

TABLE III
Percentage of Gossypol and Oil (on Dry Delinted Basis) of Cotton Seeds Collected During Three Years from Early and Late Developing Bolls and from Bolls of Poorly Developed Plants.

Source of Seeds	First Year		Second Year		Third Year	
	Gossypol	Oil	Gossypol	Oil	Gossypol	Oil
Early developed bolls	0.632	24.30	0.518	23.88	0.443	23.89
Late developed bolls	0.542	23.69	0.508	23.88	0.498	22.69
Bolls of poorly developed plants	0.535	24.27	0.458	23.60	0.407	23.12

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FOOTNOTES

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²The age of the seeds was calculated from the date of flowering. Usually less than 50 per cent of the cotton bolls were open 40 days after flowering and less than 1 per cent at 34 days.

³The seeds obtained the third year, although produced in the same general vicinity as those of previous years, were from different experimental plots and for that reason their analysis should be considered apart from the others.

TEA SEED OIL— A TEST FOR ITS DETECTION IN OLIVE OIL

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THE close similarity between tea seed oil and olive oil in both physical and chemical constants (1, 2, 3, 4), makes it impossible to distinguish readily between the two oils. For sixteen years it has been known that tea seed oil is used as an adulterant of olive oil. In 1920 Ernest Millian (5) noted: "Adulteration of olive oil is practiced on a large scale in England." Since both oils have practically the same physical and chemical constants some additional test is necessary to detect the presence of tea seed oil.

Furthermore, as this adulterant has become more highly refined, none of the tests previously proposed for its detection remains sufficient. Cofman-Nicoresti suggested a color test (6) which H. A. Caulkin (7) showed was of no value with highly refined tea seed oil.

Another color test was proposed by several investigators, Biebers Test (3), H. Blins' Test (8), Dybowski & Millia (4), the only apparent difference between them being the volume of reagents used. This color test, quoted from Allen's Organic Analysis, Vol. II (4), is: "4 cc. of oil are shaken with a mixture of 5 cc. H₂SO₄, 3 cc. HNO₃ and 3 cc. water for 30 seconds: immerse the whole mixture at 5°C for 5 minutes and note color after 15 minutes. The test as applied should result in a deep black and turbid layer for pure tea seed oil and light straw and clear for olive oil." The authors of this article have applied this test to a sample of pure tea seed oil and

substantiated the conclusion of E. Richard Bolton (3), and Allen (4), that "the test is of little value in detecting tea seed oil in olive oil."

The above two color tests are not the only methods which have been published and subsequently proven practically useless in view of the present degree of refinement of tea seed oil. Other methods have been proposed since.

One of these, the Bolton and Williams (9) method of grouping fatty oils with reference to the determination of the iodine number of the Unsaponifiable Matter, divides the oils into four groups. The fact that olive oil is the only oil in one of these groups is supposed to distinguish it from all other oils. A report of the Olive Oil Committee of the American Oil Chemists Society (9), however, finds the method to be inaccurate.

Both E. Richard Bolton (3) and George S. Jamieson (2) state that 25% or more of tea seed oil would reduce notably the Titer below that given by olive oil alone. Olive oil, quoting Jamieson, has a Titer range of 18°C-25°C, tea seed oil has a range of 13°C-15°C. The authors have found 17.0°C to be the Titer on the tea seed oil they used. The U. S. P. XI gives the following Titer range for olive oil, "Not less than 17°C nor more than 26°C." With this wide range for olive oil and its slight variation from tea seed oil, it does not seem probable that even 50% tea seed oil in olive oil could be detected by the Titer test.

Constants of the samples of oils

used in the experiments reported herewith were as follows:

Tea Seed Oil—	
Iodine Value	84.3
Saponification Value	191.5
Acid Value	0.2
Titer Test	17.0° C.

This sample of refined tea seed oil conforms to the requirements of the U. S. P. XI FOR A PURE OLIVE OIL.

Italian Olive Oil—	
Iodine Value	83.8
Saponification Value	191.7
Acid Value	1.80
Titer Test	22.6° C.
Spanish Olive Oil—	
Iodine Value	84.8
Saponification Value	191.0
Acid Value	1.15
Titer Test	19.6° C.
French Olive Oil—	
Iodine Value	83.7
Saponification Value	191.0
Acid Value	0.88
Titer Test	21.6° C.
California Olive Oil—	
Iodine Value	84.0
Saponification Value	192.2
Acid Value	2.56
Titer Test	19.65° C.

Examination of the foregoing figures shows that from the constants alone it is practically impossible for any chemist to prove that a sample of oil, which is a blend of 75% tea seed and 25% of pure olive oil, is adulterated. The acid value of olive oil varies, the U. S. P. XI permits a maximum acid value of 3.0 and oils are known which have an acid value as low as 0.4.

The authors have developed a test which they believe is positive for as little as 5% of tea seed oil in the presence of 95% olive oil. Results below this value depend upon the olive oil used. While the test will recognize the presence of tea seed oil in pure olive oil, it is not claimed that it will be specific for tea seed oil—as is shown later

in this paper. If an oil has all the constants of a normal olive oil and gives a negative reaction for other adulterants such as cottonseed, sesame and peanut oils, then the following test will detect the presence of as little as 5% of tea seed oil.

Preliminary Investigation

S. Fachini (12), using equal volumes of oil and acetic anhydride, heating, and adding a drop of sulphuric acid, produced a red coloration on extracted oil. The authors based their investigation on this test. The addition of conc. H_2SO_4 drop by drop to an unheated mixture of equal volumes of tea seed oil and acetic anhydride showed in the first five seconds of addition, a fine red color which soon changed to dark blue green. This red was more persistent in tea seed oil than in case of an Italian olive oil, while all other olive oils did not yield the red color. This initial red color was the basis of the test. Failure of the color to persist for more than a few seconds did not warrant calling this a final test.

Attempts to prolong the duration of this color were made either by adding less sulphuric acid or diluting the oil and acetic anhydride with some inactive solvent. Reducing the proportion of acid did not help. Diluting the oil and acetic anhydride with a solvent, however, proved more promising. Experimenting with chloroform, carbon tetrachloride, ether and petroleum benzene, the authors found that chloroform was most suitable. This was substantiated by later tests. The authors then proceeded to try different concentrations of acid with chloroform as a fixed diluent. Too little acid produced only the momentary change, too much acid produced deep permanent red in both oils—due possibly to the sulphonation at the high temperature produced on addition of the acid.

When acid was added to the mixtures both gave an initial fading red color which in the case of tea seed oil subsequently changed to dark green, while the olive oil became light green. When these were allowed to stand at room temperature the color of the tea seed turned deep red and the olive oil turned a darker green.

Here was the initial difference between the two oils. Tea seed oil under controlled conditions produced the red; olive oil under the same controlled condition was com-

paratively inert with regard to color. The "controlled" conditions of the "sulphonation," in the case of the tea seed oil yielded a red colored end-product, whereas olive oil produced a light yellow green (when diluted with alcohol). Both oils apparently reacted, since a uniform solution without separation of chloroform layer resulted when alcohol or water was used as a further diluent.

At this time chloroform showed its superiority over carbon tetrachloride and other solvents, by not separating out in the test. The change from straight concentrated sulphuric to glycerol-sulphuric acid mixture was the result of an attempt to counteract somewhat the effect of the Italian oil.

The question of time was studied by a long series of experiments. Too short a time caused incomplete reaction as shown by the separation of a chloroform layer. Too long a period produced a light red brown in case of olive oil, which was somewhat similar to a slight adulteration with tea seed oil.

B. W. Van Eldick Thieme (11), working with 100% fatty acids, reports that "Glycerol sulphuric acid reacted with fatty acids to form mono-di or tri-glycerides at temperature of 70°C for 3 hours." In view of the influence of rancid olive oil on the reaction, and since the Italian oil had an acid value slightly higher than either the Spanish oil or French oil used, it was believed that the addition of glycerol-sulphuric acid would improve the test. It was found, however, that the higher fatty acids apparently were not responsible for the reaction, for the California olive oil with an even higher acid value, did not give the test.

Procedure of the Test

The test developed by the authors for the detection of tea seed oil in unheated, non-rancid olive oil, not otherwise adulterated, is as follows: 3 cc. of oil in a test tube (1 x 6) are mixed with 3 cc. chloroform C.P., and 3 cc. acetic anhydride C.P. The mixture is immersed in an ice-bath for 5 minutes. Then drop by drop slowly add, with constant shaking and immersing in the ice water bath, 0.8 cc. of glycerol-sulphuric acid mixture (i.e., 100 cc. of conc. H_2SO_4 + 10 cc. of glycerol C.P. allowed to cool. This mixture of glycerine and sulphuric acid must be made fresh). Remove the test tube from the ice

bath and let stand exactly 1 hour at room temperature, shaking every five minutes until nothing appears to separate out—usually within ½ hour. During this time the mixture will change from a deep blue green to deep red for tea seed and will remain dark green for olive oil. Various mixtures of tea seed oil in olive oil will cause intermediate colors to appear. At the end of one hour again immerse the test tube in an ice bath for 5 minutes. Very slowly and with constant shaking and immersion in ice bath—(the reaction mixture tends to get very hot)—add 3 cc. H_2O and let stand at room temperature 5-10 minutes until maximum color develops. Note appearance of mixture.

The resulting mixture may not be clear and care must be exercised in reading a test when the percentage of tea seed oil is as low as 5%. Nevertheless this is the best stage at which to judge the test. The addition of alcohol clears the solution and yields the red-brown-to-red color in contrast to the light yellow-brown of pure olive oil. The test fades too rapidly for establishing the admixture of less than 5% tea seed oil with olive oil. Pure olive oil will show a color varying from a dark dirty green to a very light green, but distinctly no red or brown coloration. As little as 5% tea seed will show a distinct red-brown color easily distinguished from a control test of pure olive oil. The red color will increase and the dirty-green color correspondingly decrease in proportion to the percentage of the tea seed present in the olive oil.

Results of the Test

The test was tried out by preparing various mixtures of olive and tea seed oil. The olive oils used were of different origins and, according to physical and chemical tests, were pure olive oil. The Spanish and California oils were obtained direct from the producers; the French and Italian oils were purchased on the open market. The tea seed oils used were obtained from reliable sources, and were further checked as to their physical and chemical constants. All were subjected to the test herein proposed. Unknowns were prepared by one of the authors and checked by the other to determine whether the test was positive or negative. The tests were repeated several times in order to be sure that the

results were consistent. (Table I.) of chloroform equal to the volume

Brand of Olive Oil	Per cent Tea Seed Oil	Per cent Olive Oil	Reaction
Spanish Carmen A*	100	0	Heavy red
" " B*	25	75	Distinct red
" " C*	0	100	Somewhat dark, Dirty green
" " D*	50	50	Red
" " E*	75	25	Somewhat heavy red
Spanish Carbonell	0	100	Negative
" "	25	75	Positive
" "	50	50	Positive
Spanish Sole Vita	0	100	Negative
" "	25	75	Positive
" "	50	50	Positive
French	0	100	Negative
" "	25	75	Positive
" "	50	50	Positive
Italian	0	100	Negative**
" "	25	75	Positive
" "	50	50	Positive
California	0	100	Negative
" "	25	75	Positive
" "	50	50	Positive

*Tests with these samples were repeated the next day with similar results.
 **The Italian brand showed a darker green than other oils with just a suggestion of a positive reaction. In a subsequent experiment the faint red color proved to be decidedly less intense than for the 5% sensitivity, claimed for the test.

The test was repeated with a smaller percentage of adulteration in order to determine how little tea seed oil could be detected. It was desired also to compare results with the Italian olive oil with tests in which 5% tea seed oil was added to other olive oils. (Table II.)

of the sample and the chloroform solution was water washed.

The test for tea seed oil then was applied to the clear solution without any further addition of chloroform. The results showed that the interfering action of sesame oil was reduced considerably.

Brand of Olive Oil	Per cent Tea Seed Oil	Per cent Olive Oil	Reaction	Trials
			1st	2nd
California	0	100	N	N
"	5	95	P	P
"	10	90	P	P
"	20	80	P	P
French	0	100	N	N
"	5	95	P	P
"	10	90	P	P
"	20	80	P	P
Spanish	0	100	N	N
"	5	95	P	P
"	10	90	P	P
"	20	80	P	P
Italian	0	100	N	N
"	5	95	P	P
"	10	90	P	P
"	20	80	P	P

Interfering Substances

To determine the value of the method in the presence of other oils the following oils were tested and the reactions observed:

	Reaction
Cottonseed 100%	Very deep red
Peanut 100%	Very light green
Sesame 100%	Very dark green, almost black
Strongly Rancid } Olive Oil }	Deep red

From these results it is apparent that cottonseed oil and rancid olive oil both yield a positive test. Peanut oil presents no interference. Sesame oil under careful observation does not give a positive reaction, as the color produced is very dark there may be some interference depending on the percentage of sesame oil present.

The test then was modified slightly so as to observe the reaction of sesame oil more clearly. The pure sesame oil first was shaken out with two portions of conc. HCl, letting each shaking stand 10 minutes before withdrawing the acid layer. The oil then was shaken with an amount

The above modification also was tried on the following oils, which gave the various reactions listed:

	Reaction
Sesame Oil	Negative
Cottonseed Oil	Positive
Tea Seed Oil	Positive
Olive Oil	Negative
Peanut Oil	Negative

A final series of tests were made with oils that had been maintained at a high temperature for 15 minutes and then cooled. Set A was heated to 150-160°C, Set B to 195°C-205°C and set C to 240°C-250°C (Table III). Upon the addition of acid to the oil-chloroform-acetic-anhydride mixture, an initial deep red coloration was produced, but all samples changed rapidly to their respective greens. Heating of Set C caused a separation of a chloroform insoluble material; only the portion soluble in chloroform was used for the test.

In Set A, the olive oils gave a positive reaction with the color not as intense as in tea seed. According to this result it would be difficult to establish adulteration with tea seed oil in a sample of olive oil, which had been heated. The results obtained with Sets B and C showed that the positive reaction became more intense as the temperature was increased. This applied to the pure tea seed as well as the olive oil. All tests produced a clearer solution than with the unheated oils. The test on the heated oils tends to produce a red coloration somewhat similar to one produced by a mixture of tea seed oil and olive oil. The clarity of the solution, however, indicates that the oils may have been heated.

Jaques Sonol (10) states "the objection that most of these tests are negative in superheated seed oils is immaterial, since the latter are never used as adulterants, on account of their dark color and unpleasant odor and taste."

DISCUSSION

The constants of cottonseed, sesame, rancid olive and heated oils are sufficient for the detection of any amount used for adulterating a pure olive oil. The flavor of rancid olive and heated oils is such that there is little likelihood of these oils being used for adulteration. The Halphen test for cottonseed oil and the Modified Villivecchia test for sesame oil definitely will identify any admixture of these oils with olive oil. The specific tests for the cottonseed or sesame oils, when these oils are combined with tea seed oil to adulterate olive oil, are sufficient to establish the adulteration of the olive oil. The great demand for tea seed oil as a substitute for olive oil has been due to the fact that until now, means of differentiating between these two oils were not available.

CONCLUSIONS

- I. The test presented permits detection of as little as 5% of tea seed oil in 95% olive oil.
- II. Cottonseed oil or sesame oil interfere with the test. The interference of sesame oil can be overcome to a great extent through the modification recommended.
- III. Rancid olive oil interferes

	Reaction SET A	Reaction SET B	Reaction SET C
Sesame Oil	Negative	Negative	Negative
Peanut Oil	Negative	Sli. Positive	Sli. Positive
Italian Olive Oil	Positive	Positive	Positive
Spanish Olive Oil	Sli. Positive	Positive	Positive
Tea Seed Oil	Very Str. P.	Very Str. P.	Very Str. P.

with the test. Heated oils may interfere. However, such oils are not likely to be present in olive oil sold for food purposes.

Note:

Since this paper was written there has been reported in the July issue of Food Industries,¹³ a method used by the Federal Food and Drug Administration for the last few months.

This method has been tried by the authors who find that the color resulting does not last long enough to permit of easy comparison with standards, and second that some olive oils give a pink color reaction which can be mistaken as indicating the addition of some tea seed oil.

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PHOTOCHEMICAL STUDIES OF RANCIDITY: INDUCTION PERIOD OF PROTECTED AND NON-PROTECTED OILS*

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THE induction period of oils or fats and its significance in determining their keeping quality have long been active subjects for investigation. Usually in the process of refining and in handling for the market, oils are more or less exposed to deleterious agents, especially finely divided metals, as well as unfavorable temperature and light conditions, all of which play an important part in altering the induction period.

Under normal conditions, an oil of good keeping quality is characterized by a long induction period and a low peroxide value. Under such conditions, when the oil is not in contact with metals, the peroxide value will increase to about 30 or 60 before the oil becomes rancid. This usually takes from one week to one month. If an oil is exposed to light of more than ordinary intensity, under certain conditions the induction period may be shortened to a few days only. In general, as rancidity develops the peroxide value increases. Consequently, many investigators have undertaken to correlate rancidity and the induction period with the peroxide value. Numerous observations have shown, however, that oils or fats protected from light, for example, by means of a green wrapper or container, may have a peroxide value equal to or even greater than an unprotected oil or fat that has

become rancid and still be organoleptically free from rancidity.¹

Preliminary Experiment

Corn oil through which moisture-laden air¹ had been bubbled for six months, during which time it had been protected from light with opaque black paper, was transferred to two tubes, one of which was wrapped with protective green (delimited by 4,900 to 5,800 Angstrom units) paper while the other was unprotected. The tubes were then exposed to the light in an east window. After such exposure for about seven weeks, it was found that, although the peroxide value of the two samples was about the same, the unprotected sample was rancid while the oil protected by green paper was still free from rancidity. This preliminary experiment indicated that the reaction which gives rise to the rancid taste or odor may have no connection with the formation of peroxides. That the development of rancidity in these experiments is a photochemical change is also indicated by the fact that when oils are protected from harmful light the original mobility and color of the oil, as well as the taste and odor, are to a large extent preserved.

¹1934. Coe, Mayne R., and J. A. LeClerc. Photochemical Studies of Rancidity: Peroxide Values of Oils as Affected by Selective Light. Ind. Eng. Chem., Vol. 26, Page 245.

The results noted above have raised the questions: (1) "What influence does protection from harmful light have on the induction period of a fresh oil?" (2) "Does 'protective green,' as defined above, extend the keeping quality or induction period of an oil?" (3) "Does the process concerned with the development of rancidity begin when the oil is first exposed to light, i.e., does this mark the beginning of the induction period?" (4) "Does the peroxide value of a protected oil modify the induction period of that oil when the oil is exposed to light?" It may be possible to answer some, if not all, of these questions.

Experimental

An experiment was made to determine whether rancidity develops at about the same time in a fresh oil as in an oil which, for a time, had been exposed to light but had been protected by this special green wrapping. Cottonseed and corn oils were used. The initial peroxide value of the former was 1.0; that of the latter was 1.5.

Two eight-ounce bottles of clear glass containing cottonseed oil and two containing corn oil were each provided with a two-hole cork stopper. One bottle of each set was wrapped with protective green paper; the remaining bottle of each set was not wrapped. Through one hole of the stopper was inserted a

*Food Research Contribution No. 299.

¹A very adverse treatment.