THE RIBOFLAVIN AND THIAMINE CONTENT OF DIFFERENT VARIETIES OF MICHIGAN BEANS, RAW AND COOKED

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THE RIBOFLAVIN AND THIAMINE CONTENT OF DIFFERENT VARIETIES OF MICHIGAN BEANS, RAW AND COOKED

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INTRODUCTION

Navy beans (Phaseolus Vulgaris) and other legumes generally have been considered as economical sources of body building protein and energy. Their use as food has increased during the past few years, therefore their overall nutritive value is considered to be of interest. It seems that seeds of plants are one of the richer sources of thiamine and riboflavin among the plant tissues in that they are prepared for the growing of young plants. In Michigan, the navy bean is one of the most important and dependable field crops. In 1915, the Robust bean, a variety of the white pea or navy type was introduced by F. A. Spragg of Michigan State College Agricultural Experimental Station. The Michelete bean was developed by E. E. Down and J. W. Thayer Jr., in 1937 from a cross between the Robust and Early Prolific varieties. The Michelete variety possesses the desired characteristic of uniformity in size and shape and has greater resistance than other varieties to virus mosaic and other diseases.

Studies by Fixsen (1938), Arnold and Elvehjem (1939) on the chemical properties of thiamine and riboflavin showed that thiamine is relatively heat labile and that riboflavin is markedly heat stable. Extent of vitamin cooking losses depended upon the conditions under which the food was prepared. The process of cooking, the amount of cooking water added, time, temperature and many other details are all important in determining the rate of destruction of the vitamins. Since chemical and microbiological analytical methods have been developed, numerous determinations on thiamine and riboflavin of foods, raw and prepared, were made for choosing of varieties having high contents and for studying the actual retention of the nutritive value after different methods of processing.

The purpose of this experiment was to determine the difference in thiamine and riboflavin content of six varieties of Michigan beans grown under controlled conditions and to determine the effect of baking on the thiamine and riboflavin content of these beans.

REVIEW OF LITERATURE

Methods for the Determination of Thiamine

Several biological methods for the determination of thiamine were used before the chemical methods were developed. The biological methods most generally used include the rat-growth method of Sherman and Chase (1931), the pigeon weight-maintenance method and the bradycardia method. In 1934, Schopfer proposed a quantitative test using a mold growth method. A technique was described for estimating thiamine by means of the growth of mold which does not normally grow in a synthetic medium. On the addition of pure crystalline thiamine, the mold is stimulated to considerable growth. With increasing concentration of thiamine, the growth of the organism is proportionately increased up to a maximum point. He believed that the organism used thiamine to synthesize its own growth factor. Sinclair (1938) showed that the above method was not specific. Schultz, Atkins and Frey (1937) used a fermentation method based on Sinclair's idea that the rate of alcoholic fermentation is powerfully stimulated by the presence of thiamine in the fermentation mixture.

The first chemical test was published by Kinnersly and Peters (1934). A diazotized compound of thiamine and sulfanilic acid in the presence of a buffer and formaldehyde was found. This compound which is pink colored was used for a qualitative test. In 1938, the same investigators introduced the use of takadiastase to liberate free thiamine. Prebluda and McCollum (1939) proposed the use of diazotized p-amino-acetophenone the intensity of the color being a measure of the vitamin concentration. Melnick and Field (1939) extended this method, released the phosphorylated thiamine by incubation with yeast phosphate, and found that permutit was the only adsorbent that would remove the vitamin and permit a 100 percent elution with dilute sulfuric acid, yielding a solution which could be rendered suitable for analysis. Emmett, Peacock and Brown (1940) modified the Prebluda-McCollum reaction by adsorbing the thiamine on Superfiltrol. Kirch and Bergeim (1942) described a method in which the urinary thiamine was coupled with diazotized ethyl-p-aminobenzoate producing a pink to red color. The trichloroacetate of this amine was fairly stable in alcoholic solution. That the enzymatic digestion converted the cocarboxylase to free thiamine without any destruction of the thiamine molecule was also reported. Teeri (1948) mentioned that thiamine under certain conditions produces with cyanogen bromide a colored compound measurable at the wave length commonly employed for the quantitative determination of nicotinic acid.

The thiochrome method which is generally used was introduced in 1935 when Peters; Barger, Bergel and Todd found that a pure thiamine preparation could be converted by oxidation in aqueous solution to a pale yellow sulfur containing compound, thiochrome, which showed an intense sky blue fluorescence. Jansen (1936) pointed out that thiamine could be measured by oxidation to thiochrome with alkaline ferricyanide. Recovery of thiamine by this method was not quantitative. Hennessy and Cerecedo (1939) used an enzyme prepared from defatted beef kidney in acid solution for extraction, and subsequently adsorbed the vitamin on Decalso. The fluorescence was measured photoelectrically. The result obtained by this method agreed well with those using biological tests. Harris and Wang

(1939) removed the interfering materials of urine samples by washing with an equal volume of isobutyl alcohol. The washed urine was oxidized to convert thiamine into thiochrome, extracted with isobutyl alcohol and cleared with anhydrous sodium sulfate. In the measurement of fluorescence, a standard solution of thiochrome was added drop by drop to a blank used as a control, while at the same time an equal volume of isobutyl alcohol was added to the unknown until the fluorescence of the two was matched.

Pyke (1939) employed pepsin digestion followed by takadiastase to obtain a complete liberation of thiamine. Conner and Straub (1941) described the optimal conditions for the oxidation of thiamine to thiochrome. The same authors and Halliday and Deuel (1941) reported that takadiastase and other related enzymes with high phosphate activity are capable of hydrolyzing cocarboxylase quantitatively into free thiamine and phosphatase. The presence of protein interferes with the hydrolysis. The combined thiamine is in a complex form. Takadiastase was unable to separate the thiamine complex unless aided by a proteolytic enzyme.. Cheldelin, Eppright, Snell and Guirard (1942) suggested the use of both takadiastase and papain for the liberation of the free vitamin.

Johannson and Rich (1941) applied the thiochrome method to the analysis of cereal and cereal products. Andrews (1944) reported a collective study of thiamine concentration in cereals.

Numerous microbiological methods for the determination of thiamine have appeared in the literature in the last few years. These include the methods of Niven and Smiley (1943) and Sarett and Cheldelin (1944).

Since the thiochrome method was used in this study, the microbiological methods will not be reviewed.

Methods for the Determination of Riboflavin

Chick and Roscoe (1928), Sherman and his coworker (1931-35) used the rat growth method for the determination of riboflavin and defined the Bourquin-Sherman rat unit as that amount of riboflavin which when fed as daily doses induced a gain of 3 gm. per week in a standardized experimental animal. The animal is fed a basal ration which is sufficiently free from riboflavin to result in loss of weight during the test period.

Recently the microbiological method of Snell and Strong (1939) was investigated by measuring the influence on both the cell growth and the acid production of Lactobacillus Casei grown on a synthetic medium free of riboflavin. Very satisfactory results were obtained.

Kuhn and Wagner-Jauregg (1933) first noted the yellow green fluorescence of riboflavin. These same authors and Kaltschmitt (1934) studied the riboflavin distribution in plants as lumiflavin by means of a photometer. Cohen (1935) measured the fluorescence of unknown solutions with a selenium cell method. Koschara (1935) used a method based on measurement of the light absorption of the vitamin extract rather than on its fluorescence. He also introduced the use of permanganate to oxidize interfering pigments and destroyed excess potassium permanganate with hydrogen peroxide. Hodson and Norris (1939) described a fluorescent method for use with foods. Riboflavin and the interfering pigments were reduced with a mixture of sodium hyposulfite and stannous chloride. The reduced riboflavin was oxidized by bubbling air through the solution and its fluorescence measured by the use of a fluorophotometer. Sullivan and Norris (1939) measured the fluorescent reading of a sample in a photometer. The sample was then reduced with sodium hyposulfite. Reading of the reduced sample was also taken. The difference of the two photometric readings was regarded as the riboflavin value of the sample.

Farrebee (1940) introduced the use of two Fuller's earth preparations, Floridin and Supersorb, for the adsorption of riboflavin. The vitamin was eluted from these adsorbents with a solution of 20 percent pyridine in two percent acetic acid. The eluate was treated with potassium permanganate and hydrogen peroxide prior to measuring the fluorescence. Najjar (1941) applied the fluorometric method to urine and other biological fluids. The statement was made that two sources of error in the methods in which fluorescence was measured in aqueous solutions were turbidity of the solution and the formation of gaseous emulsions. The addition of potassium permanganate and hydrogen peroxide resulted in the formation of minute bubbles of oxygen which tended to remain dispersed in the medium. This gave a "whitish" tint to the riboflavin fluorescence and interfered with the accuracy of the fluorescence measurements. Najjar measured riboflavin in a non-aqueous medium by saturating the pyridine extract with anhydrous sodium sulfate. Conner and Straub (1941) modified the fluorometric method and combined it with the thiochrome method for the determination of the two vitamins, thiamine and riboflavin in food products. Andrews (1944) reported the collaborative study of riboflavin assay methods. Large variations in the results of individual collaborators were found. That variation in the efficiency of different lots of Florisil were partially responsible for the difference were indicated. Rubin and De Ritter (1945) found that riboflavin was adsorbed more efficiently by Florisil from simple solution than extracts of high potency, but found that the use of Florisil may cause errors ranging up to 30 percent; the factors influencing the adsorption included the clarity, volume and concentration of the extracts. Slater and Morell (1946) modified Najjar's method by using a larger amount of potassium permanganate and an internal standard and found good agreement between values obtained by this method and those from microbiological assays. De Ritter, Moore, Heischberg and Rubin (1948) stated that assays by a Florisil adsorption procedure including permanganate treatment of the eluate were slightly high as compared to other methods. In terms of absolute amounts of riboflavin, these differences were small, but at a very low riboflavin level, the percentage differences may be high.

The validity of the use of a mixture of takadiastase and papain for the liberation of riboflavin from foods was considered to be the most effective ones by Cheldelin, et al., (1942) and was confirmed by Rosner, Lerner and Cannon (1949).

The Effect of Processing on the Thiamine and Riboflavin Content of Foods

Concerning the effect of processing of foods on their thiamine and riboflavin content, Fixsen (1938), Arnold and Elvehjem (1939) stated that thiamine shows considerable resistance to heat in an acid or slightly acid medium. Exposure to temperatures of 100°-120° C. resulted in greater losses than were observed at lower temperatures even in an acid medium, while in an alkaline medium there was extensive destruction at low temperatures. Beadle, Greenwood and Kraybill (1943) reported that the destruction of thiamine upon heating is a function of the temperature, time of heating, the pH of the medium and the nature of other substances present. Fixsen (1938) and Munsell (1940) found that no loss of riboflavin resulted from exposure to the temperature associated with cooking unless the medium was alkaline. Solubility in water will be the cause of losses in riboflavin when vegetables are boiled and the cooking water rejected. The study of Arnold and Elvehjem (1939) reported a 60-80 percent loss of thiamine due to heating. Miller (1945) studied the thiamine content of Japanese soy bean products. Seventy-nine percent loss of thiamine in cooking was found. Fenton, et al., (1945) reported that approximately two-thirds of the thiamine and riboflavin were retained in peas during cooking.

Greenwood (1938) reported that adding of soda is an effective method of softening the seed coats of beans. Lantz (1938), Aughey and Daniel (1940), Johnson, Schauer and Daniel (1944) stated that soda may shorten the cooking time and has no effect on thiamine and riboflavin content.

Several papers were published where a higher riboflavin content was observed after cooking than was observed in the unprocessed materials. These findings were not considered as errors in the analytical procedures. Streightoff, et al., (1946) reported an average riboflavin retention totaling 122 percent for boiled carrots plus boiling water in ten batches of stored miscellaneous varieties and of 112 percent in ten batches of fresh Chantenay coreless carrots during large quantity preparation. Tucker, Hinman and Halliday (1946) stated that during braising, frying and broiling of beef there appeared to be no loss of riboflavin by any of the methods of cooking and in many cases there appeared to be a gain. The complex form in which riboflavin is formed or released during cooking is of such a nature that the vitamin can not be liberated by digesting or autoclaving under acid condition nor by enzyme hydrolysis. Watts, Peng and Esselbaugh (1948) made the statement that the enzymes within the meat itself bring about a progressive increase in the riboflavin liberated if opportunity is allowed for autodigestion in the presence of preservatives. This enzymatic liberation of riboflavin probably continues at the low temperatures of freezing storage and may be greatly accelerated at higher temperatures in cooking.

Few data relative to the thiamine and riboflavin content of navy beans appeared in the literature. Table I presents a summary of the vitamin content of navy beans as reported by several investigators. The nutritive values of the cooked beans are also included.

Table I

Thiamine and Riboflavin Content of Beans Reported by Different Investigators

Name of Investigators	Year	Name of Beans	Thiamine Content	Riboflavin Content
Baker & Wright	1935	Bean, Haricot Bean, Baked	1.2 (Brady-cardia unit) trace	
Fixsen & Roscoe	1938	Bean, Haricot Baked, & Canned	50-120 I.U.*/100 gm. trace	
Munsell	1940	Navy bean	170 I.U./100 gm.	
Aughey, Daniel	1940	Navy bean Cooked	250 I.U./100 gm. 240 I.U./100 gm.	
Kelley, Dietrich & Porter	1940 1941	Michelete Baked in soaking water	170 I.U./100 gm. 230 I.U./100 gm.	
Munsell	1942	Navy bean, dried		300,325 mcg./100 gm.
Booher, Hartzler & Hewston	1942	Michelete Robust Navy	600 mcg**/100 gm. 720 " 750 "	
Ives, Wagner Elvehjem & Strong	1945	Baked beans new English type	210 mcg./100 gm.	540 mcg./100 gm.
Daniel & Norris	1945	Robust Michelete	630 mcg./100 gm. 656 mcg./100 gm.	179 mcg./100 gm. 170 mcg./100 gm.

* The international unit is defined as the potency of 3 micrograms of pure thiamine hydrochloride.

** Mcg. is used as an abbreviation of microgram.

Among the reported studies, the work of Kelley, Dietrich and Porter (1940, 1941) and Daniel and Norris (1945) were of particular interest. Kelley, Dietrich and Porter used the rat growth method to determine the thiamine value of eight varieties of beans grown in two localities in Michigan. Results obtained indicated that the Blue pod and Robust varieties were highest in thiamine content and Michelete was of medium value. The effect of cooking on the thiamine content of Michelete and Cranberry beans was studied using the same method. Thiamine content of all samples of beans cooked by the different methods was found to be higher than that of the original raw beans. The assumption was made that the cooking processes rendered the thiamine content of the beans more readily available to the animals than was the thiamine in the uncooked beans.

Daniel and Norris studied the riboflavin, niacin and thiamine contents of dried legumes. The thiochrome method and fermentation method were used for the thiamine analysis. The fluorometric method and microbiological method were used for the riboflavin analysis. Excellent agreement was found between the two methods that were used in the assay of each vitamin in one variety of legume. Vitamin contents of Michelete and Robust beans were reported in Table I.

EXPERIMENTAL PROCEDURE

One variety and strains of five different crosses of Michigan navy beans were obtained from the Farm Crop Department in Michigan State College. In the cooking trials, Michelete was used as a standard for comparison of palatability. Other beans represent the newly crossed seeds. The distribution of the samples is shown in Table II. A total of 46 raw and the same number of baked beans were analyzed for thiamine and riboflavin content.

According to the reports of Loofbourow and Harris (1942), William and Chedelin (1942) and DeMerre and Brown (1944) riboflavin can be destroyed by visible light. Therefore all experiments in this study were carried in semidarkness.

For the determination of thiamine, the thiochrome method of Hennessy and Cerecedo (1939) as developed by Michelsen, Condiff and Keys (1945) for the analysis of thiamine in urine was used. The chemical method of Hodson and Norris (1939) with modification by Conner and Straub (1941) and Keys (1944) was used for the estimation of riboflavin. Fluorescence of the vitamins was measured with a fluorescence meter.*

Baking of Beans:

The baked beans were prepared by Miss Louise Kelley as a part of an experiment station project on the palatability of different samples of beans. The recipe for the baked beans is given in Table III.

* A Coleman photofluorometer and a Lumetron photofluorometer were used.

Table II

Distribution of Navy Bean Samples Analyzed for Thiamine and Riboflavin Content

Variety	Strains of Different Crosses				
Michelete	Robust x Early Prolific	Robust x Crawford	Robust x Darling	Robust x Mexican Tree	Robust x Hunter
Sample No.	Sample No.	Sample No.	Sample No.	Sample No.	Sample No.
801	802	803	822	866	836
811	812	804	823	872	842
821	813	805	824	873	843
831	814	806	825		
841	815	844	826		
851	816	845	832		
861		846	833		
871		852	834		
SS		853	835		
		854			
		855			
		856			
		862			
		863			
		864			
		865			

Table III

Recipe For The Baked Beans

Ingredient	Approximate measure	Weight (gm.)	Source
Dried beans	1.5 cups	310.0	Variable
Molasses	3.0 tablespoons	70.0	Grandma's Old Fashioned
Mustard	1.75 teaspoons	1.3	Bulk
Salt	0.75 teaspoons	5.0	Iodized
Salt pork	0.20 pound	70.0	
Soda	0.15 teaspoons	not weighed	Bicarbonate

Six samples of beans were baked each time, one of the six being the Michelete variety. Before baking, the selected dried beans were washed through two waters, drained and rinsed with distilled water. Three hundred and ten grams of beans were soaked in 600 cc. of distilled water overnight in a Pyrex bowl. In the morning, the beans and the soaking water were transferred to an aluminum pan and were allowed to boil for five minutes with a small amount of baking soda added to it. The soda water was then drained off and the beans were washed with distilled water. Slices of salt pork were put at the bottom of a bean pot. The beans were transferred into the bean pot. Molasses, salt, mustard were added and the beans covered with boiling distilled water, baked in a preheated oven at 250° F. for seven hours. The cover of the pot was removed during the last hour of baking.

Five samples of the beans were used to compare the cooking differences in the riboflavin and thiamine value due to the ingredients added to the baked beans. Each kind of beans were baked in two pots. One sample was cooked with all the ingredients among which pork and molasses were extremely interesting. Another sample was cooked under the same condition without any ingredient except the soda. Analyses of the vitamins were made using the same methods as described.

Moisture Determinations

Samples of raw beans and freshly baked beans were put in weighed moisture dishes and were dried in an oven between 70°-75° C. for 48 hours.

Vitamin Assay: Hydrolysis of Sample

In hydrolyzing the samples, Hennessy's method (1941) was used. Fifty grams of freshly baked beans were ground in a Waring blender for 3 minutes with 100 cc. of 0.2 N sulfuric acid. One hundred grams of the slurry was put in an amber glass flask and hydrolyzed for one hour on a steam bath. After cooling, the pH was adjusted to 4.5 with 15 percent sodium hydroxide and bromocresol green was used as an outside indicator. Ten millimeters of an acetic acid-sodium acetate buffer solution containing 0.1 gm. of takadiastase and 0.1 gm. of papain were added to the sample. The flask was incubated over night at 45° C. The following morning, the samples were heated on a steam bath for 5-10 minutes to inactivate the enzymes; the sample was cooled and filtered through No. 12 Whatman pleated filter paper. The filtrate was used for assay of thiamine and riboflavin.

For the analyses of the uncooked dried beans a twenty-five gram sample was used and 200 ml. of 0.2 N sulfuric acid were added and then blended as described above. The samples were transferred quantitatively to a 250 ml. volumetric flask after hydrolyzing with enzymes and then filtered.

The vitamin content of pork, molasses and the enzymes were analyzed separately. The enzymes were found to contain insignificant amounts of riboflavin and thiamine.

Vitamin Assay: Thiamine analyses

Five milliliters of the filtrate were allowed to pass through a column of activated Decalso and drained. The column was washed with three portions of buffered water. The adsorbed thiamine was eluted with about 20 ml. of hot 25 percent KCl in 0.1 N HCl solution. The eluate was collected in a 25 ml. graduated cylinder and made to volume. Five milliliters of the KCl-HCl eluate was oxidized in an oxidizing chamber which contained three ml. of 15 percent sodium hydroxide and 0.1 ml. of freshly prepared one percent potassium ferricyanide. Fifteen milliliters of redistilled isobutyl alcohol were added and the chamber was shaken for $1\frac{1}{2}$ minutes, centrifuged for $1\frac{1}{2}$ minutes, and the water layer removed with a suction pipette. The isobutyl alcohol layer was cleared with approximately 3 gms. of anhydrous sodium sulfate. The extract was transferred to a glass cuvette and its fluorescence was measured in the fluorophotometer which was standardized against a thiamine solution containing 0.5 mcg. of thiamine per ml. Quinine sulfate solution was used as a secondary standard because it is stable. Readings of the blank of each sample were measured in the same way without the ferricyanide solution. Andrews (1944) recommended that the highest fluorescence values were obtained when the alkali was added first and followed immediately by the ferricyanide and after $1\frac{1}{2}$ minutes of shaking.

Vitamin Assay: Riboflavin Analyses

Ten milliliters of the filtrate from hydrolysate of the raw or cooked beans were allowed to pass through a column of activated Florisil. The columns were rinsed with two successive 10 ml. portions of hot distilled water. Portions of hot pyridine-acetic acid solution of total volume 35 cc. were passed through and collected in a 50 ml. volumetric flask. One milliliter of two percent potassium permanganate solution was added and followed by a shaking of three minutes. One milliliter of three percent hydrogen peroxide was used to decolorize the excess permanganate. The sample was made to volume and read in the fluorophotometer. The fluorescence of samples was read against a standard solution containing 0.1-0.15 mcg. of riboflavin per ml. A reagent blank was prepared for each set of readings.

Recoveries of thiamine and riboflavin were measured by adding standard solutions to the slurries of both the raw and the baked beans. Results are given in Table IV.

Table IV

Recoveries of Thiamine and Riboflavin on the Raw and Baked Beans

Sample	Thiamine			Riboflavin		
	Amount in sample Mcg./gm.[1]	Amount added mcg.	Recovery %	Amount in sample mcg./gm.[1]	Amount added mcg.	Recovery %
Raw bean	2.65	1.00	98.89	1.72	1.60	96.73
Baked bean	10.25	2.00	98.54	1.55	1.60	99.21

[1] Dry basis

RESULTS AND DISCUSSION

Thiamine and Riboflavin Content of Raw Beans

Results of the study of the thiamine and riboflavin content of raw beans are presented in Table V. The thiamine values are all higher than those reported by Kelley, Dietrich and Porter (1940), of Booher, et al., (1942) and of Daniel and Norris (1945). (See Table I). The one variety and the five strains as arranged in descending order in relation to the thiamine content are Robust x Darling, Robust x Mexican Tree, Michelete, Robust x Early Prolific, Robust x Hunter and Robust x Crawford. The mean values were 11.89 ± 0.45 (standard deviation), 10.90 ± 0.41, 10.84± 0.37, 10.05± 0.55, 10.02± 0.11 and 9.67± 1.54 micrograms per gram of dried beans respectively. The range of the thiamine values was from 7.28 to 12.97 mcg. per gm. of dried beans.

The range of the riboflavin values was from 1.35 to 1.95 mcg. per gm. of dried beans. These values are generally lower than those reported in the literature, (See Table I). Some of the values agree well with those of Daniel and Norris (1945). The one variety and the five strains as arranged in relation to the riboflavin content are Robust x Darling, Robust x Mexican Tree, Michelete, Robust x Hunter, Robust x Early Prolific and Robust x Crawford. The mean values were 1.81± 0.01, 1.72± 0.01, 1.68±0.02, 1.66± 0.01, 1.63± 0.03, 1.58± 0.05 mcg. per gm. of dried beans respectively.

According to the results of this comparison, the raw samples of the Robust x Darling strain is considered to be the best source of thiamine and riboflavin in the series. The samples of Robust x Darling strain

Table V

Thiamine and Riboflavin Content of One Variety and Five Strains of Michigan Navy Beans

Variety	Thiamine		Riboflavin	
	Mean and Standard Deviation	Range	Mean and Standard Deviation	Range
Michelete	Mcg./gm.[1] 10.84±0.37	mcg/gm.[1] 9.97-11.77	mcg./gm.[1] 1.68±0.02	mcg./gm.[1] 1.53-1.84
Robust x Early Prolific	10.05±0.55	9.61-11.46	1.63±0.03	1.35-1.86
Robust x Crawford	9.67±1.54	7.28-11.62	1.58±0.02	1.35-1.78
Robust x Darling	11.89±0.45	11.06-12.97	1.81±0.01	1.63-1.95
Robust x Mexican Tree	10.90±0.41	10.16-11.34	1.72±0.01	1.63-1.83
Robust x Hunter	10.02±0.11	9.79-10.40	1.66±0.01	1.59-1.78

[1] Dry basis.

have the highest value in both thiamine and riboflavin content while the Robust x Crawford beans have the lowest value in both vitamins.

In order to separate the variation between strains from the variation due to the replication of samples within each strain of beans, analysis of variance was used. The analysis for the thiamine values is shown in Table VI.

Table VI

Analysis of Variance of Thiamine Content in Raw Beans

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	Value of F
Total	45	65.07		
Between variety	5	31.53	6.31	7.52 **
Within variety	40	33.54	0.84	

	Predicted value of F	(From Snedecor)
Probability < .05	2.45	
Probability < .01	3.51	

From the above calculation of F value, the difference in thiamine value of samples between varieties is highly significant.

The data of the riboflavin content of raw beans were also analyzed by using analysis of variance. Result is given in Table VII.

Table VII

Analysis of Variance of Riboflavin Content in Raw Beans

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	Value of F
Total	45	1.148		
Between variety	5	0.335	0.067	3.30 *
Within variety	40	0.813	0.203	

	Predicted value of F
Probability $< .05$	2.45
Probability $< .01$	3.51

There was a significant difference in the riboflavin content of different varieties.

Correlation between Thiamine and Riboflavin Content of Raw Beans

Correlation coefficient between the riboflavin and thiamine content of raw beans was calculated by using the equation:

$$r = \frac{Sxy - \frac{Sx\ Sy}{n}}{\sqrt{\left(Sx^2 - \frac{(Sx)^2}{n}\right)\left(Sy^2 - \frac{(Sy)^2}{n}\right)}}$$

where x represents the thiamine content,

y represents the riboflavin content.

The correlation coefficient obtained was 0.44. The probability of significance of this value is given below.

	Value of r (Fisher)
Probability $< .05$	0.3044
Probability $< .01$	0.3932

The correlation coefficient between thiamine and riboflavin value of raw beans is highly significant.

Losses of Thiamine and Riboflavin in Baking Navy Beans

The cooking losses in thiamine and riboflavin content were determined for five samples which were baked under the same conditions with and without molasses and salt pork. The differences in thiamine and riboflavin content between raw beans and those baked without added ingredients were used as a measure of the cooking losses. Results are shown in Tables VIII and IX. A decrease in both vitamins was shown. The average percentage loss of thiamine is 70.88 percent, and the average loss of riboflavin is 21.09 percent. Thus it is shown that in the baking process the beans lose more thiamine than riboflavin.

The thiamine and riboflavin content of baked beans are presented in Table X. The average, range and standard deviation are reported for each kind. Less varietal difference in the thiamine and riboflavin content of the baked samples appeared than in the same samples of the raw beans.

In the recipe used for the baked beans, a considerable amount of salt pork and molasses were added to each bean pot. The riboflavin content of these ingredients is high enough to affect the result of the baked beans. Table XI shows the amount of vitamins added to each bean pot.

Table VIII

Cooking Losses of Thiamine of Five Samples of Beans

No. of Sample	(A) Thiamine in Baked Beans with Ingredients	(B) Thiamine in Baked Beans without Ingredients	(C) Thiamine in Raw Beans	Difference between A & C	Difference between C & B.	Percentage Loss in Cooking $(\frac{C-B}{C} \times 100)$
	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]	%
802	2.87	2.88	9.80	6.93	6.92	70.61
804	3.22	3.12	9.16	5.94	6.04	65.94
812	2.48	2.37	9.46	6.98	7.07	74.95
814	2.75	2.71	9.34	6.59	6.63	70.99
842	2.88	2.77	9.86	6.98	7.09	71.91

Average Percentage of Cooking Loss	70.88%
Range	65.94-74.95%

[1] Dry basis.

Table IX

Cooking Losses of Riboflavin of Five Samples of Beans

No. of Sample	(A) Riboflavin in Baked Beans with Ingredients	(B) Riboflavin in Baked Beans without Ingredients	(C) Riboflavin in Raw Beans	Difference between A & C	Difference between C & B	Percentage Loss in Cooking $(\frac{C-B}{C} \times 100)$
	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]	%
802	1.84	1.42	1.79	1.05	0.37	20.67
804	1.81	1.29	1.59	0.22	0.30	18.87
812	1.70	1.28	1.66	0.04	0.38	22.89
814	1.71	1.22	1.55	0.16	0.38	21.29
842	2.30	1.26	1.61	0.69	0.35	21.74

Average Percentage of Cooking Loss	21.09%
Range	18.87-22.89%

[1] Dry basis.

Table X

Thiamine and Riboflavin Content in Baked Samples of One Variety and Five Strains of Michigan Navy Beans

Variety	Thiamine		Riboflavin	
	Mean and Standard Deviation	Range	Mean and Standard Deviation	Range
	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]	mcg./gm.[1]
Michelete	3.24±0.32	2.47-3.89	1.86±0.06	1.45-2.05
Robust x Early Prolific	3.04±0.14	2.68-3.56	1.76±0.06	1.36-2.16
Robust x Crawford	3.06±0.13	2.20-3.62	1.97±0.03	1.72-2.12
Robust x Darling	3.41±0.21	2.69-3.91	1.84±0.03	1.49-2.08
Robust x Mexican Tree	3.53±0.02	3.37-3.64	1.98±0.00	1.92-2.04
Robust x Hunter	3.30±0.21	2.86-3.77	2.15±0.02	2.03-2.32

[1] Dry basis.

Table XI

Amount of Thiamine and Riboflavin Added to Each Pot of Baked Beans

Source of added vitamin	Thiamine mcg.	Riboflavin mcg.
Salt pork	37.80	60.90
Molasses	9.80	177.80

In the difference of vitamin contents between the raw beans and the baked beans, there is a marked decrease in thiamine content and a slight increase in riboflavin content after baking. Some investigators mentioned before, Streightoff, et al., (1946) and Tuck, Hinman, and Halliday (1946) had found high retention of riboflavin after cooking of vegetables and meat. These authors suggested that there was greater liberation of riboflavin in the cooked samples than that in the raw ones. This may be one of the reasons in this study that a high value of riboflavin was obtained after baking. The addition of salt pork and molasses may be of importance in raising the riboflavin value of the cooked samples, because a decrease in both vitamin values was obtained when the beans were baked with no added ingredients. The amount of thiamine in the baked beans cooked with ingredients is approximately 141 mcg. for each serving and the riboflavin is approximately 107 mcg. for each serving.

Actual results of thiamine and riboflavin analysis of each sample of beans raw and baked are tabulated in the Appendix Tables I, II, III and IV.

SUMMARY AND CONCLUSIONS

The differences in thiamine and riboflavin content of 46 raw and cooked samples representing one variety and strains of five crosses of Michigan navy beans were determined. The method of Mickelson, Condiff and Keys (1945) was used to determine the thiamine content of the beans; the riboflavin content was determined by the method of Conner and Straub (1941), modified by Keys (1944).

Analysis of variance of the thiamine and riboflavin contents of the raw beans of one variety and strains of five crosses showed that there was a highly significant difference in the thiamine content between the various lots of beans and that there was a significant difference in the riboflavin content between lots.

The average thiamine contents were 11.89 mcg. per gm. of dry beans for Robust x Darling, 10.90 mcg. for Robust x Mexican Tree, 10.84 mcg. for Michelete, 10.50 mcg. for Robust x Early Prolific, 10.02 mcg. for Robust x Hunter and 9.67 mcg. for Robust x Crawford, arranged in descending order. The average riboflavin contents were 1.81 mcg. per gm. of dry beans for Robust x Darling, 1.72 for Robust x Mexican Tree, 1.68 for Michelete, 1.66 for Robust x Hunter, 1.63 for Robust x Early Prolific and 1.58 for Robust x Crawford.

The correlation coefficient between the thiamine and riboflavin content of the dried seeds was found to be highly significant.

Five samples of the beans were baked without the addition of salt pork, and molasses, thiamine cooking losses averaged 70.88 percent and the cooking losses of riboflavin averaged 21.09 percent for the same samples.

In the baking trials, when the beans were baked with added ingredients, a marked decrease in the thiamine content and a slight increase in the riboflavin content were observed. The increase in riboflavin content resulted from the addition of the salt pork and molasses. There appeared to be less difference in the thiamine content of different lots of baked beans than was observed in the same samples before baking.

A 150 grams serving of the baked beans reported in this study contained approximately 141 mcg. of thiamine and 107 mcg. of riboflavin.

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APPENDIX

APPENDIX TABLE I -- THIAMINE IN RAW NAVY BEANS

Variety		Sample No. and Thiamine Content															
Michelete	No. of Sample	801	811	821	831	841	851	861	871	SS							
	Thiamine mcg./gm.[1]	9.97	11.54	10.98	10.83	10.80	10.72	10.12	11.77	10.25							
Robust x Early Prolific	No. of Sample	802	812	813	814	815	816										
	Thiamine mcg./gm.[1]	9.89	9.46	11.46	10.21	9.61	9.66										
Robust x Crawford	No. of Sample	803	804	805	806	844	845	846	852	853	854	855	856	862	863	864	865
	Thiamine mcg./gm.[1]	11.55	9.82	9.39	10.14	8.91	11.62	10.38	9.20	8.62	7.28	8.08	9.41	8.90	9.33	10.58	11.47
Robust x Darling	No. of Sample	822	823	824	825	826	832	833	834	835							
	Thiamine mcg./gm.[1]	12.97	12.37	12.70	11.32	11.06	11.92	11.29	11.44	11.94							
Robust x Mexican Tree	No. of Sample	866	872	873													
	Thiamine mcg./gm.[1]	10.16	11.34	11.20													
Robust x Hunter	No. of Sample	836	842	843													
	Thiamine mcg./gm.[1]	10.40	9.86	9.79													

[1] Dry basis.

APPENDIX TABLE II -- RIBOFLAVIN IN RAW NAVY BEANS

Variety	Sample No. and Riboflavin Content																
Michelete	No. of Samples	801	811	821	831	841	851	861	871	SS							
	Riboflavin mcg./gm.[1]	1.81	1.53	1.73	1.80	1.47	1.84	1.76	1.63	1.55							
Robust x Early Prolific	No. of Samples	802	812	813	814	815	816										
	Riboflavin mcg./gm.[1]	1.79	1.66	1.86	1.55	1.35	1.56										
Robust x Crawford	No. of Samples	803	804	805	806	844	845	846	852	853	854	855	856	862	863	864	865
	Riboflavin mcg./gm.[1]	1.78	1.59	1.40	1.65	1.53	1.60	1.62	1.52	1.35	1.41	1.70	1.68	1.45	1.38	1.88	1.72
Robust x Darling	No. of Samples	822	823	824	825	826	832	833	834	835							
	Riboflavin mcg./gm.[1]	1.76	1.96	1.83	1.85	1.86	1.73	1.63	1.95	1.75							
Robust x Mexican Tree	No. of Samples	866	872	873													
	Riboflavin mcg./gm.[1]	1.83	1.63	1.69													
Robust x Hunter	No. of Samples	836	842	843													
	Riboflavin mcg./gm.[1]	1.61	1.59	1.78													

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[1] Dry Basis.

APPENDIX TABLE III -- THIAMINE IN BAKED NAVY BEANS

Variety		Sample No. and Thiamine Content															
Michelete	No. of Sample	801	811	821	831	841	851	861	871	SS							
	Thiamine mcg./gm.[1]	3.07	3.78	3.74	3.89	3.16	2.61	2.47	3.75	2.65							
Robust x Early Prolific	No. of Sample	802	812	813	814	815	816										
	Thiamine mcg./gm.[1]	3.56	2.86	2.68	2.88	2.78	3.48										
Robust x Crawford	No. of Sample	803	804	805	806	844	845	846	852	853	854	855	856	862	863	864	865
	Thiamine mcg./gm.[1]	2.78	3.32	2.71	3.08	3.30	3.21	3.21	3.18	3.02	2.75	2.20	3.16	3.44	3.35	3.62	2.70
Robust x Darling	No. of Sample	822	823	824	825	826	832	833	834	835							
	Thiamine mcg./gm.[1]	3.78	3.91	3.74	3.61	2.99	2.69	3.72	2.82	3.50							
Robust x Maxican Tree	No. of Sample	866	872	873													
	Thiamine mcg./gm.[1]	3.64	3.57	3.37													
Robust x Hunter	No. of Sample	836	842	843													
	Thiamine mcg./gm.[1]	3.27	2.86	3.77													

[1] Dry Basis.

APPENDIX TABLE IV -- RIBOFLAVIN IN BAKED NAVY BEANS

Variety		Sample No. and Riboflavin Content															
Michelete	No. of Sample	801	811	821	831	841	851	861	871	SS							
	Riboflavin mcg./gm.[1]	1.80	1.92	1.93	2.12	2.05	1.62	1.45	2.14	1.72							
Robust x Early Prolific	No. of Sample	802	812	813	814	815	816										
	Riboflavin mcg./gm.[1]	2.16	1.70	1.77	1.36	1.81	1.76										
Robust x Crawford	No. of Sample	803	804	805	806	844	845	846	852	853	854	855	856	862	863	864	865
	Riboflavin mcg./gm.[1]	1.72	1.80	1.84	1.84	2.23	2.10	1.94	2.29	2.10	1.86	1.97	1.96	2.12	2.08	1.72	1.88
Robust x Darling	No. of Sample	822	823	824	825	826	832	833	834	835							
	Riboflavin mcg./gm.[1]	1.78	2.04	1.88	1.90	1.82	1.88	2.08	1.73	1.49							
Robust x Mexican Tree	No. of Sample	866	872	873													
	Riboflavin mcg./gm.[1]	1.98	2.04	1.92													
Robust x Hunter	No. of Sample	836	842	843													
	Riboflavin mcg./gm.[1]	2.03	2.11	2.32													

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[1] Dry basis.