when the fruits are picked reaches a maximum ranging from 0.1 to 0.2 p.p.m., the many analyses of oil samples from several olive-growing areas have constantly shown the practical absence of residues of dimethoate and its metabolites.

Research has given clear evidence that a certain amount of dimethoate—almost the whole amount of the P=O metabolite and of the products of hydrolysis—is contained in the aqueous phase. The olive husks contained a part of the dimethoate (whose concentration gradually decreases with increasing elapsed time between treatment and process of oil yielding) and traces of the P=O metabolite and of compounds extracted with $CHCl_3$ (Table IV).

Treatment of eating olives with NaOH, in the same way as in the industrial process, produces further degradation and a strong extraction of the P^{32}-containing compounds possibly present in the fruits.

At the end of the soaking in NaOH solution, the amount of P^{32} extracted represents about 85 to 90% of the content (referring to the residues of dimethoate and its metabolites) present in the olives at the moment of dipping (Table V). A further soaking of olives in water for 5 days (as usually recommended in the industrial process) takes out from 98 to 99% P^{32} derived from residues of dimethoate in olives at harvest time (Table VI).

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QUALITY OF SHUCKED OYSTERS

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

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COMMERCIALLY shucked raw oysters are among the most perishable food products. Since the sanitary safeguards normally used with food products generally cannot be used with raw oysters, only good sanitary practices during harvesting, shucking, storing, and transportation can assure the consumer safe and palatable oysters. Control measures have been developed as a result of disease outbreaks in 1924–25, 1928, and 1939, attributable to bacterial contamination from oysters. The shellfish industry, local health authorities, and the Public Health Service have cooperated in setting tentative standards for permissible levels of viable bacteria and certain coliform organisms in shellfish growing waters, in the oysters before shucking, and in the finished product. Satisfactory compliance by interstate shippers is recognized by state certification.

Although no satisfactory chemical tests are available to indicate the sanitary quality of oysters, pH is used frequently as an indicator of acceptability and potential storage life. The pH of freshly shucked oysters appears, however, to be dependent in part on harvesting area and season. Freshly shucked oysters from Apalachicola Bay in the Gulf of Mexico may vary from pH 6.0 in late spring to 6.4 in winter (*5*); the pH of oysters from the East Coast may be as high as 6.8 and is apparently independent of seasonal variation (*7*). The pH of oysters from Chesapeake Bay usually decreases during optimum storage, whereas that of oysters from the Gulf Coast may not change for several days (*1, 5*).

The storage life of raw shucked oysters depends primarily on storage temperature. The manual of recommended practices (*11*) suggests that shucked oysters be cooled to 10° C. or lower within 2 hours after shucking and held at that temperature during storage. The freshly packaged oysters usually are cooled with crushed ice; dry refrigeration also is used. The time required to cool packaged oysters is dependent on the temperature and capacity of the refrigeration system, the initial temperature of the oysters (which is largely dependent on the temperature of the water used for washing and "blowing"), and the size of the container. In a crushed ice slurry over 3 hours is required to cool a 1-gallon can of oysters from 17° to 10° C., whereas less than 30 minutes is required to cool a $^1/_2$-pint can (*10*). Rigorous control of storage is very difficult because of the time and distance between the harvesting and sale of oysters. Although 3 or 4 weeks may expire before oysters at 0° C. show signs of decomposition, definite organoleptic signs of spoilage appear much earlier if oysters are allowed to reach temperatures above 10° C. during storage.

During work on other projects related to shellfish, substances capable of acting as reducing agents in a variety of oxidation-reduction reactions were observed in the liquor surrounding commercially shucked oysters. Preliminary investigations indicated that these substances included glucose, lactic acid, and

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

at least one unidentified component. The concentration of these substances increased as a function of time and temperature, indicating a possible chemical determination of the age-temperature history of oysters.

Practical requirements of a test suitable for field use include simplicity, short duration, and availability of reagents, glassware, and auxiliary equipment. Several oxidation-reduction systems were investigated. Since the color changes associated with the reduction of chromium(VI) to chromium(III) are easily discernible, this method appeared to be the most feasible.

DEVELOPMENT OF METHOD

Materials and Methods

Dichromate Solution, Containing 10 Mg. of Chromium per Ml. Potassium dichromate was dried for 2 hours at 110° to 120° C., weighed, and dissolved in distilled water using 2 ml. of concentrated sulfuric acid per liter of solution.

Trivalent Chromium Sulfate Standard Solution. A stock solution containing 23.9 grams of $Cr_2(SO_4)_3.nH_2O$ per liter of 1M H_2SO_4 was analyzed for the chromic ion. The Cr(III) was oxidized to Cr(VI) with $KMnO_4$ and reacted with an excess of a standard ferrous sulfate solution; the excess iron(II) was determined by titration with standard dichromate solution, using diphenylbenzidine as an indicator. After standardization of the stock solution, suitable dilutions were made to give working standards containing 2, 3, and 3.5 mg. of Cr(III) per ml. These solutions appeared to remain stable indefinitely.

Dextrose Solution, approximately 0.01M, using anhydrous dextrose.

Diluted Oyster Liquor. After thorough, gentle stirring of the oyster sample, a 1-ml. aliquot of the oyster liquor was diluted with 15 ml. of distilled water. A 2-ml. aliquot contained a sufficient concentration of reducing substances to react with 0.1 to 5 mg. of Cr(VI).

Dialyzed Oyster Liquor. When a refined sample was necessary, a 1-ml. aliquot of oyster liquor was pipetted to a washed dialysis bag (3/4-inch diameter) and suspended in a 50-ml. screw-cap tube containing 15 ml. of distilled water. The tube was then agitated until the dialyzable substance came into equilibrium between the two phases. Equilibrium occurred within 2 hours when the tubes were placed at a 45° angle and agitated on a variable-speed shaker at the maximum rate possible without splashing on the screw cap. After equilibration, the dialysis bag and contents were discarded, and a 2-ml. aliquot was used for the chromate color test.

Standard Curve. A standard curve was prepared by determining the absorbance at 580 mμ of solutions containing known concentrations of chromium(III) at the acidity used in the test. With a Beckman Model B spectrophotometer, Beer's law applied throughout the range of concentration from 0 to 10 mg. per tube (4 ml.).

Visual Color Standards. An 11-tube series of standards was prepared in borosilicate glass test tubes (15 × 150 mm.) which were flame-sealed or closed with ground-glass stoppers. Each tube contained 1 ml. of concentrated sulfuric acid, a combination of Cr(III) and Cr(VI) to give a total of 10 mg., and distilled water to make a total volume of 4 ml. The concentration of chromium(III) in the series ranged from 0 to 10 mg., giving colors ranging from yellow-orange to blue-violet.

Chromate Color Test

In a 15- by 150-mm. test tube, 1 ml. of dichromate solution was added to a 2-ml. aliquot of the sample capable of reducing 0.1 to 10 mg. of chromium(VI). While the tube was being rotated to ensure nearly instantaneous mixing, 1 ml. of concentrated sulfuric acid was added. Although an automatic buret equipped with drying tubes was routinely used for dispensing the acid, a dispenser calibrated to deliver 1-ml. aliquots of the concentrated acid was also satisfactory. The tube was shaken and allowed to stand at room temperature for 10 minutes ±15 seconds. The absorbance of the solution was read at 580 mμ. The instrument was adjusted to 100% transmittance with a blank containing 2 ml. of distilled water, 1 ml. of the dichromate solution, and 1 ml. of concentrated sulfuric acid. The concentration of the chromium(III) was determined from the standard curve relating absorbance to the concentration of chromium(III). The concentration of chromium(III) in the reaction mixture was determined also by comparison of its color with visual standards.

Development and Evaluation of Chromate Color Test

Selection of Acid and pH of Reaction. At the concentration of chromium used in this reaction the O-R (oxidation-reduction) potential of the system is influenced by the kind and concentration of acid. It has been reported (*8*) that at a constant pH the O-R potential of dichromate decreases in the following order of acids: $HClO_4$ > H_2SO_4 > HNO_3 > CH_3COOH. For use in a field test, sulfuric acid seemed most applicable. In the reduction of chromium(VI) to chromium(III) the acid adjusts the pH to an optimum value and provides heat to accelerate the reaction but has little effect on the absorptivity at 580 mμ. These effects are demonstrated in the oxidation of dextrose by chromium(VI) in reaction mixtures containing three different concentrations of acid (Figure 1). When 1 ml. of concentrated acid is used to make the resultant mixture 4.45M in acid, the reaction is almost complete within 10 minutes.

Effect of Order of Adding Reagents. It is essential that the acid be added to the reaction mixture containing both dichromate and the material to be oxidized and that the tube be rotated to promote mixing while the acid is being added. If the acid is allowed to form a separate layer, the results are not reproducible and are generally too high.

Time Requirement for Color Reaction. On the basis of data such as those presented in Table I, a 10-minute reaction period was chosen for the test. The reaction is almost complete within 5

minutes after addition of the acid. When the concentration of RS results in the production of less than 4 mg. of chromium(III), the increase in the concentration of chromium(III) between 5 and 10 minutes is less than the experimental error. With higher concentrations the change in absorbance readings within this time range is significant but slow enough to make the readings reproducibile. An interval timer was routinely used, so that the sample could be read within 10 minutes ± 15 seconds after addition of the acid. Since carbon dioxide is formed in many of the reactions and dissolved gases are released with increased temperature of the solution, the absorbance should be read immediately after transference of the reaction mixture to the spectrophotometric cells to minimize this source of error.

Effect of Temperature on Reaction Rate. Enough heat is liberated upon addition of the concentrated sulfuric acid to the reaction mixture that variations in the temperature of the reagents in the laboratory may be ignored. If the analysis is done in the field, however, results may be lowered by as much as 14% if the temperature of the reagents is as low as 14° to 18° C. This effect is demonstrated by the data in Table II, which are the averages for triplicate tests run on a solution containing 1.803 mg. of dextrose brought to the temperature of the reagents. After the acid was added, the mixtures were held at room temperature for 10 minutes and then read in the spectrophotometer.

Effect of Reaction Products on O-R Potential of Reagent. Although it has been reported that the concentration of chromium(III) has no effect on the O-R potential of dichromate (*8*), a determination of the applicability of this finding to the chromate color test was essential. Suitable dilutions of glucose were reacted in the test and the absorbance was observed at 580 mμ. In the concentration range studied, the relationship between the absorbance at 580 mμ and the concentration of dextrose was linear with zero concentration passing through the origin, indicating that the O-R potential is independent of the concentration of chromium(III) or other products of the reaction.

Comparability of Degree of Hydration. The color of chromium(III) solutions varies with the degree of hydration as well as with the anionic species present and the pH. Marks (*6*) prepared different hydrates by varying the acid concentration and then observing the variations in the absorbance maxima and the molar absorptivity. The comparability of the degree of hydration of the chromium(III) in the standards and that produced through the reaction of dichromate in the chromate color test

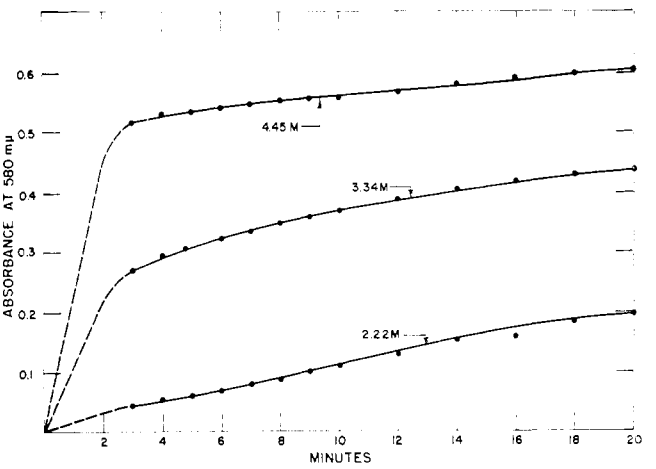


Figure 1. Effect of sulfuric acid concentration on oxidation of 3.606 mg. of dextrose by 10 mg. of chromium (VI)

Table I. Effect of Time on Oxidation of Dextrose by Dichromate in Solution 4.45M in H_2SO_4

Chromate Reduction Time, Min.	1.803 Mg. Dextrose		3.606 Mg. Dextrose	
	Density at 580 mμ	Chromium(III), mg.	Density at 580 mμ	Chromium(III), mg.
5	0.272	3.10	0.538	6.09
7	0.279	3.18	0.550	6.22
8	0.281	3.18	0.555	6.28
9	0.283	3.20	0.560	6.34
10	0.285	3.21	0.562	6.38
12	0.287	3.24	0.572	6.49

Table II. Effect of Temperature of Reagents on Oxidation of Dextrose by Dichromate in 4.45M H_2SO_4

Temp. of Reagents, ° C.	Lab. Temp., ° C.	Chromium(III) Produced by 1.803 Mg. Dextrose
14.3	27.8	2.85
16.3	27.8	2.85
18.3	27.8	2.85
20.3	27.8	2.95
22.3	27.8	3.08
22.9	27.8	3.08
24.3	27.8	3.01
26.3	27.8	3.13
28.3	27.8	3.29
30.3	27.8	3.24
32.3	26.0	3.18
34.3	26.0	3.29
36.3	26.0	3.31

Table III. Molar Absorptivities at 580 Mμ of Standard Solutions and Reaction Mixtures

Chromium(III), Molar Concn., × 10^{-2a}	Absorbance at 580 Mμ	Molar Absorptivity
Standard Solutions		
0.96	0.178	18.5
1.44	0.263	18.2
1.92	0.354	18.4
2.40	0.443	18.4
	Av.	18.4
Reaction Mixture after Oxidation of Dextrose		
1.63	0.290	17.8
3.16	0.585	18.5
	Av.	18.1

[a] Chromium(III) concentration determined by standard volumetric procedure (*11*).

Table IV. Precision of Chromate Color Test

(Results from 20 replicate tests on each reducing substance)

Reducing Substances	Chromium (III) Measured in Color Test, Mg.			
	Av.	Median	Range	Std. dev.
Dextrose solution	3.31	3.31	3.13–3.43	0.0806
Dialyzed oyster liquor	3.94	3.92	3.77–4.12	0.0929
Diluted oyster liquor[a]	4.64	4.62	4.42–4.82	0.1029
Diluted oyster liquor[b]	4.79	4.75	4.52–5.20	0.1687

[a] From shucked oysters held in deep freeze for several months.
[b] From shucked oysters held several days above 0° C.

was confirmed by two lines of evidence. Chromium(III) in the standard solutions and that produced as the result of the reaction of glucose, oyster liquor, and several other reducing agents all have an absorbance maximum at 574 to 590 mμ. Since changes in the wave length of maximum absorption for different degrees of hydration of chromium(III) are not great, a substantial line of evidence was developed through comparison of the molar absorptivities calculated from the absorbance values of the standard solutions and those from the reaction mixtures after the oxidation of dextrose. The concentration of chromium(III) in each was determined by a standard volumetric procedure (*12*) and the absorbance values were read at 580 mμ. These data (Table III) indicate that the extent of hydration in the chromate color test reaction mixture is the same as that in the chromium(III) standards.

Precision of Chromate Color Test. The precision of the chromate color test was estimated by the assay of 20 replicate samples of dextrose, dialyzed oyster liquor, and diluted oyster liquor (Table IV). The degree of precision of the chromate color test was judged to be adequate for estimating the age-temperature history of raw shucked oysters.

The precision of the test with diluted oyster liquor is in part dependent on the condition of the oyster liquor. Suspended particulates may be a source of error, unless special precautions are taken in sampling. In practice it is satisfactory to perform the analyses on duplicate aliquots of the oyster liquor. If the results check within ±0.25 mg. of chromium(III), no further tests need to be run. If the duplicates vary in excess of this amount, however, the color test should be repeated on additional aliquots until such a check is obtained. It is sometimes impossible to obtain checks, and therefore, these samples cannot be run in the color test unless the dialysis procedure is used.

Use of Dialysis. Although preparation of oyster liquor samples by the dialysis technique is more tedious than simple dilution, it affords a higher degree of precision and excludes the possibility of error through the presence of nondialyzable substances, such as glycogen, which may be present if the samples have been handled roughly.

The dilution and dialysis techniques applied to common samples of oyster liquor are summarized in Figure 2. The diluted samples consistently assay slightly higher than dialyzed samples, but the relationship between the two is linear and sufficiently precise to allow comparison of samples prepared by either technique.

Use of Visual Standards. Although all work has been based on a spectrophotometric determination of chromium(III), it is possible to estimate with reasonable accuracy the concentration of chromium(III) in the reaction mixture by comparison of its color with visual standards.

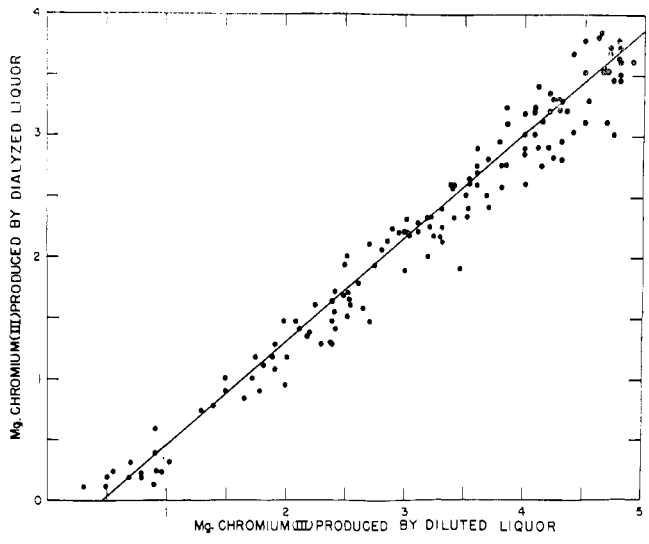


Figure 2. Results of chromate color test on diluted and dialyzed oyster liquor

When the concentration of chromium(III) is 4 mg. or less, the comparison with the visual standard appears to have a high degree of precision, giving results within ±0.5 mg. of those obtained by spectrophotometry. As the concentration of chromium(III) in the sample is increased, however, the precision of the estimate decreases. A sample containing 6 to 8 mg. of chromium, as measured spectrophotometrically, may give visual readings of 5 to 7 mg. The loss in precision for higher levels of chromium(III) does not interfere appreciably with the chromate color test, since the procedure for preparing samples has been adjusted so that most samples do not assay above 4 or 5 mg. of chromium(III) before becoming organoleptically unacceptable.

Discussion

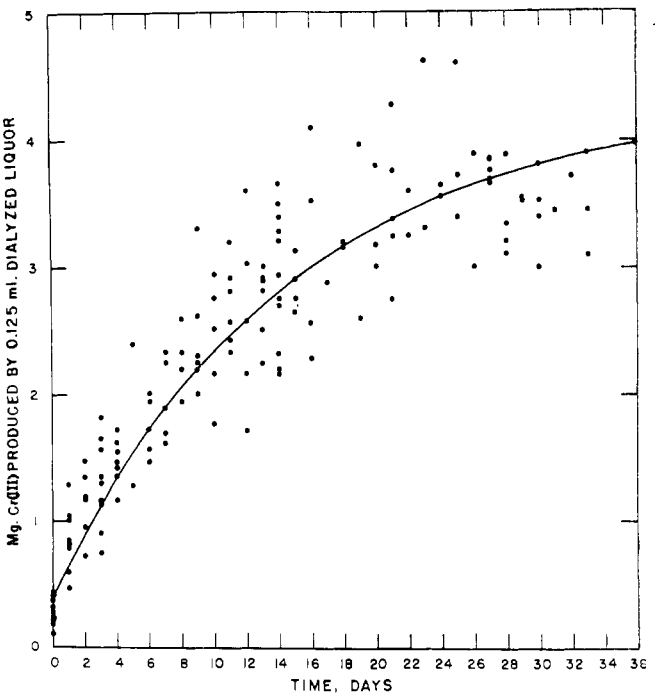
The wave length for maximum absorption of chromium(III) is dependent on the pH and the anionic species present. Bjerrum (*2*) reported the maximum molar absorptivity for hexaaquachromium(III) of chlorochromic sulfate at 564 ± 11 mμ. Chromium(III) in 0.1 to 4.5*M* sulfuric acid has maximum absorption at 580 to 590 mμ (*4*, *6*). With higher concentrations of sulfuric acid a bathochromic effect occurs that shifts the wave length for maximum absorption to 610 mμ when the reaction mixture contains 50% sulfuric acid (*9*).

The observed molar absorptivity of 18.4 is significantly higher than that reported in literature for chromium(III) in neutral or dilute acid solutions. Elving and Zemel (*3*) reported a value of 13.9 for the hexaaqua $[Cr(H_2O)_6^{+3}]$ ion in perchloric acid (up to 6.0*M*) at 575 mμ and Bjerrum (*2*) reported a value of 12.96 for the same species at 564 ± 11 mμ. Marks (*6*) reported a value of 13.8 for the violet isomer at 519 fresnels (578 mμ) in a neutral solution; however, he observed a slight increase in the absorptivity with an insignificant difference in the maximum absorption as the acid concentration was increased to 4.5*M*. Marks also studied a green hydrate of chromic sulfate; he reported that it had a maximum absorption at 508 fresnels (590 mμ) and a molar absorptivity of 20.7. The difference between the two isomeric forms is in the degree of hydration; the violet hydrate contains large amounts of water, whereas the green hydrate contains five molecules of water. Marks suggests that chromic hydroxide dissolved in sulfuric acid solution forms a mixture of the green and violet isomers and that the ratio of the two is dependent on the acid concentration. In work with known ratios of the two isomers, Marks observed an increase in the molar absorptivity from 14.43 to 18.95 as the mole fraction of the green isomer was increased from 0.25 to 0.875. Accordingly, the absorptivity of 18.4 observed for the standard solution of chromic sulfate used in this study indicates a solution containing a mixture of the two hydrates.

APPLICATION OF METHOD TO OYSTERS HELD UNDER KNOWN CONDITIONS OF TIME AND TEMPERATURE

Effect of Time

Study I. This study was an evaluation of the chromate color test for estimating the age of shucked oysters stored at 0° C. Samples of the species *Cras-*



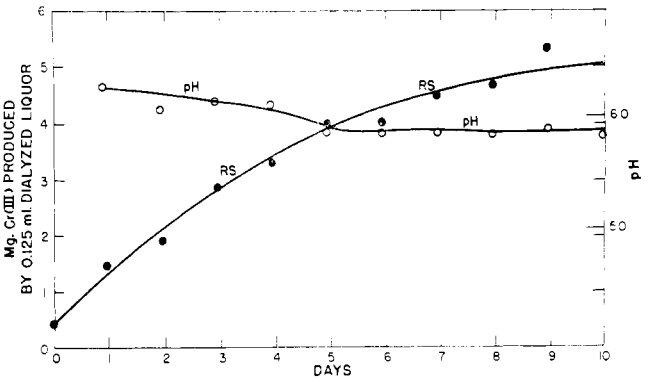


Figure 4. Comparison of concentration of organic reducing substances in liquor of shucked oysters and pH of oysters

◀

Figure 3. Increase in organic reducing substances in liquor of raw shucked oysters during storage at 0°C.

Theoretical curve constructed by substituting average values in mathematical equation

sostrea virginica were collected from 12 commercial shucking houses during the first 2 weeks of December 1958. The oysters were harvested from eight areas in Chesapeake Bay—i.e., James River, Hampton Bar, Egg Island, York River, Mobjack Bay, Great Wicomico River, Rappahannock River, and Potomac River.

Immediately after shucking, washing, and "blowing," the oysters were collected from the strainers and placed in quart polyethylene bottles with screw caps. Initial samples of liquor (4 to 5 ml.) were removed from around the oysters within 15 minutes and then placed in 5-ml. screw-cap vials and stored in crushed ice until used in the chromate color test. The samples of oysters were covered with crushed ice and transported in portable refrigerators to a central storage room, where they were maintained at 0° to 2° C. After all the samples were collected, they were transported to Cincinnati in portable refrigerators containing a crushed ice slurry. They were subsequently held for 15 to 30 days at 0° C. Aliquots of liquor were removed every 2 to 3 days for analysis. On each day that the oyster liquor was assayed for its concentration of RS, duplicate diluted samples and duplicate dialyzed samples were prepared for use in the chromate color test.

The 14 samples of liquor collected from the oysters within 15 minutes after being packaged for Study I produced between 0.11 and 0.37 mg. of chromium(III) in the chromate color test. The dialysis procedure was used for sample preparation and the results were read spectrophotometrically. Six additional samples were collected for the storage study, but aliquots of liquor were not removed immediately after packaging. Analyses of these six samples and of subsequent liquor samples collected from the initial 14 samples after various periods of storage at 0° C. showed increasing concentrations of chromium(III).

Effect of Temperature

Studies II and III. Studies IIA and IIB were an application of the chromate color test for estimating the effect of temperature on the rate of increase of RS in oyster liquor. The five samples of fresh oysters used in these studies were harvested from Mobjack Bay and processed between January and April 1959. Each sample consisted of 8 pints of commercially packaged standard oysters. They were shipped to the laboratory in crushed ice and the study was begun on their arrival. Thus, the samples had been shucked 36 to 48 hours at the beginning of the study.

STUDY IIA included four of the five samples of fresh oysters. In preparation for the study, the 8 pints in each sample were combined to make a composite sample that was then divided into four equal aliquots and stored in quart polyethylene bottles with screw caps. The bottles were suspended in water baths held at 0°, 15°, 25°, and 35° C. They were agitated gently until the center of the container was within 3° or 4° C. of the temperature of the bath. At that time an aliquot of the liquor (3 to 4 ml.) was removed for analysis. The sample in the 35° bath required 45 to 48 minutes to reach 31° C., while that in the 0° bath was sampled immediately. Subsequent aliquots of liquor were removed at regular intervals, ranging from 30 minutes for the samples held at 35° C. to 12 hours for those held at 0° C.

STUDY IIB was a similar study on the fifth sample of oysters. For this, however, it was assumed that the commercially packaged pints were aliquots of a composite sample. The unopened pint containers were placed in polyethylene bags and immersed in water baths at the temperatures used in Study IIA. They were held in each water bath for known time intervals, 3 hours at 35° C., 12 hours at 25° C., 36 hours at 15° C., and 7 days at 0° C., and were then opened and assayed.

STUDY III was also a temperature study; however, frozen oysters were used. One sample was harvested from Mobjack Bay in December 1958 and was processed in the same plant as the freshly shucked oysters. The other two samples were harvested from the James River and the Potomac River and were processed during December 1958. Each sample consisted of 3 quarts of oysters in a gallon can. The three samples were placed in a room held at −10° to −15° C. within 6 hours after they were packaged and held in the frozen state for 40 to 60 days. The gallon containers of the frozen samples were suspended in a 15° C. water bath until thawed. Each sample then was divided into four aliquots, which were stored in quart polyethylene bottles with screw caps and subjected to the experimental procedures used in Study II.

A direct relationship was observed between the rate of increase of RS, as measured by the chromate color test, and the storage temperature of all the samples used in Studies II and III. The concentration of RS doubled within 4 hours when aliquots of the freshly shucked oysters used in Study IIA were held at 35° C. In comparison, only a slight increase occurred in 4 hours at 0° C. The three samples used in Study III, which were frozen for varying lengths of time prior to the study, showed a much greater rate of

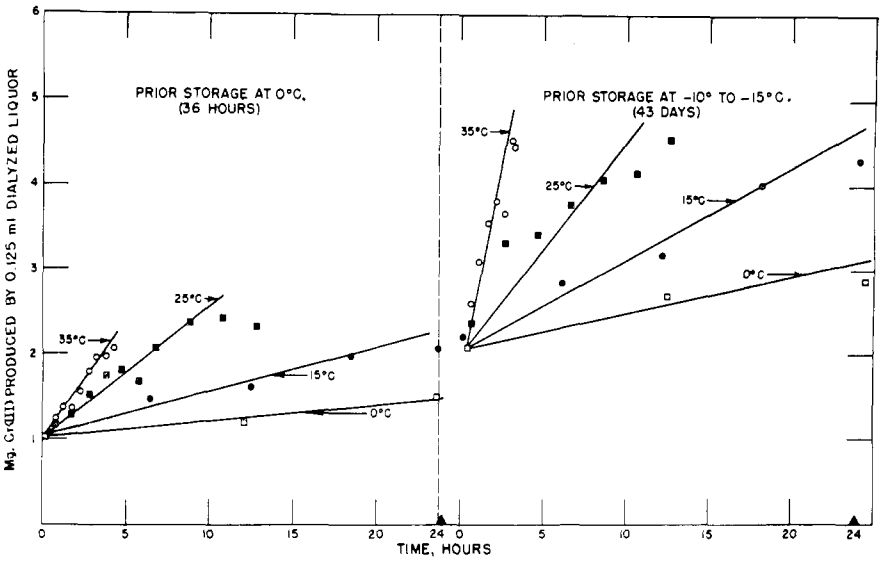


Figure 5. Effect of prior storage conditions on rate of production of organic reducing substances in shucked oysters

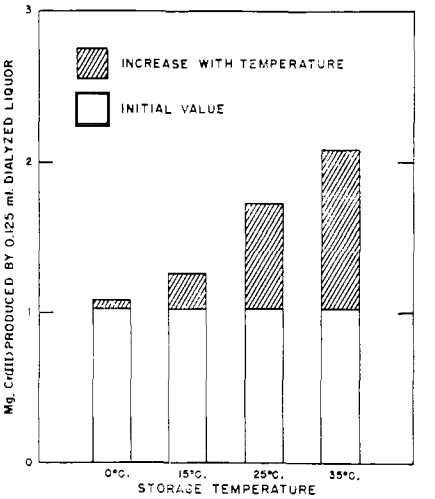


Figure 6. Increase of organic reducing substances in shucked oysters during 4 hours of storage

increase of RS at each temperature level than any of the samples of freshly shucked oysters. The initial RS value for the frozen sample was twice that for the freshly shucked sample. Undoubtedly some of this increase occurred during the initial freezing and subsequent thawing of the sample. Work with pint samples of freshly shucked oysters has indicated, however, that the concentration of the RS increases slowly during storage of shucked oysters at temperatures below 0° C. An increase in the assay for RS of about 0.5 mg. of chromium(III) [from 1 — 1.5 to 1.4 — 2.0 mg. of chromium (III)] was observed in pint samples of oysters after 2 to 3 months of frozen storage.

Discussion

For study I, a mathematical equation was developed to describe the increase in RS evidenced by increases in chromium(III) during storage of oysters at 0° C.:

$$Y_t = Y_0 + K(1 - e^{-ct})$$

The parameters in the equation are identified with the concentration of chromium(III) produced in the chromate color test. Y_t is the average chromium(III) concentration at storage time t in days; Y_0 is the average initial level; c is a parameter defining the rate of increase of the chromium(III) concentration; and K is a proportionality constant which, when added to Y_0, represents the average upper limit.

Data collected from eight samples during storage for 14 to 15 days and from seven samples stored for 4 to 5 weeks were used to estimate the three parameters in the equation. The five samples that did not reach an upper limit during the 14 days they were studied were omitted from the evaluation of the model equation. The average values of c and Y_0 and the geometric mean of K were used to construct the theoretical curve shown in Figure 3. Surrounding this theoretical curve are the data for 15 oyster samples used in this storage study. Of the individual tests on the oyster liquor, 94% are within ± two standard deviations and 61% within ± one standard deviation of the average curve defined by the model equation.

Oysters from the Gulf Coast also were used during the development of the test. Samples collected from seven shucking houses had been harvested from eight different areas—i.e., three areas in Apalachicola Bay, Florida; two areas off the Alabama coast; Biloxi Bay, Mississippi; and two areas off the Louisiana coast. These shucked oysters from the Gulf Coast were neither "blown" nor held in water. Instead they were shucked into water, washed with a spray of water, and immediately drained and packaged. The chromate color test indicated that all of these samples contained small amounts of RS immediately after shucking, and all showed an increase during storage. A direct comparison cannot be made between data from these samples and those from the Chesapeake Bay, since the Gulf Coast oysters were not sampled within 15 minutes after packaging and the storage temperature was higher than 0° C. The data indicate, however, that the chromate color test can be used to reflect the age of shucked oysters from the Gulf Coast area. One of the samples from Apalachicola Bay clearly demonstrates the increase in RS during the time that the pH remained fairly constant, as shown in Figure 4.

For the temperature studies (II and III), the four samples of freshly shucked oysters used in Study IIA were treated as reliable random samples for a statistical analysis. Because of the short time involved in this study, a straight line could be used to describe the increase of chromium(III) with time at each temperature. The mean slope representing the average hourly increase in milligrams of chromium(III) for these samples is given in Table V, along with the standard deviation of the individual slopes. The direct relationship between the mean slope and the temperature clearly demonstrates that the increase of chromium(III) concentration accelerates with rising temperatures. The standard deviation at each temperature for random samples with identical time-temperature histories may be less than that given in Table V, since the samples used in this study were held for varying lengths of time with some temperature variations before the study. At the beginning of the study the concentrations of chromium(III) produced in the color tests on these four samples ranged from 1.03 to 1.52 mg. and thus were significantly different. This variation in the initial concentration of RS can be explained for all except one sample, by the variation in the time interval between processing of the oysters and beginning of this study. The excepted sample is known to have been exposed to some unrecorded temperature variations during shipment prior to the study.

The effects of time and temperature on frozen and freshly shucked oysters are shown in Figure 5. Data from a representative sample of the freshly shucked oysters are shown in Figure 6. The concentrations of RS after 4 hours at each of the four temperatures were obtained by interpolation from the smooth curves relating concentration of chromium(III) and time (Figure 5).

Table V. Effect of Temperature on Rate of Increase of Reducing Substances in Dialyzate from Liquor of Shucked Oysters

Temp., °C.	Mean Slope, Mg. Cr(III)/Hr.	St. Dev. of Individual Slopes
0	0.0107, through 7.3 days	0.0028
15	0.0508, through 1.5 days	0.0073
25	0.1329, through 0.5 day	0.0234
35	0.2796, through 0.2 day	0.0306

This sample had been held at 0° to 5° C. prior to the study and contained sufficient RS to produce 1.03 mg. of chromium(III) when the study was started.

Study IIB was undertaken to ensure that laboratory manipulation of the oyster samples was not the causative factor in the observed time-temperature dependence of the concentration of RS in the oyster liquor. The pint sample, which was opened at the beginning of the study, produced 1.19 mg. of chromium (III). The sample suspended in the 35° C. water bath produced 1.57 mg. of chromium(III) after 3 hours; the one in the 25° C. bath, 1.89 mg. after 12 hours; the one in the 15° C. bath, 2.06 mg. after 36 hours; and the one held at 0° C., 2.54 mg. of chromium(III) after 7 days. Although these data cannot be compared directly with those obtained for the samples of freshly shucked oysters used in Study IIA, the results clearly demonstrate a similar temperature dependence.

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