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THE NUTRITIVE VALUE OF BLACK WALNUTS

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The Nutritive Value of Black Walnuts[1]

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Nuts have long been an important food of some of the peoples of the world. Even today they make up an appreciable part of the diet of the inhabitants of many countries. In America, except in the diets of vegetarians, nuts are not a staple part of daily menus but are used extensively to enhance the flavor and texture of confections, cakes, breads, salads, and other dishes; and in the preparation of nut butters, nut pastes, and margarines.

Black walnut meats with their unique and distinctive flavor, which persists even after cooking, are especially prized for culinary uses by many individuals. On a commercial scale, it is not unusual for a single processor to use 40,000 pounds of black walnut meats annually and if dealers could guarantee a steady supply even more would be used (Batchelor).

In the past, due chiefly to the tedious and time consuming methods required in the preparation of the kernels for market, most of the annual native black walnut crop has been allowed to go unharvested and limited interest has been shown in the cultivation and propagation of black walnut trees for better quality of fruit. At present, however, plants equipped with power-driven machinery for hulling, cracking, and shelling black walnuts have been established near important growing centers in Tennessee, Missouri, Virginia, Ohio, North Carolina and Illinois. Hand labor is required only for inspecting the nut meats for dark or shriveled kernels and for shells. Some of these plants handle 10 to 25 tons of walnuts annually. In Tennessee, a single plant estimates that it can shell one ton of walnuts per hour (Batchelor). One plant in southwest Missouri placed 50,000 pounds of walnut meats on the market in 1947 (Hibbard)[4]. The price offered per bushel of walnuts should make it worthwhile, in most cases, for the owners of the trees to gather the crop. It is interesting to read the report by Batchelor in which he states that in several cases individuals

[1]Data contained in this paper were taken from theses submitted by Dorothy Poertner Tyrrell and Mary Holke Jenkins in partial fulfilment of the requirements for the degree of Master of Arts in the Graduate School of the University of Missouri.

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have received more for the nuts produced by a tree in one year than they were offered for the tree as lumber. Thus we see that while the black walnut tree has been, and still is, grown chiefly for lumber, the fruits of the tree are gaining in importance economically.

With the present special interest in the planting of seedling black walnut trees[5], the development of cultivated black walnut orchards, and the improvement in methods for preparing the kernels for market, the future of the black walnut industry seems most promising. It is only natural, therefore, that inquiries should be made concerning the nutritive value of a food which is being placed on the market in increasing quantities.

Analyses of nuts have shown them to be of two types, one rich in fats and proteins and the other relatively rich in carbohydrates. With the exception of the coconut and the chestnut, most nuts, including the black walnut, belong to the first class. Chemists have pointed out that these nuts have a combination of concentrated protein and fat found in no other foodstuff. Smith (1950) in his interesting book "Tree Crops" refers to the black walnut as one of the "meat-and-butter trees".

Some information was found in the literature on the quantitative amounts of protein, fat, and minerals in black walnuts (Talbert, 1942; Bridges, 1941; and Elvehjem and Peterson, 1928), but none could be found on their vitamin content. The present investigation, therefore, was undertaken to determine quantitatively the riboflavin, thiamine, niacin, and carotene content of black walnuts (Juglans nigra L.) and to add to the information available on their protein and fat content.

EXPERIMENTAL PROCEDURE

The Sample

The five kinds of black walnuts used in this study were of the fall crop obtained the following January from a commercial nut-shelling concern at Exeter, Missouri. They included Thomas (cultivated), Stabler (cultivated), seedlings from each of two Missouri localities, and Mill-run, a mixture of nuts as shelled at the plant.

In order to secure representative samples of each kind of walnut the procedure for sampling recommended by the Association of Vitamin Chemists (1947) was followed. The mixing and dividing of a given variety was repeated until the quantity in each portion was approximately 100 grams. These portions were placed into cellophane bags, as much air as possible was expressed from the bags, they were sealed with heat, and stored in a refrigerator at 35° F. Just before an analysis was made, a sample was removed from the refrigerator and allowed to come to room temperature. It was then finely ground in a Waring Blendor for five minutes and the analysis was begun immediately.

Preliminary Assays and Review of Methods

In an effort to find the most efficient and accurate method for the fluorometric determination of riboflavin and of thiamine, many modifications

[5]Project sponsored by the U. S. D. A. to aid in the prevention of soil erosion.

of the original procedures have been reported. It is recognized that among other factors, the composition of the food itself, which is being assayed, may affect the analysis and make it necessary to modify the procedure in order that reliable results may be obtained. Bearing this in mind, preliminary tests were made to check the suitability of a procedure for determining the riboflavin and thiamine content of black walnuts.

Riboflavin. Variations included: (1) extracting the sample in 0.1 N HCl or 0.1 N H_2SO_4; (2) performing or omitting (a) autoclaving, (b) ether washing of the extract, (c) adsorbing and eluting the riboflavin, (d) oxidizing with $KmNO_4$-H_2O_2; and (3) destroying the riboflavin by means of photolysis, NaOH or $Na_2S_2O_4$ to obtain the fluorometric reading of the blank. All samples were digested with a combination of the enzymes papain and takadiastase at a pH of 4.5, after acid extraction. The procedure finally adopted is described in detail below.

Thiamine. Extraction. In this laboratory, autoclaving the sample in 0.1 N HCl followed by enzymatic digestion with takadiastase-papain was found satisfactory for extracting the riboflavin from the black walnut samples. From a review of the literature it followed that the same procedure could be used for extracting thiamine.

Purification. It has been recommended by Hennessy and Cerecedo (1939) that tests always be made to determine whether or not the purification step may be omitted.

Direct oxidation of the black walnut sample extract showed conclusively that further purification was necessary. Therefore two methods of purification were investigated: (1) adsorption-elution (Hennessy and Cerecedo, 1939; Conner and Straub, 1941); and (2) the isobutanol wash (Harris and Wang, 1941). The per cent recovery of thiamine added to the black walnut extract was used as the criterion for judging the efficiency of the method.

Adsorption of the thiamine on Decalso and its elution with hot acid KCl proved unsatisfactory. The per cent recovery of thiamine was low and variable. Other investigators (Wang and Harris, 1939; Jowett, 1940; and McFarlane and Chapman, 1941) also reported that this method of purification often gives low assay values due to incomplete adsorption or elution of thiamine.

The most consistent and reproducible results, with good recovery (90%) of added thiamine, were obtained when the extract was washed with isobutanol prior to oxidizing the thiamine to thiochrome.

After evaluating the results obtained by the various modifications of procedure for determining the thiamine content of black walnuts, the method described on page 6 was chosen as the one giving the best results.

Procedures Followed

Riboflavin. The fluorometric and microbiological methods of assay were used.

A 5-gram sample was autoclaved in 30 ml. of 0.1 N HCl at 15 pounds for 15 minutes. After the solution was cool, 0.1 gm. of takadiastase and 0.1 gm. of papain dissolved in 3 ml. of 2.5 M sodium acetate were added.

Several drops of benzene were added, as a preservative, and the sample (pH 4.5) was incubated overnight at 37° C. The solution was then filtered through moistened filter paper, and the residue was washed with distilled water. The filtrate was adjusted to pH 6.6-6.8 with 0.2 N NaOH, refiltered directly into a 100-milliliter volumetric flask and made to volume with distilled water[6].

Fluorometric Method. Oxidation of the sample extract with glacial acetic acid, potassium permanganate and hydrogen peroxide was carried out as described by the Association of Vitamin Chemists (1947).

The fluorescence of the sample extract, sample extract plus one ml. of reference riboflavin (0.5 mcg. per ml.) and the blank, obtained by adding sodium hydrosulfite to the sample extract, was measured on a Coleman Model 12 photofluorometer[7] which had been standardized with sodium fluorescein (0.05 mcg. per ml.) to give a deflection of 60 on the galvanometer scale. The amount of fluorescence produced by the enzymes, as well as that produced by the reagents used in the analysis, was determined in the same manner as for the sample extract and the correction applied in calculating the riboflavin content of the sample.

Microbiological Method. The Strong and Carpenter (1942) modification of the Snell and Strong (1939) procedure was followed. Aliquots of the same sample extract used for the fluorometric determination of riboflavin were used for the microbiological assay. The amount of riboflavin in the reagents and enzymes was determined in the same manner as for the sample. Recovery of added riboflavin was 97% by the fluorometric method and 98% by the microbiological method.

Thiamine. After washing the sample extract with isobutanol, aliquots of the aqueous solution containing approximately 0.25 mcg. of thiamine were treated with 0.03% $K_3Fe(Cn)_6$ in 15% NaOH to oxidize the thiamine to thiochrome. Three aliquots per sample were placed into each of 3 oxidizing chambers followed by the addition of the solutions indicated: to No. 1, one ml. of reference thiamine (0.1 mcg. per ml.); to No. 1 and No. 2, three ml. $K_3Fe(Cn)_6$NaOH; and to No. 3, three ml. 15% NaOH. Isobutanol (15 ml.) was then added to each of the oxidizing chambers and the procedure described by the Association of Vitamin Chemists (1947) was carried out. A Coleman Model 12 Photofluorometer[8] which had been standardized with quinine sulfate solution (0.1 mcg. per ml.) to give a deflection of 60 on the galvanometer scale was used to measure the fluorescence of these solutions.

Nicotinic Acid (niacin). The nicotinic acid content of the black walnuts was determined according to the Krehl, Strong, and Elvehjem (1943) modification of the Snell and Wright (1941) procedure.

Carotene. The technique used to determine the carotene content of black walnuts employed spectrographic measurement of the carotenoid pigments which were extracted from the sample by the "foaming mixture"

[6]This sample extract was used for riboflavin, thiamine, and niacin assays.
[7]The photofluorometer was equipped with filters B-2 and PC-2. A transformer supplied a constant voltage to the machine.
[8]Equipped with filters B-1 and PC-1.

method of Moore and Ely (1941) and chromatographically adsorbed by an adaptation of the procedure recommended by Moore (1940).

Commercial carotene (90% β-carotene and 10% α-carotene) diluted to the desired concentration with petroleum ether (1.0 mcg. per ml.) was used as the reference standard. Spectrographic measurements were made by means of a Model 14 Coleman Spectrophotometer, at a wave length of 4400 Å, the region of maximum absorption of the reference standard. The final value designated as carotene pigment includes β-carotene, α-carotene, and the neo isomers of carotene. However, it is stated by the Association of Vitamin Chemists (1947) "for all practical purposes the value may be considered as β-carotene." Therefore, the carotene pigment was considered the β-carotene content of the sample.

Protein. The nitrogen content of the black walnuts was determined by the Kjeldahl-Gunning method (A.O.A.C., 1945). The per cent of nitrogen present was multiplied by the factor 6.25 to give the percent of protein in the sample.

Moisture and Fat. Procedures recommended by the Association of Official Agricultural Chemists (1945) were followed.

RESULTS AND DISCUSSION

Values for the riboflavin, thiamine, niacin, and carotene content in micrograms per gram of the walnut samples on a room-dried basis[9] are given in Table 1. In all cases the mean is the average of four determinations, except for carotene, here it is the average of duplicate determinations in which individual values varied no more than 5.8%.

Riboflavin. It was found necessary to make corrections in the calculations for the fluorescence produced by the enzymes and reagents used in the fluorometric assay. The correction applied was equivalent to 7.57 mcg. of riboflavin per gm. of enzyme used. This amount was within the range, 1 to 8 mcg. of riboflavin per gm. of the same enzyme combination (papain and takadiastase) as published by the Association of Vitamin Chemists (1947).

In the microbiological assay, no correction was necessary since the riboflavin value of the blank was equivalent to only 1.2 mcg. per gm. of the enzymes. Cheldelin, Snell, et al (1942) reported that by microbiological assay a gram of papain and takadiastase combined in equal amounts contained 4.4 mcg. of riboflavin. They say, further, that in most cases the correction to be applied to the sample for the enzyme blank is of little significance since it represents only 4% of the total weight of the sample.

The average riboflavin content of the five kinds of walnuts was 1.15 and 1.08 mcg. per gm. with average coefficients of variation of 4.4 and 3.5 when assayed by the fluorometric and the microbiological methods, respectively (Table 1).

In all cases, except for the Thomas variety, the microbiological method gave slightly lower values than the fluorometric method. The average of

[9]The moisture content of the room-dried samples ranged from 3.14 to 3.74 with an average of 3.51%.

TABLE 1.--RIBOFLAVIN, THIAMINE, NIACIN, AND CAROTENE CONTENT* OF BLACK WALNUTS

Sample	Riboflavin						Thiamine			Niacin			Carotene
	Fluorometric			Microbiological									
	mcg./gm.	S.D.	C.V.	mcg./gm.	S.D.	C.V.	mcg./gm.	S.D.	C.V.	mcg./gm.	S.D.	C.V.	mcg./gm.
Cultivated Thomas	1.28 ±	0.03	2.3	1.30 ±	0.07	5.4	2.24 ±	0.14	6.3	9.43 ±	0.26	2.8	1.87
Stabler	1.22 ±	0.08	6.6	1.06 ±	0.03	2.8	2.29 ±	0.06	2.6	7.65 ±	0.31	4.1	2.23
Uncultivated Seedlings From Locality I	1.12 ±	0.02	1.8	1.04 ±	0.02	1.9	2.29 ±	0.10	4.4	6.17 ±	0.03	0.5	1.85
From Locality II	1.11 ±	0.06	5.4	1.05 ±	0.02	1.9	2.22 ±	0.05	2.3	6.98 ±	0.04	0.6	1.38
Mill-run	1.03 ±	0.06	5.8	0.94 ±	0.05	5.3	1.91 ±	0.12	6.3	6.34 ±	0.17	2.7	1.77
Average	1.15		4.4	1.08		3.5	2.19		4.4	7.31		2.1	1.82

* Micrograms per gram on a room-dried basis. Each value listed is the average of four determinations except for carotene, in which case it is the average of duplicate determinations.

S.D. = Standard deviation of mean.
C.V. = Coefficient of variation.

the values obtained by the two methods shows the microbiological results to be 6% lower. Jentsch and Morgan (1949) working with a similar type of medium, English walnuts, reported that their results were about 20% lower by the microbiological method than by the fluorometric method. They say the discrepancy is difficult to explain unless some fluorescent is operative to give higher results in the latter method.

Since no other quantitative analyses have been reported on the vitamin content of black walnuts, it is impossible to compare these results with those of other investigators. The results obtained by Jentsch and Morgan (1949) for English walnuts compared to the results obtained in this investigation indicate that English walnuts are very similar to black walnuts in their riboflavin content. The average number of mcg. of riboflavin per gm. of English walnuts was 1.47 by the fluorometric method and 1.11 by the microbiological method.

Thiamine. The average thiamine content of the black walnuts was 2.19 mcg. per gm. of sample (Table 1). Here again, as with riboflavin, a similarity was noted between the thiamine content of black and English walnuts. The thiamine content of the latter according to Jentsch and Morgan was 2.8 mcg. per gm. (thiochrome method).

Nicotinic Acid (niacin). Cheldelin, Snell, et al. (1942) reported that one gram of papain and takadiastase combined in equal amounts contained 17.0 mcg. of nicotinic acid. The enzymes and reagents used in the microbiological assay for niacin in these samples contained 12.1 mcg. of nicotinic acid per gm. This value was deducted from the assay results to give the corrected values for niacin reported in Table 1.

The average amount of niacin in the black walnuts was 7.31 mcg. per gm. Deviations from the mean for each of the varieties were small. The average coefficient of variation was only 2.1%. It is of interest to note that the niacin content of English walnuts (Jentsch and Morgan, 1949) was 7.1 mcg. per gm.

Carotene. Recovery of known amounts of carotene added to the walnut samples which were assayed by the chromatographic-spectrographic

method was 96%, which indicated that the procedure was satisfactory and reliable.

The black walnuts contained an average of 1.82 mcg. of carotene per gm. In terms of vitamin A, this would be approximately 3.0 I.U. per gm.

No quantitative analyses could be found on the carotene or vitamin A content of black walnuts, however, vitamin A values have been reported for some other nuts. Sherman (1941) has published a table which indicates the range within which the average vitamin A value, expressed in I.U. per gram, may be expected to fall. The estimated range is as follows: walnuts[10], 1.0 to 1.5; pecans, 1.0 to 2.0; hazelnuts, 4.4[11]; and almonds, 5.8. The average value of 3.0 I.U. of vitamin A per gram of black walnuts as found in this laboratory, is comparable to the data listed by Sherman.

Protein. The protein content of the Mill-run and the seedling walnut samples was larger than that of the two cultivated varieties, Table 2. Locality 1 had 26.0% more protein than Thomas, 17.6 more than Stabler, but only 5.2 more than Locality 2, and 2.6 more than Mill-run. The average protein content 24.95% agrees well with the values, minimum 24.9 and maximum 30.3% reported by Atwater and Bryant (1906) and 26.95% reported by Wainio and Forbes (1941).

TABLE 2.--MOISTURE, PROTEIN, AND FAT CONTENT OF BLACK WALNUTS

Sample	Moisture Content (room-dried sample) - %	Protein * (room-dried basis) %	Fat (moisture-free basis) %
Clutivated			
Thomas	3.74	21.67	64.03
Stabler	3.14	23.22	62.02
Uncultivated Seedlings			
From Locality I	3.48	27.31	57.63
From Locality II	3.64	25.96	56.82
Mill-run	3.53	26.61	58.49
Average	3.51	24.95	59.80

* N X 6.25

Since the quality as well as the quantity of the protein in a food is important, it would be well to know the amino acid make-up of the protein in black walnuts. Again such reports could not be found. Osborne (1907) studied the supplementary value of hazelnuts on cereals and legumes and reported that the protein of hazelnuts had a good supplementary value when combined with these foods. Mitchell and Beadles (1938) state that nuts (peanuts, English walnuts, and pecans) are inadequate as a substitute for meat.

Fat. The per cent of fat in these samples averaged 59.80. This is in close agreement with 60.79%, the average oil content as given by Talbert (1942) for eleven varieties of black walnuts. In this study the walnuts with the lower amount of protein contained the higher amount of fat.

It is of interest to compare the nutritive value of black walnuts to some commonly used foods (Watt and Merrill, 1950). As a source of ribo-

[10]Presumably English walnuts.
[11]Only one value when not enough data available for range.

flavin they are similar to whole wheat flour and other whole grain cereals. They are about one-half as rich in thiamine as whole wheat flour. In niacin they are comparable to dried whole milk, mustard greens, and asparagus. Although black walnuts are high in fat, containing about two times as much fat as egg yolks, they do not equal eggs in vitamin A content, rather their vitamin A content corresponds to that of bananas, rutabagas, canned salmon, and evaporated milk. Black walnuts compare favorably to lean meat, cheddar cheese and dried whole milk in the amount of protein which they contain. How they rank in efficiency as a protein food needs further investigation.

SUMMARY

The black walnuts used in this study were obtained from a commercial nut-shelling plant in southwest Missouri. They included Thomas (cultivated), Stabler (cultivated), seedlings from each of two Missouri localities, and Mill-run, a mixture of nuts as shelled at the plant.

Average values per gram of sample for the nutrients investigated were as follows: riboflavin, 1.08 mcg. (microbiological), 1.15 mcg. (fluorometric); thiamine, 2.19 mcg.; niacin, 7.31 mcg.; carotene, 1.8 mcg.; fat, 59.80%; and protein, 24.95%.

While the number of samples assayed was not sufficient to allow definite conclusions to be drawn, results in general indicate that there are significant differences in some of the nutrients of different varieties of black walnuts.

Black walnuts are a fair source of the B vitamins. They rank well with lean meat and whole dried milk in the amount of protein which they contain but, according to studies made on other nuts, it appears that they would be less efficient than these as a protein food. Since they are consumed in relatively small amounts by individuals in this country, they do not contribute materially to the daily requirement for essential nutrients. At the present time, their chief value lies in their use as an accessory food to enhance the flavor of prepared dishes and delicacies; in this way thousands of pounds are used annually by single processors. The economic importance of the black walnut meat industry, in the regions where the tree grows well, is greater than generally recognized.

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