Final Report

BASF Sodium metabisulphite laboratory trial

By

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Materials and method

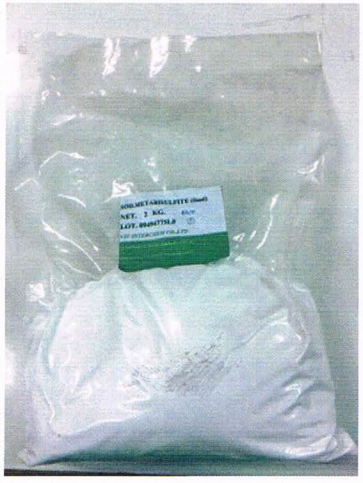
Sample preparation

The shrimp (size 20 gram) used was from a farm in Chantaburi Province, East of Thailand. Live shrimp was transported to Aquaculture Business Research Center, Kasetsart University, Bangkok, Thailand.

Shrimp was divided into 4 groups

1. The control group (without sodium metabisulphite), shrimp (1kg.) were dipped into water with ice (0-3°C) for 1 minute followed by draining and then kept in the refrigerator at temperature 2-4°C.
2. Unknown 1, this group was divided into 6 subgroups. Shrimp (6 kg.) were dipped into sodium metabisulphite solutions at the concentrations of 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3% (1 kg.for each concentration)
3. Unknown 2, this group was divided into 6 subgroups. Shrimp (6 kg.) were dipped into sodium metabisulphite solutions at the concentrations of 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3% (1 kg.for each concentration)
4. Unknown 3, this group was divided into 6 subgroups. Shrimp (6 kg.) were dipped into sodium metabisulphite solutions at the concentrations of 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3% (1 kg.for each concentration)

At 1, 2 ,3 ,4 and 5 days after storage, fifteen shrimp from the control group and fifteen shrimp from each subgroup were taken randomly for sensory, microbiology and chemical analyses.



Unknown 1

Unknown 2

Unknown 3

Fig 1) Three sodium metabisulphite products (unknowns 1,2 and 3) used in this experiment

Sensory analyses

Sensory assessment of shrimp freshness (odour) and melanosis formation (black spot) were observed. Higher scores represent a gradual loss of quality associated with deterioration.

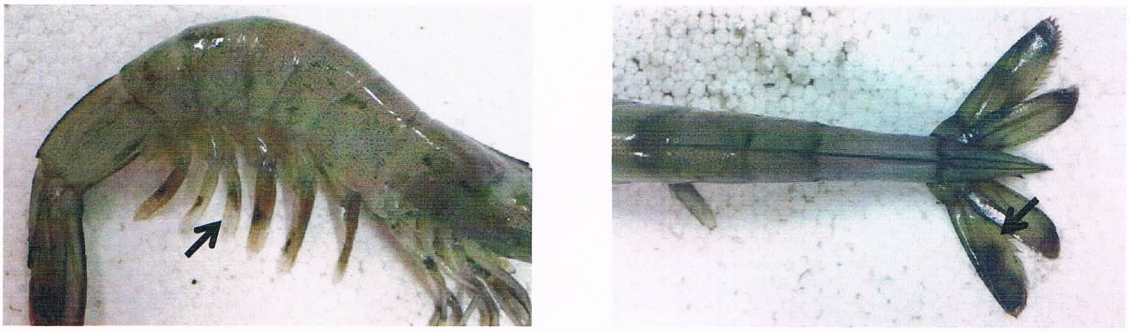


Fig 2) The black spots that indicate melanosis on the body, legs and tails of shrimp (black arrow).

Microbiological analyses

The total bacteria count was determined by the spread plate method using Nutrient agar (NA) followed the method of Collins *et al.*,1995. The spread plates were prepared in triplicates and incubated at 25° C for 1 day. At the end of incubation period, white colony were counted and the number of colony-forming units (CFU) per gram were calculated and data were recorded.

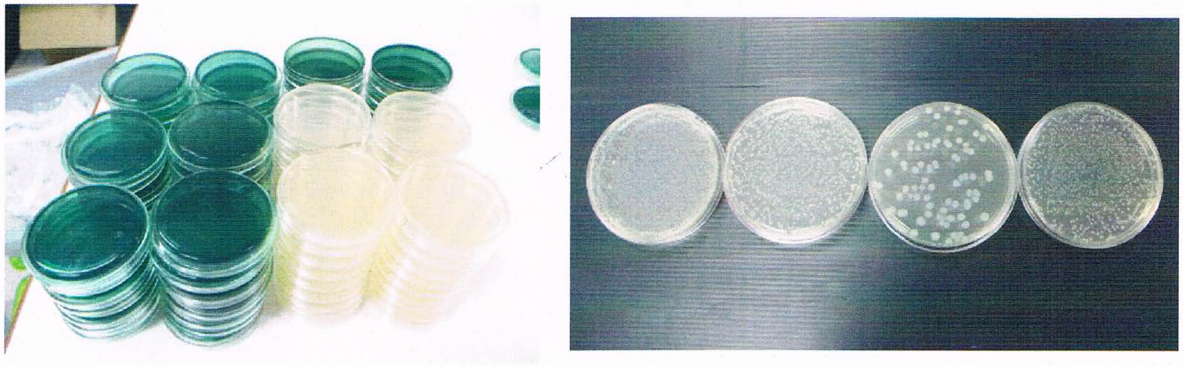


Fig 3) The total bacteria count was used to determine the total number of bacteria in the sample

Chemical analyses

Sulfites residues in a sample were analyzed using ALERT® Sulfites Detection Kit from NEOGEN Corporation 620 Lesher Place, Lansing, MI 48912, USA. (www.neogen.com)

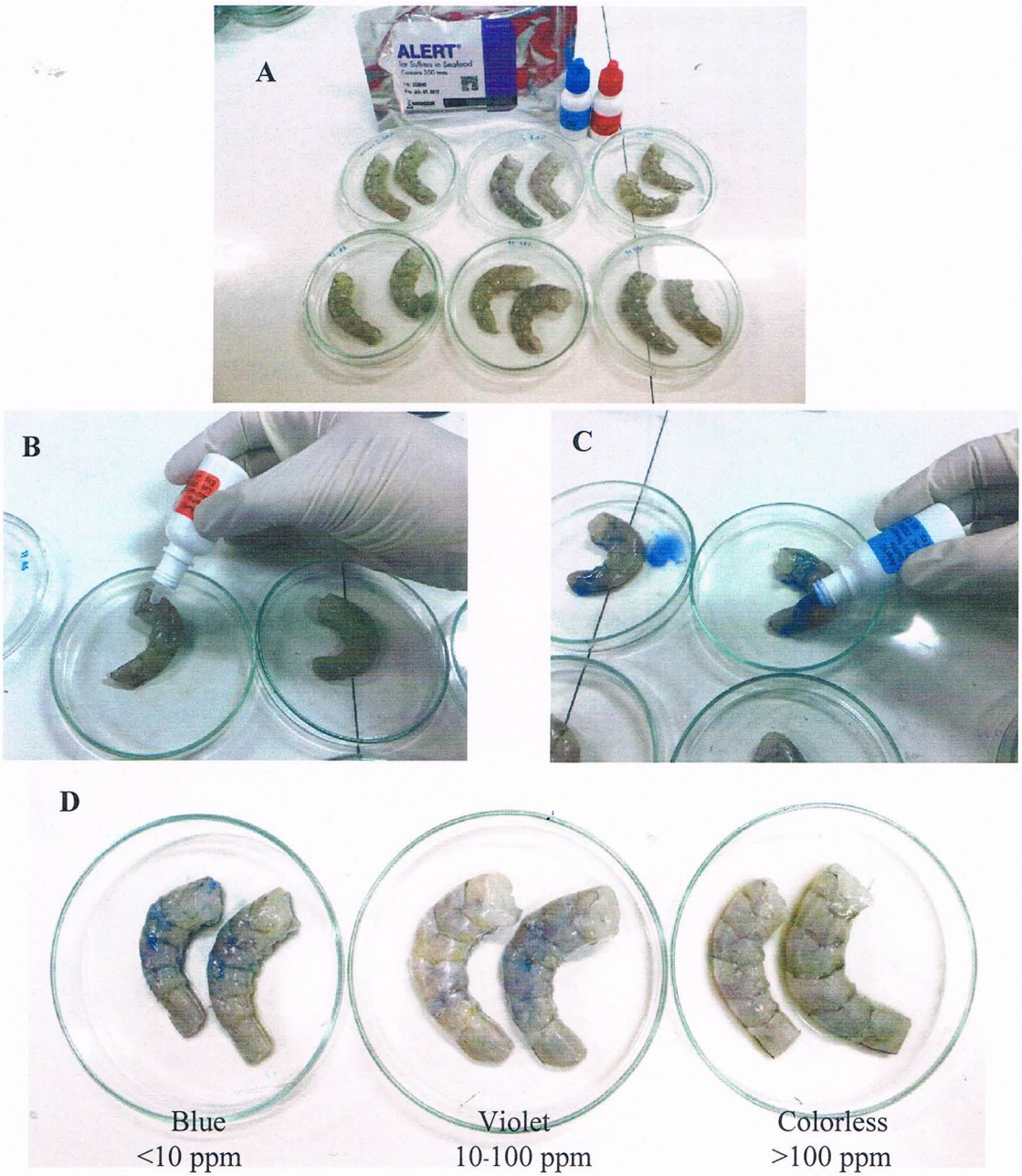


Fig 4) A: Sulphite determination was analyzed using ALERT® Sulfites Detection Kit
B: Added 1 drop of activator solution.
C: Added 1 drop of dye reagent.
D: Observed color changes.

Results

Table 1 Sensory, Microbiology and Chemical evaluation during storage of frozen shrimp (day 1)

Sources	Concentration	1 days			
		Sensory		Microbiology test (10^7 CFU/g)	Sulphite determination
		colour	odour		
Control	-	Melanosis	1	2.58 ± 0.01^{a}	-
Unknown 1	0.5%	Normal	0	0.24 ± 0.02^{fg}	10-100 ppm
	1.0%	Normal	0	0.23 ± 0.01^{fg}	10-100 ppm
	1.5%	Normal	0	0.23 ± 0.01^{gh}	10-100 ppm
	2.0%	Normal	0	0.21 ± 0.01^{hi}	>100 ppm
	2.5%	Normal	0	0.21 ± 0.01^{hi}	>100 ppm
	3.0%	Normal	0	0.19 ± 0.01^{i}	>100 ppm
Unknown 2	0.5%	Normal	0	1.01 ± 0.01^{b}	10-100 ppm
	1.0%	Normal	0	0.97 ± 0.01^{c}	10-100 ppm
	1.5%	Normal	0	0.97 ± 0.01^{cd}	10-100 ppm
	2.0%	Normal	0	0.95 ± 0.01^{d}	>100 ppm
	2.5%	Normal	0	0.95 ± 0.02^{d}	>100 ppm
	3.0%	Normal	0	0.95 ± 0.01^{d}	>100 ppm
Unknown 3	0.5%	Normal	0	0.28 ± 0.00^{e}	10-100 ppm
	1.0%	Normal	0	0.28 ± 0.01^{e}	10-100 ppm
	1.5%	Normal	0	0.26 ± 0.01^{f}	10-100 ppm
	2.0%	Normal	0	0.25 ± 0.01^{fg}	>100 ppm
	2.5%	Normal	0	0.25 ± 0.01^{f}	>100 ppm
	3.0%	Normal	0	0.21 ± 0.04^{fg}	>100 ppm

The data are presented as mean ± standard deviation. Means in the same column with different superscript are significantly different from each other ($p<0.05$).

Remark: level of odor

0 Odorless
1 Intense
2 Very intense

A

Melanization was observed in the body of shrimp in the control group.

B

For the unknown 1, No melanization was observed.

C

For the unknown 2, No melanization was observed

D

For the unknown 3, No melanization was observed

Fig 5) Melanosis observation during storage of frozen shrimp (day 1)

Table 2 Sensory, Microbiology and Chemical evaluation during storage of frozen shrimp (day 2)

Sources	Concentration	2 days			
		Sensory		Microbiology test (10^7 CFU/g)	Sulphite determination
		colour	odour		
Control	-	Melanosis	1	3.25 ± 0.02^{a}	-
Unknown 1	0.5%	Normal	0	2.21 ± 0.02^{f}	10-100 ppm
	1.0%	Normal	0	2.02 ± 0.02^{gh}	10-100 ppm
	1.5%	Normal	0	2.01 ± 0.01^{g}	10-100 ppm
	2.0%	Normal	0	2.01 ± 0.01^{hi}	10-100 ppm
	2.5%	Normal	0	2.00 ± 0.01^{i}	>100 ppm
	3.0%	Normal	0	2.00 ± 0.01^{hi}	>100 ppm
Unknown 2	0.5%	Normal	0	2.75 ± 0.02^{d}	10-100 ppm
	1.0%	Normal	0	2.74 ± 0.0^{d}	10-100 ppm
	1.5%	Normal	0	2.73 ± 0.01^{d}	10-100 ppm
	2.0%	Normal	0	2.73 ± 0.00^{de}	10-100 ppm
	2.5%	Normal	0	2.72 ± 0.01^{e}	>100 ppm
	3.0%	Normal	0	2.72 ± 0.01^{e}	>100 ppm
Unknown 3	0.5%	Normal	0	2.85 ± 0.01^{b}	10-100 ppm
	1.0%	Normal	0	2.84 ± 0.02^{bc}	10-100 ppm
	1.5%	Normal	0	2.83 ± 0.01^{c}	10-100 ppm
	2.0%	Normal	0	2.83 ± 0.01^{c}	10-100 ppm
	2.5%	Normal	0	2.82 ± 0.01^{c}	>100 ppm
	3.0%	Normal	0	2.82 ± 0.01^{c}	>100 ppm

The data are presented as mean ± standard deviation. Means in the same column with different superscript are significantly different from each other ($p<0.05$).

Remark: level of odor

0 Odorless
1 Intense
2 Very intense

A

Melanization was observed in the body of shrimp in the control group.

B

For the unknown 1, No melanization was observed.

C

For the unknown 2, No melanization was observed

D

For the unknown 3, No melanization was observed

Fig 6) Melanosis observation during storage of frozen shrimp (day 2)

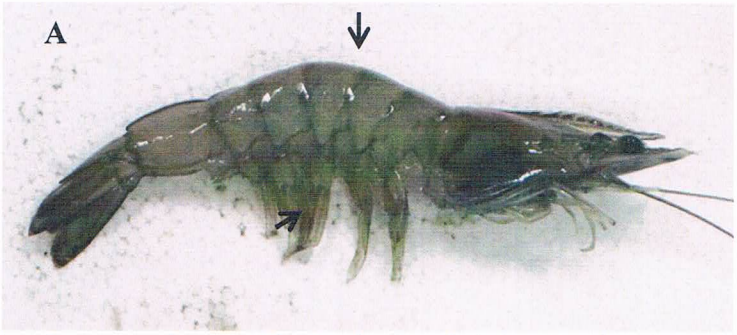
Table 3 Sensory, Microbiology and Chemical evaluation during storage of frozen shrimp (day 3)

Sources	Concentration	3 days			
		Sensory		Microbiology test (10^7 CFU/g)	Sulphite determination
		colour	odour		
Control	-	Melanosis	2	4.49±0.03[a]	-
Unknown 1	0.5%	Normal	1	3.56±0.02[de]	<10 ppm
	1.0%	Normal	1	3.42±0.01[e]	<10 ppm
	1.5%	Normal	1	3.41±0.01[e]	10-100 ppm
	2.0%	Normal	0	3.41±0.01[e]	10-100 ppm
	2.5%	Normal	0	3.40±0.01[e]	10-100 ppm
	3.0%	Normal	0	3.59±0.36[d]	>100 ppm
Unknown 2	0.5%	Melanosis	1	3.90±0.09[c]	<10 ppm
	1.0%	Melanosis	1	3.88±0.02[c]	10-100 ppm
	1.5%	Normal	1	3.88±0.01[c]	10-100 ppm
	2.0%	Normal	1	3.86±0.03[c]	10-100 ppm
	2.5%	Normal	1	3.86±0.01[c]	10-100 ppm
	3.0%	Normal	0	3.85±0.01[c]	>100 ppm
Unknown 3	0.5%	Normal	1	4.23±0.03[b]	<10 ppm
	1.0%	Normal	1	4.23±0.03[b]	10-100 ppm
	1.5%	Normal	1	4.22±0.01[b]	10-100 ppm
	2.0%	Normal	1	4.21±0.02[b]	10-100 ppm
	2.5%	Normal	1	4.20±0.02[b]	10-100 ppm
	3.0%	Normal	0	4.20±0.01[b]	>100 ppm

The data are presented as mean ± standard deviation. Means in the same column with different superscript are significantly different from each other ($p<0.05$).

Remark: level of odor

0 Odorless
1 Intense
2 Very intense



Melanization was observed in the body of shrimp in the control group.

For the unknown 1, No melanization was observed.

For the unknown 2, Melanization was observed in the body of shrimp in the group treated with 0.5% and 1.0%

For the unknown 1, No melanization was observed.

Fig 7) Melanosis observation during storage of frozen shrimp (day 3)

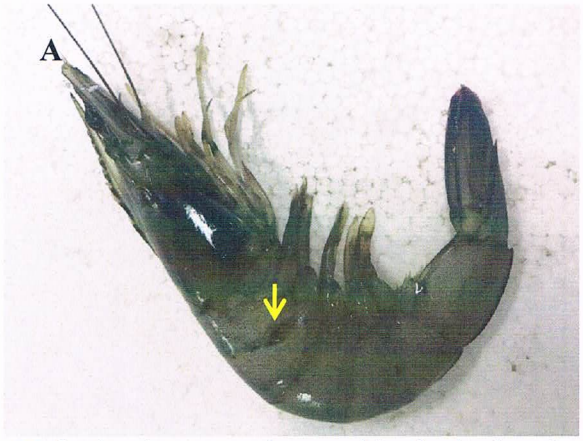
Table 4 Sensory, Microbiology and Chemical evaluation during storage of frozen shrimp (day 4)

Sources	Concentration	4 days			
		Sensory		Microbiology test (10^7 CFU/g)	Sulphite determination
		colour	odour		
Control	-	Melanosis	2	5.92 ± 0.02^{a}	-
Unknown 1	0.5%	Normal	1	4.67 ± 0.02^{i}	<10 ppm
	1.0%	Normal	1	4.52 ± 0.02^{j}	<10 ppm
	1.5%	Normal	1	4.50 ± 0.02^{j}	10-100 ppm
	2.0%	Normal	0	4.50 ± 0.01^{j}	10-100 ppm
	2.5%	Normal	0	4.46 ± 0.01^{k}	10-100 ppm
	3.0%	Normal	0	4.45 ± 0.02^{k}	>100 ppm
Unknown 2	0.5%	Melanosis	1	5.12 ± 0.02^{g}	<10 ppm
	1.0%	Melanosis	1	5.10 ± 0.02^{g}	10-100 ppm
	1.5%	Melanosis	1	5.10 ± 0.02^{g}	10-100 ppm
	2.0%	Melanosis	1	5.07 ± 0.02^{h}	10-100 ppm
	2.5%	Normal	1	5.07 ± 0.01^{h}	10-100 ppm
	3.0%	Normal	0	5.05 ± 0.02^{h}	>100 ppm
Unknown 3	0.5%	Melanosis	1	5.89 ± 0.02^{b}	<10 ppm
	1.0%	Melanosis	1	5.87 ± 0.03^{bc}	<10 ppm
	1.5%	Melanosis	1	5.85 ± 0.01^{cd}	10-100 ppm
	2.0%	Melanosis	1	5.84 ± 0.01^{de}	10-100 ppm
	2.5%	Melanosis	1	5.82 ± 0.02^{ef}	10-100 ppm
	3.0%	Normal	0	5.80 ± 0.01^{f}	>100 ppm

The data are presented as mean ± standard deviation. Means in the same column with different superscript are significantly different from each other ($p<0.05$).

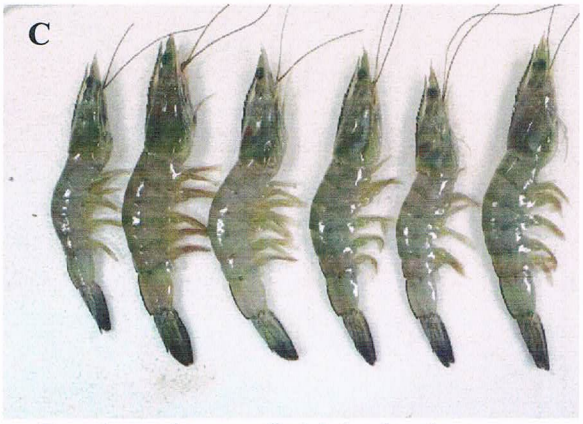
Remark: level of odor

0 Odorless
1 Intense
2 Very intense

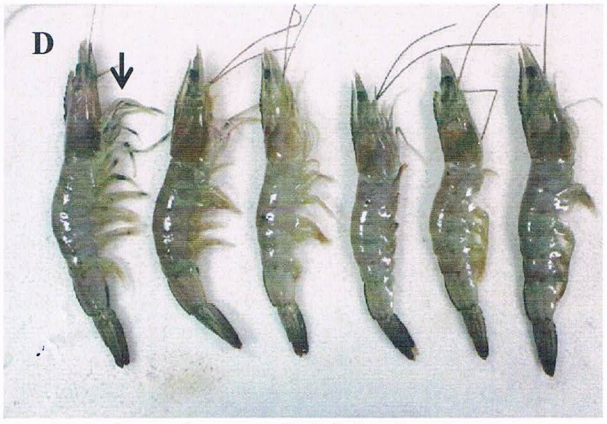


Melanization was observed in the body of shrimp in the control group.

For the unknown 1, No melanization was observed.



For the unknown 2, Melanization was observed in the body of shrimp in the group treated with 0.5%,1.0%,1.5% and 2.0%



For the unknown 3, Melanization was observed in the body of shrimp in the group treated with 0.5%,1.0%,1.5%, 2.0% and 2.5%

Fig 8) Melanosis observation during storage of frozen shrimp (day 4)

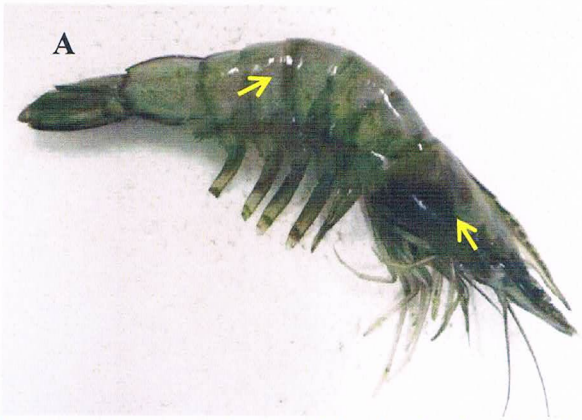
Table 5 Sensory, Microbiology and Chemical evaluation during storage of frozen shrimp (day 5)

Sources	Concentration	5 days			
		Sensory		Microbiology test (10^7 CFU/g)	Sulphite determination
		colour	odour		
Control	-	Melanosis	2	6.24 ± 0.01^{a}	-
Unknown 1	0.5%	Normal	1	4.81 ± 0.01^{l}	<10 ppm
	1.0%	Normal	1	4.77 ± 0.02^{m}	<10 ppm
	1.5%	Normal	1	4.75 ± 0.01^{m}	<10 ppm
	2.0%	Normal	1	4.75 ± 0.02^{n}	<10 ppm
	2.5%	Normal	1	4.72 ± 0.03^{n}	10-100 ppm
	3.0%	Normal	1	4.71 ± 0.03^{o}	>100 ppm
Unknown 2	0.5%	Melanosis	2	5.88 ± 0.02^{f}	<10 ppm
	1.0%	Melanosis	2	5.86 ± 0.02^{fi}	<10 ppm
	1.5%	Melanosis	1	5.85 ± 0.01^{ij}	<10 ppm
	2.0%	Melanosis	1	5.84 ± 0.01^{ij}	10-100 ppm
	2.5%	Melanosis	1	5.83 ± 0.01^{j}	10-100 ppm
	3.0%	Melanosis	1	5.80 ± 0.01^{k}	>100 ppm
Unknown 3	0.5%	Melanosis	2	6.12 ± 0.02^{b}	<10 ppm
	1.0%	Melanosis	2	6.10 ± 0.02^{bc}	<10 ppm
	1.5%	Melanosis	2	6.09 ± 0.01^{c}	<10 ppm
	2.0%	Melanosis	2	6.07 ± 0.01^{cd}	10-100 ppm
	2.5%	Melanosis	1	6.05 ± 0.01^{d}	10-100 ppm
	3.0%	Melanosis	1	6.00 ± 0.02^{e}	>100 ppm

The data are presented as mean ± standard deviation. Means in the same column with different superscript are significantly different from each other ($p<0.05$).

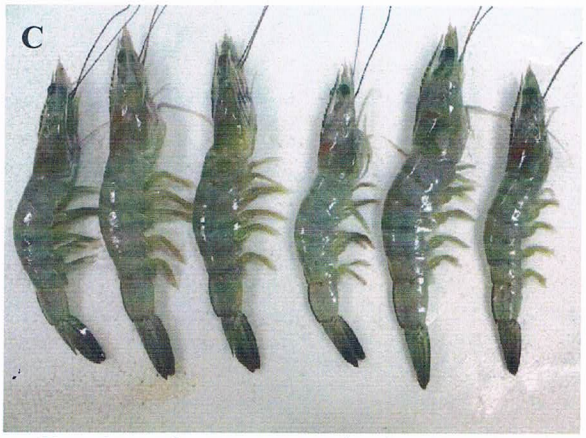
Remark: level of odor

0 Odorless
1 Intense
2 Very intense



Melanization was observed in the body of shrimp in the control group

For the unknown 1, No melanization was observed.



For the unknown 2, Melanization was observed in the body of shrimp from all samples.

For the unknown 3, Melanization was observed in the body of shrimp from all samples.

Fig 9) Melanosis observation during storage of frozen shrimp (day 5)

Sensory analyses

During storage days sensory results showed that the untreated shrimp control had a high occurrence of melanosis including dark head, black spot on body and tail. The melanosis was found since day 1 after storage. It was clear that sodium metabisulphite had a strong effect on delaying melanosis development on shrimp. Shrimp dipped in unknown 1 showed the best result in controlling melanosis formation. No melanosis was observed in all samples from this group throughout 5 days study period. Shrimp treated with unknown 2 at the concentration of 0.5% and 1.0% had melanosis at day 3 after storage and melanosis formation was found in all samples in day 5 after storage. For shrimp treated with unknown 3, the melanosis was found in day 4 after storage at the concentration of 0.5%, 1.0%, 1.5%, 2.0% and 2.5%. At day 5 after storage, the melanosis was found in all samples. (Table 1-5)

In control group, ammonia odour (level 1) developed on the 1st day and the odour increased to level 2 in the 3rd after storage. In the unknown 1 group, ammonia odour (level 1) developed on the 3rd day at the concentration of 0.5%,1.0% and 1.5%. After storage for 5 days, ammonia odour (score 1) was found in all samples. In the unknown 2 group, ammonia odour (level 1) developed on the 3rd day at the concentration of 0.5%,1.0% ,1.5%, 2% and 2.5%. On the 5th day, ammonia odour was developed in all samples. The result also showed that the ammonia odour increased to level 2 in the sample treated with 0.5% and 1.0%. In the unknown 3 group, ammonia odour (level 1) developed on the 3rd day at the concentration of 0.5%,1.0% ,1.5%, 2% and 2.5%. After 5 day of storage, the ammonia odour increased to level 2 in the sample treated with 0.5%, 1.0% ,1.5% and 2% while, the ammonia odour level 1 was detected in the sample treated with 2.5% and 3%.(Table 1-5)

Microbiological analyses

Bacterial counts are used as indicators of freshness. The total number of bacteria in all four groups had increased with storage time. There was no difference between the total number of bacteria in the sample treated with different concentrations of each sodium metabisulphite product during each storage time. Shrimp in the unknown 1 group had the lowest number of bacteria followed by the unknown 2 group and the unknown 3 group throughout the study period. Shrimp in the control group had the highest total number of bacteria among the four experimental group. (Table 1-5)

Chemical analyses

There was a decrease in sulphite level with storage time. Shrimp treated with unknown 1 showed the best result in sulphite reduction. At 5th day, shrimp treated with unknown 1 at the concentration of 0.5%,1.0%,1.5% and 2.0% had sulphite residue less than 10 ppm. While, shrimp treated with unknown 1 at the concentration of 2.5% and 3% had sulphite residue of 10-100 ppm and over 100 ppm, respectively. There was no difference between the sulphite reduction of shrimp in the unknown 2 and the unknown 3 groups. At 5th day, shrimp treated with unknown 2 and 3 at the concentration of 0.5%,1.0% and 1.5% had sulphite residue less than 10 ppm. While, shrimp treated with unknown 2 and 3 at the concentration of 2.0%, 2.5% and 3% had sulphite residue of 10-100 ppm , 10-100 ppm and over 100 ppm, respectively.

Conclusion

This study has demonstrated the effectiveness of sodium metabisulphite product as an agent in delaying melanosis development in shrimp. It's antimicrobial effect was confirmed. The unknown 1 product showed the best results in delaying melanosis development, control the total number of bacteria and decrease in the sulphite residue among the 4 groups.