and carbohydrates as well as ethanolic extracts of tomatoes.

From 1 to 14 mg. of galacturonic acid could be determined in an aliquot of standard solution or tomato extract containing up to 276 mg. of organic acids and 100 mg. of carbohydrate with an accuracy within 0.12 mg. using these procedures. Galacturonic acid content of Queen's tomatoes did not appreciably change with storage time. No increase in galacturonic acid, which would occur if the pectic substances were degraded to galacturonic acid, was found.

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IMPURITY MEASUREMENT IN FATS

Spectrophotometric Determination of Melamine and Formaldehyde in Lard

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Ultraviolet and visible spectrophotometric methods for the determination of micro amounts of melamine and formaldehyde in aqueous media have been modified in order to obtain the desired detectability in fats such as lard. These methods were used in the investigation of the degree to which melamine and formaldehyde might be extracted from wet-strength paper by lard.

PROPOSALS FOR THE USE OF MELAMINE-FORMALDEHYDE RESINS to impart wet strength to paper intended for food wrapping have prompted an investigation of the degree to which melamine and formaldehyde might be extracted from the paper by the food. Ultraviolet (*4*) and visible (*1, 5*) spectrophotometric methods were available for the determination of micro quantities of these compounds in aqueous media; however, modifications of these methods were required in order to achieve the necessary detectability for their application to fats such as lard. The present paper describes a procedure of extraction of the lard with dilute hydrochloric acid and a hydrocarbon, which yields as increase in detectability and eliminates certain interferences.

Apparatus and Materials

A Cary automatic recording spectrophotometer, Model 11, No. 67, and fused quartz cells of various light path lengths were used for the ultraviolet spectrophotometric work. A modified General Electric recording spectrophotometer, equipped with a photomultiplier tube, and 10-mm. glass cells were used for the visible spectrophotometric work. "Spectroscopically pure" isooctane and 12N and 0.1N hydrochloric acid were used. Solutions of phenylhydrazine hydrochloride (1%) and of potassium ferricyanide (5%) in distilled water were prepared fresh daily. Raw prime steam lard (Swift), containing no antioxidants or inhibitors, was used in the recovery and storage tests. (Commercial antioxidants have ultraviolet absorption which interferes with the detection of melamine.) Wet-strength wrapping paper, treated with PAREZ resin 607 (*2*) (a commercial trimethylolmelamine, American Cyanamid Co.) and a non-resin-treated wrapping paper were used in the storage tests.

Ultraviolet Spectrophotometric Method for Melamine

The strong absorption band of melamine at 235 mμ in dilute acid solution has been used for the determination of melamine in wet-strength paper, (*4*), and the theoretical detectability has been calculated to be 4 γ. The detectability of melamine in a material such as lard may be improved by an extraction procedure which serves to separate the melamine from interfering materials and to concentrate the melamine in a minimum volume corresponding to the volume of the absorption cell to be used. Although melamine and melamine resin (trimethylolmelamine) may be distinguished from one another by their spectra and a two-component analysis may be performed (*3, 4*) when these compounds are present in appreciable amounts, it is not possible to distinguish them in the extremely low concentrations encountered in the lard samples. As the resin and the melamine have very nearly the same absorptivity at the analytical wave length, 235 mμ, the data may be reported as "melamine and/or resin."

It was found that some absorbing material was present in the 0.1N hydrochloric acid extraction of an iso-octane solution of lard which had not been exposed to wet-strength paper. In order to compensate for this interfering absorption, a suitable correction term had to be devised. A simple subtractive term using absorbance at some longer wave length where melamine did not absorb (*3, 4*) was ineffective. Examination of 17 extracts of fresh lard solutions showed that the ratio of absorbances at 235 and 260 mμ was constant (Table I).

This allowed the use of a correction term based on the experimentally determined ratio of absorbances of "blank" lard samples stored under the same conditions as the test samples.

Procedural Details

Approximately 15 grams of lard were weighed by difference on an analytical balance, dissolved in 100 ml. of iso-

octane, and transferred to a separatory funnel. Ten milliliters of 0.1N hydrochloric acid were added, and the funnel was shaken well and allowed to stand for 20 minutes for separation to take place. The bottom (acid) layer was removed, and the extraction procedure repeated with another 10-ml. acid portion, which was added to the first portion. The sample was transferred to a 20-mm. absorption cell and examined versus a matched cell filled with 0.1N hydrochloric acid, scanning from 270 mμ to the lower wave-length limit of the instrument. Absorbance readings were taken at 235 and 260 mμ. A shorter cell length was used if the absorbance exceeded the accurate range of the instrument. Both blank and exposed samples were examined in this manner. The ratio of the absorbance at 235 to that at 260 mμ was calculated for each blank, and averaged. The concentration of melamine and/or resin was calculated from

$$c_m = \frac{A_{235} - (R_{av.} \times A_{260})}{(b)(a_{235})}$$

where c_m is the concentration of melamine in grams per 100 ml., A_{235} and A_{260} are the observed absorbances at the subscript wave lengths of the extracts of the exposed lard samples, b is the cell light path length in millimeters, a_{235} is the absorptivity of melamine at 235 mμ (81.0), and $R_{av.}$ is the average of the ratio of the observed absorbances at 235 and 260 mμ of the extracts of the blank lard samples. The concentration of the melamine in the lard is given by

$$C_m = \frac{(c_m)(V)}{W}$$

where C_m is the concentration of melamine in the lard, V is the volume of the extracting hydrochloric acid in milliliters, and W is the weight of the lard sample in grams.

Visible Spectrophotometric Method for Formaldehyde

A red color is formed when dilute solutions of formaldehyde phenylhydrazone are treated with potassium ferricyanide (*7*, *5*) in the presence of an excess of hydrochloric acid. Formaldehyde phenylhydrazone is formed by the reaction of formaldehyde and phenylhydrazone hydrochloride. Time was found to be an important factor in this reaction and in the color development. Ten minutes were allowed between the addition of the phenylhydrazone hydrochloride and the addition of the remaining reagents; 10 minutes were also allowed for full color development after the addition of the hydrochloric acid. A reagent blank was run with each set of samples. The detectability of the method was found to be 0.3 γ. The developed color obeyed Beer's law.

Table I. Reproducibility of Ratio of Absorbances for Extracts of Lard Solutions

(20-mm. cell)

A_{235}	A_{260}	A_{235}/A_{260}
0.395	0.288	1.37
1.150	0.782	1.47
1.070	0.718	1.47
1.135	0.788	1.44
0.365	0.257	1.42
1.150	0.757	1.52
1.560	1.120	1.39
1.365	0.973	1.40
2.31	1.570	1.47
2.30	1.580	1.46
2.34	1.602	1.46
1.68	1.224	1.38
1.975	1.340	1.47
1.975	1.340	1.47
1.735	1.240	1.40
2.000	1.500	1.33
1.945	1.452	1.34
	Average	1.42
	Standard deviation	0.05

Procedural Details A sample of lard of approximately 15 grams was weighed by difference on an analytical balance and dissolved in 30 ml. of iso-octane. This solution was extracted four times with 3 ml. of distilled water using a separatory funnel. The first two extracts were combined and the third and fourth extracts were combined prior to the development of the color. These two sets of combined extracts were examined separately. The volumes of the extracting medium were kept to a minimum in order to improve the detectability through concentration, as in the melamine procedure.

The water extract (of not more than 6-ml. volume) was treated with 1.0 ml. of 1% phenylhydrazone hydrochloride and allowed to stand for 10 minutes. Then 0.5 ml. of 5% potassium ferricyanide and 2.0 ml. of 12N hydrochloric acid were added in rapid succession. The solution was made up to 10-ml. volume, and the color was measured after 10 minutes, in a glass cell of 10-mm. light path length. The analytical wave length was 520 mμ. The concentration of formaldehyde was calculated from

$$c_f = \frac{A_{520} - A_{blank}}{(b)(a_{520})}$$

where c_f is the concentration of formaldehyde in grams per 100 ml., A_{520} is the observed absorbance at 520 mμ of the sample, A_{blank} is the observed absorbance of the reagent blank, b is the cell light path length in millimeters, and a_{520} is the absorptivity of the formaldehyde (in the formaldehyde-phenylhydrazone), which is 660. The concentration of formaldehyde in the lard sample is given by

$$C_f = \frac{(c_f)(V)}{W}$$

where C_f is the concentration of the formaldehyde in the lard sample, V is the volume of the extract in milliliters, and W is the weight of the lard sample in grams.

Sample Calculations

To illustrate the application of these methods, a typical sample calculation is given. A lard sample weighing 13.504 grams gave absorbances of 0.736 and 0.332 at 235 and 260 mμ, respectively, in a 20-mm. cell. The ratio of the absorbances at 235 to 260 mμ, determined from the blanks, was 1.85. The extract volume was 20 ml.

$$c_m = \frac{A_{235} - (R_{av.} \times A_{260})}{(b)(a_{235})} = \frac{0.736 - (1.85)(0.332)}{(20)(81.0)} =$$

0.000075 gram/100 ml. = 0.753 γ/ml.

$$C_m = \frac{(c_m)(V)}{W} = \frac{(0.753)(20)}{13.504} =$$

1.12 p.p.m.

A lard sample weighing 15.217 grams gave absorbances of 0.083 and 0.023 at 520 mμ for the sample and the blank extracts, respectively, in a 10-mm. cell, with a 10-ml. extract volume.

$$c_f = \frac{A_{520} - A_{blank}}{(b)(a_{520})} = \frac{0.083 - 0.023}{(10)(660)} =$$

0.0000009 gram/100 ml. = 0.09 γ/ml.

$$C_f = \frac{(c_f)(V)}{W} = \frac{(0.09)(10)}{15.2177} =$$

0.06 p.p.m.

Recovery Tests

In order to test the efficiency of the extractions and the quantitative recovery of known amounts of melamine and formaldehyde, these materials were added to lard and the described methods were applied. The results are shown in Table II.

These recovery test results appeared satisfactory enough to warrant the carrying out of storage tests with lard exposed to wet-strength and non-wet-strength paper.

Exposure Tests

In order to determine if melamine and/or formaldehyde migrated from wet-strength paper into lard, exposure tests were carried out. The area of paper used in wrapping a commercial 1-pound package of lard was measured and found to be 65 sq. inches. Two sets of samples were prepared using 65 and 32.5 sq. inches of wet-strength

Table II. Results of Recovery Tests

Material	Added, P.P.M.	Found, P.P.M.	Recovery, %
Melamine	100.0	100.4	100.4
	100.0	99.5	99.5
	100.0	99.1	99.1
	10.0	10.28	102.8
	10.0	9.99	99.9
	10.0	10.43	104.3
	1.00	0.95	95.0
	1.00	0.46	46.0
Melamine resin	10.0	8.70	87.0
	10.0	8.27	82.7
Formaldehyde	0.070	0.077	110
	0.070	0.064	92
	0.130	0.116	89
	0.130	0.113	87
	0.130	0.094	72
	0.20	0.18	90
	0.20	0.17	84
	0.20	0.18	91
	0.33	0.324	98
	0.33	0.287	87
	0.33	0.287	87
	0.33	0.36	109

paper to 45.36 grams of lard to give paper-to-lard ratios of 10 and 5 times the normal package ratio. A set of samples was prepared using 65 sq. inches of non-wet-strength paper and another set using no paper, for use as blanks. Ten sheets were selected at random from a stock of 18-pound sulfite wrapping paper which had been treated with PAREZ resin 607, as the acid colloid, added to the slush stock. This paper was a commercial product which contained 0.73% resin, as determined by Kjeldahl nitrogen analysis and by the ultraviolet spectrophotometric method (*3*) and was identical to the "specialty wrap" paper previously tested by that method (*4*). The sheets were cut into 5 × 6.5 inch sizes, and these were rolled into loose cylinders and inserted into 25 × 150 mm. test tubes. The lard was melted, poured into the test tubes, and weighed by difference.

Each set of tubes was placed in a large beaker in a vacuum desiccator while the lard was still melted. The desiccator was evacuated to remove air bubbles from the tubes. The air in the desiccators was removed by alternate evacuation and filling with dry nitrogen; four such flushing cycles were used. The desiccators were stored with an atmosphere of dry nitrogen; this was done to reduce oxidation of the lard, as it contained no inhibitor.

The desiccators were stored for 4 weeks in a forced-circulation oven at 120° F. At the end of this period, two samples of approximately 15 grams were weighed out from each test tube, one for the melamine and one for the formaldehyde analysis. The results of the determinations are shown in Table III.

Two types of blanks—one containing resin-free paper and the other lard only—had been stored along with the lard exposed to the wet-strength paper. Both sets of blanks were extracted and examined spectrophotometrically, and the absorbance ratio average at 235 and 260 mμ were calculated for both sets. As these values were not the same, both values were used in calculating the melamine concentrations; these are shown in the columns of Table III which are labeled "lard-only blank" and "lard + paper blank." It is unlikely that any significance can be attached to the slightly different values obtained with the two different blank corrections.

Both sets of blanks were similarly extracted and examined spectrophotometrically for formaldehyde. All samples of both sets assayed less than 0.01 p.p.m. of formaldehyde, and hence were not included in data in Table III.

It may be seen from examination of Table III that only very small quantities of melamine and/or resin and formaldehyde are able to migrate from the wet-strength paper into the lard when stored for 1 month at 120° F. (conditions which are not normally encountered commercially), even when the paper-to-lard ratios were 5 and 10 times the normal ratio. The concentrations encountered are roughly those of the smallest amounts that could be satisfactorily recovered when added to lard, as shown in Table II.

These extraction procedures and spectrophotometric determinations can be applied to the determination of melamine and formaldehyde in other foodstuffs similar to lard, with probably only minor modifications in the techniques. The presence of interfering materials which would extract along with the melamine or the formaldehyde would be the greatest source of difficulty.

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Table III. Extraction of Melamine and Formaldehyde from Paper by Lard

(120° F., 4 weeks)

Paper/Lard Ratio	Melamine in Lard, P.P.M.		Formaldehyde in Lard, P.P.M.
	Lard-only blank	Lard + paper blank	
10 × normal	1.04	1.52	0.06
	0.98	1.50	0.07
	1.15	1.74	0.07
	1.12	1.66	0.05
	0.96	1.46	0.08
	0.97	1.44	0.07
	1.78	2.46	0.05
	0.85	1.32	0.06
Average	1.11	1.64	0.064
Standard deviation	0.27	0.33	0.010
5 × normal	0.51	0.87	0.10
	0.54	1.00	0.07
	1.06	1.50	0.03
	0.83	1.27	0.05
	0.68	1.06	0.05
	1.08	1.58	0.04
	0.49	0.92	0.04
	0.54	0.99	0.06
	0.86	1.29	0.05
	0.78	1.21	0.05
Average	0.74	1.17	0.054
Standard deviation	0.21	0.23	0.018