



OPEN Evaluation of the nutritional quality of food composites developed from local ingredients to target the needs of persons experiencing nodding syndrome in Northern Uganda

Bonny Aloka^{1,2✉}, Solomon Olum¹ & Duncan Ongeng¹

Undernutrition is widespread in developing countries and exacerbated by conditions such as nodding syndrome, with a prevalence of 4.5% in northern Uganda. To combat this problem, nutrient-rich formulae were developed using locally sourced ingredients. Preferred formulae were analysed for nutritional, physico-chemical, antinutritional, and functional properties using standard methods. The composites were developed from two main energy lines (maize and sorghum), with ingredients at ratios of 70:2:15:5:3:5 for maize/sorghum: silver fish: soybean: orange-fleshed sweet potato (OFSP): beans: chia seeds, respectively. Compared with the sorghum-based formula, maize-based formula had significantly higher amounts of selenium, potassium, and vitamins A and D; the bioavailability of iron and zinc; and in vitro protein digestibility. Unlike the sorghum-based formula, the maize-based formula also had lower levels of antinutritional factors and a better fatty acid profile ($p < 0.05$). The sorghum-based formula had significantly higher levels of ash, crude protein, magnesium, calcium, and vitamin B6 and a higher oil absorption capacity and bulk density. Whereas maize-based formula is good in levels of selenium, vitamins A and D, bioavailability of zinc and iron, protein digestibility and antinutritional factors, the sorghum-based formula is better in terms of crude protein, ash, oil absorption capacity and bulk density. Therefore, the choice of each formula should depend on the benefits targeted.

Keywords Composite, Functional property, Nodding syndrome, Northern Uganda, Nutritional quality

Malnutrition remains a major challenge in both developing and industrialized countries. Globally, pooled estimates show that the prevalence rates of overweight and obesity among children and adolescents are 14.8% and 22.2%, respectively¹. It is projected that approximately 167 million people worldwide will experience compromised health due to being overweight or obese by 2025. In addition, 47 million people are underweight, and 340 million suffer from micronutrient deficiencies worldwide². Although developing countries face a double burden of malnutrition, undernutrition outweighs overnutrition in its impact³. The prevalence is higher in Southeast Asia and Sub-Saharan Africa than in other regions^{4,5}. In Uganda, the national prevalence of undernutrition is 9% overall, with 14% among women of reproductive age (15–49 years) and men (15–54 years), respectively⁶. In northern Uganda, the prevalence of wasting was 9% among women, while Pader district recorded up to 12% prevalence of wasting among women of reproductive age⁷.

There is a link between malnutrition and nodding syndrome (NS). NS is a condition of unknown cause, characterized by frequent atonic seizures. It is complicated by tonic clonic, focal, myoclonic or atypical absence seizures; cognitive and motor decline; malnutrition; and behavioural and emotional difficulties⁸. It affects many people in Uganda and across other sub-Saharan African countries^{9–11}. The condition typically manifests

¹Department of Food Science and Postharvest Technology, Faculty of Agriculture and Environment, Gulu University, Gulu, Uganda. ²Department of Science and Vocational Education, Faculty of Education, Lira University, Lira, Uganda. ✉email: alokabonny@gmail.com

with undernutrition, both as wasting and stunting^{9,12,13}. In such cases, deficiencies in micronutrients such as selenium, vitamin D and vitamin B6 are common^{14–17}. The situation is worsened by high levels of food insecurity in the region, as most families feed on staples known to be less nutritious. A number of studies have reported significant association between NS and malnutrition prevalence, especially undernutrition, among affected individuals. For example, Spencer et al.¹⁶ reported a case-control study in the Kitgum district in northern Uganda, where more cases than controls were associated with vitamin B6 deficiency. Enyagu and Bazira¹³ reported that undernutrition was associated with NS cases. In this study, 100% were wasted, 90% were stunted and 78% were underweight. Significant factors associated with undernutrition being a lack of food, seizures at food and early weaning. A retrospective review of 32 NS children admitted to health facilities in northern Uganda revealed that moderate and severe undernutrition among cases improved following 13 months of regular nutritional, multivitamin and antiseizure supplementation¹⁸. Nodding syndrome, especially in parents, can nutritionally affect children. For example, Nyakato et al.¹⁹ reported that children born to mothers with NS had significantly poorer nutritional and growth outcomes than those born to NS-free mothers. A study conducted in northern Uganda by Kitara et al.⁸ also linked several anthropometric indicators, such as head circumference, which depended on the duration of NS sickness. In terms of minerals, the serum concentrations of potassium and sodium were lower in NS patients than in controls. Furthermore, a case series reported by Idro et al.¹² revealed that undernutrition, especially wasting and stunting, was associated with NS in Ugandan children admitted to Mulago National Referral Hospital.

Most of the interventions to manage nodding syndrome in northern Uganda have been primarily medical^{20,21}, although proper nutrition is crucial for treatment effectiveness²². Because nodding syndrome cases involve people who will be future parents, they require adequate and well-tailored interventions. Nutritious and acceptable foods for any nutritional condition can be formulated by combining locally available food items to meet particular nutritional needs of the target group^{23–25}. Northern Uganda is endowed with nutritious food items that, if used well, can address the nutritional needs of affected individuals²⁶. However, these locally available nutritious foods have been largely unexplored for use in the dietary management of NS. Furthermore, these foods are often consumed without value addition, which limits their application in managing medical conditions such as NS. In addition, the lack of value added to these nutrient-rich items means that these foods have a long preparation time, which consumes energy and makes them impractical as dietary interventions in resource-limited communities affected by NS. Despite the evidence linking NS with undernutrition, the literature on food-based approaches-particularly the development of nutrient-rich composites from locally available ingredients for development of nutrient-rich composites from locally available ingredients for dietary management of NS in poor communities-remains scarce in the context of northern Uganda.

Importantly, processing different food ingredients into a formulation can affect a number of product attributes. This includes the bioavailability of the target nutrients, due to interactions among the ingredients and their physicochemical and functional properties^{27–30}. As such, it is important to evaluate the quality attributes of newly developed food products before they are recommended for use by the target group. This study evaluated the nutritional and functional quality of locally developed food composites to target the nutritional needs of individuals with nodding syndrome in northern Uganda. The region is known to be agricultural and produces many food items despite having a high prevalence of NS, which is associated with malnutrition. For example, the seasonal food production level has been 55 metric tons (MT) for maize, 23 MT for millet, 25 MT for sorghum, 10 MT for beans, 16 MT for sweet potato and 6 MT for soybeans, in the Acholi subregion of Northern Uganda, where the study was conducted³¹.

Results
Proximate composition of the formulated composite

As shown in Table 1, significant differences were observed between the two formulations in moisture content, ash content and crude protein content. The moisture content of the maize-based formula was significantly higher than that of the sorghum-based formula ($p < 0.05$), at 8.92% versus 7.99%, respectively. On the other hand, the sorghum-based formula had a significantly higher ash content than the maize-based formula ($p < 0.05$), at 2.23% versus 2.08%, respectively. Similarly, for crude protein, the sorghum-based formula had a significantly higher value than the maize-formula, at 7.85% versus 7.45%, respectively. However, the main energy source did not

Parameter	Main energy source		t (p value)
	Maize-based formula	Sorghum-based formula	
Moisture (%)	8.920 ± 0.016	7.987 ± 0.022	58.470 ($p < 0.001$)
Ash (%)	2.079 ± 0.022	2.234 ± 0.013	−10.383 ($p < 0.001$)
Crude fat (%)	15.622 ± 2.869	12.292 ± 2.197	1.596 (0.186)
Crude protein (%)	7.449 ± 0.227	7.852 ± 0.062	−2.971(0.041)
Crude fibre (%)	1.050 ± 0.044	1.860 ± 0.660	−2.121(0.101)
Total carbohydrate (%)	64.881 ± 2.636	67.777 ± 2.860	−1.290(0.267)
Total energy (kcal)	429.919 ± 14.373	413.138 ± 8.382	1.747(0.156)

Table 1. Proximate composition of nutrient-dense food composites formulated from local food materials for nodding syndrome segregated by main energy source. Note: All the results are expressed as the means ± SDs ($n = 3$) and reported in different units depending on the parameter.

Parameter	Main energy source		t (p value)
	Maize-based formula	Sorghum-based formula	
Sodium (mg)	74.553 ± 0.479	74.370 ± 0.902	0.311(0.771)
Potassium (mg)	351.693 ± 1.213	314.377 ± 1.842	29.305(<i>p</i> < 0.001)
Magnesium (mg)	105.073 ± 1.747	115.827 ± 1.625	−7.806(0.001)
Calcium (mg)	134.522 ± 0.878	144.352 ± 0.876	−19.420(<i>p</i> < 0.001)
Phosphorus (mg)	269.443 ± 32.853	247.543 ± 33.878	0.807(0.465)
Zinc (mg)	2.073 ± 0.015	2.053 ± 0.015	1.604(0.184)
Iron (mg)	2.740 ± 0.020	2.763 ± 0.015	−1.606(0.184)
Copper (mg)	0.260 ± 0.036	0.270 ± 0.026	−0.387(0.718)
Manganese (mg)	0.433 ± 0.058	0.523 ± 0.023	−2.507(0.066)
Selenium (µg)	18.433 ± 0.252	17.833 ± 0.208	3.182(0.033)
Vitamin A (µg)	42.370 ± 0.415	36.177 ± 0.419	25.751(<i>p</i> < 0.001)
Vitamin D (µg)	334.793 ± 5.998	195.61 ± 32.771	7.236(0.016)
Vitamin B6 (mg)	21.147 ± 0.215	26.247 ± 0.598	−13.903(<i>p</i> < 0.001)

Table 2. Minerals and vitamin profile of nutrient-dense food composites formulated from local food materials targeting nodding syndrome cases in Northern Uganda. Note: All the results are expressed as the means ± SDs (*n* = 3) and reported in different units depending on the parameter.

Parameter	Main energy source		t (p value)
	Maize-based formula	Sorghum-based formula	
Phytate (mg/100 g)	0.495 ± 0.011	0.870 ± 0.008	−47.409(<i>p</i> < 0.001)
Total tannins (mg/100 g)	5.472 ± 0.330	8.125 ± 0.810	−5.253(0.006)
Total polyphenol (mg/100 g)	2.970 ± 0.502	19.595 ± 0.394	−45.118(<i>p</i> < 0.001)
Trypsin inhibitor (mg/100 g)	5.718 ± 0.689	18.430 ± 1.331	−14.693(<i>p</i> < 0.001)
Bioavailability of iron (%)	50.013 ± 1.324	22.920 ± 0.543	32.801(<i>p</i> < 0.001)
Bioavailability of zinc (%)	54.933 ± 0.850	33.533 ± 0.252	41.791(<i>p</i> < 0.001)
In vitro protein digestibility (%)	0.374 ± 0.001	0.350 ± 0	31.571(<i>p</i> = 0.001)

Table 3. Antinutritional factors, micronutrient bioavailability and protein digestibility of nutrient-dense food composites formulated from local food materials for nodding syndrome, segregated by main energy source. Note: All the results are expressed as the means ± SDs (*n* = 3) and reported in different units depending on the parameter.

significantly affect crude fat (*p* = 0.186), crude fibre (*p* = 0.101), total carbohydrate (*p* = 0.267) or total energy content (*p* = 0.157) when the two formulae were compared.

Minerals and vitamin contents of the formulated composite by main energy source

The micronutrient contents of the composites are presented in Table 2. The maize-based formula contained significantly higher potassium (351.69 mg/100 g) than the sorghum-based formula (314.38 mg/100 g) (*p* < 0.001). In contrast, the sorghum-based formula had significantly higher magnesium (115.83 mg/100 g versus 105.07 mg/100 g) and calcium (144.35 mg/100 g versus 134.52 mg/100 g) than maize-based formula (*p* < 0.05). However, there was no significant difference in the sodium and phosphorus contents between the two formulae (*p* > 0.05). For trace elements, a significant difference was observed only for selenium, where the maize-based formula had significantly higher amounts than the sorghum-based formula, with 18.43 µg/100 g versus 17.83 µg/100 g, respectively. There were no significant differences in zinc, iron, copper or manganese levels between the two formulae (*p* > 0.05). Compared with the sorghum-based formula, the maize-based formula contained significantly higher levels of vitamins A (42.37 µg/100 g versus 36.18 µg/100 g) and D (334.79 µg/100 g versus 195.61 µg/100 g) (*p* < 0.05). The sorghum-based formula, however, had a significantly higher level of vitamin B6 than did the maize-based formula (*p* < 0.05), at 26.25 mg/100 g versus 21.15 mg/100 g, respectively.

Antinutritional factors, micronutrient bioavailability and protein digestibility of nutrient-dense food composites formulated from local food materials

Table 3 shows significant differences in the levels of all antinutritional factors studied between the maize- and sorghum-based formulae. In this regard, the sorghum-based formula had significantly higher values of antinutritional factors than the maize-based formula (*p* < 0.05). The values were 0.87 mg/100 g versus 0.50 mg/100 g for phytate, 8.13 mg/100 g versus 5.47 mg/100 g for total tannins, 19.60 mg/100 g versus 2.97 mg/100 g for total polyphenols and 18.43 mg/100 g versus 5.72 mg/100 g for trypsin inhibitors. The proportion of bioavailable zinc was higher than that of iron. The proportion of bioavailable zinc in the maize-

Parameter	Main energy source		t (p value)
	Maize-based formula	Sorghum-based formula	
Oil absorption (%)	1.355 ± 0.591	3.002 ± 0.605	−3.373(0.028)
Water absorption (%)	1.338 ± 0.560	1.378 ± 0.571	−0.087(0.935)
Bulk density (g/cm ³)	0.779 ± 0	0.891 ± 0.032	−6.100(0.004)
Peak time (minute)	5.930 ± 0	3.777 ± 0.081	46.143(<i>p</i> < 0.001)
Pasting temperature (°C)	87.983 ± 0.757	81.583 ± 0.325	13.451(<i>p</i> < 0.001)
Peak viscosity (RVU)	861 ± 26.963	339 ± 13.454	30.005(<i>p</i> < 0.001)
Viscosity (RVU)	861 ± 26.963	292.333 ± 13.031	32.881(<i>p</i> < 0.001)

Table 4. Functional properties of nutrient-dense food composites formulated from local food materials targeting nodding syndrome cases, segregated by main energy food sources. RVU: Rapid Visco Unit; Note: All results are expressed as the mean ± SD (*n* = 3) and reported in different units depending on the parameter.

Fatty acid category (w/w)	Main energy source		t (p value)
	Maize-based formula	Sorghum-based formula	
Palmitic acid	0.113 ± 0.006	0.190 ± 0	−23.0(0.002)
Stearic acid	1.947 ± 0.167	1.957 ± 0.015	0.104(0.927)
Oleic acid	61.570 ± 1.732	4.903 ± 0.238	56.137(<i>p</i> < 0.001)
Linoleic acid	6.457 ± 0.309	1.857 ± 0.078	24.988(0.001)
Linolenic acid	50.883 ± 7.609	3.153 ± 0.189	10.861(0.008)
Saturated fatty acids (SAFAs)	2.063 ± 0.175	2.143 ± 0.012	−0.791(0.511)
Monounsaturated fatty acids (MUFAs)	61.570 ± 1.732	4.903 ± 0.238	56.137(<i>p</i> < 0.001)
Polyunsaturated fatty acids (PUFAs)	57.343 ± 7.298	5.007 ± 0.122	12.420(0.006)

Table 5. Fatty acid profile of nutrient-dense food composites formulated from local food materials for nodding syndrome by main energy source. Note: All results are expressed as the mean ± SD (*n* = 3) and reported in w/w of sample.

based formula (54.93%) was significantly higher than that in the sorghum-based formula (33.53%) (*p* < 0.05). In terms of iron bioavailability, the proportion of bioavailable minerals was also significantly higher (*p* < 0.05) in the maize-based formula (50.01%) than in the sorghum-based formula (22.92%). Finally, protein digestibility was significantly higher in the maize-based formula (37.4%) than in the sorghum-based formula (35%).

Functional properties of the formulated composites

Table 4 presents the results of the variations in the functional properties of nutrient-dense formulae from two main energy food sources, maize and sorghum. Compared with the maize formula, the sorghum-based formula showed significantly higher oil absorption (3.0% versus 1.36%) and bulk density (0.89 g/cm³ vs. 0.79 g/cm³) (*p* < 0.05). In contrast, the maize-based formula had higher values of peak time (5.93 min vs. 3.78 min), pasting temperature (87.98 °C versus 81.58 °C) and viscosity (861 RVU versus 292.3 RVU) than sorghum-based formula (*p* < 0.05).

Fatty acid profile of nutrient-dense food composites

As shown in Table 5, the fatty acid compositions of the two composites differed significantly in relative weight. The dominant fatty acids detected included palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). Compared with the sorghum-based formula, the maize-based formula contained significantly higher values of oleic acid, linoleic acid and linolenic acid. On the other hand, sorghum-based formula had significantly higher palmitic acid than maize-based formula (*p* = 0.002). There was no significant difference in the mean stearic acid composition when the two formulae were compared (*p* = 0.927). Finally, the maize-based formula contained significantly higher monounsaturated fatty acids (MUFAs) (62%) and polyunsaturated fatty acids (PUFAs) (57%) than the sorghum-based formula (*p* < 0.05). There was however, no significant difference when the composite formulae were compared for SAFAs (*p* = 0.511).

Discussions

Nodding syndrome is associated with undernutrition and deficiencies in essential nutrients such as iron, zinc, and selenium³². These deficiencies are attributed to the routine consumption of carbohydrate-rich diets³³, a common practice in northern Uganda. One approach to addressing these problems is to develop nutrient-rich food rations of public health importance. However, food formulations can negatively affect the levels and bioavailability of target nutrients due to interactions among different ingredients³⁰. This study evaluated the nutritional quality of nutrient-dense food composites formulated from local food materials that targeted the nutritional needs of nodding syndrome patients in northern Uganda.

Proximate composition is often used as an indicator of nutritional quality and shelf life in food products, which reflects their suitability for use³⁴. The low moisture content of the formulated formulae suggests good storage stability because they prevent mold growth and other chemical reactions²⁸. The moisture content of a food is influenced by factors such as the type of food, variety, and storage conditions³⁶. The moisture content of the maize-based formula was higher than that of the sorghum-based formula, probably because of the roasting of sorghum. This processing technique is known to lower the moisture content of cereal flours³⁷. However, both formulae had moisture contents within the acceptable limits for cereal flours, which is less than 10%. The ash content of food indicates its total mineral content such as iron, calcium, and potassium^{38,39}. The high ash content observed is likely due to the incorporation of ingredients such as silverfish and iron-rich beans which are known to be rich in minerals⁴⁰. The sorghum-based formula had a higher ash content than the maize-based formula, possibly because sorghum, the main ingredient, was malted and roasted, which positively impact the ash content^{41,42}. On top of that, sorghum not being dehulled unlike maize makes sense to have higher ash content since dehulling reduces the level of minerals which make up the ash⁴³. This implies that it could potentially supply more minerals than the maize-based formula. The crude protein level in both formulae was higher than previously reported values of approximately 7% and 4.2% obtained in sorghum and maize-based composites in Kenya and Uganda, respectively^{44,45}. The levels were, however, below other findings, which were above 10%^{46,47}. The high protein content of the two formulations could be due to silver fish, beans and soybeans. These all have high protein contents above 20%. Compared with the maize-based formula, the sorghum-based formula had a significantly greater level of crude protein. This is due to the reduction in fats and carbohydrates, as they are utilized during respiration as well as the synthesis of other amino acids⁴⁸.

In terms of minerals and vitamins, sorghum-based formula could contain relatively high levels of magnesium and calcium. This is because sorghum, the dominant ingredient, was not dehulled, unlike maize. Dehulling has been found to reduce the level of mineral nutrients in cereals, which could explain this observation⁴³. In addition, sorghum grain has been previously found to have higher levels of calcium than maize does, which is another probable explanation⁴³. However, the higher potassium level of maize-based formula than sorghum-based formula could be because malting has been found to significantly reduce the potassium concentration in cereal grains⁴⁹. Although the vitamin A content of cereal grains can increase during germination⁵⁰, maize-based formula (not malted) had higher vitamin A and D contents than sorghum. This was likely because roasting reduces vitamin D concentration in foods⁵¹. Carotenoids are concentrated in the corneous endosperm (74–86%), followed by the endosperm (9–23%) and germ (2–4%)⁵². Milling maize could increase the composite's carotenoid content, and thus its vitamin A level compared with sorghum-based formula^{53,54}. Compared with the maize-based formula, the sorghum-based formula resulted in significantly higher levels of vitamin B6. This could be due to the malting of sorghum, the main ingredient. Such a process results in the synthesis of a number of B vitamins⁵⁵. On the other hand, the maize-based formula had higher amounts of selenium than the sorghum-based formula. The differences could be due to the higher total selenium content of maize than sorghum, and maize contains more selenium than sorghum does⁵⁶. Selenium plays key protective roles against a wide range of diseases, such as cancer, cardiovascular disease, neurodegenerative disease, and infertility⁵⁷. This important trace element has been found to be deficient among nodding syndrome patients^{12,58}.

Although malting is generally known to reduce the antinutritional components of plant-based foods⁵⁹, the malted sorghum-based formula had higher levels of these components than the maize-based formula. This could be because maize flour is known to contain lower concentrations of tannins and phytic acid than sorghum flour does. This in turn, has lower concentrations of total polyphenols and trypsin inhibitor activity than maize flour does^{60–63}. In addition, maize was dehulled once. This processing technique is known to reduce the concentration of antinutritional factors, which are always concentrated in the maize bran⁶⁴. The levels of tannins and trypsin inhibitory activity observed in this study were higher than those reported in previous studies involving maize-soybean composites^{65–67}. This could be because the maize and soybeans in those formulations were steeped in water. This further reduced the composition of antinutritional factors. In contrast, the maize-based formula in the present study had lower phytate concentrations than those reported in the above studies, probably because of dehulling. The higher bioavailability of iron and zinc in the maize-based formula than in the sorghum formula could be due to the high level of antinutritional factors in sorghum. These factors are known to cause a significant reduction in the bioavailability of such micronutrients⁶². The bioavailability of iron and zinc obtained in this study is far higher than the values obtained from a previous study. That previous study involved a blend of millet-sesame-soybean, which also responded to nutrition gaps in northern Uganda⁶⁸. This could be because the present study included more ingredients rich in the two minerals mentioned above. For example, silver fish, which contain 2.6–10.7 mg/100 g of iron and 4.1–10.3 mg/100 g of zinc⁶⁹; chia seeds, which contain 7.7–24.4 mg/100 g of iron and 4.6–6.9 mg/100 g of zinc⁷⁰; and iron-rich beans, which contain 7.0 mg/100 g of iron⁷¹. The protein *in vitro* digestibility in the current study was, however, lower than values obtained from previous studies^{68,72}. This could be because, in previous studies, there were few ingredients with few protease inhibitors and food matrices, which could increase the bioavailability of protein, unlike in the current study.

The functional properties determine the suitability of a given food product for targeted end use⁷³. The oil absorption capacity indicates the rate at which proteins bind to lipids in food formulations³⁵. This makes flour suitable for improving flavour and mouthfeel when it is used in food preparation²⁸. The oil absorption capacity (OAC) of sorghum-based formula could have been higher than that of maize-based formula due to malting of sorghum. This processing technique is known to increase the OAC of sorghum, peaking on the third day⁷⁵. This increase in OAC during germination is likely due to the change in protein quality. During such a process, some amino acids are synthesized, and this protein promotes oil absorption⁷⁶. The OAC for the maize-based formula in this study is lower than the OAC obtained in a previous study²⁸ of different formulations: maize-millet, soybean-wheat, and rice-wheat at ratios of 70:30, 50:50, and 30:70, respectively. This could be due to the dehulling of maize in the current study, a processing step known to have a significant effect on OAC⁷⁷. However,

sorghum-based formula had higher OAC scores than other composite products with sorghum as the dominant ingredient⁷⁸. This implies that sorghum should have better organoleptic properties, such as mouth feel, flavour, and texture, which positively influence overall product acceptability⁷⁹. In foods, oil is usually bound to proteins, particularly amino acids²⁸. This may explain why sorghum-based formula had higher crude protein values than maize-based formula.

The bulk density, which measures the heaviness of flour, can be affected by the particle size and flour density⁷⁶. Dehulling of maize was previously found to decrease the bulk density from 0.76 g/mL to 0.65 g/mL⁷⁷. This could explain why the maize-based formula had a lower bulk density than the sorghum-based formula in the present study. However, the bulk density of the composites in this study was generally higher than that reported in other studies that investigated the bulk density of maize and sorghum malted flours^{74,78}. This is possibly because of the different treatments the ingredients in this study underwent. Unlike those two studies, the maize in this study was not malted. Even in the case of sorghum-based formula where malting was performed, it took only two days, unlike in some cases in those studies where malting was performed for three days. Notably, higher bulk density is desirable for nutrient-dense food composite targeting undernutrition as higher energy and nutrient density per volume is always the goal⁸⁰.

The water absorption capacity (WAC) determines the ability of flour or starch to hold or retain water against gravity²⁹. It is important in the bulking and consistency of products as well as in baking applications⁷⁹. The good water absorption capacity of composite flours can prove useful in the manufacture of products where good viscosity is required⁸¹. The WAC in both composite formulae in this study is higher than that obtained in a previous study⁷⁹, which ranged from 0.7% to 1.32%, probably due to differences in ingredient composition.

The peak viscosity indicates the water binding capacity as well as the ease with which the starch granules disintegrate, and it is correlated with the final product quality⁸². In other words, it is the maximum viscosity achieved after the temperature has risen above the pasting temperature⁸³. The peak viscosity is closely associated with high starch damage, which in turn enhances viscosity⁸⁴. The pasting temperature (PT) refers to the temperature at which the viscosity of starch begins to increase during heating. Hence, it is the minimum temperature required for cooking⁸⁵. The pasting temperature here is higher than the values previously obtained in other studies while using Rapid Visco Analyser (RVA), which were 74.28–76.67 °C and 73.48–75.10 °C⁸⁶, 70.00–80.33 °C⁸⁷ and 75.02–76.72 °C for different composites involving cereals. The high pasting temperatures in this study indicate the resistance potential of the ingredients against swelling. This could be correlated with the amount of starch (amylose and amylopectin) present in the flours^{86,87}. The high pasting temperature of flour is important for creating a strong and elastic dough and improving the texture and mouthfeel⁸⁷. This makes the products in the current study suitable for food applications as thickeners and gelling agents⁸⁸. Finally, sorghum-based formula had lower pasting temperatures than maize-based formula. This implies that it requires less energy to prepare. This is more suitable for resource-constrained beneficiaries, such as caregivers, for nodding syndrome cases. The higher pasting temperature and viscosity of maize-based formula than sorghum-based formula implies that it will require more energy or fuel to cook it unlike for sorghum-based formula. This is a significant barrier to its use by resource-limited households with nodding syndrome cases.

The peak time offers an indication of the minimum time required to cook the flour⁸⁶. The shorter peak time in the sorghum-based formula than in the maize-based formula could be due to malting. This processing technique has previously been found to reduce the peak time⁸⁹. This implies that maize-based formula require more energy to cook than sorghum-based formula do because the intermolecular bonds in maize need to be broken to achieve gelatinization⁹⁰.

In terms of the fatty acid profile, the maize-based formula generally had significantly higher monounsaturated and polyunsaturated fatty acids than the sorghum-based formula did ($p < 0.05$). Maize flour has been reported to have a lower fatty acid concentration than sorghum flour in previous studies^{66,91}. The sorghum-based formula could have had a lower fatty acid composition because of soaking and malting, which sorghum underwent⁹². Because it is rich in polyunsaturated fatty acids, the maize-based formula is preferable for supporting health and brain function, which is particularly important for NS patients^{93,94}. Unsaturated fatty acids are essential and play a number of functions in the human body. For example, oleic acid has been found to have a small blood pressure-lowering effect⁹⁵ and is likely to improve glucose control and insulin sensitivity⁹⁶. Furthermore, human cell membranes contain high amounts of linoleic acid. This is known to lower blood cholesterol and low-density lipoprotein (LDL) cholesterol concentrations, hence lowering cardiovascular disease (CVD) incidence and mortality⁹⁷. Linolenic acids, commonly found in crops such as chia seeds⁹⁸, are known to have cardiovascular and anti-inflammatory health benefits⁹⁹.

A summary of the difference between maize-based and sorghum-based formulae has been highlighted in Table 6. In summary, it can be noted that maize-based formula outperformed sorghum-based formula in key nutrition and anti-nutritional factors like levels of selenium, vitamins A and D, bioavailability of zinc and iron, protein digestibility and antinutritional factors. On the other hand, the sorghum-based formula performed better than maize-based formula in terms of crude protein, ash, oil absorption capacity and bulk density. Therefore, the choice of each formula should depend on the benefits targeted.

Conclusion and recommendations

Food formulations have previously been developed to address the nutritional needs of different population groups. However, such interventions remain underexplored for chronic conditions such as nodding syndrome (NS), which is strongly associated with undernutrition. This study evaluated the nutritional and functional quality of nutrient-dense food composites formulated from locally available foods to address the nutritional needs of NS patients in northern Uganda. The results demonstrate that both sorghum- and maize-based composites are suitable for NS patients due to their rich nutrient profiles. The maize-based formula performed better than sorghum-based formula in terms of minerals, fat soluble vitamins, antinutritional factors, fatty

Attribute	Maize-based formula	Sorghum-based formula
Minerals and vitamins	Superior in selenium, vitamins A and D	Has lower levels of selenium, vitamins A and D
Bioavailability of nutrients	Superior in the bioavailability of zinc and iron	Has lower bioavailability of zinc and iron
Protein digestibility	Has superior protein digestibility	Has lower protein digestibility
Fatty acid profile	Has superior fatty acid profile	Has lower fatty acid profile
Antinutritional factors	Has lower antinutritional factors	Has higher antinutritional factors
Crude protein	Has lower crude protein content	Has superior crude protein quality
Ash	Has lower ash content	Has higher ash content
Oil absorption capacity	Has lower oil absorption capacity	Has higher oil absorption capacity
Bulk density	Has lower bulk density (lower energy/nutrient density per volume)	Higher bulk density (higher energy/nutrient density per volume)

Table 6. Comparison of the key advantages and disadvantages of formulations designed to Meet the nutritional needs of patients with nodding syndrome in Northern Uganda

acid profile, protein digestibility and the bioavailability of zinc and iron. Conversely, the sorghum-based formula outperformed maize-based formula in proximate composition, water-soluble vitamins and functional properties. These differences are important for guiding public health practitioners in selecting appropriate nutrient-dense composites for managing NS. To maximize benefits of sorghum-based composites, strategies to reduce their high antinutritional factors, which limit nutrient bioavailability, should be explored. To guide future interventions, efficacy trials of these composites among the target population should be conducted to assess nutritional responses and determine optimal conditions for maximising their benefits. Future studies should also investigate how alternation in processing methods e.g., malting duration, and roasting parameters, can contribute to the reduction of the antinutritional factors from the food ingredients. This should be carefully done to preserve the important nutritional and functional benefits already highlighted in this study.

Materials and methods
Selection of food materials for composite development

The nutrient-dense formulations were developed from locally sourced food ingredients, including biofortified orange-fleshed sweet potato variety OFSP-NASPO 8 (*Ipomoea batatas*), soybean variety MAKSOY 3 N (*Glycine max*), silver fish (*Rastrineobola argentea*), iron-rich beans-NARO Bean 1 (*Phaseolus vulgaris*), maize of local variety (*Zea mays*), sorghum of local red color variety (*Sorghum bicolor*), and chia seeds-local variety (*Salvia hispanica*). These samples were chosen because of their nutrient profiles. For example, maize and sorghum mainly supply 358 kcal/100 g and 329 kcal/100 g of energy, respectively¹⁰⁰, whereas beans and soybeans, with approximately 45% protein¹⁰¹, provide a low-cost source of protein for households that cannot afford animal protein regularly. Soybeans with 25% fat were also supplied with fatty acids in the composite¹⁰¹. Silver fish with 53.35% protein⁶⁹ complemented proteins from beans and soybeans, as its amino acid profile is different from that of beans. Chia seeds with 66.6 µg/100 g of selenium⁷⁰, OFSP with 276.98 µg/100 g of β-carotene¹⁰² and iron-rich beans with 6.98 mg/100 g of iron⁷¹ provide rich sources of some of the most limiting micronutrients in the diets of the communities where most NS cases reside.

Treatment of ingredients

The maize and sorghum grains were cleaned following local practices to remove impurities and other unwanted grains. The sorghum was malted for 48 h to reduce antinutritional factors^{63,103}, sun-dried for 24 h and then oven-roasted. Maize was polished/dehulled once to reduce the aflatoxin concentration^{104,105} and improve acceptability as locally practiced. Soybean and chia were each cleaned and roasted in an oven to reduce antinutritional factors such as trypsin inhibitors¹⁰⁶ as well as to increase the protein, carbohydrate and ash contents of the product¹⁰⁷. Roasting also improves the organoleptic characteristics and overall acceptability of soybean products¹⁰⁷. Iron-rich beans were cleaned to remove foreign bodies and then kept in a dry place. Orange-fleshed sweet potato (OFSP) was peeled with a stainless-steel knife, washed, grated, and dried in sunshine on a clean tarpaulin for 48 h¹⁰⁸. Finally, the silver fish were cleaned and oven-roasted to reduce undesirable odours^{109,110} before use in the composite formulation.

Composite formulation

The ingredients were selected from different food groups to provide a nutrient balance. Theoretical formulations were established via Microsoft Excel to optimize the nutrient values of the different combinations. This was done with the aid of the food composition table developed by Harvest Plus¹¹¹ to match the nutrient content of selected foods with the intake recommendations for the target age group. The proportions were based on the recommended daily allowance (RDA) for macronutrients for the target group, specifying that 50–70% of daily energy should come from carbohydrates, 10–35% from protein, and 20–35% should from fats¹¹². Therefore, a total of 8 formulations were developed (3 for each energy line and a control purely maize or sorghum) with varying proportions of the ingredients (60:2:20:10:3:5, 65:5:3:15:5:7, 70:2:15:5:3:5) in the ratio of maize/sorghum: silver fish: soybean: OFSP: beans: chia seeds. The formulations are detailed in Table 7. These formulations were subjected to a preliminary sensory evaluation which led to the choice of one most preferred formulation of each energy line. This chosen formulation (70:2:15:5:3:5) was subjected to nutrient and functional analyses in this paper. The intention was to recommend it for uptake.

Product code	Order of ingredient proportion	Actual proportion, respectively (%)
205	Maize	100
715	Maize: silver fish: soybean: OFSP: beans: chia seeds	60:2:20:10:3:5
522	Maize: silver fish: soybean: OFSP: beans: chia seeds	65:3:15:5:5:7
270	Maize: silver fish: soybean: OFSP: beans: chia seeds	70:2:15:5:3:5
200	Sorghum	100
510	Sorghum: silver fish: soybean: OFSP: beans: chia seeds	60:2:20:10:3:5
420	Sorghum: silver fish: soybean: OFSP: beans: chia seeds	65:3:15:5:5:7
277	Sorghum: silver fish: soybean: OFSP: beans: chia seeds	70:2:15:5:3:5

Table 7. Variations in composite formulations designed to Meet the nutritional needs of patients with nodding syndrome in Northern Uganda

Laboratory analysis of the composite

The most accepted composites from both energy lines (maize and sorghum) were analysed at the National Agriculture Research Organization (NARO) institutes in Uganda; Namulonge and Kawanda Food Science laboratories. This involved the determination of physicochemical characteristics, proximate, mineral composition and antinutritional factors.

Proximate analysis

For the two most preferred composites, all the analyses were performed in triplicate according to AOAC standards as previously described by Ngoma et al.¹¹³ to determine the moisture content, crude protein, crude fat, ash, crude fibre, total carbohydrate, and crude energy contents. For moisture content, the crucible and food samples were weighed and placed in an oven at a temperature of 105 °C, and the sample was dried until a constant weight was achieved via an Oven plus II Gallenkamp model OPL225.WD1. C SG96/06/320, United Kingdom. Crude protein was determined via the Kjeldahl method according to method 981.10 of the AOAC International. Crude fat content was determined via the hexane Soxhlet extraction method as previously described¹¹⁴. In this case, a Soxhlet extraction machine with the specification 2055 SOXTECH Model Foss Tecator by Food Technology Ltd., Sweden, was used. The crude fibre content was determined following the method of Sani et al.¹¹⁵. The ash content was also determined following the method of Sani et al.¹¹⁵, using a Naber Furnace D-2804 muffle furnace model (Bremen, Germany). The total carbohydrate content (%) was calculated as: 100- (moisture + crude protein + crude fat + crude fibre + ash)¹¹⁶. Finally, total energy was determined following the AOAC standards above by multiplying the mean values of crude fat, crude protein and carbohydrate by 9, 4, and 4, respectively, and taking the sum of the products⁸⁶.

Macro- and micronutrient analyses

For minerals, calcium was determined via the flame photometric method as previously described by Slyke and Cleaver¹¹⁷, whereas phosphorus, iron, magnesium, and zinc were determined via the atomic absorption spectrophotometric method¹¹⁸. Selenium was analysed via high-resolution continuum source atomic absorption spectrometry¹¹⁹. The levels of vitamin D, phytic acid and tannins were determined via High-Performance Liquid Chromatography (HPLC), according to Poitevin¹¹⁸.

Determination of functional properties

The determination of bulk density followed procedures of Adeleke and Odedeji¹²⁰. To a graduated cylinder of 25 mL, 10 g of flour was added. The cylinder was then tapped until a constant volume was reached, which was then recorded. The bulk density was then calculated via the following formula:

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of the sample}}{\text{Volume of the sample after tapping}}$$

Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC) were determined following previous procedures¹²¹. Here, 2 g of flour was mixed with 10 mL of distilled water for 5 min with a magnetic stirrer. The mixture was then stirred at room temperature for 30 min and centrifuged at 3500 rpm for 30 min. After this, the volume of the supernatant was measured via a 10 mL measuring cylinder on each sample. The water absorption capacity was then calculated via the following formula:

$$\text{WAC (\%)} = \frac{\text{Weight of water bound} \times 100\%}{\text{Weight of the dry sample (basis)}}$$

To determine the Oil Absorption Capacity (OAC), 1 g of flour was mixed with 10 mL of sunflower cooking oil. The mixture was then stirred for 30 min at room temperature before being centrifuged at 4500 rpm for 10 min. After this, the excess water or clear supernatant was decanted and discarded. Finally, the OAC was calculated as the amount of oil absorbed per gram of flour sample via the following formula:

$$OAC(\%) = \frac{W_3 - W_2}{W_1}$$

where:

W_1 = weight of flour, W_2 = weight of empty tube + flour used, W_3 = weight of empty tube + flour + oil absorbed.

For the pasting property (peak viscosity, final viscosity, pasting time and temperature), a Rapid Visco Analyser (RVA), which was directly connected to a computer system, was used to record the parameters automatically¹²². This machine analyses the ability of a sample to form a viscous gel on the basis of its rheology under controlled conditions. In this case, 3 g of each sample was weighed, and 25 mL of water was added to form a suspension, which was then spun and heated under controlled time-temperature conditions, as described in a previous study¹²³.

Antinutritional factors

The antinutritional factors, such as trypsin inhibitors, phytate, tannins, and total polyphenols, were determined following a number of established procedures. For trypsin inhibitor analysis, the sample was sieved (No. 100, 150 μ m), and 1.0 g was extracted with 50.0 mL of 10 mM NaOH via a magnetic stirrer for 1 h. While a No. 50 sieve was used, extraction took 3 h, and the suspension was diluted for 30–70% trypsin inhibition. Reagents were added in sequence, and the assay was run in a 37 °C water bath. After 10 min of trypsin addition with mixing, the reaction was stopped with 1.0 mL of 30% acetic acid. The mixture was centrifuged or filtered, and the absorbance at 410 nm (A410S) was used to measure trypsin activity in the presence of the inhibitors¹²⁴.

In brief, the total phenolic content was measured by adding 50 mL of sample, 20 μ L of Na_2CO_3 solution and 20 μ L of Folin–Ciocalteu reagent to a 96-well microplate in triplicate. The mixture was left in the dark for 30 min, and the relative absorbance of the samples was measured at 765 nm in a spectrophotometer via Magellan™ data analysis software. Gallic acid was used as the reference standard, and the data were expressed as gallic acid equivalents (GAE), expressed as mg GAE/10 mL¹²⁵.

Tannin levels were determined according to Herald et al.¹²⁶ via the vanillin-HCl method. The reagents were prepared by mixing equal volumes of 8% HCl in methanol and 1% vanillin in methanol. A 0.2 g ground sample was extracted with 10 mL of conc. HCl in methanol, shaken for 20 min, and centrifuged at 2500 rpm for 5 min. Then, 1.0 mL of the supernatant was mixed with 5 mL of vanillin-HCl. The absorbance at 450 nm was measured after 20 min at 30 °C. A standard curve was used to express the results as catechin equivalents, which were corrected for the blank and reported as tannic acid equivalents as follows:

$$\text{Tannin}(\%) = \frac{Cx10}{\text{Sample weight}} \times 100$$

where:

C = Concentration corresponding to the optical density.

10 = Volume of the extract (mL).

For phytate analysis, 2.0 g of the sample was mixed with 50 mL of Na_2SO_4 -HCl solution (100 g/L Na_2SO_4 , 1.2% HCl) in a 100 mL conical flask and shaken for 2 h at room temperature. The supernatant was filtered, and 10 mL of the extract was diluted to 30 mL with distilled water after adding 1 mL of 0.75 M NaOH. The mixture was passed through an anion resin column (AG1-X4, 100–200 mesh). The column was washed, and phytic acid was eluted with 0.7 M NaCl. After FeCl_3 -sulfosalicylic acid reagent was added, the mixture was centrifuged, and the absorbance was measured at 500 nm via a spectrophotometer¹²⁷. Nutrient digestibility and bioavailability, especially for iron and zinc, were analysed according to Kiers et al.¹²⁸, where 5 g of sample was suspended in 30 mL distilled water and digested under simulated gastrointestinal conditions using amylase, lipase, pepsin, pancreatin, and bile. After digestion, the suspension was centrifuged at $3600 \times g$ for 15 min. The supernatant was decanted, pooled, and filtered through a 0.45 μ m filter. A blank sample (distilled water) was processed similarly. Both filtered supernatants were analysed for Fe and Zn via a Micro Plasma Atomic Emission Spectrophotometer (MP-AES 4200). The mineral content was corrected by subtracting the blank values, and soluble Fe and Zn were considered bioavailable. The percentage of soluble minerals was calculated as the bioavailability %.

$$\text{Bioavailability}\% = \frac{\text{Amount of Fe or Zn (supernatant)} - \text{amount of Fe or Zn (blank)}}{\text{Amount of Fe or Zn (undigested sample)}} \times 100$$

Lipid profile analyses

The fatty acid profile was assessed via High-Performance Liquid Chromatography (HPLC) as previously described¹²⁹. A 0.5 ± 0.001 g sample was weighed into a saponification flask and mixed with 7.0 mL of 0.5 M methanolic KOH. The mixture was incubated at 80 °C with periodic shaking for 1 h. The sample was washed five times with 14.0 mL of ultrapure water (HPLC grade). Dichloromethane (10.0 mL) was added, and the upper layer containing fatty acid methyl esters (FAMES) was collected and pooled. The extract was dried over magnesium sulfate and filtered, and volatiles were removed under reduced pressure. The dried extract was dissolved in diethyl ether, filtered through a 0.22 μ m PTFE filter, and transferred into a 1.5 mL amber vial for HPLC analysis as described by Carvalho et al.¹³⁰.

Data analysis

The laboratory data were analysed via Stata software version 16. Descriptive statistics such as the mean, standard deviation and median were used to present the proximate, nutritional, and physicochemical characteristics and functional properties of the composites. Independent-sample t-tests were used to establish significant differences in the means of the proximate, functional and nutritional properties of the maize and sorghum composite lines. All

the assumptions were tested before parametric tests were conducted. The differences were considered statistically significant at $p < 0.05$.

Data availability

All the data used and presented in this paper will be made available from the corresponding author upon reasonable request.

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Author contributions

B.A.: conceptualisation (lead) and writing original draft (lead). S. O: supervision (support), writing – review, and editing. D. O: supervision, writing, review, and editing.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

The study was approved by the Gulu University Research Ethics Committee (GUREC) with registration number GUREC-2022-258. After clearance, the protocol was registered with the Uganda National Council for Science and Technology (UNCST) with registration number A234ES, in compliance with the UNCST guidelines 2014 on the protection of human subjects.

Additional information

Correspondence and requests for materials should be addressed to B.A.

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