1970

Development of a Total Reducing Substance Test for Ascertaining Oyster Quality.

Stephen Charles Lagarde
Louisiana State University and Agricultural & Mechanical College

71-6585

LAGARDE, Stephen Charles, 1943-
DEVELOPMENT OF A TOTAL REDUCING SUBSTANCE TEST FOR ASCERTAINING OYSTER QUALITY.

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1970
Food Technology

University Microfilms, Inc., Ann Arbor, Michigan

DEVELOPMENT OF A TOTAL REDUCING SUBSTANCE TEST FOR ASCERTAINING OYSTER QUALITY

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

Food Science

by
Stephen Charles Lagarde
B.S., Louisiana State University, 1965
M.S., Louisiana State University, 1967
August, 1970

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to his major professor, Dr. Joseph A. Liuzzo, for his advice and guidance during the course of this investigation. He is also indebted to Dr. Arthur F. Novak, Professor and Head of the Department of Food Science, for extending the facilities to make this research possible.

Acknowledgments are also extended to Dr. Robert M. Grodner and Dr. Ramachandra M.R. Rao of the Department of Food Science, to Dr. Alworth D. Larson of the Department of Microbiology and to Dr. B. Hall Davis of the Poultry Science Department for serving as members of the examining committee.

The author wishes to express his thanks to Dr. Prentiss E. Schilling of the Department of Experimental Statistics, for his assistance in the statistical analysis of the data. Acknowledgment is also made to the United States Public Health Service for the financial assistance throughout the course of this research.

Above all, the author wishes to express his love and indebtness to his parents, and to his wife, Kathy, whose encouragement and support made this undertaking possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF FIGURES

Lagarde, Stephen Charles, B.S. Louisiana State University, 1965
M.S., Louisiana State University, 1967
Doctor of Philosophy, Summer Commencement, 1970
Major: Food Science; Minor: Microbiology
Development of a Total Reducing Substance Test for Ascertaining Oyster Quality
Dissertation directed by Professor Joseph A. Liuzzo
Pages in Dissertation, 106. Words in abstract 457.

ABSTRACT

A new test for oyster quality has been developed. The test procedure measures the group of compounds designated as total reducing substances (TRS). The analysis for TRS is simple and utilizes the quantitative determination of both volatile and non-volatile substances in oysters which are capable of reducing an alkaline solution of potassium permanganate. By virtue of comparisons with other indices of quality, the total reducing substance test appears to offer the most rapid and reliable means of ascertaining oyster quality.

The applicability of the total reducing substance test was determined by comparison with existing chemical indices of quality, namely pH, trimethylamine-nitrogen, total volatile bases, and indole. Microbiological studies on mesophilic and psychrophilic plate counts and organoleptic evaluations were performed in conjunction with the chemical tests during a 15 day iced-storage period. Where applicable determinations were made on both oyster meat and oyster liquor.

Statistical analyses were performed on the data from

each chemical, microbiological, and organoleptic test to determine if significant changes occurred during the storage period and to evaluate the reliability of each test procedure. Correlation coefficients were then determined between the various tests, using organoleptic quality as a basis for comparison.

The results showed that there were highly significant differences ($P < 0.01$) in total reducing substances, pH, trimethylamine-nitrogen, total volatile bases, psychrophilic plate counts, and organoleptic evaluations. In addition, significant changes ($P < 0.05$) were found to occur in mesophilic plate counts. No significant changes were found in the indole content.

The total reducing substance test for oyster quality was found to give highly reproducible results. The results returned for pH were also reproducible, but to a lesser degree than was total reducing substance concentration. Trimethylamine-nitrogen, total volatile bases, indole, mesophilic plate counts and psychrophilic plate counts were not found to be reproducible, and their reliability as quality indices for oysters is therefore questionable.

Highly significant, positive correlations ($P < 0.01$) were obtained between organoleptic quality versus pH measurements of both oyster meat and liquor and mesophilic plate counts of oyster meat. Highly significant, negative correlations existed between organoleptic evaluations and

the total reducing substances content of both oyster meat and liquor. Significant, negative correlations ($P < 0.05$) were found between the psychrophilic plate counts of oyster meat and liquor and organoleptic quality. Correlations between organoleptic quality versus trimethylamine-nitrogen and total volatile bases were not found to be significant.

It is important to note that the highest correlations obtained with organoleptic quality were with total reducing substances and pH. This fact illustrates the potential use of these tests as quality indicators of oysters.

By virtue of the statistical analyses and correlations of the various chemical and microbiological tests with organoleptic quality, it would appear that the total reducing substance test provides a useful, reliable means of ascertaining the quality of ice-stored oysters.

INTRODUCTION

Food quality is largely the sum of the characteristics which register favorably or adversely on an individual's senses. These characteristics include flavor, odor, texture, color, size, shape, and the presence or absence of defects. The degree of freshness, the nutritive value, the presence of chemical residues and the presence of pathogenic organisms are also involved with food quality, although they may not be measured by the senses.

When one visualizes a spectrum of food quality ranging from good to poor, the extremes are more easily differentiated than the central part of the spectrum. This is especially true for those foods where processing may tend to mask differences.

The consumer often depends upon organoleptic factors for evaluating food quality. These factors, however, are qualitative and may vary between individuals. The need for more adequate evaluation has focused attention upon the use of chemical compounds which can be used to differentiate the stage of freshness or the degree of decomposition. Since food decomposition is usually the result of microbiological action combined with autolytic processes, certain compounds arising from the metabolism of the dominant spoilage flora may answer this need.

The dominant spoilage flora varies within and between

species of various foods and under different environmental and storage conditions. For these reasons the metabolic behavior of the microbiological flora in foods is unpredictable to a large extent. Almost all accurate metabolic studies on microorganisms have been made with pure cultures, and the experiments have been carried out under controlled conditions which will never be found in commercial foods. The phenomena of symbiosis and antagonism, coupled with a variable initial microflora, are always present and are perhaps the main factors in the control of chemical changes, especially in determining the final end products. In addition, variations in environmental conditions such as pH, temperature, humidity, and available oxygen may alter the course of events in microbiological metabolism and a different flora may exist at different stages of decomposition.

For many years the food industry has been trying to find new and better methods which will enable the technologist to assess the stage of freshness of a certain comodity and to estimate the remaining shelf life. Most freshness tests are based on measuring major chemical and physical alterations from the original or fresh condition of the product. Since these alterations are brought about primarily by microorganisms which are using the food as a substrate, nearly all freshness tests are based on the detection and measurement of degradation compounds which

are produced by microbiological growth.

The preferred principle in the development of a chemical test for measuring the stage of decomposition of a food is to measure one or more of the compounds which together are responsible for the smell or taste that influences an individual's evaluation of that particular food. This simple principle has been very difficult to put into actual practice, because in some instances the organs of smell and taste are far more sensitive than the corresponding chemical tests, while in other cases the particular compound measured in a chemical test may increase rapidly without a corresponding increase in sensory detection. This latter case leads to another alternative in the development of a chemical index of freshness. That is, find and measure one or more compounds that are always present in the decomposition, but which may not be important factors in organoleptic judgment.

In actual practice the chances are that several tests may be tried before a promising one is found, and then a substantial number of samples must be analyzed before it can be accepted.

The objective of this research was to develop a rapid modified chemical test for ascertaining oyster quality and compare it with other tests that have been developed for this purpose. It should be possible to correlate data from these various tests with organoleptic tests with the aim of determining which test or combination of tests is

able to give the fastest and most reliable information concerning oyster quality.

REVIEW OF LITERATURE

The words "decomposition," "quality," "freshness," and "spoiled" are widely used in relation to fish and shellfish. The actual meaning, however, of each of these words will depend upon the context with which it is used. In actuality, decomposition begins the moment the fish or shellfish dies or is removed from its natural environment, and consists of a breaking down into constituent parts. This breakdown may be the result of autolytic mechanisms or microbiological action, and the extent and type of the decomposition will determine the quality or freshness with respect to the discriminating consumer. When the food is decomposed to a degree which this consumer thinks it unfit for consumption, the term "spoiled" may be applied to it, denoting poor quality.

Poor quality in a particular product may be the result of pathological conditions or seasonal variations in chemical composition and physical properties. Most often, however, quality is synonymous with freshness, or the degree of microbiological spoilage which the product has undergone, although the level of freshness at which "good quality" becomes "poor quality" is obviously subjective and quite variable.

Many characteristics of fish and shellfish which are related to quality are innately immeasurable and can only be defined in terms of an attribute which the product does

or does not possess. However, aspects of quality such as weight, size, organoleptic class, or concentration of a particular substance in the flesh are measurable, and together with the number of organisms per gram or milliliter, constitute the class of properties known as variables. Methods for the determination of relevant product variables are frequently available and fall into three general categories: chemical, microbiological, and sensory.

Once the product has been harvested, autolytic degradation begins, the microorganisms associated with the product invade the tissues, and decomposition advances.

Chemical compounds which indicate deterioration due to microorganisms may be defined as metabolic by-products which are produced by the dominant spoilage organisms as a result of their growth in the product. Westerdijk (1949) suggested that the term "association" be used for the characteristic flora of a food. The term "dominant" may be used to denote the species which determines the spoilage, although these organisms may not necessarily be the most numerous.

We must recognize the fact that such microbiological associations can proceed in succession, that is, they may change under varying conditions. Under practical conditions the genesis of such associations is determined by several groups of factors: (1) the initial infection of the substrate; (2) intrinsic factors which depend upon the properties of the substrate, such as chemical composition

and physical properties; (3) extrinsic factors which are functions of the storage conditions; and (4) the characteristics of the dominant microorganisms such as rate of development, synergism, and antagonism. As we review these factors, we begin to see the complexity of utilizing metabolic by-products as indices of quality.

Fields et al. (1968) proposed several criteria which a chemical indicator for food quality should possess: (1) the compound should be present in low levels or absent in sound foods; (2) there should be an increase in the concentration of the compound with an increase in spoilage; (3) the compound should make it possible to differentiate low-quality raw materials from poor processing techniques; and (4) the indicator should be produced by the dominant spoilage flora. To be useful as an index of quality, the test for the compound should be rapid and the analysis simple (Novak et al., 1956; and Rogers and McCleskey, 1961). Farber (1952) stated that a spoilage index should be as reliable as organoleptic evaluations and should indicate stages of decomposition which cannot be established definitely by organoleptic testing. Patterson (1945) emphasized that the most rigorous requirement which an acceptable test must fulfill is that it should never give a false positive. Theoretically, this possibility is never entirely eliminated from a method, for there is always the chance of an exception. If a second test can be found, even though it may be longer or more

difficult, to serve as a check on the first, then the chances of a "double false positive" are lessened drastically. The corroborating test must either measure a different compound from the first or not be subject to the same predictable interferences as the first.

It must be admitted that in actual practice such a test is indeed rare. If one were asked to explain the poor showing of chemical tests as indicators of quality, the answer might be that the chief factor has been the lack of specific quantitative tests for those decomposition products which can be detected sensorily. A contributing factor may well be the wishful effort to find a simple and universal indicator of decomposition. Certainly the chemical complexity of foods and the equal complexity of microbiological changes do not make the task easier. Even in a single type of food, such as shellfish, it would probably be impossible to find one method which will detect all stages of freshness and cases of decomposition. However, a test that will catch the majority of poor samples and find no false positives would be a useful advance.

Perhaps the best way to develop a new chemical test which measures the microbiological decomposition of a particular food is by a thorough investigation of the dominant spoilage flora, with regard to their specific metabolic by-products from certain nutrients, be they carbohydrate, fat, or protein.

Research on the quality of high protein foods as indicated by the metabolic by-products of microorganisms has centered on fish and shellfish, probably because they are so highly perishable. Since this investigation is concerned primarily with oysters, chemical indicators of quality for foods of high protein content will receive priority over others.

Although the high protein foods contain significant amounts of fats and carbohydrates (Fields et al., 1968) our primary concern would seem to be with compounds derived from the protein. It must not be overlooked, however, that substances arising from components other than protein may have potential as indicators of quality for fish and shellfish.

The chemical composition of the spoiling substrate is decisive, because it determines the quantities of vital nutrients available for the developing microorganisms. It must be remembered that the nutritive requirements of an organism may change with a change in the conditions under which it is growing. For this reason, environmental conditions strongly influence the metabolism of microorganisms and thus their metabolic by-products.

As the food decomposes there will be changes in pH, redox potential, available oxygen, etc. These changes will select for certain members of the association, permitting only a limited number of organisms to develop in the

spoilage process. This selection may not continue to develop as a totality, since one or more members may be able to dominate and supress the others. The character and extent of these influences naturally depend upon the micro-organisms constituting the primary selection.

Mossel and Ingram (1955) found that nonmesophilic bacteria comprise the bulk of the microbial population of shellfish. Similar reports are provided by Alford et al. (1942); Cambell and Williams (1952); and Frazier (1958). The general composition of the microbial flora of oysters has been investigated by Eliot (1926) and by Colwell and Liston (1960). Gram-negative rods predominate while Gram-positive forms constitute a minor portion of the flora. However, there is a larger percentage of Gram-positive organisms in oysters than in fish (Liston, 1957; and Georgala, 1958). This is accounted for by the fact that Gram-positive organisms are typically terrigenous in origin, and oysters, due to their close proximity to land, are exposed to considerable terrigenous contamination.

Colwell and Liston (1960) found that the microbial flora of oysters exhibited a high degree of similarity from area to area. Moreover, this similarity extended beyond taxonomic groupings to biochemical groupings. It seems probable that only those organisms which are well adapted to the micro-environment provided by the oyster will be able to establish themselves as a significant part of the oyster flora.

In a product such as raw oysters, which is normally stored in containers which are held under iced conditions, the temperature will undoubtedly select for the growth of some organisms over others. At cool temperatures, psychrophilic strains of Pseudomonas, Achromobacter, Flavobacterium, and Micrococcus characterize the initial spoilage associations (Tanikawa, 1937; and Mossel and Ingram, 1955). The Pseudomonas/Vibrio group has been found to be the largest single group, with members of the Achromobacteriaceae next largest (Colwell and Liston, 1960). Jay (1970) reports that Pseudomonas and Achromobacter predominate during the early stages of oyster spoilage, with streptococci, lactobacilli, and yeasts dominating the later stages of spoilage.

A resemblance has been found between the physiological characteristics of the oyster flora and that of the fish flora (Liston, 1957). In both cases the proteolytic activity is high while the saccharolytic activity is generally low. However, there is a high incidence of organisms in oysters which are capable of fermenting carbohydrates. This is due to the high levels of carbohydrates in molluscan meats, which are largely in the form of glycogen. With levels of the type that exist in oysters, fermentative activities may be expected to occur as a part of the microbial spoilage (Jay, 1970). It has also been shown that oysters are more susceptible to microbial degradation than are vertebrate fish due to high amounts of free amino acids

their tissues contain (Jay, 1970).

Evidently, the association which dominates the spoilage of a particular food under certain storage conditions will have a most complex genesis, and it is this genesis which will ultimately be responsible for determining the compounds which could be used as quality indicators for that particular food.

Many of the chemical quality tests which were developed for vertebrate fish have been suggested as chemical indicators for the quality of oysters and other shellfish, although their applicability depends to a large part on the particular variety of shellfish. Some of the tests which have been investigated include indole, trimethylamine-nitrogen, total volatile bases, ammonia nitrogen, volatile reducing substances, pH and volatile acids. Quite often these tests are accompanied by organoleptic and microbiological examination of the product.

The use of indole as an index of quality in high protein foods has been investigated by a number of workers. Indole reacts under certain conditions with para-dimethylaminobenzaldehyde to give a red to pink color (King et al., 1945). This aldehyde is known as "Ehrlich's aldehyde" and is the principle ingredient in Ehrlich's and Kovaks' reagents, which are used by microbiologists to detect the presence of indole-producing organisms in a tryptone medium. The measurement of this color was made quantitative for the

determination of indole in butter (Clarke et al., 1937) by the use of a photometer. Duggan and Strasburger (1946) further modified the procedure, and their final modification has been accepted as an official method of analysis for indole in oysters and shrimp by the Association of Official Agricultural Chemists (1960). This procedure uses a steam distillation for the extraction of the indole from an homogenate followed by chloroform washes to recover the indole from the distillate. Para-dimethylaminobenzaldehyde is then mixed with the chloroform wash and the resulting color is measured in a photometer at 560 mu.

In 1903 it was proposed that tryptophan gives rise to indole and skatole upon its breakdown (Hopkins and Cole, 1903). It is believed that intermediate products, such as indole-propionic acid and indole-acetic acid are also formed (King et al., 1945). Okuda et al. (1919) have demonstrated the presence of tryptophane among other hydrolysis products of crustacean and mollusk proteins.

Fields et al. (1968) compiled information from Bergey's "Manual of Determinative Bacteriology" (Breed et al., 1957) concerning indole producers. Of eight genera, including Pseudomonas, Achromobacter, Flavobacterium, Sarcina, Serratia, Alcaligenes, Proteus, and Micrococcus, 14 species were producers of indole and 101 were non-producers. ZoBell and Upham (1944) tested 60 marine bacteria, only two of which formed indole in detectable amounts.

Clough (1922) stated that only 31 percent of 229 different cultures of bacteria isolated from raw or canned salmon gave positive results for indole production.

The results of different studies on indole in seafoods varied widely in the amounts of indole associated with good and with poor organoleptic quality. King et al. (1945) considered shucked, raw oysters slightly stale when the indole concentration reached 1.5 micrograms per 100 grams, whereas 1.9 micrograms per 100 grams was associated with the class of drained, canned oyster meat categorized as good. They further indicated that 34.0 micrograms per 100 grams of canned oysters was associated with a slightly putrid odor. Beacham (1946) described canned oysters which contained as much as 33 micrograms per 100 grams as having an odor which was slightly "off". It was also noted that unwashed oysters produced large quantities of indole.

Duggan and Strasburger (1946) investigated the relationship of indole content and organoleptic quality of shrimp. They concluded that absolutely fresh shrimp do not contain indole in detectable amounts; the indole content increases as decomposition increases; storage of raw shrimp at commercial holding temperatures for extended periods of time has no appreciable effect on the indole content; and that shrimp may decompose without the formation of appreciable amounts of indole.

Farber (1952) found indole to be of limited use as an

indicator of spoilage for tuna, mackerel, and sardines, because relatively few of the indigenous microflora were able to form the compound. Barry et al. (1956) stated that the amount of indole in canned shrimp decreased after storage periods of several months. In addition, odors associated with decomposition were less recognizable as storage time increased.

Lartigue et al. (1960) evaluated the indole test for oyster quality. Indole concentrations showed no definite pattern during iced-storage, and therefore were not recommended for the assessment of oyster quality. Similar trends of increasing, then decreasing, followed by increasing amounts of indole with decreasing organoleptic quality were found by King et al. (1945) in raw and canned oysters. This fluctuation in indole concentration, together with a very low potential occurrence of indole-producing organisms, are major obstacles in the use of indole as an index of quality. In addition, Snow and Beard (1939) noted instances where processing may be responsible for various cleavages which cause false positive indole tests.

Quality analysis for fish and shellfish often utilize the determination of volatile bases. Beatty (1938) found that the volatile bases consisted of ammonia, and primary, secondary, and tertiary amines, with trimethylamine comprising about 95 percent of the total. Trimethylamine and ammonia are often determined separately, and have received

more attention than the other volatile bases. Little attention has been given to dimethylamine since Shewan (1938) showed its poor potential as an index of quality. Methods for isolating the volatile bases by aeration, distillation, and microdiffusion have been described by Stansby et al. (1944) and by Conway (1957). Dyer's method for the colorimetric determination of trimethylamine (TMA) as the picrate salt also has wide acceptance (Bethea and Hillig, 1965).

Trimethylamine may be synthesized from creatine, betaine, choline, acetylcholine, and trimethylamine oxide (TMAO) (Fields et al., 1968). Trimethylamine and other amines may arise from the process of decarboxylation of amino acids (Bramstedt, 1957), but according to Beatty (1938) most of the TMA apparently comes from TMAO.

Suwa (1909) was the first to show that the TMAO now known to be common to almost all marine creatures, is reduced to the corresponding amine in the course of bacterial action. Later work by Beatty and Gibbons (1937) showed that autolysis plays only a minor role in the formation of TMA and other volatile bases, and that the specific bacterial species which possess the enzyme triamineoxidease use this respiratory catalyst in the system where TMAO is activated as the hydrogen acceptor. Triamineoxidease has been found in the cells of bacteria from widely different sources (Tarr, 1940), and several common bacteria were

found to have TMAO-reducing enzyme systems which were less active at 0°C than at 37°C.

Castell and Snow (1951) found cultures of Achromobacter and non-fluorescent Pseudomonas to be active reducers of TMAO. The enzyme systems involved with this reduction were found to be sensitive to acidic conditions and were relatively inert below pH 6.0. According to Elliot (1952) the optimum pH for triamineoxidease is from 7.2 to 7.4. Furthermore, Neilands (1945) found that indole, skatole, and hydrogen sulfide caused partial inhibition of the enzyme system. It was found that Pseudomonas putrefaciens will reduce six times as much TMAO at pH 7.8 as compared to pH 5.9 (Castell and Snow, 1951). Achromobacter sp. showed similar responses to pH change.

None of the Micrococci examined by Baird and Wood (1944) reduced TMAO. In a study by Castell and Mapplebeck (1952) on the importance of Flavobacterium in fish spoilage, only 16 percent of 245 cultures had the ability to reduce TMAO, although 50 percent were found to be proteolytic.

Beatty and Gibbons (1937) reported the separation of a fraction of the volatile bases from decomposing cod, which was found to develop only as the result of bacterial action. This fraction was believed to consist entirely of TMA derived from the reduction of TMAO, because ammonia and the other amines were removed during the analytical procedure. Castell et al. (1961) reported that haddock had greater

deterioration than did cod for a given TMA level. There were also seasonal variations in the amount of TMA found in various fish.

Although Dyer (1952) found the amounts of TMAO to be similar in the same or related species from different parts of the world, Anderson and Fellers (1952) presented evidence of the variations in the TMAO content among different species of marine fish and shellfish. It is interesting to note that Dyer (1952) found no TMAO in clams, mussels, or oysters, yet TMA levels in these products often increase with storage time, suggesting another source of TMA.

Fieger and Friloux (1954), while working with shrimp, found that significant increases in bacterial plate counts preceded increases in the TMA content. Bailey et al. (1956) stated that in most cases a TMA level of 1.5 mg per 100 grams of shrimp and a bacterial count of 1×10^7 or greater indicated an unacceptable product.

Lartigue et al. (1960) determined the TMA content of oysters which had been treated with chlorotetracycline. During a 30 day iced-storage period no obvious or definite trend could be found in the results, and it was concluded that the TMA test was not useful in predicting oyster quality, nor is it of value as a measure of the preservative qualities of chlorotetracycline in oysters.

Farber and Lerke (1961) used TMA values to evaluate the freshness and to estimate the storage life of raw fishery

products such as sole fillets, rockfish, salmon steaks, albacore, and unpeeled, raw shrimp. In general, higher TMA values were obtained with higher plate counts. Later work by Farber (1963) compared sensory judgements and TMA content of a number of white- and red-fleshed species of fish at seven different laboratories. Correlations of TMA level tended to parallel those of the sensory judgements of the white-fleshed fish but were not related to the stage of spoilage of red-fleshed fish.

Spinelli et al. (1964) noted large increases in the TMA content of English sole fillets after 9 days of iced-storage. It was also found that less time would be required for the formation of large amounts of TMA when contamination due to improper handling was in evidence.

There are conflicting views as to the reliability of TMA as an index of quality for marine products. It is generally agreed that TMA determinations would be useful in indicating whether spoilage had occurred or not, but would be of considerably less value in yielding information on quality changes (Fieger and Friloux, 1954). The general opinion seems to be that TMA tests are not conclusive but might be useful in combination with other tests.

Ammonia is another volatile base which has received a great deal of attention as an index of quality for marine products. Ammonia is a metabolic by-product of most proteolytic bacteria. According to ZoBell (1946) marine bacteria

are actively proteolytic, and they rapidly attack most types of proteinaceous materials with nearly all of them liberating ammonia from amino acids and peptones. All of the 60 species described by ZoBell and Upham (1944) produced ammonia. Of 36 species of marine bacteria on which information on ammonia production was given by Breed et al. (1957), 29 were positive. These included species of Achromobacter, Pseudomonas, Flavobacterium, Proteus, and Micrococcus.

Bandemer and Schaible (1936) developed a microdetermination method for ammonia-nitrogen in eggs. This consisted of a microdiffusion procedure where ammonia was liberated by the addition of potassium carbonate and trapped in standard hydrochloric acid. After an incubation period to insure complete liberation of the ammonia, the excess hydrochloric acid was titrated with standard sodium hydroxide and the ammonia was calculated as the milligrams of ammonia-nitrogen per 100 grams of eggs. Additional methods on microdiffusion analyses are given by Conway (1957) and Budd and Spencer (1968).

Burnett (1965) used a method based on the color reaction between ammonia, thymol, and bromine to determine the ammonia content of fresh and frozen crabmeat. Good reproducibility was obtained with this method, but Burnett recommended further study on crabmeat and application to shrimp and other marine products.

Ammonia was found to increase rapidly during the decomposition of canned salmon, but Clough (1922) pointed out

that it may have been the result of processing and therefore was of little value as an indicator of quality. Ammonia has been used by Stansby and Lemon (1933) as a quality index for fresh haddock. Crooks and Ritchie (1938) were able to set standards for haddock quality based on the ammonia content as they were able to show the increase in ammonia content with storage at 4-5°C.

Ammonia and other volatile bases were found to be of little value as quality indices of frozen breaded shrimp by Gagnon and Fellers (1958) due to extreme variation between samples. Hughes (1959) demonstrated that the fresh flesh of herring contained some ammonia and that the concentration increased continuously during a test period of 100 hours at 10-13°C. Thus the use of ammonia as a quality indicator for herring was suggested. The ability to detect ammonia in crabmeat before organoleptic spoilage was evident caused Burnett (1965) to suggest it as a likely index of quality.

The major limiting factor in the use of ammonia as an index of quality is its volatility which would introduce a large degree of error in testing some products. Another disadvantage is the frequent variation in the ammonia content within the same organoleptic class of food.

While ammonia and trimethylamine are the most widely used volatile bases for evaluating the quality of various marine products, several workers suggest the use of total volatile bases for measuring the stages of decomposition.

The biosynthesis of volatile bases and the microorganisms which produce them are similar to those for ammonia and trimethylamine. The methods for the determination of total volatile bases include microdiffusion, distillation and aeration and are similar to those used for ammonia and TMA (Stansby et al., 1944). Tomiyama et al. (1956) suggested that a vacuum distillation procedure for the recovery of volatile bases from fish and shellfish be applied.

Volatile bases were found to be highly correlated with the organoleptic quality of cod (Hillig et al., 1958), ocean perch (Hillig et al., 1960), and pollock and whiting (Hillig et al., 1961). Cambell (1962) reported that total volatile bases also held promise in the analysis of fresh or frozen shrimp.

Spinelli et al. (1964) studied irradiated crabmeat in relation to volatile base production, bacterial counts, and TMA. Results indicated that total volatile bases content could be used with only a fair degree of accuracy to assess the quality of vacuum-packed crabmeat. It was found that the bacteria surviving the irradiation possessed reduced ability to form volatile bases.

The disadvantages which apply to the use of TMA or ammonia as indices of quality also apply to the mixture of compounds assayed as total volatile bases. However, the fact that total volatile bases are a mixture of compounds is a definite advantage, since not all spoilage organisms

produce large amounts of a single indicator. In this case the multiple by-products may more nearly indicate the extent of decomposition by a mixed culture.

The group of compounds designated as volatile reducing substances (VRS) includes compounds such as acetylmethylcarbinol (AMC), diacetyl, and other volatile components which have the ability to reduce alkaline potassium permanganate during an aeration procedure (Fields et al., 1968). The amount of reduction is then determined as the microequivalents of reduction per unit of sample. Even though AMC, diacetyl and other volatile products of microbial metabolism may normally be determined in the spoilage of high-carbohydrate foods, they may serve as an indicator of quality for fish and shellfish, where fermentation processes may be involved in spoilage.

Much of the work using VRS as an indicator of the quality of fish has been performed by Farber (1952), who found that VRS correlated better with the organoleptic quality of raw and canned fish than did TMA or indole. Subsequent work by Farber and Ferro (1956) showed that the VRS content of canned tuna, anchovies, mackerel, herring, and sardines correlated closely with organoleptic quality, but total volatile bases and TMA did not. Farber and Lerke (1958) concluded that the VRS determination was a more useful test than TMA or total volatile bases for judging the quality of all types of fish and fish products and their diverse spoilage

patterns under varying storage conditions.

Moorjani et al. (1958) reported on the changes in VRS and bacterial counts as indices of the spoilage of fresh water fish. At the time that definite spoilage had set in, there was a corresponding rise in VRS content, thus showing the relationship of bacterial counts, VRS, and organoleptic quality.

The determination of VRS requires an aeration procedure which is a disadvantage in many laboratories due to the length of aeration and the cost of the equipment. However, since VRS include several compounds there would be a greater chance of metabolites from a mixed flora being present than would be true if a single compound were measured. One must bear in mind, however, that the compounds which may accumulate as the result of microbial degradation may not be volatile enough to be measured by the VRS test.

Unlike the pH of most fish and shellfish, that of oysters falls progressively with spoilage, owing to the breakdown of glycogen, in which they are rich. Rosen (1966) noted a corresponding increase in the concentration of lactic and other acids with a decrease in the glycogen content of ice-stored oysters. Several investigators, among them Hunter and Linden (1925), Pottinger (1948), and Abbey et al. (1957), have thus advocated the use of pH as an index of freshness for oysters. Hoff et al. (1967) showed that the pH of oysters declines more rapidly at 10°C than at 3°C or in ice. However, pH changes generally show poor correlation

with microbial changes. Conversely, Liebman et al. (1957) reported good correlation between pH and plate counts during the storage of Pacific oysters. However, it was noted that autolytic activity of the oysters, as well as microbiological degradation, can cause changes in the pH. Jay (1970) has reported that pH is apparently a better test for detecting spoilage in oysters and other molluscan shellfish than total volatile bases. In addition to its possible use as an indicator of quality, the change in pH is of great importance due to the effect it may have on the production of certain metabolites, such as TMA (Castell and Snow, 1951).

Since the production of various acids causes a decrease in the pH of oysters, one might think that the concentration of volatile acids would be a reliable index of freshness. However, volatile acids have been found to be unreliable as an index of oyster quality (Beacham, 1946; and Cambell, 1962).

Other chemical substances which have been investigated as indicators of quality in fish and shellfish include histamine (Williams, 1954; Hillig, 1956; and Hillig, 1963), hydrogen sulfide (Snow and Beard, 1939; King et al., 1945; and Sands et al., 1949), hypoxanthine (Spinelli et al., 1964; Jones, 1965; and Lerke and Farber, 1969), and ethyl alcohol (Hillig et al., 1959; and Hillig, 1963). However, these tests have not received a great deal of attention.

As mentioned previously, organoleptic evaluations and determination of microbial populations may accompany the

chemical indices of quality, or they may be used alone in an attempt to assess the freshness. The use of bacterial counts after a period of incubation of the product at 15°C has been suggested by Tarr (1943) to indicate the potential keeping quality at lower temperatures. Fieger and Friloux (1954) measured organoleptic values, total plate counts, volatile acids, amino nitrogen values, and TMA content of shrimp. Significant increases in bacterial plate counts preceded similar increases in volatile acids and TMA values. Amino nitrogen values decreased with increasing storage time and correlated well with organoleptic values for flavor and quality.

Novak et al. (1956) discussed procedures for the rapid determination of bacterial numbers in oysters and shrimp, whereby the degree of acid formation in a standardized medium or the time required for the reduction of methylene blue by the organisms present in a sample were compared to total plate counts. Gagnon and Fellers (1958), in working with biochemical methods for determining shrimp quality, obtained fairly good correlations between log of total bacteria per gram versus volatile bases, taste panel scores, and storage time.

Moorjani et al. (1958) found organoleptic evaluations, volatile reducing substances, and bacterial counts to be interrelated during the storage of fresh water fish. Lartigue et al. (1960) determined indole, TMA, bacterial counts, and organoleptic scores on oysters during a 30-day iced-

storage period. No definite pattern could be found with indole or TMA concentrations, but bacterial numbers closely paralleled organoleptic ratings.

Farber and Lerke (1961) suggested the measurement of the changes in the types rather than in the total plate counts for evaluating the quality of raw fishery products. Later work by Adams et al. (1964) determined the incidence of spoilers during spoilage of fish muscle. The results indicated that the bacterial flora of spoiling fish muscle consists of a wide variety of organisms, of which only a small percentage cause spoilage. The remainder probably exist as free riders, or perhaps are involved in some synergism with weak spoilers.

Hoff et al. (1967) studied the effect of storage in crushed ice at 3° and 10°C on the bacteriological quality of shucked Pacific oysters and Olympia oysters. Determinations were made on 35°C plate counts, coliform MPN, fecal coliform MPN, and pH. The 35°C plate count showed the best correlation with time and temperature of storage. Fecal coliform MPN's showed the greatest stability under all storage conditions, while the coliform MPN's increased at all three temperatures. However, the patterns of change were more uniform with the 35°C plate count.

Several workers have employed the use of direct, microscopical counting techniques for the rapid assessment of microbial numbers and freshness. (Blanchard et al., 1951). Lerke and Farber (1969) found initial direct bacterial

counts of fresh fish to correlate well with future keeping quality. They were also able to reliably predict remaining shelf life for fish fillets in this manner.

The sensory evaluation of fish and shellfish has usually been made by methods based on either hedonic scales (Hansen, 1960; and Cohen and Peters, 1962) or on descriptions of the various sensory attributes of the product such as color, texture, odor or flavor. These descriptions may be limited to the general changes distinguishing the fresh product from the spoiled product, or they may be highly detailed descriptions of the entire gamut of changes from absolute freshness to putridity.

Shewan et al. (1953) devised a detailed descriptive scheme for the correlation of organoleptic scores with chemical indices of freshness. Castell and Greenough (1958) have investigated a further approach to the sensory evaluation of freshness. This approach results in the classification of the product into several groups or grades, viz. no spoilage present, signs of early spoilage evident, and spoiled. These grades were found to correlate well with the TMA content in fish although species and seasonal variations were in evidence. A final approach to the sensory evaluation of freshness involves the use of the flavor-profile method developed by Cairncross and Sjostrom (1950), whereby all flavor components can be considered in relation to one another. This method aims at giving complete flavor information, not only information about differences, but

also definition of differences, similarities, and likenesses (Caul, 1957).

Few of the methods for assessing the freshness or the degree of decomposition of marine products have been generally adopted (Fields et al., 1968). Certain tests will provide better information than others, depending upon the particular product, processing and storage conditions. The chemical test may estimate a group of compounds, i.e. total volatile bases, or an individual compound, such as ammonia or trimethylamine. In addition, microbiological tests and organoleptic evaluations of the product may be an aid in giving additional information about the stage of decomposition.

The opinion has often been expressed that research should be directed toward developing a single objective quality test that will be applicable to a wide variety of products. However, there are so many variables in a food product, that it may be impossible to develop a single reliable test. Rather, it may be better to determine which tests, when run in conjunction with one another, will give the best overall view of the decomposition which has taken place.

MATERIALS AND METHODS

The potential usefulness of a total reducing substance (TRS) test for oyster quality was determined by comparison with previously available chemical indices, microbiological quality, and organoleptic evaluations. Statistical analyses were then used to show which tests correlated well with the total reducing substance method.

These studies originated in May, 1969, with freshly shucked oysters that were obtained from a commercial packing plant in New Orleans, Louisiana.

Oysters used in this investigation were collected from oyster beds near New Orleans and transported to New Orleans in a refrigerated truck at 40°F. The oysters were shucked upon arrival by professional shuckers using facilities which conformed to FDA regulations (FDA Definitions and Standards for Shellfish, Title 21, Part 36, Section 36.10). The oysters were washed in running tap water for 2 minutes and allowed to drain for a period of 5 minutes. All draining was done on FDA approved, stainless steel skimmers, which had perforations of at least 0.25 inch in diameter located not more than 1.25 inches apart. The oysters were distributed evenly over the surface of the skimmer but were not otherwise agitated during the draining period. After the oysters were washed and drained, they were packed in one-gallon cans, placed into ice chests, covered with ice and then transported to the Food Science Department of LSU in

Baton Rouge. No more than 2 days had elapsed from the time the oysters were removed from the water until initiation of testing.

Several chemical tests have been investigated for the purpose of ascertaining the quality of oysters and other shellfish. Most notable among these are the tests for pH, trimethylamine-nitrogen, total volatile bases, and indole. Microbiological studies and organoleptic evaluations are often performed in conjunction with these chemical tests. The test for total reducing substances (TRS) is a new modification of an old test. Therefore it must be compared with existing tests to determine its potential usefulness as an index of quality.

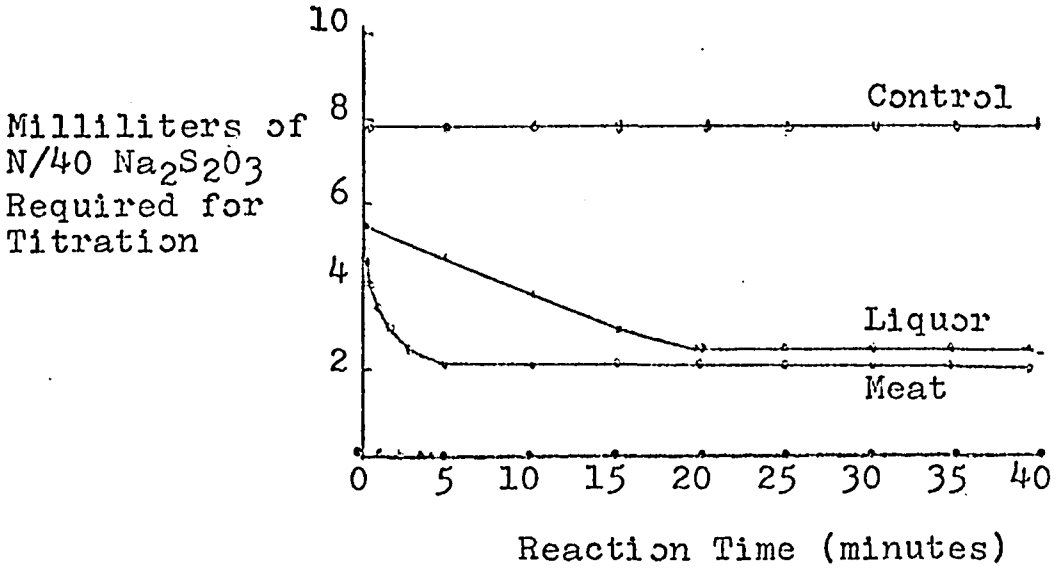
The possibility existed that the chemical compounds which are used to measure oyster quality are affected by iced-storage. To ascertain if any changes did occur during a 15 day iced-storage period, duplicate samples were withdrawn from each of two gallons of oysters at 0, 1, 3, 5, 7, 9, 11, 13, and 15 days. It was often thought that the results obtained with stored homogenates were a valid representation of what ensues in the whole oyster. However, preliminary investigations by this author and by Digirolamo et al. (1970) showed it inadvisable to generalize from homogenate studies to whole oysters. Therefore testing was performed on whole oysters and, where applicable, the oyster liquor.

Total Reducing Substance Determination- The use of total reducing substances (TRS) as a quality index for oysters was developed while experimenting with the volatile reducing substance (VRS) test of Farber (1952). The VRS test involves an aeration procedure in which air is circulated through a sample homogenate and then standard alkaline potassium permanganate solution for a specified length of time, usually 45 minutes or 1 hour. The reduction of the permanganate by volatile compounds from the sample is manifested by a change from the purple potassium permanganate to the green potassium manganate. This change can then be measured either gravimetrically or spectrophotometrically. Results are expressed as microequivalents of reduction per unit of sample. The results obtained with oysters using the VRS procedure were found to be extremely variable and the use of aeration equipment and the length of the aeration period prevented a large number of samples from being run.

The measurement of total reducing substances (TRS) is essentially a modification of the VRS test in that it uses no aeration procedure. Either oyster meat homogenate or oyster liquor may be analysed. Preparation of the homogenate consisted of blending 50 g. of drained oyster meat in a Waring Blendor for 3 minutes. Two-tenths of a milliliter of the homogenate was then pipetted into a 50 ml. Erlenmeyer flask for testing. For testing the oyster liquor, 0.2 ml. of liquor was pipetted directly from the

container to the 50 ml. Erlenmeyer flask. Ten milliliters of N/50 $KMnO_4$ in N NaOH was then pipetted into the reaction flask. From this point on the procedure for both oyster meat homogenate and oyster liquor was the same except for the reaction time. Less reaction time was required for the oyster meat homogenate than for the oyster liquor (Figure 1). It is recommended that a time standardization procedure be determined for each sample.

Figure 1. TRS reaction time standardization.



After the reaction was essentially complete, 5 ml. of 6/N H_2SO_4 was pipetted into the reaction flask. The contents were stirred and 3 ml. of 20 percent KI was added. The liberated iodine was then titrated with N/40 $Na_2S_2O_3$, using starch solution as an indicator. The control for the unreacted permanganate was treated in the same manner as the sample.

The total reducing substance concentration of the oyster

meat homogenate or oyster liquor was then calculated using the following formula:

$$\text{Microequivalents of reduction per 0.2 ml. of sample} = \frac{\text{Titration for control} - \text{Titration for sample}}{} \times 25$$

where: 25 = microequivalents per ml. of N/40 $Na_2S_2O_3$.

pH Determination- The pH of both the oyster meat homogenate and oyster liquor was determined using a Corning pH meter Model 10 with expanded scale. It was found that similar results could be obtained for oyster meat or oyster liquor by using a 1:1 or a 1:10 dilution with deionized water. Due to the small amounts of oyster liquor available, the 1:10 dilution was used. Oyster meat homogenate was treated accordingly. The pH meter was standardized before and after each reading with a standard buffer solution of pH 7.0.

Trimethylamine-nitrogen Determination- Trimethylamine-nitrogen (TMAN) was determined using a modification of the microisothermal distillation method of Beatty and Gibbons (1937). This method allows the determination of the tertiary amine in the presence of ammonia by reducing the vapor pressure of the latter with the addition of formaldehyde (Budd and Spencer, 1968). Budd and Spencer have conducted experiments on the percent recovery of various amines and ammonia by isothermal distillation in the presence and absence of formaldehyde. As shown by their work, the results returned for TMAN will only be approximate if comparable

amounts of the other amines are present (Table 1).

TABLE 1. Recovery of amines by isothermal distillation.[1]

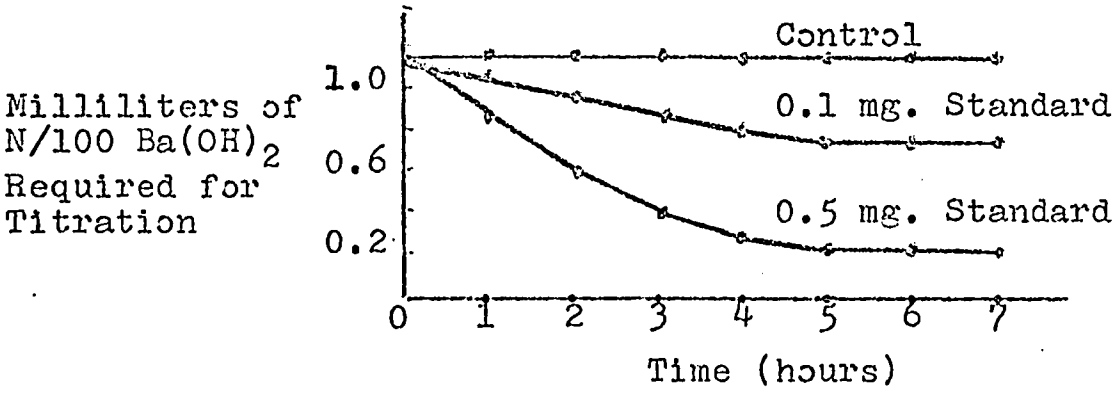
Compound	Percent Recovery	
	KOH alone	KOH + HCHO
Ammonia	97	2
Methylamine	99	6
Dimethylamine	100	11
Trimethylamine	100	100
Ethylamine	91	21
Diethylamine	97	21
Triethylamine	100	99

[1] From Budd and Spencer (1968).

Trimethylamine-nitrogen was determined on both oyster meat homogenate and oyster liquor. Twenty-five grams of oyster meat was homogenized with 100 ml. of 5 percent trichloroacetic acid and 25 ml. of distilled water in a Waring Blendor for 2 minutes. The contents were then transferred to a 250 ml. volumetric flask and made up to volume with distilled water. This mixture was then transferred to a centrifuge bottle and centrifuged at 2500 r.p.m. for 15 minutes. The supernatant was saved for TMAN determinations. For determination of the TMAN content of oyster liquor, 2.5 ml. of liquor was pipetted into a centrifuge tube with 10 ml. of 5 percent trichloroacetic acid and 2.5 ml. of distilled water. The contents were mixed using a vortex mixer and then centrifuged at 2500 r.p.m. for 15 minutes. The supernatant was saved for TMAN determinations.

In place of a No. 1 Conway cell, a 150 ml. screw top bottle containing a 10 ml. beaker was used for ease of manipulation. The 10 ml. beaker contained 2.0 ml. of 0.005 N HCl with Tashiro's indicator. For analysis of the oyster meat, 0.5 ml. of the supernatant was placed in the bottom of the bottle. For oyster liquor analysis, 1 ml. of supernatant was used. The bottle tops were replaced after the addition of sample and each reagent. Two milliliters of 10 percent formaldehyde was added to the bottom of each bottle and then 1 ml. of 40 percent KOH (KOH was used rather than K_2CO_3 because the amine-containing supernatant was strongly acidic (Conway, 1957)). The bottles were then sealed and incubated at room temperature. The distillation was found to be essentially complete after 5-6 hours (Figure 2).

Figure 2. Isothermal distillation standardization.



After the incubation period the beaker was removed from the bottle and the excess standard HCl was titrated with N/100 $Ba(OH)_2$ using a microburette. Such small

amounts of $Ba(OH)_2$ were required for the titrations that a micromagnetic stirrer was used to mix the solutions.

Control blanks for unreacted standard HCl were treated the same as were the samples. The concentration of trimethylamine-nitrogen was calculated as follows:

$$\text{Milligrams of TMAN per 100 g. or 100 ml. of sample} = \frac{(\text{Control} - \text{Sample})(14)(N)(D)}{(\text{Sample weight}) \times 1000} \times 1000 \times 100$$

where 14= molecular weight of nitrogen.
N= normality of $Ba(OH)_2$.
D= dilution factor.

Total Volatile Base Determination- Conway (1957) states that this group of compounds includes ammonia, the amines, amino acids, and other compounds which yield ammonia upon breakdown by the reagents employed. Budd and Spencer (1968) reported this group as total amines and ammonia, since almost complete recovery of all the amines tested and ammonia was achieved.(Table 1).

The procedure used for the determination of total volatile bases (TVB) is essentially the same as that used for the determination of trimethylamine-nitrogen except that no formaldehyde is added to the isothermal distillation bottle. The amount of TVB was determined in both oyster meat and oyster liquor, with the results expressed in mg. per 100 g. of meat or 100 ml. of liquor.

Indole Determination- Indole was determined using the Official Methods of Analysis of the A.O.A.C. (9th ed), with the following modifications: (1) a chloroform trap was

used to collect the steam distillate, and (2) an additional 5 ml. HCl wash (1:5) was used to clean the color reagent before reading in the spectrophotometer. Due to the lack of sufficient oyster liquor, only the oyster meat was analyzed for indole content. Results are expressed in micrograms of indole per 100 g. of oyster meat.

Microbiological Plate Counts- Microbiological plate counts were determined on both the oyster meat and oyster liquor using the following procedure. Sample preparation for oyster meat consisted of homogenizing 50 g. of meat with 450 ml. of sterile phosphate buffer in a sterile Waring Blendor jar for 2 minutes. For oyster liquor, 1 ml. of liquor was pipetted into a sterile 9 ml. buffer blank. Serial dilutions of the samples were then made and aliquots were plated in quadruplicate on Eugonagar (BBL). Duplicate plates were incubated for 2 days at 37°C for mesophilic plate counts while duplicate plates for psychrophilic plate counts were incubated for 7 days at 5°C. Total colony counts were made with the aid of a Quebec colony counter and the results expressed as the log of the number of organisms per gram of oyster meat or milliliter of oyster liquor.

Organoleptic Evaluations- Organoleptic evaluations were carried out on raw, ice-stored oysters using both an hedonic scaling system (Peryam and Pilgrim, 1957) and a class designation system (Castell and Greenough, 1958).

A sample organoleptic scoring sheet is shown in Figure 3. Panelists consisted of 12 laboratory personnel who had previous experience in organoleptic testing. On each test day, each panelist evaluated 3 oysters for odor, appearance, flavor and texture. Each of these characteristics was rated from 10 to 1 for the hedonic scaling. For the class designation each panelist rated the product as good quality (Class I), showing evidence of spoilage (Class II), or spoiled (Class III).

When a test day fell on a weekend, the organoleptic evaluations were performed on the nearest week day. Consequently, tests were run on days 0, 1, 3, 6, 7, 9, 10, 13, and 15 for one replicate and days 0, 1, 4, 5, 7, 8, 11, 13, and 15 for the other replicate.

Statistical Analysis and Correlations- Data compiled from the aforementioned chemical, microbiological, and organoleptic tests were subjected to an analysis of variance and correlation coefficients were determined. All analyses were conducted at the Computer Science Center at Louisiana State University with the aid of the Experimental Statistics Department.

Figure 3

Organoleptic score sheet

Product_____ Date_____

Judge's Name_____

Sample	No.1	No.2	No.3
Odor			
10. Excellent 8. Good 6. Fair 4. Poor 2. Inedible			
Appearance			
10. Excellent 8. Good 6. Fair 4. Poor 2. Inedible			
Flavor			
10. Excellent 8. Good 6. Fair 4. Poor 2. Inedible			
Texture			
10. Excellent 8. Good 6. Fair 4. Poor 2. Inedible			

Do you grade this product as:

_____ Class I (good quality)
_____ Class II (evidence of spoilage)
_____ Class III (spoiled)

RESULTS AND DISCUSSION

The average values for total reducing substances, pH, trimethylamine-nitrogen, total volatile bases, indole, mesophilic plate counts, psychrophilic plate counts, and organoleptic evaluations on ice-stored oysters are reported in Tables 2 through 9. Graphic presentation of this data and comparison with organoleptic quality classes can be found in Figures 4 through 11. Each of these figures shows the relationship of the test results to organoleptic quality- Class I (good quality), Class II (evidence of spoilage), and Class III (spoiled). Class II products were judged to be organoleptically acceptable, but not of superior quality, whereas Class III oysters exhibited such intense evidences of spoilage as to render the product organoleptically unacceptable.

The individual test results for each test and their respective summaries of statistical analysis are given in Tables A through V of the Appendix. Major correlation coefficients are reported in Tables 10 and 11, while minor correlations can be found in Table W of the Appendix.

The average concentration of total reducing substances (TRS) was found to increase uniformly in both oyster meat and oyster liquor throughout the 15 day iced-storage period (Table 2). This increase was found to be highly significant ($P < 0.01$). The average TRS values for oyster meat increased

from 135.50 microequivalents of reduction per 0.2 ml. of sample at day 0 to 144.57 microequivalents of reduction at day 15. The TRS values for oyster liquor increased from 108.27 to 138.23 microequivalents of reduction per 0.2 ml. of sample over the same storage period. By virtue of the fact that oyster liquor exhibits a larger range of increase in TRS content than does oyster meat, there would be less chance of a false positive due to experimental error or seasonal variation with the oyster liquor. The range of values with the liquor is large enough so that a borderline or incipient spoilage stage would be sufficiently distinct from the fresh product to make its detection possible. In addition, the TRS concentration is more easily measured in oyster liquor than in oyster meat.

As seen in Table 0, there was a highly significant difference ($P < 0.01$) between the TRS values for meat and for liquor. However, it is important to note that there was no significant difference between gallons on each test day or between replicates, showing that the TRS test gives results that are reproducible both within and between replicates.

As shown in Figure 5, the pH of both oyster meat and oyster liquor was found to decrease uniformly throughout the iced-storage period. The decrease is in close agreement with the observations of Piskur (1947), Pottinger (1951), Hoff et al. (1967), and Digirolamo et al. (1970). The

TABLE 2

The effect of iced-storage on the total reducing substance content of oysters.[1]

Storage time (days)	Oyster Meat[2]	Oyster Liquor[2]
0	135.50	108.27
1	136.17	117.49
3	136.30	127.37
5	138.66	130.72
7	142.12	134.59
9	141.41	136.49
11	143.37	137.71
13	144.01	137.92
15	144.57	138.23

[1] Total reducing substances are expressed as micro-equivalents of reduction per 0.2 ml. of sample. The values given are the averages of 2 replications which were not significantly different from one another.

[2] The increase in the total reducing substance content of oyster meat and liquor during the storage period was significant ($P < 0.01$).

Figure 4. Changes in the concentration of total reducing substances in oysters during iced-storage.

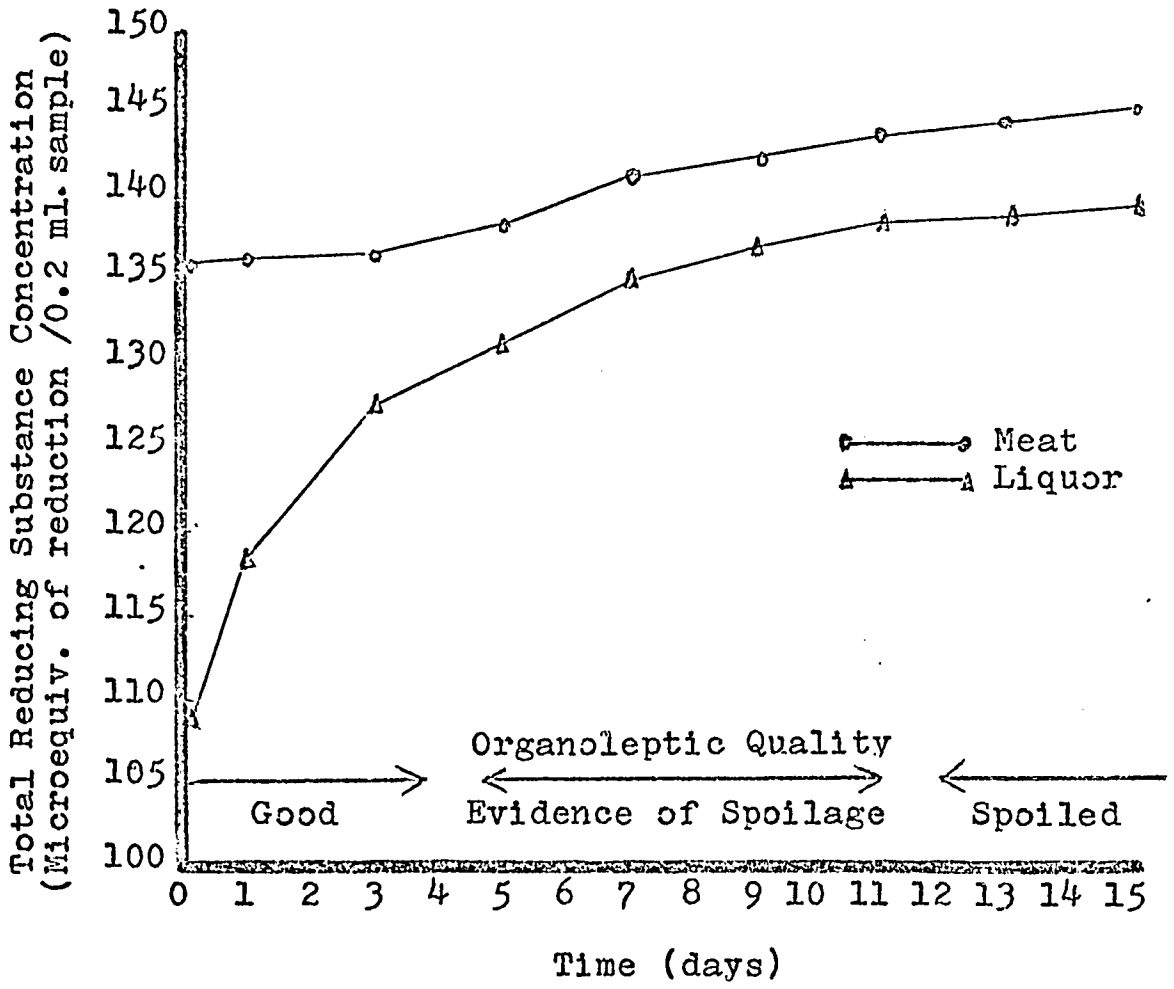


TABLE 3

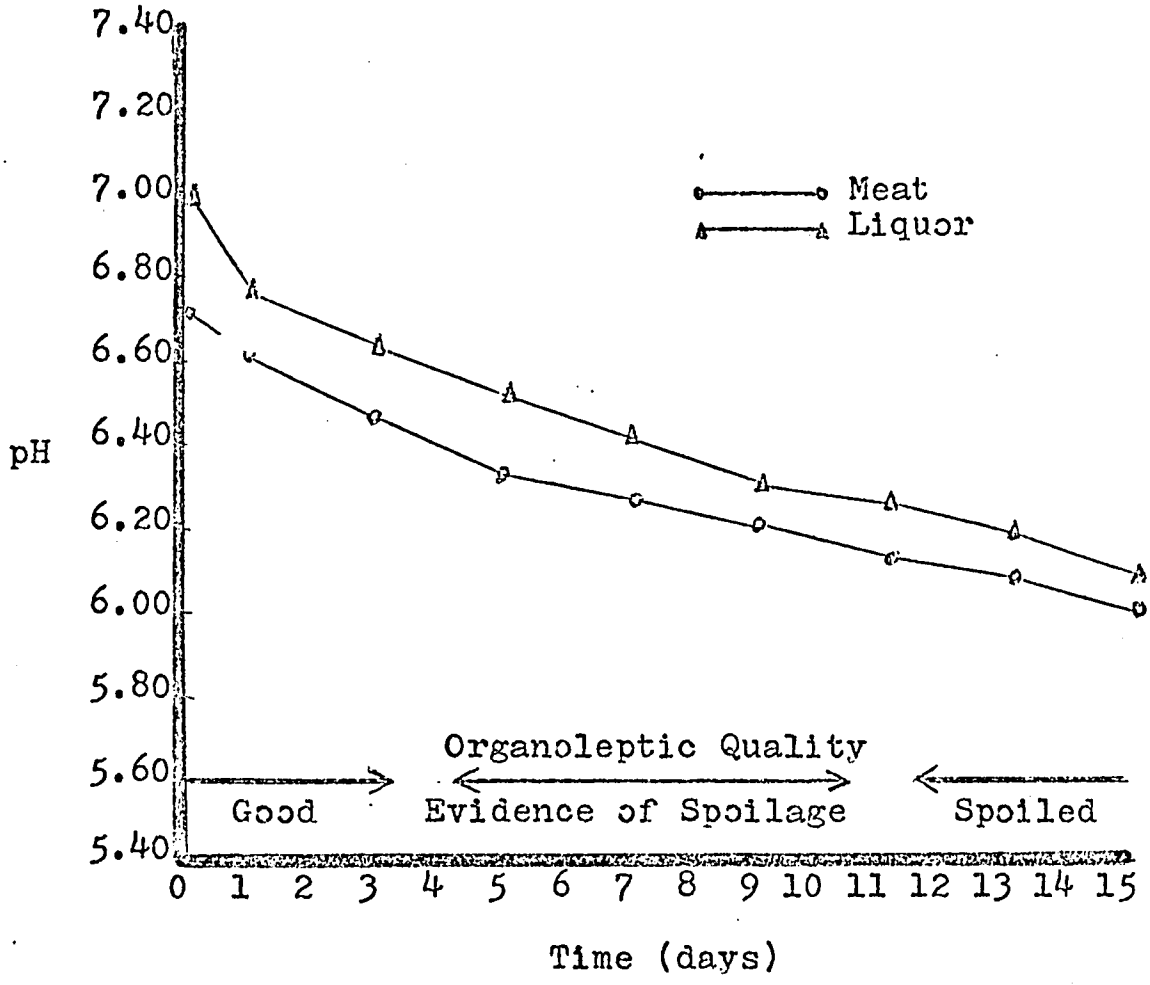
The effect of iced-storage on the pH of oysters.[1]

Storage time (days)	Oyster Meat[2]	Oyster Liquor[2]
0	6.69	6.97
1	6.57	6.74
3	6.49	6.60
5	6.32	6.49
7	6.24	6.40
9	6.21	6.28
11	6.11	6.22
13	6.05	6.13
15	5.96	6.02

[1] The pH values given are the averages of 2 replications which were significantly different from one another ($P < 0.05$).

[2] The decrease in pH of oyster meat and liquor during the storage period was significant ($P < 0.01$).

Figure 5. Changes in the pH of oysters during iced-storage.



average pH of oyster meat decreased from 6.69 to 5.96 during the storage period, whereas the pH of the liquor decreased from 6.97 to 6.02 (Table 3).

As reported in Table P, the drop in pH was highly significant with respect to storage time. There was also a highly significant difference between the pH of the oyster meat and the liquor. It is important to note that there was no significant difference between gallons within replicates. However, there was a significant difference ($P < 0.05$) between replicates which makes pH less reproducible than total reducing substances.

No definite pattern could be established for the trimethylamine-nitrogen (TMAN) content of either oyster meat or oyster liquor during iced-storage (Table 4). As shown in Figure 6, there was a general increase in TMAN content which was found to be highly significant. Unfortunately, this increase was not consistent throughout storage. Similar changes in the TMAN content of oysters have been reported by Lartigue et al. (1960).

The TMAN content of oyster meat increased from 4.16 mg. per 100 g. at the beginning of the storage period to 13.02 mg. per 100 g. at the end of storage. TMAN values for oyster liquor increased from 4.72 mg. to 14.43 mg. per 100 ml. during the storage period. It is thought that the ability of microorganisms to elaborate TMA is decreased by iced-storage and by a lowering of the pH (Castell and Snow, 1951), thus

limiting the use of TMAN as an index of oyster quality.

No significant differences were found between the TMAN content of oyster meat and liquor, or between gallons within replicates (Table Q). However, there was a highly significant difference between replicates which emphasizes another disadvantage in using TMAN as an index of oyster quality.

A highly significant increase was found in the concentration of total volatile bases (TVB) in both oyster meat and liquor (Table 5). As seen in Figure 7, there are fluctuations in the TVB content during the first week of iced-storage, but there is a uniform increase after that time.

The average TVB content of oyster meat increased from 19.68 mg. per 100 g. at day 0 to 30.05 mg. per 100 g. after 15 days. During the same period the TVB content of oyster liquor increased from 15.23 mg. to 42.00 mg. per 100 ml.

As reported in Table R, there was a highly significant difference between the the TVB content of oyster meat and liquor. There were no significant differences between gallons within replicates, but the highly significant difference found between replicates is a distinct disadvantage in the use of TVB as an index of oyster quality.

Indole was detected in minute quantities throughout the storage period (Table 6). As shown in Figure 8, there is a slight decrease in indole concentration from day 0 to day

TABLE 4

The effect of iced-storage on the trimethylamine-nitrogen content of oysters.[1]

Storage time (days)	Oyster Meat [2]	Oyster Liquor [2]
0	4.16	4.72
1	9.73	12.60
3	12.96	12.34
5	14.95	14.43
7	11.26	11.81
9	13.46	15.22
11	15.00	16.27
13	13.45	13.38
15	13.02	14.43

[1] The values for trimethylamine-nitrogen are expressed in mg. per 100 g. of oyster meat or 100 ml. of oyster liquor. The values given are the averages of 2 replications which were significantly different from one another ($P < 0.01$).

[2] The increase in the trimethylamine-nitrogen content of oyster meat and liquor during the storage period was significant ($P < 0.01$).

Figure 6. Changes in the concentration of trimethylamine-nitrogen in oysters during iced-storage.

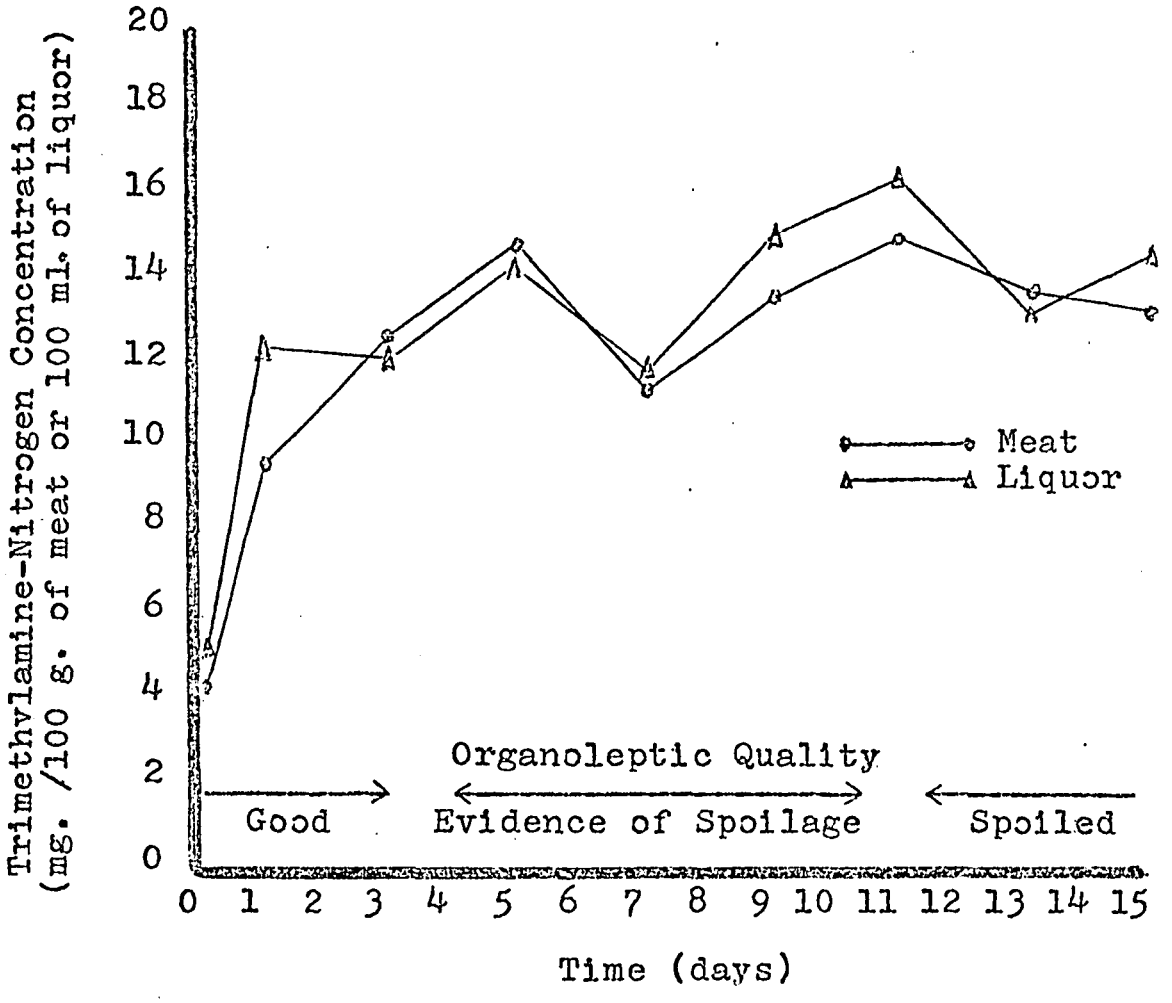


TABLE 5

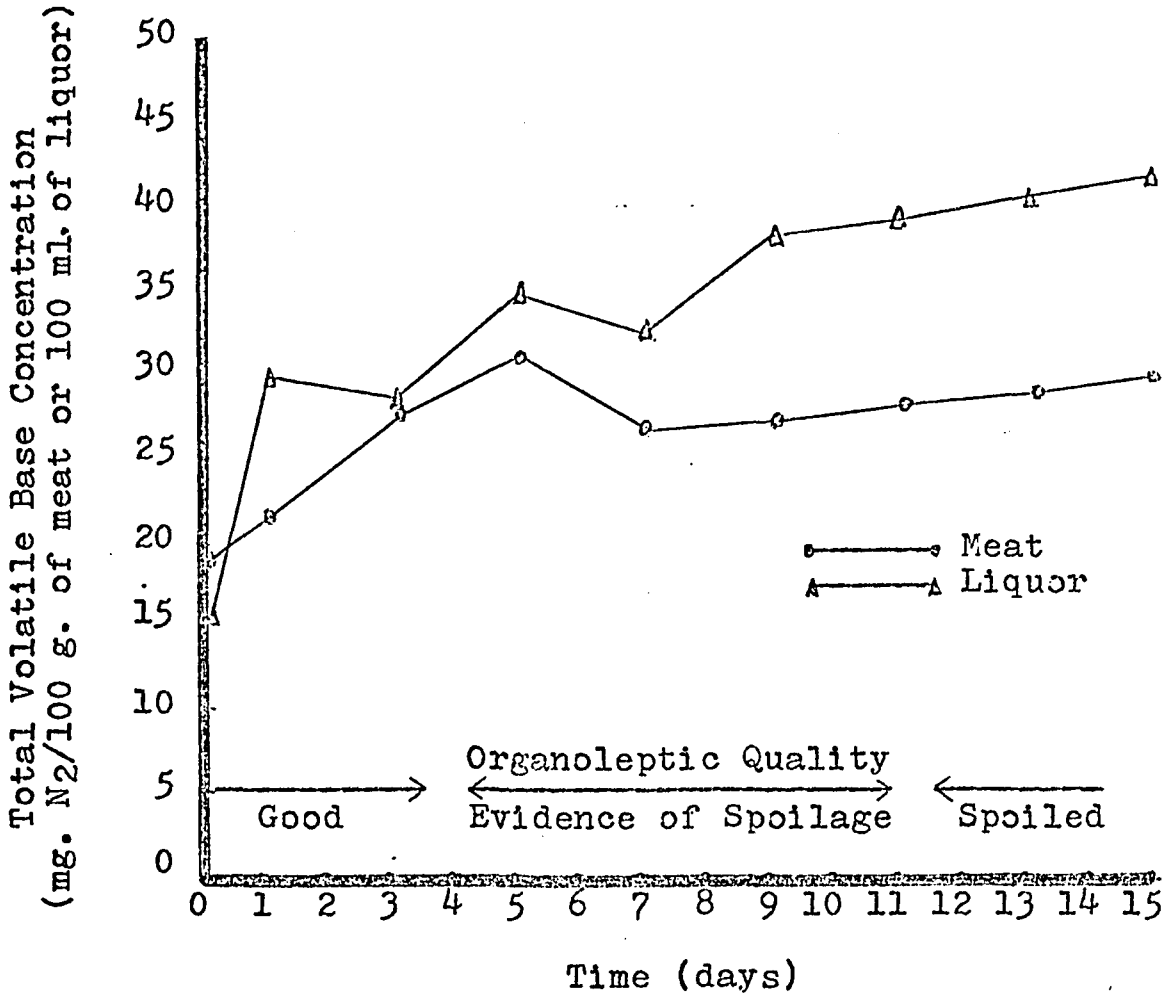
The effect of iced-storage on the total volatile base content of oysters.[1]

Storage time (days)	Oyster Meat[2]	Oyster Liquor[2]
0	19.68	15.23
1	22.64	30.19
3	28.33	28.61
5	32.05	35.18
7	27.16	33.08
9	27.82	38.85
11	28.11	39.90
13	28.83	40.95
15	30.05	42.00

1 The values for total volatile bases are expressed in mg. per 100 g. of oyster meat or 100 ml. of oyster liquor. The values are the averages of 2 replications which were significantly different from one another ($P < 0.01$)

2 The increase in the total volatile base content of oyster meat and liquor during the storage period was highly significant ($P < 0.01$).

Figure 7. Changes in the concentration of total volatile bases in oysters during iced-storage.



9, with a slight increase after that time. This pattern was not found to contain any significant trends, and is in agreement with the work of Duggan (1948) and Lartigue et al. (1960). The uselessness of indole as a quality index of oysters is further increased by the fact that significant differences occurred between gallons (Table S).

The decrease in the log of mesophilic plate counts of both oyster meat and liquor during the 15 days of iced-storage was found to be highly significant (Table 7). These decreases closely parallel one another for the first 11 days of storage. After the 11th day the mesophilic plate counts of oyster liquor increased slightly, while those for the oyster meat decreased more rapidly (Figure 9). The general decrease in mesophilic plate counts is in direct opposition to the results reported by Rosen (1966) but is in apparent agreement with the work of Colwell and Liston (1960).

As reported in Table T, there was a significant difference between counts obtained with oyster meat and liquor, and highly significant differences between gallons within each replicate. There were no significant differences between replicates.

In contrast to the decreasing trend found with mesophilic counts, it is interesting to note that the psychrophilic plate counts of oysters increased steadily during the 15 days of iced-storage (Table 8). This increase was found to be highly significant, as were psychrophilic plate

TABLE 6

The effect of iced-storage on the indole content of oysters. [1]

Storage time (days)	Oyster Meat
0	6.32
1	5.73
3	5.42
5	5.70
7	5.25
9	5.07
11	6.05
13	5.60
15	6.62

[1] The values for indole are expressed in micrograms per 100 g. of oyster meat.

Figure 8. Changes in the concentration of indole in oysters during iced-storage.

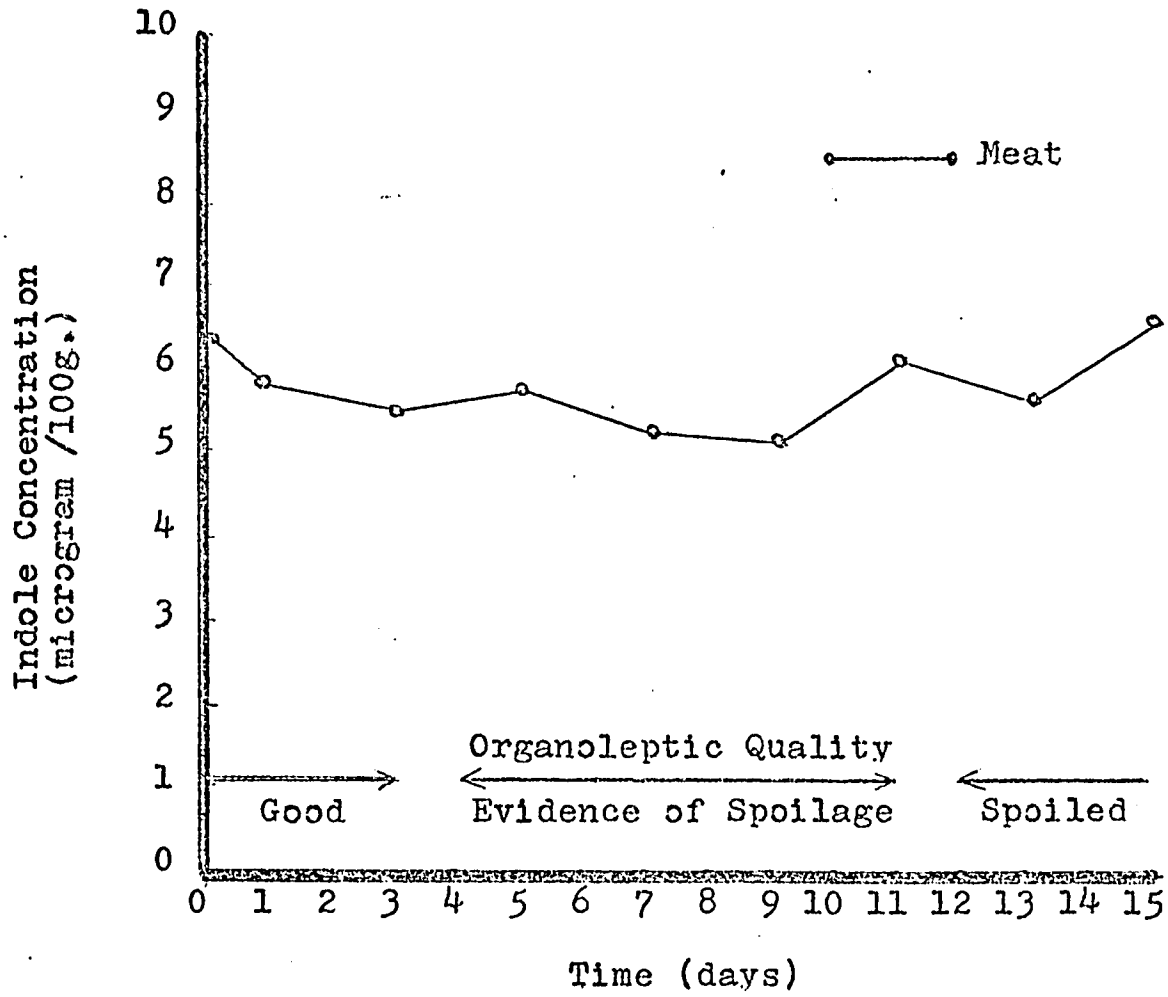


TABLE 7

The effect of iced-storage on the mesophilic plate counts of oysters. [1]

Storage time (days)	Oyster Meat [2]	Oyster Liquor [2]
0	4.3942 (2.48×10^4)	4.4215 (2.64×10^4)
1	4.3902 (2.46×10^4)	4.3063 (2.02×10^4)
3	4.3325 (2.15×10^4)	4.1936 (1.56×10^4)
5	4.3013 (2.00×10^4)	4.2279 (1.69×10^4)
7	4.0929 (1.24×10^4)	4.0857 (1.22×10^4)
9	4.0408 (1.10×10^4)	4.1794 (1.51×10^4)
11	4.0329 (1.08×10^4)	4.0842 (1.21×10^4)
13	3.5389 (3.46×10^3)	4.2294 (1.70×10^4)
15	3.7473 (5.59×10^3)	4.3788 (2.39×10^4)

1 Mesophilic plate counts are expressed as the log of the number of organisms per 100 g. of oyster meat or 100 ml. of oyster liquor. The actual number is given in parentheses. The values given are the averages of 2 replicates which were not significantly different from one another.

2 The decrease in the mesophilic plate counts of oyster meat and liquor during the storage period was significant ($P < 0.01$).

Figure 9. Changes in the mesophilic plate counts of oysters during iced-storage.

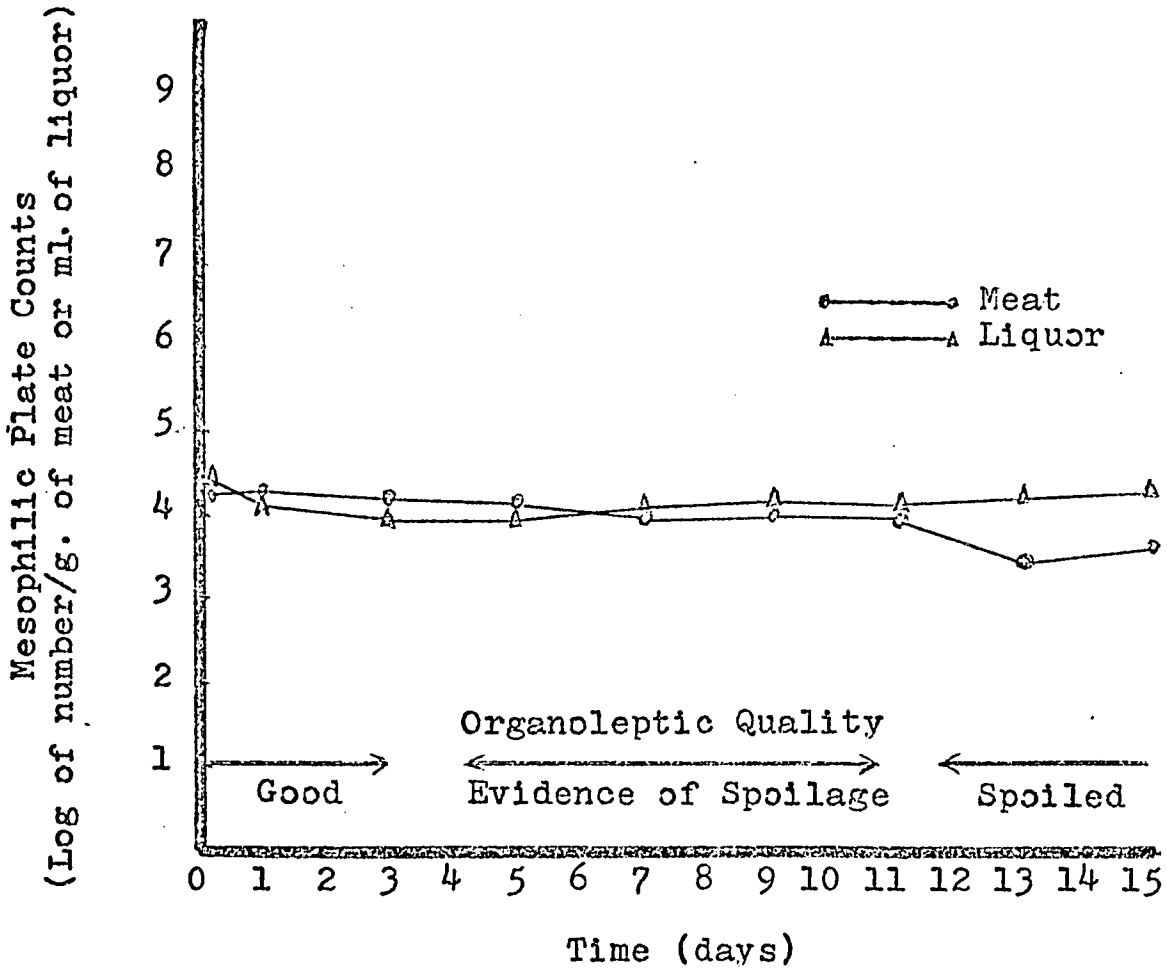


TABLE 8

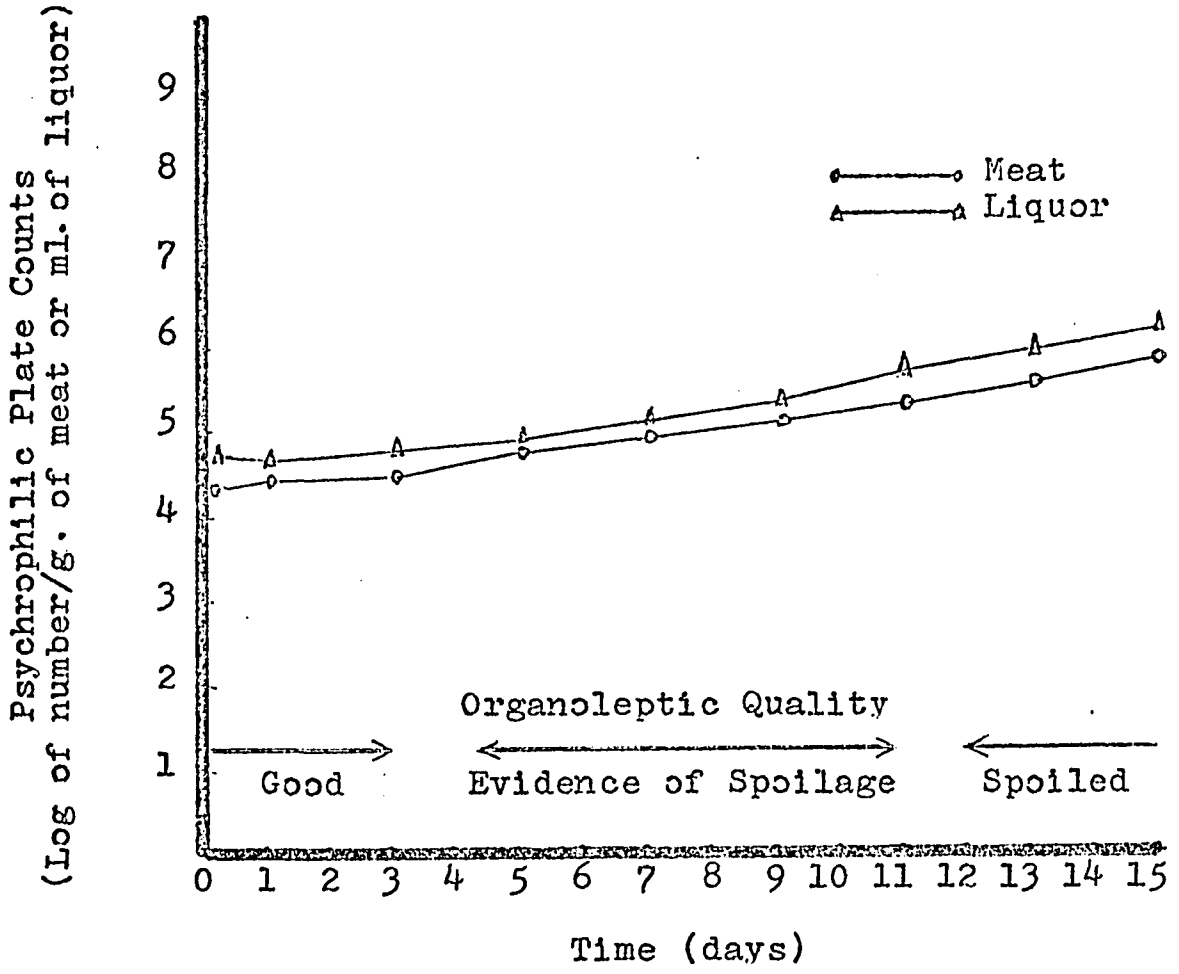
The effect of iced-storage on the psychrophilic plate counts of oysters. [1]

Storage time (days)	Oyster Meat[2]	Oyster Liquor[2]
0	4.3699 (2.34×10^4)	4.6347 (4.31×10^4)
1	4.4774 (3.00×10^4)	4.6074 (4.05×10^4)
3	4.4983 (3.15×10^4)	4.7359 (5.44×10^4)
5	4.7790 (6.01×10^4)	4.8118 (6.48×10^4)
7	4.8942 (7.84×10^4)	5.0608 (1.15×10^5)
9	5.0523 (1.13×10^5)	5.3860 (2.43×10^5)
11	5.3785 (2.39×10^5)	5.7744 (5.95×10^5)
13	5.5574 (3.61×10^5)	5.9965 (9.92×10^5)
15	5.8841 (7.66×10^5)	6.2452 (1.76×10^6)

1 Psychrophilic plate counts are expressed as the log of the number of organisms per 100 g. of oyster meat or 100 ml. of oyster liquor. The actual number is given in parentheses. The values given are the averages of 2 replicates which were not significantly different from one another.

2 The increase in the psychrophilic plate counts of oyster meat and liquor during the storage period was significant ($P < 0.01$).

Figure 10. Changes in the psychrophilic plate counts of oysters during iced-storage.



counts between meat and liquor and between gallons within each replicate (Table U). As with mesophilic plate counts, there were no significant differences between replicates.

The difficulty in obtaining reproducible counts for either mesophiles or psychrophiles poses a definite disadvantage for their use as an index of quality. In addition, the times required for incubation seriously limit the use of microbiological counts as indices of oyster quality.

The decrease in each of the organoleptic characteristics of odor, appearance, flavor, and texture during the storage period was found to be highly significant (Table 9). The cumulative quality obtained by grouping all of these characteristics was also found to be highly significant. As shown in Figure 11, the same trend was evident for all of the various characteristics, with flavor grading lower than any other characteristic throughout the storage period.

There were highly significant differences for appearance and texture, and significant differences for cumulative quality between the 2 replicates (Table V). However, it is worthwhile to note that there were no significant differences for odor or flavor between the replicates. This is important because odor and flavor are the most valuable organoleptic characteristics for evaluating the results of various chemical tests. There is a strong possibility that some of the compounds measured might be contributors to odor or flavor.

Although the data for class designations was not

TABLE 9

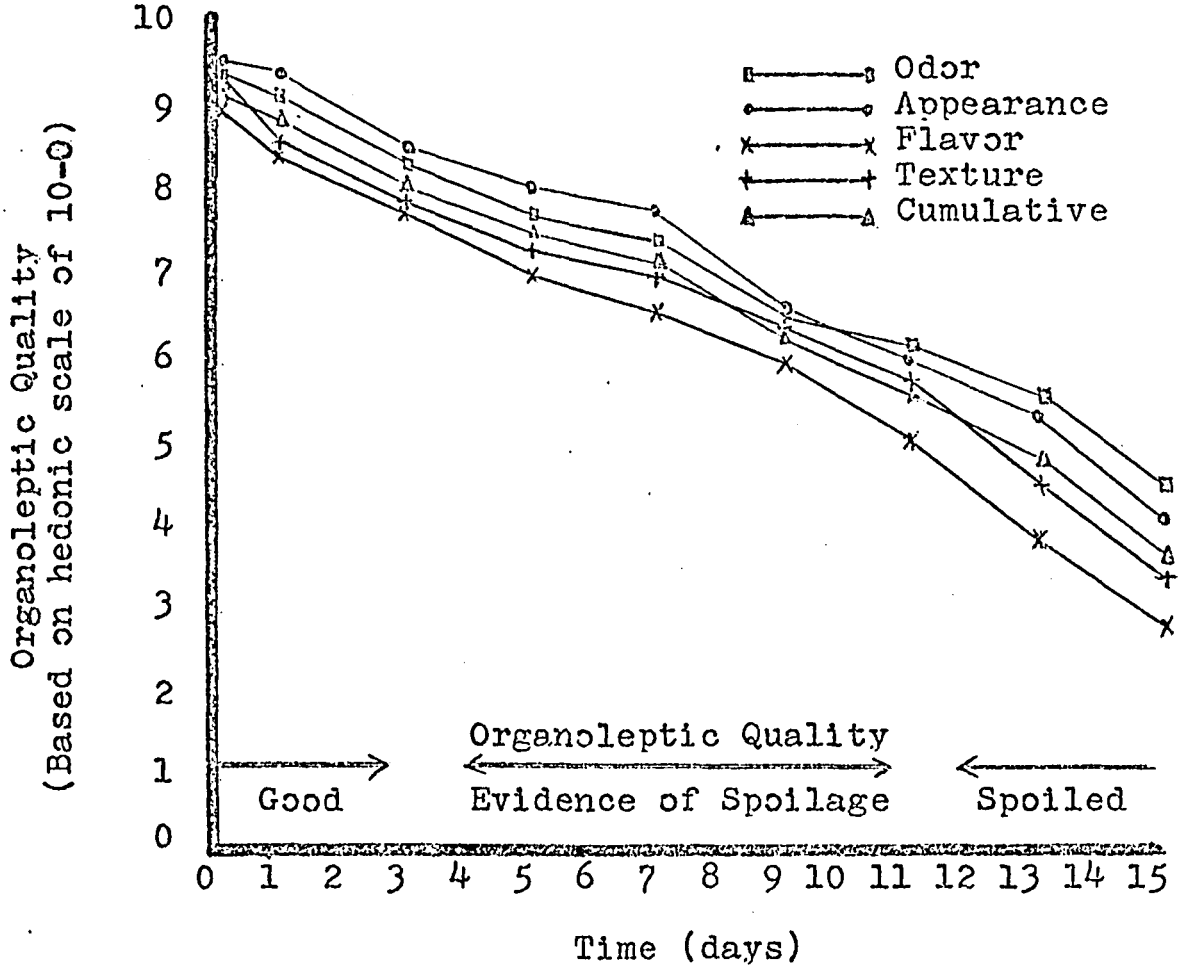
The effect of iced-storage on the organoleptic quality of oysters. [1]

Storage time (days)	Odor[2]	Appearance[2]	Flavor[2]	Texture[2]	Cumulative[2]
0	9.28	9.43	8.77	9.23	9.17
1	8.92	9.07	8.16	8.25	8.59
3	8.08	8.15	7.56	7.57	7.84
5	7.51	7.77	6.74	7.07	7.27
7	7.14	7.53	6.49	6.74	6.97
9	6.23	6.37	5.76	6.26	6.14
11	5.98	5.93	4.84	5.61	5.59
13	5.38	5.06	3.61	4.44	4.62
15	5.36	3.94	2.54	3.07	3.48

1 The values given for organoleptic quality are expressed on a hedonic scale of 10 to 1. The values given are the averages of 2 replicates which contained significant differences for appearance and texture ($P < 0.01$) and cumulative quality ($P < 0.05$).

2 The decrease in the organoleptic quality during the storage period was significant ($P < 0.01$).

Figure 11. Changes in the organoleptic quality of oysters during iced-storage.



subjected to statistical anaylsis, it allows one to estimate the time required for the product to change from "good" to "beginning to spoil" to "spoiled". The main reason for the use of the class designation system was therefore as a visual aid in graphing data from other tests. As seen in Figure 11, the oysters were classed as "good" from day 0 to day 3. From day 4 to day 11, the oysters showed signs of spoilage, but were still organoleptically acceptable (Class II). All panelists agreed that the oysters were organoleptically "spoiled" (Class III) after the 12th day of iced-storage.

The primary objective of this research was to show which tests correlated well with the organoleptic evaluations of ice-stored oysters. Correlation of total reducing substances with other chemical and microbiological tests was also of primary importance so that an evaluation could be made of TRS as an index of oyster quality.

Only those tests which demonstrated significant ($P < 0.05$) or highly significant ($P < 0.01$) correlation coefficients with organoleptic quality hold promise as indices of oyster quality. However, it must be remembered that a high correlation does not necessarily mean that a particular test will be feasible, since fluctuations in a general trend may severely limit the use of a test as a quality index.

Correlations of organoleptic evaluations with other

test results are reported in Table 10. Because of the extremely high correlations between cumulative organoleptic quality and the individual characteristics of odor, appearance, flavor, and texture, cumulative quality was used for the correlations with chemical and microbiological tests. Due to the lack of sufficient data, correlation coefficients were not determined for indole.

Highly significant, positive correlations were obtained between cumulative organoleptic quality versus pH (meat and liquor) and mesophilic plate counts (meat). Highly significant, negative correlations existed between cumulative quality versus total reducing substances (meat and liquor). Significant, negative correlations were obtained between cumulative organoleptic scores and psychrophilic plate counts (meat and liquor). It is important to note that the highest correlations obtained with organoleptic quality were with pH (meat and liquor) and total reducing substances (meat and liquor). This fact illustrates the potential use of pH and total reducing substances as quality indicators of oysters.

Correlations between total reducing substances and the other chemical and microbiological tests can be found in Table 11. There was a highly significant, positive correlation between TRS values for meat and for liquor. There were highly significant, positive correlations between the TRS content of oyster meat versus psychrophilic plate counts

(meat and liquor) and the total volatile base content of oyster liquor. Highly significant, negative correlations existed between TRS (meat) versus pH (meat and liquor), and significant, negative correlations were found between TRS (meat) versus mesophilic plate counts of oyster meat.

The total reducing substance content of oyster liquor gave a highly significant, positive correlation with pH (meat and liquor), and a significant, negative correlation with the mesophilic plate counts of oyster meat.. In addition, psychrophilic plate counts (meat and liquor) and total volatile base concentrations of oyster liquor showed significant, positive correlations with the TRS content of oyster liquor.

The results of this investigation suggest that the total reducing substance content of either oyster meat or oyster liquor could be used as an index of oyster quality. The pH of oyster meat and liquor also has merit as an index of oyster quality. Both the TRS test and pH compare favorably in several aspects: (1) they are both relatively rapid and simple to determine, (2) they are both reproducible, with TRS being more so than pH, and (3) no false positives were obtained with either test.

Although there was good correlation between organoleptic scores versus mesophilic plate counts (meat) and psychrophilic plate counts (meat and liquor), one must be wary of using plate counts as an index of oyster quality. The

TABLE 10

Correlation coefficients (r) of organoleptic quality versus chemical and microbiological data on ice-stored oysters.

Cumulative Organoleptic Score vs	r [1]
Odor	+0.991**
Appearance	+0.993**
Flavor	+0.993**
Texture	+0.993**
Total reducing substances (meat)	-0.846**
Total reducing substances (liquor)	-0.835**
pH (meat)	+0.907**
pH (liquor)	+0.880**
Trimethylamine-nitrogen (meat)	-0.101
Trimethylamine-nitrogen (liquor)	-0.106
Total volatile bases (meat)	-0.067
Total volatile bases (liquor)	-0.395
Mesophilic plate counts (meat)	+0.779**
Mesophilic plate counts (liquor)	+0.133
Psychrophilic plate counts (meat)	-0.547*
Psychrophilic plate counts (liquor)	-0.521*

[1] For 18 and 18 degrees of freedom, the following correlation coefficients are significant: 5 percent level (± 0.468); 1 percent level (± 0.590).

**Significant at the 0.01 level of probability.
*Significant at the 0.05 level of probability.

TABLE 11

Correlation coefficients (r) of total reducing substances versus chemical and microbiological data on ice-stored oysters.

Total Reducing Substances (Meat) vs	r [1]
Total reducing substances (liquor)	+0.844**
pH (meat)	-0.942**
pH (liquor)	-0.937**
Trimethylamine-nitrogen (meat)	+0.414
Trimethylamine-nitrogen (liquor)	+0.419
Total volatile bases (meat)	+0.356
Total volatile bases (liquor)	+0.635**
Mesophilic plate counts (meat)	-0.545*
Mesophilic plate counts (liquor)	+0.056
Psychrophilic plate counts (meat)	+0.787**
Psychrophilic plate counts (liquor)	+0.751**

Total Reducing Substances (Liquor) vs	r [1]
pH (meat)	-0.876**
pH (liquor)	-0.927**
Trimethylamine-nitrogen (meat)	+0.308
Trimethylamine-nitrogen (liquor)	+0.287
Total volatile bases (meat)	+0.220
Total volatile bases (liquor)	+0.491*
Mesophilic plate counts (meat)	-0.526*
Mesophilic plate counts (liquor)	-0.089
Psychrophilic plate counts (meat)	+0.545*
Psychrophilic plate counts (liquor)	+0.529*

1. For 18 and 18 degrees of freedom, the following correlation coefficients are significant : 5 percent level (± 0.468); 1 percent level (± 0.590).

**Significant at the 0.01 level of probability.

*Significant at the 0.05 level of probability.

individual and seasonal variations are thought to contribute a large degree of error to plate counts, which may at times have no relationship with quality.

Trimethylamine-nitrogen and total volatile bases showed little merit as indices of oyster quality. Undoubtedly the organisms present in the product were not able to elaborate these compounds in large enough quantities under the environmental conditions. Although correlations were not made for indole, the lack of any definite trend in the indole content of oysters severely limits its use as an indicator of quality.

Organoleptic evaluations have several disadvantages, not the least of which is personal preferential judgements. The panelists also knew the approximate age of the samples, which may have further biased their evaluations. However, the purpose of the organoleptic evaluations was to gather information which could be used in determining correlations so that each objective test could be compared to subjective evaluations of oyster quality.

By virtue of the results of statistical analyses 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. It is concluded that of all the methods which have been tested as indices of oyster quality, the TRS method most nearly

approaches the criteria which a chemical indicator for food quality should possess.

SUMMARY

A new test has been developed for determining the quality of oysters. This test measures the group of compounds designated as total reducing substances (TRS) and utilizes the determination of both volatile and non-volatile substances which are capable of reducing an alkaline solution of potassium permanganate. The applicability of the TRS test has been demonstrated by comparison with other chemical tests which have been used for estimating oyster quality. Microbiological studies and organoleptic evaluations were performed in conjunction with the chemical tests during a 15 day iced-storage period.

Determinations were made on oyster meat and, where applicable, on oyster liquor for total reducing substances, pH, trimethylamine-nitrogen, total volatile bases, indole, mesophilic counts, psychrophilic counts, and the organoleptic characteristics of odor, appearance, flavor, and texture. In addition, the oysters were evaluated for organoleptic class. Oysters being of good quality were rated in Class I; those which exhibited evidence of spoilage were placed in Class II; and spoiled oysters were rated Class III. The oysters were placed in Class I from day 0 to day 3, Class II from day 4 to day 11, and Class III after day 12.

Statistical analyses were performed on all data to evaluate storage changes and to determine the reproducibility of each test procedure. Correlation coefficients were then determined between the various tests.

The tests for trimethylamine-nitrogen, total volatile bases, and indole were not found to offer reliable information concerning oyster quality. Since these substances showed no definite patterns during storage, they are not recommended for the assessment of quality.

Psychrophilic counts were found to increase uniformly during the storage period, whereas mesophilic counts decreased. However, the use of microbiological counts are not recommended as indices of quality due to the lack of adequate reproducibility and the time required for incubation.

The measurement of the pH decrease in oysters is apparently a better test for quality then are trimethylamine-nitrogen, total volatile bases, indole, or microbiological counts. The results returned for pH were fairly reproducible and gave high correlations with organoleptic evaluations.

The concentration of total reducing substances was found to increase steadily throughout the storage period. Good reproducibility and high correlations with organoleptic quality predicate the use of total reducing substances as an index of oyster quality.

By virtue of the statistical analyses and correlations of the chemical and microbiological tests with organoleptic quality, it is concluded that total reducing substance and pH measurement provide the most reliable means for ascertaining oyster quality.

LITERATURE CITED

Abbey, A., Kohler, R.A., and Upham, S.D. 1957. Effect of aureomycin chlortetracycline in the processing and storage of freshly shucked oysters. Food Technol. 11, 265-271.

Adams, R., Farber, L., and Lerke, P. 1964. Bacteriology of spoilage of fish muscle. II. Incidence of spoilers during spoilage. Appl. Microbiol. 12, 277-279.

Alford, J.A., Tobin, L., and McCleskey, C.S. 1942. Bacterial spoilage of iced fresh crabmeat. Food Res. 7, 353-359.

Anderson, D.W., Jr., and Fellers, C.F. 1952. The occurrence of trimethylamine and trimethylamine oxide in fresh water fishes. Food Res. 17, 472-474.

AOAC. 1960. "Official Methods of Analysis." 9 th ed. Assoc. Offic. Agr. Chemists, Washington, D.C.

Bailey, M.E., Fieger, E.A., and Novak, A.F. 1956. Objective tests applicable to quality studies of ice stored shrimp. Food Res. 21, 611-619.

Baird, E.A. and Wood, A.J. 1944. Reduction of trimethylamine oxide by bacteria. 2. The Micrococcaceae. J. Fish. Res. Bd. Can. 6, 243-244.

Bandemer, S.L. and Schaible, P.J. 1936. Microdetermination of ammonia nitrogen in eggs. Ind. Eng. Chemistry. 8,201.

Barry, H.C., Weeks, J.F., Jr., and Duggan, R.E. 1956. Effect of storage on decomposed canned shrimp. J. Assoc. Offic. Agr. Chemists. 39, 801-805.

Beacham, L.M. 1946. A study of decomposition in canned oysters and clams. J. Assoc. Offic. Agr. Chemists. 29, 89-99.

Beatty, S.A. 1938. Studies of fish spoilage. II. The origin of trimethylamine produced during the spoilage of cod muscle press juice. J. Fish. Res. Bd. Can. 4, 63-68.

Beatty, S.A. and Gibbons, N.E. 1937. The measurement of spoilage in fish. J. Fish. Res. Bd. Can. 3, 77-91.

Bethea, S. and Hillig, F. 1965. Decomposition—determination of trimethylamine-nitrogen in extracts and in volatile fractions of fish. J. Assoc. Offic. Agr. Chemists. 48, 731-735.

Blanchard, L., Pantaleon, J., and Prudhomme, M. 1951. The use of direct microscopical counts as an index of potential keeping quality. Bull. Acad. Vet. Fr. 24, 87.

Bramstedt, F. 1957. Geschmacks-und geruchsstoffe in fischfleisch. Arch. Fischerelwissenshaft 8, 94-103. Van Klaveren, F.W. translater, 1959. Substances responsible for taste and smell in fish. J. Fish. Res. Bd. Can., Translation Series, No. 235.

Breed, R.S., Murray, E.G.D., and Smith, N.R. 1957. "Bergey's Manual of Determinative Bacteriology." The Williams & Wilkins Co., Baltimore, Md.

Budd, J.A. and Spencer, C.P. 1968. The utilisation of alkylated amines by marine bacteria. Marine Biology 2, 92-101.

Burnett, J.L. 1965. Ammonia as an index of decomposition in crabmeat. J. Assoc. Offic. Agr. Chemists. 48, 624-627.

Cairncross, S.E. and Sjostrom, L.B. 1950. Flavor profiles-a new approach to flavor problems. Food Technol. 4, 308-311.

Cambell, C. 1962. Decomposition in shellfish. J. Assoc. Offic. Agr. Chemists. 45, 731-732.

Cambell, L.L., Jr., and Williams, O.B. 1952. The bacteriology of Gulf Coast shrimp. IV. Bacteriological, chemical and organoleptic changes with ice storage. Food Technol. 6, 125-126.

Castell, C.H. and Greenough, M.F. 1958. Grading fish for quality. 3. Grading of recently cut fillets. J. Fish. Res. Bd. Can. 15, 729-748.

Castell, C.H. and Mapplebeck, E.G. 1952. The importance of Flavobacterium in fish spoilage. J. Fish. Res. Bd. Can. 9, 148-156.

Castell, C.H. and Snow, J.M. 1951. Reduction of trimethylamine oxide by bacterial enzymes. J. Fish. Res. Bd. Can. 8, 195-206.

Castell, C.H., Elson, M.F., and Giles, J.G. 1961. Grading fish for quality. 4. Variations in the relation between TMA values and grades for gutted, trawler-caught Atlantic cod and haddock. J. Fish. Res. Bd. Can. 18, 303-310.

Caul, J.F. 1957. The profile method of flavor analysis. in Advances in Food Research. Academic Press N.Y. 1-40.

Clarke, J.W., Cannon, J.H., Coulter, E.W., Goodman, M.S., Greene, W.S., Milstead, K.L., Vandaveer, R.L., and Wildman, J.D. 1937. Determination of decomposition products in butter and cream. J. Assoc. Offic. Agr. Chemists. 20, 475-505.

Clough, R.W. 1922. A biochemical study of Pacific Coast salmon with particular reference to the formation of indole and skatole during decomposition. Univ. Wash. Publ. Puget Sound Biol. Sta. 3, 195-272.

Cohen, E.H. and Peters, J.A. 1962. Storage of fish in sea water. I. Quality changes in ocean perch as determined by organoleptic and chemical analyses. Fish. Indust. Res. 2, 41-43.

Colwell, R.R. and Liston, J. 1960. Microbiology of shellfish-bacteriological study of the natural flora of Pacific oysters (Crassostrea gigas). Appl. Microbiol. 8, 104-109.

Conway, E.J. 1957. "Microdiffusion Analysis and Volumetric Error." Crosby Lockwood & Sons, Ltd., London.

Crookes, G.C. and Ritchie, W.S. 1938. A study of rate of decomposition of haddock muscle at various temperatures as indicated by ammonia content. Food Res. 3, 589-597.

Digirolamo, R., Liston, J., and Matches, J. 1970. The effects of freezing on the survival of Salmonella and E. coli in Pacific oysters. J. Food Sci. 35, 13-16.

Duggan, R.E. 1948. Report on decomposition in shellfish-indole in shrimp, oysters, and crabmeat. J. Assoc. Offic. Agr. Chemists. 31, 507-510.

Duggan, R.E. and Strasburger, L.W. 1946. Indole in shrimp. J. Assoc. Offic. Agr. Chemists. 29, 177-188.

Dyer, W.J. 1952. Amines in fish muscle. VI. Trimethylamine oxide content of fish and marine invertebrates. J. Fish. Res. Bd. Can. 8, 314-324.

Eliot, C. 1926. Bacterial flora of the market oyster. Am. J. Hyg. 6, 755-776.

Elliot, R.P. 1952. Reduction of trimethylamine oxide in dogfish flesh. Food Res. 17, 225-234.

Farber, L. 1952. A comparison of various methods for the determination of spoilage in fish. Food Technol. 6, 319-324.

Farber, L. 1963. Quality evaluation studies of fish and shellfish from certain northern European waters. Food Technol. 17, 476-480.

Farber, L. and Ferro, M. 1956. Volatile reducing substances (VRS) and volatile nitrogen compounds in relation to spoilage in canned fish. Food Technol. 10, 303-304.

Farber, L. and Lerke, P.A. 1958. A review of the value of volatile reducing substances for the chemical assessment of the freshness of fish and fish products. Food Technol. 12, 677-680.

Farber, L. and Lerke, P.A. 1961. Studies on the evaluation of freshness and on the estimation of the storage life of raw fishery products. Food Technol. 15, 191-196.

Fieger, E.A. and Friloux, J.J. 1954. A comparison of objective tests for quality of Gulf shrimp. Food Technol. 8, 35-37.

Fields, M.L. 1964. Acetylmethylcarbinol and diacetyl as chemical indicators of microbial quality of apple juice. Food Technol. 18, 114-118.

Fields, M.L., Richmond, B.S., and Baldwin, R.E. 1968. Food quality as determined by metabolic by-products of microorganisms. in Advances in Food Research, Academic Press N.Y. 161-229.

Frazier, W.C. 1958. "Food Microbiology." McGraw-Hill, New York.

Gagnon, M. and Fellers, C.R. 1958. Biochemical methods for determining shrimp quality. I. Study of analytical metnods. Food Technol. 7, 340-343.

Georgala, D.L. 1958. The bacterial flora of the skin of North Sea cod. J. Gen Microbiol. 18, 84-91.

Hansen, W.P. 1960. New testing technique tells what consumers think of products. Biscuit and Cracker Baker. 49, 23-25.

Hillig, F. 1956. Volatile acids, succinic acid, and histamines as indices of decomposition in tuna. J. Assoc. Offic. Agr. Chemists. 39, 773-800.

Hillig, F. 1963. Report on decomposition in foods (chemical indexes). J. Assoc. Offic. Agr. Chemists. 46, 22-24.

Hillig, F., Shelton, L.R., Jr., and Loughrey, J.H. 1959. Chemical indices of decomposition in haddock. J. Assoc. Offic. Agr. Chemists. 42, 702-708.

Hillig, F., Shelton, L.R., Jr., Loughrey, J.H., and Bethea, S. 1961. Chemical indexes of decomposition in pollock and whiting. J. Assoc. Offic. Agr. Chemists. 44, 499-507.

Hillig, F., Shelton, L.R., Jr., Loughrey, J.H., and Eisner, J. 1958. Chemical indicies of decomposition in cod. J. Assoc. Offic. Agr. Chemists. 41, 763-776.

Hillig, F., Shelton, L.R., Jr., Loughrey, J.H., and Fitzgerald, B.F. 1960. Chemical indices of decomposition in ocean perch. J. Assoc. Offic. Agr. Chemists. 43, 433-438.

Hoff, J.C., Beck, W.J., Ericksen, T.H., Vasconcelos, G.J., and Presnell, M.W. 1967. Time-temperature effects on the bacteriological quality of stored shellfish. 1. Bacteriological changes in live shellfish: Pacific Oysters (Crassostrea gigas), Olympia oysters (Ostrea lurida), native littleneck clams (Protothaca staminea), and Manila clams (Venerupis japonica). J. Food Sci. 32, 121-124.

Hopkins, F.G. and Cole, S.W. 1903. A contribution to the chemistry of proteids. II. The constitution of tryptophan, and the action of bacteria upon it. J. Physiol. 29, 451-466.

Hughes, R.B. 1959. Chemical studies on the herring (Clupea harengus), I. Trimethylamic oxide and volatile amines in fresh, spoiling, and cooked herring flesh. J. Sci. Food Agr. 10, 431-436.

Hunter, A,C. and Linden, B.A. 1925. Microorganisms in decomposed oysters. J. Agr. Research. 30, 971-976.

Jay, J.M. 1970. "Modern Food Microbiology." Van Nostrand Reinhold Co., New York.

Jones, N.R. 1965. Hypoxanthine and other purine-containing fractions in fish muscle as indices of freshness, in "The Technology of Fish Utilization," ed. by R. Kreuzer, Fishing News, Ltd., London. 179-183.

King, W.H. Flynn, F.F., and Gowanlock, J.N. 1945. Experimental studies on decomposition of oysters used for canning. J. Assoc. Offic. Agr. Chemists. 28, 385-398.

Lartigue, D., Novak, A.F., and Fieger, E.A. 1960. An evaluation of the indole and trimethylamine tests for oyster quality. Food Technol. 14, 109-112.

Lerke, P. and Farber, L. 1969. Direct bacterial count as a rapid freshness test for fish fillets. Appl. Microbiol. 17, 197-201.

Liebman, H.L,, Kuda, G., Chapel, J.G., and Stein, J.A. 1957. Effects of temperature upon the storage life of fresh shucked Pacific oysters (Ostrea gigas). Comm. Fish. Rev. 19, 1-3.

Liston, J. 1957. The occurrence and distribution of bacterial types on flatfish. J. Gen. Microbiol. 16, 205-216.

Moorjani, M.N., Iyengar, J.R., Visweswariah, K., Bhatia, D.S., and Subrahmanyan, V. 1958. Changes in the total volatile base, volatile reducing substances and bacterial count as indices of fresh water fish spoilage. Food Technol. 12, 385-386.

Mossel, D.A.A. and Ingram, M. 1955. The physiology of the microbial spoilage of foods. J. Appl. Bact. 18, 232-268.

Neilands, J.B. 1945. Factors affecting triamineoxidease I. Inhibition of the enzyme. J. Fish. Res. Bd. Can. 6, 368-379.

Novak, A.F., Fieger, E.A., and Bailey, M.E, 1956. Rapid procedures for approximation of bacterial counts in shrimp and oysters. Food Technol. 10, 66-67.

Okuda, J., Uematsu, J., Sakata, J., and Fujikawa, J. 1919. Observations on the amino acids of shellfish proteins. J. Col. Agr. Imp. Univ. Tokyo. 7, 39.

Patterson, W.I. 1945. Report on decomposition in foods. J. Assoc. Offic. Agr. Chemists. 28, 233-239.

Peryam, D.R. and Pilgrim, F.J. 1957. Hedonic scale method of measuring food preference. Food Technol. 11, 9-14.

Piskur, F.T. 1947. Preliminary study of correlation of pH and quality of shucked Pacific oysters. Comm. Fish. Rev. 9, 22-24.

Pottinger, S.R. 1948. Some data on pH and freshness of shucked Eastern oysters. Comm. Fish. Rev. 10, 1-3.

Pottinger, S.R. 1951. Results of some tests with frozen oysters. Comm. Fish. Rev. 13, 8-10.

Rogers, R.E. and McCleskey, C.S. 1961. Objective tests for quality of ground beef. Food Technol. 15, 210-212.

Rosen, B. 1966. Accumulation of organic acids during cold storage of shucked soft clams, Mya arenaria (L.), in relation to quality. Fish. Ind. Res. 3, 5-11.

Sands, A.E., Grafius, M.A., Wainwright, H.W., and Wilson, M.W. 1949. The determination of low concentrations of hydrogen sulfide in gas by the methylene blue method. U.S. Dept. Bur. Mines, Report of Invest. 4547.

Shewan, J.M. 1938. The spoilage of haddock. Rept. Food Invest. Bd. Gt. Brit. 75-78.

Shewan, J.M., Macintosh, R.G., Tucker, C.G., and Ehrenberg, A.S.C. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J. Sci. Food Agr. 4, 283-298.

Snow, J.E. and Beard, P.J. 1939. Studies on bacterial flora of North Pacific salmon. Food Res. 4, 563-585.

Spinelli, J., Eklund, M., and Miyauchi, D. 1964. Measurement of hypoxanthine in fish as a method of assessing freshness. J. Food Sci. 29, 710-714.

Stansby, M.E. and Lemon, J.M. 1933. An electrometric method for detection of relative freshness of haddock. Ind. Eng. Chem. 5, 208-211.

Stansby, M.E., Harrison, R.W., Dossow, J., and Sater, M. 1944. Determining volatile bases in fish. Ind. Eng. Chem. Anal. Ed. 16, 593-596.

Suwa, A. 1909. Trimethylamine oxide in fish. Pfluger's Arch. Ges. Physiol. 128, 421-426.

Tanikawa, E. 1937. Bacteriological examination of oysters stored at low temperatures. Zentr. Bakteriol. Parasitenk. Abt. 2, 97, 133-147.

Tarr, H.L.A. 1940. Specificity of triamineoxidease. J. Fish. Res. Bd. Can. 5, 187-196.

Tarr, H.L.A. 1943. Rise and fall of bacterial populations in fish muscle. J. Fish. Res. Bd. Can. 6, 119-128.

Tomiyama, T., da Costa, A.A., and Stern, J.A. 1956. A rapid vacuum distillation procedure for the determination of volatile acids and volatile bases in fish flesh. Food Technol. 10, 614-617.

Westerdijk, J. 1949. The concept "association" in mycology. Leeuwenhoek ned. Tijdschr. 15, 187.

Williams, D.W. 1954. Report on chemical indices of decomposition in fish. J. Assoc. Offic. Agr. Chemists. 37, 567-572.

ZoBell, C.E. 1946. "Marine Microbiology." vol. 15. Chronica Botanican Co., Mass.

ZoBell, C.E. and Upham, H.C. 1944. A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanog. Univ. Calif. 5, 239-292.

APPENDIX

TABLE A

Total reducing substance concentration of oyster meat as affected by iced-storage of 2 replications of oysters. [1]

Storage time (days)	Replicates 1				Replicates 2			
	1 Gallon 1		1 Gallon 2		1 Gallon 1		1 Gallon 2	
	1	2	1	2	1	2	1	2
0	135.50	135.00	135.50	136.25	135.83	135.00	135.41	135.00
1	135.75	135.50	137.00	139.50	135.41	135.00	135.80	135.41
3	136.75	136.75	137.00	137.50	135.41	135.83	135.41	135.83
5	141.75	141.75	138.75	138.75	136.66	135.83	137.50	138.33
7	143.00	142.50	143.25	142.50	141.66	140.41	140.00	140.83
9	140.00	143.00	142.50	142.50	142.08	140.41	140.00	140.83
11	145.00	144.25	144.50	144.50	142.50	142.08	141.67	142.51
13	144.25	143.25	143.75	143.75	144.58	143.75	144.17	144.58
15	143.25	145.00	145.00	143.75	144.58	145.00	145.00	145.00

[1] Total reducing substance values are expressed as microequivalents of reduction per 0.2 ml. of oyster meat.

TABLE B

Total reducing substance concentration of oyster liquor as affected by iced-storage of 2 replications of oysters. [1]

Storage time (days)	Replicates							
	1				2			
	1 Gallon		2		1 Gallon		2	
	1	2	1	2	1	2	1	2
0	112.50	111.25	109.50	110.00	109.71	107.08	103.75	102.92
1	120.00	121.25	119.25	118.75	119.19	117.15	112.50	111.67
3	126.25	127.00	127.50	127.00	127.50	127.92	127.92	127.92
5	129.50	130.75	129.25	129.50	131.25	132.18	131.67	131.67
7	131.75	132.00	134.50	134.25	135.42	136.25	135.83	136.76
9	133.75	134.25	135.25	136.25	137.50	138.33	138.33	138.33
11	138.75	138.25	135.50	135.50	138.75	138.33	138.33	138.33
13	136.25	138.75	136.50	136.50	138.33	138.75	139.17	139.17
15	136.50	137.50	136.25	136.50	140.00	139.58	140.41	139.17

[1] Total reducing substance values are expressed as microequivalents of reduction per 0.2 ml. of oyster liquor.

TABLE C

The pH of oyster meat as affected by iced-storage of 2 replications of oysters.

Storage time (days)	Replicates 1				Replicates 2			
	1 Gallon 1		1 Gallon 2		1 Gallon 1		1 Gallon 2	
	1	2	1	2	1	2	1	2
0	6.73	6.70	6.70	6.73	6.65	6.70	6.67	6.71
1	6.66	6.71	6.55	6.50	6.53	6.55	6.57	6.53
3	6.54	6.50	6.52	6.50	6.50	6.48	6.44	6.47
5	6.18	6.26	6.34	6.37	6.38	6.38	6.38	6.37
7	6.14	6.22	6.23	6.15	6.33	6.31	6.24	6.28
9	6.15	6.11	6.21	6.18	6.28	6.23	6.27	6.24
11	5.93	5.92	6.16	6.14	6.22	6.19	6.19	6.17
13	5.84	5.96	6.12	6.10	6.16	6.14	6.08	6.07
15	5.85	5.83	5.90	5.92	6.07	6.07	6.06	6.03

TABLE D

The pH of oyster liquor as affected by iced-storage of 2 replications of oysters.

Storage time (days)	Replicates							
	1				2			
	1 Gallon		2		1 Gallon		2	
	1	2	1	2	1	2	1	2
0	7.00	6.95	6.95	6.95	6.97	6.95	7.03	7.02
1	6.57	6.53	6.63	6.65	6.85	6.87	6.94	6.92
3	6.54	6.50	6.56	6.50	6.73	6.68	6.66	6.66
5	6.43	6.41	6.46	6.45	6.57	6.58	6.50	6.52
7	6.34	6.34	6.38	6.38	6.47	6.45	6.45	6.42
9	6.20	6.22	6.24	6.20	6.34	6.36	6.33	6.33
11	6.15	6.16	6.22	6.21	6.27	6.25	6.29	6.26
13	6.12	6.12	6.12	6.12	6.15	6.17	6.14	6.17
15	6.13	6.12	6.00	5.96	6.08	6.09	6.08	6.06

TABLE E

Trimethylamine-nitrogen content of oyster meat as affected by iced-storage of 2 replications of oysters. [1]

Storage time (days)	Replicates 1				Replicates 2			
	1 Gallon 1		2		1 Gallon 1		2	
	1	2	1	2	1	2	1	2
0	4.27	7.78	2.03	5.80	2.54	2.30	4.86	3.74
1	10.61	9.89	21.00	22.88	3.18	2.86	3.59	3.89
3	24.39	22.34	17.17	18.82	6.42	4.27	4.88	5.45
5	28.00	27.02	28.00	22.74	3.33	3.92	3.29	3.36
7	23.03	18.53	18.98	17.03	3.37	4.99	1.35	2.82
9	19.05	20.42	14.85	17.39	8.84	10.17	10.12	6.86
11	19.65	24.56	23.84	21.00	7.80	8.39	7.26	7.53
13	25.20	22.40	13.66	16.15	7.91	7.63	7.48	7.20
15	18.98	21.88	14.43	16.67	7.04	7.95	9.18	8.05

[1] Trimethylamine-nitrogen content is expressed in mg. per 100 g. of oyster meat.

TABLE F

Trimethylamine-nitrogen content of oyster liquor as affected by iced-storage of 2 replications of oysters.[1]

Storage time (days)	Replicates							
	1				2			
	1 Gallon		2		1 Gallon		2	
	1	2	1	2	1	2	1	2
0	6.30	4.20	2.10	4.20	4.20	4.20	6.30	6.30
1	25.20	18.90	23.10	12.60	4.20	6.30	4.20	6.30
3	16.80	16.80	25.20	16.80	6.30	6.30	4.20	6.30
5	21.00	25.20	27.30	27.30	4.20	4.20	4.20	2.10
7	23.10	18.90	21.00	16.80	4.20	4.20	2.10	4.20
9	23.10	21.00	18.90	18.90	8.40	10.50	10.50	10.50
11	23.10	23.10	25.20	25.20	8.40	8.40	8.40	8.40
13	21.00	18.90	16.80	18 90	6.30	8.40	6.30	10.50
15	21.00	21.00	21.00	18.90	8.40	8.40	8.40	8.40

[1] Trimethylamine-nitrogen values are expressed in mg. per 100 ml. of oyster liquor.

TABLE G

Total volatile base concentrations of oyster meat as affected by iced-storage of 2 replications of oysters. [1]

Storage time (days)	Replicates 1				Replicates 2			
	1 Gallon 1		1 Gallon 2		1 Gallon 1		1 Gallon 2	
	1	2	1	2	1	2	1	2
0	17.07	17.51	16.23	23.20	25.36	28.54	14.58	14.97
1	25.45	24.73	37.33	38.88	12.73	12.86	16.15	12.96
3	48.73	42.81	38.14	35.75	17.13	12.78	16.28	14.98
5	50.40	46.67	50.40	42.96	16.67	17.65	14.82	16.76
7	48.35	41.17	33.22	34.05	15.14	14.96	14.86	15.52
9	38.09	37.92	25.45	21.74	21.21	20.35	30.34	27.45
11	36.84	44.21	34.67	35.00	18.71	18.47	18.88	18.06
13	42.00	42.00	29.02	40.38	17.80	19.07	20.59	19.79
15	37.96	38.88	38.48	41.66	19.35	19.89	22.05	22.13

[1] Total volatile base values are expressed in mg. of nitrogen per 100 g. of oyster meat.

TABLE H

Total volatile base concentrations of oyster liquor as affected by iced-storage of 2 replications of oysters. [1]

Storage time (days)	Replicates							
	1				2			
	1 Gallon		2		1 Gallon		2	
	1	2	1	2	1	2	1	2
0	8.40	12.60	8.40	8.40	18.90	21.00	21.00	23.10
1	39.90	44.10	37.80	42.00	16.80	18.90	21.00	21.00
3	35.70	29.40	42.00	44.10	14.70	18.90	23.10	21.00
5	50.40	52.50	48.30	48.30	21.00	18,90	21.00	21.00
7	42.00	46.20	42.00	46.20	21.00	21.00	23.10	23.10
9	42.00	46.20	44.10	44.10	31.50	35.70	33.60	33.60
11	50.40	58.80	48.30	48.30	29.40	31.50	25.20	27.30
13	54.60	58.80	44.10	50.40	31.50	29.40	27.30	31.50
15	58.80	56.70	48.30	46.20	31.50	33.60	29.40	31.50

[1] Total volatile base values are expressed in mg. of nitrogen per 100 ml. of oyster liquor.

TABLE I

Indole concentration of oyster meat as affected by iced-storage. [1]

Storage time (days)	1 Gallon		2	
	1	2	1	2
0	5.8	5.5	7.9	6.1
1	4.6	6.4	5.6	6.3
3	4.6	5.3	7.0	4.8
5	4.1	6.0	6.7	6.0
7	5.9	4.5	5.9	6.6
9	4.6	4.6	6.5	4.6
11	7.0	4.6	6.4	6.2
13	4.6	6.6	6.6	4.6
15	6.0	6.6	7.0	6.9

[1] Indole values are expressed in micrograms per 100 g. of oyster meat.

TABLE J

Mesophilic plate counts of oyster meat as affected by iced-storage of 2 replications of oysters.[1]

Storage time (days)	Replicates							
	1				2			
	1 Gallon		2		1 Gallon		2	
	1	2	1	2	1	2	1	2
0	4.3802	4.4216	5.2967	5.0792	4.0899	4.3304	3.7924	3.7634
1	4.4314	4.5353	5.1038	5.0828	4.1173	4.1584	3.7482	3.9445
3	4.1614	3.8603	5.1303	5.1673	4.4771	4.4281	3.8129	3.6232
5	3.9956	4.1523	5.1072	5.2480	4.3747	4.2175	3.6435	3.6721
7	3.0792	3.3711	5.0828	5.5224	4.2833	4.2405	3.6021	3.5441
9	3.2430	3.1614	5.2342	4.8692	4.2945	4.2695	3.6532	3.6021
11	3.5502	3.3858	4.7993	4.8357	4.2695	4.2480	3.6435	3.5315
13	2.8827	2.1303	4.3856	4.5798	3.9590	3.1173	3.5441	3.6721
15	3.3284	3.0043	4.3598	4.4031	3.8808	3.9956	3.5441	3.4624

[1] Mesophilic plate counts are expressed as the log of the number of organisms per g. of oyster meat.

TABLE K

Mesophilic plate counts of oyster liquor as affected by iced-storage of 2 replications of oysters.[1]

Storage time (days)	Replicates 1				Replicates 2			
	1 Gallon 1		2		1 Gallon 1		2	
	1	2	1	2	1	2	1	2
0	4.5198	4.6721	5.6721	5.7308	3.8261	318633	3.5315	3.5563
1	4.1987	4.1673	5.7160	5.6314	3.9638	3.9777	3.3979	3.3979
3	3.8513	3.7993	5.3694	5.4031	4.1004	4.0755	3.4314	3.5185
5	3.5441	3.6232	5.4314	5.4771	4.2504	4.2625	3.6128	3.6232
7	3.4249	3.4502	5.0934	5.1367	4.2227	4.1367	3.6902	3.5315
9	3.4014	3.4150	5.2253	5.1584	4.4771	4.4669	3.6812	3.6990
11	3.1584	2.9638	5.1271	5.2253	4.3617	4.3365	3.7160	3.7853
13	3.8573	3.7324	5.0453	5.1875	4.2553	4.2878	3.6532	3.8195
15	4.3820	4.4150	4.9138	4.9542	4.4914	4.5065	3.7243	3.6435

[1] Mesophilic plate counts are expressed as the log of the number of organisms per ml. of oyster liquor.

TABLE L

Psychrophilic plate counts of oyster meat as affected by iced-storage of 2 replications of oysters.[1]

Storage time (days)	Replicates 1				Replicates 2			
	1 Gallon 1		2		1 Gallon 1		2	
	1	2	1	2	1	2	1	2
0	4.3617	4.3858	5.4116	5.2227	4.1430	4.4249	3.5051	3.5051
1	4.4099	4.5185	5.3560	5.4487	4.1875	4.3075	3.7853	3.8062
3	4.3222	4.5705	5.7782	5.6180	4.2330	4.1492	3.5991	3.7242
5	5.0719	5.0064	6.1004	5.9004	4.3264	4.3201	3.7709	3.7324
7	5.0021	5.0021	6.1584	6.0828	4.7559	4.3858	3.9345	3.8325
9	5.1818	4.6232	6.3962	6.3054	4.5563	4.6532	4.4624	4.2405
11	5.0191	5.5527	6.0021	6.4232	5.1673	5.4082	4.6232	4.8325
13	5.0719	5.0645	6.2430	6.4624	5.6232	5.6721	5.1703	5.1520
15	5.5740	5.6232	6.2841	6.2011	6.0414	5.9294	5.6721	5.7482

[1] Psychrophilic plate counts are expressed as the log of the number of organisms per g. of oyster meat.

TABLE M

Psychrophilic plate counts of oyster liquor as affected by iced-storage of 2 replications of oysters.[1]

Storage time (days)	Replicates 1				Replicates 2			
	1 Gallon 1		2		1 Gallon 1		2	
	1	2	1	2	1	2	1	2
0	4.9031	4.8195	5.9823	5.9638	4.1761	4.0334	3.6812	3.5185
1	4.7993	4.8692	5.9823	5.9345	4.2355	4.1004	3.4473	3.4914
3	5.0792	5.1761	6.0414	6.1173	4.1239	4.2014	3.5682	3.5798
5	5.1931	5.1673	6.2405	6.0934	4.1430	4.1367	3.8129	3.7076
7	5.1987	5.2095	6.3729	6.3304	4.5185	4.5911	4.1072	4.1584
9	5.2330	5.2718	6.5539	6.5635	5.1004	5.0792	4.6532	4.6335
11	5.3139	5.2504	6.6532	6.6191	5.8325	5.9243	5.3054	5.2967
13	5.3858	5.4624	6.7559	6.6990	6.1038	6.0864	5.7160	5.7634
15	5.5276	5.4997	6.8325	6.8129	6.4378	6.4183	6.2455	6.1875

[1] Psychrophilic plate counts are expressed as the log of the number of organisms per ml. of oyster liquor.

TABLE N

The organoleptic quality of oysters as affected by iced-storage of 2 replications. [1]

Storage time (days)	Replicate 1				
	Odor	Appearance	Flavor	Texture	Cumulative
0	9.29	9.46	9.00	9.46	9.30
1	9.26	9.22	8.59	8.52	8.90
3	8.52	8.48	7.74	7.85	8.14
6	8.17	8.42	7.75	7.53	7.96
7	7.52	7.78	7.07	7.48	7.46
9	6.57	6.93	6.07	6.70	6.56
10	5.88	6.33	5.25	5.83	5.82
13	5.21	5.28	3.47	4.60	4.64
15	4.22	4.11	2.00	3.00	3.33
Storage time (days)	Replicate 2				
	Odor	Appearance	Flavor	Texture	Cumulative
0	9.27	9.40	8.53	9.00	9.05
1	8.58	8.91	7.73	7.97	8.29
4	7.63	7.81	7.37	7.29	7.53
5	6.85	7.12	5.73	6.61	6.58
7	6.76	7.29	5.91	5.99	6.48
8	5.89	5.81	5.45	5.74	5.72
11	6.09	5.52	4.43	5.38	5.35
13	5.54	4.83	3.75	4.29	4.60
15	4.48	3.76	3.09	3.14	3.62

[1] The values for organoleptic quality are based on an hedonic scale of 10 to 1.

TABLE O

Summary of analysis of variance for total reducing substance concentrations of ice-stored oysters.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Replicates	1	2.0929	1.355
Gallons/Replicate	2	1.5443	0.4862
Material (meat and liquor)	1	3871.1196	1219.058**
Replicates X Material	1	27.0227	8.509**
Days	8	768.7119	242.075**
Material X Days	8	226.9072	71.455**
Replicates X Days	8	11.8208	3.723**
Replicates X Material X Days	8	12.1176	3.816**
Error	34	3.1755	

* Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

TABLE P

Summary of analysis of variance for pH of ice-stored oysters.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Replicates	1	0.2704	22.937*
Gallons/Replicate	2	0.0117	2.141
Material (meat and liquor)	1	0.6778	123.115**
Replicates X Material	1	0.0128	2.333
Days	8	1.1879	215.762**
Material X Days	8	0.0164	2.987*
Replicates X Days	8	0.0060	1.095
Replicates X Material X Days	8	0.0272	4.491**
Error	34	0.0055	

* Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

TABLE Q

Summary of analysis of variance for trimethylamine-nitrogen content of ice-stored oysters.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Replicates	1	5780.0469	430.701**
Gallons/Replicate	2	13.4200	1.218
Material (meat and liquor)	1	23.0719	2.094
Replicates X Material	1	0.0058	0.001
Days	8	175.9325	15.971**
Material X Days	8	5.2192	0.473
Replicates X Days	8	140.4728	12.752**
Replicates X Material X Days	8	3.4417	0.312
Error	34	11.0152	

* Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

TABLE R

Summary of analysis of variance for total volatile base content of ice-stored oysters.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Replicates	1	11341.1917	132.089**
Gallons/Replicate	2	85.8651	2.492
Material (meat and liquor)	1	1468.6780	42.634**
Replicates X Material	1	0.0633	0.001
Days	8	536.0529	15.561**
Material X Days	8	136.6268	3.966**
Replicates X Days	8	442.4094	12.842**
Replicates X Material X Days	8	35.1145	1.019
Error	34	34.4481	

* Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

TABLE S

Summary of analysis of variance for indole content of ice-stored oysters.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	35		
Gallons	1	5.8402	7.584*
Days	8	0.8681	1.127
Error	26	0.7700	

*Significant at the 0.05 level of probability.

TABLE 7

Summary of analysis of variance of mesophilic plate counts of ice-stored oysters.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Replicates	1	10.0335	0.497
Gallons/Replicate	2	40.3539	158.903**
Material (meat and liquor)	1	0.8458	6.661*
Replicates X Material	1	0.7492	5.900*
Days	8	0.4248	3.345 *
Material X Days	8	0.3787	2.982*
Replicates X Days	8	0.4965	3.910**
Replicates X Material X Days	8	0.0698	0.549
Error	34	0.1269	

* Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

TABLE U

Summary of analysis of variance of psychrophilic plate counts of ice-stored oysters.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Replicates	1	34.1981	2.556
Gallons/Replicate	2	13.3774	403.300**
Material (meat and liquor)	1	2.4793	74.747**
Replicates X Material	1	2.0319	6.125*
Days	8	5.2721	158.943**
Material X Days	8	0.0718	2.166
Replicates X Days	8	1.3527	40.782**
Replicates X Material X Days	8	0.1567	4.726**
Error	34	0.0331	

* Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

TABLE V

Summary of analysis of variance of organoleptic quality of ice-stored oysters.

Odor

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	17		
Replicates	1	0.7001	3.91
Days	8	5.4010	30.24**
Error	8	0.1786	

Appearance

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	17		
Replicates	1	1.7174	21.47**
Days	8	6.7707	84.67**
Error	8	0.0799	

Flavor

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	17		
Replicates	1	1.3612	3.56
Days	8	8.6362	22.56**
Error	8	0.3827	

*Significant at the 0.01 level of probability.

TABLE V (continued)

Summary of analysis of variance of organoleptic quality of ice-stored oysters.

Texture

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	17		
Replicates	1	1.7174	16.17**
Days	8	7.2199	67.99**
Error	8	0.1061	

Cumulative

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	17		
Replicate	1	1.3284	10.48**
Days	8	6.9104	54.55**
Error	8	0.1266	

**Significant at the 0.01 level of probability.

TABLE W

Correlation coefficients (r) of chemical and microbiological tests on ice-stored oysters.

pH (Meat) vs	r [1]
pH (liquor)	+0.949**
Trimethylamine-nitrogen (meat)	-0.428
Trimethylamine-nitrogen (liquor)	-0.435
Total volatile bases (meat)	-0.374
Total volatile bases (liquor)	-0.673**
Mesophilic plate counts (meat)	+0.629**
Mesophilic plate counts (liquor)	+0.015
Psychrophilic plate counts (meat)	-0.729**
Psychrophilic plate counts (liquor)	-0.652**

pH (Liquor) vs	r [1]
Trimethylamine-nitrogen (meat)	-0.482*
Trimethylamine-nitrogen (liquor)	-0.484*
Total volatile bases (meat)	-0.410
Total volatile bases (liquor)	-0.695**
Mesophilic plate counts (meat)	+0.516*
Mesophilic plate counts (liquor)	-0.094
Psychrophilic plate counts (meat)	-0.772**
Psychrophilic plate counts (liquor)	-0.745**

Mesophilic Plate Counts (Meat) vs	r [1]
Mesophilic plate counts (liquor)	+0.603**
Psychrophilic plate counts (meat)	-0.084
Psychrophilic plate counts (liquor)	-0.029
Trimethylamine-nitrogen (meat)	+0.288
Trimethylamine-nitrogen (liquor)	+0.273
Total volatile bases (meat)	+0.287
Total volatile bases (liquor)	-0.054

[1] For 18 and 18 degrees of freedom, the following correlation coefficients are significant: 5 percent level (± 0.468); 1 percent level (± 0.590).

**Significant at the 0.01 level of probability.
*Significant at the 0.05 level of probability.

TABLE W (continued)

Correlation coefficients (r) of chemical and microbiological tests on ice-stored oysters.

Mesophilic Plate counts (Liquor) vs	r [1]
Psychrophilic plate counts (meat)	+0.537*
Psychrophilic plate counts (liquor)	+0.607**
Trimethylamine-nitrogen (meat)	+0.559*
Trimethylamine-nitrogen (liquor)	+0.543*
Total volatile bases (meat)	+0.570*
Total volatile bases (liquor)	+0.384
Psychrophilic Plate Counts (Meat) vs	**r [1]**
Psychrophilic plate counts (liquor)	+0.974**
Trimethylamine-nitrogen (meat)	+0.745**
Trimethylamine-nitrogen (liquor)	+0.735**
Total volatile bases (meat)	+0.692**
Total volatile bases (liquor)	+0.798**
Psychrophilic Plate Counts (Liquor) vs	**r [1]**
Trimethylamine-nitrogen (meat)	+0.683**
Trimethylamine-nitrogen (liquor)	+0.667**
Total volatile bases (meat)	+0.631**
Total volatile bases (liquor)	+0.710**
Trimethylamine-nitrogen (Meat) vs	**r [1]**
Trimethylamine-nitrogen (liquor)	+0.983**
Total volatile bases (meat)	+0.971**
Total volatile bases (liquor)	+0.915**
Trimethylamine-nitrogen (Liquor) vs	**r [1]**
Total volatile bases (meat)	+0.946**
Total volatile bases (liquor)	+0.938**
Total Volatile Bases (Meat) vs	**r [1]**
Total volatile bases (liquor)	+0.884**

VITA

Stephen Charles Lagarde was born in Baton Rouge, Louisiana on September 27, 1943. He attended the local schools and graduated from Baton Rouge High in 1961. In the same year he enrolled in Louisiana State University, Baton Rouge, where he received his B.S. in May, 1965.

The author was accepted into the Graduate School through the Department of Food Science and Technology in January, 1966. He received the degree of Master of Science from that department in August, 1967, and was awarded a Fellowship from the United States Public Health Service for studies leading to the Doctorate.

On August 23, 1969, he married the former Kathleen Anne Ermert of New Orleans, Louisiana.

The author is currently a candidate for the degree of Doctor of Philosophy in Food Science.

EXAMINATION AND THESIS REPORT

Candidate: Stephen Charles Lagarde

Major Field: Food Science

Title of Thesis: Development of a Total Reducing Substance Test for Ascertaining Oyster Quality.

Approved:

Joseph A. Lionetti
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

A. D. Larson

Robert M. Grodner

Hall Davis

A. J. Novak

Date of Examination:

July 16, 1970