COMMUNICATIONS

Methods for Analyzing Percentage Lipid of Ground Beef and Beef-Soy Blends

Raw and cooked ground beef and beef-soy blends (15 and 30% soy) were analyzed for percentage lipid by: (1) ether extraction, (2) chloroform-methanol-water extraction, (3) acid predigestion with ether extraction, and (4) thermal extraction (for raw meat only). For raw meat, the chloroform-methanol-water extraction method gave the highest ($P < 0.01$) values for percentage lipid, ether extraction gave intermediate values, and acid predigestion with ether extraction and thermal extraction (similar to each other) gave lowest values. For cooked meat, values obtained by chloroform-methanol-water and ether extraction were similar to each other and higher than those obtained by acid predigestion with ether extraction. For repeated analyses of raw meat, percentage lipid values obtained by thermal extraction varied the least; for cooked meat those obtained by ether extraction varied least. Based on values obtained, the precision of the method, and the laboratory procedure, we recommend ether extraction for percentage lipid analysis, when time for obtaining results is not a factor.

Soy is being used increasingly as a meat extender. Information on different methods to determine percentage lipid of meat-soy blends is needed. Generally, the official AOAC (1970) method for lipid determination (ether extraction) is used for ground meat, but other methods include acid predigestion with ether extraction, polar solvent extraction, and thermal extraction (used only for uncooked ground meat).

Mize (1972) reported that samples with and without added soy were nearly the same in lipid content when determined by acid hydrolysis or chloroform-methanol extraction but that they differed when he used ether extraction; ground beef containing 2% added soy had 4% less fat than did ground beef with no added soy. He did not report on statistical analysis of data and the number of replications in his study.

Because some lipids in animal products are bound to proteins and carbohydrates, not all lipids may be extracted by nonpolar solvents such as ether (Pomeranz and Meloan, 1971). If meat products are acid hydrolyzed before extracting the lipids, values may be higher. Sheppard et al. (1973), who compared eight methods for percentage fat analysis in various meat-containing products, recommended use of acid predigestion with ether extraction.

Polar solvents extract more bound lipids than nonpolar solvents. Lipids from animal tissue can be isolated and purified rapidly by a chloroform-methanol-water extraction process proposed by Folch et al. (1957) and modified by Bligh and Dyer (1959). That method has been adapted to use for red meat (Ostrander and Dugan, 1961).

Thermal extraction processes for measuring crude fat in meat and meat products have been developed. Bellis et al. (1967), in a comparison study, found that the Hobart and AOAC procedures gave similar results for samples containing between 15 and 29% crude fat.

We determined the percentage lipid in raw and cooked ground beef and beef-soy blends (15 and 30% soy) by several methods and compared the values obtained and the precision of those methods.

MATERIALS AND METHODS

Materials. Ground beef containing approximately 25% fat was obtained from the Department of Animal Science and Industry at Kansas State University and soy (Ultrasoy Minced), which contained approximately 1% fat, was obtained from Far-Mar-Co., Inc., Hutchinson, Kan. Mixtures containing 0, 15, and 30% rehydrated soy by weight were prepared.

The 15% rehydrated soy mixture contained 5% soy, 10% distilled, deionized water, and 85% ground beef, and the 30% mixture contained 10% soy, 20% water, and 70% ground beef. Blends were mixed with a Hobart mixer for 2 min at 113 rpm.

Five 150-g samples of ground beef or beef-soy blends (15 and 30% soy) were packaged in aluminum foil and held frozen (−17°) until analyzed for lipid. In addition, five 180-g patties of ground beef and of each beef-soy blend were packaged in aluminum foil and held frozen (−17°) until cooked. Patties were thawed for 15 hr at 6° and 2 hr at 25° and then modified broiled on wire racks (7 cm high) in metal pans, in a rotary hearth electric oven maintained at 177° to an internal temperature of 75°. Each cooked pattie was ground twice (through a ⅛-in. plate), before being analyzed for lipid.

Methods of Lipid Analysis. *Ether Extraction.* Samples were dried in a C.W. Brabender semi-automatic moisture tester at 121° (60 min for cooked samples and 90 min for raw) and extracted with petroleum ether on a Goldfisch extraction apparatus according to AOAC (1970) methods.

Acid Predigestion with Ether Extraction. The procedure used was developed by the Division of Nutrition of the Food and Drug Administration to comply with nutrition labeling requirements or regulations for total lipid (Sheppard, 1973). Samples were digested 30 min with 4 *N* hydrochloric acid at 60° and then 30 min at 90°—all under nitrogen (to protect the polyunsaturated fatty acids)—before being extracted with diethyl ether.

Table I

Source of variation	DF
Method of lipid analysis (ML)	3
Percentage soy (PS)	2
ML × PS	6
Error	48
Total	59

Table II

Source of variation	DF
Method of lipid analysis (ML)	2
Heat treatment (HT)	1
Percentage soy (PS)	2
ML × HT	2
ML × PS	4
HT × PS	2
ML × HT × PS	4
Error	71
Total	89

Table III. Mean Values[a] for Percentage Lipid in Ground Beef and Beef-Soy Blends

Treatment	Method: Chloroform–methanol–water	Method: Ether extraction	Method: Acid predigestion	Method: Thermal extraction	Blend mean	ANOVA, significance of F value[b]: Method	% soy	Heating	Method × heat
Raw									
0% soy	28.22	27.35	23.81	24.70	26.02 *	**	**	**	**
15% soy	23.80	21.92	18.87	19.00	20.90 *				
30% soy	20.93	19.38	15.06	16.03	17.85				
Method mean	24.32 *	22.88 *	19.25 ns	19.91					
Cooked									
0% soy	19.52	20.58	19.60		19.90 *				
15% soy	16.01	16.12	14.49		15.54 *				
30% soy	14.52	14.16	10.87		13.19				
Method mean	16.68 ns	16.95 *	14.99						

LSD[c] for: Raw — Method 0.86; % soy 0.86. Cooked — Method 1.17; % soy 1.04. Method × heat 1.08.

[a] Mean of five replications. [b] * = $P < 0.05$; ** = $P < 0.01$; ns, not significant. [c] LSD = least significant difference, $P < 0.05$.

Table IV. Variances for Methanol–Chloroform–Water Extraction, Ether Extraction, Acid Predigestion with Ether Extraction, and Thermal Lipid Extraction Methods in Raw and Cooked Ground Beef and Beef-Soy Blends[a]

Treatment	Method: Methanol–chloroform–water	Ether extraction	Acid predigestion	Thermal extraction
Raw	3.89 ns	1.54 ns	1.04 ns	0.41
Cooked	7.44 **	0.53 **	8.38	

[a] * = $P < 0.05$; ** = $P < 0.01$; ns = not significant.

Methanol–Chloroform–Water. After samples were extracted with methanol, chloroform, and water, zinc acetate was added to the extract to precipitate the protein. The residue (containing protein) was reextracted with chloroform and that extract was combined with the previous extract to determine percentage lipid (Ostrander and Dugan, 1961).

Thermal Extraction (Raw Samples Only). A Hobart Model F101 Fat Percentage Measuring Kit designed specifically for ground beef was used. A 56.7-g sample was placed under a heating element for 15 min and fat and juices collected in a test tube. The amount of lipid in the test tube was measured and converted to percentage lipid by a calibrated scale. Values were interpolated to the nearest quarter of a percentage.

Analysis of Data. The experimental design provided data suitable for analysis of variance from raw samples (Table I) and on data for both raw and cooked samples, omitting the data from the Hobart thermal extraction of raw meat (Table II). One replication of raw samples analyzed by acid predigestion with ether extraction was lost during extraction and therefore was not included in the analysis of variance. That reduced the degrees of freedom in the error term and the total degrees of freedom by three for both analyses. Bartlett's test of homogeneity and a two-by-two F test were used for determining the precision of the methods.

RESULTS AND DISCUSSION

Percentage Lipid Values. Mean values of five replications for percentage lipid in raw and cooked ground beef and beef-soy blends (15 and 30% soy) are presented in Table III. Generally, chloroform-methanol-water extraction and ether extraction methods gave higher values than did either acid predigestion with ether extraction or thermal extraction methods. However, there was a significant interaction between method of lipid analysis and heating treatment since the same differences between methods were not found for both raw and cooked samples. For raw meat, chloroform-methanol-water extraction gave the highest ($P < 0.01$) values; but for cooked meat ether extraction gave values similar to chloroform-methanol-water extraction, perhaps because of greater crust formation on raw meat particles as they dry (making it more difficult to extract lipids from the sample) or because of volatile losses during drying. For both raw and cooked meat, ether extraction gave higher ($P < 0.01$) percentage lipid values than the acid predigestion with ether extraction. For raw meat, values for percentage lipid determined by thermal extraction and the acid predigestion with ether extraction were not significantly different. Bellis et al. (1967) reported similar values for percentage lipid, as determined by ether extraction and by thermal extraction; we found values that were about 3% higher for ether extraction. Values for the thermal extraction and acid predigestion methods were similar for both beef-soy blends, so lower cooking losses that have been observed with such blends (Bowers and Engler, 1974) were probably caused by moisture retention not by lipid retention.

Raw ground beef, with or without soy, contained more ($P < 0.01$) lipid than did cooked. That was expected, in that during cooking meat loses both moisture and lipids.

Because the soy product contained only about 1% lipid, adding rehydrated soy to ground beef reduced total per-

centage lipid in beef-soy blends in relation to amount added. One purpose of this study was to determine if presence of soy in ground beef would influence percentage lipids as determined by different methods. Generally it was not, in that no significant interaction was found between method of lipid analysis and percentage of soy.

Precision of Methods. Variances of values for the four methods of lipid analysis for raw and cooked ground beef and beef-soy blends are presented in Table IV. For raw meat, thermal extraction was more precise than was the methanol-chloroform-water or the ether extraction method, but it was not significantly more precise than the acid predigestion with ether extraction method. Perhaps the reason for low variance with the thermal extraction method is the insensitive reading scale (values were read only to the nearest quarter of a percent). When the thermal extraction method was eliminated from the comparisons, the other three methods used for raw meat had essentially the same precision. For cooked meat, values varied more for the acid predigestion with ether extraction and the chloroform-methanol-water extraction methods than for ether extraction.

CONCLUSIONS

Before selecting a lipid extraction method, three factors to consider are the values obtained, the variance of the values, and suitability of the method for laboratory conditions. Because it obtains values that vary little and that are only slightly lower than those of methanol-chloroform-water extraction, we recommend ether extraction. That method requires a longer extraction time, but less laboratory handling time, than do the other methods; it is suitable for a laboratory that does not require immediate results. Thermal extraction, which requires only 15 min per sample, is useful in quality control of beef-soy blend products.

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Pamela P. Engler
Jane A. Bowers*

Department of Foods and Nutrition
Kansas Agricultural Experiment Station
Manhattan, Kansas 66506

Received for review September 16, 1974. Accepted January 10, 1975. Contribution No. 311 from Kansas Agricultural Experiment Station.

Residue Determination of Thompson-Hayward 6040 in Bovine Manure by High Performance Liquid Chromatography

Residues of TH 6040, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea, were determined in bovine manure at levels between 2.0 and 0.5 ppm. Samples were cleaned up by liquid-liquid partition and elution through a Florisil column. Analysis was performed with reverse-phase high performance liquid chromatography.

One of the new "insect growth regulator" insecticides is Thompson-Hayward 6040, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea. TH 6040 inhibits the synthesis and deposition of cuticle during the molting process as insects mature through immature stages to become adults (Mulder and Gijswijt, 1973). When this compound is applied to larval growth media, it prevents emergence of adults of the house fly, *Musca domestica* L. (Wellinga et al., 1973), and the stable fly, *Stomoxys calcitrans* (L.) (Wright, 1975). In both cases the adult fly is the pest, especially in the case of the stable fly as both sexes are blood-sucking and feed upon livestock and man.

Since conditions in cattle feedlots are ideal for the production of stable flies (the large amounts of manure offer an excellent environment for larvae, and cattle are available for adult feeding), an insecticide such as TH 6040 that would kill the nonpestiferous immature stages in manure would be desirable (Wright, 1975). However, before a compound such as TH 6040 can be used as a control agent, its residual properties must be investigated. No suitable methods have been available for quantifying TH 6040 at low parts per million levels since TH 6040 is refractory to GLC analysis and methods employing hydrolysis followed by derivatization have not proved satisfactory (Oehler, 1973). We therefore attempted to develop a method for determining residues of TH 6040 using high performance liquid chromatography (HPLC) to quantify TH 6040 extracted from bovine manure. The results of these investigations are reported here.

MATERIALS AND METHODS

Fresh samples of bovine manure were frozen immediately after collection and stored at −18°. Fortified samples were prepared by adding the appropriate amount of a dichloromethane solution of TH 6040 to 20 g of manure to produce TH 6040 levels of 0.5, 1.0, and 2.0 ppm. The solvent was removed with a stream of dry nitrogen.

The samples were homogenized in 75 ml of acetonitrile for 2 min with a Polytron Model PT-10 homogenizer. Insolubles were removed by filtration through a 150-ml coarse fritted glass funnel. The homogenizer was rinsed three times with 50-ml aliquots of acetonitrile, and the rinses were filtered and pooled with the initial filtrate. After evaporation of the sample to dryness with a rotary evaporator at 50°, the residue was taken up in 50 ml of acetonitrile and partitioned twice against 200 ml of hex-