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Determination of Sugars in Yogurt by Gas–Liquid Chromatography

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Individual sugar contents of yogurt sold in the United States were determined by gas–liquid chromatographic analysis of the trimethylsilylated sugar derivatives of freeze-dried samples from 72 composites. Yogurts were obtained from three major grocery stores in each of three cities, Houston, Los Angeles, and Philadelphia, and included 30 brands in three styles of the 10 most popular flavors. Contents of the individual sugars and their sums (total sugar) are reported. Total sugar ranged from 4 to 6% for plain yogurts and from 12 to 18% for flavored yogurts.

Annual per capita consumption of yogurt has risen steadily from 0.25 lb in 1960 to 2.3 lb in 1976 (Kroger, 1978). SAMI (Selling Areas Marketing, Inc.) data indicate an 11% increase in tonnage sales of yogurt in 1982 (*Food Ind. Newsl.*, 1983). The increasing popularity of yogurt among American consumers is reflected in the frequent appearance of new brands and expanded shelf space in supermarkets across the country. This popularity is not surprising in view of the image of yogurt as a convenient, wholesome, high-protein food. Numerous studies, reviewed by Shahani and Chandan (1979) and Deeth and Tamime (1981), have reported the beneficial effects of yogurt in the diet. Recently, Hargrove and Alfrod (1978) and McDonough et al. (1982) found, in controlled studies with rats, that those fed yogurt had increased growth over milk-fed rats.

Carbohydrates, as sugars, are major ingredients in all types of yogurt. Plain yogurt, like milk, contains lactose but also contains galactose produced during fermentation. Flavored yogurts contain other sugars contributed by fruit, fruit preserves, and/or sweeteners. Typically six sugars are found in fruit-flavored yogurts. Relatively few data have been published on the individual sugar contents of yogurts. Southgate et al. (1978) analyzed a small number of plain and flavored yogurts, purchased in Great Britain, using ion-exchange chromatography. Birkhed et al. (1980), in Sweden, included a plain and fruit-flavored yogurt in foods analyzed for sugars and sugar alcohols by gas–liquid chromatography (GLC) but did not report galactose values. Goodenough and Kleyn (1976) used thin-layer chromatography to determine the qualitative and quantitative changes in carbohydrates during the manufacture of unflavored yogurt.

Many analysts consider high-performance liquid chromatography (HPLC) to be the most reliable and rapid method for the determination of individual sugars in foods (Ugrinovits, 1980; Tweeten and Euston, 1980), but the six sugars present in most fruit-flavored yogurts have not been completely separated by any one of the commercially available HPLC columns. Richmond et al. (1982) summarized the current capability for analysis of carbohydrates in dairy products by HPLC. Quantitation of sucrose and lactose is at best uncertain on resin-based columns, and galactose and glucose have not been separated on bonded-phase columns. Barton et al. (1982) have recently reported quantitative separation of glucose and galactose in the neutral sugar hydrolysate of forage cell walls by HPLC. Also, samples of yogurt or other dairy products require considerable cleanup before injection onto either type of HPLC column. We were able to separate and quantitate all the sugars in yogurt in whatever combination they occurred using GLC derivatives (Li and Schuhmann, 1981) prepared from dried samples with no cleanup or extraction. We now report the results of sugar determinations of a nationwide sampling of yogurt including various brands of the most popular flavors.

MATERIALS AND METHODS

Samples. Initial investigation of the market for nonfrozen yogurt indicated that few brands were sold nationwide and that regional brands held a significant share of the market. Because so many brands were available, we prepared composited samples for analysis. We defined three main styles of yogurt on the basis of appearance and label information. In sundae style, plain yogurt is layered above fruit preserves and generally contains active cultures. Swiss style is a stirred product, containing fruit bits and other flavoring throughout. It is usually thickened with gelatin, gums, or modified starch and has been heat-treated after fermentation to inactivate the cultures. A third style, which may be called either blended or French-style yogurt, is a softer gel with uniformly distributed fruit and other flavorings and usually contains active cultures. The sundae and Swiss contain various sweeteners including sugar (Sucrose), corn syrup, honey, fructose, and others. The blended style contains only sucrose as an added sweetener.

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Yogurts, representing 30 brands, were purchased from three major grocery stores in each of three cities; Houston, Los Angeles, and Philadelphia. Buyers were instructed to purchase one container (single-serving size) of each of 10 designated flavors, in every available brand and style, in each store. The number of brands, styles, and flavors varied from store to store, resulting in a chance distribution of numbers of containers per composite (see Table I). Samples, packed with ice, were shipped by air and arrived at our laboratory in Beltsville, MD, during May 1982. Upon apprival, they were composited by homogenizing the contents of all containers of the same flavor and style from each city. An aliquot of each composite was taken for sugar determination. Replicate samples (30–70 mg) were immediately weighed into sample vials, freeze-dried and stored frozen until the time of analysis.

Standard Solutions and Reagents. Sugar standards (Sigma Chemical and Supelco, Inc.) were dried under vacuum before use. Three standard sugar solutions were prepared: standard 1 contained only galactose and lactose in the proportion usually found in plain yogurt; standard 2 contained, in addition to the two sugars, an amount of sucrose similar to that found in coffee and vanilla yogurts; standard 3 contained, fructose, galactose, glucose, lactose, maltose, and sucrose in concentrations approximating those found in fruit-flavored yogurts. Aliquots of the above standard solutions were dried in small vials (Wheaton Scientific) and kept frozen until needed. Pyridine reagent was prepared by dissolving hydroxylamine hydrochloride (25 mg/mL) and an internal standard, phenyl β-D-glucopyranoside (1 mg/mL), in a 1-qt, freshly opened bottle of pyridine (Burdick and Jackson Laboratories, Inc.). Aliquots of this solution were pipetted into 50-mL serum bottles, sealed under nitrogen, and stored below −15 °C. Hexamethyldisilazane (PCR Research Chemicals, Inc.) was similarly transferred from a 500-mL bottle into serum bottles for use as needed. Trifluoroacetic acid (Aldrich Chemical) was used as purchased.

Derivatization. Pyridine reagent (0.5 mL) was added to each vial containing freeze-dried yogurt. Samples were heated at 75 °C for 30 min with vigorous mixing and sonication at intervals. After the samples were cooled briefly, 0.5 or 1.0 mL (depending on the amount of sugars in the sample) of hexamethyldisilazane and 4–6 drops of trifluoroacetic acid were added. The contents were mixed and centrifuged; after 30 min the derivatives were ready for GLC analysis without further treatment.

GLC Analysis. The trimethylsilylated derivatives of sugars were injected automatically onto a 6 ft × $^1/_8$ in. stainless steel column packed with 3% SP2250 on 80–100-mesh Supelcoport. A Hewlett-Packard Model 5840A gas chromatograph equipped with a flame ionization detector was used. Operating conditions were as follows: injection port temperature, 200 °C; detector temperature, 325 °C; column oven temperature programmed from 140 to 300 °C at 5 °C/min or 170 to 300 °C at 10 °C/min; helium carrier flow, 30 mL/min at the starting temperature; hydrogen flow, 40 mL/min; air flow, 300 mL/min; injection volume, 1-μL setting on an autosampler.

RESULTS AND DISCUSSION

Preliminary investigation of sugar contents of yogurt (four brands, two styles, and three flavors) purchased locally indicated that container-to-container variation within brand, style, and flavor for a given lot was no greater than the experimental error. Another study was conducted in order to assess the lot-to-lot variability in each of two brands and three flavors. On three separate occasions, 18 containers of yogurt bearing the same expiration date for each brand and flavor were combined to give six composites, each consisting of three containers. The result of this study, as shown in Table II, indicate that the lot-to-lot variability was low. In a further study, the total sugar values for four brands of blueberry, strawberry, vanilla, and plain yogurt (eight analyses per flavor) showed relative standard deviations of 10, 13, 11, and 19%, respectively. We concluded that within a style, the sugar contents of various brands were similar enough to permit compositing of brands by style and flavor.

Table I is a summary of the sugar contents of 72 composites of yogurts sampled according to the scheme described earlier. The yogurts are listed according to flavor and style. In terms of total sugar content, the yogurts that we analyzed fell into two groups: plain (4–6%) and flavored (12–18%). As for the individual sugar contents, we found three distinct groups (Figure 1): first, plain yogurts that contained only galactose and lactose; second, coffee and most vanilla yogurts that contained glactose, lactose, and sucrose; third, all other fruit-flavored yogurts that contained five or six sugars. Of the last group, none of the blended style yogurts contained any detectable amount (<0.1%) of maltose.

To determine an appropriate sample preparation procedure for GLC analysis of sugars in yogurts, we first tried sequential extraction of dried sample (~1 g wet weight) with *n*-hexane followed by either 80% methanol or deionized water. The extracts containing sugars were again dried and derivatized. Alternatively, homogenized samples (30–70 mg) were weighed directly into GLC sample vials, dried, and derivatized along with the extracts. For individual sugars the values from GLC were very similar between the water extract and the corresponding freeze-dried sample. Values were all (slightly) lower for the methanol extract. The total sugar value for a plain yogurt was 5.01% from the methanol extract, 5.3% from the water extract, and 5.33% from the freeze-dried sample. We concluded that yogurt samples could be prepared for derivatization with no treatment except drying.

For GLC analysis, sugars can be converted to their volatile derivatives with a number of reagents. Of these, trimethylsilylating compounds are used most frequently because the procedure is rapid and simple. However, most trimethylsilylated ethers of sugars give multiple peaks corresponding to various isomeric forms. This problem can be minimized by first converting the reducing sugars (namely, the aldoses and ketoses) to their oximes before trimethylsilylating the polyhydroxy groups (Mason and Slover, 1971). Of the six sugars found in most fruit-flavored yogurts that we analyzed, all except galactose gave single peaks on the SP2250 column. The smaller of the two peaks of galactose coeluted with glucose. For quantitation of each individual sugar, particularly those two hexoses, we prepared several standard solutions containing varying amounts of each sugar. The concentrations of the sugars in these standards covered the ranges found in the flavored yogurts. The average relative standard deviation (RSD) of the response factors for all six sugars in each of the three mixtures was about 1%. Even when response factors were compared among standards, the average RSD was no more than 1.5% except for glucose, which was of course affected by the amount of the coeluting galactose. Since the area of the minor galactose peak in the pure standard is consistently one-third that of its major peak, the calculated peak area due to galactose can be subtracted from a given glucose peak area. When this correction was made, the RSD for glucose response factor among the standards was 2.5%. The amounts of galactose and glucose

Table I. Sugar Contents of Yogurts

flavor	style[a]	no. of containers	no. of composites	g/100 g wet wt						
				fructose	galactose	glucose	sucrose	lactose	maltose	total sugar
blueberry	A	17	3	3.67 ± 1.17[b]	0.86 ± 0.07	4.11 ± 1.12	2.63 ± 2.33	3.29 ± 0.06	0.56 ± 0.11	15.1 ± 0.31
	B	15	3	3.38 ± 0.97	0.82 ± 0.03	3.66 ± 1.12	4.42 ± 1.76	2.99 ± 0.56	0.49 ± 0.39	15.8 ± 0.40
	C	12	3	1.44 ± 0.29	0.84 ± 0.16	1.45 ± 0.24	7.55 ± 0.06	3.93 ± 0.21	ND[c]	15.2 ± 0.80
cherry	A	7	3	2.98 ± 2.06	0.87 ± 0.12	3.12 ± 1.90	6.46 ± 2.52	3.62 ± 0.46	0.61 ± 0.02	15.3 ± 0.14
	B	9	3	2.31 ± 0.98	0.89 ± 0.16	2.78 ± 1.11	5.84 ± 1.95	2.80 ± 0.11	0.41 ± 0.29	14.9 ± 0.32
	C	7	2	2.18 ± 1.15	0.99 ± 0.12	2.28 ± 1.02	6.53 ± 1.28	3.83 ± 0.19	ND	15.8 ± 1.11
coffee	A*	5	2	ND	0.88 ± 0.16	ND	9.47 ± 0.35	4.06 ± 0.45	ND	14.4 ± 0.14
lemon	A	9	3	3.49 ± 0.16	1.11 ± 0.12	4.02 ± 0.17	9.91 ± 0.26	3.60 ± 0.37	ND	13.8 ± 0.97
	B	7	3	1.61 ± 0.81	0.82 ± 0.14	2.44 ± 0.65	7.00 ± 0.86	3.69 ± 1.01	0.44 ± 0.00	15.7 ± 0.57
	C	6	2	1.45 ± 0.45	1.01 ± 0.11	1.47 ± 0.40	10.7 ± 1.09	4.58 ± 0.26	ND	19.2 ± 0.72
peach	A	15	3	2.25 ± 0.83	0.89 ± 0.07	2.84 ± 0.67	5.10 ± 2.88	2.79 ± 0.26	0.52 ± 0.24	14.4 ± 1.60
	B	16	3	1.79 ± 1.11	0.92 ± 0.11	2.17 ± 1.28	7.44 ± 2.23	2.30 ± 0.19	0.34 ± 0.22	14.8 ± 0.82
	C	9	3	0.87 ± 0.28	0.92 ± 0.07	0.97 ± 0.25	10.8 ± 0.97	3.27 ± 0.04	ND	16.8 ± 0.62
pineapple	A	7	2	3.31 ± 0.76	0.89 ± 0.10	3.88 ± 0.46	3.28 ± 3.61	3.94 ± 0.29	0.53 ± 0.42	15.8 ± 2.48
	B	10	3	1.71 ± 1.01	0.80 ± 0.02	1.87 ± 1.00	7.86 ± 1.75	3.39 ± 0.39	0.26 ± 0.004	15.8 ± 0.39
	C	4	2	1.51 ± 0.09	0.81 ± 0.17	1.49 ± 0.04	9.25 ± 0.19	4.32 ± 0.19	ND	17.4 ± 0.34
plain	A*	16	3	ND	1.44 ± 0.07	ND	ND	3.68 ± 0.12	ND	5.12 ± 0.08
	B	2	1	ND	1.57 ± 0.02	ND	ND	2.91 ± 0.01	ND	4.53 ± 0.01
	C	1	1	ND	1.33 ± 0.01	ND	ND	4.93 ± 0.12	ND	6.26 ± 0.11
raspberry	A	20	3	3.00 ± 0.62	0.88 ± 0.07	3.54 ± 0.49	3.68 ± 1.76	3.99 ± 0.11	0.69 ± 0.19	15.8 ± 0.92
	B	18	3	2.89 ± 0.50	0.80 ± 0.04	3.09 ± 0.51	4.78 ± 0.78	3.76 ± 0.59	0.32 ± 0.09	15.6 ± 0.59
	C	12	3	1.27 ± 0.48	0.87 ± 0.12	1.33 ± 0.38	9.32 ± 1.53	4.43 ± 0.14	ND	17.2 ± 1.00
strawberry	A	17	3	2.56 ± 0.79	0.98 ± 0.09	3.40 ± 0.68	4.45 ± 1.81	3.28 ± 0.29	0.68 ± 0.20	15.3 ± 0.70
	B	19	3	2.23 ± 1.52	0.99 ± 0.17	2.65 ± 1.79	7.51 ± 2.70	2.92 ± 0.33	0.54 ± 0.04	16.5 ± 0.90
	C	19	3	0.57 ± 0.21	0.91 ± 0.25	0.87 ± 0.11	10.1 ± 0.23	4.17 ± 0.45	ND	16.3 ± 0.37
vanilla	A*	10	3	ND	1.12 ± 0.12	ND	7.79 ± 0.19	3.58 ± 0.17	ND	12.5 ± 0.23
	B	4	2	1.78 ± 0.03	0.89 ± 0.05	2.76 ± 0.05	7.53 ± 1.26	2.81 ± 0.16	ND	13.5 ± 1.29
	C	1	1	ND	0.37 ± 0.00	ND	9.30 ± 0.32	3.57 ± 0.14	ND	13.2 ± 0.47

[a] A = sundae; A* = similar to sundae style but without any fruit on the bottom; B = Swiss; C = blended (or French). [b] Mean and standard deviation. [c] ND = not detectable; less than 0.1 g/100 g.

Table II. Sugar Contents of Yogurts: Variation between Lots[a] and Brands

flavor	brand	g/100 g wet wt fructose	galactose	glucose	sucrose	lactose	maltose	total sugar
plain	X	ND[b]	1.21 ± 0.08[c]	ND	ND	3.65 ± 0.29	ND	4.86 ± 0.33
	Y	ND	1.35 ± 0.11	ND	ND	4.17 ± 0.45	ND	5.52 ± 0.35
vanilla	X	ND	0.98 ± 0.04	ND	7.75 ± 1.23	3.22 ± 0.15	ND	11.90 ± 1.20
	Y	ND	1.02 ± 0.09	ND	8.16 ± 0.30	3.79 ± 0.27	ND	13.00 ± 0.42
strawberry	X	3.58 ± 0.43	0.79 ± 0.08	4.70 ± 0.48	5.48 ± 0.52	2.86 ± 0.09	1.28 ± 0.04	18.70 ± 0.40
	Y	2.57 ± 0.18	0.98 ± 0.03	3.01 ± 0.21	5.24 ± 1.33	3.21 ± 0.15	1.33 ± 0.28	16.40 ± 1.10

[a] Three lots; six composites per lot. [b] ND = not detectable, i.e., less than 0.1 g/100 g. [c] Mean and standard deviation.

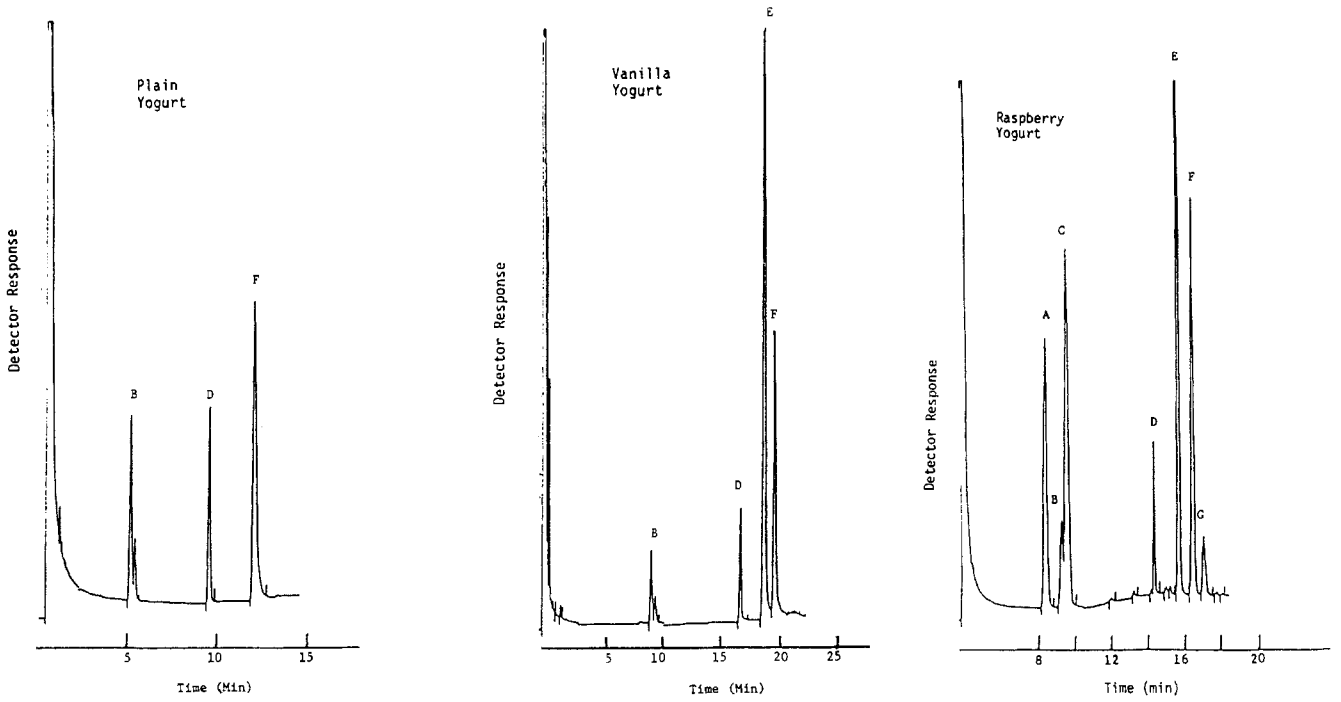

Figure 1. Gas chromatograms of Me_3Si derivatives of sugars in yogurts. Column, SP-2250 on 80–100-mesh Supelcoport, 6 ft × 1/8 in. stainless steel; flow rate, 30 mL/min, He; detector, FID; temperature, 170–300 °C at 10 °C/min (plain) and 140–300 °C at 5 °C/min (vanilla and raspberry). (A) Fructose; (B) galactose; (C) glucose (galactose); (D) phenyl β-glucopyranoside; (E) sucrose; (F) lactose; (G) maltose.

in a given flavored yogurt sample were comparable whether the values were obtained by manual subtraction of the galactose peak area contribution to glucose or by direct calibration of a closely matched standard mixture. The standards for plain yogurt contained only galactose and lactose in the expected proportion. In quantitating galactose, we used only its major (first) peak.

Additional trials were made to assess the reliability of the separation of glucose from galactose in concentrations outside of those encountered in the yogurt samples. Known amounts of glucose were added to plain yogurt samples. The apparent amount of glactose fell from 98% to 85% of the expected amount when the added glucose was increased from 3 to 7 mg/100 mg. The glucose value (corrected for galactose) averaged 103.8 ± 1.2%. In trials of recovery of extra galactose added to a blueberry yogurt sample, the apparent galactose value rose from 99 to 108% of theoretical as the added galactose increased from 0.8 to 10 mg/100 mg. The glucose measured was 99.5 ± 1.3% of the expected value.

In conclusion, we believe that the method described is convenient and reliable when the following points are kept in mind. Samples must be weighed while fresh; after freeze-drying, they may be stored and derivatized at a later time. Derivatization is reproducible if sufficient shaking is applied during the first step. Quantitation of glucose and galactose is dependent upon a closely matched standard. Using the procedures described above, we analyzed a large number of composited yogurt samples and, based on sugar profiles, identified three types among the 10 most popular flavors.

Registry No. Fructose, 57-48-7; galactose, 59-23-4; glucose, 50-99-7; sucrose, 57-50-1; lactose, 63-42-3; maltose, 69-79-4.

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Nutritional Value of the Fluted Pumpkin (*Telfaria occidentalis*)

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The proximate, mineral, fatty acid, amino acid, and carbohydrate compositions of several parts of the fruit of the fluted pumpkin were analyzed. Meals were prepared from raw full-fat (RFM), cooked full-fat (CFM), raw defatted (RDM), and cooked defatted (CDM) seeds without testa and their nutritive values determined by using rats. The seed contained 53% fat and 27% crude protein. Oleic and linoeleic acids were the predominant fatty acids while glutamic acid, arginine, and aspartic acid were the most abundant amino acids. RFM and RDM gave low digestibility values which were improved by cooking and oil extraction. Metabolizable energy (ME), which was 20.5 and 13.4 kJ/g for RFM and RDM, respectively, increased on cooking.

The search for lesser known crops, many of which are potentially valuable as human and animal foods, has been intensified to maintain a balance between population growth and agricultural productivity, particularly in the tropical and subtropical areas of the world. The value of cucurbit seeds as useful sources of proteins and oils has been reviewed by Jacks et al. (1972), Bemis et al. (1967, 1975), and Tu et al. (1978).

The fluted pumpkin (*Telfaria occidentalis*), a tropical cucurbit, may be a possible source of such nutrients. It is a fast growing, climbing annual that bears heavy fruits which are furrowed. Mature fruits, weighing between 2 and 5 kg, contain many seeds. The young green leaves form a very delicious vegetable when cooked, and the young seeds are sometimes eaten when cooked. The seed contains a smooth flowing, golden-yellow oil.

While extensive work has been accomplished to elucidate the nutritional qualities of cucurbits (Zucker et al., 1958; Oyenuga and Fetuga, 1975; Berry et al., 1976), little information is available on the composition or nutritive value of the fluted pumpkin.

The present report accounts for the chemical composition of various parts of the fruit and the nutritional quality of the seed.

EXPERIMENTAL SECTION

Materials. Mature, fresh fluted pumpkin fruits were obtained from the International Institute of Tropical Agriculture and National Horticultural Research Institute of Nigeria. The fruits were carefully separated into seeds, pulp, and husk and sun-dried. The testa was removed from the cotyledons of the dried full-fat seeds (RFM) and milled while some other fresh seeds were boiled for 2 h in water. The deep purple water produced was changed after 1 h of boiling to obtain the cooked full-fat sample (CFM) which was also dried and milled. Seed meals (RFM and CFM) were ether extracted for 7 h and defatted meals (RDM and CDM, respectively) obtained were spread on trays and air-dried to expel residual ether.

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Methods. All samples were analyzed for proximate composition by methods of the Association of Official Analytical Chemists (1970). Gross energy was determined in a Gallenkamp oxygen ballistic bomb calorimeter by using thermochemical-grade benzoic acid as a standard. The ground samples were wet-ashed in concentrated sulfuric acid–concentrated perchloric acid–concentrated nitric acid (0.5:1.0:5.0 by volume), and the metallic elements were determined after dilution by using a flame atomic absorption spectrophotometer (Model No. 703, Perkin-Elmer). Phosphorus was determined colorimetrically as the phosphomolybdovanadate complex. Chemical interference due to PO_4^{3-} on calcium and magnesium was eliminated by the addition of lanthanum chloride (Perkin-Elmer, 1980). The carbohydrates and lignin were fractionated as outlined by Southgate (1969a,b), and sugars were quantified as total sugars (Dubois et al., 1956).

The fatty acid components of the total lipid extract was determined by converting an aliquot into methyl esters (Metcalfe and Schmitz, 1961) which were separated by gas–liquid chromatography. Amino acids of seed samples were determined in a Technicon automatic sequential amino acid analyzer after hydrolysis of test materials in 6 M hydrochloric acid at 110 °C for 24 h. For the determination of sulfur amino acids, the samples were first oxidized with performic acid for 18 h according to the method of Lewis (1966) before acid hydrolysis. Tryptophan was chemically determined by the method of Miller (1967).

Animal Feeding Experiment. Weanling albino rats of the Wistar strain weighing between 45 and 50 g were distributed into treatment groups of four male rats each on the basis of weight and litter origin such that mean group initial weights were identical. They were individually housed in cages that allowed for separate fecal and urinary collection and the measurement of food intake. The protein quality of the raw and cooked seed meals was assayed by using the digestibility procedure. The experiment lasted 21 days, the first 7 days being the preliminary period. True protein digestibility was calculated by the method of Mitchell and Carman (1926). The energy trial was carried out according to the procedures followed by

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