Comparisons of Methods for Calculating Retentions of Nutrients in Cooked Foods

Elizabeth W. Murphy,* Patricia E. Criner,[1] and Brucy C. Gray

Six types of weight changes that occur when food is cooked by different methods are described. For five of these types, true retentions of nutrients (defined as calculations based on nutrient content of known weights of food before and after cooking) were compared with apparent retentions (defined as calculations based on nutrient content of moisture-free raw and cooked foods). Comparisons included retentions of proximate components, minerals, and vitamins. Apparent retentions overestimated the true retentions in nearly all instances. To avoid bias, true retentions should be reported whenever it is feasible to obtain data on weights of foods before and after cooking.

Accurate knowledge of the nutrient intake of individuals and groups of people requires information on the nutrient content of cooked foods. Many dietary calculations are made on the basis of foods as brought into the kitchen. Factors are needed that can be applied to weights of raw foods to correct for nutrient losses or changes in preparation. This paper presents some of the problems encountered in establishing accurate retention factors.

A true retention should measure the proportion of nutrient remaining in the cooked food in relation to the amount of that nutrient originally present in a given weight of the food before cooking. Thus, the direct measure of true retentions requires data on the weights of the food both before and after cooking, as well as the contents of the nutrient per gram (or other unit of weight) of raw and cooked food.

To provide maximum useful data, studies on retentions of nutrients in foods should be planned so that analyses are made on comparable raw and cooked samples. For meats, fish, and poultry, anatomically matched cuts representing opposite sides of the same carcass should be analyzed raw and after being cooked. From well-mixed lots of raw foods such as vegetables, legumes, and shellfish, subsamples for cooking should be carefully drawn, and similarly chosen subsamples should be analyzed raw. Weights of products before and after cooking should be recorded, along with weights of drippings, cooking water, or other discard. Weights and analyses of discard are needed if a total accounting for all nutrients originally present in the raw food is sought, so that solubility losses, as well as destruction, are known. Keeping records of weights may not always be feasible in studies involving production-line processing, but should be possible in research involving institutional and home cooking. Unfortunately, few studies have been reported which were designed to provide the information just described.

To circumvent problems associated with obtaining weights, many researchers have reported apparent retention values. The apparent retention is here defined as the ratio of nutrient content in the cooked food without discard to nutrient content in the raw food, with both values expressed on the moisture-free basis. The use of apparent rather than true retentions involves the assumption that solids are not lost to any practical extent with cooking. This assumption is clearly not valid for meats, which give up both fat and protein to the drippings when cooked; it is probably not valid for vegetables, legumes, and many cereal products, either.

Nutrient Data Research Center, Consumer and Food Economics Institute, Agricultural Research Service, U.S. Department of Agriculture, Federal Building, Hyattsville, Maryland 20782.
[1] Deceased.

Several types of weight changes occur when food is cooked. These are: (type 1) volatiles (primarily moisture) lost; example, vegetables cooked by steaming; (type 2) moisture gained; example, rice cooked so that all of the water is absorbed; (type 3) solids lost but moisture gained; example, dry legumes cooked in water which is not completely absorbed; remaining liquid is discarded; (type 4) solids and moisture both lost; example, organ meats cooked in water; (type 5) solids and moisture lost from more than one tissue; example, roasted poultry, which contains lean muscle, skin, and sometimes depot fat; (type 6) moisture lost and fat or other solids gained; example, doughnuts and other foods fried in deep fat.

Data from research done under the sponsorship of the Agricultural Research Service have provided the opportunity to compare true retentions with apparent retentions on the same food samples for a number of foods which show changes with cooking of types 1 through 5. These data compare apparent retentions (AR), calculated as follows:

$$\% \text{ AR} = [\text{nutrient content per g of cooked food (dry basis)}]/[\text{nutrient content per g of raw food (dry basis)}] \times 100$$

with true retentions (TR), calculated as follows:

$$\% \text{ TR} = (\text{nutrient content per g of cooked food} \times \text{g of food after cooking})/(\text{nutrient content per g of raw food} \times \text{g of food before cooking}) \times 100$$

Data for cooked foods used in the calculations of retentions did not include the nutrient content of any cooking discard such as drippings. Table I gives references to the analytical methods used in obtaining the nutrient data from which retentions were calculated.

TYPE 1 CHANGE, MOISTURE LOSS ONLY

Retentions were calculated for five nutrients in 4 to 27 vegetables which had been cooked by steaming. Vegetables were cooked in aluminum pans over boiling water. No salt, fat, or other ingredients were added. The few grams of water that condensed in the cooking pan during steaming were included as part of the cooked sample. Information on cooking time and degree of doneness was not available.

Table II shows data comparing true retentions with apparent retentions. For these vegetables, retentions were essentially complete, and differences between the two calculation procedures were not significant, as indicated by paired "t" tests.

Data on true retentions for 13 nutrients in six lots of oven-roasted peanuts have been published (Derise et al., 1974). In addition, the present authors calculated apparent retentions for these same samples. Peanuts were roasted in the shell in an electric oven at 177 °C (350 °F) for 35 min. The shelled kernels, including skins, of both raw and roasted peanuts from the same lot were weighed and analyzed. Retentions calculated from the results of the analyses are shown in Table III. For peanuts, unlike steamed vegeta-

Table I. References to Methods of Analysis for Nutrients Used in Retention Calculations

Nutrient	Reference
Proximate components (protein, fat, ash, crude fiber)	AOAC (1970)
Mineral elements	
Ca, Cu, Fe, Mg, Mn, K, Na, Zn	Perkin-Elmer (1968)
P (in turkey)	AOAC (1970)
P (in peanuts and legumes)	Fiske and Subbarow (1925)
B vitamins (thiamine, riboflavine, niacin)	AOAC (1970)
Cholesterol	Tu et al. (1967)
Ascorbic acid	Freed (1966)
Carotene	AOAC (1970)[a]
Retinol	Ames et al. (1954)

[a] Extraction procedure 39.015 modified for analysis of liver.

Table II. Apparent and True Retentions of Selected Nutrients in Steamed Vegetables (Type 1[a] Weight Change with Cooking)

Nutrient	No. of samples	Apparent retention Mean, %	Apparent retention C.V.[c]	True retention[b] Mean, %	True retention[b] C.V.
Ash	4	102	1.7	99	1.6
Calcium	4	97	7.2	94	3.8
Magnesium	19	96	1.9	96	1.2
Potassium	27	103	2.8	101	2.3
Sodium	20	97	6.0	97	5.5

[a] Volatiles (primarily moisture) lost. [b] Differences between apparent and true retentions not significant. [c] Coefficient of variation.

bles, differences between apparent and true retentions were significant ($P = 0.05$). In all cases, the apparent retention value was higher than the true retention value.

TYPE 2 CHANGE, MOISTURE GAIN ONLY

Data were available for three nutrients in one sample of brown rice. The rice was rinsed once with tap water and drained before being cooked by boiling. All cooking water was absorbed by the rice. Time of cooking was not reported.

Retentions calculated from these data on cooked brown rice are shown in Table IV. In contrast to retentions for type 1 foods, the apparent retentions for brown rice tended to be lower than the true retentions. Because data were available for only one sample, it is not possible to tell whether or not apparent and true retentions differed significantly for this type 2 change.

TYPE 3 CHANGE, SOLIDS LOST AND MOISTURE GAINED

For the type 3 cooking change, data were available on three lots each of ten different dry legumes—Great Northern, navy, pinto, red kidney, large lima, baby lima beans, cowpeas (blackeyes), chickpeas (garbanzos), green split peas, and lentils. The legumes were purchased in local food markets in Virginia, and were simmered in glass cooking pans in 885 to 1189 g of deionized water, the amount of water varying with the kind of legume. Time of cooking ranged from 20 min for green split peas to 140 min for chickpeas. The ratio of weights for the cooked to the dry forms ranged from 2.1:1 for chickpeas to 2.9:1 for lentils.

Table III. Apparent and True Retentions of Nutrients in Six Samples of Oven-Roasted Peanuts (Type 1[a] Weight Change with Cooking)

Nutrient	Apparent retention Mean, %	Apparent retention C.V.[c]	True retention[b] Mean, %	True retention[b] C.V.
Protein	100	1.4	97	1.5
Fat	99	0.9	96	1.1
Ash	129	5.1	124	5.0
Crude fiber	105	2.5	102	2.2
Calcium	102	1.2	98	1.6
Copper	102	2.7	98	2.6
Iron	101	2.9	98	2.8
Magnesium	102	1.2	98	2.6
Manganese	107	2.4	104	2.7
Phosphorus	113	2.0	109	2.4
Potassium	99	0.4	96	0.8
Sodium	84	3.6	82	3.1
Zinc	104	1.3	100	1.2

[a] Volatiles (primarily moisture) lost. [b] All differences between apparent and true retentions significant ($P = 0.05$). [c] Coefficient of variation.

Table IV. Apparent and True Retentions of Selected Nutrients in One Sample of Brown Rice (Type 2[a] Weight Change with Cooking)

Nutrient	Apparent retention, %	True retention, %
Protein	101	105
Crude fiber	115	117
Potassium	93	96

[a] Moisture gained.

Table V gives data on apparent and true retentions for nutrients in the cooked dry legumes. Differences between the two methods of calculation were significant ($P = 0.01$) according to paired "t" tests. For all 12 nutrients, the apparent retention gave a value significantly higher than the true retention. Average differences between the two methods ranged from 6 to 11 percentage points, mean difference 8%, for the different nutrients.

TYPE 4 CHANGE, SOLIDS AND MOISTURE BOTH LOST

Retentions were calculated on nine lots each of turkey gizzard, heart, and liver, which had been cooked by simmering. The giblets were cooked in distilled water in one pan. Because the time of cooking required was greater for gizzards than for livers, and because cooking of all giblets was started at the same time, livers were probably overcooked.

Apparent and true retentions of 19 nutrients in turkey livers are given in Table VI. These data indicate a considerable difference between the two methods of calculation. Apparent retentions ranged from 6 to 24 percentage points, with a mean of 14 percentage points, higher than true retentions. All differences between calculation methods were significant ($P = 0.01$). Thus, apparent retentions were not reliable measures of the true retentions for this food showing type 4 changes.

For turkey gizzards and hearts, differences between calculation methods were even greater than for livers. (Data are not tabulated, but available from authors on request.) Comparisons of 16 nutrients in gizzards (protein, fat, ash, three B-vitamins, nine mineral elements, and cholesterol) showed a range of 6 to 22 percentage points, with a mean of

Table V. Apparent and True Retentions of Nutrients in 30 Samples of Boiled Mature Dry Legumes (Type 3[a] Weight Change with Cooking)

Nutrient	Apparent retention		True retention[b]	
	Mean, %	C.V.[c]	Mean, %	C.V.
Protein	103	0.9	96	1.6
Fat	111	3.8	102	3.4
Ash	86	1.9	80	3.2
Fiber	134	3.4	123	3.0
Calcium	108	2.2	100	2.6
Copper	97	2.3	90	2.9
Iron	120	2.7	111	2.7
Magnesium	85	3.6	79	4.1
Manganese	104	3.4	97	4.0
Phosphorus	92	3.4	86	4.1
Potassium	98	4.9	91	5.2
Zinc	120	2.2	112	2.9

[a] Solids lost, but moisture gained. [b] All differences between apparent and true retentions significant ($P = 0.05$). [c] Coefficient of variation.

13 percentage points, in differences between the two methods. For all 16 nutrients, differences were significant ($P = 0.01$). For turkey hearts, differences between the two calculation methods were significant ($P = 0.01$) for 10 of the 16 nutrients. For the remaining six nutrients (riboflavine, niacin, cholesterol, copper, manganese, and zinc), the number of comparisons involved was small, ranging from two to five. Had there been a larger number of comparisons, it is likely that differences between calculation methods would have been significant for these six nutrients also, as the differences were large, ranging from 15 to 40 percentage points. Thus, for foods undergoing these type 4 changes, apparent retentions consistently overestimated true retentions, and these overestimates were frequently very large.

TYPE 5 CHANGE, SOLIDS AND MOISTURE LOST FROM MORE THAN ONE TISSUE

Data were available for calculating retentions of proximate components, B-vitamins, cholesterol, and minerals in carcasses of turkeys of nine different age–sex groups. For 6 of the 41 replications, carcasses were separated into the major parts prior to roasting. Parts from one side of each bird were reserved for analysis in the raw state, and parts from the other side were roasted. For the remaining 35 replications, carcasses were split in half, with one half being analyzed raw and the opposite half being analyzed after roasting. Each of the first six replications consisted of ten half-carcasses, and each of the remaining 35 replications included four half-carcasses. Roasting was done in aluminum pans in ovens set at 145 °C (325 °F) until the temperature of the meat reached 85 °C (185 °F). Drippings were weighed and saved for analysis. Analyses showed that moisture, fat, protein, and ash were all lost to the drippings.

Comparisons of apparent and true retentions were made for meat and for meat plus skin in the turkey carcasses on the same 16 nutrients as were determined for turkey giblets. Table VII compares apparent and true retentions for 16 nutrients in the meat only from the turkey carcasses. Both calculation procedures have allowed for proportions of light meat to dark meat as determined by weights of these tissues in the carcass. As can be seen in the table, there was little difference between results from the two calculation procedures for meat only. Of the 16 nutrients determined, only fat showed significant differences between the two calculation methods, and these differences were numerically small. For all 16 nutrients, the differences between the two calculation procedures ranged from 0 to 2, with a mean of 1, percentage points.

Table VI. Apparent and True Retentions of Selected Nutrients in Simmered Turkey Livers (Type 4[a] Weight Change with Cooking)

Nutrient	No. of samples	Apparent retention		True retention[b]	
		Mean, %	C.V.[c]	Mean, %	C.V.
Protein	9	103	1.7	85	3.8
Fat	9	131	5.7	107	4.7
Ash	9	71	3.8	59	5.3
Thiamine	9	70	11.6	58	13.3
Riboflavine	9	57	9.6	47	10.9
Niacin	9	53	8.6	43	8.1
Cholesterol	9	112	4.7	92	4.6
Ascorbic acid	9	36	9.6	30	9.2
Carotene	9	109	11.2	89	9.8
Retinol	9	62	13.4	51	15.0
Calcium	9	123	6.3	102	8.2
Copper	8	95	5.7	78	5.1
Iron	9	65	7.8	54	10.4
Magnesium	9	66	8.7	55	9.3
Manganese	8	74	5.7	61	7.1
Phosphorus	9	76	4.4	63	5.9
Potassium	9	57	6.2	47	7.9
Sodium	9	57	5.8	47	7.0
Zinc	8	108	3.0	89	3.6

[a] Solids and moisture both lost. [b] All differences between apparent and true retentions significant ($P = 0.01$). [c] Coefficient of variation.

However, when retentions were calculated in meat plus skin of the turkey carcass, rather than in meat alone, different results were obtained (Table VII). In the same way as calculations on meat only were made, calculation procedures allowed for proportions of light meat to dark meat to skin, as determined by weights of these tissues in the carcass. For all 16 nutrients, differences between the two calculation procedures were significant ($P = 0.05$). The differences ranged from 3 to 17 percentage points, with a mean of 6 points, and in every case the apparent retention was higher.

TYPE 6 CHANGE, SOLIDS GAINED AND MOISTURE LOST

No data were available for calculating retentions of nutrients in foods undergoing type 6 changes, such as doughnuts or french-fried potatoes, which take up fat while losing moisture.

DISCUSSION

For a number of nutrients posted in Tables III through VII, retentions appeared to be unusually high or low. For instance, the retention of ash in oven-roasted peanuts, Table III, was high, and the retention of sodium was low. Derise et al. (1974) have suggested that the low sodium value might be explained by loss of sodium into the peanut shells and hulls with heating. The high ash retentions were not explained; possibly they represent problems in methodology of determining ash in raw compared with roasted peanuts. Crude fiber retentions shown in both Tables IV and V were also high. Data for fiber were obtained from two widely separated laboratories which were not in communication with each other. Therefore, if the high fiber retentions indicate inaccurate methodology, the difficulty is likely to be a general problem in applying the method for

Table VII. Apparent and True Retentions of Selected Nutrients in Roasted Turkey Carcasses (Type 5[a] Weight Change with Cooking)

Nutrient	No. of samples	Meat only				Meat plus skin			
		Apparent retention		True retention[b]		Apparent retention		True retention[b]	
		Mean, %	C.V.[c]	Mean, %	C.V.	Mean, %	C.V.	Mean, %	C.V.
Protein	41	99	0.4	101	0.6	105	0.6	101	0.5
Fat	41	128	1.8	130	2.4	94	1.4	90	1.8
Ash	41	81	1.0	82	1.1	87	0.8	84	1.0
Thiamine	41	68	3.9	68	3.6	71	3.2	68	3.5
Riboflavine	41	82	2.7	83	2.6	90	2.6	83	3.4
Niacin	41	89	2.0	90	2.2	96	2.2	92	2.3
Cholesterol	41	89	2.3	90	2.2	92	1.9	88	2.1
Calcium	41	130	2.8	132	2.8	137	2.5	131	2.6
Copper	9	71	12.8	71	12.2	84	11.3	72	11.8
Iron	41	96	3.8	97	3.9	101	3.9	97	4.0
Magnesium	41	79	0.9	80	1.0	87	0.9	83	0.9
Manganese	9	83	8.7	84	8.4	102	7.4	87	8.1
Phosphorus	41	81	1.2	82	1.2	88	1.0	84	1.2
Potassium	41	75	1.0	76	1.1	81	0.9	77	1.0
Sodium	41	76	1.2	77	1.2	82	1.1	79	1.2
Zinc	9	100	3.5	101	3.1	118	3.0	101	2.8

[a] Solids and moisture lost from more than one tissue. [b] For meat only, differences between apparent and true retentions significant only for fat ($P = 0.05$); for meat plus skin, all differences significant ($P = 0.05$). [c] Coefficient of variation.

crude fiber to both raw and cooked foods, rather than improper adaptation of a satisfactory method by a particular laboratory. High fat and calcium retentions in Table VII could possibly be caused by cooking of nutrients out of skin or bone into the meat. Low retentions in Tables V, VI, and VII could be attributed to loss of nutrients to cooking water or drippings, or, for labile nutrients such as thiamin and ascorbic acid, to inactivation by heat. Regardless of whether or not the retention data indicated problems in methodology, comparisons of apparent and true retentions, which were calculated on the same sample, are valid, and conclusions about the relative accuracy of the two methods of calculation can be drawn from the data reported here.

Data in Tables II, III, and IV indicate that for foods undergoing type 1 or type 2 changes (loss or gain of moisture), apparent retentions, calculated on the dry basis, may or may not be significantly different from true retentions, which take into account weight changes with cooking. Retention data evaluated for foods exhibiting more complex changes with cooking showed that apparent retentions were not reliable estimates of true retentions. Apparent retentions, which tended to give false high values, did not allow for loss of solids. Even for turkey carcass meat, the one example of a complex cooking change in which the two calculation procedures agreed well, weighting of the different tissues in the food was necessary to arrive at retentions reasonably representative of all of the edible part of the carcass. If weights of tissues are available, it would seem reasonable to calculate true retentions rather than apparent retentions, which may be less accurate.

Even in those instances in which differences between the two calculation methods were not significant, apparent retentions were almost always higher than true retentions. The use of apparent retentions thus introduces a source of bias which could be eliminated by the use of true retentions.

The usefulness of a calculation system can be affected by the amount of variability it allows, in addition to its accuracy. Coefficients of variation for apparent and true retentions were therefore reviewed to see whether or not they were within reasonable bounds and if they differed appreciably. For 115 comparisons, the average coefficients of variation were 5.3% for true retentions and 4.9% for apparent retentions. Furthermore, as was shown in several of the previous examples, few individual comparisons of coefficients of variation showed appreciable differences between the two methods of calculation.

With the data at hand, it is not possible to predict either the significance or the magnitude of differences between true and apparent retentions. Therefore, it is not now possible to establish correction factors which could be applied to apparent retentions so that they would more closely estimate true retentions. Because of the improvement in accuracy, with little change in variability of the data, true retentions are preferable to apparent retentions in evaluating the effects of cooking. The improved data to be obtained by using true retentions would be well worth the additional effort required to weigh the raw and cooked foods. Of course, in cases where it is not feasible to obtain batch weights before and after processing, such as on canning lines, true retentions cannot be calculated, and some other approach to their estimation needs to be developed.

These findings, that apparent retentions in many instances are not reliable estimates of true retentions, are not new. Streightoff et al. (1946), Dodds et al. (1944, 1946), and Hewston et al. (1948) all judged that measurement of true retentions requires information on weight changes with cooking, if solids are lost with cooking or ingredients are added to the raw food. Watt and Attaya (1945), in a review of published and unpublished data then available on retentions of vitamins in quantity cooking of vegetables, also warned that inaccuracies could result from using retentions calculated on the dry basis. Few present-day food scientists are familiar with the older literature on experimental cookery, including that on retentions of nutrients, except through such reviews as Harris and Von Loesecke's "Nutritional Evaluation of Food Processing" (1960). Unfortunately, this publication contains no discussion of problems in calculating retentions.

Many research reports do not explain how retentions were calculated, nor is it always possible, with the data given, for the reader to know what procedures were used.

The findings reported here, which are based on new data, confirm the judgments of the earlier researchers. It is

hoped that this paper will encourage food scientists to make their data more accurate and meaningful to others by reporting procedures used for calculating retentions as part of the description of analytical methods. True retentions, rather than apparent retentions, should be reported whenever possible.

ACKNOWLEDGMENT

Apparent and true retentions reported in this paper were calculated from results of laboratory research made under the sponsorship of the Agricultural Research Service and conducted at the following locations: the University of Hawaii (R. L. Van Reen, Principal Investigator, and Mrs. Nao S. Wenkam), the University of Nebraska (T. E. Hartung, Principal Investigator), and Virginia Polytechnic Institute and State University (S. J. Ritchey, Principal Investigator).

LITERATURE CITED

Ames, S. R., Risley, H. A., Harris, P. L., *Anal. Chem* **26**, 1378–1381 (1954).
Association of Official Analytical Chemists, "Official Methods of Analysis", 11th ed, Washington, D.C., 1970.
Derise, N. L., Lau, H. A., Ritchey, S. J., Murphy, E. W., *J. Food Sci.* **39**, 264–266 (1974).
Dodds, M. L., MacLeod, F. L., Carr, J. S., Tennessee Agricultural Experiment Station, Progress Note 2, University of Tennessee, Knoxville, Tenn., June 1946.
Dodds, M. L., MacLeod, F. L., Smith, J., Tennessee Agricultural Experiment Station Progress Note 1, University of Tennessee, Knoxville, Tenn., Oct 1944.
Fiske, C. H., Subbarow, Y., *J. Biol. Chem.* **66**(2), 375–400 (1925).
Freed, M., "Methods of Vitamin Assay", 3rd ed, Interscience, New York, N.Y., 1966.
Harris, R. S., Von Loesecke, H., "Nutritional Evaluation of Food Processing", Avi Publishing Co., Inc., Westport, Conn., 1960, reprinted 1971.
Hewston, E. M., Dawson, E. H., Alexander, L. M., Orent-Keiles, E., *U.S. Dep. Agric. Misc. Publ.* **No. 628** (1948).
Perkin-Elmer Corp., "Analytical Methods for Atomic Absorption Spectrophotometry", Norwalk, Conn., 1968.
Streightoff, F., Munsell, H. E., Ben-Dor, B. A., Orr, M. L., Cailleau, R., Leonard, M. H., Ezekiel, S. R., Kornblum, R., Koch, F. G., *J. Am. Diet. Assoc.* **22**(2), 117–127 (1946).
Tu, C., Powrie, W. D., Fennema, O., *J. Food Sci.* **32**(1), 30–34 (1967).
Watt, B. K., Attaya, M. B., *J. Home Econ.* **37**(6), 340–344 (1945).

Received for review March 21, 1975. Accepted July 23, 1975.

Biotin Content of Feedstuffs

Jacob Scheiner* and Elmer De Ritter

The biotin contents of a variety of feedstuffs are reported. Preliminary experiments using hydrolysis for 2 hr at 121° with 2 *N* and 6 *N* H_2SO_4 indicated that higher results were obtained with 2 *N* acid for feedstuffs of plant origin and with 6 *N* for feedstuffs of animal origin. On the basis of these results, all subsequent extractions were made with 2 *N* acid for plant materials and with 6 *N* acid for samples of animal origin. Peanut meal, safflower seed meal, streptomyces meal and solubles, brewers' yeast, dried liver, and a whey–yeast product had relatively high biotin contents. Other samples have been grouped in order of decreasing biotin contents.

A number of investigators (Patrick et al., 1942; McGinnis and Carver, 1947; Roblee and Clandinin, 1953; Slinger and Pepper, 1954) have reported biotin deficiency in poults fed rations containing practical feed ingredients. However, the occurrence of this deficiency in commercial flocks was either not recognized or not reported until recently. It had been generally believed that the feedstuffs in use, combined with biotin arising from intestinal synthesis, supplied sufficient biotin to meet the poults' requirement. Recently, however, the occurrence of biotin deficiency in commercial flocks has been reported (Brown, 1966; Wilson, 1967; Richardson and Wilgus, 1967; Johnson, 1967). Marusich et al. (1970) encountered biotin deficiency symptoms in poults fed a commercial ration in the laboratory. Apparent biotin deficiencies in swine under commercial conditions have also been reported (Adams et al., 1967; Cunha et al., 1968). As a consequence of these findings, a reevaluation of the biotin content of feedstuffs was desirable, particularly since the available published data cover only a limited number of feedstuffs and some of the results were obtained by methods whose validity could be questioned. The present study was undertaken to provide more comprehensive data on the biotin content of a variety of feedstuffs. Biotin determinations were made by microbiological assay using *Lactobacillus plantarum* (*arabinosus* 17-5, ATCC no. 8014), the test organism considered to yield the most reliable results.

For the preparation of extracts for microbiological assay, no single hydrolytic procedure is universally effective for maximum liberation of bound biotin. Table I summarizes the results of various acid extraction procedures employed by a number of investigators. These studies indicate that stronger acid concentrations are required to liberate bound biotin from animal tissues than from plant tissues. In the extraction of plant tissues, biotin is less stable in relation to autoclaving time and acid concentration than in extraction from animal tissues.

METHODS

The microbiological assay procedure for biotin was that of Wright and Skeggs (1944) with the exception that the test organism was grown on the liver–tryptone agar of Nymon and Gortner (1946). Inocula were prepared from stab cultures transferred the previous day.

In view of the demonstrated effects of acid concentration and conditions of hydrolysis on yields of biotin from different materials, two hydrolytic procedures were used in the present study, namely, autoclaving for 2 hr at 121° with either 2 *N* or 6 *N* H_2SO_4. In each case, 20 ml of acid was used per gram of sample. Similar conditions were used for extraction of a number of samples with water as a means of estimating the content of free biotin. Recovery tests were

Hoffmann-La Roche Inc., Nutley, New Jersey 07110.