(Patton—*J. Dairy Sci. 33,* 680). In one series of tests by Zollikofer & Fuchs (*Proc. 12th Internat'l. Dairy Congr. 2,* 634) on the butter fat hydrolyzing action of nine microorganisms the hydrolysis varied from 0.4 to 8.7%. Various organisms liberated various mixtures of fatty acids from the butter fat.

Burgoyne & Thomas (*J. Soc. Chem. Ind. 68,* 300), as part of a study on decomposition of palm kernel oil by mold, determined the hygroscopic equilibra between methyl bromide gas and palm kernels, jute sacking contaminated with palm kernel fat, and uncontaminated sacking of the same type. The methyl bromide gas was used as a sterilizing agent. Only jute bags absorbed moisture more readily above 71% humidity and were more prone to microorganism attack than uncontaminated bags.

A method of preservation of suet comprised immersion in a solution of acetic acid and sodium acetate in respective concentrations of 10.5 ml. and 12.3 g. per liter (Francois & Sergent—*Ann. nutrition et aliment. 3,* 441). The preservation was further improved by adding 5 g. of potassium dichromate to the solution (Francois & Sergent—*Bull. mens. ITERG 4,* 151).

DECOMPOSITION BY ELECTRICAL DISCHARGE. Menzel *et al.* (*Chem. Ber. 82,* 418) recorded the reaction taking place on exposure of fatty materials to electric discharges. Stearic acid was converted to an unsaturated acid, and some moisture was split off and this corresponded to a decrease in the acid number. Methyl oleate subjected to electric-glow discharges in hydrogen atmosphere was hydrogenated, and some acidity developed. In nitrogen atmosphere the change in iodine value was less but more acidity developed. Splitting, condensation, and polymerization were also evident in the processes.

Estimation of Monocarbonyl Compounds in Rancid Foods[1]

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A METHOD has been developed for the determination of monocarbonyl compounds in the benzene-soluble fraction of rancid foods. The determination involves the formation of the 2,4-dinitrophenylhydrazones of monocarbonyl compounds in benzene solution, removal of unreacted 2,4-dinitrophenylhydrazine reagent and any hydrazones of dicarbonyl compounds with alumina, and colorimetric measurement of the remaining hydrazones in alkaline solution.

Since monocarbonyl compounds (aldehydes) follow as secondary reaction products of the hydroperoxides initially formed in fat oxidative deterioration and contribute greatly to the off odors and flavors in rancid food (11, 17), there is, of course, a definite need for a convenient and reliable method for the estimation of these compounds. At the low levels of aldehydes found in rancid fats, previously available methods, such as differential titrations with sodium bisulfite (5) or hydroxylamine (6), or quantitative modifications of the Schiff test (2, 8, 13, 18), have failed to yield consistent results and occasionally have given high results with fresh foods.

2,4-Dinitrophenylhydrazine has been used in aqueous solution for the gravimetric determination of aldehydes and ketones (3) and in the colorimetric determination of dicarbonyl compounds (9, 10). Chromatographic separations of various aldehydes and ketones as their 2,4-dinitrophenylhydrazones have been reported (1, 7, 12, 14, 16, 19). No account of the use of this compound as a colorimetric reagent for simple aldehydes and ketones has been noted in the literature. The method as described below is applicable to many aldehydes, and particularly to the aliphatic, saturated or unsaturated, aldehydes which arise in fat deterioration.

Reagents and Apparatus.[3] a) Dissolve 500 mg. of 2,4-dinitrophenylhydrazine in 1 liter of benzene by heating gently and shaking occasionally. b) Dissolve 60 g. of potassium hydroxide in 1 liter of 99% aldehyde-free ethyl alcohol (15) and filter through a fritted-glass funnel or glass wool. The alcohol may be used without purification if the reagent is made up fresh daily. c) Activated alumina, F-20 grade, 80-200 mesh supplied by the Aluminum Ore Company, East St. Louis, Ill., was used throughout this work. It is too active as received and must be modified by mixing with 15% of fully hydrated material prepared by exposing the alumina in a thin layer to water vapor in a vacuum desiccator. Allow the mixed alumina to stand in a closed container several hours before using. If kept in an air-tight container, this mixed alumina is stable indefinitely. d) Chromatograph tubes, 7 mm. inside diameter, 110 mm. long, with a short piece of 1-mm. capillary tubing sealed to the lower end and a 110-mm. length of 10-mm. tubing to the upper end. Insert a small loose plug of glass wool in the upper end of the capillary section to retain the alumina column. e) Twenty-five-ml. glass-stoppered graduated cylinders. f) Photoelectric colorimeter for measurements at 435 mμ. A Coleman photoelectric colorimeter was used in these studies.

Method of Analysis. Prepare the chromatograph column by pouring in alumina to a depth of 3 cm. Pipette in 10 ml. of the dinitrophenylhydrazine reagent and immediately sprinkle in sufficient additional alumina to make the total depth 10 to 11 cm. After all of the reagent solution has entered the top of the alumina column, add 5 ml. of fresh benzene. When all of this has entered the column, add the sample (containing the equivalent of 0.05-0.50 micromole of aldehyde) dissolved in 3-4 ml. of benzene and begin collection of the solution issuing from the column in

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[3] Use of commercial names does not imply recommendation by the Department of Agriculture over other products of similar nature not mentioned.

a 25-ml. graduated cylinder. Add fresh benzene to the column until 19 ml. have been collected. Dilute to 25 ml. with alcoholic KOH, mix, and read the absorbancy [4] at 435 mμ immediately. The solution of dinitrophenylhydrazones in benzene is stable indefinitely, but the red color of the alkaline solution fades at a rate of 0.5% to 0.6% per minute. The importance of a uniform minimum delay in reading is apparent. A blank should be run with each series of determinations. Dilute the blank benzene solution, after passage through the alumina column, to 25 ml. with alcoholic potassium hydroxide and use for the comparison solution in setting the colorimeter to zero absorbancy (100% transmittance), thus automatically correcting for the small amount of carbonyl found in the usual reagent grade of benzene.

The simple procedure of carrying out the reaction between aldehyde and hydrazine on the alumina, in contrast to reaction in benzene solution followed by passage through the column, was adopted because it gave more nearly quantitative recovery of known aldehydes.

In the preparation of samples for analysis, fats and oils are easily dissolved in benzene and the appropriate aliquots used. Solid foods (e.g., potato chips) are ground as finely as is practical in a mortar or a mill. One- to two-gram samples are weighed into small beakers, benzene is added with stirring, and the solid plus benzene extract are transferred to the space above the alumina in the chromatograph tube with several small portions of benzene. The remainder of the procedure is the same as for fats and oils.

Standardization and Evaluation of Method. In order to express the absorbancies obtained by this method in terms of moles of carbonyl compounds or in terms of equivalent amounts of some known stable monocarbonyl compound, the spectral absorption of the 2,4-dinitrophenylhydrazones of a wide range of monocarbonyl compounds was measured. The 2,4-dinitrophenylhydrazones of butyraldehyde, isovaleraldehyde, heptaldehyde, nonylaldehyde, undecylaldehyde, crotonaldehyde, 2-decenaldehyde, and acetone were prepared and recrystallized several times. Various concentrations of these hydrazones in benzene solution were treated with alcoholic potassium hydroxide as described in the above method and the absorbancy at 435 mμ determined. The resulting data, which are plotted in Figure 1, demonstrate the extreme dilution at which these substances will yield a measurable color, the satisfactory adherence to Beer's law over a considerable range of concentrations, and the relative constancy of the molar absorbancy index with change in the size and type of the carbonyl moiety. The molar absorbancy index calculated from the regression line for the saturated aldehydes is 19,200. The factor relating absorbancies to concentrations of hydrazones (and hence monocarbonyl compounds) will depend on cell thickness and spectral band width. With a Coleman photoelectric colorimeter (Model 11) and 1.3-cm. cells, the absorbancy is taken to be equal to the number of equivalent micromoles of saturated aliphatic aldehyde per 25 ml. of final volume of the test solution. This equality is based on a standard average value for the molar absorbancy index of the hydrazones

[4] Terminology of the National Bureau of Standards, as given in its circular 484, "Spectrophotometry," is used.

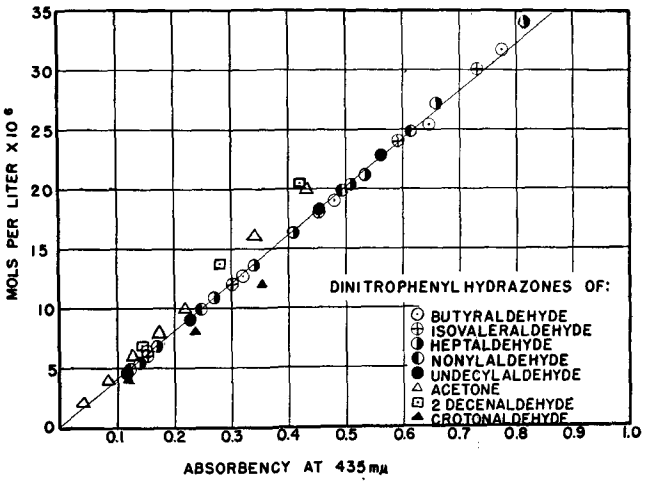

FIG. 1.

of 19,200, with which the saturated aldehyde hydrazones agree quite closely, but from which hydrazones of certain unsaturated aldehydes and acetone show appreciable deviations. Since a complete qualitative determination of the carbonyl compounds in rancid fats has not yet been accomplished, it is impossible to determine the error introduced by these deviations. However spectral absorption curves, in the visible region, of hydrazones of the total aldehydes in rancid fat closely resembled curves for the saturated aldehyde-hydrazones and differed markedly from the curves of unsaturated-aldehyde-hydrazones. This suggests a proportionately small contribution by the unsaturated aldehydes and a correspondingly small error.

The spectral absorption curves over the range 400 to 500 mμ of the alkaline solution of the dinitrophenylhydrazones of heptaldehyde and of the carbonyl compounds isolated from rancid turkey fat are shown in Figure 2. The absorption maximum is in the region of 430 to 435. The measurements reported here were made at 435 mμ, which is near the maximum of the curve and at a point where the instrument sensitivity is slightly greater than at shorter wavelengths.

The specificity of the method for monocarbonyl compounds was demonstrated by recovery experiments on known amounts of heptaldehyde, with and

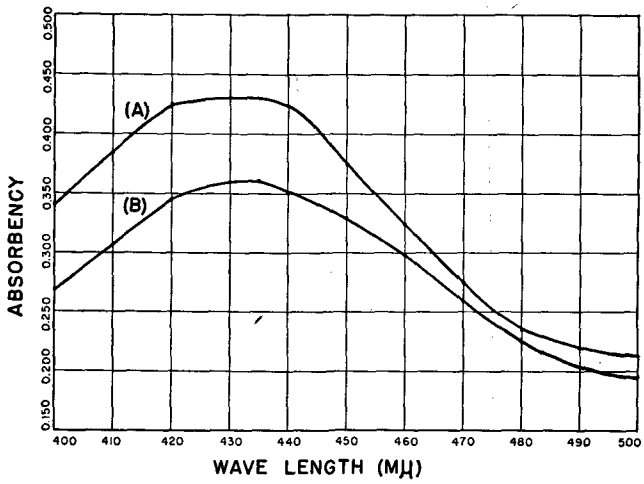

FIG. 2. Spectral absorption curve of the alkaline solution of the dinitrophenylhydrazones of (A) heptaldehyde (B) carbonyl compounds isolated from rancid turkey fat.

without dicarbonyl compounds present. As shown in Table I, there was essentially no interference by

TABLE I

Non-Interference by Dicarbonyls

Added				Found
Heptaldehyde (micrograms)	Diacetyl (micrograms)	Acetyl benzoyl (micrograms)	Fat (grams)	Calc. as heptaldehyde (micrograms)
30.7	.....	.....	.....	30.0
30.7	25.4	.....	.....	30.2
30.7	.....	.....	1.0 (fresh)	35.8
30.7	25.4	.....	1.0 (fresh)	33.8
.....	25.4	.....	.....	1.2
46.0	.....	.....	.....	44.2
46.0	38.2	.....	.....	44.2
.....	.....	.....	1.0 (rancid)	35.3
.....	.....	14.8	1.0 (rancid)	34.2
.....	.....	14.8	.....	0.0

diacetyl or acetylbenzoyl. Similar results were obtained with less pure preparations of glyoxal. The effect of a double bond in the 2-position was tested in two pairs of aldehydes—butyraldehyde-crotonaldehyde and 2-decen-1-al—undecanal. As indicated in Table II, both saturated and unsaturated aldehyde-hydrazones passed through the alumina and were determined with quantitative recovery.

TABLE II

Recovery of 2,4-Dinitrophenylhydrazones of Saturated and Unsaturated Aldehydes

Aldehyde	Determination of 2,4-dintrophenyl-hydrazones of aldehydes (absorbency units)	
	Before adsorption step	After adsorption step
2-Decen-1-al	0.141 (2)[a]	0.141 (2)
Undecanal	0.111 (2)	0.110 (2)
2-Decen-1-al + undecanal	0.258 (2)	0.255 (2)
Crotonaldehyde	0.182 (3)	0.174 (3)
Butyraldehyde	0.148 (3)	0.149 (3)
Crotonaldehyde + butyraldehyde	0.340 (3)	0.335 (3)

[a] Number in parentheses indicates number of replicates run.

The reproducibility and quantitative nature of the method were established by analyzing standard solutions of heptaldehyde prepared from freshly distilled heptaldehyde assayed by the method of Iddles, *et al.* (4). Results appear in Table III.

If the overall adsorption strength of the alumina is increased very much over that of the recommended material (15% hydrated), the recovery falls, and there is a slightly greater loss in the case of the unsaturated aldehydes. At a 10% level of hydrated material the decrease is appreciable. Care should therefore be taken in adjusting the adsorption strength of the alumina, and it should be checked with standard solutions of 2,4-dinitrophenylhydrazones. The 2,4-dinitrophenylhydrazone of cinnamaldehyde is useful for this purpose since it is more strongly adsorbed than most of the compounds to be determined. Twenty-five to 50 micrograms of the substance dissolved in 1 or 2 ml. of benzene are added to the top of a column prepared in the regular way and eluted until the volume collected totals 19 ml. This solution is made alkaline, read in a colorimeter, and the reading compared with that of the same weight of the material made to the same volume but not chromatographed. Alumina, deactivated to the extent that quantitative recovery of cinnamaldehydedinitrophenylhydrazone and its separation from dinitrophenylhydrazene is permitted, is of about optimum activity for use in this method.

TABLE III

Recovery of Known Amounts of Heptaldehyde

Heptaldehyde added (micrograms)	Heptaldehyde found (micrograms)	% Recovered
19.1	18.9	99
	19.9	104
38.2	38.8	101
	38.9	102
	38.9	102
	38.8	101
	38.0	99
57.3	56.0	98
	58.2	101
	56.0	98
0.0+1.0 g. fat	≅12.0	....
38.2+1.0 g. fat	50.8	(Net) 101
	49.7	(Net) 99

Application of the method has been made to rancid poultry fat and rancid potato chips, peanuts, and crackers. Some illustrative results are given in Table IV. The carbonyl contents of rancid foods so far tested do not show any consistent correlation with peroxide contents, the ratio apparently being dependent on the stage of oxidative deterioration and the conditions under which the deterioration took place.

The extent to which these mono-carbonyl determinations correlate with organoleptically determined rancidity is under investigation. An accurate, simple method for determining the gross aldehydic content in rancid foods might well be superior to the peroxide test for chemical tests of fat deterioration since aldehydes contribute considerably more to the off odors and flavors present in rancid fat than do the relatively non-volatile peroxides.

TABLE IV

Illustrative Data on Monocarbonyl Determinations in Rancid Foods

Sample		Monocarbonyl compounds,[a] millimols/kg.		
Turkey fats Stored 1 yr., —30°F.	(1	0.05	0.06	0.05
	(2	0.14		
Turkey fats of Varying stabilities Stored 6 mo., 0°F.	(3	0.42	0.43	0.47
	(4	0.54	0.59	0.59
	(5	7.34	.....	.....
Potato chips[b]				
Stored 0 days		0.15		0.15
Stored 4 days		0.22		0.21
Stored 6 days		0.29		0.29
Stored 7 days		0.90		0.87
Crackers[b]				
Stored 0 days		0.06		0.06
Stored 9 days		0.07		0.07
Stored 21 days		0.22		0.22
Peanuts[b]				
Stored 0 days		0.16		0.16
Stored 4 days		0.17		0.17
Stored 9 days		0.24		0.20

[a] Peroxide values, millimoles/kg.: 1, 0.0; 2, 4.4; 3, 10.9; 4, 19.5; 5, 240.0.

[b] Storage at room temperature (Ca 23°C.)

Summary

A convenient method for the determination of monocarbonyl compounds in rancid foods is described. The quantitative procedure is based on the formation of the 2,4-dinitrophenylhydrazones of monocarbonyl compounds in benzene solution, the removal of excess hydrazine reagent and the hydrazones of dicarbonyl compounds with alumina, and the colorimetric determination of the remaining hydrazones of monocarbonyl compounds in alkaline solution. Applicability to aldehydes varying in molecular size and degree of unsaturation has been demonstrated. The method may be used on the crude benzene extracts of rancid foods. Illustrative data are presented.

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The Equilibrium Moisture Content of Tung Fruit and Its Components at Different Relative Humidities

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THE drying of tung fruit and seeds so that they can be milled or stored with safety has been an important problem of the tung oil industry. The tung fruit falling from the trees contains about 65% moisture and will heat, mold, and sprout if stored before being dried to about 25% moisture. Since the hulls contain more than half of the moisture present in the whole fruit, their removal before drying or storage is desirable to minimize the heat required for drying and to reduce the space required for storage. However it has been found that the moist, broken seed produced by the usual commercial hulling operation will develop free fatty acids rapidly and heat spontaneously unless dried to about 10% moisture.

Tung fruit and its products tend to assume a moisture content in equilibrium with that of the surrounding atmosphere, hence knowledge of their equilibrium moisture content at different relative humidities is of considerable importance in the drying and storage of these products. Also, in the spontaneous heating of ground hulls and press cake, equilibrium moisture contents are important because it is believed by certain workers in this field that the relative humidity of the interstitial air determines whether mold growth and heating takes place or not, rather than the moisture content of the material *per se*.

Holmes and Pack (1) determined the equilibrium moisture contents of tung seeds and kernels at different relative humidities for two different temperatures, but the temperatures could not be controlled accurately.

The following experimental work was carried out by placing samples of the test materials in desiccators containing saturated solutions of different salts and storing them in a room held constant at 25° C., using essentially the same equipment and procedures described by Karon and co-workers (2, 3) in determining the hygroscopic equilibrium of rice and peanuts. The nine salts used and the relative humidities maintained by their saturated solutions at 25° C. are given at the top of Table I. These values are taken from Technical Report No. 40 of the American Paper and Pulp Association (4).

Outer hulls, inner hulls, shells, kernels, intact seeds, press cake, and whole fruit were used in these experiments. A batch of fresh tung fruit was divided by hand into outer hulls, inner hulls, shells, and kernels. The seeds (composed of kernel plus shell) and whole fruit were from the same batch of fruit after it had been dried to the equilibrium moisture content at the prevailing humidity of the laboratory. Samples of each material were placed in trays with wire screen false bottoms, made to fit the desiccators and divided radially into four compartments. The experiments on the outer hulls, inner hulls, shells, and kernels were carried on simultaneously in the same desiccators; those on the seeds and press cake were conducted simultaneously; but those on the whole fruit were made alone.

Before placing the materials in the desiccators, the hulls, shells, and press cake were ground in a Wiley mill to pass a 0.25-inch screen. Because tung oil oxidizes rapidly and the materials had to be stored for several weeks, the kernels, seeds, and fruit (all products high in oil content) were used without grinding to minimize oxidization.

In addition to the material contained in trays in the desiccators, small baskets made of very fine screens were filled with the same materials (except whole fruit), placed in the desiccators, and weighed every few days. From the changes in weight and the original moisture content of samples, the approximate moisture content could be calculated as a check on the establishment of equilibrium.

For the determination of moisture in the hull, shell, and press cake, portions of about five grams each were rapidly transferred to a previously weighed moisture dish. The dish was immediately covered and weighed, then with the cover removed was dried to

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