Report of the Olive Oil Committee

THIS committee, since the Fall of 1937, has been investigating two quite recently devised methods for the detection of teaseed oil in olive oil: the Fitelson or so-called government test and the Siebenberg-Hubbard test. The former was altered to run at cold temperature, and as modified reads now as follows:

"Measure into a test tube exactly 0.8 cc acetic anhydride, 1.5 cc chloroform and 0.20 cc concentrated sulphuric acid. Mix and cool in a water bath to 5° C. Then add 7 drops of the oil to be tested, mix and cool again to 5° C. Allow the test tubes to remain cold for five minutes, then add 10 cc cold anhydrous ether and mix immediately by inverting. Teaseed oil will change to an intense red within a minute or so, the color reaching a maximum and then fading away. Some olive oils pass through a fleeting faint tinge of pink. Mixtures of teaseed oil and olive oil give a red coloration proportional to the amount of teaseed present. Standards containing known amounts of teaseed oil should be run simultaneously with the sample and the deepest red color produced used as a basis of comparison."

Samples of pure olive oil and mixtures with teaseed oil were prepared and sent out to the members, but because their compositions were known to the chairman, Mr. R. R. Lewis of the same laboratory was asked to collaborate in the required testing.

The list is as follows:

1. Edible olive oil containing 10% refined deodorized edible teaseed oil.
2. Edible olive oil containing 15% refined deodorized edible teaseed oil.
3. Edible olive oil giving the silver benzoate sulphur reaction.
4. Commercial olive oil of 5% free fatty acidity.
5. Oil extracted from the skins of over-ripe olives. This was accomplished by soaking the crushed skins with warm negative-testing olive oil for a few days, pressing out the oil through a muslin bag and mixing this with the oil extracted from the residual pulp by carbon bisulphide after the solvent was evaporated off. The whole was then filtered for use.
6. Sample No. 1 diluted one-half or 50/50 with negative testing olive oil.
7. Sample No. 2 likewise diluted one-half or 50/50 with olive oil.
8. Edible olive oil of a well-known brand.
9. Sample No. 8 to which teaseed oil was added to a content of 20% teaseed oil.
10. Sample No. 9 diluted 50/50 with olive oil.
11. Sample No. 9 diluted 25/75 with olive oil.

The first five samples and numbers 8 and 9 were sent out, the members making the diluted mixtures with olive oil of their own choice.

Table I embodies the results of the members of the committee on these oils, according to the Fitelson (marked F) and the Seibenberg Hubbard (marked S-H) tests.

INDIVIDUAL COMMENTS & REPORTS

HUBBARD: "It is my opinion that both the government test and the test developed in this laboratory have value in determining the presence of teaseed oil in olive oil. The government test is very rapid and does not require a great deal of experience to carry out. However, it is not conclusive as some adulterated olive oils will give the pink color reaction. The test developed in this laboratory takes more time and requires some experience in manipulation, but when the reaction is positive, there is no doubt but what teaseed oil is present. One of the chemists in the case at Philadelphia, stated if olive skins were present in large quantity in otherwise pure olive oil, the government test would show a color reaction equivalent to 50% or more adulteration with teaseed oil."

JAMIESON: "It should be noted that the Fitelson test as you have described it, has been modified as to temperature from 25° C. to 5° C. at certain stages of the test. Dr. McKinney also tested these oils with the original Fitelson test and obtained comparable results and states that he is inclined to like the modified test the better of the two. However, we have been informed by the Food and Drug Administration that they do not believe the red color will develop fully if the first part of the test is conducted at 5° C. Our experience here is that the Fitelson test is superior to that proposed by Hubbard. I recommend that the Fitelson methods (qualitative and quantitative) be made official.

TABLE I

		No. 1 10% T/S Mixture	No. 2 15% T/S Mixture	No. 3 Edible Olive	No. 4 Com'l Olive	No. 5 "Skin" Oil	No. 6 5% T/S Mixture	No. 7 7.5% T/S Mixture	No. 8 Edible Olive	No. 9 20% T/S Mixture	No. 10 10% T/S Mixture	No. 11 5% T/S Mixture
Sheely	F	10	15-20	5-10	0-5	0	5-10	10-15	a. 0 b. 0	a. 25-30 b. 30-35	a. 10-15 b. 15-20	a. 5-10 b. 10
	S-H	10-20	20	5	0	10-20	0-5	5-10	a. 0 b. 0 c. 0	a. 0 b. 0 c. 0	a. 0 b. 0 c. 0	a. 0 b. 0 c. 0
Hubbard	F	5-10	5-10	0	0	0	5-10	5-10				
	S-H	Positive	Pos.	Pos.	Negative	Pos.	Pos.	Pos.				
Reese	F	Positive slight pink	P> No. 1	? very slight	? Neg.	Neg.	Pos.	P> No. 1	Neg.	Pos. pink	Pos. light pink	Neg.
	S-H	Pos.	P> No. 1	P> No. 2	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	? Neg.	Neg.
Lewis	F	5-10	15	0-5	0	0	2.5-5	8.10	0	20	10	0-5
McKinney	F	12	15	2	6	0	6	12	(Color = 1-27) 0	25	12	6
Stillman	F	5	a. 15 b. 10	0-5	0-5		<5	a. 10 b. 5	a. 0 b. 0	a. 25 b. 20	a. 10 b. 10	a. 5 b. 5-10

"Sample No. 8 gave a color distinctly fainter than that given by the negative Testing olive oil containing 3% of refined teaseed oil. We have found some supposedly pure olive oil giving about the same color. As we understand that any results below 10% teaseed oil with the Fitelson test are uncertain, I would say that sample No. 8 probably contains no teaseed oil.

No positive reactions could be detected with these oils using the Siebenberg-Hubbard test. The mixture after addition of the glycerol-sulphuric acid and shaking at five minute intervals for one hour, became so turbid that no red color could be detected, nor could such a color be seen after adding water. Furthermore, the addition of alcohol, as suggested by the authors, was not helpful."

LEWIS: "The Fitelson test offers no difficulty; it is easy to do and takes little time. However, some olive oils will give a fleeting pink tinge, but as this color in any case does not last very long, it occurred to me to delay somewhat and possibly hold it for sufficient time to permit leisurely estimates by comparison with standards. By chilling the solutions before the addition of ether, to about 30° below zero C. with a dry ice and kerosene bath, the reddish color slowly produced could be kept for almost half an hour. In some cases, a reddish percipitate forms, which, if time is allowed, will settle out, leaving a clear colorless liquid above."

REESE: "Prior to making tests on the cooperative samples, in order to familiarize ourselves with the details and limitations, a number of mixtures of olive oil with known amounts of teaseed oil were tested and we were unable to obtain consistent quantitative results. As for instance, a 10% admixture did not consistently give a more positive indication than one containing 5%.

"Attempting to make a quantitative interpretation of these dates, it would appear that olive oil containing ¼ as much teaseed oil as sample No. 9 approaches the lower limit of positive detection. Judging from the relative depth of the colors developed and placing the color No. 9 equal to 100, sample No. 10 would place about 66 and No. 11 in the neighborhood of 10; this would be due to the masking effect of the blue component over the red. Placing the color developed by pure teaseed oil equal to 100, sample No. 9 would be judged as containing not more than 25% nor less than 10% teaseed oil. Should the chromogenic substance in teaseed oil be found to be relatively constant in amount, it would be possible to set limiting values on unknowns by comparison with mixtures of known composition."

SHEELY: "Our results indicate that the Dept. of Agriculture qualitative method is fairly reliable for the detection of teaseed oil in olive oil. We may state that whenever the test is unquestionably positive we may be sure that teaseed oil is present. A negative test, however, does not necessarily indicate the absence of this oil. We found that the original color of the various lots of olive oils tested has a pronounced effect on the sensitiveness of the test."

"For regular commercial inedible olive oils, an adulteration of 10% or more of teaseed oil can be definitely detected. A quantitative determination in commercial oils is of little value due to the variation of colors produced by different oils.

"In the edible oils tested, the pure oil gives a slightly positive test which makes the detection of small adulterations (0 to 5%) impossible. Fairly good quantitative results can be obtained on edible grade olive oils.

"In the instruction for the Fitelson test, it is asserted "Teaseed oil will show a brown color changing to intense red in a minute or so." Actually a dark reddish brown was obtained for 100% teaseed oil and for mixtures of 50% or less the color was light red or pink. It is also stated that "on rare occasions olive oil may pass through a fleeting faint tinge of pink." It was found, however, that this pink color was not fleeting but in every trial it lasted as long as the adulterated oils. This pink color produced by pure olive oil was noticeably less than the 5% teaseed mixture. However, it would be difficult to detect less than 5% of teaseed oil.

"In connection with the test, it should be stressed that the ether used be anhydrous. Very erratic and unsatisfactory results were obtained using ether (USP XI) which had been redistilled over calcium chloride. This difficulty was eliminated when ether conforming to A. C. S. specification distilled over sodium was used." (In sending out the samples, the chairman had cautioned the use of anhydrous ether, from his own experience, subsequently confirmed by Dr. Fitelson's verbal communication to him to that effect.)

"Positive tests by the Siebenberg-Hubbard method could not be considered definite unless the nature of the preparation of the oil were known. The authors state that their method is applicable only for unheated, non-rancid and not otherwise adulterated oil. Inasmuch as commercial olive oil for soap making may be processed under various methods, the method to be adopted should be applicable to any type of olive oil.

"Number 5 shows a considerable amount of adulteration by the S-H method while giving a negative test by the Fitelson. The likely explanation for this would be that this particular sample has either been intentionally heated or is of a commercial grade. Its dark color would indicate the latter."

Mr. Sheely also ran the Fitelson test on samples of his own of peanut, sunflower, soyabean, cottonseed and sesame oils and obtained negative results. They were tested to show the possibility of the interference of these oils with the test.

It is significant to note that Mr. Sheely gets negative results by the S-H method in three trials on samples nos. 8, 9, 10 and 11 though known mixtures containing as low as 10% teaseed oil will give a very markedly positive test by both methods.

Supplementing the general report, two individual ones are herewith given: one (Table II) to illustrate the development of the color in the Fitelson test and the other (Table III) to show the different reactions this test gives with commercial olive oils as compared with the edible grades.

It has been stated by Fitelson that the preliminary colors before the addition of ether as seen through transmitted and reflected lights are significant indications of the presence of teaseed oil, i.e., that teaseed oil will exhibit a deep green color by reflected light and a brown color by transmitted light, while olive oil will show a green color by both lights.

The above proves that in quite a number of instances final adjudication is directly contrary to first indications. Your chairman has examined a great number of samples during the past two years and corroborates Mr. Reese's findings.

The noting of these preliminary colors only serves to confuse and possibly prejudice final results. It seems therefore best, since they are of no particular value, to disregard them completely and let the color developed from the ether speak for itself.

THE SEIBENBERG-HUBBARD METHOD

Early in the investigation, it became apparent that this method required, as Dr. Hubbard himself states, a great deal of experience to carry out and to interpret.

Colors yielded by the Fitelson test were so clear as compared with the cloudy and rather murky colors of the S-H test that the members found considerable difficulty in reading them. Moreover the Fitelson test was easy to perform, requiring no skill and took less time. For these reasons the work of the committee continued with the government test, without prejudice to the Siebenberg-Hubbard method.

THE FITELSON METHOD

The committee did not go into the nature of the chromogenic substance giving the characteristic Fitelson reaction. It is obvious that the test itself is an adaption of the Liebermann-Burchard test, which gives in almost all cases a final green or blue with the vegetable oils, though at the first quick sight, some of the oils will give a reddish coloration. Oils like cocoanut, corn, rice bran and quite a few others when dissolved in a little chloroform and acetic anhydride give fleeting reds with a drop of concentrated sulphuric. The unsaponifiable matter from these oils, especially cocoanut oil, give the reddish color more markedly, and the color can be delayed somewhat by cold temperature. However, the chairman ran through the Fitelson test on all these oils, including palm oil, olive with carotene, with chlorophyll, with carbon bisulphide and with extracted olive skin oil, on olive oil foots, peanut, soya, sunflower, sesame, cottonseed and other oils which might be used as possible adulterants of olive oil and found in no case any definitely positive reddish reaction, except in some olive oils which gave a color under 5% teaseed oil by comparison with standards.

I have found the test to work on olive oil after caustic refining, after concentrated acid washings, after heating and in rancid olive oils. It reacts with the fatty acids liberated from olive oil soaps. While the color of commercial olive oils used for soap making masks to some degree the final color characteristic of this test, nevertheless, if teaseed is present to at least ten per cent, the Fitelson test is positive and roughly approximate to the depth of coloration, the time of development and final disappearance.

TABLE II. (Reese)

Sample No.	Color by Light Transmitted	Color by Light Reflected	Indication	Color after adding Ether	Final Indication
1.	Darker Green	Deeper Green	Negative	Slight Pink	Positive
2.	Brown green	Deeper Green	Negative	Pink	Positive
3.	Lt. Br. green	Yellow Green	Negative	Vy. slight Pk.	Vy. slight
4.	Yellow-green	Increased Grn.	Negative	Vy. slight Pk.	Doubtful
5.	Crimson	Brown-red	Positive	Lt. grey brown	Negative
6.	Green	Green	Negative	Pink	Positive
7.	Yellow-green	Green	Negative	Pink> No. 1	Positive
8.	Green	Green	Negative	Gray	Negative
9.	Brown	Green	Negative	Red	Positive
10.	Brown	Green	Negative	Red to Pink	Positive
11.	Green (brownish)	Green	Negative	Slight Pink	Doubtful

TABLE III (Sheely)

Samples			Fitelson Test	Siebenberg-Hubbard Test
Commercial Olive Oil		(soap-making)	Green-practically no pink cast	Dark red brown
6.	50% AOCS No. 1 /50%	Edible 0.0.	5-10	0-5
6A	AOCS /50%	Commercial 0.0.	Positive*	20%
7	50% AOCS No. 2 /50%	Edible 0.0.	10-15	5-10
7A	AOCS /50%	Commercial 0.0.	Positive*	20-25

5-10-25-50% Teaseed Oil in Commercial oil; black green tinge predominates but pinkish gradation apparent in the Fitelson test; dark red brown in all cases in the S-H test.

*Owing to the blackish cast, estimation of teaseed oil is difficult.

"It may be seen that in the case of a commercial oil, the final color had a blackish cast. This cast varies for different oils and makes it difficult to compare the color of an unknown to the colors produced by an oil of standard adulteration and thereby reduces the quantitative results to rough estimates. For edible type oils, these quantitative estimates are much more accurate." Sheely.

A scrutiny of Table I will show results on the Fitelson test to agree both qualitatively and quantitatively remarkably well among the different collaborators with the prepared mixtures. Sample 3, 4, 5 and 8 of olive oil showed some variation in color estimates. However, the first testing brings out a wider variation due to the personal factor, one chemist having a tendency to underrate a color intensity and another inclining to exaggeration. Repeated trials on known mixtures familiarize the chemist in time to the right measurement of color. In view of the fact that some olive oils will give a positive though weak reaction, it would appear reasonable to underrate the color ntensity when making standard comparison.

Sample No. 5 is important because of its bearing on the defense in a celebrated court case at which it was stated that the skins of the olive contained the substance which would give a strong coloration in the Fitelson test. Curiously enough, the Siebenberg-Hubbard test showed strongly positive as compared with a completely unanimous negative Fitelson. I have since conducted trials on the other samples of "skin" oil, some made by extracting the skins of over-ripe olives and, separately, of green olives with carbon bisulphide, and other samples by soaking these two types of olives in negative-testing olive oil, squeezing the mixture as dry as possible in a hand press, to obtain the alleged reacting substance from the skins. The carbon-bisulphide extracted samples were of course heated on the steam bath to remove all but traces of the solvent, but the oil leachings were not heated. In no case did I get any positive reaction above a very slight pink equal at most to under 5% teaseed oil. In fact, most of the tests were negative, not the slightest pink appearing.

CONCLUSION

The Fitelson test for the detection of teaseed oil in olive oil is distinctive and reliable and can be used to determine the presence of teaseed oil in olive oil, both edible and inedible.

In applying the test for quantitative purposes, it gives a rough approximation of the amount of teaseed oil in commercial olive oils, being masked somewhat by the varied colors produced by these oils.

For edible olive oils, the test gives quantitative results proportional to the depth of color produced and is capable of detecting teaseed oil in mixture with olive to the extent of ten per cent or more with a fair degree of accuracy.

As some olive oils may show the characteristic reaction given in this test, estimates below 10% would be considered speculative, although these olive oils as a rule show a coloration under 5%.

SUMMARY

The two recently devised tests for the detection of teaseed oil in olive oil, i.e. the Siebenberg-Hubbard and the Fitelson or so-called government tests, have been quite thoroughly investigated by this committee.

Data are given in a general report from the members, showing in detail the collaborative results, with individual comments on the behavior and accuracy of the methods.

From this work, the conclusion is reached that, of the two tests, the government one is by far the easier to perform, the clearer to interpret, and is applicable to any type of olive oil and teaseed oil mixture.

The committee recommends this to the Society for adoption as a tentative method for the detection of teaseed oil in olive oil, thus, incidentally, helping the government in its campaign to ferret out adulteration.

The test reads as follows:

The Fitelson Test for Teaseed Oil

(Lieberman-Burchard test modified)

Apparati: Test tubes of the same size, preferably flat-bottomed color tubes, 18 x 150 mm.

Pipettes and pieces of glass-tubing, 4 mm. outside and 2 mm. inside diameter designed to deliver 7 drops of oil equivalent to approx. 0.22 grams.

Reagents: C. P. Chloroform, conc. sulphuric acid, acetic anhydride.

A. C. S. anhydrous ether, redistilled over sodium and not over calcium chloride.

Method: Measure into a test tube, exactly 0.8 ml. acetic anhydride, 1.5 ml. chloroform and 0.2 ml. conc. sulphuric acid. Mix and cool in a water and ice bath to 5°C. Then add 7 drops of the oil to be tested. If the solution of oil in the reagents is cloudy, add acetic anhydride drop by drop, shaking each time, until clear. Let stand at 5°C. for five minutes. Now add 10 ml. cold anhydrous ether and mix immediately by inverting the stoppered test tube.

Teaseed oil will change to an intense red within a minute or so, the color reaching a maximum and then fading away. Some olive oils pass through a fleeting faint pink. Mixtures of teaseed and olive oils give a red coloration proportional to the amount of teaseed oil present. To arrive at an estimate of the amount of teaseed oil in a given sample, standards made up with known amounts should be run simultaneously and their colors compared.

This test will give reliable quantitative results above 10% of teaseed oil in the case of edible olive oils, but because of their variation in color, the inedible grades mask the end-point somewhat, so that the results are only roughly approximate. Occasionally olive oils of unquestionable purity are encountered, which test slightly pink, usually less than that equivalent to the presence of 5% of teaseed oil. For this reason, any color indicating less than 10% teaseed oil is uncertain and speculative, although not necessarily erroneous.

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ABSTRACTS

Oils and Fats

Edited by
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New British standards for whale oil and cod liver oil. *Fette u. Seifen 46*, 401 (1939). New Norwegian standards for whale oil, N. S. 487. L. Erlandsen. Ibid. 402.

Purity and purity determination of the higher fatty acids. H. Kurz. *Fette u. Seifen 46*, 397-400 (1939). Various methods of fractionating the mixed fatty acids of oils are reviewed. Development of a simple partial sepn. method to yield a characteristic to be called the sepn. no. (VK no.) is suggested. Sepn. may be by 2 immiscible solvents as alc. and ligroin. The portion of acids in each layer is detd. by sepg. the solns. and titrating.

Extraction of soap soln. with ethyl ether. H. W. Weedon. *Fette u. Seifen 46*, 400-1 (1939). In shaking a soap soln., to ext. unsaponifiable, with ethyl ether no detectable amt. of soap dissolves in the ether; some free fatty acids enter the ether phase. If the extn. is washed with water, the hydrolysis of the soap in soln. will increase the free fat acids in the ether phase.

Iodometric acid determination on spoiled fats. H. P. Kaufmann and Matty Lund. *Fette u. Seifen 46*, 390-1 (1939). The KI-KIO_3 method for detg. the acid no. of fats gives higher results than the usual method of titrating with KOH. The data and work points out that this difference is not due to the peroxide or active oxygen content of the fats.

The constitution of the linoleic acid of seed fats. T. P. Hilditch & H. Jasperson. *J. Soc. Chem.* Ind. *58*, 233-41T (1939). The yields of cryst. tetrabromostearic acid, m.p. 114°, and of the various tetrahydroxystearic acid producible from natural linoleic acid of cottonseed oil, and from the isomeric varieties produced by chemical means, have been compared. In addn. to the natural acid, "∝"- and "β"-linoleic acids were prepd. by debromination resp. of the cryst. and of the viscous liquid tetrabromostearic acids which are formed when bromine unites additively with the natural acid; further, the cis-trans isomerised lineoleic acids produced by the action of 0.3% of Se at 220° on the "∝"- and "β"-linoleic acids were also studied. The results confirm the view, already expressed by several workers, that natural and "∝"-linoleic acids are stereochemically identical. "β"-Linoleic acid is more probably a mixt. of about equal parts of the cis-Δ^9-cis-Δ^{12}-acid and the cis-Δ^9-trans-Δ^{12}-acid, whilst