SPECTROPHOTOMETRIC TECHNIQUES FOR THE EVALUATION OF FROZEN PINK SALMON

by

GOPI NATH GUPTA

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APPROVED:

Professor of Food Technology Department

In Charge of Major

Head of Department of Food Technology

Chairman of School Graduate Committee

Dean of Graduate School

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CHAPTER I

INTRODUCTION

There are five distinct species of salmon (Oncorhynchus) in the Pacific Ocean and the rivers flowing into it. John N. Cobb, formerly dean of the college of fisheries at the University of Washington, lists them as follows: (5)

1. Oncorhynchus tschawytscha (Walbaum). This species is frequently classified by the trade as Tyee, Quinnat, Chinook, Spring or King salmon.

2. Oncorhynchus nerka (Walbaum). The trade names for this salmon are the Red, Sockeye, or Blueback salmon.

3. Oncorhynchus kisutch (Walbaum). This salmon is often referred to as either the Silver, Coho, or Medium Red salmon.

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

5. Oncorhynchus gorbuscha (Walbaum). The most common designation for this species is Pink or Humpback salmon.

Of these five species of Pacific salmon just

mentioned above, three species, viz., King or Chinook, Silver or Coho and Fall or Chum, have been preserved by freezing on a large scale in commercial practice.

The summary and comparison of stocks of frozen fishery products held on December 1, 1949, are as follows: (8)

Species Salmon	Pounds (December 1, 1949)
King or Chinook	4,540,974
Silver or Coho	4,474,443
Fall or Chum	1,702,018

Pink salmon (Oncorhynus gorbuscha), the fifth species of the Pacific salmon, is not generally frozen commercially. The freezing preservation of this species has been limited because of development of discoloration and rancidity of the frozen fish after comparatively short storage periods. If a successful method of storing the fish in the frozen state could be developed, it could be preserved by freezing on a large scale with greater consumers' demands in the market.

In an effort to prolong the storage period of frozen pink salmon, without any appreciable changes in color and flavor, pink salmon samples were prepared by packaging them as fillets into different kinds of paper cartons along with different treatments of some selected and supposedly most effective antioxidants (Table I).

Preservation of pink salmon by freezing had been a problem due to a rapid deterioration, but an equally important problem was to measure the extent of deterioration by some suitable methods. Peroxide value as an index of oxidation of the fish has been used quite frequently. Nevertheless, a neat, rapid and simple method was desired to measure accurately the deterioration of fish for routine work in the freezing industries. Hence, a spectrophotometric method was employed in this analysis of frozen pink salmon.

The objectives in this investigation, then, are twofold. First, to investigate the extent and nature of the spectral changes in the extracts of frozen pink salmon, when treated differently physicochemically under supposedly different conditions of oxidation, and second, to develop a spectrophotometric method to estimate quantitatively the degree of deterioration and its corresponding discoloration of some antioxidant treated frozen pink salmon fillets in successive periods of storage.

CHAPTER II

LITERATURE REVIEW

Estimation of the Quality of Fish

1. Organoleptic method: Organoleptic opinion of an investigator is always a physiological reaction which is a result of past training, experience of the individual, influence of personal preference and powers of perception. Organoleptic methods are universally applied in estimating quality on the market and at the table for consumers' preference purposes. The works of Mathers (16) and Brock (3) should be referred to for further detailed information of the use of this method in evaluation of frozen pink salmon.

2. Bacteriological test: Bacteriological methods may give a fairly good picture of the sanitary history and handling of the product but a high bacterial count does not always mean that the product is spoiled. Hunter (11) stated that "attempts to correlate the total count with the physical condition of the salmon failed". Others have come to similar conclusions. Moreover, bacterial growth is checked to a great extent in freezing conditions. Such methods, therefore, are not widely used for evaluation of frozen fish.

3. Chemical tests:

a. Volatile acid and trimethylamine tests: Friedemann (10) showed a chemical method for the determination of volatile acid as an index for different stages of spoilage of fish. In 1937, Beatty and Gibbons (1) observed that the increase in trimethylamine in the muscle of marine fishes runs closely parallel to the degree of decomposition and concluded that "trimethylamine value of fish muscle fulfills all the conditions required of an effective test for freshness". Later, Sigurdsson (18) stated clearly, after a complete review of most of the chemical tests used for the quality of fish at different temperatures, that at temperatures below 0°C. neither the volatile acids nor the trimethylamine is an adequate test for the condition of the fish, although both are reliable when the product is stored at higher temperatures.

b. Bradley and Bailey (2) used the Folin and Ciocalteu (9) colorimetric determination of tyrosine as a measure of the disintegration of fish muscle. Sigurdsson (18) observed that at low temperatures, especially if there was a possibility that the fish might have been stored at temperatures below 0°C or frozen, tyrosine determination would be of considerable value but a serious objection to use of this method

could be its inconsistent fluctuations in the results reported thus far.

c. Hydrogen sulfide is formed progressively during putrefaction of meat and fish products, and its use as an index of spoilage has been attempted. Sigurdsson (18) compared the validities of different chemical methods and observed that hydrogen sulfide does give a certain indication of the state of fish, but sometimes the changes in hydrogen sulfide, especially at low temperatures, are so small that it becomes difficult to evaluate small changes in fish analysis.

d. Peroxide values have been widely used as an index of rancidity of oils and fatty tissues. A number of different procedures are available for making such determinations, but Stansby (19) concluded that

> "the results by different methods do not always show good agreement, and in using a given method, results are often not reliable. At least with the fish oils, precision is often poor, and points are vague, and correlation between peroxide number and organoleptic rancidity, so far, has not been established".

Within a decade or so, a considerable amount of work on peroxide values has been done by many leading workers as Lea (13), Tarr (22) and others. The necessary modifications in this method were being made from time to time. The peroxide test still suffered certain limitations

which could not be improved upon. However, further experiments on frozen pink salmon for peroxide values were carried on by Brock (3), this author and Mathers (16). A few definite improvements in the method were made. The method and procedure are well described by Brock (3).

4. Physical tests: Stansby and Lemon (20) developed an electrometric method based on buffer capacity measurement for detection of relative freshness of fish. They observed certain changes in buffer capacity of the fish muscle as decomposition proceeds. Sidaway (17) (1941) introduced a method based on refractive index of the juice. Charnley and Bolton (4) attempted to test the quality of fish by its firmness, but with fresh fish none has met with success. Stansby and Dassow (21) recorded the color changes in frozen pink salmon by the use of photographic color transparencies and their spectral distribution curves were obtained, using a photoelectric spectrophotometer. Errors due to variations in illumination during exposure and in processing of the film are eliminated by taking pictures of objects to be compared on the same negative. This method seems to be quite unique and interesting. Dassow and Stansby (6) further recorded and compared the color changes of freshly cut interior tissue with that of surface tissue of frozen pink salmon. The method employed

for recording and evaluating the color of the flesh was the same as described above. In each sample, they considered that the color changes were confined to the surface of the fillet during the entire storage period, and the color of freshly cut surface closely approximated the initial color of the fillet before storage.

The use of surface pH as a measure of spoilage is described by Dyer, et. al. (7). These workers emphasize the importance of using samples from a thin layer of the surface in detecting incipient spoilage of the fillets, since all deteriorative changes begin at the surface long before they can be detected in the interior.

Spectroscopic Analyses in Rancidification of Lard and Vegetable Oils

Lundberg, et. al., (15) conducted spectrophotometric measurements on lard, which had been oxidized to various degrees by exposure to air in an oven at 63°C. It was found that in the ultra-violet region there occurred increases in the absorption of light that were approximately proportional to the peroxide values of the sample, at least in the earlier stages of rancidification.

Williams, et. al., (24) studied the absorption spectra of chloroform solution of the crude, refined and

bleached oils of cotton seed. They showed a correlation between the increase in bleach color and the absorption spectra of the crude oils. The decrease in the height of the absorption band of the crude hydraulic-pressed oils at 378 to 370 mm. with concurrent increase in bleach color indicates that deterioration of the pigments in the crude oils or their precursors in the seed is responsible for the development of high bleached color during storage of crude oil and seed. The term "bleach color" is used to designate the residual color, in terms of Lovibond red and yellow units, of an oil which has been alkali refined.

CHAPTER III

EXPERIMENTAL PROCEDURES

A. Preparation of the Extracts

About 120 to 150 g. of the freshly cut interior tissue (deeper than 1 cm), of the pink salmon fillets were cut into small cubes, on a clean surface with a stainless steel knife. The diced tissue was placed in a Waring blender with about 175 ml. of chloroform and 45 g. of sodium sulfate (anhydrous), and stirred for 40 seconds. The mixture was transferred to a small screw-top glass jar. It was kept in a zero degree freezer room for half an hour to break an emulsion formed in mixing. Then, the mixture of the tissue and chloroform was squeezed tightly through a fine piece of nylon cloth into a 250 ml. Erlenmeyer flask to get almost a clear extract of the fish tissue. About 15 g. of sodium sulfate (anhydrous) was added again to the extract and allowed to stand for two hours in the dark to absorb any moisture present in it. Finally, the extract was filtered through the filter paper by use of the Buchner funnel and water suction pump into a clean 250 ml. conical flask. About 150 ml. of clear, and pink colored extract was obtained. The extract from each sample of salmon fillet was

obtained in this way.

From 150 ml. of extract of each sample, one 10 ml. aliquot was withdrawn at once for oil content determination and the rest of it was used for further spectrophotometric analyses.

One 10 ml. aliquot was placed in a previously tared 120 ml. Erlenmeyer flash. The chloroform was evaporated on a water bath without any use of inert gas, and dried for a few minutes (10 minutes) to a constant weight in an oven at 100°C ± 1°C. This weight of the oil was used to determine the oil concentration in the extract of the sample as well as for the peroxide value determination and for the purpose of dilution to be made in absorption analysis.

B. Controlled Oxidation Experiments in Vitro

The experiments were designed to estimate, in vitro, the extent of oxidation and decomposition of the oil and its associated pigments in the extract, when treated physicochemically under different conditions. Moderate and extreme chemical changes were brought about and estimated by their corresponding spectral changes.

The rest of the extract after determination of the oil content, was diluted to a desired volume (in this case 200 ml.) for appropriate concentration (in this case

1.68 g./100cc.) of oil in it, so that the optimum value of optical desity(d) of the solution might be near the range of 0.2 to 0.8, the best range to be used without any considerable loss of accuracy (14).

In order to determine if the oil and pigment changes were due to free O_2, were photochemical, or were due to thermal effects, the following experiments, in vitro, were carried out. From the diluted extract, four 35-ml. portions were withdrawn to be treated differently in the following ways:

1. The first control portion had no treatment. It was held at room temperature for the same length of time as the rest of the portions.
2. Oxygen gas treatment - The second portion of the extract was refluxed in a 125 ml. Erlenmeyer flask, on a water bath and a slow stream of oxygen gas was bubbled through the solution for 16 minutes, which seemed to be a convenient length of time.
3. Infra-red lamp treatment - The third portion was refluxed in a 125.ml Erlenmeyer flask by infra-red lamp (250 w, 120 v.), placed beneath the flask also for 16 minutes.
4. Sunlight treatment - The fourth portion, in a 125 ml. conical flask, was placed outside and

the flask was exposed to direct sunlight for two hours.

After the above treatments, 5 ml. of each of the treated portions were taken for absorption spectra analyses.

The remaining 30 ml. of each of the treated portions was used for peroxide values, determined by the modified Tarr's (22) method described by Brock (3).

C. Investigation of the Extent of Deterioration, in situ, of some Antioxidant Treated Samples of Frozen Pink Salmon

On the basis of observations of spectral changes, in vitro, the chemical changes taking place, in situ, in frozen fish, presumably at a very slow rate, were estimated quantatively by absorption spectrum analysis. It was decided to compare the extent of deterioration of the surface tissue with that of interior tissue of the frozen pink salmon. The freshly cut interior tissue was assumed to be in the original fresh state, both in color and condition of the flesh.

Recently, Dassow and Stansby (6) compared the color differences between the surface and interior tissues, assuming that alterations in color were confined to the surface tissue and the color of the freshly cut interior tissue was the same as before storage.

Also, Sigurdsson, et. al., (18) showed by three chemical tests, (trimethylamine value, tyrosine value and pH value), that the spoilage changes begin at the surface and proceed there at a much greater rate than in the interior. In the light of this surface concept of spoilage it has been suggested that determination designed to detect incipient spoilage in fish fillets should be based on the sample taken from the surface of the product rather than on cross-sectional segments as are ordinarily employed for chemical analysis.

Hence, extractions of the surface (1 cm. deep from outer surface) and interior tissues (deeper than 1 cm.) of the antioxidant treated samples were made as previously described in Chapter III, Section A, to get 35 ml. to 45 ml. of clear extracts. Absorption spectra analyses of the extracts of both surface and interior layers of each sample were carried out. The difference of optical density of two interior and surface layers at their maxima in each sample was obtained, which was evaluated arbitrarily in terms of per cent optical density change for measurement of the extent of deterioration taking place in the surface tissue of frozen pink salmon as compared to that of interior layer. In this way, values of per cent optical density change of six duplicate antioxidant treated samples of frozen pink salmon were estimated (Table III).

CHAPTER IV

RESULTS

A. Absorption Spectrum Analysis

All spectrophotometric measurements were made with Beckman-quartz D.U. spectrophotometer, using fused silica cells with an optical light path of 1.000 ± 0.005 cm. The slit width was 0.013 mm. to 0.036 mm. Absorption spectrum of each sample was measured over the range 400 mu. to 570 mu. Optical density readings were made every 10 mu. The concentration of the oil in all the extracts of the samples was kept the same. Absorption spectra curves of all four differently treated portions of the extract of each sample in vitro, were then plotted against wavelengths on the semi-logarithmic, 3 cycle paper. The highest maxima of these curves were found to be at 490 mu. wavelength. The gradual lowering of the maxima and changes in the shape of the curves were observed. The chloroform extract of the salmon tissue consists of mostly the fish oil and its associated pigment. About the chemical nature of the oil Lavern (12) states "that typical fish oils are the penta and hexa ethylenic acid of 20 and 22 C atoms". Tunison (23) pointed out that astacin and astaxanthin are two carotenoid pigments responsible for the colora-

tion of many fishes. Thus, it can be reliably stated that the fish oil and its associated carotenoid pigments both have polyenic molecular structures (possess a large number of double bonds). Chemically, the two components of the extract are very labile. Depending upon the conditions, there could be slow or rapid oxidation, decomposition and polymeric changes of the original, long-chain, and unsaturated molecules of the oil and its associated color pigments in vitro and in situ as well. Under moderate conditions, little changes have taken place and in the extreme cases severe changes have occurred. The spectral changes are due to chemical changes taking place in the components of the extract of the tissue of the fish. The total optical density change, at 490 mu. wavelength, from fresh state (control portion) to the extreme altered state (supposedly completely oxidized state) was considered to be 100 per cent optical density change. The extract of the fresh state tissue has the highest optical density at 490 mu. wavelength. Successive chemical alterations in the differently physicochemically treated portions of the extract have taken place with the result that gradual lowering of optical density was observed. Also, it was considered that in the extremely severe state there would be zero optical density at 490 mu. wavelength. With the view of 100 per cent optical density change in between

the two above mentioned states (fresh and extremely oxidized), the intermediary changes brought about in the different portions of the extract of each of three samples were computed arbitrarily in terms of per cent optical density change to determine quantitatively the extent of deterioration occurring in differently treated portions of the extract of three different samples (Table II).

The same procedure was followed in antioxidant treated samples of frozen pink salmon. The two absorption curves of surface and interior tissue extracts of each sample were plotted on the semi-logarithmic, two-cycle paper due to better spreading of the curves. It could be plotted on three-cycle paper as well. Figure 3 shows a typical absorption curve of the sample A^1.

Here the difference of optical density at the maxima of two curves was estimated arbitrarily in terms of per cent optical density change, considering 100 per cent change taking place from fresh state to completely altered state.

Table I gives identity of frozen antioxidant treated, pink salmon fillet samples stored for 200 days at $0^{o}C$.

B. Peroxide Value

After determination of the concentration of the oil of each extract, a measured portion of the extract was placed in 250 ml. conical flask and titration was carried out as in the method employed by Tarr (22). The amount of the oil by weight was kept constant in all samples. There was 0.2798 g. of the oil in each measured portion of the extract for determination of peroxide value. The method and procedure are well described by Brock (3).

TABLE I

Identity of Frozen Antioxidant Treated Pink Salmon Fillet Samples Stored for 200 days at 0°C.

Code	
A^1	Fillets frozen in Canco container, dry.
A^2	Duplicate of A^1.
B^1	Fillets plus water frozen in Canco.
B^2	Duplicate of B^1.
C^1	Fillets plus 0.5% NDGA antioxidant solution frozen in Canco.
C^2	Duplicate of C^1.
E^1	Fillets plus 3.0% Na Cl solution frozen in Canco.
E^2	Duplicate of E^1.
N^1	Fillets frozen in waxed carton with waxed overwrap after a 15-20 second dip in 0.5% Krim Ko Gel plus) .3% ascorbic acid, plus Tweens 81 (wetting agent) in conc. of 0.001%.
N^2	Duplicate of N^1.
P^1	Fillets frozen in waxed carton with waxed paper overwrap after a dip of 15-20 seconds in a 0.5% Krim Ko Gel and 0.5% NDGA solution. The temperature of solution was 40-45 degrees F.
P^2	Duplicate of P^1.

TABLE II. Optical Density, Per Cent Optical Density Change and Peroxide Number of the Extracts of Three Samples, in vitro, Stored for 200 days at 0°C.

Sample Number	Treated Portions	d Optical Density at 490 mu wavelength	d Optical Density difference from control portion	$\frac{d \times 100}{d}$* Per Cent Optical Density change	Peroxide Number**
1	a. control	0.114	0	0	6.38
	b. oxygen-gas	0.113	0.001	0.88	6.89
	c. infra-red	0.102	0.012	10.52	8.2
	d. sunlight	0.003	0.111	97.35	105.0
2	a. control	0.147	0	0	6.18
	b. oxygen-gas	0.141	0.006	4.08	8.7
	c. infra-red	0.091	0.056	38.1	17.25
	d. sunlight	0.012	0.135	92.0	129.0
3	a. control	0.221	0	0	7.45
	b. oxygen-gas	0.217	0.004	1.82	12.90
	c. infra-red	0.194	0.027	12.20	10.30
	d. sunlight	0.006	0.215	97.30	84.2

* Computed arbitrarily from optical density measurement.

** ml. of .002 N sodiumthiosulfate required/g. of oil.

TABLE III. Optical Density, Per Cent Optical Density Change and Peroxide Number of the Extracts of Duplicate Antioxidant Treated Samples, in vitro, Stored for 200 days at 0°C.

Sample# Code	d Optical Density of interior tissue extract at 490 mu.	d^1 Optical Density of surface tissue extract at 490 mu.	$d - d^1$	$\frac{d \times 100^*}{d}$ Per Cent Optical Density change	Peroxide Number** of surface tissue extract
A^1	0.221	0.148	0.073	33.0	28.60
A^2	0.211	0.142	0.069	32.6	13.42
			Average =	32.8 =	21.01
B^1	0.296	0.147	0.149	50.3	22.3
B^2	0.391	0.292	0.099	25.3	12.0
			Average =	37.8 =	17.15
C^1	0.097	0.072	0.025	25.8	6.64
C^2	0.221	0.097	0.124	56.2	4.56
			Average =	41.0 =	5.6
E^1	0.371	0.238	0.133	35.8	9.4
E^2	0.362	0.247	0.115	31.8	12.5
			Average =	33.8 =	10.9
N^1	0.172	0.104	0.068	39.6	6.36
N^2	0.283	0.217	0.066	23.4	6.68
			Average =	31.5 =	6.52
P^1	0.288	0.124	0.164	56.8	15.63
P^2	0.247	0.173	0.074	29.9	13.70
			Average =	43.3 =	14.66

TABLE I.

* Computed arbitrarily from optical density measurements.

** ml. of .002 N sodiumthiosulfate required/g. of oil.

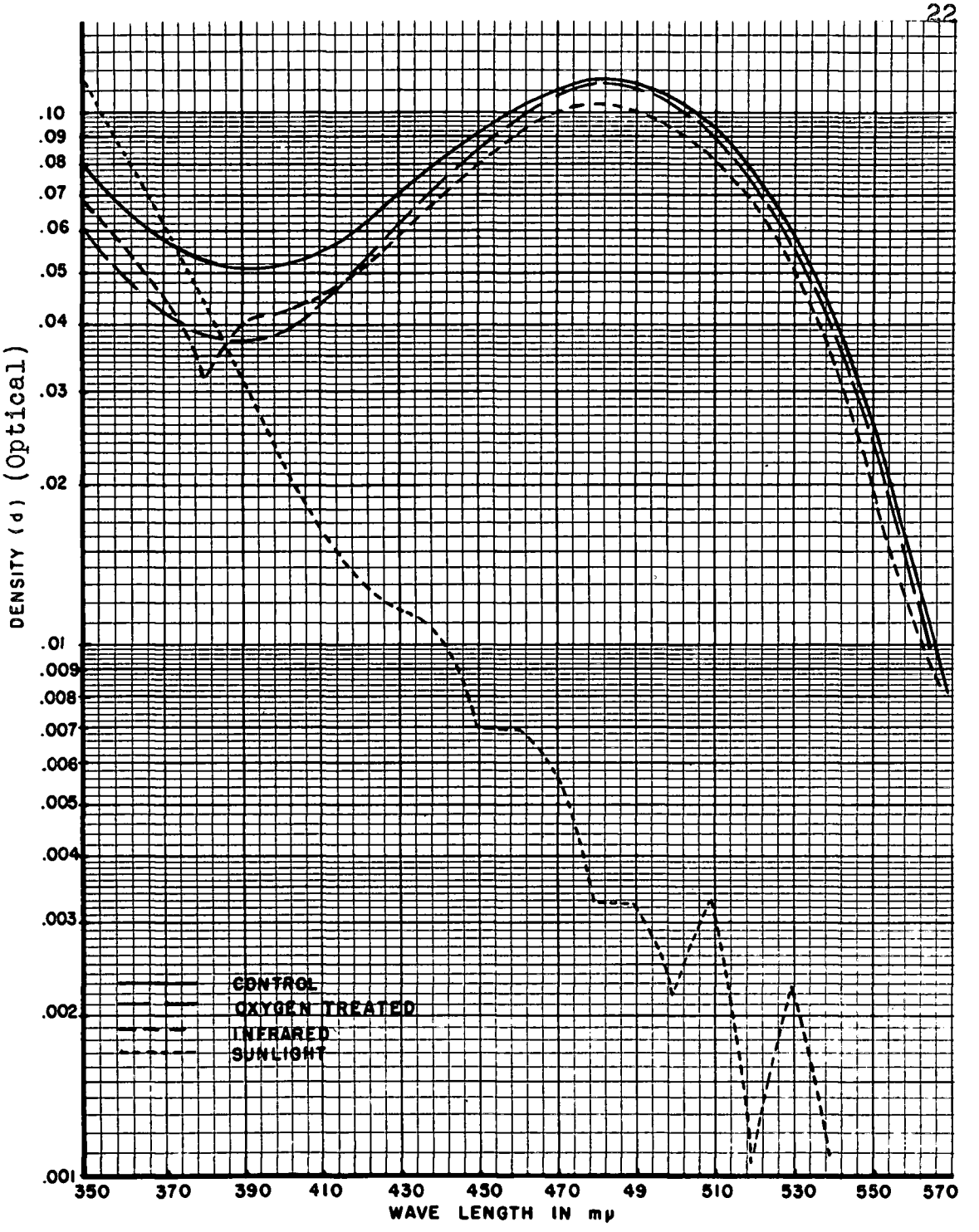

FIG. 1

Typical absorption curves of differently treated portions of the extract of the sample no. 1.

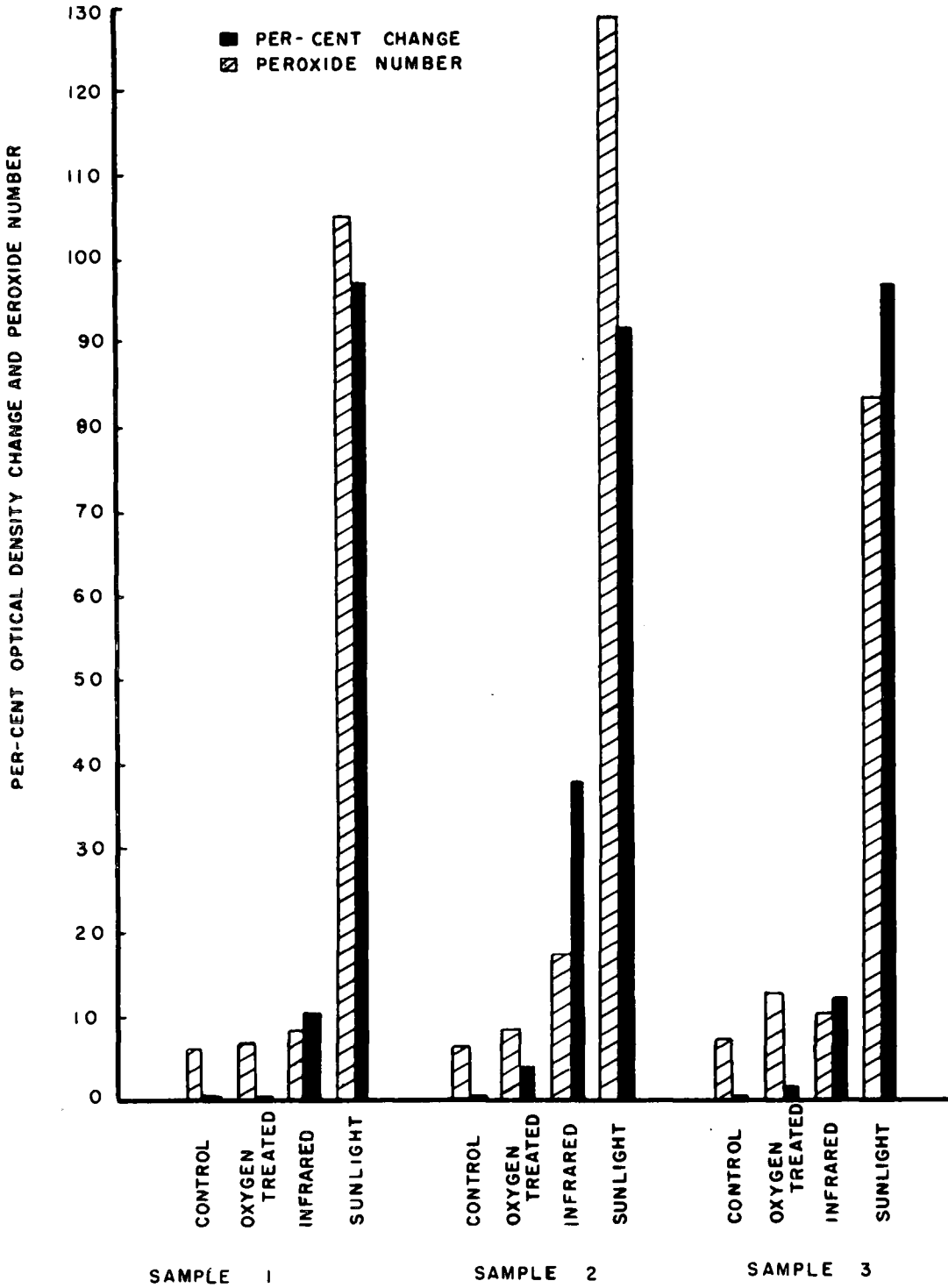

FIG. 2

Per-cent optical density change and peroxide number of different portions of the extracts of three samples in vitro.

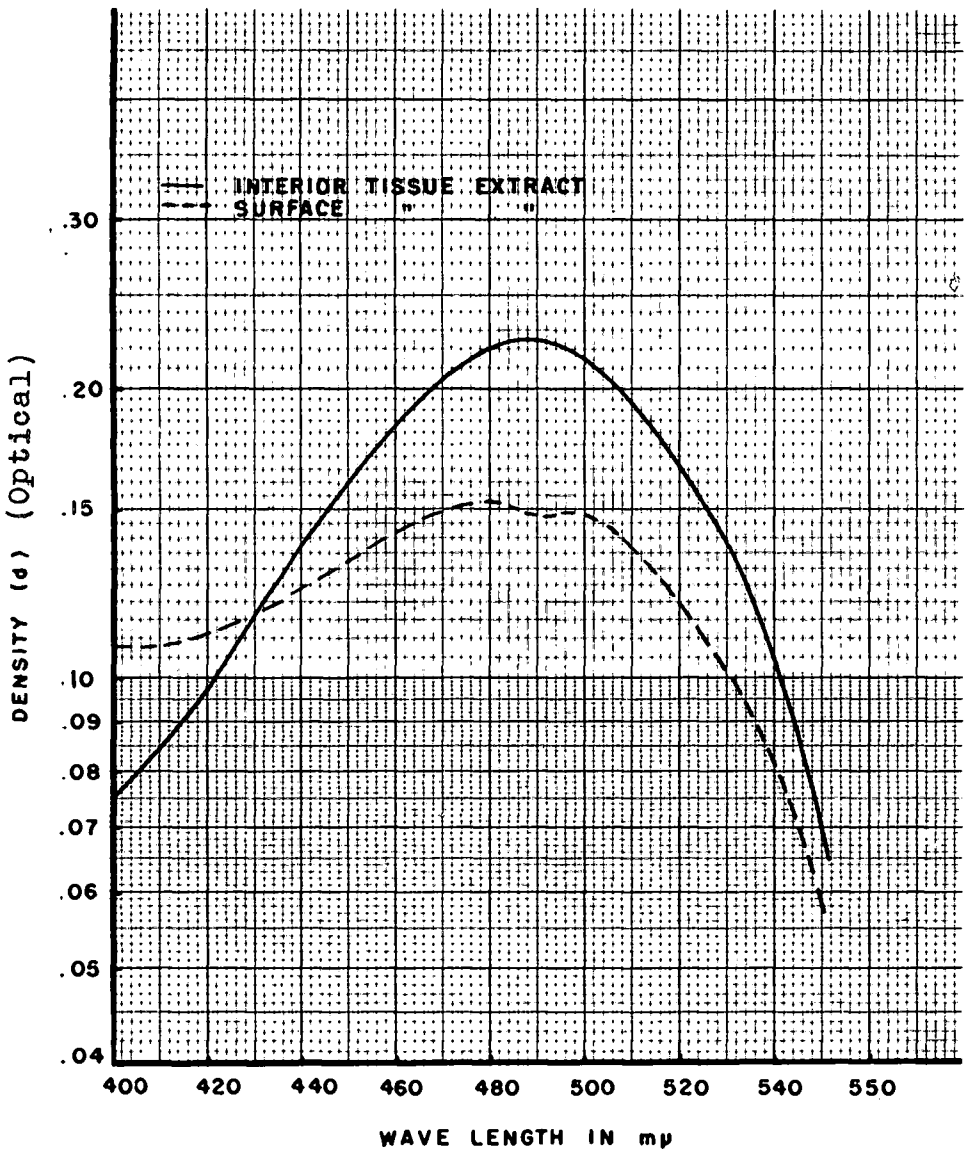

FIG. 3

Typical absorption curves

of interior and surface tissue extracts of

anti-oxidant treated sample "A'"

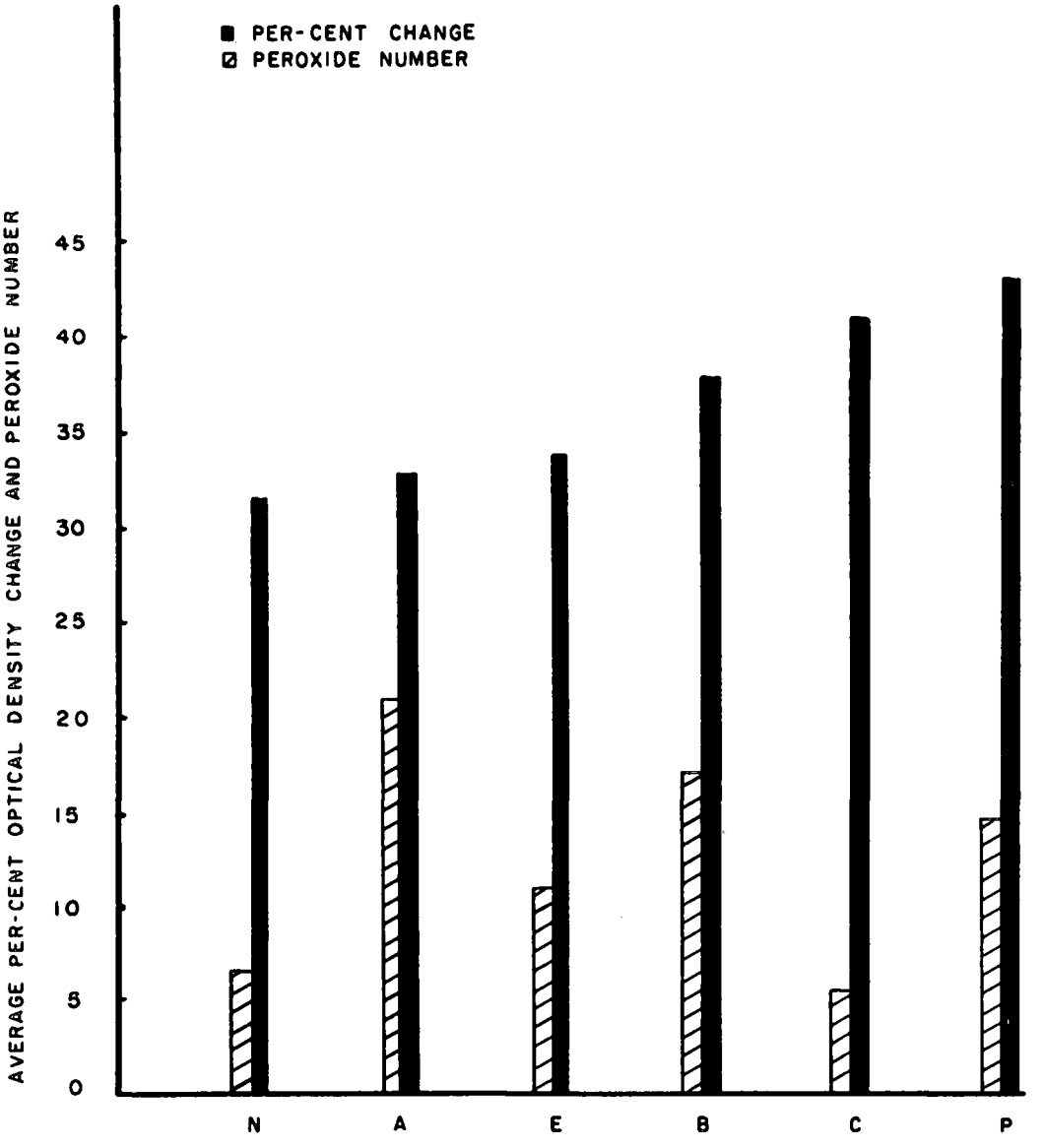

FIG. 4

Comparative view of
average per-cent aptical density change
and average peroxide number af
six duplicate antiaxidant treated samples, in situ.

CHAPTER V

DISCUSSION

Figure 1 illustrates the typical absorption curves of the physicochemically treated portions of the extract of the sample No. 1.

As chemical alteration finds its specific expression in the visible region and also in certain parts of ultra-violet region in spectrophotometric analysis, different absorption curves of differently treated portions of the extracts, in vitro, engender the following comments:

1. The first absorption curve (Figure 1) is of the first control portion of the extract of the sample. It has the highest absorption maximum at 490 mu. wavelength. This absorption curve depicts the existing chemical nature of the oil and its associated carotenoid pigments in that specific condition.

2. Effect of oxygen gas treatment - The absorption curve of the second treated portion of the extract is very slightly changed. Under such specific conditions, there seems to be very little change. The change is only 0.88 per cent, estimated arbitrarily by the difference of maximum of optical density of this curve from that of control - portion - curve at 490 mu. wave-

length. The change in peroxide number was also very slight (Figure 1, Table II).

3. Effect of the infra-red lamp - The absorption curve of the third portion of the extract has changed a little more. The absorption maximum at 490 mu. wavelength is much lower than the control portion. The change in maxima between this curve and that of the first - control - curve at 490 mu. wavelength is 10.52 per cent and so is the higher value of peroxide number, which was in this case 8.2 (Table II).

4. Effect of sunlight - The absorption curve of the fourth portion of the extract of the same sample is remarkably changed. The absorption peak is very low at 490 mu. wavelength. It is interesting to note that a severe chemical change in the extract and bleaching of carotenoid pigments have taken place. The pink colored extract is almost white. The change in optical densities between this curve and that of first (control portion) at their maxima, at 490 mu. wavelength, is 97.35 per cent, which is estimated arbitrarily from optical density measurements. Peroxide number is highest as compared to other portions of the extract of the sample (Figure 1, Table II). Besides, when chloroform was evaporated completely from this portion of the extract, the oil was found to be gummy and quite plastic

in nature. It had a very strong, repulsive odor. Presumably, it was extremely rancid and oxidized as indicated by very high peroxide value.

Identical experiments were carried out with two other samples. The spectral changes were observed to be in the same manner. Per cent optical density change computed arbitrarily from optical density measurements at the maxima at 490 mu. wavelength and peroxide number of each of the treated portions of the three samples were recorded (Table II). It was found that there was a distinct trend of increase of per cent optical density change with successive increase of peroxide number in the increasingly oxidized portions of the extracts of all three samples, in vitro.

On the basis of preceding observations of three samples, in vitro, the absorption curves of the interior and surface tissue extracts of all antioxidant treated samples were obtained. Figure 3 shows typical absorption curves of interior and surface tissue extracts of sample A^1. Similar curves could be plotted for all other samples. Both interior and surface tissue extracts had the same concentration of oil. It was accomplished by proper dilution. The absorption maxima of both curves in each sample were observed to be at 490 mu. wavelength. The changes between two curves

could be attributed to characteristic chemical changes of the oil and its associated pigments of frozen fish taking place, in situ. Difference between optical densities of the two curves at their maxima in each sample was evaluated arbitrarily in terms of per cent optical density change to indicate the extent of deterioration taking place in the surface tissue as compared to its interior tissue, which was supposed to be rather in fresh and original state. For example, Table III, shows that optical density difference between two layers of the sample A^1 at their maxima was 0.073, which was converted arbitrarily into the value of 33.0 per cent optical density change. That is, chemical changes in the extract of surface tissue of this particular sample had occurred 33.0 per cent in situ, as compared to that of interior tissue in 200 days' storage period at $0^{o}C$.

Thus, optical density changes of six antioxidant treated samples were determined in duplicate and the optical density difference of the exterior and interior layers of each sample was computed in per cent optical density change to show the extent of deterioration taking place in the surface tissue of the fish. The average values of per cent optical density change of all duplicate antioxidant treated samples were summarized in Table III.

Peroxide numbers of the extract of surface tissue of each antioxidant treated sample were also obtained. In all differently antioxidant treated samples, the range of average per cent optical density changes of all duplicate antioxidant treated samples was found to be from 31.5 per cent to 43.3 per cent in 200 days' storage period at 0°C. The range of average peroxide number of the surface tissue extract in all duplicate antioxidant treated samples was from 5.6 to 21.01.

Figure 4 gives a comparative view of average values of per cent optical density change and peroxide number of all duplicate antioxidant treated samples.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The method is a new spectophotometric method for the determination of oxidative changes taking place in frozen salmon fillets.

Controlled oxidation experiments, in vitro, were carried out with chloroform extracts of three samples of frozen pink salmon, which had been stored for a period of 200 days at $0^{\circ}C$. Different degrees of changes were brought about physicochemically in different aliquots of the chloroform extracts. The optical density - (d) of each aliquot was measured at 490 mu. wavelength. Peroxide numbers of each aliquot were also obtained. It was found that there was a distinct trend of increase of per cent optical density change ($\frac{\Delta d \times 100}{d}$) with successive increase of peroxide numbers in the increasingly oxidized portions of the extracts of all the three samples.

On the basis of observations in the three samples, in vitro, the values of per cent optical density changes of six duplicate antioxidant treated samples were obtained which had also been stored for 200 days at $0^{\circ}C$. The optical density (d) of the extracts of interior and surface tissues of each sample were obtained. The maxima

of optical density of both interior and surface tissue in each sample were observed to be at 490 mu. wavelength as previously. The optical density (d) of the extract of the interior tissue was always higher than surface tissue at 490 mu. wavelength. As a result of the in-vitro experiments, the optical density of the completely oxidized state of the extract of the sample was regarded to be zero at 490 mu. wavelength. Difference of optical density ($\triangle$ d) of interior tissue of any sample to zero optical density was considered arbitrarily to be 100 per cent optical density change. Difference of optical densities ($\triangle$ d) between interior tissue to that of surface tissue was thus computed arbitrarily into per cent optical density change to indicate the extent of deterioration taking place in the surface tissue during storage period of 200 days at $0^{o}C$.

The values of all duplicate antioxidant treated samples were averaged. All antioxidant treated samples had the range from 31.5 to 43.3 per cent optical density change during 200 days' storage period at $0^{o}C$. With this in view, it could be concluded that chemical changes taking place in the surface tissues of different anti-oxidant treated samples have the range of 31.5 to 43.3 per cent as compared to their interior tissues which were assumed to be in original fresh state during

storage period of 200 days. The more oxidative changes had taken place in the surface tissue during longer storage period, the greater would be the value of per cent optical density change.

For example, in all antioxidant treated samples, sample A which was frozen in canco container, dry, had undergone 32.8 per cent change. Other antioxidant treated samples had slight variations in their per cent optical density change from that of control sample A. Sample N had lower value than sample A and others had slightly higher values than sample A. Also, the average peroxide number of duplicate antioxidant treated samples were shown by bar-graph (Figure 4). It was observed that there were considerable fluctuations in peroxide number from one sample to another in all antioxidant treated samples.

Based on the results of this investigation it may be concluded that:

1. On the basis of per cent optical density change the extent of deterioration in the surface tissue of the frozen pink salmon as compared to its interior tissue could be estimated during the frozen periods. The author believes that more work needs to be done to validate the applicability of this method in frozen fish industry.

2. The highest value of per cent optical density change among all antioxidant treated samples was 43.3 in 200 days' storage period at 0°C. To what extent the value of per cent optical density change for any frozen pink salmon fillet would be accepted or rejected, could be determined further organoleptically.

3. The peroxide number of only surface tissue extract of all duplicate antioxidant treated samples was determined. This shows the outer surface condition in 200 days' storage period but not a comparative view with regard to its interior layer as in the case of per cent optical density change of a sample. Perhaps this might be the cause of inconsistency of peroxide number in all antioxidant treated samples.

4. The method is neat, simple, rapid, and involves only a few determinations. Preparation and standardization of chemical solutions are not required. No high dilution is necessary. The extract of the tissue should be clear.

The precision of the method depends upon the method of sampling and the instrument itself. This spectrophotometric technique could be used in routine analysis work as the spectrophotometer is becoming very common in many places because of its versatile applications.

In fatty fish, like pink salmon, oxidation and

polymerization of the oil and its associated pigments are most important chemical reactions taking place in storage period. The overall chemical changes, in situ or in vitro, were evaluated by the use of the spectrophotometeric method described in the foregoing pages.

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