EFFECTS OF VARYING STORAGE CONDITIONS ON B-VITAMIN COMPOSITION OF WHEAT

by

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B.S., Universidade Federal De Pelotas, Brazil, 1972

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Cereal grains are produced and consumed all over the world. The nutritional contribution of cereals to the diet include carbohydrates, proteins, minerals and vitamins. Those nutrients can be present in different amounts depending on environmental factors, soil, species and variety.

The production of the grains is seasonal, but consumption is continuous, so that grains produced during the growing season have to be preserved as food and seed, until the next growing season. During this preservation period, depending on conditions of storage, many changes occur. For some cereals (barley, rice, wheat) some of the changes will improve the quality of the grains, making them more suitable for use. On the other hand, especially under adverse conditions, high moisture content being the most important, changes often result in losses of quality and nutritional value.

Specific tests exist which help to predict behavior during storage and to test the extent of damage incurred. These tests include moisture determination, number and kind of molds present, viability of the seeds, fat acidity and functional uses (baking tests, flour extraction, starch and oil extraction, etc.).

Very little definite information appears to be available concerning changes of B vitamins, other than thiamin, found in grains. This study was undertaken to measure changes in the content of some B vitamins in wheat, when stored under three different relative humidities and three different temperatures.

REVIEW OF LITERATURE

Factors That Influence Biochemical and Nutritive Changes During Storage.

Moisture and Temperature:

The rates of growth and development of micro-organisms and insects, and rates at which chemical and physical changes happen are well related to the moisture content of the grain and temperature of storage. A product can safely contain more moisture if the temperature of the product is low and uniform. Moisture content controls the metabolic process while temperatu largely determines the rate of the reactions involved.

Grain in storage will sorb or give up moisture until it reaches an equilibrium with the surrounding atmosphere. This equilibrium relative humidity of the product is important because it is a measure of the availability of water to microorganisms and insects. Hunt and Pixton, (1974) reviewing the effect of moisture content on grains in storage, considered "safe" storage moisture content as being accepted as that in equilibrium with 70 percent relative humidity or less. Above 75 percent relative humidity molds will develop rapidly during storage, and heating of the product will occur with subsequent deterioration and loss. Pixton and Warburton, (1971) observed that the moisture content in equilibrium with 70 percent relative humidity varies with different products.

It is now generally recognized that equilibrium relative humidity values or "active water" is more closely related to food product stability than total moisture content. Pixton and Warburton (1971), stressed that moisture content gives an estimate of the amount of water in a product but that it does not, by itself, indicate the biological activity, or potential activity, of the product.

Acker (1969), pointed out that while whole grain can be stored for a relatively long period of time at 13 percent moisture content the milled whole grain would deteriorate in a few weeks at the same moisture content, since the disruption of the cellular structure and contact between enzymes and substrate would increase the rate of deterioration. In this case water activity plays a role in the enzyme activity of the grains, because in the disrupted structure of the milled whole grain, the water serves as the vehicle for the reactions.

Respiration:

Hummel et al (1954), studied the respiratory rate of mold-free wheat and concluded that the respiratory rate was constant at moisture contents of 16 to 31 percent and temperatures up to 35°C.

The work of many authors, Bottomley et al (1950 and 1952); Hilner et al (1947); Milner and Geddes (1946), indicate that the presence of molds on or within the kernel caused an increased rate of respiration measured in grains with different and increasing moisture content and temperature. Geddes (1958), observed that high moisture grain produced a respiration time curve, measured as production of CO_2, similar in form to a microbiological respiration curve during population growth.

Respiration, regardless of its origin, depends upon chemical reactions and is therefore accelerated by an increase in temperature. Milner et al (1947), described three factors that can retard the rate of respiration: thermal inactivation of the enzymes involved, exhaustion of the substrates or accumulation of inhibitory concentration of carbon dioxide.

Molds growing on a simple substrate of carbohydrates and fatty acids, yield as end product of aerobic respiration water, carbon dioxide and heat and as end product of anaerobic respiration, carbon dioxide and several simple organic compounds.

Effects on Nutrients

Carbohydrates:

Bottomley et al (1952), using samples of corn aerated and non-aerated (sealed jars) found decreased quantities of non-reducing sugars. The aerated samples, at high moisture content, had a reduction to one-seventh of their original value of non-reducing sugars. The loss of non-reducing sugars could be correlated with increasing mold count. The non-aerated sample had relatively little change in mold count and non-reducing sugar content.

Since fungi are largely responsible for the deterioration of grain, it was suggested that the spoilage of moist grain might be prevented if it were stored in carbon dioxide or nitrogen to prevent the mold growth. Glass et al (1959), using wheat stored in air atmosphere and nitrogen, found at all moisture contents, an increase in reducing sugars. Non-reducing sugars decreased in all samples above 13 percent moisture content and changes were somewhat greater at higher moisture contents. Mold count increased rapidly at moisture content of 15 percent and above in the grain stored in air, but the grain stored in nitrogen remained virtually mold-free. The decrease in non-reducing sugars was almost exactly compensated for by the increase in reducing sugar. When the damp wheat was stored in air, extensive mold growth occurred and the increase in reducing sugars was only about one-fourth as great as the decrease in non-reducing sugars owing to the utilization of the former by molds.

Early workers expressed the non-reducing sugars as sucrose and reducing sugars as maltose, but Glass and Geddes (1960), using wheat stored under nitrogen, isolated myo-inositol, galactose and glycerol which are not present in the free state in sound wheat. Lynch et al (1962), applied quantitative paper chromatography to investigate mono- and disaccharides in wheat stored under air, nitrogen and carbon dioxide. In air the sucrose content of the wheat decreased markedly, whereas little change occurred in glucose, fructose, galactose or maltose content. In carbon dioxide and nitrogen the sucrose content decreased and there was rather large increases in glucose, fructose and galactose.

Linko et al (1960), studying formation of reducing sugars during storage of wheat germ, under conditions which product germ-damaged ("sick") wheat in commercial storage, suggested that the reducing sugar formed in the germ would react with free amino acids to develop the characteristic brown color of the germ of "sick" wheat.

Proteins:

Jones and Gersdorf (1941), stored wheat flour and whole wheat flour for two years at 76°F. and 30°F. and studied the alterations occurring on proteins during that period. The changes included a decrease in soluble proteins, in true protein content, in amount of protein preciptable by trichloroacetic acid and in digestibility. The amino nitrogen increased. The extent of changes depended on temperature and nature of the stored material. Jones et al (1941), also studied the effect of storage on whole shelled corn and ground corn. The results showed a decrease in soluble protein and a partial breakdown of proteins indicated by an increase in

amino nitrogen. All the changes occurred much more rapidly in the milled products of grain than in whole grain.

The protein content when calculated from the nitrogen content of grain, is generally assumed to remain the same during normal storage, but Shutt (1909), working with wheat and Daftary et al (1970), working with flour, found slightly but consistently higher values of protein content in those stored products. The relative increase in percentage basis, can be explained by respiratory losses of carbohydrates. In samples stored at 37°C. protein values determined by a dye binding method were lower than protein values determined by the Kjeldahl method.

Ferrel et al (1970), used lightly scarified wheat infused with up to 15 percent of lysine hydrochloride to prepare blends of wheat with lysine enrichment at any practical desired level. Studies were undertaken to determine the storability of the added lysine (the limiting amino acid in wheat). The studies indicated that lysine hydrochloride added to wheat is stable under reasonable conditions of storage for at least one year (10 percent reduction) whether held as highly fortified wheat or practical blends ready for the consumer.

Proteolytic enzymes in grain and microorganisms associated with grains hydrolyse the proteins into polypeptides and finally into amino acids. These reactions proceed very slowly and are not readily measurable until the grain has reached an advanced stage of deterioration.

De Vay (1952), compared free amino acids composition of sound and moldy wheat, using paper chromatography, and concluded that based on the size and color intensity of the spots, alanine, gamma-amino butyric acid, proline,

serine, and lysine had apparently increased while histidine had decreased in moldy wheat compared to the sound wheat.

When the grain is moistened to insufficient levels to produce germination but enough moisture exists to activate certain enzymes, some physiological and biochemical changes will occur. Dampening ground whole wheat to moisture contents up to 40 percent, Linko and Milner (1959), observed a decrease in the content of glutamic acid accompanied by an increase in gamma amino butyrate and a slower increase in alanine. The destruction of glutamate by decarboxylation resulted in the high values of gamma aminobutyrate, transaminase activity was indicated by a rapid drop in alanine and increase in levels of glutamate. Moisture levels as low as 18 percent activated both enzyme systems and activity increased rapidly with moisture content up to that required for germination of the grain. Transamination occurred at moisture levels as low as 15 percent. Glutamic acid decarboxylase was located almost entirely in the embryo.

Fats:

Deterioration of grain and cereal products in storage is accompanied by increased acidity, including free fatty acids, acid phosphates and free amino acids. Pomeranz and Shellenberger (1966), observed that at early stages of deterioration fat acidity increases at a much greater rate than either of the other two types or all types of acidity combined.

Rancidity may be caused by either hydrolytic or oxidative changes in the fat, resulting in development of objectionable odor and flavors. Grains contain fairly active antioxidants and fats in unbroken kernels are rather effectively protected against oxidative rancidity.

The fats in grains are readily broken down by lipases into free fatty acids and glycerol during storage, particularly when the temperature and moisture content are high and thus favorable to general deterioration. Pomeranz (1971), observed that this type of change is greatly accelerated by mold growth because of high lipolytic activity of molds.

Barton Wright (1938), working with flour, found that during storage the fraction soluble in petroleum ether decreased rapidly and this was correlated with increased fungi number. When the fatty acids were not utilized by the fungi and flours with normal oil content were stored under conditions to arrest fungi development, the increase in free fatty acids resulted in breaking down of the washed gluten. He further observed that removal of the fat from aged flour returned the gluten to its original condition.

Morrison (1963), studied the amount and composition of free fatty acids in flour. The amount of free fatty acids increased with age, spring wheat flours contained less palmitic acid and more linoleic acid than those from winter flours but there was no correlation with flour strength. Recoveries of phosphorous-free lipids were nearly constant during storage experiments and it was assumed that most of the free fatty acids originated from triglycerides. Palmitic, oleic, linoleic and linolenic acids were liberated at a steady rate, in proportion close to those of total lipids.

Daftary and Pomeranz (1965), studied changes in lipids of wheat stored at elevated temperatures and high moisture levels. Increase in mold count from 1,000 to about 200,000 was accompanied by a 40 percent decrease in total lipids (extractable with petroleum ether). Non polar lipids decreased about 25 percent and the damaged wheat contained only one-third as much polar lipids as the sound wheat. Grain deterioration was accompanied by rapid

disappearance of glycolipids and phospholipids. The breakdown of polar lipids (extractable with water satured butanol after petroleum ether extraction) was more rapid and extensive than formation of free fatty acids or disappearance of triglycerides.

The development of fat acidity in wheat or wheat flour has been considered an index of bread making quality. Morrison (1963), reported decrease of loaf volume when more than one-third of flour lipids were hydrolysed to free fatty acids. However, Shellenberger et al (1958), found no correlation between bread quality and fat acidity of flour stored at 40°F.

Vitamins:

Cereal grains are generally good sources of thiamin, niacin, pyriodoxine, inositol, biotin and Vitamin E. Vitamin A activity occurs in yellow corn, but is practically absent in all other cereal grains. Losses in vitamin content that may occur during storage are of considerable practical importance.

Investigations dealing with the effect of storage on vitamin content of cereals have been limited, owing in part to the assumption that vitamins are stable during storage.

Bayfield and O'Donnell (1945), reported that wheat stored under normal and high moisture content under two different atmospheres, (air and ethylene gas) stored for a period of five months, showed a definite decrease in thiamin with increasing age. The high moisture wheat (17 percent), which was treated with ethylene gas showed slightly less loss of thiamin than the untreated wheat. The normal moisture wheat, 12 percent, lost less thiamin than the high moisture wheat, but it was still an appreciable loss of the

vitamin during favorable storage conditions. They also analyzed wheat samples ranging from 3 to 51 years old that had been stored under normal laboratory conditions in closed jars. As the original thiamin content of that wheat was unknown, the comparison was based upon the average thiamin content of that year's crop, but it was evident that a considerable loss of thiamin had occurred. However, some of these samples that were as much as 21 years old still had a fairly high thiamin content and appeared to have lost little vitamin during the long storage period.

Fifield and Robertson (1945), also reported fairly high values of thiamin in wheats, stored under dry conditions, for 14 to 21 years.

Pomeranz et al (1956), stored wheat at high moisture (up to 23.5 percent) and normal moisture content (11 percent) at two different temperatures (1-2°C and 20-21°C) and analyzed for changes in thiamin, riboflavin and niacin. During storage of the wheat with 23.5 percent moisture at 20-21°C, thiamin and nicotinic acid content decreased, whereas the content of riboflavin increased.

Hollenbeck and Obermeyer (1952), conducted storage tests on samples of enriched flour under both normal and accelerated conditions. The relative stability of thiamin mononitrate in flour was less influenced by the temperature and moisture conditions of the flour than it was the stability of thiamin chloride hydrochloride. The increased stability of thiamin mononitrate was attributed to its relatively low hygroscopicity compared to thiamin chloride hydrochloride.

Pelshenke (1960), reported no loss of thiamin or tocopherol in wheat stored below 20°C for 3 to 4 months but there was a 10 percent loss of thiamin after 19 months in storage.

Hunt et al (1950), investigated differences in vitamin content of normal and moldy corn. The moldy corn had higher values for niacin and pantothenic acid than the normal corn. Rhizopus sp. was isolated from the inside of the moldy grain and grown in sterile vitamin-free medium for a period of 3 to 4 days, then the mycelia was collected and analyzed, showing that this particular mold had the ability to synthesize niacin and pantothenic acid.

MATERIALS AND METHODS

Material: Lancota hard wheat from the 1976 Kansas wheat crop. Fifty grams of wheat was placed in 125 ml jars. The jars were stored in sealed plastic boxes for relative humidity control and then in three different temperature controlled chambers, at 15°C, 25°C, and 35°C.

Methods:

Relative Humidity Control:

Saturated solutions of three different salts were used for relative humidity control. The salts and relative humidity created are indicated in the following table:

	Relative Humidity in Percentage			
Temperature	15°C	25°C	35°C	Reference
Salt				
KNO_3	94.4	92.0	89.3	Wexler and Hasegawa(195
NaCl	75.3	75.8	75.5	Wesler and Hasegawa(195
$K_2CO_32H_2O$	44.0	43.8	43.4	O'Brien(1948)

The solution was prepared with chemically pure salts and distilled water, with an excess of undissolved crystals. Care was taken so that no crystals protruded above the surface, thereby creating a lower humidity than desired.

The relative humidity created in the vapor space is determined by the water vapor pressure and the saturation water vapor pressure at the temperature of the vapor. The relative humidity created in the vapor space of the box was not measured, only the effect, as moisture content, in the grain.

Vitamin Analysis:

There are three general methods for vitamin analysis: biological, microbiological and chemical methods. Biological methods are, in general, time consuming and expensive, but they actually measure the quantity of the vitamin available to the animal. Microbiological methods are faster and require less space, labor and material; they also have good biological specificity. These methods, unlike chemical methods, can be used even before the chemical nature of the vitamin being assayed has been determined. Chemical analysis can be carried out rapidly and economically and are more applicable to routine determination than most of the other methods.

Thiochrome procedure for Thiamin: Association of Vitamin Chemists (1966)

Thiamin occurs in natural foods and other biological materials, either in the free or in a combined form: as a protein complex or as phosphorus-protein complex, or as pyrophosphoric acid ester, cocarboxylase. The relative amounts of any of these forms vary considerably in different sources.

Thiochrome procedure depends upon the oxidation of thiamin to thiochrome, which fluoresces in ultraviolet light. Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present, and hence to the thiamin originally in solution.

Thiamin was extracted from the sample by 0.1N H_2SO_4 in a boiling water bath. The acid extraction will solubilize all forms of thiamin that may be present and in the resulting pH, thiamin is very stable. The acid extraction was followed by an enzymatic digestion to hydrolyse any phosphate esters of thiamin and to digest the starch material.

Purification through Decalso was not necessary and the filtrate of the extraction was used directly for the oxidation reaction. Thiamin was oxidized by potassium ferricyamide in strong alkali to yield thiochrome. This substance is soluble in isobutyl alcohol and when irradiated with ultraviolet light fluoresces, emitting a blue light. Fluorescence was measured in a Coleman 14 Universal Spectrofluorometer, using a quinine sulfate solution as standard. The blank value was obtained by adding one drop of concentrated hydrochloric acid to the cuvette mixing and reading, following the procedure of McRoberts, (1958).

Calculations: Thiamin content of the sample in γ/g

$$\frac{U - UB}{S - SB} \times \frac{1}{5} \times \frac{100}{\text{wt of sample}}$$

where

U = deflection of unknown

UB = deflection of unknown blank

S = deflection of standard solution (0.2 γ/g)

SB = deflection of standard solution blank

Fluorescence procedure for Riboflavin: Association of Vitamin Chemists (1966)

Riboflavin is a yellow-green, fluorescent, water soluble pigment widely distributed in plant and animal cells. Williams and Cheldeline (1942), observed that riboflavin is very sensitive to both visible and ultraviolet light and that low light intensity should be used during the analysis.

The intensity of fluorescence is proportional to the concentration of riboflavin in dilute solutions. The riboflavin is measured in terms of the difference between the fluorescence before and after chemical reduction.

The vitamin was extracted with 0.1N hydrochloric acid in an autoclave for 30 minutes at 15 lb. of pressure. After autoclaving and cooling the sample, the interferring substances were precipitated first at pH 6.0 with 0.1N sodium hydroxide then at pH 4.5 with 0.1N hydrochloric acid.

The filtrate was used for the oxidation reaction according to the following scheme:

	TUBE A	TUBE B
Wheat extract (ml)	10	10
Standard riboflavin solution (ml)	1	--
Water (ml)	--	1
Acetic acid (ml)	1	1
KMO4 3% (ml)	0.5	0.5
Time of reaction (min)	2	2
H_2O_2 3% (ml)	0.5	0.5

The addition of potassium permanganate reduces interferring fluorescent substances to a non-fluorescent form. Shaking after the addition of H_2O_2 oxidizes the riboflavin to the fluorescent form.

The fluorescense was measured in a Coleman Universal 14 Spectrofluorometer, using sodium fluorescein as standard. Tubes A and B were read, then 20 mg of sodium hydrosulfite, that reduces the riboflavin to a non-fluorescent form, was added to tube B to have a blank reading.

Calculation: Riboflavin content of the sample in γ/g

$$\frac{B - C}{A - B} \times \frac{\text{riboflavin increment}}{\text{10 ml aliquot}} \times \frac{\text{dilution factor}}{\text{sample wt.}} =$$

where

A = fluorescence of the sample plus riboflavin standard increment

B = fluorescence of the sample

C = blank of filtrate

Colorimetric procedure for pyridoxine: Hochberg, Melnick and Oser, (1944)

Vitamin B_6 occurs in natural foods in various forms, mainly as pyridoxol, pyridoxamine, pyridoxal, pyridoxamine 5-phosphate and pyridoxal 5 phosphate. It has been established that all these various vitamin B_6 forms have the same biological activity in man and higher animals. For the enrichment of food and feed only pyridoxine is used.

Pyridoxine was extracted using 4N hydrochloric acid for one hour in boiling water bath. This procedure constitutes an effective mean for both the hydrolysis of bound pyrodoxine and extraction of the vitamin.

The free vitamin is absorbed from solution with Lloyd's reagent at pH3 and eluted with sodium hydroxide. The eluate is clarified by precipitation with isopropanol. Three aliquots of the supernatant are then tested

with 2.6-dichloroquinonechloromide reagent, one alone, another in the presence of added pyridoxine (internal standard), and the third with boric acid which renders the vitamin non-reative without affecting the ability of other reacting compounds to couple with the reagent.

For each series of tubes the blank is used for setting zero absorbance at 620 mm and then the unknown and internal standards are measured. All readings should be done exactly 60 seconds after the color reaction.

Calculations:

$$\frac{A_2}{A_3 - A_2} \times \frac{10}{6} \times \frac{30}{5} \times \frac{20}{\text{weight of sample}} = \gamma/g$$

A_2 = absorbance of tube 2

A_3 = absorbance of tube 3

A_2-A_3 increment in absorbance due to the added 10 of pyriodoxine.

Niacin Procedure: Pelletier and Campbell, (1959)

Niacin and niacinamide are widely distributed in foods. Meat, fish, yeast, enriched and whole grain cereals are good dietary sources. In natural products, niacin is usually bound to other chemical compounds and must be freed by hydrolysis or enzymatic treatment before analytical methods can be applied.

The sample was digested with 1.5 percent calcium hydroxide, which yields light-colored extracts, as compared with the darker extracts produced by acid or stronger alkalis. Moreover, sugars and starch are precipitated as calcium salts.

Clarification of the extract is done by centrifuging, precipitating with ammonium sulfate at 55-60°C and filtering.

Color reaction at 2°C, with cyanogen bromide develops a stable color that is measured at 470 mm, setting zero absorbance with the standard blank, 12 to 15 minutes after the color development reaction.

One reagent blank and five levels of standard nicotinic acid solution are treated the same way as the unknown.

Calculations:

Plot reference curve of absorbance of standard minus that of reagent blank, against concentration of niacin in nmg/100 ml drawing straight line that is best fit. From this curve read concentration, C, corresponding to absorbance of samples corrected for sample blank and reagent blank.

$$\text{mg niacin/100 g sample} = \frac{\text{concentration}}{10 \times \text{wt of sampling}}$$

RESULTS AND DISCUSSION

Thiamin and Riboflavin:

Every two months two samples were drawn from storage and analyzed for thiamin and riboflavin content. The sample was first analyzed for moisture content, then it was air dried, cleaned of excess molds (when necessary), ground through a 20 mesh screen and frozen until the time of analysis.

Results for thiamin and riboflavin content are provided in Table I and II.

Table I. Thiamin, γ/g*

Time (months)	0		2		4		6	
Low Relative Humidity								
15°C	4.88	4.69	4.42	4.51	4.35	4.41	4.38	4.51
25°C	4.51	4.79	4.68	4.31	4.30	4.35	4.08	4.85
35°C	5.26	4.69	4.10	4.10	4.32	4.39	4.25	4.31
Medium Relative Humidity								
15°C	4.94	4.52	4.40	4.00	4.24	4.13	4.02	4.57
25°C	4.94	4.94	4.73	4.54	4.49	4.43	3.68	3.46
35°C	4.94	5.16	4.56	3.93	4.28	4.14	3.71	4.27
High Relative Humidity								
15°C	4.85	4.51	4.14	5.02	4.79	5.21	4.51	4.52
25°C	5.36	5.36	5.17	6.03	5.17	5.28	3.00	3.26
35°C	4.93	4.79	4.79	5.17	4.93	4.99	2.62	2.34

*Moisture free basis.

Table II. Riboflavin, γ/g*

Time (months)	0		2		4		6	
Low Relative Humidity								
15°C	1.63	1.64	1.36	1.16	1.42	1.55	1.93	1.97
25°C	1.39	1.28	1.19	1.28	1.30	1.44	1.16	1.40
35°C	1.54	1.36	1.35	1.34	1.38	1.18	1.77	1.44
Medium Relative Humidity								
15°C	1.90	1.25	1.47	1.35	1.41	1.22	1.50	1.27
25°C	1.50	1.44	1.36	1.36	1.25	1.20	1.47	1.42
35°C	1.35	1.44	1.31	1.41	1.24	1.33	1.77	1.82
High Relative Humidity								
15°C	1.39	1.26	4.46	4.28	4.99	4.59	8.84	9.81
25°C	1.26	1.33	3.65	3.87	4.96	4.40	12.34	10.05
35°C	1.54	1.36	3.87	3.91	6.39	7.45	18.24	18.06

*Moisture free basis

The average values for thiamin and riboflavin, in the wheat, before storage (time zero) were:

thiamin 4.89 ± 0.55 γ/g s.d. = 0.263

riboflavin 1.46 ± 0.37 γ/g s.d. = 0.175

These values are in agreement with known values for those vitamins in whole wheat.

The experiment was based on a split split plot design, where whole plots were defined by temperature, subplots by relative humidity and sub. subplots by time. The analysis of variance for the six months storage period were as follows:

Table III. Analysis of Variance for Thiamin

Source of variance	df	mean square	F values
Total	53		
temperature	2	0.4352	
error A	0		
relative humidity	2	0.3915	5.50**
relative humidity x temperature	4	0.1225	1.72
error B	9	0.0712	
time	2	2.6793	34.03**
relative humidity x time	4	1.6154	20.52**
temperature x time	4	0.6452	8.19**
relative humidity x time x temperature	8	0.3273	4.16**
error C	18	0.7878	

There was no degree of freedom for error A because the temperature factor was not replicated. The interaction of the three factors studied, time x relative humidity x temperature, was significant (P>.001), meaning that the three factors and their combined effects acted upon the grain to cause the changes in the thiamin content. The interaction of these same factors was also significant for the riboflavin content of the stored wheat.

Table IV. Analysis of Variance for Riboflavin

Source of variance	df	mean squares	F values
Total	53		
temperature	2	7.7331	
error A	0		
relative humidity	2	219.1342	1346.28**
relative humidity x temperature	4	7.2040	44.28**
error B	9	0.1627	
time	2	50.9690	340.43**
relative humidity x time	4	42.7514	285.54**
temperature x time	4	4.3693	29.18**
relative humidity x temperature x time	8	4.0295	26.91**
error C	18	0.1497	

Effect of Time of Storage on Vitamin Content

The thiamin content of the wheat decreased with increasing time of storage. The decrease was greater for the wheat stored under high relative humidity than for the wheat stored under lower relative humidities. At high relative humidity the vitamin content, at the end of the sixth month, varied from less than half of the amount present at time zero to 90 percent of the initial value, depending on the temperature of storage.

The riboflavin content of the wheat increased with the time of storage. Changes were greater at high relative humidity than at lower relative humidities. For the high relative humidity, temperature of storage had a markedly effect on riboflavin content.

Effect of Temperature of Storage on Vitamin Content

By means of regression analysis, tests were run to determine the effect of temperature on the vitamin content.

At low relative humidity the decrease in thiamin content was the same at 15°C, 25°C, and 35°C, meaning that temperature of storage did not affect the amount of change. The change in riboflavin content was also the same for the three different temperatures.

At medium relative humidity the change in thiamin was independent of of temperature of storage, and it was the same at the three different temperatures. There was not a significant change in riboflavin value at medium relative humidity for any of the temperatures.

At high relative humidity, for both thiamin and riboflavin, temperature had a highly significant effect on the changes of the vitamins. The greatest decrease of thiamin occurred at 35°C and the greatest increase in riboflavin occurred also at 35°C.

For thiamin content the lowest temperature, 15°C, had a stabilizing effect, and the decrease in thiamin, at this temperature, was very small, for the high relative humidity stored grain, when compared to the changes at 25°C and 35°C.

Effect of Relative Humidity on Vitamin Content

All the changes in vitamin content were markedly affected by the relative humidity of storage and the subsequent equilibrium moisture content.

At low and medium temperatures changes in vitamin content were small during the period of storage. For high relative humidity, changes were

highly significant and the effect of time and temperature of storage was much more important than under the lower relative humidities.

Pyridoxine and Niacin

The wheat was analysed for pyridoxine and niacin content before the storage period (zero time) and after 6 months of storage. The pyridoxine analysis is not specific for this form of the vitamin, and the chloromide reagent, used for color reaction, will react to a certain extent with pyridoxal and pyridoxamine, two other forms of B_6 vitamin, but since most of the B_6 vitamin in wheat is in the pyridoxine form, the values are relatively precise.

Results for niacin and pyridoxine are provided in Table V and VI.

Table V. Niacin, γ/g*

Time (months)	0		6	
Low Relative Humidity				
15°C	64.9	67.1	49.3	70.5
25°C	63.2	71.6	73.0	67.1
35°C	63.2	67.1	77.2	72.6
Medium Relative Humidity				
15°C	61.0	71.7	60.4	67.8
25°C	60.9	71.6	65.6	70.5
35°C	59.3	70.6	88.1	75.9
High Relative Humidity				
15°C	71.6	70.5	184.2	143.7
25°C	62.0	70.5	78.7	62.0
35°C	64.9	66.0	70.5	69.0

*Moisture free basis.

Table VI. Pyridoxine, γ/g*

Time (months)	0		6	
Low Relative Humidity				
15°C	3.57	3.57	3.68	3.72
25°C	2.83	3.07	3.63	3.71
35°C	3.72	3.57	3.69	3.90
Medium Relative Humidity				
15°C	3.95	3.14	4.93	4.95
25°C	3.98	3.57	3.88	3.86
35°C	3.57	3.57	3.93	3.85
High Relative Humidity				
15°C	3.95	3.75	5.95	5.89
25°C	3.60	3.48	4.62	4.40
35°C	3.71	3.56	4.93	5.23

*Moisture free basis.

The average value for pyridoxine and niacin in the whole wheat before storage was:

pyridoxine 3.56 ± 0.63 γ/g s.d. = 0.299

niacin 66.1 ± 10.27 γ/g s.d. = 4.877

Polansky and Toepper (1969), reported values for total B_6 vitamin in five hard wheats as being from 2.71 γ/g to 3.75 γ/g. Of the total B6 vitamin 75 percent was identified as pyridoxine, 13 percent as pyridoxal and 12 percent as pyridoxamine, by microbiological analysis.

The analysis of variance are given in Table VII and VIII.

Table VII. Analysis of Variance for Niacin

Source of variance	df	Mean square	F values
Total	17		
temperature	2	1675.908	
error A	0		
relative humidity	2	1689.345	13.22**
relative humidity x temperature	4	2197.482	17.33**
error 3	9	126.800	

There was no degree of freedom for error A because temperature was not replicated. The interaction relative humidity x temperature was significant (P>0.001), meaning that those factor and their combined effect acted upon the grain to cause the changes in niacin content.

The analysis of variance for pyridoxine was also significant (P>0.001), for the interaction of relative humidity x temperature.

Table VIII. Analysis of Variance for Pyridoxine.

Source of variance	df	Mean square	F values
Total	17		
temperature	2	1.1148	
error A	0		
relative humidity	2	3.2368	289.43**
relative humidity x temperature	4	0.3245	29.02**
error B	9	0.0111	

Effect of Time of Storage on Vitamin Content:

Pyridoxine content increased during time of storage for the higher levels of relative humidity, but more for the highest relative humidity. There was a significant increase ($P>0.001$) for the sixth month pyridoxine content for the medium and high relative humidity, when compared to the wheat before storage.

Niacin content increased with increasing time of storage, especially for the highest relative humidity. There was a significant difference ($P>0.001$) for the sixth month average content, when compared to the average content before storage.

Effect of Temperature of Storage on Vitamin Content:

Pyridoxine increased with decreasing temperature, more for the higher humidity values than for the lower relative humidity. There was a highly significant difference ($P>0.001$) for the values of pyridoxine at 15°C, at the higher relative humidities, when compared to the vitamin content before storage.

Niacin, also, increased with decreasing temperature, but differences in value were significant ($P>0.001$) only for high relative humidity, when compared to the average niacin content before storage. Effects were more pronounced at 15°C than any other temperature.

Effect of Relative Humidity of Storage on Vitamin Content:

Changes for niacin and pyridoxine were highly affected by the relative humidity of storage and the equilibrium moisture content of the wheat.

Niacin and pyridoxine content were slightly effected at low or intermediary relative humidity but they were highly effected under the highest relative humidity, when comparing to the average values before storage.

Presence of Molds:

The wheat stored at the lowest relative humidity remained without any visible molds during the storage period. The initial moisture content (average 11.49 percent) dropped slightly during the first two months of storage and then started to increase. After 6 months, the moisture content averaged 14.5 percent, the lowest value was obtained at 35°C and the highest value at 15°C.

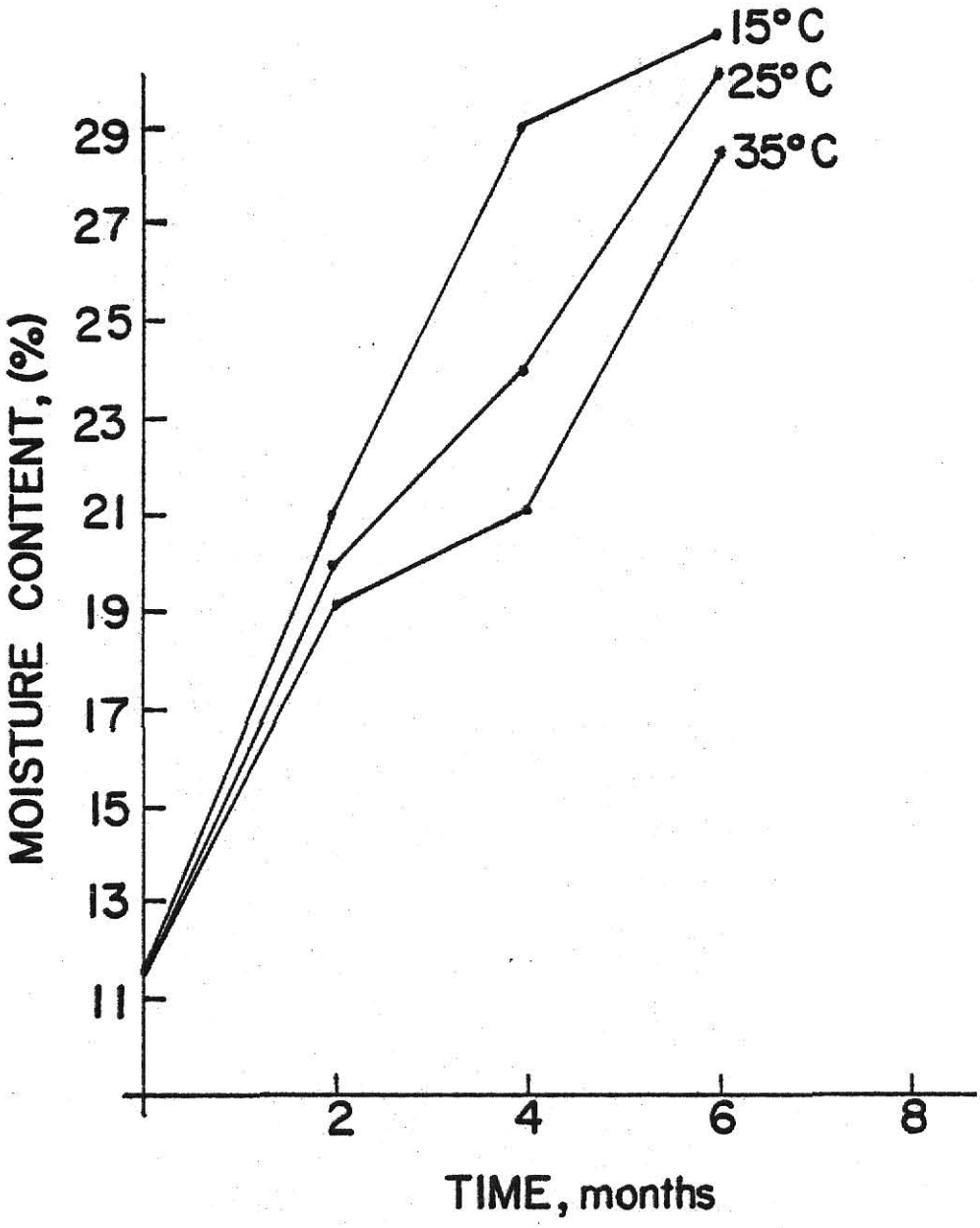
The wheat stored at the intermediary relative humidity remained without any visible molds for the first five months of the experiment, but at the sixth month some mold could be observed on the germ of the kernels, especially at 35°C. The average moisture content remained around 14.5 percent during part of the storage time, but there was a jump of two percent in average, in all temperatures, as soon as molds start to develop. The lowest moisture content were found in samples stored at 35°C and the highest in samples stored at 15°C.

The wheat stored at the highest relative humidity had signs of mold growth after the second month of storage. The moisture content increased rapidly and by the end of the sixth month some samples had moisture content as high as 30 percent. After the samples were air dried and cleaned, the discoloration caused by the molds was visible and the kernels were reduced mostly to bran, were very light in weight and friable.

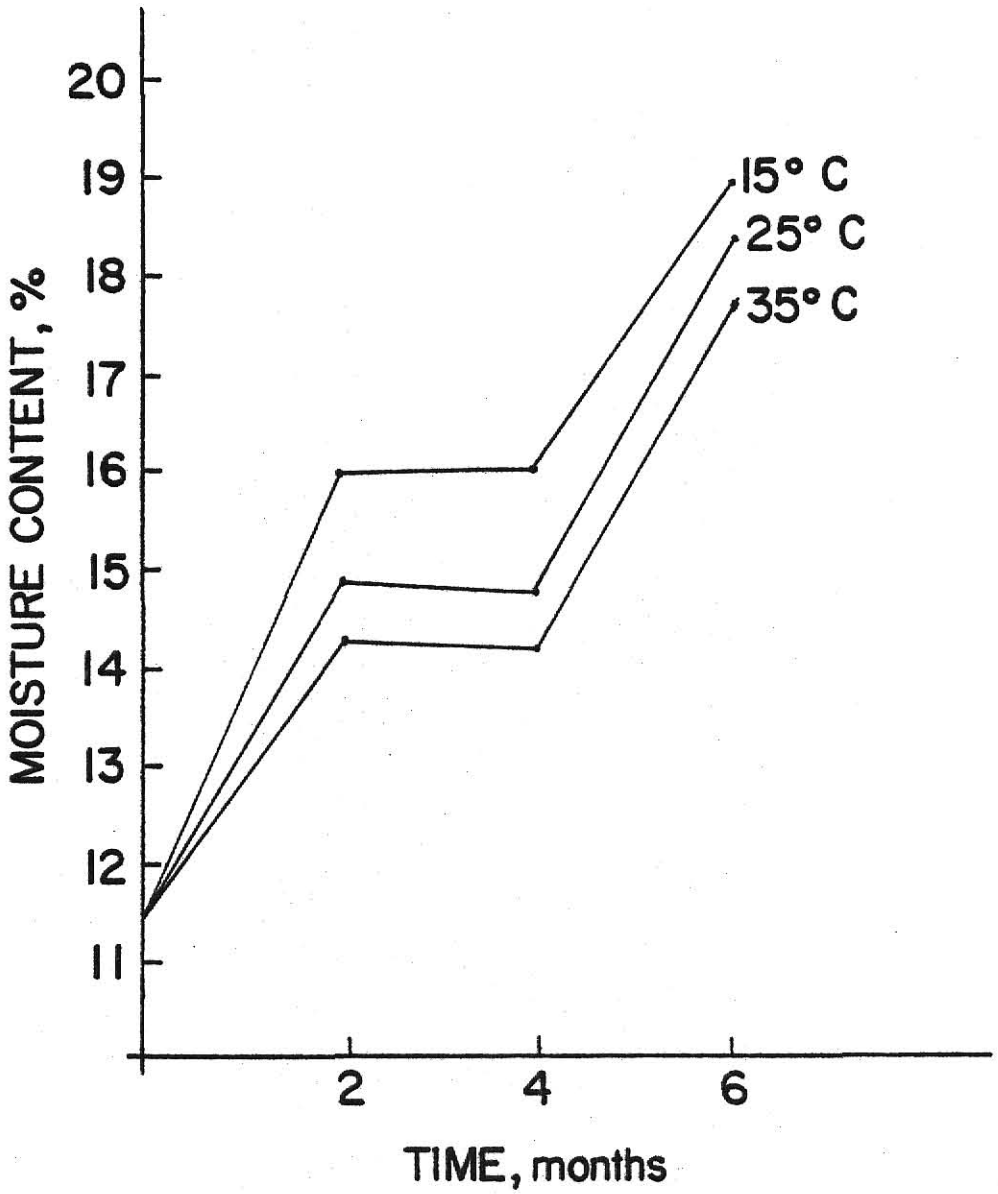
Under the aerobic conditions of the experiment oxygen is absorbed and such nutrients as carbohydrates and fats are oxidized, with the release of energy and the formation of carbon dioxide and water as end products, both by the grains and the microorganisms on or within the grain. This would explain the light weight and almost total absence of endosperm in the highly infested grain.

Samples stored at 25°C were surface desinfected and planted in agar substrate, to observe what kind of molds would grow. For the grain stored at low relative humidity, 62 percent of the grains, still yielded field fungi, mostly Alternaria. The grain stored at medium relative humidity yielded most Aspergillus glaucus, a storage fungi. For the high relative humidity stored grain, the molds were Aspergillus versicolor, Aspergillus candidus and Aspergillus glaucus, all storage fungi.

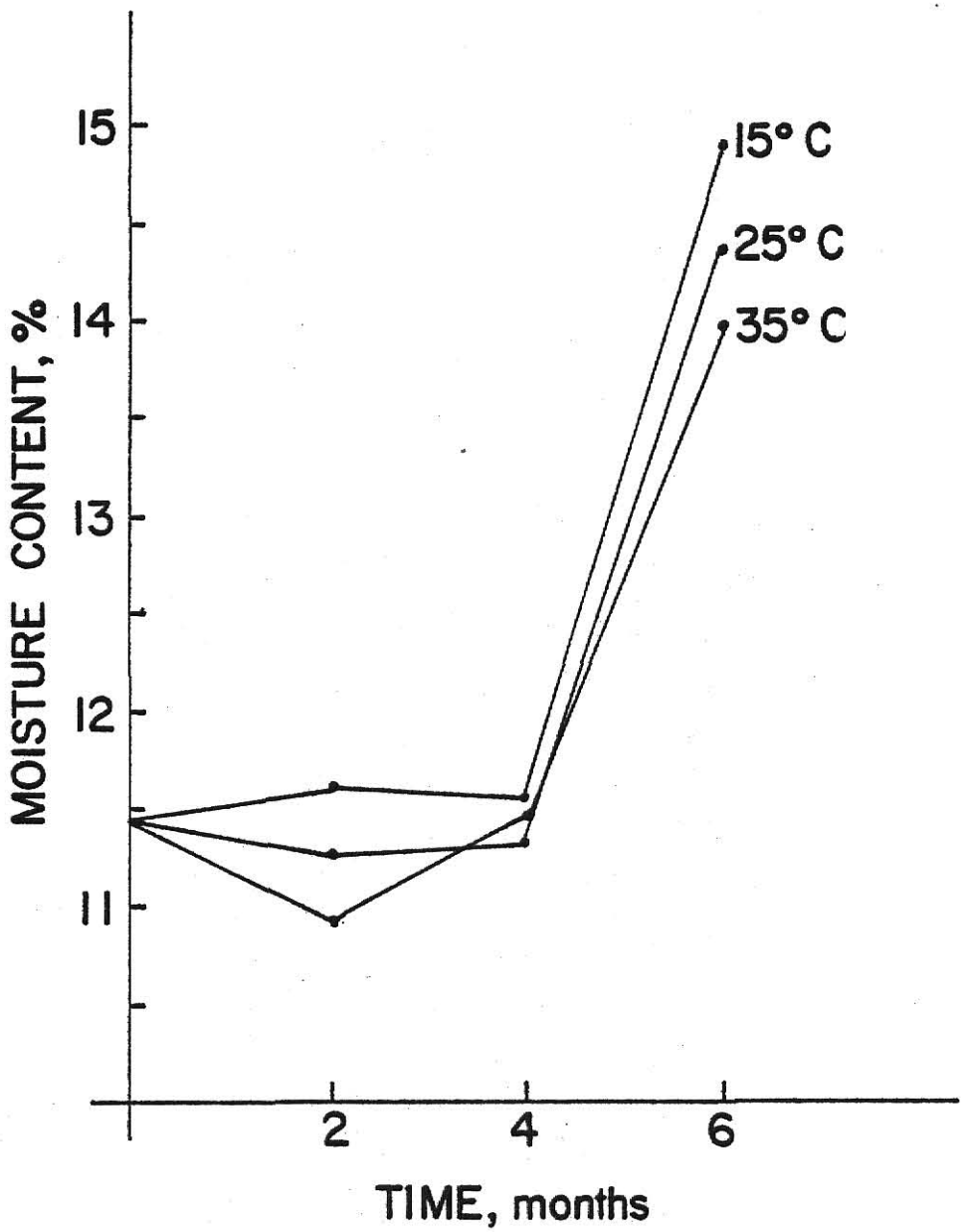
EFFECT OF STORAGE ON MOISTURE CONTENT HIGH RELATIVE HUMIDITY

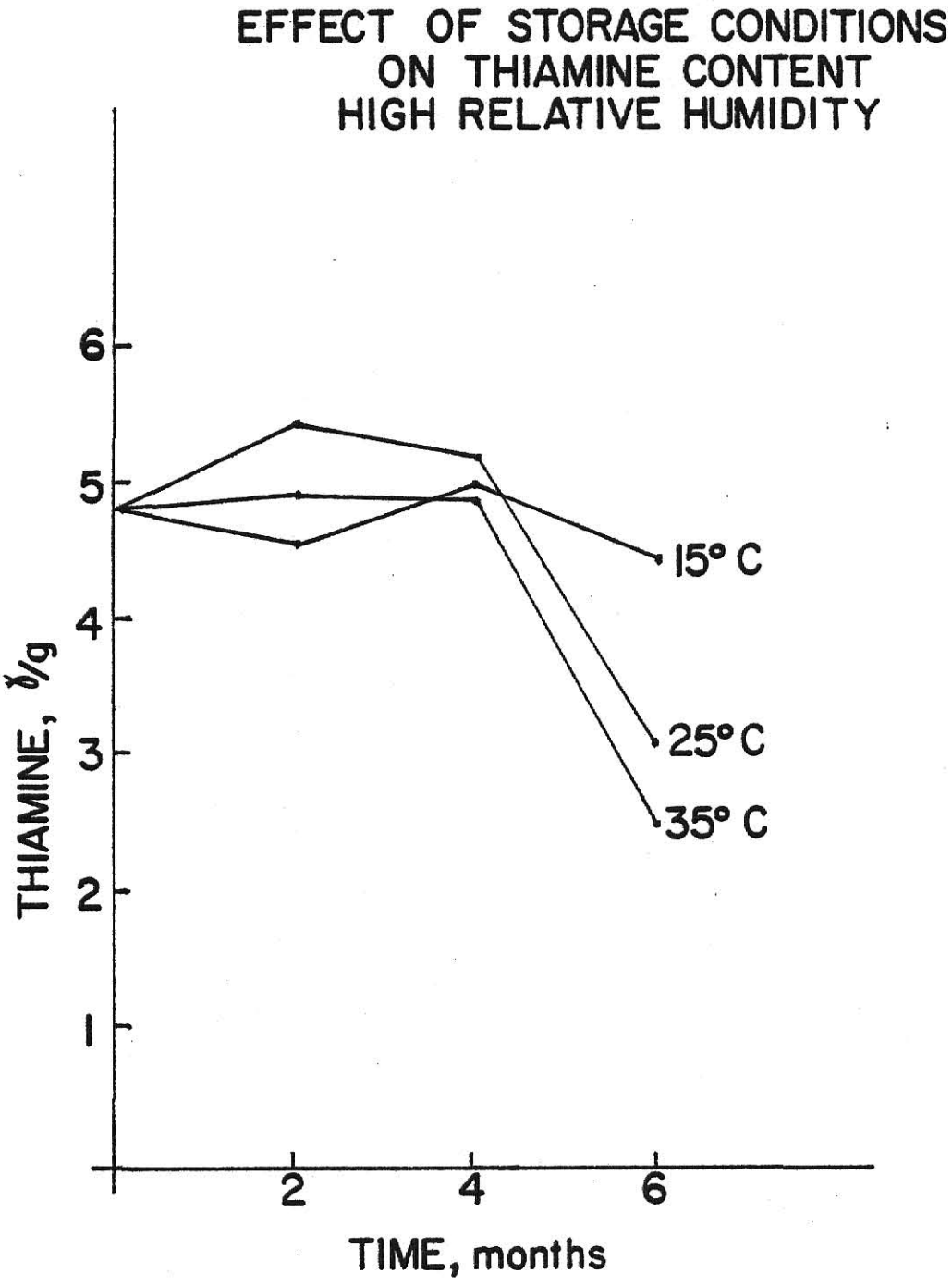


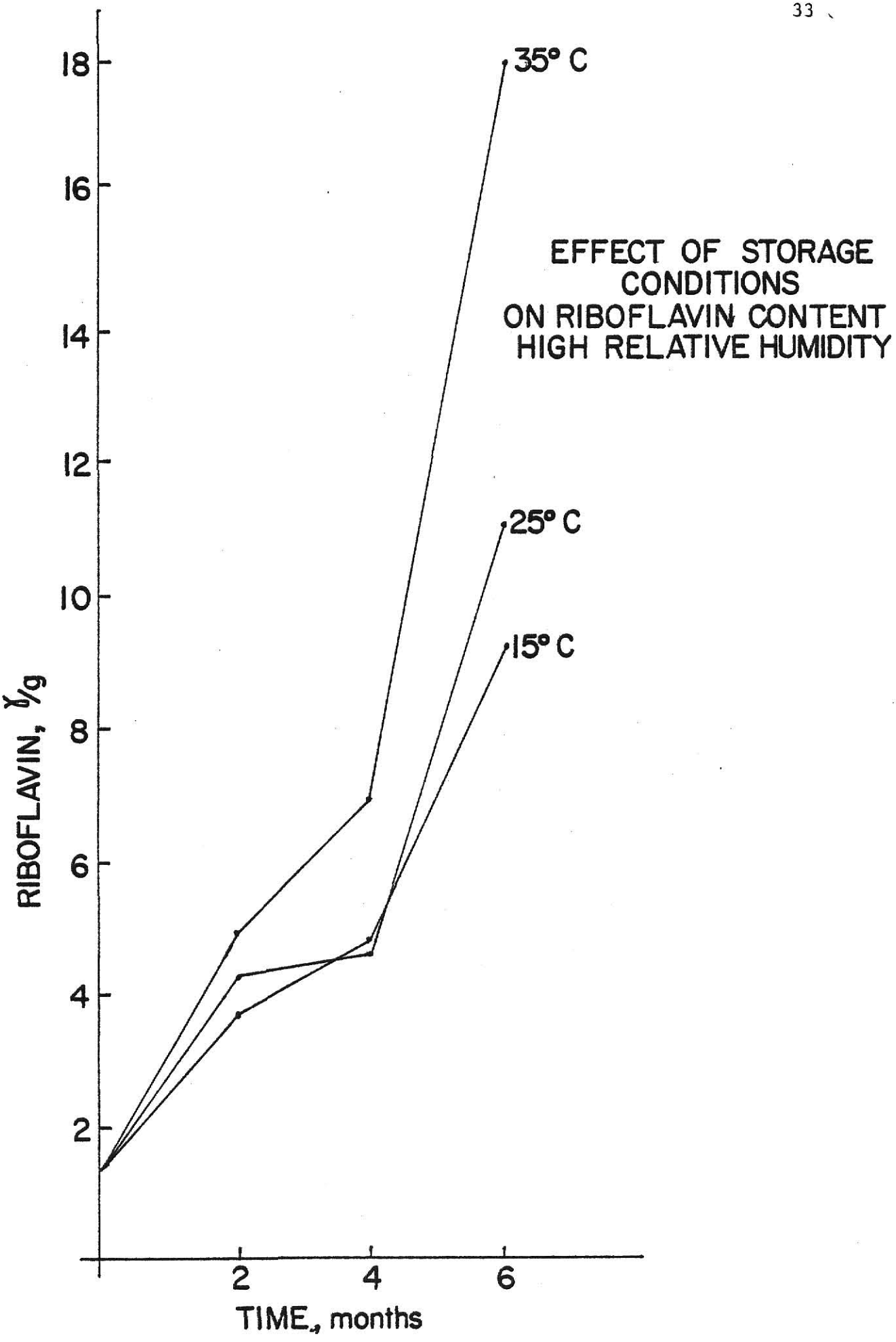
EFFECT OF STORAGE
ON MOISTURE CONTENT
MEDIUM RELATIVE HUMIDITY



EFFECT OF STORAGE
ON MOISTURE CONTENT
LOW RELATIVE HUMIDITY




EFFECT OF STORAGE CONDITIONS
ON THIAMINE CONTENT
HIGH RELATIVE HUMIDITY
THIAMINE, γ/g
6
5
4
3
2
1
15° C
25° C
35° C
2
4
6
TIME, months


EFFECT OF STORAGE CONDITIONS ON RIBOFLAVIN CONTENT HIGH RELATIVE HUMIDITY
35° C
25° C
15° C
RIBOFLAVIN, γ/g
TIME, months
18
16
14
12
10
8
6
4
2
2
4
6

SUMMARY AND CONCLUSIONS

Riboflavin, thiamin, pyridoxine and niacin content in wheat were analyzed before storage and after varying period of time. The grain was stored under three different temperatures and three different relative humidities.

Riboflavin, niacin and pyridoxine content increased with storage time specially under high relative humidity, while thiamin content decreased under the same conditions.

Thiamin and riboflavin changes were more noticeable at 35°C while niacin and pyridoxine changes were more noticeable at 15°C.

Pomeranz et al (1956), reported losses of thiamin and niacin for wheat stored at high moisture content. Hunt et al (1950), and Kao (1970), reported a niacin increase for corn and wheat when the grains were infested by molds.

Pomeranz et al (1956), and Bayfield and O'Donnell (1945), reported a thiamin decrease and riboflavin increase for wheat with high moisture content. Kao (1970) reported pyridoxine increase in moldy wheat.

These reports of vitamin changes involved high moisture content grain and the presence of molds. It is probable, that under these conditions, the molds synthesized some of the vitamins.

Sixty-two percent of the thiamin found in wheat is in the scutellum, eighty-two percent of the niacin and sixty-one percent of the pyridoxine are in the aleurone layer, thirty-seven percent of the riboflavin is in the aleurone layer, while thirty-two percent is in the endosperm. Thiamin that

* is present mostly in the germ area of the grain was the only vitamin to show a decreased value, while the vitamins that are present, mostly, in the aleurone layer and endosperm showed an increased value.

One important conclusion that should not be overlooked is the stability of the vitamins at proper storage conditions, namely at seventy percent relative humidity or less. Under normal storage conditions, only small changes in the vitamin content of the wheat were found after the storage period.

ACKNOWLEDGMENT

The author would like to acknowledge Professor Joseph Ponte Jr., major advisor and Mr. John Pedersen, who provided valuable time and experience in assisting her in these studies. Further thanks go to Empresa Brasileira de Pesquisa Agropecuaria, EMBRAPA, for the scholarship enabling the completion of her Masters program.

The author is most deeply appreciative of the support offered by her family Sr. and Sra. João Beleia and Mr. and Mrs. David P. McCrate Sr. Last, yet foremost, thanks to her husband Andrew McCrate.

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EFFECTS OF VARYING STORAGE CONDITIONS ON B-VITAMIN COMPOSITION OF WHEAT

by

ADELAIDE DEL PINO BELEIA

B.S., Universidade Federal De Pelotas, Brazil, 1972

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

ABSTRACT

Changes in the concentration of B vitamins during wheat storage were studied. Thiamin, riboflavin, pyridoxine and niacin were analyzed before storage and after varying periods of time. The wheat was stored under three different relative humidities and three different temperatures.

The thiamin content of the whole wheat decreased with time of storage, specially when stored under high relative humidity and high temperature. Niacin, pyridoxine and riboflavin content increased with time of storage. The greatest changes were observed for the wheat stored under high relative humidity. Niacin and pyridoxine showed the greatest increase under the lowest temperature, 15°C, while riboflavin showed the greatest increase under the highest temperature, 35°C.

Molds developed in samples that had fourteen percent moisture content or higher. The grain stored at the highest relative humidity was completely out of condition by the end of the sixth month.