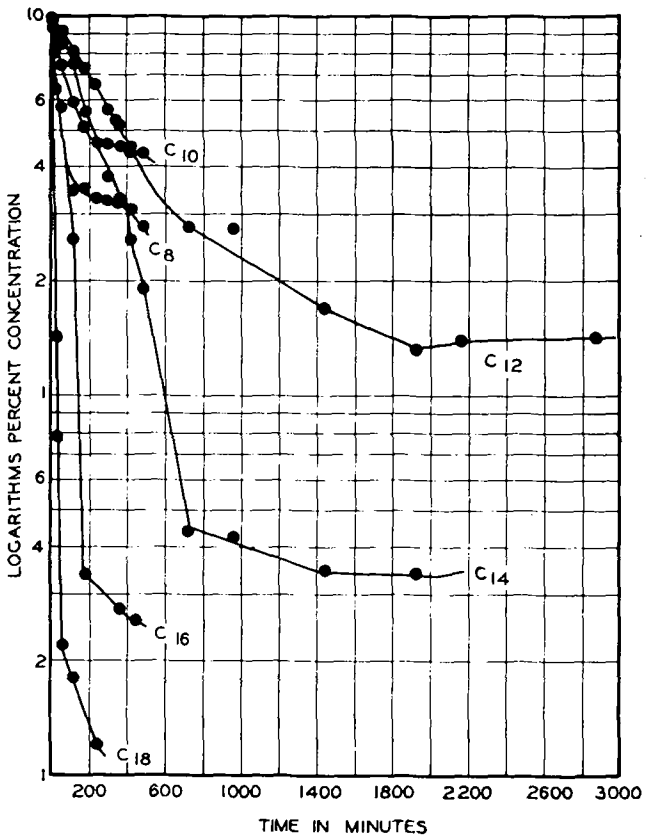

FIG. 3. Hydrolysis of saturated acid chlorides in water at 25° C.

ant to hydrolysis than either the chlorides of the lower molecular weight octanoic and decanoic acids or the chlorides of the higher molecular weight myristic to stearic acids.

The chlorides of the unsaturated acids, oleic, elaidic, and linoleic, are hydrolyzed less rapidly than stearyl chloride. However, elaidyl and myristyl chlorides exhibit the same relative rates of hydrolysis during the first two hours of reaction. Myristyl chloride hydrolyzes more rapidly than elaidyl chloride after the first two hours.

The addition of either hydrochloric acid or free fatty acids to the reaction mixture was found to have no pronounced effect on the hydrolysis of the acid chlorides.

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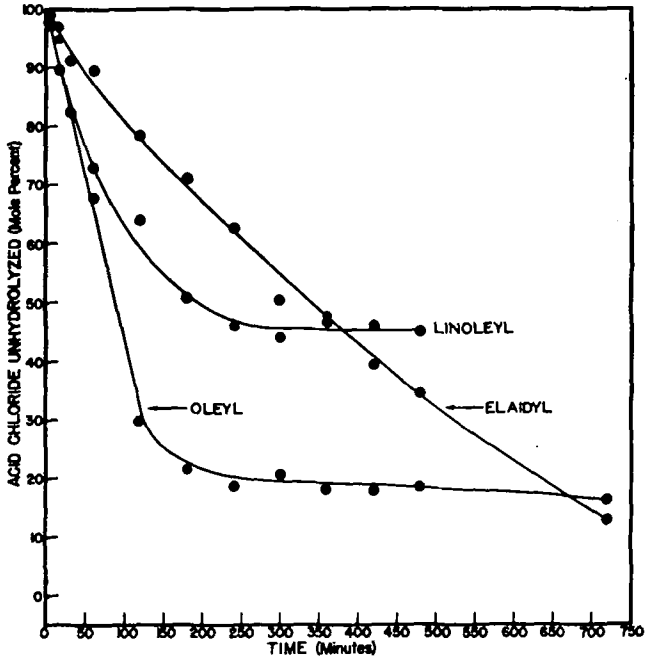

FIG. 4. Hydrolysis of unsaturated acid chlorides in water at 25° C.

A Study of Rancidity of Olive Oils

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INVESTIGATION of the problem of rancidity is rendered difficult by the fact that the ultimate standards to which all data must be referred are based on taste and smell. Among the aims of these experiments were: a) the comparison of the various known chemical tests for rancidity of fats and oils with the organoleptic test for the purpose of selecting the most reliable one for further use as a standard of reference; b) determination whether olive oils from different localities have different susceptibility to fat oxidation and the study of the effect of various factors and treatments upon such susceptibility to oxidation; and c) determinations whether anise seeds and mustard seeds have antioxidant value for olive oil.

The methods used were the following:

Oxidizability-value developed by Issoglio and modified by Kerr (1), which gives the amount of water-soluble oxidizable substances expressed in O_2/100 gm. of oil.

The Kreis (2) test which is based on the presence in rancid fats of epihydrin aldehyde, an oxidation product of acrolein. It consists in the treatment of the oil with hydrochloric acid and a solution of phloroglucinol in ether.

Fellenberg (3) test based on the presence of aldehydes in the oxidized fats. Vintilesco and Popesco (4) test based on the presence of peroxides and oxides formed during the development of rancidity.

The Wheeler's (3) iodimetric method of determining the peroxides formed in rancid fats.

The last method was carried out as follows: five g. of olive oil were dissolved in 50 ml. of a mixture of glacial acetic acid and chloroform (3:2 by volume), after which one ml. of saturated potassium iodide solution was added. The mixture was stirred by giving a rotary motion to the flask. Exactly one minute after addition of the potassium iodide, 100 ml. of water were added and the liberated iodine titrated with 0.01 *N* sodium thiosulphate, using starch as

indicator. The titration figures were expressed in ml. *N* thiosulphate per 100 gm. of oil.

Eighty-eight samples of olive oils collected during two seasons from the olive oil mills of different localities of Greece were tested by the above methods in the Research Experiment Station for Agricultural Technology in Athens. The results are shown in Tables I and II.

From the examination of the above tables it is evident that no one of the chemical tests used gives satisfactory results. The Wheeler method, however, gave values which agreed more closely with the organoleptic test than did any other of the chemical tests considered and therefore was selected as the standard test in our investigation. The acid value which at present is the official method in Greece for determining the commercial value of the olive oil and its suitability for human consumption, proved to be very unreliable from the point of view of rancidity.

The Issoglio test, although of no value for testing rancidity, can be of help in differentiating the natural olive oils from the refined oils if the latter are not too old. The treatment of aeration for the measurement of the susceptibility of the oils to rancidity was carried out by bubbling at constant temperature (30° C.) through the oil a constant flow of air washed by passage through permanganate solution. The factors examined were: a) the method of extraction (extraction by pressure, extraction by the use of solvent, extraction of the oil from the flesh and from the kernels separately), b) the sanitary condition of the olives, and c) the effect of two natural antioxydants extracted from anise seeds and mustard seeds. The results appear in the following Tables III, IV, and V, and seem to warrant the conclusion that there is a great variation in the susceptibility to fat oxidation of the oils of different localities. For instance No. 7 oil from Nafpactos (Table III) although very high in acid value and characterized by official Greek standards as unfit for human consumption, shows a very high stability to oxidation. Determination of the unsaponifiable fraction of this oil gave a very high value mounting to 2.88% in comparison with the other samples whose values ran from 1.74 to 1.99%.

The process of extraction of the oil and the sanitary condition of the fruit are factors which affect considerably the resistance of the oil to fat oxidation as is shown from the figures in Table IV. The introduction in the olive oil industry, therefore, of the methods of extraction by solvents applied at present only in the seed oil industry appears, in the light of this investigation, to be advisable, as it would help to create more stable oils as far as rancidity is concerned and reduce the cost of extraction since this method needs less capital, labor, and space for operation.

In Table V are given the results of the experiment conducted for the purpose of determining whether anise seed and mustard seed have any antioxidant value for olive oil. The idea was suggested by the fact that in the book "Geoponica" (5) of an ancient Greek writer "Kassianos Vassos" is found a recipe for the improvement of the quality of olive oil by the use of the above seeds. The results show that alcoholic extracts of anise seeds and anise seed flour have some effect in increasing the stability of the olive oil. Mustard seed extracts so far do not have any effect.

TABLE I.

Comparison of Rancidity Tests With Organoleptic Examination.

Sample	Source	Acid Value mg. NaOH / g. oil	Issoglio Test O_2/100 g.	Kreis Test	Vintilesco and Popesco Test	Fellenberg Test	Organoleptic Test
A. Raw Olive Oils							
1	Avia	0.72	35.20	p.[1]	f. p.[2]	f. p.	excellent
2	Kardamyli	1.00	20.48	p.	f. p.	p.	excellent
3	Kalamata	1.28	28.80	p.	f. p.	p.	very good
4	Kalamata	3.20	21.50	p.	p.	f. p.	good
5	Kalamata	3.60	12.80	p.	p.	f. p.	good
6	Corfu	1.44	12.80	p.	f. p.	f. p.	very good
7	Corfu	1.52	14.40	p.	p.	p.	good
8	Corfu	3.56	19.30	p.	p.	p.	good
9	Kampos	3.20	31.30	p.	p.	p.	no good
10	Chalkis	5.08	13.90	p.	p.	f. p.	good
11	Chalkis	5.20	14.40	p.	p.	f. p.	good
12	Atalante	5.48	22.40	p.	p.	p.	no good
13	Atalante	10.40	54.40	p.	p.	p.	no good
14	Atalante	10.80	22.40	p.	p.	p.	no good
15	Chania	3.20	24.00	p.	f. p.	f. p.	very good
16	Chania	10.84	29.10	p.	p.	p.	no good
17	Chania	17.20	51.20	p.	p.	p.	very bad
18	Scopelos	5.80	31.40	p.	p.	p.	no good
19	Scopelos	7.20	22.40	p.	p.	p.	no good
20	Stylis	6.92	12.10	p.	p.	f. p.	no good
21	Skiathos	7.60	14.80	p.	p.	f. p.	no good
22	Limni	9.20	21.30	p.	p.	f. p.	no good
23	Amphissa	9.36	11.00	p.	p.	p.	no good
24	Pelion	10.00	19.30	p.	p.	p.	no good
25	Pteleos	9.40	25.60	p.	p.	p.	no good
26	Pteleos	10.20	18.00	p.	p.	f. p.	no good
27	Peta	13.60	18.70	p.	p.	p.	no good
28	Marmari	14.48	16.60	p.	p.	p.	very bad
29	Marmari	18.00	19.50	p.	p.	p.	very bad
30	Sparta	13.20	18.30	p.	p.	p.	no good
31	Sparta	18.00	26.20	p.	p.	p.	very bad
32	Rombion	6.80	19.80	p.	p.	f. p.	no good
33	Kranidi	1.60	22.40	p.	p.	p.	very good
34	Skiathos	7.60	14.80	p.	p.	f. p.	no good
B. Refined Olive Oils							
1	Chanion	0.20	.58	p.	n.[3]	p.	no good
2	Chanion	0.40	.96	p.	n.	p.	no good
3	Rethymnis	0.28	.65	p.	n.	p.	no good
4	Corfu	0.16	.93	p.	n.	p.	no good
5	Lesvou	0.36	2.56	p.	d[4]	p.	no good
6	Elais	0.28	.94	p.	n.	p.	no good
7	Greek Refineries	0.33	1.60	p.	d.	p.	no good
8	Paranteli	0.20	6.30	p.	d.	p.	no good
9	Paranteli	0.24	12.80	p.	p.	p.	8 examined 7 months later.

[1] Positive. [2] Faintly positive. [3] Negative. [4] Doubtful.

TABLE II.

Comparison of Acid Number and Wheeler Test for Rancidity With Organoleptic Examination of Olive Oils From Various Localities of Greece.

Sample	Source	Acid Value mg. NaOH / g. oil	Wheeler Test ml. N thiosulfate / 100 g. oil	Organoleptic Test
1	Corfu	8.47	0.10	good
2	Corfu	3.49	0.28	good
3	Creta	12.83	3.00	slightly rancid
4	Creta	5.85	2.25	slightly rancid
5	Creta	3.75	3.80	rancid
6	Creta	21.04	6.30	rancid
7	Creta	7.33	3.07	rancid
8	Creta	5.15	1.46	good
9	Creta	3.05	0.11	good
10	Creta	4.64	0.29	good
11	Creta	3.67	2.05	good
12	Creta	11.96	2.10	good
13	Creta	0.96	0.20	good
14	Peloponessos	2.80	0.60	good
15	Peloponessos	2.96	0.60	good
16	Peloponessos	3.44	0.60	good
17	Peloponessos	3.20	0.60	good
18	Peloponessos	4.64	4.00	good
19	Peloponessos	3.60	7.20	good
20	Peloponessos	3.20	6.40	good
21	Peloponessos	1.84	4.00	good
22	Peloponessos	1.52	7.80	rancid
23	Peloponessos	2.48	11.00	rancid
24	Peloponessos	4.40	7.80	rancid
25	Peloponessos	4.16	9.00	rancid
26	Peloponessos	6.88	6.60	rancid
27	Peloponessos	3.20	8.00	rancid
28	Peloponessos	2.64	10.00	rancid
29	Peloponessos	2.08	6.00	good
30	Peloponessos	2.72	1.00	good
31	Peloponessos	1.60	5.00	good
32	Peloponessos	4.64	4.00	good
33	Peloponessos	4.00	5.00	good
34	Peloponessos	2.32	22.00	rancid
35	Peloponessos	4.64	14.60	rancid
36	Peloponessos	2.64	10.60	rancid
37	Peloponessos	2.88	7.80	rancid
38	Peloponessos	2.48	10.60	rancid
39	Peloponessos	2.16	12.00	rancid
40	Peloponessos	3.28	13.00	rancid
41	Peloponessos	4.48	9.80	rancid
42	Peloponessos	3.36	10.00	rancid
43	Peloponessos	2.80	9.00	rancid
44	Peloponessos	1.44	7.00	rancid
45	Peloponessos	4.00	7.00	rancid

Summary

The Wheeler test for peroxides proved to be relatively the most reliable method for the examination of olive oils for rancidity.

The Issoglio test, although of no value for testing rancidity, can be of use in differentiating the natural olive oils from the refined olive oils.

There is a wide variation in the susceptibility to fat oxidation of the oils from different sources.

Infestation of the olives by mold induces the susceptibility of the oils to rancidity.

Alcoholic extracts of anise seed showed antioxidant properties. Negative results were obtained using alcoholic extracts of mustard seed.

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TABLE III.

Susceptibility of Various Olive Oils to Oxidation as Determined by the Swift Stability Test (3).

Specification of Olive Oil	Acid Value mg. NaOH / g. oil	Wheeler Test ml. N thiosulfate / 100 g. oil
1. Olive oil from Lesvos extracted from olives not ripened	1.57	0.72
The same sample after aeration for ½ hour	1.56	1.12
2. Olive oil from Lesvos extracted from ripe olives	2.70	1.12
The same sample after aeration for 3 hours	2.66	1.44
3. Olive oil from Corfu extracted from olives infested by the olive fly	8.45	0.20
The same sample after aeration for 2 hours	8.20	0.70
4. Olive oil from Corfu extracted from healthy olives	3.49	0.35
The same sample after aeration for 2 hours	3.49	0.35
The same sample after aeration for 4 hours	3.48	0.45
5. Olive oil from Greta extracted from olives infested by the olive fly	4.63	1.40
The same sample after aeration for 2 hours	4.36	1.60
The same sample after aeration for 4 hours	.....	2.30
6. Olive oil from Greta extracted from healthy olives	3.05	0.25
The same sample after aeration for 2 hours	3.05	0.80
The same sample after aeration for 4 hours	.....	2.50
7. Olive oil from Nafpactos	10.12	0.00
The same sample after aeration for 5 hours	10.12	0.00
The same sample after aeration for 10 hours	10.01	0.00
8. Olive oil from Agion	8.72	0.00
The same sample after aeration for 2 hours	7.84	0.25
The same sample after aeration for 5 hours	.....	0.40
9. Oil from olive kernels from Agion	1.22	0.00
The same sample after aeration for 2 hours	0.95	0.00
The same sample after aeration for 5 hours	0.52	0.27
The same sample after aeration for 7 hours	0.43	0.60
10. Oil from olive kernels from Lesvos	1.65	0.15
The same sample after aeration for 4 hours	1.00	1.50
The same sample after aeration for 7½ hours	1.04	5.65

TABLE IV.

Susceptibility to Oxidation of Oils Extracted by Various Methods From Mold Infested and Healthy Olives

Specification of the Olive Oil	Acid Value mg. NaOH / g. oil	Wheeler Test ml. N thiosulfate / 100 g. oil
1. Olive oil from healthy olives extracted by pressure	0.56	0.6
The same after aeration for 4 hours	.....	0.6
The same after aeration for 8 hours	.....	0.6
The same after aeration for 12 hours	.....	1.6
2. Olive oil from healthy olives taken by extraction with ether	2.40	1.0
The same after aeration for 4 hours	.....	1.0
The same after aeration for 8 hours	.....	1.0
The same after aeration for 12 hours	.....	1.0
The same after aeration for 18 hours	.....	1.0
The same after aeration for 24 hours	.....	1.0
3. Olive oil from olives infested by mold	3.60	0.6
The same after aeration for 4 hours	.....	7.2
The same after aeration for 8 hours	.....	8.0
The same after aeration for 12 hours	.....	12.4
4. Olive oil from olive flesh heavily infested by mold	8.40	0.4
The same after aeration for 8 hours	8.24	310.0

TABLE V.

Effect of Some Natural Antioxidants Upon the Susceptibility of Olive Oil to Oxidation.

Sample	Specification of the Oil and Treatment	Acid Value mg. NaOH / g. oil	Wheeler Test ml. N thiosulfate / 100 g. oil
1	Olive oil from Kalamata	0.8	3.0
	The same after aeration for 4 hours	0.72	10.4
	The same after aeration for 4 hours, but with a drop of alcoholic extract from anise seed	0.72	4.4
	The same after aeration for 2 hours	.....	4.6
	The same after aeration for 2 hours but with a drop of alcoholic extract from mustard seed	.....	4.6
2	Olive oil from Messenia	3.76	0.34
	The same after aeration for 1½ hours	.....	0.90
	The same after aeration for 1½ hours, but with the addition of anise seed powder	3.76	0.78