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The Resazurin Test for Estimating Bacteriological Quality of Shrimps

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Abstract - Significant inverse correlations between viable bacterial counts and 0.0025% resazurin reduction times were observed in fresh and refrigerated shrimp. The derived regression equation for predicting bacterial counts is given. The results indicate that resazurin reduction test can be used to determine the bacteriological quality of raw shrimp.

Shrimp constitute one of the most important seafood commodities in international trade. Shrimp-importing countries have very stringent bacteriological standards and therefore it is imperative that the shrimp processing industries adopt very strict quality control measures. Although in-plant sanitation facilities are often good, processors often have no control on preprocess handling of the raw material. The processors do not have a rapid method to assess the bacteriological quality of the raw material as it arrives at the plant. Some workers used a dye reduction test for estimating bacterial counts in seafood with equivocal results. Novak et al. (1955) successfully employed the methylene blue reduction test for approximation of bacterial counts in shrimps and oysters, while Cavallone (1959) found this method unreliable. Uno and Tokunaga
(1954) noted that the resazurin test was useful for herring but not for mackerel. Shewan and Liston (1957) reported the usefulness of a tetrazolium derivative in assessing the quality of iced whitefish, while Moorjani et al. (1957) observed that this test was of less value in fishes with a high content of trimethylamine oxide (TMA-O). Recently, Kummerlin (1982) noted that resazurin reduction time and viable plate count correlated well in deep frozen shrimp.

Against this background, we evaluated some of the dyes for determining the quality of raw shrimp. Preliminary experiments indicated that methylene blue and tetrazolium chloride were not useful. Further studies were done only with resazurin.

Strips measuring 2.5 x 0.6 cm were cut from Whatman No. 1 filter paper and dipped in dye solution for 5 minutes. Two concentrations of the resazurin were tried, 0.005% and 0.0025%. The filter paper strips were dried overnight at 37°C, transferred to polythene bags and stored in a refrigerator.

Shrimp samples, mainly *Penaeus indicus, Metapenaeus dobsoni* and *Parapeneopsis stylifera*, were obtained from Mangalore fish landing center. Samples were brought to the laboratory within 30 minutes of collection. Some were analyzed immediately. The remainder were stored in refrigerators (14 samples at 3-4°C and 11 samples at 9-10°C) and examined at intervals to get samples at various stages of spoilage. All samples were analyzed in duplicate for total bacterial count (Speck 1976) at ambient temperature (about 27°C), alpha amino nitrogen (Pope and Stevens 1939), trimethylamine nitrogen (AOAC 1975), total volatile base nitrogen (Conway and Byrene 1933) and resazurin reduction. The latter was performed as follows: 50 g of shrimp samples were homogenized into 450 ml of physiological saline and about 10 ml of homogenate was transferred to two sterile petri plates. Ten filter paper strips containing 0.005% resazurin were dipped in one and strips containing 0.0025% resazurin in the other. Similar strips dipped in physiological saline served as control. After one minute the strips were removed, packed separately in polythene pouches, sealed and incubated at 37°C. Initially strips were observed every 30 minutes. As reduction progressed, the frequency was increased. Strips were observed till the dye was completely reduced.

The data were subjected to correlation and regression analysis using both model I and model II regression technique (Ricker 1973).

The reduction time of 0.0025% resazurin correlated inversely with viable bacterial count (Table 1) in both fresh and refrigerated

Table 1. Correlation coefficient between resazurin (0.0025%) reduction time (RRT) and various parameters of shrimp.

Sample	Correlation coefficient (r)			
	RRT and VBC	RRT and TMA-N	RRT and TVB-N	RRT and α amino nitrogen
Fresh shrimp	-0.9471** (n = 6)	-0.5121 (n = 6)	-0.4029 (n = 6)	-0.3051 (n = 6)
Stored shrimp	-0.8809** (n = 20)	-0.8150** (n = 19)	-0.82.61** (n = 19)	+0.4339 (n = 19)
Fresh and stored shrimp	-0.8839** (n = 26)	-0.6602* (n = 25)	-0.6817** (n = 25)	+0.1454 (n = 25)

*Significant at 5% level.
**Significant at 1% level.

shrimp. All values were significant at the 1% level. A similar correlation coefficient (r = -0.88) between bacterial counts and resazurin reduction time was reported by Kummerlin (1982) for deep frozen shrimp. With 0.005% resazurin, however, the correlation coefficient (r = -0.8469) was significant only at the 5% level only for fresh shrimp, and is not considered further here. When results for all samples combined (fresh and stored) were combined, the r value (-0.8282) was significant at the 1% level.

Fig. 1 shows the relation between log number of bacteria and resazurin (0.0025%) reduction time. The regression equations have been fitted by employing both simple linear regression (model I) and functional or geometric mean regression model (model II) for predicting log bacterial counts (Y) based on resazurin reduction times (X). The standard error of estimate of regression coefficients has not been calculated for model II regression since no appropriate formula was available (Ricker 1973).

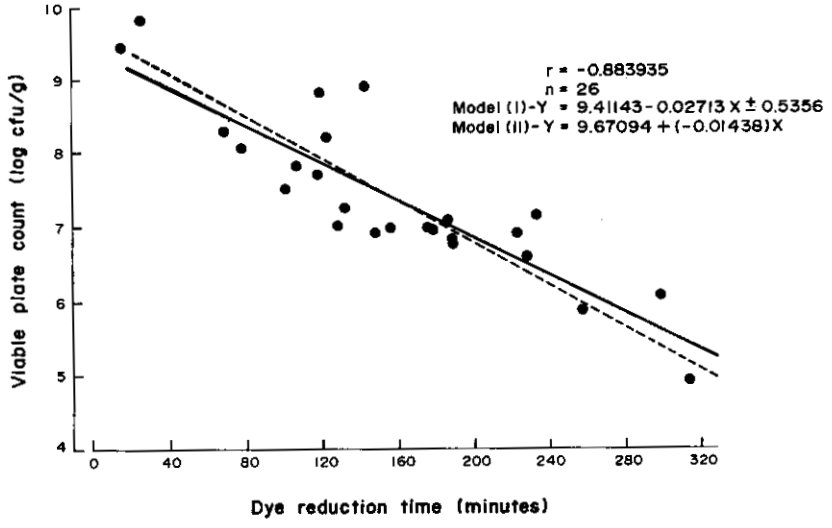

Fig. 1. Relation between resazurin (0.0025%) reduction time and viable bacterial count in shrimp.

The relation between resazurin reduction time and some of the biochemical parameters are presented in Table 1. There was a significant inverse relationship between resazurin reduction time and trimethylamine nitrogen (TMA-N) as well as for total volatile base nitrogen (TVB-N). The correlation was better in stored shrimp than in fresh shrimp. This is to be expected since the levels of TMA and

TVB-N are indicators of an advanced degree of spoilage. There was no correlation between levels of α-amino nitrogen and resazurin reduction times.

Equations for predicting levels of TMA-N and TVB-N based on resazurin reduction times are presented in Table 2. These equations have been constructed using both linear regression (model I) and functional or geometric mean regression (model II). The standard errors of estimate were very large. Thus, resazurin reduction test is not helpful in predicting levels of TMA-N and TVB-N accurately. However, as pointed out, both parameters are only of significance in later stages of spoilage.

Table 2. Regression equation for predicting various parameters based on 0.0025% resazurin reduction time in shrimp samples.

Variable to be estimated (Y)	Regression equation using Model I (± S.E.)	Model II
a. Viable bacterial count	Y = 9.411 - 0.0272 X ± 0.536	Y = 9.671 - 0.0144 X
b. TMA (mg%)	Y = 28.445 - 0.1076 X ± 9.478	Y = 37.239 - 0.1629 X
c. TVB-N (mg%)	Y = 108.583 - 0.3878 X ± 32.406	Y = 136.708 - 0.5690 X

Since there was good correlation between resazurin reduction time and viable bacterial count and the standard error of estimate was within acceptable limits (0.53 log units), this test could be used by the shrimp processing industry to estimate bacterial counts in raw material. The test is rapid, does not involve expensive equipment or highly trained personnel, and would therefore be of great value to the industry.

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