THE THIAMIN CONTENT OF MEATS AND CEREALS

By

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INTRODUCTION

Thiamin or Vitamin B_1 is one of the best known of the vitamins needed in our diet. Its importance is recognized universally. The Committee on Foods and Nutrition of the National Research Council (Washington) (1941) recommends a daily intake of 1.8 mg. for men and 1.5 mg. for women. The Canadian Council on Nutrition also approves these recommendations. Common sources of this vitamin are yeast, pork, liver, organs and muscles of many animals, nuts, eggs, legumes, whole grains and most vegetables. Thiamin is found in greatest amounts in yeasts, meats, cereal foods, and legumes. A tabulation of thiamin in foods in the average American diet reveals both cereals and meats supply one-quarter, dairy products and vegetables each one-fifth, and fruits one-tenth of the thiamin. The principal sources of thiamin in the average diet are lean pork, milk and bread. This was determined by Lane, Johnson and Williams (1942) using the food consumption study records of Stiebeling and Phipard (1939).

According to dietary surveys carried on under the Department of Pensions and National Health, in larger Canadian cities,

thiamin is the vitamin most lacking in the diet of the average Canadian. Improvement would come with more careful food selection on the part of the consumer. This condition would be helped if food processing companies were to give more explicit information on the amounts of the nutritionally important ingredients, in terms of the daily requirement, in the products they market.

Breakfast cereals may be a worthwhile source of thiamin. The variety on the Canadian market is extensive. Advertising has popularized many types whose thiamin content is as variable as the different methods of processing employed. In many cases cereals may be stored a considerable time before consumption. This might affect their thiamin content. So might any cooking necessary to prepare them for consumption. It appeared worthwhile to determine the value of breakfast cereals as sources of thiamin and the effect of storage and cooking on them.

It is known that the thiamin content of various meats shows considerable variation. The purpose of this study is to

illustrate the variation between meats of different animals as well as the variations in thiamin content within a single carcass. A wide variety of meats available and popular in Manitoba retail markets was selected for this purpose. It was decided to determine the thiamin content of an average serving of meat as served, excluding all waste and visible fat. In order that the housewife might be able to select meats which contain less waste the percent of meat originally purchased which is actually edible, was to be calculated.

Review of Literature

In 1914 Dr. Casimir Funk theorized that beriberi, scurvy and pellagra were caused by absence from the diet of special substances of the nature of organic bases which he called "vitamines". The beriberi "vitamine" he believed to be a pyrimidine base analogous with thymine. Jansen and Donath (1926) isolated the vitamin as a crystalline hydrochloride. The composition was determined by Windaus and his co-workers (1932) who gave it the formula C_{12} H_{17} N_4 OS. Windaus, Tschesche and Grewe

(1935) established its chemical constitution, Cline, Williams, and Finkelstein (1937) succeeded in synthesizing the vitamin, to which the name thiamin has been given.

A variety of methods for determining amounts of thiamin in food have been used, both before and after the chemical nature of the vitamin was established. Osborne and Mendel (1926) utilized the rat growth method to indicate the strength of concentrates added to an adequate diet. The rats are maintained on a thiamin deficient diet until depleted, and then given daily, weighed quantities of test material. Subsequent gain in weight is a measure of the thiamin content of the test material. The rat curative method devised later has proved to be more specific. In this method rats are maintained on a thiamin-free diet until they show declining weight, symptoms of paralysis, or both. Samples to be assayed for thiamin are adminstered orally or by injection. The length of time before a recurrence of the symptoms is noted indicates the potency of the sample in thiamin.

In England Carter and Drury (1929) observed a condition of bradycardia preceding symptoms of polyneuritis in pigeons

fed polished rice. The low rate of heart beat which was due to accumulation of excess lactic acid, was increased to normal and the lactic acid disposed of by administration of thiamin. The rate of recovery to normal forms the basis of the bradycardia method of thiamin assay.

Microbiological methods for the thiamin assay of foods have been described and used to quite an extent. Orr-Ewing and Reader (1928) assayed antineuritic extracts by growth promoting tests on Streptothrix corallinus. Schopfer (1935) found the growth of Phycomyces blakesleeanus sensitive to the presence of thiamin, others have not considered this test specific so it has not been used extensively. The yeast fermentation method of Schultz, Atkin and Frey (1937) is the most widely used microbiological method today. In this method the influence on rate of gas production of test materials is compared with that of standard thiamin solutions. The greater the concentration of thiamin, the greater is the increase in gas production.

A new chemical method of measuring thiamin was developed by Kinnersley and Peters (1938). In 1935 they reported that one of

the most nearly pure preparations of thiamin available was converted by oxidation in aqueous solution into a substance showing intense sky blue fluorescence. Kuhn, Wagner-Jauregg, van Klaveren and Vetter (1935) gave the name "thiochrome" to the sky blue fluorescence in ultra violet light produced when thiamin is subjected to mild oxidation as by alkaline potassium ferricyanide. It has been shown that the intensity of fluorescence is proportional to the concentration of thiochrome and therefore to the concentration of thiamin from which it is formed by oxidation. Several modifications of this method have been reported. One of these modifications is that of Hennessy and Cerecedo (1939). Two modifications of the Hennessy and Cerecedo method were used in this laboratory for the analysis of cereals and meats.

Both biological and chemical methods have been used to determine the thiamin content of a variety of foods. Less information has been published for breakfast cereals than for meats.

Nordgren and Andrews (1941) found that the thiamin content of wheat is influenced by the type, variety and environ-

ment during growth of the wheat. Durum and spring wheats have the highest thiamin content followed by hard winter and soft varieties. The same variety of wheat grown in different locations frequently differs in thiamin content. So breakfast cereals from the same variety of wheat grown and packaged in different sections of Canada or in Canada and the United States, might vary widely in thiamin content. Golberg and Thorpe (1942), on analysis of samples of South African wheat, discovered that varieties of wheat grown on irrigated land were 15% higher in thiamin than similar varieties grown on swamp land. The use of potassium and nitrogen fertilizers caused an increase in thiamin content. When water, manure, and phosphorus fertilizers were used thiamin content increased from 264 mcg./100 gm. to 336 mcg./100 gm.

Allen (1943) in New Zealand found variations in cereal foods from packet to packet according to age, storage conditions and thiamin content of the grain. This was the first indication that the value for a particular cereal product cannot be considered universal. A study of thiamin content of various parts of the grain by Geddes and Levine (1942)

showed that thiamin is transferred from the stem to the leaf, glume and rachis. There are at the same time changes in the thiamin content of the different parts of the seed. Thus the degree of maturation and portion of the grain used would determine the thiamin content of the cereal product.

The thiochrome method was used by Slater and Rial (1942) for the analysis of Australian biscuits and breakfast foods. Brand names were not given and soft Australian wheat is not of the same thiamin content as Canadian hard wheat, so that results are not comparable. Jackson and Malone (1943) in Canada have reported on breakfast cereals as have Kitzes and Elvehjem (1943,1944) in the United States. The following are typical of the values they found, the results being reported as, or calculated by us to, mcg./gm.

	Allen (1943)	Slater and Rial(1942)	Jackson and Malone (1943)	Kitzes and Elvehjem (1943)
Bran Flakes	3.55	3.9	1.38 - 2.44	4.6 - 5.5 *
Corn Flakes			0.00 - 0.13	4.0 - 4.5 *
Oatmeal	4.32-4.55	1.32 - 5.9	6.13 - 7.51	5.8 -15.0
Puffed Rice	negligible		0	15.0 *
Puffed Wheat			0	5.4 *
Shredded Wheat			1.83 - 3.24	1.6 - 2.4
Wheat Flakes			1.18 - 2.87	0.8

The American values marked with asterisks represent cereals fortified with thiamin concentrates and therefore are not comparable with those of other countries. Fixsen and Roscoe (1940) in England report the thiamin content of oatmeal to be 9.75 mcg./gm. they used the rat growth method of assay. These results suggest that there are marked differences between the thiamin contents of different breakfast cereals and between different samples of the same kind of cereal. A recent study of cooking losses of thiamin in breakfast cereals as related to pH values has been made by Lincoln, Hove and Harrel (1944). They found that cooking losses increased at higher pH values.

Several investigators have reported on the thiamin content of different basic cuts of meat. Values found are presented below on a wet basis as micrograms per gram of meat:

	Miltz et al (1943)	Bacharach (1942)	Schweigert et al(1943)	Lane et al(1942)	Miller et al (1943)	McIntire et al (1944)	Reedman et al (1943)	Cover et al (1944)
Pork loin chops	5.34-8.67			14.84			419-52.0	
Pork loin roasts	6.10-7.78	0.6-0.84	7.4-15.2		9.5-23.1			
Pork shoulder roast					7.9-17.3			
Luncheon meat	2.26-4.71						9.6-20.7*	

Continued

	Miltz et al(1943)	Bacharach (1942)	Schweigert et al(1943)	Lane et al(1942)	Miller et al (1943)	McIntire et al (1944)	Reedman et al (1943)	Cover et al (1944)
Bacon	3.51-5.26							
Chicken		0.6-1.02						
Lamb		2.7-4.2	1.28-2.32					
Ham	5.52-8.20	0.6-1.02	7.7-14.8	11.81	10.3-23.9			
Beef rare								0.7-1.1
" well done								0.5-0.9
Liver beef						2.3		
" baby beef						1.9		
Veal heart						4.0		
Veal			1.35-2.0					

*moisture - free basis % moisture in fresh sample ranged from 57.1 - 64.8 (mean)

There are variations in the thiamin content for individual basic cuts. Miller, Rence, Dutcher, Ziegler and McLarty (1943) found that the thiamin content of pork may be directly influenced by the level of thiamin intake of the pig and suggested thiamin high feed for the hog dietary.

Schweigert, McIntire and Elvehjem (1943, 1943a) have also presented data on cooking losses. They found these losses varied with the size of the cut and cooking method used. Braising losses were greater than those for roasting or broiling, due to the extraction of thiamin by the cooking water.

Hiltz, Robinson and Levinson (1943) found cooking losses for pork to vary from 7 to over 50%. They used paired cuts in their study. They suggest that adjacent cuts might be better standards for comparison in estimating cooking losses.

Bacharach (1942) suggested that further analyses of meats should present values as consumed, rather than as gathered, slaughtered or purchased. The study of causes of variation from breed to breed or within the breed or within the carcass of a single animal, was also recommended.

PART A CEREALS

M E T H O D S

Samples of varieties of breakfast cereals available on the Winnipeg market were purchased for analysis. Ready - to - eat cereals were analysed as purchased and uncooked or partially cooked varieties were cooked according to the manufacturers directions as outlined on the package. For rolled oats the two steps referred to in Table III is the

overnight method of cereal cooking. Cereal was added to rapidly boiling water and cooked over direct heat five minutes. It was then covered and cooked twenty-five minutes the next morning over a double boiler. Uncooked cereals were ground and analysed in duplicate for moisture content by the vacuum oven method. Similarly the moisture content of cooked cereals was determined.

After the first sampling the packages were sealed and stored at room temperature for one year. Some packages were stored without preliminary analysis. Then samples were ground and tested for moisture and thiamin. The analyses before storage were made by Miss Abigail Levinson. Those after storage were made by the author. The same methods for breakfast cereals were used by both investigators.

As stated previously the method used for thiamin determination was that of Hennessy and Cerecedo with slight modifications. The details of the method were:- Duplicate five gm. samples of ground cereal was mixed with about 90 cc. of 2% acetic acid solution in an Erlenmeyer flask, shaken

thoroughly and placed in a boiling water bath for one hour. Then the flask was cooled. To it was added 2 cc. of 36% sodium acetate solution to bring the pH to 4.0 to 4.5. This was checked with bromcresol green on the spot plate. 0.4 gm. takadiastase in 2 cc. water were added and the mixture incubated for two hours at 50° C with occasional shaking 1 cc. of normal sulfuric acid solution was added and the mixture brought to a boil. Then it was cooled and made up to 100 cc. in a volumetric flask. A portion was centrifuged, 20 cc. of the extract was heated to boiling and allowed to seep through a column of activated 60-80 mesh Decalso in a base exchange tube. The liquid was passed through the column a second time. The Decalso absorbed the thiamin and so separated it from the extract. It was removed from the Decalso by passing boiling 25% potassium chloride solution through the column. The eluate was made up to 25 cc. A 5 cc. aliquot was placed in a reaction vessel. To it was added 3 cc. of a mixed reagent made by diluting 1 cc. of 3% potassium ferricyanide solution to 100 cc. with 15% sodium hydroxide solution. After one minute 16 cc. of isobutyl alcohol were added and the

reaction vessel shaken vigorously for one and a half minutes. It was next centrifuged at low speed for 45 seconds. The water layer was drawn off. 1.5 gm. of anhydrous sodium sulfate was shaken with the isobutyl alcohol layer for twenty seconds. The layer was then poured off into a cuvette. This was placed in a Coleman Electronic Photofluorometer, model twelve, and the reading taken. A blank was run using 5 cc. of the eluate but adding 3 cc. of 15% sodium hydroxide instead of the mixed reagent. The difference between the readings for the sample and that for the blank is a direct measure of the amount of thiamin in the sample. The apparatus was calibrated by the use of thiamin.

Each result reported is the average of two determinations. Most of these checked within one division on the photofluorometer scale - in terms of thiamin this is within 0.03 micrograms per gram. It represents a difference of from zero to 6% of the total thiamin in the material being tested. Duplicate results showing differences of 10% or more were discarded.

A standard thiamin solution was prepared in this laboratory. It was checked against a commercial standard - Winthrop's Vitamin B_1 standard - and against a solution provided by Dr. Hoffer of the Western Canada Flour Mills Co. Ltd. It checked with each of these outside standards.

The results are reported as micrograms of thiamin per gram of material, and are calculated to two places of decimals. For cooked cereals, the results are calculated to three places of decimals. Since the content of solids in a cooked cereal is quite low - usually about 20% - the third place of decimals is of the same order of accuracy as the second place is for uncooked cereals.

A rapid method has been proposed by Andrews and Nordgren (1941). Hoffer, Alcock and Geddes (1943) have modified this as follows:- One gm. ground cereal is weighed into a centrifuge tube and to this is added 20 cc. of 25% KCl in 2% acetic acid. The tube is swirled rapidly to disperse lumps. It is next covered with a boiling tube and set in a water bath at 70° C. for half an hour. Then it is centrifuged