rations was observed in three investigations (Ney & Newell—*Poultry Sci. 33*, 297; March *et al.—Ibid.* 300; Balloun—*Ibid.* 1041), whereas in another investigation six synthetic detergents and one soap failed to give an increased growth response over the control (Branion & Hill—*Ibid.* 62). Detergents do not improve digestibility of feed nutrients by steers (Lassiter *et al.—J. Animal Sci. 13*, 991). In superphosphate manufacture, detergents tend to accelerate the primary reaction between phosphate rock and sulfuric acid but had no significant effect on the extent of the over-all reaction in a period of one to 24 hours after mixing (Fox *et al.—J. Agr. Food Chem. 2*, 618). In another report the over-all experience of using detergents in factory production of superphosphate mixed fertilizer is related (Fox *et al.—Farm Chemicals 117*, No. 9, 43).

An Instrumental Method for Measuring the Degree of Reversion and Rancidity of Edible Oils[1, 2]

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THE EVALUATION of an edible oil or fat by organoleptic means has served as a very useful method for measuring its degree of flavor reversion and rancidity. However the selection, training, and maintenance of an elaborate testing panel is time-consuming and expensive. Further, comparatively small laboratories may lack qualified personnel to serve as panel members, or the qualified personnel may be too close to the research program to render a completely unbiased opinion. Therefore it would seem desirable to have available an instrumental method to collect data which could be correlated with those obtained by organoleptic means.

The phenylhydrazone derivatives of the carbonyl compounds which developed in rancid oils have been used as a means of estimating their degree of rancidity (1). However no satisfactory method of general acceptance based on carbonyl compounds has been developed because of two main difficulties. First, oils and fats often contain components which are not responsible for the odor and flavor of the oil or fat but may either interfere or respond to the test. Second, an accurate method for the quantitative measurement of the micro amounts of hydrazones formed had not been developed until recently.

In the present method these difficulties were overcome by removing the volatile carbonyl compounds from the oil with the aid of a stream of nitrogen and collecting them in a cold trap cooled with solid carbon dioxide. The amount of carbonyl compounds condensed in the cold trap was determined by the procedure of Lappin and Clark (2). Carbonyl compounds could be quantitatively determined to a concentration as low as 5 x 10^{-6} molar. The results obtained by this method were correlated with the scores given to the oil or fat by an experienced testing panel.

Experimental

Analytical Procedure. The specifications for the apparatus are shown in Figure 1. A 38-mm. diameter tube was suspended in an oil bath which was kept at 80 ± 0.2°C. by means of a Fenwal thermoswitch control unit and a knife-type of immersion heater. The cold trap, made of a 20-mm. diameter tube, was suspended in a Dewar flask. A Cenco orifice-type Pyrex glass gas flowmeter, calibrated with the aid of a gas burette, was connected to outlet C. The capillary tip B was made by heating a 7-mm. (inside diameter) tube in a flame and allowing it to shrink evenly to 0.5 mm. (inside diameter) and 4.0 mm. in length.

High purity dry nitrogen was passed through the apparatus from A through B to C at a rate of 84 ml. per minute at room temperature for 15 min. to displace air and to remove any trace of solvent in the apparatus. The rate of nitrogen flow was regulated by means of a Hoke metering valve (No. 2RB285), which was connected to a gas reducing valve set at 5 p.s.i. The Dewar flask was then filled with finely powdered solid carbon dioxide. Next 101 g. of an oil or fat were weighed into a 250-ml. beaker, which contained a clean 8-cm. glass funnel. The tempera-

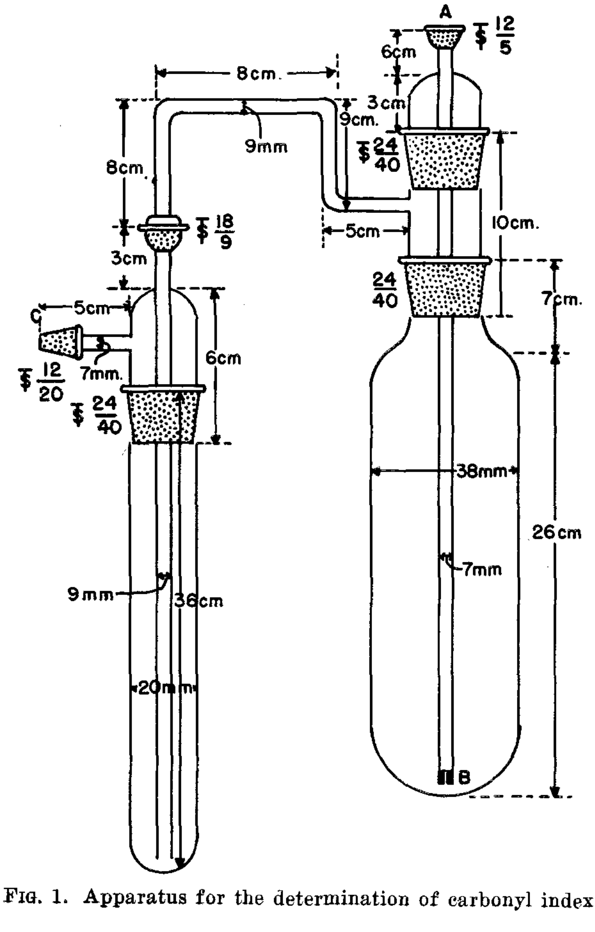

FIG. 1. Apparatus for the determination of carbonyl index.

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[1] Funds for this study were furnished by a grant-in-aid from Swift and Company, Chicago, Ill.

[2] Presented at the American Oil Chemists' Society meeting, Minneapolis, Minn., Oct. 11-14, 1954.

[3] Present address, Swift and Company, Chicago, Ill.

ture of a fat was kept approximately 5°C. above its melting point, and an oil was kept at room temperature during the weighing operation. In order to obtain reproducible results great care had to be taken to avoid overwarming of the sample. Tube A was carefully removed from the apparatus, the 8-cm. glass funnel was suspended on top of the ₸ 24/40 joint, and the sample was transferred into the 38-mm. tube. The funnel was transferred to the beaker, and Tube A was immediately replaced. Nitrogen was bubbled through the oil for exactly 2 hrs. at a rate of 84 cubic centimeters per minute (S.T.P.) as indicated by the flowmeter connected to the apparatus at C. The timing with an electric "Time It" stopwatch graduated to seconds was begun as soon as the first nitrogen bubble came through B. The empty beaker and glass funnel were reweighed, the difference in weight represented the weight of the sample used. At the end of exactly 2 hrs. the ₸ 18/9 ball joint was disconnected, and the condensate in the cold trap was quantitatively dissolved in 5 ml. of carbonyl-free methyl alcohol.

One ml. of this solution was transferred into a 22- x 90-mm. test tube which was fitted with a ₸ 24/40 ground glass joint, 1.0 ml. of the 2,4-dinitrophenylhydrazine reagent and 0.01 ml. of concentrated hydrochloric acid were added, the tube was stoppered with a hollow ₸ 24/40 stopper and heated for 30 minutes in a water bath kept at 50 ± 0.5°C. The tubes were cooled quickly in cold water, the inner wall of the tube was washed down with 5.0 ml. of potassium hydroxide solution, and the resulting colored solution was allowed to stand for 10 minutes. A blank solution was prepared with 1.0 ml. of the carbonyl-free methyl alcohol in place of the sample. The absorbance of the solution at 480 mμ was determined with the aid of a Beckman Model DU spectrophotometer, which was adjusted at zero absorbance for the blank solution. If the reading was above 0.4, the original solution was properly diluted with carbonyl-free methyl alcohol and the test repeated with the diluted solution. The absorbance of a 1.0-ml. aliquot of the 5-ml. methanolic wash in the cold trap of the volatile material from 100 g. of the sample times 1,000 was defined as the carbonyl index of the oil, as given by the following equation:

$$\text{Carbonyl index} = \text{Absorbance}\,\frac{100\text{ g.}}{\text{Wt. of sample (g.)}} \times 1{,}000$$

PREPARATION OF REAGENTS

Carbonyl-free methyl alcohol. Ten g. of 2,4-dinitrophenylhydrazine and 1 ml. of concentrated hydrochloric acid were added to 1 liter of reagent grade methanol and refluxed for 2 hrs. The methanol was cooled and filtered to remove excess 2,4-dinitrophenylhydrazine, and the filtrate was distilled twice through a Vigreaux column.

The 2,4-dinitrophenylhydrazine solution. Reagent grade 2,4-dinitrophenylhydrazine was recrystallized twice from carbonyl-free methanol. A saturated solution of this recrystallized 2,4-dinitrophenylhydrazine in carbonyl-free methanol was prepared. This solution was not considered to be stable for more than one week.

Potassium hydroxide solution. Ten g. of reagent grade potassium hydroxide were dissolved in 20 ml. of distilled water, and the solution was made up to 100 ml. with carbonyl-free methanol.

Cleaning of apparatus. All glassware was scrupulously cleaned by the following procedure: It was degreased thoroughly with Skellysolve B, washed with hot running water to remove the solvent, soaked in chromic acid cleaning solution for 2 hrs., rinsed thoroughly with water at least six times, rinsed with redistilled acetone, flushed with clean air to remove acetone, and dried in a vacuum oven at 140°C. for 2 hrs.

Reversion of samples. All samples were aged in 600-ml. beakers. Approximately 300 ml. of each sample were measured into a 600-ml. beaker and covered with a watch glass. The beakers were suspended in an oil bath to the depth of the sample in the beaker, and the temperature of the oil bath was kept constant within ± 0.2°C. The carbonyl index of reverted samples was either determined immediately, or the samples were stored at —20°C. in glass stoppered bottles. If stored at 0°C., the carbonyl index of reverted soybean oil increased markedly during storage. The organoleptic evaluations of the reverted samples were conducted according to the method designed by the Northern Regional Research Laboratory (3).

Results and Discussion

A direct correlation was noted between the carbonyl indices and the organoleptic scores of seven samples of soybean oil which had been aged for 0 to 6 days at 60°C. (Table I). However half of the members of the panel had difficulty when asked to arrange the samples in an increasing order of reversion. It was difficult for some members of the panel to differentiate between the samples which had been aged at 60°C. for 1 to 2 and 3 to 4 days, respectively. On the other hand, the carbonyl indices of these samples differed sufficiently to demonstrate a distinctive degree of reversion between them.

TABLE I

Correlation Between Carbonyl Indices and Organoleptic Evaluation of Soybean Oil Aged at 60°C.

Days aged at 60°C.	Carbonyl index	Organoleptic score
0	28	9.0
1	52	8.0
2	258	7.5
3	611	7.0
4	870	6.0
5	1125	4.2
6	2320	2.7

The accuracy and sensitivity of the carbonyl index method for evaluating the degree of reversion and rancidity of edible oils was further tested by the method designed by Handschumaker (4). In this method a series of blends of hydrogenated soybean oil and cottonseed oil were selected at random. Five samples ranging from all soybean oil to all cottonseed oil were combined in 25% steps and reverted at 140°C. for 4 hours. Panel members, who were acquainted with the odor of reverted soybean oil, were asked to rate the samples in order of increasing reversion odor. Six observers were found who could consistently place these specimens in the proper order. The percentage of correct placements by these six selected members in routine performance ranged from 76.7 to 95.7%. On the other hand, when this method was adopted to test the accuracy and sensitivity of the carbonyl index determination with various combinations of oil and

TABLE II

A Test of the Sensitivity of the Carbonyl Index Method

Composition of sample		Condition of aging		Carbonyl index
Soybean oil	Cottonseed oil	Time (days)	Temperature	
100%	0%	6	60°C.	2024
75	25	6	60	1185
50	50	6	60	714
25	75	6	60	474
0	100	6	60	113
Soybean oil	Shortening[a]			
100%	0%	6	60	2024
75	25	6	60	1356
50	50	6	60	783
25	75	6	60	274
0	100	6	60	72
Hydrogenated soybean oil	Hydrogenated cottonseed oil			
100%	0%	2	100	1743
75	25	2	100	1550
50	50	2	100	1317
25	75	2	100	1167
0	100	2	100	850

[a] Commercial product made from hydrogenated vegetable oils.

various methods of reversion (Table II), the percentage of correct placement was 100.

As some oils and fats evolve odor and flavor components which are not carbonyl in character, the carbonyl index may not reflect the organoleptic characteristics of such oils and fats as well as a testing panel. Oleo oil and lard for example had a lower flavor score than their carbonyl indices would indicate (Table III). However, if the carbonyl index of a specific oleo oil or lard is compared with those of other oleo oils and lards rather than soybean or cottonseed oil, it may be possible to set up an "internal standard" technique which could serve as a quick method of evaluation and could compare favorably with organoleptic scores.

TABLE III

Carbonyl Indices of Oils and Fats Aged at 60°C. for Six Days

Sample	Carbonyl index	Organoleptic score
Soybean oil	2320	2.7
Lard	315	5.5
Corn oil	204	7.2
Oleo oil	157	4.6
Peanut oil	125	6.5
Hydrogenated soybean oil (I. V. = 84)	127	7.0
Cottonseed oil	113	7.5
Shortening I[a]	72	8.0
Shortening II[a]	64	8.0
Hydrogenated cottonseed oil	63	8.2

[a] Shortening I was prepared from hydrogenated vegetable oils. Shortening II was prepared from animal and vegetable fats.

When very stable oils and shortenings were aged at 60°C. for 6 days, only a small increase in the carbonyl index was noted. For such oils and fats, reversion at 100°C. for 2 days gave a more satisfactory index of stability than reversions at 60°C. for 6 days (Table IV).

TABLE IV

Carbonyl Indices of Oils and Fats at 100°C. for Two Days

Sample	Carbonyl index	Organoleptic score
Cottonseed oil	5810	1.0
Hydrogenated soybean oil (I. V. = 84)	828	5.4
Shortening I[a]	618	6.1
Shortening II[a]	458	6.4
Deodorized oleo oil (I. V. = 46.3)	1002	5.1
Hydrogenated oleo oil (I. V. = 43.0)	239	7.0

[a] Shortening I was prepared from hydrogenated vegetable oils. Shortening II was prepared from animal and vegetable fats.

Samples of various oils and fats which were purchased from local sources had relatively low carbonyl indices and compared favorably with the freshly deodorized oils and fats. However the carbonyl indices of the purchased oils all increased markedly after they had been aged at 60°C. for 6 days (Table V).

TABLE V

A Comparison of the Flavor Stability of Soybean Oil, Cottonseed Oil, and a Hydrogenated Shortening by the Carbonyl Index Method

Sample	Carbonyl Index		
	Freshly deodorized	A sample obtained from local market	Aged at 60°C. for 6 days
Soybean oil	28	152	2320
Cottonseed oil	17	45	113
Shortening (prepared from hydrogenated vegetable oils)	less than 10	22	72

For the actual determination of the carbonyl index a temperature of 80°C. was chosen, as above this temperature unstable oils, such as soybean, tended to yield volatile carbonyl compounds even under an inert atmosphere. For example, a bland soybean oil gave a relatively high carbonyl index if 100°C. was used (Table VI). The high carbonyl index obtained by using a temperature greater than 80°C. indicated that the carbonyl index was not indicative of the total amount of carbonyl compounds in the oil but rather represented a proportional amount of the volatile carbonyl compounds which were present in the oil. However the volatile carbonyl compounds gave a better correlation with the odor and flavor of the oil than the total carbonyl. For example, a bland corn oil gave a high carbonyl value if the oil itself rather than the volatile carbonyl compounds was used to run the Lappin and Clark test.

TABLE VI

Effect of Temperature on the Carbonyl Index Determination

Temperature, °C.	Carbonyl Index	
	Deodorized soybean oil, organoleptic score = 9.0	Reverted soybean oil, organoleptic score = 6.5
50	28	124
80	28	735
100	103	3132

TABLE VII

Reproducibility of the Carbonyl Index Method

Experiment	Sample I	Sample II	Sample III
1	251	534	1140
2	256	543	1140
3	258	532	1116
4	254	537	1146
5	255	541	1124

The analytical procedure was found to have a reproducibility of 3% (Table VII). This percentage error corresponded to less than 0.1 of a point in a panel scoring system in which 1 point represented very objectionable oil and 10 points a very good oil. No attempt was made to correlate the carbonyl index with the organoleptic score as the scale used by this panel was not standardized and cross-checked with the panels of other laboratories. Recently Foster (5) pointed out a great need for standardizing the method and scoring system of testing panels. If an elaborate cooperative panel could be set up and a large number of samples could be studied statistically, the carbonyl index may help to establish a standardized scale for evaluating the reverted and rancid odor of edible oils.

Summary

The amount of volatile carbonyl compounds diffused from 100 g. of an oil into a stream of nitrogen bubbled through the oil under specified conditions was determined by converting the carbonyl compounds into their 2,4-dinitrophenylhydrazones and then measuring the absorption of the wine-red color of the quinoidal ions at 480 mμ. From the absorbance thus obtained, a carbonyl index was calculated and assigned to the oil.

The carbonyl indices of a number of edible oils, such as soybean, cottonseed, and hydrogenated vegetable, were found to correlate with the degree of reversion and rancidity of the oils as determined by organoleptic means. The reproducibility of the carbonyl index determination was 3%. This accuracy corresponded to approximately ± 0.1 point in an organoleptic testing panel in which 1 point represented a very unacceptable oil and 10 points a very good oil. The carbonyl index method may also be used as a means of evaluating the flavor stability of edible oils. The carbonyl index gave a good indication of the flavor stability after aging at 60°C. for the less stable and 100°C. for the more stable oils and fats.

REFERENCES

1. Kilgore, L. B., Oil and Soap, *9*, 269 (1932).
2. Lappin, G. R., and Clark, L. C., Anal. Chem., *23*, 541 (1951).
3. Moser, H. A., Jaeger, C. M., Cowan, J. C., and Dutton, H. J., J. Am. Oil Chemists' Soc., *24*, 291 (1947).
4. Handschumaker, E., J. Am. Oil Chemists' Soc., *25*, 54 (1948).
5. Foster, D., Food Tech., *8*, 304 (1954).

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Development of Buckeye Continuous Moisture Meter for Oil-Bearing Materials[1]

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The need for rapid accurate moisture determinations in the oil milling industry has long been recognized. This has been partially met by electric moisture meters which indicate the moisture content on single samples. Their speed and simplicity permit plant operators to make frequent tests and to improve moisture control. However certain disadvantages remain. Mill operators are not always sufficiently careful to take representative samples, operate the instrument carefully, and determine temperature and readings accurately. Obvious advantages would be obtained by a meter installation which would automatically and continuously sample the material and determine and record the moisture content. A continuous record which shows trends is greatly superior to single tests at intervals.

The development of a continuous moisture recorder has paralleled that of the batch type in the Buckeye Cotton Oil Company. Six years were required to carry theory into design and to field-test the instruments until a successful model was evolved. As it is an asset in the processing of oil seeds, the Buckeye Cotton Oil Company wishes to make the meter available to the industry.

Theoretical Considerations

A. *Moisture Detection and Recording*

A continuous recording moisture meter consists of three parts:

1. The detection circuit which measures a change in a characteristic of the material which is dependent upon its moisture content.
2. The test cell through which a stream of the material flows.
3. The recorder which translates the signal from the detection circuit to a chart or graphic record.

Detection Circuit. In developing the batch type meter it was found that the dielectric constant of vegetable oil materials varied with the moisture content, also that it was possible to compensate for other influencing variables as their effects upon the dielectric constant are consistent and measurable.

As the basic considerations in the measurement of the dielectric constant have been discussed in a preceding paper, "Development of the Buckeye Moisture Meter for Use on Oil-Bearing Materials," they will not be repeated.

Test Cell. The primary problem in developing a continuous meter was the design of a test cell through which the sample flows. A cylindrical cell was chosen as it would be self-cleaning and more uniformly filled by a flowing sample. Also the test cell must be a precision electrical condenser; cylindrical design tends to eliminate edge effects. Three types were considered: a) coaxial design consisting of two cylindrical electrodes of different diameter; b) single tube split lengthwise, the two halves forming the two electrodes, being mounted on a non-conducting tube; c) a non-conducting cylinder wrapped spirally with two strips of metal spaced equidistant at all points.

The dimensions of the test cell were calculated for coaxial design and determined experimentally for the other types.

Recording Instrument. The recording instrument must translate the signal from the detection circuit to a graphic record indicating percentage of moisture. There are a number of manufacturers of suitable recorders. The choice depends upon the type and magnitude of the signal, the range, and required speed of response to signal changes.

B. *Compensation for Variables Other Than Moisture.*

Temperature compensation must be built into the circuit of a continuous meter. It was neces-

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[1] This paper covers work carried out by the A. D. Little Company under contract C-57803 in 1947 and 1948 with The Buckeye Cotton Oil Company. It also covers Buckeye investigations carried out since 1930. The paper was presented at the fall meeting of the American Oil Chemists' Society, Cincinnati, O., in October, 1952.