

AUTOXIDIZED OILS FROM SAURY OIL AND ITS HIGHLY UNSATURATED ACID FRACTION

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Properties of the autoxidized oils extracted from variously sun-dried saury fish were reported in a separate paper by Shimo-oka and Toyama.¹⁾ In this connection, the present study has been undertaken with the object of knowing that whether properties of the autoxidized oils differ in any respect or not depending upon whether the oil as contained in saury body has undergone autoxidation during the sun-drying of saury or the oil separated from saury has been autoxidized under exposure to the sun, and also that whether properties of autoxidized saury oils depend on any way upon whether the oil has been autoxidized under exposure to the sun or it has been autoxidized by air-blowing without exposure to the sun. Further, since the highly unsaturated acids among the fatty acids of saury oil are considered to participate preferentially in the autoxidation of saury oil, a highly unsaturated acid fraction separated from saury oil was autoxidized to give autoxidized samples containing peroxides. On decomposing the peroxides by heating autoxidized samples, volatile decomposition products and the non-volatile residue were then separated and analyzed.

1. Autoxidized saury oils

A sample of saury oil, produced in Sanriku District in October, 1956, was variously autoxidized in the following way.

A. Autoxidation under exposure to the sun. Each 100 g.-portion of the oil was placed in four flat-bottomed dishes of the same size, 12 cm. in diameter, and the dishes containing oil were placed outdoors and exposed to the sun in the bright day-time and placed indoors in the cloudy or rainy day and night-time. The content of the dishes was prevented from being contaminated with dust by using a frame covered with a film of transparent cellophane. In this way, four samples, which had been autoxidized for different periods, were prepared.

B. Autoxidation by air-blowing. One hundred g. of the oil was placed in a flask, and air was blown into the oil at 50°C to effect the autoxidation of oil. By varying the period of air-blowing, four samples of autoxidized oil were prepared.

Properties of the original saury oil and autoxidized oils are given in Tables 1 A and 1 B.

As is seen from Tables 1 A and 1 B, characteristics of saury oil undergo changes with the progress of autoxidation. The ether-insoluble bromide from the fatty acids is on the decrease with the progress of autoxidation, indicating that highly unsatu-

TABLE 1 A. Properties of the Saury Oil and the Autoxidized Saury Oils Prepared under Exposure to the Sun

Oil sample	Original saury oil	Autoxidized saury oils			
		A-1	A-2	A-3	A-4
Period of autoxidation (day)	0	10	18	32	64
Period of exposure to the sun (hr.)	0	27	33	46	109
d_4^{20}	0.9138	0.9325	0.9335	0.9348	0.9651
n_D^{20}	1.4739	1.4754	1.4762	1.4791	1.4842
Specific viscosity (30°C)	28.9	62.8	75.1	136	—
Peroxide value (meq./kg.)	5.9	186.2	221.7	288.5	394.1
Acid value	3.9	5.4	7.7	9.7	11.9
Saponification value	185.0	189.7	190.6	191.6	194.5
Iodine value (Wijs)	155.5	141.3	134.5	119.8	99.6
Hydroxyl value	6.9	24.9	36.6	40.5	91.1
Unsaponifiable matter (%)	0.50	0.53	0.64	0.98	1.08
Conjugated diene (%)	0.7	2.7	3.0	3.1	4.0
Fatty acids					
Neutralization value	193.2	192.9	195.4	196.8	196.5
Iodine value	159.0	144.7	137.7	122.4	102.6
Ether-insoluble bromide (%)	49.9	36.1	24.6	13.6	8.2

Notes: The color of oil was not noticeably varied by the autoxidation of oil. Peroxide values were determined by the method of Wheeler. The content of conjugated diene was calculated from the value of k_{233} for the oil in *n*-heptane by applying the equation: diene (%) = $0.84(k_{233} - 0.07)$. The effect of oxidized fatty acids on the absorption value was neglected.

TABLE 1 B. Properties of the Autoxidized Saury Oils Prepared by Air-blowing

Oil sample	B-1	B-2	B-3	B-4
d_4^{20}	0.9227	0.9242	0.9284	0.9287
n_D^{20}	1.4742	1.4758	1.4769	1.4783
Specific viscosity (30°C)	41.1	41.7	50.2	51.1
Peroxide value (meq./kg.)	130.9	157.6	273.6	365.6
Acid value	5.1	5.2	5.7	5.8
Saponification value	186.8	188.1	193.9	192.3
Iodine value	149.0	147.1	146.3	139.2
Hydroxyl value	17.3	18.9	39.9	48.2
Unsaponifiable matter (%)	0.64	1.05	1.35	1.53
Conjugated diene (%)	1.6	1.9	2.0	2.6
Fatty acids				
Neutralization value	193.7	192.5	196.4	194.2
Iodine value	155.9	154.2	150.8	145.6
Ether-insoluble bromide (%)	45.9	44.9	41.7	37.4

rated acids among others in saury oil have undergone autoxidation to a marked extent. The fatty acids from the autoxidized oils A-4 and B-4 were isomerized under the condition of 21% KOH-ethylene glycol, 180°C and 15 min., and the ultra-violet absorption spectra of alkali-isomerized acids were determined as shown in Fig. 1. The absorption spectra all exhibited a characteristic maximum at 374 $m\mu$ besides other characteristic maxima at shorter wavelengths; k_{374} being 1.1 for the sample A-4 and 2.1 for the sample B-4. Although the content of hexaenoic acid

in the fatty acids containing oxidized acid can not be estimated from the value of K_{374} , it is seen from Fig. 1 that hexaenoic acid still remains intact in some measure in the sample A-4 as well as in the sample B-4.

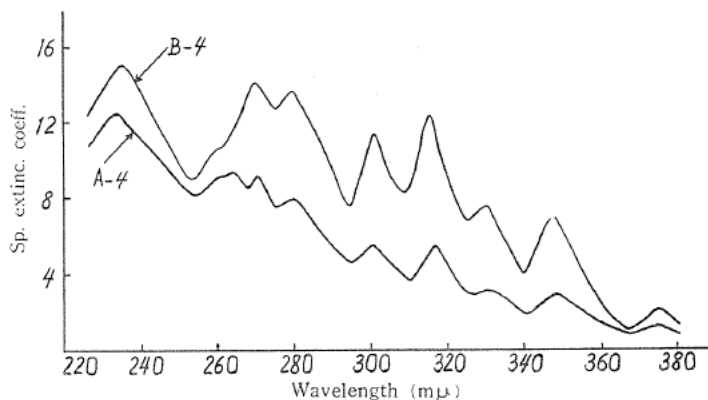


FIG. 1. Ultraviolet absorption spectra of the alkali-isomerized fatty acids from the autoxidized samples A-4 and B-4.

In respect to iodine value, the samples A-1 (I.V. 141.3) and A-2 (I.V. 134.5) obtained by the autoxidation under exposure to the sun correspond to the samples B-3 (I.V. 146.3) and B-4 (I.V. 139.2) obtained by the autoxidation by air-blowing. However, the samples A-1 and A-2 show lower peroxide values than the samples B-3 and B-4, suggesting that the ratio of the decomposition of peroxide to the formation of peroxide is greater in the autoxidation under exposure to the sun than in the autoxidation by air-blowing. In other respects, the samples obtained under exposure to the sun show more or less smaller hydroxyl values and yields of ether-insoluble bromide than the corresponding samples obtained by air-blowing. Comparing the autoxidized saury oil samples obtained in the present study and those extracted from sun-dried saury in a separate study,¹⁾ it may be noted here that the latter have generally more or less larger acid and saponification values and smaller peroxide values than the former. As a reason for increased acid and saponification values of the oils extracted from sun-dried saury, it is conceivable that the oil in sun-dried saury might have undergone more or less enzymatic hydrolysis to give free fatty acids and also that some oil-soluble acids might have been formed from some substances other than fat contained in the sun-dried saury. That the oils extracted from sun-dried saury have smaller peroxide values is partly due to the decomposition of peroxides in the course of the extraction procedures, although it is conceivable, too, that the decomposition of peroxides is accelerated by some substances contained in sun-dried saury.

2. Autoxidation of a highly unsaturated acid fraction of saury oil

The fatty acids from the saury oil were segregated as urea adduct using 300 g. of urea and 750 cc. of methanol for 100 g. of the fatty acids. After removal of crystalline urea adducts containing saturated and less unsaturated acid components, a highly unsaturated acid fraction was separated from the methanol filtrate. In total,

1,400 g. of fatty acids gave 400 g. of a highly unsaturated acid fraction having n_D^{20} 1.4900, neutralization value 181.1 and I.V. 355.3. Four portions taken out from this fraction were placed in flasks, and the content of each flask was shaken in an atmosphere of oxygen for a different period at a room temperature of 5°-18°C. The autoxidized samples thus prepared were then heated at 90°C while a current of nitrogen was passed into the flasks containing samples with the aid of suction. Volatile compounds formed by the decomposition of peroxides in the samples were carried off with the current of nitrogen and passed successively through a 7% solution of potassium carbonate and a 0.2% solution of 2,4-dinitrophenylhydrazine in 2 N-hydrochloric acid to catch volatile acid and carbonyl compounds.

The period of autoxidation, the period of heating for the decomposition of peroxides, peroxide values of the autoxidized samples before heating, and characteristics of the autoxidized samples after heating are given in Table 2.

TABLE 2. Autoxidation of a Highly Unsaturated Acid Fraction from Saury Oil and Heat Decomposition of the Peroxides in Autoxidized Samples

Expt. No.	1	2	3	4
Period of autoxidation (hr.)	0.3	8	15	55
Peroxide value of autoxidized sample before heating (meq./kg.)	125	486	1,380	2,210
Period of heating of autoxidized sample at 90°C (hr.)	1.0	2.0	2.5	3.5
Autoxidized sample after heating				
Peroxide value (meq./kg.)	6.9	13.9	17.7	17.2
d_4^{20}	0.9444	0.9662	0.9731	1.0420
n_D^{20}	1.4911	1.4937	1.4968	1.5100
Neutralization value	180.5	169.9	162.0	146.3
Saponification value	207.4	213.0	222.2	241.7
Iodine value	351.2	333.9	318.7	239.3
Unsaponifiable matter (%)	1.11	1.24	1.46	1.65
Hydroxyl value	—	—	38.9	46.7
Carbonyl value	—	—	50.1	85.9
Elementary analysis				
C (%)	—	—	74.35	70.56
H (%)	—	—	9.37	9.01
O (%)	—	—	16.28	20.43
Molecular weight (Rast)	360	410	480	640

Notes: The highly unsaturated acid fraction showed a peroxide value of 99.3 immediately before the experiments on autoxidation. It had Mol. Wt. (Rast) 340. In Expts. 1 and 2, a 80 g.-portion of the material was used. In Expts. 3 and 4, a 100 g.-portion of the material was used. The viscosity of the material increased with the period of autoxidation. The original material was reddish orange. The color of the material became lighter with the increasing period of autoxidation, but the color of the material after heating for the decomposition of peroxides became darker with the increasing period of autoxidation.

As is seen from Table 2, the specific gravity and refractive index of autoxidized sample after heating become increasingly higher with the period of autoxidation. The specific gravity for the sample in Expt. 4 is higher than 1. The neutralization value decreases while the saponification value increases with the period of autoxidation, resulting in an increase of the difference between these two values. The

molecular weight increases until it becomes nearly twice as large as the molecular weight of the original sample in Expt. 4. While the highly unsaturated acid used in this study is considered to have a mean composition roughly corresponding to $C_{20}H_{32}O_2$ (Calcd., C 78.89%, H 10.59%, O 10.52%) from its neutralization and iodine values, the oxygen contents of the samples in Expts. 3 and 4 are found markedly larger than the theoretical value for $C_{20}H_{32}O_2$. Although the presence of hydroxyl and carbonyl groups in these samples, as indicated by their hydroxyl and carbonyl values, is responsible for this large content of oxygen, it alone can not account for this. The samples in Expts. 3 and 4, particularly that in Expt. 4, appear to contain some oxygen other than carbonyl, hydroxyl and carboxyl types.

3. Volatile compounds formed by the decomposition of peroxides in the autoxidized samples of a highly unsaturated acid fraction from saury oil

As described above, the volatile acids and carbonyl compounds formed by the decomposition of peroxides in the autoxidized samples were collected in the potassium carbonate solution and 2,4-dinitrophenylhydrazine solution, respectively.

(i) **Volatile acids.** The potassium carbonate solutions used for Expts. 1-4 in Table 2 were united and evaporated to dryness. The residue was acidified with the least excess of dilute sulfuric acid, and then extracted with an adequate amount of ether. The ether solution was washed with a saturated solution of sodium chloride, dehydrated over anhydrous sodium sulfate, and filtered. On removal of ether from the filtrate, there remained 20 mg. of a thin liquid having a pungent odour. This was converted to hydroxamic acid in ethanol, and the ethanol solution was paper chromatographed with the results shown in Table 3, by which saturated monobasic acids from formic to caproic acids are identified as their hydroxamic acids. Judging from the color intensity of spots, formic, acetic and propionic acids are present in a relatively large proportion while butyric acid in the smallest proportion.

TABLE 3. Paper Chromatography of Hydroxamic Acids

Hydroxamic acid from	R_f	
	Sample under examination	Pure acid for reference
Formic acid	0.35	0.37
Acetic acid	0.50	0.49
Propionic acid	0.66	0.66
Butyric acid	0.78	0.77
Valeric acid	0.83	0.84
Caproic acid	0.93	0.91

Notes: Ascending method; developer: butanol saturated with water; chromogenic reagent: ferric chloride in ethanol.

(ii) **Volatile carbonyl compounds.** The precipitates of DNPHs (2,4-dinitrophenylhydrazones) formed in the solutions of 2,4-dinitrophenylhydrazines were filtered and analyzed as follows.

Expts. 1 and 2. The DNPHs obtained in Expts. 1 and 2 were united, and the united material (590 mg.) was fractionally crystallized from ethanol to give twelve

fractions. The 1st fraction (38 mg.), most difficultly soluble in ethanol, had m.p. 235°-240°C. The absorption curve for this fraction in ethanol showed a peak in the region of 400-410 m μ , and the solution of this fraction in ethanol developed a deep blue color with a 10% solution of potassium hydroxide in ethanol, suggesting this fraction to consist substantially of dialdehyde DNPHs. The 2nd (10 mg.) and 3rd (31 mg.) fractions were recognized as unpurified crotonaldehyde DNPH and pentanal DNPH, respectively (properties for these fractions are listed in Table 4). The united material (188 mg.) of the 4th to 6th fractions appeared to contain substantially saturated aldehyde DNPHs. This material was fractionated by elution chromatography on silica gel, and fractions of propionaldehyde, butyraldehyde, pentanal and hexanal (or methylbutylketone) DNPHs were separated. The 7th and succeeding fractions had relatively low melting points and were contaminated with resinous matter. No uniform DNPH could be separated from these fractions.

Expt. 3. The DNPHs (594 mg.) were fractionally crystallized from methanol to give nine crystalline fractions and a resinous fraction of low melting point. The 1st to 3rd fractions (36 mg.) were considered to contain dialdehyde DNPHs and unsaturated aldehyde DNPHs, but they could not be closely studied. The 4th to 9th fractions (220 mg.) consisted chiefly of saturated aldehyde DNPHs among which butyraldehyde DNPH was separated (see Table 4).

Expt. 4. The DNPHs (1,110 mg.) were fractionated by fractional crystallization from methanol and by elution chromatography on silica gel, by which nine crystalline fractions (293 mg.) together with a relatively large amount of resinous fractions of low melting points were obtained. On analyzing the crystalline fractions for their melting point, nitrogen content and ultraviolet absorption spectrum, the 1st and 2nd fractions were recognized chiefly as dialdehyde DNPHs, the 3rd to 6th fractions chiefly as unsaturated aldehyde DNPHs and the 7th to 9th fractions chiefly as saturated aldehyde DNPHs. But all fractions were still a mixture from which any uniform DNPH could not be separated.

TABLE 4. Properties of DNPHs

DNPH	m.p. (°C)		N (%)		Remark
	Observed	Reported	Observed	Calcd.	
Propionaldehyde	150-152	154-155 ²⁾	23.3	23.5	Expts. 1 and 2
Butyraldehyde	122-124	120-121 ²⁾	22.8	22.2	"
"	121-123	"	22.1	"	Expt. 3
Pentanal	98-100	104-105 ²⁾	20.9	21.0	Expts. 1 and 2
Hexanal*	106-108	107-108 ²⁾	19.6	20.0	"
Crotonaldehyde	188-191	190 ²⁾	22.2	22.4	"
Pentenal	157-163	163 ²⁾	21.7	21.2	"

Notes: * The absorption spectrum, in ethanol, for this hexanal DNPH exhibited an absorption maximum at 366 m μ while the characteristic absorption maximum for other saturated aldehyde DNPHs obtained in these experiments were observed at 359-360 m μ . Braude and Jones⁴⁾ reported m.p. 108°C and the absorption maximum at 365 m μ for methylbutylketone DNPH. Hence, the substance obtained in these experiments might be not hexanal DNPH but methylbutylketone DNPH.

Summary

1. Saury oil was autoxidized to different extents under exposure to the sun and also by air-blowing at 50°C, and autoxidized samples were analyzed for their characteristics. Comparing autoxidized samples having iodine values of an approximately same level, samples obtained under exposure to the sun had smaller peroxide values than samples obtained by air-blowing.

2. A highly unsaturated acid fraction separated from saury oil was autoxidized with molecular oxygen for different periods at a room temperature of 5°-18°C. The autoxidized samples were heated at 90°C to effect the decomposition of peroxides. The samples, after the decomposition of peroxides, and the volatile compounds formed by the decomposition of peroxides were analyzed. Among volatile acids, the saturated monobasic acids from formic to caproic acids were identified. As volatile carbonyl compounds, propionaldehyde, butyraldehyde, *n*-pentanal, *n*-hexanal or methylbutylketone, crotonaldehyde and pentenal were identified as their 2,4-dinitrophenylhydrazones. The presence of dialdehyde was also indicated.

References

- 1) T. Shimo-oka and Y. Toyama : *Memoirs Faculty of Engineering, Nagoya University* **9**, 360 (1957).
- 2) G. D. Johnson : *J. Am. Chem. Soc.* **75**, 2720 (1953).
- 3) O. C. Johnson, S. S. Chang and F. A. Kummerow : *J. Am. Oil Chemists' Soc.* **30**, 317 (1953).
- 4) E. A. Braude and E. R. Jones : *J. Chem. Soc.* **1945**, 498.