the quantity of the two parameters with a delay in icing.

Changes in biochemical quality parameters during initial handling
Total Volatile Bases-Nitrogen (TVB-N)
TVB-N is one of the most widely used methods to estimate the degree of decomposition of volatile nitrogenous compounds in seafood spoilage (Malle and Poumeyrol, 1989; Riquixo, 1998). TVB-N occurs due to continuous production of volatile bases caused by a breakdown of proteins through the action of microbes (Babu *et al.*, 2005). TVB-N scale of acceptability has been reported to range from <12 mg N/100 g for fresh raw shrimps, 12 – 20 mg N/100 g for edible but lightly decomposed, 20 – 25 mg N/100 g for borderline and > 25 mg N/100 g for inedible and decomposed (Lannelongue *et al.*, 1982; Bono and Badaluco, 2012; Okpala *et al.*, 2014). Several reports (Boee *et al.*, 1992; Mahmud *et al.*, 2007) observed an even increase in TVB-N during shrimp storage, indicating continued decomposition leading to quality loss. Similarly, in this study, there was a continuous significant

increase in TVB-N values with the control sample giving the least TVB-N value and the 6-hr sample giving the highest value. These changes were attributed to the delay in icing of shrimp during initial handling. However, as much as TMA-N values increased with a delay in icing, they remained within standard limits of acceptability (< 25mgN/100g) for the six hour period.

Trimethylamine –Nitrogen (TMA-N)
TMA-N is formed in spoiling fish by the action of certain species of bacteria on the substance trimethylamine oxide (TMAO). Determination of TMA content is a measure of bacterial activity and spoilage (Aitken et al. 1982). TMAO is said to be an important compound for maintenance of physiological functions in fish and shellfish and also a key substance in the spoilage of raw or processed seafood (Norman and Benjamin, 2000). In this study TMA-N values ranged between 1.58 mg N/100 g and 4.38 mg N/100 g for the control and 6-hr sample, respectively. Similar results were reported in other studies (Mahmud *et al.*, 2007; Okpala *et al.*, 2014). The increase in these values were mainly attributed to a delay in icing. However, the standard limit of acceptability for TMA-N (5mgN/100g) was not surpassed within the six hour delay in icing. The shrimps were therefore still edible despite the delay before icing.

Peroxide Value (PV)
Lipids contain high levels of polyunsaturated fatty acids (PUFA) which are highly susceptible to oxidation. According to Boran *et al.* (2006), the degree and rate of lipid oxidation is influenced by the composition of fatty acids, oxygen concentration, temperature, surface area and water activity. In this study, significant change in PV with increased delay in icing period was observed similar to those observed by Nirmal and Benjakul (2009) as well as Chaijan (2011). This was attributed mainly to a delay in icing favouring the oxidation process. The increasing PV value observed from the sample preserved immediately after catch to the 6-hr sample indicates decreasing quality with time. Taking in consideration that the standard limit of acceptability has been set at 10-16 meq O_2/kg (Okpala *et al.*, 2014), the six hours delay in icing lowered its quality to the borderline limit of acceptability. This indicates that rancidity increased with delay in icing.

Anisidine Value (p-AV)
Typically, quantification of primary lipid oxidation products (peroxides) is done using peroxide value (Chaijan, 2011). Alternatively, the increase in concentration of hydro-peroxides results in a wide variety

of secondary oxidation products such as aldehydes (Sephan and Benjakul, 2012) measured using Anisidine Value. In this study, there was a significant increase in Anisidine value with the highest value being observed in the 6-hr sample, while control gave the lowest value. Similar observations with storage time was reported by Okpala *et al*. (2014) who associated this with the generation of secondary lipid products, largely non-volatile compounds (aldehydes). The increase in secondary products indicates continued lipid oxidation leading to rancidity. In relation to the control sample, delayed icing enhanced lipid oxidation leading to the observed increase in values of p-Anisidine. With the limits of acceptability being ≤ 20 meqO2/kg (Codex Alimentarius Commission, 2015), the delay in icing of shrimp beyond 2 hours resulted in quality loss beyond the acceptability limit.

In general, delayed icing during initial handling by artisanal fishermen had a major effect on the quality of penaeid shrimps, while icing at the point of catch gave the best quality. The period at which biochemical quality parameters remained within acceptable ranges for all parameters tested was 2 hours. However, TVB-N, TMA-N and PV were within limits of acceptability up to the six hour period. It is recommended that delay in icing of shrimps for more than 4 hours after landing be discouraged and that further research on other quality factors such as microbial load and melanosis should be conducted to provide more information on the general quality attributes of shrimps during initial handling. At the same time, sensitization of fishers on the extent of spoilage due to delay in icing at the initial handling stage should be continually carried out to ensure maintenance of shrimp quality in the small-scale shrimp fishery.

Acknowledgements

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