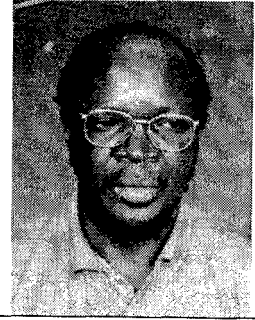


EFFECT OF MALTING ON PROTEIN DIGESTIBILITY OF SOME SORGHUM (*Sorghum bicolor*) VARIETIES GROWN IN KENYA

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ABSTRACT

Protein digestibility of sorghum is generally low. Malting is one of the processing methods which can be applied to improve this digestibility. It is a method whose technology is well known by local communities in Kenya. The objective of this study was to investigate the effect of malting on the digestibility of some varieties of sorghum grain grown in Kenya. Protein digestibility in the grain and malt was determined using porcine pepsin. In raw unmalted sorghum, the protein digestibility ranged from 0% in the high tannin varieties of Essuti, IS 8613, Nakhadabo and Seredo to 66.4% in the low tannin IESV 91022. Cooking decreased the digestibility of all the sorghum grain whose digestibility was above 0%, mostly the low tannin

varieties. When the sorghum grain was malted, the digestibility ranged from a minimum of 45.5% in Essuti to 88.7% in KM 1 in the raw sorghum. In the cooked malted sorghum, the digestibility ranged from 23.7% in Seredo to 100% in the low tannin varieties of KM1, IESV 91022 and KAT 386. There were significant differences ($P < 0.001$) in digestibility due to variety. The protein digestibility of very high tannin sorghum varieties increased with germination period between 72 and 144 hours during malting. Further investigation is required on the mechanisms through which malting influences protein digestibility.

Keywords: sorghum, malting, protein digestibility

L'EFFET DU MALTAGE SUR LA DIGESTIBILITE PROTEINIQUE DE CERTAINES VARIETES DE SORGHO (*Sorghum bicolor*) CULTIVÉES AU KENYA

RÉSUMÉ

La digestibilité protéinique du sorgho est généralement basse. Le maltage est l'une des méthodes de traitement qui peut être appliquée pour améliorer cette digestibilité. Il s'agit d'une méthode dont la technologie est bien connue par les communautés locales au Kenya. L'objectif de cette étude était de chercher l'effet du maltage sur la digestibilité de certaines variétés de graines de sorgho cultivées au Kenya. La digestibilité protéinique dans la graine et le malt a été déterminée en utilisant la pepsine porcine. Dans du sorgho cru sans malt, la digestibilité protéinique variait de 0% dans les variétés de haut tannin d'Essuti, IS 8613, Nakhadabo et Seredo à 66,4% de

bas tannin IESV 91022. La cuisson a diminué la digestibilité de toutes les graines de sorgho dont la digestibilité était au-dessus de 0%, surtout les variétés de bas tannin. Lorsque les graines de sorgho étaient maltées, la digestibilité variait d'un minimum de 45,5% en Essuti à 88,7% en KM 1 dans le sorgho cru. Dans le sorgho cuit et malté, la digestibilité variait de 23,7% en Seredo à 100% dans les variétés de bas tannin de KM1, IESV 91022 et KAT 386. Il y avait de grandes différences ($P < 0,001$) dans la digestibilité selon la variété. La digestibilité protéinique des variétés de sorgho de très haut tannin augmentait avec la période de germination entre 72 et 144 heures pendant le maltage. Des recherches plus approfondies sont nécessaires sur les mécanismes par lesquels le maltage influence la digestibilité protéinique.

Mots clés: sorgho, maltage, digestibilité protéinique.

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INTRODUCTION

Digestibility of sorghum protein is of immense interest, particularly to communities in Kenya and elsewhere who depend on sorghum as their staple food. In such situations, the cereal is often also the main source of dietary protein. Of even more importance, sorghum and finger millet porridge is widely used in Kenya as a weaning food for children, where it is again the main source of protein for such children. Improving protein digestibility in such situations is one way of alleviating protein-energy malnutrition, which is relatively high.

Sorghum's protein content is more variable than maize protein and can range from 7 to 15 % [1]. As is the case with other cereals, sorghum protein is generally low in lysine, which is its first limiting amino acid. To be of adequate nutritional quality for human beings, it therefore needs supplementing with lysine. For growing children, it may additionally require supplementation with threonine and methionine [2]. The average protein content of 522 varieties from world sorghum collection was found to be 12.61% [3]. Lysine was the limiting amino acid and its average content was found to be 2.1% of protein [3]. Threonine was found to be the second limiting amino acid [4]. This deficiency in essential amino acids (AA) is not unique to sorghum, but common to all cereals.

Sorghum protein is also unusually high in the nutritionally valueless prolamines [2]. This is an alcohol soluble cross-linked form of protein that humans cannot easily digest. It has been reported that prolamines account for 59 % of the total protein in normal sorghum [2]. This is higher than in major cereals, and it considerably lowers the protein digestibility and food value of sorghum considerably.

The protein digestibility of sorghum grain has been reported to be lower than that of other cereals such as maize, rice and wheat [5]. It has also been reported that cooking may further lower the protein digestibility of sorghum grain [6]. Some workers have concluded that the lower digestibility of cooked sorghum was due to the formation during cooking of a starch fraction that is resistant to digestion and to the content of the endosperm protein, kafirin, that binds with tannin [7].

Though differences in protein digestibility between sorghum grain and other cereal grains in man has been evident, it was observed that there was no measurable difference between the digestibility of any of these cereal proteins for laboratory animals such as weanling rats [5]. An *in-vitro* digestibility method using porcine pepsin was developed that gave results which were more similar to human feeding test results than were those from weanling rats [8].

A few varieties of sorghum that have relatively low prolamines levels have been identified. Some of these are grown in Ethiopia and Sudan. Two varieties found in Ethiopia contained over 30% protein, and had about twice the normal level of lysine [9]. The grains of these sorghum varieties were roasted over a fire, and they were then eaten like peanuts. In Sudan, a remarkable sorghum variety called Karamaka also has high protein content, and its protein is of high nutritional value [1]. Its lysine content is 62 % above that of ordinary sorghum. Its protein has a chemical score of 62 rather than the 30 to 40 figure of regular sorghum protein [3].

The existence of these high protein quality sorghum grain types provides an opportunity of selecting and breeding for this type of grain on a larger scale. There could also be the opportunity of improving the protein content and quality through biotechnology.

The problem of low protein digestibility can, however, be partly solved through processing. Several ways of improving sorghum protein digestibility have been reported [5]. One such method involves cooking sorghum in the presence of an appropriate reducing agent. This method increased protein digestibility by 25% [10]. In contrast, there was no improvement in the digestibility of barley, rice or maize when cooked in this way. The reducing agents found suitable included 2-mercaptoethanol, sodium bisulphite and L-cysteine.

The use of decortication and low cost extrusion processing has also been reported to markedly improve sorghum protein digestibility [11]. Similarly, fermentation also improved the pepsin digestibility of the sorghum protein, though the effect varied with different varieties of sorghum [12]. Unfermented ugali (a thick paste prepared by mixing maize, sorghum, millet or cassava flour in hot water) in East Africa is of comparatively low digestibility [13]. Aliya and Geervani [14] found that the digestibility of fermented sorghum increased significantly, but not that of finger millet or pearl millet. Another report stated that fermentation of sorghum raised its protein digestibility from 59% to 65.5%, while it raised that of pearl millet protein from 74.8% to 85.5% [7].

Malting has been reported to be effective in raising the protein digestibility in sorghum [15]. One simple method practised in Ugandan villages is described by Mukuru [16]. It involves use of equal amounts of clean wood ash and water to make a slurry which is alkaline (pH 11). About one kilogram of grain is mixed with 150 ml of the wood ash slurry in a basket. The basket is then submerged in a well for 12 - 15 hours. Thereafter, the grain is then covered with grass, under which it germinates for 3 - 4 days. When the radicals are 2.5 - 4 cm long, the grain is dried in the sun, then pounded in a traditional way using a mortar and pestle.

Winnowing is then used to remove the dry ash and other chaff, while the remaining grain is ground to flour. This flour can be used to make thin porridge or beer. In both products, the tannin level is considerably reduced, and the digestibility increased.

Malt flour with the highest levels of digestibility as measured by soluble nitrogen, soluble sugars and thiamine was obtained from wheat, followed by maize, while sorghum had the lowest digestibility [17]. Dreyer [18], however, reported that malting increased the digestibility of sorghum protein by 7%. There was no comparable improvement shown on malting maize. Effect of malting on sorghum was attributed to the fact that the corneous protein matrix of the endosperm is more effectively digested by the phytoenzyme liberated during the malting than by the enzymes of the gastrointestinal tract (GIT).

This study aimed at determining the effect of malting on the digestibility of some of the varieties of sorghum grown in Kenya, using the *in vitro* porcine pepsin method. It also aimed at investigating the effect of germination period during malting on the protein digestibility of high tannin sorghum.

MATERIALS AND METHODS

Samples: Sorghum grain samples, harvested in 1998, were obtained from Kenya Agriculture Research Institute (KARI), Katumani Research Station. Both low and high tannin varieties were selected for digestibility tests. The low tannin sorghum varieties were Kari Mtama 1 (KM 1), Mahube, IESV 91022, KAT 412, KIB 3 and KAT 386. The high tannin sorghum varieties were Essuti, IS 8613, Nakhadabo, Seredo and Red Nyoni. They were kept in a cold room between 5°C and 10°C, to avoid deterioration and insect damage. The grains were cleaned using sieves before malting.

Nitrogen (protein): Determination of nitrogen in the sorghum grain and malt was done using the Kjeldahl method according to the AOAC [19].

Steeping and Malting: Malting of the sorghum grain was done as described by Gomez *et al.* [20]. About 100 g of sorghum grain of each variety was steeped for 24 hours, then germinated for 96 hours. The germinated grain was dried in an air oven at 50°C for 48 hours. The samples were kept in airtight bottles at between 5°C and 10°C.

Tannin Determination: This was done using the Vanillin Hydrochloric acid method [21,22].

Protein digestibility: This was done using the Porcine pepsin method [7] as adapted by Gomez *et al.* [20]. The initial protein content of the samples was determined using the micro-Kjeldahl nitrogen determination method

[19]. The second stage involved pepsin digestion, where 0.2 g of the sample was weighed in duplicate into centrifuge tubes. To determine the digestibility of a cooked sample, 2 ml of distilled water was added to the sample and shaken, then placed in boiling water for 20 minutes. This step was omitted for determination of protein digestibility of raw samples. To the cooked or raw sample, 20 ml of buffered pepsin solution was added and mixed thoroughly. A blank tube was prepared in a similar manner, but did not contain a sample. The tubes were placed in a water bath at 37°C, and shaken gently every 20 minutes for 2 hours. After this period, the tubes were placed in an ice bath for 30 minutes to attain a temperature of 4°C. The tubes were then centrifuged at 6 000 revolutions per minute (RPM) for 15 minutes. The supernatant was removed with a dropper and discarded. To each tube was added 10 ml of the buffered pepsin solution. It was then well shaken and centrifuged as before. The supernatant was removed and discarded again. Using a spatula, the residue was removed from each tube and placed in the centre of a piece of the filter paper on the Buchner funnel. Suction was applied to the filter flask, and the remaining residue was rinsed from the tube into the funnel using 5 ml of the buffer. The filter papers were rolled and inserted into Kjeldahl flasks. The flasks were dried in the oven for a minimum period of 15 minutes. In the Kjeldahl flask containing the dried filter paper and sample, 10 ml of concentrated H₂SO₄, 1 g potassium sulphate, and 1 ml of 10% copper sulphate solution were added. Digestion, distillation and titration were done as for the micro-Kjeldahl nitrogen determination. % Protein digestibility was calculated as follows:

$$(A-B)/A$$

Where A = % protein in the sample before digestion
B = % protein after pepsin digestion.

ANALYSIS OF DATA

Statistical analysis of the data was done to compare the protein digestibility of malted and unmalted sorghum grain. The protein digestibility of the different varieties of sorghum grain was also compared. Differences were considered to be significant between means when the probability that the differences occurred due to chance was less than 0.05 ($P < 0.05$).

RESULTS

Protein Digestibility of Unmalted Sorghum

Protein digestibility in unmalted sorghum grain is presented in Table 1. The digestibility was determined for both raw and cooked samples of 11 sorghum grain varieties. Six of these varieties - Kari Mtama 1 (KM 1), Mahube, IESV 91022, KAT 412, KIB 3 and KAT 386 were low tannin varieties. The other five: Essuti, Seredo, Nakhadabo, IS8613 and Red Nyoni were high tannin varieties.

In both raw and cooked samples, there were significant differences ($P < 0.001$) in protein digestibility among varieties. In the raw sorghum samples, the protein digestibility ranged from a minimum of 0% in the high tannin Essuti, IS 8613, Nakhadabo and Seredo varieties to a maximum of 71.2% in the low tannin KAT 412. All the high tannin samples, with levels of tannin above 2% catechin equivalents (CE), were found to have indigestible protein (protein digestibility of 0%). The observations imply that high tannin content may completely eliminate protein digestibility in unmalted sorghum grain. However, it was observed that Red Nyoni, with a tannin content of 1.3 % CE, had a relatively modest protein digestibility of 35.6%. Among the six low tannin varieties, the protein digestibility ranged from a minimum of 46.6% in Mahube to a maximum of 71.2% in KAT 412. This range is comparable to that observed by other workers such as Ejeta et al. [6], who reported a mean sorghum protein digestibility of 56%.

In the cooked unmalted sorghum samples, the protein digestibility was very much reduced ($P < 0.001$) when compared to that of the raw samples. The mean protein digestibility was 15.4%, and it ranged from a minimum of 0% in the high tannin varieties to 55.7% in IESV 91022.

Protein Digestibility of Malted Sorghum

The protein digestibility of the malted sorghum samples is shown in Table 2. The digestibility of ten malted sorghum grain varieties was determined. Five of these - KM1, KAT 412, Mahube, IESV 91012 and KAT 386- were low tannin varieties. The others; Red Nyoni, Seredo, Nakhadabo, IS 8613 and Essuti were high tannin varieties. The mean digestibility of the raw malted sorghum samples was 65.4%. The digestibility ranged from a minimum of 40.3% in Red Nyoni to a maximum of 88.7% in KM 1. Malting significantly increased ($P < 0.001$) protein digestibility. The increase was particularly pronounced in the high tannin sorghum varieties of Seredo, Nakhadabo, IS 8613 and Essuti. Each of these had a digestibility of 0% in the unmalted state.

The increase in protein digestibility after malting was even more dramatic in the cooked samples. These had a high mean digestibility of 69.2%. Three samples of the low tannin varieties had 100% digestibility. This implies that for some low tannin sorghum grain varieties, germination for 96 hours during malting may be adequate to obtain optimum protein digestibility. However, other low tannin varieties had relatively low protein digestibility. Mahube, for instance, had a protein digestibility of 33.6%. It was also observed that for malted samples, cooking increased the digestibility of the other four low tannin varieties except Mahube. This

was also true for the high tannin Red Nyoni. This is the reverse of what was observed in the raw samples. However, for the high tannin varieties other than Red Nyoni, cooking resulted in a decrease of protein digestibility.

Effect of Malting on Protein Digestibility of High Tannin Sorghum

The effect of germination period on protein digestibility of high tannin sorghum varieties is shown in Table 3. Three high tannin sorghum varieties were germinated for 72, 96, 120 and 144 hours. Before malting, the protein in all the samples was indigestible. The mean protein digestibility of the raw samples after 72, 96, 120 and 144 hours was 49.7%, 61.1%, 64.0% and 74.8%, respectively. The digestibility increased with germination time. It was also noted that Essuti, with a much higher tannin content than the other varieties, had a lower digestibility at all the germination periods.

The protein digestibility increased from a mean of 42.6% after 72 hours of germination to 60.9% after 144 hours of germination for the cooked samples. The digestibility increased with increasing germination period. However, the rate and extent of the increase between 72 and 144 hours for the cooked samples was much less than that observed in the raw samples. This was particularly true for Nakhadabo, whose protein digestibility increased by 11.7% between the two periods for the cooked sample, while it increased by 20% during the same period for the raw samples.

DISCUSSION

Cooking reduced protein digestibility in the sorghum grain. The reduction in digestibility due to cooking was particularly evident in the low tannin varieties. This indicates that the effect of cooking on reducing protein digestibility is not due to tannins. Bach Knudsen *et al.* [13] observed that the lower digestibility of cooked sorghum was due to the formation during cooking of a protein-starch fraction that is resistant to digestion, and to the content of the endosperm protein, kafirin, that binds with tannin. It has also been reported that when sorghum is cooked, the solubility of the protein is altered [8]. It was observed that the amount of the soluble kafirins was reduced from 42 to 6%, hence reducing the overall digestibility of sorghum protein [10]. The same researchers also observed that cooking sorghum caused the formation of high molecular weight disulphide linked polymers. These protein polymers formed by cooking may also contribute to limiting the protein digestion.

Sorghum has been reported to have higher levels of cross-linked proteins called kafirins (part of the prolamines) in comparison to other cereals. It has been

suggested that these type of proteins are responsible for the low protein digestibility of sorghum following cooking [1]. Sorghum contains about twice the quantity of the indigestible cross-linked kafirin than it does the soluble kafirin protein. This is in contrast with other cereals such as maize and pearl millet which contain more of the soluble kafirin protein than the insoluble fraction [23]. These proteins show only a slight decrease in protein digestibility after cooking.

Ejeta *et al.* [6] showed that the pepsin test estimated the digestibility of uncooked maize and pearl millet to be similarly high: 82 - 91%. The cooked grain maize and millet showed protein digestibility of 82 - 87%, while that for sorghum was only 56%. However, high lysine sorghum gave digestibility of 73% for the cooked grain. This difference between the two types of sorghum grain implied that the prolamine protein was the main source of protein indigestibility in normal sorghum. These researchers suspected that the problem lay in the formation of protein polymers linked by disulphide bonds.

Malting increased the protein digestibility of both raw and uncooked sorghum grain, though the increase was more pronounced in the cooked grain. The effect of malting on sorghum digestibility has been attributed to the fact that the corneous protein matrix of the endosperm is more effectively digested by enzymes released during malting than by the enzymes of the gastro-intestinal tract [18]. However, the increase in digestibility due to malting observed in these results is much more than the 7% reported by the same researcher. The results are also comparable to those reported by Mosha [15], who worked with two white low tannin and two brown high tannin Tanzanian sorghum varieties. He observed higher *in vitro* protein digestibility in the raw ungerminated low tannin varieties than in the high tannin varieties. However, in his case malting improved digestibility to a greater extent in the low tannin varieties than it did in the high tannin varieties.

Malting was particularly effective in increasing protein digestibility in the high tannin sorghum varieties. However, the rate and extent of increase differed among the varieties. For Nakhadabo, there was very little increase in protein digestibility of the cooked samples after 96 hours of germination. This indicates that prolonging the germination time beyond 96 hours may not have a significant impact on their digestibility. However, for Essuti the protein digestibility nearly doubled from 26.5% to 50.0 % when the germination period increased from 72 to 144 hours. There was also a steady increase in the digestibility between consecutive germination periods. This implies that for Essuti, it is probable that a significant increase in digestibility may be observed if the germination period is further extended beyond 144 hours.

The observations about the high increase in protein digestibility when high tannin sorghum grain is germinated are similar to those made by Ahmed [24]. This researcher reported that germination reduced the polyphenols and tannins during malting. One explanation for the reduction was that the tannins were released from the complexing moieties of either sugar or polypeptides by the malting process [25].

CONCLUSION

Unmalted sorghum grain varieties had low protein digestibility. Those with tannin levels above 2% CE such as Essuti, IS 8613, Nakhadabo and Seredo had protein digestibility of 0%. Cooking decreased the sorghum digestibility further, particularly for the low tannin varieties. There were differences due to variety in the protein digestibility of sorghum. Malting raised protein digestibility, particularly in the cooked high tannin sorghum varieties. The extent of the increase differed with varieties. It is therefore recommended that consumers use malted sorghum in the preparation of porridge or weaning foods for their children. Further work is required on mechanisms through which malting influences the protein digestibility in sorghum grain, since it was observed that grain with similar tannin levels differed significantly in their protein digestibility.

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Table 1
Protein Digestibility (PD) of raw and cooked unmalted sorghum grain

Variety	Protein (%)	Tannins (CE)	PD (%) Raw	PD (%) Cooked
KM1	11.5b	0.0L	53.9ab	6.0a
Mahube	11.3ab	0.0L	46.9ab	23.9ab
IESV 91022	12.2b	0.0L	66.4b	55.7b
KAT 412	13.2b	0.0L	71.2b	26.5ab
KIB3	8.8ab	0.1L	63.6b	13.6ab
KAT 386	10.0ab	0.2L	62.0b	4.0a
Red Nyoni	12.4b	1.3H	35.6ab	9.3a
Seredo	6.5a	2.3H	0.0a	0.0a
Nakhadabo	14.6b	3.8H	0.0a	0.0a
IS 8613	14.2b	5.4H	0.0a	0.0a
Essuti	8.9ab	13.8H	0.0a	0.0a
Mean	11.2±2.4		36.3±29.0	12.6±16.3

Values in columns 2,4 and 5 followed by the same letter, or without a letter, are not significantly different ($P<0.05$) from each other. However, they differ significantly with values that do not share a similar letter

In column 2: CE - Catechin equivalent L - Low tannin H - High tannin

Table 2
Protein Digestibility (PD) of raw and cooked malted sorghum grain

Variety	Protein (%)	Tannins (CE)	PD (%) Raw	PD (%) Cooked
KM1	11.5	0.0L	88.7b	100.0b
KAT 412	13.2b	0.0L	78b	82.7b
MAHUBE	11.3ab	0.0L	57.5ab	33.6a
IESV 91022	12.2ab	0.0L	73ab	100.0b
KAT 386	10ab	0.2L	82b	100.0b
Red Nyoni	12.4ab	1.3H	40.3a	89.5b
Seredo	9.7ab	2.3H	51.5ab	23.7a
Nakhadabo	14.6b	3.8H	74.0ab	66.9ab
IS 8613	14.2b	5.4H	63.8ab	57.7ab
Essuti	8.9a	13.8H	45.5a	37.5a
Mean	11.8±1.8		65.4±15.5	69.2±28.1

Values in columns 2,4 and 5 followed by the same letter, or without a letter, are not significantly different ($P<0.05$) from each other. However, they differ significantly with values that do not share a similar letter

In column 2: CE - Catechin equivalent L - Low tannin H - High tannin

Table 3
Protein digestibility (%) of high tannin sorghum at different germination periods (hours)

Variety	Tannin (%CE)	Period of germination for raw (R) or cooked (C) sorghum grain (hours)									
		0,R	0,C	72,R	72,C	96,R	96,C	120,R	120,C	144,R	144,C
Essuti	13.8	0.0	0.0	37.5	26.5a	45.5a	37.5	51.1	42.9	62.5a	50.0a
IS 8613	5.4	0.0	0.0	45.4	43.1ab	63.8ab	57.7	66.2	59.4	74.8ab	63.8ab
Nakhadabo	3.8	0.0	0.0	66.1	58.3b	74.0b	66.9	74.8	67.3	86.1b	69.0b
Mean		0.0	0.0	49.7	42.6	61.1	54.0	64.0	56.5	74.5	60.9
				14.8	15.9	14.4	15.0	12.0	12.5	11.8	9.8

Values in columns 5 to 12 followed by the same letter, or without a letter, are not significantly different ($P < 0.05$) from each other. However, they differ significantly with values that do not share a similar letter.