PHOTOCHEMICAL STUDIES OF RANCIDITY: A NOTE ON THE POSSIBILITY OF USING "CHLOROPHYLL VALUES" AS MEANS OF ESTIMATING THE STABILITY OF AN OIL OR FAT.*

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THE term "rancid" is descriptive of odor and taste and consequently "rancidity" is detected and estimated chiefly by means of the sense preceptions. However, for practical laboratory work, the need for a reliable chemical test for replacing organoleptic tests is apparent. For a test to be most satisfactory, it should give a reaction at the first stages of rancidification so that one may estimate the stability of an oil or fat before rancidity can be detected organoleptically.

The Kreis test is perhaps the best known test for indicating rancidity, yet this test is also given by certain nonrancid cottonseed oils. The Schiff test with fuchsin-sulphurous acid for the aldehyde grouping has been applied to the detection of rancidity in oils, but always in a qualitative or at best a very rough quantitative manner. Naturally there has been much controversy as to the ability of the various chemical tests to parallel the organoleptic tests for rancidity. Experience with various methods, especially the peroxide test and the Kreis test, shows that there are no values above which oils and fats, in general, may be invariably regarded as rancid and below which they may be considered sweet. So much depends on the nature of the oil and also in no small degree on the conditions of temperature and light to which the oil has been subjected. One of our problems has been to find a quantitative chemical test which will correspond more closely to organoleptic rancidity than do the existing ones and which will go still further by indicating the relative stability of an oil before organoleptic rancidity appears. The method presented in this paper gives promise of fulfilling these requirements.

The new method for following the autoxidation of an oil or fat depends upon the relative absorption of magnesium chlorophyll as indicated by the red fluorescence of chlorophyll when placed under an ultra-violet lamp equipped for fluorescence studies. Fresh oils and fats, without the addition of this chlorophyll, fluoresce strongly with a bright grayish-blue. As oils or fats approach organoleptic rancidity their fluorescence decreases greatly in intensity, i.e., to a dull grayish-blue. Light energy is, through absorption, changed to chemical energy, and a rancid oil is the final product. This transition in oils is difficult to follow, unless some means is found to intensify the difference in fluorescence between a fresh and a rancid oil. The addition of magnesium chlorophyll apparently fulfills this purpose. Oils, however, absorb or combine with chlorophyll to a considerable extent without fluorescing red, depending upon the degree of oxidation, but as the oil becomes oxidized, it absorbs less and less chlorophyll and the color, which is indicative of the beginning of rancidity, increases to a brilliant red under the light as soon as the oil reaches the organoleptic state of rancidity.

—THEORY—

An explanation of this phenomenon seems to be that the oil contains a compound or radical which reacts with the chlorophyll. When the oil is exposed to ordinary light and this compound or radical is gradually used up by oxidation, less chlorophyll reacts with this compound and the light grayish-blue fluorescence peculiar to the oil is overcome by the red fluorescence of the chlorophyll. Advantage has been taken of this phenomenon to devise the following method:

A standard solution "A" is made with 0.3 grams of magnesium chlorophyll[1] in 200 cc. of non-fluorescing mineral oil.

A second standard "B" is made by adding chlorophyll solution A, drop by drop, to each variety of oil until when examined under the ultra-violet lamp each oil gives a neutral color, i.e., is just short of giving a pink fluorescence; in other words, to the point where, if one more drop of the chlorophyll solution were added to the standard oil, there would appear a pink fluorescence. Add, by means of a burette, the oil to be tested to 10 drops[2] of the standard chlorophyll solution A contained in a small (5 to 10 cc. capacity) crucible and match the color of this oil under the ultra-violet lamp with that of standard B. Duplicate tests within less than 0.2 cc. of oil are easily possible.

According to the theory here proposed stable oils contain a greater amount of a yet unnamed compound which reacts with chlorophyll than do rancid oils. Hence the smaller the amount of the oil needed to quench the red fluorescence of the chlorophyll, the longer induction period will the oil have. Since each oil has a slightly different fluorescence, it is well to have a standard made up with each oil for the purpose of more accurate comparisons.

EXPERIMENTAL

In order to show what effect the progress of rancidity has on the oil absorption of chlorophyll, the following experiment was conducted:- Fourteen glass color filters, covering the visible spectrum, were placed over crystalizing dishes, of which contained about 25 cc. of non-rancid cottonseed oil. These samples were then exposed to irradiation from four CX Mazda lamps for intervals of 2, 6, 10, and 14 days respectively. Results will be found in Table I.

[1]The chlorophyll used in this investigation was a highly refined, oil-soluble, magnesium chlorophyll.

[2]A definite volume, e.g. one cc., of standard A could be used just as well if found more convenient.

*Food Research Division Contribution No. 426

TABLE I — CHLOROPHYLL VALUES AND PEROXIDE VALUES OF "FINISHED" COTTONSEED OIL IRRADIATED UNDER COLOR FILTERS. (Light from CX lamps.)

FILTERS	Two days irradiation		Six days irradiation		Ten days irradiation		Fourteen days irradiation	
	Oil drops Chph.V	Peroxide value	Oil drops Chph.V	Peroxide value	Oil drops Chph.V	Peroxide value	Oil drops Chph.V	Peroxide value
(initial values)	12	44.5	12	45.5	12	45.5	12	45.5
Corex A	25 R	80.5	84 VR	322.0	....	....	....	....
Violet	19 R	72.0	45 VR	109.0	75 VR	218.0	....	....
Signal Blue	25 R	75.0	70 VR	180.0	75 VR	355.0	....	....
Noviol "O"	18 R	74.5	45 R	130.0	.... VR	....	....	....
Noviol "A"	12	69.0	21 R	100.0	.... VR	....	....	....
Noviol "C"	14 R	66.0	18 R	91.5	24 R	146.5	....	....
Sextant Green	12	65.5	12	86.5	15	110.0	21 R	248.5
Traffic Yellow	12	64.5	12	83.0	20 R	121.0	21 R	290.0
Lantern Yellow	12	66.5	12	89.5	18 R	115.0	21 R	278.0
Shade Red	12	66.5	12	91.5	15 R	108.0	21 R	248.0
Traffic Red	12	65.5	12	84.0	18 R	103.0	21 R	181.0
Signal Red	12	65.0	12	84.0	18 R	106.0	21 R	236.0
Pyrometer Red	12	62.0	12	83.5	15.5 R	104.0	21 R	207.5
Sextant Red	12	58.0	12	79.0	15	101.0	21 R	193.5

R-rancid, VR-very rancid, Chph.V-Chlorophyll Value.

It has been noted before (**"Photochemical Studies of Rancidity,"** Oil and Soap *11,* No. 9, pages 189-90, 1934,) that the peroxide values of oils under certain filters may be higher than those under other filters and yet the oils in the first case may be fresh while in the second case they are rancid.

The figures under "chlorophyll value," in Table I represent, in this case, the number of drops (instead of cubic centimenters) of the irradiated oils needed to quench the red fluorescence of one drop of chlorophyll. The more drops of oil used, the nearer the oil is to being rancid.

In another experiment, fresh cottonseed oil and fresh corn oil separately were added from a burette to 10 drops of standard chlorophyll solution A until they matched their respective standard B. The amount of oil added was 2.65 cc. and 0.50 cc. respectively. These are the initial "chlorophyll values" as shown in Table II, viz., the number of cubic centimeters of oil that are necessary to neutralize the red fluorescence of ten drops of standard magnesium chlorophyll when placed under a mercury quartz lamp equipped for fluorescence studies. Another sample of each of these oils was then irradiated under CX Mazda lamps for varying lengths of time, for the purpose of showing how the chlorophyll values increase as the oils approach rancidity. The lower the number of cc. of oil needed to reduce the red fluorescence the less susceptible the oil is to rancidity and the longer the induction period of the oil.

DISCUSSION

The development of rancidity is indicated by the chlorophyll absorption test or "chlorophyll value" in that the more oil used to reduce the red fluorescence, the nearer it is to being rancid and vice versa. It will be observed (Table I) that the amount of oil required to quench this red fluorescence is much greater in oils that become rancid quickly, e.g. under Corex A, Violet, Signal Blue, Noviols "O", "A", and "C", than in the oils which become rancid slowly, e.g. under Sextant Green, Yellow or Red filters.

Thus, it seems that when samples of oil have been exposed to ultraviolet, violet and blue light, the amount of oil needed to match the standard B is much greater than in the case of oils which have been irradiated through filters which transmit little or no blue light. Likewise, it will be noted that in any one oil the chlorophyll value increases with the onset of rancidity. (See Table II.)

It is believed that the stability of an oil may be estimated with this test, but before any definite statement can be made numerous samples of oils must, of course, be analyzed by this method in order to establish its usefulness. An average chlorophyll value then should be ascertained for oils that are known to be strictly fresh, which value may be taken as a standard for freshness. An average chlorophyll value for organoleptically rancid oils should also be established. A minimum value could well be adopted as a standard of the refinement or of the quality of an oil for the market.

CONCLUSIONS

No. 1—The "chlorophyll value" of an oil gives promise of replacing the tests for rancidity now in common use because it seems to indicate a progressive change in the oils from the time it begins to be oxidized up to and beyond the point when organoleptic rancidity is observed.

No. 2—Such a chemical test would eliminate the personal equation which is so objectionable in organoleptic tests.

No. 3—This chemical test is based on natural constituents contained in oils that are eliminated during oxidation and not on new substances which are formed as a result of oxidation.

No. 4—This test is simple and may be made in a few minutes without complicated manipulation.

No. 5—The chlorophyll value may thus afford a new and reliable means to detect the oxidation of an oil long before organoleptic rancidity can be noted.

TABLE II — CHLOROPHYLL VALUES AND PEROXIDE VALUES OF COTTONSEED AND CORN OIL RESPECTIVELY AS THEY APPROACH RANCIDITY WHEN IRRADIATED BY CX MAZDA LAMPS.

Time (minutes)	Cottonseed Oil: Cc. of oil to 10 drops of chlorophyll-Chph.V	Peroxide values	Corn Oil: Cc. of oil to 10 drops of chlorophyll-Chph.V	Peroxide values
(initial values)	2.65 cc.	40.3	0.50 cc.	31.0
35	2.90 cc.		0.60 cc.	
65	3.40 cc. R		0.70 cc.	
83	3.65 cc. VR	347.5	0.80 cc. R	57.5

Chph V-Chlorophyll value, R-Rancid, VR-Very rancid.