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Protein Modification during Germination of *Sorghum Bicolor*

Malomo Olu*, and Alamu E A

Bells University of Technology, Km 9 Idiroko road, Ota, Ogun State, Nigeria.

ABSTRACT

In other to determine the protein modification of *sorghum bicolor* during germination, the relationship between protein, amino nitrogen and other protein quality parameters in Sorghum were investigated. Also to determine the amount of protein reserves with suitability of Sorghum malt for brewing process in Africa, especially in Nigeria and finally to assist in the selection of *sorghum bicolor* for brewing process 13 varieties of Sorgum species were selected for the project obtained from Institute of Agricultural Research (IAR) of the Ahmadu Bello University Zaria. The study showed little change in the crude protein content, but appreciable changes in the amino nitrogen as the germination period advanced, although there were varietal differences. The KSV, SRN, and NVW varieties showed outstanding increase of more than double in composition. This was also observed with the soluble nitrogen components. The correlation co efficient between crude protein and amino acid was 0.47 while that with soluble nitrogen was 0.23.However with a higher correlation coefficient of 0.59 for protease activity. The SSV varieties were found with lower Kolbachs index, making them more suitable for brewing as a result of their tendencies in haze formation.

Keywords: Crude protein, Kolbach index, Correlation coefficient, Soluble nitrogen, Amino nitrogen.

Corresponding author

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INTRODUCTION

The grain of sorghum is an important world cereal crop usually ranking fifth in terms of total production. About 20% of the world produce is consumed as human food. Trends are now towards the utilization of sorghum in beer and malt production for which this project is all about.

Malt is a grain which has been allowed to germinate for a limited period of time under controlled conditions of temperature, aeration, etc. During malting, diastatic proteolytic and other enzymes develop and accumulated in the grains. The use of malt in many industrial food and beverage manufacturing process lies in its being a rich source of these enzymes.

A lot of alcoholic and nonalcoholic beverages have been prepared from sorghum or sorghum-maize, for example, Cameroonian angba, Nigeria otika and South Africa Kaffir beer. The activity of protease enzyme is manifested by protein degradation during malting. This activity increases during germination especially after four or five day's growth. No adequate method have been deviced for estimating this activity, since the nature and mode of action of the enzymes present are not closely understood.(Wang and Fields, 1978;Malomo et.al., 2013)

MATERIALS AND METHODS MATERIALS

The main materials used for this project are fifteen varieties of sorghum bicolor supplied by the Institute of Agricultural Research (IAR) of the Ahmadu Bello University, Samaru Zaria. The varieties are coded, SSV3, SSV9, SSV9, SSV10, SSV11, SSV12, KSV4, KSV7, KSV8, KSV11, KSV12, KSV13 and SRN 484, based mainly on.

1. Yield per acre.
2. Adaptation to specific ecological zones and resistance to diseases.

A white local variety obtained from Noek village was also used.

Experimental Method

Steeping

1kg of the grains were washed in tap water to remove dirt and then steeped in 2 litres of water for 18 to 48 hours depending on the result of preliminary investigations with respect to water uptake and length of steeping period.

Germination

The stepped grains were germinated at 30 + 2 ^{2} C (ambient temperature) in a plastic vessel lined with adsorbent papers. The vessels were placed in the germinating cupboard provided with some vents. They were watered twice daily. Germination started for the KSV,

SRN and NVW varieties at the 18th hour while germination for the SSV varieties was more noticeable at 24th hour.

Sampling

Samples were removed every 24 hours for the following analysis.

- Crude protein and total Nitrogen determination.
- Soluble nitrogen determination.
- Amino nitrogen determination.
- Protease activity estimation.

Kilning

After samples for protease estimation are removed. A measured quantity suitable for the aforementioned analysis were removed and Kilned at $48^0 + 2^0$C for 10 hours in an oven.

Analysis

After trying, the roots and shoots are removed and the malt was milled using the Bra bender Quadrant experimental will. All analysis except protease estimation are then carried out.

Determination of Total Nitrogen and Crude Protein Content

These are carried out by the standard method of kjehdal as officially stated in the A.O.A.C. The crude protein content was obtained by multiplying the total nitrogen value by a factor of 6.25. Results are expressed as percentage dry basis.

Soluble Nitrogen Determination

5g of the malt was milled and soaked in 25mls of distil water at ambient temperature. The entire portion was centrifuged for 30 minutes. 1ml of this portion was pipethed into the Kjehdahl digestion tube and analysed for total nitrogen as previously carried out in step 4.2.5.1. The results are expressed as percentage dry weight basis.

Amino Nitrogen Determination

This was carried out by method of Sorensen (1909).

Procedure: 20g of the milled sample was put into a conical flask unto which 100ml of distill water was added. The mixture was allowed to stand for 1hour at ambient temperature, with occasional shaking. It was filtered using whatman number 2 paper.

10ml of the filterate was then titrated against 0.14N NaOH to an end point previously established by addition of phenol red to a buffer solution of pH 8.0.

A further 10ml of 405 formaldehyde was added and allowed to stand for 15mins and titrated againt to pH 8.0. Reading (in ml gives) the milligram of titratable Nitrogen in the aliquot. The results are expressed on dry weight basis.

Protease Activity Estimation

Extraction of Enzyme

75-100 seeds were withdrawn at intervals up to five days after steeping, extracted with 30ml of 0.1M sodium acetate (pH 4.4) in an homogenizer and stirred for 1hour. After centrifugation (35,000 x g), the pellet was stirred for one hour with 30ml of buffer and centrifuged.

Protease Activity Estimation

Protease activity was determined by the method of Kunitz (1946). A 1% (w/v) case in solution was prepared in 0.02m citrate phosphate buffer (pH 7.0) and was heat denatured at 100^0C for 15minutes in a boiling water bath, cooled and filtered through four layers of Muslim cloth and used as the substrate.

One ml of enzyme filterate was added to 1ml of the substrate, thoroughly missed and incubated in a water bath at 35^0C for 1hour. The reaction was terminated by adding 5ml of cold 5% TCA. The control tubes were then provided with 1ml of the enzymes filterate and the tubes allowed standing for 1hour at 4^0C to allow undigested protein to precipitate completely after which separation was done a 6.780g for 1hour. The supernatant was carefully decanted and the non precipitable (TCA) protein in it was measured by the Lowry method (Lowry et al (1951).

Enzyme Protein Determination

The protein in the enzyme solution was determined by the method of Lowry et al (1951) was follows:

50ml of 2% Na_2CO_3 in 0.IN NaoH were mixed with 1ml of 0.55 $CuSo_4$. $5H_2O$ in 1% sodium – potassium tartrate and the mixture was labeled as reagent “C”. Follins regaent (BDH) was diluted twice with distilled water and labeled as reagent “D”. a standard 0.01% protein solution was prepared from egg albumin powder (BDh) dissolved, with continuous agitation in distilled water, 1ml of the enzyme solution was taken in a clean sterile test tube 1ml of the standard protein solution was also taken in another test-tube. To each was added 5ml of reagent C and the content mixed thoroughly. After 10minutes of incubation at room temperature, 0.5ml of reagent D was added to test tube and incubated at room temperature for 30minutes. The

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optical density was taken by 670mm with a Gallenkamp Colorimeter. The optical density at this wavelength was used to estimate the extent of protease activity in the sample.

(Calculation – optical density of sample at 670mm) - (Optical density of control at 670mm) = Extent of protease activity in terms of optical density

RESULTS AND DISCUSSION

RESULTS

Changes in Crude protein during germination

Table 1 showed the trend in protein changes during germination. As can be seen on the table, the crude protein decreased slowly during the first three days of germination and then steeply between fourth and fifth days, the fall appeared more gradual in the SSV varieties than the KSV, SRN and NVW varieties.

Table 1: Changes in Percentage Crude Protein during Germination (dry matter basis)

SAMPLES	DAYS OF GERMINATION					
	0	1	2	3	4	5
SSV 3	10.89	10.31	9.7	9.02	8.32	7.52
SSV 9	8.20	7.71	7.73	6.35	6.05	5.27
SSV 10	10.33	9.64	9.48	8.77	8.18	7.20
SSV 11	9.07	8.82	7.66	6.75	6.02	5.44
SSV 12	9.04	8.36	8.20	7.49	6.98	6.55
KSV 4	8.62	7.54	6.95	6.63	6.07	5.29
KSV 7	8.46	7.45	7.05	6.49	5.70	4.72
KSV 8	8.057	8.04	7.16	6.54	6.49	6.15
KSV 11	8.85	8.06	8.03	7.73	7.41	6.54
KSV 12	5.61	5.40	4.94	4.78	4.54	4.34
KSV 13	8.69	8.40	8.26	7.92	7.34	6.37
KSV 14	8.01	7.78	7.35	6.87	5.99	4.79
SRN 484	10.64	8.17	7.59	6.99	6.41	5.81
NVW	7.70	5.99	5.10	4.42	4.21	3.80

The residual crude protein for the SSV varieties ranged from 5.27% to 7.52%. While percentage residual crude protein for the KSV, SRN and NVW ranges from 3.80% to 6.54%.

Changes in Total Nitrogen During Germination

As previously pointed out that there was a decrease in the percentage crude protein during germination, this result also showed that there is a corresponding decrease in the total nitrogen content. This decrease could be attributed to the degradation of protein to supply the

embryo with amino acid. Table 2 showed the trend in nitrogen changes with germination, with the residual total nitrogen in raw sorghum, falling gradually to the fifthl day of germination. The final nitrogen percentage for the SSC varieties ranged from 0.84% to 1.37% while the KSV, SRN and NVW varieties had a range of 0.80% to 1.16%.

Table 2: Percenatge Total Nitrogen in the Germinating Grain on dry matter basis.

SAMPLES	DAYS OF GERMINATION					
	0	1	2	3	4	5
SSV 3	1.74	1.59	1.50	1.43	1.38	1.35
SSV 9	1.32	1.26	1.14	1.01	0.96	0.84
SSV 10	1.55	1.53	1.52	1.40	1.31	1.51
SSV 11	1.73	1.64	1.61	1.52	1.43	1.37
SSV 12	1.44	1.33	1.30	1.20	1.11	1.04
KSV 4	1.37	1.20	1.11	1.06	0.97	0.84
KSV 7	1.34	1.19	1.13	1.04	0.91	0.78
KSV 8	1.36	0.87	0.74	0.65	0.64	0.58
KSV 11	1.42	1.40	1.32	1.27	1.18	1.04
KSV 12	1.18	1.15	1.08	1.03	0.96	0.69
KSV 13	1.39	1.34	1.32	1.74	1.55	1.16
KSV 14	1.70	1.44	1.30	1.24	1.14	1.02
SRN 484	1.70	1.31	1.20	1.11	1.52	0.94
NVW	1.40	1.20	1.12	1.07	0.94	0.80

Changes in Amino Nitrogen

As can be seen in table 3, there was an increase in the amino nitrogen as the days of germination advanced. The final proportion of the amino nitrogen to the original was more than double at the fifth day of germination. This increase might be mainly attributable to the formation of amino acids from protein degradation. This increase was found to occur earlier in the KSV, SRN and NVW variety than the SSV varieties. The final proportion of the amino nitrogen for the SSV ranged from 0.210% to 0.2405, the SRN and NWV had ranged in the neighborhood of 0.260% to 0.310%. This indicated that more amino nitrogen were formed in these lot than the SSV varieties. These trends also coincided with the rapid growth noticed in the KSV, SRN and NVW compared with the SSV varieties.

Changes in Soluble Nitrogen

Table 4 showed an increase in the proportion of soluble Nitrogen as germination advanced. This increase was particularly considerable during the active growth stage. Majority of the KSV varieties had soluble Nitrogen content ranging between 0.015% to 0.02155 while the SSV varieties ranged between 0.0090% and 0.0180% at the fifth day of germination.

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Table 3: Changes In Percentage Amino Nitrogen During Germination (Dry matter basis).

SAMPLES	DAYS OF GERMINATION					
	0	1	2	3	4	5
SSV 3	0.093	0.120	0.140	0.160	0.170	0.210
SSV 9	0.090	0.110	0.140	0.190	0.230	0.240
SSV 10	0.090	0.130	0.160	0.210	0.220	0.230
SSV 11	0.090	0.130	0.170	0.180	0.190	0.220
SSV 12	0.090	0.120	0.160	0.190	0.230	0.210
KSV 4	0.090	0.120	0.160	0.190	0.230	0.270
KSV 7	0.090	0.120	0.170	0.180	0.210	0.260
KSV 8	0.090	0.160	0.190	0.250	0.280	0.310
KSV 11	0.090	0.160	0.190	0.250	0.280	0.310
KSV 12	0.090	0.140	0.230	0.260	0.280	0.290
KSV 13	0.092	0.150	0.190	0.230	0.260	0.270
KSV 14	0.090	0.120	0.210	0.240	0.250	0.270
SRN 484	0.090	0.130	0.170	0.210	0.230	0.250
NVW	0.090	0.160	0.210	0.230	0.260	0.290

Table 4: Changes In Percentage Soluble Nitrogen During Germination.

SAMPLES	DAYS OF GERMINATION					
	0	1	2	3	4	5
SSV 5	0.0060	0.0068	0.0093	0.0101	0.0152	0.0180
SSV 9	0.0060	0.0061	0.0073	0.0092	0.0110	0.0127
SSV 10	0.0044	0.0047	0.0062	0.0070	0.0083	0.0090
SSV 11	0.0052	0.0056	0.0090	0.0109	0.0132	0.0167
SSV 12	0.0059	0.0063	0.0069	0.0080	0.0093	0.0104
KSV 4	0.0054	0.0079	0.0104	0.0123	0.0187	0.0240
KSV 8	0.0056	0.0071	0.0071	0.0087	0.0171	0.0233
KSV 11	0.0055	0.0067	0.0086	0.0099	0.0107	0.0158
KSV 12	0.0050	0.0062	0.0086	0.0099	0.0107	0.0158
KSV 13	0.0053	0.0065	0.0087	0.0091	0.0106	0.0201
KSV 14	0.0052	0.0071	0.0092	0.0107	0.0203	0.0215
KSV 15	0.0056	0.0067	0.0087	0.0102	0.0124	0.0175
SRN	0.056	0.0068	0.0093	0.0101	0.0148	0.0173
KSV 7	0.0060	0.0071	0.0083	0.0091	0.0102	0.0115

Changes in Protease Activity

Table 5 showed the relationship between protease activity and days of germination..

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Table 5: Changes In Optical Densities (670nm) During Germination As An Index Of Protease Activity.

SAMPLES	DAYS OF GERMINATION					
	0	1	2	3	4	5
SSV 3	0.013	0.038	0.073	0.098	0.107	0.103
SSV 9	0.017	0.045	0.063	0.071	0.83	0.110
SSV 10	0.021	0.041	0.067	0.087	0.093	0.110
SSV 11	0.025	0.078	0.079	0.062	0.092	0.110
SSV 12	0.018	0.033	0.067	0.088	0.098	0.101
KSV 4	0.012	0.037	0.048	0.040	0.077	0.088
KSV 8	0.013	0.041	0.052	0.097	0.087	0.099
KSV 11	0.018	0.062	0.083	0.080	0.92	0.120
KSV 12	0.016	0.073	0.058	0.079	0.120	0.124
KSV 13	0.017	0.041	0.053	0.093	0.103	0.101
KSV 14	0.011	0.031	0.071	0.079	0.095	0.098
KSV 15	0.012	0.029	0.058	0.062	0.095	0.102
SRN 484	0.023	0.047	0.089	0.060	0.075	0.093
NVW	0.020	0.045	0.063	0.062	0.093	0.120

The extent of protease activity in the entire varieties did not follow the previous trend in the crude protein and total nitrogen results. One possible explanation to this non conformation could be attributed to the fact that the extracted enzyme were more active on a synthetic substrate rather than the natural substrates, that is, endosperm substrate rather than the natural substrate, this is the. endosperm protein, In addition a suitable method had need to be devised to estimate the extent of the activity of the enzymes, this method was utilized on the ground that the substrate was the one likely to be acted upon by the enzyme.

Kolbach Index Value

This value is the ratio of total Nitrogen to the soluble Nitrogen. This reflects the extent of protein degradation. It is desirable that the soluble Nitrogen content must be as small as possible as this increases the value of the index, thus a higher total nitrogen value and a corresponding small soluble nitrogen value is highly desired in sorghum to be used in malt and beer making.

Higher content of soluble nitrogen would lead to haze formation which is detrimental to the quality of a typical beer. The higher the kolbach's value the more suitable is the sorghum for brewing. On this basis the SSV varieties were considered more suitable for brewing purposes as can be seen in Table 6.

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Table 6: Kolbachs Index Values.

SAMPLES	KOLBACHS INDEX T.N. / S.N.
SSV 3	75.00
SSV 9	66.14
SSV 10	127.00
SSV	82.03
SSV 12	100.00
KSV 4	35.00
KSV 7	67.82
KSV 8	24.89
KSV 11	68.42
KSV 12	43.67
KSV 13	57.71
KSV 14	48.37
KSV 15	42.85
SRN 484	46.77
NVW	69.56

Correlation factors between residual protein amino nitrogen, soluble nitrogen and protease activities.

If a linear relationship is assumed between two variables the coefficient of correlation is given by:

$$R = \frac{\Sigma \; x \; y}{(\Sigma x^2)\,(\Sigma y^2)}$$

Where $x = x - \bar{\times}$ and $y = y - \bar{y}$

This formular which gives the sign of r is known as PRODUCT – MOMET FORMULA and clearly shows the symmetry between two variables. Denoting the average percentage protein by $\bar{\times}$ and average percentage amino nitrogen, soluble nitrogen and optical density at 670mm by y, the coefficient of correlation was calculated.

Correlation coefficient between Crude Protein and Amino Nitrogen

$$r = \frac{-0.175}{\sqrt{(15.44)^2 \times (0.0425)^2}} = -0.47$$

The values are shown on Table 7.

Correlation coefficient between crude protein and soluble nitrogen

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$$r = \frac{0.63}{\sqrt{(15.44)^2 \times (0.00047)^2}} = -0.23$$

The values are shown on Table 8.

Table 7: Correlation Coefficient Of The Residual Crude Protein And Amino Nitrogen At Fifth Day Of Germination.

SAMPLES	X	Y	$x=x-\bar{x}$	$y=y-\bar{y}$	X^2	Y^2	Xy
SSV 3	7.52	0.21	1.79	-0.05	3.20	0.0025	-0.008
SSV 9	5.27	0.24	-0.46	-0.02	0.21	0.004	0.0092
SSV 10	7.20	0.23	1.47	-0.03	2.16	0.009	-0.044
SSV 11	5.44	0.22	-0.29	-0.04	0.084	0.0016	0.0116
SSV 12	6.55	0.21	0.82	-0.05	0.67	0.0025	-0.041
KSV 4	5.29	0.27	-0.44	0.01	0.19	0.0001	-0.0044
KSV 7	4.72	0.26	-1.01	0	1.02	0	0
KSV 8	6.15	0.26	0.42	0	0.18	0	0
KSV 11	6.54	0.31	0.81	0.05	0.66	0.0025	0.0040
KSV 12	4.34	0.29	-1.39	0.03	1.93	0.009	-0.0417
KSV 13	6.37	0.27	0.64	0.02	0.41	0.004	0.0128
KSV 14	6.08	0.27	0.35	0.02	0.12	0.004	0.007
KSV 15	4.79	0.29	-0.94	0.04	0.88	0.0016	-0.0316
SRN 484	5.81	0.25	0.08	-0.01	0.0064	0.0001	-0.0008
NVW	3.81	0.29	-1.93	0.04	3.72	0.0016	-0.0772

$\bar{x} = 5.73$, $\bar{Y} = 0.26$ x = Residua crude protein at fifth day germination

$\Sigma xy = 0.175$ y = Residual amino Nitrogen at fifth day germination

$\Sigma x^2 = 15.44$ $\Sigma y^2 = 0.0425$

Table 8: Correlation Coefficient of The Residual Crude Protein And Soluble Nitrogen At Fifth Day Of Germination.

SAMPLES	X	Y	$x=x-\bar{x}$	$y=y-\bar{y}$	X^2	Y^2	Xy
SSV 3	7.52	0.018	1.79	0.002	3.20	4	5.73
SSV 9	5.27	0.013	-0.46	-0.003	0.21	9	0.097
SSV 10	7.20	0.009	1.47	-0.007	2.16	4.9	-3.18
SSV 11	5.44	0.017	-0.29	0.001	0.084	1	-0.0029
SSV 12	6.55	0.010	0.82	-0.005	0.67	2.5	-0.0041
KSV 4	5.29	0.024	-0.44	0.008	0.19	6.4	-0.035
KSV 7	4.72	0.023	-0.01	0.007	1.02	4.9	-0.007
KSV 8	6.15	0.015	0.42	-0.001	0.18	1	-0.00042
KSV 11	6.54	0.016	0.81	0	0.66	0	0
KSV 12	4.34	0.020	-0.39	0.004	1.93	1.6	-0.0056
KSV 13	6.37	0.022	0.64	0.006	0.41	3.6	0.0038
KSV 14	6.08	0.018	0.35	0.002	0.12	4	0.0007
KSV 15	4.79	0.201	-0.94	0.004	0.88	1.6	-0.0038
SRN 484	5.81	0.017	0.08	0.001	0.0064	1	0.00008
NVW	3.81	0.012	-1.93	-0.004	0.0016	1.6	-0.0077

$\bar{x} = 5.73$, $\bar{Y} = 0.016$, x = Residua crude protein at fifth day germination

$\Sigma xy = 0.63$ y = Residual amino Nitrogen at fifth day germination

$\Sigma x^2 = 15.44$, $\Sigma y^2 = 0.00047$

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Correlation coefficient between crude protein and protease activity

$$r = \frac{-0.014}{\sqrt{(15.44)^2 \times (0.00153)^2}} = -0.59$$

The values are shown in Table 9

Table 9: Correlation Coefficient of The Residual Crude Protein And Protease Activity At Fifth Day Germination.

SAMPLES	X	Y	$x=x-\bar{x}$	$y=y-\bar{y}$	X^2	Y^2	Xy
SSV 3	7.52	0.103	1.79	-0.003	3.20	0.9	-0.0054
SSV 9	5.27	0.110	-0.46	0.004	0.21	-1.6	-0.0018
SSV 10	7.20	0.110	1.47	0.004	2.16	1.6	0.0059
SSV 11	5.44	0.110	-0.29	0.004	0.084	1.6	-0.0012
SSV 12	6.55	0.101	0.82	-0.005	0.67	2.5	-0.0041
KSV 4	5.29	0.088	-0.44	-0.018	0.19	32.4	0.0079
KSV 7	4.72	0.099	-1.01	-0.007	1.02	4.9	0.0070
KSV 8	6.15	0.120	0.42	0.014	0.18	19.6	0.0059
KSV 11	6.54	0.124	0.81	0.018	0.66	32.4	0.015
KSV 12	4.34	0.115	-1.39	0.009	1.93	08.1	-0.013
KSV 13	6.37	0.101	0.64	-0.005	0.41	2.5	-0.0032
KSV 14	6.08	0.098	0.35	-0.008	0.12	6.4	-0.0028
KSV 15	4.79	0.102	-0.94	-0.04	0.88	1.6	0.0038
SRN 484	5.81	0.093	0.08	-0.013	0.0064	16.9	-0.0010
NVW	3.81	0.120	-1.93	0.014	3.72	19.6	0.27

$\bar{x} = 5.73$, $\bar{Y} = 0.106$,x = Residua crude protein at fifth day germination
$\Sigma xy = 0.014$, y = Residual amino Nitrogen at fifth day germination
$\Sigma x^2 = 15.44$, $\Sigma y^2 = 0.00153$

DISCUSSIONS

Fifteen locally available sorghum bicolor have been shown to possess some chemical characteristics suitable for malt and beer production. High emphasis was however laid on the total nitrogen and soluble nitrogen content since this had a considerable influence on the quality of the overall product. The general results on this study appeared to agreed with the findings of previous workers such as Okafor, (1980), Aisien (1980) and Noveillie (1966) who made some observations on the malting of sorghum grain.

Previous studies by Fleming et al (1960), Hwang and Bushuk (1973) had shown that large increases in the general levels of proteolytic activity occurred concomitantly with storage protein hydrolysis. This falls in trend with what was observed during the course of the project though our approach did not distinguish between endo and exoproteolytic enzyme.

From these findings about the responsiveness of the SSV varieties to germination, protein degradation result indicated that enhancement of proteolytic activity during germination may be necessary.

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The protein content of the malt ranged from 4.97% to 7.5% and this was in consonance with what previous worker obtained. However the values for the KSV varieties were generally low. This could be attributed to the rapid growth of these lot resulting to rapid degradation of the protein reserve compared with the SSV varieties whose growth appeared rather slow.

Based on these results and the correlation coefficient there was a negative correlation between the crude protein content and amino nitrogen, soluble nitrogen and protease activity. The implication could be that as the crude protein content decreases there is a corresponding increase in the values of the amino nitrogen, soluble nitrogen and protease activity.

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