only 8 per cent. with a sixfold multiplication of the concentrations of the former salts, and has very nearly the value, 0.0100.

Assuming that the two silver compounds are completely dissociated in their saturated solutions, the requirement of the Mass-Action Law and the Ionic Theory that the ratio of the squares of their solubilities be equal to the concentration-ratio just referred to, leads to the conclusion that the solubility of silver oxide in water is ten times that of silver chloride. The value for silver oxide determined directly by us is, however, 14.4 times that obtained for silver chloride by Kohlrausch and Rose by the conductivity method. It is pointed out that this divergence would be accounted for by the assumption that silver hydroxide in saturated solution is only 70 per cent. dissociated.

An exact method is described of deriving the dissociation of two dissolved salts in the presence of each other, which consists essentially in considering the dissociation of each salt to be that which conductivity measurements show it to have when it is alone present at a concentration of its ions equal to the square-root of the product of the concentrations of its ions in the solution of the mixed salts.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY,
BOSTON, MASS.

SOME NOTES CONCERNING HALPHEN'S TEST FOR COTTONSEED OIL.

BY ELTON FULMER.

Received September 3, 1902.

SINCE Halphen's reaction for cottonseed oil was first published in 1897,[1] it has been submitted to more or less rigid test by a considerable number of investigators. Although some differences of opinion have arisen as to its merits and general applicability, it seems to have been quite generally accepted as the most reliable method at hand for detecting the presence of cottonseed oil in admixture with other oils or fats.

K. Mazher,[2] after using nitric acid, Hirschsohn's reagent, Becchi's test, and Halphen's reaction, concludes that only the latter is to be depended upon.

---

[1] *J. Pharm. Chim.*, **9**, 392 (1897).

[2] *Ibid.*, **17**, 159.

J. Wauters[1] reviewed the Becchi test at some length, and concluded that Halphen's reaction is more reliable. He found it to be very delicate, and reported his ability to detect, by its use, 0.25 per cent. of cottonseed oil in a mixture. He also found it possible to determine the approximate amounts of oil in a mixture by comparison with known mixtures. R. D. Oilar[2] gave it extended trial and found it to be reliable for 0.50-0.25 per cent., and that it was even possible to detect such small amounts as $^1/_{16}$ of 1 per cent. Raikow and Tscherweniwanow[3] found it to be sensitive to 0.50 per cent. Many others have found the test as proposed by Halphen to be very satisfactory, and it seems to have great favor among chemists at the present time.

There is, however, some lack of agreement as to its reliability in the case of old or rancid oils.

J. Wauters[4] claims that old oils also respond to the test. P. Soltsien[5] states that the age and rancidity of the oil have no influence upon the characteristic reaction. R. D. Oilar,[6] on the other hand, found that old and rancid oils do not produce the crimson coloration.

The object of this article is not to discuss the sensitiveness nor reliability of Halphen's reaction when applied to normal oils, but rather to call more special attention to two points in connection with its use which require some emphasis. It is manifest that the diagnostic value of the test must be dependent upon all the conditions which affect it. The first point to be considered is the effect of heat upon cottonseed oil in its relation to Halphen's reaction, and the effect of high temperatures upon the palatability of the oil. It is conceded that the substance in the oil to which the characteristic coloration is due, can be eliminated by heat. Whether it is volatilized unchanged, or whether it is decomposed by the heat, is immaterial.

Raikow[7] has shown that the active substances in Becchi's and Halphen's reactions are not identical, and that by oxidation with

---

[1] *Bull. Assoc. Belg. Chim.*, **10**, 404-416 (1899).

[2] *Am. Chem. J.*, **24**, 353-373.

[3] *Chem. Ztg.*, 1899, pp. 1025-1028.

[4] *Bull. Assoc. Belg. Chim.*, **10**, 404-416 (1899).

[5] *Ztschr. öffentl. Chem.*, **5**, 106-107.

[6] *Am. Chem. J.*, **24**, 355-373.

[7] *Chem. Ztg.*, 1900, pp. 562-563.

potassium permanganate an oil can be rendered inactive to the latter, while responding with undiminished intensity to the former. This would seem to indicate that the color-producing substance in Halphen's test is easily oxidizable, possibly aldehydic in character, although Gill and Dennison[1] were unable to detect the presence of bodies of this nature in cottonseed oil. But whatever may be the nature of this active substance, it cannot withstand high temperatures. Raikow and Tscherweniwanow[2] found that it was destroyed by heating with superheated steam, or at a temperature of 220° C.

Allen[3] states that oil heated to 245° C. still gives the Halphen reaction but with diminished intensity. Gill and Dennison[4] state, as the result of their own experiments, that when heated to 260° C., an oil will no longer respond to the test. P. Soltsien[5] observes that the reaction still takes place when oil is heated to 200° C., but that a temperature of 250° C. destroys its sensitiveness to the test. D. Holde and R. Pelgry[6] found that a temperature of 200°-210° C. served to lessen the intensity of the coloration produced, and that when oil is maintained at 250° C. for ten minutes, it is no longer capable of producing a color change.

R. D. Oilar[7] concludes from his experimental work that the temperature at which the oil is rendered inactive, depends upon the nature of the containing vessel, and duration of the heating. He found that in glass vessels a temperature of 315°-320° C. was necessary in order to destroy the capability of color reaction, but that the same result was produced by heating the oil for one hour in a steel frying pan, at 140°-150° C.

Some experiments recently carried out in our laboratory showed that a temperature of 265°-270° C. is necessary to render the oil *wholly* inactive. The results given below were obtained with a sample of cottonseed oil which had been sold upon the market for olive oil. It was yellow in color, and had an iodine number of 106.3. It was not in the slightest degree rancid. The tests were

---

[1] This Journal, **24**, 397-398 (1902).

[2] *Chem. Ztg.*, **23**, 1025-1028 (1899).

[3] "Commercial Organic Analysis," Vol. II, Part I, third edition, p. 143.

[4] This Journal, **24**, 397-398 (1902).

[5] *Ztschr. öffentl. Chem.*, **5**, 135-136.

[6] *Chem. Rev. Fett. u. Harz. Ind.*, **6**, (4) 67-68.

[7] *Am. Chem. J.*, **24**, 355-373.

applied as follows: Eight test-tubes were prepared, each containing 10 cc. of oil, 10 cc. of colorless amyl alcohol, and 10 cc. of carbon disulphide containing 2 per cent. of sulphur. In one tube unheated oil was used; in another, oil that had been previously heated to 180° C. for ten minutes; in the other tubes oil was used which had been heated ten minutes at the following temperatures: 200°, 210°, 220°, 240°, 260° and 280° C. The test-tubes and contents were heated in a bath of glycerol and water to 110° C. for fifteen minutes. Results as follows: The unheated oil gave a deep crimson coloration. That which had been previously heated to 180° C. gave a crimson coloration which was very perceptibly lighter than that given by the unheated oil. There was a gradual lessening of intensity of the color produced by the oil which had been heated to 200° and 210° C., while that which had been submitted to a temperature of 220° C. gave only a light crimson coloration; a slight pink color resulted from the sample heated to 240° C., and only a very faint tinge of pink from that heated to 260° C., while the contents of the tube containing the oil which had been previously heated to 280° C. showed no trace of color.

Soltsien[1] and others believe that cottonseed oil which has been heated to 250° C. is no longer fit for edible purposes. Our work, although by no means disproving this belief, seems to indicate that possibly it is erroneous. The oil was raised gradually to a temperature of 280° C. by us, by heating it in open test-tubes in a paraffin bath. It was kept at this temperature for ten minutes and the only observable difference between it and the unheated oil was an almost imperceptible deepening of the yellow color. At this date, three months after the heating, the heated and unheated samples show no difference in taste or odor. It is probable that oils produced by different methods of extraction and refining may be more or less dissimilar, and hence results from a single sample cannot be regarded as conclusive, although the presumption is strong in favor of the view that oils would differ but little in the characteristic just mentioned. It is, of course, superfluous to remark that if cottonseed oil can be heated to a temperature sufficient to destroy its color reaction without injuring its palatability, Halphen's test cannot be wholly reliable for detecting its presence

---

[1] *Ztschr. öffentl. Chem.*, **5**, 135-136.

when mixed with other oils or fats. However, it is not necessary to completely destroy the capability of color production, and hence not necessary to heat the oil to 265°-270° C., in order to render the test untrustworthy. Heating to 220°-230° C. would be sufficient. This fact brings us to the second point to be emphasized; *viz.*, that the active substance in cottonseed oil to which the characteristic coloration in Halphen's reaction is due, is transmitted apparently unchanged into the fat of animals fed on cottonseed meal.

J. Wauters[1] says that when Halphen's test is applied to butter from cows fed on cottonseed meal, a coloration is obtained equivalent to about 1 per cent. of cottonseed oil. F. Werenskiold[2] is responsible for the assertion that when cows were fed 1 kilo per day of peanut cake, no test for arachidic acid could be obtained in the butter they produced; but that when an equal amount of cottonseed meal was fed, the butter gave a distinct reaction for cottonseed oil with Halphen's reagent.

Allen[3] states that lard and lard oil obtained from animals which have been fed on cottonseed meal, will often respond faintly to Halphen's test. The same statement is repeated by Leffmann and Beam.[4] P. Soltsien[5] stated that he had met with American lards which gave a very weak reaction with Halphen's test, equivalent to about 1 per cent. of cottonseed oil. The same authority reported at a later date[6] that he had obtained a color reaction, also with American lards, equivalent to that given by a mixture containing 25 per cent. of cottonseed oil. The lards in question were obtained by a member of the German Consulate at Chicago, at Armour & Co's establishment, from hogs which had been liberally fed on cottonseed meal. This most astounding result reported by Soltsien has not been corroborated by any other worker. If correct, it almost shakes one's belief in animal metabolism.

In order to satisfy ourselves as to the accuracy of the several statements above quoted, a series of feeding experiments were planned by us so that samples of lard might be obtained from hogs

[1] *Bull. Assoc. Belg. Chim.*, **10**, 404-416 (1899).

[2] *Jahresber. öffentl. Veranstalt. Förd. Landw. Norwegen 1, J.*, 1897.

[3] "Commercial Organic Analysis," Vol. III, Part I, third edition, p. 143.

[4] "Food Analysis," p. 169.

[5] *Ztschr. öffentl. Chem.*, **7**, 25.

[6] *Ibid.*, **7**, 140 (1901).

which had received varying amounts of cottonseed meal as a part of their daily rations. Before we were ready to begin these, it was learned that Director John Fields, of the Oklahoma Experiment Station, was already engaged in a feeding experiment with cottonseed meal, and his cooperation was therefore solicited and secured. The samples of lard mentioned below were rendered from the fat of the hogs he was using in the feeding test, the following details of which were furnished by him. Our thanks are due Director Field for his cooperation. Four lots of hogs were used, each lot containing six animals. All of them had been fed uniformly before the beginning of the experiment, and all of them had received small amounts of cottonsed meal in their ration. The feeding experiment covered a period of fifty-six days, during which time the following rations were eaten:

LOT NO. 1.
2026 lbs. wheat chop.

LOT NO. 2.
$675\frac{1}{3}$ lbs. wheat chop.
$337\frac{2}{3}$ lbs. wheat bran.
1013 lbs. wheat middlings.

LOT NO. 3.
$135\frac{1}{15}$ lbs. cottonseed meal.
$1890\frac{14}{15}$ lbs. wheat chop.

LOT NO. 4.
$66\frac{1}{8}$ lbs. cottonseed meal.
$1928\frac{1}{3}$ lbs. wheat chop.

When the animals were slaughtered, a piece of fat about two inches square was taken from each one. Some of the pieces were taken from the head, and some from other portions of the body, but no leaf fat was included. The samples taken from the animals belonging to each lot were mixed and rendered, and the resulting lard sent to us. These lard samples were received and work upon them completed, before receiving any information concerning the rations fed to each lot, and hence our observations were free from any unconscious bias that might have resulted had such information been at hand. The results of our tests are as follows:

		Lot No. 1. Lab. No. 893.	Lot No. 2. Lab. No. 894.	Lot No. 3. Lab. No. 895.	Lot No. 4. Lab. No. 896.
Becchi's test		No reduction	Very slight reduction	Slight reduction	Very slight reduction
Halphen's test		Faint pink	Pink	Light crimson	Light pink
Approximate amount cottonseed oil[1]		1.2%	2.8%	4.0%	2.0%
Iodine number		55.48	59.96	52.96	53.03
Maumené's test	Rise in temp.	25.9°	25.7°	23.5°	26.0°
	Sp. temp. reaction[2]	0.64	0.63	0.58	0.64

[1] Obtained by comparison with known mixtures. [2] Rise in temperature of water, 40.5°.

The cottonseed oil used in the known mixtures for comparison, was the same as previously mentioned. Check tests were made upon lard from animals which had never been fed cottonseed meal, and in no case was the slightest coloration manifested.

These results confirm the observations of others that cottonseed meal will impart the characteristic color-producing power of the corresponding oil, to the fat of animals which eat it. They show a more decided reaction with Halphen's test than indicated by Allen, Wauters, and other authorities quoted, but are very far from corroborating the recent statement of Soltsien. They further show that the color-producing power of the cottonseed oil is very persistent when once introduced into the fat of an animal. Lots 1 and 2 had received no cottonseed meal in their ration for fifty-six days, and yet the lard from their fat showed a coloration with Halphen's reagent equivalent to 1.2 per cent. and 2.8 per cent. of cottonseed oil, lot No. 2 showing even a greater coloration than lot No. 4 which had eaten $66^1/_6$ pounds of cottonseed meal during the feeding period.

We propose carrying on, as soon as practicable, the feeding experiments originally planned, in order to continue our study of the effect of feeding cottonseed meal, upon lard in its relation to Halphen's test.

Summarizing the points above emphasized we have the following:

First.—Cottonseed oil is rendered inactive toward Halphen's reagent by heating to 260°-270° C.

Second.—The intensity of the reaction is very greatly diminished by heating the oil to 220°-240° C.

Third.—It is possible that cottonseed oil may be heated to 280° C. without injuring it, and reasonably certain that a temperature of 220°-240° C. will not render it unfit for use as an article of food, either alone, or as part of a mixture.

Fourth.—Lard from animals fed on cottonseed meal may respond to Halphen's reagent with an intensity of coloration equivalent to several per cent. of unheated cottonseed oil.

It naturally follows from these facts that a mixture of fats or oils may be prepared containing at least 25 per cent. of cottonseed oil which has been heated to 220°-240° C., or a much larger percentage if heated to 250°-260° C., which will give a coloration with Halphen's reagent not more intense than that obtained with lard from cottonseed meal-fed hogs. Under such conditions the value of the test for diagnostic purposes is somewhat questionable, especially in its application to the analysis of lard. It is claimed by lard manufacturers and cottonseed oil refiners that the oil is never heated to such a high temperature. This may be true, but it is nevertheless desirable that chemists should be on their guard, and not place too much reliance upon this comparatively new test. For normal, unheated oil, its value is unquestioned; but in view of the facts above discussed, its limitations should not be overlooked.

The analytical work in this paper was performed by Mr. R. W. Thatcher, assistant chemist of the Washington Experiment Station, to whom our thanks are due.

FIXATION OF ATMOSPHERIC NITROGEN BY ALFALFA ON ORDINARY PRAIRIE SOIL UNDER VARIOUS TREATMENTS.[1]

BY CYRIL G. HOPKINS.

Received August 18, 1902.

MANY different farmers have tried to grow alfalfa in various sections of Illinois and adjoining states, but, in most cases, it has been pronounced a failure. Where alfalfa has been grown with success in Illinois, it has usually been necessary to sow it on very rich ground or to keep it well manured.

In theory, alfalfa ought to grow and do well on these prairie soils, and it ought not to require heavy and frequent applications of manures, because alfalfa is a very deep rooting crop and is thus capable of drawing upon the soil to great depths for the necessary mineral elements of plant food, and, being a leguminous plant, it has the power of "gathering" nitrogen from the inexhaustible supply of the air, by means of bacteria which inhabit its roots.

Numerous observations made on several fields of alfalfa in different sections of the country during the past few years led the

---

[1] From advanced sheets of Bulletin No. 76 of the University of Illinois Agricultural Experiment Station.