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# The Use of Indole as an Indicator of Spoilage in Fresh Shrimp

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SOME OBJECTIVE TEST of shrimp quality is urgently needed by the industry to assist buyers and to help in the establishment of quality standards, and in the proper application of regulatory laws. One of the tests used has been to measure the quantity of indole produced during storage periods.

Tryptophane, an amino acid which is produced by the catalytic action of the enzyme tryptose upon the hydrolysis of albuminous matter is subject to various changes which eventually yield indole, among other substances.

The presence of tryptophane in crustacean and mollusk proteins was demonstrated by Jones and Okuda (Winton, 1939).

Para-dimethylaminobenzaldehyde, the main ingredient of Ehrlicks reagent, is known to react with indole under certain conditions, to form a red color. Chernoff (1940) developed a method to determine this color quantitatively, by setting up a series of standard solutions of varying concentrations for visual comparison. Clarke et al (1937) adapted the method for the determination of indole in dairy products, and introduced the use of the photometer.

King, Flynn and Gowanloch (1945) proposed the use of indole to detect decomposition in oysters. They also noted in fresh oysters the presence of some interfering substance that reacted with p-dimethylaminobenzaldehyde similarly to indole. Beacham (1946) found larger quantities of this indole reacting material in clams. Duggan and Strasburger (1946) observed that there was a relation between the indole content and the stage of decomposition organoleptically detected in shrimp. In later studies with shrimp, oysters and crabmeat, using samples containing amounts of indole that might be encountered in commercial samples, it was found that the greatest variations were in the shrimp samples (Duggan 1948). These inconsistencies were attributed to sampling errors and to the inexperience of the chemist with the method.

## Experimental Procedure

The shrimp used in these tests were the pink spotted shrimp, *Penaeus duorarum,* caught in the Key West-Tortugas Grounds. All samples were collected personally by the investigator on board the fishing vessels, and their precise history is known. The shrimp were deheaded immediately after capture, carefully washed with sea water and stored in the hold in boxes containing snow-type ice. The shrimp were re-iced a number of times en route to The Marine Laboratory. Upon arrival at the Laboratory they were transferred to insulated storage bins. In the first experiment the shrimp were stored in crushed "Rickey" type ice, and in the second experiment in flake ice. Indole determinations were made on shrimp samples drawn every two days, starting with the second day of iced storage. Samples were drawn at regular intervals and tested throughout a nineteen day period in the first experiment, (Figures marked "A") and over a twenty-two day period in the second (Figures marked "B"). Organoleptic and bacterial tests were made at more irregular intervals. Indole content was compared to organoleptic scores, as determined by an experienced test panel, and to bacterial scores. The organoleptic criteria used were odor and taste. Bacterial counts were made by the direct count method as modified by Tarr (1941). To minimize sampling errors which had been experienced by previous investigators, simultaneous determinations were made with two sets of apparatus. The indole quantities represented here are the averages of two determinations, which has resulted in a marked smoothing of the original lines. Figures 1-3 compare indole production with odor, taste and bacterial scores.

As stated above, the basic method used by all workers was that of Clarke (1937) et al. King, Flynn and Gowanloch (1945) modified Clarke's method in that no Reichert-Meissel bulb and no stop cock lubricant were used, water instead of alcohol was used in the distilling flask, and steam rather than alcohol was used to wash the condenser. Beacham (1946) further modified the method in that an all-glass apparatus was used, thereby eliminating

any interference from rubber stoppers. The distillate was made alkaline before extraction with chloroform and sodium sulfate was added to prevent the formation of emulsions. Before the addition of the color reagent the extract was shaken with HCl to partly eliminate the apparent indole. Acetic acid alone was used to develop the color of the phosphoric acid-aldehyde extract rather than an acetic acid-ether mixture. Duggan and Strasburger (1946) followed essentially the same procedure as Clarke except that all rubber stoppers were covered with tin foil, as unprotected natural or synthetic rubber connections may cause variable distillation blanks. Actual distillation time was reduced from the 90 minutes of Clarke et al to approximately 45 minutes. Also the following experimentally determined facts were observed by Clarke and his co-workers.

(1) No differences in recovery of indole are discernible when water is substituted for alcohol, except that less total distillate is required.
(2) No indole is obtained in the first 100 ml of distillate when 100 ml or more of alcohol is present in the distilling flask.
(3) Approximately 60 per cent of the indole present is found in the first 100 ml distillate coming over after the alcohol has distilled off.
(4) When not more than 150 ml of alcohol is present in the distilling flask 95-100 per cent of the indole present is contained in the first 450 ml of distillate.
(5) Continued distillation beyond the 450 ml of distillate required in the procedure does not form any indole in fresh shrimp (raw or cooked).
(6) Continued distillation of decomposed shrimp beyond 450 ml does not form any indole. A small amount (3-4 per cent of the total) of indole is found in the second 450 ml distillate. Only one or two micrograms are found in the third and fourth 450 ml distillate. This amount was not considered significant when the original concentration of approximately 500 micrograms was considered.
(7) Traces of chlorine and bromine in the water will destroy the indole in the determinations.

The technique used by the present investigators was that of Clarke, as modified by the above workers. The procedure follows:

I. Preparation of Reagents:

(a) Color Reagent
Dissolve 0.4 g of p-dimethylaminobenzaldehyde in 5 ml of acetic acid and mix with 92 ml of phosphoric acid and 3 ml concentrated HCl. Mix the color reagent in 100 ml or 500 ml lots. Purify p-dimethylaminobenzaldehyde according to A.O.A.C. *20*, 477 (1937). The purity of this reagent exerts a strong influence on the intensity of the reagent blank.

(b) Acetic Acid
If this reagent gives a pink reaction with the color reagent, purify by distilling in an all glass still 500 ml with 25 g $KMNO_4$ and 20 ml $H_2SO_4$.

(c) Hydrochloric Acid
A 5 per cent solution.

(d) Sodium Sulphate
A saturated aqueous solution.

(e) Chloroform
U.S.P.

(f) Standard Indole Solution

Weigh accurately 20 mg indole into a 200 ml volumetric flask and dilute to mark with 95 per cent alcohol. Prepare working solutions fresh daily. Keep stock solution refrigerated and do not use after two weeks. If Eastman White Label (M.P. 52°-53°C) indole is used the standard solutions are reported to be stable for months if kept refrigerated and protected from the light.

II. Distillation Apparatus:

Use a separate steam generator for each unit. The generator itself may be made from a one liter Erlenmayer flask and connected to an all-glass still by means of polyethylene tubing. The 500 ml distillation flask is connected to a vertical straight bow condenser through a spray trap. Use foil-covered rubber stoppers in the steam generator.

III. Procedure:

(a) Distillation

Weigh two or three shrimp and place them in a blendor; add 80 ml alcohol and mix the sample for several minutes until a smooth homogeneous mixture is obtained. The mixture is then transferred to the distillation flask, and a minimum amount of alcohol used to rinse the blendor.

A few drops of Dow Corning Antifoam "A" are added and the flask then connected to the steam distillation apparatus. Sufficient heat is applied to the distillation flask to maintain an 80-90 ml volume. About 450 ml of distillate should collect in approximately 45 minutes. Steam should be used to wash the condenser.

(b) Extraction

Transfer the distillate to a 500 ml separatory funnel, add 5 ml dilute HCl and 5 ml sodium sulfate solution. Extract successively with 25, 20 and 15 ml portions of chloroform. Shake each portion vigorously for at least one minute. Combine and wash the 25 and 20 ml extracts in a 500 ml separatory funnel with 400 ml water, five ml sodium sulfate and five ml dilute HCl. Save the wash water. Filter the combined extracts through a plug of cotton into a dry 125 ml separatory funnel. Wash the 15 ml portion with the same wash water, and combine with the other portions in the 125 ml separatory funnel.

IV. Development of the Color:

Add 10 ml of the color reagent to the combined extracts and shake vigorously for exactly two minutes, and allow to settle as completely as possible. Drain off 9.0 ml and dilute to 50 ml with acetic acid. Mix well and transfer to a suitable photometer cell and measure the color photometrically at 567 millimicrons. The color solutions may be diluted with acetic acid provided that the blanks are determined at the same dilution. Calculate indole in terms of micrograms per 100 grams of shrimp.

V. Determination of the Standard Curve and Blank:

Prepare a standard curve by steam distilling a series of known amounts of indole. Determine the distillation blank in a like manner omitting the addition of indole.

***Experimental Results***

Fresh shrimp (up to two days in iced storage) contained little or no indole (0 to 0.5 gammas/100 g of shrimp): A general increase in the amount of indole present occurred with increased storage time in ice (Figures 1-3). The

trend is unmistakable in all of the graphs drawn, but the curves show irregularities in indole production. Inconsistencies and fluctuations in the yield of indole have presented a special problem. For example, shrimp nine days in storage contained less indole than did those five days in storage, and shrimp stored sixteen days exhibited less indole than those stored twelve days. As extremes it may be noted that in one case shrimp twelve days in storage, and in another case shrimp fourteen days in storage showed no indole in one of the determinations.

The above-mentioned anomalies could be attributed to the following factors:

(1) Sampling

(2) Size of the apparatus

(3) Experience of the investigator

(1) To test the extent of the sampling error two simultaneous determinations were made in these experiments. Normal variability among individual shrimp, with the resulting difficulty in obtaining an accurate and representative sample, certainly contributed to the sampling error and may, in fact, be the main factor. For example, in producing the inconsistencies noted difference in indole yields of the two samples from the same storage bin are shown in Figure 4.

Obviously the differences in indole production in individual shrimp cannot be avoided. They could possibly be minimized if a larger sample (up to 10 shrimp) were to be used.

To test this possibility a much larger sample (10 shrimp instead of three) was drawn from the storage bin for analysis. It is evident that the use of this larger sample has resulted in a much smoother curve. (Figure 5)

The use of 50 grams of shrimp per determination from the larger, blended sample has yielded results which are more consistent.

(2) By chance the two distillation units used in the first experiments had spray traps and condensing tubes of different sizes.

SIZE OF APPARATUS USED IN THE EXPERIMENTS

| Part | Apparatus A | Apparatus B |
|---|---|---|
| Length of Water Jacket | 580 | 580 |
| * Inner Tube Diameter | 10.9 | 18.2 |
| * Outer Tube Diameter | 17.8 | 24.6 |
| Spray Trap | 61 | 72 |

* Outer diameter

About half-way through the experiment it was suggested that the interchanging of the different-sized parts might be contributing to the anomalies in the results. A record was therefore kept of the yield of indole for each distilling unit. Where such data were available the largest yields were invariably obtained from the smaller apparatus. Verification of this was obtained by running known quantities of indole in two distilling units.

TABLE I

PERCENTAGE RECOVERY USING TWO DIFFERENT SETS OF APPARATUS

| *Concentration gamma/ml* | *Percentage Recovery unit A* | *unit B* |
|---|---|---|
| 40 | 96.0 | 88.7 |
| 60 | 91.5 | 90.0 |
| 80 | 97.6 | 94.0 |
| 100 | 95.5 | 93.0 |
| Average | 95.2 | 91.4 |

These differences were found to be statistically significant. It was therefore concluded that the size of the apparatus also has an appreciable influence on the indole yields. Clarke (1938) and Hillig (1942) in their work with volatile fatty acids mention similar difficulties with apparatus.

(3) It is thought that personal error, characteristic of individual investigators, is a factor determining to some extent the results obtained by this technique. However, the consistency of results which were obtained in the present experiments after a certain facility was attained as a result of experience, encourages the belief that this factor can be made insignificant.

***Conclusion:***

Sampling errors, characteristics of the apparatus, type of equipment, personal error and the time involved in distillation, extraction and spectrophotometric procedures prevent the use of the indole method as a universal criterion for shrimp freshness and as a means of rapid detection of spoilage of shrimp.

However, the results of these experiments suggest that indole can be useful as an indicator of spoilage in fresh shrimp, provided that a constant procedure is established and certain precautions are taken to minimize sampling errors. The method can probably be usefully applied within a given laboratory for comparative results.

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## Underwater Television in Commercial Fisheries Research

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THERE ARE NUMEROUS problems in fisheries investigations which might be better answered by direct visual underwater observations rather than by techniques of sampling and analyses. This is particularly true in research in fishery methods and equipments, long hampered by limited access to actual observations of gear in action. Traditionally, fishermen have tended to use proven types of gear. Advances in the design and construction of nets, trawls and other gears have come about largely as the result of trial and error, based upon catch returns. Information obtained by the use of models or divers working with underwater cameras has been helpful and at times rewarding. The operation of such equipment is often difficult and the data obtained cannot always be completely evaluated.

Perhaps the first successful use of underwater television was during the Bikini atom bomb tests by the United States Navy in 1947. Shortly afterward some experiments were carried out in Canada, in the United Kingdom, and in this country at Cornell University. Early in 1951, the British Admiralty using a standard television broadcast unit was able to locate and salvage a sunken submarine. In 1952 the Canadian Wildlife Service applied underwater television to limnological studies in British Columbia.

The initial success with television in marine research prompted the Fish and Wildlife Service to develop equipment for operating fishing gear. This work was assigned to the Service's Exploratory Gear Development Station at Coral Gables, Florida.

Preliminary investigations were conducted in cooperation with the Navy Bureau of Ships, The Marine Laboratory of the University of Miami and