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UTILIZATION OF SODIUM METABISULPHITE FOR PRESERVATION OF FROZEN-THAWED SHRIMP (*PANDALEUS BOREALIS*)

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ABSTRACT

Whole frozen-thawed shrimp (*Pandaleus borealis K.*) was dipped into 1.25% of sodium metabisulphite solution and stored in ice and ice slurry for 7 days at 2-3°C. The effect of sodium metabisulphite on melanosis development was assayed by sensory analyses and bacterial counts. Spoilage bacterial growth, pH, TVB-N, TMA and sulphite were monitored for comparison. Sodium metabisulphite showed strong properties of delaying melanosis development and its antimicrobial effect was demonstrated when the shrimp was held in ice. However no effect on microbial reduction was observed when sodium metabisulphite treated shrimp was kept in ice slurry.

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1 INTRODUCTION

The export of shrimp is an integral part of Mozambique seafood industry. In recent years the contribution made by fisheries products to the trade balance has been about 40%, of this shrimp exports contribute over 90%. Most of the exported shrimp is frozen on board. Shrimp for frozen on shore come from small artisanal and semi-industrial boats. These boats fish far, one to five days, some without appropriate refrigeration. The result is a high level of post-harvest losses and loss of quality of the raw material used in land plants. Mozambican fisheries policy supports the growth of land plants, improvement of the quality of their products and reduction of post-harvest losses. The usual method of preserving catches of shrimp, in Mozambique, between the time the shrimp is caught and it is landed and sold, is to store the catch in crushed ice. Shrimp is highly perishable and the maintenance of quality depends upon number of factors, including storage time, storage conditions and temperature. Therefore training personnel in shelf life assessment of shrimp and studies on alternative methods to prolong the shelf life are important.

Sulphiting agents in fisheries products are forbidden in several countries. In others limits enforced on residual sulphites are due to a sulphur sensitive group of people. There is no evident risk for the general population when sulphites are used at permitted amounts. Sulphiting agents are used in the Mozambique shrimp industry and its use is also reported in countries like Australia (Smith, 1980 and Slattery *et al*. 1990). India (Chakrabarti *et al*. 1992) and Indonesia (Suparno and Mulyanah, 1991).

The product affected by blackspot is not unsafe or unfit because melanosis does not affect the eating quality. Bacteria do not cause the melanin production that results in blackspot. It is due to a natural and irreversible enzymatic process. Studies on Norway lobster (Yan *et al*. 1989) suggest that the blackspot development is result of oxidation of tyrosine or its derivates to melanin. Consumers tend to reject a product when undesirable sensory characteristics are present.

There are several alternatives to sulphite, but according to Smith (1980) sulphite is the cheapest and easiest quite and effective method to prevent the occurrence of melanosis. Six types of sulphites are commonly used, of those sodium metabisulphite is the most used in the Mozambican shrimp industry.

Sodium metabisulphite has been considered as an antimicrobial food additive (Lueck, 1980) and Suparno and Mulyanah (1991) concluded that it has a reduction effect on total bacterial and H_2S producing bacteria growth. Pyle and Koburger (1984) reported that microbial numbers were reduced immediately by as much as 50% after dipping shrimp in a sodium bisulphite solution.

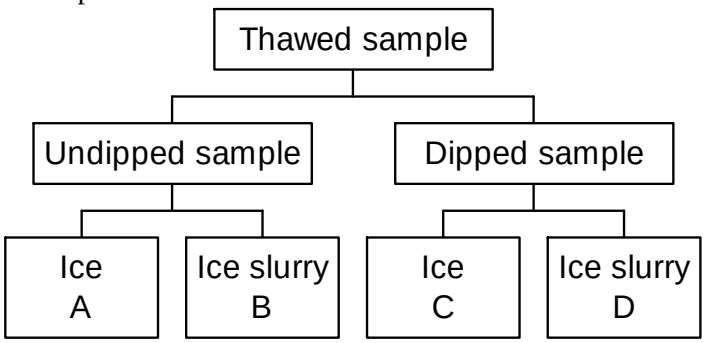
Oxygen plays an important role in melanosis development. Smith (1980) reported that prawn held in refrigerated slurry takes about 3 days to develop melanosis. Ogawa *et al.* (1983) stated that the absence of air-oxygen retarded blackening. Several other studies showed that ice slurry is more effective than crushed ice in inhibiting blackspot formation due to lack of oxygen (Krishnasany, 1995).

The aim of this study was to assess and compare the changes in sensory quality of shrimp preserved with sodium metabisulphite, and stored in ice or ice slurry. Microbiological changes, total volatile base nitrogen (TVB-N), trimethylamine (TMA) and sulphite residue were monitored for comparison.

2 MATERIALS AND METHODS

2.1 Sample preparation

The trial was carried out using thawed-frozen shrimp (*Pandaleus borealis K.*). The shrimp used was from one batch, caught in the North East of Iceland during the period 1st-7th October 1998. On board the shrimp were individually quick frozen and packed into large sacks. It was stored at –24°C until used for the trial in the 3rd week of January 1999. In the laboratory part of the sample, 5 kg was kept frozen at –18°C for control. Another part was thawed overnight at 5°C. The thawed sample was divided into groups, 8 kg each. One group was dipped into 1.25% sodium metabisulphite solution (1.5 kg/100 litres) for 1 minute with moderate agitation followed by draining. The shrimp were then split into two groups, group C kept iced (at an approximate ratio of 2 parts ice to 1 part shrimp) and D was kept in ice slurry (ice and salt water with strength of seawater, 3:1). The other eight kilos were kept untreaded in two ways. A held on ice and B in ice slurry. The samples were re-iced as necessary during storage. All boxes were housed in a refrigerated room at 2° to 3°C to slow ice melting. At 0, 3, 5 and 7 days after thawing, samples were taken randomly from all areas of the box for microbiological, chemical and sensory analyses. Analyses were done in samples of whole shrimp.



2.2 Sensory analyses

Sensory assessment of the whole shrimp freshness and the blackspot formation were done using a Quality Index Method (QIM) where a rating scale (0 to 3) based on an external examination of different attributes of whole shrimp, such as darkening of head (extent of melanosis), colour of the roe, odour, and colour of whole shrimp (Appendix 1). Higher scores represent a gradual loss of quality associated with deterioration. Twelve assessors from the Icelandic Fisheries Laboratories (IFL) panellist group participated in each panel section.

2.3 Microbiological analyses

The number of H_2S-producers organisms and total bacteria count were determined by the spread (surface) plate method using iron agar (Gram *et al.* 1987). Iron agar was prepared as follows: 20.0 g peptone (Difco); 3.0 g Lab lemco powder; 3.0 g yeast extract (Difco); 0.3 g ferric citrate; 0.3 g sodium thiosulphate; 10 g NaCl; 15 g agar; for each 1000 ml of distilled water. The pH was adjusted to 7.4 with 2 N NaOH and the iron agar sterilized at 121°C for 15 minutes. Just before being poured on to the plate 10 ml of 4% filter sterile L-cysteine was added to the melted agar. Samples for microbiological analyses were prepared by homogenising 25 g of minced shrimp in a stomacher bag with 225g of Butterfield´s buffer (Vanderzant and Splittstoesser, 1992) using a stomacher blender (Seward Stomacher 400 Lab system) for 60 seconds. The samples were further diluted decimally as needed and spread uniformly onto surface of predried iron agar plates. The spread plates were prepared in duplicate and incubated at 15°C for 5 days. At the end of incubation period, H_2S-producers

(black colonies on iron agar) and white colonies were counted separately with a colony plate counter and the number of colony-forming units (CFU) per gram were calculated and data were recorded. Total bacteria count were performed using the spiral plate method on plate count agar (Standard Agar, Difco) with 0.5% NaCl (w/v) added as outlined in the "Compendium of Methods for the Microbiological Examination of Food" (Vanderzant and Splittstoesser, 1992). A known volume of sample was dispensed onto a rotating agar plate in an Archimedes spiral. All plates were incubated at 15°C for 5 days. At the end of the incubation period, PCA plates containing 25-250 colonies were counted using Laser Colony counter (Model 500A) with CASBA data processor (Model 800E) (Donnelly *et al*. 1976, Fung and Goetsch, 1991 and Manninen *et al*. 1991) (Spiral System Instruments, Bethesda, MD) and the data recorded.

2.4 pH

On each sampling day the pH of homogenized samples was measured using a calibrated glass electrode pH meter. The pH meter was calibrated using the buffer solution of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/s, Bagsvaerd Denmark PHM 80)

2.5 Chemical analyses

2.5.1 Total volatile bases -nitrogen (TVB-N)

Total volatile bases of nitrogen (mgN/100g) was estimated in trichloroacetic acid (TCA) extracts of whole minced shrimp, by steam distillation method using Struers-type distillatory (Malle and Tao, 1987). Twenty-five ml of filtrate with 10 ml of 10% NaOH were distilled in 10 ml of 4% aqueous boric acid solution.

2.5.2 Trimethylamine (TMA)

To assay TMA (mgN/100g) in TCA extract, the steam distillation method was used (Malle and Tao, 1987). Twenty ml of 35% formaldehyde was added to the distillation tube to block the primary and secondary amines.

2.5.3 Sulphite

Extracts of whole shrimp were analyzed using the enzymatic method, also known as UV-method, for sulphite determination according to procedures described by Edberg (1993). Sampling for sulphite determination was done only for groups C and D, on days 3 and 5 of storage.

2.6 Statistical analyses

Microbiological results were compared using the log 10 transformations of the bacterial counts. A paired students' t-test (two-tailed distribution) was carried out to compare total bacterial count, iron agar vs. plate count agar.

3 RESULTS AND DISCUSSION

3.1 Sensory analyses

During storage days sensory results showed that untreated shrimp (A and B) had a high occurrence of melanosis, especially the sample kept only with ice (A) (Fig. 1). Over 75% of the heads were dark, the colour of the body was green-yellowish and roes were discoloured. Treated shrimp (C and D) had developed slight melanosis for almost all storage period and the sample kept in slurry (D) had the lowest scores (0-1). It was quite clear that $Na_2S_2O_5$ had a strong effect on delaying melanosis development on shrimp. Previous studies (Suparno and Mulyanah, 1991; Yamagata and Low, 1995) confirm that $Na_2S_2O_5$ treated shrimp stored in ice has an extended shelf life compared to untreated shrimp stored in ice. When comparing iced shrimp and shrimp in ice slurry the results showed slight difference between them, but shrimp in ice slurry was of better quality. According to Chinivasagam *et al*. (1996) ice slurry gives better protection against melanosis development than ice because of the lack of oxygen in ice slurry.

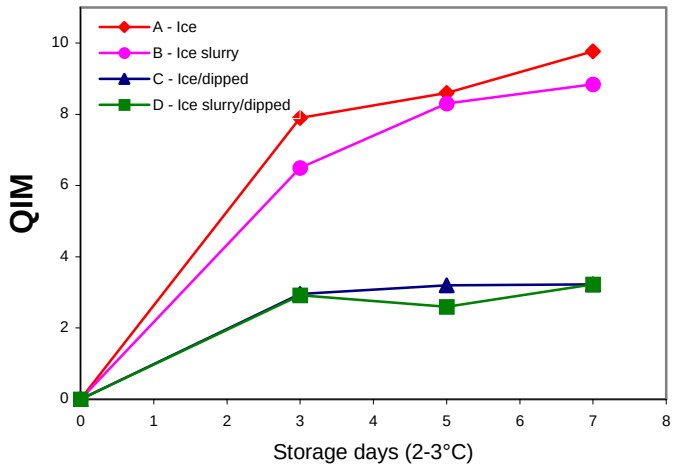


Figure 1: Sensory evaluation during storage of frozen-thawed shrimp dipped with sodium metabisulphite ($Na_2S_2O_5$) and undipped, kept in ice and ice slurry.

In untreated groups (A and B), ammonia odour (scores 2-3) developed on the 3rd day, whereas in treated groups (C and D) no ammonia odour was detected (scores 0-1). At the end of the trial period perceivable ammonia odour had developed (scores 3) in undipped groups (A and B) while in the group (C) kept in ice a week ammonia odour was reported (scores 1-2) and in D ammonia odour did not developed (scores 0-1). It might be due to type of spoilage flora growing. Chinivasagam *et al*. (1996) reported *Shewanella putrefaciens* growth in ice slurry and the sulphur odour could not be detected until close to the point where the prawn was rejected. The ammonia odour detected in untreated groups might be the result of enzymatic reaction since total bacterial count and number of H_2S producing bacteria was lower in these groups (Fig. 1). Finne (1982) studied the enzymatic ammonia production in Penaied shrimp held in ice. In his study, Finne showed that there is some increase of ammonia production with storage time in sterile shrimp muscle. On the other hand the difference could be explained by the fact that the Quality Index Method was developed for normal shrimp spoilage not bearing in mind shrimp treatment with preservatives that could mask the ammonia odour.

3.2 Microbiological analyses

Results of H_2S-producing bacterial growth are showed in Fig. 2. The number of H_2S producing bacteria had not reached 100.000 (Log.5)/g. (Fig. 2). At rejection time by sensory analyses results, for group A and B, and at the end of the trial for the other groups, There is a gradual increase in the number of H_2S-producing bacteria. This could be explained by the fact that the experiment was done using thawed shrimp. Higher H_2S-producing bacterial growth is found in unfrozen fillets than in thawed and in much lower numbers if fish was kept frozen for longer period (≥ 14 wk) (Magnússon and Marteinsdóttir 1995). The number of H_2S-producing bacteria were higher in the sample kept in ice slurry (B and D) in contrast to the others although A had the typical lag in logarithmic bacterial growth that could be due to mode of storage, e.g. storage in ice and storage in ice slurry made the difference. According to Chinivasagam *et al*. (1996) there is higher growth of H_2S-producing bacteria in ice slurry with time than in ice-stored prawn. Sodium metabisulphite has been considered as an antimicrobial food additive (Lueck, 1980, Suparno and Mulyanah (1991) concluded that it has a reduction effect on total bacterial and H_2S producing bacteria growth. Pyle and Koburger (1984) reported that microbial numbers were reduced immediately by as much as 50% after dipping shrimp in a sodium bisulphite solution. In the present study the combined effect of $Na_2S_2O_5$ and ice slurry did not show a reduction on growth of spoilage organisms while $Na_2S_2O_5$ with ice did.

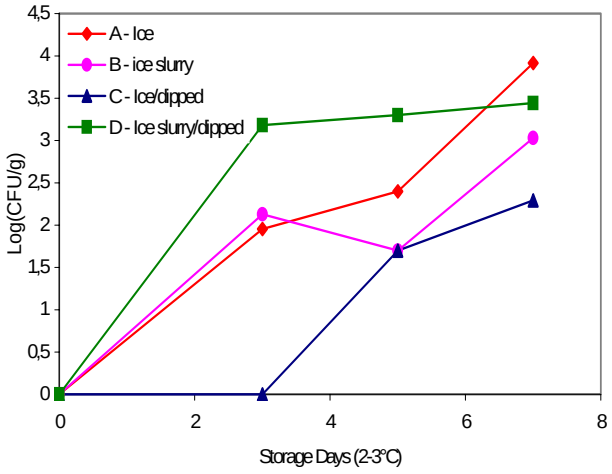


Figure 2: Growth of H_2S producing bacteria on iron agar (count at 15°C) during storage of frozen-thawed shrimp dipped with sodium metabisulphite ($Na_2S_2O_5$) and undipped, kept in ice and ice slurry.

Although total counts (PCA), showed in Fig 3, do not exceed 10^6 CFU/g in any of four groups gradual increase was observed with time. Many investigators have reported bacterial counts of 10^6 to 10^8 g^{-1} or cm^{-1} when sensory spoilage was detected in food (Simpson *et al*., 1997). The low total count observed might be due to the fact the shrimp used for the trial was frozen-thawed shrimp and had been kept frozen for more than fourteen weeks. Neither should it be forgotten that melanosis development is the result of enzymatic action not bacteria activity. In their study of storage quality of fresh and frozen-thawed fish in ice Magnússon and Martinsdóttir (1995) concluded that long-term freezer storage reduced total bacterial growth of cod fillets. B and C are useful as an objective indicator of gross spoilage. Although it is not

recommended that bacterial counts are used as indicators of freshness (Ryder *et al*., 1993) because melting ice could severely affect bacterial loading. In spite of the advanced stage of melanosis in groups A and B the eating quality was not affected. The shrimp was within the recommended parameters for human consumption.

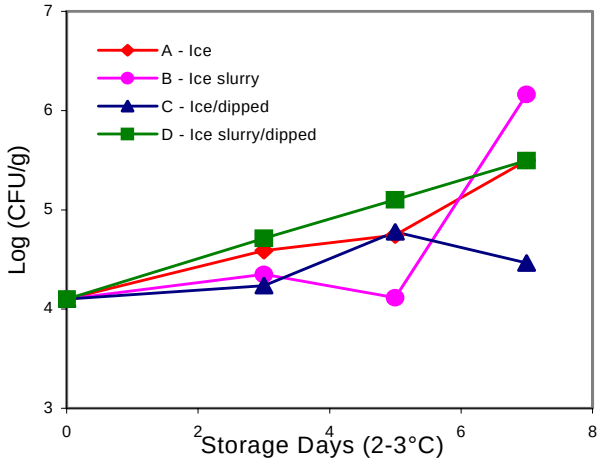


Figure 3: Growth of bacteria on plate count agar with 0.5% NaCl (total count at 15°C) during storage of frozen-thawed shrimp dipped with sodium metabisulphite ($Na_2S_2O_5$) and undipped, kept in ice and ice slurry.

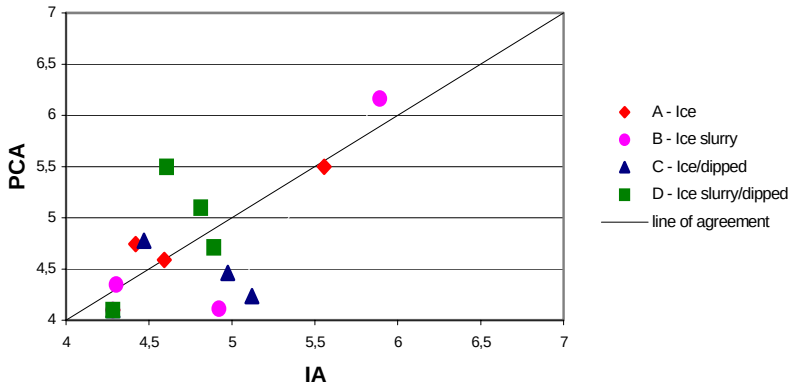


Figure 4: Relationship between total count on iron agar (IA) and plate count agar (PCA) of frozen-thawed shrimp dipped with sodium metabisulphite ($Na_2S_2O_5$) and undipped, kept in ice and ice slurry, during storage days.

Results of total bacterial count on iron agar plate and plate count agar are shown in Appendix 2. There is no significant difference ($P > 0.05$) between total count in the two media (Fig. 4). Furthermore, observations of the results of each treatment group did not reveal any systematic difference in total bacterial count between the two media. Therefore, the results do not indicate that the microflora present in the samples grew better in one media than in other.

3.3 pH

In addition to microbial numbers (TVB-N and TMA levels) pH has been also used as an

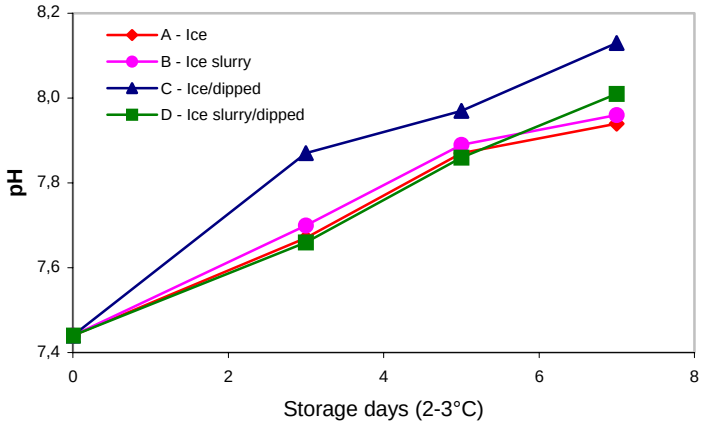


Figure 5: Results of pH measurements during storage days of frozen-thawed shrimp dipped with sodium metabisulphite ($Na_2S_2O_5$) and undipped, kept in ice and ice slurry.

index of shrimp quality. In the present study pH of the samples increased steadily with time for all studied groups (Fig.5). The initial pH was 7.4, but at the end of the trial after 7 days from 7.9 to 8.1. A pH of 7.4 for zero-days of old shrimp (*Peneaus azetecus*) was reported by Flick and Lovell (Flores and Crawford, 1973) and the same authors mentioned a pH of 8.4 after 8 days of ice storage of Pacific shrimp. In the present study the pH does not appear to be affected by the different types of treatment. The pH was slightly higher in the treated sample C, but we could not find an explanation for that difference.

3.4 Chemical analyses

3.4.1 Total volatile basic nitrogen (TVB-N)

All groups showed an increase of TVB-N values in the 3 first days followed by a reduction (Fig. 6) with TVB-N exceeding 30 mgN/100g in all groups on day 3 (Fig. 6). After day 3 the TVB-N values decreased progressively on groups kept in ice slurry (B and D) while in the iced groups the values remained constant. Montgomery *et al*., cited by Hanpongongkittikun *et al*. (1995) considered fishery products with TVB of 30 mgN/100g values or above as unfit for human consumption. Although TVB-N values are high, the microbiological results registered are not high. Possibly the total volatile bases detected were the result of endogenous enzymatic activity. Finne (1982) in his study of enzymatic ammonia production in Penaied shrimp held in ice refers to a demonstration made by Cobb and Vanderzant on a progressive increase in total volatile nitrogen during storage of sterile shrimp extract. The reduction observed on TVB-N values could be explained by the fact that the volatile bases leake evaporated away with the melt water from ice, it is more evident on those groups B and D where samples were in ice slurry whereas ice melted quicker.

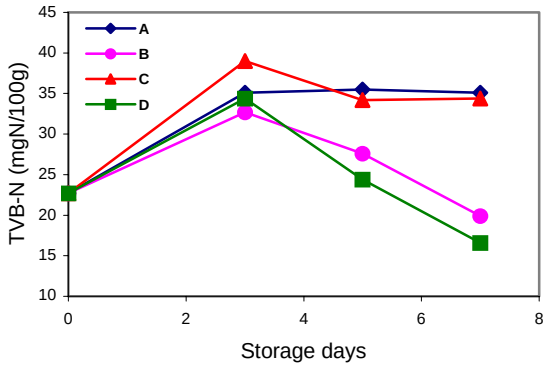


Figure 6: Development of TVB-N during storage of frozen-thawed shrimp dipped with sodium metabisulphite ($Na_2S_2O_5$) and undipped, kept in ice and ice slurry.

3.4.2 Trimethylamine (TMA)

After 3 days of ice storage, TMA in dipped groups rose to 5.7-6.9 mg/100g (sample C and D respectively), while in undipped group TMA had not reached 1 mgN/100g (Fig. 7). No TMA was detected in the group B after initial sampling (thawed sample). Possibly a very low amount of TMA formed and leaked out into the ice slurry and was not detected in the analyses or the method was not accurate enough to detect very low levels of TMA. In the present study higher values of TMA were found in the dipped sample D compared to the others. TMA is well documented as a product of bacterial spoilage (Gram *et al*., 1987 and Gram and Huss, 1996) and the higher values, compared to the others, of total and H_2S-producers bacterial count found in the dipped sample D might be the explanation for this results. We could not find an explanation for slightly raised TMA in the sample B. For groups A and B, as we pointed out in chapter 3.2 and 3.5.1 the low spoilage organisms found in samples from those groups plus dissolution factor of melting ice can be the reason for the very low amount detected.

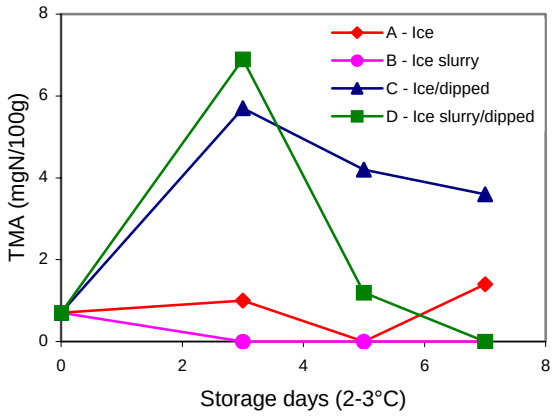


Figure 7: Development of trimethylamine (TMA) during storage of frozen-thawed shrimp dipped with sodium metabisulphite ($Na_2S_2O_5$) and undipped, kept in ice and ice slurry.

In the present study, TMA does not appear to have any value in monitoring the loss of freshness that can occurs in shrimp during ice and ice slurry storage.

3.4.3 Sulphite

The sulphite results are shown in Appendix 2. In groups C and D there is a decrease in sulphite level with storage because sulphite is readily soluble in water and melted water from ice and ice slurry removed most sulphite during storage. Data from Australia reports a loss of over 90% of sulphite residue in 10 days of ice stored prawn (Slattery *et al*. 1990). In this study shrimp kept in slurry (D) lost 35% of it is sulphite while the iced shrimp lost (C) only 8.37%. It would be more appropriate to measure sulphite residue in edible parts of shrimp to compare allowable limits instead of measuring the whole shrimp.

4 CONCLUSIONS

This study has demonstrated the effectiveness of sodium metabisulphite as an agent in delaying melanosis development in shrimp. Its antimicrobial effect was confirmed, but this property was lost in combination with ice slurry. Changes in TVB-B values were likely more due to endogenous enzymatic activity than due to bacterial activity since the numbers of spoilage organisms detected were low.

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APPENDIX 1:

Quality Index Method (QIM-scheme) used for evaluating freshness and blackspot formation of whole shrimp (method developed and used in Icelandic Fisheries Laboratory (IFL)) (Martinsdóttir *et al*., unpublished)

Attribute	Description	Score
Darkening of head	No	0
	Some (25%)	1
	Many (50-75%)	2
	Every (75-100%)	3
Colour	Red-pink	0
	Pink	1
	Yellowish	2
	Green-Yellowish	3
Colour of roe	Cooper, blue-green	0
	Discoloured	1
	Dark	2
Odour	Strong seaweed, marine odour	0
	Weak shrimp odour, reminiscent of tar	1
	Weak ammonia	2
	Distinct ammonia, sour, putrid	3
Quality index		0-11

Source: IFL

APPENDIX 2:

Results from sensory, microbiological and chemical analyses during ice and ice slurry storage of dipped ($Na_2S_2O_5$) and undipped frozen-thawed shrimp.

		Test								
Days	Samples	QIM	PCA total count (log CFU/g)	IA total count(log CFU/g)	Iron Agar (log CFU/G)		pH	TVB-N (mgN/100g)	TMA (mgN/100g)	$S0_2$
Day 0					Black	White				
	Frozen	0								
	Thawed	0	3.89		0			20.3	0.0	
			4.10	4.28	0	4.28	7.4	22.7	0.7	
Days3										
	A	7.96	4.59	4.59	1.95	4.59	7.7	35.1	1	
	B	6.5	4.35	4.30	2.13	4.30	7.7	32.7	0	
	C	2.96	4.24	5.12	0	5.12	7.9	39	5.7	526
	D	2.92	4.71	4.89	3.18	4.88	7.7	34.4	6.9	418
Day 5										
	A	8.6	4.75	4.42	2.39	4.41	7.9	35.5	0	
	B	8.3	4.11	4.92	1.69	4.92	7.9	27.6	0	
	C	3.2	4.78	4.7	1.69	4.47	8.0	34.2	4.2	
	D	2.6	5.10	4.81	3.30	4.79	7.9	24.4	1.2	
Day 7										
	A	9.77	5.49	5.56	3.92	5.54	7.9	35.1	1.4	
	B	8.84	6.16	5.89	3.03	5.89	8.0	19.9	0	
	C	3.23	4.46	4.98	2.29	4.97	8.1	34.4	3.6	341
	D	3.23	5.49	4.6	3.44	4.54	8.0	16.6	0	383