Quantification of Carbonyl Compounds in Oxidized Fats as Trichlorophenylhydrazones[1]

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ABSTRACT

A rapid determination of the carbonyl compounds in oxidized fats and oils was devised based on formation of trichlorophenylhydrazones (TCPH). Fat in cyclohexane/ether (99:1) was passed through a Florisil column to remove interfering hydrocarbons, and the fat and carbonyl compounds were eluted with ether. The eluate was reacted with trichlorophenylhydrazine with Florisil as a catalyst, and the TCPH were isolated from the fat by chromatography on a Florisil column. The TCPH were separated and quantified by gas chromatography on a 10-m SE 30 capillary column. The method was applied to soybean oils stored under various conditions. The method also was applied to linoleic acid hydroperoxide to determine the extent to which decomposition of peroxides gave rise to carbonyl artifacts during the procedure. Although artifacts were minimal, they were reduced even further by passing the fat through a column of Celite impregnated with stannous chloride to reduce hydroperoxides before the carbonyl analysis.

INTRODUCTION

Autoxidation of unsaturated fatty acids produces a variety of scission products that have been implicated in off-flavor development (1). Most of these are carbonyl compounds which may be quantified as hydrazones, especially as 2,4-dinitrophenylhydrazones (DNPH), and a number of procedures have been suggested (2-7). A recurring problem in this kind of analysis is the possibility that during the preparation and isolation of the DNPH from the fat, hydroperoxide breakdown will occur and cause the formation of additional amounts or kinds of DNPH (7-10). Attempts have been made to avoid this problem by prior reduction of the hydroperoxides (11,12), reaction at low temperature (13), and the use of DNPH-phosphoric acid reaction columns (5), but it is not certain that any of these methods are effective in avoiding artifacts. In addition, these methods require a long time to complete. They tend to be rather insensitive and their sensitivity is frequently limited by the problem of preparing solvents sufficiently free of interfering carbonyls. The DNPH usually are made in polar solvents which prevents the quantitative formation of the DNPH of compounds such as vinyl ketones. These ketones have been implicated in the flavors of oxidized milk fat and soybean oil (14).

An alternative procedure for the quantification of carbonyl compounds uses 2,4,6-trichlorophenylhydrazine (15). Both DNPH and 2,4,6-trichlorophenylhydrazones (TCPH) can be quantified by direct gas chromatography with a packed column, but poor resolution and double peaks remain problems (16-18). Recently, a 10-m capillary column coated with SE-30 has been used for the quantification of TCPH (19). Rapid results, elimination of double peaks, and sensitivity to nanogram amounts were reported. For this method to be used in analyses of fat oxidation, a way to separate the TCPH from the fat had to be developed. This was accomplished in the following study.

METHODS

For purifying cyclohexane, Florisil (60-100 mesh, Floridin Co.) was activated at 300 C overnight. For column chromatography, Florisil was activated at 250 C overnight, and 10% of its weight of water was added and allowed to equilibrate for 24 hr.

Silica Gel H for thin layer chromatography (TLC) was cleaned before plate preparation by slurrying 100 g of silica gel with 200 mL distilled water. The silica gel was filtered through a Buchner funnel and air was pulled through it until fairly dry. The silica gel was then washed twice with 200 mL methanol and three times with 200 mL of distilled ether. The silica gel was dried until no odor of ether was detectable. Cyclohexane was purified by slow distillation through a 100-cm column of porcelain saddles to remove high boiling impurities. A variable take-off head was set for 50% collection and 50% reflux. It was then passed through a column of activated Florisil (ca. 30 mL/g) to remove carbonyl impurities. The column was shielded from light to minimize photoxidation. Ether was reacted overnight with $LiAlH_4$ and distilled fresh each day. Tetrachloroethane was purified by heating with lauroyl peroxide at 100 C for 1 hr, distillation at reduced pressure, and a second distillation at reduced pressure from 1,5-diphenylcarbohydrazide. Ethanol was refluxed with magnesium turnings and a trace of iodine and distilled.

To purify the trichlorophenylhydrazine, 5 g was dissolved in 100 mL warm ethanol. Charcoal was added to remove the yellow color, and the charcoal was removed by hot filtration through a Buchner funnel. The charcoal addition and hot filtration was repeated. The filtrate was reduced to 20 mL, cooled to room temperature, and the trichlorophenylhydrazine crystals were removed by filtration.

Crude soybean oil was obtained from Anderson Clayton and Company. It was deodorized using an apparatus similar to that described by Schwab and Dutton (20). The oil was deodorized for 1.3 hr at 235 C.

The TCPH were analyzed on a Varian Aerograph Series 1520 gas chromatograph equipped with a hydrogen flame detector and modified for use with capillary columns. Glass capillary columns (10-m) were coated with SE-30 (Supelco, Bellefonte, PA). The column temperature was programmed from 40 to 250 at 10 C/min. Representative samples and model compounds were also analyzed on a Finnigan Model 400 GC-MS to aid in the identification of some compounds. The ionizing voltage was 70 eV.

Linoleic acid hydroperoxide was produced using the lipoxygenase procedure of Gardner (21), except Tween 20 was omitted to avoid excessive foaming. Linoleic acid and soy lipoxygenase were obtained from Sigma Chemical Company (St. Louis, MO). An aliquot of the linoleic acid hydroperoxide mixture equivalent to 6 μmol was streaked on a 0.25-mm silica gel plate. After development, the linoleic acid hydroperoxide was identified by using a spray containing ammonium thiocyanate and ferrous sulfate, as described by Gunstone et al. (22).

To reduce hydroperoxides in the samples tested, two reducing columns were developed. The first was based on the use of $SnCl_2$ as described by Egerton et al. (23). The sample to be reduced was passed through an 11-mm id × 26-cm column containing 4 g Celite impregnated with 1 g $SnCl_2$ in 2 mL 0.1 N NaOH. The second method was devised using the reagents from the AOCS Official Method for peroxide value (PV) (24). Column dimensions were the same as for the first procedure; however, a two-tiered pack-

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ing was employed. Ca. 4 g Celite impregnated with 1 mL saturated KI and 1 mL 85% H_3PO_4 was packed over 2 g Celite impregnated with 1 mL 1 N $Na_2S_2O_3$. The thiosulfate reduced the iodine produced in the reduction of hydroperoxides. Removal of residual HI in the sample after reduction was necessary if accurate PV determinations were to be made. In this case, the sample was collected in a tube containing 2 mL of a 10% solution of Na_2CO_3. The addition of Na_2SO_4 and centrifugation removed the added water from the sample. With both reduction procedures, suction was used to pull the oil through the column. Ca. 10–12 g of oil could be reduced by each column. Both methods were checked for complete reduction by determining the peroxide value immediately after reduction (24).

The following method was developed for the quantification of carbonyl compounds from fat. To remove interfering hydrocarbons in soybean oil, ca. 0.3 g fat and 1.2 μg of 2-pentanone internal standard were applied to an 11-mm id × 33-cm column filled with 10 g Florisil in cyclohexane. Hydrocarbons which represent only ca. 0.002% of the oil, but which interfere with the gas chromotography of the TCPH were eluted with 80 mL cyclohexane/ether (99:1). Pure ether was then applied to the column. The first 10 mL of eluate was discarded and the next 18 mL collected. The remaining lipid constituents, including the carbonyls, were present in this fraction. To form TCPH from the carbonyls, the ether eluate was placed in a 50-mL round-bottom flask with 0.1 g trichlorophenylhydrazine and 0.5 g Florisil. Florisil catalyzed the reaction, allowing immediate derivatization of the carbonyls with no added acid. The ether was evaporated in a rotary evaporator at a temperature below 25 C to avoid thermal breakdown of hydroperoxides. To separate the TCPH from the lipids, the dry Florisil-TCPH-lipid mixture was slurried with cyclohexane and packed on top of a column containing 9.5 g Florisil. The column was washed with 30 mL cyclohexane/ether (99:1) which was discarded, and the TCPH were collected in the next 45 mL. The solvent containing the derivatives was concentrated to ca. 3 mL by rotary evaporation and transferred to a centrifuge tube where it was evaporated to 50 μL under nitrogen in a 30 C water bath. A 1-μL sample was injected into the GC. For quantification, sample peak heights were compared with an internal standard, 2-pentanone. For identification, retention times were compared with those of known compounds. Because the TCPH are unstable, a series of 2-ketone-TCPH served as working standards. The 2-ketone-TCPH were made fresh at least weekly. Various aldehydes and vinyl ketones were tested, and their retention times recorded in relation to the 2-ketones.

Peroxide values of oil samples were determined by the method of Hamm et al. (25).

RESULTS AND DISCUSSION

The TCPH method for analysis of carbonyls was based on the procedure of Hammond et al. (19) in which a Celite column impregnated with trichlorophenylhydrazine in phosphoric acid was used as a reaction column to convert carbonyls into TCPH. Recovery of the TCPH from the Florisil columns was tested using a series of 2-ketones, 2-propenal (acrolein), and 2-*trans*-butenal (crotonaldehyde) as standards. During these tests, we discovered that Florisil would catalyze the formation of TCPH and that this method was simpler and gave more complete recovery of the standards than the Celite-trichlorophenylhydrazine column of Hammond et al. Elimination of the Celite-trichlorophenylhydrazine column removed acid and water from the derivatization step, which may reduce artifact formation. Recovery was determined by measuring GC responses for

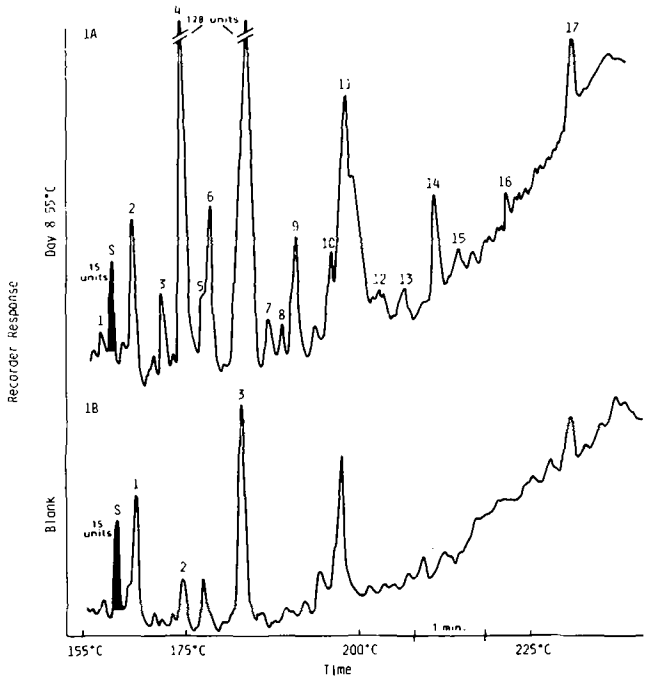

FIG. 1. (A) Chromatogram of TCPH obtained from soybean oil oxidized for 8 days at 55 C. Ten-m SE-30 capillary column, attenuator 16, electrometer 10^{-12}. Peak numbers are the same as in Table I. Peak S is pentanone, the internal standard. (B) Chromatogram of a solvent blank. Conditions same as in Fig. 1A. Peak S is pentanone, the internal standard.

three different amounts (1, 3 and 9 μg) of model compounds. To minimize measurement errors and differences due to individual GC runs, a 2-pentanone-TCPH preparation was used as an internal standard. The recovery of TCPH was linear with the amount introduced and ranged from 84 to 100% with an average of 93%.

A chromatogram of a typical blank, prepared as one would a fat sample, but without the addition of fat, appears in Figure 1B. Peak S is the internal standard, 2-pentanone. Peak 3 was identified by GC-MS as either 2-cyclohexenone-TCPH or 1-formylcyclopent-2-en-TCPH.

Peaks 1 and 2 are probably pentanal and hexanal from the solvent. Such blanks are quite reproducible in background, but the amount of peak 3 varies. When fat alone, without TCPH, was subjected to the procedure, little contamination was apparent; however, one peak at ca. 169 C appeared in the fat blank. This was identified by GC-MS as a long-chain ester or hydrocarbon having a MW of 358-360. It is shown in Figure 1A between peaks 2 and 3.

A chromatogram of a typically oxidized oil (55 C, day 8) appears in Figure 1A. Again, peak S is the internal standard, 2-pentanone. The size of the peaks in comparison with the peaks in the blank should be noted. Peaks eluting earlier than butanal tended to be obscured by large impurity peaks in the blank, which arise from the ether. They are not represented in Figure 1A or 1B. Fortunately, short-chain carbonyl compounds have high flavor thresholds and probably contribute little to the flavor of oxidized oils.

This method was sensitive to ca. 0.1 ppm (1×10^{-7} g) of carbonyl in the fat. This amount is represented by a peak 0.025 the height of peak S in Figure 1B. Below this level, impurities from the blank tended to overwhelm the compounds being measured. Better purification of the solvents and reagents should result in even better sensitivity. The method reported here for purifying cyclohexane is much better at removing carbonyls than previously reported methods based on DNPH (26) and H_2SO_4 (27) reaction columns. Shielding the Florisil column from light during

TABLE I

Amounts[a] and Identifications of Carbonyls Produced from Soybean Oil Stored under Various Conditions

Peak no.	Identification	55 C Storage PV=0.24 Day 0	PV=1.40 Day 3	PV=9.27 Day 5	PV=16.34 Day 8	PV=30.44 Day 10	30 C Storage PV=15.82 Day 44	Light-oxidized PV=15.45 Day 8
1	Butanal	0	0	1.74	1.65	0.39	6.33	1.55
2	Pentanal	0	0	0.16	3.52	3.65	17.60	5.75
3	2-*trans*-Pentenal	0.32	0.97	2.20	5.17	2.00	5.98	3.39
4[b]	Hexanal	0	2.42	34.56	44.90	55.49	33.75	26.74
5	Unknown	0	0	3.07	2.91	6.36	2.87	0
6[b]	2-*trans*-(or 3-*cis*-)Hexenal	0.42	1.20	6.88	9.85	10.56	11.37	5.14
7	2-*trans*-4-*trans*-Hexadienal	0.39	0.42	1.10	1.30	1.34	6.01	4.75
8	Octanal	0.29	0.94	2.03	2.03	2.29	15.83	1.29
9	2-*trans*-Heptenal	0.29	0.61	3.46	5.49	8.04	4.33	10.27
10	2-*trans*-4-*trans*-Heptadienal	0	0	0.90	1.62	3.52	4.01	1.20
11	Nonanal or 2-*trans*-octenal	0	0	6.36	10.30	10.63	6.72	5.14
12	2-*trans*-4-*trans*-Octadienal	0	0	0.81	1.07	1.32	9.95	1.74
13	Decanal or 2-*trans*-nonenal	0.23	0.39	1.10	1.59	2.26	2.23	1.55
14	2-*trans*-4-*trans*-Nonadienal	0	0	2.91	6.85	1.68	3.33	8.72
15	2-*trans*-Decenal	0	0	0	0.42	0.84	2.20	0.90
16	2-*trans*-4-Decadienal	0.16	0.14	0.48	0.73	0.81	5.04	0.90
17	2-*trans*-4-*trans*-Dodecadienal	0	0	1.13	1.78	2.10	15.12	3.30

[a]Amounts listed as μg of TCPH-derivative found in 1 g oil.
[b]It has not been established whether 3-*cis*-hexenal would rearrange to 2-*trans*-hexenal in this procedure. If not, 3-*cis*-hexenal TCPH probably would migrate with the TCPH of hexanal.

cyclohexane purification drastically reduced the amount of peak 3 in the blank; however, we have not been able to reduce impurities further. Our results suggest that the background in Figure 1B may represent carbonyls formed by reaction of the solvents with Florisil during the chromatographic separations. Minimizing the amount of Florisil minimizes the blank background. Attempts to improve the Florisil by various heat and reduction treatments were ineffective.

In this study, deodorized soybean oil was analyzed at various stages of oxidation during storage at 55 C in the dark. In addition, oil was oxidized at 30 C in the dark for 44 days and was oxidized at room temperature (25 C) for 8 days under a fluorescent light. Amounts representing the average of duplicate runs, and tentative identifications of carbonyls produced under these conditions appear in Table I. The PV of the oil, as measured by the Stamm test (25) also is listed for each treatment. The amounts of any interfering compounds found in the fat or solvent-trichlorophenylhydrazine blanks have been substracted from the amounts appearing in Table I.

GC-MS was helpful in positively identifying butanal, hexanal, 2-*trans*-(or 3-*cis*-)hexenal, and 2-*trans*-heptenal. Mass spectral data for these compounds are given in Table II. Carbonyl-TCPH larger than C_7 gave no response in the GC-MS analyses, so the identity of compounds having a greater MW than this could not be varified by GC-MS.

Injection of TCPH standards revealed incomplete resolution of 2-*trans*-octenal and nonanal, as well as pent-1-en-3-one and pentanone. Possibly, overlapping peaks of other carbonyls occurred. A longer GC capillary column might result in better resolution.

Many of the carbonyl compounds found in the oxidizing soybean oil in this test were those that had been identified

TABLE III

Amounts[a] and Identifications of Carbonyls Present in Linoleic Acid Hydroperoxides Using Reduced and Unreduced TCPH-Florisil Procedures

Peak no.	Identification[b]	Unreduced	Reduced by $SnCl_2$	Reduced by HI	Blank unreduced[c]
1	Butanal	1.12	0.00	0.00	0.99
2	Pentanal	0.39	0.26	0.28	0.87
3	2-*trans*-Pentenal	1.16	9.74	0.61	0.00
4[d]	Hexanal	1.97	0.36	1.97	0.00
5[d]	2-*trans*-(or 3-*cis*-)Hexenal	0.31	0.19	0.00	0.00
6	Unknown	0.00	0.00	1.34	0.00
7	2-*trans*-Heptenal	0.00	0.05	0.08	0.00
8	2-*trans*-4-*trans*-Heptadienal	0.00	0.11	0.01	0.00
9	Unknown	0.00	0.00	0.05	0.00
10	2-*trans*-4-*trans*-Octadienal	0.00	0.00	0.03	0.00
11	Decanal	0.28	0.00	0.00	0.00
12	2-*trans*-Nonenal	0.00	0.43	1.65	0.00
13	2-*trans*-4-*trans*-Nonadienal	3.18	0.05	0.17	0.00
14	2-*trans*-4-*trans*-Decadienal	0.00	0.15	0.17	0.00

[a]Amounts expressed as μg of TCPH-derivatives found in 6 μmol peroxide.
[b]Compounds larger than C_7 were tentatively identified by relative retention times. Smaller compounds were identified by GC-MS.
[c]Blank made as in the unreduced sample except linoleic acid was omitted.
[d]It has not been established whether 3-*cis*-hexenal would rearrange to 2-*trans*-hexenal in this procedure. If not, 3-*cis*-hexenal TCPH probably would migrate with the TCPH of hexanal.

by previous workers, either from oxidized soybean oil or from oxidized fatty acids commonly found in soybean oil (1,28). The rapid increase in amount of hexanal should be noted during 55 C storage. The green plant flavor, often associated with oxidizing soybean oil, has been attributed to its presence (29). Peak 6, possibly 2-*trans*-or 3-*cis*-hexenal, grew as oxidation at 55 C progressed. 3-*cis*-Hexenal has been said to be responsible for the "green-beany" flavor in autoxidized soybean oil (30). Oil stored at 30 C and light-oxidized oil also contained significant amounts of hexanal and peak 6. The detection of oct-1-en-3-one, although possible by the TCPH method, was not noted. When the standard, pentanone, was omitted from the samples, no peak appeared at that location, indicating that there also was no pent-1-en-3-one. It was mentioned earlier that pent-1-en-3-one and pentanone are incompletely resolved. Peak 5, unknown, and peak 11 (nonanal or 2-*trans*-octenal) grew steadily as oxidation at 55 C proceeded. Present in small to moderate amounts in the oil stored at 55 C were compounds identified as pentanal, 2-*trans*-4-*trans*-hexadienal, octanal, 2-*trans*-4-*trans*-heptadienal, 2-*trans*-4-*trans*-octadienal, 2-*trans*-nonenal or decanal, 2-*trans*-4-*trans*-nonadienal and 2-*trans*-4-*trans*-decadienal. Overall, increases in peak number and size can be noted as oxidation at 55 C proceeded. The PV generally increased as the peak sizes increased.

Similar kinds of carbonyls were produced during 55 C oxidation, 30 C oxidation, and light-oxidation, but the amounts of a carbonyl produced differed. Similarly, in a study in which pure hydroperoxides were decomposed, Frankel et al. (31) found that the same major volatile products were formed from autoxidized esters and from photosensitized oxidized esters, although in different relative amounts.

The PV was not a good indicator of peak size. For example, oxidation in the dark at 30 C for 44 days and at 55 C for 8 days and oxidation in the light gave comparable PV and produced several peaks of considerable size, yet the amounts of these peaks differed. The oil oxidized at 30 C generally had larger peaks than the light-oxidized sample and the oil stored at 55 C. The unknown peak 5 was not produced by oxidation in the light.

The sample oxidized at 30 C contained large amounts of several carbonyls compared with 55 C oxidation at similar PV, namely, butanal, pentanal, 2-*trans*-4-*trans*-hexadienal, octanal, 2-*trans*-4-*trans*-heptadienal, 2-*trans*-4-*trans*-octadienal, 2-*trans*-decenal, 2-*trans*-4-*trans*-decadienal and 2-*trans*-4-*trans*-dodecadienal. Perhaps oxidation at 30 C allowed the buildup of large amounts of these carbonyls compared with 55 C because the further oxidation of these carbonyls is slowed more than the reactions leading to their formation at 30 C. Other carbonyls were produced in similar amounts at both temperatures at similar PV.

In the light-oxidized sample, 2-*trans*-heptenal and 2-*trans*-4-*trans*-nonadienal were produced in larger amounts than in dark samples of similar PV. Pentanal and 2-*trans*-4-*trans*-hexadienal were produced in greater amounts in the lighted sample than in the 55 C dark sample but not in the 30 C dark sample.

It is possible that carbonyl artifacts are produced from hydroperoxides during reaction of a fat with Florisil and trichlorophenylhydrazine. To investigate this, $SnCl_2$ or HI reducing columns were used to reduce pure linoleic acid hydroperoxide to its alcohol before analysis by the TCPH method. An aliquot of pure hydroperoxide previously determined to be ca. 6 μmol was used in each case. This value was chosen to correspond to the upper limit of PV found in the soybean oils that were analyzed. No hydroperoxide could be detected by PV after passage through either of the reducing columns.

A comparison of artifact production in the reduced and unreduced linoleic acid hydroperoxide samples after TCPH analysis is shown in Table III. The amount and identification for each carbonyl produced by the different procedures are listed. A blank was prepared in the same manner as the regular, unreduced sample except without the addition of linoleic acid. Duplicates of each treatment were run. All three procedures, including the reduced and unreduced samples, resulted in some artifact production.

TABLE II

Mass Spectral Data for Carbonyl-TCPH Identified by GC-MS

Identification	Characteristic fragments m/e (relative abundance)
Butanal	194(100), 196(84), 195(49), 167(47), 169(46), 97(43), 197(42), 62(31), 61(28), 198(28), 264[a](20)
Hexanal	194(100), 196(88), 195(69), 197(62), 167(54), 169(47), 201(45), 61(34), 236(33), 238(33), 198(33), 124(32), 292(14)
2-*trans*-(or 3-*cis*)Hexenal	28(100), 96(97), 69(34), 290(28), 194(25), 79(25), 292(23), 196(22), 81(21), 169(18), 159(18)
2-*trans*-Heptenal	68(100), 110(58), 194(54), 67(42), 196(39), 304(39), 93(35), 306(33), 167(28), 169(28)

[a]This parent ion is underlined.

Although both reduction methods resulted in increases in some carbonyl compounds, other peaks were decreased by an initial reduction procedure, indicating that some hydroperoxide scission had occurred in the TCPH procedure. In most cases, the $SnCl_2$ reduced the peak size more than the HI and produced fewer artifacts. The amount of carbonyls found that had chain lengths greater than six carbons in all of the three treatments was small when compared with the soybean oil samples. One must suppose that some of the carbonyl compounds that were found may have been formed by decomposition of the linoleic acid hydroperoxide during its isolation. When only the compounds likely to have come from linoleic acid hydroperoxide are considered, the $SnCl_2$ reduction column did an excellent job in removing them.

Samples of soybean oil were reduced and tested in a manner similar to the linoleic acid hydroperoxide. Similar evidence of a small but detectable artifact production by the reducing columns was noted (32). We conclude that use of reducing columns might be helpful when analyzing oils at early stages of oxidation. However, in advanced stages of oxidation, the amounts of the carbonyls are so large in comparison with the artifacts that prior reduction would not be necessary.

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Automatic Karl Fischer Titration of Moisture in Sunflower Seed[1]

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ABSTRACT

An automatic Karl Fischer (KF) titrator of the motor-driven buret type was applied to the determination of moisture in sunflower seed. A study of the effect of sample size on KF moisture analysis showed a significant decrease in moisture content with increase in sample size from 1 to 5 g. In the moisture range of 5.5-10.5%, a sample size of 3-4 g gave moisture values closest to those obtained by the AOCS official oven method for sunflower seed. Comparison of KF moisture analysis with oven methods on 6 samples with moisture contents ranging from 5.4 to 12.7% showed that KF moisture values were not significantly different from air oven and vacuum oven methods. The mean standard deviation of KF determinations of whole sunflower seed was 0.11% moisture content, whereas for forced draft oven moistures, the mean standard deviation was 0.05%. KF moisture values generally were slightly lower than the air oven moisture values. Using KF and vacuum oven methods to measure moisture contents, sunflower seed were found to lose 0.6% moisture from a sample containing 8.9% moisture when grinding the sample with Hyflo Super Cel as in the AOCS official method.

INTRODUCTION

In a study of the application of near infrared reflectance (NIR) spectroscopy for determining moisture in sunflower seed, a calibration method was needed to determine as accurately as possible the "true" moisture content of the seed.

The Karl Fischer reagent titration method (KF) is specific for water and has the added advantage that it is much more rapid for many materials than official oven methods (1). The KF method has been applied to a wide variety of organic materials such as butter and margarine (2), cereal and cereal products (1), ground cottonseed (3), grain (4,5) and soybean meal (6).

Fosnot and Haman (1) determined the moisture in cereals and cereal products by the KF method and reported that the two most important factors in the analyses were the length of time that the material was in contact with the Fischer reagent and the particle size of the sample. Ground cereal was placed in flasks with methanol and brought to boiling. After cooling to room temperature, the sample was titrated to excess with Fischer reagent, allowed to stand for a specified time, and then the endpoint was determined electronmetrically according to the "dead-stop" procedure using a cathode ray tube. They found a contact time of 30 min and a grind of ½ mm or less gave complete extraction of moisture. With a wide variety of cereal products ranging from 1% to 80% moisture, agreement with the oven method was in most instances very good.

Hoffpauir and Petty (3) compared oven drying with KF titration for determination of moisture in ground cottonseed products. Ground cottonseed samples were extracted with anhydrous methanol by allowing the sample to stand for 3 hr at room temperature, with frequent shaking, and then titrating directly with KF reagent. For fumed and ground cottonseed and ground cottonseed meal, moisture values by the KF method were lower than that by both vacuum and air oven at 101 C. They concluded that the lower values might be due to inadequate extraction of water with methanol or that some volatile components other than water are lost in the oven method.

Krober and Collins (6) used the KF procedure to determine the moisture content of extracted soybean meal. Soybean meal was extracted with dry methanol by allowing the sample to stand for 1 hr and then shaking it for 15 min with a mechanical shaker. The mixture was titrated directly with KF reagent to a visual endpoint. The results agreed with oven analysis within 0.1% for samples of moisture content between 1.5% and 16.8%.

Although the KF method, in the absence of interferences, is specific for water and can be used to analyze materials with volatile components and heat labile substances, the method required considerable technical skill

[1] Presented at the 74th annual AOCS meeting, Chicago, 1983.