THE VALIDITY OF PEROXIDE VALUES AND OPTICAL DENSITIES AS MEASURES OF THE QUALITY OF FROZEN CHINOOK SALMON

by

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THE VALIDITY OF PEROXIDE VALUES AND OPTICAL DENSITIES AS MEASURES OF THE QUALITY OF FROZEN CHINOOK SALMON

CHAPTER I

INTRODUCTION

Pacific coast salmon is of the genus Oncorhynchus and is divided according to Cobb (9) and Davidson (14) into five different species:

1. Oncorhynchus tschawytscha (Walbaum). This species is commonly known as Tyee, Quinnat, Spring, King or still better as Chinook salmon.

2. Oncorhynchus nerka (Walbaum). Its common names are Red, Sockeye, or Blueback salmon.

3. Oncorhynchus kisutch (Walbaum). This salmon is known as Silver, Coho or Medium Red salmon.

4. Oncorhynchus keta (Walbaum). This salmon is referred to as the Chum or Dog salmon.

5. Oncorhynchus gorbuscha (Walbaum). This type is commonly known as Pink or Humpback salmon.

Three of these five species have been preserved by freezing on a commercial scale. They are the King or Chinook salmon, the Silver or Coho and the Chum salmon. The amounts of chinook salmon frozen annually exceed those of the other two species.

Aims of the Investigation:

The success of every industry is very much enhanced when a reliable and precise method for the determination of the quality of its products is available. The frozen salmon industry is no exception and, consequently, will find it to its benefit if such a method is offered to it for use.

There are a number of objective methods which have been proposed for that purpose, but which thus far have been found to be inadequate in one respect or another. Examples of these methods fall under physical and chemical categories. Of the first category are changes in electrical conductivity, in refractive index and in firmness, while chemical tests would include colorimetric determination of tyrosine, hydrogen sulfide test, volatile reducing substances, trimethylamine, peroxide value.

Reay and Shewan (30), reviewing the spoilage of fish, wrote "Attempts to measure changes in the physical state of the fish flesh as, for example, alteration in electrical conductivity (Stansby and Lemon 1933) (39); in refractive index of muscle juice (Sidaway, 1941) (31); and in firmness (Charnley and Bolton, 1938) (7): Tauti et al (1931) (45) have not been successful in providing a sensitive method of assessing quality."

The colorimetric method for tyrosine was developed by Bradley and Bailey (5). They regarded it as a broad index of autolytic and bacterial degradation of protein. Reay and Shewan (30) regard the tyrosine estimation as unsuitable as the basis for a system of quality grading.

As for the hydrogen sulfide test proposed by Boury (4), it was stated by Reay and Shewan (30) that the determination of hydrogen sulfide cannot be recommended for the estimation of quality. They go on to say that the original values for this substance in fresh fish vary considerably and its production is irregular.

The production of volatile reducing substances claimed by Lang et al (22) in 1944 to be correlated fairly closely with recognizable stages of deterioration, was later looked upon by one of his co-workers (Farber) (17) as a means of chemically evaluating the amount of odoriferous substances in a sample, regardless of subjective interpretation. At any rate it is ruled out for quality control work on account of complexity of apparatus and length of time required for an analysis (30).

Wood et al (49) and Dyer et al (15) advocated the measurement of the pH of the surface of fish by contact glass electrode as a suitable index of general quality. Elliott (16) has examined the suitability of this method for quality grading and rather doubts the general

reliability of the surface pH test in routine examination of fish.

The titration test proposed by Stansby and Lemon (1933) (38), where they followed spoilage by measuring a titration value (A) between pH 6 and pH 4.3 and a corresponding rise in titration value (B) between the original pH of the fish and pH 6, was found to be irregular by Nickerson and Proctor (28) and by Cutting (11, 12).

As for the trimethylamine test, Sigurdsson (32) has shown that rancidity may result in marked loss of quality before trimethylamine production commenced.

It is of no surprise then that Farber (17), in reviewing the methods for the determination of spoilage in fish, states that "None of the tests have proved of sufficient general usefulness to warrant their widespread adoption."

Since peroxide value is considered the most important test for oily products (32), although never proven reliable enough to detect rancidity in all types of fats and oils (7), it was chosen to represent the chemical side of this investigation.

A spectrophotometric method for the determination of optical density of the oil extract was chosen for investigation and elucidation, due to its rapidity and promising nature (18).

Another aim of this work was to investigate the effectiveness of ascorbic acid as an anti-oxidant and as a preservative of the edible quality of chinook salmon. Several investigators (3, 39, 40, 41, 42, 43, 44) have claimed that ascorbic acid treated samples had lower peroxide values and better keeping quality than salmon samples which received no treatment, but these results were at variance with the results reported by other workers (6, 26). In either case, the results were not tested statistically, hence it is evident that a test designed and analyzed statistically is a necessity to settle this point. The design of this experiment allowed carrying out such a test.

Several investigators (3, 18, 29, 36, 37, 41, 42) have reported color and flavor changes during the frozen storage of salmon. So the fourth phase of the aims of this work was to follow closely such changes.

One of the phases which arose during the course of this work was the investigation of the validity and precision of some of the methods proposed for the determination of peroxide values (6, 19).

CHAPTER II

REVIEW OF LITERATURE

Methods for Peroxide Determination:

It is well known that such factors as light, temperature, moisture, metals and others have a definite effect on the extent of oxidative rancidity. Brocklesby (7), in listing several methods for the detection of rancidity, stated that they are unreliable due to the effect of various other oxidation accelerators besides peroxides.

Wheeler (48) proposed a method for the determination of peroxide content, but stated that it was not an infallible index but rather an indication of the extent of oxidation. His method (48) dissolves the oil or fat in a 6:4 mixture of acetic acid and chloroform. Iodine liberated upon the addition of saturated potassium iodide to the mixture is estimated by titration against sodium thiosulfate of known normality using starch as an indicator.

Stansby (34) adapted Wheeler's method to the determination of rancidity in fish by extracting finely ground fish flesh with ethyl ether in presence of anhydrous sodium sulfate. Then he determined peroxide value in this ether extract according to Wheeler's method.

Stansby (35) in 1941, investigating the determination of peroxide values in fish oils, called attention to some precautions such as:

1. Do not allow the oil to stand with solvent before adding potassium iodide.

2. Use the best grade of acetic acid available.

He also suggested the following modifications to Wheeler's (48) method:

1. Use only 10 ml. of solvent in place of 50 ml. as recommended by Wheeler.

2. Allow reaction mixture to stand for one minute in the dark rather than to swirl the flask as suggested by Wheeler (48).

3. Use same weight of oil for all tests.

4. Add 50 ml. of 0.1 N hydrochloric acid in place of water at the end of the reaction. The end point is then characterized by a more rapid break in the emulsion, giving a water clear upper layer.

Tarr (41) in 1947, for peroxide value determination in fish, extracted the oil from the minced flesh with chloroform in presence of pure anhydrous sodium sulfate. After leaving it to stand in the dark for 15-30 minutes, he filtered the mixture through filter paper with suction. He then estimated the peroxide values using Wheeler's (48) method.

Brock (6) introduced some modifications on Tarr's method for his work on pink salmon, and claimed the use of these modifications were to an advantage. This method is described in the chapter of procedures.

Hartman and White (19) in 1952 proposed some modifications and claimed better results by using their modifications.

Brocklesby (7) stated that the peroxide values as used by several investigators have the disadvantage of lack of consistent agreement with organoleptic results.

Naumann et al (27), working on frozen ground pork, concluded that flavor, odor and peroxide values were not found to be closely associated with each other.

Stansby (35) stated that at least with fish oils precision is often poor, end points are vague and correlation between peroxide number and organoleptic rancidity is uncertain. He also listed several factors which affect the peroxide determination. Some of these are: sample size, exposure to air, light, decreasing ratio of chloroform to acetic acid and time elapsed between addition of solvent to the oil and the addition of the iodide.

Brock (6) found no conclusive evidence that a correlation exists between peroxide values and taste test scores in frozen pink salmon.

Pigments of Salmon:

Bailey (2) states that Sorensen isolated astacin, or astaxanthin, from salmon oil in 1935. Bailey (1) has shown, in 1937, that the red color of both sockeye salmon (Oncorhynchus nerka) and red spring salmon--chinook (Oncorhynchus tshawytscha), and also the steelhead (Salmo gairdnerii) is due to the presence of two astacin-like pigments.

Bailey et al (2) and Tunison et al (46) state that astacin and astaxanthin are carotenoids responsible for the coloration of many marine products and their oils. Bailey et al (2) states that astacin, tetraketo-β-carotene occurs in fish oils in the form of esters. According to Lederer (23), the free pigment is insoluble in water, very slightly soluble in ethyl ether, petroleum ether and methyl alcohol, somewhat more soluble in benzene and ethyl acetate, and very soluble in chloroform, carbon disulphide dioxane and pyridine. Astacin bleaches slowly when an oil containing it is exposed to light and is rapidly destroyed by aeration of the oil at a high temperature. The absorption spectrum of astacin has a single maximum at approximately 500 mu, although the exact position varies slightly in different solvents.

Color Changes in Frozen Salmon:

In 1944, Stansby and Harrison (37) reported that during the storage of frozen fillets, drastic changes in the color of pink salmon occurred. It became yellow to gray in two months. Chum salmon was badly discolored in six months. Silver salmon showed some fading in six months but not nearly so much as the other salmon fillets. They also reported changes in flavor with all species.

They stated that "Alteration in the appearance of frozen pink salmon fillets occurs in both the pink muscle and in the dark muscle and associated oil, the latter being the more phenomenal. Two types of changes develop:

1. There is an intensification in the yellow pigment which tends to give a generally yellow appearance to the fish, and

2. A deepening of the color of dark muscle tissue."

They explained the intensification of the yellow pigment and later development of the brown color as due to oxidation by air.

They noticed that chum salmon showed similar changes but at a slower rate while changes in frozen silver salmon fillets were less pronounced.

Tarr (41), in 1947, wrote "The development of rancidity in red salmon is accompanied by superficial bleaching of the red pigments, which is particularly evident in regions where the flesh is exposed to atmospheric oxygen." In this experiment he was working with coho salmon and red spring salmon.

In another paper published in the same year, Tarr (42) states "In fatty fish such as herring, pilchards and some species of salmon, the oil which exudes on the surface becomes yellow or brownish in color and of gummy consistency, a condition referred to as 'rusting'. In salmon, the red pigments of the flesh may become badly bleached, while in white fleshed fish a slight brown discoloration may develop on prolonged storage."

Bauernfein (3), in 1948, noticed extremely yellow discoloration and rancidity in pink salmon after six months storage at 10°F.

Dassow and Stansby (13), in 1949 used the method proposed by them earlier in 1942 (36) to follow the color changes in red (or sockeye) salmon. This method depends on taking a color transparency of a standard object with that of the object under investigation, then analyzing the spectrographic transmission of these color transparencies. They assumed in their work that no changes in the color of the interior of the fish occurs. The literature offers no

substantial evidence for this assumption. Also, the method included some technical difficulties (36) such as the necessity of using only one roll of film and standardizing light exposures.

Gupta (18), in 1951, using a spectrophotometric method for the determination of color, reported color changes in the surface of frozen pink salmon fillets as compared to the color of the interior of the fillets. He also assumed--as Dassow and Stansby (13) did--that the color of the interior is stable throughout the storage period.

Another observation in this effect was made by Norton et al (29) in 1952 when they were investigating the use of monosodium glutamate in frozen foods. They reported that color changes took place as the storage period of frozen silver salmon steaks increased. They also reported degradation in flavor during the storage period.

Ascorbic Acid Treatment:

In 1945, Tarr (39) reported lower peroxide values for ascorbic acid treated red spring salmon and herring than those of the control. He then concluded that ascorbic acid retarded rancidity. In 1946, Tarr (40) claimed that either dipping or glazing red spring salmon with ascorbic acid retarded rancidity. Again, in 1947, Tarr (41) found

that ascorbic acid retarded rancidity in coho and red spring salmon flesh. In 1948, Tarr (43) reported that l-ascorbic acid and d-isoascorbic acid and their sodium salts (pH 6.0) gave approximately equal protection to fish against rancidity. In 1948, Bauernfein (3) reported using l-ascorbic acid effectively in the prevention of rancidity and discoloration of pink salmon by either dipping or glazing.

Mathers (26) reported, in 1950, that a pretreatment consisting of dipping in a solution of Krim Ko Gel, l-ascorbic acid and Tweens 81 was evaluated by a panel as being higher in flavor quality than the controls.

Tarr and his associates (44), in 1951, using a 1% ascorbic acid treatment and another treatment of 0.3% ascorbic acid and 0.5% high viscosity carrageen by dipping, reported that they apparently were equal in their effectiveness in retarding rancidity.

CHAPTER III

PROCEDURES

Source of Fish:

In the second half of September, 1951, chinook salmon steaks were obtained already cut and eviscerated from a commercial source. Two to three steaks were taken from the middle portion of the fish. The thickness of the steak was about 1-3/8 inch and weighed about 12 ounces depending on the size of the fish.

Preparation of the Samples:

To allow direct comparison between ascorbic acid treatment and no treatment, each steak was cut in half; one half was treated with ascor ic acid by dipping it in a 2% solution of l-ascorbic acid for one minute, then it was drained, wrapped in a polyethylene bag, the air squeezed out of the bag and then put in a waxed carton. The non-treated half-steaks were packed in the same manner without the ascorbic acid treatment. The fish was immediately quick frozen and stored at 0°F. This was carried out on the same day that the fish was obtained from the fishery at The Sea Foods Laboratory in Astoria. Later they were transferred to the frozen storage room of The Food Technology Department.

Oil Extraction from the Fish:

It was found necessary to use a sample unit of two half-steaks to be able to obtain enough fish material to carry out the different tests. The cartons containing the half-steaks were drawn from the freezer according to the table of random numbers. The method used for the preparation of the fish for the different tests was essentially that followed by Brock (6) and Gupta (18) with some modifications. The different steps were as follows:

1. A representative sample weighing from 130-140 gr. was obtained by cutting off portions of the abdominal and dorsal fat as well as the other parts of the steak while it was still in the frozen stage, and then left to thaw over night in glass beakers. The remaining parts of the steaks were then put back in the polyethylene bags and these in the waxed carton and returned to the freezer room till the next night when they were withdrawn to run the taste tests.

2. The thawed fish for the laboratory tests were chopped into small cubes put in a waring blender with 175 ml. U.S.P. chloroform and 45 grams of anhydrous sodium sulfate, and blended for 40 seconds.

3. It was then transferred to a Mason jar, covered and put at 0°F. for 45 minutes to break the emulsion.

4. This blended sample was then filtered through nylon cloth with the help of squeezing by the hand. The resultant extract was received through a glass funnel in a 250 ml. conical flask.

5. To absorb any excess moisture in the extract, about 20 grams anhydrous sodium sulfate were then added to the extract and then the extract was allowed to stand in the dark for two hours.

6. Then the extract was filtered, using suction through a filter paper moistened with chloroform and fitted to a Buchner funnel.

7. The extract obtained this way was clear and to determine the percentage of oil in it, a 10 ml. sample was put in a previously weighed 125 ml. conical flask and the chloroform was then driven off on a steam-bath. The flasks containing the oil were then dried in an oven at 100°C. to a constant weight. This took about 10-15 minutes.

Optical Density Measurements:

1. Solutions of the original extracts were made up in volumetric flasks so that they would contain 1.68 grams of oil per 100 ml. (18). This concentration gives optical densities in the range of 0.2 to 0.8 approximately where reasonable accuracy is attained (25).

2. Optical density readings were then observed on a Beckman-quartz D.U. spectrophotometer using fused silica cells. The slit width used was about 0.01 mm.

Peroxide Values:

The method used by Brock was approximately followed in the beginning of this investigation. The steps followed were:

1. Twenty ml. of the clear chloroform extract filtrate were transferred to a 250 ml. conical flask.

2. Thirty ml. of pure glacial acetic acid were added, followed by two drops of saturated potassium iodide solution. The flask was then kept stoppered in the dark for 10 minutes.

3. At the end of the 10-minute period, 50 ml. of distilled water were added and the liberated iodine was titrated with 0.002 N sodium thiosulfate using approximately 2 ml. of acid starch as indicator.

4. The amount of oil used for each determination was calculated from the weight of oil obtained after evaporating the chloroform as previously described.

5. The peroxide values were calculated according to the following formula and were expressed as ml. of 0.002 N sodium thiosulfate per gram of oil:

$$\frac{\text{N. } Na_2S_2O_3 \times \text{Ml } Na_2S_2O_3}{0.002 \times \text{weight of oil in grams}}$$

Hartman and White's method:

During the course of this investigation (1952), Hartman and White (19) proposed changes in the method of determining the peroxide values. The author applied this new method with some modifications to adapt it to the fish-oil-extract along with the originally used method and this originally used method with an atmosphere of nitrogen in the subsequent openings. Hartman and White's (19) method as used by the writer is as follows:

1. A citric acid reagent was prepared by dissolving 10 grams of citric acid B.P. quality in 60 ml. of hot tertiary-butyl alcohol followed by the addition of 35 ml. of carbon tetrachloride.

2. Ten ml. of the oil extract were transferred to a conical flask, dissolved in 20 ml. of solvent and de-aerated with nitrogen. One ml. of saturated potassium iodide solution was added and the flow of nitrogen was discontinued.

3. The flask was stoppered and left in the dark for 15 minutes.

4. The contents of the flask were then diluted with 50 ml. distilled water and titrated with 0.002 N sodium thiosulfate, using starch as indicator.

5. The peroxide values were expressed as ml. of 0.002 N sodium thiosulfate per gram of oil using the previously mentioned formula.

Comparison between these three methods for determining the peroxide values will be embodied with the general discussion on peroxide values.

Flavor Testing:

The remaining portions of the samples were withdrawn from the freezer, allowed to thaw and then prepared for the taste panel consisting of four members of the Food Technology Department whom Brock (6) had found to be able to detect differences in salmon. The four members participated in all the flavor tests carried out with the exception of one member who was unable to participate in the last flavor test only. The procedure comprised the following steps:

1. The thawed chinook salmon samples were seasoned by dipping in a 5% sodium chloride solution for two minutes.

2. The samples were then allowed to drain, followed by rolling them in cracker crumbs.

3. For baking, the samples were placed in pyrex plates, and baked in an oven at 550°F. for about 35-40 minutes.

4. The samples were then macerated, the bones removed and the samples submitted to the flavor test panel as homogeneous as possible. At each sitting, the panel tested six sample units--three treated with ascorbic acid and three not treated. The two sample units--treated and not treated--coming from the same steaks (by splitting each steak into two halves as previously described) were put on the same plate and the panel were asked to test them in comparison to each other and also in comparison to the rest of the sample units. This allowed direct comparison of the two treatments represented by the scores of the three sample units in each treatment. Since the members of the panel were already found by Brock (6) to be consistently able to detect the differences in salmon flavor, no attempt was made to validate this point. The members of the panel scored the salmon according to the following scheme commonly used in the Food Technology Department at that time:

10 - ideal	5 - acceptable
9 - excellent	4 - fair
8 - very good	3 - poorly fair
7 - good	2 - poor
6 - fairly good	1 - very poor
	0 - repulsive

Experimental Design:

As mentioned before, a sample unit of two half-steaks was used. Six of the ascorbic acid treated samples and six of the non-treated samples were withdrawn in each opening giving us three observations for each test run on the ascorbic acid treated and non-treated samples in each opening. The time lapse between two consecutive openings was eight weeks. There were five openings giving 30 observations for each test. The statistical design of the experiment is a split plot and is shown as follows:

Source of Variation	No. of degrees of freedom
Storage period	4
Error (a)*	10
Treatment	1
Interaction between treatment and storage period	4
Error (b)**	10
Total	29

*Error (a) is the variation among the whole salmon steaks within the respective storage periods (includes treated and non-treated).

**Error (b) is variation between half-steaks comprising one whole steak used in the whole investigation.

The 5% significance level is used throughout the experiment unless otherwise indicated.

CHAPTER IV

RESULTS AND DISCUSSION

Data on the three variables peroxide value, optical density, and flavor scores were obtained. These results are shown in Tables 1, 2, 3, 4, and 8. The appearance and color of the frozen steaks remained normal for the first and second openings, that is, for the first 16 weeks, but the writer noticed some changes in these qualities in the third opening, that is, after 24 weeks of frozen storage. These changes included darkening of the color of the fatty portions of the steaks. The protein appeared as fibrous and ice crystals covered the steaks. The appearance of the ice crystals could be due to irregularities in the freezing room to which the samples were transferred from the old Food Technology building. These changes remained practically the same through the following openings, that is, up until 10 months of frozen storage.

The reader, upon looking on the peroxide values in Table 1, will notice some discrepancies. These discrepancies rendered the statistical analysis of these values over all the investigation pointless. However, a statistical comparison of the different methods for determining the peroxide values that were used in the latter

part of this investigation was carried out and will be mentioned in the general discussion of the peroxide values.

A statistical analysis was carried out on both the optical densities and flavor scores. The correlation between these two variables was also investigated. The writer will start the discussion with the optical density.

Optical Density:

The maximum absorption of the extract in the first opening was at 460 mu. However, this maximum absorption shifted to lower wave-lengths as storage proceeded. Figure 1 shows the absorption curve for two sample units from the first and last openings respectively and illustrates this shift. The shift made desirable the determination of the optical density at more than one wave-length in order to determine which wave length would be most efficient. The optical densities for the wave lengths 450 mu, 460 mu and 470 mu common to all storage periods are shown in Tables 2, 3, and 4. The results of the analysis of variance of the optical densities at these three wave lengths are summarized in Tables 5, 6, and 7. Examination of these results indicate that:

1. The storage period has a definite significant effect on the optical densities at any of the three different wave lengths mentioned above, and the longer the

TABLE 1

PEROXIDE VALUES DETERMINED BY THE METHOD USED BY BROCK

	Treated		Not Treated	
First	0.00	0.00	3.05	3.05
	3.62	3.03	4.53	4.53
	3.04	1.39	6.04	11.27
Second	--	--	4.78	7.59
	34.18	29.02	16.32	14.23
	37.17	36.92	33.73	34.42
Third	3.62	2.86	10.12	9.71
	3.64	3.55	19.98	10.51
	6.09	2.26	6.91	14.82
Fourth	19.89		26.24	
	41.99		73.43	
	52.69		36.45	
Fifth	43.09		86.18	
	11.90		39.87	
	16.67		26.77	

storage period the greater the change in optical density.

2. The optical densities measured at 450 mu. and 460 mu. show no significant difference between the samples treated with ascorbic acid and the non-treated samples, while the optical densities obtained at 470 mu. show a significant difference between the treated and non-treated samples. This can be due to higher sensitivity of optical density measurements at this particular wave length than at the other two. Another possibility is that it is due to chance error the magnitude of which is 5%.

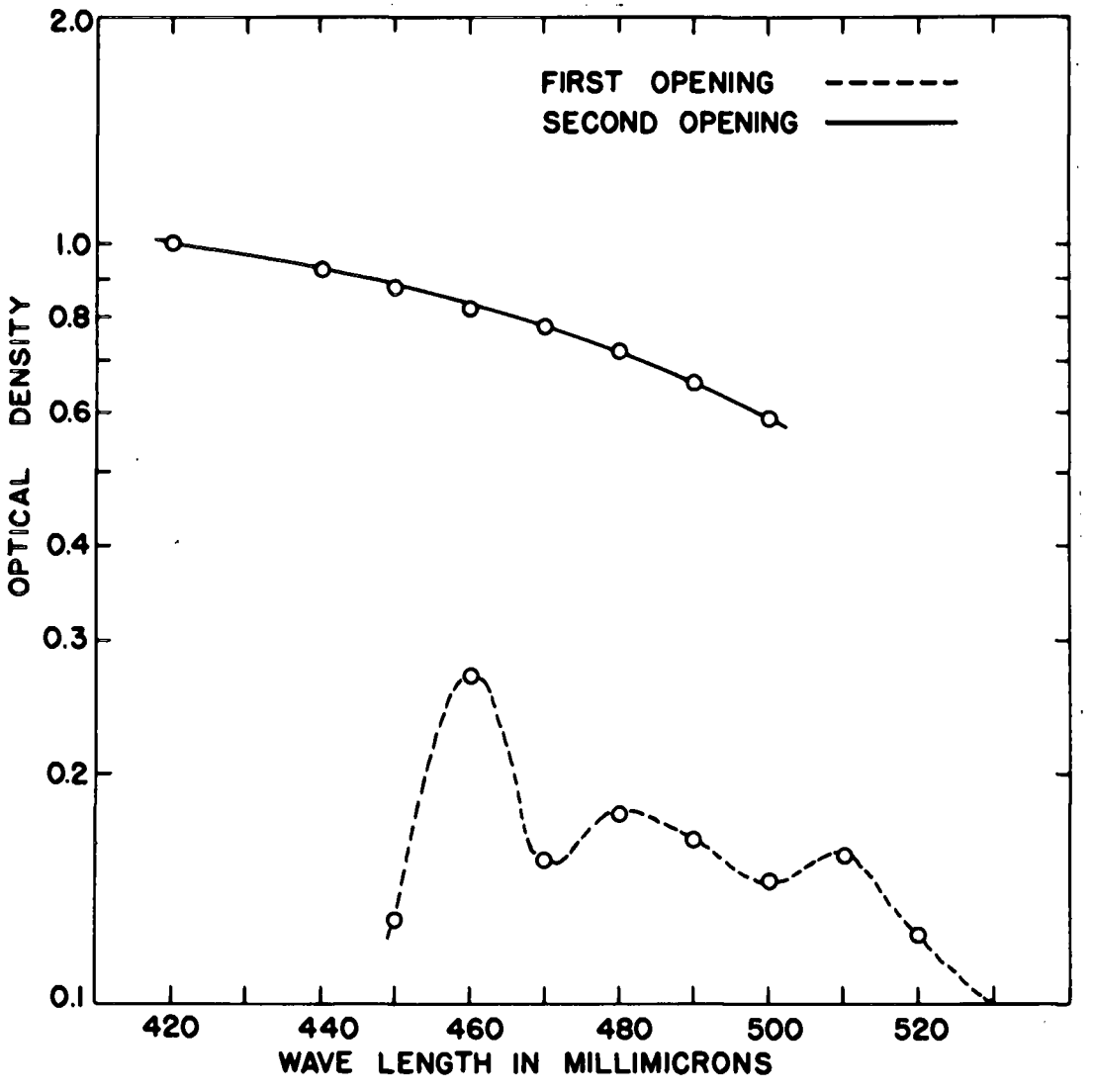

FIGURE 1. REPRESENTATIVE OPTICAL DENSITY CURVES OF CHLOROFORM EXTRACTS.

TABLE 2

OPTICAL DENSITIES OF CHLOROFORM-EXTRACT AT 450 MU.

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	0.144	0.184	0.650	0.878	0.895
	0.150	0.266	0.588	0.942	0.860
	0.238	0.370	0.564	0.861	0.921
Not Treated	0.138	0.184	0.612	0.920	0.882
	0.164	0.223	0.534	0.852	0.838
	0.128	0.445	0.502	0.876	0.890

Averages* for each opening

	Based on 3 observations		Based on 6 observations
Opening	Treated	Not treated	
First	0.177	0.143	0.160
Second	0.273	0.284	0.279
Third	0.601	0.549	0.575
Fourth	0.894	0.883	0.888
Fifth	0.892	0.870	0.881

*Averages rounded out to third decimal point

TABLE 3

OPTICAL DENSITIES OF CHLOROFORM-EXTRACT AT 460 MU.

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	0.183	0.201	0.625	0.858	0.845
	0.218	0.270	0.548	0.938	0.818
	0.367	0.242	0.524	0.842	0.895
Not Treated	0.176	0.195	0.608	0.910	0.828
	0.198	0.222	0.506	0.830	0.782
	0.207	0.278	0.472	0.866	0.850

Averages* for each opening

	Based on 3 observations		Based on 6 observations
Opening	Treated	Not treated	
First	0.256	0.194	0.225
Second	0.238	0.232	0.235
Third	0.566	0.529	0.547
Fourth	0.879	0.869	0.874
Fifth	0.853	0.820	0.836

*Averages rounded out to the third decimal point

TABLE 4

OPTICAL DENSITIES OF CHLOROFORM-EXTRACT AT 470 MU.

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	0.138	0.077	0.600	0.790	0.792
	0.182	0.145	0.508	0.870	0.761
	0.293	0.178	0.485	0.780	0.855
Not Treated	0.122	0.068	0.570	0.840	0.785
	0.154	0.096	0.460	0.760	0.726
	0.154	0.188	0.428	0.795	0.805

Averages* for each opening

	Based on 3 observations		Based on 6 observations
Opening	Treated	Not treated	
First	0.204	0.143	0.174
Second	0.133	0.117	0.125
Third	0.531	0.486	0.509
Fourth	0.813	0.798	0.806
Fifth	0.803	0.772	0.787

*Averages rounded out to third decimal point

TABLE 5

ANALYSIS OF VARIANCE OF OPTICAL DENSITIES AT 450 MU.

Variation Due to:	Degrees of Freedom	Mean Square	F	Remarks**
Storage period	4	0.67467687	96.43	Significant
Error (a)*	10	0.00699690		
Treatment	1	0.00347764	2.66	Not Significant
Treatment X period interaction	4	0.00082180	0.63	Not Significant
Error (b)*	10	0.00130917		
Total	29			

*For definition of error a and b see page 21

**At the 5% significance level

TABLE 6

ANALYSIS OF VARIANCE OF OPTICAL DENSITIES AT 460 MU.

Variation Due to:	Degrees of Freedom	Mean Squares	F	Remarks**
Storage period	4	0.58788222	153.28	Significant
Error (a)*	10	0.00383543		
Treatment	1	0.00663053	3.95	Not Significant
Treatment X period interaction	4	0.00076912	0.46	Not Significant
Error (b)*	10	0.00167910		
Total	29			

*For definition of error a and b see page 21

**At the 5% significance level

TABLE 7

ANALYSIS OF VARIANCE OF OPTICAL DENSITIES AT 470 MU.

Variation Due to:	Degrees of Freedom	Mean Square	F	Remarks**
Storage period	4	0.63143959	131.74	Significant
Error (a)*	10	0.00479323		
Treatment	1	0.00843364	6.37	Significant
Treatment X period interaction	4	0.00057938	0.44	Not Significant
Error (b)*	10	0.00132463		
Total	29			

*For definition of error a and b see page 21

**At the 5% significance level

TABLE 8

AVERAGE SCORES OF SELECTED PANEL OF 4

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	5.00	5.00	6.00	3.00	5.00
	5.75	4.75	5.60	4.75	5.00
	5.75	4.75	3.60	5.00	4.66
Not Treated	6.00	5.00	5.50	4.00	5.00
	6.00	5.75	5.00	4.50	5.00
	6.50	6.00	4.50	4.00	5.33

Averages for each opening

	Based on 3 half-steaks		Based on 6 half-steaks
Opening	Treated	Not Treated	
First	5.50	6.17	5.83
Second	4.83	5.58	5.21
Third	5.07	5.00	5.03
Fourth	4.25	4.17	4.21
Fifth	4.89	5.11	4.998

TABLE 9

ANALYSIS OF VARIANCE OF FLAVOR SCORES

Variation Due to:	Degrees of Freedom	Mean Square	F	Remarks**
Storage period	4	2.024745	3.92	Significant
Error (a)*	10	0.516587		
Treatment	1	0.666030	2.71	Not Significant
Treatment X period interaction	4	0.234072	0.95	Not Significant
Error (b)*	10	0.245713		
Total	29			

*For definition of error a and b see page 21

**At the 5% significance level

3. The optical densities are not affected by any interaction between treatment and storage period, that is to say that these two factors are independent (10,33).

Flavor:

The average scores of the four members of the flavor panel assigned to chinook salmon samples submitted to them are given in Table 8. The analysis of variance of these scores is shown in Table 9. Examination of this analysis indicates that:

1. There are significant differences in the flavor of the salmon in the different openings, that is, after different storage periods. Our conclusion would be that the freezing storage of chinook salmon steaks affects its flavor adversely.

2. There is no proven difference between the ascorbic acid treated chinook salmon and the non-treated samples. That is to say that the ascorbic acid dip--as used here--had no significant effect on preserving the quality of the fish in frozen storage. This might appear to be contradicting the results reported by some other workers (3, 39, 40, 41, 43, 49) but since those workers did not induce their conclusions from a statistical analysis, there is no way of direct comparison and maybe if

they had analyzed their data statistically, they would have arrived at the same conclusion that the writer found. It may be observed that some of those workers used thickening agents in applying ascorbic acid to the fish. Tarr (44), using a 1% ascorbic acid treatment and another treatment of 0.3% ascorbic acid and 0.5% high viscosity carrageen, by dipping, reported that they were approximately equal in their effectiveness in retarding rancidity. The writer's treatment of the salmon consisted of dipping in a 2% ascorbic acid solution.

3. The flavor scores are not affected by any interaction between the storage period and treatment. Hence, these two factors are independent.

Relationship Between Optical Densities and Flavor Scores:

If we compare the results of flavor scores and those of the optical densities, we find them in agreement in all cases except one where the optical densities measured at 470 mu. showed a significant difference between the ascorbic acid treated salmon and the non-treated salmon, although the flavor scores showed no significant difference. This could be attributed to higher sensitivity of the spectrophotometer than the flavor panel or to chance error. At any rate, this agreement would indicate that these two variables are affected by the same factors

to a large extent and point out the possibility that optical density measurements might be used to measure the organoleptic status of the salmon. Correlation coefficients between these two variables--optical densities at the three different wave length and flavor scores--were calculated and are shown in Table 10. Examination of this table indicates that these correlation coefficients are not significant. Figure 2 shows the graph obtained by plotting optical densities at 460 mu. versus their corresponding average flavor scores. This graph is representative of the graphs which would be obtained by plotting optical densities at any of the three wave lengths 450 mu., 460 mu., or 470 mu., and illustrates the results obtained statistically.

It is well known that regression theory--at the present time--deals only with sampling from specific populations which have the following characteristics: (24)

(A) Each array of y follows the normal distribution.

(B) All arrays of y have the same variance.

(C) The regression function is linear.

The reason for these non-significant correlation coefficients is probably due to a departure from the last assumption.

TABLE 10

CORRELATION COEFFICIENTS BETWEEN
OPTICAL DENSITIES AND FLAVOR SCORES

	Correlation Coefficient	Degrees of Freedom	Remarks*
1. Between optical densities at 450 mu. and flavor scores:			
a.**	0.4010	9	Not significant
b.***	0.0998	9	Not significant
2. Between optical densities at 460 mu. and flavor scores:			
a.**	0.5778	9	Not significant
b.***	0.0658	9	Not significant
3. Between optical densities at 470 mu. and flavor scores:			
a.**	0.5645	9	Not significant
b.***	0.0653	9	Not significant

*At the 5% significance level.

**a, is the correlation coefficient within storage periods, i.e., among the three whole steaks in each storage period, and represents their relationships in any of the five storage periods, after elimination of the effect of treatment, storage period, error b and treatment X storage period interaction.

***b, is the correlation coefficient between the optical densities and flavor scores assigned to the halves within each steak, after removal of the effects of storage period, error a and treatment X storage period interaction.

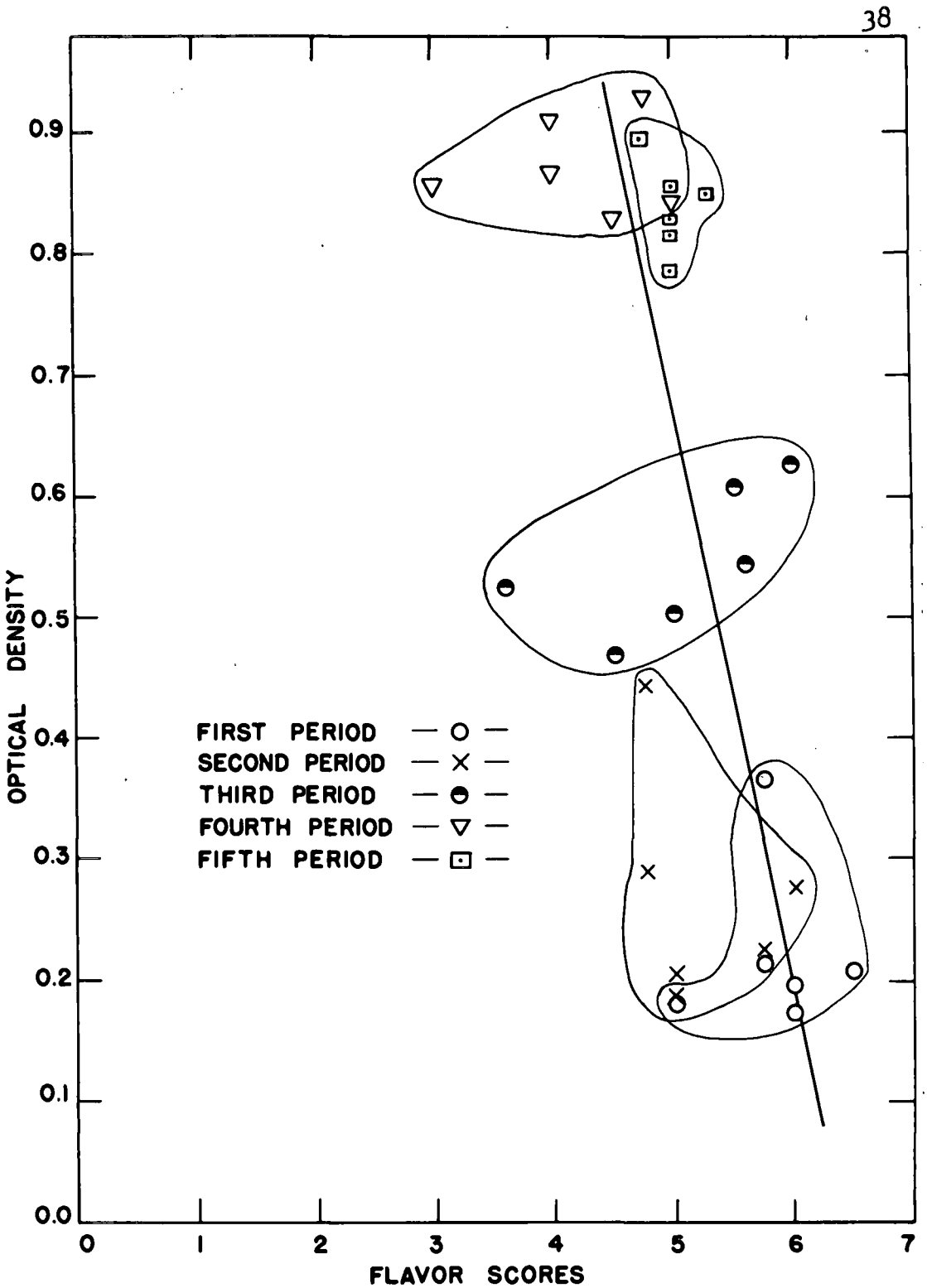

FIGURE 2. GRAPH OF OPTICAL DENSITIES AT 460 MILLIMICRONS VERSUS FLAVOR SCORES OF TREATED AND NON-TREATED SALMON HALF-STEAKS.

Kendall, in his book The Advanced Theory of Statistics, v. I., p. 383 (20), writes that ranked data arise in two ways of which one is "From material which is believed to be capable of measurement theoretically but cannot be measured in practice, e.g. human preference for food or intelligence."

It is of significance to mention here that The Statistics Department of The Virginia Polytechnic Institute, which is engaged on developing "Statistical Methods for Sensory Differences; Tests of Food Quality" with the Bureau of Agricultural Economics, United States Department of Agriculture, has been basing their methods on rank analysis. This is evident from examining their research papers embodied in the five biannual reports they have published thus far and distributed through The Virginia Agriculture Experiment Station. These reports are mentioned in the bibliography at the end of this thesis (47).

Due to the above mentioned factors, it was felt that the use of rank correlation which does not assume linearity is more appropriate to this work than ordinary correlation. The writer hence transformed the individual observations into ranks, according to the desirability. Table 11 shows the ranks of the flavor scores, the rank 1 being assigned to the lowest score. Tables 12, 13, and 14 show the ranks of the optical densities at the three

TABLE 11

RANKS OF AVERAGE FLAVOR SCORES

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	1	3	3	1	2.5
	2.5	1.5	2	2	2.5
	2.5	1.5	1	3	1
Not Treated	1.5	1	3	1.5	1.5
	1.5	2	2	3	1.5
	3	3	1	1.5	3

Ranks of average scores for each opening

	Based on 3 half-steaks		Based on 6 half-steaks
Opening	Treated	Not Treated	
First	5	5	5
Second	2	4	4
Third	4	2	3
Fourth	1	1	1
Fifth	3	3	2

1: lowest flavor score

TABLE 12

RANKS OF OPTICAL DENSITIES AT 450 MU.

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	3	3	1	2	2
	2	2	2	1	3
	1	1	3	3	1
Not Treated	2	3	1	1	2
	1	2	2	3	3
	3	1	3	2	1

1: highest optical density

Ranks of averages of optical densities for each opening

	Based on 3 observations		Based on 6 observations
Opening	Treated	Not Treated	
First	5	5	5
Second	4	4	4
Third	3	3	3
Fourth	1	1	1
Fifth	2	2	2

TABLE 13

RANKS OF OPTICAL DENSITIES AT 460 MU.

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	3	3	1	2	2
	2	1	2	1	3
	1	2	3	3	1
Not Treated	3	3	1	1	2
	2	2	2	3	3
	1	1	3	2	1

1: highest optical density

Ranks of averages of optical densities for each opening

	Based on 3 observations		Based on 6 observations
Opening	Treated	Not Treated	
First	4	5	5
Second	5	4	4
Third	3	3	3
Fourth	1	1	1
Fifth	2	2	2

TABLE 14

RANKS OF OPTICAL DENSITIES AT 470 MU.

	Opening				
	First	Second	Third	Fourth	Fifth
Treated	3	3	1	2	2
	2	2	2	1	3
	1	1	3	3	1
Not Treated	3	3	1	1	2
	1.5	2	2	3	3
	1.5	1	3	2	1

1: highest optical density

Ranks of averages of optical densities for each opening

	Based on 3 observations		Based on 6 observations
Opening	Treated	Not Treated	
First	4	4	4
Second	5	5	5
Third	3	3	3
Fourth	1	1	1
Fifth	2	2	2

different wave lengths. The rank 1 was assigned to the highest optical density. Upon examining the ranks of the optical densities with the corresponding ranks of flavor scores, the reader will notice that these ranks within a storage period agree at times and disagree at others. This reflects lack of correlation within a storage period and is probably due to individual differences among salmon fish.

The picture changes completely, however, when the reader examines the ranks of the optical densities for the storage periods based on six observations with the corresponding ranks of flavor scores. The reader here will find perfect agreement of the ranks of optical densities at 450 mu. and 460 mu. with the ranks of flavor scores. This gives a perfect correlation with either wave length and Spearman's coefficient of rank correlation (20, 21) is 1 for either wave length as calculated from the formula

$$\rho = 1 - \frac{6 \Sigma (d^2)}{n^3 - n}$$

where: ρ is Spearman's coefficient of rank correlation

Σ is the sum of

d is the difference between two corresponding ranks

n is number of ranked pairs

Since these ranks can occur in $5! = 120$ different combination, the probability of getting a complete

agreement between them by chance is 1 out of 120, and hence these two correlation coefficients are significant even at the 1% significance level.

When the storage period ranks of optical densities at 470 mu. are examined with the corresponding ranks of flavor scores, we find that they differ only in one case out of five. Spearman's coefficient of rank correlation as determined by the above formula in this case is 0.9. Upon examining Table 162 p.397 of Kendall's book "The Advanced Theory of Statistics" (20), published also in his book "Rank Correlation Methods" (21) as appendix table 2 p.142, a Spearman's rank correlation coefficient of 0.9 for n = 5 will be found significant.

The conclusion which can be reached from the above discussion is that the optical density of the chloroform extract of chinook salmon measured at any of the three wave lengths--450 mu., 460 mu. or 470 mu.--can be used as a measure of the change in the organoleptic quality of frozen chinook salmon. Since freezing is just a method utilized to extend the edible life of chinook salmon, it is felt that this method would probably prove adequate as an objective method for determining the organoleptic quality of chinook salmon preserved by methods other than freezing.

Bailey (2), on p.78, writes "Of the various carotenoid pigments, astacin, or astaxanthin as it is sometimes called, appears to be the most common in oils of marine origin." He (2) stated that Sorensen isolated it from salmon oil, and Bailey (1) has shown that the red color of both sockeye salmon (Oncorhynchus nerka) and red spring salmon (Oncorhynchus tschawytscha) and also the steelhead (Salmo gairdneri) is due to the presence of two astacin-like pigments. Hence, this method might prove applicable not only to salmon species other than chinook but also to other fatty or oily fish that contain this astacin pigment. Further investigation is recommended to elucidate this point.

Peroxide Values:

Hartman and White (19) proposed a method in March, 1952, for determining the peroxide values in fats and oils. This method was adopted by the writer for the determination of these values in fish oils in his present work. This method was used in obtaining the peroxide values of the salmon in the last two openings along with the method used by Brock (6) and this latter method as run in an atmosphere of nitrogen. These values are reported in Table 15. The analysis of variance was used to compare these three methods. Its results are shown in Tables 16,

TABLE 15

PEROXIDE VALUES OBTAINED BY THREE METHODS
IN THE FOURTH AND FIFTH OPENINGS

	Treated			Not Treated		
	Method			Method		
Opening	1	2	3	1	2	3
Fourth	19.89	18.09	9.77	26.24	29.74	11.13
	41.99	17.88	10.89	73.43	18.00	12.60
	52.69	10.78	7.78	36.45	17.48	11.24
Fifth	43.09	0.00	0.00	43.09	0.00	8.30
	11.90	11.24	5.51	39.87	13.07	8.71
	10.67	9.60	4.002	26.77	20.35	10.71
	180.23	67.59	37.95	245.85	98.64	62.69

1: The method used by Brock

2: The method used by Brock in an atmosphere of N_2

3: The method proposed by Hartman and White

TABLE 16

ANALYSIS OF VARIANCE OF THE PEROXIDE VALUES OBTAINED BY THE METHOD USED BY BROCK, IN THE FOURTH AND FIFTH OPENINGS

Variation Due to:	Degrees of Freedom	Mean Square	F	Remarks**
Storage Period	1	472.50750	1.02	Not Significant
Error (a)*	4	462.774467		
Treatment	1	358.83203	1.87	Not Significant
Treatment X Period Interaction	1	42.26254	0.22	Not Significant
Error (b)*	4	191.844325		
Total	11			

*For definition of errors a and b see page 21

**At the 5% significance level

TABLE 17

ANALYSIS OF VARIANCE OF THE PEROXIDE VALUES OBTAINED BY THE METHOD USED BY BROCK WHEN RUN IN AN ATMOSPHERE OF NITROGEN IN THE FOURTH AND FIFTH OPENINGS

Variation Due to:	Degrees of Freedom	Mean Square	F	Remarks**
Storage Period	1	277.537003	3.17	Not Significant
Error (a)*	4	87.6516675		
Treatment	1	30.341875	4.83	Not Significant
Treatment X Period Interaction	1	2.891011	0.17	Not Significant
Error (b)*	4	16.634066		
Total	11			

*For definition of errors a and b see page 21

**At the 5% significance level

TABLE 18

ANALYSIS OF VARIANCE OF THE PEROXIDE VALUES OBTAINED BY HARTMAN AND WHITE'S METHOD IN THE FOURTH AND FIFTH OPENINGS

Variation Due to:	Degrees of Freedom	Mean Square	F	Remarks**
Storage Period	1	57.116033	12.86	Significant
Error (a)*	4	4.4415835		
Treatment	1	51.005633	25.26	Significant
Treatment X Period Interaction	1	11.368534	5.63	Not Significant
Error (b)*	4	2.01888325		
Total	11			

*For definition of errors a and b see page 21

**At the 5% significance level

17 and 18. Examination of these tables show that:

1. Hartman and White's method is capable of showing the differences in the peroxide values while the other two methods fail to do so.

2. The error in Hartman and White's method (19) is much smaller in magnitude than in both other methods.

3. That the magnitude of both errors a and b of the method used by Brock (6) is very much lowered by determining the peroxide values by this method in an atmosphere of nitrogen indicating that the air, or rather the oxygen in the air, has an unfavorable effect on these determinations, using acetic acid.

4. The fact that the method used by Brock (6)--even when run in an atmosphere of nitrogen--still is incapable of detecting the differences in peroxide values indicates that there is an inherent difference between the two methods. Since the major difference between these two methods--after the exclusion of the air in the method used by Brock (6)--is the use of a citric acid solvent--its preparation previously described--in Hartman and White's method (19) and of glacial acetic acid in the method used by Brock (6), it could be concluded that this difference is the cause of the failure of the method used by Brock (6) to detect peroxide value differences.

Table 1 shows the peroxide values as determined

by the method used by Brock (6) for all the storage periods. Fluctuations in these values appear upon examining this table. These fluctuations render the use of peroxide values as determined by this method without value in predicting the quality of frozen chinook salmon. As mentioned above, these irregularities may be attributed to the following:

1. The effect of oxygen in the air on peroxide value determination by the method used by Brock (6).

2. The use of glacial acetic acid in the method used by Brock (6), instead of the citric acid solvent as proposed by Hartman and White (19).

CHAPTER V

SUMMARY AND CONCLUSIONS

This investigation dealt with several phases of the quality of frozen chinook salmon. Peroxide values and optical densities were examined as objective measures of the quality. Changes in chinook salmon during frozen storage were followed closely. Also, the effectiveness of an ascorbic acid dip in preserving the edible quality of chinook salmon during frozen storage was determined. Some methods of determining peroxide values in fish oils were compared. The salmon steaks used in this investigation were prepared as follows:

Each steak was halved, one-half being dipped in a 2% solution of l-ascorbic acid for one minute and then wrapped in polyethylene bag and this was put in a waxed carton. The other half was wrapped similarly without ascorbic acid treatment. In both cases, the salmon was then quick frozen and stored at 0°F. for a maximum period of 10 months. At intervals of eight weeks, six cartons from each treatment were withdrawn from the freezing storage room according to a table of random numbers. A sample unit of two half-steaks was used and hence each opening gave three observations for each treatment and for each test run. Optical density measurements and peroxide determinations were

run on a chloroform-extract of the salmon. The wave lengths at which the optical density readings were obtained--common to all the storage periods--were 450 mu., 460 mu. and 470 mu. The methods used for the determination of peroxide values were compared statistically. Flavor scores representing the edible quality of the salmon were obtained by baking the chinook salmon steaks, after seasoning in a 5% salt solution, at 550°F. for 35-40 minutes and submitting the macerated homogeneous salmon flesh to a panel of four reliable fish tasters.

Analysis of variance was used to find out any differences in the salmon samples. Rank correlation, being appropriate for this type of work, was utilized to find out the relation between the objective and subjective tests.

The conclusions of the investigation are as follows:

I. Concerning the Quality of Fish:

1. The quality of the frozen salmon steaks is affected adversely by the length of the storage period. Flavor scores indicating the quality had a standard deviation of ±0.72 of a flavor score unit. This means that if a chinook salmon steak is scored an infinite number of times by this panel, two-thirds of the scores will be within a range of ±0.72 of the mean. It should be noted

here that this is not adduced from true replicates as the salmon steaks came from different fish.

2. The ascorbic acid dip as used in this investigation is not more effective in keeping the edible quality of frozen chinook salmon than no treatment.

3. There was no interaction between storage period and treatment, meaning that these two factors affect flavor scores independently.

II. Concerning Optical Densities:

1. The wave length at which the maximum absorption of the chloroform-extract occurred shifted from 460 mu. in the first opening to lower wave-lengths in the subsequent openings.

2. The longer the storage period, the higher the optical density of the chloroform-extract measured at 450 mu., 460 mu., or 470 mu. Optical densities at these wave-lengths had standard deviations of 0.084, 0.062 and 0.069 respectively.

3. The ascorbic acid treatment as used here did not cause significantly different optical densities than those obtained from non-treated chinook salmon.

4. The storage period and treatment affect the optical densities independently. This is reflected from the absence of interaction of these two factors in optical

density measurements.

III. Concerning the Utilization of Optical Density as a Measure of the Quality of Salmon:

Optical density of the chloroform-extract of chinook salmon at 450 mu., 460 mu. or 470 mu. is a valid measure of the quality of frozen chinook salmon. Spearman's rank correlation coefficients between flavor scores and optical densities at 450 mu., 460 mu. and 470 mu. were 1, 1 and 0.9 respectively.

Further investigation is recommended to adapt this method to the special circumstances that it might be used in, and to prove its universality for salmon preserved by methods other than freezing, and for other species of fish that contain the same pigment as salmon.

IV. Concerning Peroxide Values and the Methods Used Here for their Determination:

1. The peroxide values as determined by the method used by Brock (6) are liable to fluctuations which render these data valueless in predicting the quality of chinook salmon. The fluctuations could probably be attributed to:

a. The effect of the oxygen in the air on these

determinations.

b. The use of pure glacial acetic acid instead of citric acid solvent which affects its capability of detecting differences in peroxide values even after the exclusion of the air.

2. The method proposed by Hartman and White (19) is capable of reliably detecting differences in peroxide values. Further investigation may prove peroxide values, as determined by this method, to be a valuable objective method in determining the quality of salmon.

BIBLIOGRAPHY

1. Bailey, B. E. The Pigments of Salmon. Journal of Biological Board of Canada, 3, 469-472, 1951.

2. Bailey, B. E. Marine Oils with Particular Reference to Those of Canada. Revised from bulletin No. 59 with editorial assistance of N. M. Carter and L. A. Swain. Fisheries Research Board of Canada Bulletin No. 89, 1952, 413p.

3. Bauernfein, J. C., E. G. Smith, O. M. Batcher and G. F. Seemens. Retardation of rancidity in frozen fish by ascorbic acid. Quick Frozen Foods 10, No. 8, 68-70, 72, 1948.

4. Boury, M. and Schvinte, J. L'alteration du poisson. Rev. trav. office sci. et tech. peches maritimes 8, 282-333, 1935. Through Reay and Shewan (30).

5. Bradley, H. C. and Bailey, B. E. Estimation of decomposition of fish muscle. Food Research 5, 487-493, 1940.

6. Brock, James Richard. Comparison of taste test scores with peroxide values of frozen pink salmon. M.S. thesis, Oregon State College, 1951.

7. Brocklesby, H. N. (ed.) The Chemistry and Technology of Marine Animal Oils with Particular Reference to Those of Canada. Fisheries Research Board of Canada, Bulletin No. 59, 1941, 442p.

8. Charnley, F. and Bolton, R. S. The measurement of canned salmon and other semi-rigid bodies by the dynamic penetrometer method. I. Experiments with multi-needle penetrometer. Journal of Fisheries Research Board of Canada 4, 162-173, 1938.

9. Cobb, J. N. Pacific Salmon Fisheries, 3rd ed. Department of Commerce, Government Printing Office, Washington D. C. 1921 (Bureau of Fisheries Document No. 902).

10. Cochran, William C. and Cox, Gertrude M. Experimental Designs. John Wiley and Sons, New York, 1950, 454p.

11. Cutting, C. L. Electrometric titration of haddock's muscle. Annual Reports of Food Investigation Board (Britain) p.78, 1937.

12. Cutting, C. L. The electrometric titration of fish's muscle. Annual Reports of Food Investigation Board (Britain) p.39-40, 1938.

13. Dassow, J. A. and Stansby, M. E. Recording color changes in frozen pink salmon. United States Department of Interior, Fish and Wildlife Service Fishery Leaflet No. 332, January 1949.

14. Davidson, F. A. and Hutchinson, S. J. Geographic distribution and environmental limitations of Pacific Salmon (genus Oncorhynchus). United States Government Printing Office, Washington, D. C. 1938, 692p.

15. Dyer, W. J., Sigurdsson, G. J. and Wood, A. J. A rapid test for detection of spoilage in sea fish. Food Research 9, 183-187, 1944.

16. Elliott, R. P. Evaluation of surface pH as a freshness index for fish fillets. Food Research, 12, 87-98, 1947.

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

18. Gupta, Gopi Nath. Spectrophotometric techniques for the evaluation of frozen pink salmon. M.S. Thesis, Oregon State College. 1951.

19. Hartman, Leopold and White, M.D.L. Reagents for iodometric determination of peroxides in fats. Analytical Chemistry, 24 (3) 527, 1952.

20. Kendall, Maurice G. The Advanced Theory of Statistics, v. 1. Charles Griffin and Company Limited, London 1943, 457p.

21. Kendall, Maurice G. Rank Correlation Methods. Charles Griffin and Company Limited, London, 1948, 160p.

22. Lang, V. W., Farber, L., Beck, C. and Yerman, F. Determination of spoilage in protein foodstuffs with particular reference to fish. Industrial and Engineering Chemistry, Analytical Edition 16, 490-494, 1944.

23. Lederer, E. Les Carotenoides des Animaux. Herman et Cie, Paris 1935.

24. Li, Jerome C. R. Notes on Statistical Methods, 1950-51, 136p.

25. Lothian, G. F. Absorption Spectrophotometry 3rd ed. Hilger and Watts Limited, London 1949.

26. Mathers, John Hamilton. Processing treatments to extend the storage time of frozen pink salmon. M. S. Thesis, Oregon State College, 1950.

27. Naumann, H. D., Bradley, D. E., Palmer, A. Z. and Tucker, L. N. Odor, flavor and peroxide value as measures of rancidity in frozen ground pork. Food Technology 5, 496-499, 1951.

28. Nickerson, J. J. R. and Proctor, Bernard E. Some chemical changes exhibited in sterile and contaminated muscle stored at different temperatures. Journal of Bacteriology 30, 383-394, 1935.

29. Norton, K. B., Tressler, D. K. and Farber, L. D. The use of monosodium glutamate in frozen foods. Food Technology 6, 405-411, 1952.

30. Reay, G. A. and Shewan, J. M. Spoilage of fish and its prevention by chilling. Advances in Food Research 2, 343-398, 1949.

31. Sidaway, E. P. Light-bending properties of "drip" from stored halibut. Fisheries Research Board (Canada) Progress Reports, Pacific Station No. 49, 3-5, 1941.

32. Sigurdsson, J. Comparison of chemical tests of the quality of fish. Analytical Chemistry 19, 892-902, 1947.

33. Snedecor, George W. Statistical Methods. The Iowa State College Press, 1946, 485p.

34. Stansby, M. E. Analysis of fish. Journal of The Official Agricultural Chemists 18, 616-621, 1935.

35. Stansby, M. E. Determination of peroxide values for rancidity in fish oils. Industrial and Engineering Chemistry, Analytical Edition 13, 627-31, 1941.

36. Stansby, M. E. and Dassow, J. A. Recording color of opaque objects. Industrial and Engineering Chemistry 14, 13-15, 1942.

37. Stansby, M. E. and Harrison, R. V. Preliminary investigation of methods for freezing and storing of fillets of some Pacific Northwest fish. United States Department of Interior. Special Scientific Report No. 15, 1944.

38. Stansby, M. E. and Lemon, J. M. An electrometric method for detection of relative freshness of haddock. Industrial and Engineering Chemistry. Analytical Edition 5, 208-211, 1933.

39. Tarr, H. L. A. Control of rancidity in stored fish. Fisheries Research Board (Canada), Progress Reports, Pacific Coast stations, No. 64, 57-61, 1945.

40. _____ Control of rancidity in stored fish. Fisheries Research Board (Canada) Progress Reports, Pacific Coast Stations, No. 68, 52-54. 1946.

41. _____ Control of rancidity in stored flesh. Chemical anti-oxidants, Journal of Fisheries Research Board (Canada), 7, 137-154, 1947.

42. _____ Preservation of quality of edible fish products. Fisheries Research Board (Canada). Progress Reports, Pacific Coast Stations No. 71, 15-20, 1947.

43. _____ Control of rancidity in fish flesh. II Physical and chemical methods. Journal of Fisheries Research Board (Canada) 7, 237-247, 1948.

44. _____, Southcott, B. A. and Bissett, H. M. Control of rancidity in stored fish IV. Fisheries Research Board (Canada). Progress Reports, Pacific Coast Stations, No. 88, 67-68, 1951.

45. Tanti, M., Hinose, I. and Wada, H. A physical method of testing the freshness of raw fish. Journal of the Imperial Fisheries Institute (Japan), 26, 79-86, 1931.

46. Tunison, A. V. et al. Coloration of fish. Progressive Fish Culturist, United States Fish and Wildlife Service, 9, 53-60, 1947.

47. Virginia Agricultural Experiment Station, Blacksburg, Virginia. Statistical Methods for Sensory Difference. Tests of Food Quality. Bi-Annual Reports Nos. 1, 2, 3, 4, and 5. December 1950-December 1952.

48. Wheeler, D. H. Peroxide formation as a measure of auto-oxidative deterioration. Oil and Soap 9, 89, 1932.

49. Wood, A. J., Sigurdsson, G. J. and Dyer, W. J. The surface concept in measurement of fish spoilage. Journal of Fisheries Research Board (Canada) 6, 53-62, 1942.

APPENDIX

TABLE 1

Optical Densities of Chloroform - Extracts at the 1st Opening

Wave Length	Not Treated	Treated	Not Treated	Treated	Not Treated	Treated
m.u.						
450	.144	.138	.150	.164	.238	.128
460	.183	.176	.218	.198	.367	.207
470	.138	.122	.182	.154	.293	.154
480	.169	.156	.218	.183	.336	.179
490	.173	.159	.206	.186	.326	.170
500	.166	.154	.180	.174	.294	.145
510	.155	.135	.202	.167	.294	.157
520	.100	.087	.166	.111	.235	.123
530	.107	.096	.136	.118	.184	.098

TABLE 2

Optical Densities of Chloroform - Extracts at the 2nd Opening

Wave Length	Not Treated	Treated	Not Treated	Treated	Not Treated	Treated
m.u.						
440	.169	.174	.520	.466	.295	.321
450	.184	.184	.266	.223	.370	.445
460	.201	.195	.270	.222	.242	.278
470	.077	.068	.145	.096	.17[illegible]	.188

TABLE 3

Optical Densities of Chloroform-Extracts at the 3rd Opening

Wave Length	Not Treated	Not Treated	Not Treated	Not Treated	Not Treated	Not Treated
m.u.						
400	.850	.820	.820	.790	.770	.760
420	.672	.670	.648	.628	.633	.605
440	.650	.612	.600	.580	.585	.562
450	.650	.612	.588	.534	.564	.502
460	.625	.608	.548	.506	.524	.472
470	.600	.570	.508	.460	.485	.428
480	.585	.521	.472	.408	.441	.370
490	.594	.492	.367	.472	.446	.330

TABLE 4

Optical Densities of Chloroform-Extracts at the 4th Opening

Wave Length	Not Treated	Not Treated	Not Treated	Not Treated	Not Treated	Not Treated
m.u.						
400	1.03	1.25	1.03	1.17	.998	1.16
420	.998	.980	1.04	.915	.990	.922
440	.864	.938	.918	.866	.852	.880
450	.878	.920	.942	.852	.861	.876
460	.858	.910	.938	.830	.842	.866
470	.790	.840	.870	.760	.780	.795
480	.790	.715	.888	.648	.788	.674
490	.682	.660	.780	.601	.688	.632
500	.646	.640	.748	.580	.648	.600

TABLE 5

Optical Densities of Chloroform-Extracts at the 5th Opening

Wave Length	Not Treated	Not Treated	Not Treated	Not Treated	Not Treated	Not Treated
m.u.						
400	1.045	1.08	1.04	1.08	1.05	1.07
420	.980	1.00	.970	.979	.995	.980
440	.904	.925	.895	.885	.945	.921
450	.895	.882	.860	.838	.921	.890
460	.845	.828	.818	.782	.895	.850
470	.792	.785	.761	.726	.855	.805
480	.745	.726	.728	.666	.804	.752
490	.678	.660	.642	.601	.746	.695
500	.604	.590	.575	.532	.670	.624