Abstracts of Methods Used to Assess Fish Quality

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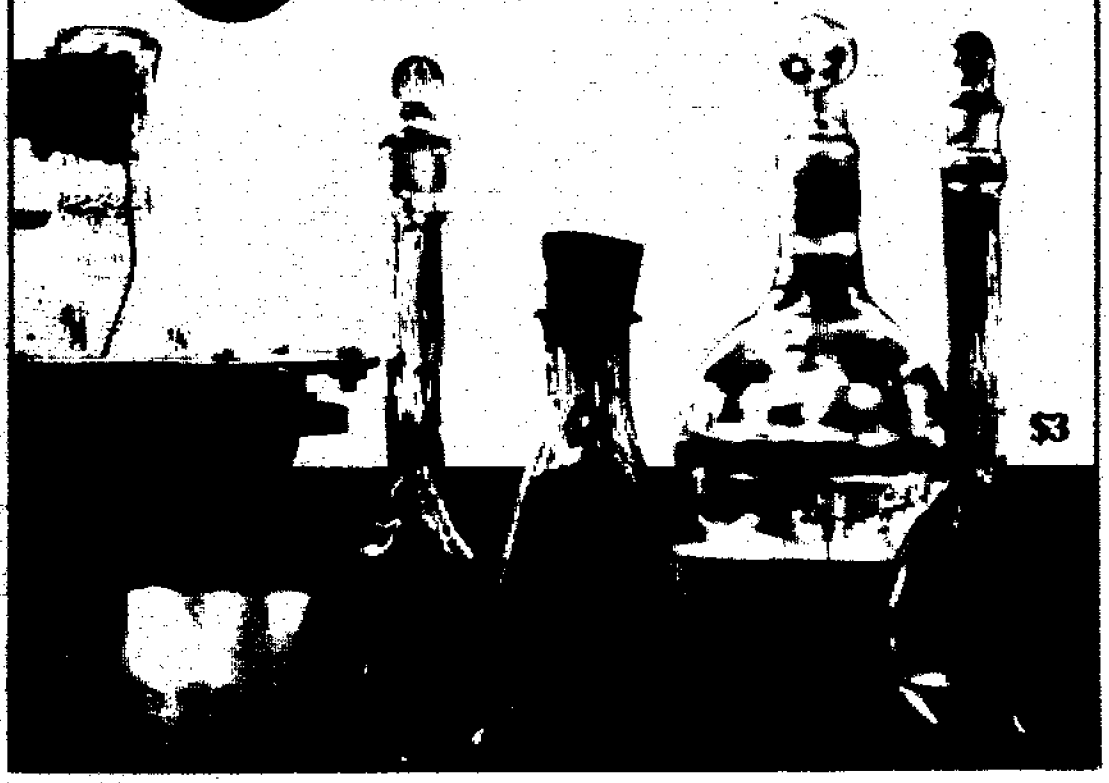

University of Rhode Island Marine Technical Report 69

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We are truly grateful to the many people who provided assistance and substance to make this publication a reality. Though the authors researched and assembled the abstracts, without the dedicated effort of those involved in fisheries research this publication could not have taken form. We are especially grateful to them for their work over the years, and this publication is a tribute to that effort.

A note of special thanks is in order to the National Fisheries Institute for their support, guidance and patience.

As with many publications, the proper preparation of a manuscript is an essential, time-consuming task which requires the cooperation of many. This was handled expertly by Mrs. Geraldine Karotkin, Department of Food Science and Nutrition, Mrs. Deborah Prefontaine, Marine Advisory Service, and Vicki Desjardins, Publications Office.

For everyone's long hours of work and unending patience we are deeply indebted.

INTRODUCTION

In the fall of 1976, the National Fisheries Institute decided that an up-to-date compilation of relevant available material on fish quality was needed. This could be used as an aid to the seafood industry and provide some standardization in methods.

The National Fisheries Institute awarded the Marine Advisory Service at URI a scholarship grant in January 1977 to coordinate the research of the literature for this publication. The expertise of Dr. Spiros Constantinides in the Department of Food Science and Nutrition was used to research and assemble all the available data. The efforts of his group focused on reputable publications, international journals, trade journals, and reports presented at technological conferences. The entire spectrum of methods presented in this publication was collected and reviewed, and organized into five major categories: Chemical, Biochemical, Microbiological, Organoleptic and Miscellaneous. Review articles on the methods of determining fish quality are listed in the section that follows these categories. Each abstract is quoted in as complete a form as possible in order to provide a reference for further information. We in no way feel that the abstract can act as a substitute for the article and encourage the reader to review the articles for a greater understanding of the methods practiced. The abstracts are given in chronological order to point out the most recent material.

It is our sincerest hope that this publication will provide a valuable reference and tool for those interested in assessments of fish quality. Additionally, we hope some standardization may result and that needs for future work may be recognized and ultimately realized.

CHEMICAL METHODS FOR DETERMINING FISH QUALITY

Chemical Quality Index of Canned Tuna As Determined by High Pressure Liquid Chromatography

John L. Mietz
Food and Drug Administration, Department of Health, Education and Welfare,
Philadelphia District Office, Philadelphia, PA
and Endel Karmas Department of Food Science, Rutgers University, New Brunswick, NJ
Journal of Food Science 42:155-158, 1977

A chemical quality index of canned tuna was established for estimating the extent of decomposition in fresh tuna prior to canning. Histamine has frequently been used as such an indicator, but by itself it has not always proved useful. The relationship of 5 amines (histamine, putrescine, cadaverine, spermine and spermidine) was studied to generate a chemical index of tuna decomposition. The amines were extracted from authentic pack and commercially prepared canned tuna samples. The dansyl derivatives were formed and determined by reverse phase, linear gradient elution, high pressure liquid chromatography. An index was developed from the individual amines and the resulting chemical indices scores compared favorably to organoleptic and authentic pack value scores.

A Total Reducing Substance Test For Ascertaining Oyster Quality

Joseph A. Liuzzo, Stephen C. Lagarde, Robert M. Grodner and Arthur F. Novak
Department of Food Science, Louisiana State University, Baton Rouge, LA
Journal of Food Science 40:125-128, 1975

From the results of statistical analysis and correlations between the various chemical, microbiological and organoleptic tests, it would appear that the total reducing substance test offers a rapid and reliable means of ascertaining the quality of ice stored oysters. Of all the methods tested as indices of oyster quality, the TRS (total reduced substances) method most nearly approaches the criteria which a chemical indicator for food quality should possess.

Development of a Chemical Test For Shrimp Quality

Bryant F. Cobb III and Carl Vanderzant
Department of Animal Science, Texas A&M University, College Station, TX
Journal of Food Science 40:121-124, 1975

The total volatile nitrogen/amino-nitrogen (TVN/AA-N) ratio may be a more accurate indicator of shrimp quality (organoleptic) than bacterial counts. In tests on frozen imported shrimp, some samples with TVN/AA-N ratios > 1.5 mg. N/mM had bacterial counts ranging from 11,000 to 95,000 per gram. These shrimp, which were from freezer boats, were rejected as spoiled by a trained taste panel.

The Gas Chromatographic Determination of Trimethylamine and Dimethylamine in Fish, Fishery Products and Other Foodstuffs

T.M. Ritskes
Journal of Food Technology 10:221-228, 1975

Baseline separation of trimethylamine (TMA) and dimethylamine (DMA) is obtained on a Carbowax 400/polyethylene imine column provided with an alkaline pre-column. Based on this separation, a method of analysis for these amines in fish and other foodstuffs was worked out. Quantitative distillation of traces of DMA could be achieved by addition of ethylamine prior to distillation. A sensitivity increase up to the ppm range was found to be possible. Amine oxides, after reduction with titanous chloride, can be determined in the same way. The automation of the gas chromatographic injection procedure is described.

Simultaneous Measurements of Trimethylamine and Dimethylamine in Fish, and Their Use for Estimating Quality of Frozen-Stored Gadoid Fillets

C.H. Castell, Barbara Smith, and W.J. Dyer
Department of the Environment, Fisheries and Marine Service, Halifax Laboratory, Halifax, N.S.
Journal of the Fisheries Research Board of Canada 31:383-389, 1974

On the basis of some of the recent modifications of Dyer's colorimetric tests for measuring trimethylamine (TMA) in fish, a procedure is described that gives both the TMA and the dimethylamine (DMA) contents. Measurements on 84 frozen-stored fillets of haddock, cod, pollock,

cusk, and hake showed a correlation coefficient of 0.94 between DMA values obtained by the copper-dithiocarbamate test and those obtained by the picric acid procedure. The simultaneous measurements of TMA and DMA are useful in estimating the quality of frozen-stored gadoid fillets, the TMA value indicating the extent of microbial spoilage before the muscle was frozen and the DMA value the extent of deterioration through action of tissue enzymes during frozen storage.

Hypoxanthine Measurement in Assessing Freshness of Chilled Canned Catfish *(Ictalurus punctatus)*

Larry R. Beuchat
Journal of Agricultural and Food Chemistry 21(3):453-455, 1973

Existing methods were modified for the inexpensive, objective measurement of hypoxanthine in muscle of channel catfish *(Ictalurus punctatus)* stored at 2° postmortem for up to 22 days. Sensory ratings were performed by appearance, color, aroma, texture, and flavor and compared to the rate of hypoxanthine accumulation in the same fish. Measurement of the purine, especially prior to noticeable spoilage, shows potential value as an index in assessing freshness of chill-stored catfish.

An Automation Analysis of Trimethylamine in Fish Muscle

Noboru Kato and Hitoshi Uchiyama
Bulletin of the Japanese Society of Scientific Fisheries
39(8) 899-903, 1973

The conditions of automatic analysis of trimethylamine (TMA) were examined by using a Technicon autoanalyzer. To the trichloroacetic acid (TCA) extract of fish muscle, 10% formaldehyde and 30% potassium hydroxide solutions are successively added, and then the mixture is heated in an oil bath of 60°C. The TMA thus liberated from the reaction mixture is captured in a gas trap, then mixed with a solution of bromothymol blue, and finally determined colorimetrically at 620 mμ.
Within the range of 3-50 μg N/ml, there was a linear relation between optical density and TMA concentration. Results obtained using this method correlate very well with those obtained by the picrate method in which potassium hydroxide was substituted for potassium carbonate.

Quantitative and Selective Gas Chromatographic Analysis of Dimethyl- and Trimethylamine in Fish

Alexander Miller III, Richard A. Scanlan, Jong S. Lee, and Leonard M. Libbey
Journal of Agricultural and Food Chemistry 29(3):709-711, 1972

The selective gas chromatographic separation of methylamines was accomplished using columns containg Graphon and tetraethylenepentamine with an alkali flame ionization dectector (AFID). Trimethylamine (TMA, 10 ppm) and dimethylamine (DMA, 50 ppm) added to fish were easily detected by equilibrium vapor analysis. Greater sensitivity (25 ppb TMA and 100ppb DMA) was obtained by using the AFID in conjunction with a gas entrainment, on column trapping procedure.

Detection of Frozen Fish Deterioration by An Ultraviolet Spectrophotometric Method

A.A. Danopoulos and V.L. Ninni
Athens Graduate School of Economics and Business Science, Chemistry and Technology Laboratory, Athens, Greece
Journal of Food Science 37:649-651, 1972

Ultraviolet absorption has been found to give a reasonably satisfactory measure of the extent of oxidation for tissue oil in frozen fish. This method is applicable not only at the early stages of oil oxidation but also at the advanced stages, when peroxide value determination cannot be used because of rapid decomposition of hydroperoxides.

The Separation of Aliphatic Amines in Dilute Aqueous Solution by Gas Chromatography and Application of This Technique to the Quantitative Analysis of Tri- and Dimethylamine in Fish

J.N. Keay and R. Hardy
Department of Trade and Industry, Torry Research Station, Aberdeen, Scotland
Journal of the Science of Food and Agriculture 23:9-19, 1972

Gas chromatography on an alkaline Dowfax 9N9 column has been found to be suitable for the qualitative and quantitative analysis of alkylamines in dilute aqueous solution. The method has been applied to the analysis of trimethylamine (TMA) and dimethylamine (DMA) in fish and has been used to throw further light on uncertainties concerning the use of the "picrate" procedure for TMA determination.

An Investigation of the Method of Determining Trimethylamine in Fish Muscle Extracts by the Formation of Its Picrate Salt-Part II

C.K. Murray and D.M. Gibson
Department of Trade and Industry, Torry Research Station, Aberdeen, Scotland
Journal of Food Technology 7:47-51, 1972

Thin layer chromatography, gas liquid chromatography and colorimetric procedures were used to identify and determine amines present in standard solutions, cod muscle extracts, and in the toluene phase of a modified TMA procedure. These tests show that the use of potassium hydroxide solution to liberate TMA from formalized trichloracetic acid extracts of fresh or frozen cod is superior to potassium carbonate solution.

Volatile Basic Nitrogen (VBN) as a Freshness Indicator of Fish For Canning

M. Akiba and E. Tanikawa
Laboratory of Marine Food Technology, Faculty of Fisheries, Hokkaido University, Hakodate, Japan
and Y. Fujii
Tokai Regional Fisheries Research Laboratory, Tokyo, Japan
Food and Agricultural Organization 115:39-43, 1971

This study deals with the relationship between the decomposition products of ATP and volatile basic nitrogen content VBN on fresh and canned salmon. The VBN content can be used to determine the acceptability of the raw material. It is recommended that the fish must be processed before the VNB index reaches 20mg%. Fish with a greater content of VBN results in poor quality canned products.

Quality Assessment of Iced Cod and Prepackaged Cod Fillets

K.J.A. van Spreekens
Institute for Fishery Products TNO, Ijmuiden, The Netherlands
Food and Agricultural Organization 115:83-94, 1971

Deterioration of fresh cod and of iced fillets were studied using total bacteria counts, total volatile bases, a hypoxanthine method and organoleptic methods. The effect of hygienic conditions, quality of the raw material and temperature of storage were studied.

Significance in Measuring Volatile Base and Trimethylamine Nitrogen and Nucleotides in Fish Muscle as Indices of Freshness of Fish

Hitoshi Uchiyama, Shigeo Ehira, Hiroshi Kobayashi, and Wataru Shimizu
Bulletin of the Japanese Society of Scientific Fisheries
36(2):177, 1970

Among the various tests for elucidating the freshness of fish, measurements of volatile base and trimethylamine nitrogen in the muscle have been used widely by many investigators. However, little information is available on the relation between the value of these tests and the freshness evaluated commercially by organoleptic tests. Typical uncooked Japanese seafoods, "Sashimi", or sliced raw fish, and "Sushi" condiments were prepared from fish which were selected with the traditional severe scrutiny for flavor. Using these samples obtained from local markets, and fish killed immediately after being caught, the significance in measuring volatile base, trimethylamine nitrogen, and nucleotides and related compounds for estimating the freshness was studied. The main features are as follows:

1. The distribution of value of volatile base and trimethylamine nitrogen, far from showing linear correlation among the fish killed immediately after being caught, "Sashimi" and "Sushi" condiments of several grades of qualities, appeared to be nearly random, and no trend can be discerned. In measuring the amounts of these bases, we were only able to estimate the limit of acceptability or the onset of spoilage of the foods.
2. From the results of statistical examinations, the nucleotide decomposition ratio, K value, was found to be a useful index for *"Ikino yosa"*, or freshness in the true sense of the word, which was used as a sensory criterion in the commercial purchase and sales of fish.

Effect of Dimethylamine on the Value of Trimethylamine Determined by the Dyer's Method

Harumi Tozawa, Kazuko Enokibara, and Keishi Amano
Bulletin of the Japanese Society of Scientific Fisheries
36(6):606, 1970

It has been known that the picrate colour reaction is not specific for trimethylamine (TMA) because the reaction also occurs with many types of amines formed during the spoilage of fish. Thus, the TMA level in the muscle of gadoid fish are considered to be over-estimated by the DYER's picrate method, since the muscle often contains a considerable amount of dimethylamine (DMA) even in a good grade of quality.

By chance the authors observed that the picrate value of cod muscle, obtained by HASHIMOTO's method using KOH instead of K_2CO_3, was too low for the expected level from the amount of DMA contained, and therefore, examined the colour development of DMA picrate in relation to the reagents employed in the picrate method.
Optical density of DMA picrate was much smaller with the combined use of KOH and 1 ml of 10% formaldehyde (FA), than the O.D. obtained with K_2CO_3 and FA. The ratio of the O.D. of DMA picrate to the O.D. of the equivalent TMA picrate (ratio of colour development) was approximately 4% for the KOH method, while the ratio was about 50% in the case of K_2CO_3.
The amount of FA showed markedly different effects in the two methods: with K_2CO_3, the colour intensified in proportion to the increase of FA, while with KOH, a miximum value occurred in the absence of FA and abruptly dropped with the addition of 0.5 to 1.0 ml of FA, followed by a gradual intensification in colours as FA increased.
The use of KOH as well as K_2CO_3 was examined to determine their effect on the picrate values of fish samples. Cod and Alaska pollock fillets, including irradiated or salted, were analyzed after chilled or frozen storage. The picrate values with the use of KOH were found close to the "real" TMA values measured by gas-liquid chromatography (GLC), except one sample which contained a larger amount of DMA. However, the picrate values obtained with K_2CO_3 were much higher than the GLC values.
With regard to the results mentioned above, the authors propose that KOH be used instead of K_2CO_3 as the alkali reagent, and the amount of 10% FA added be kept to 1 ml, when gadoid fishes are subjected to TMA analysis by the picrate method.

Formation of Dimethylamine in Stored Frozen Sea Fish

C. H. Castell, Wanda Neal, and Barbara Smith
Fisheries Research Board of Canada. Halifax Laboratory, Halifax, N.S.
Journal of Fisheries Research Board of Canada 27(10):1685-1690, 1970

In cod fillets undergoing deterioration during frozen storage, the dimethylamine content increases (and not the trimethylamine content as previously reported by us). There was no evidence to show an accumulation of dimethylamine in the muscle of frozen scallops, lobster, or shrimp that were purposely held at relatively high storage temperatures. It is suggested that for fish of the family Gadidae dimethylamine might be used as a measure of "frozen-storage deterioration" in much the same way as trimethylame has been used as a measure of microbial spoilage in the unfrozen fish.

Evaluation of Muscle Hypoxanthine and Volatile Bases as Potential Quality Indices for Industrial Bottomfishes From the Gulf of Mexico

Enrique J. Guardia and Gerhard J. Haas
Fisheries Industrial Research 5(3):117-122, 1969

Croaker *(Micropogon undulatus)* and spot *(Leiostomus xanthurus)* are the two species of fish found most commonly in catches of industrial bottomfish in the Gulf of Mexico. Hypoxanthine increased linearly in both species during the first 2 weeks that these fish were stored in ice. This test for hypoxanthine could thus indicate the quality of both croaker and spot and presumably that of the whole catch. Only after the fish had been stored 1 week in ice, however, did the total amount of volatile bases increase. Consequently, this latter test could not be used as an index of freshness, although it might be used as an index of spoilage.

Studies on Freshness Determination of Fish Meat by the Distillation Ratio of Volatile Acids-IX.
On the Applicability of D.R. Value to the Determination of The Freshness of Oyster

Suezo Asakawa
Bulletin of the Japanese Society of Scientific Fisheries 24(9):714, 1969

The distillation ratio of volatile acids (D. R. value) was measured on the Japanese oyster, *Ostrea (Crassostrea) gigas* Thunberg, during the course of the deterioration of its freshness. The results indicated that (1) the D.R. value of very fresh oyster is smaller than that of fish flesh, (2) the value decreases rapidly as the freshness lowers, and (3) there is no relationship between D.R. value and the freshness.
(4) D.R. value was decreased by the presence of such a large amount of glycogen as is found in oyster, (5) there exists much differences between the composition of lower volatile acids produced during spoilage of oyster and that of ordinary fish flesh, (6) in the case of oyster, higher volatile acids precipitated in the distillate, causing the D.R. value to decrease rapidly with the deterioration of freshness. Thus, D.R. value and freshness showed no correlation in oyster on account of the high glycogen content of the body and the various kinds of acids distilled out.
From the above, it was concluded that the "D.R. standard scale" previously proposed for fish flesh can not be applied to oyster, and no substitute standard scale was proposed for oyster.

An Improved Automated Analysis of Hypoxanthine

J. R. Burt, J. Murray and G. D. Stroud
Journal of Food Technology 3:165-170, 1968

The level of hypoxanthine in fish muscle is a good indicator of fish quality. Its routine determination can be carried out reliably and quickly with the Auto Analyzer using a redox indicator dye. Interference from formaldehyde and acetaldehyde in extracts of fish muscle, at concentrations greater than would be expected there, is not significant. Results obtained using this method correlate very well with those from the standard manual enzymatic assay procedure.

Production of Trimethylamine in Frozen Cod Muscle

C. H. Castell, D. M. Bishop, and Wanda E. Neal
Journal of the Fisheries Research Board of Canada 25(5):921-933, 1968

Trimethylamine (TMA) was produced in frozen cod fillets and in scallop muscle under conditions where bacterial activity could not take place. The amounts formed were smaller than those which usually accompany bacterial deterioration of unfrozen fish. Decreases in storage temperature between -3° and -26°C reduced the rate of TMA formation. At -26°C no measurable increase of TMA was produced in cod fillets during storage periods up to 700 days.
TMA formation appeared to be related to other chemical changes taking place in the frozen muscle. It followed shortly after the formation of free fatty acids and was almost simultaneous with changes taking place in the amounts of extractable protein.

Determination of Volatile Amines in Fish Muscle by Gas-Liquid Chromatography-I. Trimethylamine

Junsadu Nonaka, Hitomi Mitani, and Chiaki Koizumi
Bulletin of the Japanese Society of Scientific Fisheries 33(8):735-757, 1967

A convenient method of trimethylamine (TMA) determination has been studied by gas-liquid chromatography (G.L.C.). The recommended procedure is as follows: A 2 ml portion of 5% trichloracetic acid extract of fish muscle is placed in a test tube with a 20 ml capacity. To the test tube, 4 ml of n-heptane and 2 ml of 50% potassium hydroxide are added successivley, and the tightly stoppered test tube is kept 5 minutes at 55°C in a water bath. The test

tube is vigorously shaken for 2 minutes, and after standing for 10 minutes, an aliquot of the supernatant n-heptane layer is applied to a G.L.C. equipped with both hydrogen flame ionization detector and 1.5m x 4mm column packed with 20% acetylalcohol +2% potassium hydroxide on C-22 firebrick of 60 - 80 mesh. Column temperature is adjusted at 52°C and nitrogen is used as carrier gas. The average recovery of TMA was estimated as more than 98%. Comparative determinations of TMA were run by the G.L.C. and the Dyer method on the horse mackerel and the mackerel muscles which were stored at 1-4°C for 0, 2, 5, 8 and 11 days, respectively. It was confirmed that the former always gave better than the latter especially when the fish became unsound.

Hypoxanthine in Iced Freshwater Fish

L. C. Dugal
Fisheries Research Board of Canada, Freshwater Institute of Winnipeg, Canada
Journal of Fisheries Research Board of Canada 24(11):2229-2239, 1967

The formation of hypoxanthine in ordinary muscle was followed in 36 individual yellow walleye *(Stizostedion vitreum)* and in 22 individual whitefish *(Coregonus clupeaformis)* stored in ice. At the time of death, the average hypoxanthine content was approximately the same for the two species (0.25 µmole/g); it increased gradually to 1.52 µmole/g in 22 days and to 2.54 µmole/g in 18 days in yellow walleye and in whitefish respectively. The average rate of formation in yellow walleye (0.06 µmole/g per day) was the same as in ordinary muscle of swordfish; the rate of formation in whitefish (0.13 µmole/g per day) was slightly higher than that of Atlantic salmon, but lower than that of haddock, petrale sole, and several other marine species. Large variation in the rate of formation was noticed between individual yellow walleyes. The average hypoxanthine content of both yellow walleyes and whitefish taken as groups was found to be proportional to the number of days in storage. No difference in average rate of formation was noticed between whitefish fillets from opposite sides of the fish, nor between fresh and thawed fish. The hypoxanthine content appears to be suitable as an index of freshness of groups of fish, not of individual fish.

Measurement of Hypoxanthine Concentration in Canned Herring as an Index of the Freshness of the Raw Material, With a Comment on Flavour Relations

R. B. Hughes and N. R. Jones
Journal of the Science of Food and Agriculture 17:434-436, 1966

Hypoxanthine concentration in skeletal muscle increases during the storage of raw herring at 7-12°C or in ice. No further increase occurs during canning and subsequent short-term storage. Consequently, the concentration of the purine in the canned material affords an index of the quality of the fish at processing. Relative differences in hypoxanthine concentration at points of inedibility are discussed in relation to current flavour theory.

Measurement of Hypoxanthine in Fish as a Method of Assessing Freshness

J. Spinelli, M. Eklund and D. Miyauchi
Bureau of Commercial Fisheries Technological Laboratory, Seattle, Washington
Journal of Food Science 29:710-714, 1964

The hypoxanthine contents of fresh fillets taken from three species of fish in Pacific Northwest waters were found to be almost zero, and increased at a fairly uniform rate during the first 8-10 days of storage in melting ice. The hypoxanthine content reached maximum values in about 8-10 days. Total nucleotides reached a minimum in about 6-8 days. Fillets stored at -20°F showed practically no change in hypoxanthine content during four months of storage.
Hypoxanthine can be rapidly measured and the data can be used to judge the length of time fish has been held in storage.

Rapid Estimations of Hypoxanthine Concentrations as Indices of the Freshness of Chill-Stored Fish

N. R. Jones, J. Murray and (in part) Mrs. E. T. Livingston and C. K. Murray
Journal of the Science of Food and Agriculture 15:763-774, 1964

Rapid estimates of hypoxanthine concentration in muscle extracts by xanthine oxidase reaction agreed well with those obtained by ion exchange chromatography. Assay by precipitation as the silver salt was subject to some inaccuracy. Concentrations of hypoxanthine increased throughout the period of useful storage of a number of species. The increases correlated well with most evaluations of quality by taste panel.

Ammonia as an Index of Decomposition in Crabmeat

James L. Burnett
Food and Drug Administration, Seattle, WA
Journal of the Association of Official Agricultural Chemists 48(3):624-627, 1964

A method based upon the color reaction between NH3, thymol, and bromine, uses ammonia as an index of decomposition in fresh and frozen crabmeat. The color is extracted into n-butyl alcohol and net absorbance is determined by subtracting absorbance at 475 mμ from that at 682 mμ. Sensitivity is about 1 ppm NH_3. The method is very rapid and requires no special apparatus. Samples of crabmeat decomposed under controlled conditions were examined and graded organoleptically. Ammonia content was determined and found to increase uniformly and rapidly with spoilage. Good reproducibility and low results were obtained on fresh crabmeat. Ammonia can be detected by this method before spoilage can be detected organoleptically.

Rancidity in Lean Fish Muscle I. A Proposed Accelerated Copper-Catalyzed Method for Evaluating the Tendency of Fish Muscle to Become Rancid

J. MacLean and C. H. Castell
Fisheries Research Board of Canada, Technological Research Labortory, Halifax, N.S.
Journal of the Fisheries Research Board of Canada 21(6): 1345-1359, 1964

A method that can be carried out within 24-72 hr is suggested for determining the tendency of fish muscle to become rancid. It consists of adding measured, trace amounts of copper ion to muscle that has been blended with water (1:3) followed by storage at 0°C. Rancidity is observed subjectively by noting the odours that develop and objectively by means of the thiobarbituric acid reaction.

Determination of Adenine, Hypoxanthine, Adenosine and Inosine by Ion Exchange Chromatography

Ken-ichi Arai and Tsuneyuki Saito
Bulletin of the Japanese Society of Scientific Fisheries 29(2):168, 1963

The assay method is based on the separation of adenine (A), hypoxanthine (H), adenosine (AR), inosine (HR), and nucleotides by using the ion exchange resins, DIA-ION SA-100 (formate type) and Dowex 1x2 (chloride type). The former is used for adsorption of nucleotides and the

latter for adenine, hypoxanthine, adenosine and inosine. It is possible to differentiate adenine, hypoxanthine, adenosine and inosine from Dowex 1x2 quantitatively owing to their differences in the rate of elution by using the solvent system, NH_4OH-NCI-$Na_2B_4O_7$, in which A is 0.1N NH_4OH-0.035N HCI-0.005N $Na_2B_4O_7$, B is 0.001N HCI-0.0002N $Na_2B_4O_7$ in the stepwise elution system.

Quality Evaluation Studies of Fish and Shellfish from Certain Northern European Waters

Lionel Farber
Seafood Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, California
Food Technology 17110-114, 1963

Sensory judgements and determinations of trimethylamine nitrogen (TMAN) and of volatile reducing substances (VRS) were made on the white-fleshed fish cod, haddock, whiting, plaice, sole, and gurnard, on red-fleshed Atlantic herring, and on shrimp in fisheries research laboratories in Aberdeen, Paris, Ijmuiden, Copenhagen, Goteborg, Bergen, and Reykjavik. In general, correlations of the contents of TMAN and the VRS with sensory judgements for the white-fleshed fish were parallel, with coefficients of correlation of 0.8-0.9. In contrast were the results for the herring: the VRS values increased and the TMAN values were not related to the progress of spoilage. The VRS values for the shrimp sampled paralleled the sensory judgements. The methods used in California to evaluate the quality of Pacific Coast fish and shellfish were useful for the varieties caught in northern Euopean waters.

A Fluorometric Method for Determining The Freshness of Fish - I

Toyoki Ono, Fumio Nagayama, Takuichiro Yoshikane and Yoshinori Muto
Bulletin of the Japanese Society of Scientific Fisheries 28(9):936, 1962

As the technique for measurement of fluorescence is simple and not time-consuming, this method has been proposed by several workers for food inspection which requires rapidity. However, there is no established method for fish. The present investigation was undertaken to see whether fluorometry could be used for determining the quality of fish. Sole flesh gave a clear solution extracted with 5 volumes of 70% ethanol, but gave a turbid solution which is not suitable for fluorometry when extracted with water. Maximum fluorescence of the extracts was found at 460 mμ.

Therefore, the measurement could be standardized with quinine sulfate solution. Heat treatment of flesh increased fluorescence of the extracts especially in the presence of glucose. Fluorescence from spoiled flesh was greater than that from fresh flesh.
Development of fluorescence was closely related to the storage time. Fluorescence from flesh which was steam boiled with glucose seemed to be a better indication of quality of the flesh than VBN value.

Summary of Chemical Data on Progressive Decomposition Studies of Cod, Haddock and Perch

Fred Hilling, L.R. Shelton, Jr. and J.H. Loughrey
Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D.C.
Journal of the Association of Official Agricultural Chemists 45:724, 1962

Previously reported results by chemical methods for detecting decomposition are summarized to set limiting values for fish which are judged organoleptically satisfactory, with cod, haddock and perch.

Comparison of pH, Trimethylamine Content, and Picric Acid Turbidity as Indices of Iced Shrimp Quality

Sammie Bethea and Mary E. Ambrose
Commercial Fisheries Review 24:7-10, 1962

As possible indices of quality during iced storage, a study was made of the changes in pH and trimethylamine nitrogen content of raw headless brown shrimp. Results were compared with those obtained from picric acid turbidity tests conducted simultaneously on the same shrimp and previously reported. The quality ratings of the shrimp as determined by a taste panel were used as standards for all tests. Changes in color and viscosity of shrimp homogenates were also observed for possible use as accessory tests of quality.

Chromate Color Test For Estimating Age-Temperature History of Raw Shucked Oysters

M. L. Schafer, J. E. Campbell, and K. H. Lewis
Journal of Agricultural and Food Chemistry 10(3):261-267, 1962
Public Health Service, Robert A. Taft, Sanitary Engineering Center, Department of Health, Education and Welfare, Cincinnati, Ohio

In less than 15 minutes with little or no preliminary preparation the concentration in oyster liquor of organic substances capable of reducing chromate ion to chromium(III) is measured in a highly acidic medium. Between 0 and 10 mg. of chromium (III) in residual chromium (VI) is determined from the absorbance at 580 mm (standard deviation 0.08). By comparison with visual standards the concentration chromium(III) in the same concentration range is estimated within ±0.5 mg. of that obtained by spectrophotometric measurement. During storage at 0°C the concentration of the organic reducing substances (RS) gradually increased in freshly shucked oysters from Chesapeake Bay. A direct relationship was observed between storage temperature (from 0° to 35°C) and the rate of RS increase. Of individual tests at 2- to 3-day intervals on 15 samples held at 0°C for 14 to 30 days, 94% fell within ± two standard deviations of the average curve defined by an equation describing the increase and 61% within ± one standard deviation. Shucked oysters frozen and then thawed showed a much greater rate of increase in RS at corresponding storage temperatures than did unfrozen samples.

Formation of Acetoin in Cod and Other Bottom-Fish Fillets During Refrigerated Storage

Herman S. Groninger
Technological Laboratory Bureau, Commercial Fisheries, Seattle, Washington
Food Technology 15(1):10-12, 1961

Acetoin and the total volatile bases were measured in samples of commercial bottom fish fillets that were stored at 34°F. The content of acetoin increased from <1 to 7-10 mg/100 g after 4 to 8 days storage. The usefulness of the acetoin content as a measure of quality of commercial fillets appears to be quite limited, because the increase of acetoin does not occur until just before the time the sample would be considered unacceptable.

Chemical Methods for the Determination of the Freshness of Fish

Dardjo Somaatmadja, John J. Powers, and Dan E. Pratt
Department of Food Technology, University of Georgia, Athens, Georgia
Journal of Milk and Food Technology 24:2-6, 1961

Various objective methods to estimate the degree of freshness of fish and fishery products were compared. The total volatile nitrogen (TVN) and ammonia-nitrogen of carp flesh reached values of 30-33 mg% when carp stored at 0-4°c and 25-27°C began to have a bad odor. For catfish stored at 0-4°C and 25-27°C these values reached 20-30 mg%. Trimethylamine was not found in fresh and spoiled carp and catfish. The determination of trimethylamine as an index of spoilage cannot be used for these fresh-water fish. The determination of tryosine and volatile reducing substances (VRS) cannot be used to estimate the degree of freshness of fish if the temperature at which the fish had been stored is not known. If these two methods were combined with TVN and ammonia-nitrogen one could determine the degree of freshness of fish and the temperature at which the fish were stored. The determination of tyrosine combined with TVN ammonia-nitrogen can give further support as to whether fish are absolutely fresh. If the temperature under which fish have been stored is known each of the tests used in this study can be applied individually to determine the degree of freshness of fish.

Studies on the Evaluation of Freshness and the Estimation of the Storage Life of Raw Fishery Products

Lionel Farber and Peter Lerke
Fisheries Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, California
Food Technology 15191-196, 1961

Determinations of the contents of volatile reducing substances (VRS) and trimethylamine nitrogen (TMN), and of the percentage of pigmented bacteria before and after 5 hours of incubation of 31°C (88°F) are suggested as useful criteria for evaluating the freshness of raw fish samples and estimating the deeping quality at refrigerated storage temperatures above freezing. Applications to flatfish and rockfish fillets, salmon steaks, tuna, and unpeeled beheaded shrimp are given for both experimentally stored and commercial samples.

Physical and Chemical Properties of Shrimp Drip as Indices of Quality

Sammie Bethea and Mary E. Ambrose
Commercial Fisheries Review 23:9-13, 1961

Physical and chemical characteristics of drip obtained from frozen--thawed shrimp were studied to determine if changes in these characteristics could be correlated with quality as determined by a taste panel. Shrimp were tested that had been stored (1) on ice followed by a minimum of frozen storage for the formation of drip, (2) at -10°F, and (3) both on ice and at -10°F.
The pH of the drip appeared to be a satisfactory objective quality index. Drip from shrimp considered "good" by the taste panel gave pH readings of 7.50 to 8.25, from shrimp considered "acceptable," from 8.26 to 8.40, and from shrimp considered "unacceptable," 8.41 and higher. The color and optical density of the drip changed correspondingly with quality, and objective measurements of the optical density could be made with a photoelectric colorimeter. Trimethylamine nitrogen content of shrimp drip showed good correlation with spoilage but gave no indication of the state of freshness of the unspoiled shrimp. The volume of drip collected and the nitrogen content of the drip were of little or no value as a quality index.

Studies On the Method For Testing The Spoilage Of Food-XVI
A Method for Determination of VRS by Micro-Diffusion Technique

Tetsuo Tomiyama and Seiya Fujino
Bulletin of the Japanese Society of Scientific Fisheries
87(7):678-683, 1961

A method which was developed in this study is as following: Place to the outer chamber of CONWAY unit a 2-cc. aliquot of sample flesh extract which can be prepared by mixing a 25-cc aliquot of 7% trichloracetic acid solution with a mixture of 3.8g. of ground flesh and 10 cc. of distilled water. Place to the inner chamber exactly 1 cc.-aliquot of alkaline solution of 0.05 N $KMnO_4$. Then, add 1 cc. of saturated K_2C03 solution to the sample extract in the outer chamber, followed by immediately sealing the unit with a cover, and leave the unit stand for two hours at room temperature. Remove the cover, add 0.55cc. of 20% KI to the inner chamber, and further add few drops of sulfuric acid (2:5), followed by immediate addition of 0.05 N $Na_2S_2O_3$ while being stirred with a magnetic stirrer. Titrate the excess thiosulfate by 0.05 N KHI_2O_6, using a KIRK-type capillary micro-burette and a starch solution as indicator.

VRS obtained by the present method was about one-half as much as that by the distillation method. It is to be noted that VRS value of canned flesh obtained by the present method could not be employed for the estimate of the freshness of its material.

The Separation and Determination of Free Purines, Pyrimidines and Nucleoside in Cod Muscle

N. R. Jones
Department of Scientific and Industrial Research, Torry Research Station, Aberdeen, Scotland
Analyst 85:11-118, 1960

A procedure is described for the quantitative separation of purines pyrimidines and nucleoside present in extracts of cod muscle. After the removal of nucleotide at pH 6.5 by a strongly basic anion-exchange resin in the formate form, the purines, etc., are exchanged on to a similar resin in chloride form at pH 11 to 12. They are then eluted by a gradient of increasing chloride and tetraborate concentrations, and diminishing pH, for evaluation by ultra-violet spectrophotometry.

Studies On The Method For Testing the Spoilage of Food-XV An Improved Aeration Method for Determination of VRS

Tetsuo Tomiyama, Shigenobu Oyama and Seiya Fujino
Lab. Fish. Chem., Fac. Agric., Kyushu Univ., Fukuoka
Bulletin of the Japanese Society of Scientific Fisheries 26(5):520, 1960

The present study deals with the improvement of the Farber method of employing deproteinized extract for the aeration in place of the red muscle juice and on aeration of pH 9.3 rather than unadjusted pH of sample tissue.
The VRS value of mackerel and albacore obtained by the present method amounts approximately one-half as much as that by the Tomiyama et al's steam distillation method. This infers that VRS determined by the aeration method involves volatile substances possessing higher volatility. It was found that the volatility of VRS varied with the freshness of sample flesh and that trimethylamine could hardly be removed at pH 7.0-7.7 whereas nearly one-half as much removed at pH 9.3. It was also noted that the Farber method was capable of determining rather a small fraction (29%) of the neutral substances as compared with the Tomiyama et. al. method.

Studies on The Methods for Testing The Spoilage of Food-XVII. Fractionation of Volatile Reducing Substances (VRS) With Ion-Exchanger

Tetsuo Tomiyama and Kazuya Ikeura
Bulletin of the Japanese Society of Scientific Fisheries
26(2):128-135, 1960

Fractionation with Amberlite IRC 50 and IRA 400 was carried out of VRS in steam distillates obtained from a deproteinized extract of mackerel flesh whose pH's were adjusted at 2.0, 6.0 and 9.3 before the distillation. Data clearly indicates that VRS which are distilled off at pH 6.0 are contaminated with basic substances and appreciably increase with a slight rise of pH of the sample extract. The fractionation into acidic, basic and neutral VRS with ion-exchange resulted in a fairly good conformity with respective VRS values obtained by the steam distillation. It is clearly seen that the basic VRS increases after 1-day storage and nearly stops its increase at 3 to 4 day storage while the neutral VRS increases after 2-day storage and continues to increase up to a 4-day storage.

Studies on The Method For Testing The Spoilage Of Food-XI. Determination Of Volatile Reducing Substances of Mackerel Flesh By Steam Distillation

Tetsuo Tomiyama, Kazuya Ikeura and Shigenobu Oyama
Bulletin of the Japanese Society of Scientific Fisheries
26(1):33-38, 1960
Lab. Fish. Chem., Fac. Agr., Kyushu Univ.. Fukuoka

In Strochecker's method of VRS determination difficulties were experienced due to foaming in the distillation step and in removing protein coagulum adhered to the distillation flask. Volatile reducing substance (VRS) can be determined on an extract which is freed from protein by magnesium sulfate, eliminating the difficulties inherent in Strochecker's method.
Since it was found that both neutral and basic volatile reducing substances were increased at incipient spoilage the steam distillation of the extract is to be carried out in alkaline reaction (about pH 9.3). This improvement in the method gives rise to a more pronounced increase in VRS at incipient spoilage. VRS is, among several chemical indices, the most sensitive one for bacterial spoilage of mackerel flesh.

The present method in which a six-minute distillation followed by a 5-minute boiling with potassium permanganate is employed, enables us to run six determinations with a single apparatus within one hour.

Chemical Indexes of Decomposition in Flounder

F. Hillig, L.R. Shelton, Jr., J.H. Loughrey, B.F. Fitzgerald and S. Bethea
Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D.C.
Journal of the Association of Official Agricultural Chemists 43:755-760, 1960

The techniques of previous studies were applied to flounder. Analyses during progressive decompn. showed that volatile acid no., HCO_2H, AcOH, volatile bases and amines, and $(CH_3)_3N$ gave a high degree of correlation with organoleptic judgment.

Studies on The Influence of Treatments Immediately After Catching Upon The Quality of Fish Flesh-V. Determination of Lactic Acid in Fish Muscle

Yasuhiko Tsuchiya and Kiyoshi Kunii
Bulletin of the Japanese Society of Scientific Fisheries 26(3):284-288, 1960

The development of lactic acid in fish is not only influenced by the degree of muscular exercise in life, but also by the condition of treatment after death. This report deals with the results conducted by the authors on the determination of lactic acid in fish muscle. The dorsal muscle of bonito, *Katsuwonus vagans*, showed the highest value of 1,170 mg. per cent of lactic acid, while the ventral muscle of flat fish, *Xystrias grigorjewi*, showed the lowest 34 mg. per cent among 14 kinds of fish purchased from the fish shops at Sendai City, Miyagi Prefecture. The muscle of the fish employed in this experiment is roughly classified into three groups as red, medium red and white muscle fish. The amount of lactic acid was found in the range of 283 to 1,170 in the red muscles of bonito, tunny, sardine etc., 283 to 639 in the medium red in carp, *Clupanodon punctatus* and *Lateolabrax japonicus* and 34 to 185 mg. per cent in the white in codfish, flatfish etc. These differences are discussed from an ecological point of view. The lactic acid content in dark muscle is generally lower than that in normal one.

The highest concentration of lactic acid in carp muscle killed immediately after catching was found at 55 hours in storage at 0°C and at 25 hours at 10°C. When fish were killed after being left to weaken in the air at room temperature for 5 hours it was 5 to 15 hours at 0°C and 1 to 5 hours at 10°C.

Studies On The Determination of Spoilage In Brined Fresh-Water Fishes-I.
Comparison of Various Methods For The Determination of Spoilage in Brined *Carassius carassius L.*

Eiichi Kuroda
Bulletin of the Japanese Society of Scientific Fisheries 26(9):944-524, 1960

A comparison of various chemical methods for the determination of spoilage in freshwater fish, especially *Carassius carassius L.* in Lake Biwa, was made. The chemical methods included $HgCl_2$ test, pH test, volatile basic nitrogen (VBN) and volatile reducing substances (VRS). The $HgCl_2$ test was unfavorable and the pH test uncertain for the fresh-water fish. VRS was measured by steam distillation on 10 ml of water extract from flesh for 20 minutes, the reagents and titration were the same with Farber's method. VRS is a more sensitive method in evaluating the degree of spoilage of fresh or brined fresh-water fish, and its values are closely correlated with those of organoleptic judgement.

Assessment of the Progressive Spoilage of Ice-stored Shrimp

J. R. Iyengar, K. Visweswariah, M. N. Moorjani and D. S. Bhatia
Central Food Technological Research Institute, Mysore, India
Journal of Fisheries Research Board of Canada 17(4):475-485, 1960

Freshly caught shrimp, stored in ice, were sampled for chemical and bacteriological tests. Data has been presented to correlate the results of these tests with the quality and spoilage of shrimp. Rapid methods based on use of test papers have been developed as an index of shrimp spoilage.

A Simple and Rapid Method for the Determination of Histamine in Fish Flesh

Toshiharu Kawabata, Yutaka Uchida and Taeko Akano
Bulletin of the Japanese Society of Scientific Fisheries
26(12):1183, 1960

The method described employs a cationic exchanger, Amberlite CG-50 type 1 (100-200 mesh) for separating histamine (Hm) from such interfering substances as histidine (Hd), tyrosine, tyramine, etc., in a trichloroacetic acid extract (TCA-extract) of fish flesh. A 10ml portion of a TCA-extract is adjusted to pH 4.6 and added to top of a cationic exchanger column (8x55mm; flow rate, 2-3ml/min.; pH of the resin is pre-conditioned to pH 4.6 with 0.2N acetate buffer). The column is rinsed with 80 ml of 0.2N acetate buffer at pH 4.6 and the Hm absorbed on the resin column is eluted with 8ml of 0.2N HCL. The reaction of the elute is adjusted to pH 7 and the volume is filled to 10 ml with distilled water.
A 2ml aliquot of the elute is distributed in a colorimetric tube containing 5 ml of 1.1N Na_2CO_3, coupled with 2 ml of Paury's diazo reagent, and the absorbance is determined at 510 mμ by a Coleman spectrophotometer.
The recovery of Hm by this method was 99 to 101%. The maximum exchangeable capacity for Hm of the column was 7,000γ with pure Hm-0.2NJCl solution and 6,000γ with decomposed fish extract. The presence of Hd, tyrosine, or tyramine in TCA-extract did not influence the Hm value.

An Evaluation of the Indole and Trimethylamine Tests for Oyster Quality

Donald Lartigue, Arthur F. Novak, and Ernest A. Fieger
Department of Agricultural Chemistry and Biochemistry,
Louisiana State University, Baton Rouge, Louisiana
Food Technology 14(2):109-112, 1960

Indole and trimethylamine tests were investigated for applicability as quality tests for freshly-shucked and refrigerated oysters. Some modifications were made which were necessary for adapting these tests to oysters, and these modified tests were found to be accurate and reproducible. Both are sensitive and capable of detecting microgram quantities of compounds.

These determinations along with bacterial count liquid measurements, and organoleptic ratings were employed in oyster quality tests, and their value indices of oyster spoilage was determined. Since indole and TMA concentrations showed no definite pattern during storage, these tests are not recommended for assessment of oyster quality. Bacterial counts and liquid measurements closely parallel organoleptic ratings. Spoilage was usually evident from the eighteenth to the twentieth day of storage, and the oysters at this time had bacterial counts of about 10 million per gram, and had increased in weight by about 13% due to the loss of fluid from the meats.
The effects of CTC (Chlorotetracycline) on these tests and on extending the storage life of oysters were also investigated. The antibiotic did not interfere with the tests, nor did it suppress the formation of indole and TMA; but it did limit the bacterial count, and the development of odors.

Chemical Studies on the Herring *(Clupea harengus)*-I. Trimethylamine Oxide and Volatile Amines in Fresh, Spoiling and Cooked Herring Flesh

R.B. Hughes
Journal of the Science of Food and Agriculture 10:431-436, 1959

Gas chromatography was applied to a study of the production of volatile amines and ammonia in herring flesh under various conditions. Fresh flesh contained ammonia and a small quantity of trimethylamine, but no monomethylamine, kimethylamine or higher amines in the range of ethylamine to pentylamine were detected. The trimethylamine oxide content varied according to the season, being higher in winter than in summer. During storage at 10-13°C, ammonia and trimethylamine were formed, and also smaller quantities of di- and mono-methylamine. No higher amines were detected after 4 days. The trimethylamine oxide content dropped over the period. Cooking in sealed glass tubes at 120° resulted in breakdown of trimethylamine oxide, and formation of ammonia, tri- di- and mono-methylamine (trace). No higher volatile amines were detected in the cooked flesh. The possible significance of the results in relation to the development of flavour in canned herring is discussed.

Report on Chemical Indices of Decomposition in Fish (Histamine)

David W. Williams, Associate Referee
Food and Drug Administration, Department of Health, Education, and Welfare, San Francisco, California
Journal of the Association of Official Agricultural Chemists 42:287-289, 1959

A comparative study on the methodology of determining histamine was evaluated. Recommendations for changes in methods were proposed by the Associate Referee.

Inosinic Acid Content of Foods-I. Determination Method

Takao Fujita, Yoshiro Hashimoto and Takajiro Mori
Bulletin of the Japanese Society of Scientific Fisheries 25(2):149, 1959

Inosinic acid, an alleged tasting substance of foods, was determined by the column chromatographic method using an ion-exchange resin, Dowes 1. The separation of nucleoside monophosphates is attained by adopting a longer column and a slightly modified eluting agent. It was also found that the time required for elution of inosinic acid is reducible without any decrease in accuracy by changing the flow rate and composition of eluting agent.
The content of inosinic acid determined on the perchloric acid extracts of several kinds of foods is listed together with the results of recovery tests on the added inosinic acid. The fish meat reveals higher values, whereas the acid is not detected in the foods of vegetable origin, dried mushroom and miso (bean paste).

Studies On The Method For Testing The Spoilage Of Food-X. Errors Involved In Ota's Method for Determination of Histamine

Atsushi Tsuda, Kenji Mori and Testuo Tomiyama
Bulletin of the Japanese Society of Scientific Fisheries 25(5):361, 1959

The chemical estimate of fish freshness is not always in accord with the organoleptic observation. Tomiyama et al. showed that the histamine (Hm) content of sardine flesh ran parallel with the organoleptic grade. A prerequisite for the study on the applicability of this objective test to judge quality of mackerel is to devise an appropriate practical method for the determination.

Ota reported a practical method in which he employed p-nitrobenzene diazonium ion (p-NBD) as diazotizing reagent and ethyl acetate as extractant of Hm azo-pigment. However, when Hm determination of mackerel flesh was made by this method in our laboratory, it was found that high Hm values were always obtained on fresh fish extract and that the absorption specturm of the Hm azo-pigment from a pure Hm solution was different from that from Hm in the presence of flesh extract. The present paper deals with the improvement of the Ota's method by removing interfering substances with Amberlite IRA400 (OH-form) and introducing nitrous acid treatment prior to diazotization. Data herein presented revealed that (1) a maximal formation of the Hm pigment occurs at a pH range of 9 to 9.5, (2) the determination is greatly interfered by the presence of various amino acids in flesh extract and mechanism of the interference involved is due to consumption by p-NBD by amino acids giving an orange pigment, and (3) the interfering substances can be eliminated by percolating a trichloracetic acid extract of sample flesh through Amberlite IRA-400 (OH-form) leaving Hm in effluent, histamine being absorbed on Amberlite IRC-50 and eluted with 0.2N HCI, followed by the nitrous acid treatment and coupling with p-NBD.

Studies on the Method for Testing the Spoilage of Food-XI.
A New Method for Determination of Histamine in Tissues

Atsushi Tsuda and Testuo Tomiyama
Bulletin of the Japanese Society of Scientific Fisheries
25(6):451, 1959

The method described employs Amberlite IRC-50 (H-form) for separating histamine (Hm) from interfering substances in a trichloracetic acid extract of sample tissue. Hm which is absorbed on the ion exchanger at pH 4.6 is eluted with 0.2N HCl, treated with nitrous acid, and freed from ammonia by vacuum distillation in alkaline reaction. Hm azo pigment which is formed by coupling with p-nitrobenzene diazonium chloride at pH 9 can be extracted with ethylacetate, the extract being dehydrated with anhydrous sodium sulfate. Shortly after a small amount of ammonia is added, the absorbance of the azo pigment solution is determined at 550 mu.
The recovery of Hm included in several muscle tissue extracts was found to be 96 to 106%. It was found that the Hm content of mackerel flesh ran parallel with bacterial counts and showed a marked change with quality of the flesh as compared with total volatile bases nitrogen.

Comparison of Certain Scottish and Canadian Experiments in Respect of Grading Fish for Quality

J. M. Shewan and A. S. C. Ehrenberg
Journal of Fisheries Research Board of Canada 16(4):555-557, 1959

It was found that sensory assessments of quality for fish such as cod and haddock under normal conditions of storage tend to be highly correlated with the TMA content of fish muscle. However, the relationship between quality and TMA differed from one catch of fish to another, even when the catches were made under very similar conditions and subsequent storage was carefully controlled.

2-Thiobarbituric Acid Method for the Measurement of Rancidity in Fishery Products II. The Quantitative Determination of Malonaldehyde

Russel O. Sinnhuber and T. C. Yu
Oregon Agricultural Experiment Station, Seafoods Laboratory
Astoria, Oregon
Food Technology 12(7):9-12, 1958

Oxidative rancidity in fat-containing foods leads to the formation of malonaldehyde or derivatives of this compound. The red reaction product formed by the reaction of malonaldehyde and 2-thiobarbituric acid (TBA) is an effective means of measuring the extent of autoxidation. The TBA procedures previously described, although useful in studying the development of oxidative rancidity, are of empirical usage. A quantitative, 2-thiobarbituric acid procedure for measurement of malonaldehyde, using the stable compound TEP (1,1,3,3-Tetraethoxypropane) as a standard, is proposed. Acid hydrolysis of TEP yields malonaldyde which reacts with 2-thiobarbituric acid, under the conditions described to afford a quantitative method for the determination of malonaldehyde. The term, TBA number of milligrams of malonaldehyde per 1000 grams of material, is suggested. The reaction time, total recovery, and spectral characteristics of this reaction compound are presented. The method is sensitive to 10^{-8} moles of malonaldehyde in 100 ml. of solution. The autoxidation that occurs in frozen tuna scrap and the effect of the antioxidants, DPPD (N,N'-diphenyl-p-phenylenediamine and Tenox IV) in preventing oxidative rancidity was followed with this procedure. The present paper, although presenting a quantitative method for malonaldehyde, does not definitely establish that free malonaldehyde exists in rancid fish products. The mechanism of the development of malonaldehyde and the role of this compound in fat oxidation awaits positive identification and isolation of this material from oxidized fat. Research on the isolation of this material and characterization of the TBA-malonaldehyde pigment is in preparation.

Review of the Value of Volatile Reducing Substances for the Chemical Assessment of the Freshness of Fish and Fish Products

Lionel Farber and Peter A. Lerke
Fisheries Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, California
Food Technology 12(12):677-680, 1958

Applicability of the VRS (Volatile Reducing Substances) procedure for the determination of spoilage in a number of commercial samples of fish and fish products has been shown. Products tested included raw flat fish fillets, rockfish fillets, halibut, salmon, swordfish, white sea bass and corbina and whale meat, salted cod and herring, raw and canned smoked yellowtail, canned kipper snacks, and canned abalone, jack mackerel and Pacific sardines in tomato sauce. The correlation of the VRS content with the organoleptic judgments has been pointed out. The significance of the VRS method as a generally-applicable test for fish spoilage has been discussed in the light of a number of criteria and applications. It has been concluded that of all the fish spoilage tests reported to date the VRS method most closely approaches the specifications of a generally useful test for all kinds of fish and fish products and their diverse spoilage patterns under varying storage conditions.

Chemical Indices of Decomposition in Cod

Fred Hillig, L. R. Shelton, Jr., J. H. Loughrey, and Jerome Eisser
Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC
Journal of Association of Official Agricultural Chemists 41(4):763-776, 1958

A study of the individual analyses leads to the conclusion that volatile acid number (VAN), formic acid, acetic acid, volatile bases, volatile amines and trimethylamine (TMA) show the highest degree of correlation with organoleptic judgement, while succinic acid and alcohol show less correlation.

On the Formation of Amine in Fish Muscle-III. Simple Method for the Detection of Histamine in Fish Muscle

Fuyuo Ota
Bulletin of the Japanese Society of Scientific Fisheries
24(1):37, 1958

A simple method for the detection of histamine in fish muscle, based on the extraction of histamine azo-compound with organic solvent, is presented. When p-nitroaniline was used for the preparation of diazoreagent, using ethyl-acetate as a extracting agent, histamine azo-compound was easily transferred into the ester and separated from histidine. The azo-compound of almost all the diazo-reaction positive substances presumably contained in fish muscle was also prevented from being transferred into the ester. At high concentrations of histidine, histidine azo-compound passed slightly into the ester but it was possible to remove this by treatment with dilute alkaline solution.
Detection procedure was summarized as follows: Fish muscle extract was obtained by shaking and filtering after being added 20 volumes of water (or successively 10 volumes of water and of 5% CCl_3COOH). To 1.0cc of the extract was added 2.0cc of 2.0% Na_2CO_3. To the above solution, was added 1cc of diazo-reagent. After brief standing, 7cc of ethylacetate was added to it and its solution was shaken vigorously. Histamine concentration was measured roughly from the intensity of rose color of ester layer. Diazo-reagent was freshly prepared by adding 0.1cc of 5% $NaNO_2$ to 5cc of 0.1% p-nitroaniline in 0.1N HCl solution, and it was used immediately after preparation.

Studies on the Buffering Capacity of Fish Muscle-I. The Buffering Capacity as a Measure of Freshness

Michizo Suyama and Tadashi Tokuhiro
Bulletin of the Japanese Society of Scientific Fisheries
24(4):267, 1958

The buffering capacities of fish muscle and muscle extractives were studied to test its value as a criterion of the degree of freshness of fish muscle. Although judgement of freshness by buffering capacities was not proved to be accurate enough, measurement of the so-called B-value proposed by Stansby et al was found to be promising.
As a rule, the muscle of elasmobranch shows a higher degree in the capacity than muscle of common fish. It may be ascribed to the abundance of trimethylamine oxide contained in the muscle of elasmobranch.

Carbonyl Compounds in Fish as Related to the Deterioration-I. Detection of Volatile Carbonyl Compounds Formed in Fish Flesh

Fuyuo Ota
Bulletin of the Japanese Society of Scientific Fisheries
24(5):334, 1958

A simplified method for estimating volatile carbonyl compounds (VC) in fish is presented. This method proved to show good results in the recoveries of VC added on the one hand to fish flesh and to its steam-distillate on the other.
A modified procedure for the paper chromatographic analysis of VC, by which lower VC were found to be easily identified, is also presented. In fresh fish, VC were very small in amount. Their contents increased with the length of storage-time, and tended to decrease in the stage of advanced spoilage. The rate of VC formation varied according to the difference of state of flesh stored and also with varying parts of fish. By the modified paper chromatographic method, the formation of acetaldehyde, butyl-aldehyde and acetoin in fish flesh during storage was revealed.

Carbonyl Compounds in Fish as Related to the Deterioration-II. Thermal Production of Formaldehyde in Fish Flesh

Fuyuo Ota
Bulletin of the Japanese Society of Scientific Fisheries
24(5):338, 1958

After a short time of steam heating no appreciable amount of formaldehyde (FA) was produced from fish flesh, but by heating under pressure, it was produced to a measurable extent together with a considerable amount of dimethylamine (DMA). The amount of DMA thus produced was almost proportional to trimethylamine oxide (TMAO) contents in the flesh, whereas that of FA was not.
Thermal breakdown of TMAO was accelerated by the presence of fish extracts or certain kinds of amino acids. The degree of decomposition of TMAO in the presence of cysteine was proportional to the concentrations of TMAO and cysteine: Quantitative ratio between these two decomposition products, however, was not theoretical in any case of experiments, which might be attributed to some subordinate reaction of FA with certain kinds of amino acids.
In several marine canned foods, content of FA was much less than that of DMA.

The Use Of Tetrazolium Salts For Assessing The Quality of Iced White Fish

J. M. Shewan and J. Liston
Journal of the Science of Food and Agriculture 8:222-226, 1957

2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride is used in a colorimetric method of assessing the quality of iced fish. The results are compared with those obtained from organoleptic examination, viable bacterial counts and the contents of trimethylamine and volatile bases.

2-Thiobarbituric Acid Method for the Measurement of Rancidity in Fishery Products

T. C. Yu and Russel O. Sinnhuber
Oregon Agricultural Experiment Station, Seafoods Laboratory, Astoria, Oregon
Food Technology 11(2):104-108, 1957

A modified TBA method was found to be effective means for the determination of oxidative rancidity in a wide variety of fishery products. The oxidation of fat in fish meal, fish oil, fresh, and frozen fish was satisfactorily determined by this procedure. The reaction is performed on the intact sample eliminating the difficult extraction which is necessary in many rancidity methods. Conditions which affect the sensitivity of the reaction were investigated. Preliminary results indicate that it may be used as an index of quality of stored fishery products.

Chemical Changes Occurring in Cod Muscle During Chill Storage and Their Possible Use as Objective Indices of Quality

J. M. Shewan and N. R. Jones
Journal of the Science of Food and Agriculture 8:491-497, 1957

Studies have been made on the extractives and volatile substances (amino-acids, amines and other nitrogenous compounds, sugars, etc.) of freshly caught cod muscle and of some of the changes that occur during autolysis (storage at 0° under sterile conditions) along or combined with bacterial spoilage as in normal trade practice (storage in ice). The merits of some of these changes as objective indices of quality, particularly in relation to taste panel assessment, are discussed.

Studies on Freshness Determination of Fish Meat by the Distillation Ratio of Volatile Acids-VI. On the Form of Volatile Acids in Fish Meat

Suezo Asakawa
Bulletin of the Japanese Society of Scientific Fisheries
23(7&8):463, 1957

The present study was carried out to survey the form of volatile fatty acids and its change during the deterioration of fish meat. Using the minced meat of a kind of blue fin tuna as a test material, volatile acids were fractionated into free, salt and ester forms. The fractions obtained were further examined on their component fatty acids by paper chromatography.
Among the three forms found, the salt form was most abundant through all stages of deterioration. The proportion of these forms and the pattern of component fatty acids were observed to vary according to the freshness of meat. In the chromatographic study it was suggested that the component fatty acids in the free and salt forms were gradually replaced by the higher volatile acids with the progress of deterioration. In the ester form, the presence of higher acids ($>C_6$) was presumed.

Reporting on Organic Acids as Indices of Decomposition Chromatographic Separation

Halver C. VanDame, Associate Referee
Food and Drug Administration, Department of Health, Education, and Welfare, Kansas City, MO
Journal of the Association of Official Agricultural Chemists
40(2):404, 1957

Several organic acids are useful indices of decomposition in foods. Among the ones of most value are the volatile acids (formic, acetic, propionic, and butyric), lactic, and succinic. The methods for the determination of these acids now in Official Methods of Analysis are varied and somewhat empirical. The volatile acids are steam distilled, and acetic, propionic, and butyric acids are then separated by chromatography. Formic acid is determined on a fraction of the distillate by the weight of Hg_2CL_2 formed when the formic acid is refluxed with Hg_2Cl_2. Latic acid is determined by a ferric chloride colorimetric method. Succinic acid is determined after separation from lactic acid by a chromatographic procedure.

On the Determination of Trimethylamine and Trimethylamine Oxide. A Modification of the Dyer Method

Yoshiro Hashimoto and Tomotoshi Okaichi
Faculty of Agriculture, Tokyo University
Bulletin of the Japanese Society of Scientific Fisheries
23(5):269, 1957

The extraction of trimethylamine (TMA) with toluene was found to be affected by temperature, sorts of alkali and number of times shaken, whereas the color development with picric acid was not influenced by temperature. From these observations, the extraction procedure was modified to be carried out at a standard temperature (30°C). In an incubator, test solutions, reagents and funnels were previously warmed before extraction and the funnels shaken 60 times were again left for 5 minutes in it before the separation of toluene layer.
In the second place, the same volume of 25% potassium hydroxide solution was adopted instead of the original 50% potassium carbonate solution, which was confirmed to be not suitable for the TMA solution resulting from the reduction of trimethylamine oxide (TMO) with Devarda's alloy and hydrochloric acid. Results indicate that some constituents of the alloy may disturb the extraction of TMA in the case of potassium carbonate, but not in the case of the hydroxide. This agrees well with the fact that the carbonate forms white colloidal precipitates, perhaps carbonates of Al and Zn, in the aliquot of the heated reducing mixture, and the precipitates were suspected to absorb or occlude TMA. The hydroxide does not give such precipitates.
The modification above-mentioned facilitated the determination of the smaller amount of TMAO and the modified method gave over 97% recovery down to 0.025mg N which is far lower than 0.4 mg N in the method. The recovery of TMO added to carp muscle extracts was also satisfactory.

A Chemical Method for the Determination of Histamine in Canned Tuna Fish

O.S. Sager and William Horwitz
Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D.C.
Journal of the Association of Official Agricultural Chemists
40(3):892-904, 1957

Discrepancies between the biological and chemical methods for histamine were found to be eliminated by modifying the bioassay method so that the acid extracts were neutralized with sodium bicarbonate and Ringer-Locke solution rather than with solid sodium carbonate. When the extracts were neutralized in this manner, the pH was uniformly about

7.5; thus alkaline simulation of the guinea pig ileum was avoided, and results checked well with those obtained by the chemical method.

Report on Chemical Indices of Decomposition in Fish (Histamine)

David W. Williams, Associate Referee
Food and Drug Administration, Department of Health, Education, and Welfare, San Francisco, California
Journal of the Association of Official Agricultural Chemists 39(3):609, 1956

A biological method for the determination of histamine in fish was submitted to collaborative study by the Associate Referee in accordance with the recommendation of Subcommittee.
Each collaborator was sent 5 samples of ground mixed fish in hermeically sealed containers. Sample A consisted of Class 1 canned bonito; Sample B, Class 1 canned tuna; Sample C, Class 2 canned tuna; Sample D, Class 3 canned tuna; and Sample E, Class 4 canned tuna. The Associate Referee prepared Samples A and E by grinding the material in a food chopper, mixing it mechanically, and sterilizing the product in glass jars. Samples B,C, and D were a part of a canned experimental pack prepared by Hillig in 1950. Each collaborator was sent a copy of the procedure published in 1956.

Volatile Reducing Substances (VRS) and Volatile Nitrogen Compounds in Relation to Spoilage in Canned Fish

Lionel Farber and Michael Ferro
Fisheries Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, California
Food Technology 10:303-304, 1956

The VRS apparatus was changed to provide a closed system with a recirculating pump and spherical ball and joint connections.
The content of volatile reducing substances (VRS); total volatile and trimethylamine nitrogen (TVN, TMN) in canned California anchovies, California and Atlantic herring, California mackerel, California sardines in brine and in tomato sauce and tuna was determined for material judged organoleptically passable and not passable. The content of VRS correlated quite closely with the organoleptic judgement, whereas the content of TVN and TMN did not show any definite correlation. The content of trimethylamine nitrogen apparently varies with the species of fish canned and is the same in fish of different states of freshness for any single species.

Studies on the Method for Testing the Spoilage of Food-VI. A New Turbidimetric Method for Determination of Freshness of Cooked Fish-paste "Kamaboko"

Tetsuo Tomiyama, Yasuo Yone and Norisuke Sugawara
Bulletin of the Japanese Society of Scientific Fisheries
21(8):954, 1955

During spoilage of cooked fish-paste, it was observed that the surface is gradually decomposed by various micro-organisms. It follows that, on washing the surface with water by using brush, a turbid washing will be resulted, its turbidity being proportional to the degree of development of the micro-organisms.
Data presented in this paper have revealed that freshness of cooked fishpaste can readily be estimated by determining the turbidity of the washing which is made with 250 cc water by employing a brush of soft hair. The turbidity measurement has been carried out by the electrophotometer with a filter of 470 mμ. A marked increase in the value for the turbidity during the spoilage has been found to be in good conformity with a marked increase in the catalase activity of the washing which was already reported as an index of incipient spoilage.

The Effects of Iced and Frozen Storage Upon the Trimethylamine Content of Flounder *(Parophrys vetulus)* Muscle

Charlie M. Good and Joseph A. Stern
School of Fisheries, University of Washington, Seattle, Washington
Food Technology 9:327-332, 1955

English sole *(Parophrys vetulus)* were held in iced storage for periods ranging from 0 to 15 days. At intervals during this period samples were removed from the ice and filleted. Fillets were packaged, frozen and held in frozen storage at 0°F for periods up to 24 weeks. Samples were subjected to various spoilage tests prior to freezing and at intervals during the period of frozen storage. Of the various tests investigated, only the determination of trimethylamine was found to be of value in estimating the quality of English sole prior to freezing. The trimethylamine content of the fillets did not change during frozen storage, and it was concluded that the test could be used, after frozen storage, to indicate the quality of the flesh at the time it was frozen.

A Comparison of Objective Tests for Quality of Gulf Shrimp

E. A. Fieger and J. J. Friloux
Department of Agricultural Chemistry and Biochemistry,
Louisiana State University, Baton Rouge, Louisiana
Food Technology 8:35-38, 1954

Freshly caught Gulf shrimp stored in crushed ice were sampled daily for chemical, bacteriological and organoleptic tests. The correlation of the results of the chemical and bacteriological data to quality and spoilage is discussed.

Individual Volatile Acids, Succinic Acid, and Histamine as Indices of Decomposition in Atlantic "Little Tuna" *(Euthynnus alleteratus)*

Fred Hillig
Food and Drug Administration, Department of Health, Education,
and Welfare, Washington, DC
Journal of the Association of Official Agricultural Chemists
37:927-931, 1954

Progressive decomposition studies on "little tuna" demonstrate that volatile acid number, content of formic, acetic, propionic, butyric, and succinic acids, and histamine are indices of decomposition, and that these values correlate with the progress of decomposition of the raw material from which the canned product was prepared.

Studies On Freshness Determination of Fish Meat by the Distillation Ratio of Volatile Acids-VII. Proposition of a New Scale for the Freshness Determination

Suezo Asakawa
Bulletin of the Japanese Society of Scientific Fisheries
20(2):158, 1954

The correlation coefficient (r) between the amount of volatile basic nitrogen and the 2nd distillation ratio was -0.83. Moreover volatile fatty acids at each degree of freshness or spoilage were detected by the paper chromatography. Therefore, the distillation ratio determines the degree of deterioration of fish meat.
Various teleost fish-meats were classified as to their freshness according to the author's sensory tests, and their pH-values, volatile basic nitrogen amounts, and the 2nd distillation ratios measured.

A new standard scale is proposed for freshness determination of fish meat by means of the 2nd distillation ratio (D.R.) as follows:-

D. R.	>130	Excellent freshness
	130-100	Freshness
	99- 90	Inferior freshness
	89- 80	Early stage of spoilage
	79- 70	Spoilage
	< 70	Advanced stage of spoilage

this scale may be applied not only to many kinds of teleost fish but also to shark-fish, ray-fish and cuttle-fish.

Studies on Freshness Determination of Fish Meat by the Distillation Ratio of Volatile Acids-VI.

Suezo Asakawa
Bulletin of the Japanese Society of Scientific Fisheries
20(2):157, 1954

Block, minced, and brayed meat prepared from fresh or spoiled fish muscle were compared to determine the degree of crush using the steam distillate method. In view of the practical treatment, sample meat must be brayed in a mortar. Often the brayed meat from a complete putrid fish muscle gives a larger ratio than its exact value when it must be suspended in the distillate flask liquid. An all titrating alkaline solution can be used in about 0.02N concentration.
The preservative conditions in a dilute H_2SO_4 solution are also determined.

Colorimetric Method for Measuring the Quantities of Ammonia in Fish Meat on the Rapid Method

Fuyuo Ota and Zentaro Oshiro
Bulletin of the Japanese Society of Scientific Fisheries
19(12):1150, 1954

Some experiments were carried out to determine the best method for measuring ammonia in fish meat.
The instrument, which was specially devised by us to simplify the process of weighing the fish meat, proved very suitable for the present purpose.
Some precautions on the preparation and use of the reagents for the determination were shown.
By shaking vigorously with water for about one minute, ammonia was completely extracted from fish meat.
Mixture of potassium dichromate and cobalt nitrate was suitable as a standard color solution for the visual measurement of color intensity in Nessler's method.

Analytical procedure is as follows: Five grams of finely minced fish meat was weighed with the above described instrument and extracted with 10 volumes of water by vigorous shaking by hand for about one minute and filtered through a gauze or a cotton cloth. To 10cc of the extract was added 2.5cc of 15% sulphosalicylic acid containing 5% of sulphuric acid and 1.5g of activate carbon, and then it was filtered after being adequately mixed.
To 1.0cc of the filtrate taken in the colorimetric tube was added 4.8cc of the mixture of 1.2% sodium hydroxide with 4.2% Rochelle salt (1 : 1) and further to the mixed solution was added 0.2cc of Nessler's reagent. Color-intensity was immediately compared with the standard color solution.

The Determination of Volatile Reducing Substances (V.R.S.) as an Aid in Quality Control of Fish Products

Lionel Farber and Anne Cederquist
Fisheries Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, California
Food Technology 7:478-480, 1953

The concept of quality is discussed from two points of view; namely, the distinction between an acceptable or wholesome product and one that is not, and the extent to which a given commodity meets some ideal condition or standard. Illustrative data have been presented showing the possible application for the determination of the content of volatile reducing substances as an aid in evaluating the aforementioned aspects of quality. Data for the content of volatile nitrogen compounds have also been included.

The Quantitative Determination of Histamine with Cotton Acid Succinate

Hajime Kadota and Juntaro Inque
Bulletin of the Japanese Society of Scientific Fisheries 19(8):916, 1953

McIntire's method for the purification of histamine was applied to the quantitative determination of histamine produced in decomposed meat extract of fish.
It was found that this method is very suitable for rapid determination of histamine in a large number of samples. The essential features of this method are as follows: A decomposed meat extract of fish which contains histamine is extracted with n-butanol under conditions such that 83% of the histamine is removed from the aqueous phase in one extraction.

The histamine is recovered from the butanol by means of a new action exchange medium, cotton acid succinate. The histamine is eluted from cotton acid succinate with a small volume of diluted hydrochloric acid and the elute is neutralized with sodium carbonate and diluted with water to give a suitable concentration for colorimetrical determination with diazo-reaction.

Fundamental Studies on the Determination of Volatile Basic Nitrogen by Aeration Method-IV. Special Factors Affecting the Velocity Constant in Removing the Volatile Base

Toshiharu Kawabata
Bulletin of the Japanese Society of Scientific Fisheries
19(7):819, 1953

This paper deals with some of the factors affecting the velocity constant in removing the volatile base by aeration method, with special reference to the freshness of the fish meat examined.
The fresh meat from several species of fish showed no difference in the velocity constant, but showed a considerable difference in the velocity constant upon spoilage. The curves of volatile base obtained were not in complete agreement with the first order reaction. The cause of this error was found to be due to the high content of trimethylamineoxide in the muscle.
The curves obtained from the samples of spoiled fish meat which have relatively small amounts or no oxide, such as albacore, mackerel, carp and whale meat, were found to have curves nearly or completely in agreement with the theoretical curves.
A shortened procedure is presented, which is based on obtaining a 50 per cent removal of total volatile base and is able to determine within a short period of 15 minutes aerating at 45°C, 50 1/hr.

Fundamental Studies on the Determination of Volatile Basic Nitrogen by Aeration Method. Studies on Some Determinative Factors Influencing the Volatile Bases Obtainable from Fish Meat

Toshiharu Kawabata and Hiroshi Terui
Bulletin of the Japanese Society of Scientific Fisheries
19(6):746, 1953

The experiments were carried out on the trichloracetic acid extracts of fresh and spoiled mackerel meat. The results obtained on some determinative factors were not in complete agreement with those obtained from the equations

described in our previous report, especially on the aeration temperature. The curves of the amount of volatile basic nitrogen obtained when plotted by temperature showed two irregular increases at the temperatures of 45° and 70°C, which seemed to have no direct relation to the freshness of the material examined.

Studies on the Method of Determination for Freshness of Fish-flesh by "Distillation Ratio" of Volatile Acids in Steam Distillate-I.

Suezo Asakawa
Bulletin of the Japanese Society of Scientific Fisheries
19(2):118, 1953

In the determination of freshness of tuna, the "Distillation ratio" - % by acidity of the 2nd fraction (50 cc) to the 1st fraction (50cc) at the steam distillation with sample solution H_2SO_4 acidic condition - was changed proportionaely by the freshness of the fish flesh.

Studies on a Simplified Method for Estimating Freshness of Fish-I. On the Inhibitory Nature of Fish Muscle Extract Revealed in Kruger's Reaction of Acetic Acid

Taneko Suzuki
Bulletin of the Japanese Society of Scientific Fisheries
19(2):106, 1953

Volatile acids, such as formic, acetic, iso-valeric and butyric acid increased during fish spoilage. So it was tried to estimate the freshness of fish by detecting volatile acid, especially acetic acid. The results obtained would be summarized as follows:
1. It was proved by paper chromatography that acetic acid was not detected in newly caught fish muscle, while it was found in the period descending in freshness.
2. It was found that the detective reaction of acetic acid, so called lanthanblue reaction by Kruger, was inhibited by fish muscle extract.
3. A remarkable relation between the degree of the inhibition of the reaction and the freshness of fish was noticed.

Determination of Volatile Acids for Judging the Freshness of Fish

Taneko Suzuki
Bulletin of the Japanese Society of Scientific Fisheries
19(2):102, 1953

Friedemann's method for estimating the amount of volatile acids was adopted to determine freshness of several kinds of fish.

At high temperature 20°C-30°C, the increasing curves of volatile acids were sharper than those of volatile basic nitrogen which were usually taken as an index of spoilage. For fish stored above 0°C, the determination of volatile acids was a reliable test for quality.
By using Behrens method, it was disclosed that formic acid was found in the distillate of fresh fish while butyric, iso-valeric and acetic acid was found in the distillate of spoiled fish.

Studies on Freshness Determination of Fish Meat by the Distillation Ratio of Volatile Acids-V. Differences in the Distillation Ratio by Anatomical Parts

Suezo Asakawa
Bulletin of the Japanese Society of Scientific Fisheries
19(3):167, 1953

In order to see if the ratio of steam distillation of volatile acids is feasible in determining freshness of fish meat, a series of tests have been carried out using samples taken from the bonito, *Euthynnus yaito* Kishinouye. Fresh meat sampled from the dorsal, caudal, ventral, and dark muscle of a carcass was separately comminuted. The spoiled samples were prepared from similar parts of another fish which had been left under natural condition for three days. The ratios obtained from steam distillation of the samples were compared with the values of pH and volatile basic nitrogen (V-N) measured for each sample.
The quality of the fresh samples tested with V-N or pH revealed little difference among the anatomical parts. However, when expressed in the ratio of second distillation, the fresh samples had different values in accordance with the parts as:100 for dorsal, 93 for caudal, 82 for ventral, and 74 for dark muscle.
Spoilage measured with V-N and pH of the deteriorated samples was fairly distinct between the parts. The second distillation ratios of the samples were 75 for dorsal, 75 for caudal, 59 ventral, and 56 for dark muscle. Neglecting individual variance between the two specimens, the spoilage of the samples caused by three days' standing may be expressed as 75% for dorsal, 81% for caudal, 72% for ventral, and 75% for dark muscles. The greatest spoilage took place in the ventral muscle.
Freshness of two fish belonging to the same species can be examined by comparing the distillation ratios obtained from similar parts of them, although quality of a round fish cannot be determined from the ratio of its parts.

Studies on Freshness Determination of Fish Meat by the Distillation Ratio of Volatile Acids-IV. The Cause of the 2nd Distillation Ratio Exceeding 100 Percent

Suezo Asakawa
Bulletin of the Japanese Society of Scientific Fisheries
19(3):162, 1953

On the basis of the results obtained from the previous experiments the author has made the assumptions as follows:
1. Volatile acids in fish meat may consist of two kinds, one being free acids, and the other bound acids.
2. The second distillation ratio of the acids from fresh meat of fish exceeds 100 percent because the bound acids are greater than the free acids in amount so that few acids could be obtained in the first distillation.
3. However, the amount of the free acids increase, while the bound acids decrease, in response to deterioration of the meat. Such fluctuations in the acids seem to account for the second distillation ratio lowering below 100 percent in case of a spoiled meat.
In order to prove the above assumptions the following experiments have been carried out.
Prepare three kinds of samples from fresh meat of fish; minced meat, its water exudation, and a protein-free solution by treating the sample with 20% sodium tungstate. The similar group of the samples is prepared from the spoiled meat of a fish. Apply steam distillation to each sample. It was found as a result that the free acids were almost removed by exudation, for volatile acids contained in the residue of the exudation were measured. When free volatile acids were wrapped as a reagent together with gelatin, they were formed into bound acids to some extent.

Studies on Methods for Determining Freshness of Foods-VI. A Comparative Study of Methods for Determining Freshness of "Kamaboko"

Tetsuo Tomiyama and Yasuo Yone
Bulletin of the Japanese Society of Scientific Fisheries
18(10):521, 1953

No consistent value for the volatile base-nitrogen has been observed at the start of the spoilage of "Kamaboko" as reported by Kimata who gave 30-40 mg% as a criterion for spoilage. Data have also been presented showing that, contrary to Uchixama's report, a 60 mg% value for volatile acid seems to be inadequate as the criterion for beginning of spoilage.
It has been clearly shown that the catalase activity on the surface of "Kamaboko" shows a parallel relationship to the degree of growth of micro-organisms. This data infers a possibility of presenting a new method for expressing freshness of "Kamaboko".

Report on Fish (Indole in Crab) Indole as an Index of Decomposition in Dungeness Crab

David W. Williams
Food and Drug Administration, Department of Health, Education, and Welfare, San Francisco, California
Journal of the Association of Official Agricultural Chemists
35(3):525-526, 1952

A method to determine indole was investigated. Difficulty was experienced with foaming in the distillation and this greatly extended the time of analysis. This difficulty was corrected by using a silicone antifoam (D.C. Antifoam A). Standards and blanks were run using the antifoam and it was found to have no effect on the results.

Studies on the Method for Testing the Spoilage of Foods - V. A Study on the Aeration Method for the Determination of Volatile Base

Tetsuo Tomiyama
Bulletin of the Japanese Society of Scientific Fisheries
17(12):405, 1952

This paper deals with a study of factors governing the rate of removal of volatile base by aeration.
Only a slight increase in the rate of removal of the base was observed when the aeration tube was completely emerged in a water bath and by changing the dimensions of the aeration tube and drop catcher. This rate decreases with decreased freshness of the sample. A considerable increase in this rate, however, has been made by increasing the amount of potassium carbonate which is added prior to aeration. Raising the temperature also remarkably reduced the length of time needed for complete determination. By raising temperature of the aeration, mackerel flesh gave an excess amount of base while whale meat did not give any excess amount even at 80°C.

Studies on the Method for Testing the Spoilage of Foods - III. A New Steam-Distilling Method for the Determination of Volatile Base in Fish Flesh

Tetsuo Tomiyama, Katsumi Ide and Tsutomu Akiyama
Bulletin of the Japanese Society of Scientific Fisheries
17(7):1, 1952

A new steam-distilling method has been presented for the determination of the volatile base-N in fish flesh. Data is presented showing that the fish muscle brei on direct steam distillation at either pH 7.6 or 9.0 gave a value of about 145% of that obtained by the vacuum method. A 1-3% trichloracetic acid extract of the brei gave a value of nearly the same as that by the vacuum method, irrespective of the freshness of the sample used.

Simple Colorimetric Method for Measuring the Quantities of Ammonia in Fish Meat-II. An Application of Nessler's Colorimetric Method

Fuyuo Ota
Bulletin of the Japanese Society of Scientific Fisheries
17:309, 1951

The adaptability of Nessler's method to the estimation of ammonia in fish meat with the aid of some preliminary treatments was reported in the previous paper.
In the present paper experimental conditions in practicing this method were discussed with the following results.
1. The increasing rate of ammonia determined by this method, in the decrease of freshness of fish and in the fish meats heated under pressure, was nearly parallel with that of volatile basic nitrogen determined by distillation method usually employed.
2. This method made the determination of ammonia contents of various marine products and other fleshes very easy and simple.

Simple Colorimetric Method for Measuring the Quantities of Ammonia in Fish Meat-I. A Device to Remove Obstructing Substance in Nessler's Reaction

Fuyuo Ota
Bulletin of the Japanese Society of Scientific Fisheries
16(6):264, 1950

Some experiments were carried out to apply Nessler's colorimetric method for measuring the quantities of ammonia in fish meat. The colour reaction by Nessler's reagent for extracts of fish meat, does not indicate characteristic colour which is to be induced by ammonia, but colours differently; and it was shown that some substances which obstruct Nessler's reaction are removed by adding the adsorbent which does not influence ammonia in acidified solutions.
Therefore, Nessler's method may be adapted as a simple method for the estimation of ammonia in fish meat, by the following items:
1. Treating the aqueous extracts of fish meat with protein-precipitants charged with definite acidity and adsorbent,
2. Changing alkali earth metal ions into complex ions, dissolved out to filtrate from adsorbent,
3. Preparing a durable standard solution.

The Color Reaction Method for the Judgement of the Fish-Meat Freshness 3. Application of the Color Reaction of p-Quinone Induced by the Putrefaction Products of Fish-Meat to the Quantitative Determination of the Fish-Meat Freshness

Y. Obata and M. Ishida
Department of Fisheries, Faculty of Agriculture, Hokkaido University, Hakodate, Japan
Bulletin of the Japanese Society of Scientific Fisheries 16(4)147, 1950

In the previous paper we have reported that the p-quinone reaction was applicable qualitatively as a simple determination method of the fish-meat freshness.
The present experiments were carried out to see whether the same reaction would be applicable or not as a quantitative determination method. The results so far obtained are as follows.
The reddish brown color of the reagent shows a tendency to become darker, in proportion as the loss of the fish-meat freshness advances. But it is impossible to detect precisely by the color reaction method the critical grade of the loss in the fish-meat freshness considered from the sanitary point of view.
Furthermore, the color reaction of p-quinone proves hardly applicable to a quantitative colorimetric determination of the fish-meat freshness on account of a lot of technical difficulties in preparing necessary standard colors.

The Color Reaction Method for the Judgement of the Fish-Meat Freshness 2. The Judgement of the Fish-Meat Freshness by Means of p-Quinone

Y. Obata and K. Zama
Bulletin of the Japanese Society of Scientific Fisheries 16(1):10, 1950

The color reaction pf pyperidin, of the bacterial decomposition product of Lysin, against p-quinone has been proved applicable as a simple method for the judgement of the fish meat freshness, as it was recognized that the fish meat treated with p-quinone assumed a red color more rapidly in proportion to the progress of its putrefaction. This method is applicable also for freshess evaluation of boiled fish paste and smoked fish. In the case of smoked fish, the color reaction is affected by the wood-tar component which is contained in the sample as shown by its alcohol extract.

The New, Simplified Method of Determination for Freshness of Fishes (I)

T. Mori and M. Hata
Bulletin of the Japanese Society of Scientific Fisheries 15(8): 407, 1949

The periodical variation of reducing power of the 11 fish meats by the Anson-Mirsky's Ferricyanide method was investigated.
Reducing power increases in proportion to the lowering of the freshness.
The increasing rate of reducing power of various fish are nearly equal.
The reducing power at the beginning of putrefaction is approximately equal among various fish.
The increase of reducing power runs nearly parallel with that of volatile basic nitrogen. Therefore, it is possible to use this method as a standard for the determination of freshness in fish.

Simplified Test on the Freshness of Fish Meat-III. Non-Precipitability of Hexon Base with Mercuric Chloride

K. Amano, H. Uchiyama and F. Tomiya
Bulletin of the Japanese Society of Scientific Fisheries 15(6):262, 1949

As a delicate and characteristic method for detecting the early stages of decomposition of fish flesh, we reported the coagulation test of soluble protein-like substances with mercuric chloride solution. In the previous paper, it was described that the coagulum probably contains soluble protein, and also mercuric-aminochloride but in lesser amounts. However, some questions have been left as to whether the coagulum contains hexon bases. To solve this problem, the precipitability of mercuric chloride with hexon bases was examined in aqueous extracts of fish muscle.

The Color Reaction Method for the Judgement of the Fish Meat Freshness 1. The Color Reaction of the Component Responsible for the Fish-odor against p-Quinone.

Y. Obata and K. Zama
Bulletin of the Japanese Society of Scientific Fisheries 15(9):499, 1949

The color reaction against p-quinone was examined with the dilute aqueous solutions of ammonia, methylamine, trimethylamine and pyperidin, which have hitherto been thought as

the components responsible for the fish-odor. Ammonia and trimethylamine assumed a brown color while methylamine and pyperidin a red color. By the fact that albumin showed no reaction against p-quinone, the color reaction in question proved to have nothing to do with protein. Lastly, by examining the odor producing conditions of fresh salmon mucus and the reaction against p-quinone of the substance responsible for the odor, it was recognized that the color reaction appeared more rapidly in proportion to the production of fish-odor.

Simplified Test on the Freshness of Fish Meat-IV. Effect of Freshness of Fish Meat on the Stability of Its Aqueous Extract Against Alcohol

K. Amano and F. Tomiya
Bulletin of the Japanese Society of Scientific Fisheries
15(12:753, 1949

In the present report the stability of water soluble protein of fish meat against alcohol was examined concerning freshness of the fish meat by the following method: 1.0cc of water extract of the meat (10gms of meat: 100cc of distilled water) was put into a 50cc beaker and absolute alcohol was added dropwise from a burette with a rate of 20cc per minute; and the amount of alcohol required to form both turbidity and precipitation was determined. The concentration of alcohol necessary to coagulate protein in extract of fresh meat lies between 70 to 80%, while in the case of decaying meat 30 to 40% alcohol is enough to aggregate the protein. Probably this phenomenon involves the strength of hydration of the soluble protein.

It was found that coagulation of protein occurs in lesser concentration of alcohol in the presence of $HgCl_2$ than in the absence of the salt. Also it was proved that alcohol coagulable nitrogen increased in parallel with the amount of $HgCl_2$ contained in the solution. Materials employed in the above experiments were mackerel, tuna fish and skipjack.

In the light of the results obtained, one of the reasons for nonprecipitability of mercuric chloride in the extract of fresh fish meat may be due to the strength of hydration of the protein enough to prevent precipitation between protein and mercuric chloride.

On the Method of Microdetermination of Formaldehyde in the Smoked Foods

E. Tanikawa
Bulletin of the Japanese Society of Scientific Fisheries
14(1):56, 1948

To determine the quantity of formaldehyde in smoked fish by Iodometry, N/100 Iodine and Sodium Thiosulfate solutions were prepared, and the accuracy of the method was tested with diluted formaline solutions of definite concentration. About 5/100,000 diluted formaldehyde could be detected. To determine the quantity of formaldehyde in smoked fish meat, distillates of ground smoked fish meat prepared by steam distillation serves for Iodometry. Each 50 cc of the distillate is separately obtained at every 5 minutes and must be prepared in this way up to the 6th distillate. All the 6 distillates are mixed together for the next Iodometry. The maximum quantity of formaldehyde in the smoked fish meat can be obtained when the meat is ground and soaked immediately in distilled water. This micro-determination of formaldehyde in the smoked foods demands careful attention in the experiment, otherwise the exact amount can not be ascertained.

Simplified Test on the Freshness of Fish Meat-I. An Application of Walkiewicz's Method for Measuring the Freshness of Whale Meat

K. Amano and H. Utiyama
Bulletin of the Japanese Society of Scientific Fisheries
14(1):48, 1948

A rapid and simple method of measuring the freshness of raw beef, which was introduced by W. Walkiewicz, was applied to fish and whale meat. The Walkiewicz tests may be applicable as a rapid and simple method in detecting the first stages of decomposition of some fish meat.

Differential Spectrophotometry of Purine Compounds by Means of Specific Enzymes I. Determination of Hydroxypurine Compounds

Herman M. Kalckar
Division of Nutrition and Physiology, The Public Health Research Institute of the City of New York, Inc., New York
Journal of Biological Chemistry 167:729-742, 1947

Methods are given for the determination of very small amounts of hypoxanthine, inosine, xanthine, guanine, guanosine, and uric acid. These methods are based on

changes in the ultraviolet absorption of these compounds resulting from the action of specific enzymes. The procedures combine marked sensitivity with high specificity and seem to be well suited for the study of these purines and purine derivatives in biological materials.

Comparisons of Chemical Tests of the Quality of Fish

G.J. Sigurdsson
Food Technology Laboratories, Massachusetts Institute of Technology, Cambridge, MA
Industrial and Engineering Chemistry 19:892-902, 1947

The literature on the spoilage of fish is critically reviewed and a method not previously used for determining volatile acids in fish products is described. Methods of measuring the rate of spoilage of herring are compared for fish stored at different temperatures. For fish stored above 0°C determination of the total volatile acids or trimethylamine is found to be the most reliable, but these tests alone do not give full information about the state of all constituents in the muscle. For fish stored below zero these tests are unreliable, and a measurement of the protein breakdown is necessary.

Amines in Fish Muscle I. Colorimetric Determination of Trimethylamine as the Picrate Salt

W.J. Dyer
Atlantic Fisheries Experimental Station, Halifax, Nova Scotia
Journal of the Fisheries Rdsearch Board of Canada 6(5):351, 1945

A sensitive accurate colorimetric method for trimethylamine determination is presented, based on the extraction with toluene of an alkaline sample containing 0.002 to 0.02 mg. trimethylamine nitrogen, and the formation of the yellow coloured picrate by mixing with a picric acid reagent. The application of the method in fishery products and effects of interfering substances have been investigated.

Histamine Content of Unprocessed and Canned Fish. A Tentative Method for Quantitative Determination of Spoilage

E. Geiger
Van Camp Laboratories, Terminal Island, California
Food Research 10:293-297, 1944

It was reported in an earlier paper by Geiger, Courtney, and Schnakenberg (1944) that the muscle tissue of marine fish contains a biologically very active substance which

was identified as histamine. The histamine content of the fresh fish was found to be very low, but it increases post mortem rapidly. The changes in the histamine content were so evident and so regular that it seemed to be promising to use the histamine content as a criterion for the freshness of fish.

Determining Volatile Bases in Fish Comparison of Precision of Certain Methods

Maurice E. Stansby, Roger W. Harrison, John Dassow, and Mary Anne Sater
Technological Laboratory, U.S. Fish and Wildlife Service, Seattle, WA
Industrial and Engineering Chemistry, Analytical Edition 16(9):593-596, 1944

Methods were investigated for determining total volatile base and tertiary volatile base in fish flesh as an index of spoilage. Sampling methods tested included use of press juice, protein-free press juice, 60% ethanol-leached samples, samples "liquidized" with 60% ethanol, and samples of ground fish suspended directly in solution. Volatile base was removed by microdiffusion, distillation, and aeration. Most precise results were obtained for total volatile base by extracting the fish flesh with 60% ethanol and removing the volatile base by distillation from the solution made alkaline with borax.

The Pungent Principles of Fishes Produced by Decrease in Freshness Part III. On the Colorimetric Determination of Histamine in the Canned Fishes and Several Food Stuffs

Hikomi Igarasi
Bulletin of the Japanese Society of Scientific Fisheries 8(1-6):161, 1939-40.

In Parts I and II the writer described the isolation of histamine from fish with decreased freshness and from fish which were treated with bleaching powder, α-and β-naphthol and kept at 24-25°C for two days. By applying the method of R. Yokoyama the writer determined the amount of histamine in canned fish as well as other food-stuffs.

The Pungent Principles of Fishes Produced by Decrease in Freshness. Part II.

Hikomi Igarasi
Bulletin of the Japanese Society of Scientific Fisheries
8(1-6):158, 1939-40.

In the previous communication, the writer has shown that the pungent principle of fish produced by a decrease in freshness is histamine and has given a method of isolation of the amine. Biological observations on the formation of histamine have been made by many workers. The writer could isolate histamine from *Scomber japonicus* (Houttuyn) treated with bleaching powder containing 0.04% chlorine, α- and β-naphthol 0.5% and kept at 24-25°C for two days.

The Alcohols as a Measure of Spoilage in Canned Fish

Duncan A. Holaday
Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC
Journal of the Association of Official Agricultural Chemists
22(2):418-420, 1939

A determination of the amount of alcohol present is proposed as a measure of the extent of decomposition of canned fish.

A Chemical Procedure for Evaluating Spoilage in Canned Fish, Especially Salmon and Tuna Fish

F. Hillig and E. P. Clark
Journal of the Association of Official Agricultural Chemists
21:688-695, 1938

Suspended a 50-g sample of canned fish in water and phosphotungstic acid, filter off the solids, and steam-distil the resulting liquid by the modified Dyer method; collect the distillate in 2-100-cc portions and titrate separately as proposed by Dyer, unite the portions and det. formic acid. These data are sufficient to det. the extent of spoilage in canned fish, as is well shown by tabulated data. With modification the method may be used on sauce-packed fish. Preliminary work has shown that the volatile acid no. on such material (sardines) is of little value because of the excessive quantities of acids in the sauce (essentially AcOH): however, if the formic acid no. is detd., the method becomes valuable as an index of the condition of any sample under investigation.

The Measurement of Spoilage in Fish

S. A. Beatty and N. E. Gibbons
Atlantic Fisheries Experimental Station, Halifax, Nova Scotia
Journal of Biological Research Board of Canada 3(1):77, 1937

The increase in volatile nitrogenous bases in codfish muscle between the pre-rigor period and the first appearance of odour is approximately 6mg. per 100 grams of tissue, and is due almost entirely to the action of bacteria. It can be used to follow the course of spoilage only if the original value of the fish in question is known, as the range in variation of the original values is as great as the increase to the appearance of odour.
A method for the rapid determination of "trimethylamine" in cod muscle has been devised. Its increase parallels the increase in bacterial population. Odours always appear at approximately the same level of "trimethylamine". The increase resulting from autolysis is negligible. The increase during the development of spoilage is fifteen to twenty times the original value. Spoilage can be followed in fish preserved with borates as well as in untreated fish.

Relation Between the Rate of Decomposition of Fish Muscle and Chemical Constituents

Yarokuro Yamamaura
Bulletin of the Japanese Society of Scientific Fisheries
1(1-6):75, 1932-33

The following empirical formula is obtained between the ammonia-content at the beginning of decomposition of fish muscle, A_0, and that after D-days A: $A=A_0 e^{KD}$ where K is a constant.
The values of K are calculated by the formula for a number of types of fish. It is shown that the value of K has a remarkable correlation with total nitrogen, N; fat, F; and water, W.

BIOCHEMICAL METHODS FOR DETERMINING FISH QUALITY

A Rapid Visual Enzyme Test to Assess Fish Quality

F. D. Jahns, J. L. Howe, R. J. Coduri, Jr., A. J. Rand, Jr.
Department of Food Science and Technology, University of Rhode Island, Kingston, RI
Food Technology 30:27, 1976

A visual test strip has been developed to assess fish freshness. The visual method closely resembles the colorimetric data making it possible to predict the practical use of the test strip as a semi-quantitative analysis to detect hypoxanthine concentrations in a given sample of fish.

Relation Between the Quality of Canned Fish and Its Content of ATP-Breakdown-III. ATP-Breakdown in Canned Albacore and Skipjack in Relation to the Organoleptic Inspection

Yutaka Fujii, Katsuo Shudo, Kunisuke Nakamura, Senji Ishikawa and Minoru Ikada
Bulletin of the Japanese Society of Scientific Fisheries 39(1):69-84, 1973

The ratios of Hx and IMP content to total Hx, HxR and IMP content in canned albacore and skipjack meat were measured and correlated with organoleptic judgements by Japanese and American inspectors in order to establish a useful chemical index of meat "decomposition". A linear relation expressed by a simple equation and a high correlation coefficient (over 0.9) between the Hx or IMP ratios in raw and canned meat was obtained. As the inspection scores (odour judgements by Japanese inspectors) passed from high rating a' through b, d' down to low ratings C and D, there is a definite increase in the Hx ratios of the respectively rated canned meats. From this it was inferred that the Japanese inspection standard of odour is closely related to the freshness of fish material. The Japanese inspection scores based on odour and the American inspection judgements of "decomposition" of canned albacore and skipjack prepared from frozen fish agreed fairly well with each other. There was, however, a pronounced disagreement in inspection judgements of canned "winter albacore" and canned skipjack prepared from iced fish.

A Rapid Method for Determination of Inosine, Hypoxanthine, Uric Acid, and Nucleotides in Fish Muscle by Continuous Gradient Column Chromatography

Noboru Kato, Hitoshi Uchiyama, and Fumiaki Uda
Bulletin of the Japanese Society of Scientific Fisheries
39(10):1039-1044, 1973

In a previous paper, the authors reported that a rapid method for determination of ATP-related compounds in fish muscle by concave gradient column chromatography is useful for judgement of enzymatic freshness of fish. However, by this method, inosine and hypoxanthine could not be separated from each other. When sodium borate is added to an inosine solution, the hydroxyl radicals at the cis-position of ribose in inosine react with the borate and a complex compound, having an affinity toward an ion exchange resin greater than that of inosine, is produced. By adding sodium borate to the extract of fish muscle, inosine was completely separated from hypoxanthine in an appropriate pH range. Hypoxanthine, inosine, uric acid, AMP, IMP, ADP, and ATP can be separated completely from each other within 3.5hr with satisfactory recoveries. The distribution patterns of these compounds in muscle of sea bass, dark muscle of tuna and chicken muscle during ice storage were examined by the improved method.

Hypoxanthine and Nucleotide Levels in Pacific Halibut Stored in Refrigerated Sea Water, In Ice, and Plait Frozen

D. E. Kramer
Vancouver Laboratory, Fisheries Research Board of Canada,
Vancouver, British Columbia, Canada
Food and Agricultural Organization, Fisheries Report
115:28-32, 1971

Pacific halibut was frozen, kept in refrigerated sea water or kept in ice. The first sample has the control (time 0) and the others were stored for 5-10-15-20 day periods. Determination of the hypoxanthine index and total nucleotides were made.

Simple and Rapid Method for Estimating the Freshness of Fish

Hiroshi Kobayashi and Hitoshi Uchiyama
Bulletin of the Tokai Regional Fisheries Research Laboratory
61:21, 1970

The K value proposed as an index of freshness of fish has been recognized to be effective by many workers. A simple and rapid determination method for the K value was developed. The main features are as follows:

1. Using column chromatography of Dowex 1x4 chloric type and authentic ATP and its related compounds, the recoveries of 97-100% were obtained by eluting the mixture of HxR and Hx with 50 ml of 0.001 N HCl solution, and that of AMP, IMP, ADP and ATP with the same volume of 0.6 N NaCl in 0.01 N HCl solution.
2. ATP and its related compounds were eluted faster on Dowex 1x4 chloric type column than on Dowex 1x8 formic type column.
3. By using a simple apparatus the method presented here was practical for the rapid estimation of the freshness of fish and the values determined by this method agreed with those obtained by the column chromatography of Dowex 1x8 formic type resin.

A Rapid Method for Determination of the Acid-soluble Nucleotides in Fish Muscle by Concave Gradient Elution

Shigeo Ehira, Hitoshi Uchiyama, Fumiaki Uda, and Hiroyuki Matsumiya
Bulletin of the Japanese Society of Scientific Fisheries 36(5):491-496, 1970

Many workers have investigated to elucidate the relation between the degradation of nucleotides and freshness of fish. We have also reported that the ratio of nucleotide decomposition (K value) has been found to be a more useful index for freshness of fish than the amount of bacterial decomposition products such as volatile base and trimethylamine. From the results of these studies, it may be safely said that pursuing the changes of nucleotides in the fish muscle is a prerequisite for estimating the freshness of fish. Ion-exchange chromatography with stepwise elution is usually used in this field. The method is, however, so time consuming and troublesome that it is inappropriate for rapid determination of nucleotides and their related compounds. The procedure described in the present report used Dowex 1x4 (Cl) column with concave gradient elution system by employing a simple apparatus. The results were a rapid separation of HxR, Hx, IMP, AMP, ADP and ATP with adequate resolution and ease of detection.

Premortem Stress and Postmortem Biochemical Changes in Skipjack Tuna and Their Relation to Quality of the Canned Product

Ladell Crawford and Enid J. Irwin, John Spinelli and W. D. Brown
Journal of Food Science 35:849-851, 1970

Live skipjack were caught, brought to shoreside and held in tanks 24-48 hours. At this time some were exercised to simulate stress during commercial capture, sacrificed,

held at 74° and 32°F for 6 hours, sampled for chemical analyses, then canned. Unstressed (rested) skipjack were treated similarly. Some autolytic degradation products postmortem were measured and the differences noted. Organoleptic evaluation was made on canned fish from the various treatment groups. While there were differences in various organoleptic parameters among the groups subjected to different treatments, there was no overwhelming evidence connecting stress or temperature of holding to quality in the canned product.

Alpha-Glycerophosphate Dehydrogenase as an Index of Iced-Storage of Fresh, Gutted Haddock *(Melanogrammus Aeglefinus)*

Edith Gould
Bureau of Commercial Fisheries, Technological Laboratory, MA
Journal of Fisheries Research Board of Canada
26(12):3175-3181, 1969

A new test described for judging the iced-storage age of unspoiled gutted haddock, from early loss of quality to the first gross signs of spoilage. The test is a measure of the changing properties of alphaglycerophosphate dehydrogenase in tissue fluid with time after death, and is interpreted as an indication of leaching of tissue during the first 7 days of storage. Such functional changes in an enzyme system are a particularly sensitive criterion of freshness in fish during the first 4 or 5 days of iced storage.

Rapid Estimation of Freshness of Fish by Nucleoside Phosphorylase and Xanthine Oxidase

Shigeo Ehira and Hitoshi Uchiyama
Tokai Regional Fish Research Laboratory, Chuo-Ku,
Tokyo, Japan
Bulletin of the Japanese Society of Scientific Fisheries
35(11):1080-1085

A rapid estimation of freshness of fish muscle has been modified for application to the muscle of fish where inosine instead of hypoxanthine is accumulated after death. The method involves the use of nucleoside phosphorylase besides xanthine oxidase. Perchloric acid extract of the muscle tissue is neutralized with potassium hydroxide and the precipitated perchlorate is removed. The above extract is buffered with phosphate buffer (pH 7.6) and mixed with an aqueous solution containing both

nucleoside phosphorylase and xanthine oxidase. After the mixture is incubated at 37°C for 30 minutes., the uric acid produced is determined by measuring the absorption at 293 mμ. Another aliquot of the extract is treated in the same way, except that the solution containing xanthine oxidase only is used as the enzyme solution, and the uric acid derived from hypoxanthine in the tissue is determined. This amount is subtracted from the total uric acid to yield the content of uric acid originated from inosine in the tissue. Recoveries of inosine and hypoxanthine added to the extracts of fish muscle were fairly good, being 92-106% and 95-106%, respectively. The values obtained by this method agreed well with those by ion-exchange chromatography. In the course of this experiment, it was found that jack mackerel was of the inosine accumulating type, as is the already known Pacific salmon.

Nucleotide Degradation and Organoleptic Quality in Fresh and Thawed Mackerel Muscle Held at and Above Ice Temperature

D.I. Fraser, D.P. Pitts and W.J. Dyer
Fisheries Research Board of Canada, Halifax Laboratory, Halifax, N.S.
Journal of the Fisheries Research Board of Canada
25(2):239-253, 1968

In mackerel, by the time of initial sampling, adenine nucleotides had been deaminated to inosine monophosphate (IMP) in the ordinary muscle; in the red muscle the degradative sequence was even more advanced, as indicated by high initial levels of inosine. Postmortem rates of degradation of IMP to hypoxanthine through inosine were similar in both types of muscle; at ice temperature the rates were slower than in cod but faster than in swordfish. A delay in icing of 6-8 hours after catching accelerated the gradual decline in eating quality with replacement of the characteristic fresh mackerel flavor by tastelessness. IMP dephosphorylation paralleled development of tastelessness while spoilage (organoleptic) had developed prior to accumulation of appreciable amounts of hypoxanthine. At higher temperatures, 5-20° C, rates of IMP dephosphorylation, hypoxanthine accumulation, and quality loss were markedly increased. Thawing did not influence subsequent deterioration rates, but ascorbic acid dips delayed darkening of the flesh in thawed samples.

Excellent correlation of taste with both IMP and hypoxanthine content, and with various simple measures if IMP dephosphorylation was obtained under the various handling conditions investigated, including delayed icing, holding at elevated temperatures, and after thawing. The simple tests - ultraviolet absorption at 248 mμ of a Dowex treated perchloric acid extract, and ratio of ultraviolet absorption of extracts at 251 mμ after Dowex treatment to that before treatment - proved as good indices of progressive quality loss to the unacceptability level as the more complex estimation of IMP or hypoxanthine.

Nucleotide Degradation, Monitored by Thin-Layer Chromatography, and Associated Postmortem Changes in Relaxed Cod Muscle

Doris Fraser, J.R. Dingle, J.A. Hines, S.C. Nowlan and W.J. Dyer
Fisheries Research Board of Canada, Halifax Laboratory, Halifax, NS
Journal of the Fisheries Research Board of Canada
24(8):1837-1840, 1967

Cod muscle relaxed at death, containing high initial levels of CP and ATP and very low levels of lactate, maintained its elevated level of ATP during iced storage at 24 hr postmortem; as a result, nucleotide catabolism, glycolysis, and onset of rigor were delayed. The thin-layer chromatographic procedure used proved a useful qualitative and semiquantitative tool for following the pattern of nucleotide degradation quickly and easily.

A Study on the Change in Nucleotides and Freshness of Carp Muscle During Chill- Storage

Tetsuo Tomiyama, Kunio Dobayashi, Keiko Kitahara, Etsuko Shiraishi and Nobuyoshi Ohba
Lab. Fish. Chem., Fac. Agr., Kyushu University, Fukuoka
Bulletin of the Japanese Society of Scientific Fisheries
32(3):262-266, 1966

A number of studies have been reported on the change in the acid soluble nucleotide of muscle tissue. In fish muscle, however, few works have been done on the relation between the change in the nucleotides and the freshness based on the bacterial spoilage. It is proposed recently from a consumer's standpoint that the flavor quality index of muscle tissue is more desirable as freshness rather than the bacterial spoilage index. The present paper deals with the relationship between the change in the concentrations of various nucleotides, VBN and the number of bacteria in carp muscle during chill-storage.

The concentration of IMP is quite important as the index of flavor quality of fish muscle, because IMP is the major nucleotide in fish flesh as the flavor-enhancing constituent. The amount of IMP reached the maximum level (5.5 μ mole/g) at 0-4°C one day after slaughter of carp. IMP stays about three days without appreciable change under the chill-condition. Following 4-day storage, however, IMP was decreased rather rapidly. On the other hand, the bacterial spoilage was found to occur after 11-day storage. Therefore, the maintenance time of IMP, namely, the time of keeping passable flavor quality of carp muscle was one third to one half of the time of incipient spoilage.

Nucleotide Measurement: Rapid Measurement of Inosine Monophosphate and Total Adenosine Nucleotides in Fish Tissue

John Spinelli and Barbara Kemp
Bureau of Commercial Fisheries, Technological Laboratory, Seattle, Washington
Journal of Agricultural and Food Chemistry 14(2):176-178, 1966

A method is described for the rapid determination of inosine monophosphate (IMP) and total adenosine nucleotides in fish tissue. Perchloric acid extracts of the tissue are made, and the nucleotides are absorbed and separated for nucleosides and purine bases on a Dowex 1-X4-(Cl) resin. After elution from the resin with H_2SO_4, total adenosine and inosine nucleotides are determined by measuring the absorbancy of the effluent at 250 mμ. Adenosine nucleotides in the effluent are then determined chemically and subtracted from the total nucleotides to yield the IMP content of the extract. The method is useful over varying concentrations of adenosine and inosine nucleotides, and values are in close agreement with those obtained by using classical ion exchange systems.

Studies on Relation Between Freshness and Biochemical Changes of Fish Muscle During Ice Storage

Hitoshi Uchiyama, Taneko Suzuki, Shigeo Ehira and Elizabro Noguchi
Tokai Regional Fisheries Research Laboratory, Chuo-Ku, Tokyo, Japan
Bulletin of the Japanese Soceity of Scientific Fisheries 32(3):280-285, 1966

Studies have been made on the relation between the lowering of freshness and the biochemical changes in the fish muscle during ice storage using plaice and skipjack. The changes in total amounts of free amino acids, salt-

soluble proteins, volatile bases and trimethylamine, together with bacterial count, pH and "K" value (absorption ratio in percentage of 250 mμ of inosine plus hypoxanthine fraction to the perchloric-acid extract of muscle) which had been proposed for an index for freshness. Psychlorphylic bacteria were little increased during the first 10 days for plaice and throughout the storage time (9 days) for skipjack. The solubility of 0.6M KCl-soluble proteins was not changed significantly in both cases. The amount of volatile bases was fairly well increased at the earlier stage and thereafter kept constant. However, trimethylamine was not increased until 14 days and 9 days for plaice and skipjack, respectively. The amount of free amino acids was observed to increase during the storage for plaice, and on the tendency to increase was rather unexpectedly for skipjack. The freshness was organoleptically observed to be markedly lowered at the 14th days for plaice, and at the 4th day for skipjack, earlier than bacterial counts and substances such as volatile bases and trimethylamine were not increased so much. Based on these results, it may be said that in the early stage of the storage the deterioration of freshness of fish is effected by autolysis rather than bacterial action. Also in the present study, of fish, i.e. amounting to 40-50% when freshness was recognized to be markedly lowered in both cases.

Nucleotide Degradation and Quality in Ordinary and Red Muscle of Iced and Frozen Swordfish (*Xiphias gladium*)

W. J. Dyer, Doris I. Fraser, and Dianne P. Lohnes
Fisheries Research Board of Canada, Halifax Laboratory, Halifax, N.S.
Journal of Fisheries Research Board of Canada 23(12):1821-1834, 1966

In iced dressed swordfish, inosine monophosphate, initially the predominant nucleotide (5.2 μmole/g), was dephosphorylated to inosine during 19 days of storage. Hypoxanthine increased very slowly to about 1 μmole/g while quality (taste panel) showed no significant decrease up to 15 days but was near borderline at 19 days. These changes occurred more slowly than in cod and related species. The sequence of nucleotide changes occurred much earlier in the red muscle. Rapid freezing and storage at -26C for 4-5 months inhibited nucleotide enzymic activity, and quality remained unchanged. Slow freezing and storage for 1 week at 4°C significantly reduced quality to borderline or unacceptable levels, but only slightly affected the nucleotide degradation, indicating that other

factors were responsible for the loss in quality. Dephosphorylation and hypoxanthine accumulation continued during further storage at -4C. The levels of hypoxanthine reached during 19 days iced storage or 4-5 months frozen storage were not sufficiently high enough to impart bitter flavors, except possibly in the red muscle. A simple measure of inosine (+ hypoxanthine) may be useful as a quality test; a supplementary hypoxanthine test could be used to confirm spoilage.

Sugar Phosphates as Indicators of Fish Quality

J.R. Burt
Technology of Fish Utilization, R. Kreuzer, ed., pp. 176-179, Fishing News, London, 1965

The considerable contributions made by hexose phosphates, pentose phosphates and nucleotides to fish quality, particularly in the early post-mortem states, is reviewed. The lack of suitable methods for determining single compounds of the above classes would appear to preclude their use as indicators of quality. It is concluded that two general, comparatively nonspecific, chemical tests might have some practical applications. The resorcinol reaction for ketohexose and ketohexose phosphate, when carried out on extracts of frozen fish muscle, could give indications of time elapsed prior to freezing and of time and temperature of storage. The "barium, alcohol nonprecipitable pentose" index of Shewan and Jones is of use in deciding on the suitability for freezing of certain fish.

Estimation of Hypoxanthine Concentrations of Fish Muscle by a Rapid, Visual Modification of the Enzymatic Assay Procedure

J.R. Burt, G.D. Stroud, and N.R. Jones
Technology of Fish Utilization, R. Kreuzer, ed., pp. 367-370, Fishing News, London, 1965

The several existing methods of determining hypoxanthine concentrations in extracts of fish muscle differ in their accuracy, their ease and speed of execution and the amount of instrumentation required. The enzymatic assay procedure, which using xanthine oxidase monitors hypoxanthine as its oxidation product uric acid, requires complex instruments for its operation. A method of simplifying this technique has been developed by incorporating the oxidation-reduction

indicator, 2,6-dichlorophenolindophenol, into the reaction mixture. This is decolourized to an extent proportional to the amount of hypoxanthine present. Amounts of dye, whose total decolourization would correspond to a pre-set rejection limit and to levels just above and below that limit are used. Extracts of fish muscle can then rapidly be screened and classified as (a) well below, (b) just below, (c) just above, or (d) well above the rejection limit. A large number of extracts have been examined by this procedure and the fish from which they were prepared were classified with a high degree of accuracy. After comparing the results of this test with the correct hypoxanthine concentrations it was found that 94 and 95 percent of the samples would have been correctly rejected and accepted respectively.
The advantages that the hypoxanthine test has over many other tests of fish freshness, particularly during the early post-mortem stages of autolytic deterioration makes it potentially of great use in screening iced fish prior to freezing ashore. In addition, hypoxanthine concentrations in stored frozen fish are known to be a good index of pre-freezing quality.

Hypoxanthine and Other Purine-Containing Fractions in Fish Muscle as Indices of Freshness

N.R. Jones
Technology of Fish Utilization, R. Kreuzer, ed.,
pp. 171-180, Fishing News, London, 1965

Adenosine 5'-triphosphate (ATP) is degraded to hypoxanthine in chill-stored muscle, and thereafter the purine ring is cleaved. The early reactions, of the sequence producing these changes, are autolytic. Bacterial enzymes become progressively more active in later stages. Analogous autolytic reactions can occur in the frozen state.
This degradation of mononucleotides results in a deterioration in flavour. Two key stages in the reaction-sequence are slow enough to allow their measurement as objective indices of freshness:
The dephosphorylation of inosine 5'-monophosphate (IMP) is predominantly autolytic. Consequently, its estimation can be a measure of freshness for fish before the onset of bacterial attack. Methods for the estimation are discussed. A rapid procedure, based on selective ion-exchange from muscle extracts on to resin suspensions, is described.

The accumulation of hypoxanthine results from both autolytic and bacterial activation in muscle so that measurement can be valuable throughout storage "life". Relative performances of a number of procedures for estimating the purine are discussed. A modified xanthine oxidase assay, suitable for multiple analyses in quality control laboratories, is described, together with an assessment of its potential value as an index of storage time and of quality.
It was ascertained that the injection of the mercurous chloride solution resulted in the increase of both the hematocrit value and the erythrocyte count, while the rearing in the same solution were followed by the marked decrease in oxygen consumption. Treatment in the natrium chloride solution resulted in a remarkable increase in both the erythrocyte count and the erythrocyte resistance.

Degradation of Adenine- and Hypoxanthine- Nucleotide in the Muscle of Chill-Stored Trawled Cod *(Gadus Callarias)*

N.R. Jones and J. Murray
Journal of the Science of Food and Agriculture 13:475-480, 1962

The muscle of trawled cod contains little adenosine 5'-triphosphate and much inosine 5'-monophosphate at death. The adenosine 5'triphosphate remaining in the muscle is rapidly converted to inosine 5'monophosphate during chill storage. This is dephosphorylated to inosine, which is itself cleaved to hypoxanthine and either ribose or ribose I-phosphate. The stoicheiometry and nature of the reactions liberating the free base, ribose and the isomeric ribose phosphates are discussed in relation to their technological implications.

A New Method for Estimating the Freshness of Fish

Tsuneyuki Saito, Ken-ichi, Arai and Minoru Matsuyoshi
Department of Fisheries, Hokkaido University, Hakodate, Japan
Bulletin of the Japanese Society of Scientific Fisheries
24(9):749-750, 1950

In spite of the extreme importance of phosphate esters as metabolic intermediates, published reports on studies of their changes in fish muscle especially such dealing with acid soluble nucleotides, are few in number. According to the facts related to the role of these esters in living muscle, it may be considered that the freshness of fish must be closely related to the changes in the acid soluble

nucleotides. By determining the content of ribose derived from nucleotides, this principle was applied in estimating the freshness of fish. On the other hand, in the previous paper it has been reported that when carp muscle is frozen at a slow rate there occur rapid splitting of ATP and instantaneous accumulation of IMP. Furthermore, IMP thus formed is slowly converted to inosine and then to hypoxanthine. At room temperature these changes are accelerated and as a result inosine and hypoxanthine, especially the latter, are predominantly accumulated. Using these results as a basis, it has been decided that the freshness of raw or frozen fishes may be judged by estimating their content of inosine and hypoxanthine.

Rapid Measures of Nucleotide Dephosphorylation in Iced Fish Muscle. Their Value as Indices of Freshness and of Inosine 5'-Monophosphate Concentration

N.R. Jones and J. Murray
Journal of the Science of Food and Agriculture 15:684-690, 1964

Ion-exchange chromatographic procedures were modified so that nonphosphorylated derivatives in muscle extracts could be separated rapidly from nucleotide precursors. Separations were effected either by filtration through a resin bed or by the shaking of extracts at appropriate pH with resin. Nucleotide dephosphorylation was calculated from ultraviolet absorption measurements before and after treatment. The performances of the procedures were compared with that of the fractionation of extracts with barium. The validities of these rapid analyses as indices of absolute nucleotide breakdown, inosine 5'-monophosphate concentration and freshness are discussed.

Nucleotide Degradation in the Muscle of Iced Haddock *(Gadus aeglefinus)*, Lemon Sole *(Pleuronectes microcephalus)*, and Plaice *(Pleuronectes platessa)*

Bugn-Orn Kassemsarn, B. San Perez, J. Murray, and N.R. Jones
Torry Research Station, Aberdeen, Scotland
Journal of Food Science 28:28-32, 1963

The muscle of trawl-caught haddock, lemon sole, and plaice contained little adenosine 5'-triphosphate (ATP) and much inosine 5'-monophosphate (IMP) at death. ATP, adenosine 5'-diphosphate (ADP), and adenosine 5'-monophosphate (AMP) levels changed rapidly after the fish died. IMP was lost from the muscle more slowly, with

liberation of inosine, which was, in turn, degraded to hypoxanthine. A little adenine was formed by an alternative pathway of ATP degradation in lemon sole. A relatively high initial level of guanine was found in plaice muscle. Traces of xanthine was detectable in spoiling muscle from the three species. Implications of the findings are discussed in relation to quality testing and flavor changes in iced fish.

Method for Judging the Physiological Condition of Fish by the Quantitative Changes of the Blood Characters-I.

Osamu Tamura, Masato Yasuda and Tetsuo Fujiki
Fac. of Fish., Nagasaki University, Nagasaki, Japan
Bulletin of the Japanese Society of Scientific Fisheries
28(5):504, 1962

An attempt is made in the present paper to select a technique estimating environmental and physiological conditions of fish by means of some blood characters. The differences between the first and second blood samples from the intact carp were used as a standard in the values of hematocrit, erythrocyte count, erythrocyte resistance and oxygen consumption. Then the differences between the blood samples before and after treatment were compared with the standard obtained above. The treatments tried were as follows: (group 1) the injection of 0.2cc of mercurous chloride solution (0.2-0.4 ppm) into the lateral muscle; (group 2) keeping in the same solution (0.2 ppm) for ten days; (group 3) keeping in a natrium chloride solution (1%) for one or two days.

Enzymatic Method for the Estimation of Freshness of Fish. - II. Relationship between Freshness of Fish and Succinic Dehydrogenase Activity

Hironari Fukuda
Bulletin of the Japanese Society of Scientific Fisheries
23(768):490, 1957

The change of succinic dehydrogenase activity of fish tissues with incubation of 25°C are discussed. Although great variations in the enzymatic activity were observed among various tissues, a decrease in the enzyme activity of a certain sample tissue showed a significant correlation with decrease of its freshness. Therefore, the freshness of sample fish can be judged from decrease in the enzyme activity (freshness-index value E) of various sample tissues, and the values were compared with those values of fresh fish of the same kind.

Enzymatic Method for the Estimation of Freshness of Fish-I. Estimation of Freshness of Fish by Determining Activity of Succinic Dehydrogenase of Fish-Tissues

Hironari Fukuda
Bulletin of the Japanese Society of Scientific Fisheries
23(7&8), 486, 1957

The activity of succinic dehydrogenase in fish organs was found to decrease with deterioration of their freshness. The determination of succinic dehydrogenase activity with 2,6-Dichlorophenolindo-o-chlorophenol, was applied to the estimation of freshness of fish.
The freshness can be expressed as "Freshness-index", which is equal to the value of succinic dehydrogenase activity per one gram of fresh tissue as previously described. "Freshness-index" of various organs or tissues can be conveniently expressed as Ek, El, Edm, etc. for kidney, liver, dark muscle, etc. depending on each tissue tested. The "freshness-index" value of various tissues will indicate the actual freshness of the fish sample as a whole.

MICROBIOLOGICAL METHODS FOR DETERMINING FISH QUALITY

Spoilage and Spoilage Indicators in Queen Scallops *(Chlamys opercularis)* II. Effects of Processing

A.B. Thomson, H.K. Davis, J.C. Early and J.R. Burt
Torry Research Station, Aberdeen, Scotland
Journal of Food Technology 10, 81-89, 1975

Queen scallops were shucked and held as prepacked meats. Some of the prepacked meats were frozen and held frozen together with some shell on queens. At various times of storage, organoleptic descriptions of the meats were given along with determinations of TMA, TVB, VRS, glycogen, hypoxanthine, ribose fractions and optical density ratios before and after treatment with ion-exchange resin. Of these tests, only hypoxanthine showed any promise as a freshness indicator under all the conditions covered.

Rapid Methods for the Determination of Faecal Contamination in Oysters

R.B. Quadri, K.A. Buckle, and R.A. Edwards
Department of Food Technology, University of New South Wales, Kensington, Australia
Journal of Applied Bacteriology 37:7-14, 1974

Two methods for the rapid detection and estimation of numbers of faecal coliforms and *Escherichia coli* type I in oysters have been developed. That for faecal coliforms involves incubation of tubes of MacConkey broth for 2 h at 37°C and then for 22-24 h at 44°C. The second method is specific for *E. coli* type I and makes use of the same system of incubation, but requires the inoculation of tubes of peptone water as well as MacConkey broth, the former tubes being used for subsequent testing for indole formation. Both methods take only 24-26 h and are as sensitive and accurate as the Most Probable Number methods which are in common use and which take upwards of 72-96 h to complete.

Bacterial Counts and Quality of Iced Fish Retailed at a Lusaka Market, Zambia

K. Watanabe and Grete Ulstrup
Central Fisheries Research Institute, Chilanga, Zambia
Journal of Applied Bacteriology 36:513-522, 1973

The counts of total viable, coliform, streptococcal and sulphite reducing anaerobic bacteria and the presence of salmonellae were determined on 134 iced fish obtained from Luburma Market, Lusaka, Zambia, during June-December 1970. The quality of the uncooked fish was also assessed by appearance and odour. The purpose of these determinations was to obtain a picture of the variations of the bacterial counts in relation to season, origin, fish species and market quality. Total viable and coliform counts were of the order of millions and tens of thousands/cm of skin surface, respectively. Higher counts were obtained in the hot season during September-October but with little change in appearance of the fish.
There was a significant correlation ($P<0.01$) of both total via coliform bacteria with quality scores. A maximum permissible level of 10^7 cells/cm^2 of skin surface was proposed for total viable counts and 105/cm^2 for coliform bacteria, for iced fish of acceptable quality in Zambia.

A 24-Hour Method for the Detection of Coagulase-Positive Staphylococci in Fish and Shrimp

N. F. Insalata, C. W. Mahnke, W. G. Dunlap and C. C. Beazley
Food Technology 26:78-82, 1972

Of the 14 diagnostic tests evaluated, the coagulase slide, catalase activity, gram stain and the coagulase tube tests proved the most successful with the 143 total samples tested. The coagulase tube, gram stain and the catalase activity gave 78.9, 86.8 and 88.7% agreement, respectively, for staphyloccocal actions in 24 hours.

Bacteriological Aspects of Frozen Prawn Products and Their Significance in Quality Evaluation

T.S.G. Iyer, D.R. Chaudhuri and V.K. Pillai
Central Institute of Fisheries Technology, Ernakulam, Cochin, India
Food and Agricultural Organization 115:59-69, 1971

Bacteriological aspects of frozen prawn products have been studied. During freezing, 92-95% of *E. Coli* and 25-35% of streptococci are destroyed. Subsequent storage at -20°C (-4°F) has a greater effect on the *E. coli* in comparison to *Streptococci*.

Bacteria Active in the Spoilage of Certain Sea Foods

R.A. Herbert, Margaret S. Hendrie, D.M. Gibson and J.M. Shewan
Torry Research Station, Ministry of Technology, Aberdeen, Scotland
Journal of Applied Bacteriology 34(1):41-50, 1971

Spoilage of certain sea foods is caused by the activities of some groups of gram negative bacteria. The characteristic of odours and flavours of naturally spoiling cod and haddock have been reproduced in blocks of sterile cod muscle by organisms identified as *Pseudomonas putida, Ps. fragi, Ps. putrefaciens* and other *Pseudomonas spp.*

An Accelerated System for Screening of Process Variables and Freshness Indices of Irradiated Fishery Products

M.D. Alur, V.N. Madhavan, N.F. Lewis and U.S. Kumta
Biochemistry and Food Technology Division, Bhabha Atomic Research Center, Trombay, Bombay, India
Journal of Food Technology 6:73-83, 1971

Incubation of fish homogenates at 30°C led to enhancement of microbial growth of other freshness indices like TMA and TVBN values enabling rapid screening of process variables. This accelerated test system was found to be successful when applied to shrimps and four species of locally available fish, viz. Indian Salmon *(Eleutoeronesma tetradactylum)*, Surmai *(Scomberomorus gettatus)*, Pomfret *(Stomateus cinereus)* and Bombay duck *(Harpondon nehereus)*; which were either untreated or subjected to gamma radiation with or without combination of sodium nitrite and benzoic acid.

The Bacteriology of "Scampi" *(Nephrops norvegicus)*. I. Preliminary Bacteriological, Chemical and Sensory Studies

P. Walker, D. Cann and J.M. Shewan
Journal of Food Technology 5:375-383, 1970

A bacteriological, chemical and sensory study has been made of iced and un-iced scampi *(Nephrops norvegicus)* stored at an ambient temperature of 2.2°C. After 8-10 days storage, the scampi became inedible due to the presence of strong, ammoniacal, sour odours and flavours. The bacterial counts, of iced and un-iced scampi, both of which were made at 20°C and 37°C, rose sharply after the fourth day reaching values of 10^6/g at 20°C and 10^4/g at 37°C at the end of the storage period. The initial bacterial flora, consisting mainly of coryneforms (80%), gradually changed during storage until it finally consisted mainly of *Achromobacter* species (70%). The total volatile base and trimethylamine content of the flesh also increased considerably during storage, reaching values of approximately 70 and 20 mg/N100 g flesh after 10-12 days; initial values were about 20 and 0.5 mg n/100 g flesh, respectively.

Direct Bacterial Count as a Rapid Freshness Test for Fish Fillets

Peter Lerke and Lionel Farber
Seafood Research Laboratory,
George Williams Hooper Foundation, University of California
San Francisco, California
Applied Microbiology 17(2):197-201, 1969

Comparison of various indices of deterioration of refrigerated fish fillets showed that the direct bacterial count can be used to predict the storage life of the foodstuff. For direct counts, a thin film made from fillet surface material was spread on a microscope slide, stained, and read. Initial counts were found to correlate well with keeping quality; a period of freshness of 24 or 48 hr at 5 C could be reliably predicted. Preliminary data indicated that hypoxanthine estimation could probably also be used for the prediction of shelf life but that the relative complexity of the procedure detracted from its usefulness.

Detection and Incidence of Specific Species of Spoilage Bacteria of Fish I. Methodology

R.E. Levin
Department of Food Science and Technology, University of Massachusetts, Amherst, Massachusetts
Applied Microbiology 16(11):1734, 1968

The ability of *Pseudomonas putrefaciens* to form H_2S was found to serve as a singularly useful criterion of identity for this species and was used to directly enumerate the organism for haddock fillets by the use of pour plates of Peptone-Iron Agar. Subsurface colonies appear intensely black, whereas surface colonies are black or gray. A highly sensitive soft-agar-gelatin overlay technique has been found useful for directly determining the numbers of weakly and strongly proteolytic organisms from fish tissue.

Bacteriology of Spoilage of Fish Muscle-II. Incidence of Spoilers During Spoilage

R. Adams, L. Farber, and P. Lerke
Seafood Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, California
Applied Microbiology, 12(3):277-279, 1964

A test medium consisting of a sterile raw press juice from fish muscle was used to determine the incidence of spoilage bacteria on stored fillets of English sole *(Parophrys vetulus)*. The initial load of spoilers was shown to be consistently below 10%. This percentage rose but slightly toward the middle of the spoilage runs, and actually declined when spoilage was most apparent both organoleptically and chemically. Further evidence implicating the *Pseudomonas* and *Achromobacter* groups in the spoilage of fresh fish is presented.

Bacteriological Survey of Filleting Process in the Pacific Northwest 1. Comparison of Methods of Sampling Fish for Bacterial Counts

Wayne I. Tretsven
Bureau of Commercial Fisheries, Technological Laboratory, U.S. Department of the Interior, Seattle, Washington
Journal of Milk and Food Technology 26(9):302-306, 1963

Lots of incoming commercial cod were sampled by six different methods to determine the best one for measuring the bacterial load on the fish. Rinses of similar whole

cod gave bacterial counts that were relatively low and quite variable, whereas spraying a rinse against the surface resulted both in higher and in more uniform counts. When similar 5-cm areas were sampled, bacterial counts obtained by swabbing represented 35% and scraping represented 46% of that obtained from the excised surface. Counts differed more between the lots of fish than between fish or between portions of the same fish. Swabbing appears to be a practical method of sampling in-line processing of fish, as it is non-destructive and relatively efficient in removing large and fairly uniform numbers of bacteria.

Bacteriology of Spoilage of Fish Muscle-I. Sterile Press Juice as a Suitable Experimental Medium

P. Lerke, R. Adams and L. Farber
Seafood Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, California
Applied Microbiology 11:458, 1963

A sterile raw fish muscle press juice, diluted 1:4 with saline, has been prepared. This dilution greatly facilitated Seitz filtration and affected the spoilage properties of the medium only negligibly. At 5.5°C, the spoilage pattern of naturally contaminated diluted juice was almost identical to that of naturally contaminated fillets. This was shown by comparing the quantitative and qualitative aspects of the bacterial flora on the two substrates and by measuring the production of volatile reducing substances (VRS) and of trimethylamine (TMA). With the sterile raw muscle press juice, some preliminary data showed that individual members of the genera *Achromobacter* and *Pseudomonas* differ markedly in their spoilage capabilities: some grew but did not produce spoilage detectable either organoleptically or chemically; others gave rise to strong off odors and to higher levels of VRS and TMA.

Storage Changes in Frozen Fish: Comparison of Objective and Subjective Tests

Kerstin Andersson and Carl Erik Danielson
Findus Research Laboraotry, Bjuv, Sweden
Food Technology 15:55-57, 1961

Herring fillets, treated by dipping in 0.5% ascorbic acid and untreated as reference, were frozen and analyzed at monthly periods using the TBA method accompanied by organoleptic estimations. The chemical changes ascertained by the TBA method exhibit good agreement with the changes in taste. The untreated samples became rancid after 2 months' storage, whereas the treated samples remained palatable for 11 months.

The Action of *Pseudomonas* on Fish Muscle. 3. Identification of Organisms Producing Fruity and Oniony Odours

C.H. Castell, Maxine F. Greenough and Jacqueline Dale
Fisheries Research Board of Canada, Technological Station, Halifax, N.S.
Journal of the Fisheries Research Board of Canada
16(1):13-19, 1959

Bacteria capable of producing fruity and onion-like odours have been isolated from Atlantic cod and haddock fillets that had developed off odours of this type. These organisms have been identified as being nonproteolytic strains of *Pseudomonas fragi*. Compared to other fish-spoiling bacteria, including cultures of *Serratia*, *Proteus*, *Achromabacter*, and green pigmented *Pseudomonas*, *Ps. fragi* is quite sensitive to the bacteriostatic action of antibiotics of the tetracycline group.

The Action of *Pseudomonas* on Fish Muscle. 1. Organisms Responsible for Odours Produced During Incipient Spoilage of Chilled Fish Muscle

C.H. Castell and Maxine F. Greenough
Fisheries Research Board of Canada, Technological Station, Halifax, N.S.
Journal of the Fisheries Research Board of Canada
14(4):617-625, 1957

Many of the odours characteristic of the earlier stages of spoilage of chilled fish muscle have been reproduced by inoculating sterile fish and fish media with pure cultures of bacteria isolated from fish. These organisms belong to the *Pseudomonas* and the majority are neither proteolytic nor break down trimethylamine oxide. They are chiefly achromogenic, although a few green pigmented species are included. Odour production of these organisms appears not to be inhibited by sodium nitrite. Similar odours, produced by similar types of organisms, have been observed in the past on dairy products, eggs, meat, poultry and other protein foods held in cold storage.

Rapid Procedures for Approximation of Bacterial Counts in Shrimp and Oysters

A.F. Novak, E.A. Fieger and M.E. Bailey
Department of Agricultural Chemistry and Biochemistry, Louisiana State University, Baton Rouge, Louisiana
Food Technology 10:66-67, 1956

Two methods for rapid approximation of bacterial counts in shrimp and oysters are described. Measured samples of

products to be tested are added to media capable of supporting definite physiological conversions. These transitions occur rapidly and are measured by observing color changes. Time required to reach the end point is indicative of the bacterial count.

The Bacteriology of Gulf Coast Shrimp. IV. Bacteriological, Chemical, and Organoleptic Changes With Ice Storage

L. Leon Campbell, Jr. and O.B. Williams
Department of Bacteriology, University of Texas,
Austin, Texas
Food Technology 6:125-126, 1952

Headed, washed shrimp in crushed ice retained acceptable commercial quality for at least 16 days. There was an increase in *Achromobacter*, and in the several chemical substances for which changes in amount were determined.

Bacterial Fish Spoilage and Its Control

E. Hess
Food Technology 4:477-480, 1950

It is generally accepted that the changes occurring in fish flesh, which lower its palatability and consequently its consumer acceptance, are caused primarily by bacterial action. It is also accepted that the flesh of healthy, live fish is sterile, but that soon after death bacteria begin to ivade the muscle tissues from the surface slime and intestinal contents where they occur in large numbers. Recent studies on the effects of temperatures near the freezing point upon fish spoilage bacteria and their activity have emphasized their psychrophilic nature. The main avenues of controlling the bacteria responsible for fish spoilage, namely the reduction of the initial contamination and the inhibition of bacterial growth and activity, are under investigation in various countries. The first is achieved by means of improved sanitary methods of handling fish aboard vessels and in fresh fish plants. In the achievement of the second, the most important single factor, low storage temperature, is emphasized through improved construction of vessels' holds and more effective methods of storage and shipping.

Relation of Bacterial Counts to Quality of Cod Fillets

C.H. Castell, C.W. Anderson and Hilliard Pivnick
Atlantic Fisheries Experimental Station, Halifax, N.S.
Journal of the Fisheries Research Board of Canada 7(6):378-388, 1948

Bacterial counts are valueless as a measure of the degree of spoilage in fresh fillets. There is very close correlation between the number of psychrophitlic Gram negative organisms of fillets and their keeping time in cold storage. This correlation degenerates into a "general tendency" which cannot always be appled to individual samples, if the counts used include all the organisms growing on plates incubated at 25°C. Counts made on plates incubated at 37°C are of no value for estimating the keeping quality of fillets stored at low temperatures.

ORGANOLEPTIC METHODS FOR DETERMINING FISH QUALITY

Organoleptic Technique Predicts Refrigeration Shelf Life of Fish

S.E. Charm, R.J. Learson, L.J. Ronsivalli, and M. Schwartz
National Marine Fisheries Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Atlantic Fishery Products Technology Centre, Gloucester, MA
Food Technology 26(7):65-68, 1972

Temperature is a critical variable in the quality of fresh foods, especially fish. It would be useful if food scientists could estimate the shelf life of such foods at any given refrigerated temperature, and since storage temperatures for fish in the commercial distribution chain are variable, at any given set of varying storage conditions. To evaluate the quality of foods, food scientists use either organoleptic methods or so-called objective methods which must ultimately be correlated with organoleptic data to establish their reliability. Variability in sensory panels - and consequently in chemical and physical indices-has implied an erratic process of spoilage for most fish species. The question is often asked whether it is the spoilage process or the sensory panel that is erratic. Based on our results with cod fillets, we believe that the latter is the case.

A New Approach for Evaluating the Quality of Fishery Products

R.J. Learson and Louis J. Ronsivalli
Bureau of Commercial Fisheries Technological Laboratory, Gloucester, MA
Fisheries Industrial Research 4(7):249-259, 1969

Although organoleptic panels lack precision, they are the only instrument that, at present, can integrate all the factors that affect quality. Described here is a new approach to improving panel precision. Using the approach, a panel expresses quality in terms of the estimated storage time of the sample rather than in such ambiguous terms as "excellent," "very good," and "borderline." The approach obviates the need for arbitrary terms to describe quality and assists the panelist in making his evaluations objectively. Statistical analysis of the results obtained when a panel used the method on samples of fresh cod fillets indicates that the storage age of such samples can be estimated to within ±2.2 days with a reliability of 95 percent.

A Taste Panel Technique for Evaluating the Eating Quality of Frozen Cod

C.R. Baines, J.J. Connell, D.M. Gibson, P.F. Howgate, Evelyn I. Livingston and J.M. Shewan
Freezing and Irradiation of Fish, R. Kreuzer, ed., pp. 361-370, Fishing News, London, 1969

The final eating quality of frozen fish reaching the consumer will depend in general upon the sum of, or interaction between, the deteriorations occurring in both the unfrozen and frozen states of the fish throughout its history.
Detailed descriptions of the organoleptic changes occurring during unfrozen storage of fish are available, but similar comprehensive descriptions of the changes occurring during freezing and frozen storage are lacking. A new study is therefore being made of the changes in organoleptic characteristics of frozen fish, and taste panel score sheets based on the findings are being constructed. This study is confined to white fish, and to date only cod *(Gadus morhua)* has been surveyed.
It is now clear from this and previous work at this Station that two distinctive types of change of equal importance occur during frozen storage. These are changes in texture and flavour (including odour), and it has been found possible to clearly differentiate these changes for those occurring during ordinary chill storage. It has been established that the organoleptic characteristics described in the Shewan-Ehrenberg score sheet are not appreciably modified by frozen storage, and that the initial freshness does not affect the nature of the changes occurring during frozen storage. Thus it is possible to score reasonably accurately any sample of frozen cod in terms of the deteriorations occuring during both the unfrozen and frozen stages of its history.

Objective Measurement of the Hardness Parameter in Cooked Fish Muscle

A.H. Sutton and G. Main
Freezing and Irradiation of Fish, R. Kreuzer, ed., pp. 371-375, Fishing News, London, 1969

There are two principal physical parameters which characterize cooked fish texture. These are hardness and succulence. Chewiness is also of importance but since chewiness and hardness are generally closely associated, a separate measurement of this is rarely required. A combination of hardness, succulence and chewiness produce a major proportion of the possible cooked fish textures.

The hardness of muscle has a simple physical significance, and the problems associated with its objective measurement are purely instrumental. A penetrometer-type instrument was constructed which measures the pressure build-up when the sample is compressed to a fixed distance. In designing the equipment portability was considered to be an important factor, and our total instrument weight is less than 20 pounds.
A good correlation has been found between the instrument and taste panel assessment of hardness. Statistical analysis of the results show that the instrument produces a more precise mean value of hardness than the taste panels, and it is hoped to improve this further. The main problem has been in obtaining uniform packing of the sample, this causing fairly large standard mean deviations in the results. This is overcome at present by taking a large number of samples, but various other preparative procedures are being investigated.
The instrument is proving to be a very useful tool where more precise texture measurements are required, and will be invaluable where trained taste panels are not available.

Sensory and Objective Measurements of the Quality of Frozen Stored Haddock of Different Initial Freshnesses

J. J. Connell and P.F. Howgate
Ministry of Technology, Torry Research Station, Aberdeen, Scotland
Journal of the Science of Food and Agriculture 20:469-476, 1969

The quality of haddock fillets cut from fish held for different periods in melting ice and then stored in the frozen state of three different temperatures has been assessed on the same samples by a taste panel and by a series of objective tests. The measured changes in the texture and flavour of the fish were compared with changes in objective parameters in order to determine the value of the latter in predicting eating quality. The results for fish caught in the North Sea show that haddock fillets keep less well than cod fillets during frozen storage.

Sensory and Objective Measurements of the Quality of Frozen Stored Cod of Different Initial Freshnesses

J.J. Connell and P.F. Howgate
Journal of the Science of Food and Agriculture 19:342-354, 1968

The eating quality of cod kept for different periods in melting ice before being frozen and stored at three different temperatures has been evaluated by a taste panel using a new score sheet. Objective measurements

of both initial freshness before freezing and deterioration during frozen storage were carried out on the same samples. Correlations were obtained between the objective measurements and various aspects of eating quality. The relative contributions of the various aspects of eating quality to the final overall acceptability were obtained, and the value of the objective measurements in predicting overall acceptability assessed.

Nucleotide Degradation and Organoleptic Quality in Fresh and Thawed Mackerel Muscle Held at and above Ice Temperature

Doris I. Fraser, Dianne P. Pitts, and W.J. Dyer
Fisheries Research Board of Canada, Halifax Laboratory, Halifax, N.S.
Journal of the Fisheries Research Board of Canada
25(2):239-253, 1968

In mackerel, by the time of initial sampling, adenine nucleotides had been deaminated to inosine monophosphate (IMP) in the ordinary muscle; in the red muscle the degradative sequence was even more advanced, as indicated by high initial levels of inosine. Postmortem rates of degradation of IMP to hypoxanthine through inosine were similar in both types of muscle; at ice temperature the rates were slower than in cod but faster than in swordfish. A delay in icing of 6-8 hr after catching accelerated the gradual decline in eating quality with replacement of the characteristic fresh mackerel flavor by tastelessness.

IMP dephosphorylation paralleled development of tastelessness, and spoilage (organoleptic) had developed prior to accumulation of appreciable amounts of hypoxanthine. At higher temperatures, 5-20°C, rates of IMP dephosphorylation, hypoxanthine accumulation, and quality loss were markedly increased. Thawing did not influence subsequent deterioration rates, but ascorbic acid dips delayed darkening of the flesh in the thawed samples.

Excellent correlation of taste with both IMP and hypoxanthine content, and with various simple measures of IMP dephosphorylation, was obtained under the various handling conditions investigated, including delayed icing, holding at elevated temperatures, and after thawing. The simple tests - ultraviolet absorption at 248 mμ of a Dowex treated perchloric acid extract, and ratio of ultraviolet absorption of extracts at 251 mμ after Dowex treatment to that before treatment - proved as good indices of progressive quality loss to the unacceptability level as the more complex estimation of IMP or hypoxanthine.

Development of Standard Rating Scales for Mechanical Parameters of Texture and Correlation Between the Objective and the Sensory Methods of Texture Evaluation

Alina Surmacka Szczesniak, Margaret A. Brandt, and Herman H. Friedman
General Foods Corporation, Technical Center, Tarrytown, New York
Journal of Food Science 28:397-403, 1963

Standard rating scales of hardness, brittleness, chewiness, gumminess, viscosity, and adhesiveness were established for quantitative evaluation of food texture. The scales cover the entire intensity range found in food products and may be expanded at any desired point for greater precision in a narrower range. Each point on the scale is represented by a food product selected on the basis of availability, familiarity, constancy of textural characteristics, and other criteria. Using the developed scales, correlation was good between sensory and instrumental (texturometer and viscosimeter) evaluations of texture.

Rapid Determination of the Quality of Whole Eviscerated Haddock

B.E. Proctor, J.T.R. Nichlos, T.L. Fazzina, L.J. Ronsivalli, R.K. Smith and J. Stern
Department of Food Technology, Massachusetts Institute of Technology, Cambridge, MA
Food Technology 13(4):224-228, 1959

Optimal density tests on haddock eye fluids gave promising results but were not as suitable as were the refractive index measurements for quality indication. Changes in the physical properties of haddock eye fluids during storage at refrigerator temperatures above freezing are probably due to enzyme action, since there was no evidence that bacterial composition is involved in such changes.

Post-Mortem Changes in the Lenses of Fish Eyes
II. Effects of Freezing, and Their Usefulness in Determining the Past History of the Fish

R.M. Love
Journal of the Science of Food and Agriculture 7:220-226, 1954

It is possible to show if a fish has been frozen or not by examining the eye lenses. A positive reaction is shown by opacity in the lens and the fish must be cooled to at least -4.8°C for the effect to occur. It is only masked by storage in ice for more than 16 days, or by salting the fish for 1 day or more before freezing. The mechanism of the phenomenon is deduced.

The Development of a Numerical Scoring System for the Sensory Assessment of the Spoilage of Wet White Fish Stored in Ice

J.M. Shewan, R.G. MacIntosh, C.G. Tucker and A.S.C. Ehrenberg
Journal of the Science of Food and Agriculture 4:283-298, 1953

An attempt is made to classify the sensorily perceptible quality-factors of cod fish, stored in ice, throughout the spoilage chain from absolute freshness to putridity. The classification is given numerical form to ease the handling and interpretation of the resulting data. A panel has been trained to agree in assessing any one sample, and its internal consistency is discussed on the basis of some experimental data.
The importance of the method would seem to be that those factors are classified which would give rise to opinions of preference in the ordinary consumer, were the food presented to him. In practice the accuracy obtained by the panel was such that samples could be clearly differentiated, in terms of periods of storage in ice under certain standard conditions, to within a day or two.

The Objective Approach to Sensory Tests of Food

A.S.C. Ehrenberg and J.M. Shewan
Journal of the Science of Food and Agriculture 4:482-490, 1953

The use of a technique, previously described, is discussed. Results are given to show that the explicit training of a panel of assessors to agree is a chief factor in achieving accuracy. The validity of comparing results from different investigations is then examined, with reference to such factors as the internal consistency of the panel, the use of control samples or standards and of certain physicochemical criteria.

MISCELLANEOUS METHODS FOR DETERMINING FISH QUALITY

Methods for Quality Assessment in Fishery Products

George W. Chang
University of California, Berkely, California
IMR Reference Number 78-101, Sea Grant Publication Number 61:117, 1977

The major objective of this research was the development of more convenient ways of detecting spoilage in seafoods. Efforts were directed at the detection of hypoxanthine (HX), since this substance is a good indicator of seafood quality. There is almost no HX in the muscle of living fish, but after death its level rises and if the fish are kept under adverse conditions it rises at a faster rate. In order to simplify the measurement of HX, a prototype specific electrode has been developed.

Trimethylamine Specific Electrode for Fish Quality Control

George W. Chang, Wai Lin Chang and Kitty B.K. Lew
Department of Nutritional Sciences, University of California, Berkeley, California
Journal of Food Science 41:723-724, 1976

Trimethylamine (TMA) is one of the major components of the smell of spoiled marine fish. An increased TMA level is so characteristic of spoilage that the TMA levels have been used as an objective index of fish quality. A specific electrode was developed in order to simplify the measurement of TMA. The Orion ammonia electrode was made specific for TMA by replacing the inner filling solution with 0.01M TMA - HCI in 0.04M KCl and by adding enough formaldehyde to the sample solution to obtain a concentration of 0.22% (0.075M). The electrode is suitable for the measurement of TMA in aqueous solutions and in homogenates of fish muscle. The use of this electrode is much simpler than the methods now used for TMA analysis and it may be applicable in commercial practice.

Comparison of Methods of Freshness Assessment of Wet Fish. II. Instrumental and Chemical Assessments of Boxed Experimental Fish

J.R. Burt, D.M. Gibson, A.C. Jason and H.R. Sanders
Journal of Food Technology 11:73-89, 1976

Samples of cod were obtained from different fishing grounds at different seasons. They were stored in boxes with ice for periods of up to 20 days. At regular intervals measurements by Torry Fish Freshness Meter and Intelectron Fish Tester V and determinations of hypoxanthine and trimethylamine concentrations were made. Linear relationships with length of time of storage were established and calibrations with sensory tests are presented.
Ground and seasonal effects were found in the relationships with days of storage and with sensory assessment. The amounts of spoilage measured by the different tests are correlated.

Comparison of Methods of Freshness Assessment of Wet Fish. III. Laboratory Assessments of Commercial Fish

J.R. Burt, D.M. Gibson, A.C. Jason and H.R. Sanders
Journal of Food Technology 11:117-128, 1976

Samples of cod were obtained during four commercial fishing trips and stored in boxes and in bulk storage. After landing, freshness assessments were made by a sensory panel, two instrumental methods - Torry Fish Freshness Meter and Intelectron Fish Tester V - and two chemical methods-the determination of hypoxanthine and trimethylamine indices. The results from the chemical methods were in close agreement with those previously obtained on experimental fish. The instrumental methods were strongly affected by the type of storage.

Comparison of Methods of Freshness Assessment of Wet Fish. IV. Assessment of Commercial Fish at Port Markets

J.J. Connell, P.F. Howgate, I.M. Mackie, H.R. Sanders and G.L. Smith
Journal of Food Technology 11:297-308, 1976

Two sensory methods (General Appearance and Raw Odour) and two instrumental methods (Torry Fish Freshness Meter and Intelectron Fish Tester V) were tested on Aberdeen and Hull Markets. The variability of each method, comparisons between the methods and the time required to carry them out under market conditions were obtained. The instrumental methods (particularly the averaging version of the Torry instrument) are the most economical to operate.

The Construction of Grading Schemes Based on Freshness Assessment of Fish

H.R. Sanders and G.L. Smith
Journal of Food Technology 11:365-378, 1976

Standard acceptance sampling schemes can be modified for use in grading where grades are defined by one or two boundaries and a percentage permitted to fall below the lower boundary. Schemes are constructed to grade fish by freshness, using sampling by attributes or by variables, where the method of assessment is Raw Odour. When another method of testing, such as the Torry Fish Freshness Meter, is used, it is not sufficient in the attributes scheme to convert to new grade boundaries; the corresponding percentages falling below the grade boundaries for the new test must be determined. The procedures for deriving a scheme are not restricted to grading of fish, but may be applied to any similar quality control situation.

How the GR Torrymeter Aids Quality Control in the Fishing Industry

Anne Cheyne
Torry Research Station, Aberdeen, Scotland
Fishing News International 14:71, 1975

The torrymeter measured systematic changes in certain physical properties of fish muscle and skin during storage in the wet state, and different methods of handling and processing will affect these measurements. The torrymeter has been found to give a satisfactory and rapid indication of freshness.

Comparison of Methods of Freshness Assessment of Wet Fish. Part I. Sensory Assessments of Boxed Experimental Fish

J.R. Burt, D.M. Gibson, A.C. Jason and H.R. Sanders
Journal of Food Technology 10:645-656, 1975

Samples of cod were obtained from different fishing grounds at different seasons. They were stored in boxes with ice for periods of up to twenty days. Freshness assessments by sensory and non-sensory methods were carried out at regular intervals.
Results obtained on four freshness factors by a sensory panel are reported in this paper. The different factors give similar results, but considerable variations are found in fish from different catches.

Effect of Special Handling of Haddock on the Postirradiation Shelf Life of Haddock Fillets

Vincent G. Ampola and Louis J. Ronsivalli
Fisheries Industrial Research 4(3):109-111, 1969

Improved techniques for handling eviscerated haddock after capture resulted in superior quality of the fish prior to irradiation and a significant extension in the postirradiation shelf life of fillets cut from them.

The "Intelectron Fish Tester V" A New Electronic Method and Device for the Rapid Measurement of the Degree of Freshness of "Wet" Fish

Christian Hennings
Technology of Fish Utilization, R. Kreuzer, ed., pp. 154
Fishing News, London, 1965

The comparison of the AC resistances or impedances of a fresh cell tissue measured at two different frequencies can give some information about the capacitance of a cell wall. The cell walls of the dead fish become increasingly permeable due to enzymatic breakdown of the protein and consequently lose their capacitance gradually during storage. Thus the differences in the impedances measured in fresh tissue become less and less during storage and finally disappear so that these differences would seem to be correlated with freshness and therefore could be used as indices of freshness.
The method of measurement and the operation of the instrument are explained and results of investigations are discussed. Only unskinned fish can be used and measurements have to be made at areas where the skin is undamaged. The measurements made during storage of cod at various temperatures resulted in a fairly good agreement with experience gained by other means in relation to the loss of freshness during storage. Each species of fish has its own characteristic curve differing from others both in control value and with the change of slope. The "Q" values of fish which have reached the borderline of acceptability differed from species to species.
The readings on the "freshness scales" of the instrument correctly reflect the true condition of the fish, but as with other methods, the device cannot simultaneously give an indication of the rate of loss of freshness. Various factors which influence the measurements are discussed e.g. mechanical damage of the skin and tissue, role of the interstitial liquid, texture of cell tissue of different species, formation of ice crystals in the tissue, electrolytic substances such as salt.

Post-Mortem Changes in the Lenses of Fish Eyes: Assessment of Storage Time and Fish Quality

R.M. Love
Torry Research Station, Aberdeen, Scotland
Journal of the Science of Food and Agriculture 5:566-572, 1954

The eye lenses of fish stored for increasing lengths of time in ice become progressively turbid. The cause of this turbidity is shown to be the diffusion of water through the corneas into the intra-ocular fluid, followed by gradual hydration of the lens. In iced fish kept in a room of fairly constant air temperature, it was found possible by examination of 20 or more lenses to assess the storage time with an error of not more than 1 day. The limitations of the method are discussed.

Changes in the pH and Buffering Capacity of Fish During Spoilage

C.L. Cutting
Department of Scientific and Industrial Research, Torry Research Station, Aberdeen, Scotland
Journal of the Science and Food and Agriculture 4:597-603, 1953

Claims have recently been made once again for a test for freshness of fish based on a decrease in buffering capacity with increasing staleness. This decrease has been shown to be due chiefly to the bacterial reduction of trimethylamine oxide. However, under commercial conditions the results appear to be too irregular for such a test to be reliable. The buffering capacity of fish flesh in various ranges of pH and its variation with degree of spoilage have been almost completely accounted for by the contributions of proteins and water-soluble constituents. The pH changes in fresh and spoiling fish, which depend in large measure on buffering capacity, are also too irregular to be satisfactorily correlated with freshness.

An Assay Method for Freshness of Fishes by the Estimation of pH Value

Makoto Yamamoto and Masanori Sonehara
Bulletin of the Japanese Society of Scientific Fisheries 9(6):761, 1953

The present paper is concerned with the accuracy and usefulness of pH determination in terms of freshness of fish. A special glass electrode connected with an amplifier capable of reading exactly 0.50 mV intervals was used.

The results obtained are shown and may be summarized as follows: (1) The pH value of fish meats will be adopted as a criterion of the freshness of fish to some extent. (2) However, the pH value varies according to the part of body. And some divergencies are also observed according to the fishing season and species of fish. (3) The pH value of fish meats at early spoilage was 6.85 in the white meat of fish and 6.27 in red meat.

Evaluation of Surface pH as a Freshness Index of Fish Fillets

R. Paul Elliott
Fishery Technological Laboraotry, U.S. Fish and Wildlife Service, Seattle, Washington
Food Research 12(2):87-98, 1946

The organoleptic data was classified as fresh, flat, sweet, stale, and putrid. A fillet designated as "fresh" had the normal odor of freshly caught fish. If it was "flat" there was an absence of odor-normal or otherwise. A "sweet" fillet had an odor not especially unpleasant but reminiscent of watermelon. A "stale" fillet had a characteristic ammonia-like odor (odor of ammonia and other mixed amines) but had not reached the "putrid" stage at which point the odor became obnoxious (hydrogen sulfide, indole, skatole, etc.) All fish examined in this study spoiled in the above manner except whiting and rosefish. At the sweet stage whiting developed a perfume-like aroma instead of the usual watermelon-like odor. Rosefish did not become sweet but passed directly from flat to slightly stale.
The author and those who co-operated with him considered the fillets edible through the "sweet" stage, of questionable edibility at "very sweet" and "slightly stale," and inedible at "stale" and at more advanced stages of decomposition.

The "Stinkometer" - New Tool

O.W. Lang, L. Farber and F. Yerman
Seafood Research Laboratory, George Williams Hooper Foundation, University of California Medical Center, San Francisco, California
Food Industries 17(8):78, 1945

The "Stinkometer" develops a new and reliable means of evaluating freshness in foods by measuring volatile odors. It also can be used to measure the loss of desirable flavors.
The method apparently is applicable to meats, prunes and raisins, as well as fish, in the first instance, and to coffee, spices and perfumes in the second.

A Rapid Test for Detection of Spoilage in Sea Fish

W.J. Dyer, G.J. Sigurdsson and A.J. Wood
Atlantic Fisheries Experimental Station, Halifax, Nova Scotia
Food Research 10:183, 1944

A satisfactory routine practical method for the determination of the index of spoilage for several sea fish has been developed. The pH at the surface of the fillet or fish is measured by placing a glass electrode of a Beckman pH meter in contact with the moist surface of the tissue. It has been found that fresh fish gave a pH range of 6.2 to 6.8; pH levels above 6.8 are indicative of spoilage; and the higher the pH above 6.8 the more extensive have been the spoilage changes. This method is simple, rapid, and adapted for use as a commercial test. Correlation with other spoilage tests is satisfactory. A much better indication of conditions of incipient spoilage is afforded than that obtained by currently used methods. The standard scale of the direct reading pH meter may be replaced by one showing the degree of freshness of the fisheries product.

On the Use of the pH Value as a Measure of the Freshness of Fish Muscle Tissue

F. Charnley and D.H. Goard
Canned Salmon Inspection Laboratory, Department of Fisheries, Vancouver, BC
Canadian Journal of Research 20:20-32, 1942

The pH value of the aqueous liquors derived from fish muscle tissue is connected through statistical relations with the buffer action of the liquor, the log bacterial count of the sample, and with a subjective estimate of freshness determined on the basis of odour. In the case of pH and odour rating the relation is not a correlation but, instead, a linear relation between the means of a series of populations. By means of the latter it is possible from observations of the pH of the aqueous liquor in the sample to determine objectively the freshness of a parcel of canned chum salmon to any desired degree of accuracy by increasing the size, n, of the sample taken for examination.

An Electrometric Method for Detection of Relative Freshness of Haddock

Maurice E. Stansby and James M. Lemon
U.S. Bureau of Fisheries, Gloucester, MA
Industrial and Engineering Chemistry, pp. 208, May 15, 1933

A reliable test for the freshness of fish has been described, based on buffer capacity measurements. The test requires less than one hour to perform. It gives information, not only as to the accumulation of bacterial end products, but, what is even more important, as to the amount of protein breakdown taken place.

REVIEW ARTICLES ON METHODS OF DETERMINING FISH QUALITY

Quality Assessment of Fresh Fish and the Role of the Naturally Occuring Microflora

R.E. Martin, R.J.H. Gray and M.D. Pierson
Food Technology, 32(5):188-192, 1978

Methods of Assessment of the Freshness of Fish

A. Swaney
JBL Seafood Ltd., Auckland
Fishing Industry Board Report on Quality in Fish Products 3: 1971

Methods of Quality Assessment V

Fish Inspection and Quality Control, R. Kreuzer, ed., pp. 172-215, Food & Agricultural Organization, 1971
Organoleptic Assessment of Quality pp. 172-182
Objective Methods of Quality Assessment pp. 183-215

The Microbiology of Fish and Fishery Products-A Progress Report

J.M. Shewan
Journal of Applied Bacteriology 34(2):229-315, 1971
Address of the President of the Society of Applied Bacteriology delivered at a meeting of the Society of 13 January 1971

Bacteriological Standards of Fish and Fishery Products

J.M. Shewan
Ministry of Technology, Torry Research Station, Aberdeen, Scotland
Chemistry and Industry 7:193-199, 1970

The Bacteriology of Fresh and Spoiling Fish and Some Related Chemical Changes

J.M. Shewan
Recent Advances in Food Science 1:167-193, 1962

Freshness Tests

Lionel Farber
Seafood Research Laboratory, George Williams Hooper Foundation, University of California, San Francisco, CA
Fish As Food, Borgstrom, ed., pp. 65-126, Academic Press, New York, 1961

The Spoilage of Fish and Its Preservation by Chilling

G.A. Reay and J.M. Shewan
Torry Research Station, Aberdeen, Scotland
Fish As Food, G. Borgstron, ed., pp. 343-398, Academic Press, New York, 1961

A Determinative Scheme for the Identification of Certain Genera of Grama-Negative Bacteria, With Special Reference to the Pseudomonadaceae

J.M. Shewan, G. Hobbs, and W. Hodgkiss
Torry Research Station, Aberdeen, Scotland
Journal of Applied Bacteriology 23(3):379-390, 1960

APPENDIX

LIST OF JOURNALS

Analyst

Applied Microbiology

Aquatic Sciences and Fisheries Abstracts

Australian Fisheries

Bulletin of Hokkaido University Faculty of Fisheries

Bulletin of the Japanese Society of Scientific Fisheries

Bulletin of the Tokai Regional Fisheries Research Laboratory

Canadian Journal of Research

Chemistry and Industry

Commercial Fisheries Review

Fisheries Industrial Research

Fishing Gazette

Fishing Industry Board

Fishing News International

Food and Agricultural Organization

Food Industries

Food Product Development

Food Research

Food Science and Technology Abstracts

Food Science and Technology Proceedings

Food Technology

Indian Journal of Fishery

Industrial and Engineering Chemistry

Journal of Agricultural and Food Chemistry

Journal of Applied Bacteriology

Journal of Biological Chemistry

Journal of the Association of Official Agricultural Chemists

Journal of the Fisheries Research Board of Canada

Journal of Food Science (Food Research)

Journal of Food Technology

Journal of Lipid Research

Journal of Microbiology

Journal of Milk and Food Technology

Journal of Association of Public Analysts

Journal of the Science of Food and Agriculture

Process Biochemistry

Quick Frozen Foods

Recent Advances in Food Science

LIST OF BOOKS

1976 Recommended International Code of Practice for Fresh Fish, Joint FAO/WHO Food Standards Programme. Codex Alimentarius Commission CAC/RCP 9

1975 Connell, J.J., Control of Fish Quality, Fishing News (Books) Ltd., London

1971 Gould, E., and Peters, J.A., On Testing the Freshness of Frozen Fish, Fishing News (Books) Ltd., London
Kreuzer, R., ed., Fish Inspection and Quality Control, F.A.O.

1969 Kreuzer, R., ed., Technical Conference on the Freezing and Irradiation of Fish, Fishing News (Books) Ltd., London

1965 Kruezer, R., ed., Technology of Fish Utilization, Fishing News (Books) Ltd., London

1962 Herschdoerfer, S.M., ed., Quality Control in the Food Industry, Academic Press, New York

1961 Borgstrom, G., ed., Fish as Food, Academic Press, New York

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