

Proximate analysis and hydrolysis of malted millet (*Panicum miliaceum*) blended with maize and rice flour

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ABSTRACT

The physico-chemical result of the analysis of proso millet (*Panicum miliaceum*) showed the optimum water uptake of the millet to be 96 hours, optimum malting time 96 hours, ash content 3.26 ± 0.03 , moisture content $11.37\% \pm 0.33$, crude fat content $1.30\% \pm 0.09$, crude protein content $13.87\% \pm 0.5$, carbohydrate content $71.46\% \pm 0.80$, reducing sugars $5.08\% \pm 0.80$, hydrolysable sugars $54.29\% \pm 0.70$ and fibre content $6.37\% \pm 0.30$. The mineral composition of proso millet in mg/100g showed that the millet contains K (2.14), Fe (1.04), Mg (0.85), Ca (0.65), Na (0.17), Al (0.13), Mn (0.013), Zn (0.04), Pb (Lead) was not detected in the milled. Temperature and pH studies on the malted millet showed the optimum values to be $50-60^\circ\text{C}$ and 5-6 respectively. The blending ratio of the gelatinized maize flour ground with the chaff with malted millet was found to be 50%, while the blending ratio of gelatinized maize after removing the chaff with malted millet was determined to be 60%. The blending ratio of the gelatinized rice flour with malted millet was also found to be 60%. The decrease of about 10% in the value of reducing sugar production between the two maize flours could be due to the fact that, the unprocessed seeds have more lignified materials which could inhibit access to amylase during hydrolysis. The absence of heavy metal lead (Pb) and high carbohydrate content of proso millet indicates that it could be used in the brewing industry as an adjunct to barley.

Keywords: *Panicum miliaceum*; water; carbohydrate; protein; fat; sugar; fibre; minerals

1. INTRODUCTION

Proso millet (*Panicum miliaceum*) belongs to the family Gramineae, which is a large family of grasses with about 620 genera and 1,000 species dispersed in many parts of the world where the plants can survive. Gramineae is economically the most important family of flowering plants, the grasses provide diet of most of the world population.

This is the reason for the extensive studies of the chemical composition of cereals, optimum water uptake, optimum malting time and also the determination of whether the amylases of the malted cereals could be used to hydrolyse starches of other cereals such as maize flour before and after removing the chaff and also rice flour.

Maize (*Zea mays*) is one most important cereals in the world apart from wheat and rice. It also belongs to the family Gramineae. It is also an adjunct needed in the brewing industry as raw material for the production of starch and other beverages. It is ground into powdered form and made into porridge called Kpekple in Ghana, Kunu (Hausa) in Northern Nigeria.

Bidia in Zaire, Sadza in Zimbabwe, puto in Zulu land. The meal obtained from maize is cooked with water to provide a thick meal called two (Hausa) Northern Nigeria.

Rice (*Oryza sativa*) is the seed of the monocot plant. Rice is the most important grain with regard to human nutrition and caloric intake. It provides more than one fifth (1/5) of the calories consumed worldwide by humans. Rice is usually cooked by boiling and is eaten mostly with fish, vegetable or meat. Raw rice may be ground into powder (flour) and converted into many uses including rice milk, rice wine etc.

Carbohydrates belong to the class of organic compounds having the general molecular formula of $C_nH_{2n}O_n$. Starch, glucose, cellulose and glycogen all belong to this class of organic compounds. Starches are substances which constitute the major part of the human diet for almost half of the world population as well as animals. Starch molecules are glucose polymers linked together by α 1,4- and α -1,6-glycoside bonds as opposed to the β -1,4-glycoside bonds for cellulose. Enzymes are capable of hydrolyzing starch usually to form oligosaccharides and other smaller units e.g disaccharides and monosaccharides.

This is possible with a special type of enzymes called alpha amylase which is present in the saliva of human beings and can hydrolyse starch to maltose and glucose. Partial hydrolysis of starch usually produces Dextrin which is a mixture of D-glucose and branched oligosaccharide.

Dextrin is often a component of infant diet because it is easily digestible starch granules are quite resistant to penetration to both water and hydrolytic enzymes due to the formation of intermolecular and intramolecular hydrogen bonds.

However the hydrogen bonds can be weakened as the temperature of the suspension is raised. When this happens, water absorption becomes possible and the starch granules swell. This process is called gelatinization.

2. MALTING

The process of malting actually refers to all the changes that take place during the development of the seed into seedling. In which water is absorbed by the seed through the micropyle, thus causing the entire seed to swell up. The cells of the cotyledon become active and turgid and begin to make use of the water to dissolve and digest the food solution stored in them.

The solubilised foods reserved are then transported to the growing plumule and radicle. Malting also called germination is affected by a number of factors such as temperature, water, air, enzymes, and β amylase provide an appropriate medium for the degradation of the starch inside the grain into simple sugars such as glucose and maltose.

In addition to starch degradation, the reserved protein inside the seed is also degraded into appropriate mixture of polypeptide and amino acid by specific enzymes called protein degrading enzymes.

3. MATERIALS AND METHODS

3. 1. Collection of samples and preparation for analysis

The millet seeds (*Panicum Miliaceum*) were obtained from Kadamo in Jengre, Bassa Local Government Area of Plateau State. The feeds were winnowed to remove dust and pebbles. The seeds were ground into powder and stored in a dessicator for the analysis, but maize seeds were divided into two, one part ground with the chaff and the other part ground

after removing the chaff, while some millet seeds were kept aside for water uptake and malting quality analysis.

3. 2. Optimum water uptake determination

5.0g of millet grains was weighed at room temperature 25-28 °C and steeped in twelve (12) pre-weighed petri dishes for various hours from 12 hours to 144 hours. Each of the petri dishes was removed after pre-determined time interval of 12 hours and the water drained off and was allowed to dry at temperature of 25-28 °C for exactly 2 hours. The sample was then re-weighed and the increase in weight was then expressed as the percentage water uptake of the millet grains.

3. 3. Determination of optimum malting time

5.5 g millet grains were each placed into five different pre-weighed petri dishes containing filter paper inside them in order to keep the grains wet and were steeped at room temperature. The millet grains were allowed to germinate (i.e. green malting) for various number of hours by changing the water at time intervals of 16 hours. Each of the samples was milled after a time interval of 24 hours, 48 hours, 72 hours, 96 hours and 120 hours.

Each of the milled samples was transferred into 100 ml standard volumetric flask and acetate buffer solution pH 5.5 was added to each millet sample and hydrolysed at various temperatures of 30 °C, 50 °C, 60 °C, 70 °C and 80 °C respectively for 2 hours using thermostat water bath. The hydrolysed sample was then centrifuged and assayed for reducing sugar by Nelson Method.

3. 4. Determination of ash content

About 3.0 g, 4.0 g and 5.0 g of ground moisture free millet samples were placed inside pre-weighed porcelain crucibles. The crucibles were then placed in a muffle furnace and the temperature maintained at 600 °C for about 8 hours. The crucibles were then removed and reweighed. The weight of the residue was expressed as the % ash content of the millet samples.

Table 1 Determination of Ash Content.

S/No	weight of sample (g)	weight of empty crucible	weight of sample and crucible	weight before ashing	Weight after ashing	lost in weight	% ash content
1	3.00	19.4482	21.4482	21.4482	21.34%	0.0986	3.2867
2	4.00	19.5460	23.5460	23.5460	23.4168	0.1292	3.2300
3	5.00	19.1512	24.1512	24.1512	23.9874	0.1638	3.2760

% Ash Content = 3.26 ±0.03

3. 5. Determination of moisture content

5.0 g, 6.0 g and 7.0 g of ground moisture free millet samples were weighed into 3 pre-weighed dry clean small size beakers (50 ml) and were allowed to dry inside a hot air Gallen – kanp oven for about 24 hours maintained at a temperature of 105 °C. After drying, the samples were allowed to cool in dessicators for about 2 hours after which they were reweighed. The % loss in the weight was expressed as the moisture content of the millet.

Table 2. Determination of moisture content.

S/No	weight of sample (g)	weight of empty beaker	sample + beaker (before)	sample + beaker (after)	lost in weight	% moisture content
1	5.00	22.2043	27.2043	26.6492	0.5551	11.10
2	6.00	20.3392	26.3392	25.6288	0.7104	11.84
3	7.00	21.6546	28.6546	27.8724	0.7822	11.17

% moisture content = 11.37 ± 0.33

3. 6. Determination of crude fat

Ground moisture free millet samples of 8.0 g, 9.0 g and 10.0 g were weighed into 3 different pre weighed fat – free white cloths and wrapped separately. The wrapped samples were then weighed into 3 different fat – free thimbles. This was followed by extraction using petroleum ether (60-80 °C) for 8 hours after which the thimbles were carefully removed and allowed to dry at room temperature. The thimbles and the samples inside were reweighed after drying. The difference in the weight was expressed as the percentage crude fat content of millet.

Table 3. Determination of crude fat.

S/No	weight of sample (g)	weight of white cloth	weight of thimble	total weight before extraction	total weight after extraction	lost in weight	% crude fat
1	8.00	1.6724	9.6724	19.4748	19.3538	0.1210	1.513
2	9.00	2.0748	11.0748	21.0882	21.9865	0.1017	1.130
3	10.00	2.1678	12.1678	24.2724	24.1602	0.1122	1.122

% Crude fat content = 1.12 ± 0.2

3. 7. Determination of crude protein and nitrogen

0.5 g of ground moisture free millet samples were weighed on different ashless filter papers wrapped together and quantitatively transferred into 3 different Kjeldahl digestion flasks labeled A, B, C with the fourth flask labeled D, blank sample 0.2 g of the catalyst (mixture of anhydrous Na₂SO₄, CuSO₄ and SeO₂) was added to each flask followed by 10ml of concentrated H₂SO₄ and few pieces of anti-bombing granules was added to each of the flask. The Kjeldahl flasks were heated gently at first. As the fumes ceased, the heating is increased and would continue for about 2-4 hours after which a clear solution is obtained. The solution was allowed to cool and diluted with distilled water with the flasks immersed inside the bath. The diluted solution was then transferred into a 100 ml volumetric flask and made up to the mark for all the samples with distilled water. Kjeldahl distillation apparatus (Markehan still) was assembled over a Bunsen burner and 10 ml of the digested sample was transferred into the flask through the funnel connected by means of a spray bulb to the condenser. 20 ml of 40 % NaOH was added to the flask containing the digested sample while the tip of the condenser was deeped into 20 ml of 2 % Boric acid containing few drops of the indicator (mixture of methylred and methylene blue indicators) was added. Ammonia

released was passed through the condenser to the Boricacid which changed the colour from purple colour to green. The heating was stopped after about 75 ml of the solution was collected. The solution was then titrated against 0.05 M H₂SO₄ until the purple colour of the Boricacid reappeared. This is the end point. Percent protein was expressed according to the formula

$$\% N_2 = \frac{14(T_s - T_b) \times M \times 100 \times 2}{1000 \times 10 \times \text{weight of sample}}$$

where T_b = volume of the standard acid of blank

T_s = Volume of the standard acid in the titration

M = Molarity of the H₂SO₄

10 = Volume of the sample used

100 = Dilution factor used

14 = Atomic weight of Nitrogen.

Table 4. Determination of Crude protein.

S/No	weight of sample (g)	Titre value of sample (CM ³)	volume of sample- volume of blank	% Nitrogen	% protein
1	sample 0.5027	1.20	0.80	2.13	14.25
2	sample 0.5027	1.13	0.76	2.12	13.26
3	sample 0.5027	1.17	0.80	2.23	13.93

% Crude Nitrogen = 2.22 ± 0.08 Titre value %

% Crude protein = 13.87 ± 0.5 Blank sample = 0.

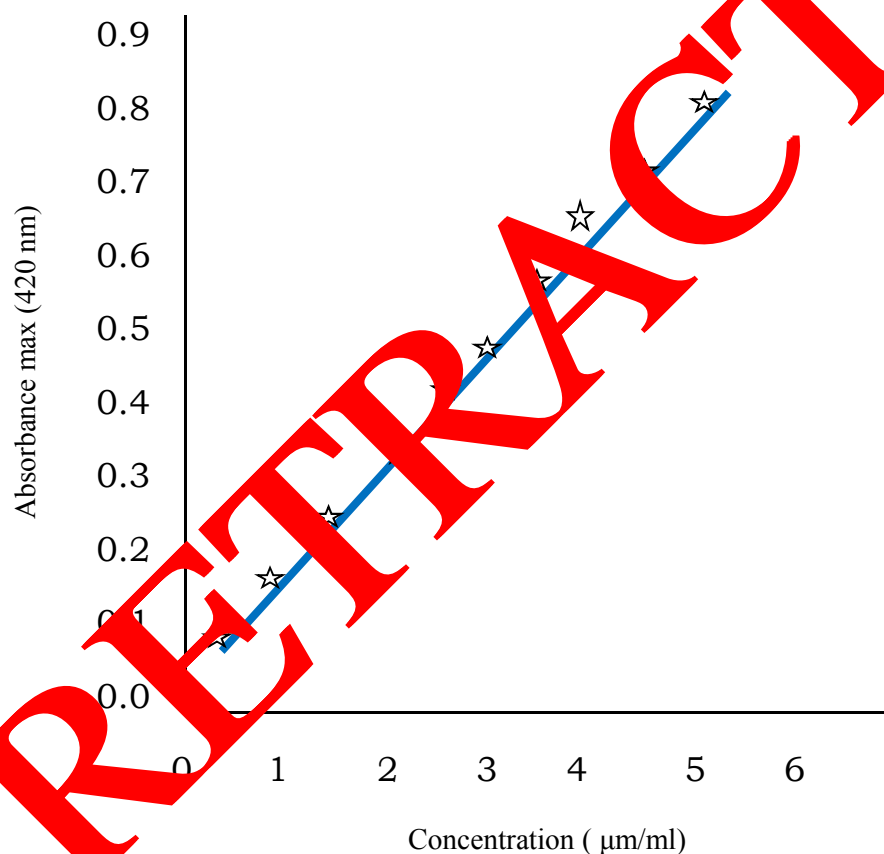
3. 8. Preparation of standard curve for total

Carbohydrate using D (+) glucose

0.1802 g Vacuum dried D (+) glucose was weighed and quantitatively transferred into a 100 ml volumetric flask and dissolved and made up to the mark with distilled water producing 0.01 M glucose solution (10 µm/ml). From the solution 5 ml were each pipetted and transferred into a 100 ml standard volumetric flask and made up to the mark with distilled water with the concentrations as 0.5 µm/ml, 1.0 µm/ml, 1.5 µm/ml, 2.0 µm/ml, 2.5 µm/ml, 3.0 µm/ml, 3.5 µm/ml, 4.0 µm/ml, 4.5 µm/ml and 5.0 µm/ml respectively. To each of the above standards, 1ml was taken and placed in a test tube standing inside ice bath and 5ml of L-Cystine, sulphuric acid was added in the ratio 1:5. The test tubes were then covered with aluminium foils and then heated inside boiling water for 3 minutes after which they were allowed to Cool to room temperature and the absorbance immediately taken at 420 nm using colorimeter. The reading of the absorbance was then plotted against the corresponding values of the sugar concentration.

Table 5. Standard curve values of D (+) glucose by L-Cysteine sulphuricacid method.

No of test tube	Dilution of glucose and dist. water (ml)	Standard conc. of sugar ($\mu\text{m}/\text{ml}$)	Absorbance max 420 (nm)
1	5/95	0.50	0.08
2	10/90	1.00	0.16
3	15/85	1.50	0.24
4	20/80	2.00	0.32
5	25/75	2.50	0.40
6	30/70	3.00	0.48
7	35/65	3.50	0.56
8	40/60	4.00	0.64
9	45/55	4.50	0.72
10	50/50	5.00	0.80

**Fig. 1.** Total carbohydrate by L – Cysteine sulphuricacid method.

3. 9. Determination of mineral content

2.0 g each of the ground millet sample was weighed and placed into two different crucibles and ashed at temperature of 600 °C for 8 hours. The crucibles were then removed after ashing and cooled in dessicators. The ash was quantitatively transferred into 150 ml beakers. To the ashes in the beakers, 10 ml of distilled water, 10 ml of concentrated perchloricacid, 10 ml of concentrated hydrochloricacid and 10 ml of concentrated

trioxonitrate (v) acid were added. The beakers were covered and heated at a temperature of 120 °C on a hot plate until the emission of brown fumes stopped. The sides of the beakers were rinsed with distilled water and then heated further to concentrate the solution to 5 ml. The beakers were then removed and cooled before adding 10 ml of concentrated HNO₃. To the solution, 5 ml of distilled water was added and boiled for 5 minutes. The solutions were then filtered into 100 ml standard volumetric flask and made up to the mark with distilled water. The solutions of the samples prepared were then used to determine the elements of interest using atomic absorption spectroscopy machine (AAS). The metals determined were; Ca, Fe, Se, Mg, Ni, Mn, K, Cd, As, Na, Pb, Cu, Zn, Al.

Table 6. Determination of mineral content.

S/No	Element	mg/L mg/100 g Sample/		dry weight		Mean value
		A	B	A	B	
1	Al	65.39	66.55	0.1367	0.129	0.1290
2	As	13.25	14.85	0.0277	0.0271	0.0274
3	Ca	331.0	335.0	0.6509	0.65102	0.6511
4	Cd	0.321	0.291	0.0007	0.0005	0.0006
5	Cr	5.501	5.551	0.0115	0.0101	0.0108
6	Cu	15.59	15.59	0.0326	0.0288	0.0307
7	Fe	535.9	537.5	1.1202	0.9681	1.0442
8	K	1112	1069	2.3265	1.9472	2.1369
9	Mg	4.9	4.7	0.8668	0.8246	0.8457
10	Mn	6.455	6.293	0.0135	0.0115	0.0125
11	Ni	87.83	90.89	0.1834	0.1656	0.1745
12	Ni	0.132	0.146	0.0003	0.0003	0.0003
13	Pb	N.D	N.D	N.D	N.D	N.D
14	Se	2.147	2.237	0.0045	0.0041	0.0043
15	Zn	18.45	17.44	0.0386	0.0318	0.0352

3. 10. Determination of optimum blending ration of malted millet and rice flour

Rice flour and malted millet were blended in the following ratio to make up 5g; 0.5: 4.5, 1:4, 1.5: 3.5, 2:3, 2.5, 3:2, 3.5:1.5, 4:1,

The rice flour was gelatinized first in 100 ml of 0.05 M acetate buffer pH 5.5 then cooled to a temperature of 50 °C before adding the malted millet. The solutions were then allowed to hydrolyse at a temperature of 50 °C for exactly 2 hours. Samples of the solutions

were withdrawn, centrifuged and assayed for reducing sugar by Nelson method, max 520 nm using a colorimeter.

Table 7. Determination of optimum blending ratio of malted millet and rice flour.

S/No	Weight of rice flour (g)	Weight of malted millet (g)	Absorbance at max 520 nm	Concentration mg/ml	% hydrolyted sugar
1	0.5	4.5	0.11	180	0.65
2	1.0	4.0	0.14	240	0.84
3	1.5	3.5	0.18	320	1.15
4	2.0	3.0	0.24	440	1.59
5	2.5	2.5	0.25	460	1.66
6	3.0	2.0	0.25	460	1.66
7	3.5	1.5	0.20	350	1.30
8	4.0	1.0	0.16	280	1.01
9	4.5	0.5	0.12	200	0.72

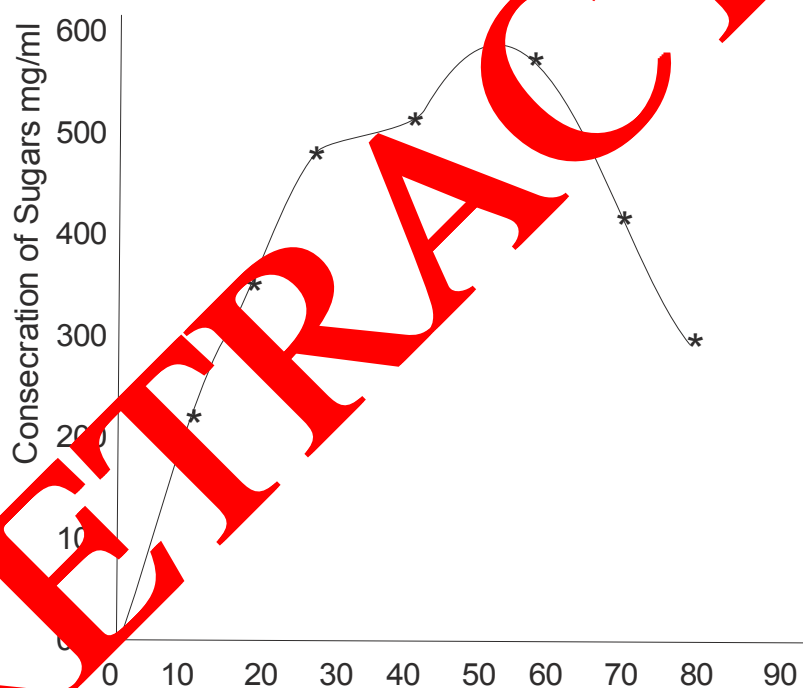


Fig. 2. Percentage of blended malted millet and maize flour without chaff

3. 10. Determination of optimum blending ration of malted millet and maize flour ground with the chaff (unprocessed)

Maize flour ground with the chaff was blended with malted millet in the following ratios to make 5 g; 0.5:4.5, 1.0:4.0, 1.5:3.5, 2.0:3.0, 2.5:2.5, 3.0:2.0, 3.5:1.5, 4.0:1.0, 4.5:0.5. The maize flour was gelatinized first in 100 ml of 0.05 M acetate buffer pH 5.5. It was cooled to a temperature of 50 °C before the malted millet was added. The solution was hydrolysed at a temperature of 50 °C using a thermostat water bath for about 2 hours.

Samples were withdrawn, centrifuged and assayed for reducing sugars by Nelson method max 520 nm using a colorimeter.

Table 8. Determination of optimum blending ratio of malted millet and maize flour ground with chaff (unprocessed).

S/No	weight of maize flour (g)	weight of malted millet (g)	Absorbance (520 nm)	concentration (mg/ml)	% hydrolyzed
1	0.5	4.5	0.08	120	0.43
2	1.0	4.0	0.12	200	0.72
3	1.5	3.5	0.17	300	1.08
4	2.0	3.0	0.20	360	1.30
5	2.5	2.5	0.22	400	1.45
6	3.0	2.0	0.20	360	1.30
7	3.5	1.5	0.17	300	1.08
8	4.0	1.0	0.14	240	0.86
9	4.5	0.5	0.10	180	0.58

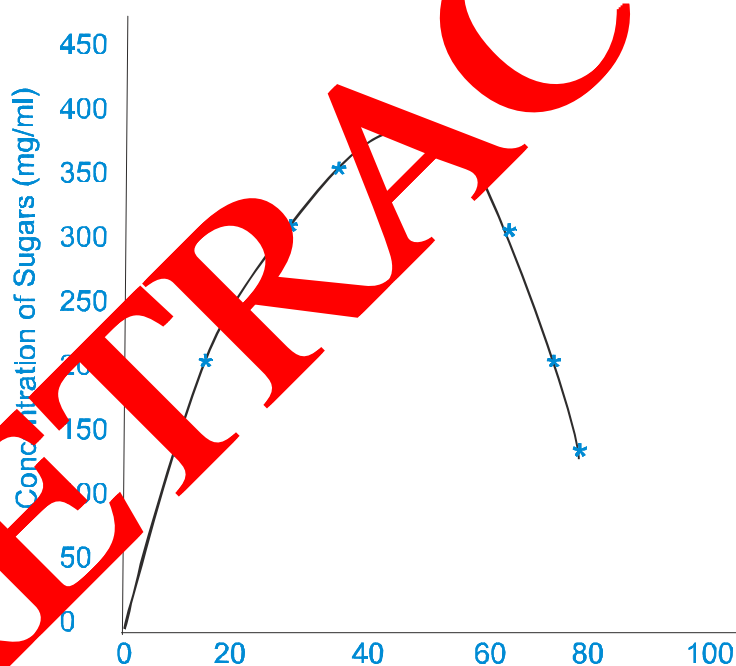


Fig. 3. Percentage of blended malted millet and maize flour with the chaff.

3. 11. Determination of optimum blending ration of malted millet and maize flour ground without the chaff

Maize flour obtained after removing the chaff was blended with malted millet in the following ratio to make up to 5g; 0.5:4.5, 1.0:4.0, 1.5:3.5, 2.0:3.0, 2.5:2.5, 3.0:2.0, 3.5:1.5, 4.0:1.0, 4.5:0.5, the maize flour was gelatinized first in 100 ml 0.05 M acetate buffer pH 5.5, allowed to cool to a temperature of 50 °C before malted millet was added, the solutions were hydrolyzed at a temperature of 50 °C using a thermostat water bath for 2 hours. Samples were withdrawn, centrifuged and assayed for reducing sugar by Nelson method using a colorimeter with max 520 nm.

Table 8. Determination of optimum blending ratio of malted millet and maize flour ground without chaff.

S/No	weight of maize flower (g)	weight of malted millet (g)	absorbance at 520 nm	concentration (mg/dl)	hydrolyzed
1	0.5	4.5	0.06	80	0.29
2	1.0	4.0	0.07	100	0.36
3	1.5	3.5	0.08	120	0.43
4	2.0	3.0	0.10	160	0.58
5	2.5	2.5	0.14	140	0.86
6	3.0	2.0	0.17	280	1.01
7	3.5	1.5	0.13	220	0.79
8	4.0	1.0	0.11	160	0.58
9	4.5	0.5	0.07	100	0.36

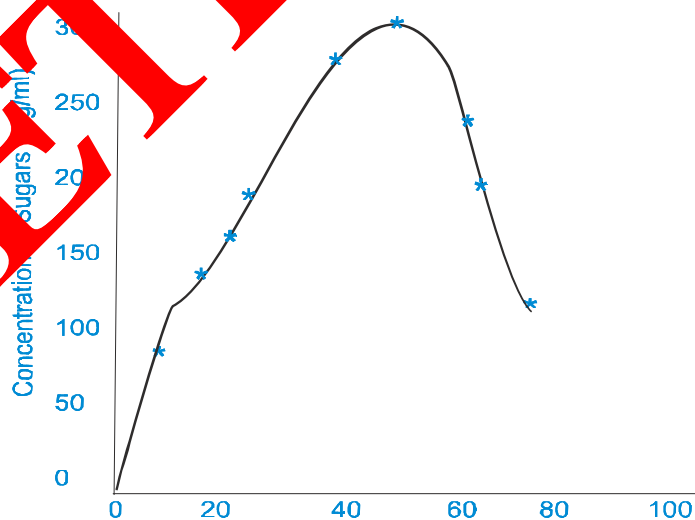


Fig. 4. Percentage of blended malted millet and maize flour without chaff.

4. DISCUSSION

The result of the water uptake of the millet shows that there was rapid absorption of water at the initial steeping time but shows no significant change at 96 hours. This shows that, at 96 hours there is maximum Saturation of water by the seeds. Results for the hydrolysis of malted millet for various hours (24-120) for temperatures between 30-80 °C shows that amylase activity was higher at 96 hours of malting time. The temperatures at which the amylase functions best was found to be 50 °C. At higher temperature, there was decrease in the activity of the amylase. This could be due to the denaturation of the enzymes. In Table 1, the ash content of the millet grains was determined to be 3.26 % \pm 0.03. This indicates a high mineral content in the millet grain. Table 2 shows that the moisture content of the millet is 11.37 % \pm 0.03. The moisture content of Cereals usually depends on factors such as harvesting time as well as storage conditions. Adequate moderation of these conditions is needed to prevent deterioration due to high moisture content especially insect attack and fungal growth. However, moisture content of 10-12 % is reported to be safe for grains intended to be stored for a long period of time. The Crude fat content shown in table 3 was determined to be 1.3 % \pm 0.2. generally, the total lipid (fat) content of cereals is about 5% of the dry grains. Though fats are useful nutritionally they can constitute a problem in the brewing industries. This is because the degradation of fatty acid may give rise flavor defect in beer and milk drinks. Table 4 shows the Crude protein of the millet to be 13.37 % \pm 0.5. In general, lower values of protein content is associated with cereals with higher carbohydrate content and vice-versa. Such cereals with higher carbohydrate content are said to produce extract which is rich in malt. The carbohydrate content of the millet was determined to be 71.46 % \pm 0.08. The reducing sugar content was found to be 35.08 % \pm 0.80 while the hydrolysable sugar was determined to be 54.19 % \pm 0.70. The lower values suggest that the use of acid hydrolysis could have resulted in the conversion of some carbohydrates to furans and pyrans. Tables 7-9 show the blending ratio of millet with gelatinized rice flour, maize flour with the chaff and maize flour with the chaff respectively. The values are 60 %, 50 % and 60 % respectively. The increase of 10 % of reducing sugar production of the gelatinized maize with the chaff compared to the one without the chaff could be due to the fact that chaff of the seeds contain some lignified materials such as proteins fats and fibre, which are found in the outer seed coat of the grains and could constitute hindrance to enzyme activity. Table 6 shows the presence of metals in the proso millet. Absence of lead metal (Pb) is an indication of the safety of consumption of the grain.

5. CONCLUSION

The research work shows that. Proso millet (*panicum millaceum*) is rich in protein, carbohydrate and also contains some important minerals such as calcium, potassium, magnesium and iron hence it is a good source of food and as an adjunct in the brewing industry.

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