

Effect of Germination on Oats and Oat Protein¹

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ABSTRACT

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Lysine content of germinated oats increased after eight days, from 4.4 to 5.3 g per 16 g of nitrogen. Increases in nonprotein nitrogen, albumin, and residue nitrogen (all rich in lysine) and decreases in globulin and prolamin (poor in lysine) accompanied sprouting. The percent nitrogen in oats

germinated for three days or longer was greater than that in the initial grain as a result of dry matter loss in the grain during germination, but the absolute amount of nitrogen per kernel was decreased.

Oats have good-quality protein and a high protein content compared with other cereal grains (Jones et al 1948, Robbins et al 1971). Although the lysine content of oats is relatively high in relation to other cereals, it is still deficient as compared with the National Academy of Sciences (1980) pattern of high-quality protein for human consumption. Hamad and Fields (1979) reported an increase in available lysine in germinated oats by *Tetrahymena pyriformis* W. assay. Dalby and Tsai (1976) found an increase in lysine content expressed as percent of dry weight of oats during germination. Robbins and Pomeranz (1971) observed an increase in lysine content of oat sprouts compared with that of the grain. Kim et al (1979) studied the effects of germination on protein fractions of oat endosperm. Jahn-Deesbach and Schipper (1980) determined the protein fractions and amino acid compositions of germinated oats during the first 84 hr.

The purpose of this study was to examine the potential for improving nutritional quality of oats by sprouting and other effects of germination on oats. The present study emphasizes the effects of length of germination, temperature, and light, and answers questions raised by earlier workers. Oats were germinated for one to eight days. Dry matter and nitrogen changes per 100 kernels as well as nitrogen content of germinated oats were determined. The protein from sprouted oats was fractionated into different solubility classes, and essential amino acid compositions of the sprouted oats and protein fractions were obtained.

MATERIALS AND METHODS

Oats

R. I. H. McKenzie of Agriculture Canada, Winnipeg, Manitoba, supplied terra oats. The oats were hullless, and they had protein and oil contents comparable to groats of other Canadian varieties. The grain was soaked in distilled water overnight, briefly surface-sterilized with 0.2% formaldehyde solution to retard mold growth during germination, and then thoroughly rinsed and soaked in distilled water to remove residual formaldehyde. The wet oats were spread thinly on Whatman filter paper saturated with distilled water in trays having plenty of air space. Details on treatment of grain were reported previously (Wu and Wall 1980).

The percentage of sprouted oats generally increased with time of germination and ranged from 79 to 96 under the various conditions used. No mold was observed after three days, but 5–10% of the grain was moldy after six days, and 10% of the grain was moldy after eight days. All moldy grain was discarded. Entire sprouted grains and incubated, unsprouted grains were freeze-dried separately, then were ground once in a Mitey mill and once in a Weber pulverizing mill equipped with a 0.3-mm slotted screen. Additionally, 100 kernels of sprouted oats (in triplicate) were dried

at 105°C to constant weight and were compared with the weight of 100 kernels of untreated grains (in triplicate) for dry matter loss during germination.

Protein Extraction

Each sample (5 g) was put in a stainless steel cup with 100 ml of solvent at room temperature and blended for 5 min in a Waring Blender. The blended sample was centrifuged at room temperature for 10 min at $10,400 \times g$, the supernatant solution was decanted, and the residue was extracted with the next solvent. The solvents used sequentially were water (twice), 1M NaCl (twice), 70% ethanol, and 0.005N NaOH plus 0.1% dithiothreitol (DTT). These solvents extracted albumin plus nonprotein nitrogen, globulin, prolamin, and glutelin, respectively. The combined water extracts, combined NaCl extracts, NaOH + DTT extract, and residue were freeze-dried separately. The ethanol extract was first dried in a rotoevaporator and then freeze-dried. A portion of freeze-dried combined water extracts (0.11–0.26 g) was dissolved in 5 ml of distilled water, and 5 ml of 20% trichloroacetic acid was added to precipitate protein. The mixture was centrifuged at $34,800 \times g$ for 20 min, and the supernatant was analyzed for nitrogen by micro-Kjeldahl. The supernatant nitrogen is nonprotein nitrogen, and the precipitated nitrogen is albumin.

Composition

Nitrogen was determined by micro-Kjeldahl in quadruplicate according to AACC approved method 46-13 (1976). For amino acid analysis, each sample was hydrolyzed for 24 hr by refluxing in boiling 6N hydrochloric acid under atmospheric pressure. The hydrolyzed sample was evaporated to dryness in a rotoevaporator, and the residue was dissolved in pH 2.2 citrate buffer. A portion of the acid hydrolyzate was analyzed in a Beckman Spinco model 121 amino acid analyzer, and the data were computed automatically by the method of Cavins and Friedman (1968).

RESULTS AND DISCUSSION

Dry Matter and Nitrogen

Weight and nitrogen changes of 100 kernels of oats during germination at 20 and 25°C in the dark and at 23°C in room light are listed in Table I. The dry matter loss was relatively small for the first three days of germination and amounted to 6% or less, but the loss increased to 14–20% after six to eight days of germination. Loss was higher at 25 than at 20°C, but was not significantly influenced by darkness or daylight during germination. The percent nitrogen content of germinated oats decreased at first, probably because water-soluble nitrogen was lost, but it increased after three days so that the nitrogen content was higher than in the untreated oats after six and eight days of germination. The absolute amount of nitrogen in 100 kernels of oats, however, was reduced during germination because of dry matter loss through respiration and loss of soluble matter on wet filter paper. The percent nitrogen content of germinated oats was higher at higher temperatures, but light did not have any significant effect. The incubated, unsprouted oats had a lower percentage of nitrogen at three days, and higher

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values at eight days compared with untreated oats, but the values were lower than in sprouted oats (not shown in table).

Bartlett (1917) reported 13 and 17% loss in dry matter and 4 and 3% loss in total crude protein for oats after six and eight days of germination, respectively. Although the temperature during germination was not given, dry matter loss was comparable, and crude protein loss was lower than that shown in Table I. Kim et al (1979) found about 90% loss in dry matter and about 90% loss in nitrogen of oat endosperm during 10 days of germination at 20°C. Because their result was based on endosperm rather than on whole grain, any comparison would be meaningless. Dalby and Tsai (1976) sprouted wheat, triticale, barley, rye, and oats for five days at 28°C in the dark and reported that percent protein (as percent of dry weight) increased steadily with time of sprouting, except for oats, which showed an initial increase in percent protein content that leveled off after one day.

Protein Fractions

The nitrogen distribution of oats in various protein fractions as germination proceeded at 23°C in daylight is shown in Table II. The globulin fraction accounted for 41% of total nitrogen of untreated oats. Peterson and Smith (1976) found that globulins were the major component of oats and accounted for 46 and 50% of total nitrogen in two varieties. After eight days of germination, globulin and prolamin fractions decreased, whereas nonprotein nitrogen, albumin, and residue nitrogen increased. The nitrogen distribution of germinated oats after eight days in the dark at 25°C was similar to that at 23°C in daylight, except the latter was lower in nonprotein nitrogen and globulin but higher in prolamin. These differences were relatively small, however. Oat globulin extracted at 5°C (Kim et al 1979) accounted for only 13% of total oat nitrogen instead of 41%, as shown in Table II. They reported decreases in glutelin, prolamin, globulin, albumin, and residue fractions after seven days of germination, based on the amount of nitrogen in the endosperm. Comparison would be difficult because of their low globulin fraction and higher protein fractions following globulin extraction, about 73% loss in total nitrogen, and their protein fractions were calculated differently than in Table II. Jahn-Deesbach and Schipper (1980) germinated oats for 84 hr and observed large increases in the water-soluble fraction but decreases in globulin, prolamin, and glutelin fractions compared with untreated oats. Their globulin fraction, however, accounted only for 13% soluble nitrogen or 12% of total oat nitrogen. The rest of the globulin must be contained in subsequent fractions, and therefore only their water-soluble fraction can be compared with the values in Table II. Dalby and Tsai (1976) observed that prolamin contents of wheat, triticale, barley, rye, and oats decreased as germination time increased from one to five days. Wu (1982) reported an increase in nonprotein nitrogen and a decrease in prolamin, glutelin, and residue nitrogen as time of germination of triticale increased to eight days. Wu and Wall (1980) observed an increase of water-soluble nitrogen (albumin plus nonprotein nitrogen) but decreases in prolamin and cross-linked prolamin after sorghum was germinated for 10 days. Nielsen et al (1978) reported three- to sixfold increases in the water-soluble proteins for two varieties of wheat after 10 days of sprouting. Hwang and Bushuk (1973) observed a marked increase in the amount of the acetic acid-soluble fraction and a decrease in the residue proteins of flours from germinated wheat.

Amino Acid Composition

The essential amino acid composition of oats as germination proceeded at 23°C in daylight is listed in Table III. Lysine content of germinated oats increased after three days and continued to increase to a value of 5.3 after eight days. The amino acid composition of oats after six and eight days of germination, however, meets the National Academy of Sciences pattern. In addition to the increase in lysine with germination, increases in isoleucine and threonine also occur, as do decreases in methionine plus cystine and in phenylalanine plus tyrosine. Table IV shows the essential amino acid composition of solubility fractions from oats. Prolamin has the lowest lysine content, whereas residue has the

highest. Water extractables included both albumin and nonprotein nitrogen. The lysine content of albumin calculated from Tables II and IV is 5.6. The increase in lysine of germinated oats can be explained by decreases in prolamin (low in lysine) and in globulin (relatively low in lysine) and increases in nonprotein nitrogen, albumin, and residue fractions (all higher in lysine). Nonprotein nitrogen showed increases in threonine, valine, isoleucine, and leucine, and a decrease in methionine plus cystine as germination proceeded (not shown in table). The amino acid composition of germinated oats at 20 and 25°C was almost identical to the values at 23°C (Table III) when the same germination time was compared (not shown in table).

Jahn-Deesbach and Schipper (1980) germinated oats for 84 hr at 25°C and reported an 8% increase in lysine and a 9% decrease in methionine plus cystine. Robbins and Pomeranz (1971) malted

TABLE I
Weight and Nitrogen Changes of 100 Kernels
of Oats During Germination

Days Sprouted	Temperature ^a (°C)	Weight (g)	Weight Loss (%)	Nitrogen Content (%)	Nitrogen (mg)	Nitrogen Loss (%)
0	...	2.577	...	2.84	73.2	...
1	20	2.515	2	2.77	69.7	5
	23	2.537	2	2.46	62.4	15
	25	2.482	4	2.35	58.3	20
2	20	2.513	2	2.53	63.6	13
	23	2.545	1	2.76	70.2	4
	25	2.481	4	2.80	69.5	5
3	20	2.448	5	2.78	68.1	7
	23	2.420	6	2.91	70.4	4
	25	2.413	6	2.97	71.7	2
6	20	2.210	14	2.99	66.1	10
	23	2.169	16	3.02	65.5	11
	25	2.089	19	3.07	64.1	12
8	20	2.138	17	2.92	62.4	15
	23	2.054	20	3.09	63.5	13
	25	2.074	20	3.19	66.2	10

^a Kernels were germinated in the dark at 20 and 25°C and in daylight at 23°C.

TABLE II
Nitrogen Distribution of Oats with Germination in Daylight at 23°C

Fraction	Percent Total Nitrogen of Fraction at Day of Germination				
	0	3	6	8	8 ^a
Nonprotein nitrogen	10	15	23	23	26
Albumin	6	7	4	10	10
Globulin	41	36	27	23	25
Prolamin	14	10	10	9	7
Glutelin	13	13	15	14	15
Residue	13	11	20	18	17
Total	97	92	99	97	100

^a In the dark at 25°C.

TABLE III
Essential Amino Acid Composition of Oats
as Germination Proceeded at 23°C^a

Amino Acid	Days of Germination						NAS ^b
	0	1	2	3	6	8	
Threonine	3.4	3.4	3.4	3.5	3.5	3.7	3.5
Valine	5.5	5.0	5.7	4.9	5.3	5.4	4.8
Methionine plus cystine	4.2	4.4	4.3	4.2	3.8	3.8	2.6
Isoleucine	3.9	4.1	4.1	4.0	4.1	4.3	4.2
Leucine	7.5	7.7	7.6	7.5	7.7	7.6	7.0
Phenylalanine plus tyrosine	9.2	9.7	9.4	9.2	8.9	8.5	7.3
Lysine	4.4	4.4	4.3	4.9	5.1	5.3	5.1

^a Grams per 16 g of nitrogen recovered.

^b 1980 National Academy of Sciences pattern of high-quality protein for human consumption.

TABLE IV
Essential Amino Acid Composition of Solubility Fractions^a from Oats

Amino Acid	Non-protein		Globulin	Prolamin	Glutelin	Residue
	Nitrogen	Water Extractable				
Threonine	2.9	3.9	3.9	2.1	3.6	4.4
Valine	0	4.6	6.0	6.7	5.5	6.6
Methionine						
plus cystine	9.0	4.9	1.3	4.9	2.6	2.3
Isoleucine	0	2.9	4.9	3.4	4.5	5.1
Leucine	2.8	5.8	8.1	10.9	7.9	9.1
Phenylalanine						
plus tyrosine	6.1	8.0	10.6	9.2	9.2	9.6
Lysine	4.5	4.9	2.7	1.5	4.0	5.6

^aGrams per 16 g of nitrogen recovered.

oats for five days at 16°C and found a small increase in lysine for malt, but this small increase may be within experimental uncertainty. Dalby and Tsai (1976) also reported increases in lysine and tryptophan, based on dry weight of oats after five days of germination. Hamad and Fields (1979) found that the increase in available lysine was highly significant in germinated oats based on *Tetrahymena pyriformis* W. growth assay. Folkes and Yemm (1958) reported that lysine in germinated barley increased 65% over ungerminated grain after 10 days. Miller (1978) observed increases in lysine content of 15–27% after seven days of sprouting wheat. Wu and Wall (1980) reported large increases in lysine content of germinated sorghums after seven to 10 days. Large increase in lysine content of germinated triticale after eight days was observed (Wu 1982). The relatively large increases in lysine for wheat, sorghum, barley, and triticale compared with oats after germination may be a result of the relatively low prolamin fraction for the latter compared with the former cereals.

CONCLUSION

A significant increase in lysine was observed when oats were germinated for six to eight days. The amino acid composition of germinated oats after six to eight days meets or exceeds the National Academy of Sciences pattern of good-quality protein for human consumption.

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