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A Direct Microscopic Count Procedure for the Rapid Estimation of Bacterial Numbers on Green-Headless Shrimp[1,2]

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ABSTRACT

A Direct Microscopic Count (DMC) procedure, utilizing the Gram-stain, was used to estimate bacterial numbers on 149 samples of green-headless (shell-on) shrimp. A correlation of 0.876 (significant at $P < 0.01$) existed between the log DMC and log agar plate counts done at 25 C for 2 days. Samples judged questionable or unacceptable organoleptically had DMC's of 10^8 or more.

In 1953, Lane and Whittaker (*11*) stated, "One of the most urgent needs in the shrimp industry is a rapid and accurate method of testing the quality of iced shrimp. This method must be free from the errors of personal opinion. The industry suffers through the inability of buyers to recognize spoiling shrimp with sufficient accuracy. Lacking objective tests of quality, penalities in the form of lower prices cannot be imposed for poorly handled catches. On the other hand, bonuses cannot be offered careful fishermen or other workers for high quality raw material." Today, over 20 years later, the statement is still basically true. Acid-soluble orthophosphate (*2*), alcoholic turmeric solution (*10*), amino nitrogen (*2, 4, 8, 11*), ammonia (*9*), B-vitamin content (*2*), catechol-ferric cholride (*10*), dimethylamine (*4*), free fatty acids (*4*), glycogen (*2, 11*), hydrogen sulfide (*4*), hydration capacity (*2, 15, 18*), inosine monophospahte (*16*), indole (*4, 7, 9, 11*), iodine titration (*2, 7, 9,*), lactic acid (*2*), methylene blue reductase (*14*), peroxide number (*4*), pH (*2, 3, 10, 11, 18*), phenol red test paper (*10*), photoelectric reflection number (*7*), picric acid (*3*), skatole (*11*), total fat (*11*), total nitrogen (*11*), trimethylamine (*2, 3, 4, 7, 8, 9, 10*), total volatile nitrogen/total nitrogen (*7, 9*), tyrosine (*8*), ultraviolet light-change in fluorescence (*11*), volatile acids (*2, 4, 8*), and volatile nitrogen (*10*) are some of the chemical and physical tests that have been evaluated over the years. None of these are presently used by the industry on a routine basis.

In most chemical tests, results can vary with the age of the shrimp, size, species, area of catch, and handling conditions. Many of the tests only indicate the onset of spoilage thus providing only limited information before that state and have been shown to vary independently of the quality as scored by a sensory panel (*11*). With the exception of pH (*2, 3, 18*) and ratios of volatile nitrogen with total or amino nitrogen (*5, 7, 9*), none of the tests have proved to be rapid, simple, or accurate enough for routine testing of shrimp at unloading or processing facilities. The industry still depends on visual observation, smell, and bacteriological testing for evaluating quality. The purpose of this investigation was to evaluate the use of a direct microscopic count (DMC) as a rapid means of assessing the bacteriological quality of shell-on shrimp tails.

MATERIALS AND METHODS

Green-headless shrimp samples (shrimp tails with shell-on) were either from commercial processing plants along the Texas coast or shrimp held until spoilage under various laboratory storage conditions. Depending on the size of the shrimp, either three shrimp or 50 g were weighed and placed in 100 ml of sterile phosphate buffer. The sample was shaken vigorously and 0.01 ml was transferred with a platinum-rhodium inoculating loop to a fluorescent antibody slide with two etched 10-mm diameter circles. Smears were air-dried in an oven at 50 C for 15-20 min. They were then Gram stained and examined under oil immersion. Twenty fields were examined if the number of bacteria per field was less than 10, 10 if the number per field was 10-50, and five if the number per field was greater than 50. The average number of bacteria per field, dilution factor, and microscopic factor (MF) were used to calculate the direct microscopic count (DMC) per gram. The Microscopic Factor (MF) was determined as prescribed in *Standard Methods for the Examination of Dairy Products* (*1*).

Agar plate count (APC) was determined by spread-plating decimal dilutions of the same sample on Standard Methods Agar (BBL) and incubating plates at 25 C for 2 days. The log numbers of bacteria from agar plate count and direct microscopic count from 149 samples were subjected to standard regression and correlation analysis.

RESULTS AND DISCUSSION

Correlation between the logarithm of DMC and APC (Fig. 1) was 0.876 (significant at $P < 0.01$). A linear relationship Log DMC = 1.79 + 0.82 Log APC was established and the coefficient of variations for DMC and APC were 0.14 and 0.17, respectively.

Lerke and Farber (*12*) utilized a direct bacterial count from scrapings of fish fillets as an indicator of freshness. They substituted a 30-sec staining with safranin for the complete Gram stain because micrococci were the only gram-positive organisms found and then only on very fresh fillets. Since the bacterial flora of freshly landed Gulf of Mexico shrimp consists primarily of coryneforms, *Achromobacter, Flavobacterium,* and *Bacillus* (17), the

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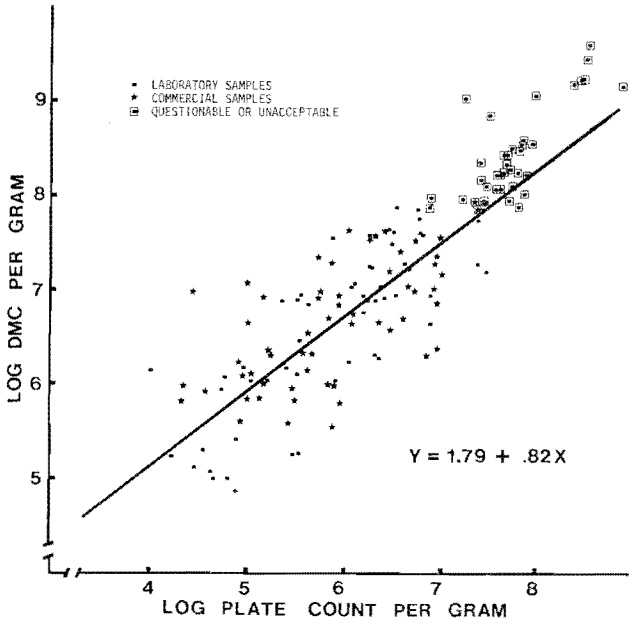

Figure 1. *Direct Microscopic Count (DMC) and Agar Plate Count (APC) of 149 samples of green-headless shrimp.*

entire Gram stain was used in the present study. It proved acceptable and provided useful information as to the types of bacteria present.

It is realized that there are certain inaccuracies inherent to the DMC. Levowitz (*13*) indicated that the direct microscopic count could not be used in estimating the number of bacteria in market grade raw milk because of the small number of cells available. Other investigators (*6*) have used a microscopic counting technique on fish and reported it to be of value only in the later stages of spoilage, when numbers were approaching 10^5 per gram. Bacterial counts below the MF are difficult to estimate because the number of fields examined must be increased and fractions are used to express average number per field. These limitations do not pose a great handicap in using the DMC to estimate the bacterial counts and quality of Gulf of Mexico shrimp because shrimp of poor or questionable quality usually have APC's in excess of $10^6 - 10^7$ per gram. APC's (25 C for 2 days) of commercial shrimp samples in the study ranged from 2.3×10^4 to 3.2×10^7 per gram and DMC's from 2.0×10^5 to 7.3×10^8 per gram. As judged organoleptically only two of the commercial samples were questionable or unacceptable and both had APC's in excess of 10^7 per gram. In addition, onset of spoilage in samples held under laboratory storage conditions was not noted until APC's exceeded 10^7 per gram. Questionable or unacceptable samples in either event had DMC's near or in excess of 10^7 per gram.

High counts may result from bacterial multiplication on shrimp or from contact with heavily contaminated surfaces. The degree of quality deterioration depends to a large extent on the biochemical activity of the microflora upon the chemical constituents of the shrimp. Although the DMC may not separate live from dead cells, high counts are undesirable because they indicate improper handling and/or storage between catch and processing.

The DMC procedure for green-headless shrimp should not be substituted for the agar plate count but does provide a rapid simple means of assessing the storage history and quality of green headless shrimp when used in conjunction with conventional sight and smell evaluations.

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