

**Table III. Mean Scores<sup>a</sup> for Flavor of Selected Beef Cuts from Untreated Animals and Animals Treated with Co-Ral**

Treatment	Rib Roasts <sup>b</sup>	Rib Patties <sup>b</sup>	Round Patties <sup>b</sup>	Liver <sup>b</sup>	Kidney <sup>b</sup>
Control, untreated	4.7	4.5	4.5	4.6	4.7
Co-Ral	4.4	4.3	4.5	4.3	4.6
Test difference <sup>c</sup>	0.8			0.3	

<sup>a</sup> Score of 5 represents no off-flavor; 4, slight off-flavor; 3, moderate off-flavor; 2, strong off-flavor; and 1, very strong off-flavor.

<sup>b</sup> Means were based on 48 scores (4 judges × 6 replications × 2 animals).

<sup>c</sup> Difference between two means is significant at the 5% level, if it equals or exceeds the test difference [*t* test (17)].

The similarity in the mean scores for rib roasts and rib patties representing long and short cooking periods, respectively, indicated that cooking time was not a factor in flavor quality. Scores of 4.3 or above indicated that only slight off-flavors were present.

Results of the Co-Ral study are in general agreement with studies carried out at the University of Pittsburgh in which flavor evaluations indicated no off-flavors in round steak from beef animals treated with 0.25% of Co-Ral or in liver from beef animals treated with 0.5% of Co-Ral (2).

These investigations should be considered exploratory in nature because of the limited number of animal replicates. Further research is needed before definite conclusions can be drawn. Results

should be interpreted in connection with other research on this subject.

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## FOOD ADDITIVES ANALYSIS

### Turbidimetric Determination of the Extractability of Polyethylene Food Packaging Film in Vegetable Oil

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The extractability of polyethylene food packaging film in vegetable oil is determined by the amount of turbidity produced when the extract is treated with a mixture of ethyl and isopropyl alcohols. The turbidity, in nephelos, is applied to a calibration curve prepared with standard hexane solutions obtained by digesting the film in this solvent at various temperatures. The extractability of polyethylene in vegetable oil at 57° C., as specified by the Food and Drug Administration, coincides with the extractability of the polymer in hexane at 37° C.

POLYETHYLENE, like other materials used in the food packaging industry, must meet certain specifications regarding migration of components into the contents of the food package. The migrating species of polyethylene has been identified as primarily short chain polymer and is thus referred to as the low molecular weight fraction (LMWF) of the polymer.

Because of difficulties encountered in determining the migrating substances

directly in a food commodity, certain simulated food solvents, including water, 3% acetic acid, and vegetable oil, have been used for migration studies. Solvents were chosen to represent the different classes of foods. Other solvents such as ethyl alcohol may also be required, depending on the proposed use of the material under study.

Conditions for studying the extractability of a packaging material were proposed by Food and Drug Administra-

tion scientists (1). Briefly, the material—in the form of a thin film—is exposed to solvents at the ratio of 0.5 sq. inch per ml. The mixture then is digested at 57° C. for 1 week, after which the solvent is removed and analyzed for extracted substances.

Analysis of aqueous and alcoholic extracts generally involves evaporation of the solvent and a gravimetric measurement of the residue. Variations of this technique have been applied, but in

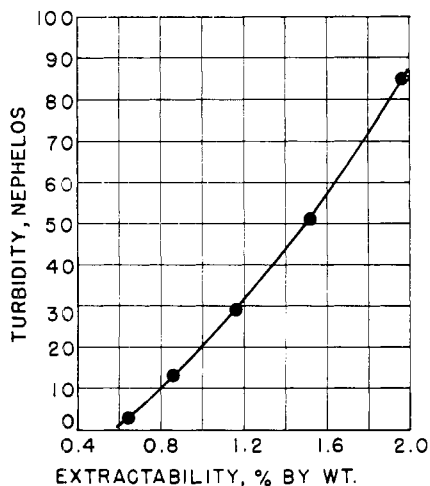


Figure 1. Typical calibration curve for turbidimetric determination of the extractability of a polyethylene polymer in vegetable oil

general the determination is relatively simple.

Vegetable oil, however, presents a much more difficult problem. Its high boiling point precludes direct determination of the polyethylene extractables by the evaporation technique. The problem is further complicated by the absence of distinctive functional groups in the migrating species, thus preventing the use of conventional colorimetric techniques or a direct infrared approach as a solution to the problem. It is necessary, therefore, to rely on the physical characteristics of the polymer as a means of determining its fat extractability.

Among the first attempts was a test involving addition of a small piece of polyethylene film to a definite volume of oil. If, upon digestion under prescribed conditions, the film was not entirely dissolved, its extractability was specified as an amount less than that added. This test, however, was invalidated by subsequent characterization studies which revealed that instead of the entire polymer being soluble in the oil, a LMWF which constitutes only a small percentage is actually the migrating species.

Attempts to determine loss of weight as an indirect measurement of the extractability of the film in vegetable oil have met with little or no success. The inability to remove residual oil quantitatively from the film without possibly also removing additional polymer has been the chief obstacle in this approach.

Johnson and Kvalnes (2) expanded this principle by following oil extraction with an extraction of the same film in hexane. An infrared technique was then used to determine the amount of oil in hexane and hence, by difference, the amount of polymer extracted by hexane. The difference between the latter value and the hexane extracta-

bility of a sample of virgin film represents the extractability of the film in vegetable oil.

Radioactive tracer techniques have also been reported, but the time and cost involved make this approach undesirable.

The present method is likewise based on the physical properties of the migrating polymer. In this procedure, the oil extract is treated with a mixture of ethyl and isopropyl alcohols which precipitates portions of the extractables. The resulting turbidity is then measured with a Coleman photonephelometer and, by reference to a calibration curve, the concentration of extractables in oil is determined

### Experimental

**Preparation of Sample.** Cut exactly 200 sq. inches of 1-mil film into strips and weigh to the nearest milligram. Place the film in a wide-mouthed glass bottle and add 200 ml. of vegetable oil. Digest this mixture, along with a blank portion of the oil, at 57° C. for 168 hours. While hot, decant the oil from the film and allow it to cool to room temperature. Pipet 10-ml. portions of the blank and the extract into separate 25-ml. graduated cylinders and add exactly 10 ml. of hexane to each. Keep these solutions for turbidity determination along with the calibration standards as described under "Turbidity Measurement."

**Preparation of Calibration Standards.** Prepare five wide-mouthed bottles, each to contain 200 sq. inches of the 1-mil film weighed to the nearest milligram and cut into narrow strips. Add exactly 200 ml. of hexane to each bottle; close the bottles tightly and immediately place them in a series of water baths

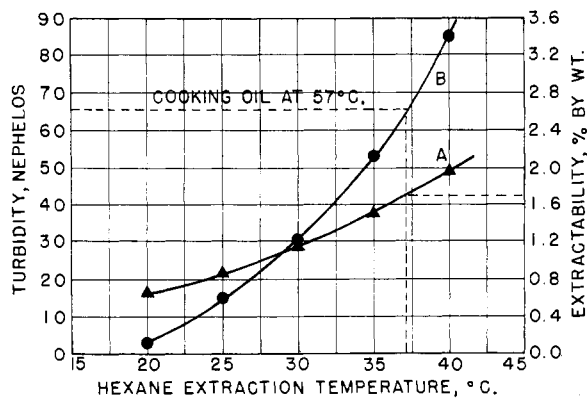


Figure 2. Turbidimetric determination of the extractability of polyethylene food packaging polymer in vegetable oil

- Turbidity vs. hexane extraction temperature
- ▲ Hexane extractability vs. extraction temperature

carefully controlled at 20°, 25°, 30°, 35°, and 40° C., respectively, for 2 hours. Decant the hexane immediately from the film, taking adequate precautions to prevent evaporation during the transfer. Allow the extracts to attain room temperature and then transfer 100 ml. of each to tared culture dishes. Evaporate the hexane almost to dryness on heating mantles or a steam bath, using a nitrogen current to aid in the evaporation. Complete the evaporation under vacuum and determine the weight of the residue. Calculate the weight per cent of film extracted by the hexane in each case.

Into each of a series of 25-ml. glass-stoppered graduated cylinders, pipet 10 ml. of the remainder of the hexane extracts. To each add, with a pipet, 10 ml. of the blank vegetable oil prepared in the previous section and mix well. Process each of these, along with the oil extracts, for turbidity measurement as described below.

**Turbidity Measurement.** Depending on the relative extractability of the film, pipet 2 to 4 ml. of the blank, samples, and standards into a series of 125-ml. glass-stoppered Erlenmeyer flasks. Add to each, with a pipet, 20 ml. of a 25% (v./v.) solution of ethyl alcohol (SD2B grade; Union Carbide Chemicals Co.) in anhydrous isopropyl alcohol. Mix well to effect complete solution of the oil and allow the flasks to stand together at room temperature for 15 minutes with occasional swirling. Using a Coleman Model 7 photonephelometer, determine the turbidity, in nephelos, of the blank, sample, and each standard as quickly as possible, allowing the solutions to remain in the sample chamber for only a few seconds. Determine net turbidity in the sample and standards by subtracting the reading of the blank from each.

**Table I. Extractability of Polyethylene Polymers in Vegetable Oil**

Polymer	Extractability		Deviation, P. P. M.
	% by wt.	P. p. m. <sup>a</sup>	
A	0.30	31	-3.8
	0.35	36	+1.2
	0.37	38	+3.2
	0.33	34	-0.8
		Av.	±2.2
B	0.91	73	-0.5
	0.89	71	-2.5
	0.93	74	+0.5
	0.95	76	+2.5
		Av.	±1.5
C	1.72	211	+1.7
	1.71	210	+0.7
	1.68	207	-2.3
	1.70	209	-0.7
		Av.	±1.4
D	2.70	335	-6.0
	2.80	347	+6.0
		Av.	±6.0

<sup>a</sup> Calculated at ratio of 0.5 sq. inch of film/ml. of oil.

Prepare a calibration curve by plotting the net turbidity of the standards against corresponding weight per cent extractabilities as determined in the preceding section. Determine the per cent extractability of the polymer in vegetable oil by applying the turbidity of the sample to the calibration curve. Using the following equation, convert the percentage extractability to parts per million at the ratio of 0.5 sq. inch per ml. as specified by the FDA.

$$E \times \frac{F}{400} \times 10^4 =$$

p. p. m. of LMWF in vegetable oil

where

$E$  = per cent extracted in the oil  
 $F$  = weight of film, grams  
 400 = number of 0.5-sq. inch units in 200 sq. inches

### Discussion

The LMWF of polyethylene is defined, for these purposes, as that portion of the polymer which is soluble in hexane at 50° C. Characterization studies in which this material was fractionated according to molecular weight have shown that it is composed of various subfractions, each having different solubility properties, depending on temperature and solvent. Approximately 10% of the LMWF is soluble in absolute ethyl alcohol at -60° C., while at 60° C. approximately 15% is soluble. Therefore, in this method, the degree of turbidity is a direct function of both temperature and solvent, thus making it essential that the calibration curve and the samples be processed for turbidity measurement under identical conditions. Not only must they be allowed to attain

temperature equilibrium, but solvent proportions must be precisely controlled.

It is also essential that the same alcohol mixture be employed throughout a single set of determinations. A slight change in water content of this solvent has a profound effect on the degree of turbidity.

Based on characterization studies, only that portion of the extractables above a certain molecular weight will precipitate in the alcohol-vegetable oil-hexane medium. All material below that weight will have no influence on degree of turbidity, but inasmuch as it is accounted for in the total per cent extractability, this fraction does affect the position of the calibration curve. Figure 1 is a typical calibration curve showing that the curve is elliptical and does not project through the origin. Instead, by extrapolation to the abscissa, it is shown that a fraction constituting, in this case, 0.58% of the resin is soluble, but produces no turbidity under the conditions of the method. The molecular weight of this fraction, therefore, is below that necessary to cause precipitation.

To maintain a constant concentration of the nonprecipitating portion of the LMWF and thus prevent an inherent inconsistency in the method, calibration standards and oil extracts are prepared at the same film to solvent ratio. Therefore, to obtain standards containing different concentrations of the LMWF, each standard is extracted separately at a different temperature. Because the nonprecipitating portion is quantitatively soluble at all of these temperatures, the concentration of this fraction is the same in each standard.

The film to solvent ratio does not necessarily have to be 0.5 sq. inch per ml. during the extraction, provided that maximum extractability is achieved at the ratio selected. However, the specified ratio is reflected in final calculations, simply by obtaining the weight of 0.5 sq. inch of film and applying the percentage oil extractability value to determine the micrograms extracted. The latter figure thus represents the parts per million (w./v.) extracted into vegetable oil under recommended extraction conditions.

A graphic illustration of the operation of the method is shown in Figure 2. Here, the calibration curve of Figure 1 has been broken down to show two curves. Curve A was produced by plotting the temperature at which the standards were extracted against the per cent of the polymer extracted. Curve B, on the other hand, reflects the relationship between extraction temperature and resulting turbidity.

This figure illustrates a relationship

that exists between the extractability of polyethylene in vegetable oil and in hexane. By applying the turbidity produced by the vegetable oil extract to these curves, both the per cent extractability and the temperature at which the hexane extractability of the film coincides with that of the oil at 57° C. can be determined. Without exception, oil extractability, under conditions set forth by the FDA, corresponds to extractability of the film in hexane at approximately 37° C.

**Proof of Method.** To prove the performance of the turbidimetric method, a modification of the Du Pont infrared method previously mentioned was used. Good agreement was shown between the two methods.

An indication of the precision of the turbidimetric method is shown by data (Table I) which represent nonconditioned polymers with a wide range of extractabilities. Extractabilities are calculated both in per cent and in parts per million according to FDA specifications. The irregular relationship between parts per million and per cent values is explained by the varying thicknesses of the films. This factor does not affect the percentage figure, but inasmuch as the parts per million calculation is based on film surface area, the value obtained is directly affected by film thickness.

**Scope of Method.** Although the present method was designed specifically for base polyethylene, it can also be applied to finished virgin polyethylene food packaging material, provided that the various conditioning additives are soluble in both hexane and vegetable oil. These additives will not produce turbidity under the conditions of the test; however, like the nonprecipitating portion of the LMWF, they will influence the position of the calibration curve and thus are accounted for in the final calculation.

Studies have shown that the performance of the method is not affected by film thickness up to approximately 3 mils. Beyond 3 mils, however, the extraction period in both hexane and oil must be appropriately adjusted to effect complete extractability.

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