QUANTITATIVE CHANGES IN CERTAIN CONSTITUENTS OF CORN GRAIN DURING GERMINATION

by

Maysoon Najeeb Jassim

B.S., University of Baghdad, IRAQ, 1964

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

College of Agriculture

Department of Food Science

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1980

Approved by:

H. L. Mitchell

Major Professor

TABLE OF CONTENTS

INTRODUCTION

Food nutrients are the building blocks of the human body. Nutrients are needed for growth and are needed to maintain and repair the body tissues, to regulate body processes, and to furnish energy for the body's functions. The nutrients that must be supplied in the daily food to keep man in good health are classified as proteins, fats, carbohydrates, vitamins, minerals, and water. Proteins are very important to the human diet, and are obtained from both animal and plant sources. Animal protein, which has greater nutritive quality than plant protein and is the choice of most humans, is becoming increasingly more expensive to produce, and the high price makes it less available to many people.

The low nutritive quality of plant proteins, coupled with the low protein content of most grains, may result in a particularly tragic disease called Kwashiorkor. Improving the nutritive value of existing crops by increasing their essential amino acid contents and identifying new sources of plant proteins have become very important. The discoveries of mutant genes in corn, which result in higher levels of lysine have opened the door to the development of improved protein quality of corn (23, 29), similar genes have now been found in barley and sorghum. The mutant gene of corn provides protein of sufficient quality to support good growth and development in feeding tests with laboratory rats and human children without supplemental protein in the diet (2).

Certain organic substances in plants are known to cause deleterious effects when ingested by man or animals. Among them is a class of substances called trypsin inhibitors, which are also proteins. They are found distributed widely in plant species such as soybeans, sweet

potatoes, mung beans and corn. Some of these inhibitors, cause growth depression in young animals (5).

A major protein of most corn grain is zein, which is almost devoid of lysine and tryptophan. If a certain gene, called the opaque-2 gene, is present in corn, zein synthesis is greatly depressed, and the protein mixture synthesized by the developing grain will have greater lysine and tryptophan contents than protein mixtures of genotypes not containing the gene (23). Also, it is known that genetic sources containing the opaque-2 gene have about twice the amount of trypsin inhibitor as is present in normal corn (14).

Seeds undergo marked chemical change during germination. Some of the changes are beneficial nutritionally, such as enhancement of certain of the vitamins (5, 15). Little is known about protein and trypsin inhibitor changes. This study was conducted to determine the relation of germination to the latter. Three genetic sources of corn were used: A normal hard endosperm hybrid, an opaque-2 soft endosperm hybrid, and a hard endosperm opaque-2 synthetic variety from CIMMYT in Mexico. The latter may be used widely as a food in some developing countries. Changes in sugars and starch also were included in this attempt to evaluate germinated corn as a food product.

REVIEW OF LITERATURE

Maize endosperm protein is deficient in lysine and tryptophan. Since mutations can affect the amino acid sequence of a protein, or change the proportions of protein mixtures, scientists have searched for genetic sources of corn with greater contents of these amino acids. The opaque-2 gene has been shown to cause changes in amino acid composition, resulting in increased amounts of lysine and tryptophan in the storage protein of the endosperm (23, 29).

Lodha, *et al.* (21) indicated that the poor protein quality of maize is a direct result of greater accumulation of zein (alcohol soluble protein), which is deficient in lysine and tryptophan, and that improvement in opaque-2 maize is a direct result of depressed zein synthesis. Mehta, *et al.* (22) found that nucleic acids disappeared from the endosperm of opaque-2 corn during the first 31 days after pollination. They speculated that this might be responsible for depressed zein synthesis in opaque-2 corn.

Osborne (30) classified vegetable proteins into three main groups on the basis of chemical composition: simple, conjugated and derived proteins. He further classified the simple proteins into subgroups based on their extractibility by the following sequence of solvents: (1) water, albumin; (2) salt solution, globulin; (3) 70 percent alcohol, prolamine; and (4) dilute alkali (or acid), glutelin. Murphy and Dalby (27) studied the amino acid patterns of the protein fractions extracted by salt, alcohol, and alkali, and in the insoluble protein, and compared lysine distribution in normal and opaque-2 corn fractions. They found that the lysine contents of the salt, alcohol, alkaline and insoluble proteins in normal corn were 8.5, 8.2, 32 and 47 percent, respectively, and of opaque-2 corn were 14, 2, 50 and 35 percent. In both normal and opaque-2, percentage of protein decreased while protein per kernel increased with kernel development (12, 21). Also Pukrushpan, *et al.* (32) reported that in sweet corn total protein decreased with increased maturity, albumin-globulin and glutelin fractions decreased, while the zein fraction increased.

Germination

Rapid uptake of water is the most obvious change associated with the initiation of germination. Germination generally increases the nutritive value of seed, but some constituents increase whereas others decreases (5, 41). Nass and Crane (28) found that some mutants germinate sooner and may

have faster growth rates than normal. Taba (37) concluded that germination of seeds in lysine solutions at 18-19° C was not effective for selecting higher lysine and tryptophan corn seeds. Changes in the free amino acid content of germinated seeds have been observed. Tsai, *et al.* (38) found that during germination of normal maize, concentrations of lysine and tryptophan increased and the zein content decreased. He concluded that germination may offer a method for converting nutritionally poor quality seed protein to a higher quality for human and animal use. Jones and Tsai (18) reported that the total levels of lysine and tryptophan increased rapidly in normal corn during germination. However, the increase of these two amino acids in opaque-2 was minimal. But Chibber, *et al.* (6) reported that germination was accompanied by an increase in total and free lysine levels in normal maize seedling, while in opaque-2 seedlings, total lysine declined over an 11 day period, accompanied by an increase in the free lysine pool. Wang and Fields (41) also reported that germination of corn and sorghum increased the levels of lysine, tryptophan and methionine. In somewhat similar fashion, other workers have noted increases in the lysine and tryptophan content of barley (9). Ingle, *et al.* (17) found that soluble protein content of the whole seedling increased progressively, while the insoluble protein decreased during germination. Fujimaki, *et al.* (11) found that degradation of zein during germination of corn is eventually followed by an increase of free amino acids.

During germination, Tsai, *et al.* (38) found the amount of starch in maize decreased about 50 percent in 5 days. Meanwhile, reducing sugars and sucrose increased about 60-fold and 3-fold, respectively. Also, Jones and Tsai (18) reported that starch content was reduced 42 percent in normal corn and 50 percent in opaque-2 corn. As the amount of starch decreased, the amount of soluble carbohydrate increased.

Although during early development, more starch was synthesized in opaque-2 than in normal corn, at maturity normal endosperm had 14.6 percent higher starch per endosperm than opaque-2 corn (12). Ingle, *et al.* (17) reported that soluble carbohydrate decreased initially in both endosperm and scutellum (within 24 and 48 hours). During the last 72 hours of the germination period, the soluble carbohydrate content increased in all parts of the seedling. Hasim and Fields (15) found more niacin and riboflavin in corn meal made from germinated corn than in corn meal made from ungerminated corn.

Trypsin Inhibitor

Proteinase inhibitors are widely distributed in the plant kingdom. They have been studied by several investigators in food crops generally, but most extensively in legumes (20, 40). The distribution of trypsin inhibitors within the plant structure varies from crop to crop. For example, sweet potatoes have the inhibitors in the leaves and tubers, and they are high in cotyledons and leaves of many beans (17). On the other hand, trypsin inhibitors of soybeans are confined to the seeds (3). Although earlier studies had indicated cereals lack trypsin inhibitors (4), later investigations have shown that cereal grains do contain them (8, 19).

A dormant seed contains proteolytic enzymes. They are kept inactive by some unknown mechanism, and become activated only on germination. It has been suggested that this behavior is due to the presence of proteolytic enzyme inhibitors in dormant seeds, which disappear on germination (24, 36).

Halim, *et al.* (13) reported that trypsin inhibitor activities varied within corn sources when plants were subjected to differential water stress at different growth stages. Pusztai (33) reported that total trypsin inhibitor in dormant kidney bean was 2.2 mg per g and rose to about 3.6 mg by the seventh day of germination and declined slowly after the tenth day.

Freed and Ryan (10) reported that discontinuous gel electrophoresis of crude 0.01 N NaOH extracts of soybean seed, after various periods of germination, showed that a number of changes took place in the seed proteins. Some bands decreased, others increased, and some showed little change.

MATERIALS AND METHODS

Corn Samples

Three genetically different sources of corn were used: A Kansas experimental normal hard endosperm hybrid, K41 X H28; a soft endosperm opaque-2 source, HL206; and a hard endosperm opaque-2 source from CIMMYT in Mexico, Lote 191. They will be designated hereafter as NHE (normal hard endosperm), SEO_2 (soft endosperm opaque-2) and HEO_2 (hard endosperm opaque-2).

Germination

The seeds were soaked in alcohol for 2 minutes, washed with deionized water, and then soaked for 15 minutes in a solution containing 16 ml of 40% formaldehyde and 4 ml of methanol per liter of water. After being washed with deionized water several times, 30-seed samples were placed on moistened filter paper in petri dishes. The dishes were placed in a growth chamber operating at 22° C, and appropriate samples were removed after 0, 1, 3, 5 and 7 days of germination.

After germination, seeds were freeze-dried, the dried samples were ground to pass a 20 mesh screen, and then were pulverized to find powders in a Wig-L Bug amalgamator. The samples were defatted with ether in a Soxlet apparatus. Moisture was determined on the defatted powders.

Fractionation

A half-gram sample of the dried, pulverized powder, was weighed into a centrifuge tube. Successive extractions were made by adding 10 ml of the solutions shown below, in the order shown, and rotating in a rotator at about 33 rpm for the times indicated. After extraction with each solution, the sample was centrifuged at 1500 x g for 10 minutes. The clear solution was decanted into a 25 ml volumetric flask. The residue was washed with about 10 ml of the same solution, and the centrifuging was repeated. The extracts for a given solvent were combined and made to 25 ml.

After extraction with 0.2% NaOH, 10 ml of acetone were added to the centrifuge tube, the suspension was mixed well, and was transferred to a weighed filter paper. The residue was washed with 10 ml of acetone, air dried at room temperature overnight, and its weight was determined. All determinations were done in duplicate.

Solution	Time (hr)	Fraction obtained
5% NaCl	1	Saline soluble proteins and free amino acids
70% Alcohol	4	Prolamins
0.2% NaOH	1	Glutelins

Protein Determination

Nitrogen was determined by a micro-Kjeldahl method outlined by Mitchell (26). Five ml of each fraction or 0.1 g of the residue or of the original sample were digested. Percent nitrogen of the dried defatted sample was calculated, and this value was converted to percent protein by multiplying by the factor 6.25.

Total Sugar Determination

Total sugar was determined by a modification of the method of the A.O.A.C. (1) by combining 0.5 g sample with 10 ml of 80% alcohol and rotating in a rotator at about 33 rpm for 4 hours. After extraction with alcohol, the sample was centrifuged at 1500 x g for 15 minutes, and 5 ml of the clear solution was heated in a 100 ml beaker on a steam plate for a few minutes to drive off alcohol. A little water (10-15 ml) was added and the sample again was heated for a few minutes. The solution was transferred to a 100 ml flask, 0.1 ml saturated lead acetate was added, the solution was made to volume, and after 15 minutes was filtered. Solid sodium oxalate was added to precipitate excess lead ions, and the solution again was filtered. Twenty-five ml of the solution was transferred to a 100 ml flask, 5 ml of 20 percent HCl was added, and the solution was left standing overnight. The solution then was neutralized with NaOH and diluted to 100 ml. Reducing sugars in 50 ml of the solution were determined by adding potassium ferricyanide solution, heating in a boiling water bath for 10 minutes, partially neutralizing with 2 N H_2SO_4, adding arsenomolybdate reagent to develop a blue color, diluting to 100 ml, and measuring absorbance after 30 minutes at 745 nm.

Starch Determination

Starch was determined by a modification of the A.O.A.C. method (1). One-tenth gram of sample was weighed into a 250 ml beaker and a few drops of alcohol were added to moisten the particles. Saturated $CaCl_2$ (50 ml) was added and the sample was boiled for one hour. During boiling, the sides of the beaker were washed with water occasionally. The sample was cooled, transferred to a 100 ml flask, 3 ml of uranyl acetate solution were added to precipitate the proteins, and the solution was diluted to 100 ml. Part of the solution was centrifuged at 1500 x g for 20 minutes.

Ten ml of clear solution were transferred into a centrifuge tube and 5 ml of 20 percent NaCl and 2 ml of I_2-KI solution were added to precipitate starch. The tube was centrifuged and the liquid decanted. The residue was washed by adding 5 ml of alcoholic NaCl solution, centrifuging and discarding the liquid. Two ml of alcoholic NaOH was added to the precipitate to discharge the blue color. The wall of the tube was washed with 5 ml of alcoholic NaCl and the tube was centrifuged. The residue was washed again with alcoholic 20 percent NaCl and centrigued. Two ml of 0.7 N HCl were added to the residue and the tube was stoppered with a one hole rubber stopper containing a short piece of glass tubing to act as a condenser. The tube was placed in a boiling water bath for 2.5 hours to hydrolyze the starch to glucose. The solution was transferred to a 100 ml volumetric flask and neutralized with 0.7 NaOH by adding 2-3 drops of methyl red as an indicator. The determination of glucose was done by the sugar reduction procedure described earlier. Sucrose was converted to its starch equivalent by multiplying by 0.9.

Trypsin Inhibitor

One-tenth gram of defatted samples were weighed into 15 ml polycarbonate centrifuge tubes and 5 ml of 0.05 M Tris buffer (PH 8.2, containing 0.02 M $CaCl_2$ were added. The stoppered tubes were rotated at a speed of 30 rpm for 30 minutes. The samples were centrifuged at 2,000 x g for 30 minutes. One ml of the crude extract from each sample was transferred to a test tube containing 1 ml of trypsin stock solution. The stock solution consisted of 0.5 mg of trypsin (1:300) per ml of 0.001 M HCl. Suitable controls without trypsin and one with trypsin were prepared. Substrate solutions of benzoyl-DL-arginine-p-nitroanilide (BAPA) were prepared (43.5 mg BAPA dissolved in 80-90 ml H_2O, heated at 80° C until BAPA dissolved, and diluted to 100 ml with H_2O). All tubes were equilibrated at 25° C for

5-10 minutes in a thermostatically controlled water bath. Five ml of BAPA substrate were added to each tube and the reactions were allowed to run for 10 minutes. One ml of 30% acetic acid was added to stop the reaction, and the quantity of p-nitroaniline released by enzyme hydrolysis was estimated spectrophotometrically at 410 nm, using a Beckman DU spectrophotometer.

Statistical Analysis

Data were calculated and analyzed statistically by using Duncan's multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Germination

Table 1 compares the germination behavior of 30-seed samples of each of three sources of corn. Experiment 2 was performed about eight months after experiment 1. Germination is characterized by a rapid uptake of water, which activates the enzymes necessary for mobilization of reserve materials and utilization of the reserves for growth. As germination progresses, the radicle appears first, followed by the hypocotyl and finally by the root. At the end of the first day, there was no apparent change other than swelling of the seeds due to uptake of water. Thereafter, germination of HEO_2 proceeded more rapidly (number of radicles and hypocotyls per day) and more completely (number of roots) than NHE and SEO_2. SEO_2 seeds apparently lost some viability during the interval between the two experiments.

Figure 1 shows the loss of dry matter during germination. Losses were greatest with HEO_2, and were least with NHE corn. SEO_2 losses were intermediate. Dry weight measurements were made on each intact sample, and therefore included losses by ungerminated seeds also. Smaller losses

should occur from endosperms than from live embryos. Hence, lower dry matter losses in SEO_2 and NHE grains probably reflect lower extent of germination, and hence less embryo respiration.

Table 1. Germination behavior of three sources of corn (counts per 30-seed sample).

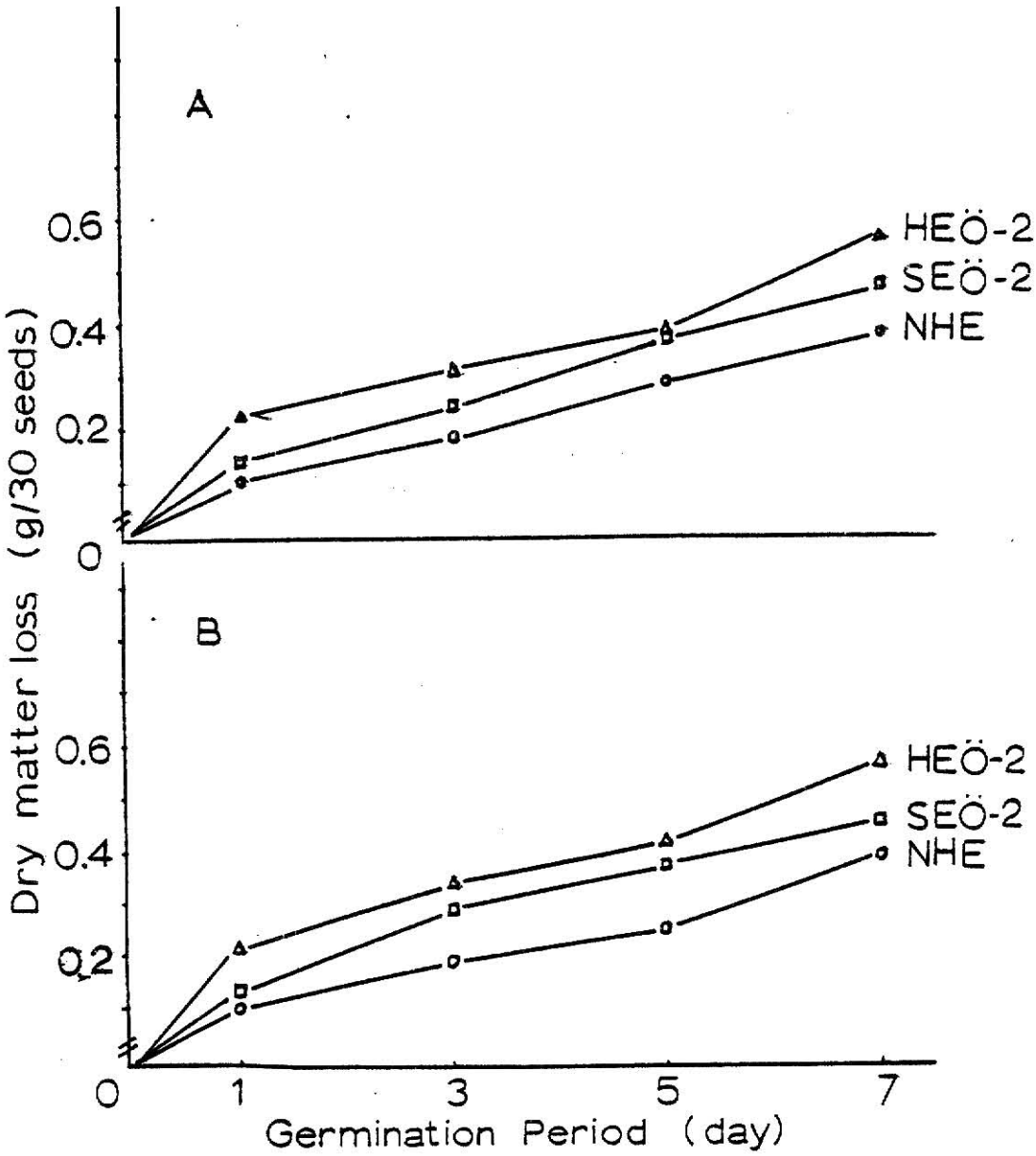
		Radicle		Hypocotyl		Root	
Days	Variety	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
	HEO_2	-	-	-	-	-	-
1	NHE	-	-	-	-	-	-
	SEO_2	-	-	-	-	-	-
	HEO_2	29	25	22	6	1	6
3	NHE	15	7	1	-	-	-
	SEO_2	14	7	5	1	-	-
	HEO_2	30	27	30	27	30	26
5	NHE	18	19	16	10	13	6
	SEO_2	22	21	19	19	17	10
	HEO_2	30	28	29	28	30	28
7	NHE	22	24	20	22	18	20
	SEO_2	26	15	25	9	25	9

- = no observed change

Fig. 1. Loss of dry matter during germination

A. Experiment 1

B. Experiment 2


A
B
0.6
0.4
0.2
0
HEÖ-2
SEÖ-2
NHE
Dry matter loss (g/30 seeds)
1
3
5
7
Germination Period (day)

Protein Components

Data for percent crude protein, presented in Table 2, show that crude protein varied from source to source. Proteins were significantly higher in NHE corn and lower in SEO_2 corn, while HEO_2 corn was intermediate. There were no significant differences in percentage of protein within a source at the various stages of germination. At zero days, percent protein in both experiments of HEO_2 and in the second experiment of SEO_2 appears to be a little low.

Total lysine levels of the three sources are shown in Fig. 2. At the dormant stage, SEO_2 corn contained the most, with 4.22 g/100 g protein, HEO_2 corn was next highest with 3.73 g/100 g protein, and NHE corn was lowest with 2.38 g/100 g protein. The data are similar to those obtained by Halim (14) for normal and opaque-2 corns. The differences in total lysine of corn are due to variations in the synthesis of proteins having greater contents of basic amino acids in the acid-soluble fraction of mutant endosperm (23).

Fig. 2 also indicates that germination did not alter appreciably the total lysine of a given source. These data do not agree with those of others (18, 38) who, however, used an agar medium for seed germination. Comparison of Table 2 and Figure 2 show an inverse relationship between crude protein and total lysine: when crude protein was high, the total lysine was less, and when protein was low, lysine was higher.

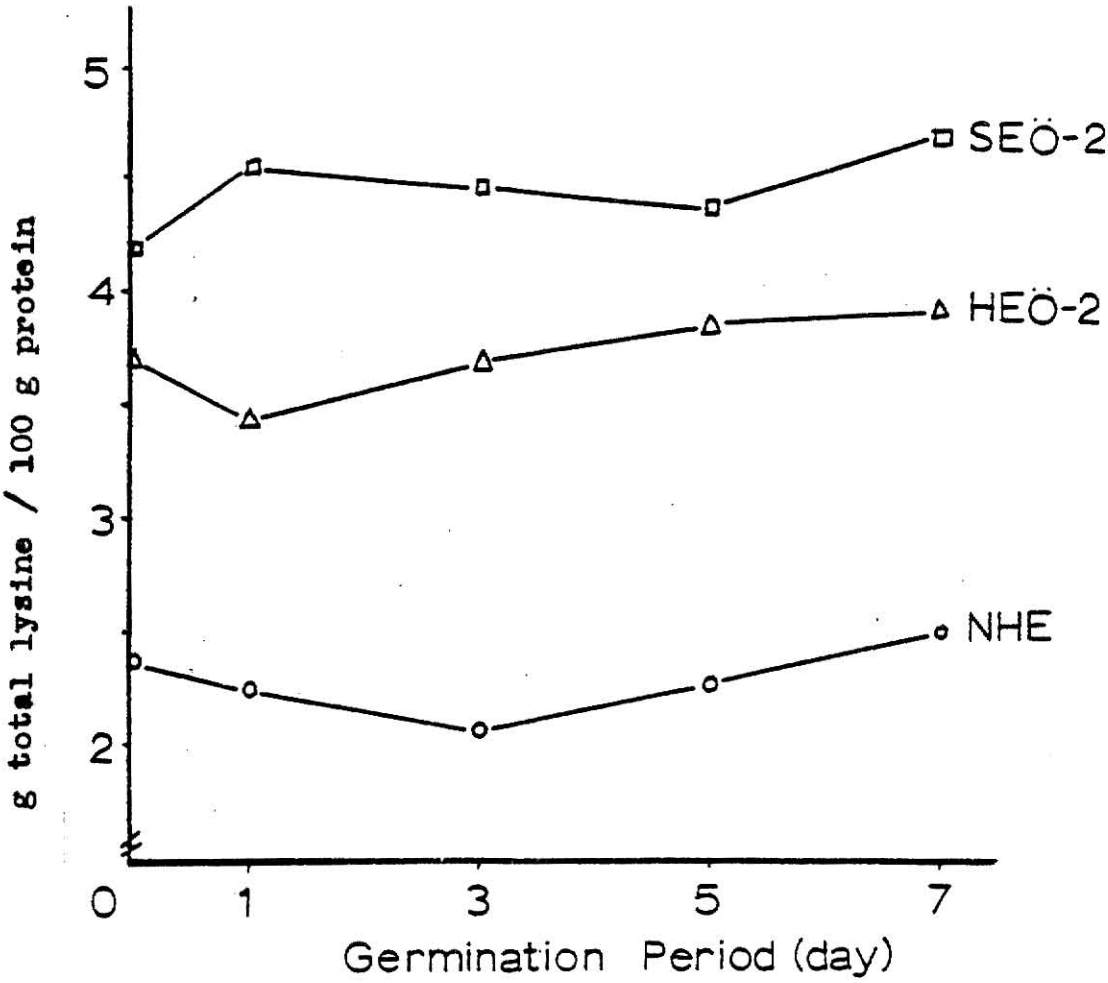
Table 2. Effect of germination on percentage of total protein.

	HEO_2		NHE		SEO_2	
Days	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
0	10.42 i	10.53 i	13.80 a	13.16 cd	8.30 jkl	7.31 n
1	11.40 efg	11.50 ef	13.22 acd	13.18 d	8.00 kl	7.50 mn
3	11.58 e	11.50 gh	13.55 abc	13.18 cd	8.66 j	8.19 kl
5	11.07 egh	10.70 hi	13.91 a	13.53 abc	8.43 jk	8.10 kl
7	11.49 efg	11.13 fgh	13.63 abc	13.01 d	8.27 jkl	7.88 lm

Means followed by the same letter were not statistically different, at the 5% level.

Presented in Tables 3a and 3b are changes in saline soluble protein, prolamin, glutelin and insoluble protein which occurred during germination. The protein which is extracted by salt solution is a mixture of at least two proteins; namely, globulin and albumin. However, globulin is the main protein in salt soluble fractions of corn. The data show that the saline fraction in HEO_2 and SEO_2 were significantly greater than in NHE corn at zero day (control). There were significant differences during germination for all three sources. HEO_2 showed marked increases up to the seventh day, and the amounts were significantly greater than those of NHE and SEO_2. NHE was significantly less than SEO_2 and HEO_2 during germination, with SEO_2 being intermediate. NHE decreased slightly in albumin and globulin up to three days, then increased thereafter. In contrast, SEO_2 and HEO_2 increased markedly and continuously throughout the germination period. The latter two corns thus contained more albumin-globulin than normal corn at all stages.

Fig. 2. Changes during germination in total lysine of three sources of corn


g total lysine / 100 g protein
5
4
3
2
0
1
3
5
7
Germination Period (day)
SEÖ-2
HEÖ-2
NHE

Prolamins are major proteins of the cereal grains. Zein, the prolamin of corn, is the most abundant protein in mature corn and is readily peptized by 70% ethanol. It functions as a storage protein, mainly. Zein is extremely deficient in lysine, and the greater concentration of this amino acid in SEO_2 and HEO_2 corns is a direct result of depressed zein synthesis. The data in Table 3a show that in the dormant grains zein is significantly lower in SEO_2 than in the other sources. It was greatest in NHE corn, while HEO_2 had a intermediate level. It was shown by Dalby and Davies (7) that in opaque-2 maize, there is an increase of ribonuclease activity during endosperm maturation, which may account for the observed reduction in zein synthesis. The three levels were significantly different from each other. There were no significant zein changes in SEO_2 and HEO_2, but in NHE corn zein tended to increased from the first day to the fifth day, and then declined to the seventh day.

Glutelin, another major protein class in corn, contains most of the nitrogenous constituents remaining in the grain after extraction with saline and aqueous ethanol solvents (31). The data from Table 3b show that HEO_2 and NHE corns are significantly greater than SEO_2 at zero day (control). Glutelin changes in the three sources generally were comparable, tending to increase for the first three days, and declining thereafter. However, the glutelin changes were not marked.

The data from Table 3b show that insoluble protein of NHE corn was significantly greater than that of HEO_2 and SEO_2 at zero days (control). During germination, this fraction decreased significantly in all three sources.

Table 3a. Effect of germination on protein fractions of three sources of corn (percent dry weight).

Days	Source	Albumin-globulin		Zein	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
Zero	HEO_2	2.88 gh	2.94 fg	1.00 j	0.99 j
	NHE	2.22 i	2.19 i	3.61 d	2.96 g
	SEO_2	2.88 g	2.63 h	0.64 k	0.49 k
1	HEO_2	2.96 fg	3.22 d	1.47 h	1.30 i
	NHE	1.96 jk	2.15 i	3.87 c	3.14 e
	SEO_2	2.94 fg	2.72 h	0.64 k	0.54 kl
3	HEO_2	3.96 ef	3.21 de	0.96 j	1.28 i
	NHE	1.74 l	1.94 k	4.28 a	2.85 g
	SEO_2	3.02 fg	2.89 f	0.59 kl	0.55 kl
5	HEO_2	4.28 b	3.49 c	0.96 j	1.13 ij
	NHE	2.18 i	2.06 ijk	4.10 b	3.19 f
	SEO_2	3.22 d	3.15 def	0.44 l	0.59 kl
7	HEO_2	4.41 ab	4.50 a	1.00 j	0.97 j
	NHE	3.59 c	2.99 fg	3.43 e	2.22 g
	SEO_2	3.64 c	3.25 d	0.59 kl	0.55 kl

Mean followed by the same letter were not significantly different, at the 5% level.

Table 3b. Effect of germination on protein fractions of three sources of corn (percent dry weight)

Days	Sources	Glutelin		Insoluble Protein		Total of Fractions	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Zero	HEO_2	3.84 defg	3.67 fgh	2.82 e	3.09 d	10.53 gh	10.68 fgh
	NHE	3.81 efg	3.85 def	4.12 ab	4.11 ab	13.76 a	13.11 bc
	SEO_2	2.88 mno	2.22 g	2.02 hi	2.16 fghi	8.40 ij	7.45 l
1	HEO_2	4.78 a	4.16 bc	2.25 fgh	2.78 e	11.45 d	11.46 d
	NHE	4.13 bc	3.53 hi	3.49 c	3.93 b	13.43 ab	12.76 c
	SEO_2	2.97 lm	2.45 p	1.54 kl	1.89 ij	8.09 jk	7.60 l
3	HEO_2	4.60 a	4.33 b	2.31 f	2.27 fg	10.41 h	11.09 def
	NHE	4.03 cd	3.85 def	3.53 c	4.31 a	13.57 a	12.94 c
	SEO_2	3.15 kl	3.04 lm	2.04 ghi	1.74 jk	8.79 i	8.21 j
5	HEO_2	3.97 cde	3.92 cde	2.07 fghi	2.19 fgh	11.27 de	10.73 fgh
	NHE	4.03 cd	3.63 gh	3.42 c	3.52 c	13.78 a	13.40 ab
	SEO_2	2.75 no	3.08 lm	1.93 ij	1.32 lm	8.34 j	8.13 jk
7	HEO_2	3.37 ij	3.30 jk	2.20 fgh	2.19 fgh	10.97 ef	10.76 efg
	NHE	3.53 hi	3.50 hij	3.09 d	3.39 c	13.64 a	12.69 c
	SEO_2	2.91 mn	2.68 o	1.10 m	1.23 m	8.24 j	7.70 kl

Means followed by the same letter were not significantly different, at the 5% level.

Carbohydrate Components

Germination begins with the imbibition of water, and is accompanied by a rapid rise in respiratory rate. The increased metabolic activity is confined mainly to the embryo, and the respiratory substrates in these early stages are sugars produced from starch in the endosperm. Table 4 shows the percent total soluble sugar of SEO_2 was significantly greater than that of HEO_2 and NHE. The total soluble sugar decreased to the third day of germination in all sources, and then increased. There were no significant differences between the three sources during the germination period. The data are similar to those of Jones and Tsai (18). Thus, the early decrease of sugars is evidence that respiration initially consumed them more rapidly than they were produced from starch. However, after the third day amylase activity was great enough to produce sugars from starch faster than they were metabolized.

Table 4. Effect of germination on percent total soluble sugars of three corn sources

	HEO_2		NHE		SEO_2	
Days	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
0	1.96 ef	1.95 ef	2.13 d	2.17 d	2.64 b	2.42 c
1	1.68 i	1.55 j	1.94 ef	1.73 hi	1.97 ef	1.97 ef
3	0.99 lm	0.96 mn	0.88 n	0.99 lm	0.87 n	1.10 kl
5	1.99 e	1.97 ef	1.08 kl	1.07 kl	1.89 fg	1.83 gh
7	2.68 b	2.65 b	2.71 b	2.79 b	2.93 a	2.93 a

Means followed by the same letter were not statistically different, at the 5% level.

Table 5 shows the changes in the percentage of starch of the three strains during germination. Starch was significantly greater in NHE than in HEO_2 and SEO_2 prior to germination. Starch decreased markedly in all three sources during germination, in agreement with the results of others (18, 38). The pattern of decrease was quite similar among the three sources, and NHE corn still contained the most starch after seven days. Approximately half of the starch in each strain was utilized for the germination process. The decreases in starch content occurred because of increased amylase activity during germination.

Table 5. The effect of germination on percent starch of three corn sources

	HEO_2		NHE		SEO_2	
Days	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
0	61.39 cd	59.97 d	67.92 a	67.31 a	59.48 d	59.86 d
1	59.02 de	56.82 ef	64.33 b	63.16 bc	56.88 ef	56.31 f
3	52.79 g	48.77 h	51.36 g	56.75 ef	48.73 h	48.86 h
5	41.48 i	40.53 i	41.95 i	47.59 h	39.88 i	39.89 i
7	31.67 k	31.02 k	36.71 j	36.63 j	30.55 k	30.10 k

Means followed by the same letter were not statistically different, at the 5% level.

Trypsin Inhibitor

The effect of germination on trypsin inhibitor of the three sources is shown in Table 6. Throughout the germination period, the inhibitor content was significantly greater in HEO_2 than in the other sources.

Table 6. Effect of germination on the trypsin inhibitor content of three corn sources (micrograms/gram)

	HEO_2		NHE		SEO_2	
Days	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
0	23.2 fg	24.3 de	13.1 o	13.2 o	20.5 i	20.6 i
1	27.5 a	28.0 a	14.3 mn	14.8 m	21.0 hi	21.8 h
3	27.0 ab	26.0 bc	16.4 l	16.9 kl	22.0 gh	22.8 gh
5	24.1 ef	25.7 c	18.0 jk	18.5 j	23.3 ef	23.1 ef
7	24.0 ef	25.2 cd	12.1 o	13.4 no	23.1 ef	23.1 ef

Means followed by the same letter were not significantly different, at the 5% level.

The trend in HEO_2 corn was an increase in inhibitor content at the first day and a slow decline thereafter. NHE corn increased through the fifth day and decreased sharply to the seventh day. In contrast, the inhibitor of SEO_2 increased continuously during the germination period. After seven days the inhibitor contents of HEO_2 and SEO_2 corns were similar, and were much greater than the amount in normal corn. These results are similar to those of Halim (14), who analyzed only the endosperms excised from germinating corn seeds. A similar pattern of changes in kidney beans was reported by Pusztai and Duncan (34), who found that inhibitor increased for seven days, but had decreased at ten days. Our data show a decrease or

a leveling between the fifth and seventh days in most instances. If the germination period had been extended, obvious decreases likely would have been observed. The sharp decrease between the fifth and seventh days in normal corn is noteworthy, suggesting that it is more susceptible to metabolic change than are the inhibitors in the other sources.

The significance of the initial increase of this minor constituent of the seed, and the subsequent decline as germination progresses, is not clear. Suggestions of its possible role in the seed have been: (a) as a regulator of endogenous proteinases; (b) as a protective agent by inhibiting the proteinases of invading insects and micro-organisms; and (c) as a storage protein which only coincidentally inhibits trypsin (35).

GENERAL DISCUSSION

Partially germinated seeds have been used as food for a long time. Mature corn grain is a major food item in many countries, but corn protein has a low nutritive value because it is deficient in lysine and tryptophan. This research was initiated because it was thought that protein quality of corn might be enhanced by germination. This might happen if the zein fraction, which is almost devoid of lysine, was utilized preferentially during germination, or if synthesis of lysine from other amino acids occurred. It was considered desirable also to examine other constituents which might undergo change and would influence use of germinated corn as a food.

Lysine content of the three sources did not increase during germination, nor did the percentage of the total protein which was zein change appreciably. Hence, it appears unlikely that protein quality of these strains could be improved by germination. Although available carbohydrate (sugars plus starch) decreased appreciably, the amount remaining would make germinated corn an adequate source of energy.

One report (25) failed to show a deleterious effect of corn trypsin inhibitor on rat growth. A comparable study has not been done with humans, and therefore its effect in human diets has not been established. If the inhibitor should be nutritionally objectionable, limited germination of corn would increase its content. Unlike soybean trypsin inhibitor, the corn inhibitor is heat stable, and its effect would persist in cooked foods.

CONCLUSIONS

1. NHE corn contained a greater percentage of protein than in other sources. There were no significant changes during germination.
2. Total lysine was highest in SEO_2 corn, was lowest in NHE, and was intermediate in HEO_2.
3. Albumin-globulin fraction was higher in HEO_2 and SEO_2 corns than in NHE corn. There were significant differences during germination for all three sources.
4. Zein was lower in SEO_2 than in other corns. There were no significant changes in SEO_2 and HEO_2 during germination.
5. Glutelin significantly higher in HEO_2 and NHE corns than in SEO_2.
6. Insoluble protein decreased significantly during germination in all three sources.
7. Starch decreased markedly in all three sources during germination, while total sugar decreased initially, and then increased, in all three sources.
8. Trypsin inhibitor was significantly greater in HEO_2 than in other. In all three sources, trypsin inhibitor increased during germination.

LITERATURE CITED

1. Association of Official Analytical Chemists. 1975. "Official Methods of Analysis," p. 48. 12th ed. Assoc. Offic. Agric. Chem., Washington, D.C.

2. Baker, L. R. 1977. In "Nutritional evaluation of food processing," Second edition. By Harris, R. S., and Karmas, E. The Avi Publishing Company, Inc. Westport, Connecticut. p. 19-32.

3. Birk, Y., and M. Waldman. 1965. Amylolytic, Trypsin inhibiting, and urease activity in three varieties of soybeans and in soybean plant. Qualitas Plant Mater. Vegetabiles. 12:199-209.

4. Borchers, R., C. W. Ackerson, and L. Kimmet. 1947. Trypsin inhibitor IV. occurrence in seeds of the Leguminosae and other seeds. Arch. Biochem. Biophys. 13:291-293.

5. Chem, L. H., C. E. Wells, and J. R. Fordh. 1975. Germinated seeds for human consumption. J. of Food Sci. 40:1290-1293.

6. Chibber, B. A. K., E. Voicu, E. T. Mertz, and D. V. Glover. 1977. Studies on corn proteins. VI. Distribution of lysine during germination of normal and opaque-2 maize. Cereal Chem. 54(3):558-564.

7. Dalby, A., and J. AB. I. Davies. 1967. Ribonuclease activity in the developing seeds of normal and opaque-2 maize. Sci. 155:1573-1575.

8. Desikachar, H. S. R., and S. S. De. 1947. Role of inhibitors in soybeans. Sci. 106:421-422.

9. Folkes, B. F., and E. W. Yemm. 1958. The respiration of barley plants. X. respiration and the metabolism of amino acids and proteins in germinating grain. New Phytol. 57:106-131.

10. Freed, R. C., and D. S. Ryan. 1978. Note on modification of the Kunitz soybean trypsin inhibitor during seed germination. Cereal Chem. 55(4):534-538.

11. Fujimaki, M., M. Abe, and S. Arai. 1977. Degradation of zein during germination of corn. Agric. Biol. Chem. 41(5):887-891.

12. Gupta, N. D., M. L. Lodha, and S. L. Mehta. 1977. Changes in protein, starch and free and protein lysine in normal and opaque-2 *Zea mays* endosperm during development. Indian J. of Exp. Biol. 15:781-783.

13. Halim, A. H., C. E. Wassom, and H. L. Mitchell. 1973. Trypsin inhibitor in corn (*Zea mays* L.) as influenced by genotype and moisture stress. Crop Sci. 13:405-407.

14. Halim, A. H. 1972. Genotype and environmental relationships of protein, lysine and trypsin inhibitor in corn (*Zea mays* L.). A Doctor's dissertation. Kansas State University, Manhattan, Kansas.

15. Hasim, N. B., and M. L. Fields. 1979. Germination and relative nutritive value of corn meal and corn chips. J. of Food Sci. 44:936-937.

16. Honavar, P. M., and K. Sohoie. 1955. Distribution of trypsin inhibitors in different organs of plants of sweet potatoes (*Ipomea batata*) and green gram (*Phaseolus aureus*). J. Univ. Bombay. 24B:64-69.

17. Ingle, J., L. Beevers, and R. H. Hageman. 1964. Metabolic changes associated with the germination of corn. I. Changes in weight and metaboites and their redistribution in the embryo axis, scutellum, and endosperm. Plant Physiol. 39:735-740.

18. Jones, R. A., and C. Y. Tsai. 1977. Changes in lysine and tryptophan of normal and mutant maize seed. Cereal Chem. 54(3):565-571.

19. Learmonth, E. M., and J. C. Wood. 1963. A trypsin inhibitor in wheat flour. Cereal Chem. 40:61-65.

20. Liener, I. E., and M. L. Kakade. 1969. Protease inhibitors in "Toxic constituents of plant foodstuffs". Liener, I. E. ed. Academic Press N.Y. 7-68.

21. Lodha, M. L., P. C. Ram, H. O. Gupta, S. L. Mehta, and J. Singh. 1977. Changes in protein, lysine and tryptophan in normal and opaque-2 *Zea mays* kernels during development. 1977. Indian J. Exp. Biol. 15:1080-1082.

22. Mehta, S. L., K. N. Srivastava, P. C. Mali, and M. S. Naik. 1972. Changes in the nucleic acid and protein fractions in opaque-2 maize kernels during development. Phytochemistry. 11:937-942.

23. Mertz, E. T., L. S. Bates, and O. E. Nelson. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm. Sci. 145:279-280.

24. Mikola, J., and E. M. Suolinna. 1969. Purification and properties of trypsin inhibitor from barley. European J. Biochem. 9:555-560.

25. Mitchell, H. L., D. B. Parrish, M. Cormey, and C. E. Wassom. 1976. Effect of corn trypsin inhibitor on growth of rats. J. Agric. Food Chem. 24(6)1254-1255.

26. Mitchell, H. L. 1972. Microdetermination of nitrogen in plant tissues. J. of the AOAC. 55:1-3.

27. Murphy, J. J., and A. Dalby. 1971. Changes in the protein fractions of developing normal and opaque-2 maize endosperm. Cereal Chem. 48:336.

28. Nass, H. G., and P. L. Crane. 1970. Effect of endosperm mutants on germination and early seedling growth rate in maize (*Zea mays* L.). Crop Sci. 10:139-140.

29. Nelson, O. E., E. T. Mertz, and L. S. Bates. 1965. Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Sci. 150:1469-1470.

30. Osborne, T. B. 1924. Vegetable proteins. P. 1. Monographs on biochemistry. Longman, Green and Co., London.

31. Paulis, J. W., and J. S. Wall. 1977. Fractionation and characterization of alcohol-soluble reduced corn endosperm glutelin proteins. Cereal Chem. 54(6):1223-1228.

32. Pukrushpan, T., H. L. Mitchell, and J. K. Greig. 1977. Effect of stage of maturity on protein fractions of sweet corn cultivars. J. of Food Sci. 42(3):851-852.

33. Pusztai, A. 1972. Metabolism of trypsin inhibitory proteins in the germinating seeds of kidney bean (*Phaseolus vulgaris*). Planta (Berl.). 107:121-129.

34. Pusztai, A., and I. Duncan. 1971. Changes in proteolytic enzyme activities and transformation of nitrogenous compounds in the germinating seeds of kidney bean (*Phaseolus vulgaris*). Planta (Berl.). 96:317-325.

35. Ryan, C. A. 1973. Proteolytic enzymes and their inhibitors in plants. Ann. Rev. Plant Physiol. 24:173-196.

36. Shain, Y., and A. M. Mayer. 1965. Proteolytic enzymes and endogenous trypsin inhibitor in germinating lettuce seed. Physiol. Plantarum. 18:853-859.

37. Taba, S. 1972. Corn seed germination and seedling elongation as influenced by amino acids. M. S. Thesis, Kansas State University, Manhattan, Kansas.

38. Tsai, C. Y., A. Dalby, and R. A. Jones. 1975. Lysine and tryptophan increases during germination of maize seed. Cereal Chem. 52:356-560.

39. Virupaksha, T. K., and L. V. S. Sastry. 1968. Studies on the protein content and amino acid composition of some varieties of grain sorghum. J. Agr. Food Chem. 16(2):199-203.

40. Vogel, R., I. Trautshold, and E. Werle. 1968. Plant Inhibitors. In "Natural proteinase inhibitors". Academic Press. N.Y. 9-42.

41. Wang, Y. Y. D., and M. L. Fields. 1978. Germination of corn and sorghum in the home to improve nutrient value. J. of Food Sci. 43:1113-1115.

ACKNOWLEDGMENTS

The writer wishes to express her sincere gratitude to Dr. H. L. Mitchell, major professor, Department of Biochemistry for his valuable advice, guidance, suggestions, and encouragement throughout preparation of this thesis.

To my guidance committe, Dr. C. Setser, Department of Food and Nutrition, and Dr. R. Bassette, Department of Animal Science, I wish to express my gratitude for making helpful suggestions.

Thanks are also due to Dr. C. E. Wassom, Department of Agronomy, for his suggestion.

Recognition is given also with the greatest appreciation to the writer's husband, Dr. Mohammed Al-Izzi for his encouragement and helpfulness. Thanks are also due to my sons Kaied and Ali, and daughter Zainab for their patience and happiness they brought to the family.

QUANTITATIVE CHANGES IN CERTAIN CONSTITUENTS OF CORN GRAIN DURING GERMINATION

by

Maysoon Najeeb Jassim

B.S., University of Baghdad, IRAQ, 1964

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

College of Agriculture

Department of Food Science

KANSAS STATE UNVIERSITY
Manhattan, Kansas

1980

ABSTRACT

Corn grain has a low protein nutritive value because it is deficient in lysine and tryptophan. This research was conducted to test the possibility that protein quality might be improved by germination. Certain other changes, which might influence the use of germinated corn as a food, were studied also.

Three genetically different sources of corn were used: A Kansas experimental normal hard endosperm, K41 X H28; a soft endosperm opaque-2 source, HL206; and a hard endosperm opaque-2 source from CIMMYT in Mexico, Lote 191. They will be designated hereafter as NHE (normal hard endosperm), SEO_2 (soft endosperm opaque-2), and HEO_2 (hard endosperm opaque-2). Sterilized 30-seed samples were germinated for 1, 3, 5, and 7 days in a growth chamber operating at 22° C. The samples then were vacuum-dried, defatted and ground. Protein fractionation was accomplished by sequential extraction with 5% NaCl (albumin-globulin fraction), 70% ethanol (zein fraction) and 0.2% NaOH (glutelin fraction).

Prior to germination, NHE corn contained a greater amount of protein than SEO_2 or HEO_2 corns, with SEO_2 being the least. There were no significant changes during germination. Total lysine was greatest in SEO_2 corn, least in NHE, and was intermediate in HEO_2 corn. There was an inverse relationship between protein and lysine levels: when percent protein was high, lysine was low, and when protein was low, lysine was higher.

The albumin-globulin fraction was greater in HEO_2 and SEO_2 corns than in normal corn. There were significant differences during germination for all three sources. The zein, fraction which is deficient in lysine, was lower in SEO_2 than in the other corns. There were no significant zein changes, although in NHE corn, zein tended to increase during germination. The

glutelin fraction was significantly higher in HEO_2 and NHE corns than in SEO_2. Insoluble protein decreased significantly during germination in all three sources.

Starch decreased markedly in all three sources during germination. Total soluble sugars decreased initially, and then increased, as germination progressed, in all three sources.

Trypsin inhibitor concentration was greater in HEO_2 corn than in the other sources prior to germination. In all three sources, there was an increase in trypsin inhibitor during germination.