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Expanding the uses of phane, a nutritionally rich local food in Southern Africa

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Abstract

Dried mophane worms (phane) are a common but small part of the diet in Botswana. Proximate analysis of phane showed it to be a very good food source, with high protein content (approximately 50%) and a significant amount of fat (approximately 15%). The traditional method of preparing phane, which is degutting, cooking, salting and drying, should produce a product suitable for long-term storage. Therefore, we explored several ways to use phane as a high protein food component, with a view to promote its use year round. The most promising of these was the addition of phane to weaning food. Sorghum was mixed with phane at a ratio of 80:20 to give a protein mixture of about 18%, and with ditloo, bambara nuts and phane at 40:40:20 (sorghum:ditloo:phane) to give a protein content of about 20%. In the case of weaning food, microbiological quality becomes especially important. It was found that phane purchased from street vendors in Botswana was contaminated with coliforms/faecal coliforms at levels that might be acceptable when the phane was to be cooked further, but unacceptable for infant food. Therefore, a post-processing method to reduce these organisms to acceptable levels was tested.

Key words: Mophane worms, phane, *Acanthocampa belina* larvae, sorghum, *Sorghum bicolor*, marama bean, gemsbok bean, *Tylosema esculentum*, soybean, *Glycine max*, cowpea, V*igna unguilata*, bambara groundnut, ditloo, *Vigna subterranea*, enzymes, complementary (weaning) foods.

Introduction

Throughout human history, insects have served as a supplementary food source. The entire major insect orders, including Lepidoptera (moths and butterflies), Hymenoptera (bees and ants), Isoptera (termites), Coleoptera (beetles), Hemiptera (true bugs) and Orthoptera (locusts and grasshoppers) have been eaten, principally in Africa and Australia [9, 11]. The native people of southern Africa have used a number of insects as food, including caterpillars, locusts/grasshoppers, ants, termites and beetles [4, 11]. Many of these are consumed fresh at the time of harvest either because the small number collected don't warrant the effort of further processing or because they are too high in fat to dry and store well [11]. However, mophane worms, the larvae of *Acanthocampa belina* (formerly *Gonimbrasia belina* [10]) reach the appropriate stage for consumption in large number over a short period each year. Processing for preservation is therefore routinely carried out, by essentially the same method throughout the area.

The collected larvae are squeezed by hand to remove the intestinal contents, boiled with salt added, then dried by spreading in the sun, usually on the ground [4, 11]. The traditional method of preparation should result in a product that can be safely stored for long periods, especially if sufficient salt is added during cooking. Possible sources of concern are contamination of the caterpillars during the drying process, and insufficient drying or improper storage, which could lead to growth of spoilage organisms. In addition, the growth of mycotoxin–producing moulds might occur if the processed caterpillars were improperly stored.

Muller and Tobin [9] found that insects can provide useful amounts of protein (20-30%) and fat (from a low of 2-4% to as high as 30%). The nutritional composition values for some Lepidoptera insects have been reported. Mayhew and Penny [7] stated that silkworm pupae contain 14.2% fat and 23.1% protein. Quin [11] quotes a report of Richards that dried caterpillars from northern Zimbabwe contain 65% protein and 5% fat, and Platt that fresh caterpillars from Malawi contain 14% protein and 3% fat. He reported his own analysis of several caterpillars eaten fresh by the Pedi of the Northern Transvaal, in which protein ranged from 7.8 to 13.5% and fat (ether extract) from 3.3 to 13.2%. For dried mophane, he reported the following values: 19.1% protein and 51.1% fat (ether extract).

Studies of the traditional lifestyles of southern Africa in the middle of this century suggest that insects may have supplied a substantial amount of "animal" protein in the diet [4, 11]. Currently, however, many insects previously eaten in Botswana are no longer common in the diet. Mophane worms have become more of a delicacy, especially for the southern part of the country where they are not locally processed. Most people in this area do not eat the caterpillar, either because they find it objectionable (due to lack of exposure to it), or it is just not available. Given that insects are traditionally acceptable to the Botswana, it seems unfortunate that this potentially nutritious and relatively non-perishable food source is not being fully exploited.

Phane is seasonal, occurring only during the rains. Furthermore, it is only found in certain parts of the country, mainly in the Central and North East Districts. Current key issues are to find ways of increasing the yields per season, such that phane is available throughout the year. The South African animal feed market, which offers good prices for the caterpillar, largely affects phane availability. This competition with animal feed thus needs to be given the consideration.

Investigations on how phane could be better utilized in the local diet were carried out. Since it seemed likely to supply good amounts of "animal-type" protein (i.e. high quality), its usefulness as a protein-balancing addition for weaning food was tested. It is important to use cereals such as sorghum (which is a staple food in Botswana) in composite flours, and fortify these with locally available protein sources such as phane and legumes.The proximate analysis of phane, its amino acid profile, digestibility of phane:sorghum and phane:sorghum:ditloo mixtures were carried out.We examined phane for possible pathogenic and spoilage microorganisms that might make it unsafe or limit its storage life, particularly in the context of a child weaning food. Also post-processing treatment to reduce these organisms to acceptable levels, through treatment of the phane with vinegar, was tested.

Materials and Methods

Sample collection: Samples of fresh and dried phane were supplied or purchased from Francistown (first batch) and street vendors in the Palapye area (second batch) respectively. Each dry sample of 500 g was transported in a new plastic bag and stored at room temperature (25°C) until analysis was carried out. The fresh samples were cleaned immediately after they were received.

Microbiological analysis: Samples of dried phane, 50 g, were suspended in 500 ml (1:10) of Butterfield's buffer (0.31 mM KH_2PO_4, pH 7.2) and blended for 2 min at high speed to produce the homogenate for analysis. To aid homogenization, samples were soaked in the Butterfield's buffer for 5 minutes before proceeding with blending. Aerobic plate counts were enumerated by making pour plates of dilutions in the plate count agar. Yeasts and moulds were enumerated by making pour plates of potato dextrose agar containing 40 mg/ml chlortetracycline. *E. coli* and coliforms were estimated by the MPN technique and *E. coli* identified according to the Bacteriological Analytical Manual [3].

Two vinegar treatments were used to clean the phane. These treatments were 1:1 vinegar in water and 1:100 (1 tablespoon in a litre) vinegar in water. In both vinegar treatments, 50 g samples were weighed into a 250 ml wide mouth flask. A 1:1 (or 1:100) solution of vinegar and water was added to cover the sample. This solution had a pH of 2.5 (pH 4.5 for the 1:100), a pH level that has been shown sufficient to kill *E. coli* [5]. Each sample was submerged in the vinegar solution for 30 minutes with occasional swirling. The vinegar solution was then poured off and the sample rinsed with five changes of tap water. The analysis then proceeded as outlined in the preceding omitting the soaking step, since the samples were already slightly softened by exposure to liquid.

Chemical analyses: Samples were prepared for analysis by milling in a hammer mill (200 Lab Mill; 1.5 kW). Analytical grade reagents were used. These were purchased from 'Glassworld' and 'NT Laboratory Supplies', Johannesburg, Republic of South Africa. Proximate and sugar analyses were assayed by the A.O.A.C. [14] methods. Protein digestibility was done by an *in vitro* procedure[6]. Values for protein digestibility were calculated as the percentage of total nitrogen, which was digested by this process.

Amino acid analysis was performed at the University of Pretoria by the Department of Biochemistry. The PICO.TAG rapid method for amino acid analysis, which involves pre-column derivatization, was used.

The FAO/WHO recognized CODEX-approved Aflascan (Rhone-Poulenc Diagnostics Ltd) semi-quantitative method of total aflatoxin detection was used to determine levels of aflatoxins in phane. The kits' reliability was tested with a VICAM peanut paste standard (MICROSEP Pty Ltd). The enzymes used were alpha-amylase (BAN), neutral protease (neutrase) and amyloglucosidase (AMG). All powdered enzyme samples were supplied by NOVO-NORDISK, Denmark.

Results and Discussion

Microbiological quality: Tables 1a and 1b show results of microbiological analyses carried out on samples from the first batch of phane samples and the lab respectively. These analyses were intended to evaluate levels of microbial contamination in phane samples from different sources, presumably prepared and stored under different conditions and levels of hygiene. Sample 002, prepared and supplied by a villager in Kanye had no detectable presence of coliforms, including *E. coli*, and relatively low levels of aerobic microorganisms and yeasts and moulds. Samples 003 and 004 obtained from street vendors were grossly contaminated with aerobic organisms, coliforms, yeasts and moulds.

Samples 014 and 015 were collected fresh, degutted and washed with 1/100 ml vinegar before drying. Analysis of these samples showed no detectable levels of coliforms and low counts of aerobic microorganisms. There were no detectable levels of *Salmonella* and *Staphylococcus* spp. in all the variously processed samples 002, 003, 004, 014 and 015.

Compared to published standards, phane is most similar to meat that has been cooked, salted and dried, which may be eaten without further cooking. Published limits for microorganisms for meat treated as such are: APC, $1x10^5$; coliforms, $1x10^2$ and *Salmonella*, 0 per 25 g. The phane purchased from street vendors exceeds the APC and coliform limits, even when washed with vinegar, as in the case of sample 004. The source of contamination could not be determined from the analysis. Therefore, it could not be established with certainty the contamination was due to post-process drying and storage. The scope of the analysis could also not show whether the contamination was due to organisms on the surface, in the gut of the phane or both.

The work done on the second batch of dry samples of phane from the Palapye area (north of Gaborone,the capital) sought to measure the effect of two post-process treatments on the microbial load of the phane in respect of aerobic micro-organisms, total coliforms (including *E. coli*), yeasts and moulds. The two treatments were soaking in vinegar of two strengths, B (1:100) and C (1:1).

The results in Table 2, for samples 008, 009, 010, 011 and 013, show appreciable reduction in counts of the micro-organisms under test between untreated and treated samples. The reductions in the counts of aerobic microorganisms due to treatments B and C are generally close, so that it would be economical to use the lower strength vinegar for the post-process treatment for aerobic microorganisms. There is a marked difference in the effects of the two treatments in three of the five samples on the total coliforms, and in four of the five samples on the yeast and mould count.

Chemical quality: The proximate analysis of some raw materials found in Botswana is shown in Table 3. This table compares these values to those of phane. The protein content of phane is 55.25%, while morama and soya are in the 33% range, cowpea 22.9%, ditloo 17.3% and sorghum 10% respectively. Because phane is animal protein, it therefore follows that it is of good quality. Phane, morama and soya have a high fat content i.e. 15, 37 and 20% respectively. This makes these food samples, when they are in season, very important in the everyday diet of the people.

Table 4 shows the proximate data of possible mixtures with sorghum at about 12 to 20 % protein. Ideally, 17-18% will support the growth of young children. The presence of phane seems to increase the ash content as compared to that of sorghum. This can be attributed to the keratinized heads found in phane.

Tables 5-8 show the amino acid content of phane mixtures and compare them to the requirements of infants, pre-school children, school going children and adults. These requirements are age group dependent as noted in Table 5.

Dietary amino acid requirements

Phane: *Infants:* Table 6 shows that histidine, threonine and phenylalanine + tyrosine were adequate. Methionine + cystine were also within the range, though less than the average requirement. The rest of the essential amino acids were lower than the minimum requirements, which are also shown in the table.

Pre-school children (2-5 years): Histidine, threonine, phenylalanine + tyrosine, valine, isoleucine and tryptophan were adequate (Table 6). Methionine + cystine, although also adequate, were less than the average requirement. Leucine and lysine were lower than the minimum requirement. The lysine deficiency could be supplemented through the incorporation of a legume such as ditloo.

School children (6-12 years): All essential amino acids, plus histidine, cystine and tyrosine were adequate (phenylalanine + tyrosine actually more than 4.5 times the requirement). Tryptophan, on the other hand, was very inadequate, at less than eight times the requirement (Table 6).

Adults: For this category, phane showed excellent value, with all amino acids existing in adequate amounts (Table 6). Even tryptophan was in this case double the required amount (1.1 vs 0.5 g/100 g protein). Threonine and phenylalanine + tyrosine were outstanding, with values five times the requirement. Lysine was also very high, being three times the requirement.

Two-way mixture(s) sorghum:phane (80:20): *Infants*: Amino acids that were found to be in adequate amounts were threonine and phenylalanine + tyrosine (Table 7). Although histidine, methionine + cystine and leucine were lower than the average requirement, they were within the range of requirement. Valine, isoleucine and lysine were low and tryptophan very low (about a quarter of the requirement).

Pre-school children: This mixture had an essential amino acid profile in amounts adequate for this category, with the exception of lysine and tryptophan (Table 7).

School children: Table 7 indicates that all amino acids, except tryptophan, were adequate (tryptophan was very inadequate, being less than 20 times the requirement). Leucine was more than double the requirement, while phenylalanine + tyrosine were more than 4.5 times the needed amount.

Adults: For adults, all amino acid requirements were met, except for tryptophan, for which the requirement was met only up to 90%. With the exception of histidine, the rest of the amino acids was more than double the required amounts (Table 7).

Sorghum:phane (80:20) is comparable to sorghum:soya (60:40), despite a lower proportion of the protein source (phane) in the former. This emphasizes the superiority of phane over soya, which is pleasing to note, because phane is a locally available raw material and soya an import material. Sorghum:phane (80:20) favourably compares with the average household's proportion of cereal to relish in Botswana. The highest ash and fibre value in this mixture are desirable, coupled with a 3 times lower fat value than in the sorghum: soya mixture (i.e. 5.96 vs. 17.52%).

Three-way mixture(s) sorghum:ditloo:phane (40:40:20): *Infants:* In the three-way mixtures (Table 8), adequate amounts of amino acids were recorded for histidine, threonine, phenylalanine + tyrosine. Lower than average but within range of requirement values were for valine, metionine + cystine, leucine and lysine. Table 8 shows that isoleucine was low (2.8 vs. 3.72 g/100 g protein), while tryptophan was very low (about three times less than the lower limit (0.55 vs. 1.6 g/100 g protein). It can be deduced that this mixture was found to generally meet the requirements for infants, because even low amino acid values were mostly within the requirement range. A rather low value of tryptophan was recorded, i.e., 0.55 g/100 g protein, which is about 2-3 times less than the required amount.

Pre-school children: For this age group, all amino acid values were high, with the exception of tryptophan (0.55 g/100 g protein), being half the required amount for this group (Table 8).

School children: All amino acids except tryptophan were adequate. Phenylalanine + tyrosine were outstanding, being about 5 times the required amount (Table 8). Tryptophan was very inadequate, 16 times below the required 9 g/100 g protein.

Adults: All amino acids were adequate for adults. Most of them were more than three times the adult requirement (Table 8).

The results show that it would be desirable to supplement the lysine content of the mixtures with a legume component as found in the case of the sorghum:ditloo:phane (40:40:20) mixture. It can also be deduced from these results that the three-way mixture was adequate for people of 10 years age and older.

The complementarily of sorghum and ditloo would be particularly good in terms of lysine content, found at high levels in legumes but at lower levels in cereals, and methionine + cystine (the sulphur-containing amino acids), percentages of which are, on the other hand, high in cereals and low in legumes. The results obtained show that the highest level of the sulphur-containing amino acids (3.46 g/100 g protein) is found in the case of the sorghum:phane (80:20) mixture. This confirms the findings of Muller and Tobin [9] on the complementarity of cereals and legumes. Lysine, which is commonly limiting in foods, is usually used as a standard for determining the quality of proteins. However, the amino acid ratio, which is computed on the basis of the most limiting amino acid in a particular food, would in the above sample

Table 1a. Microbiological analysis of phane from the first batch of samples.

Sample number	Aerobic plate count colonies/g	Total coliforms MPN/g	Yeast and mould colonies/g	*E.coli* MPN/g	*Salmonella* spp. cfu/25 g	Coagulase positive Staphylococci
002 prepared in the lab	270	< 3	100	< 3	ND	< 3
003 street vendor	> 2.5 x 10^5	> 1100	240	9	ND	< 3
004 street vendor (washed with 1/100 vinegar)	> 2.5 x 10^5	> 1100	< 100	4	ND	< 3

Table 1b. Preliminary microbiological tests on phane prepared in the laboratory.

Sample	Aerobic plate count colonies/g	Total coliforms MPN/g
washed with (1:100) vinegar	< 250	< 3
washed with (1:100) vinegar	< 250	< 3

Table 2. Microbiological analyses of the second batch of phane samples.

Sample number	Treatment	Aerobic plate count cfu/g	Total coliforms MPN/g	Yeasts and moulds cfu/g	*E.coli* MPN/g
1	A	1.8 x 10^7	2.4 x 10^3	2.5 x 10^3	< 3
	B	4.6 x 10^6	> 1100	8.0 x 10^2	< 3
	C	2.8 x 10^6	< 3	< 100	< 3
2	A	6.8 x 10^4	23	3.2 x 10^4	< 3
	B	x 10^3	< 3	1.4 x 10^2	< 3
	C	2.6 x 10^2	< 3	1.5 x 10^2	< 3
3	A	1.6 x 10^7	> 1100	2.5 x 10^3	14
	B	3.6 x 10^2	240	1.5 x 10^2	9
	C	2.8 x 10^2	< 3	< 100	< 3
4	A	1.8 x 10^7	93	4.7 x 10^3	< 3
	B	5.9 x 10^4	43	7.3 x 10^2	< 3
	C	5.6 x 10^4	< 3	<100	< 3
5	A	4.5 x 10^4	93	2.1 x 10^2	< 3
	B	5.8 x 10^3	< 3	2.0 x 10^2	< 3
	C	3.9 x 10^2	< 3	< 100	< 3

Index to treatments: A – raw phane, dried
B – raw phane, dried and treated with 1-tablespoon vinegar/litre
C – raw phane, dried and treated with a 1:1 mixture of vinegar and water.

Table 3. Proximate analysis data for raw materials found in Botswana (Baseline data D.M.B.).

No.	Sample	Moisture %	Protein %	Ash %	Crude fibre %	Crude fat %
1	Phane	10.24	55.25	9.02	9.59	14.57
2	Marama	7.50	31.78	4.10	N/A	36.70
3	Ditloo	10.10	17.30	3.40	N/A	6.70
4	Cowpea	12.47	22.90	3.10	N/A	1.32
5	Soya	6.85	33.20	4.90	N/A	19.90
6	Sorghum	10.01	10.62	1.64	N/A	2.79

Key: CFb = crude fibre, Cft = crude fat, DMB = dry matter basis, N/A = not available

Table 4. Proximate analysis for mixtures (DMB).

Sample No.	Components	Ratio	Moisture (%)	Protein (%)	Ash (%)	Crude Fibre (%)	Fat (%)
1	Sor: Soy	60:40	13.20	18.79	2.47	2.32	17.52
2	Sor: Ph	80:20	11.12	17.11	3.31	2.74	5.96
3	Sor: Dit	50:50	9.47	11.76	1.73	1.28	6.43
4	Sor: Dit: Ph	40:40:20	9.45	20:50	3.56	2.54	8.30
5	Sor	100	10.01	10:62	1.64	N/A	2.79
6	Soy	100	6.85	33.20	4.90	N/A	19.90

Key: Sor = sorghum, Soy = soya, Ph = phane, Dit = ditloo

Table 5. Amino acid assay of sorghum/phane/ditloo mixtures (g/100g protein).

Amino acid	Phane	Sorghum: Phane (80:20)	Suggested pattern of requirement A {A"}	B	C	D	Sorghum: Ditloo:Phane (40:40:20)
Aspartic acid	8.58	8.35					9.89
Glutamic acid	11.67	16.20					16.46
Serine	8.42	7.69					7.95
Glycine	4.33	3.85					4.18
Histidine@	2.92	2.47	2.6 (1.8-3.6)	1.9	1.9	1.6	3.13
Arginine	4.74	4.72					5.79
Threonine*	4.60	4.40	4.3(4.0-4.5)	3.4	2.8	0.9	4.60
Alanine	4.71	6.59					5.75
Proline	4.51	5.99					5.33
Valine*	4.07	4.34	5.5 (4.4-7.7)	3.5	2.5	1.3	4.46
Methionine*	1.50	1.76					1.89
Total S AAs			}4.2(2.9-6.0)	}2.5	}2.2	}1.7	
Cystine	1.63	1.70					1.15
Isoleucine*	3.02	3.35	4.6(4.1-5.3)	2.8	2.8	1.3	3.72
Leucine*	5.03	9.12	9.3 (8.3-10.7)				8.28
Phenylalanine*	3.82	4.78	7.2(6.8-11.8)	6.6	4.4	1.9	5.52
Tyrosine	6.21	5.27		6.3	2.2	1.9	5.15
Lysine*	4.72	4.62	6.6 (5.3-7.6)	5.8	4.4	1.6	6.25
Tryptophan*_	1.10	0.44	1.7(1.6-1.7)	1.1	(9)	0.5	0.55

Key: * 'Essential' amino acids
@ Also required by children
_ Most limiting in all treatments
} Combined value
AA Amino acid

A Infant
Mean (A" = Range)
B Pre-School Child (2-5 yrs)
C School Child (10-12 yrs)
D Adult

be based on tryptophan. It was observed, though, that the tryptophan values were rather low (Table 5), and it would thus be misleading to base formulations on them. Hence, it would be advisable to base food formulations on lysine, which is highly limiting in cereals [1]. FAO [2] cites lysine, the sulphur-containing amino acids, threonine and tryptophan as the more important amino acids to consider in practical diet formulation, as they are often first-, second- or third-limiting in many food sources. Under the present circumstances, threonine is excluded, while the sulphur-containing amino acids are in very few cases second- or third-limiting. This suggests good quality protein in both phane and the mixtures. Of the limiting amino acids, the FAO report says that cystine, threonine and tryptophan usually disappear to a significant extent in the large intestine of a pig. It is therefore necessary to consider this when making food formulations for humans (who like pigs, are non-ruminants).

The sorghum:ditloo:phane mixture of 40:40:20 is good for the complementarity of the sulphur-containing amino acid. The protein quantity in all the above mixtures is more than 15 g protein/ 100 g required by the FAO [2].

Table 6. Amino acid adequacy – phane.

Amino acid	Actual amount	Required amount	Remarks
Infants			
Histidine	2.92	2.6 (1.8-3.6)	Adequate
Threonine	4.60	4.3 (4.0-4.5)	Adequate
Valine	4.07	5.5 (4.4-7.7)	Low
Methionine + Cystine	3.13	4.2 (2.9-6.0)	Lower than average, but within range
Isoleucine	3.02	4.6 (4.1-5.3)	Low
Leucine	5.03	9.3 (8.3-10.7)	Low
Phenylalanine +Tyrosine	10.03	7.2 (6.8-11.8)	Adequate
Lysine	4.72	6.6 (5.3-7.6)	Low
Tryptophan	1.10	1.7 (1.6-1.7)	Low
Pre-school children			
Histidine	2.92	1.9	Adequate
Threonine	4.60	3.4	Adequate
Valine	4.07	3.5	Adequate
Methionine + Cystine	3.13	2.5	Adequate
Isoleucine	3.02	2.8	Adequate
Leucine	5.03	6.6	Low
Phenylalanine +Tyrosine	10.03	6.3	Adequate
Lysine	4.72	5.8	Low
Tryptophan	1.10	1.1	Adequate
School children			
Histidine	2.92	1.9	Adequate
Threonine	4.60	2.8	Adequate
Valine	4.07	2.5	Adequate
Methionine + Cystine	3.13	2.2	Adequate
Isoleucine	3.02	2.8	Adequate
Leucine	5.03	4.4	Adequate
Phenylalanine +Tyrosine	10.03	2.2	Adequate (>4.5 times the requirement)
Lysine	4.72	4.4	Adequate
Tryptophan	1.10	9	Very inadequate (<8 times the requirement)
Adults			
Histidine	2.92	1.6	Adequate
Threonine	4.60	0.9	Adequate (5 times higher)
Valine	4.07	1.3	Adequate
Methionine + Cystine	3.13	1.7	Adequate
Isoleucine	3.02	1.3	Adequate
Leucine	5.03	1.9	Adequate
Phenylalanine +Tyrosine	10.03	1.9	Adequate (>5 times the requirement)
Lysine	4.72	1.6	Adequate ($\pm$ 3 times the requirement)
Tryptophan	1.10	0.5	Very inadequate (2 times the requirement)

Aflatoxin: The level of total aflatoxin was for both the sorghum: ditloo:phane (40:40:20) and phane 8-20 ppb, while that of the sorghum:phane (80:20) mixture was 4-8 ppb (Table 9). The lower level in the sorghum:phane mixture is due to the higher proportion of sorghum. Under dry conditions, sorghum normally does not harbour aflatoxin-producing moulds.

Protein digestibility: In general terms, digestibility values were high (above 95%). The digestibility value for M and N in Table 10 were lower. These low values could be due to the keratinized heads, posterior ends and the thorns covering the body of the caterpillars (phane). To ameliorate this phenomenon in the use of phane as a weaning food, the heads and posteriors could be removed before adding to sorghum. However, if this preliminary removal of heads and posteriors proves to be laborious, then fermentation of mixtures incorporating phane might be a possible option, judging from the high digestibility value obtained for ting (i.e. fermented sorghum 99.62%). The digestibility values thus compare very well with those of casein (99%) [2] and tsabana (99.4%) (Table 10).

The addition of enzymes to mixtures before cooking was done to increase the nutrient density and nutrient availability. Tsabana with enzyme was a case in point, with protein content as high as 28.7% on dry matter basis (Table 10). This occurred due to the fact that the enzyme made the porridge less viscous, thus more nutrients were dissolved.The starch was largely hydrolysed, resulting in a high total sugar content of 34.3% for the sorghum: phane (80:20) mixture, which had the highest level of starch (in the form of the sorghum, (Table 10). Adding enzymes to the mixtures prior to cooking resulted in a more runny porridge and did not necessarily improve the digestibility significantly. There appears to be no great difference between prototypes that were cooked with enzyme and those cooked without. Despite this, it should be noted that cooking in it did improve the digestibility of the mixtures. The practice of enzyme addition is appropriate when formulating weaning foods, which should be as "soft" as possible. From experience, it has been found that the more runny the porridge, the more objectionable it is to adults. Therefore, the current situation, whereby tsabana weaning food is being consumed by an increasing number of adults instead of the age 4-36 months target group, would be alleviated. Austin *et al.*[1] noted that weaning foods "are often watered down to stretch further, shared by the whole family, or used to substitute for rather than supplement a child's basic consumption."

The high digestibility values obtained for tsabana (not cooked 99.4%) outclass the digestibility values for the sorghum:phane (80:20) and sorghum:ditloo:phane (40:40:20) mixtures, which were 82.0 and 81.8%, respectively. This superiority is possibly due

Table 7. Amino acid adequacy – sorghum:phane (80:20).

Amino acid	Actual amount	Required amount	Remarks
Infants			
Histidine	2.47	2.6 (1.8-3.6)	Lower than average, but within range
Threonine	4.40	4.3 (4.0-4.5)	Adequate
Valine	4.34	5.5 (4.4-7.7)	Low
Methionine + Cystine	3.46	4.2 (2.9-6.0)	Lower than average, but within range
Isoleucine	3.35	4.6 (4.1-5.3)	Low
Leucine	9.12	9.3 (8.3-10.7)	Lower than average,but within range
Phenylalanine +Tyrosine	10.05	7.2 (6.8-11.8)	Adequate
Lysine	4.62	6.6 (5.3-7.6)	Low
Tryptophan	0.44	1.7 (1.6-1.7)	Very low
Pre-school children			
Histidine	2.47	1.9	Adequate
Threonine	4.40	3.4	Adequate
Valine	4.34	3.5	Adequate
Methionine + Cystine	3.46	2.5	Adequate
Isoleucine	3.35	2.8	Adequate
Leucine	9.12	6.6	Adequate
Phenylalanine +Tyrosine	10.05	6.3	Adequate
Lysine	4.62	5.8	Low
Tryptophan	0.44	1.1	Low (2.5 times less)
School children			
Histidine	2.47	1.9	Adequate
Threonine	4.40	2.8	Adequate
Valine	4.34	2.5	Adequate
Methionine + Cystine	3.46	2.2	Adequate
Isoleucine	3.35	2.8	Adequate
Leucine	9.12	4.4	Adequate (>2 times the requirement)
Phenylalanine +Tyrosine	10.05	2.2	Adequate (>4.5 times the requirement)
Lysine	4.62	4.4	Adequate
Tryptophan	0.44	9	Very inadequate (<20 times the requirement)
Adults			
Histidine	2.47	1.6	Adequate
Threonine	4.40	0.9	Adequate (± 5 times higher)
Valine	4.34	1.3	Adequate (> 3 times higher)
Methionine + Cystine	3.46	1.7	Adequate (2 times the requirement)
Isoleucine	3.35	1.3	Adequate (> 2 times the requirement)
Leucine	9.12	1.9	Adequate (± 5 times the requirement)
Phenylalanine +Tyrosine	10.05	1.9	Adequate (>5 times the requirement)
Lysine	4.62	1.6	Adequate (± 3 times the requirement)
Tryptophan	0.44	0.5	Inadequate (0.9 times the requirement)

Table 8. Amino acid adequacy – sorghum: ditloo: phane (40:40:20).

Amino acid	Actual amount	Required amount	Remarks
Infants			
Histidine	3.13	2.6 (1.8-3.6)	Adequate
Threonine	4.60	4.3 (4.0-4.5)	Adequate
Valine	4.46	5.5 (4.4-7.7)	Lower than average, but within range
Methionine + Cystine	3.04	4.2 (2.9-6.0)	Lower than average, but within range
Isoleucine	3.72	4.6 (4.1-5.3)	Low
Leucine	8.20	9.3 (8.3-10.7)	Lower than average but just within range
Phenylalanine +Tyrosine	10.67	7.2 (6.8-11.8)	Adequate
Lysine	6.25	6.6 (5.3-7.6)	Lower than average, but within range.
Tryptophan	0.55	1.7 (1.6-1.7)	Low (± 3 times less than the requirement)
Pre-school children			
Histidine	3.13	1.9	Adequate
Threonine	4.60	3.4	Adequate
Valine	4.46	3.5	Adequate
Methionine + Cystine	3.04	2.5	Adequate
Isoleucine	3.72	2.8	Adequate
Leucine	8.28	6.6	Adequate
Phenylalanine +Tyrosine	10.67	6.3	Adequate
Lysine	6.25	5.8	Adequate
Tryptophan	0.55	1.1	Low (half the requirement)
School children			
Histidine	3.13	1.9	Adequate
Threonine	4.60	2.8	Adequate
Valine	4.46	2.5	Adequate
Methionine + Cystine	3.04	2.2	Adequate
Isoleucine	3.72	2.8	Adequate
Leucine	8.28	4.4	Adequate
Phenylalanine +Tyrosine	10.67	2.2	Adequate (±.5 times the requirement)
Lysine	6.25	4.4	Adequate
Tryptophan	0.55	9	Very inadequate (± 16 times less than the requirement)
Adult			
Histidine	3.13	1.6	Adequate
Threonine	4.60	0.9	Adequate (± 5 times higher)
Valine	4.46	1.3	Adequate (>3 times higher)
Methionine + Cystine	3.04	1.7	Adequate
Isoleucine	3.72	1.3	Adequate (± 3 times the requirement)
Leucine	8.28	1.9	Adequate (>4 times the requirement)
Phenylalanine +Tyrosine	10.67	1.9	Adequate (>5 times the requirement)
Lysine	6.25	1.6	Adequate (± 4 times the requirement)
Tryptophan	0.55	0.5	Adequate

Table 9. Sugar content of mixtures with added enzyme (DMB), and aflatoxin levels of phane-containing mixtures.

Sample	Ratio	Brix	Reducing sugar	Total sugar	Aflatoxin (ppb)
Sor: Soy	60:40				
Phane					8-20
Sor: Dit: Ph	40:40:20	27.40	7.95	18.21	8-20
Sor: Ph	80:20	31.60	13.13	34.34	4-8

Key: DMB= dry matter basis, Sor = sorghum, Ph = phane, Soy = soya, Dit= Ditloo

Table 10. Percent protein and digestibility values of tsabana and prototypes.

Parameter	Casein (Std) A	Sorghum B	TBN (Std) C	TBN (not cooked) D	TBN (moist-heated) E	TBN (Cooked ≥ 15min) F	TBN 250 g+BAN 2g +AMG 2 g +neutrase 1g (cooked 30min) G
% Protein	94.7	9.8 ϕ	15 (min)	15.8	15.4	18.6	28.7♣
% Protein dig'ty	99	♦♦♦ 60-65% dehulled 73% Fermented	Not Specified	99.4	98.8	99.6	99.8

Parameter	Ting H	Phane I	Ditloo J	Sorghum: Ditloo Cake (50:50) K	Ting and Ditloo (70:30) L	Sorghum: Phane (80:20) M	Sorghum: Ditloo: Phane (40:40:20) N
% Protein	17.2	55.3	17.0	17.3	15.4	18.2	21.8
% Protein dig'ty	99.6	To be done	75.0	61.7	99.8	82.0 (89.1 cooked with enzymes)	81.8 (90.6)

TBN = Tsabana = 17.0% protein at 60% moisture conten
t= Averaged from internal lab results
() = Values for cooked mixtures in brackets

to extrusion on the part of tsabana. This food processing technique is also known to improve flavour. Similar and cheaper applications such as roasting could therefore be lent to these mixtures as well.

It would appear from results obtained with ting:ditloo (70:30) that the traditional process of fermentation would serve purposes of digestibility improvement very well. The low digestibility values for dehulled sorghum (60-65%) and ditloo (61.7%; 75%) are enhanced by fermentation. Ting:ditloo (70:30) had a protein digestibility of 99.75%, exactly that the sorghum component in the phane mixtures tested would benefit from fermentation.

Conclusions and Recommendations

Phane is contaminated with aerobic microorganisms, coliforms, yeasts and moulds, and this depends on the methods of preparation and storage. The microbial load of contaminants in phane can be effectively reduced by vinegar treatment during processing and in the post-processing stage. Phane has high levels of high quality protein. The sorghum:phane (80:20) and sorghum:ditloo:phane (40:40:20) mixtures are best suited for people that are of 10 years age and older.

i) Additional work to further validate the effects so far observed should be done using a greater range of vinegar strengths in order to optimize the efficacy of the vinegar treatments. This can also include variations in the soaking time. The scope of the work should also be broadened to include vinegar treatments during processing of fresh phane.

ii)The microbial loads in composites of phane and cereal mixtures for weaning food preparation should also be evaluated to establish the suitability of phane as a component of infant food mixtures.

iii) Further assays should be done with differing proportions of sorghum:phane:ditloo to optimize nutrient requirements for infants and pre-school children. The use of fermented sorghum should be tried in composite flour formulations.Ways of assuring an all-year availability of phane should be explored.

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