AN ENZYMATIC ASSAY FOR THE MEASUREMENT OF AMMONIA IN SEAFOOD PRODUCTS

Cindy B. Knight and Paul M. Toom
Department of Chemistry
University of Southern Mississippi
Hattiesburg, MS 39401

Organoleptic examination by trained inspectors is the most common method for detecting decomposed fish and fishery products. Although this method is a rapid, inexpensive technique to quickly evaluate seafood quality, it is only semi-quantitative, and hence only estimates the degree of spoilage which has taken place. Since organoleptic examination relies on human sensory organs, such tests are subjective and can vary from one inspector to another.

As early as 1937, Beatty and Gibbons (3) recognized the need for a quantitative method to quantitate decomposition in seafood. These investigators developed a technique to quantitate volatile amines and demonstrated that the concentration of these amines could be correlated to decomposition in seafood. Since the work of Beatty and Gibbons, numerous investigators have developed methodologies for quantitating various nitrogen containing components and have shown that concentrations of these compounds in fishery products can be correlated to the extent of decomposition (2).

During postmortem ice storage of panaeid shrimp, it has been shown that ammonia is produced at the rate of approximately one mg/100g/day (8). This enzymic production of ammonia results in a pH change of from about seven to eight in shrimp muscle (6), which in turn causes an increased production of ammonia, since the pH optimum of the ammonia-producing enzymes is approximately 8.5 (5). In addition, urease activity has also been detected in commercially caught shrimp (7). This enzyme is believed to be of bacterial origin and is probably important in the production of ammonia during the latter stages of ice storage when urea concentrations (another decomposition product) increase (5).

Although much of the ammonia formed is leached from the shrimp, the quantitation of free ammonia has been used as a measure of decomposition, and numerous countries have established limits for ammonia in shrimp (15). This use of ammonia as a measure of decomposition is not limited to shrimp, but rather is widely used as a means to detect and quantitate decomposition in a variety of seafood products ranging from shellfish (4) to dogfish (24).

Various methods for determining ammonia, and hence decomposition in fishery products, have been proposed including Conway's microdiffusion technique coupled with Nesslerization, the Okaloff

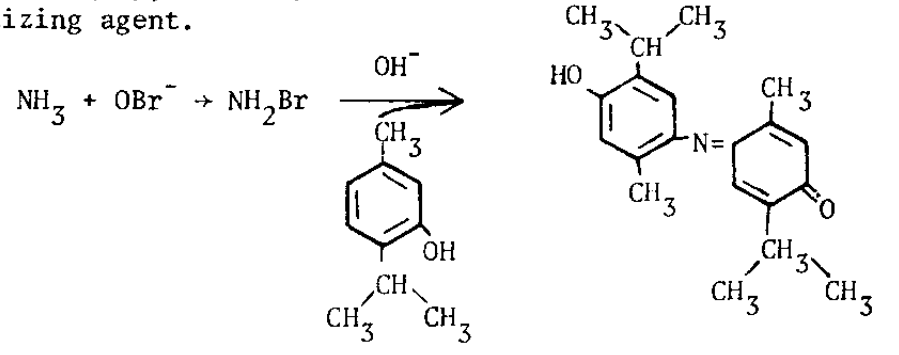
magnesium oxide distillation volumetric method and the Berthalot reaction. Nessler's reagent (potassium mercuric iodide) readily reacts with NH_3 in alkaline conditions to form colloidal dimercuric ammonium iodide. This product is yellow to orange-brown depending on concentration, and hence the amount of ammonia can readily be calculated spectrophotometrically.

$$NH_4^+OH + 2(KI)_2HgI_2 + KOH^- \rightarrow NH_2HgI_3 + 5\ KI + 2\ H_2O$$

However, prior to reaction with Nessler's reagent, free ammonia must first be removed from other amines in the fishery product. A number of techniques have been suggested, all of which rely on a Conway diffusion technique (10) or a similar method for volatilizing and trapping ammonia in the sample (9,24). Hence, the major disadvantage of the method is the time-consuming volatilization of ammonia prior to the Nesslerization reaction.

The Okaloff magnesium oxide distillation volumetric method is recommended by many food standards authorities for the determination of ammonia (15). As the name implies, this procedure relies on the separation of ammonia from interferring substances by distillation of an alcohol/aqueous extract in the presence of magnesium oxide. Titration of the distillate following the addition of formaldehyde completes the quantitation for ammonia (26). Not only is the procedure lengthy (the extraction step itself requires 24 hours) but the multiple titrations in addition to the distillation step each add potential sources of error, resulting in a method of low precision.

The Berthelot analysis for ammonia involves the formation of a blue chromagen (indophenol) when ammonia and aromatic amines react under alkaline conditions with phenol and hypochlorite (14,17). A number of variations using alternate oxidizing agents and various aromatic alcohols have been suggested as preferred modifications of the technique. One such modification was proposed by Burnett (4) for use as an indicator of decomposition in crabmeat. This modification, which subsequently was adopted by the AOAC as the official method for the analysis of ammonia in crabmeat (21), uses thymol as the alcohol and bromine as the oxidizing agent.



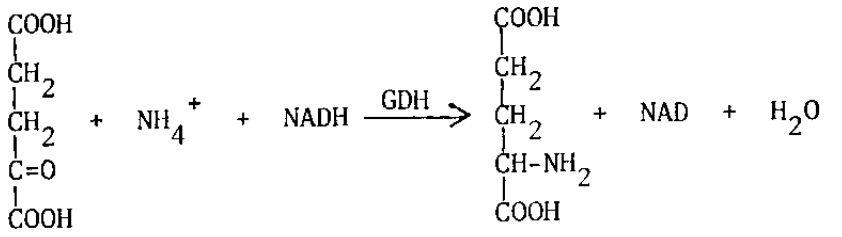
Thymol (Blue)

Due to the lengthy extractions required throughout this procedure,

the requirement for the addition of reagents in small aliquots with mixing between additions and the overall length of the procedure, this method has found only limited use in routine, quality control situations.

The use of enzymes as reagents is an area receiving considerable attention in quality control laboratories associated with both the halth care and food industries (11,22). Unlike the chemical methods which must utilize distillations, extractions, and/or chromatography steps to make a method specific for a specific compound, enzymatic analyses utilize the specificity of selected enzymes to react only with the substance of interest.

The enzyme glutamate dehydrogenase (GDH) has been used to measure ammonia in a number of biological samples (18-20,23). An aliquot of sample to be analyzed is added to a solution of the substrate α-ketoglutarate, the coenzyme NADH and the enzyme GDH. Reaction of the sample ammonia with substrate forms glutamic acid with the simultaneous oxidation of NADH to NAD. The extent of reaction (which is dependent on the amount of ammonia added) is readily measured by following the decrease in absorbance at 340 nm due to the oxidation of NADH.



Although this technique is widely utilized for the analysis of ammonia in both clinical samples and waste materials (18,20), no studies on the potential use of this method for ammonia determinations in food products have been undertaken. It is the purpose of this report to evaluate the potential of this technique as a quality control method for quantitating ammonia in seafood samples.

MATERIALS AND METHODS

For the enzymatic assay, a ten to twelve gram portion of shrimp muscle was weighed to the nearest 0.1 gram, placed in a blender, and homogenized for two minutes with 200 ml of phosphate buffer (0.1 M, pH 7.3). The homogenate was then certrifuged for five minutes at 1000 X G and the supernate used directly as the test sample.

Substrate (2.4 ml), consisting of 3×10^{-3} M α-ketoglutaric acid, 1×10^{-4} M ADP and 1×10^{-4} M EDTA in 0.1 M phosphate, pH 7.3, was added to a 1 cm disposable cuvette and mixed with 0.3 ml of NADH (1×10^{-3} M) and 0.2 ml of supernate to be assayed. The absorbance of the mixture was recorded, and the reaction initiated by the addition of 0.1 ml glutamate dehydrogenase

(110-150 IU/ml). After 15 minutes, the absorbance was once again read and the difference between initial and final readings calculated. A reference solution in which 0.2 ml of buffer replaced the shrimp supernate was run with each set of cuvettes. Standards consiting of 0.05, 0.1, 0.2, 0.3, and 0.4 µg NH_3/0.2 ml were run with each assay, and a standard curve was constructed. Ammonia concentrations of each sample were read from the standard curve and divided by the weight of shrimp to express results as µg NH_3/g shrimp.

The AOAC procedure for analyzing ammonia in crabmeat was followed (1). Twenty grams of shrimp were homogenized in 180 ml of 2.5% phosphotungstic acid and the homogenate filtered through Whatman #1 filter paper. To separatory funnels, 2 ml of sample filtrate, 8.0 ml of deionized/distilled water, 1 ml 2.5 N NaOH, 2 ml thymol and 5 ml bromine solution were added. After thorough mixing, and following a 20 minute reaction time, 20 ml of n-butanol was added, the flasks swirled, and the phases allowed to separate. The aqueous layer was then removed and discarded, and the alcohol layer was passed through anhydrous Na_2SO_4, the absorbance read at 680 nm, and the concentration of NH_3 determined from a standard curve in which ammonia standards had been treated in the same manner.

NADH, α-ketoglutaric acid, ADP, GDH (ammonium sulfate free), EDTA, spermine, spermidine, trimethyl amine and putrescine were all purchased from Sigma Chemical Company, Saint Louis, MO. Ammonium chloride, sodium sulfate, sodium bisulfite, sodium hypochlorite, phosphotungstic acid, butanol, bromine, and thymol were all the products of J. T. Baker.

RESULTS AND DISCUSSION

The selection of an optimum time for incubation of fish extract with substrate and enzyme is critical for an analysis such as this. An incubation time too short to permit complete reaction will not only result in an inaccurate calculation of ammonia concentration, but the precision will also suffer unless all test vials are sampled at the exact time. On the other hand, excessive incubation times severely limit the number of samples which can be processed. As shown in Figure 1, the enzymatic conversion of ammonia to glutamic acid under the assay conditions employed is rapid. Within two minutes, 50% of the ammonia has reacted, while the reaction is complete within eight minutes. A reaction time of 15 minutes was thus selected for all subsequent assays. Not only is this time short enough that numerous assays can be run in a workday, but the seven minute interval between completion of reaction and time of analysis is more than adequate to compensate for day to day variations in enzymatic activity and minor fluctuations in substrate concentrations.

As can be seen in Figure 2, the assay, as described, is linear for ammonia concentrations ranging from 0 to 600 µg/g of sample. Since permissible levels of ammonia if fishery products are between 400 and 500 µg/g, this test will readily differentiate

acceptable from unacceptable products. For those unacceptable samples where ammonia concentrations are calculated to be more than 600 μg/g, a simple 1:2 or 1:4 dilution of sample extract followed by a second enzymatic determination will readily yield a total ammonia concentration.

In addition to ammonia, decomposing seafood also contains elevated levels of other nitrogen containing compounds. These include volatile, primary and secondary amines, as well as numerour amines arising from the decarboxylation of amino acids. Thus, it is important that any assay for ammonia in seafood not only be specific for ammonia, but also not be inhibited by these other nitrogen containing compounds. As shown in Figures 3 and 4, a number of nitrogen containing compounds typically found in decomposing seafood were added to spiked shrimp extracts prior to analysis. Although concentrations far in excess of naturally occurring concentrations of these compounds were used, none of the added amines produced results significantly different from the spiked ammonia samples. Thus, the assay is specific for ammonia, and other amines present in the sample do not interfere with the analysis.

It is common for shrimpers to add sodium bisulfite to their catch to retard black spotting (melanosis) (25). Thus, any assay for ammonia in shrimp must be insensitive to this additive. While the Codex International Standard for quick frozen shrimp has recommended a tolerance of not more than .003% total SO_2 in shrimp (12), a one minute dip in a 1.25% $NaHSO_3$ solution is the recommended application technique (13). As shown in Figure 5, extracts containing 0.15% $NaHSO_3$ did not interfere with the assay at any ammonia concentration. However, higher concentrations of $NaHSO_3$ (1.25% and 5%) did partially inhibit the enzyme, resulting in calculated ammonia values approximately 10% below controls. Although instances of shrimp containing such high $NaHSO_3$ levels are extremely rare, this inhibition can be overcome by the addition of 0.05% hypochlorite to the sample extract as shown in Figure 5. It should be pointed out that excessive treatment of shrimp with $NaHSO_3$ severely affects the appearance of the shrimp. Hence, the addition of hypochlorite to the shrimp extract would be suggested by their physical appearance prior to processing.

The expected precision of the assay is presented in Table 1. As can be seen from the table, multiple analysis of a homogeneous extract of an acceptable shrimp extract results in a coefficient of variation of under 7%. Analysis of five different, clearly decomposed samples still produced a coefficient of variation of well under 15%.

TABLE 1

Precision of Assay

	NH_3 Concentration (µg/g shrimp)		
	Mean	Standard Deviation	CV %
Single Sample (n = 10)	165	11	6.7
Multiple Samples (n = 5)	1163	152	13.0

In order to compare the enzymatic method to the present AOAC method, shrimp containing various levels of ammonia were required. To obtain such samples, shrimp were placed on ice and samples removed and assayed by both the AOAC and enzymatic methods every two days for a month. As shown in Figure 6, a correlation coefficient of 0.86 was obtained between the two methods. However, as the figure illustrates, ammonia concentrations were consistently 15-25% lower with the enzymatic assay. This was not unexpected, however, since other investigators have demonstrated that, unlike the enzymatic assay, the AOAC method is not specific for ammonia. Thus, the enzymatic method would appear from this study to be the more accurate of the two methods.

CONCLUSIONS

Use of the enzyme glutamate dehydrogenase for the quantitation of ammonia in seafood products appears to overcome many of the limitations of techniques presently used for such analyses. The method is fast, reproducible and does not require sophisticated instrumentation or techniques. In addition, unlike other methods, the enzymatic technique is specific for ammonia and other decomposition products and food additives do not interfere with the assay.

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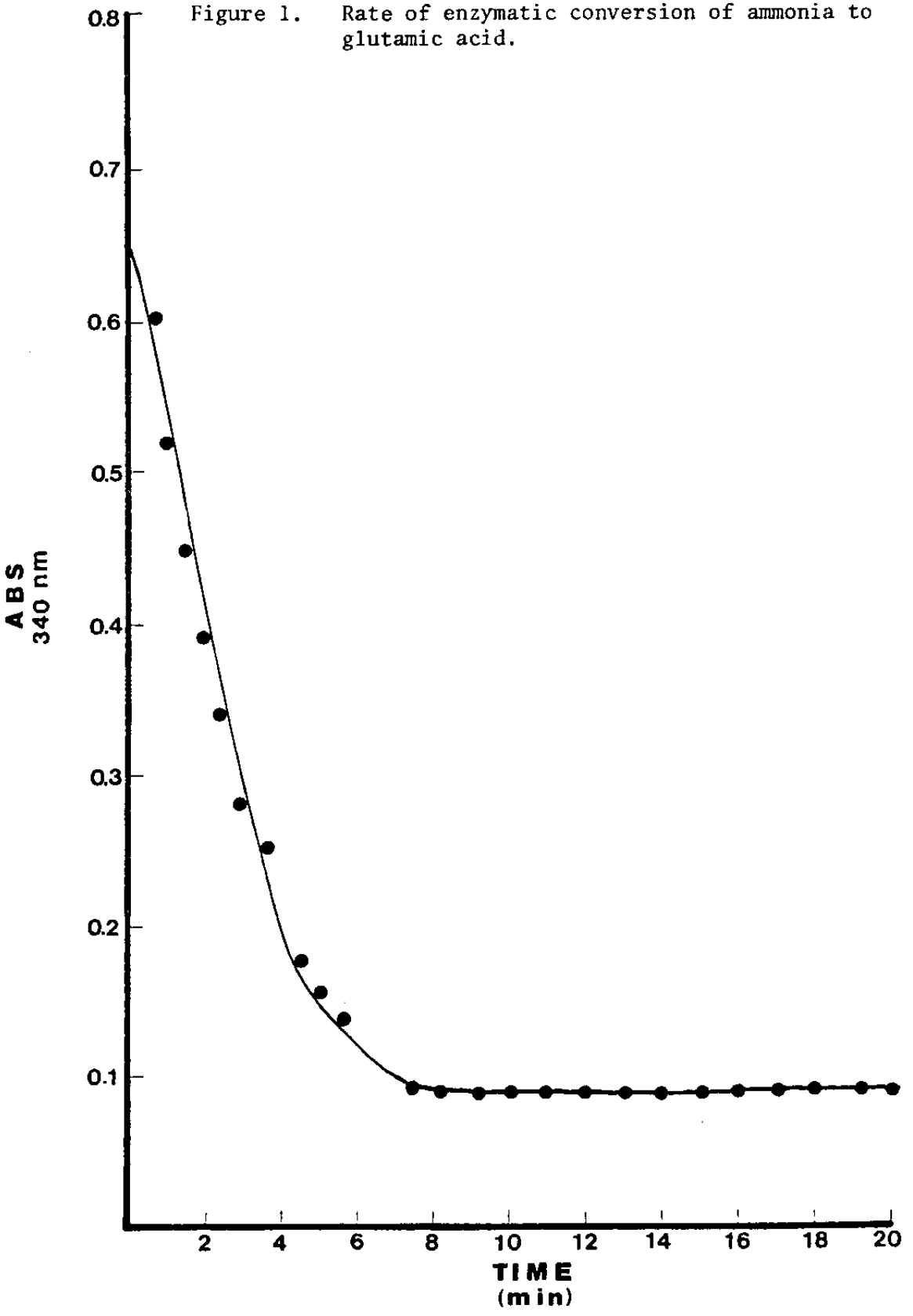
Figure 1. Rate of enzymatic conversion of ammonia to glutamic acid.



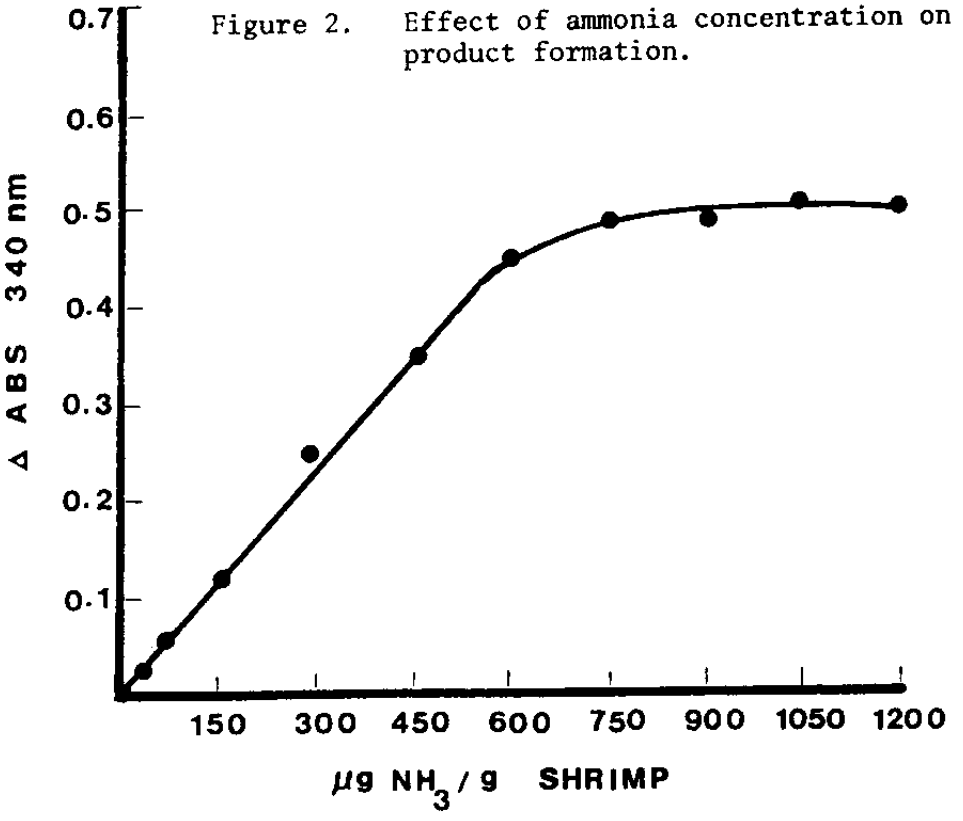
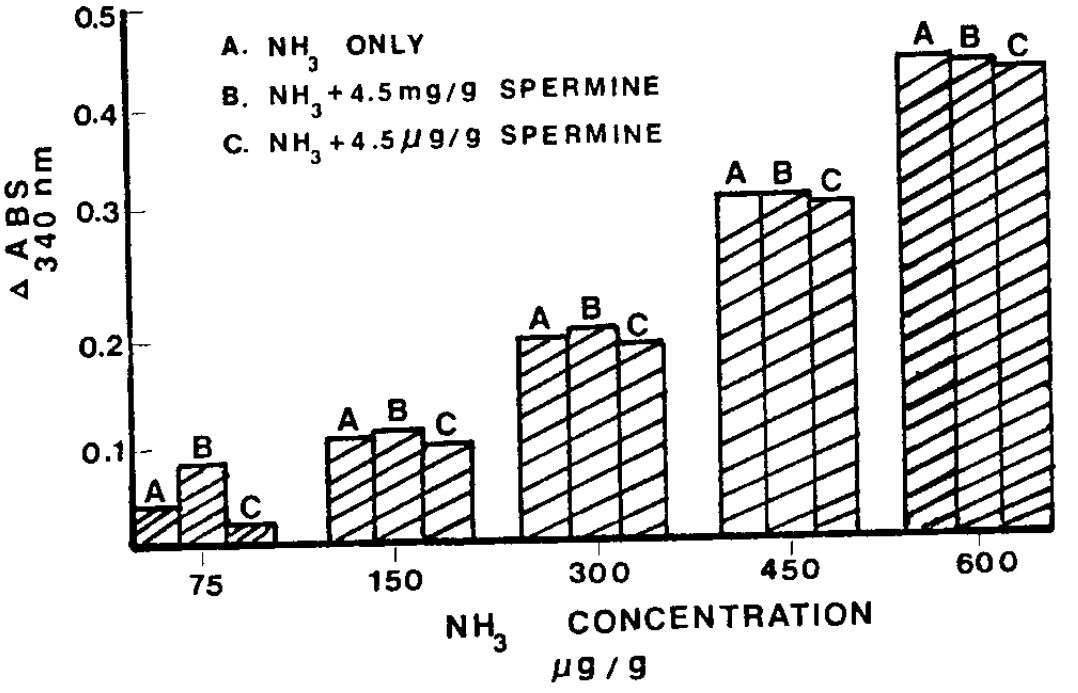
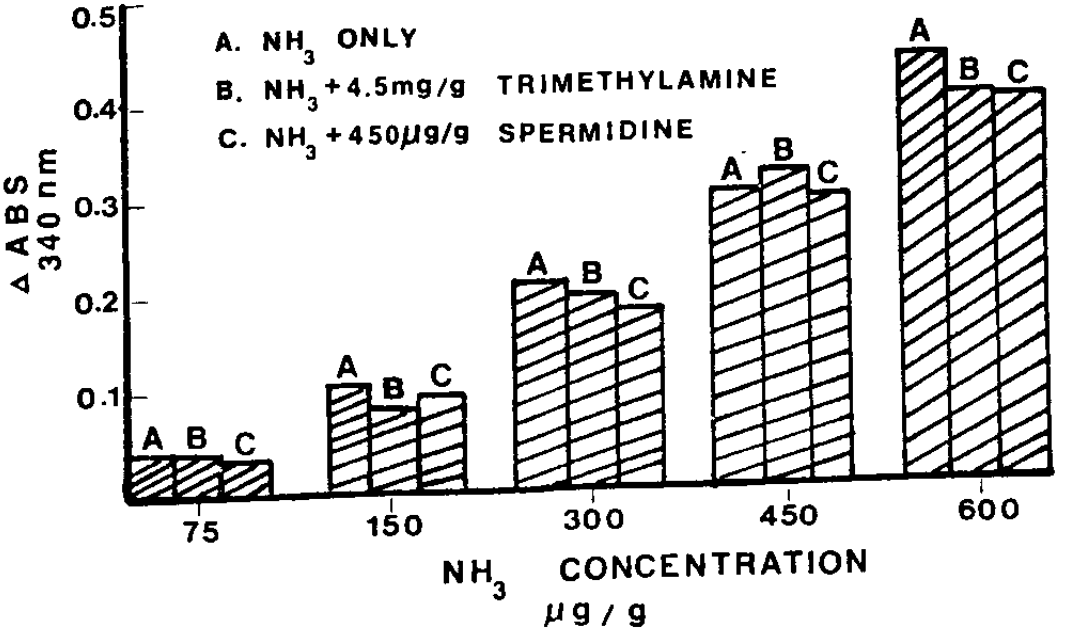


Figure 2. Effect of ammonia concentration on product formation.

Figure 3. Effect of naturally occuring amines on enzymatic determination of ammonia.





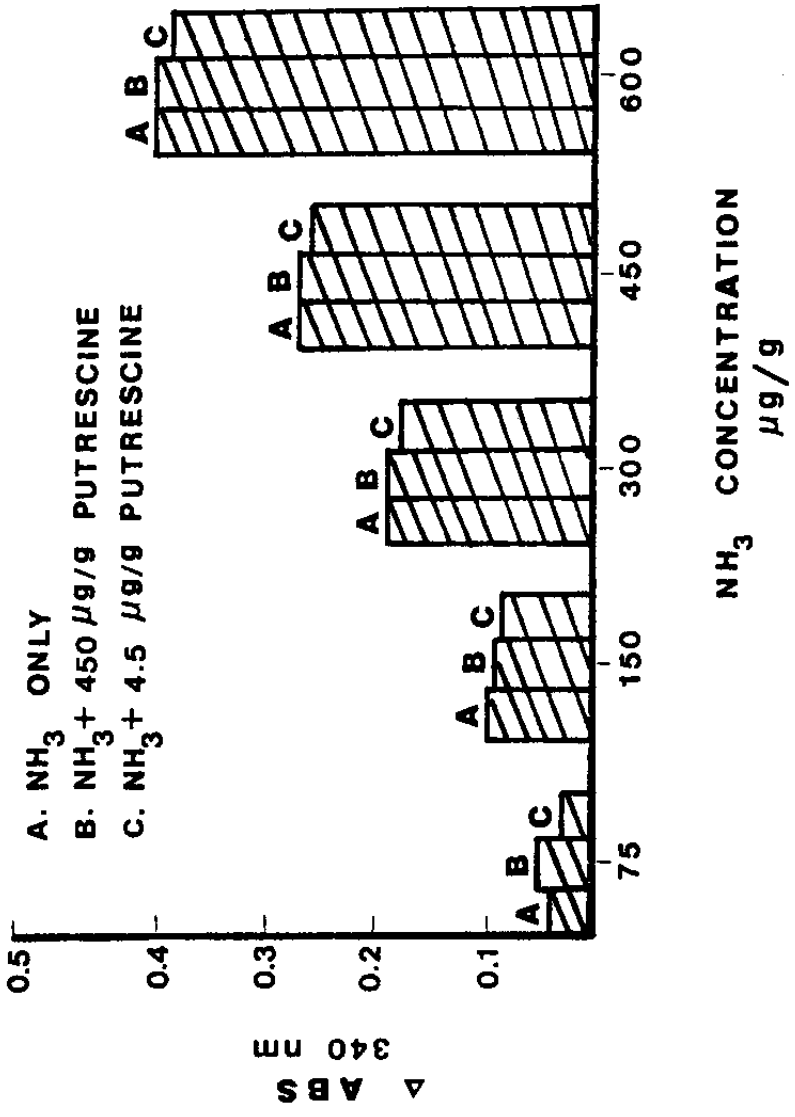
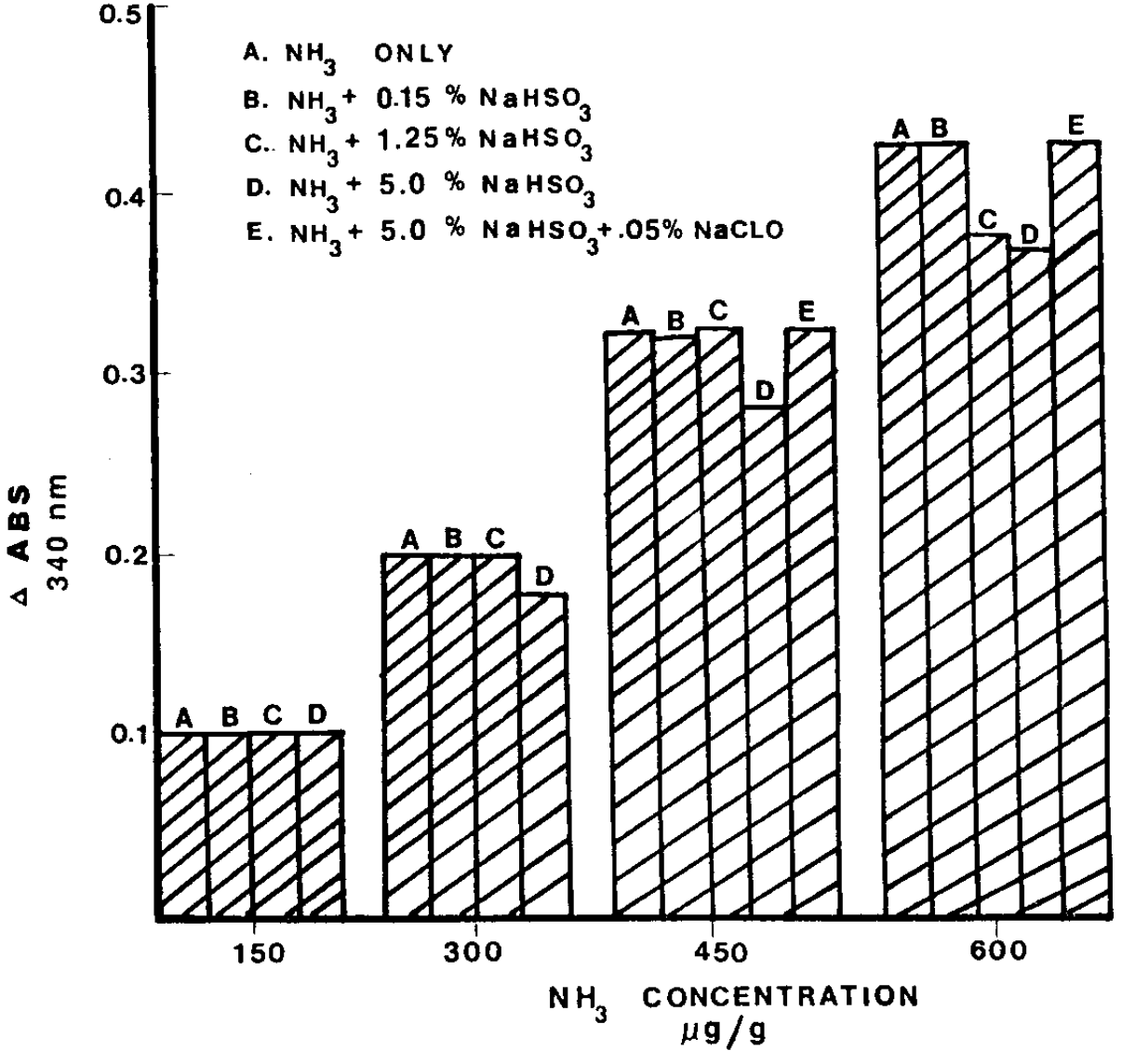


Figure 4. Effect of putrescine on enzymatic determination of ammonia.

Figure 5. Effect of added sodium bisulfite on enzymatic determination of ammonia.



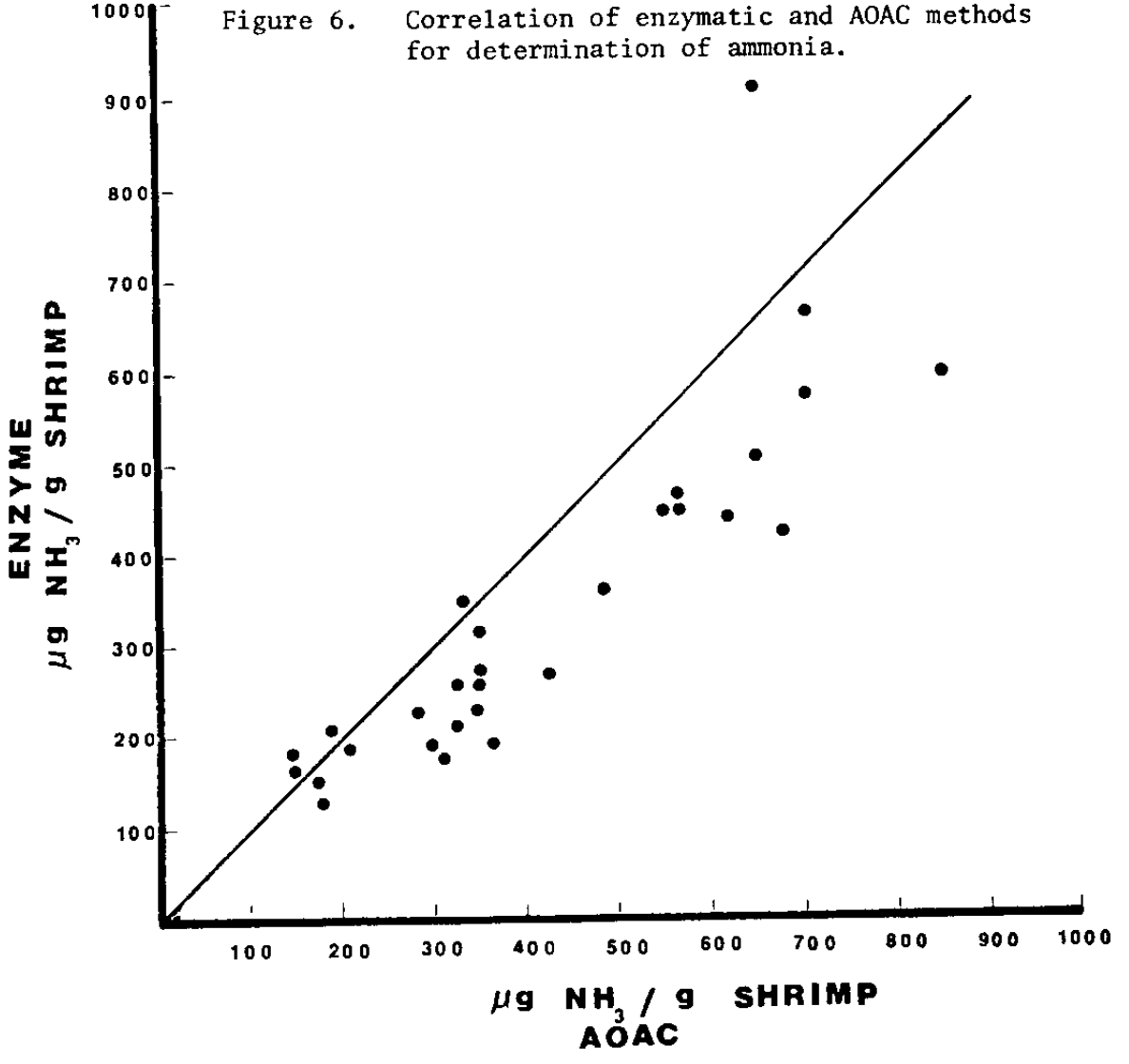


Figure 6. Correlation of enzymatic and AOAC methods for determination of ammonia.

DETERMINATION OF PHOSPHOROUS IN SHRIMP TREATED WITH SODIUM TRIPOLYPHOSPHATE

Vickie Tenhet, Gunnar Finne, Ranzell Nickelson II and Don Toloday*
Seafood Technology Section
Animal Science Department
College Station, Texas 77843
and Singleton Packing Corporation*
Tampa, Florida 33601

Polyphosphates are being used extensively by the seafood industry for both fresh and frozen products. Among many advantages claimed, the most important reasons for using polyphosphates include: limited weight loss during frozen storage, less drip loss, less toughening of the product when stored in the frozen state and less loss in weight in cooking (Ellinger, 1972). Since polyphosphates, according to FDA, (Food and Drug Administration) have GRAS (Generally Recognized As Safe) status, it is up to the individual processor to formulate treatment mixtures and application techniques.

To determine the mechanism of action of phosphates on foods, it is essential that added phosphates be accurately determined at a later stage. For many proteinacious foods such as meats, poultry, processed cheese, and seafoods this is difficult because of the high and variable endogenous phosphate levels. These foods contain phosphorous in the form of nucleotides, phospholipids, etc. together with naturally occuring orthophosphates. Recommended methods for the analysis of phosphorous in foods involve a nitric acid digestion which will convert total phosphorous into orthophosphate which subsequently is determined. With varying background levels, added phosphates are thus difficult to determine accurately.

The objectives of this study were to: (a) determine the phosphorous content in peeled and deveined shrimp after different STP treatments, (b) determine the stability of STP during treatments and subsequent frozen storage using a P^{32} labeled STP isotope.

METHODS AND MATERIALS

Phosphorous Determination

Alkalimetric. Brown shrimp, (Penaeus aztecus), frozen as shell-on tails (green-headless) in 5 pound boxes, were used for this portion of the study. The shrimp were thawed, peeled and deveined prior to

polyphosphate treatment. Aqueous sodium tripolyphosphate (STP) solutions were prepared using purified granular STP (Fisher Scientific Company). Solutions of 0.5%, 1.0%, 5.0% and 12.0% were employed and dip times at each concentration were 20 sec, 1 min, 5 min and 20 min. Shrimp from each of the 16 different treatments were digested in nitric acid and analyzed for phosphorous according to the alkalimetric ammonium molybdophosphate method (AOAC, 1970). Untreated shrimp were analyzed as controlled samples.

Spectrophotometric. Shrimp used in this phase of the study were of the same species and size as those above. In this case, however, shrimp were obtained fresh from shrimp trawlers, put on ice and transported to the laboratory in College Station. The shrimp were divided into two lots of which one was peeled, deveined and polyphosphate treated immediately while the other was frozen as green-headless in 5 pound boxes. After two weeks of storage at -26°C, the shrimp were thawed in running cold water, peeled, deveined and polyphosphate treated. The phosphate treatments were the same as described for the alkalimetric method. After treatment, shrimp were digested in concentrated nitric acid and the digests determined for phosphate according to the method of Halmann (1972) using a Perkin-Elmer Coleman 124 double beam spectrophotometer with untreated shrimp as reference cell blank.

Hydrolysis of Sodium Tripolyphosphate

To determine the stability of STP in frozen shrimp during storage, peeled and deveined fresh brown shrimp were soaked in a 10% P^{32} labeled STP isotope solution for 20 minutes according to the method of Tenhet et al. (1980). After treatment, the shrimp were frozen in plastic bags and held at -26°C. After two weeks of storage, two 50 g portions were removed from the freezer and thawed at room temperature. One portion was blended in a Waring blender with 100 ml of a 7% trichloroacetic acid solution while the other was blended with 100 ml of distilled water. The slurries were centrifuged at 5000 x g for 20 min. at 4°C and the supernatants analyzed for phosphates using thin layer chromotography (TLC). Ascending chromatography was carried out on 20 x 20 cm glass plates coated with cellulose MN 300 HR (Machery Nagel Co.) using a mixture containing 150 ml isopropanol, 125 ml H_2O, 100 ml ethanol, 75 ml n-propanol, 50 ml n-butanol, 10 g trichloroacetic acid, and 0.5 ml concentrated ammonia (0.88) as irrigant. This is a modified compositon of one reported by Gibson and Murray (1973) and another suggested by Stahl (1969). After 10 min heat activation at 110°C the cooled plates were prepared by spotting a band of extract across the entire plate. To determine correct Rf values, two standard plates each spotted with 5ul of a 2% STP solution, a 2% sodium pyrophosphate solution and a 2% sodium orthophosphate solution were run concurrently with the active phosphate analysis. One of the two standard plates was developed using the ammonium molybdate-stannous chloride method described by Gibson and Murray (1973) while the other was sprayed with the phosphate stain suggested by Kates (1972). The two plates containing the isotope extracts were dried but not color developed. One centimeter bands of coating, beginning at the solvent front and working downwards, were

scraped off across the entire plate and collected in scintillation vials. Thirteen one centimeter fractions were collected from each plate and Rf values are reported as the mid-point of each fraction. After additon of scintillation fluid and thorough mixing, each fraction was counted for ten min using a Packard-Tri Carb Liquid Scintillation Spectrometer Model 3330.

All subsequent TLC analyses related to frozen storage stability of STP were performed on water extracted samples. These samples were analyzed after five, eight and ten weeks of frozen storage of the treated shrimp.

RESULTS AND DISCUSSION

Phosphorous Determination

The phosphorous concentration, reported as P_2O_5, in the first set of shrimp treated with STP determined by the alkalimetric AOAC method are shown in Table 1. The data indicates that when treating shrimp with diluted STP concentrations (0.5% and 1.0% STP dips) the phosphate uptake is very low. In only two of eight treatments at these concentration levels did the phosphorous concentrations in treated shrimp exceed the range found in untreated samples. The wide phosphorous range found in the control samples is in agreement with the data by Sidwell et al. (1977). In a review article they reported an average phosphorous level of 239 mg P/100 g shrimp and prawns (mixed species) with a range from 127 mg P/100 g to 912 mg P/100 g shrimp based on 44 analyses. Reported as percent P_2O_5 these values correspond to an average of 0.55% P_2O_5 with a range of 0.29% to 2.1% P_2O_5. Bailey et al. (1956) showed that acid soluble orthophosphate dropped during storage on ice from approximately 650 mg $PO_4^{\equiv}$/100g shrimp after two days to 400 mg $PO_4^{\equiv}$/100g shrimp after 14 days. Reported as P_2O_5 the data indicate a drop from 0.49% P_2O_5 to 0.30% P_2O_5 during storage on ice.

The low polyphosphate uptake on low treatment concentrations is in agreement with the findings of Tenhet et al. (1980; paper in press) who showed low polyphosphate penetration into shrimp muscle during sodium tripolyphosphate treatments at 0.5% and 1.0% STP. Low polyphosphate uptake after treatment of fish fillets was also demonstrated by Gibson and Murray (1973).

The phosphorous concentrations in the second set of shrimp treated both fresh and prefrozen with STP are shown in Tables 2 and 3. A sample of untreated shrimp was in this case used as instrument blank achieving automatic corrections for natural background phosphorous levels. Even though the polyphosphate uptake in the second set of shrimp was higher than in the first set, it was again evident that polyphosphate uptake at weak dip concentrations was bery low. No significant difference in polyphosphate uptake between fresh and prefrozen shrimp was determined.

Thin layer chromatography of phosphates

Shrimp treated with a P^{32} labeled STP isotope were kept in frozen storage at -26°C and a first sample was removed after two weeks. To determine the effect of extraction media on the polyphosphates present in the shrimp, the sample was divided into two equal parts. One part was extracted with 7% TCA and the other with distilled water. Immediately after extraction and centrifugation, the samples were analyzed by thin layer chromotography. Standard phosphate solutions were analyzed simultaneously with the shrimp extracts.

The thin layer chromatography (TLC) system separated the phosphates well. Rf values obtained from the developed standard plates were: 0.32 for tripolyphosphate, 0.47 for pyrophosphate and 0.71 for orthophosphates. For the pyrophosphate standard, a faint spot could be seen at the Rf value of orthophosphate and for the tripolyphosphate faint pyrophosphate and orthophosphate spots were evident. This indicates that some hydrolysis of the condensed phosphates will occur during the TLC analysis.

Although color development of the phosphates worked well at the high concentrations used on the standard plates, the method was not sensitive enough for analyzing added phosphates in treated shrimp. At treatment levels used in these experiments only faint spots could be seen after extraction and TLC analysis. Using a STP isotope makes the TLC method sensitive and low levels of phosphates could be detected. Figures 1 and 2 show the radioactive distribution of TCA and water extracted samples respectively. For the TCA extracted samples two distinct Rf values of 0.47 and 0.75 indicate complete hydrolysis of STP to pyro and orthophosphate. This was not unexpected since polyphosphates undergo hydrolysis under acid conditions. In the water extracted sample on the other hand, three high activity Rf values could be detected on the TLC plates (Figure 2). These three peaks represented according to Rf values from the standard plates: STP, pyrophosphate and orthophosphate.

The total plate activity of the TCA extracted samples was 962 counts/min as compared to a total plate activity of 1074 for the distilled water extracted sample. Since sample size, extraction and spotting values were the same for the two samples, there was apparently little difference in the phosphate extraction efficiency between the two solvents used.

To determine the stability of STP in frozen treated shrimp over a prolonged time interval, samples were analyzed after five, eight, and ten weeks of frozen storage. The samples were extracted with distilled water and the extracts analyzed by TLC and scintillation counted as described above. Figure 3 shows the distribution of active phosphates after different storage periods. As indicated, there was an initial drop in STP down to approximately 12% of total activity during treatment and the first two weeks of storage. During the same time, pyrophosphate increased to 23% and orthophosphate to 27%. During

continued frozen storage STP appeared to remain fairly constant at around 12% while pyrophosphate dropped down to 10% after 5 weeks and 2% after 10 weeks. Orthophosphate increased gradually and showed 45% of total activity after 10 weeks of frozen storage.

Activity observed on the plates at unidentifiable Rf values most likely represents phosphates tied up with compounds present in shrimp. This was specifically evident after 10 weeks of frozen storage where 15% of total activity was found to be completely immobile at Rf = 0. This immobile fraction could well represent phosphates complexed with high molecular water soluble proteins.

CONCLUSION

Because of the natural variation in the phosphorous level in shrimp and the breakdown of STP during treatment and frozen storage, it is not possible to determine STP added to shrimp. The study has shown however, that at high treatment concentrations there is enough of an increase in phosphorous so that potential overtreatments can be detected.

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Table 1.

Phosphorous in treated shrimp as determined by alkalimetric method.

% STP in treatment solution	% phosphorus (reported as P_2O_5) in shrimp muscle			
	Time of Treatment			
	20 sec	1 min	5 min	20 min
0.5	0.78	0.80	0.59	0.59
1	0.66	0.67	0.57	0.49
5	0.57	0.72	1.42	1.70
12	0.91	1.28	1.49	1.61

Note: Untreated shrimp gave a value of 0.57% P_2O_5 with a range for five samples of 0.41% to 0.76%.

Table 2.

Phosphorous in treated fresh shrimp as determined by spectrophotometric method.

% STP in treatment solution	% phosphorus (reported as P_2O_5) in shrimp muscle			
	Time of Treatment			
	20 sec	1 min	5 min	20 min
0.5	0.27	0.32	0.53	0.42
1	0.47	0.52	0.27	0.23
5	0.56	0.65	1.10	1.37
12	1.03	1.35	2.43	2.37

Table 3.

Phosphorous in treated prefrozen shrimp as determined by spectrophotometric method.

% STP in treatment solution	% phosphorus (reported as P_2O_5) in shrimp muscle			
	Time of Treatment			
	20 sec	1 min	5 min	20 min
0.5	0.56	0.64	0.37	0.10
1	0.52	0.47	0.53	0.45
5	0.79	1.09	1.40	1.80
12	1.19	1.75	1.76	2.70

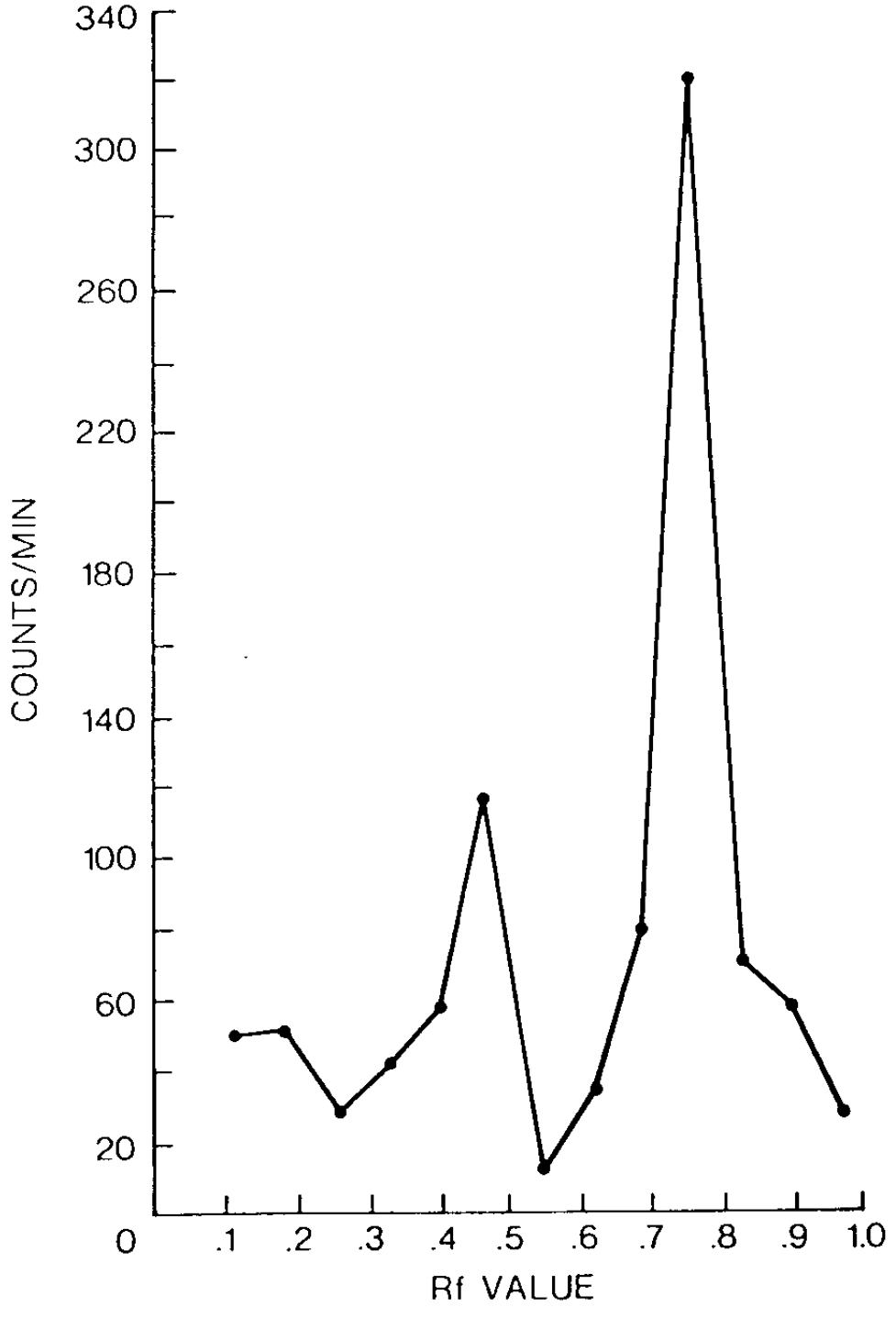

Figure 1. Distribution of activity on TLC plate for TCA extracted sample.

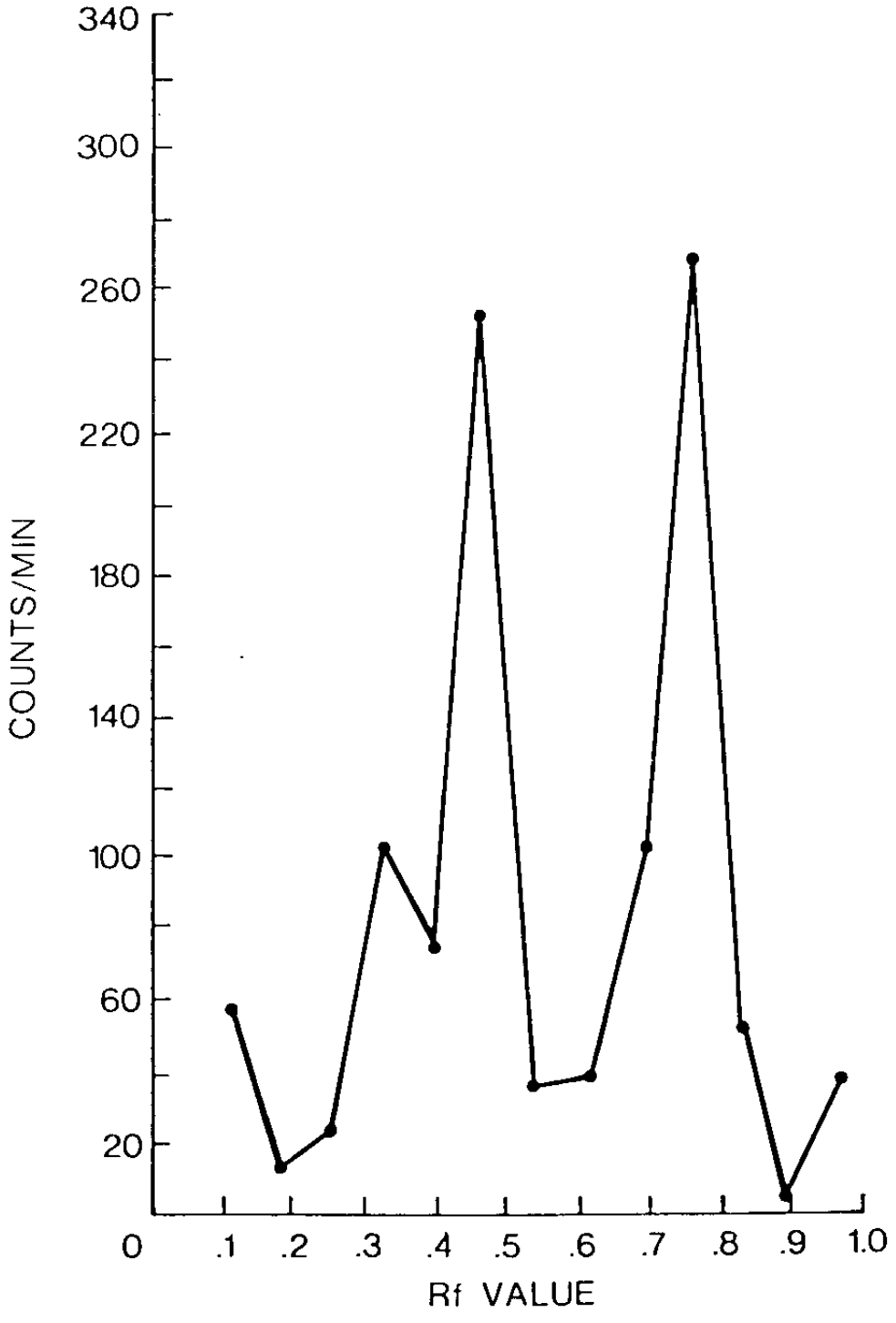

Figure 2. Distribution of activity on TLC plate for distilled water extracted sample.

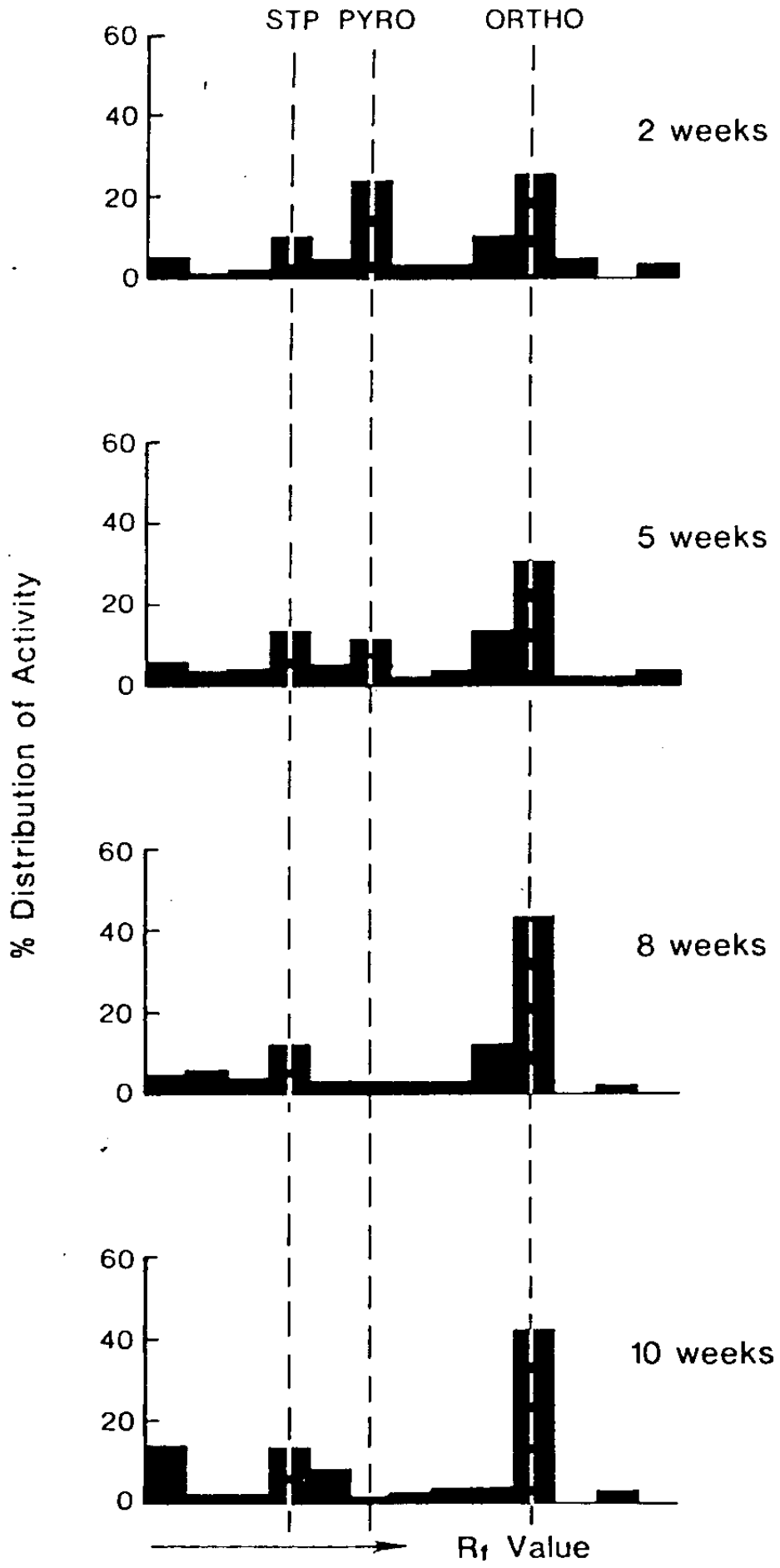

Figure 3. Distribution of activity on TLC plates as a function of frozen storage time.

TEXTURAL VARIABILITY IN FISH FILLETS

E. A. Johnson[1], R.A. Segars[2], J.G. Kapsalis[2], M.D. Normand[1] and M. Peleg [1]

[1]Department of Food Engineering
University of Massachusetts
Amherst, MA 01003

[2]U.S. Army Research and Development Command
Natick, MA 01760

INTRODUCTION

The growing cost of marine food products has created potential markets for fish species that have been underutilized. The U.S. Department of Commerce has initiated studies that will create the data basis for establishing policies and priorities regarding the introduction of new species to the domestic market. One such study focuses on the quality and consumer acceptability of such "new" fishes as compared with traditional and popular species. One of the more specific objectives of this project has been to monitor and evaluate the textural components of fish quality . This has been done through organoleptic assessment by a trained tasting panel and through objective mechanical analysis based on compression tests. The latter has introduced some unusual procedural and interpretation problems. The reason is that obtaining proper specimens for mechanical analysis is frequently very difficult. Even when tests can be performed,at acceptable degree of accuracy, there are still interpretation problems that are associated with the possibly inherent error of the test vis a vis the natural textural variability within and among the different fillets. In this report, this problem of variability is addressed and its implications assessed.

MATERIALS AND METHODS

Refrigerated fillets of a variety of fish species were brought to the laboratory within 24 hours after commercial filleting. Some of the fillets were cooked in plastic bags, submerged in a hot water bath, with circulating water at a temperature of 69°C. The process was stopped when the center temperature reached 65°C. The required cooking time was 6 to 35 minutes

depending on the thickness of the fillet. The samples were then removed from the bath and left to cool at ambient temperature.

Specimens of both raw and cooked fillets were carefully cut using an electric carving knife. During cutting the surrounding tissues were held in place by a device constructed of a dense array of needles which guided the knife and at the same time prevented the disintegration of the outer wall of the specimen. The specimens were always cut in a direction perpendicular to the fillet plane regardless of the local orientation of the flakes.

The cross-sectional area of each specimen was 4x4 cm and the height varied between 0.8 to 3 cm depending on the species.

The compressive deformation tests were performed by an Instron Universal Testing Machine equipped to provide force deformation output in a digital form. The data were converted to true stress-strain relationships from which the deformability modulus was calculated. The calculation procedure is described below.

Calculation of the Modulus

The apparent modulus of deformability M (Fig. 1) is defined as:

$$M_{APP} = \frac{\sigma_T}{\varepsilon_T} \qquad (1)$$

where σ_T and ε_T are the true stress and strain respectively.

For an incompressible material and with the assumption that the specimen retains its general shape during deformation:

$$\sigma_T = \frac{F(t)}{A(t)} = \frac{F(t)\,[H_o - \Delta H]}{A_o\ H_o} \qquad (2)$$

where F(t) is the force, A(t) the actual cross-sectional area, A_o and H_o are the original cross-sectional area and height of the undeformed specimen and ΔH the absolute deformation.

The true compressive strain under these conditions is:

$$\varepsilon_T = \ln \left[\frac{H_o}{H_o - \Delta H} \right] \qquad (3)$$

Since the specimens, for obvious reasons, were rather flat in shape the apparent moduli, as calculated by Eq. 1, were supposed to depend not only on the tissue properties but also on the dimensions of the specimens. This phenomenon, well recognized in mechanics, makes it necessary to correct the calculated values for the characteristic shape effects. This was done by adapting the method described by Lindley (1) which was originally developed for rubbery materials. According to this method, the corrected or independent modulus (M_{CORR}) is calculated from:

$$M_{CORR} = \frac{M_{APP}}{1+2kS^2} \qquad (4)$$

where k is a constant that is related to the hardness of the specimen and S is the specimen shape factor. The magnitude of S is determined from the ratio between the loaded area and the free (lateral) area. Since all the reported specimens had a 4x4 cm loaded area, the shape factor was:

$$S = \frac{4x4}{2(4+4)H_o} = \frac{1}{H_o} \qquad (5)$$

where H_o is the initial thickness of the specimen.

RESULTS AND DISCUSSION

The Shape of the Stress-Strain Curve

Schematic representation of prefailure stress relationships of raw and cooked fish fillets are shown in Figure 1. The figure demonstrates that up to a certain strain, usually in the range of 20 to 40%, the relationship was linear, supported by correlation coefficients of 0.95 to 0.99 as reported by Johnson et al. (in press). This enabled the calculation of the apparent deformability modulus from the slope of the straight line for each individual specimen.

The correction of the modulus magnitude by Eq. 4 requires knowledge of the hardness of the specimen. (Hardness in this context is the resistance to penetration which is not identical to the deformability modulus). Since this parameter was not measured, and it is still an open question whether it can meaningfully be determined in materials like fish flesh, the correction procedure was applied using three levels of k covering the whole hardness range as reported in Ref. 1.

Typical values of the apparent and corrected moduli for fillets of three fish species are demonstrated in Tables 1 to 3. (Compiled results of a variety of species have been shown during the oral presentation of this paper).

Analysis of the data revealed that the variability of the moduli among and between fillets is real and not a result of experimental artifacts resulting from specimen shape differences and imperfections. Although these certainly do contribute to the variability to some extent they cannot account for such large differences, especially in the corrected moduli. Similarly, cooking of the fillets did not produce consistent results with regard to the magnitude of the deformability moduli. In some cases, considerable softening has been observed while in other cases the texture has become firmer. Again, only part of the phenomenon can be attributed to variations in the time-temperature histories of the cooked fillets that resulted from their dimensional variability. The irregularity with regard to the overall trend is an indication that the individual fish were in different states from a biochemical standpoint (3,4).

It is interesting to note, however, that from a mechanical point of view all the cooked specimens demonstrated the same deformation pattern which was clearly distinct from that of the raw specimen. This is clearly expressed in the shape of the true stress-strain curve continuation after the end of the linear region (Fig. 1).

The concave downwards shape in the case of cooked fillets is an indication of a progressive disintegration of the tissue as a result of fibers and flakes separation and breakdown. In the raw fillets the concave upwards continuation is most likely the result of hydrostatic pressure buildup as well as some compressibility of the tissue itself.

CONCLUSIONS

A method for monitoring and evaluating textural variability in fish fillets has been demonstrated. Its application revealed that natural mechanical variations in commercially available fillets is considerably large within and among specimens. Mainly this is due to biochemical factors resulting from seasonal, and feeding differences. It is also most likely, however, that the age and postmortem history of the fish are also major factors. The latter is also true with regard to the filleting operation itself that introduces differences in the mechanical history of the various regions of the fillet.

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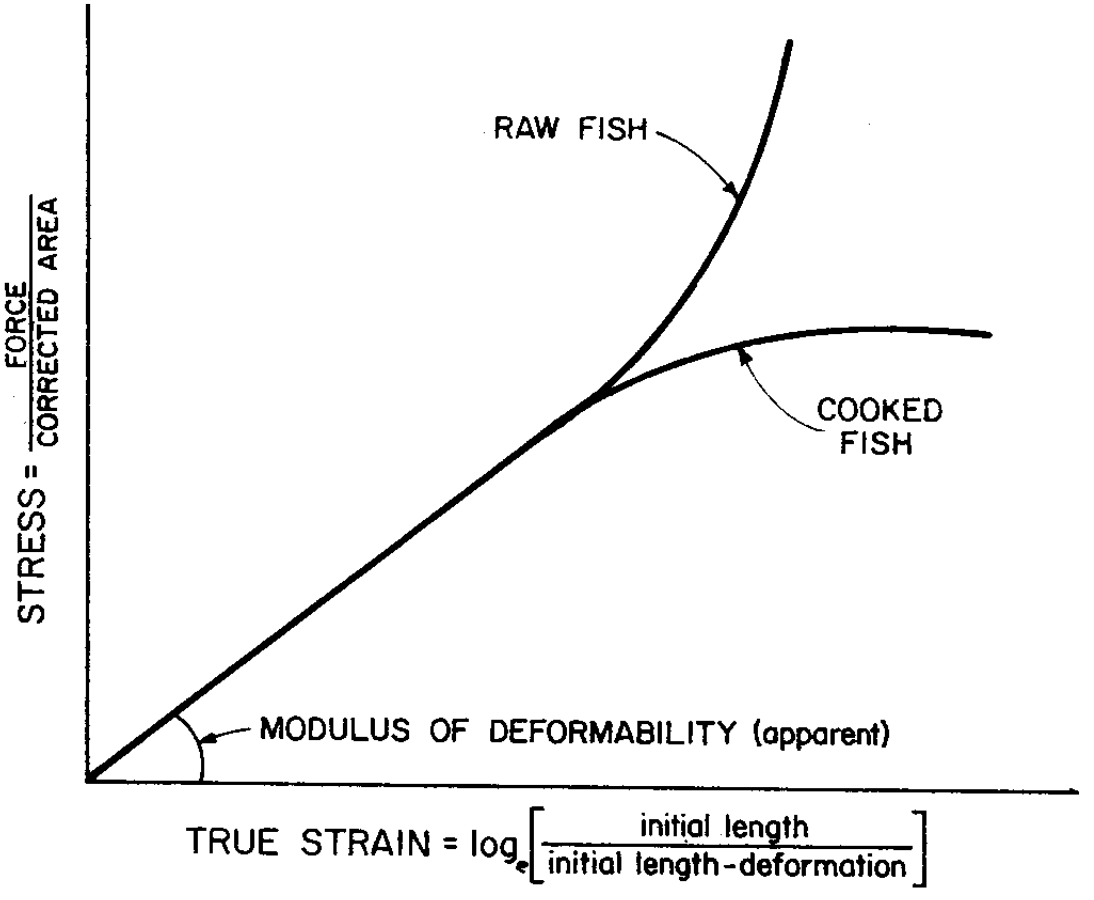

Fig. 1 Compressive stress-strain relationships of fresh and cooked fish fillets (schematic view).

State	Specimen Location	Apparent Modulus ($N.cm^{-2}$)	Shape Factor S	Corrected Modulus ($N.cm^{-2}$)		
				k=0.5	k=0.7	k=0.9
Raw	front	0.86	0.35	0.77	0.73	0.70
Raw	front	1.08	0.39	0.94	0.89	0.84
Raw	front	0.66	0.45	0.55	0.51	0.48
Cooked	front	1.59	0.63	1.14	1.02	0.92
Cooked	middle	1.11	0.55	0.85	0.78	0.72
Cooked	front	0.97	0.56	0.73	0.67	0.62

Table 1. Compressive deformability moduli of fresh and cooked bluefish fillets.

State	Specimen Location	Apparent Modulus ($N.cm^{-2}$)	Shape Factor S	Corrected Modulus ($N.cm^{-2}$)		
				k=0.5	k=0.7	k=0.9
Raw(I)	front	4.53	0.29	4.18	4.06	3.94
	middle	4.78	0.67	3.31	2.94	2.65
Cooked(I)	front	2.64	0.35	2.36	2.26	2.17
	middle	2.09	0.41	1.79	1.69	1.61
	tail	1.87	0.45	1.56	1.46	1.37
Raw(II)	front	2.08	0.36	1.84	1.76	1.68
	middle	1.69	0.46	1.39	1.30	1.22
Cooked(II)	front	2.80	0.41	2.39	2.26	2.14

Table 2. Compressive deformability moduli of fresh and cooked fillets from same individual codfish.

State	Specimen Location	Apparent Modulus ($N.cm^{-2}$)	Shape Factor S	Corrected Modulus ($N.cm^{-2}$)		
				k=0.5	k=0.7	k=0.9
Raw	front	1.20	0.53	0.94	0.86	0.80
Raw	front	2.24	0.51	1.79	1.65	1.54
Raw	front	2.08	0.28	1.92	1.87	1.82
Raw	middle	1.71	0.42	1.46	1.37	1.30
Cooked	front	2.15	0.60	1.59	1.44	1.31
Cooked	front	1.92	0.60	1.42	1.28	1.17

Table 3. Compressive deformability moduli of fresh and cooked hake fillets.