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Studies on Phenoloxidase Activity and its Sensitivity to Metabisulphite in Frozen *Metapenaeus Monoceros*

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The incidence of black spot in frozen prawns was observed in many cases during thawing at tropical ambient temperatures. A dip in 0.5% (100% purity basis) sodium metabisulphite solution for 30 seconds controlled black spot in frozen *Metapenaeus monoceros* upto 10 months. Phenoloxidase activity in the shell extract of prawn showed a decreasing trend during frozen storage (-18°C). The activity was lower in the sulphite treated sample when compared with the untreated sample. Residual sulphur dioxide in thawed meat was within permitted limits.

Key words: Phenoloxidase activity, frozen prawn, metabisulphite

Phenoloxidase, responsible for black spot formation in raw prawns, is mainly present in cephalothroax, shell and tail part of Indian prawn (Antony & Nair, 1968, 1975; Savagaon & Sreenivasan, 1978; Chakrabarti, 1993). Removal of head followed by thorough washing helps to reduce blackening. Several methods have been suggested to control black discolouration in prawn by using sulphiting agents (FAO, 1982; Anon, 1976; ISI, 1985). Commonwealth department of primary industry permits 30 ppm as upper limit of residual sulphur dioxide in treated prawn meat (Anon, 1976). This permissible limit is the lowest among the limits set by developed countries.

Inhibiting effect of sulphiting agent on phenoloxidase varies in different types of tropical prawns. Among the three major marine prawns i.e., *Penaeus indicus*, *Penaeus monodon* and *M. monoceros*, the enzyme extract from *M. monoceros* is least sensitive to metabisulphite (Chakrabarti, 1993). The present investigation was undertaken to study the residual phenoloxidase activity in sulphite treated frozen *M. monoceros* during storage and also to find out the optimum level of sulphite treatment to keep residual sulphite within permitted limits in the final product.

Materials and Methods

M. monoceros (50-60 pieces per kg) caught by shrimp trawlers operating from Kakinada, were immediately beheaded and kept in direct contact with ice for 4-6 h on board. The iced headless prawns were brought to the nearby freezing plant and divided into three lots. Each lot was washed thoroughly with water. First lot was frozen in a plate freezer at -40°C after adding 200 ml of cold glazing water without any further treatment. Second lot was dipped in 0.4% (100% purity basis) sodium

metabisulphite solution for 30 s and the third lot was dipped in 0.5% sodium metabisulphite solution for 30 s. The treated prawns were stored in direct contact with crushed ice for 90 min, then frozen as in the case of the fresh sample. After freezing, the blocks were packed in master cartons and stored at -18°C.

Phenoloxidase was extracted from prawn shell (including tail) and meat separately by the method of Savagaon & Sreenivasan (1978). Specific activity of the enzyme preparation was measured by modified Horowitz method (Savagaon & Sreenivasan, 1978). Protein concentration in the enzyme extract was determined by method of Lowry *et al.* (1951). Specific activity of the enzyme was taken as change in optical density per mg protein in 15 min at 30°C.

The stored frozen samples were analysed at a regular interval of two months. Frozen sample of each lot was kept in leak proof polyethylene bags and kept in running water till the completion of thawing. The percent of black discolouration was calculated as percent of affected prawns in total number of prawns in a block. The thawed samples were cooked in 2% sodium chloride solution for 10 min and the organoleptic evaluation of these samples was carried out by a five member panel. Overall quality of meat was rated on a five point scale from 5 to 1; samples receiving a score of 3 and above were considered acceptable.

Moisture content and residual sulphur dioxide content in thawed meat were determined by standard methods (USFDA, 1975). Total volatile base nitrogen content in thawed meat was estimated by the modified Conway micro diffusion method (Beatty & Gibbons, 1937), using trichloroacetic acid extract of meat. Salt soluble protein was extracted by the method of Ironside & Love (1958). Nitrogen content of the extract was determined by micro kjeldahl method. Soluble protein content was calculated after

Table 1. Changes in phenoloxidase activity in shell extract, residual sulphur dioxide content of meat and percent of black discolouration in frozen headless *M. monoceros* during storage at -18°C.

Sample	Parameter	Before freezing	Storage in month								
			0	2	4	6	8	10	12	14	16	18
Untreated	Phenoloxidase activity	$80x10^{-3}$	$43x10^{-3}$	$28x10^{-3}$	$27x10^{-3}$	$26x10^{-3}$	$26x10^{-3}$	$24x10^{-3}$	$23x10^{-3}$	$22x10^{-3}$	$21x10^{-3}$	20x10-3
	Black discolouration (%)*	Nil	Nil	Nil	4.5	13.5	14.3	18.5	25.3	33.0	36.5	40.0
	Residual SO_2 (ppm)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
0.4% metabisulphite	Phenoloxidase activity	$32x10^{-3}$	$25x10^{-3}$	$23x10^{-3}$	$23x10^{-3}$	$22x10^{-3}$	$21x10^{-3}$	$22x10^{-3}$	$21x10^{-3}$	$21x10^{-3}$	$20x10^{-3}$	$18x10^{-3}$
	Black discolouration (%)*	Nil	Nil	Nil	Nil	Nil	Nil	4.0	12.5	21.0	22.5	25.0
	Residual SO_2 (ppm)	32.0	20.8	18.5	16.1	14.4	11.2	9.6	9.6	6.6	6.4	6.4
0.5% metabisulphite	Phenoloxidase activity	$24x10^{-3}$	$19x10^{-3}$	$18x10^{-3}$	$17x10^{-3}$	$18x10^{-3}$	$17x10^{-3}$	$18x10^{-3}$	$16x10^{-3}$	$15x10^{-3}$	$15X10^{-3}$	$14x10^{-3}$
	Black discolouration (%)*	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2.5	10.0	10.0	12.5
	Residual SO_2	25.8	25.6	22.5	20.2	18.6	15.3	12.8	11.2	9.6	9.6	6.4

* Percent of affected number in total prawns in a block. All values are mean of four determinations

making corrections for non protein nitrogen. Total plate count of frozen meat was determined by standard pour plate method using tryptone glucose agar medium and incubating at 37°C (AOAC, 1978).

Results and Discussion

It can be seen from Table 1 that bisulphite treatment was effective in controlling the development of black discolouration in frozen *M. monoceros* during storage at -18°C. Black discolouration was noticeable in the untreated sample after 2 months, where as the treated samples remained unaffected for 8-10 months. The level of residual sulphur dioxide in all frozen samples was well within the permitted limits and there was a gradual loss of sulphur dioxide as storage progressed. There was significant reduction in the activity of phenoloxidase in the shell extract of *M. monoceros* as a result of bisulphite treatment. The enzyme activity showed a decreasing trend in both treated and untreated samples as storage progressed. Phenoloxidase activity in the meat of all samples was below detectable limit. Chakrabarti (1993) reported that the shell extract of *M. monoceros* had a high phenoloxidase activity and this activity decreased during storage at refrigerated temperature (10±1°C).

Data on biochemical and organoleptic parameters are given in Table 2. The overall quality of all types of cooked samples was acceptable and slow deteriorative changes occurred during storage. Bisulphite treatment has not effected these parameters to any significant extent.

The results show that a dip in 0.5% sodium metabisulphite solution for 30 seconds could control black discolouration in frozen *M. monoceros* upto 10 months and this treatment does not affect the acceptability of the product to any significant extent.

Table 2. Changes in moisture, salt soluble nitrogen (SSN), total volatile nitrogen (TVBN), total platecount (TPC) and overall quality (after cooking) of frozen headless *M. monoceros* during storage at -18°C.

Sample	Parameter	Before freezing	Storage in month								
			0	2	4	6	8	10	12	14	16	18
Untreated	Moisture %	80.8	80.5	79.7	79.8	79.6	79.8	79.4	79.3	79.1	78.9	78.6
	SSN%	2.3	2.3	2.2	2.2	2.0	1.9	1.8	1.6	1.6	1.5	1.5
	TVBN mg%	11.5	12.0	18.3	20.5	21.6	25.8	28.1	28.6	29.1	29.5	29.8
	TPC per gm	$32x10^3$	$18x10^3$	$6x10^3$	$32x10^2$	$22x10^2$	$17x10^2$	$16x10^2$	$15x10^2$	$12x10^2$	$6x10^2$	$3x10^2$
	Overall quality	5.0	4.8	4.8	4.4	4.4	4.0	3.4	3.2	3.2	3.0	3.0
0.4% metabisulphite	Moisture %	80.9	80.6	79.8	79.7	79.4	79.2	79.1	79.2	79.0	78.8	78.7
	SSN%	2.3	2.3	2.2	2.2	2.0	1.9	1.9	1.8	1.8	1.6	1.5
	TVBN % mg	10.2	11.2	15.6	17.8	19.9	24.7	26.8	27.1	27.9	28.2	28.8
	TPC per gm	$22x10^3$	$15x10^3$	$4x10^3$	$24x10^2$	$18x10^2$	$15x10^2$	$13x10^2$	$13x10^2$	$10x10^2$	$5x10^2$	$2x10^2$
	Overall quality	5.0	4.8	4.8	4.4	4.4	4.0	4.0	3.8	3.4	3.2	3.2
0.5% metabisulphite	Moisture %	80.9	80.8	80.3	80.1	80.2	79.9	79.8	79.6	79.5	79.1	78.9
	SSN %	2.3	2.3	2.2	2.1	2.0	1.9	1.0	1.8	1.8	1.7	1.6
	TVBN mg %	10.8	11.9	16.3	18.6	20.1	24.5	26.4	26.9	27.5	27.8	28.1
	TPC per gm	$20x10^3$	$12x10^3$	$3x10^3$	$21x10^2$	$15x10^2$	$13x10^2$	$11x10^2$	$12x10^2$	$8x10^2$	$3x10^2$	$2x10^2$
	Overall quality	5.0	4.8	4.8	4.4	4.4	4.0	4.0	3.8	3.4	3.4	3.2

All values are mean of four determination

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