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ASCORBIC ACID AS AN ANTIOXIDANT FOR FROZEN OYSTERS AND EFFECT OF COPPER-CHELATING ABILITY OF OYSTER TISSUE ON ASCORBIC ACID OXIDATION [1/]

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ABSTRACT

This study reports (1) the antioxidant effect of ascorbic acid on rancidity in frozen raw and cooked oysters and (2) the copper-chelating ability both of raw and of cooked oyster tissue as it may affect oxidative rancidity and the efficacy of ascorbic acid as an antioxidant.

INTRODUCTION

In general, fishery products are susceptible to oxidative rancidity, owing to their content of highly unsaturated oil; and ways and means of protecting fishery products from rancidity are being sought.

Several primary oil antioxidants have been used with success in preventing or retarding rancidity in oils and in foods containing oil. Among these antioxidants are butylated hydroxyanisole (BHA) and nordihydroquiaretic acid (NDGA), which are phenolic compounds that have been reported as being effective antioxidants for lard and other animal fat (Dugan et al 1950, Kraybill et al 1949, and Lehmann and Watts 1951). Using a commercial mixture, Tenox N, containing 74 percent propylene glycol, 20 percent BHA, 4 percent anhydrous citric acid, and 2 percent NDGA, Gardner and Watts (1957) were able to inhibit oxidative changes characterized by a "rancid fish" odor in cooked oysters. The phenolic antioxidants, however, are almost insoluble in water, and to distribute them uniformly over the surface of the oysters is difficult. A water-soluble antioxidant would offer advantages in ease of application.

Tarr (1946 and 1947), using ascorbic acid, was able to inhibit rancidity in frozen herring, black cod, and several varieties of salmon. Use of the dip and

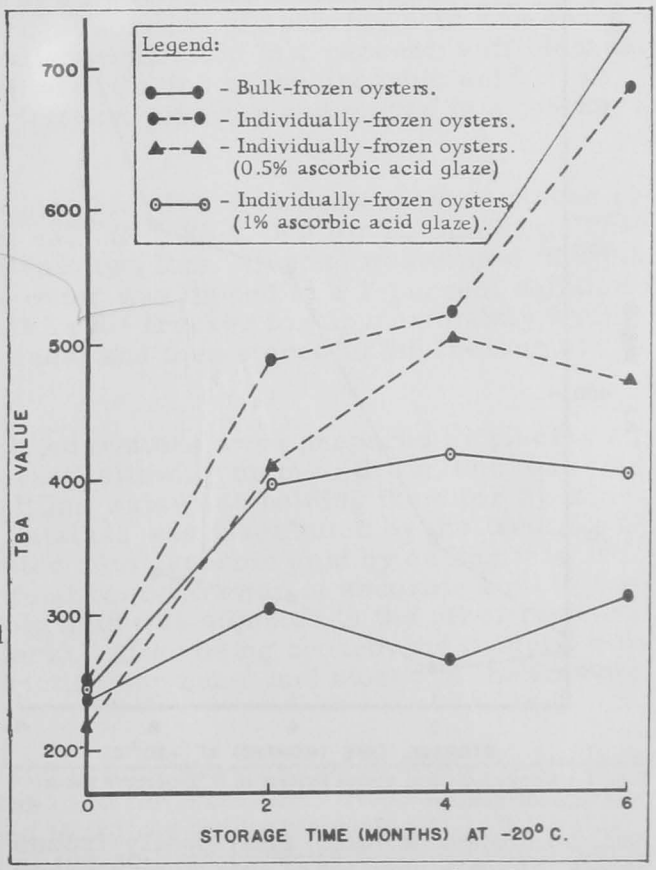

Fig. 1 - TBA value versus storage time of raw frozen oysters.

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spray methods of applying ascorbic acid to fish fillets has been reported to be commercially practical for retarding rancidity (Bauernfeind, Smith and Siemers 1951). Oxidative changes--loss of color, odor, and flavor--occurring in stored frozen shrimp, were inhibited by the use of ascorbic acid (Faulkner and Watts 1955).

On the other hand, ascorbic acid acts as a strong prooxidant in artificial systems in which fat is brought into contact with an aqueous phase (Scarborough and Watts 1949). Its activity in complex food thus is seemingly inconsistent; that is, it may accelerate as well as inhibit rancidity. Watts and Lehmann (1952) have observed acceleration of rancidity in frozen meat to which ascorbic acid was added. Krukovsky and Guthrie (1945 and 1946) have shown that ascorbic acid definitely accelerates development of oxidized flavor in milk. Banks (1952) has observed inconsistent results using ascorbic acid for protecting fish fillets. In a number of experiments in our laboratory, the addition of ascorbic acid to meats sometimes has accelerated and sometimes has inhibited rancidity.

There is considerable evidence in the literature implicating copper in cases where ascorbic acid acts as a prooxidant. Kelley and Watts (1957) suggest that the free radical formed in the copper-catalyzed oxidation of ascorbic acid can cause the dehydrogenation of the active methylene group of the fat, which is believed to initiate fat rancidity. Using an aqueous fat system, they found that only those compounds with marked ability to chelate copper synergized with ascorbic acid to retard rancidity. In the absence of copper chelators, rancidity was invariably accelerated by ascorbic acid.

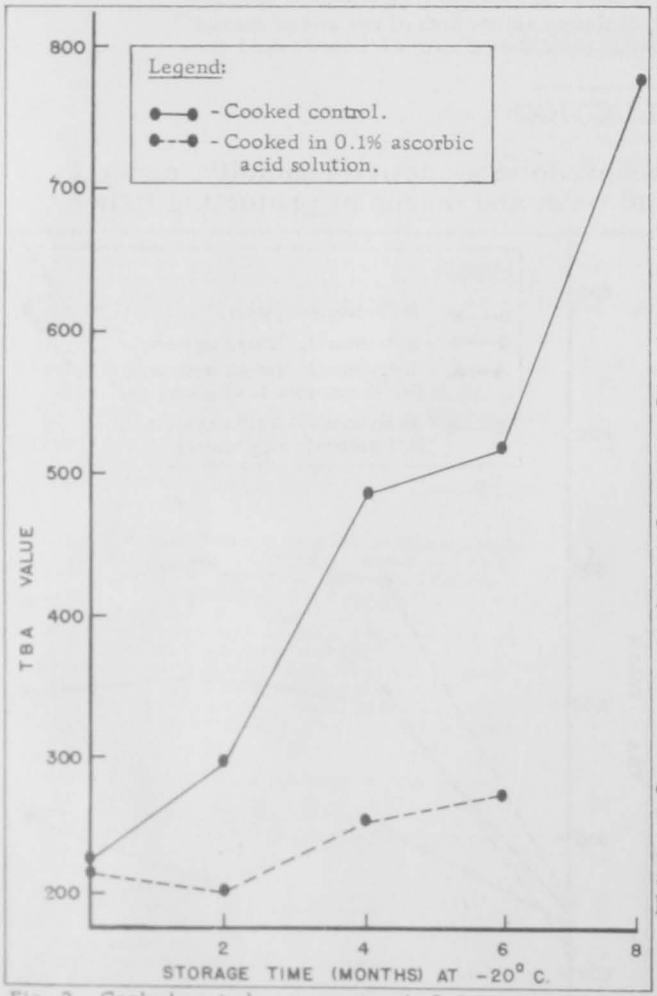

Fig. 2 - Cooked control versus oysters in 0.1-percent ascorbic acid solution.

Since the copper content of oysters varies between 0.1 and 0.2 milligrams per gram (Galtsoff and Whipple 1931 and Galtsoff et al 1947), one would expect ascorbic acid to accelerate rancidity in the oyster unless the copper is bound within the oyster tissue. The objectives of the present investigation therefore were (1) to study the antioxidant effect of ascorbic acid on oysters and (2) to measure the copper-chelating ability of oyster tissue.

SAMPLES

All oysters used in this study were of the species Crassostrea virginica, tonged from Cat Point Reef in the Gulf of Mexico. They were purchased freshly shucked from a commercial packing house in Apalachicola, Fla.

ANTIOXIDANT EFFECT OF ASCORBIC ACID

Raw oysters, if frozen, ordinarily will be frozen in bulk. Individually-frozen oysters, however, might offer advantages, since a product of this nature would aid the consumer in selecting desired quantities without having to thaw the entire pack. In previous work on cooked oysters in this Laboratory, observations indicated that the cooked refrigerated oysters

are subject to oxidative change. The experiments described in this section therefore were designed to show the antioxidant effect of ascorbic acid (1) on raw oysters (a) frozen in bulk and (b) frozen individually and (2) on oysters frozen after cooking.

PROCEDURE: Freshly-shucked oysters were divided into two groups: I and II; and those in group I were further divided into two subgroups: A and B. The oysters in group A were treated with ascorbic acid, packed in bulk, and placed in frozen storage. Those in group B were further subdivided into groups B_1 and B_2. Those in group B_1 were frozen individually, treated with a 0.5-percent solution of ascorbic acid, and placed in frozen storage. Those in group B_2 were given the same treatment except that the concentration of ascorbic acid was 1 percent. The oysters in group II were cooked in water containing ascorbic acid (this also served to inactivate catalase) and were placed in frozen storage. Suitable controls were prepared for all groups.

Table 1 - Copper-Chelating Ability of Oyster Tissue

Sample	O_2 Consumed/Hr.	Inhibition
	Microliters	Percent
Raw tissue only	16	-
Cooked tissue only	0	-
Ascorbic acid + copper	170	-
Ascorbic acid + copper + raw tissue	89	48
Ascorbic acid + copper + cooked tissue	15	91

Two methods of measuring the antioxidant effect of ascorbic acid were used: organoleptic test and thiobarbituric acid (TBA) test, with the principal emphasis being placed on the TBA test.

The details of the procedure are given in the following subsections.

Preparation of Raw Oysters Frozen in Bulk (Oysters Packed and Frozen in Their Own Liquor): One part of a 10-percent solution of ascorbic acid was added to 100 parts of raw oysters to bring the final concentration to 1 percent; sufficient natural liquor was present to insure uniform distribution of the ascorbic acid. The oysters were then packed in pint, metal, friction-top cans and stored in a freezer at -20° C. (-4° F.).

Preparation of Raw Oysters Frozen Individually: Raw oysters were placed individually on aluminum foil and frozen at -38° C. (-36.4° F.) for 25 minutes. These individually-frozen oysters were divided into two lots. One lot was dipped in a 0.5-percent solution of ascorbic acid and the other was dipped in a 1-percent solution of ascorbic acid. The oysters were put back in the freezer for approximately 2 minutes; packed in pint, metal, friction-top cans; and then stored in the freezer at -20° C. (-4° F.).

Preparation of Cooked Oysters: Cooked oysters were prepared by placing 1 pint of oysters in an aluminum frying basket, allowing them to drain, and then immersing them in $1\frac{1}{2}$ quarts of boiling distilled water and holding them for $2\frac{1}{2}$ minutes at 90° to 95° C. (194° - 203° F.). Catalase was inactivated by the treatment. The oysters, prior to cooking, were treated with ascorbic acid by adding it to the water in sufficient quantity to make the final concentration of ascorbic acid in the water 0.1 percent. The pH of the ascorbic acid was adjusted to the pH of the oysters (seasonal variation of pH occurs in oysters). After being cooked, the oysters were allowed to cool; packed in pint, metal, friction-top cans; and stored in the freezer at -20° C. (-4° F.)

Sampling and Testing: Samples of 100 grams were removed from the freezer every 2 months over a period of 6 months, and the TBA test, described elsewhere (Schwartz and Watts 1957), was employed to follow oxidative changes. Odor and flavor were evaluated by a small group of two to four persons actively engaged in

oyster research. The small size of the panel and the lack of uniform score cards and rating technique, however, did not permit statistical analysis of the results.

RESULTS AND DISCUSSION OF ANTIOXIDANT EFFECT: Raw, untreated oysters frozen in bulk showed no consistent increase in rancidity as measured by TBA value. Application of ascorbic acid therefore was not measurably beneficial. Ascorbic acid, however, did give protection against rancidity to raw oysters frozen individually and to cooked frozen oysters. The detailed results are given in the following subsections.

Raw Oysters Frozen in Bulk: Pottinger (1951) observed that the use of ascorbic acid in frozen raw oysters did not retard darkening or other quality changes in oysters during frozen storage at 0° F. for a period of 12 months. Osterhaug and Nelson (1957), working with Crassostrea gigas, the Pacific Coast oyster, found that use of ascorbic acid did not consistently or significantly extend the shelf life or prevent pigment changes in frozen raw oysters stored for 13 months.

Raw oysters frozen in bulk do not develop the typical "rancid fish" odor (Gardner and Watts 1957). In the present work, the raw oysters did not show a consistent increase in TBA value (fig. 1) but fluctuated somewhat erratically, and the addition of 1-percent ascorbic acid to the raw oysters frozen in bulk resulted in no significant differences in the TBA value between treated and untreated samples. These frozen raw oysters, with and without ascorbic acid, developed what has been described as a "grassy" odor. When the oysters were cooked, the odor disappeared, and a fresh cooked "oyster" odor was present.

Raw Oysters Frozen Individually: In contrast to the results obtained with bulk-frozen oysters as discussed above, individually-frozen raw oysters were observed to develop the "rancid fish" odor, together with an increase in TBA value (fig. 1) similar to that of cooked oysters. This odor was believed to be due to the greater exposed surface area, a condition that also exists in the cooked oysters. After 2 months' storage, a slight "rancid fish" odor was detected in the ascorbic acid-treated individually-frozen oysters along with a slight rise in TBA value. Neither the intensity of the "rancid fish" odor nor the TBA value approached that of the untreated individually-frozen oysters. Although no difference in odor could be detected between the oysters with the 0.5-percent ascorbic acid glaze and the 1-percent ascorbic acid glaze, higher TBA values were observed for oysters treated with the lower concentration of ascorbic acid (fig. 1). It is quite possible that higher concentrations of ascorbic acid employed as a glaze may inhibit the oxidative change to an even greater extent.

Cooked Oysters: Previous investigations (Gardner and Watts 1957 and Schwartz and Watts 1957) in this Laboratory indicated that cooked refrigerated oysters are subject to oxidative change with a concomitant rise in TBA value. The results obtained in the present experiment on cooked frozen oysters followed the same general pattern. Figure 2 illustrates the TBA values of cooked frozen oysters with and without 0.1-percent ascorbic acid added to the cooking water. As can be observed, oysters treated with ascorbic acid showed very little rise in TBA value. After storage for 6 months, cooked oysters treated with ascorbic acid still retained an "oyster" odor. In contrast, a slight "rancid fish" odor developed in the untreated oysters after only 2 months of storage, and the "rancid fish" odor developed in intensity, along with increased TBA values, during subsequent periods of storage.

COPPER-CHELATING ABILITY

The experiments reported in this section were devised to determine if there is a difference in copper-chelating ability between raw and cooked oysters, as had been indicated in earlier experiments in this Laboratory.

PROCEDURE: Cooper-chelating ability was determined by measuring the degree of inhibition of the copper-catalyzed oxidation of ascorbic acid in a Warburg

apparatus in the presence and absence of fresh-raw or freshly-cooked oyster tissue. A modification of the procedure employed by Frieden and Alles (1958) was used.

Homogenates of raw and cooked tissue were prepared by adding an equal amount of glass-distilled water to a known weight of oysters and blending the mixture in a Waring blendor. The homogenates were added to 0.1-percent ascorbic acid solution buffered at pH 5.8 with 0.01 M phosphate buffer in the presence and absence of 5×10^{-6} M copper solutions.

Double side-arm Warburg vessels were used. All solutions were placed in the main compartments with the exception of the copper solution, which was added to one side arm, and a 20-percent solution of sulfuric acid, which was added to the other side arm to absorb any basic volatile substances. A 30-percent solution of sodium hydroxide was added to the center well to absorb the carbon dioxide produced. Earlier trials employing single side-arm Warburg vessels consistently resulted in evolution of gas. When double side-arm vessels were used with the addition of sulfuric acid to absorb any basic volatile substances, oxygen uptake by the tissue homogenate was observed.

The vessels were attached to their specific manometers and placed in the constant temperature bath (23° C. or 73° F.). Air was the gas phase. Ten minutes was allowed for equilibration before the stopcocks were closed; the copper solution in the side arm was then tipped into the main compartment of those vessels to which it was added, and the zero reading was taken. Readings were observed at intervals of 10 minutes for 1 hour.

RESULTS AND DISCUSSION OF COPPER-CHELATING ABILITY: Cooked oysters were found to have greater copper-chelating ability than raw oysters. The fact that the cooked oysters also apparently have greater exposure of the sulfhydryl group is offered as a suggested explanation of the greater copper-chelating ability of the cooked oysters. The degree of binding or chelating of the copper by the meat determines to a large extent the efficacy of the ascorbic acid as an antioxidant. Details of the findings are given in the following two subsections.

Raw Versus Cooked Oysters: That ascorbic acid in the presence of copper becomes a strong prooxidant rather than an antioxidant has been noted (Kelley and Watts 1957). Table 1 illustrates the relative effects of raw and of cooked oyster tissue on the copper-catalyzed oxidation of ascorbic acid. Other experiments performed by the authors indicate that raw tissue exhibits a 50- to 60-percent inhibition of this reaction. Observations on cooked tissue showed an inhibition of 80 to 90 percent.

Sulfhydryl Groups: It was therefore reasonable to assume that some change occurs during the cooking of oysters that imparts to the cooked tissue its increased copper-complexing ability. Barbu, Lessian, and Macheboeuf (1949) have shown that treatment of proteins with strong alkali produces sulfhydryl groups, which then combine with copper. The presence of sulfhydryl groups in oysters was indicated by a test described by Chinard and Hellerman (1954). A drop of concentrated ammonium hydroxide followed by a drop of 5-percent nitroprusside was added to the oyster surface. In the presence of free sulfhydryl groups, a deep rose color developes. This deep rose color was noted in the cooked oysters; the raw oysters exhibited only a light pink. Greater exposure of sulfhydryl groups thus may account for the greater copper-chelating ability of cooked oysters.

CONCLUSIONS

1. Untreated raw oysters frozen in bulk and stored at -20° C. (-4° F.) showed no consistent increase in organoleptic value or TBA value, and treatment with a 1-percent ascorbic acid solution resulted in no appreciable difference in organoleptic value or TBA value between treated and untreated samples.

2. Untreated raw oysters frozen individually and stored at -20° C. (-4° F.) developed a rancid odor and increased TBA value. Use of 0.5-percent and 1-percent ascorbic acid glazes retarded, to some extent, oxidative change. The 1-percent ascorbic acid glaze was seemingly more effective than was the one of lower concentration.

3. As was to be expected, the oysters frozen in bulk showed lower TBA value on storage at -20° C. (-4° F.) than did the oysters frozen individually. The untreated bulk-frozen oysters also showed lower TBA value in storage than did the individually-frozen oysters that had been given an ascorbic acid glaze.

4. Untreated cooked oysters, frozen and stored at -20° C. (-4° F.) developed a rancid odor and increased TBA value. Cooking the oysters in 0.1-percent solution of ascorbic acid resulted in a definite decrease in rancidity and lower TBA value.

5. In confirmation of earlier experiments in this Laboratory, cooked oyster tissue was observed to be a more effective inhibitor of the copper-catalyzed oxidation of ascorbic acid than was raw oyster tissue. It is suggested that a greater exposure of sulfhydryl groups in cooked tissue may be responsible for its greater copper-chelating ability.

6. The effectiveness of ascorbic acid as an antioxidant in oysters is largely determined by the degree to which the physiological copper is "bound" by the meat and by the marketing form in which the oyster is offered to the consumer.

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