The unsaturated fractions obtained by low temperature crystallization were isomerized, and the ultraviolet absorption spectra were studied to investigate the presence of unsaturated acids.

The compositions of the oils were calculated from the methyl ester fractionation and the analytical data according to the procedure of Hilditch (3). The results are presented in Table II.

TABLE II
Composition of Body Fats of Some Marine Fish

Acids	White Pomfret		Black Pomfret		Pala	
	Wt.	MOL	Wt.	MOL	Wt.	MOL
	%	%	%	%	%	%
Saturated						
Myristic	4.75	5.68	4.37	5.3	5.32	6.34
Palmitic	20.57	21.92	13.29	13.56	23.48	24.81
Stearic	11.15	10.69	7.26	7.01	8.87	8.49
Arachidic	.....	.....	0.46	0.41	0.02	0.02
Unsaturated						
Tetradecenoic (—2H)	1.40	1.69	2.44	3.02	1.29	1.55
Hexadecenoic (—2H)	9.19	9.86	18.80	19.75	6.82	7.30
Octadecenoic (—2H)	33.16	32.08	33.16	31.98	32.88	31.68
Octadecadienoic (—4H)	3.56	3.46	0.44	1.23	1.68	1.63
Octadecatrienoic (—6H)	3.62	3.55	1.73	1.74	9.67	9.45
Eicosenoic (—2H)	7.54	6.63	4.53	4.09	9.01	7.90
Eicosatetrenoic (—8H)	5.06	4.54	6.74	6.21	0.48	0.43
Docosapentaenoic (—10H)	.....	.....	3.39	2.89	0.48	0.40
Docosenoic (—2H)	.....	.....	3.39	2.81	.....	.....

Discussion

The calculated composition of the oils indicate their complex nature. The percentages of saturated acids in the body fats of white and black pomfret and of pala fish were found to be 36.5, 25.4, and 37.7, respectively. Palmitic and stearic were the major saturated acids present.

The higher percentage of unsaturated acids in the black pomfret oil was in conformity with its higher iodine value. It also contained clupanodonic and cetoleic acids. It is noted that it was abnormally high in palmitoleic acid. Oleic acid was present in about equal amounts in all three oils.

The totals of stearic and oleic acids were about the same in the black pomfret and pala oils. This may be because of the result of biohydrogenation reaction as noted by Lovern (5). This reaction did not seem to have proceeded so markedly in white pomfret oil. The totals of palmitic and palmitoleic acids in all three oils were fairly constant, suggesting interconvertibility of one of them to the other (6).

Of the three oils the black pomfret contains the least linoleic and linolenic acid. The low content of linoleic may be attributed to its presence as a step in the hydrogenation of linolenic to oleic and then to stearic acid or to a rapid metabolism of it. Lovern (1) observed the absence of linoleic acid in halibut liver oil.

Gadoleic acid was found in all three oils; it was highest in pala oil. However arachidonic and clupanodonic acids were present in only small amounts in pala oil. These two acids were not found in white pomfret oil.

The black pomfret oil was the most typical of the three studied. It was not as highly unsaturated as the liver and depot fats studied by Lovern (1), in which more than 10% of C_{22} acids occurred. The high temperatures of Indian waters may be a contributing factor to the presence of less polyunsaturated acids.

Summary

The component fatty acids of the body fats of three typical species of Bombay marine fish were investigated. The saturated acid contents of pala and black pomfret oil were about the same. The black pomfret oil was abnormally high in palmitoleic acid. Linoleic acid was found to be remarkably low in pala and black pomfret oils. In white pomfret the amount of it present was about the same as that of linolenic. Polyunsaturated acids were present in black and white pomfret oils but only present in traces in pala oil.

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[Received November 30, 1954]

Determination of Butylated Hydroxy Anisole and Propyl Gallate in Food Antioxidants

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BUTYLATED hydroxy anisole and propyl gallate are used extensively, both alone and in combination, as antioxidants in food products. Many commercial preparations consist of one or both of these antioxidants and citric acid dissolved in propylene glycol. Analytical procedures for the determination of butylated hydroxy anisole and/or propyl gallate in such preparations should therefore be of value in controlling the addition of the antioxidants in their end-use.

Mahon and Chapman (2, 4) have reported visible spectrophotometric methods for the determination of propyl gallate and butylated hydroxy anisole in lard and shortening. After separation of the antioxidants by selective extraction from the fat, colors were developed, using a ferrous tartrate reagent for propyl gallate and a ferric chloride plus 2,2′-bipyridine reagent for butylated hydroxy anisole. These authors have reported (3, 4) another colorimetric method, using 2,6-dichloroquinone chlorimide as the reagent, which is applicable to the determination of butylated hydroxy anisole in commercial antioxidant preparations. While the interference of propyl gallate is not serious in the latter procedure, no means are provided

for its determination if both antioxidants are present.

In the present work it has been found that the ultraviolet absorption of antioxidant preparations can be used for the direct determination of butylated hydroxy anisole and propyl gallate occurring either alone or in mixtures.

Apparatus and Materials

SPECTROPHOTOMETER. A Beckman Model DU spectrophotometer equipped with an ultraviolet accessory set and a photomultiplier attachment was used for all absorbance measurements. The wavelength scale of the instrument was calibrated with a mercury lamp and found to be accurate within the tolerances stated in the manufacturer's literature (1). Silica cells of 1.00-cm. thickness were used throughout with cell corrections being applied when necessary.

BUTYLATED HYDROXY ANISOLE. Purified samples of both 2- and 3-tertiarybutyl-4-hydroxy anisole were available from an earlier research project on the preparation of the antioxidant. The melting point of the 2-isomer was 65°C. and that of the 3-isomer 64°C. Infrared spectra showed that the samples were free of the impurities normally found in crude butylated hydroxy anisole.

PROPYL GALLATE. Recrystallized material, melting point 148.5-150.0°C.

ISOPROPANOL. Distilled commercial isopropyl alcohol.

Procedures

Butylated Hydroxy Anisole and Propyl Gallate. Place about 0.7-g. of sample into a tared weighing bottle, stopper the bottle, and accurately determine the weight of the sample. Prepare a stock solution by dissolving the sample in isopropyl alcohol, transferring it quantitatively into a 100-ml. volumetric flask and diluting to the mark with isopropanol. Pipet a 1.00-ml. aliquot into a 100-ml. volumetric flask and dilute to the mark with isopropyl alcohol. Determine the absorbance at 232.1 mμ, using 1.00-cm. silica cells, pure solvent in the reference beam, and a slit-width of less than 0.70 mm.

Pipet a 5.00-ml. aliquot of the stock solution into a 100-ml. volumetric flask and dilute to the mark with isopropyl alcohol. Determine the absorbances of this solution at 241.0 and 252.0 mμ, again using 1.00-cm. cells and pure solvent as the reference and maintaining a slit-width of less than 0.56 mm. for the 241.0 mμ measurement.

After applying any necessary cell corrections, calculate the sample absorptivity at each of the analytical wavelengths by the equation:

$$a = A/C \qquad (1)$$

where a is the absorptivity, A is the observed absorbance, and C is the concentration in grams per liter. Use the calculated absorptivities to solve equations 2 through 5. The averages of the results given by equations 2 and 4 and by equations 3 and 5 are taken as the concentrations of propyl gallate and BHA, respectively.

$$\%\ \text{PG} = 6.34\ (a \text{ at } 252\ \text{m}\mu) - 0.194\ (a \text{ at } 232.1\ \text{m}\mu) \qquad (2)$$

$$\%\ \text{BHA} = 3.52\ (a \text{ at } 232.1\ \text{m}\mu) - 6.34\ (a \text{ at } 252\ \text{m}\mu) \qquad (3)$$

$$\%\ \text{PG} = 6.34\ (a \text{ at } 252\ \text{m}\mu) - 0.874\ (a \text{ at } 241.0\ \text{m}\mu) \qquad (4)$$

$$\%\ \text{BHA} = 15.86\ (a \text{ at } 241.0\ \text{m}\mu) - 6.34\ (a \text{ at } 252\ \text{m}\mu) \qquad (5)$$

The calculations can be shortened somewhat by combining equations 2 and 4 and equations 3 and 5 to give equations 6 and 7, into which the calculated absorptivities are substituted directly.

$$\%\ \text{PG} = 6.34\ (a \text{ at } 252\ \text{m}\mu) - 0.097\ (a \text{ at } 232.1\ \text{m}\mu) - 0.437\ (a \text{ at } 241.0\ \text{m}\mu) \qquad (6)$$

$$\%\ \text{BHA} = 7.93\ (a \text{ at } 241.0\ \text{m}\mu) + 1.76\ (a \text{ at } 232.1\ \text{m}\mu) - 6.34\ (a \text{ at } 252\ \text{m}\mu) \qquad (7)$$

Butylated Hydroxy Anisole in Absence of Propyl Gallate. Dissolve an accurately weighed sample of about 1.0 g. in isopropyl alcohol and dilute to volume in a 100-ml. volumetric flask. Pipet a 1.00-ml. aliquot into a 100-ml. volumetric flask and dilute to the mark with isopropyl alcohol. Measure the absorbance at 291 mμ, using 1.00-cm. cells and pure solvent in the reference beam. Calculate the absorptivity of the sample at 291 mμ and determine the concentration of butylated hydroxy anisole by the equation:

$$\%\ \text{BHA} = 4.95\ (a \text{ at } 291\ \text{m}\mu) \qquad (8)$$

Propyl Gallate in Absence of Butylated Hydroxy Anisole. Accurately weigh about 0.6 g. of sample and proceed just as in the case of butylated hydroxy anisole except measure the absorbance at 274 mμ instead of 291 mμ. From the absorptivity at 274 mμ, calculate the concentration of propyl gallate as follows:

$$\%\ \text{PG} = 1.97\ (a \text{ at } 274\ \text{m}\mu) \qquad (9)$$

Results

Spectra. The spectra of isopropyl alcohol solutions of butylated hydroxy anisole and propyl gallate are shown in Figure 1. Two isoabsorptive points are found near 232 and 241 mμ, thus indicating that it should be possible to determine the total concentration of antioxidant at these wavelengths. At all wavelengths greater than 241 mμ, propyl gallate is the stronger absorber with the ratio of absorbances reaching a maximum of slightly over 20:1 in the 252 to 258 mμ region. This represents a highly favorable condition for the determination of propyl gallate once the total antioxidant concentration is known.

In order to find the exact position of the isoabsorptive points, the absorption of butylated hydroxy anisole and of propyl gallate was measured at 0.5 mμ intervals from 230 to 234 mμ, using 0.0015% solutions and from 239 to 242 mμ, using 0.0075% solutions. The wavelength settings were approached from the short wavelength side throughout these measurements. The sensitivity knob was set at three turns from counterclockwise throughout these measurements with the slit-width being varied as necessary to maintain instrument balance. This experiment was repeated three times over a period of several days, and the average absorbance at each wavelength was calculated. A plot of these average values on an expanded scale (Figure 2) showed that the isoabsorptive points occurred at 232.1 and 241.0 mμ. Comparison of the plot of the averages with the plots of the individual runs indicated that the uncertainty in the positions of the isoabsorptive points was less than 0.1 mμ. The average deviations of the absorbances at 232.1 and 241.0 mμ were less than 1% for the propyl gallate and butylated hydroxy anisole.

Neither citric acid nor propylene glycol show meas-

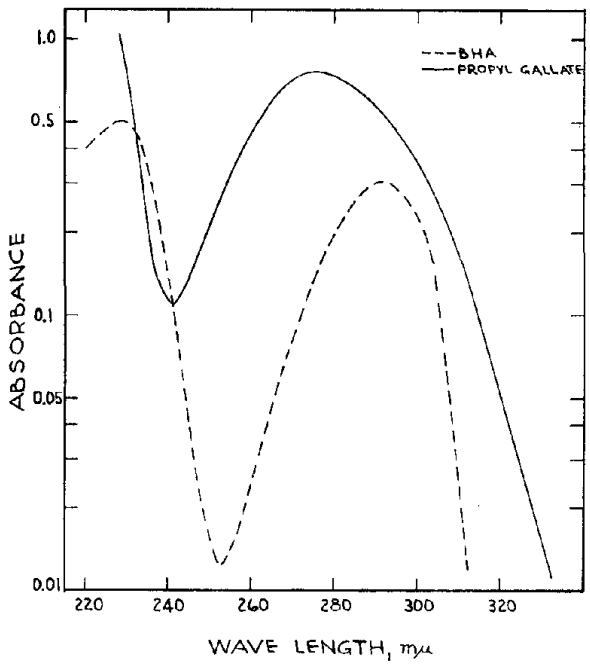

FIG. 1. Absorption Spectra
----- Butylated Hydroxy Anisole
——— Propyl Gallate
(0.0015% in Isopropanol; 1.00-cm. cell)

urable ultraviolet absorption at many times the concentrations which would be possible in the analysis of antioxidant preparations.

Stability. Neither propyl gallate nor butylated hydroxy anisole solutions showed a measurable change in absorbance at 232.1, 241.0, 252, 274, or 291 mμ upon standing for several hours.

Slit-Width. The absorbances of propyl gallate and of BHA at 232.1 and 241.0 mμ were measured with the slits being varied over the entire range possible by changing the sensitivity control of the instrument. It was found (Table I) that variation of the slit-width from 0.32 to 0.70 mm. at 232.1 mμ and from 0.27 to 0.56 mm. at 241.0 mm. resulted in less than 0.5% variation in any of the absorbance values. Wider slit-widths appeared to cause a significant lowering of the absorbances of both propyl gallate and BHA at 232.1 mμ and of BHA at 241.0 mμ. All further work was carried out with slit-widths of 0.70 mm. or less at 232.1 mμ, and 0.56 mm. or less at 241.0 mμ. The sensitivity control setting was kept at approximately three turns from counterclockwise.

Calibration Data. Standard absorbances and standard absorptivities for butylated hydroxy anisole and

TABLE I
Effect of Slit-Width on Absorbance

Sensitivity Control	232.1 mμ[a]			241.0 mμ[b]		
	Slit-Width, mm.	APG	ABHA	Slit-Width, mm.	APG	ABHA
Counterclockwise	0.26	0.453	0.453	0.21	0.495	0.497
1 turn from CCW	0.32	0.447	0.453	0.27	0.494	0.497
2 turns from CCW	0.40	0.446	0.452	0.31	0.494	0.496
3 turns from CCW	0.42	0.446	0.453	0.36	0.494	0.496
4 turns from CCW	0.48	0.446	0.452	0.39	0.494	0.495
5 turns from CCW	0.52	0.446	0.452	0.41	0.494	0.495
6 turns from CCW	0.58	0.446	0.452	0.46	0.494	0.495
7 turns from CCW	0.62	0.445	0.452	0.51	0.495	0.495
8 turns from CCW	0.70	0.445	0.452	0.56	0.495	0.495
9 turns from CCW	0.78	0.442	0.450	0.63	0.494	0.491
Clockwise	0.88	0.440	0.449	0.70	0.495	0.487

[a] 0.0015% solutions; 1.00-cm. cell.
[b] 0.0075% solutions; 1.00-cm. cell.

TABLE II
Absorbances and Absorptivities[a] of Butylated Hydroxy Anisole and Propyl Gallate in Isopropanol[b]

Wave Length, mμ	BHA		PG	
	A	a	A	a
232.1[c]	0.451	30.1	0.451	30.1
274.0[c]	.....	.....	0.762	50.8
291.0[c]	0.303	20.2	.....	.....
241.0[d]	0.500	6.67	0.500	6.67
252.0[d]	0.069	0.92	1.254	16.7

[a] Absorptivities, *a*, obtained by dividing observed absorbances, *A*, by concentration expressed in g./l.
[b] Average of three runs.
[c] 0.0015% solutions, 1.00-cm. cells.
[d] 0.0075% solutions, 1.00-cm. cells.

propyl gallate are shown in Table II. The absorbances at 232.1, 274, and 291 mμ were obtained with 0.0015% solutions and those at 241.0 and 252 mμ with 0.0075% solutions. Absorptivities were calculated by substitution of these data into Equation 1.

Equations 2 and 3 in the procedure section were derived by the simultaneous solution of equations 10 and 11, and equations 3 and 4 were derived by the solution of equations 11 and 12.

$$a \text{ at } 232.1 \text{ m}\mu. = 30.1\ C\,(\text{BHA}) + 30.1\ C\,(\text{PG}) \quad (10)$$

$$a \text{ at } 252 \text{ m}\mu. = 0.92\ C\,(\text{BHA}) + 16.7\ C\,(\text{PG}) \quad (11)$$

$$a \text{ at } 241 \text{ m}\mu. = 6.67\ C\,(\text{BHA}) + 6.67\ C\,(\text{PG}) \quad (12)$$

where C (BHA) and C (PG) represent the fractions of the total absorptivity at a given wavelength due to butylated hydroxy anisole and propyl gallate, respectively.

Mixtures covering the entire concentration range were prepared by diluting appropriate aliquots of 0.15% stock solutions so that the total antioxidant concentration was 0.0015%. The absorbances of all solutions at 232.1 mμ were within ± 0.5% of the average. Similar data were obtained with 0.0075% solutions at 241.0 mμ, and in this case all absorbances fell within ± 1.2% of the average. These data showed that the absorbances of the individual antioxidants are additive at these wavelengths.

The absorbances of the mixtures at the 0.0075% concentration level were measured at 252 mμ and the

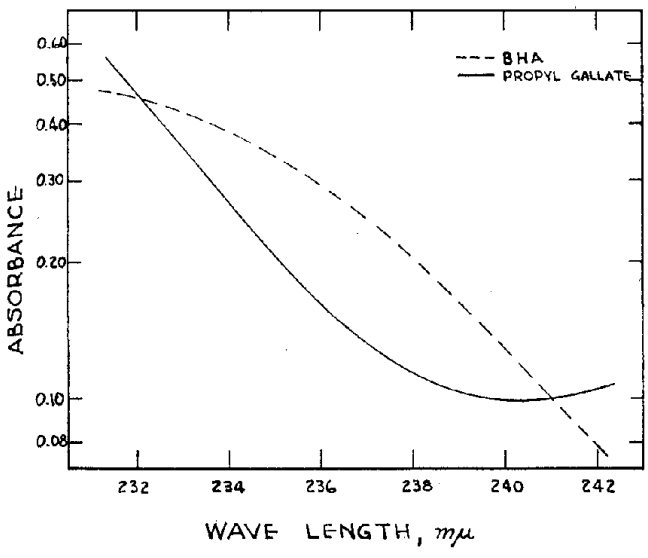

FIG. 2. Location of Isoabsorptive Points
----- Butylated Hydroxy Anisole
——— Propyl Gallate
(0.0015% in Isopropanol; 1.00-cm. cells)

values plotted against relative concentration of propyl gallate. When a straight line was drawn between the points for the two pure compounds, the points for the mixtures did not deviate from the line by more than 1% propyl gallate, thus showing that the absorbances at 252 mμ are also additive in mixtures.

Plots of absorbance at 291 mμ against concentration of butylated hydroxy anisole showed that Beer's law was followed up to a concentration of 0.0030%. The same was true for propyl gallate at 274 mμ up to a concentration of 0.0023%.

Synthetic Mixtures. Three synthetic mixtures were prepared by dissolving weighed portions of butylated hydroxy anisole, propyl gallate, citric acid, and propylene glycol in isopropyl alcohol. Both the relative concentrations of butylated hydroxy anisole and propyl gallate and the absolute concentrations of all the components were varied over wide ranges to simulate the composition of commercial antioxidant preparations. After suitable dilution with isopropyl alcohol, the absorbances of the samples were measured at the appropriate wavelengths and the antioxidant concentrations were calculated.

The results in Table III show that the actual and calculated concentrations of both butylated hydroxy anisole and propyl gallate were in good agreement. Thus the presence of citric acid and propylene glycol does not affect the absorbance values characteristic of simple isopropyl alcohol solutions of the antioxidants.

TABLE III

Synthetic Antioxidant Preparations

Component	Sample 1		Sample 2		Sample 3	
	Added, %	Found, %	Added, %	Found, %	Added, %	%, Found,
BHA	20.0	19.9	20.0	20.1	0.0	.....
Propyl Gallate	6.0	6.1	0.0	.....	20.0	20.4
Citric Acid	4.0	.....	20.0	.....	8.0	.....
Propylene Glycol	70.0	.....	60.0	.....	72.0	.....

Precision. Two production samples of antioxidant preparations were analyzed six times and a third sample seven times by three different operators. Duplicate analyses of a given sample by any one operator were carried out on different days. One of the samples contained both propyl gallate and butylated hydroxy anisole, the total antioxidant concentration being about 26%. One sample contained only propyl gallate and the other only butylated hydroxy anisole. All samples contained citric acid and propylene glycol in concentrations ranging from 4 to 20% and 60 to 72%, respectively.

Standard deviations of approximately 1.5% of the amount of antioxidant present were found for all of the determinations except that of propyl gallate in samples containing both antioxidants; in this case the standard deviation was 3.3% of the propyl gallate concentration. These results of the precision tests are summarized in Table IV.

Discussion. All of the absorbance values presented in this paper for butylated hydroxy anisole were obtained with the pure 3-isomer. It was established early in the work however that the spectra of the two isomers are substantially identical throughout the ultraviolet region of the spectrum.

TABLE IV

Precision of Determinations

Sample	Aver. % BHA	Aver. % PG	Std. Dev. %	No. of Detns.
1	19.70	5.97	0.27 (BHA) 0.20 (PG)	6 6
2	.....	20.03	0.23	6
3	19.32	.....	0.32	7

The absorbance of propyl gallate at 241 mμ varied as much as 15% when different lots of untreated commercial isopropyl alcohol were used as the solvent. The values were always higher than the one obtained with distilled alcohol. At 274 mμ, the variation in absorbance varied about 3%, using undistilled solvent, but at this wavelength the highest reading was obtained with purified solvent. The absorbance of propyl gallate at 232.1 mμ and the absorbances of butylated hydroxy anisole at all of the analytical wavelengths were unchanged by using distilled solvent. No particular effort has been made to identify the factor responsible for the varying absorbances since reproducible results were easily obtained by distilling the solvent through a short Vigreux column. In routine control work it may sometimes be desirable to use commercial isopropyl alcohol as the solvent and eliminate the measurement at 241.0 mμ. If readings at 274 mμ are required, a sample of known composition can easily be checked to insure that the particular lot of solvent will give results having the required accuracy.

Nordihydroguairetic acid interferes seriously with the determination of butylated hydroxy anisole at 291 mμ, where the absorption of the acid is about two-thirds as strong as that of the anisole derivative. If the concentration of nordihydroguairetic acid is known from some other source however, the total concentration of acid and butylated hydroxy anisole can be determined at 287.5 mμ, using a standard absorptivity of 18.9.

The procedures have been used over three years as quality control measures in the preparation of commercial antioxidant preparations, and it is believed that they will be of value in controlling the addition of such antioxidant preparations to food products. For the most accurate results with samples containing both butylated hydroxy anisole and propyl gallate, it is recommended that the standard absorptivities and the isoabsorptive points be redetermined with the instrument which is to be used in the analysis. If this is not feasible, the wavelength scale of the instrument should be calibrated to an accuracy of ± 0.1 mμ in the 230 to 265 mμ region. The standard absorptivities at 232.1 and 241.0 mμ should also be checked if the instrument is equipped with a blue-sensitive phototube rather than a photomultiplier tube since considerably wider slits may be required. When only one antioxidant is present, slit-width control and wavelength calibration are less critical.

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[Received December 1, 1954]