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Effect of Washing on Polyphenoloxidase Activity in *Metapenaeus monoceros*

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The enzyme, polyphenoloxidase, is responsible for the black spot formation in prawn. Sulphiting agents such as sodium metabisulphite and sodium bisulphite are widely used in prawn processing factories to control black spot, but sensitive persons are allergic to these chemicals. Repeated washing with optimum volume of potable water helped to leach out polyphenoloxidase from prawn considerably. 92% of the enzyme was leached out in the first two washing in the case of headless shell-on prawn and 53% was leached out in case of shell on whole prawn.

Key words: Melanosis, polyphenoloxidase, *Metapenaeus monoceros*

The formation of black spot in prawn was studied in detail by many workers (Antony & Nair, 1968; Baily *et al.*, 1960; Kakimoto & Kanaswa, 1956). As soon as prawn is caught, it should be immediately kept in ice to maintain its quality. Exposure to tropical ambient temperature and air helps the formation of black spot. The initial black spots on the shell are not objectionable organoleptically and these spots can be removed by peeling. However, comparatively higher proportion of melanin formation affects the appearance with black spot stain on the meat. The enzyme, polyphenoloxidase, responsible for black spot formation, is present in the head portion of prawn and its activity in meat is minimum (Antony & Nair, 1975; Savagaon & Sreenivasan, 1978; Chakrabarti, 1993). Removal of the head initially, followed by washing of prawn, minimize melanin formation, but both the shell-on whole prawn and the shell-on headless prawn are major products of export. Various methods are commercially adopted to control melanosis e.g. (a) by reduction of the concentration of active enzyme, (b) by cutting off the source of oxygen supply by keeping the prawn under chilled water (Antony *et al.*, 1968), (c) by treating with some permitted reducing agents such as sodium bisulphite, sodium metabisulphite, ascorbic acid, etc. (Baily *et al.*, 1960; CSIRO, 1976; FAO, 1982) and (d) by combination of the above three methods. The sulphites are widely used by prawn processing factories. An allergic or sensitive person may develop asthma from the exposure to sulphur dioxide liberated from suhlphiting agents. King *et al.* (1991) reported the use of 4-hexylresorcinol and Taoukis *et al.*, (1990) reported the use of sulphydryl protease, ficin as alternatives to sulphiting agents in controlling melanosis in shrimps. The alternate chemicals are yet to be accepted and tested by prawn processing factories; but effect of residual chemical to human body is yet to be fully understood. Removal of the enzyme from the tissue is another effective way of controlling melanosis. Therefore an investigation was carried out to study the effectiveness of simple washing in the reduction of polyphenoloxidase activity in prawn.

Materials and Methods

Metapenaeus monoceros was collected from the landing place and brought to the laboratory under ice. Weighed amount of

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head portion of prawn before and after washing with water, was taken in a mortar and ground with purified sea sand in ice cold condition and extracted with two parts its weight of cold 10 mM sodium phosphate buffer (pH 7.0) containing 0.5% Triton and 0.5% polyvinyl pyrrolidone (pH 4.0). The extract was strained through cloth. The homogenate obtained was subjected to cold centrifugation at 10000 x g for 15 min. The supernatant was used as the crude extract of polyphenoloxidase (Kakimoto & Kanazawa, 1956). The enzyme was analysed by the modified Horowitz method (Savagaon & Sreenivasan, 1978). 3 ml of assay mixture, containing 1.5 ml of 100 mM sodium phosphate buffer, pH (6.5), 1 ml of 30 mM DL-3,4 dihydroxy phenyl alanine and 0.2 ml of enzyme preparation, was incubated at 28°C for 5 min. The formation of dopachrome was measured at 470 nm using a zero blank. The change in optical density in 5 min was expressed as the enzyme activity. The specific activity was calculated as the change in optical density per mg of protein. The protein concentration in the assay mixture was measured by the method of Lowry *et al.* (1951). The enzyme activity was also determined in the presence of inhibitors, such as L-ascorbic acid, L-cystein and sodium metabisulphite at different concentrations.

Shell-on whole prawns were washed in potable water in the ratio 1:0.5, 1:1, 1:2, 1:3, 1:5 and 1:7 separately by applying whirling motion for 10 min. After each wash, the wash-water was collected by draining. The polyphenoloxidase activity was estimated in the drained water.

Shell-on whole prawn was washed in potable water in the ratio 1:1 by applying whirling motion for 5, 10, 20 and 30 min separately. In each case, the wash-water was drained out, collected and used as enzyme source for the determination of polyphenoloxidase activity. Headless shell-on prawn and potable water in the ratio 2:1 were taken in a container and whirling motion was applied for 10 min for thorough washing. The wash-water was then collected by draining. The process was repeated consecutively four times. The drained water was collected separately and used for determination of polyphenoloxidase activity.

Results and Discussion

Table 1 showed that the polyphenoloxidase activity in the washed fraction was highest when the ratio between shell-on whole prawn and water was maintained at 1:2. Table 2 indicated that specific activity of polyphenoloxidase in water was highest after washing for 30 min. Table 3 showed that specific activity of polyphenoloxidase was about 30% lower in the extract of head portion of washed whole prawn, after washing (1:1) for 10 min. It was observed that during repeated washing the polyphenoloxidase activity in the water remained almost the same at the sixth and seventh washing (Table 4). Of the total released polyphenoloxidase activity, 37%

Table 1. Polyphenoloxidase activity in the drained fractions obtained by washing shell-on whole *M.monoceros* with different proportions of water

Prawn: Water ratio	Activity/ml (O.D.)	Amount of protein/ml (mg)	Specific activity
1:0.5	0.89	4.54	0.196
1:1	0.59	3.52	0.167
1:2	0.38	1.36	0.284
1:3	0.35	1.40	0.250
1:5	0.25	1.02	0.240
1:7	0.13	0.68	0.191

Table 2. Effect of time of exposure on the release of polyphenoloxidase from shell-on whole *M. monoceros* during washing with water

Time given for washing (minutes)	Activity/ml (O.D.)	Protein/ml (mg)	Specific activity (O.D./mg protein)
5	0.12	1.20	0.100
10	0.9	5.33	0.168
20	1.0	6.83	0.148
30	2.6	9.66	0.260

Table 3. Polyphenoloxidase activity in the extracts of head portions of *M. monoceros*

Head portion	Activity*/ ml (O.D.)	Protein/ ml (mg)	Specific activity**
Unwashed whole prawn	2.6	13.25	0.196
Washed whole prawn	1.8	13.25	0.136

* The activity is expressed as the change in optical density (O.D.) per ml of enzyme in 5 minutes at 20°C.

** The specific activity is expressed as the change in O.D. per mg of protein in 5 minutes.

Table 4. Release of polyphenoloxidase from shell-on *M. monoceros* during successive washing.

Successive stages of washing	Activity/ ml (O.D.)	Protein/ml (mg)	Specific activity	% enzyme released in washing
Whole shell-on prawn				
1st washing	1.000	3.66	0.27	37.0
2nd washing	0.425	1.66	0.25	15.7
3rd washing	0.325	1.64	0.19	12.0
4th washing	0.300	1.33	0.22	11.1
5th washing	0.275	1.25	0.22	10.1
6th washing	0.255	1.25	0.13	8.3
7th washing	0.150	1.25	0.12	5.5
Headless shell-on prawn				
1st washing	0.800	5.0	0.16	66.6
2nd washing	0.300	2.5	0.12	25.0
3rd washing	0.125	1.2	0.10	8.3
4th washing	0.100	1.2	0.08	3.3

Table 5. Effect of different inhibitiors upon polyphenol odidase isolated from *M. monoceros*

Inhibitors	Concentration of the inhibitor (mM)	% of inhibition
Ascorbic acid	0.025	36.6
	0.05	70.0
	0.1	91.1
L. cystein	0.025	37.1
	0.05	53.7
	0.1	88.5
Sodium metabisulphite	1	22.2
	2	50.0
	4	72.7

came in the first washing followed by 16% in the second washing. Table 4 also shows that about 67% of the total released polyphenoloxidase activity appeared in the first washing of shell-on headless prawn, and 25% in the second washing.

It was also observed that 0.05 mM ascorbic acid or L-cystein was sufficient to bring about 50% inhibition of polyphenoloxidase (Table 5), while the requirement for sodium metabisulphite was at least 2 mM to have the same effect.

The result showed that 92% of the enzyme leached out in the first two washings in case of headless shell-on prawn, but it was 53% in case of shell-on whole prawn. Washing shell-on whole and headless prawn twice with water in the ratio 1:2 for 10 min removed the polyphenoloxidase content considerably. Residual enzymatic activity in washed prawn can be controlled by maintaining cold chain system up to consumer's end. As a precautionary measure, the dipping of the washed prawn in the solution of permitted chemicals will effectively prevent black spot/melanosis.

References

Antony, P.D. & Nair, M.R. (1968) *Fish. Technol.* **5**, 31

Antony, P.D. & Nair, M.R. (1975) *Fish. Technol.* **12**, 112

Baily, M.E., Fieger, E.A. & Novak,A.F. (1960) *Food Res.* **25**, 557

Chakrabarti, R. (1993) *Indian J. Fish.* **40**, 247

CSIRO (1976) *Australian Fisheries* **35**, (July) 34

FAO (1982) *Reference Manual to Codes of Practice for Fish and Fishery Products*, p.44 Food and Agriculture Organisation of United Nations, Rome

Kakimoto, D. & Kanazawa, A. (1956) *Bull. Jap. Soc. Sci. Fish.* **22**, 476

King, J.M., McEvily, A.J. & Iyengar, R. (1991) J. *Assoc. off. Analyt - Chem* **74**, 1003

Lowry, O.H., Rosebrough, N.J., Farr, A.K. & Randall, R.L. (1951) *J. Biol. Chem.* **193**, 265

Savagoan, K.A. & Sreenivasan, A. (1978) *Fish. Technol.* **15**, 49

Taoukis, P.S., Labuza, T.P., Lillemo,J.H. & Lin, S.W. (1990) *Lebensmittel Wissenchaft and Technologie* **23**, 52