

STABILITY OF OLIVE OIL¹

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INTRODUCTION

Some years ago one of the writers reported the results of an experiment to determine the effect (4)³ of air, light, and moisture at room temperature on butter fat. The test was planned to show the action of the three agents, singly and in combination, and was continued for a year and a half. The experiment furnished considerable information relative to the changes that take place in such materials, but proved faulty in that a fat, solid and opaque at ordinary temperature, was a poor medium for measuring such changes, which evidently were not uniform throughout the mass, but greatest at the surface; furthermore, the conditions surrounding the fat were not under satisfactory control.

Conceding the limitations of the previous experiment, but recognizing the economic value as well as scientific interest of such investigations, the writers deemed it advisable to conduct another series of tests, under more definite conditions. For this purpose all oils procurable in quantity at a reasonable price were carefully considered. Olive oil was finally selected for the reason that it is a well-known edible product of fair keeping properties and of the composition desired.

THE OIL EMPLOYED

A few letters of inquiry to Federal and Experiment Station officials elicited the information that pure olive oil, both foreign and domestic, was readily obtainable. As an American oil could be procured directly from the pressers in a comparatively short time, with details of production and treatment, an order was placed, specifying an absolutely pure product that had not been bleached, sterilized, or refined in any way except as to filtration and having a low content of free fatty acids.

A 5-gallon can of California olive oil was received on February 25, 1910. The manufacturer stated that the oil was cold-pressed from an average run of hand-picked, washed, and ground ripe California-grown olives of the season of 1908. After extraction, the oil was pumped into settling tanks and from there to storage tanks, whence it was filtered four to six times through French filter paper in a special press and was not put on the market until it was at least a year old.

¹ From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

² Mr. Reed was associated with the senior writer in the earlier stages of the work and Mr. Buckley in the later.

³ Reference is made by number (*italic*) to "Literature cited," p. 366.

ORGANOLEPTIC TESTS

The appearance of the oil as determined by the unaided eye varied with the depth of stratum and character of the light from transparent olive-green to opaque, almost black. All efforts at color differentiation without an instrument proved unsatisfactory, but were continued throughout the experiment, as a tintometer was not available for the first four years. In amounts of 6 ounces, the basis employed, the oil will be designated a dark olive-green. The green greatly exceeded that in most olive oils offered in local markets, probably due, as the manufacturer claims, to differences in soil and climatic conditions, together with possibly small variations in manufacturing methods. The oil had an excellent body and a pronounced olive odor.

PHYSICAL TESTS

Specific gravity $\frac{20^{\circ}}{20^{\circ}}$ C.	0.91308
Specific gravity $\frac{20^{\circ}}{4^{\circ}}$ C. (calculated).....	0.91152
Specific gravity 25° C. (U. S. P. standard).....	0.910- 0.915
Refractive index $n \frac{20^{\circ} \text{C.}}{D}$ (Abbe).....	1.4687
Viscosity 70° F. (Redwood).....	12.0
Valenta test (B. and A. 99.5 per cent acid).....	87.5° C.
Elaidin test.....	Green, semisolid

CHEMICAL TESTS

Saponification number.....	190.636
Saponification number (U. S. P. standard).....	190-195
Acid number (a).....	1.990
Ether number (e).....	188.646
Total fatty acids (1.00-0.00022594e).....	95.74 per cent
Neutralization number (n).....	199.12
Mean molecular weight.....	281.78
Free fatty acids as oleic acid and as $\frac{a}{n}$	1.00 per cent
Glycerol (0.00054703e).....	10.32 per cent
Reichert-Meissl number.....	None
Polenske number.....	0.13
Insoluble acids.....	95.40 per cent
Iodin number (Wijs).....	83.45
Iodin number (U. S. P. standard).....	79-90
Acetyl number.....	5.69

COLOR TESTS

Baudouin test for sesame oil.....	Nil
Bechi silver nitrate test for cottonseed oil.....	Nil
Halphen test for cottonseed oil.....	Nil
Nitric-acid test for seed oils.....	Brown, slight coagulation

All organoleptic, physical, chemical, and color tests indicated a pure olive oil, with the exception of the nitric-acid test which may be disregarded, as it is no longer designated by the Pharmacopœia.

PLAN OF THE EXPERIMENT

The object of the investigation primarily was to ascertain the nature and extent of the action of the several agents upon the oil as determined by changes in physical characteristics and chemical composition; and secondarily to deduce, if possible, from the results obtained a practical method for handling commercial oils. The experiment was planned to demonstrate the effect of air, light, and moisture, singly and in combination, which, together with control (the basis for comparison) and enzym-free samples, required nine series of tests, as follows:

Series.	Condition of the experiment.	Series.	Condition of the experiment.
A.....	Control.	F.....	Air-light.
B.....	Enzym-free.	G.....	Air-moisture.
C.....	Air.	H.....	Light-moisture.
D.....	Light.	I.....	Air-light-moisture.
E.....	Moisture.		

As the change in the oil in most cases would be comparatively slow, six years were believed necessary to obtain the maximum effect desired. The previous experiment having demonstrated that analysis oftener than once a year did not compensate for the extra labor involved, only 1 sample was allotted for each year, or 6 for each series, making a total of 54 samples. Six ounces of oil were taken for each sample which was insufficient for some physical tests, but ample for most chemical. Round flint-glass bottles of 6-ounce capacity with glass stoppers were used as containers after being carefully cleaned and dried.

Five c. c. of distilled water were pipetted into each bottle of series E, G, H, and I, after which all the bottles, with the exception of series B, were filled with the oil as received, after it was thoroughly mixed to insure uniformity. Another portion of the oil, used for series B, was heated to 70° C. on two successive days for approximately 60 minutes on the first day and 30 on the second, to destroy enzymes, if any were present. All bottles were filled to the shoulder. Where air was not a factor, the bottles were closed with glass stoppers and carefully sealed with wax. Such treatment failed as a control measure, as a small amount of air remained in the bottles; but this appeared unavoidable under the circumstances. Each series of tests was inclosed in an 8-inch Fruehling and Schultz desiccator after the porcelain plate had been removed. The bottles in an upright position were arranged in a circle and well spaced.

To exclude the action of moisture (series A, B, C, D, and F) sulphuric acid, previously heated in most cases to 212° C., or higher, was poured into the desiccators to absorb any water that might gain access. To secure a saturated atmosphere (series E, G, H, and I), distilled water was poured into the desiccators, in addition to the water in the bottles.

To exclude air (series A, B, D, E, and H), the desiccators were rarefied by means of a vacuum pump, and a small U-shaped manometer was suspended from the hook of the stopcock to indicate the rarefaction and its

permanence. The joints of these desiccators were covered with wax; but even under the best conditions leakage could not be prevented entirely, and it was found necessary to pump out the desiccators several times a year. To obtain the effect of air (series C, F, G, and I) the glass stopcocks of the desiccators were replaced by perforated rubber stoppers and straight glass tubes which passed through the stoppers and dipped into the sulphuric acid (series C and F) or into the water (series G and I).

To exclude light (series A, B, C, E, and G), the desiccators were placed in a large oblong wooden box, lined with building paper, with an overhanging cover similarly lined. The cover was held by corner posts 0.5 inch above the top of the box, projected 0.5 inch beyond the sides of the box, and overlapped 3.5 inches. In addition a strip 1.4 inches wide was nailed to the outside of the box 0.5 inch below the edge of the cover.

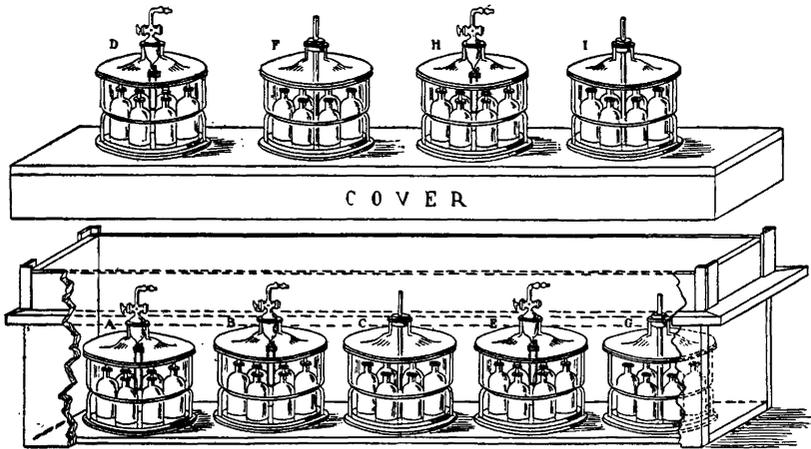


FIG. 1.—Apparatus used in the experiments to determine the stability of olive oil.

This provided a continuous air passage 0.5 inch wide under all sides of the cover and yet absolutely prevented the entrance of light, even by reflection. To obtain the effect of light (series D, F, H, and I) the desiccators were placed on the cover of the box and exposed to light from a north window. All the samples were kept in the northeast room of the Experiment Station dairy building, into which the direct rays of the sun did not enter at any season of the year. The temperature was not constant, but relative in all cases. Considerable time was consumed in obtaining the necessary supplies and in preparing the samples, so that the experiment did not actually begin until April 2, 1910.

EFFECT OF AIR, LIGHT, AND MOISTURE

ORGANOLEPTIC CHANGES

Changes of an organoleptic character are difficult to measure and even more difficult to express, particularly where the differences are slight. The results are relative, however, if not strictly accurate, and are recorded in Table I.

TABLE I.—Organoleptic changes in the olive oil

Series.	Conditions of the experiment.	As received (1910).	After 1 year (1911).	After 2 years (1912).	After 3 years (1913).	After 4 years (1914).	After 5 years (1915).	After 6 years (1916).
A	Control	Clear; dark olive green; marked olive odor.	Clear; dark olive green; olive odor.	Clear; dark olive green; color and olive odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.
B	Enzym-free	do.	do.	do.	do.	do.	do.	Do.
C	Air	do.	do.	do.	Less odor than A.	Slightly rancid odor (?).	Less green than A.	Yellowish; rancid odor.
D	Light	do.	Less green than A.	Less green than A.	Greenish yellow.	Greenish yellow.	Slightly greenish yellow.	Slightly greenish yellow; slightly rancid odor.
E	Moisture	do.	Turbid with small amount of precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with more precipitate than G, after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.
F	Air-light	do.	Less green than D.	Light yellow, color largely destroyed; rancid odor.	Yellowish, nearly colorless; rancid odor.	Yellowish, nearly colorless; rancid odor.	Nearly colorless; rancid odor.	Nearly colorless; rancid odor.
G	Air-moisture	do.	Clear with slight amount of precipitate; after filtering, color like A.	Clear with some precipitate, like E; after filtering, color like A.	Clear with least precipitate; after filtering, color like A; water acid to litmus; odor like C.	Clear with some precipitate, like E; after filtering, less green than C.	Slightly turbid with least precipitate; color less than D; rancid odor.	Slightly turbid with less precipitate than E; after filtering, slight color; rancid odor.
H	Light-moisture	do.	Turbid with more precipitate than F; after filtering, color between D and F.	Turbid with more precipitate than G; after filtering, color lighter than D.	Turbid with more precipitate than E; after filtering, color like D; slightly rancid odor (?).	Turbid with considerable precipitate; after filtering, more between D and F.	Turbid with more precipitate than E; after filtering, more yellow than D.	Turbid with more precipitate than E; after filtering, more like D.
I	Air-light-moisture	do.	Turbid with more precipitate than H; after filtering, color between H and F.	Turbid with most precipitate; after filtering, color like F; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless like F; water acid to litmus; rancid odor.	Turbid with most precipitate; after filtering, less color than F; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless; rancid odor.

The color and characteristic odor of the oil seemed to diminish gradually in the control samples. The heated samples, series B, duplicated the control samples, so far as could be observed.

Air was a negligible factor for two years; then it effected a slow but marked destruction of color fully equal to light at the close of the experiment, and caused a rancid odor on the sixth year.

Light was active in destroying color and caused a slightly rancid odor on the sixth year, probably due to a small amount of inclosed air.

Moisture caused the formation of a precipitate which rendered the oil turbid, but which effected no apparent change in color after the removal of the precipitate.

Air-light was most active and effective in destroying color, equal to air-light-moisture, and produced a rancid odor on the second year.

Air-moisture caused the formation of a slight amount of precipitate but without appreciable turbidity until the fifth year, at which time a rancid odor was produced. Air-moisture was inactive as regards color for three years, but eventually exceeded the effect of air and equaled that of light.

Light-moisture affected the color about the same as light, and caused the formation of a considerable amount of precipitate which rendered the oil turbid.

Air-light-moisture affected the color the same as air-light, produced a rancid odor the second year, and caused the formation of probably the largest amount of precipitate, which rendered the oil turbid.

The chromogenic bodies of the oil were not appreciably affected by moisture, were destroyed slowly but effectively by air, slowly but rather more effectively by air-moisture, more actively by light and light-moisture, and most actively and effectively by air-light and by air-light-moisture. Air was slowly active, light probably assisted by a small amount of inclosed air more active, and air-light the most active in destroying color. Moisture was a negligible factor except possibly in the case of air-moisture.

A rancid odor was produced on the sixth year by air and by light, on the fifth year by air-moisture, and on the second year by air-light and by air-light-moisture. Neither air nor light alone was particularly active in producing rancidity, but jointly were decidedly effective. In this connection moisture did not appear to be a factor of any consequence.

In every instance the presence of moisture caused the formation of a precipitate in a relatively slight amount by air-moisture, in small amount by moisture, in a greater amount by light-moisture, and in apparently the largest amount by air-light-moisture. Light seemingly was a factor. The so-called precipitate was first observed as dirty-white or brownish-white spots on the sides of the bottle below the surface of the oil and might be said to resemble mold. As the amount increased, the bulk of it collected near the surface of the water layer or in the water. In no case was sufficient purified material obtained to make a chemical examination.

PHYSICAL TESTS

The refractive index of the different series was determined for a number of years by means of an Abbe refractometer. The readings did not indicate any appreciable change in the oil except with air-light and with air-light-moisture, where gains of approximately 0.001 were noted. The results for the year 1912 corrected are given in Table II. Those for 1913 and 1914 gave like differences and are not reported.

TABLE II.—Refractive index for the olive oil, 1912

Series.	Conditions of the experiment.	$n_{D}^{20^{\circ}C.}$
A.....	Control.....	1.4687
B.....	Enzym-free.....	1.4687
C.....	Air.....	1.4690
D.....	Light.....	1.4690
E.....	Moisture.....	1.4690
F.....	Air-light.....	1.4700
G.....	Air-moisture.....	1.4689
H.....	Light-moisture.....	1.4689
I.....	Air-light-moisture.....	1.4702

A Lovibond tintometer was employed for determining the color of the oil in 1915 and 1916. The supply of standard glasses in 1915 was inadequate for satisfactory readings, particularly for the darker oils, and the results are merely indicative.

TABLE III.—Color of the olive oil

Series.	Conditions of the experiment.	Stratum.	Matching standards.			Color developed.	
			Red.	Yellow.	Blue.	Orange.	Yellow.
September, 1915.							
		<i>Inches.</i>					
A.....	Control.....	0.50	1.0	6.8		1.0	5.8
B.....	Enzym-free.....	.50	1.0	11.0		1.0	10.0
C.....	Air.....	1.00	.5	1.4		.5	.9
D.....	Light.....	.50	1.0	3.5		1.0	2.5
E.....	Moisture.....	.50	1.0	7.3		1.0	6.3
F.....	Air-light.....	1.00	.3	.8		.3	.5
G.....	Air-moisture.....	1.00	.5	1.3		.5	.8
H.....	Light-moisture.....	.50	1.0	3.5		1.0	2.5
I.....	Air-light-moisture.....	1.00	.1	.7		.1	.6
March, 1916.							
A.....	Control.....	.25	1.0	12.8		1.0	11.8
B.....	Enzym-free.....	.25	1.0	12.8		1.0	11.8
C.....	Air.....	1.00	.7	1.8		.7	1.1
D.....	Light.....	.50	.8	3.5		.8	2.7
E.....	Moisture.....	.25	1.0	12.8		1.0	11.8
F.....	Air-light.....	2.00	.5	1.5		.5	1.0
G.....	Air-moisture.....	1.00	.2	1.8		.2	1.6
H.....	Light-moisture.....	.50	1.2	4.6		1.2	3.4
I.....	Air-light-moisture.....	2.00	.5	1.5		.5	1.0

According to the tintometer readings, for the last two years (1915-16), the control, enzym-free, and moisture samples retained the most color; light and light-moisture next; air and air-moisture less; and air-light and air-light-moisture the least color. The organoleptic tests for the entire period rated light, and light-moisture more active than air and air-moisture, but equally effective at the close of the experiment, or nearly so. Differences between organoleptic and tintometer readings are due, partly at least, to the fact that the unaided eye is less sensitive to the yellow than to the darker colors and is unable to differentiate accurately between faint colors, but more particularly to failure in properly coordinating activeness or speed of destruction and effectiveness or completeness of destruction.

The viscosity of a "fractional" quantity of several of the samples was determined in 1912 by means of a Redwood viscosimeter.

TABLE IV.—Viscosity of the olive oil, 1912

Series.	Conditions of the experiment.	Viscosity.
A.....	Control.....	15.8
F.....	Air-light.....	20.5
I.....	Air-light-moisture.....	21.7

Air-light and air-light-moisture evidently increased the viscosity to a slight extent.

CHEMICAL TESTS

The decomposition of the olive oil as affected by air, light, and moisture, singly and in combination, was measured in terms of acid, saponification, and iodine numbers. At the outset the oil seemed to possess a certain resistance to hydrolysis, oxidation, etc., but after it began to break down to any extent, the changes were more rapid. The hydrolytic effect of air, light, and moisture on the glycerids of the oil was measured in terms of acid number, which indicates the amount of free fatty acids produced (Table V).

TABLE V.—Acid number of the olive oil

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control.....	1.99	2.18	+0.19	2.29	+0.30	2.47	+0.48	2.66	+0.67	2.97	+1.98	3.00	+1.01
B	Enzym-free.....	2.18	+0.19	2.20	+0.21	2.28	+0.29	2.39	+0.40	2.68	+1.69	2.64	+1.65
C	Air.....	2.07	+0.08	2.01	+0.02	2.03	+0.04	2.25	+0.26	2.60	+1.61	2.96	+1.97
D	Light.....	2.26	+0.27	2.38	+0.39	2.54	+0.55	2.86	+0.87	3.06	+1.07	2.39	+1.40
E	Moisture.....	2.33	+0.34	2.55	+0.56	2.93	+0.94	3.48	+1.49	4.15	+2.16	4.59	+2.60
F	Air-light.....	2.10	+0.11	2.28	+0.29	2.78	+0.79	3.58	+1.59	4.94	+2.95	6.83	+4.84
G	Air-moisture.....	2.35	+0.36	2.60	+0.61	2.97	+0.98	3.69	+1.79	4.66	+2.61	5.5	+3.68
H	Light-moisture.....	2.46	+0.47	2.81	+0.82	3.29	+1.39	4.01	+2.02	4.86	+2.87	5.59	+3.60
I	Air-light-moisture.....	2.42	+0.43	3.09	+1.10	4.08	+2.09	5.88	+3.89	8.09	+6.10	12.45	+10.46

The control samples (the basis for comparison) hydrolyzed a little more than the enzym-free, although the differences were slight.

Neither air nor light showed any appreciable action.

Moisture was moderately active and gradually effected a noticeable amount of hydrolysis.

Air-moisture and light-moisture were rather more effective than moisture, although the influence of the air or of the light must have been secondary.

Air-light was inactive for two years; then it began to affect hydrolysis and eventually exceeded air-moisture and light-moisture, probably due to the impossibility of entirely excluding moisture under the conditions of operation.

Air-light-moisture was the first to effect an appreciable amount of hydrolysis and greatly exceeded all others at the close.

Moisture effected considerable hydrolysis; air-moisture and light-moisture caused an additional amount; air-light, probably assisted by some moisture, still more, and air-light-moisture was the most active and effective. Moisture was the essential factor although air and light together greatly accelerated it.

The decomposition of unsaturated acids of olive oil as effected by air, light, and moisture may be measured in a degree by the increase in the saponification number which indicates the amount of fatty acid of high molecular weight converted into acids of lower molecular weight (Table VI).

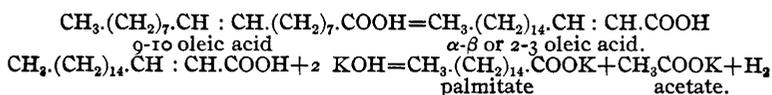
TABLE VI.—Saponification number of the olive oil

Series.	Conditions of the experiment.	Test in 1910 (as received).												
		Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.	
A	Control.....	190.64	189.86	-0.78	189.25	-1.39	190.53	-0.11	190.62	-0.02	190.57	-0.07	190.07	-0.57
B	Enzym-free.....	190.18	189.98	-0.40	189.98	-0.66	190.62	-0.02	190.79	+0.15	190.80	+0.16	190.60	-0.04
C	Air.....	190.09	189.26	-0.55	190.26	-0.38	190.73	+0.09	192.24	+1.60	194.01	+3.37	195.36	+1.47
D	Light.....	189.89	189.58	-0.75	189.58	-1.06	189.94	-0.70	189.88	-0.76	190.37	+0.27	190.45	-0.19
E	Moisture.....	189.87	189.18	-0.77	189.18	-1.46	190.12	-0.52	190.03	-0.61	190.68	+0.04	189.80	-0.84
F	Air-light.....	190.58	191.79	+1.06	191.79	+1.15	194.55	+3.91	195.10	+4.46	201.74	+11.10	203.68	+13.04
G	Air-moisture.....	189.92	190.19	+0.72	190.19	+0.45	190.93	+0.29	191.84	+1.20	194.40	+3.76	194.77	-0.13
H	Light-moisture.....	189.81	189.89	+0.83	189.89	-0.75	189.91	-0.73	189.64	-1.00	189.41	-1.23	189.82	-0.82
I	Air-light moisture.....	190.44	192.28	+1.64	192.28	+1.64	194.07	+3.43	196.16	+5.52	200.16	+9.52	203.85	+13.21

The control, enzym-free, light, moisture, and light-moisture samples were not affected in total alkali-consuming power. Air and air-moisture caused a like increase in saponification number.

Air-light and air-light-moisture effected a greater increase and of like amount. Air was undoubtedly the principal factor, although greatly intensified by light.

Fittig (1-3) has shown that unsaturated acids with the double bond in $\beta\gamma$, or 9-10 position, as in oleic acid, may undergo intramolecular changes on boiling with caustic alkali, forming an α - β , or 2-3 acid, which on fusion with alkali, according to Varrentrapp (9, p. 209-215) and Molinari (6, p. 293), splits into acetic and another saturated acid.



Schrauth (7) confirmed the reaction and claimed further that in general for each double bond two carbon atoms are split off in the form of acetic acid. Different authorities (5, p. 353-354) have shown that the unsaturated (liquid) acids of olive oil consist principally of oleic acid with a smaller amount of linolic acid.

After considering the high content of unsaturated acids and their possible decomposition as described, one might suggest as a tentative hypothesis that the increase in saponification number was due to the formation of a α - β , or 2-3 acid, from the action of air or more effectively from air-light, which on boiling with alcoholic potash broke down into acetic and palmitic acids. Either oleic or linolic acid might be affected, although the latter is unquestionably less stable and therefore more likely to undergo molecular change. Both oleic and linolic acids, on breaking down into acetic and palmitic acids, double their alkali-consuming power; therefore, the increase in saponification number would be directly proportional to the amount of acid affected. The statement made by Schrauth that one molecule of acetic acid splits off for each double bond, appeared untenable for linolic acid in this connection. The neutralization number of oleic acid is 198.709 and of linolic acid 200.138. Assuming that α - β , or 2-3 oleic acid and linolic acid, after molecular rearrangement have no appreciable iodine numbers, as determined by Wijs solution, the writers obtained the following results for the sixth year of the experiment (Table VII):

TABLE VII.—Character of the decomposition of the olive oil (1916)

Series.	Conditions of the experiment.	Increase in saponification number.	Equivalent to oleic acid.	Equivalent loss in iodine number.	Equivalent loss in linolic acid.	Equivalent loss in iodine number.	Actual loss in iodine number. ¹
C	Air.....	4.72	<i>Per cent.</i> 2.375	2.135	<i>Per cent.</i> 2.358	4.270	3.85
F	Air-light.....	13.04	6.562	5.899	6.516	11.800	11.88
G	Air-moisture.....	4.13	2.078	1.868	2.064	3.738	3.99
I	Air-light-moisture.....	13.21	6.648	5.976	6.600	11.952	11.68

¹ See Table VIII.

The close agreement of the calculated and the actual loss of iodine numbers on the basis of linolic acid would indicate that probably only linolic acid had been affected during the term of the experiment. The matter will be considered further under iodine number (Table VIII).

The decomposing action of air, light, and moisture on the unsaturated acids of olive oil may also be measured by the loss in iodine number (Table VIII).

TABLE VIII.—Iodine number of the olive oil

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control	83.45	83.97	+0.52	83.05	-0.40	82.99	-0.46	83.85	+0.40	83.86	+0.41	83.88	+0.43
B	Enzym-free	83.84	+0.39	82.87	-0.58	83.09	-0.36	83.57	+0.12	83.97	+0.52	83.73	+0.28	
C	Air	83.79	+0.34	82.52	-0.93	82.58	-0.87	81.97	-1.48	81.17	-2.28	79.60	-3.85	
D	Light	83.97	+0.52	82.84	-0.61	83.01	-0.44	83.88	+0.43	83.99	+0.54	83.67	+0.22	
E	Moisture	84.00	+0.55	83.17	-0.28	83.32	-0.13	83.80	+0.35	83.81	+0.36	83.79	+0.34	
F	Air-light	83.23	-0.22	80.80	-2.65	79.49	-3.96	77.27	-6.28	75.31	-8.14	71.57	-11.88	
G	Air-moisture	83.91	+0.46	82.79	-0.66	82.66	-0.79	82.43	-1.02	81.17	-2.28	79.46	-3.99	
H	Light-moisture	83.97	+0.52	83.15	-0.30	82.95	-0.50	84.17	+0.72	83.99	+0.54	84.00	+0.55	
I	Air-light-moisture	83.29	-0.16	80.84	-2.61	79.80	-3.65	77.29	-6.16	75.20	-8.25	71.77	-11.68	

The control, enzym-free, light, moisture, and light-moisture samples were not affected. Air and air-moisture caused considerable loss and of substantially the same amount. Air-light and air-light-moisture were equally effective and caused much greater loss than air or air-moisture; presumably moisture was a negligible factor in both instances. Air was the principal factor, although greatly intensified by light. The loss in iodine number was proportional to the gain in saponification number evidently two different measurements of the same decomposition, as shown by Table IX. The iodine number of linolic acid is 181.091.

TABLE IX.—Character of the decomposition of the olive oil (1916)

Series.	Conditions of the experiment.	Loss in iodine number.	Equivalent to linolic acid.	Equivalent gain in saponification number.	Actual gain in saponification number. ¹
C	Air	3.85	<i>Per cent.</i> 2.126	4.254	4.72
F	Air-light	11.88	6.560	13.129	13.04
G	Air-moisture	3.99	2.203	4.409	4.13
I	Air-light-moisture	11.68	6.450	12.909	13.21

¹ See Table VI.

The formation of aldehyde as a result of oxidation was manifested by the color imparted to the alcoholic potash in the determination of saponification number (Table X). The fuchsin-aldehyde reagent (7, p. 15) was employed as a confirmatory test, but proved rather too sensitive for the purpose.

TABLE X.—Production of aldehyde in the olive oil

Series.	Conditions of the experiment.	As received, 1910.	1911	1912	1913	1914	1915	1916
C	Air.....				Trace.....	Present.....	Present.....	Present.
F	Air-light.....			Trace.....	Present...	Considerable.	Considerable.	Considerable.
G	Air-moisture.....				Trace.....	Present.....	Present, like C.	Present, less than C.
I	Air-light-moisture.			Trace; more than F.	Present..	Considerable.	Considerable; less than F.	Considerable; more than F.

The relative amount of aldehyde present in the samples was estimated by the depth of color produced. Air and air-moisture produced a small amount of aldehyde, which was first noticed on the third year. Air-light and air-light-moisture produced a larger amount, first noticed on the second year. Evidently air was the essential factor accelerated by light.

The original olive oil was carefully examined for enzymes by Dr. G. H. Chapman, of this Station, and although their presence was not detected, it is impossible to say whether any of the changes noted were induced or accelerated by their action.

DISCUSSION OF RESULTS

Air effected no appreciable change in the color of olive oil for two years; then it caused a slow but marked destruction fully equal to light at the close. The tintometer for the last two years (1915-1916) showed air more destructive than light and light-moisture. Air alone was not active in producing rancidity, which was not noticeable until the sixth year. Air had no hydrolytic action but was the active factor in the decomposition of unsaturated acids and in the production of aldehyde.

Light was more active in destroying color than air or air-moisture, but according to the tintometer for the last two years it did not effect as complete destruction as air and air-moisture. Light alone was not active in producing rancidity, which was not noticeable until the sixth year, probably owing to a small amount of inclosed air. Light alone had no hydrolytic action on the glycerids or decomposing action on the unsaturated acids.

Moisture had no effect on the chromogenic bodies and did not appear a factor of any consequence in producing rancidity. Moisture caused the formation of a precipitate and a turbid oil. Moisture was the essential factor in hydrolysis and although only moderately active, gradually effected a considerable amount.

Air-light, like air-light-moisture, was the most active and effective in destroying color and in producing rancidity, which was noticeable on the second year. Air-light increased the refractive index and viscosity of the oil. Air-light had no hydrolytic action for two years; then it

gradually exceeded air-moisture and light-moisture, probably owing to the difficulty of entirely excluding moisture and at the same time permitting the entrance of air. Air-light effected more decomposition of unsaturated acids and production of aldehyde than air. Light evidently accelerated the action of air in this connection.

Air-moisture had no action on color for three years, but eventually exceeded air and equaled that of light in effectiveness. According to the tintometer, for the last two years air-moisture has exceeded light and light-moisture and has equaled that of air in destroying color. Air-moisture effected rancidity the fifth year, exceeding air and light. Air-moisture caused the formation of a slight amount of precipitate but no appreciable turbidity until the fifth year. Air-moisture effected more hydrolysis but the same amount of decomposition of unsaturated acids and formation of aldehyde as air.

Light-moisture was as effective in destroying color as light and more active than air or air-moisture. According to the tintometer, for the last two years light and light-moisture did not effect as complete destruction of color as air and air-moisture. Light-moisture caused the formation of more precipitate than moisture and a turbid oil. Light seemingly was a factor. Light-moisture effected more hydrolysis than moisture and as much as air-moisture.

Air-light-moisture, like air-light, was the most active and effective in destroying color and in producing rancidity, which was noticeable in the second year. Air-light-moisture caused the formation of apparently the most precipitate and a turbid oil. Air-light-moisture increased the refractive index and viscosity of the oil substantially the same as air-light. Air-light-moisture was the first to effect hydrolysis and exceeded all others in amount. Air-light-moisture effected the same decomposition of unsaturated acids and production of aldehyde as air-light, which greatly exceeded that of air or of air-moisture.

PRACTICAL DEDUCTIONS

From an economic standpoint air caused a slow destruction of color in olive oil, the production of rancidity, and the decomposition of unsaturated acids.

Light caused an active destruction of color and a slow production of rancidity.

Air-light caused the most active and effective destruction of color, active destruction of unsaturated acids, a rapid production of rancidity, and a slow but marked production of free fatty acids.

Moisture caused the production of a precipitate, a turbid oil, and free fatty acids.

Air-moisture practically duplicated the effect of air plus that of moisture, and light-moisture that of light plus that of moisture.

Air-light-moisture exceeded the effect of air-light plus that of moisture in the amount of free fatty acids produced; otherwise it was essentially the same.

In order to preserve olive oils in their natural state, air, light, and moisture should be excluded as completely as possible, particularly the combined action of air and light, which has proved exceedingly destructive.

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