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Changes In Reserve Protein Fractions In Germinating Millet

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ABSTRACT

The proteins in germinating millet were subjected to the Osborne-type protein fractionation. Though prolamins formed the major protein fraction in ungerminated millet, globulins became the main protein fraction after 72 hours of germination. Analysis of the amino acid composition of the protein fractions showed that millet proteins were deficient in the sulphur-containing amino acids. However, upon controlled germination these amino acids as well as the other essential amino acids were abundantly produced.

Studies on the endopeptidase activity (EPA) involved in the degradation of the millet proteins revealed that the ungerminated millet has an acidic endopeptidase, but a neutral and an alkaline endopeptidase were added in later stages of germination. The millet endopeptidase requires SH group and a divalent ion for activity.

Keywords: millet, protein degradation, endopeptidase, germination

NOMENCLATURE

EPA	endopeptidase activity
EM	2-mercaptoethanol
NEM	N-ethylmaleimide
PMSF	phenylmethylsulforylflouride
EDTA	ethylenediamine tetracetate
SH	sulfhydryl

INTRODUCTION

In most developing countries millet provides a large portion of the dietary carbohydrates, proteins and other nutrients to the large number of human population that consume it. However, the nutritional quality of millet protein is only moderate because its level is low in the grain and it is deficient in some of the essential amino acids [1]. One of the possible ways of improving this quality is through controlled germination of the grain [2], a process that has been employed for other seeds [3, 4]. Jambunathan and Mortz [5] have suggested that in order to improve the quality of cereal grains it is desirable to know the distribution and amino acid composition of protein fractions isolated by selective extraction. Indeed the improvement in baking quality of wheat has been directly related to the shift in the molecular weight distribution of endosperm protein during germination [6,7]

Breakdown of the total reserve proteins has been reported to occur during the first 18 hr of millet germination and thereafter there is a period of protein accumulation [8]. The quantitative and qualitative changes in proteins of germinating wheat [9, 10], maize [11], Jojoba seeds [12] and Chickpea seed [13] have been associated with proteolytic enzymes activity. Knowledge of some of the physiochemical properties of the various proteins that comprise the basic structure of the reserve millet proteins and the endopetidase involved in the modification of the proteins during millet germination can contribute to obtaining the desired nutritional quality of millet protein.

EXPERIMENTAL

The early maturing millet (Pennisetum americanum L) harvested in the 1984 sea-

son was obtained from the Institute for Agricultural Research Farm, Sumaru, Nigeria. Grains were surface sterlized, steeped and germinated as previously described [8].

Preparation and Assay of Endopeptidase Activity:

Defatted acetone powders were prepared [14] from the sample for the enzyme assay collected at 0, 18, 48 and 72 hr of germination. To prepare the crude enzyme extracts, 2 g of the acetone powder were homogenized in 10 ml of 25 mM citrate-phosphate buffer (pH 7.2) containing 5 mM ME. This was centrifuged (10,000 x g, 10 min) and the supernatant collected and used for EPA assay. Endopeptidase activity was assayed according to the method described by Samac and Storey[12] using azecoll as substrate. Azecoll digestion leads to the release of peptides bound to a red dye. The activity of the enopeptidase was therefore expressed as the intensity (measured at 520 nm) of the red dye per gram dry acetone powder per hour.

Prottease inhibitor studies were carried out on the 72 hour sample. The extract was prepared as before except that the homogenizing and reaction buffer did not contain ME. The assay was done at pH 5.0 in the presence of 2.5 nM NEM, 5.0 mM ME, 2.0 PMSF or 2.0 mM PMSF or 2.0 mM EDTA. These inhibitor solutions were prepared as described by Samac and Storey [12]

Protein Fractionation and Estimation:

Samples collected for protein fractionation were ground in a Wiley Laboratory mill to a 20 mm mesh. The flour was defatted with hexane and the lipid-free protein fractionated into albumins, globulins, prolamine and glutelins using deionised water, 0.5 M NaCl, 50% ethanol and borax-NaOH buffer for the extraction of the respective fractions [15]. All fractions (except the albumins) were dialysed against deionised water for 48 hr at room temperature; they (including the albumins) were then freeze-dried.

Total protein in each fraction was determined from Kjeldahl nitrogen values - N x 6.25 [16].

Amino Acid Analysis:

Samples (containing the equivalent of 2.5 mg nitrogen) were hydrolysed with 100 ml of 6 N HCl for 24 hr under reflux. Amino acid analysis was performed with Locarte Amino Acid Analyser Mark 4 equipped with an intergrater and printer.

All experiments were in triplicate and the results reported are the means of such determinations.

RESULTS

Changes in Protein Fractions In Germination

As characteristic of most cereal grains [7] there was depletion of total protein in millet as germination progressed through 18 hr. then the protein level began to increase throughout the rest of the germination period (Figure 1). It is worth recalling here that millet germinates in 18 hr after which seedling growth sets in [18].

The major storage protein in the ungerminated millet is prolamin which forms about 52% of the total reserve proteins (Figure 1). Globulin and albumin together make up about 45% while the glutelin fraction is the smallest(8%) in the millet. Prolamin and glutelin have been reported to be the major proteins (comprising more than 65% of total protein) in millet [18, 19], barley [20] and sorghum [21].

Millet glubulin, like that of peas [22] seems to be the main protein fraction preferentially hydrolysed during the inhibition of water that stimulates germination. Thereafter the glubulin starts to accumulate while the prolamin and albumin are degraded. The glutelin fraction does not seem to be metabolised during the germination and seedling growth of millet (Figure 1). The hordein (prolamin) and the glutelin are the major proteins broken down during the malting of barley [20] and sorghum [21]. Harvey and Oaks [23] have reported that the initial rate of prolamin loss in germinating maize was slow but it later became more rapid.

Amino Acid Metabolism:

Table 1 shows the effect of germina-

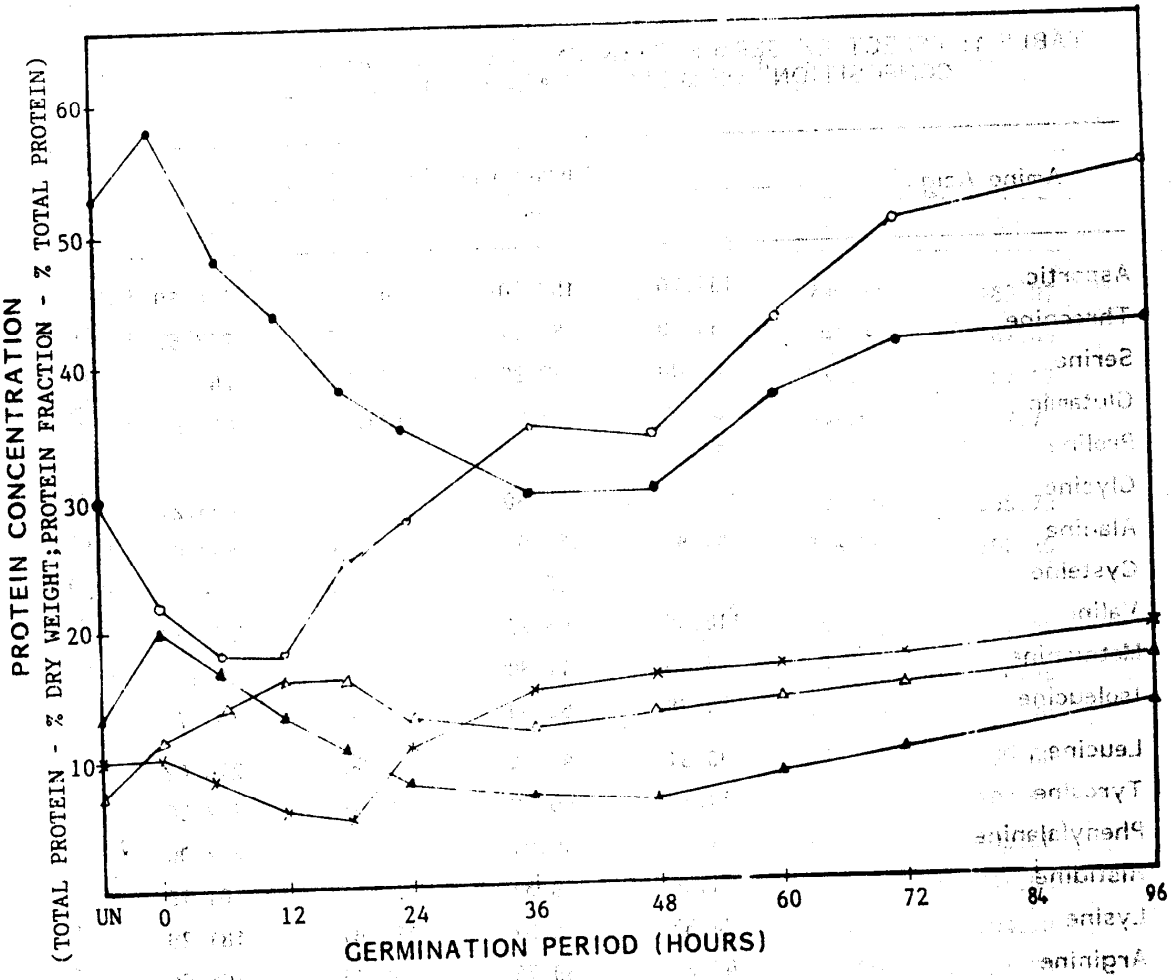

Figure 1: Effect of germination on millet proteins.

LEGEND: Total protein (x), prolamin (●), globuline (o), albumin (▲) and glutelin (△). Un - ungerminated millet

tion on the amino acid composition of millet grain. It is apparent that millet is deficient in the sulphur-containing amino acids; a situation that has been observed by several workers [1,19,24] However, these amino acids (cysteine and methionine) like most of the other amino acids increased in their levels during millet germination.

Table 2 shows the amino acid composition of the albumin and the globulin fractions extracted from germinating millet. The striking feature of this analysis was the absence of cysteine and low levels of methionine in these fractions. Otherwise the fractions contained relatively high amounts of the other amino acids. Low levels of cysteine and methionine have been reported in the albumin - globulin fractions of millet [10, 19].

The amino acid composition of the alcohol soluble proteins (prolamin) and the glutelin is shown in Table 3. The amino acid levels were generally low in the prolamin fraction but relatively this fraction was rich in Glu, Cys, Asp, Pro, Gly and Ala. Sawhney and Naik [18] and Okoh et al [19] have reported similar amino acid composition of millet prolamin. Except for the Cys that completely disappeared and the apparent absence of Met, the amino acids of millet prolamin increased during germination. Taylor [21] has reported the decrease in Met and Cys during the mal-

TABLE 1: EFFECT OF GERMINATION ON THE AMINO ACID COMPOSITION[a] OF MILLET GRAIN (nmole/mg protein)

Amino Acid	Germination Period (hr)			
	0	24	48	72
Aspartic	131.70	157.20	143.56	316.59
Threonine	63.30	88.91	74.02	213.57
Serine	61.00	89.98	76.88	247.35
Glutamic	157.64	165.64	130.96	418.55
Proline	56.05	59.51	68.84	235.42
Glycine	152.91	151.90	157.86	453.27
Alanine	165.45	151.64	155.74	530.00
Cysteine	-	16.50	40.59	-
Valine	118.45	106.22	83.45	777.79
Methionine	3.09	11.84	19.53	83.17
Isoleucine	54.78	52.94	38.76	183.72
Leucine	96.31	84.34	86.60	276.62
Tyrosine	34.12	55.99	43.20	162.30
Phenylalanine	61.12	55.91	59.22	202.00
Histidine	31.46	28.32	23.56	161.78
Lysine	74.57	78.37	138.47	181.29
Arginine	42.69	59.75	95.15	105.69
Ammonia	10.91	18.83	23.07	30.10

[a] Tryptophan was not determined. Unidentified amono acids (peaks) appeared between NH_3-Lys, and Lys-His is the 48 h samples respectively.

ting of sorghum. The glutelin fractions were rich in all the amino acids, but unlike the other fractions, the amino acids rather decreased in their concentrations during the germination process.

Endopeptidase Activity of Germinating Millet

In view of the apparent degradation of the reserve proteins and the resultant accumulation of amino acids, it was of interest to identify the EPA in millet. The activity profile of the endopeptidase in germinating millet (measured at various pH values) is shown in Figure 2. There was some EPA in the ungerminated grain. Germination was generally accompanied by increase in EPA. A neutral (pH 7) and an alkaline (pH 8) endopeptidases were absent from the ungerminated grain They apparently came into function as from the 18 hr of germination. Apparently the endopeptidase in millet is more active in the acid medium with optimum activity at pH 5. Unlike the endopeptidase of jojoba seed [12] there is no shift in the pH of optimal activity during seedling growth of millet.

TABLE 2: AMINO ACID COMPOSITION[b] OF SALT SOLUBLE PROTEINS (albumin and globulin) FOR MALTED MILLET (n moles/g protein)

Amino Acid	Germination Period (hr)			
	0	24	48	72
Aspartic	241.40	190.82	290.31	183.07
Threonine	136.68	101.11	145.43	187.03
Serine	161.77	122.19	233.97	257.17
Glutamic	225.32	208.96	342.66	427.77
Proline	116.54	85.84	148.59	179.63
Glycine	256.43	198.51	290.89	355.70
Alanine	259.35	200.74	278.75	362.80
Gysteine	-	-	-	-
Valine	211.62	155.13	266.64	321.88
Methionine	35.75	28.60	28.74	58.08
Isoleucine	96.48	83.33	124.34	174.40
Leucine	164.94	154.16	189.25	266.08
Tyrosine	102.11	60.17	111.16	109.09
Phenylalanine	122.42	88.28	159.30	193.36
Lysine	114.65	97.28	150.60	218.50
Arginine	114.96	88.05	166.41	204.18
Ammonia	11.02	15.17	17.96	21.83

[b] Trytophan was not determined. Between NH_3 and Lys of the 72 hr sample was an unidentified amino acid

The result of the effect of some known endopeptidase inhibitors on millet EPA is presented in Table 4. Millet EPA was activated by ME, inhibited by EDTA and NEM (in the presence of ME) but was not affected by PMSF.

DISCUSSIONS

It has been well established that germination and seedling growth result in the development of a number of hydrolytic enzymes which degrade the reserve food materials in cereal grains. The trend in protein metabolism coupled with the increase in EPA, as reported here for millet, has been observed in most seedlings [12, 25, 26]. The different profiles of the EPA observed at the various pH values (Figure 2) support the reports [12, 27, 29] that multiple forms of endopeptidase exist in seedlings. The inhibitor studies show that like the endopeptidases of peas [22] and castern bean [28] cleavage of disulphide bridges is necessary for activation of the millet enzyme. However, the inability of NEM to effect an inhibition without ME suggests that the disulphide group is not present at the active site but it is close enough that its cleavage could result in a more active conformation of

TABLE 3: THE AMINO ACID COMPOSITION[c] OF PROLAMIN (a) AND GLUTELIN (b) OF MALTED MILLET (n moles/mg protein)

Amino Acid	Germination Period (hr)							
	0		24		48		72	
	a	b	a	b	a	b	a	b
Aspertic	20.37	33.32	32.50	39.59	32.07	21.58	116.68	17.04
Threonine	10.12	21.05	18.05	27.43	20.32	14.16	48.93	10.81
Serine	15.30	24.86	22.16	31.69	37.96	16.08	85.68	12.63
Glutamic	42.10	65.62	87.06	76.50	127.46	34.44	210.64	24.50
Proline	22.73	15.82	36.73	40.39	46.79	12.29	110.06	8.73
Glycine	27.07	35.11	30.00	53.66	19.19	26.31	67.11	20.91
Cysteine	40.28	37.44	10.10	54.22	-	26.91	-	16.74
Valine	15.68	32.60	23.40	38.48	36.95	21.31	65.50	15.80
Methionine	-	6.37	-	7.84	-	4.18	-	3.93
Isoleucine	5.38	13.84	9.98	17.83	19.89	10.44	37.62	8.18
Leucine	11.38	32.91	13.80	34.68	52.33	20.96	87.77	19.12
Tyrosine	6.61	8.21	8.96	11.41	10.57	6.70	26.19	4.13
Phenylalanine	11.64	17.92	14.83	19.70	19.04	10.20	52.21	7.41
Histidine	7.54	9.65	7.00	12.91	8.33	5.92	22.47	4.72
Lysine	5.94	12.37	-	19.76	-	11.66	22.26	10.64
Arginine	9.08	11.08	7.68	18.98	6.34	8.56	21.63	24.22
Alanine	26.25	47.50	30.05	51.57	27.40	27.41	35.95	18.38
Ammonia	30.03	12.21	37.11	15.48	40.57	15.00	44.63	17.32

[c] Tryptophan was not determined. Unidentified amino acids appeared between His and Phe in the 72 hr prolamin sample.

the enzyme. The millet endopeptidase is not a serine protease, however, it required a divalent ion for activity.

During the germination of millet the proportion of the various nitrogenous fractions alter (Figure 1) and this reflects the degradation of some proteins and simultaneous synthesis of others. It is implied from the profile of the protein fractions that the degradation of globulin might not wholly depend on de novo enzyme synthesis. The globulin might in fact supply the amino acids necessary for the synthesis of enzymes needed for the breakdown of the prolamin and albumin fractions. While the prolamin fraction seems to be the main storage protein extensively degraded during millet germination, the glutelin does not change suggesting that it is a structural protein.

The globulin comprises over 50% of the 72 hr germinated millet protein. This fraction is rich in the basic amino acids and coupled with the improvement in the Leu; Ileu ratio will contribute, more than the other protein fractions, to improving the nutritional quality of the germinated millet. Even though the prolamins form the bulk of the reserve proteins in millet, the amino acids levels were low, a condition that was

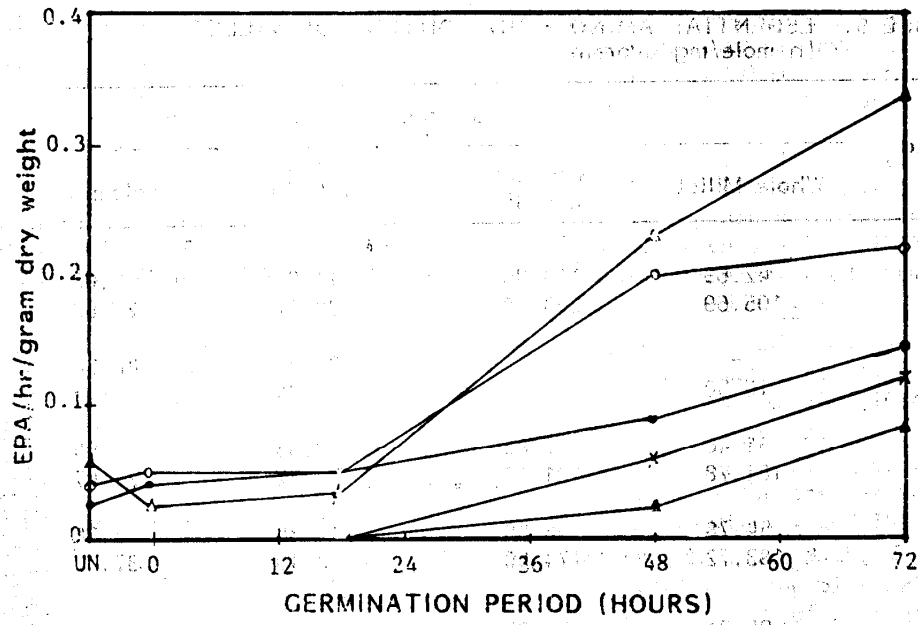

Figure 2: Endopeptidase activity during millet germination

LEGEND: Enzyme activity was measured at pH 4 (●), 5 (△) 6 (o), 7 (x) and 8 (▲). Un - ungerminated millet

TABLE 4: EFFECT OF CHEMICAL INHIBITORS ON MILLET EPA[a]

Inhibitor	Activity
None	0.12
ME	0.24
EDTA	0.00
PMSF	0.11
NEM	0.10
Control (enzyme extracted with ME in buffer)	0.332
ME + NEM[b]	0.00

[a]The 72 hr germinated sample used. Activity was measured at pH 5

[b]Enzyme was extracted with buffer containing ME.

not markedly improved upon germination. It is possible the amino acids in the prolamin fractions were hydrolysed, as the NH_3 values were quite high in these fractions, or that as already reported [19] millet prolamins are generally poor in amino acids. The prolamins do not therefore appear a promising candidate for improving millet protein quality. Unlike the prolamin, the low albumin content of millet seems to be compensated for by the quality and quantity of its (albumin) amino acid contents. Accordingly food processing technologies aimed at obtaining high salt soluble proteins (globulin-albumin) in millet products could be investigated. The decline in the amino acids levels of the glutelin fraction cannot be explained. However, the quality of the amino acids (Arg, Glu, Cys, Gly) makes the contribution of glutelin to the improved nutritional quality of germinated millet significant.

TABLE 5: ESSENTIAL AMINO ACID CONTENT OF MILLET[a]
(n mole/mg protein)

Amino Acid	Source			
	Whole Millet	Albumin-Globulin	Glutelin	Prolamin
Arginine	42.69 105.69	114.96 204.18	11.08 24.22	9.08 21.63
Cystine	- 16.50	- -	37.44 26.74	40.28 -
Histidine	31.46 161.78	57.86 101.17	9.65 4.72	7.54 22.47
Isoleucine	54.78 183.72	96.48 174.40	13.84 8.18	5.38 37.62
Leucine	96.31 276.62	146.94 266.08	32.91 19.12	11.38 87.77
Methionine	3.09 83.17	35.75 58.81	6.37 3.93	- -
Lysine	74.57 181.29	114.65 218.50	12.37 10.64	5.94 22.26
Phenylalanine	61.12 202.00	122.42 193.36	17.92 7.41	11.64 52.21
Threonine	63.30 213.57	136.68 187.03	21.05 10.81	10.12 48.93
Valine	118.45 777.79	211.62 321.88	32.60 15.30	16.68 65.50

[a] The first figures show the value of the amino acids of unmalted millet grains while the second figures show the value after 72 hr of malting.

CONCLUSIONS

The nutritional quality of a protein is better reflected in its amino acids composition. Indeed, the amino acid composition of millet (ungerminated) has been extensively reported [1, 19, 24]. The up to 10-fold increase in the limiting sulphur containing amino acids as well as the other essential amino acids (Table 5) mirrors the improved nutritional quality of malted millet [2]. Thus controlled germination (malting) presents a simpler method of improving the nutritive value of millet proteins than the genetic breeding programme of selecting variaties poor in prolamin[19]. Further detailed studies on the biological values (relative nutritive value/protein efficiency ratio) of the 72 hr germinated millet are necessary for the complete evaluation of millet proteins.

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