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The rapid column method described, unlike AOAC method 7.056, determines both neutral ("crude") and total fat in canned pet foods, and uses nonflammable solvent mixtures and simple laboratory equipment. Neutral fat values are obtained by eluting the column with dichloromethane, whereas total fat values are determined by using dichloromethane-methanol (9 + 1). For 7 samples analyzed in triplicate, fat ranged from 2.9 to 10.8%. Neutral fat values by the dry column method were significantly lower ($P < 0.05$) than were those by 7.056 (6.29 vs 6.49), although these differences were practically unimportant. Total fat determinations by the dry column method and by 7.056 yielded overall means of 7.40 and 6.49%, respectively. The 0.91% mean difference is significant ($P < 0.01$) and represents a more complete extraction of polar lipids by the proposed method.

Determination of fat content of canned pet foods by the AOAC Soxhlet method 7.056 (1) requires several hours for a complete analysis, uses flammable solvents which require fume hoods and other safety equipment, and produces a "crude" or ether-soluble extract for the fat value. Moreover, crude fat values may be misleading as indicators of fat content because they do not fully represent the potentially large amounts of phospholipids in such foods.

A method of fat extraction previously developed in this laboratory for meat and meat products (2), which alleviates many problems encountered with Soxhlet determinations, has now been modified for analysis of canned pet foods. By use of this simple, dry column procedure, either neutral (crude) or total fat values may be obtained, and the unaltered fats recovered by this method may be analyzed further (3).

METHOD

Reagents and Apparatus

Dichloromethane (DCM) and methanol were obtained from Burdick and Jackson Laboratories (Muskegon, MI 49442). Column packings were granular anhydrous Na_2SO_4 (Malinckrodt, Inc., Paris, KY 40361); $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$; and Celite 545, not acid-washed, Catalog No. C-212 (Fisher Scientific Co., King of Prussia, PA 19406). Celite 545 and $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (9 + 1) were mixed and then stored in a covered glass jar until needed.

A porcelain mortar (750 mL) and pestle (2), a glass chromatographic column ca 25 mm id \times 30 cm long with a drip tip 5 cm \times 8 mm id (prepared locally), and a tamping rod were used.

Preparation of Sample for Analysis

Samples were heated in their cans for 30 min in a 45°C water bath. The cans then were opened, and their contents were mixed in a food processor until uniform. The mixture was reheated at 45°C to ensure uniformity, and then samples for both 7.056 and dry column methods were removed and weighed on disposable tared aluminum weighing pans (use smooth-wall pans).

Procedure

(1) Place glass wool plug loosely into tip of glass column, charge column with 5 g previously prepared Celite 545- CaHPO_4 mixture, and tamp firmly in place. Place preweighed container (150 mL tall-form beaker) under drip tip.

(2) Weigh a sample of canned food (ca 2.5 g) to nearest 0.1 mg and transfer quantitatively to mortar. (Use spatula to transfer bulk of sample, then some Na_2SO_4 from step 3 to pick up remaining sample from weighing dish.)

(3) Add 10 g anhydrous Na_2SO_4 to mortar and mix thoroughly using pestle. Then add ca 7 g Celite 545 and grind until a uniform free-flowing powder is formed (ca 2 min).

(4) Transfer resultant mixture to glass column through powder funnel and firmly tamp in place.

(5) *Neutral (crude) fat determination.*—Rinse mortar with ca 25 mL DCM by use of a large disposable pipet and transfer rapidly to inner column wall. Let solvent pass through column bed until a first drop of eluant appears at drip tip, then immediately add additional 100 mL DCM and let column drip until dry. Collected solvent may be removed during elution by gentle heating and evaporation under nitrogen stream. If fat is required for further analytical studies, eluate may be collected in volumetric flask instead of beaker.

(6) *Total fat determination.*—Follow step 5 except use solvent mixture of DCM-methanol (9 + 1) to wet the column and then use an additional 100 mL to charge column.

(7) After solvent removal from either steps 5 or 6, dry residue in beaker for 30 min at 100°C as specified in sec. 7.056 (1). Determine residue weight and calculate percent (%) fat as (residue wt/sample wt) \times 100.

Results and Discussion

Although the dry column extraction method was previously applied only to meat and meat products (2), no problems were experienced when the method was attempted with canned pet foods. The present work used half the sample size and reagents used in the previous study with no effect on accuracy, and this resulted in a considerable saving in reagents. No nonlipid artifacts were found in the fat extracts, indicating that the CaHPO_4 -Celite 545 trap was effective in retaining nonlipid materials of canned foods. Total elapsed time required to complete an analysis by the dry column method is typically 1.5–2 h including the 30-min drying time needed for the fat residue, which compares favorably with that for a standard AOAC Soxhlet method 7.056 (6–8 h including sample drying time). In addition, the dry column method uses less hazardous solvents, dichloromethane and methanol, rather than flammable ethers.

AOAC method 7.056 measures crude fat, the composition of which may vary with the ether used as solvent. Small but inconsistent amounts of phospholipid are co-extracted with the neutral fat when using ethyl but not petroleum ether (4). The present study confirmed this observation where the phospholipid content of each of the ethyl ether Soxhlet extracts was measured. Each sample contained low levels (0.05–0.50%) of phospholipid (Table 1), suggesting that values obtained for fat content by Soxhlet do not represent a clearly defined portion of total fat in canned processed foods. In contrast, by proper choice of solvent with the column method, one can

Table 1. Comparison of AOAC 7.056 and dry column methods for extraction of crude and total fat and phospholipids from pet foods

Supplier	Type	Dry column			AOAC Soxhlet ^a	
		Total fat, ^{b,c} %	PL, ^{b,d} %	Neutral fat, ^{b,e} %	Crude fat, ^b %	PL, ^{b,d} %
	Dog foods:					
A	Beef	9.60 ± 0.14	0.76	8.52 ± 0.22	8.65 ± 0.15	0.48
B	Chicken	7.80 ± 0.08	0.25	7.05 ± 0.05	7.03 ± 0.06	0.05
A	Lamb	5.18 ± 0.01	0.73	4.32 ± 0.13	4.50 ± 0.25	0.32
A	Liver	6.41 ± 0.06	0.99	5.33 ± 0.03	5.63 ± 0.06	0.48
	Cat foods:					
C	Beef and liver	10.83 ± 0.08	1.37	9.33 ± 0.05	10.05 ± 0.38	0.50
D	Salmon	9.08 ± 0.12	1.43	7.58 ± 0.05	7.58 ± 0.16	0.46
E	Tuna	2.93 ± 0.03	0.89	1.91 ± 0.03	1.99 ± 0.28	0.13
Mean of 7 samples		7.40 ± 0.07	0.92	6.29 ± 0.08	6.49 ± 0.19	0.35

^aAOAC reference method 7.056 (1).

^bMean of 3 subsamples ± std dev.

^cColumn eluted with DCM-methanol (9 + 1).

^dPhosphorus content determined by method of Vaskovsky et al. (5), and expressed as % phospholipid (PL) = 25 × % phosphorus.

^eColumn eluted with DCM only.

accurately determine either the neutral or total fat in a canned food sample. When dichloromethane is used as the sole eluant, only the neutral fat is removed from the sample (3); therefore, no values for recovered phospholipid are shown in Table 1. For the reasons stated, direct comparisons between crude (Soxhlet) and neutral fat values (dry column method) cannot be made. Their values are similar (6.29 vs 6.49%), however, and although the neutral values are significantly lower ($P < 0.05$), the absolute difference between the means is only 3.1%.

The major utility of the dry column method is its ability to obtain values for the *total fat* content of canned foods. Earlier experiments with meat (3) demonstrated that *total fat* was recovered when the dry column was eluted with DCM-methanol (9 + 1). Similar results were obtained in the present work with canned pet foods, where the values for total fat (7.40 vs 6.49%) were significantly ($P < 0.01$) higher than those determined by 7.056 (Table 1). The mean difference (0.91%) between these values reflects, in part, the amounts of additional phospholipid extracted by the proposed method. Moreover, fat recovered by the dry column method is not altered by the extraction process (3) and may be used in subsequent analytical studies. Thus, the dry column fat extraction method

affords the analyst the option of attaining either the neutral (crude) or total fat content of canned pet foods by techniques that are simple to perform. In limited tests with canned processed foodstuffs other than pet foods, the proposed method worked equally well and could be considered as an alternative when these substances are analyzed for fat content.

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