THE THIAMIN CONTENT OF FLOUR
MADE FROM CERTAIN VARIETIES OF KANSAS GROWN WHEAT

by

DORIS ELAINE WHITNEY

B. S., Fort Hays Kansas State College, 1939

A THESIS

submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Food Economics and Nutrition

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1942
TABLE OF CONTENTS

INTRODUCTION ................................................................. 1

REVIEW OF LITERATURE ..................................................... 2

METHOD OF PROCEDURE ..................................................... 8

DISCUSSION OF RESULTS .................................................. 26

SUMMARY ........................................................................... 28

ACKNOWLEDGMENT ............................................................. 29

LITERATURE CITED ............................................................ 30
INTRODUCTION

Bread has an important place in the American diet. A century ago the staff of life was bread made from the whole wheat kernel crudely ground between stones. It was a rich source of vitamins and minerals. The white bread largely consumed today is a product of the modern roller milling process, which was introduced into America from Hungary about 1870. Since that time the vitamin and mineral content of the American diet has gradually decreased because in various refining processes most of these substances have been removed. As a result many American people suffer from a deficiency of some of the essential vitamins, particularly thiamin. There is a prevailing belief that white flour produces a bread superior in appearance to the whole wheat product. Also the refined flour keeps much better than that containing the bran and germ portions. As a rule 90 percent of the thiamin from the wheat kernel is lost when the bran and germ are discarded. The remaining 20 percent is retained in the endosperm or flour portion.

It is possible that certain varieties of wheat may have a higher content of thiamin in the endosperm than others and flour made from these varieties would therefore have a higher thiamin value. By determining the thiamin value of flour made from different varieties of Kansas-grown wheat, information may be
obtained which will be of value in increasing the thiamin content of American diets.

REVIEW OF LITERATURE

Only 30 years ago thiamin was one of those "unknown substances" believed to be essential to life. In 1911 Funk isolated from rice polishings a water soluble material which cured polyneuritis in birds. To this material which he believed to be a single substance he gave the name 'Vitamine' because he thought it was an amine, a compound containing nitrogen, and was vital to life. In 1913 McCollum and Davis proposed the name "water-soluble B" for this substance, while Drummond, (1920) in England, used the term Vitamin B. The work of Emmett and Luros (1920) suggested that the so-called vitamin B might consist of at least two factors, one heat-labile and the other heat-stable. Smith and Hendrick (1926) working with brewers' yeast confirmed this fact. While this work was in progress human pellagra was being studied by Goldberger and Tanner (1925) who became convinced that pellagra was prevented by some of the same foodstuffs, particularly milk, beef and yeast, that promote rat growth and prevent polyneuritis.

Fifteen years after Funk's isolation of the water-soluble vitamin from rice polishings, Jansen and Donath (1927) by concentrating the water extract of rice polishings isolated the active substance which cured polyneuritis. Kinnersley and
Peters (1928) obtained the crystalline product by a charcoal adsorption process. They extracted yeast with 0.1 N HCl, evaporated this to a small volume and adsorbed the active substance on a small amount of charcoal, thereby concentrating the vitamin five to 10 times. The chemical concentration work of Jensen and Donath (1927) was repeated in detail by Williams, Waterman and Curin (1930). The method was applied to yeast extract, and fuller's earth was substituted for the acid clay used by Jensen and Donath. The concentrates were fed to both rats and pigeons rather than to rice birds used by the former workers. Seidell and Smith (1930) further developed concentration methods by a process involving adsorption on fuller's earth and subsequent purification, and in 1931 Seidell and Brickner announced that they had obtained an as yet impure product even more active than Jansen's. In 1932 Windaus and associates, working with a crystalline preparation of vitamin $B_1$ isolated from yeast, found sulphur in the molecule.

Williams and his associates (1934) using quinine acid sulfate instead of baryta for the elution of the vitamin from fuller's earth, secured the vitamin in greater quantities than previously, getting as much as five grams per ton of rice polishings. Two years later Williams and Cline (1936), and Cline, Williams and Finkelstein (1937) synthesized the vitamin $B_1$ and established its chemical structure. This was a great step forward and pointed the way for further research.

The formula for vitamin $B_1$ established by Williams and
associates is $C_{12}H_{13}O_{4}S Cl_{2}$. The molecule contains both a substituted pyrimidine and thiazole nucleus. It is the only known vitamin to contain sulphur and this characteristic led to the adoption of the name thiamin as a substitute for the previously applied term vitamin $B_1$. Thiamin is a white crystalline substance, soluble in water and has a salty taste. The vitamin is destroyed by high temperatures and while it is stable in atmospheric oxygen it may be oxidized to thiochrome by various oxidizing agents.

During the years in which thiamin was being isolated and synthesized investigators were interested in the assay of foods for thiamin content. Williams and Seidell (1916) used the weight-maintenance method of assay in which gain in weight was noted after thiamin-deficient adult pigeons had been given a dose of test material. This test was also used by Block, Coghill and Klotz, (1932) but not all workers were certain that the response in weight was due entirely to the thiamin administered.

Williams (1919) and Bachmann (1919) noted that anti-neuritic food extracts promoted the growth of yeast. Schultz, Atkin and Frey (1937) developed an accurate means of measuring the rate of gas evolved by the yeast and detected as little as one microgram of thiamin by their method. In another paper, published in 1939, Schultz and associates report the application of the yeast fermentation method in determining the thiamin content of wheat, flour and bread.

Kinnersley, Peters and Reader (1923) using pigeons and
Smith (1930) using rats developed the polyneuritic-curative assay method, which was later modified by Kline, Tolle and Nelson (1938). On this test the animals are fed a thiamin-free diet until they show symptoms of the vitamin deficiency, then a known amount of a standard thiamin solution is given and the time of recovery noted. When these same animals are again deficient a definite amount of the test material is administered and a comparison is made as to the time of recovery with that of the standard. This test has the advantage of being specific, quick, convenient, and the animals may be used again and again for successive assays.

Chase and Sherman (1931) using rats determined the smallest amount of assay material that would promote a gain of three grams per week over a four-eight week period. This amount was called a Sherman unit. Chick and Roscoe (1929) placed rats on a thiamin-free diet and when growth ceased the test material was incorporated into the diet in graded amounts. The amount of material necessary to maintain growth at the rate of 10-14 g per week was determined. It was designated as a Chick and Roscoe unit. In 1941 the rat-growth method was improved by Kline, Hall and Morgan and in the same year was adopted by the Association of Official Agricultural Chemists for the assay of vitamin B1. The rats were kept on a diet deficient in thiamin until their weights were stationary or declining. Graded amounts of standard thiamin and varied levels of the assay material were then fed to groups of the animals. Comparisons were made
between the gain in weight of the rats fed the reference standard and those fed the assay material. Results obtained from this test were considered accurate but some investigators have pointed out that careful selection of animals is necessary as not all strains of rats grow at the same rate.

The bradycardia test proposed by Drury, Harris and Maudsley (1930) was based on the deviation of the heart rate from normal as the rat or pigeon approached the polyneuritic stage. This test used with rats has since been developed by Birch and Harris (1934) and by Harris and Leong (1937). Considerable skill is required to obtain good results with this method.

Chemical tests are also used in the assay of thiamin in foodstuffs. The oxidation of thiamin to thiochrome with diazo-tized sulfanilic acid in the presence of formaldehyde was used by Kinnorsley and Peters (1934). McCollum and Prebluda (1936) obtained a red compound by treating thiamin with p-amino acetophenone. The dye was extracted from the water solution and estimated colorimetrically. Barger and coworkers (1935) produced thiochrome by the oxidation of thiamin with alkaline potassium ferricyanide. This is the basis of the chemical method used at present to produce thiochrome for estimation by its fluorescence. Many investigators think the chemical tests should be developed further before being considered reliable for testing foods.

Flour which is the food of particular interest in this study and which contains a rather small quantity of thiamin has
not been assayed to any great extent. Harris and Leong (1937) assaying white flour by means of the bradycardia test found it to contain from 0.2 to 0.3 I.U. * thiamin per gram. Data compiled by Pixeran and Roscoe (1938) showed white straight-run flour of 70 per cent extraction to have 37 I.U. thiamin per 100 g; of 60 per cent extraction to have 24 I.U. thiamin per 100 g; while flour of 24 per cent extraction had no thiamin. Schults, Atkin and Frey (1939) using the fermentation method found that straight flour had 1.5 mcg of thiamin per gram or 0.5 I.U. per gram, and short patent flour had 0.7 mcg per gram or 0.25 I.U. per gram. Bailey (1940) found "ordinary patent flour" to contain about 0.3 I.U. of thiamin per gram, while Sherwood, Nordgren and Andrews (1941) list patent flour as having 0.29 mg thiamin per pound which is equivalent to 0.21 I.U. per gram.

At a Canadian Conference in Ottawa in 1941 Canadian second patent flour was reported to contain 136 I.U. thiamin per pound or 0.3 I.U. per gram. Taylor (1941) has reported patent flour of 63 per cent extraction as having 310 mcg of thiamin per pound or 0.22 I.U. per gram. Jackson and Whiteside (1942) by means of the thiochrome test, which was checked against bioassays using the rat growth technique, reported thiamin values ranging from 244 I.U. per pound, or 0.55 I.U. per gram, in Family flour down to 90 I.U. per pound, or 0.17 I.U. per gram, in First Patent flour. The average thiamin value of all Canadian flours tested

---

*One International Unit of thiamin chloride is equivalent in biological activity to three micrograms of crystalline vitamin B1 hydrochloride.
was 160 I.U. per pound or 0.35 I.U. per gram. Booher and Hartzler (1939) using the rat-growth method found that white, straight milled flour contained 29 I.U. thiamin per 100 g and that white patent flour had 17 I.U. per 100 g.

In the light of the above findings there seems to be a need for more work on the assay of thiamin in flour, particularly in the United States. There is also the possibility that the thiamin value of wheat grown in different parts of the United States may vary. According to the literature Canadian flours have been more extensively assayed than have the flours of our own country. No report of the thiamin content of flour made from wheat produced in Kansas was found in the literature. With this in mind the assay of flour made from different varieties of Kansas-grown wheat was chosen for this study.

**METHOD OF PROCEDURE**

Samples of long patent flour made from four varieties of Kansas-grown wheat, i.e., Turkey, Blackhull, Chiefkan and Tenmarq were obtained from the Department of Milling Industry. These samples had approximately the same water content and were of 75 per cent extraction. Biological assays of the thiamin content of these samples were carried out according to the rat-growth method described by Kline, Hall and Morgan (1941). This method in which the test material is incorporated into the diet, is applicable to such food materials as flour which contain
small amounts of thiamin.

Normal rats between 21 and 28 days of age weighing not less than 39 g nor more than 52 g at the beginning of the experiment were used for the test. Each animal was placed in an individual wire cage with an elevated screen bottom each mesh of which was \(\frac{1}{2}\)" by \(\frac{1}{2}\)" in order to prevent refection. Throughout the depletion period each rat was supplied with water and thiamin-free diet only. This diet contained:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>60</td>
</tr>
<tr>
<td>Casein</td>
<td>18</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4</td>
</tr>
<tr>
<td>Autoclaved yeast</td>
<td>5</td>
</tr>
<tr>
<td>Autoclaved peanuts</td>
<td>10</td>
</tr>
<tr>
<td>Treated liver extract</td>
<td>1</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2</td>
</tr>
</tbody>
</table>

The vitamin-free casein was purchased from the Borden Company, New York. The salt mixture was prepared according to Wesson's modification (1932) of Osborne and Mendel's method. Dried yeast was autoclaved at 15 pounds pressure for six hours and then dried at a temperature below 65°C. in order to destroy the thiamin. For the same reason raw, shelled peanuts were ground and autoclaved at 15 pounds pressure for six hours and then dried in the open air. The thiamin was removed from liver extract by the method of Kline, Tolle and Nelson (1938).

At the end of the 15- to 18-day depletion period the rats
were assembled into groups of eight animals, each group showing thiamin deficiency characterized by stationary or declining weight. For each assay material, one negative control group and more than one reference group were provided. In nearly every case the same number of rats of each sex comprised each group and not more than three rats from one litter were placed in any one group. For any one assay material the average weight of the rats in any one group did not exceed by more than 10 g the average weight of the rats in any other group. For the reference groups a water solution of standard thiamin chloride was added to a weighed amount of thiamin-free diet and mixed thoroughly. The flour was incorporated by replacing an equal amount of sucrose in the diet.

The environment was as nearly uniform as possible. The rat weights were recorded at the beginning of the depletion period, on the sixth day of depletion, and each third day thereafter until the assay was begun. During the assay period the rats were weighed every six days. A record of the amount of food consumed by each rat was made.

The first experiment was carried out on flour made from Turkey wheat. Forty-eight rats were deploted of their body thiamin by being given a thiamin-free diet for 13 days. The animals were then assembled into six groups of eight rats each. At the beginning of the assay period the average weights of the rats in each group ranged from 59 to 68 g. The reference groups were given 0, 9, 12, and 15 I.U. of thiamin in each 100 g of basal
diet. The remaining two groups were fed the basal diet in which sucrose was replaced by flour at 30 and 40 per cent levels.

A summary of the data from the assay of Turkey flour is given in Table 1 which shows the average weights of the animals in each group for each interval at which they were weighed, the average number of grams of food consumed and the average total gain or loss in weight during the test period.
Table 1. Average gain in weight and food consumption in grams of animals used in the assay of Turkey flour.

<table>
<thead>
<tr>
<th>Supplements per 100 g food</th>
<th>Depletion period (days)</th>
<th>Assay period (days)</th>
<th>Gain or loss</th>
<th>Food consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  6  9  12  15*</td>
<td>6  12  18  24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 g flour</td>
<td>48 55 62 65 62</td>
<td>67 71 74 74</td>
<td>12</td>
<td>146</td>
</tr>
<tr>
<td>30 g flour</td>
<td>45 54 60 62 59</td>
<td>59 59 59 59^c</td>
<td>0</td>
<td>101</td>
</tr>
<tr>
<td>15 I.U. thiamin</td>
<td>45 55 59 62 65</td>
<td>69 77 92 92</td>
<td>27</td>
<td>142</td>
</tr>
<tr>
<td>12 I.U. thiamin</td>
<td>48 60 67 71 66</td>
<td>65 68 73 81</td>
<td>15</td>
<td>125</td>
</tr>
<tr>
<td>9 I.U. thiamin</td>
<td>44 54 60 62 63</td>
<td>62 60 59 59^b</td>
<td>-4</td>
<td>99</td>
</tr>
<tr>
<td>Basal diet</td>
<td>48 67 69 70 68</td>
<td>65 60 58 52^a</td>
<td>-10</td>
<td>--</td>
</tr>
</tbody>
</table>

*aBeginning of assay  b^75% of animals died  c^7% of animals died  d^12% of animals died
Figure 1. Growth curves for groups of animals used in the assay of turkey flour.
Figure 1 shows the average weight gains and losses for the rats of each group during the depletion and assay periods. As shown in Fig. 1 and Table 1 the 30 per cent flour level did not allow the animals to grow and there was a gain of only 12 g on the 40 per cent level of flour during the 24-day assay period, while the group receiving 12 I.U. thiamin per 100 g food gained 15 g during this time. In Fig. 1 the line representing the average gain in weight of the animals on the 40 per cent flour level fell just below the line which represents the average gain in weight of the group fed 12 I.U. of thiamin. It was therefore estimated that 40 g of flour was equivalent to 11 I.U. of thiamin, and one gram of Turkey flour contained 0.27 I.U. of thiamin.

The second experiment was conducted on flour milled from Blackhull wheat. In the test on Turkey flour the animals did not grow on a diet containing 30 per cent flour and on the 40 per cent level gained only 12 g in 24 days. According to the method of Kline, Hall and Morgan, which is being used in these experiments, for a valid test a gain of 12 to 60 g during the test period for two-thirds of the animals in a group is necessary. The average of 12 g by the animals given 40 per cent Turkey flour was considered near the borderline. Because of this it was thought advisable to modify the method by adding to each 100 g of diet 6 I.U. of standard thiamin. This modification allowed the levels of flour to be lowered.

Forty-seven rats were depleted of their body thiamin in 15 days. The animals were then assembled into five groups of eight
each and one group of seven rats. At the beginning of the assay period the average weights of the rats in each group ranged from 65 to 69 g. The group of seven animals was used as a negative control and was given the basal diet, each 100 g of which contained 6 I.U. of thiamin. The assay groups were fed thiamin-free diets containing 15, 20, and 30 per cent flour plus 6 I.U. of thiamin per 100 g food. The two reference groups were given 15 and 18 I.U. of thiamin in each 100 g of basal diet.

A summary of the data from the assay of Blackhull flour is given in Table 2. This is a record of the averages of the weights of the animals, the food consumed, and their gains or losses in weight for this experiment.
Table 2. Average gain in weight and food consumption in grams of animals used in the assay of Blackmill flour.

<table>
<thead>
<tr>
<th>Supplements per 100 g food</th>
<th>Depletion period (days)</th>
<th>Assay period (days)</th>
<th>Gain or loss</th>
<th>Food consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 6 9 12 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 12 18 24 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 g flour + 6 I.U. thiamin</td>
<td>44 61 65 68 67</td>
<td>76 84 89 95 96</td>
<td>29</td>
<td>167</td>
</tr>
<tr>
<td>20 g flour + 6 I.U. thiamin</td>
<td>42 59 64 66 65</td>
<td>66 65 65 73 75</td>
<td>10</td>
<td>109</td>
</tr>
<tr>
<td>15 g flour + 6 I.U. thiamin</td>
<td>44 61 65 67 66</td>
<td>67 65 63 65 66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>106</td>
</tr>
<tr>
<td>15 I.U. thiamin</td>
<td>43 59 63 65 66</td>
<td>71 75 84 96 98</td>
<td>32</td>
<td>176</td>
</tr>
<tr>
<td>15 I.U. thiamin</td>
<td>44 60 67 68 68</td>
<td>72 75 81 90 94</td>
<td>26</td>
<td>160</td>
</tr>
<tr>
<td>6 I.U. thiamin</td>
<td>41 61 66 68 69</td>
<td>69 66 65 66 66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3</td>
<td>112</td>
</tr>
</tbody>
</table>

<sup>a</sup>Beginning of assay  <sup>b</sup>50% of animals died  <sup>b</sup>16% of animals died
Figure 2. Growth curves for groups of animals used in the assay of Blackhall flour.
Figure 2 records the average gains and losses in weight for the rats of each group during the experiment. The animals fed the diet containing 6 I.U. of thiamin and 50 g of flour per 100 g of food gained 29 g while the reference group receiving 18 I.U. of thiamin per 100 g diet gained 32 g. The reference group receiving 15 I.U. of thiamin per 100 g diet gained 26 g. In Fig. 2 the line representing the group receiving 30 g of flour and 6 I.U. thiamin per 100 g of food fell just between the two lines representing the animal groups given 15 and 18 I.U. of thiamin per 100 g diet.

It was therefore estimated that 30 g of flour plus 6 I.U. of thiamin was equivalent to 17 I.U. thiamin. After deducting the 6 I.U. of thiamin, 30 g of flour contained 11 I.U. of thiamin, and one gram contained 0.36 I.U.

The next assay was made with Chiefkan flour. After a 15-day depletion period 60 rats were assembled into seven groups of eight each and one group of four rats. The average weights of the rats at the beginning of the assay period ranged from 63 to 68 g. The group of four rats was given 6 I.U. thiamin per 100 g food as a supplement to the thiamin-free diet and was used as the negative control group. Supplements of 12, 15, 18, and 20 I.U. of thiamin were added to each 100 g of basal diet and given to four groups of eight rats which were called the reference groups. Three groups composed of eight rats were given supplements of Chiefkan flour at the levels of 20, 25, and 30 per cent with the addition of 6 I.U. thiamin per 100 g basal diet. Table
3 gives a summary of the data obtained in this assay. In this table is recorded the average weights of the animals in each group for each interval at which they were weighed; the average number of grams of food consumed and the average total gain or loss in weight during the assay period.
Table 3. Average gain in weight and food consumption in grams of animals used in the assay of Chieffan flour.

<table>
<thead>
<tr>
<th>Supplements per 100 g food</th>
<th>Depletion period (days)</th>
<th>Assay period (days)</th>
<th>Gain or loss</th>
<th>Food consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  6  12  15*</td>
<td>6  12  13  24  30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 g flour + 6 I.U. thiamin</td>
<td>45 57 64 67 65</td>
<td>74 73 87 97 103</td>
<td>33</td>
<td>179</td>
</tr>
<tr>
<td>25 g flour + 6 I.U. thiamin</td>
<td>46 57 61 66 65</td>
<td>72 75 79 86 88</td>
<td>23</td>
<td>147</td>
</tr>
<tr>
<td>20 g flour + 6 I.U. thiamin</td>
<td>44 55 62 65 63</td>
<td>70 72 76 82 82</td>
<td>19</td>
<td>143</td>
</tr>
<tr>
<td>20 I.U. thiamin</td>
<td>43 58 61 66 65</td>
<td>72 80 88 100 105</td>
<td>40</td>
<td>212</td>
</tr>
<tr>
<td>18 I.U. thiamin</td>
<td>46 58 63 66 65</td>
<td>70 76 84 93 98</td>
<td>33</td>
<td>163</td>
</tr>
<tr>
<td>15 I.U. thiamin</td>
<td>45 58 64 66 65</td>
<td>69 71 77 82 83</td>
<td>13</td>
<td>159</td>
</tr>
<tr>
<td>12 I.U. thiamin</td>
<td>45 58 64 66 65</td>
<td>67 68 69 70 71</td>
<td>6</td>
<td>141</td>
</tr>
<tr>
<td>6 I.U. thiamin</td>
<td>47 60 66 71 68</td>
<td>68 64 61 60 55</td>
<td>-15**</td>
<td>92</td>
</tr>
</tbody>
</table>

*Begning of assay  **75% of rats died
Figure 5. Growth curves for groups of animals used in the assay of Chiefkan flour.
Figure 3 shows the average gains and losses in weight for the rats of each group. The group of rats fed the diet containing 30 per cent flour plus 6 I.U. thiamin per 100 g food gained 38 g while the group receiving 20 I.U. of thiamin per 100 g diet gained 40 g during the assay period. Figure 1 shows that the average gain in weight of the animals on the 30 per cent flour plus 6 I.U. thiamin per 100 g of food was slightly less than of those receiving 20 I.U. of thiamin in the same amount of diet. It was therefore estimated that 30 g of flour plus 6 I.U. thiamin was equivalent to 19 I.U. thiamin, or 30 g flour contained 13 I.U. thiamin. The thiamin value of Chinaflour was 0.43 I.U. per gram.

As a further check on this test there was a difference of only one gram in the total weight gained between the group of rats fed the diet containing 20 per cent flour plus 6 I.U. thiamin per 100 g food and the group receiving 15 I.U. thiamin per 100 g diet. It was therefore estimated, in this comparison, that 30 g flour plus 6 I.U. thiamin was equivalent to 15 I.U. thiamin; or 20 g flour contained 9 I.U. thiamin. The thiamin content of Chinaflour was 0.45 I.U. per gram. These two tests agree closely.

Flour made from Temmarq wheat was used in the fourth experiment. The body thiamin of 54 rats was depleted in 16 days, after which they were assembled into six groups of eight each and one group of six rats. The average weights of the rats in each group at the beginning of the assay period ranged from 68
to 70 g. The previous experiments had shown that the basal diet plus 6 I.U. of thiamin did not support growth. Since the basal diet remained the same and this test was conducted at approximately the same time as that on Chiefkan flour, a negative control group was not considered necessary.

Three reference groups were given 15, 18, and 20 I.U. of thiamin which was added to each 100 g of basal diet. The reference group receiving 12 I.U. thiamin was made up of six animals. The supplements of flour were fed to three groups at the levels of 20, 25, and 30 per cent with the addition of 6 I.U. thiamin to each 100 g of diet.

The data from the assay of Tenmarq flour are given in Table 4 which shows the average weights of the animals in each group for each interval at which they were weighed; the average number of grams of food consumed and the average total gain or loss during the assay period.
Table 4. Average gain in weight and food consumption in grams of animals used in the assay of Tenmarq flour.

<table>
<thead>
<tr>
<th>Supplements per 100 g food</th>
<th>Depletion period (days)</th>
<th>Assay period (days)</th>
<th>Gain or food consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 6 9 12 15 18*</td>
<td>6 12 18 24 30</td>
<td></td>
</tr>
<tr>
<td>30 g flour + 6 I.U. thiamin</td>
<td>48 62 66 68 69 70</td>
<td>76 81 89 93 104</td>
<td>34 165</td>
</tr>
<tr>
<td>25 g flour + 6 I.U. thiamin</td>
<td>47 61 67 69 69 68</td>
<td>75 78 79 90 95</td>
<td>27 141</td>
</tr>
<tr>
<td>20 g flour + 6 I.U. thiamin</td>
<td>47 63 67 70 70 69</td>
<td>75 76 75 79 81</td>
<td>12 124</td>
</tr>
<tr>
<td>20 I.U. thiamin</td>
<td>47 60 67 70 70 70</td>
<td>76 88 98 116 131</td>
<td>61 205</td>
</tr>
<tr>
<td>18 I.U. thiamin</td>
<td>46 57 65 68 71 70</td>
<td>80 88 95 106 113</td>
<td>43 202</td>
</tr>
<tr>
<td>15 I.U. thiamin</td>
<td>45 55 63 68 70 68</td>
<td>74 75 77 82 81</td>
<td>13 143</td>
</tr>
<tr>
<td>12 I.U. thiamin</td>
<td>45 58 65 68 70 68</td>
<td>70 74 74 79 78</td>
<td>10 125</td>
</tr>
</tbody>
</table>

*Beginning of assay
Figure 4. Growth curves for groups of animals used in the assay of Tenmaru flour.
Figure 4 shows graphically the average gains and losses in weight for the rats in each group during the depletion and assay periods.

As recorded in Table 4 the assay group receiving 20 percent flour plus 6 I.U. of thiamin per 100 g food gained 18 g and the reference group receiving 15 I.U. of thiamin per 100 g food gained 15 g during the assay period. This showed that 20 g of flour plus 6 I.U. of thiamin in the basal diet promoted growth at a very slightly lower level than 20 I.U. of thiamin in the basal diet. It was estimated that 20 g of flour plus 6 I.U. of thiamin was equivalent to 15 I.U. of standard thiamin. Then after deducting the 6 I.U. of thiamin the 20 g of flour contained 9 I.U. of thiamin or the thiamin value of one gram of Tenmarq flour was 0.45 I.U.

DISCUSSION OF RESULTS

The results of assays of flour produced from four varieties of Kansas-grown wheat agree quite well with the data reported in the literature for several types of flour. Table 5 shows the data obtained using flour made from Turkey, Blackbull, Chiefkan and Tenmarq varieties of wheat and Table 6 gives the results obtained by other investigators.
Table 5. Thiamin values of flour milled from Kansas-grown wheat.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>I.U. per g</th>
<th>Log per g</th>
<th>I.U. per lb.</th>
<th>Log per lb.</th>
<th>%</th>
<th>per lb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>0.27</td>
<td>0.82</td>
<td>124.50</td>
<td>371.46</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Blackhull</td>
<td>0.36</td>
<td>1.08</td>
<td>103.08</td>
<td>489.24</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Chiefken</td>
<td>0.45</td>
<td>1.29</td>
<td>194.70</td>
<td>584.30</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Tenmarq</td>
<td>0.45</td>
<td>1.35</td>
<td>205.65</td>
<td>611.55</td>
<td>0.61</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Thiamin values of flour.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Type of flour</th>
<th>I.U. per g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harris and Leong (1937)</td>
<td>white</td>
<td>0.2 - 0.3</td>
</tr>
<tr>
<td>Fixsen and Roscoe (1938)</td>
<td>white straight-run</td>
<td>0.37</td>
</tr>
<tr>
<td>Schults, Atkin and Frey (1939)</td>
<td>straight</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>short patent</td>
<td>0.23</td>
</tr>
<tr>
<td>Boocher and Nartsler (1939)</td>
<td>white, straight</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>white patent</td>
<td>0.17</td>
</tr>
<tr>
<td>Bailey (1940)</td>
<td>patent</td>
<td>0.30</td>
</tr>
<tr>
<td>Sherwood, Nordgren and Andrews (1941)</td>
<td>patent</td>
<td>0.21</td>
</tr>
<tr>
<td>Canadian Conference (1941)</td>
<td>second patent</td>
<td>0.30</td>
</tr>
<tr>
<td>Taylor (1941)</td>
<td>patent</td>
<td>0.22</td>
</tr>
<tr>
<td>Jackson and Whiteside (1942)</td>
<td>Family</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>first patent</td>
<td>0.17</td>
</tr>
</tbody>
</table>
The thiamin values for Kansas produced flour varies from 0.87 I.U. for Turkey to 0.45 I.U. per gram for Tenmarq. The variation between the flour analyzed by other investigators is from 0.17 I.U. to 0.53 I.U. per gram. The average for the Kansas flour was higher with 0.36 I.U. per gram than the average of 0.27 I.U. per gram reported in the literature. The range of 0.27 I.U. to 0.45 I.U. was less than the literature range. Many more determinations should be run before any conclusions can be reached.

SUMMARY

The thiamin content of flour made from four varieties of Kansas-grown wheat was determined by means of the biological rat-growth method. It was found that flour made from Turkey wheat contained 0.27 I.U. per gram; Blackhull, 0.36 I.U. per gram; Chiefkan, 0.45 I.U. per gram; and Tenmarq, 0.45 I.U. per gram. Flour made from different varieties of wheat varied in thiamin content.

A modification of the Kline, Hall and Morgan technique is suggested.
ACKNOWLEDGMENT

Appreciation is expressed to Dr. Eaulah Westerman, assistant Professor of Food Economics and Nutrition, for directing this study; to Dr. Martha S. Pittman, Head of the Department of Food Economics and Nutrition, for helpful suggestions; and to Dr. E. G. Bayfield, Head of the Department of Milling Industry, for supplying the samples of flour.
LITERATURE CITED

Bachmann, Freda M.

Bailey, C. H.

Barger, G., Bergel, F. and Todd, A. R.

Birch, Thomas W. and Harris, Leslie J.

Block, Richard J., Cowgill, George R. and Klotz, Benjamin H.

Booher, Lela E. and Hartzler, Eva R.

Canadian Conference, Ottawa.

Chase, E. F. and Sherman, H. C.

Chick, Harriette and Roscoe, Margaret H.
A method for the assay of the antineuritic vitamin B1, in which the growth of young rats is used as a criterion. Biochem. Jour. 23: 498-503. 1929.

Cline, Joseph K., Williams, Robert R. and Finkelstein, Jacob.
Drummond, Jack C.

Irrury, Alan N., Harris, Leslie J. and Maudsley, Cecil.

Emmett, A. D. and Luros, C. O.

Fixsen, Margaret A. E. and Roscoe, Margaret H.

Funk, Casimir.
On the chemical nature of the substance which cures polynarthritis in birds induced by a diet of polished rice.
Jour. Physiol. 43: 395-400. 1911.

Goldberger, J. and Tanner, W. F.

Harris, L. J. and Leong, P. C.

Jackson, S. H. and Whiteside, A. G. O.

Jansen, B. C. P. and Donath, W. F.
The isolation of the anti-beriberi vitamin. Chem. Abs. 21: 2150. 1927.

Kinnersley, Henry W. and Peters, Rudolph A.

Kinnersley, Henry W. and Peters, Rudolph A.
Kinnersley, Henry W., Peters, Rudolph A. and Reader, Vera.

Kline, O. L., Tolle, C. D. and Nelson, E. M.

Kline, O. L., Hall, W. L. and Morgan, J. F.

McCullum, E. V. and Davis, Margeurite.

McCullum, E. V. and Prebluda, H. J.

Schultz, A., Atkin, L. and Frey, C. N.

Schultz, A. S., Atkin, L. and Frey, C. N.

Seidell, A. and Brickner, Victor.

Seidell, A. and Smith, Maurice I.

Sherwood, R. C., Nordgren, Robert and Andrews, John S.

Smith, Maurice I.

Smith, Maurice I. and Hendrick, E. C.
Taylor, Alonzo.

Wesson, Lawrence C.
Scientific apparatus and laboratory methods. A modific-
tion of the Osborne-Mendel salt mixture containing only in-

Williams, R. R. and Cline, J. K.
Synthesis of vitamin B\(_1\). Amer. Chem. Soc. Jour. 58: 1504-
1505. 1936.

Williams, R. R. and Seidell, A.
The chemical nature of the "vitamins." Jour. Biol. Chem.

Williams, R. J.
The vitamin requirement of yeast. Jour. Biol. Chem. 38:
465-486. 1919.

Williams, R. R., Waterman, R. E. and Gurin, Samuel.
The Jansen and Donath procedure for the isolation of anti-

Williams, R. R., Waterman, R. E. and Keresztesy, John C.
Larger yields of antineuritic vitamin. Amer. Chem. Soc.
Jour. 56: 1187-1191. 1934.

Windaus, A., Tschesche, R., Laquer, R. F. and Schultz, F.
The preparation of crystalline antineuritic vitamin from