PESTICIDE RESIDUES

# Modified Schöniger Combustion for Determination of Residues of Arsenic, Bromide, Chloride, Manganese, and Nickel in Pesticide-Treated Plant Material

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Use of the 5-liter modified Schöniger combustion flask has been extended to the combustion of potatoes, cherries, onions, cabbage, and oats for the determination of residues of arsenic, bromide, chloride, manganese, and nickel.

THE COMBUSTION of plant material has been carried out in a 5-liter flask (*5*) prior to the determination of residues of arsenic, bromide, chloride, manganese, and nickel.

Arsenic may be determined in potato following sodium arsenite herbicide applications by flask combustion of 10 grams of oven-dried potato tissue and absorption of arsenic in 100 ml. of 1*N* hydrochloric acid. After the flask has been rinsed twice with 25 ml. of acid, arsenic is determined (*6*) in 50 ml. of the absorbing solution; acid is omitted from the molybdate solution and the concentration of dilute stannous chloride is doubled. Recoveries of 2.5 and 5 p.p.m. of arsenic as sodium acid arsenate ranged from 81.6 to 103.2%.

Inorganic bromide in cherries, possibly resulting from Nemagon soil treatments, was determined by the following procedure.

Burn 10 grams of oven-dried cherries in the flask, absorbing hydrogen bromide in 100 ml. of water. Rinse twice with 25 ml. of water and separate bromide from chloride by ion exchange chromatography (*7*) [55 ml. of 0.6*N* sodium nitrate will remove chloride from the resin bed (12 mm. in inside diameter and 16 cm. high)]. Use 30 ml. of 1.8*N* sodium nitrate for elution of bromide and collect six 5-ml. fractions. Determine bromide in each fraction (*4*) by adding 10 ml. of water and 5 ml. of the color reagents to each and measuring the absorbance in a 2-cm. cell. Calculate bromide in the sample by subtracting the absorbance of the first fraction from each of the next four and adding the differences.

Recoveries of 5 and 10 p.p.m. of bromide as sodium bromide were 80.5, 65.8, 116.9, and 104.3 and 87.9, 86.8, 80.3, 95.9, and 133.7%, respectively.

Inorganic chloride in potatoes, resulting from heavy applications of DD to soils, was determined after flask combustion of 10 grams of vacuum-dried potato tissue and absorption of hydrogen chloride in 100 ml. of water. After the flask had been rinsed twice with 10 ml. of water, chloride was determined (*2*); the entire absorbing solution was taken for analysis. Recoveries of 2 mg. of chloride as sodium chloride were 103 and 98%.

Manzate [manganese ethylenebis(dithiocarbamate)] was determined as manganese in cabbage and onions after combustion of 10 to 15 grams of the oven-dried vegetable and absorption of gases in 100 ml. of 6*N* hydrochloric acid. After rinsing twice with 25 ml. of acid, manganese was determined (*3*), using the entire solution and making absorbance measurements in a 10-cm. cell. Recoveries of 25 $\gamma$ of manganese as manganese sulfate from cabbage and onions were 96.0, 94.0, 85.6, and 84.0 and 115.2, 101.2, 94.0, and 91.6%, respectively.

Rohm and Haas 0-3818B, a nickel-containing fungicide, was determined as nickel in oats following combustion of 1 gram of whole grain. Absorption of gases in 100 ml. of 0.1*N* hydrochloric acid and rinsing with 100 ml. of the acid and 150 ml. of water were followed by determination of nickel in the combined solutions (*7*). Recoveries of 20 $\gamma$ of nickel as nickel nitrate were 89.5, 71.0, and 74.5%.

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BUTTER ADULTERATION

# Detection of Hydrogenated Fats in Butter Fat by Measurement of cis-trans Conjugated Unsaturation

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A PERENNIAL PROBLEM to food-law enforcement agencies has been the detection of butter adulteration. As long as there exists a price differential between butter fat and possible adulterant fats, and until methods are available for the detection of all such adulterants, this problem will undoubtedly continue.

Methods for the detection of butter adulteration have been surveyed recently (*4*). Many of the methods mentioned lose their effectiveness for the detection of low levels of adulteration when the natural wide variation of pure butter fat is taken into account. Other methods are long and cumbersome and are unsuitable for the analysis of a large number of samples. One method developed in this laboratory (*12*) used the high tocopherol content of most vegetable

**Butter contains cis-trans conjugated unsaturation as well as isolated trans unsaturation, while hydrogenated fats contain only the latter. Both systems are detectable in the 940- to 990-cm.$^{-1}$ region of the spectrum. By using differential infrared spectroscopy, it was found that conjugated and isolated unsaturation are present in a constant ratio in pure butter. The addition of hydrogenated fats greatly increases the isolated trans double bonds (967 cm.$^{-1}$) but leaves the conjugated diene essentially unchanged (948 and 980 cm.$^{-1}$), thus changing the ratio. By using this technique it is possible to detect as little as 7% of a hydrogenated adulterant fat.**

oils for the detection of these oils in butter. This method, however, would not detect marine oils or vegetable oils which had been refined so as to reduce the tocopherol content. An infrared method for the detection of hydrogenated fat mentioned by Kummerow was stated "to be unreliable" (*11*).

Early in 1959, it came to the attention of the Food and Drug Directorate that a hydrogenated vegetable oil, low in tocopherol, was being used to adulterate butter in a few creameries. A sample of this "adulterant" was obtained and analyzed. From its composition and other characteristics it was obvious that at the 10 to 15% level in butter, it would escape detection by the Reichert-Meissl (*1*) and tocopherol procedures (*12*). It was necessary to develop a reliable method for the detection of this "adulterant" which could be used for a large number of samples.

Differential infrared spectroscopy, which was successful for the detection of rapeseed oil in olive oil (*3*), was applied to the butter–hydrogenated fat system. However, because of a wide variability in the spectra of pure butter samples, this method was not sufficiently sensitive to detect 10% addition. In the course of this investigation it was found that butter, which contained varying proportions of "trans" acids with an absorbance peak at 967 cm.$^{-1}$ (*7, 13*), invariably had a smaller absorption at 948 cm.$^{-1}$, which appeared to be proportional to the larger peak at 967 cm.$^{-1}$ On the basis of these observations the method described here was developed.

## Method

**Apparatus and Reagents.** The apparatus was a Perkin-Elmer Model 21 infrared spectrophotometer equipped with a scale expander. Matched 0.5-mm. sealed NaCl cells were used throughout. Dry reagent grade carbon tetrachloride and a suitable reference butter (see Discussion) are required.

**Procedure.** Sample Preparation. Melt 20 to 50 grams of butter in a small beaker in an oven at 60° to 80° C. Do not agitate or stir during the melting operation. Decant the melted fat through a fast filter paper (Whatman No. 41 or 54 or equivalent). Weigh 1.0000 ± 0.0005 gram into a 25-ml. volumetric flask. Dilute to the mark with $CCl_4$.

Infrared Examination. Using the instrumental conditions given in Table I, record the spectrum in duplicate from 1025 to 920 cm.$^{-1}$ with a 4% (w./v.) solution of a pure reference butter (see Discussion) in the reference beam. Subtract the readings on the expanded scale at 967 and 948 cm.$^{-1}$ from the reading at 920 cm.$^{-1}$. To correct these two readings from slight unbalance of the two cells, obtain a "reference spectrum" with the reference butter solution in both beams. Subtract the 967- and 948-cm.$^{-1}$ readings from 920-cm.$^{-1}$ readings, and subtract these values from the corresponding values for the sample. Plot the corrected readings for the sample against each other as in Figure 2, *a* and *b*. If this plot falls to the right of the area for pure butter as in Figure 2, *b*, hydrogenated fat is present.

## Results and Discussion

**Choice of Reference Butter.** The "trans acids" content of butter varies seasonally over a wide range (*7*). For our work a butter was chosen in the middle of the range, so that winter butter samples showed a lower absorbance and summer samples a higher absorbance at 967 cm.$^{-1}$ An attempt was made to dispense with the reference butter entirely, but the rapidly changing background absorption in this region makes resolution of the small 948-cm.$^{-1}$ peak difficult. By using a butter solution in the reference beam, a flat base line is obtained, and the accuracy of the measurements is improved.

**Spectra from 1025 to 920 Cm.$^{-1}$** Spectra of three typical butter samples are shown in Figure 1, with spectra of the same samples containing 10% hydrogenated fat (uncorrected for slight cell mismatch). The most prominent features in these spectra are the absorptions

**Table I. Instrumental Conditions for Infrared Butter Analysis on Perkin-Elmer Model 21 Infrared Spectrophotometer**

| | |
|---|---|
| Frequency range | 1025–920 cm.$^{-1}$ |
| Prism | NaCl |
| Slit program | 980 |
| Speed | 3 min./100 cm.$^{-1}$ |
| Source | 0.3 ampere |
| Filter | In position |
| Response | 1 (electrical)<br>2 (pulley position) |
| Automatic suppression | 1 |
| Gain | 4.8 |
| Ordinate expansion | 5 |
| Cells | 0.5-mm. path length, NaCl sealed type |
| Compensation | 4% (w./v.) reference butter in $CCl_4$ |
| Pen position | Set at 70% on expanded scale at 920 cm.$^{-1}$ with reference butter solution in both beams |
| Scale | 8 cm. = 100 cm.$^{-1}$ |

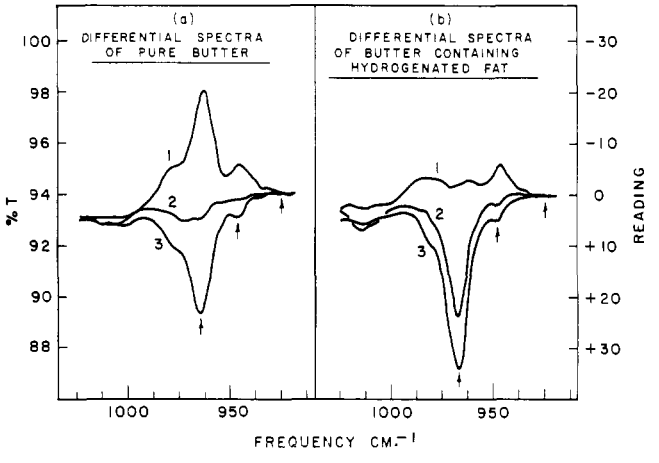


Figure 1. Differential spectra

a. Pure butters
b. Butters containing 10% hydrogenated fat

due to isolated trans unsaturation at 967 cm.$^{-1}$ (*5*) and a smaller peak at 948 cm.$^{-1}$ Spectrum 1 of Figure 1, *a*, was obtained from a sample containing less trans unsaturation than the reference butter, sample 2 contains about the same amount, while sample 3 contains more. A shoulder at about 980 cm.$^{-1}$ is also visible in these spectra. The ordinate scale at the left of the spectra is in per cent transmittance (not expanded) and the scale to the right is the chart reading on the expanded scale using the 920-cm.$^{-1}$ reading as an arbitrary zero.

Jackson *et al.* (*10*) have assigned peaks at 982 and 948 cm.$^{-1}$ to the conjugated *cis-trans*-diene structure. Other workers (*6*, *16*) have also discussed this assignment. From the appearance of the butter spectrum, it is apparent that this cis-trans conjugated unsaturation is present, and is proportional to the isolated trans unsaturation as measured at 967 cm.$^{-1}$

Partially hydrogenated fats are particularly rich in the trans isomers of oleic acid, and show strong absorption at 967 cm.$^{-1}$ but low absorption at 948 cm.$^{-1}$ Figure 1, *b*, shows this increase in absorption when 10% of a hydrogenated fat (iodine value 66) is added to the butter samples used for Figure 1, *a*. The absorption at 948 cm.$^{-1}$ remains about the same.

**Relationship of Isolated trans to cis-trans Conjugated Unsaturation for Pure Butter.** Using the procedure outlined above, 189 butter samples taken at various times of year from across Canada were examined. The apparent per cent transmittance readings at 967 and 948 cm.$^{-1}$ (on the expanded scale) were subtracted from the reading at 920 cm$^{-1}$. Corrections were made for slight differences in cells when necessary. The resultant values were then plotted with the 948-cm.$^{-1}$ value as ordinate and the 967-cm.$^{-1}$ value as abscissa, as in Figure 2, *a*. Since the points fall approximately on a straight line, the least-squares line was calculated and is shown as the central line of Figure 2. The plot was then divided into four segments, and each of the points in a segment was projected to a common ordinate for the segment by drawing a line through the point parallel to the least-squares line. The approximate 99% confidence limits at each point were then calculated using the projected abscissa. Lines drawn through these limits appear as the right and left curved lines of Figure 2.

If the isolated trans and the conjugated cis-trans systems bear a constant concentration relationship to each other, the plot should be linear in absorbance rather than per cent transmittance. However, in practice, since the authors worked over a very small range of per cent transmittance (100 to 92% *T*), the two values are very nearly linear with respect to each other. The same calculations for the least-squares line and the confidence limits were made with all readings converted to absorbance. Insignificant deviations from the lines given in Figure 2 were obtained, after conversion back to the other units.

Subsequent to the 189 spectra used for the calculation, spectra of 111 other samples of pure butter have been obtained. Of the 300 samples examined, the plot for two samples falls slightly outside the 99% confidence limits, and the plot for five other samples falls close to or on the limit. In Figure 2, *a*, the plots for 108 representative samples are given.

**Effect of Addition of Hydrogenated Fat to Plot of 967- to 948-Cm.$^{-1}$ Readings.** In the development of the method, 10% of the hydrogenated

**Table II. Detection of Hydrogenated Fat in Butter Samples**

| Level of Adulteration | No. of Samples | Outside 99% confidence limits for pure butter | Inside 99% confidence limits for pure butter |
|---|---|---|---|
| 2–4 | 2 | 1 | 1 |
| 5–7 | 8 | 5 | 3 |
| 8–12 | 26 | 26 | 0 |

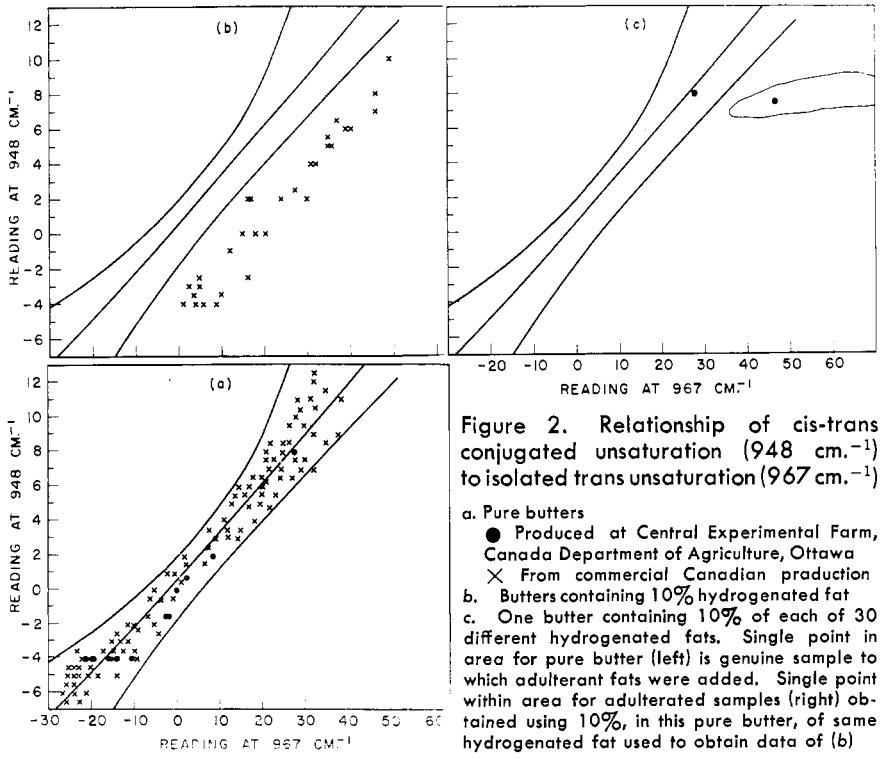


Figure 2. Relationship of cis-trans conjugated unsaturation (948 cm.$^{-1}$) to isolated trans unsaturation (967 cm.$^{-1}$)

a. Pure butters
● Produced at Central Experimental Farm, Canada Department of Agriculture, Ottawa
× From commercial Canadian production
b. Butters containing 10% hydrogenated fat
c. One butter containing 10% of each of 30 different hydrogenated fats. Single point in area for pure butter (left) is genuine sample to which adulterant fats were added. Single point within area for adulterated samples (right) obtained using 10%, in this pure butter, of same hydrogenated fat used to obtain data of (b)

**Table III. Detection Limits of Various Samples of Hydrogenated Fat**

| Fat | Iodine No. | Approx. Limit of Detection[a] |
|---|---|---|
| Margarine | | |
| 1 | 85 | 3 |
| 2 | 76 | 4 |
| 3 | 66 | 4 |
| 4 | 75 | 5 |
| 5 | 77 | 5 |
| 6 | 73 | 5 |
| 7 | 68 | 5 |
| 8 | 80 | 5 |
| 9 | 58 | 5 |
| 10 | 77 | 5 |
| 11 | 80 | 5 |
| 12 | 75 | 5 |
| 13 | 66 | 5 |
| 14 | 75 | 5 |
| 15 | 75 | 6 |
| 16 | 69 | 7 |
| 17 | 61 | 9 |
| Hydrogenated soybean | | |
| 1 | 95 | 2 |
| 2 | 83 | 2 |
| 3 | 84 | 2 |
| 4 | 78 | 5 |
| Hydrogenated herring | | |
| 1 | 64 | 3 |
| 2 | 76 | 3 |
| Hydrogenated seal | 45 | 4 |
| Hydrogenated palm | 54 | 9 |
| Hydrogenated peanut | 64 | 6 |
| Hydrogenated cottonseed | 75 | 6 |

[a] Average amount (%) of fat necessary to be added to a butter, whose plot falls on L.S. line of Figure 2, to move the plot significantly outside the 99% confidence limits for pure butter.

"adulterant" was added to 30 different butter samples. The results plotted in Figure 2, *b*, show that 10% may be easily detected. As a test of the method, the adulterant was added at the 2 to12% level to 36 samples, which were then mixed with 69 pure samples as unknowns.

The results are summarized in Table II. In every case where adulteration was not detected, the plot for the starting pure butter was to the left of the least-squares line, and the level of adulteration was less than 7%.

In addition to the adulterant fat, 27 other hydrogenated fats were added to one butter sample at the 10% level, with the results shown in Figure 2, *c*. Table III lists these fats, their iodine values, and an estimated level of detection in a butter whose plot falls on the least-squares line.

**Range of Concentration of cis-trans Conjugated Dienoic Acids and Isolated trans Acids.** Pure compounds were not available for estimation of extinction coefficients under our conditions. However, two samples at the extremes of the range encountered were dissolved in carbon disulfide, and the infrared spectra obtained using, as reference, a solution of olive oil which appeared to be free of conjugated or unconjugated trans double bonds. Using the absorptivity of 0.288 liter per gram cm. at 948 $cm.^{-1}$ found by Chipault and Hawkins (*6*) for methyl esters, the range for the conjugated *cis-trans*-diene was found to be 2.1 to 4.5%. Using the absorptivity of 0.460 liter per gram cm. for the trielaidin reported by Callen and Pace (*5*), the range for isolated trans unsaturated acids was found to be 5.8 to 12.0%. The ratio of the concentration of isolated trans to conjugated trans unsaturation is about 2.7.

When 10% of a hydrogenated fat is added, the ratio is changed to 3.1 to 3.8, with the exact value dependent on the trans acid content of the adulterant and of the butter.

**Suggested Mechanism for Formation of cis-trans and trans Unsaturation in Butter Fat.** It has been suggested (*9, 17*) that butter contains little, if any, linoleic acid (*cis*-9, *cis*-12) normally found in seed fats. Apparently the cow, in passing linoleic acid in the diet through its system to the milk, isomerizes some linoleic acid to a cis-trans conjugated dienoic acid, which then undergoes biological hydrogenation in its rumen to form a trans isomer of oleic acid such as vaccenic acid (*2, 8, 14, 15*). The first isomerization would be the rate-controlling step and would establish the amounts of these components.


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**FOOD DISCOLORATION**

# Browning in Dried Fruit Products: Non-enzymatic Browning and Its Effect on the Carotenoids in Qamareddeen, a Dried Apricot Pulp


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**A study of the effects of blanching, sulfurization, and moisture level on the development of browning and the levels of lycopene and β-carotene in dried apricot pulp has demonstrated that an improved product is obtained when the fruit is steam-blanched for 3 minutes and the pulp is sulfurized with 2000 p.p.m. of sulfur dioxide, then dried to 25% moisture content.**


One of the favorite dried fruit products in Middle Eastern countries is Qamareddeen, an apricot pulp dried in the form of thin sheets. A local variety of apricots known as Klabi is preferred for making this product.

The preparation of Qamareddeen is often carried out on a relatively small scale. The fruit used is usually of high fiber content and low eating quality, often overripe windfalls. After being washed, the fruit is mashed in straw baskets, and the pulp is spread in depths of 3 to 10 mm. on wooden trays that have been thoroughly oiled. The trays are then placed in the sun for drying, until the moisture level reaches 20 to 25%. A few producers sulfurize the apricot pulp by mixing with a dilute solution of sodium metabisulfite. The sulfuring level is critical in its influence on flavor, as the dried product is often consumed without reconstitution. The dried pulp is wrapped in yellow cellophane film or parchment paper and stored at room temperature. Considerable browning commonly occurs during storage with consequent adverse effect on quality.

The browning of dried apricots has been studied extensively by Stadtman and his associates (*8–10*), who found that sulfurization extended the storage life