Harding, P. L., Sunday, M. B., *Citrus Ind.* **45**(10), 7 (1964).
Harding, P. L., Winston, J. R., *Proc. Fla. State Hortic. Soc.* **52**, 90 (1939).
Harding, P. L., Winston, J. R., Fisher, D. F., *U.S. Dept. Agric. Tech. Bull.* **753** (1940).
Higby, W. K., *Food Technol.* **17**, 95 (1963).
Hilgeman, R. H., Dunlap, J. A., Sharp, F. O., *Proc. Am. Soc. Hortic. Sci* **90**, 103 (1967).
Hitchcock, C., Nichols, B. W., "Plant Lipid Biochemistry", Academic Press, New York, N.Y., 1971, p 201.
Kalra, S. K., Brooks, J. L., *Phytochemistry* **12**, 487 (1973).
Kefford, J. F., Chandler, B. V., "The Chemical Constituents of Citrus Fruits", Academic Press, New York, N.Y., 1970.
Miller, E. V., Winston, J. R., Fisher, D. F., *U.S.* Dept. *Agric. Tech. Bull.* **780** (1941).
Nagy, S., Nordby, H. E., *J. Agric. Food Chem.* **18**, 593 (1970).
Nagy, S., Nordby, H. E., *Phytochemistry* **10**, 2763 (1971).
Nagy, S., Nordby, H. E., Smoot, J. M., *J. Am. Oil Chem. Soc.* **52**, 121 (1975).
Nagy, S., Shaw, P. E., Veldhuis, M. K., Ed., "Citrus Science and Technology", Vol. 1, Avi Publishing Company, Westport, Conn., 1977.
Nordby, H. E., Nagy, S. *Lipids* **6**, 554 (1971).
Nordby, H. E., Nagy, S., *J. Agric. Food Chem.* **25**, 224 (1977).
Pennisi, L., Scuderi, H., Muratore, A., *Riv. Agric.* **1**, 401 (1955).
Rasmussen, G. K., Jones, J. W., *Hortic. Sci.* **4**, 60 (1969).
Rasmussen, G. K., *Plant Physiol.* **56**, 765 (1975).
Rotstein, A., Gross, J., Lifshitz, A., *Lebensm.-Wiss. Technol.* **5**, 140 (1972).
Rouse, A. H., Atkins, C. D., Moore, E. L., *J. Food Sci.* **27**, 419 (1962).
Rouse, A. H., Atkins, C. D., Moore, E. L., *J. Food Sci.* **29**, 34 (1964).
Rouser, G., Kritchevsky, G., Yamamoto, A., in "Lipid Chromatographic Analysis", Vol. 1, Marinetti, G. V., Ed., Marcel Dekker, New York, N.Y., 1967.
Roy, W. R., *J. Agric. Res.* **70**, 143 (1945).
Sacher, J. A., *Nature (London)* **195**, 577 (1962).
Schneider, H., in "The Citrus Industry", Vol. 2, Reuther, W., Batchelor, L. D., Webber, H. J., Ed., University of California Press, Riverside, 1968, p 1.
Sinclair, W. B., Ed., "The Orange: Its Biochemistry and Physiology", University of California Press, Riverside, 1961.
Sinclair, W. B., Jolliffe, V. A., *Bot. Gaz.* **119**, 217 (1958).
Sinclair, W. B., Jolliffe, V. A., *J. Food Sci.* **26**, 125 (1961).
Sites, J. W., Reitz, H. J., *Proc. Am. Soc. Hortic. Sci.* **55**, 73 (1950).
Sites, J. W., Reitz, J. H., *Proc. Am. Soc. Hortic. Sci.* **56**, 103 (1951).
Ting, S. V., *Fla. Citrus Exp. Stn. Mimeo Rpt.* **55-5** (1954).
Ting, S. V., *Proc. Fla. State Hortic. Soc.* **80**, 257 (1967).
Ting, S. V., in "Citrus Science and Technology", Vol. 2, Nagy, S., Shaw, P. E., Veldhuis, M. K., Ed., Avi Publishing Company, Westport, Conn., 1977, p 401.
Ting, S. V., Attaway, J. A., in "The Biochemistry of Fruits and Their Products", Vol. 2, Hulme, A. C., Ed., Academic Press, New York, N.Y., 1971, p 107.
United States Department of Agriculture, "United States Standards for Grades of Canned Orange Juice", 8th ed, Washington, D.C., 1969.
Vandercook, C. E., Guerrero, H. C., Price, R. L., *J. Agric. Food Chem.* **18**, 905 (1970).
Vandercook, C. E., Price, R. L., *J. Food Sci.* **37**, 384 (1972).
Wooltorton, L. S. C., Jones, J. D., Hulme, A. C., *Nature (London)* **207**, 999 (1965).
Wuthier, R. E., *J. Lipid Res.* **7**, 558 (1966).
Zamorani, A., Russo, C., Galoppini, C., *Essenze Deriv. Agrum.* **42**, 357 (1972).

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Individual Lipids and Proximate Analysis of Various Foods. 4. Commercial Cake Mixes

Theodore S. Rudolf, Willard D. Hubbard,* David R. Newkirk, and Alan J. Sheppard

Commercially prepared cake mixes were purchased from several supermarkets. Samples were analyzed for water, protein, total fat, ash, fatty acids, sterols, and *cis,cis*-methylene interrupted polyunsaturated triglycerides. The data indicate that in the process of making cake mixes the manufacturer used either vegetable oil alone or a mixture of animal and vegetable fat. Animal fat alone was not used in the mixes tested. The amount of total fat ranged from 8.2 to 15.3 g/100 g of product and the cholesterol from 0 to 22 mg/100 g of product.

Many consumers now use commercially prepared cake mixes. Therefore, information concerning the content of cholesterol and saturated vs. polyunsaturated fatty acids would be helpful to the consumer in the selection of these mixes. Some consumers may add a small amount of fat or oil to enhance flavor or texture and/or use milk instead of the water which is specified in the instructions. However, these additions probably would not significantly alter the quantity of sterols or fatty acids found in the cake mixes as prepared by the manufacturer.

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MATERIAL AND METHODS

Eighteen cake mixes were selected from seven different brands for the analyses. The brands were: Betty Crocker, Duncan Hines, Mrs. Wright's, Giant Food, Grand Union, Ann Page, and Washington. The following types of cake mixes were included in the study: yellow (No's. 2, 5, 7, 9, 13, 14, and 16), white (No's. 4, 11, and 18), chocolate (No's. 1, 6, 8, 10, 12, 15, and 17), and spice (No. 3).

The samples were homogenized in a Waring blender using chloroform–methanol extractant (2:1, v:v), as previously described by Sheppard et al. (1974). The fatty acids were esterified using boron trifluoride–methanol according to the Association of Official Analytical Chemists (AOAC, 1975) procedure as modified by Solomon et al.

Table I. Fatty Acid and *cis,cis*-Methylene Interrupted Polyunsaturated Fatty Acid Content (g/100 g of Product) of Commercial Cake Mixes[a]

Cake mix	Fatty acid methyl esters									*cis,cis*-Trilinolein
	C12	C14	C14:1	C16	C16:1	C18	C18:1	C18:2	C18:3	
1	ND[b]	0.3	ND	2.6	ND	2.3	3.9	0.5	ND	0.5
2	ND	0.3	ND	2.5	ND	2.1	3.8	0.5	Tr[c]	0.6
3	0.4	0.2	ND	1.0	ND	1.3	3.8	0.8	ND	0.8
4	ND	0.1	ND	1.0	ND	1.2	3.9	0.9	ND	0.6
5	ND	Tr	ND	1.0	ND	0.8	6.0	1.7	Tr	1.7
6	ND	Tr	ND	1.7	ND	1.3	9.2	2.5	0.1	2.6
7	0.2	0.3	ND	2.2	ND	1.9	3.3	0.3	Tr	0.5
8	ND	0.3	ND	2.5	ND	2.2	3.8	0.4	ND	0.5
9	ND	0.3	ND	2.4	ND	2.0	3.6	0.5	Tr	0.5
10	ND	0.2	ND	2.3	ND	2.2	3.5	0.4	ND	0.5
11	0.1	Tr	ND	1.3	ND	1.2	3.1	1.7	0.2	2.1
12	0.1	Tr	ND	1.3	ND	1.4	2.9	1.5	0.2	1.8
13	0.1	Tr	ND	1.2	ND	1.2	2.6	1.4	0.1	1.8
14	Tr	0.2	Tr	2.0	0.2	2.1	2.9	1.0	0.1	1.0
15	0.1	0.1	ND	1.3	Tr	1.6	3.3	0.9	ND	0.6
16	Tr	0.1	Tr	1.5	0.2	1.5	2.0	0.5	0.1	0.8
17	Tr	0.1	ND	1.6	0.1	1.7	2.2	0.8	0.1	0.7
18	Tr	0.2	Tr	1.6	0.2	1.4	2.3	0.7	0.1	0.7

[a] Average of duplicate analyses. [b] ND = none detected. [c] Trace = <0.1 g/100 g of product.

Table II. Proximate Analysis and Sterol Content of Commercial Cake Mixes[a]

Cake mix	Proximate analysis, g/100 g of product				Sterol, mg/100 g of product			
	Water	Protein[b]	Ash	Total fat	Cholesterol	Campesterol	Stigmasterol	Sitosterol
1	3.9	4.6	3.5	11.3	11	2	ND[c]	10
2	3.4	4.1	2.2	10.8	12	3	ND	9
3	3.9	4.5	2.6	9.2	1	7	4	16
4	3.9	5.0	1.8	8.6	ND	8	5	16
5	4.6	5.2	1.4	10.1	ND	7	4	18
6	3.4	5.2	1.6	15.3	ND	8	5	19
7	3.6	4.1	2.3	9.7	8	2	ND	9
8	3.7	4.7	3.2	11.7	12	2	ND	10
9	3.0	4.2	2.2	10.3	10	3	ND	10
10	3.3	4.8	3.7	9.6	10	2	ND	9
11	4.3	4.0	2.0	10.8	ND	18	17	29
12	3.9	4.7	2.5	9.5	ND	17	16	27
13	3.8	3.9	2.1	8.8	ND	16	15	26
14	3.7	4.0	2.2	11.8	22	13	ND	20
15	3.2	4.7	2.6	10.2	ND	16	Tr[d]	25
16	4.7	4.0	2.1	8.2	14	13	ND	22
17	3.9	4.6	2.4	8.5	20	14	ND	21
18	4.2	4.0	2.2	8.7	20	15	14	20

[a] Average of duplicate analyses. [b] Conversion factor for protein is 6.25. [c] ND = none detected. [d] Trace = <0.1 g/100 g of product.

(1974). A method described by Sheppard et al. (1974) was used to prepare the butyrate derivatives for sterol analysis. Official methods of AOAC (1975) were used for the proximate analysis. Methods described by Sheppard et al. (1974) were used for the lipoxidase analyses.

RESULTS AND DISCUSSION

Tables I and II present average values of duplicate analyses for each cake mix as purchased from the supermarket. (Data are given in g/100 g of product; a serving size is one-seventh of an 8-in. diameter cake, approximately 70 g.) Seven of the cake mixes contained only plant sterols, indicating that only vegetable oils were used in their manufacture. The remaining cake mixes contained both cholesterol and plant sterols, confirming the presence of both animal and vegetable fats. No product contained cholesterol alone. This demonstrates that none of the cake mixes was made solely with animal fat. Some cake mixes require the addition of one or two eggs. This would, of course, increase the cholesterol content of the cakes significantly.

There was no appreciable variation in the results of the analyses; therefore, we conclude that not only the types of cakes within brands but also the different brands are made with approximately the same formulation.

Polyunsaturated fatty acids determined by using gas–liquid chromatography compared closely with those obtained by the lipoxidase method. The largest deviation between results from the two methods was 0.3 g/100 g, which is within experimental error; this indicates that both the trans and conjugated fatty acid content were below the detectable level in the products examined.

The fatty acids and proximate analysis data obtained in this study agree with those in the U.S. Department of Agriculture Handbook No. 8 (Watt and Merrill, 1963). However, Handbook No. 8 does not provide cholesterol or other sterol data for cake mixes. The present study provides information for use by nutritionists, dietitians, and medical personnel in designing diets for specialized applications.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, D.C., 1975, Sections 28.057–28.060.

Sheppard, A. J., Hubbard, W. D., Prosser, A. R., Interim Methodology Instructions No. 2 with modification No. 1 dated

April 16, 1975, for Implementing Requirements of Section 1.18 of Title 21, Chapter 1, Subchapter A, Part I ("Labeling of Foods in Relation to Fat, Fatty Acid and Cholesterol Content") issued June 11, 1974, Division of Nutrition, Bureau of Foods, Food and Drug Administration, Washington, D.C. 20204.
Solomon, H. L., Hubbard, W. D., Prosser, A. R., Sheppard, A. J., *J. Am. Oil Chem. Soc.* 51, 424 (1974).
Watt, B. K., Merrill, A. L., U.S. Department of Agriculture Handbook No. 8, "Composition of Foods", Washington, D.C., 1963, p 20.

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Iron Absorption by Rats from Nonprescription Dietary Iron Supplements

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Absorption of iron from seven nonprescription dietary iron supplements was measured in both iron-replete and iron-depleted rats by use of the extrinsic label technique. Both ferrous and ferric forms of the radioactive extrinsic tracer iron were used; the iron was in the form of $^{59}FeSO_4$, $^{59}FeCl_3$, or ferrous-^{59}Fe gluconate. Of six products tested with both ferrous and ferric labels, significant differences in absorption measured by the two different labels were found for three products. This suggests that there is incomplete exchange between the intrinsic and extrinsic iron pools. Iron-deficient animals absorbed significantly more iron than iron replete animals when the ferrous label was used in both experiments. Although iron-replete animals discriminate among iron sources, absorption is always less than in iron-deficient animals. The results with iron deficient animals show the true bioavailability of the iron. The use of the extrinsic label may not always be valid when the metal in question can exist in more than one oxidation state.

The absorption of iron from various food products has been estimated by use of an extrinsic radioactive iron tracer (Björn-Rasmussen et al., 1972, 1973; Björn-Rasmussen, 1973; Monsen, 1974). The extrinsic iron exchanges with the intrinsic non-heme iron of the food and can thus be used to determine absorption. However, much of the iron in some foods, especially breads and cereals, is present as inorganic iron which is added as fortification, rather than being biosynthetically incorporated into the food. The use of supplementary iron salts in tablet form is widespread. We wished to examine the use of the extrinsic label method to measure absorption of such inorganic iron which is not necessarily in the same chemical form as the radioactive tracer. We chose to examine iron absorption from a number of over-the-counter dietary iron supplements that contained iron in various forms and varying amounts of vitamins, other minerals, binders, sweeteners, and alcohol. Absorption was measured in iron replete rats with the use of radioactive tracers in both the ferrous and ferric state and in iron-deficient animals with the use of a ferrous label.

MATERIALS AND METHODS

Iron supplements were purchased from local supermarkets and drug stores. Solubility of the iron in the nonliquid iron supplements was measured in HCl and in water. One tablet of a supplement was placed in 25 mL of 1.5 M HCl and shaken at 37 °C for 18 h. Disintegration of the tablets was checked at 5-minute intervals for 1 h, at 2 h and at 18 h. After 18 h, any large particles remaining were pulverized. One hour later the solutions were filtered, and iron was determined by atomic absorption. We calculated the percent solubility using the iron content per tablet given on the label as the 100% value. Solubility of the iron in water was determined by grinding the tablets with a mortar and pestle, quantitatively transferring the powder to a graduated cylinder, and mixing it with a known amount of water for 20 min. The iron in solution was measured by atomic absorption spectrophotometry and the percent solubility was calculated as for the HCl solutions.

To determine whether ferrous iron had been oxidized, solid samples were ground and mixed with water as in the solubility tests. Liquid samples were diluted with water to appropriate concentrations for analysis. Total iron was determined using 1,10-phenanthroline (Sandell, 1944) with hydroxylamine hydrochloride as a reducing agent. Ferrous iron was determined using 1,10-phenanthroline in assay mixtures without addition of hydroxylamine hydrochloride. Ferric iron was determined by difference.

The ferrous-^{59}Fe gluconate was prepared from $^{59}FeSO_4$ (ICN Pharmaceuticals, Inc., Chemical and Radioisotope Division). Dowex-1 anion-exchange resin, chloride form (Sigma Chemical Co.) was washed with 1 N NaOH until the addition of $AgNO_3$ to the wash showed no further elution of chloride ion. It was then washed with water until pH <9 and with 1 N gluconic acid until pH <2. It was rinsed with water until pH >4 before the sample was applied. A column 2 cm tall was packed in the lower portion of a pasteur pipet. Approximately 50 μg of ^{59}Fe as $FeSO_4$ was applied to the column and eluted with 0.05 M gluconic acid. To check that the iron was eluted as ferrous gluconate, a larger column (1 × 13.5 cm) was prepared and 0.14 g of $FeSO_4 \cdot 7H_2O$ in 0.5 mL of H_2O was applied and eluted in the same manner. The iron in the effluent gave a positive test for ferrous ion with potassium ferricyanide. A test for sulfate ion with 5% $BaCl_2$ was negative.

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