

Biochemical And Bacteriological Methods For Determining Shrimp Quality

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THE BIOCHEMICAL ACTIVITIES of microorganisms in marine products have been extensively studied for a number of years, but the bacteriology and the biochemical activities of bacteria in shrimp and shrimp products have received only limited attention (1, 2, 6, 14, 15).

Laboratory work was carried out to determine the validity of certain tests in detecting the sanitary conditions and changes of quality in frozen breaded shrimp due to bacterial, chemical and physical factors. The study was based on samples of frozen breaded shrimp collected at packing plants and retail outlets over a two-year period from 23 cities.

In order to determine total number of bacteria in the shrimp, the media recommended by Green (6), Williams and associates (14, 15) and Bennett *et al.* (3) were studied. The method of the American Public Health Association "Standard Methods for the Analysis of Water, Sewage and Industrial Wastes" (12) was used to determine and isolate the coliform bacteria. The enterococci (fecal streptococci) were best enumerated and isolated by the use of azide dextrose broth and ethyl violet broth, Litsky, Rosenbaum and France (10) and Larkin, Litsky and Fuller (7, 8, 9).

To obtain the total number of bacteria in frozen breaded shrimp, the Nutrient Agar, 1.5 per cent, was most satisfactory and is recommended. In determining the number of coliform bacteria, the "Most Probable Numbers" is recommended. However, the use of Violet Red Bile Agar (Difco) is equally satisfactory and much more rapid (18-24 hrs.). In no instance was the presence of *Escherichia coli* confirmed by the use of Eosin Methylene Blue Agar. Therefore, little information of value is gained from making confirmed *Escherichia coli* tests.

Out of survey samples collected from 23 cities, distribution of bacteria varied from a minimum of 21,500 bacteria per gram of shrimp to a maximum number 54 million bacteria per gram. Seven per cent contained less than 100,000 bacteria per gram. Thirty-six per cent contained less than 500,000 bacteria per gram. Out of the total samples, sixty-one per cent had a bacterial content of less than 1 million.

The number of coliform bacteria varied from a minimum of zero to 700 per gram of shrimp. Out of 144 samples, only 98 (68 per cent) contained coliform bacteria, but of these 98 samples, 90 (92 per cent), contained less than 100 coliforms per gram.

Enterococci were present in all the samples and their number varied from very few bacteria per gram to 13,500. Forty-four per cent contained less than 100 enterococci per gram of frozen breaded shrimp.

To study the biochemical aspects of the spoilage of shrimp, many analytical methods were applied. Iodine determination (4), iodine titration (13), trimethylamine (5), and Photoelectric Reflection Number appeared to be of neg-

ligible value in estimating the degree of freshness in frozen breaded shrimp. The only test that showed a definite correlation with the taste panel score and the logarithm of the total bacterial count was the ratio of total volatile bases (TVB) to total nitrogen (TN), multiplied by 100 (TVB/TN x 100).

Details of the procedure used to determine this ratio follow: *Total Volatile Bases (TVB) calculated as ammonia*: The sample of frozen breaded shrimp was passed twice through a clean food chopper. Five gram portions were weighed in waxed paper cups or beakers. Waxed cups were used to facilitate the washing of the sample from the container. Frozen breaded shrimp upon thawing became very sticky due to the presence of starch in the breading. The sample was washed into a Waring blender with a 100 ml. of CO₂-free distilled water. The mixture was blended for five minutes at high speed and quantitatively transferred to a 500 ml. Pyrex tapered glass-stoppered distillation flask by washing with approximately 100 ml. of distilled water. The distillation flask was quickly connected to a glass tapered Liebig condenser after the addition of approximately 10 ml. of a 40 per cent NaOH solution, boiling stones, and a few drops of Dow Corning AF Antifoam Emulsion¹. The tip of the condenser was dipped in approximately 15 ml. of four per cent boric acid solution in which Tashiro's indicator² has been added. The distillation was carried out with a low flame and lasted five minutes after the appearance of a green color. The tip of the condenser was rinsed with CO₂-free distilled water and the distillate was titrated with a 0.05 N standard HCl solution. The results were calculated as milligrams or grams of NH₃ per 100 grams of frozen breaded shrimp. Many trials showed the average per cent recovery of TVB was 95.0 per cent.

Total Nitrogen (TN): The total nitrogen (TN) was determined by the Kjeldahl method using a micro-Kjeldahl digestion and distillation apparatus. The digestion mixture used was prepared as recommended by Peters and Van Slyke (11), but the addition of copper sulfate was omitted and replaced by selenized granules added directly to the digestion flask. The results were calculated as g. of nitrogen per 100 g. of frozen breaded shrimp.

Ratio of Total Volatile Base (TVB) to Total Nitrogen: The results obtained for Total Volatile Base (TVB) and Total Nitrogen (TN) were used to calculate a ratio. In order to facilitate further calculations and manipulations, the ratio was multiplied by 100.

The results obtained showed a definite correlation with the average taste panel score and the logarithm of total bacterial count. Considerable variations were obtained in the Total Volatile Bases content of different lots and of individual samples within a lot. These variations could be accounted for by the combined variation in per cent breading, moisture and mainly the total nitrogen of the lots and individual samples within a lot.

The ratio (TVB/TN x 100) when plotted against time showed a curve which could almost be satisfied by a cubic equation. The ratio was found to increase in the samples undergoing the storage test, from a value of 6.0 to a value of 7.4 where it remained stationary and then increased to a ratio value of 10.0. On the basis of the correlation with the average taste panel scores, four zones were depicted in relation to a critical ratio value of 8.0 which corresponded to a critical point for quality of frozen breaded shrimp.

¹Dow Corning Corporation, Midland, Michigan.
²20.248 g. methylene blue and 0.375 g. methyl red in 300 ml. 95 per cent ethyl alcohol.

The zones were interpreted as follows for frozen breaded shrimp:

<i>Ratio Values</i>	<i>Quality</i>
Below 6.0	Very Good
Between 6.0-7.5	Good
Between 7.5-8.5	Questionable
Higher than 8.5	Unacceptable

To test how realistic the proposed ratio values for frozen breaded shrimp quality were, analyses were carried on 144 samples of frozen breaded shrimp collected from 23 cities. The ratio of TVB to TN x 100 varied between 4.5 and 12.8. On the basis of the quality scale proposed, 10.4 per cent of the samples scored higher than 8.5 (Unacceptable), 25.0 per cent between 7.5 and 8.5 (Questionable), 47.2 per cent between 6.0 and 7.5 (Good), and 17.4 per cent below 6.0 (Very Good). The results were analyzed for the skewness of their distribution and a skewness value of + 0.19 was obtained. This value for the skewness indicated that the distribution of the ratio values were very close to the symmetrical distribution that was hypothetically expected. It corroborated the realistic value of the ratio as a measure for the degree of freshness of frozen breaded shrimp. Preliminary work carried out on other shrimp products indicates that the ratio not only could be used as a measure of quality for frozen breaded shrimp, but also for seafoods and meat products in general, providing the ratio limits be correlated to quality for each specific product. It is expected that the objective values of the ratio limits will vary with the type of animal product.

SUMMARY

Tentatively a total count of more than a million bacteria per gram means frozen breaded shrimp of low sanitary quality, while one in excess of 2,000,000 means shrimp of questionable sanitary quality. It seems that the enumeration of enterococci is a better index of sanitary quality than coliform bacteria. The ratio of TVB/TN x 100 indicates the closest relationship to the quality of frozen breaded shrimp and is recommended as the objective chemical test of choice.

The following quality scale is recommended for frozen breaded shrimp: A ratio value larger than 8.5 indicates an unacceptable product. Values between 7.5 and 8.5 correspond to a questionable quality. Between 6.0 and 7.5, the product is good and below a value of 6.0, very good.

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The Effect of Chlortetracycline on Shrimp Spoilage at Various Temperatures

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AS FOR OTHER FISHERIES PRODUCTS, quality is of prime importance in the shrimp industry. In this report two aspects of the quality problem are discussed, namely, the objective assessment of the quality of shrimp and the prolongation of the quality during handling and storage.

A number of reports have been published on the assessment of the quality of shrimp, among which may be mentioned those by Campbell and Williams (1952), Duggan and Strasburger (1946), Fieger and Friloux (1953), and