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Angela Mary Filios
University of Massachusetts Amherst

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STUDY OF THE EFFECT of DIFFERENT CANNING PROCEDURES ON THE STABILITY OF CERTAIN OF THE "B" VITAMINS IN MUSHROOMS (AGARICUS CAMPESTRIS)

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A STUDY OF THE EFFECT OF DIFFERENT CANNING PROCEDURES ON THE STABILITY OF CERTAIN OF THE "B" VITAMINS IN MUSHROOMS (*Agaricus campestris*)

Angela M. Filios

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Massachusetts State College

Amherst

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INTRODUCTION

"Canning is so well established as a satisfactory method for home and commercial preservation of foods that large quantities of food are consumed in the canned state. Information on the vitamin value of these products as compared with that of the corresponding fresh foods is, therefore, of particular interest in evaluating the nutritive content of diets in many American homes", according to Adams and Smith (1944).

This investigation was made in order to obtain data on the stability and retention of certain of the B vitamins in canned mushrooms. The effects of different canning procedures, different types of containers, and different storage conditions were studied. For purposes of comparison the effects of various cooking procedures were also investigated.

The cultivated mushroom (*Agaricus campestris*), a good source of the B vitamins, was used as the test material. The vitamins studied included: riboflavin (vitamin B_2, G, lactoflavin); nicotinic acid (niacin, P-P factor); pantothenic acid (calcium pantothenate, chick antidermatitis factor, filtrate factor); and biotin (vitamin H).

The experimental work itself was divided into three parts; namely, (1) preparation of canned mushrooms in the laboratory in order to determine (a) the effect of the canning process itself and (b) the effect of storage in glass and in tin containers under different conditions on the stability of the B vitamins under consideration;

(2) the analysis of three different types of canned mushrooms as canned commercially by six different packers in order to obtain a broad picture of the B vitamin content of these products as they are available to consumers; and

(3) a laboratory study on the changes in B vitamin content of fresh

mushrooms, due to various cooking methods, such as (a) boiling, (b) broiling, and (c) sauteeing.

In order to observe the effect of container and storage conditions on the vitamin content of mushrooms this product was packed in both commercial type glass jars and tin cans. After processing, the glass jars were stored on a shelf in a South window exposed to bright sunlight; on a shelf (such as a typical store shelf) exposed to diffused daylight; and in the dark at room temperature. The tin cans were all stored at room temperatures.

Microbiological methods were employed for determining the vitamin content of canned mushrooms as they had been found particularly adaptable for use in an investigation such as this one. Since the pantothenic acid determinations are calculated from growth curves based on measured amounts of the calcium salt of pantothenic acid, the vitamin is here referred to as calcium pantothenate.

The data obtained in this study provide general information on the stability of riboflavin, nicotinic acid, calcium pantothenate, and biotin during the processing and storage of canned foods, as represented by a food rich in these vitamins; as well as the stability of these vitamins when the raw material is prepared and cooked for serving on the table.

REVIEW OF LITERATURE

Riboflavin

Although no data were found in the literature on the effect of canning on the stability of vitamins in mushrooms, information on some of the factors effecting stability of the B vitamins is helpful to an understanding of the problem.

The heat stability of riboflavin was first indicated by Smith and Hendrick (1926). In rat feeding experiments they reported an essential growth factor other than B_1 which withstood autoclaving at 15 pounds pressure for six hours. Chick and Roscoe (1927) reported that autoclaving of brewers yeast for five hours gave a preparation free from B_1, but containing the thermostable B factor. Roscoe (1931) found no loss of vitamin B_2 during the cooking of carrots for animal feed.

Hoff (1933) studied the effect of household cooking and canning on the vitamin content of vegetables. After feeding cooked and canned spinach to pigeons, he concluded that less vitamin G than vitamin B_1 was lost probably because of the greater heat stability of vitamin G. The work of Dutcher, Guerrant and McKelvey (1934), and of Cheldelin, Woods and Williams (1943) further established the heat stability of riboflavin in foods.

Recently, extensive work has been done on the retention of vitamins in meat during cooking and curing. McIntire, Schweigert, Henderson and Elvehjem (1943) reported an 85 per cent retention of riboflavin after roasting, broiling, or braising. McIntire, Schweigert and Elvehjem (1943) found that the average total retention of riboflavin after cooking lamb and veal ranged from 87 to 101 per cent. Storage, curing, and cooking

caused very little loss of riboflavin according to McIntire, Schweigert, Herbst and Elvehjem (1944) and Schweigert, McIntire and Elvehjem (1943). Cover, McLaren and Pearson (1944) reported a retention of 83 and 77 per cent of riboflavin in rare and well done rib roasts, respectively.

Morgan, MacKinney and Cailleau (1945) reported that there was but little loss of riboflavin due to cooking or steam blanching of vegetables, even when the cooking liquid was not included in the determinations.

Christensen (1936) worked on the influence of cooking and canning on meat, and reported no destruction of vitamin G. Cruess (1941) thought it likely that canning operations would cause little or no injury to riboflavin.

Grzhivo and Kondrashova (1940) studied the changes in riboflavin content during the canning of vegetables and reported losses ranging from 0 to 35 per cent on canning and storage, depending on the vegetable tested. Fellers, Esselen and Fitzgerald (1940) found the following losses of riboflavin in vegetables due to canning: peas, no loss; asparagus, two per cent; lima beans, 30 per cent; and spinach, 50 per cent. Farrell and Fellers (1942) found a very slight loss of riboflavin due to canning green snap beans. Storage for a year caused the percentage loss in glass packed beans to increase to 7.5 per cent; and in tin canned beans the loss was nine per cent after storage for a year.

Rice and Robinson (1944) reported a retention of 90 to 100 per cent of riboflavin in canned pork or beef, with little or no additional loss during storage for 219 days.

It has been apparent for some time that the hydrogen ion concentration is an important factor in the stability of riboflavin. The British Medical Research Council (1932) reported that the heat stability of

vitamin B_2 is only relative, depending largely on the acidity of the medium during heating. Williams, Waterman and Gurin (1929) found the destruction of B_2 was slight when brewers yeast was autoclaved in acid medium; but that destruction was considerable at pH 8.0 and practically complete at pH 12 to 14.

The ease of inactivation of riboflavin increases rapidly with increasing alkalinity, as shown by the work of Guerrant and Salmon (1930), Chick and Copping (1930), Halliday, Nunn and Fisher (1932), and Roscoe (1933). Chick and Roscoe (1930) and Guha and Chakravorty (1933) found that the optimum reaction for extraction from yeast and from ox kidney was a pH value of 5.0. Davis and Norris (1936) found a slight destruction of vitamin G when the pH value of milk was brought up to 9.5.

Early work on the photochemical reactions of riboflavin was conducted by Kuhn, Rudy, and Wagner-Jauregg (1933) and by Karrer, Salomon, Schopp, Schlittler and Fritsche (1934). They irradiated riboflavin solutions under different conditions, obtaining various breakdown products. Supplee, Ansbacher and Bender (1935) reported on the photochemical phenomena involved in vitamin G studies. They fed a crude milk concentrate to white rats. All samples of the concentrate kept in the dark showed typical brilliant yellow-green fluorescence with a spectral band of 3100 to 4100 Å, irrespective of whether they were saturated with air, oxygen or nitrogen. All possessed distinct growth-promoting properties. All samples exposed to light showed a progressive change in fluorescent color from the typical yellow-green to blue green and finally to blue. Disappearance of the yellow-green fluorescence and the appearance of blue fluorescence was concurrent with decrease or total destruction of growth-promoting property. Oxygen appeared to accelerate to a slight

degree the transformations noted. It was observed that substances in solution or suspension, which may act as a screen or filter for certain radiations, retarded photochemical transformations.

Bisbey and Sherman (1935) experimented upon the extraction and stability of lactoflavin. They found that oxidation by residual or occluded air did not have a very destructive influence. In acid-alcohol extractions of vitamins from skim milk powder they reported that the diminution of flavin value was probably due to combined effects of visible light penetrating the glass containers holding extraction materials, small amounts of residual oxygen and possibly a slight influence of the alcohol present.

Gyorgy (1935) found that the inactivation of lactoflavin by irradiation was greater in an alkaline than an acid medium. These findings were substantiated by Connor and Straub (1941) who worked with riboflavin solutions.

Recently, considerable work has been done on the destruction of riboflavin in milk exposed to sunlight. Peterson, Haig and Shaw (1944) and Ziegler (1944) reported that at least a 50 per cent loss of riboflavin occurred in milk during two hours exposure to sunshine. Stamberg and Theophilus (1944) reported a 50 per cent loss after four hours exposure in clear glass bottles. Holmes and Jones (1945) found that the destruction of riboflavin in milk increased fairly consistently with an increased intensity of the sunshine to which it was exposed.

Nicotinic Acid

Nicotinic acid has been known in the chemical world for over 75 years but its nutritional significance has been established only within the past decade. According to Scott (1944), nicotinic acid is non-hygroscopic and stable in air. It is a comparatively weak acid and its alkaline salts in solution show a slightly alkaline reaction. It is stable to autoclaving temperatures when in solution and shows no loss of activity when exposed to dry heat.

Cruess (1941) considered nicotinic acid stable to canning operations. Cheldelin, Woods and Williams (1943) reported nicotinic acid losses to be slight in the cooking of foods. Russell, Taylor and Beuk (1943) reported loss of nicotinic acid due to cooking vegetables varied with types and with different samples, average retention being from 75 to 87 per cent. Heller, McCay and Lyon (1943) studied large-scale cookery operations, and reported losses of nicotinic acid in vegetables ranging from two to 61 per cent. Morgan, MacKinney and Cailleau (1945) showed a wide range of retention in their study of retention of niacin in vegetables during blanching and cooking.

McIntire, Schweigert and Elvehjem (1943, 1944) in their studies on retention of nicotinic acid in meat during cooking, reported values ranging from 65 to 100 per cent retention, with most values above 90 per cent retention. Dann and Handler (1942) reported that losses due to cooking meat ranged from 33 to 50 per cent based on dry weight of meat. Cover, McLaren and Pearson (1944) found a retention of 75 to 79 per cent in well done and rare beef.

Rice and Robinson (1944) studied canned meats. Retention of

90 to 100 per cent nicotinic acid during canning was not affected by storage for 219 days. Hodson (1945) reported no loss of nicotinic acid due to the processing of irradiated evaporated and dry milk.

Pantothenic Acid

Williams, Lyman, Goodyear and Truesdail (1933), and Weinstock, Mitchell, Pratt and Williams (1939) discussed the instability of pantothenic acid in acid or alkaline media. Williams, Truesdail, Weinstock, Rohrmann, Lyman and McBurney (1938) reported that this substance is highly hydrophilic, all salts and derivatives being highly soluble in water. Pantothenic acid itself is unstable and can be handled safely only as a neutral salt. It is particularly susceptible to destruction by acidic alcohol.

Frost (1943) reported that the destruction of pantothenic acid is due to hydrolysis, the rate of which is a function of pH value and temperature and is affected also by the presence of other substances. Optimum stability is in the approximate acidity range of pH 5.5 to 7.0.

Waisman, Mickelson and Elvehjem (1939) observed that the ordinary household method of stewing decreased the potency of kidney, heart and spleen by one-third. Waisman, Henderson, McIntire and Elvehjem (1942) reported losses due to cooking and commercial processing of meats ranging from very slight to 66 per cent. Cheldelin, Woods and Williams (1943) found that pantothenic acid losses were moderate to slight due to cooking vegetables but amounted to as much as one-third in meats. Cover, McLaren and Pearson (1944) reported retentions of 91 and 75 per cent, respectively, in rare and well done beef roasts.

Cruess (1941) believed it likely that some pantothenic acid would be destroyed by canning procedures. Rice and Robinson (1944) reported a retention of 70 to 80 per cent during the canning of meat, without further change during storage up to 219 days. According to Hodson (1945) there was no apparent loss of pantothenic acid during the processing of irradiated evaporated and dry milk.

Biotin

The identity of biotin with vitamin H, the factor curative of egg white injury in rats and chicks was discovered by du Vigneaud, Melville, Gyorgy and Rose (1940) and by Gyorgy, Rose, Hofmann, Melville and du Vigneaud (1940). Thompson, Eakin and Williams (1941) found that alkaline hydrolysis gradually destroys biotin.

Schweigert, Nielson, McIntire and Elvehjem (1943) reported an average retention of 80 per cent of biotin after cooking meats. Cheldelin, Woods and Williams (1943) observed that biotin losses during cooking were moderate to negligible in vegetables, but amounted to as much as 72 per cent in meats.

EXPERIMENTAL

I. METHODS

Preparation of Samples

Fresh and blanched mushrooms were treated with as little delay as possible. Representative samples (approximately one pound) were ground in a food chopper and then in a Waring Blendor before being extracted for the vitamin determinations.

Canned mushrooms were treated according to the procedure described in the methods of analysis of the A. O. A. C. (1940). The contents of at least six cans were taken for each sample. A dial type vacuum gage was used to measure the vacuum of each container before it was opened. The contents were allowed to drain two minutes on an 8-inch sieve, and the drained weights of both solid and liquid portions recorded. The solid portions were ground in a food chopper and in a Waring Blendor.

In order to calculate the vitamin value of the total contents of a container of canned mushrooms, the drained solids and the liquid portions were separated and weighed as described above. The vitamin content per gram of solids and per milliliter of liquid was next determined. Knowing the weight of the solids and of the liquid portions of the can, the total vitamin value of each portion and then of the whole can contents could be determined.

Moisture determinations were made on all samples of solids: fresh, blanched, cooked and canned. The A. O. A. C. (1940) method employing the Bidwell and Sterling apparatus for direct distillation with toluene was used to determine the moisture content.

Vitamin Determinations

The vitamin determinations were carried out by the microbiological method of Landy and Dicken (1942). This microbiological method is based upon the growth of a bacteria (*Lactobacillus casei*) to which the vitamins assayed are essential growth factors under specified conditions. Within limited ranges, the growth of the organism is directly proportional to the amount of vitamin present. This method may be adapted for the assay of several of the B vitamins, including riboflavin, nicotinic acid, calcium pantothenate and biotin. The growth response of the organism to the vitamin in question may be measured turbidimetrically or by titration of the lactic acid produced, with alkali. In this study all determinations were made by measuring the amount of growth as indicated by lactic acid production, when titrated with 0.1 N sodium hydroxide at the end of 72 hours incubation at 37° C. (98.6° F.).

Standard growth curves were drawn from the titration values obtained when measured amounts of riboflavin, nicotinic acid, calcium pantothenate or biotin were present in the assay medium. From these standard curves, the amount of vitamin in the mushroom samples could be determined. Standard or reference curves were obtained each time the vitamin determinations were made. Typical growth curves obtained by the Landy-Dicken method are shown in figures 1 to 4 inclusive.

A question is sometimes raised regarding the reliability of the Landy-Dicken microbiological assay method, particularly in the case of certain foods. In order to determine whether or not this method might be

Note: Cultures of *Lactobacillus casei* 7469 and *Lactobacillus arabinosus* 17-5, for use in the microbiological assays were obtained from the American Type Culture Collection, Georgetown University, Washington, D. C.

Figure 1. Riboflavin Standard Curve, Landy-Dicken Method

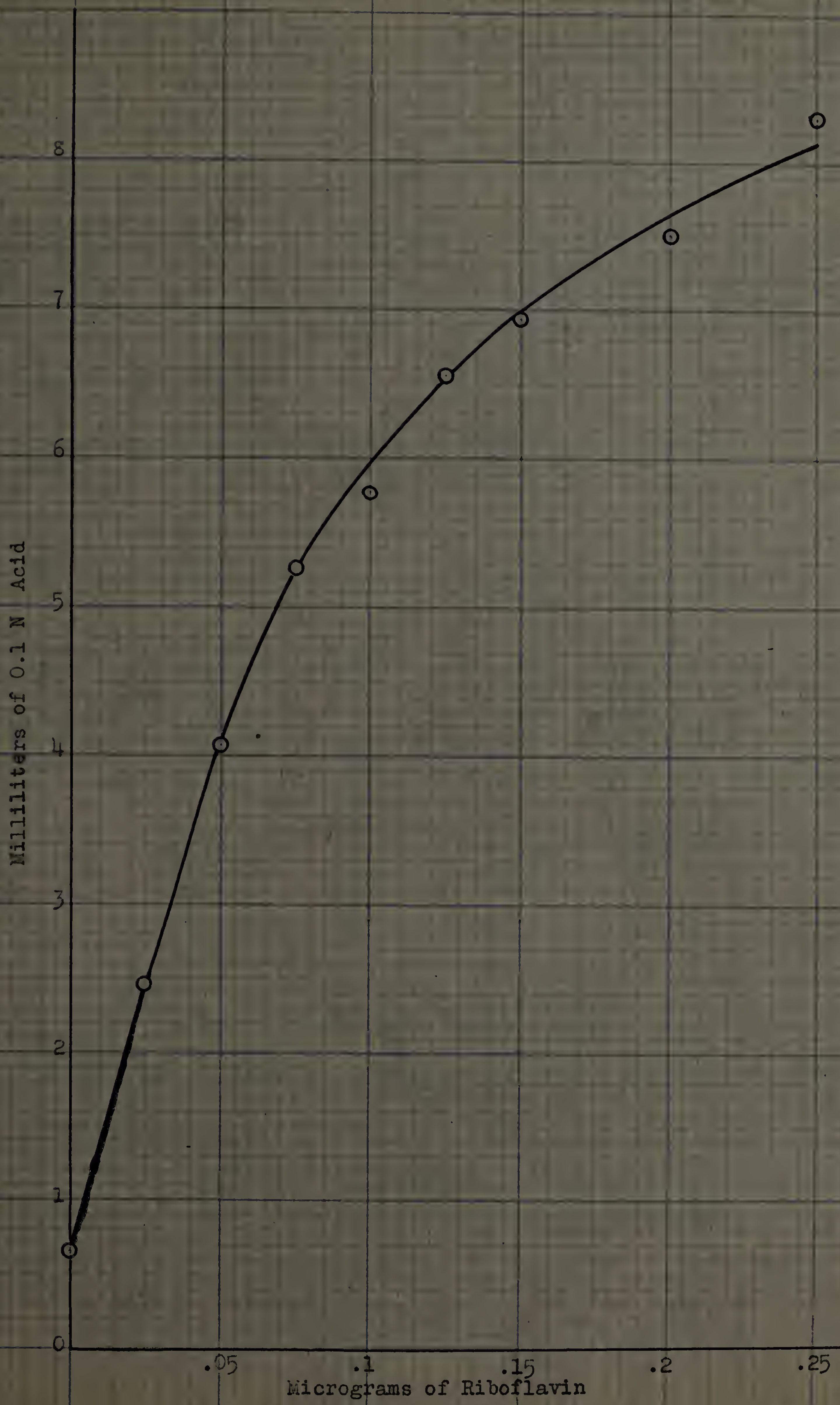


Figure 2. Nicotinic Acid Standard Curve, Landy-Dicken Method

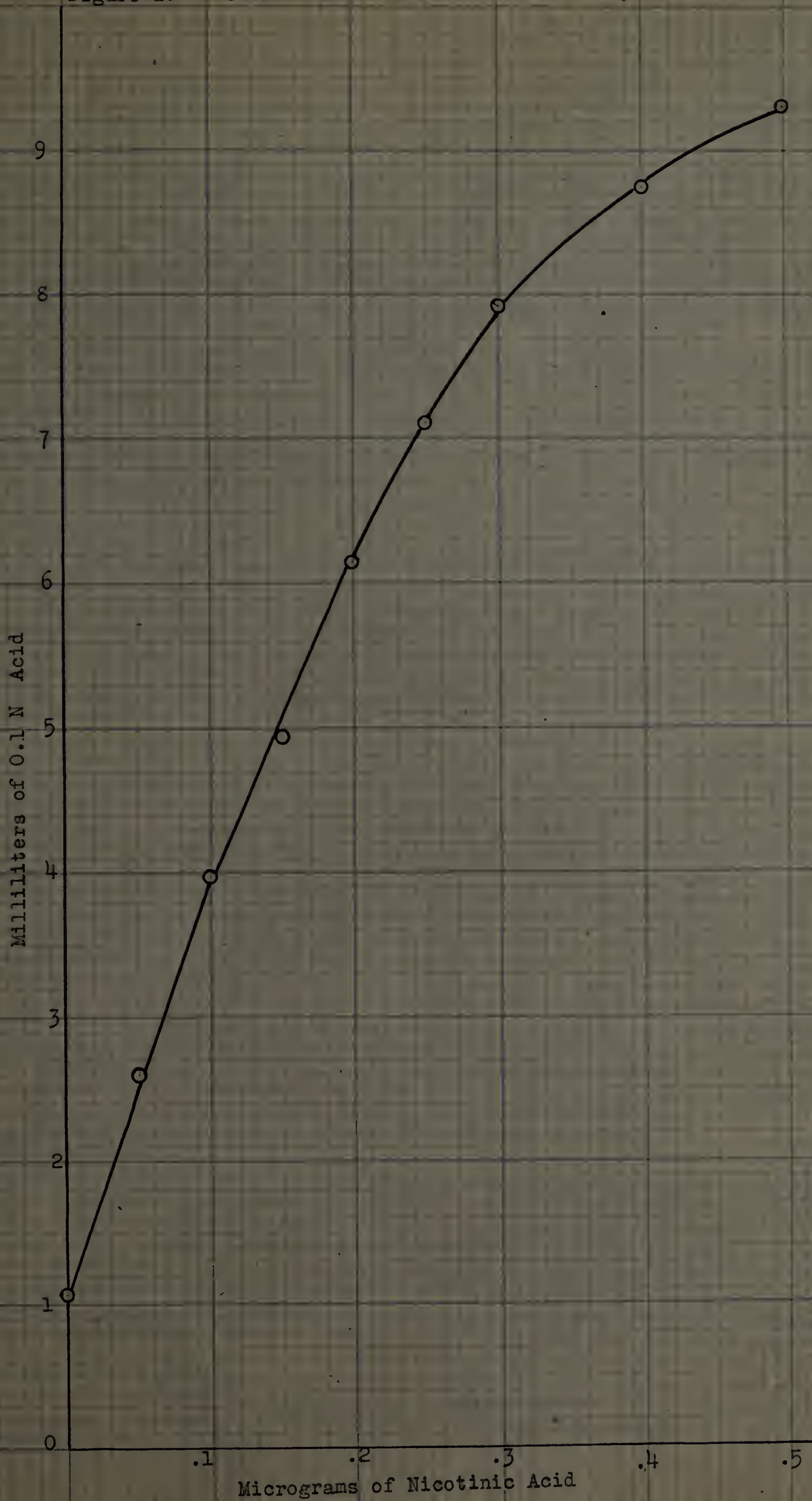


Figure 3. Calcium Pantothenate Standard Curve, Landy-Dicken Method

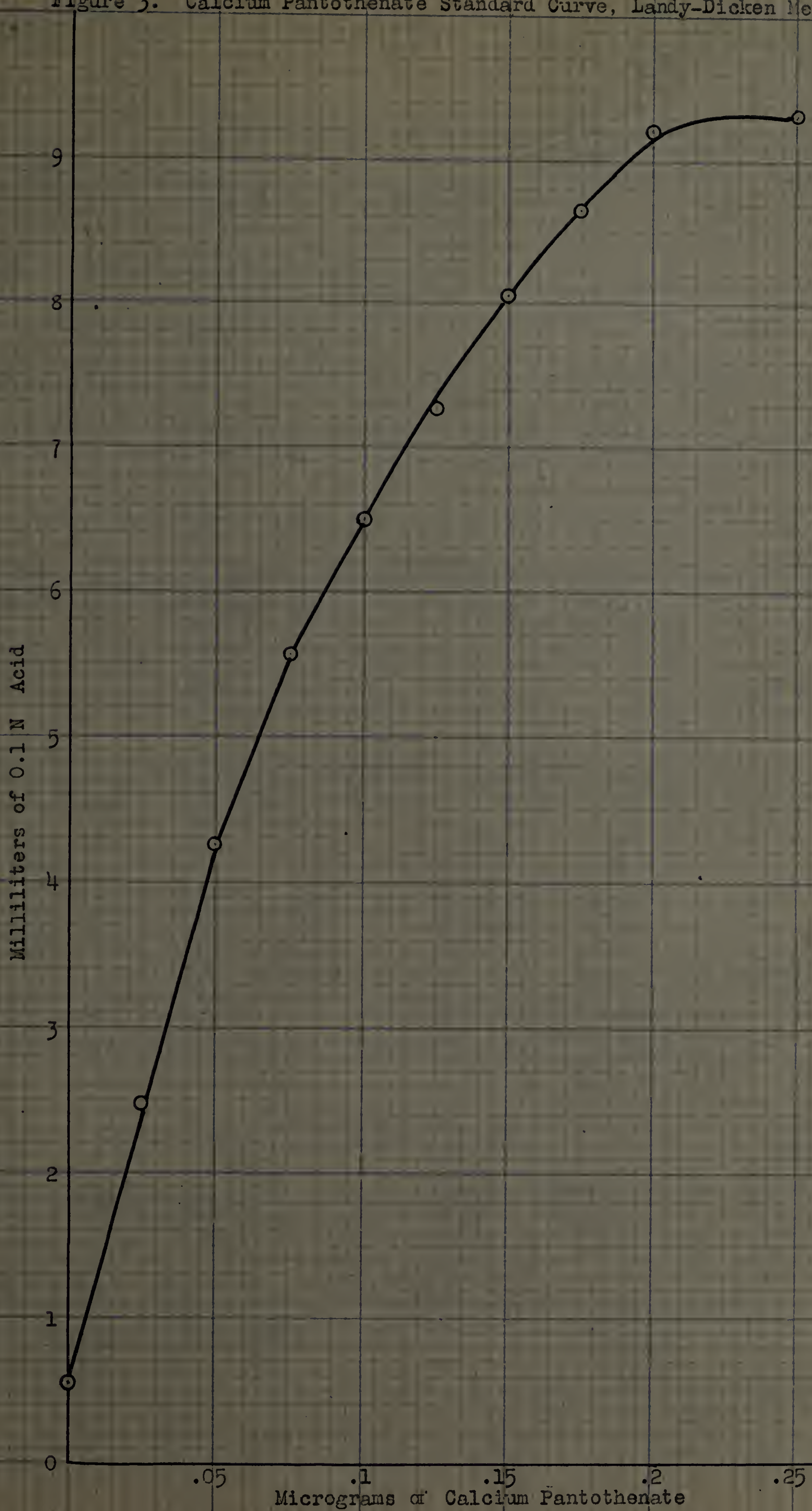
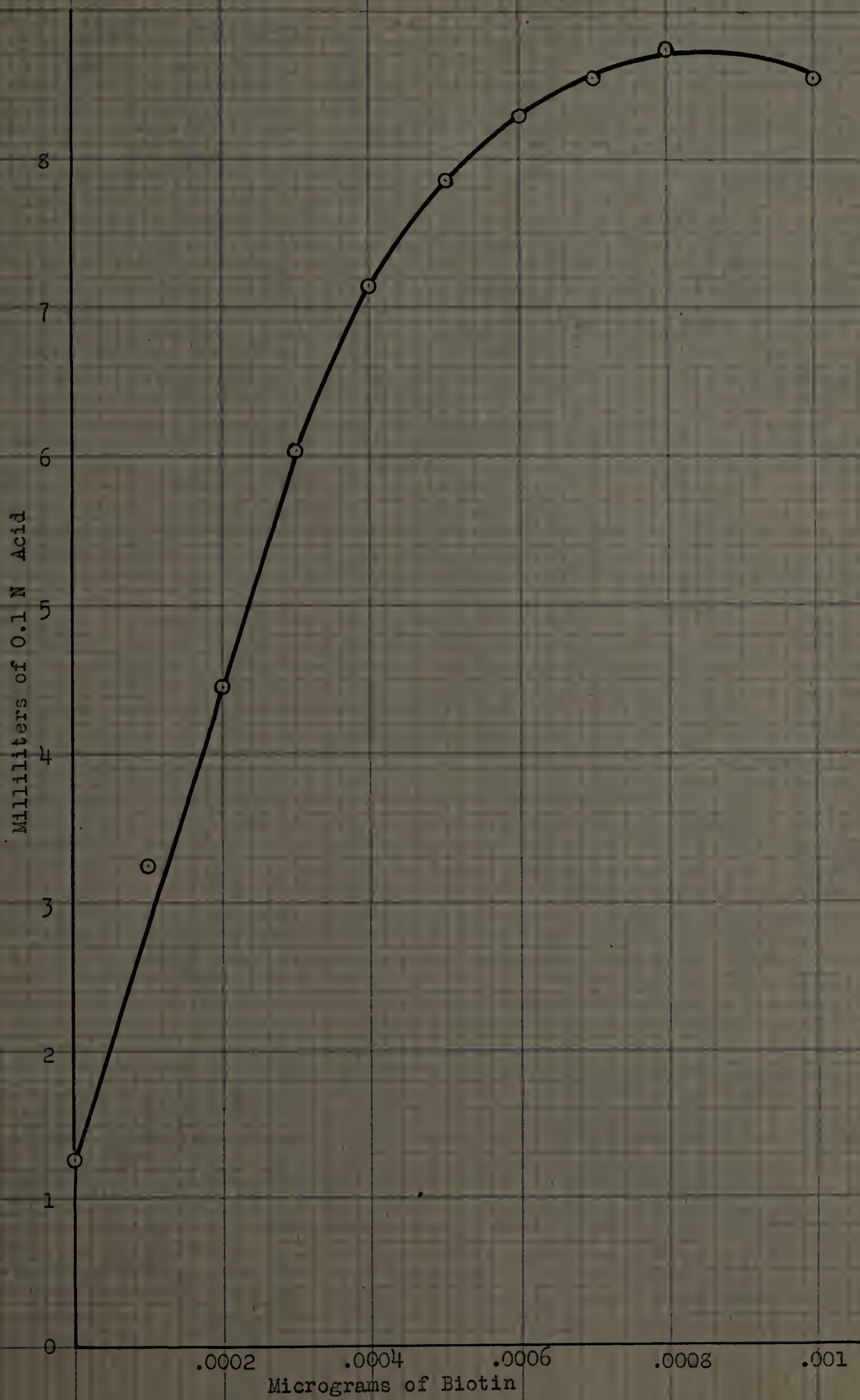


Figure 4. Biotin Standard Curve, Landy-Dicken Method



considered reliable for the assay of mushrooms, several samples were assayed by both the above method and other recent methods.

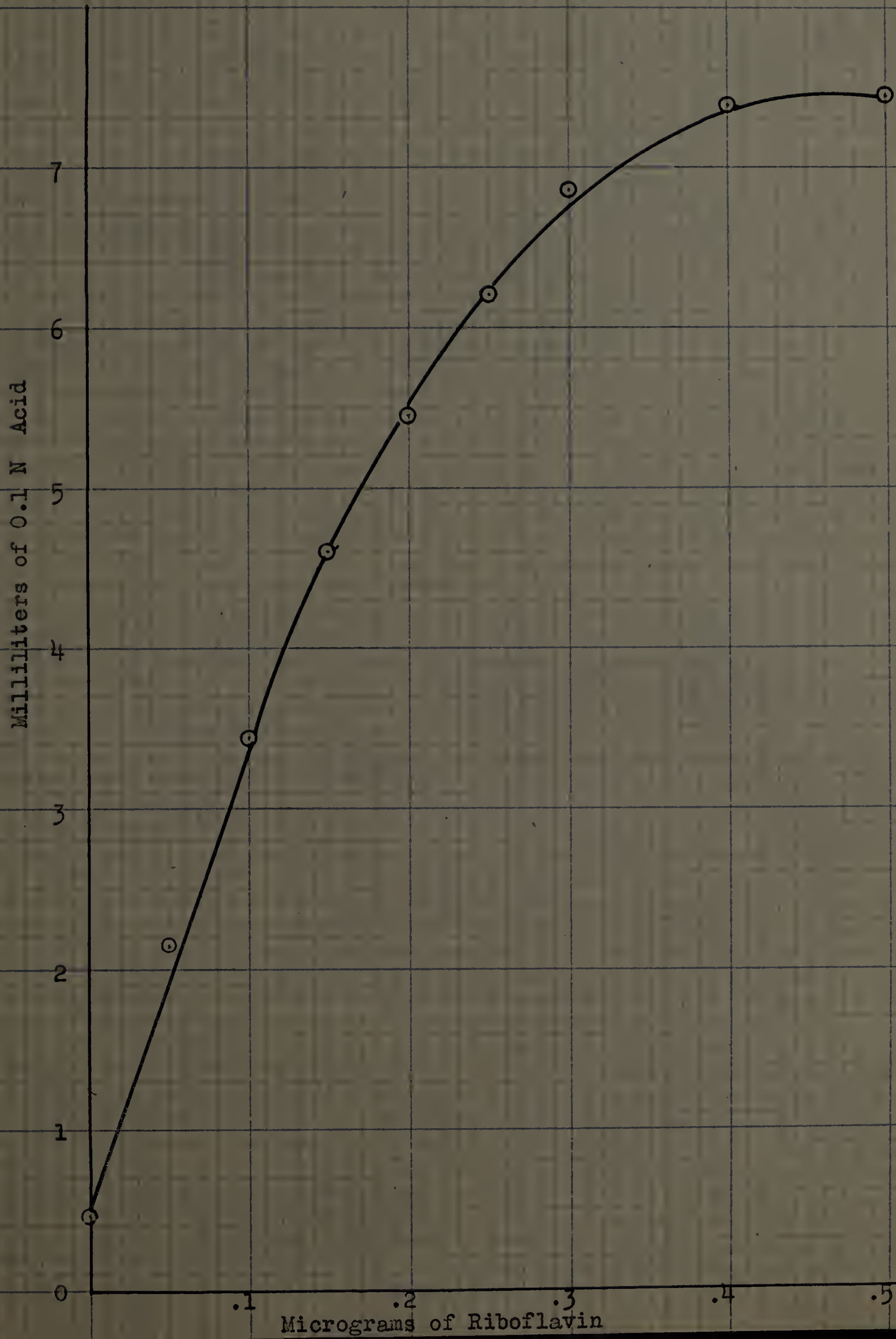
Riboflavin was determined by the microbiological method of Snell and Strong (1939) and also by the fluorometric method of Hodson and Norris (1939), (Guggenberg, 1944). Nicotinic acid determinations were compared with the Krehl, Strong and Elvehjem (1943) modification of the microbiological method of Snell and Wright (1941). The method of Neal and Strong (1943) was used as a comparative procedure in the case of calcium pantothenate.

In general, good agreement was obtained between the Landy-Dicken microbiological method and the other comparative procedures, with the possible exception of nicotinic acid comparisons, as shown in table 1. In a larger number of comparisons, it is expected that agreement would be better in the case of nicotinic acid. It was concluded that the Landy-Dicken method was satisfactory for use in the vitamin assay of mushrooms and was therefore used throughout the major portion of this investigation.

Typical reference curves of response obtained with the microbiological methods used to check the Landy-Dicken method are shown in figures 5, 6, and 7.

A comparison of the values obtained by Guggenberg (1944) by fluorometric analysis and the values obtained by the Landy-Dicken method can be seen in table 2. The microbiological values are generally higher than those obtained fluorometrically, especially in the case of low concentrations of riboflavin. This may be an indication of the higher sensitivity of the microbiological methods. The comparison shows the

Figure 5. Riboflavin Standard Curve, Snell-Strong Method



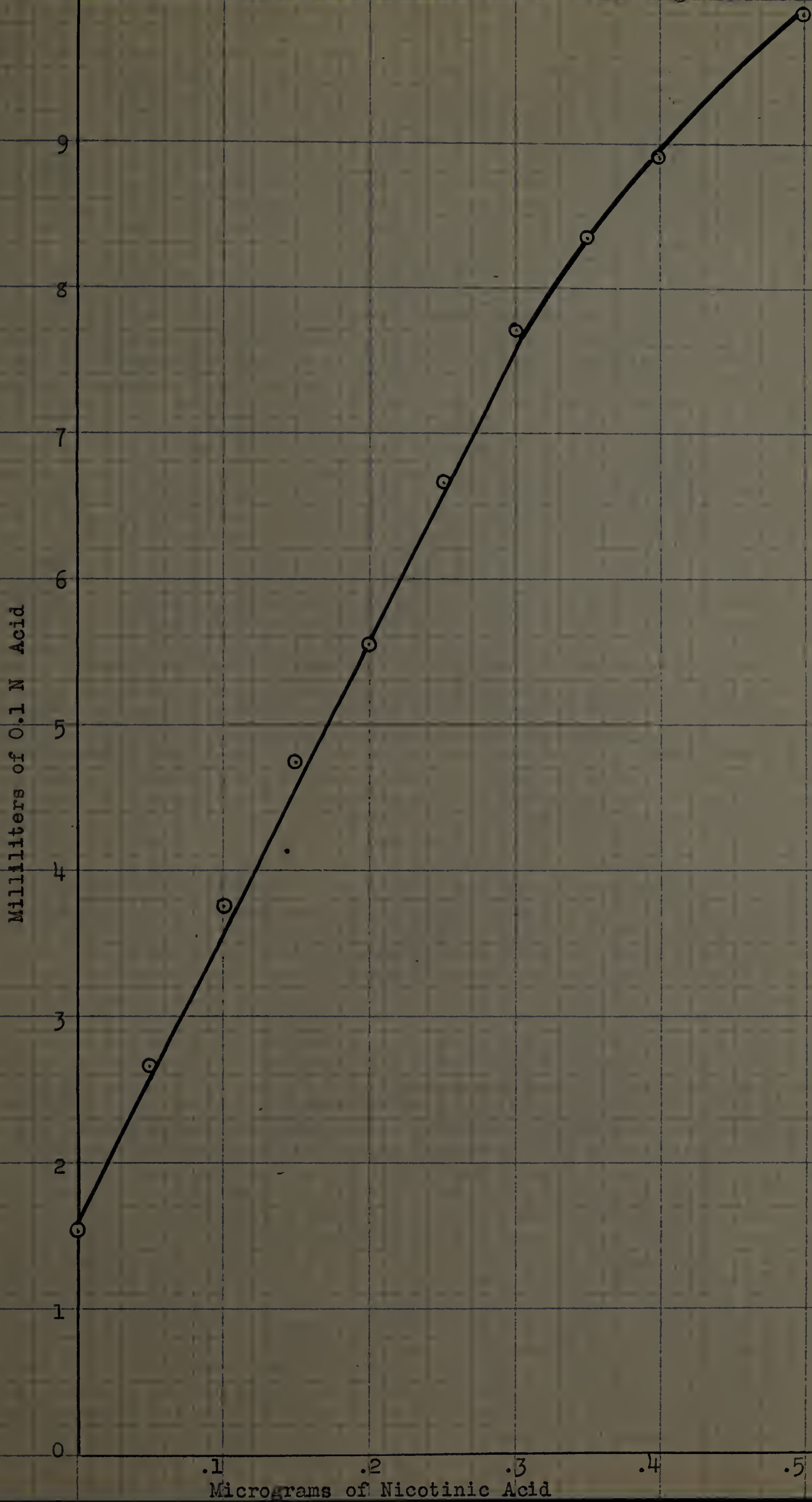


Figure 6. Nicotinic Acid Standard Curve, Snell-Wright Method

Figure 7. Calcium Pantothenate Standard Curve, Neal-Strong Method

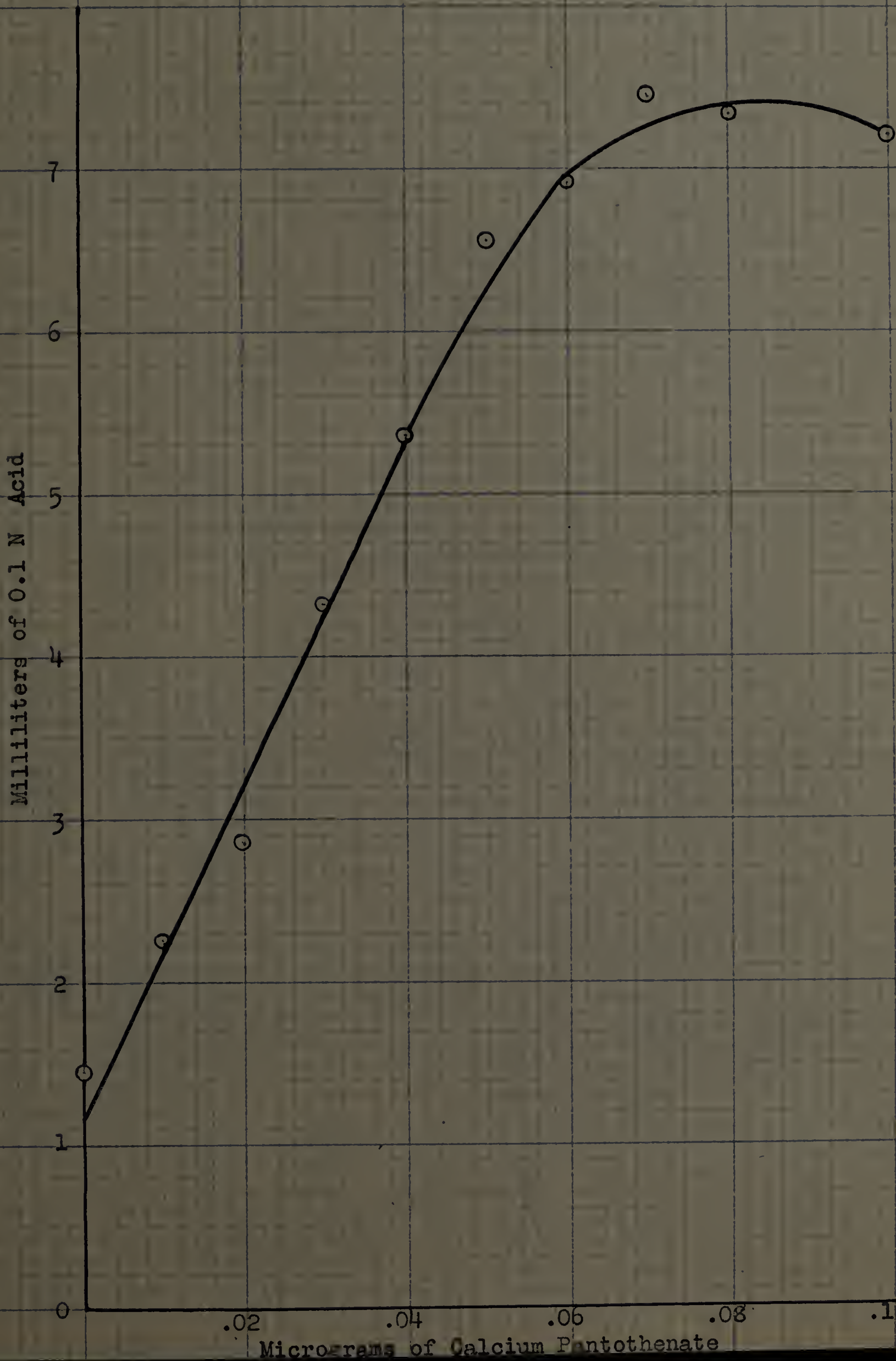


Table 1. B Vitamin Values Obtained by Different Microbiological Methods.

Description of sample	Landy-Dicken value (mg./100 g.)	Snell-Strong value (mg./100 g.)
Riboflavin		
Commercial #4, enzyme extract	0.406	0.4983
Commercial #14, enzyme extract	0.233	0.322
Commercial #1, acid extract	0.386	0.372
Commercial #7, liquid	0.303	0.294
Nicotinic Acid		**Snell-Wright**
Commercial #1, acid extract	2.368	2.92
Commercial #3, liquid	1.578	2.133
Glass packed		
South window exposed, acid extract	2.085	2.91
South window exposed, liquid	2.235	2.88
Calcium pantothenate		**Neal-Strong**
Tin packed, enzyme extract	1.64	1.69
Tin packed, liquid	1.66	1.41
Fresh mushrooms, enzyme extract	2.45	2.54
Boiled whole, enzyme extract	2.22	1.62
Broiled whole, cooking liquor	4.34	4.82
Broiled sliced, cooking liquor	3.28	3.18

Table 2. Riboflavin Values of Canned Mushrooms Fluorometrically and Microbiologically Assayed.

Description of samples	Storage time (months)	Fluorometric (mg./100 g.)	Microbiological (mg./100 g.)
South window			
Riboflavin in solids	0	0.315	0.475
	2	0.045	0.078
	6	0.0	0.029
Riboflavin in liquid	0	0.136	0.21
	2	0.0	0.028
	6	0.0	0.003
Shelf (in light)			
Riboflavin in solids	0	0.315	0.475
	2	0.175	0.299
	6	0.081	0.085
Riboflavin in liquid	0	0.136	0.21
	2	0.076	0.173
	6	0.025	0.062
Shelf (in dark)			
Riboflavin in solids	0	0.315	0.475
	2	0.269	0.487
	6	0.265	0.385
Riboflavin in liquid	0	0.136	0.21
	2	0.111	0.261
	6	0.185	0.254
Tin canned			
Riboflavin in solids	0	0.29	0.4
	2	0.321	0.345
	6	0.218	0.29
Riboflavin in liquid	0	0.121	0.152
	2	0.037	0.182
	6	0.069	0.171

same general trends in riboflavin values (losses or gains) for both fluorometric and microbiological procedures.

Methods of Vitamin Extraction.

In determining the vitamin content of foods the extraction or liberation of the vitamins from the food itself presents a serious problem in many cases. In this investigation, on mushrooms, both acid and enzyme extraction methods were carried out and compared.

For acid extractions, the procedure recommended by Krehl, Strong and Elvehjem (1943) was adopted. Five grams of mushrooms were suspended in 250 milliliters of N sulfuric acid and autoclaved at 15 pounds steam pressure. The cooled mixture was adjusted to pH 6.8 - 7.0 with approximately 1 N sodium hydroxide, and diluted to the desired volume. The solutions were not filtered. Only the enzyme extraction method was used in the case of calcium pantothenate because the vitamin is readily destroyed by acid.

Cheldelin, Eppright, Snell and Guirard (1942) studied the enzyme treatment of samples and recommended a procedure for the simultaneous release of all the B vitamins to be assayed by microbiological methods. Essentially the same procedure was used in this study except that clarase was used instead of takadiastase. The samples were incubated in acetate buffer suspensions (pH 4.5 - 4.7) containing 20 milligrams of clarase and 20 milligrams of papain per each gram of sample. Approximately 0.5 milliliter toluene was added to the suspension, in a 125 milliliter Erlenmeyer flask, which was then incubated for 24 hours at 37° C. (98.6° F.). After digestion the samples were heated in flowing steam for 30 minutes to inactivate the enzymes and to remove the toluene. The

samples were adjusted to pH 6.6 - 6.8 with approximately 1 N sodium hydroxide, filtered and diluted to the volume desired.

The enzymes themselves were assayed in order to determine their content of the vitamin being studied. When one-gram samples of clarase and of papain were tested, they were found to contain measurable amounts of B vitamins. However, when blanks were assayed, omitting the weighed mushrooms, using only the amounts of clarase and papain ordinarily used in the enzyme extractions, and carrying out the procedure and dilutions as for mushroom samples, the enzymes were found to contain only traces of any of the B vitamins tested.

The data in tables 3 and 4 show that the method of extraction has an important bearing on the vitamin values obtained by microbiological methods. In general, the enzyme extraction method proved more satisfactory, as the results obtained were more uniform and in better agreement. On the basis of this evidence, it was decided to report vitamin values for mushrooms from the data obtained by enzyme extraction methods.

II. LOSSES OF B VITAMINS IN MUSHROOMS DURING CANNING AND STORAGE.

Fresh mushrooms were secured from the Berlin Mushroom Company, Berlin, Massachusetts; and an experimental pack was prepared in the laboratory. Part of the mushrooms were packed in #303 size (16 oz.) vacuum-sealed glass jars, and the rest of the mushrooms were packed in plain tin #2 size (307 x 409) tin cans. After washing and trimming, the mushrooms were blanched for seven minutes in boiling water, packed into jars and tin cans to an approximately uniform weight and brined with a solution obtained by dissolving 54 grams of citric acid and 135 grams of salt

Table 3. Comparison of Acid and Enzyme Extraction Methods for the Determination of Riboflavin, Nicotinic Acid and Biotin in Samples of Commercially Canned Mushrooms.

Sample number	Riboflavin (micrograms/gram)		Nicotinic acid (micrograms/gram)		Biotin (micrograms/gram)	
	Acid extn.	Enzyme extn.	Acid extn.	Enzyme extn.	Acid extn.	Enzyme extn.
1	3.57-4.15	3.10	23.68	23.43	.102-.219	.063
2	3.46-4.44	3.04	18.35	21.72	.063-.359	.071
3	4.44-4.67	2.71	15.96	17.42	.044-.172	.069
4	3.39-5.72	3.84-4.27	19.12	29.9-30.7	.089-.25	.105
5	5.55	3.22-3.90	22.29	28.22-28.35	.099-.33	.12
6	5.6	2.26-2.8	11.63	17.47-22.42	.063	.094
7	2.99	3.24-3.83	14.0	24.4	.111	.08
8	2.52	2.78-3.53	14.57	19.78	.117	.084
9	1.44	2.27-2.78	7.83	11.3	.123	.049
10	0.91	3.33	11.73	17.63	.076	.051
11	1.09	2.56	14.69	20.12	.144	.041
12	3.35	2.69	20.26	17.8	.122	.04

Table 3. (Continued)

Sample number	Riboflavin (micrograms/gram)		Nicotinic acid (micrograms/gram)		Biotin (micrograms/gram)	
	Acid extn.	Enzyme extn.	Acid extn.	Enzyme extn.	Acid extn.	Enzyme extn.
13	1.24	2.24	15.67	22.13	.10	.028
14	1.79	2.33	12.25	18.04	.115	.035
15	0.71	1.69	27.11	11.72	.116	.048
16	1.88	3.62	28.98	23.98	.13	.094
17	1.03	3.97	20.03	25.97	.143	.072
18	1.86	2.04	26.63	13.23	.089	.041

Table 4. Comparison of Acid and Enzyme Extraction Methods for the Determination of Riboflavin, Nicotinic Acid and Biotin in Laboratory Canned Samples of Mushrooms.

Description of samples	Riboflavin (micrograms/gram)		Nicotinic acid (micrograms/gram)		Biotin (micrograms/gram	
	Acid extn.	Enzyme extn.	Acid extn.	Enzyme extn.	Acid extn.	Enzyme extn.
Fresh	5.50	1.50	42.0	49.3		
Blanched	1.90	6.00	36.7	45.0		
0 mo. storage						
Glass container	1.62	4.75	26.17	26.7		
Tin canned	1.0	4.00	21.0	19.0		
2 mo. storage						
South window	.265	.667-.885	22.6	29.52	.065	.032-.036
Diffused light	1.056	2.29-3.7	17.25	23.3-28.3		
In dark	2.03	4.87			.164	.032
Tin canned	3.21	3.25-4.4	13.84	26.9-42.6	.055-.18	.032-.035
6 mo. storage						
South window	.018-.066	.286	20.85	28.37	.17	.054
Diffused light	.359	.358-1.34	24.3	24.1-27.1	.197	.064-.078
In dark	1.36	3.85	22.25	24.0-29.8	.204	.104
Tin canned	1.11-1.49	2.58-3.22	17.9-22.8	24.56	.101-.15	.06-.09
12 mo. storage						
South window	.04-1.19	.33-.39	18.93	21.6	.108	.062
Diffused light	0.62	.9-.99	27.5	29.7-30.2	.139	.041-.081
In dark	1.22-1.54	2.9-3.25	23.59	25.1-32.2	.136	.034-.051
Tin canned	1.22-2.38	2.09-3.3	20.56	15.97	.09	.083

into three gallons of water. The jars and cans were processed in a retort equipped for processing glass containers, for 25 minutes at 116° C. (240° F.), and 27 pounds retort pressure (10 pounds steam pressure plus 17 pounds added air pressure). Samples were taken of the raw mushrooms, blanched mushrooms and mushrooms immediately after processing to determine the vitamin losses due to blanching and processing.

The mushrooms packed in glass jars were divided into three groups and stored as follows:

(1) Exposed on a South window shelf in the laboratory to rather severe light and temperature conditions.

(2) Exposed to diffuse light on an inside shelf at room temperature (approximately 24° C. (75° F.)) in the laboratory, simulating commercial storage conditions.

(3) Stored in cartons in the dark at room temperature (24° C. (75° F.)).

Tin cans of the experimental pack were stored on the same shelf as lot (2).

At the end of storage intervals of two, six, and twelve months, samples were taken from each lot, in tin and glass; and the solids and liquid portions assayed for vitamin content. In this way the effect of different types of containers, light and storage on the loss of vitamins in the brine or liquid as well as the solids portion of the can could be determined.

Detailed data for the fill, vacuum and moisture content of the experimental packs of mushrooms prepared in the laboratory are shown in table 5.

Table 5. Solids and Liquid Contents, Vacuum and Moisture Content of Mushrooms Canned in the Laboratory.

Type container and storage conditions	Storage time (months)	Average solids content (grams)	Average liquid content (milliliters)	Average vacuum (inches)	Moisture in solids (per cent)
		Pack I			
Fresh mushrooms					89.35
Blanched mushrooms					90.07
South window					
Glass container	0	288.2	140.0	15.5	88.90
" "	2	310.9	135.0	14.0	87.35
" "	6	312.9	126.6	15.3	90.45
" "	12	319.0	130.0	16.0	90.78
Diffuse light					
Glass container	2	300.17	141.5	14.0	90.75
" "	6	304.4	130.0	14.5	90.76
" "	12	300.7	134.3	17.4	89.63
In dark					
Glass container	2	311.8	130.0	16.0	89.0
" "	6	313.28	126.6	14.7	90.9
" "	12	319.6	124.0	19.0	90.9
Tin canned	0	342.1	207.5	6.5	89.7
" "	2	336.7	229.0	5.0	89.9
" "	6	326.15	233.0	1.5	89.1
" "	12	357.7	196.0	6.0	91.35
		Pack II			
Fresh mushrooms					88.0
Blanched mushrooms					89.1
Tin canned	0	366.5	211.6	7.75	88.0
" "	2	316.1	213.75	7.68	89.7

Table 6 shows the changes in riboflavin content of canned mushrooms during storage in tin and glass containers and exposed to varying degrees of light. From the data, it is evident that light accelerated the destruction of riboflavin in both the solids and liquid portions of canned mushrooms. It is also evident that light is not the only factor involved in the loss of riboflavin, as both the glass canned mushrooms kept in the dark and the tin canned mushrooms lost some of their riboflavin content during storage.

Table 7 shows that little or no loss of nicotinic acid occurred during storage even when the product was exposed to bright sunlight.

The calcium pantothenate content of canned mushrooms appeared to increase during storage, in both tin and glass containers, as shown in table 8. This may be due to the increased availability of the calcium pantothenate during storage, to the organisms used in the microbiological assay. Table 9 shows a similar trend in the biotin content during storage, perhaps due to the liberation of some of the bound biotin which might be present in mushrooms.

The storage figures obtained are summarized in table 10 which shows the percentage losses due to blanching and processing, as well as to the various storage conditions. Blanching seemed to increase the "per gram" content of riboflavin, but processing caused a significant loss of the original content. The apparent increase of riboflavin in blanching is attributed to difficulty in obtaining a complete extraction of riboflavin from the raw mushrooms.

In the glass containers there was 70.7 per cent retention of riboflavin after processing, while in tin containers the retention

Table 6. Changes in Riboflavin Content of Canned Mushrooms During Storage.

	Storage time (months)	Glass container						Tin canned	
		South Window		Shelf (light)		Shelf (dark)			
		Weight (grams)	Riboflavin (micrograms)	Weight (grams)	Riboflavin (micrograms)	Weight (grams)	Riboflavin (micrograms)	Weight (grams)	Riboflavin (micrograms)
Solids content per can	0	288.2	1,370	288.2	1,370	288.2	1,370	342.1	1,368.4
	2	310.9	242.5	300.17	897.5	311.8	1,518.5	336.7	1,289.6
	6	312.9	90.74	304.4	258.74	313.28	1,206.1	326.15	945.8
	12	319	114.8	300.7	285.7	319.6	984.4	357.7	962.2
Liquid content per can	0	140	294	140	294	140	294	207.5	415
	2	135	37.8	141.5	244.8	130	339.3	229	494.6
	6	126.6	3.8	130	80.6	126.6	321.6	233	398.4
	12	130	2.0	134.3	56.4	124	218.2	286.2	286.2
Total can contents	0	428.2	1,663	428.2	1,663	428.2	1,663	549.6	1,783.4
	2	445.9	280.3	441.67	1,142.3	441.8	1,857.8	565.7	1,784.2
	6	439.5	94.54	434.4	339.34	439.88	1,527.7	559.15	1,344.2
	12	449	116.8	435	342.1	443.6	1,202.6	553.7	1,248.4

Table 7. Changes in the Nicotinic Acid Content of Canned Mushrooms During Storage.

	Storage time (months)	Glass container: South window Weight (grams)	Glass container: South window Nic. acid (micrograms)	Glass container: Shelf (light) Weight (grams)	Glass container: Shelf (light) Nic. acid (micrograms)	Glass container: Shelf (dark) Weight (grams)	Glass container: Shelf (dark) Nic. acid (micrograms)	Tin canned Weight (grams)	Tin canned Nic. acid (micrograms)
Solids content per can	0	288.2	7,694.9	288.2	7,694.9	288.2	7,694.9	342.1	6,842
	2	310.9	9,177.8	300.17	7,738.4	311.8	8,848.9	336.7	11,707
	6	312.9	8,877	304.4	7,792.6	313.28	8,427.2	326.15	8,010.2
	12	319	9,557.2	300.7	8,615	319.6	5.04	357.7	7,726.3
Liquid content per can	0	140	3,276	140	3,276	140	3,276	207.5	4,565
	2	135	3,225.1	141.5	3,383.2	130	3,477.5	229	5,042.6
	6	126.6	2,829.5	130	2,593.5	126.6	2,300	233	5,860
	12	130	3,043.3	134.3	1,770.1	124	2,290.3	196	3,931.8
Total can content	0	428.2	10,970.9	428.2	10,970.9	428.2	10,970.9	549.6	11,407
	2	445.9	12,402.9	441.7	11,121.6	441.8	12,326.4	565.7	16,749.6
	6	439.5	11,706.5	434.4	10,386.1	439.9	10,727.5	559.2	13,870.2
	12	449	12,600.5	435	10,385.1	443.6	7,394.3	553.7	11,658.1

Table 8. Changes in the Calcium Pantothenate Content of Canned Mushrooms During Storage.

		Glass container							
		South window		Shelf (light)		Shelf (dark)		Tin canned	
	Storage time (months)	Weight (grams)	Ca. pantothenate (micrograms)	Weight (grams)	Ca. pantothenate (micrograms)	Weight (grams)	Ca. pantothenate (micrograms)	Weight (grams)	Ca. pantothenate (micrograms)
Solids content per can	0	288.2	1,873.3	288.2	1,873.3	288.2	1,873.3	342.1	2,138.1
	2	310.9	3,799.2	300.17	3,989.3	311.8	3,694.8	336.7	3,670
	6	312.9	3,291.7	304.4	2,888.8	313.28	3,082.7	326.15	2,592.9
	12	319	4,312.9	300.7	2,736.4	319.6	2,023.1	357.7	2,700.6
Liquid content per can	0	140	700	140	700	140	700	207.5	1,037.5
	2	135	1,908.9	141.5	1,712.1	130	1,787.5	229	2,299.2
	6	126.6	1,555.9	130	1,315.6	126.6	1,278.6	233	2,290.4
	12	130	1,448.2	134.3	741.3	124	917.6	196	1,458.2
Total can content	0	428.2	2,573.3	428.2	2,573.3	428.2	2,573.3	549.6	3,175.6
	2	445.9	5,708.1	441.67	5,701.4	441.8	5,482.3	565.7	5,969.2
	6	439.5	4,847.6	434.4	4,204.4	439.88	4,361.3	559.15	4,883.2
	12	449	5,761.1	435	3,477.7	443.6	2,940.7	553.7	4,158.8

Table 9. Changes in the Biotin Content of Canned Mushrooms During Storage.

	Storage time (months)	Glass container: South window Weight (grams)	Glass container: South window Biotin (micrograms)	Glass container: Shelf (light) Weight (grams)	Glass container: Shelf (light) Biotin (micrograms)	Glass container: Shelf (dark) Weight (grams)	Glass container: Shelf (dark) Biotin (micrograms)	Tin canned Weight (grams)	Tin canned Biotin (micrograms)
Solids content per can	2	310.9	10.57	300.17	9.91	311.8	9.98	336.7	11.11
	6	312.9	16.9	304.4	21.61	313.28	32.58	326.15	25.11
	12	319	19.46	300.7	12.93	319.6	26.53	357.7	22.18
Liquid content per can	2	135	4.59	141.5	3.96	130	4.94	229	8.02
	6	126.6	3.67	130	3.12	126.6	2.79	233	4.66
	12	130	3.77	134.3	3.36	124	3.72	196	4.31
Total can content	2	445.9	15.16	441.67	13.87	441.8	14.92	565.7	19.13
	6	439.5	20.57	434.4	24.73	439.88	35.37	559.15	29.77
	12	449	23.23	435	16.29	443.6	30.25	553.7	26.49

Table 10. Retention of B Vitamins in Canned Mushrooms During Processing and Storage for One Year.

		Glass container							
	Storage	South window		Shelf (light)		Shelf (dark)		Tin canned	
Vitamin	time (months)	mg./ 100 g.*	Per cent retention	mg./ 100 g.*	Per cent retention	mg./ 100 g.*	Per cent retention	mg./ 100 g.*	Per cent retention
Riboflavin	fresh	0.55	100	0.55	100	0.55	100	0.55	100
	blanched	0.6	109	0.6	109	0.6	109	0.6	109
	0	0.389	70.7	0.387	70.7	0.389	70.7	0.324	58.9
	2	0.063	11.45	0.259	47.1	0.42	76.4	0.316	57.2
	6	0.022	4.0	0.078	14.2	0.347	63.1	0.24	43.6
	12	0.026	4.7	0.079	14.4	0.271	49.25	0.225	40.9
Nicotinic acid	fresh	4.93	100	4.93	100	4.93	100	4.93	100
	blanched	4.50	91.2	4.50	91.2	4.50	91.2	4.50	91.2
	0	2.56	52	2.56	52	2.56	52	2.08	42.2
	2	2.78	56.4	2.52	51.1	2.79	56.6	2.96	60
	6	2.66	53.9	2.39	48.4	2.44	49	2.48	50.3
	12	2.81	57.0	2.39	48.4	1.67	33.9	2.11	42.8
Calcium pantothenate	fresh	2.0	100	2.0	100	2.0	100	2.0	100
	blanched	1.6	80	1.6	80	1.6	80	1.6	80
	0	0.6	30	0.6	30	0.6	30	0.58	29
	2	1.28	64	1.29	64.5	1.24	62	1.06	53
	6	1.10	55	0.98	49	0.99	49.5	0.87	43.5
	12	1.28	64	0.80	40	0.66	33	0.75	37.5
Biotin	2	0.0034		0.0031		0.0034		0.0034	
	6	0.0047		0.0057		0.008		0.0048	
	12	0.0052		0.0038		0.007		0.0048	

* Based on total can contents.

was only 58.9 per cent. At the end of 12 months storage, the retention of riboflavin ranged from 4.7 per cent for mushrooms stored in the South window, to 49.25 per cent retention of riboflavin in the glass containers kept in the dark. The glass canned mushrooms exposed to diffused light showed a retention of 14.4 per cent riboflavin, while the tin canned mushrooms showed a retention of 40.9 per cent after 12 months storage.

In the case of nicotinic acid, 91.2 per cent of the vitamin remained after blanching, while processing seemed to cause a more serious loss. There was 52 per cent of the nicotinic acid in the glass containers and 42.2 per cent in the tin cans immediately after processing. Storage for 12 months did not generally cause further loss of nicotinic acid.

Blanching caused a 20 per cent loss of pantothenic acid, and after processing there was only 30 per cent of pantothenic acid left. During storage, the values seemed to increase.

In order to check the validity of the figures obtained for changes during processing and storage, a second series of tests was run on a smaller group of canned mushrooms, canned in tin only. The results are shown in table 11. In general, the same trends are in evidence, except that the losses due to processing are not so great in the second series as they were in the first series of tests. For riboflavin, there was an 80 per cent retention immediately after processing. Nicotinic acid was retained to the extent of 48.4 per cent, calcium pantothenate 41.5 per cent, and biotin 51.3 per cent after processing.

Table 11. Retention of B Vitamins in Canned Mushrooms During Processing and Storage for Two Months.

Vitamin	Storage time (months)	Tin can mg./100 grams*	Tin can Per cent retention
Riboflavin	fresh	0.39	100
	blanched	0.491	126
	0	0.313	80.2
	2	0.279	71.5
Nicotinic acid	fresh	6.70	100
	blanched	4.38	65.4
	0	3.24	48.4
	2	3.58	53.4
Calcium pantothenate	fresh	3.45	100
	blanched	2.19	63.4
	0	1.43	41.5
	2	1.51	43.75
Biotin	fresh	0.0097	100
	blanched	0.0108	111.3
	0	0.005	51.3
	2	0.0068	70.1

* Based on total can contents.

III. VARIATIONS IN THE B VITAMINS IN COMMERCIALLY CANNED MUSHROOMS.

Samples of canned mushrooms were obtained from six different commercial canners. Each canner supplied samples of (1) button mushrooms, (2) sliced mushrooms, and (3) stems and pieces, all packed in 208 x 208 cans of 5.5 ounce capacity. The canned mushrooms were analyzed three to six months after they were canned.

For each vitamin determination a composite sample from six cans was taken. In each case the vacuum was measured, the drained weight of the canned mushrooms was ascertained and recorded as well as the volume of liquid. The moisture content of the drained solids was also determined. These data are shown in table 12. Considering that these mushrooms came from several sections of the country and were canned under different conditions, by different companies, the variations seem smaller than might be expected.

Vitamin determinations were made on both the solid and liquid portions of the canned product, and reported as amount of vitamin per 100 grams of the entire original contents of the can.

Table 13 shows a range of riboflavin content from 0.16 - 0.352 milligrams per 100 grams of can content. The nicotinic acid values ranged from 0.98 - 2.53 milligrams per 100 grams; the calcium pantothenate 0.312 - 1.258 milligrams per 100 grams; and the biotin results were 3.33 - 9.97 micrograms per 100 grams of can contents.

From these figures it may be seen that canned mushrooms are an excellent source of riboflavin, nicotinic acid, calcium pantothenate and biotin. Thompson, Cunningham and Snell (1944) found the riboflavin content of canned fruits ranged from 0.018 - 0.096 milligrams per 100 grams of canned food. For fish the values ranged from 0.031 - 0.2

Table 12. Solids and Liquid Content, Vacuum and Moisture Content of Commercially Canned Mushrooms.

Sample number	Average solids content (grams)	Average liquid content (milliliters)	Average vacuum (inches)	Moisture in solids (per cent)
1	121.43	71.17		88.2
2	121.3	81.08		90.5
3	130.63	67.25	7.75	90.2
4	124.2	83.67	0.67	86.2
5	122.23	82.5	1.17	83.5
6	134.57	73.3	1.83	88.1
7	126.42	92.67	1.75	89.5
8	131.24	84.6	3.5	87.5
9	131.3	90.0	2.3	91.5
10	102.72	96.63	3.67	88.1
11	113.44	90.8	3.21	89.0
12	114.84	78.72	4.71	89.5
13	110.26	94.03	9.17	90.0
14	119.12	86.27	6.83	89.8
15	108.8	102.87	5.63	89.4
16	121.19	81.0	12.75	86.4
17	132.44	71.82	6.79	87.4
18	134.08	71.4	7.0	90.2

Table 13. B Vitamin Content of Commercial Canned Mushrooms

Sample number	Type	Riboflavin mg./100 g.	Nicotinic acid mg./100 g.	Calcium pantothenate mg./100 g.	Biotin micrograms/100 g.
1	Buttons	.229	1.84	.917	6.65
4	"	.334	2.61	1.042	5.77
7	"	.352	2.09	.827	7.02
10	"	.256	1.50	.867	4.26
13	"	.183	2.08	1.179	6.15
16	"	.318	2.16	1.258	8.51
Average		.278	2.04	1.015	6.39
2	Sliced	.263	1.96	.914	4.09
5	"	.313	2.53	1.0	6.79
8	"	.279	1.63	.853	7.67
11	"	.231	1.97	.913	8.56
14	"	.191	1.56	.877	7.05
17	"	.319	2.22	1.126	9.97
Average		.266	1.98	.947	7.35
3	Stems and pieces	.227	1.69	.697	3.33
6	"	.221	1.79	.506	4.58
9	"	.213	1.0	.45	7.64
12	"	.21	1.57	.716	7.66
15	"	.16	0.98	.312	6.3
18	"	.181	1.3	.51	6.24
Average		.202	1.39	.532	5.96

milligrams per 100 grams. The same workers published the following values for calcium pantothenate content of canned foods:

Fruits and vegetables:	0.022 - 0.25 milligrams per 100 grams
Fish:	0.17 - 0.57 milligrams per 100 grams

Ives, Wagner, Elvehjem and Strong (1944) found the following values for nicotinic acid in canned foods:

Fruits and vegetables:	0.13 - 1.06 milligrams per 100 grams
Fish:	2.23 - 10.20 milligrams per 100 grams

From these figures it is evident that canned mushrooms may be considered as a richer source of these vitamins than the canned fruits and vegetables.

Canned button and sliced mushrooms appear to be a better source of the B vitamins studied than are canned mushroom stems and pieces. It is possible that the low vitamin content of the latter may be caused by a leaching of the vitamin from the product before it goes into the can.

IV. EFFECT OF COOKING METHODS ON B VITAMIN CONTENT OF MUSHROOMS.

Fresh mushrooms were cooked by several methods and the retention of the riboflavin, nicotinic acid, calcium pantothenate and biotin was determined. The mushrooms were washed and weighed into one-pound samples and cooked by the following methods:

(1) Boiled whole - whole mushrooms were boiled in one quart of water for 10 minutes.

(2) Boiled sliced - mushrooms were sliced (about 1/4 inch slices) and boiled for 10 minutes in one quart of water.

(3) Broiled whole - whole mushrooms were set on a broiling pan and broiled for 10 minutes. (The mushrooms were turned once during the broiling period).

(4) Broiled sliced - sliced mushrooms were broiled as in (3).

(5) Sauteed - mushrooms were sliced and sauteed in a frying pan for 10 minutes, with occasional turning of the mushrooms. A small amount of cooking oil was used in the pan.

The weights of the samples before and after cooking and their respective moisture contents are shown in table 14. It may be seen that, of the cooking methods employed, broiling and sauteeing caused a marked loss of moisture.

The vitamin content of the cooking liquor, as well as that of the cooked solids was determined, in order to calculate the total per cent retention of vitamin content.

Table 15 shows that there was little loss of riboflavin due to cooking procedures. The broiled sliced mushrooms suffered most, retaining but 65.8 per cent of the riboflavin content. The other samples ranged from 83.2 - 107 per cent retention of riboflavin.

The values obtained for nicotinic acid were in approximately the same range as riboflavin. According to table 16, nicotinic acid retention values were 71.8 - 98.7 per cent during cooking. Somewhat similar grouping of percentages occurred in the calcium pantothenate determinations, as shown in table 17. The percentage retention ranged from 74.6 - 106.2.

The biotin values obtained were somewhat lower, as shown in table 18. After cooking, the biotin retention ranged from 33 - 74.1 per cent.

In order to determine the adequacy of the enzyme extraction method on the samples of raw mushrooms, it was decided to try a preliminary autoclaving of the weighed fresh samples in 0.1 N acid, followed by

Table 14. Weight and Moisture Content Changes in Mushrooms During Cooking.

	Weight before cooking (grams)	Weight after cooking (grams)	Cooking liquor (milliliters)	Moisture (per cent)
Lot I				
Fresh	454			87.7
Boiled whole*	454	399.3	788	87.7
Boiled sliced*	454	349.5	760	89.9
Broiled whole	454	354.1	0	84.8
Sauteed	454	272.7	0	75.9
Lot II				
Fresh	454			89.1
Boiled whole*	454	314.4	750	87.4
Boiled sliced*	454	312.9	730	88.7
Broiled whole	454	254.6	35	83.7
Broiled sliced	454	225.1	40	81.0
Sauteed	454	215	0	75.1

* Cooked in one quart (946 milliliters) of water.

Table 15. Changes in Riboflavin Content Due to Cooking of Mushrooms

	Weight (grams)	Riboflavin micrograms/gram	Riboflavin Total (micrograms)		Riboflavin Per cent retention
Fresh	454	5.9	2,679		100
Boiled whole	399.3	4.52	1,805)	2,877	107
" " , liquor	788	1.36	1,072)		
Boiled sliced	349.5	3.43	1,199)	2,241	83.6
" " , liquor	760	1.37	1,042)		
Broiled whole	354.1	7.25	2,568		95.8
Sauteed	272.7	9.54	2,594		96.8
Fresh	454	5.44	2,469		100
Boiled whole	314.4	5.36	1,685)	2,570	104
" " , liquor	750	1.18	885)		
Boiled sliced	312.9	2.71	848)	2,111	85.6
" " , liquor	730	1.73	1,263)		
Broiled whole	254.6	6.63	1,688)	2,055	83.2
" " , liquor	35	10.49	367)		
Broiled sliced	225.1	6.03	1,358)	1,626	65.8
" " , liquor	40	6.7	268)		
Sauteed	215	10.54	2,264		91.8
			Average		90.4

Table 16. Changes in Nicotinic Acid Content Due to Cooking Mushrooms.

	Weight (grams)	Nicotinic acid Micrograms/gram	Nicotinic acid Total (micrograms)	Nicotinic acid Per cent retention
Fresh	454	56	25,424	100
Boiled whole	399.3	40	15,972)	91.5
" " , liquor	788	8.8	6,934)	
Boiled sliced	349.5	28.6	9,995.7)	81.9
" " , liquor	760	14.2	10,792)	
Broiled whole	354.1	64	22,662	89.2
Sauteed	272.7	67	18,271	71.8
Fresh	454	51	23,154	100
Boiled whole	314.4	44	13,834)	87.8
" " , liquor	750	8.7	6,525)	
Boiled sliced	312.9	31	9,700)	98.7
" " , liquor	730	18	13,140)	
Broiled whole	254.6	70	17,822)	91.2
" " , liquor	35	94	3,290)	
Broiled sliced	225.1	80	18,008)	91.3
" " , liquor	40	78	3,120)	
Sauteed	215	90	19,350	83.5
			Average	87.4

Table 17. Changes in Calcium Pantothenate Content Due to Cooking of Mushrooms.

	Weight (grams)	Calcium pantothenate Micrograms/gram	Total (micrograms)	Per cent retention
Fresh	454	27	12,258	100
Boiled whole	399.3	22	8,785)	95.4
" " , liquor	788	3.7	2,916)	
Boiled sliced	349.5	15.6	5,452)	87.2
" " , liquor	760	6.9	5,244)	
Broiled whole	354.1	33	11,685	95.2
Sauteed	272.7	35	9,544	77.8
Fresh	454	24.5	11,123	100
Boiled whole	314.4	22	6,917)	106.2
" " , liquor	750	5.7	4,275)	
Boiled sliced	312.9	13.2	4,130)	91.6
" " , liquor	730	8.3	6,059)	
Broiled whole	254.6	28	7,129)	77.6
" " , liquor	35	43	1,505)	
Broiled sliced	225.1	31	6,978)	74.6
" " , liquor	40	33	1,320)	
Sauteed	215	39	8,385	75.4
			Average	86.8

Table 18. Changes in Biotin Content Due to Cooking of Mushrooms.

	Weight (grams)	Biotin Micrograms/gram	Biotin Total (micrograms)	Biotin Per cent retention
Fresh	454	0.18	81.7	100
Boiled whole	399.3	0.094	37.5)	51.5
" " , liquor	788	0.0058	4.6)	
Boiled sliced	349.5	0.079	27.6)	44.8
" " , liquor	760	0.012	9.1)	
Broiled whole	354.1	0.104	36.8	45.0
Sauteed	272.7	0.099	27.0	33.0
Fresh	454	0.117	53.1	100
Boiled whole	314.4	0.12	37.7)	74.1
" " , liquor	750	0.0022	1.65)	
Boiled sliced	312.9	0.055	17.2)	39.7
" " , liquor	730	0.0053	3.9)	
Broiled whole	254.6	0.128	32.6)	66
" " , liquor	35	0.07	2.45)	
Broiled sliced	225.1	0.092	20.7)	41.8
" " , liquor	40	0.038	1.5)	
Sauteed	215	0.132	28.4	53.5
			Average	50

the routine enzyme treatment, to see if the vitamin values would be different from those obtained by simple enzyme treatment. In the case of riboflavin, the enzyme treatment alone had given very low values for fresh mushrooms. The preliminary autoclaving in weak acid brought the figures up to the range obtained for cooked mushrooms, indicating that the vitamin was made available to the test organism by the combined acid-enzyme extraction method. For nicotinic acid and biotin, the acid-enzyme extraction did not seem to alter the vitamin values obtained for fresh mushrooms. In this study, the riboflavin values recorded for fresh mushrooms are those obtained by acid-enzyme treatment or extraction. In all other cases, the simple enzyme extraction values are recorded.

The results indicate the importance of adequate extraction procedures in the microbiological determination of vitamins. Although the riboflavin content of blanched and processed mushrooms was evidently soluble or available to the test organism, the raw samples apparently required preliminary treatment to effect availability of the riboflavin in the determination procedure.

The question is next raised as to the adequacy of any of the present extraction procedures for microbiological determinations. The point applies especially to biotin values obtained in this study. It is believed that biotin values obtained in this investigation are liable to unknown error, very likely because of erratic availability of the vitamin content by the extraction procedures employed.

SUMMARY

A microbiological method was used for determining the riboflavin, nicotinic acid, calcium pantothenate and biotin content of mushrooms and the changes occurring during canning procedures, during storage in glass containers exposed to varying degrees of light, and during storage in tin cans. The effect of various home-cooking methods on the vitamin content of mushrooms was also investigated. Samples of commercially canned mushrooms were assayed for the same vitamins, to determine the variations found in commercial samples.

In comparing various extraction procedures for microbiological assays, it was found that enzyme extraction was satisfactory for cooked and canned mushroom samples assayed for riboflavin. Enzyme extraction was satisfactory in all cases for determination of nicotinic acid and calcium pantothenate. For biotin, the extraction procedures employed are of doubtful reliability. In the case of raw mushrooms, a combination of acid and enzyme treatment was necessary in order to liberate the riboflavin content. For foods other than mushrooms, optimum extraction procedures probably depend on the availability of the vitamin at the particular time samples are taken for determination.

Blanching caused little or no loss of the vitamins, but processing caused a considerable loss, the figures varying with the vitamin in question, and to a small extent with the type of container (tin or glass).

Storage conditions had the most effect on riboflavin values. Mushrooms in glass containers stored in the South window lost all but 4.7 per cent of the riboflavin content at the end of a year. In glass and in tin containers kept in the dark, the riboflavin retention was 49.25 and 40.9 per cent, respectively, at the end of a year. In

diffused light, glass containers retained 14.4 per cent of the riboflavin content after a year's storage.

Similar conditions of storage caused little or no loss of the other vitamins tested. In some cases, the vitamin values appeared to increase during storage for a year.

Home-cooking methods showed good retention of vitamin content, except in the case of biotin. An average of 90.4 per cent riboflavin was retained after cooking; retention of nicotinic acid, calcium pantothenate and biotin were 87.4, 86.8 and 50 per cent respectively.

Commercially canned mushrooms proved to be a good source of the B vitamins. The average content of riboflavin, nicotinic acid and calcium pantothenate were 0.249, 1.8, 0.83 milligrams per 100 grams respectively. The biotin content averaged 6.57 micrograms per 100 grams of can contents.

A comparison of microbiological assay methods showed that the Landy-Dicken method gave values in good agreement with the other microbiological methods employed. Although variations may occur in assaying some foods, mushrooms are apparently reliably assayed for riboflavin, nicotinic acid, calcium pantothenate and biotin by this method. The Landy-Dicken method has the advantage of being adaptable to the routine determinations of several B vitamins.

CONCLUSIONS

The B vitamin content of mushrooms is affected in varying degree by cooking and canning procedures and storage conditions. Exposure to extreme and to diffuse light during storage destroys the riboflavin content; otherwise, the retention of vitamins after cooking and canning procedures is generally good.

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Approved:

Francis P. Griffiths

Mary E. Garvey

Anne W. Wertz

Thesis Committee

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