Nutrient and Anti-nutrient Profile of a Local Formula from Sorghum, Peanut, Honey and Ghee (Metu2) for Treatment of Severe Acute Malnutrition

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Abstract

World over, we are still struggling with persistent acute malnutrition levels; an estimated 17 million preschool children suffer from SAM, roughly the same figures as reported in 2013, a trend depicting insufficient progress towards the 2025 World Health Assembly. One such affected area is Karamoja Region in North Eastern Uganda. Partly, the trend could be attributed to unsustainable interventions like RUTF. Formulas from locally available foods could provide not only an affordable but also a culturally acceptable and effective home based solution. Locally available sorghum, peanut, honey and ghee in North Eastern Uganda, is such a potential local formula. The nutritional and anti-nutritional profile of this local formula(metu2) was compared to plumpy-nut. Standard official analytical methods were used. Proximate composition was comparable and within the WHO recommendations for therapeutic formulas. Local formula(metu2) had a comparatively high energy content, 528kcal/100g to 509kcal in plumpynut. Vitamin A and K contents were below the WHO recommendations in local formula while Na, Mg and essential fatty acids were comparable and within the contents needed for SAM recovery. Zn was comparatively higher in plumpy-nut but levels in both formulas were below the recommendations. Trypsin inhibitors, phytates and condensed tannins were higher in local formula while aflatoxins were within the limits but not for plumpynut. Though lacking in critical K, Zn and Vitamin A, local formula(metu2) was comparable to plumpy-nut and its efficacy to sustain recovery from SAM needs to be studied.

Keywords: local formula(metu2) (sorghum, peanut, honey & ghee), plumpy-nut, severe acute malnutrition (SAM).

1. Introduction

Malnutrition is still among the main public health challenges of the 21st century despite copious advances and improvements in child health over the last decade (Black et al., 2013). A sizable wide-reaching burden of wasting exists, particularly severe acute malnutrition (SAM; weight-for-height Z score [WHZ] <-3). World over, an estimated 52 million preschool children are wasted of whom; 17 million suffer from SAM (UNICEF, World Health Organization [WHO] & World Bank Group, 2017) roughly the same figures as reported in 2013, a trend depicting insufficient progress towards the 2025 World Health Assembly target. SAM presents with an amplified risk of mortality and morbidity. An estimated 400,000 child deaths are attributed to SAM annually; i.e. the risk of death is about 10-fold greater compared to children with a z-score ≥ -1(Black et al., 2013). Deaths from under nutrition are comparable to those resulting from infectious diseases (Black et al., 2013; Bryce, Boschi-Pinto, Shibuya, Black, & Group, 2005). Developing Sub Saharan Africa remains the most affected (Food Agricultural Organization [FAO], 2014). In majority of Sub Saharan Countries; South Sudan, Uganda, Kenya, Burundi and Malawi levels are consistently above the 10% emergency levels year in year out.

Management regimens for SAM have been available for some time, and programmatic evidence shows that they have been largely effective (WHO, 2013). For treatment of uncomplicated SAM cases (without need for stabilization, infections and fluid management), community based models using ready to use therapeutic foods (RUTF) are being applied (WHO, 2013) and programmatic evidence shows that they are as effective as the standard care (Bhutta et al., 2013). This along with the cost effectiveness, and wide reach, has made community based models to grow rapidly globally (Bhutta et al., 2013). Though largely effective, RUTF present with a number of challenges in developing country contexts. Ordinarily, RUTFs are expensive, not familiar to the local beneficiaries, making them un-sustainable in low income countries with recurrent under-nutrition. For example, a package of plumpy'nut costs US 6 cents, translating in to US$ 60 for a full two-month treatment of a child (Latham, Jonsson, Sterken, & Kent, 2011). Besides, costs associated with delivery and distributions are not included in the above estimate and these could push the figure further up. Program implementation is also affected by pipeline breakdowns. The set up franchises in strategic countries have done little to bring down the cost as some ingredients and packaging materials used are imported. For example in Malawi where RUTF has been produced using the same ingredients as in Plumpy'nut formulation, the milk powder cost constituted more than half of the final RUTF cost (Collins & Yates, 2003). Thus, without United Nations agencies and other International Non-governmental Organizations who are currently footing these costs, parents of affected children cannot afford RUTF's.

To address these challenges in Sub Saharan African Countries, community based solutions; where such therapeutic foods can be made in the community or at home using locally available foods are needed. This strategy could save costs, reduce child mortality, address sustainability gaps and improve efforts to effectively manage cases at community level. A number of studies, largely in Asian contexts have tested and found positive results as far as this strategy is in managing acute malnutrition. For example, for decades, Indian hospitals have successfully used local foods to come up with formulations to treat SAM (Latham et al., 2011). A topical systematic review by Schoonees, Lombard, Musekiwa, Nel, and Volmink (2013) did not find variances in clinical outcomes between SAM children treated using home-based RUTF and   standard diet, concluding that either RUTF or flour porridge can be used depending on availability, affordability and practicality. According to Collins et al. (2006), basic ingredients for RUTF production are; a staple food preferably a cereal, a protein source; plant or animal based, vitamin and mineral mix and an energy enhancer to increase energy density. Thus, with locally available foods, communities have the capabilities to produce their own RUTF. Though evidently possible, few studies have started potential of local formulations to counter SAM in Sub-Saharan African contexts. Therefore, in this study, we formulated a diet (METU-2) from locally available foods in North Eastern Uganda; sorghum, peanut, ghee and honey and its nutrient and anti-nutrient profile was compared to that of plumpy'nut.

2. Materials and Methods

2.1 Development of Local Food Product(metu2)

Formulation; Nutri-survey software, employing linear programming, was used for formulation of the local product, METU-2 (Erhardt, 2004). The composition (quantity of each food component) of the formulation was based on WHO/UNICEF/WFP/SCN draft specifications for therapeutic foods. METU-2 contained 39% sorghum, 35% peanut, 13% honey and 13% ghee.

Preparation/Processing of ingredients; raw materials for the formulation of metu2 were locally procured from North Eastern Uganda. Sorghum, groundnuts, ghee and honey were obtained from Moroto district, Karamoja region. Sorghum and groundnuts were sun dried for five days to moisture levels below 10%. Low density material, particularly leaf, damaged kernels and stalk in sorghum were removed by winnowing. Dirt free sorghum was then milled into flour. Groundnuts were hand sorted to remove damaged kernels, foreign matter and the shriveled kernels. The groundnuts were then dry roasted to a white roast for 30 minutes using a charcoal stove before grinding to a paste. Milk from Karamajong Zebu cows was traditionally processed by fermenting it for three days in pots. Fermented milk was thereafter churned in a jerry can by hand until fat globules accumulated on top. Accumulated fat globules (ghee) were then scooped off, washed to remove the whey, and then matured for one week to develop the flavor. The ghee was then boiled using a charcoal stove for 30 minutes to remove impurities.

Plumpynut: Plumpy nut, presently used by UNICEF to treat SAM in Moroto District, Karamoja Sub-Region was used as a comparator. This Plumpy nut is manufactured by Fabrique par: JB. 24, rue Radama 1 er, BP207, Antananarivo, Madagascar.

2.2 Proximate Composition Determination

Determination of Crude Fat

Fat was determined using AOAC (2000) method number 920.39. This involved using a soxhlet apparatus to extract the fat from the dried sample (3.00g) using 60ml of petroleum ether as the extraction solvent. The percentage fat was then obtained as the ratio of the extracted fat to the original sample weight.

Determination of protein

The amount of protein was determined using Kjeldahl's method according to AOAC (2000) method number 984.13. Samples were weighed (1g) and digested in concentrated sulphuric acid with one Kjeldahl tablet followed by distillation in 40% sodium hydroxide. The resulting solution was titrated with 0.1N hydrochloric acid using a mixed indicator (methyl red and bromocresol green).

Determination of moisture

Moisture was determined using the oven drying method as described by AOAC (2000) method number 925.40. Samples were weighed (5g) in dry petri-dishes and heated in an electric oven at $105^{o}C$ for 5 hours. Dried samples were cooled in desiccators, and the weight taken. The difference in weight was then obtained.

Determination of crude fibre

Crude fiber was determined according to the ISO (2000) method number 6865. Samples were weighed (1g) and transferred to crucibles. Petroleum ether (30ml) was added and mixture filtered under vacuum. The residue was dried and quantitatively transferred to a beaker. Sulphuric acid (150 mL) was added and the mixture boiled under reflux for 30 minutes. The solutions were quickly filtered under suction and residues washed thoroughly with water until acid free. Residues were transferred back to beakers, to which 150mL of KOH was added and solution boiled under reflux for 30 minutes and quickly filtered under suction.   Residues were washed with hot water until the rinsing was neutral. Residues were then thrice washed in acetone and transferred to crucibles. They were dried to a constant weight in an oven at $105^{o}C$ for 4 hours, cooled in a dessicator and weighed. Samples were then incinerated at $550^{o}C$ for 2 hours, cooled in dessicator and reweighed. Percentage crude fiber was then computed.

Determination of ash

Ash content was determined according to AOAC (1999) method number 972.15. Samples were weighed (5g) in dry crucibles, carbonized on a hotplate, and heated in a muffle furnace at $550^{o}C$ for 6 hours. Ash content was determined after cooling samples in the desiccators to ambient temperature.

Determination of carbohydrate and energy

Carbohydrate was determined by difference in ash, moisture, fat, crude fiber and protein while energy was calculated using assessed proximate composition and the corresponding Atwater factors.

Determination of mineral content

Mineral content was analysed using an atomic absorption spectroscopy as described by AOAC (2005b) method number 975.03. Samples (2 g) were digested with concentrated nitric acid and hydrogen peroxide. magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), sodium (Na) and potassium (K) were determined at wavelengths 317.9 nm, 285.2 nm, 259.9 nm, 324.7 nm, 213.9 nm, 589.6 nm, and 766.5 nm, respectively, using an air-acetylene flame. Sodium chloride and potassium chloride were used as standards for determination of Na and K. Standard solutions of magnesium oxide and ferrous ammonium sulphate were used for determining concentrations of Mg, Ca and Fe.

Determination of Vitamin A

Vitamin A was determined according to AOAC (2001) method number 2001.13. Samples were weighed (10g) in 250mL amber glass flat bottom round flasks. Ascorbic acid (0.5g) and ethanol (50mL) were added to the sample. A 0.5M sodium sulphite solution (4 mL) and KOH solution (10 mL) were added. Samples were saponified by boiling solution under reflux for 30 minutes. After hydrolysis, distilled water was added (20mL). Solutions were cooled to ambient temperature under a stream of cold water. Samples were transferred to 250 mL separation funnels. Flasks were rinsed with distilled water (10 mL) and diethyl ether (50 mL). Funnels were swirled and left to stand to allow phases to separate. Bottom layer was collected in a flask and diethyl ether phase transferred to a separation funnel. This extraction step with diethyl ether was done three times. Diethyl ether extracts were washed with distilled water (50mL) by inverting the funnel 5 times without shaking to avoid emulsions from forming. Diethyl ether extracts were drained in a clean flask by filtering over anhydrous sodium sulphate followed by rinsing of filter paper with diethyl ether (20mL). Sample extracts were concentrated by drying in

rotavapor at 40°C and then dissolved in n-hexane (10mL). Extracts were analyzed using thin layer chromatography (TLC Silica Gel F254 plate). Retinol ester was used for preparing reference solution. The reference solution contained 0.01 mg retinol/µl ester (3.3 International Units (IU) from each ester/µl) in cyclohexane. A mobile phase was a mixture of ether and cyclohexane (20:80 V/V) stabilized with 1 g/l solution of butylhydroxytoluene. About 3 µl of each solution was spotted on the plate. Spots were examined in ultraviolet light at 254nm. A principal spot from test sample was confirmed by corresponding with that of retinol in the chromatogram of reference solution.

Determination of fatty acids

Fatty acids profile was determined using gas chromatography (GC) (PerkinElmer, Norwalk, USA) in accordance with AOCS (1998) method number Ce 1b-89. Samples (10g) were mixed with chloroform (100mL) for 2 minutes with the Ultra-Turrax followed by centrifuging at 2000 rpm for 5 minutes. The mixture was filtered over a filter paper with anhydrous sodium sulphate and evaporated (20mL) under a stream of nitrogen at 40°C.   Fat (0.5g) was dissolved in diethyl ether (2mL). A mixture of potassium hydroxide (KOH) in methanol (MeOH) solution (0.5 mL) was added to the dissolved fat solution. To the soap solution, water (2mL) and hexane (15 mL) were added. The mixture was shaken and left to stand to allow phases to separate after which the top layer was decanted. The mixture was washed four times with water (2mL) to remove residual hexane. Samples were dried using anhydrous sodium sulphate. Dried samples were transferred to a GC-auto sampler vial. Samples and standards were run on the GC.

Determination of phytates

Phytates content was determined using the Anion-Exchange method as enlisted by AOAC (2000) method number 986.11. Phosphate was used as a standard. Samples were weighed (2g) and transferred to Erlenmeyer flasks to which 2.4% HCL (40mL) was added. The mixture was homogenized for 3 hours. Columns were prepared by adding resin (0.5g) into the columns. After forming, resin beds were washed with 0.7M NaCL and distilled water. Homogenized samples were filtered and the filtrate (2mL) transferred to 25mL volumetric flasks. The Na$_2$EDTA-NaOH reagent (2mL) was added and the solution diluted to volume with water. The solution was mixed and transferred to the column and the eluate discarded. Water (15mL) and 0.1M NaCL (15mL) were eluted through column and eluate discarded. A 0.7M NaCL (15mL) was eluted through the column and eluate collected in digestion flasks. A mixture of concentrated H$_2$SO$_4$ acid (1mL) and HNO$_3$ acid (6mL) were added to the flasks, and digested until active boiling ceased. After cooling, water (10mL) was added; flasks swirled and heated at low temperature for 10 minutes to dissolve the salt. The cooled solution was transferred to a volumetric flask (50mL), molybdate solution (4mL) and solfunic acid (2mL) were added. The solution was diluted to volume with water, left to stand for 15 minutes and absorbance read at 880nm using atomic absorption spectrophotometer (PerkinElmer, Norwalk, CT, USA).

Determination of condensed tannins

Tannins were determined according to Vanillin-HCL method (Broadhurst and Jones, 1978) using atomic absorption spectrophotometer (Perkin Elmer, Norwalk, CT, USA). A standard curve was prepared using catechin (Sigma-Aldrich Chemical, St. Louis, MO, USA). Samples (0.2 g) were mixed with 70% acetone (10 ml). The tubes containing dissolved sample in acetone were homogenized in a bath containing ice and water for 10 min and then centrifuged at 3800rpm at 4°C for 30 min. The supernatant (original extract) was transferred into another tube without disturbing the residue, kept on ice and away from sunlight. The original extract (0.05 ml) was transferred into tubes and made up to 0.25 ml with 50% methanol. Vanillin (1.5 ml) and concentrated HCL (0.75 ml) were added. The tubes were homogenized and incubated at room temperature for 10 min after which absorbance was read at 500nm against a blank.

Determination of trypsin inhibitor content

The casein digestion method was used for determining trypsin inhibitor (Kakede et al., 1969). Samples (4 g) were weighed, and defatted using petroleum ether. Defatted samples were weighed (1 g) in Erlenmeyer flasks and the phosphate buffer added (20 ml). Contents were shaken on a shaker for 1 hour followed by centrifuging at 5000 rpm for 5 min. Supernatant (1 ml) was transferred to a 50 ml volumetric flask and diluted to volume with phosphate buffer. Sample aliquots (0.5 ml) were transferred to test tubes and distilled water added to make 1ml. Stock trypsin solution (1 ml) was added to each test tube and tubes placed in a water bath at 37°C. Casein solution (2%, 1 ml) previously brought to 37°C was added and then incubated at 37°C for 20 min. The reaction was then stopped by adding 6ml of 5% trichloroacetic acid (TCA). Suspensions were thoroughly mixed on a vortex mixer and left to stand at ambient temperature for 1 hour. Suspensions were filtered, and the absorbance of filtrate and trypsin standards measured at 280 nm using atomic absorption spectrophotometer (PerkinElmer,

Norwalk, CT, USA).

Determination of aflatoxins

Aflatest Fluorometer (VICAM V1 #4, Watertown, MA, USA) was used for determining aflatoxin in accordance with AOAC (2001) aflatest method number 991.31. A mixture of sample (50g) and salt, NaCl (5g) were placed in a blender jar, to which was added methanol: water solution, 80:20 (100ml), and then blended for 1 minute. Extract was filtered through a fluted filter paper and 10ml of filtered extract transferred to a clean vessel, to which purified water (20 ml) was added and homogenized. Dilute extract was filtered through a glass microfiber filter and 1 ml (equivalent to 0.167 g sample weight) of it passed through aflatest-P-affinity column at a rate of 1 to 2 drops per second, followed by 2ml purified water, 1ml at a time. The column was eluated with 1 ml HPLC grade methanol and sample eluate collected in a glass cuvette. Aflatest developer solution (1 ml) was added to eluate, mixed and then placed in a fluorometer to measure aflatoxin content.

Statistical analysis

Data was analyzed using STATA software version 12. Difference between means of proximate composition, fatty acid composition, minerals, vitamin A and anti-nutrients were tested for significance using the least significance difference (LSD) at 95% confidence level ($p<0.05$).

3. Results

3.1 Proximate Composition

Table 1 presents the comparison of the proximate profile of METU-2 to that of Plumpy nut. Analysis revealed comparable crude protein levels; 12.1% and 11.8% plumpy-nut and METU-2 respectively. Higher moisture levels, 9.8% were found in METU-2 compared to 2.7% in Plumpy nut. On the same note, crude fiber analysis revealed a higher content for METU-2, 1.24% compared to less than 0.01% levels found in plumpy nut. In terms of ash content, levels varied significantly from 3.6% in plumpy nut to 1.4% in METU-2. A superior carbohydrate profile was found for Plumpy-nut, 54.9% compared to 40.6% of METU-2. However, METU-2 had an expressively high fat content, 35.1% than Plumpy nut, 26.6%. Accordingly, a notable difference was noted in the total energy profile of the two samples; METU-2 had 528.2Kcal compared to 509 Kcal found in Plumpy nut.

Table 1. Proximate composition of Plumpy-nut and METU-2

Parameter	Plumpy-nut	METU-2	*P value*	*Recommended levels*
Moisture (%)	2.7±0.01 [b]	9.8±0.06 [b]	0.000	10
Protein (%)	12.1±0.15 [a]	11.8±0.95 [a]	0.05	10 to 12 % total energy
Fat (%)	26.9±0.06 [b]	35.1±0.21 [a]	0.0002	45 to 60 % total energy
Ash (%)	3.6±0.18 [a]	1.4±0.00 [b]	0.0016	
Crude fibre (%)	<0.01 [b]	1.2±0.93 [a]	0.0014	
Carbohydrate (%)	54.7±0.28 [a]	40.6±0.15 [b]	0.0001	
Total energy (kcal/100g)	509±1.09 [b]	528.0±1.48 [a]	0.0023	520-550

Results are expressed on dry basis except those of dietary fibre. Values in rows with different superscript letters are significantly different (p<0.05). Values are means of three replicates ±standard deviation

3.2 Micro Nutrient Profile

Table 2 presents an overview of the micro nutrient profile of the two therapeutic foods. Analysis showed that plumpy nut had higher levels of potassium (1437.5 – 420.95mg/100g), zinc (2.29 – 1.70mg/100g), copper (0.76 – 0.38mg/100g) and iron (9.23 – 5.53mg/100g) compared to METU-2. Plumpy-nut as well had superior sodium levels, 289.15mg/100g compared to 101.05mg/100g in METU-2. Both formulations statistically contained comparable magnesium contents; 119mg/100g and 114.32mg/100g plumpy-nut and METU-2 respectively. The concentration of vitamin A, 0.98mg/100g in plumpy-nut was significantly higher than that of METU-2, 0.52 mg/100g.

Table 2. Micronutrient Profile of Plumpy-nut and METU-2

Parameter (mg/100g)	Plumpy-nut	METU-2	*P value*	*Recommended levels (mg/100g)*
Potassium	1437.5±2.69 [a]	410.95±8.13 [b]	0.000	1100 to 1400
Magnesium	119.9±4.20 [a]	114.32±3.29 [a]	0.130	80 to 140
Zinc	2.29±0.09 [a]	1.70±0.02 [b]	0.006	11 to 14
Iron	9.23±0.18 [a]	5.53±0.06 [b]	0.000	10 to 14

Copper	0.76±0.17 [a]	0.38±0.010 [b]	0.046	1.4 to 1.8
Sodium	289.15±3.32 [a]	101.05±1.34 [b]	0.000	290 maximum
Vitamin A	0.98±0.01 [a]	0.52±0.14 [b]	0.000	0.8 to 1.1

Values in rows with different superscripts are significantly different (P<0.05). Values are averages of three replicates ± standard deviation.

3.3 Fat Acid Profile

Fatty acid profile of lipids extracted from plumpy-nut and METU-2 is presented in Table 3 below. Both formulations, contained comparable contents of PUFA's; METU-2 contained 2.82 g/100g of linoleic acid (18:2, n-6) while plumpy had levels of 2.87g/100g. Linolenic acid (18:3, n-3) levels ranged from 0.71g/100g to 0.75mg/100g for METU-2 and plumpy nut respectively. Oleic acid was also found in comparable levels in the two samples; 8.1396g/100g in METU-2 and 8.994g/100g in plumpy nut. On the other hand, Plumpy-nut contained expressively higher amounts of stearic acid (0.78 g/100g) and myristic acid (0.39g/100g) compared to levels found in METU-2 (0.01g/100g and 0.08g/100g stearic and myristic acid respectively). However, in terms of palmitic acid, METU-2 had significantly higher amounts, 0.35g/100g compared to Plumpy nut, 0.01g/100g.

Table 3. Fatty Acid Profile of Plumpy-nut and METU-2

Parameter (g/100g)	Plumpy-nut	METU-2	P value
Myristic acid	0.39±0.03[a]	0.079±0.18[b]	0.0008
Palmitic acid	0.35±0.02 [a]	0.04±0.00 [b]	0.0014
Stearic acid	0.78±0.20 [a]	0.01±0.02 [b]	0.0167
Oleic acid	8.99±0.21 [a]	8.14±0.63 [a]	0.1065
Linoleic acid	2.87±0.11 [a]	2.82±0.70 [a]	0.331
Gamma-linoleic acid	0.75±0.08 [a]	0.71±0.00 [a]	0.278
Arachidic acid	5.74±0.08 [a]	ND	
Erucic acid	3.73±0.43 [a]	0.85±0.008 [b]	0.0054
Lignoceric acid	0.57±0.04 [a]	0.74±0.09 [a]	0.0722

Values in rows with different superscripts are significantly different (P<0.05). Values are averages of three replicates ± standard deviation.

3.4 Levels of Anti-nutrients

All analyzed anti-nutrients; condensed tannins, trypsin inhibitors and phytates were significantly higher in METU-2 compared to plumpy-nut. Condensed tannin content ranged from 9.43mg/g in METU-2 to 6.55mg/g in Plumpy-nut while trypsin inhibitors varied from 2.54mg/g to 1.98mg/g respectively. Phytate levels ranged from 5.22mg/g in plumpy-nut to 6.72mg/g in Plumpy-nut.

Aflatoxin content: Plumpy-nut contained aflatoxin levels of 5.93ppb while METU-2 had 4.03 ppb.

Table 4. Anti-nutrient Profile of Plumpy-nut and METU-2

Parameter (mg/g)	Plumpy-nut	METU-2	P value
Condensed tannins	6.55±0.54[b]	9.43±0.09[a]	0.014
Phytates	5.22±0.02[b]	6.72±0.01[a]	0.000
Trypsin inhibitors	1.98±0.44 [b]	2.54±0.09 [a]	0.015

Values in rows with different superscript are significantly different (p<0.05). Values are averages of three replicates ± standard deviation.

4. Discussion

Proximate analysis showed comparatively high moisture content for METU-2. Scientifically, moisture levels above 10% provide a conducive environment for microbial and chemical reactions accelerating spoilage along with production of undesirable toxic components (Codex, 2006). Protein profile of both formulations was within the WHO recommendations of 10 to 12 % total energy for therapeutic diets (WHO, 2007). In SAM, mucosal enzymatic activity along with a number of transport systems within the body decrease and as these are protein based, sufficient protein is needed at rehabilitation stage to correct this deficiencies. While for plumpy nut, milk powder and peanut may equally contribute to quality of its protein profile, for METU-2, peanuts are the main contributor as protein in sorghum is not of adequate quality (Leder, 2004).

Though plumpynut had a superior carbohydrate profile, both formulations had adequate amounts to provide

sufficient energy for a child to recover from severe malnutrition. Carbohydrates are particularly needed for provision of glucose for brain functioning. About 95% of dry matter in honey is simple sugars; fructose and glucose,   (Bogdanov, Jurendic, Sieber, & Gallmann, 2008)**.**

Crude fiber levels were much higher in METU-2 and these were majorly attributed to sorghum. The milling process used for sorghum leaves much fiber. Malnourished children usually have a reduced mucosa surface and are therefore not able to fully absorb diets with high crude fiber content. Additionally, high fiber content complexes trace elements which are paramount in severely wasted children. Thus, the high crude fiber content of METU-2 is a detrimental factor.

Both formulations had a fat content within the 45 to 60 % total energy recommended amounts by WHO. However, METU-2 had a comparatively high fat content. A high fat content of METU-2 is majorly attributed to ghee. In addition to contributing profoundly to energy density, fat is also needed in the absorption of highly required vitamins, A and E (Michaelsen, 2009). Accordingly, the energy profile of METU-2 was higher than that of Plumpy nut and within the recommended 520- 550 Kcal/100g by WHO. Plumpynut was a fewer calories below this recommendation. Energy facilitates catch up growth in severely malnourished children in rehabilitation phase (Michaelsen, 2009), warranting energy dense dietary formulations.

4.1 Micronutrient Profile

For children suffering from SAM; zinc, potassium and magnesium along with vitamin A and folic acid are the most important while iron and sodium should be limited (Bhan, Bhandari, & Bahl, 2003).

Zinc is essential for the activity of more than 100 enzymes (Hotz & Brown, 2004). Deficiency impairs the working of immune system and has a direct consequence on the structure and function of mucosa (Bhan et al., 2003). Mucosa effect relates to mal-digestion. It is as well a vital component of intracellular superoxide dymutase, an enzymatic pathway system which keeps free radicals (involved in PEM manifestation) under control (Michael HN Golden & Ramdath, 1987). Zinc depletion occurs during cases of SAM (Fell et al., 1973). On this basis, WHO recommends that dietary formulations for SAM children contain zinc levels of 11 to 14 mg/100g. However, both formulations were below this recommendation. Particularly lower levels were found in METU-2. Sorghum is a good source of zinc but like other plant based foods, its bioavailability is low owing to anti-nutrients (Léder, 2004). A sustainable strategy to improve zinc content of these foods may include soil enrichment with zinc based fertilizers as research shows that zinc content of foods is largely soil dependent (Gibson, 2006).

During severe acute malnutrition cases, there is a considerable loss of potassium which in turn leads to sodium retention (Bhan et al., 2003). That is, severely wasted children have lower potassium levels, particularly intracellular concentration while total sodium levels, intracellular increase. When recovery starts, potassium concentration can drop dangerously, if adequate amounts aren't provided. In addition to promoting fluid retention leading to oedema, potassium deficiency translates in to intracellular acidosis, stimulating accumulation of Ca^{2+} deteriorates protein metabolism (M. Golden, 1988). It could as well lead to a decrease in contractile power of muscle fibers and together with hypokaliemia result in a reduction in cardiac output (M. Golden, 1988). For these reasons, higher potassium and lower sodium levels are recommended for SAM children compared normal children. While Plumpy nut contained potassium levels within the WHO recommended amounts of 1100 to 1400 mg/100g for therapeutic diets (WHO, 2007), levels in METU-2 were significantly lower. On a good note, sodium levels were below the 290 mg/100g maximum limits.

Magnesium content of the two formulations was comparable and within 80 to 140 mg/100g WHO recommendations (WHO, 2007). Peanuts contributed profoundly to the amounts in the two formulations (King et al, 2008). Sorghum is as well a good source (Léder, 2004). Magnesium is required by several enzymes involved in nucleic acid metabolism and thereby affects multiple physiologic processes (Rude, 1998). It is as well vital in ion transport systems; Ca^{2+}, pumps and Na-K ATPase.   Deficiency is particularly sensitive for Na-K pump. Magnesium is frequently low in SAM and needs to be corrected for proper running of these sensitive physiological processes.

Copper is another important micronutrient in SAM treatment, owing to its role in controlling free radicals (intracellular superoxide dymutase) and its involvement in iron metabolism (transport and oxidation in the plasma) (Michael HN Golden & Ramdath, 1987). Despite peanuts being a rich source of copper (king et al., 2008), both formulations did not meet the recommended 1.4 to 1.8 mg/100g for SAM recovery.

In malnutrition cases, iron is sequestered into storage sites, as SAM presents with infections and inflammations

yet iron promotes bacterial growth. Additionally, iron sustains radical generation process, presenting potential toxicity (Michael HN Golden & Ramdath, 1987). For these reasons, iron levels are always kept low even at the rehabilitation stage. Accordingly, WHO recommends levels of 10 to 14 mg/100g in therapeutic food formulations (WHO, 2007). However, METU-2 was way below this recommendation. Fortification explains levels found in plumpy-nut while those reported for METU-2, are largely attributed to sorghum. Sorghum is stated to have iron levels approximating 4mg/100g (Léder, 2004).

Vitamin A deficiency is common in SAM (Bhan et al., 2003). In addition to dietary deficiency, insufficiency is as well attributed to diminishing of the necessary enzymes for absorption and transportation. Dietary deficiencies of zinc, fat and proteins as well explain this deficiency. Children with SAM have more frequent infections, which increases their demand and interferes with the absorption at the gut level. Vitamin A serves a number of critical roles ranging from maintenance of the epithelial cellular integrity to promoting adequate functioning of the immune system. To realize morbidity reduction along with correction of the diminished mucosa in SAM, vitamin A is indispensable. Levels found in METU-2 were lower than those found in plumpynut and a little below the recommended 0.8 to 1.1 mg/100g WHO levels. Ghee used in METU-2 is a rich source of vitamin A.

4.2 Fatty Acid Profile

SAM affected children usually suffer essential fatty acid deficiency (EFA), depicted by the manifesting symptoms; skin changes, impaired resistance to infections, growth rate and development (Calder, 2013; Jones et al., 2015). Long chain poly unsaturated fatty acids (LC-PUFA) like docosahexaenoic acid (DHA) are chief constituent of neural lipid, and inadequacy during early childhood is linked to a range of neurodevelopmental abnormalities. Accordingly, SAM affected children are at risk of long-term cognitive and behavioral deficits. Additionally, essential fatty acid deficiency impairs nutrient absorption along with dietary calorie utilization (Calder, 2013). Thus, inadequate amounts during nutritional rehabilitation may escalate these deficits. Long chain PUFA's can be biosynthesized from n-6 linoleic acid (LA, 18:2 (n-6), and n-3 alpha-linoleic acid (ALA, 18:3 (n-3) by the sequential action of desaturase and elongase enzymes (Jones et al., 2015). Along this line therefore, WHO recommends that therapeutic formulations for SAM, contain LA and ALA at levels of 3 to 10% and 0.3 to 2.5% of total energy respectively (WHO, 2007). Both formulations not only had comparable amounts of these fatty acids, but also had levels within the WHO recommendations. The high LA could be attributed to peanuts (king et al., 2008 & Jones et al., 2015).

4.3 Anti-nutrients

Though generally present in lower levels; trypsin inhibitors, phytates and condensed tannins were higher in METU-2 compared to plumpy-nut. Literature reports considerably higher levels of these three in sorghum and peanuts (Gassem & Osman, 2003; Gibson, Bailey, Gibbs, & Ferguson, 2010; Léder, 2004). Thermal treatment reduces the levels and this mechanism could have substantially attenuated the levels in plumpynut (Hotz & Gibson, 2007). In the same line, peanut roasting could also have had a considerable effect on levels found in METU-2. Anti-nutrients have a number of nutritional effects which can jeopardize recovery from SAM. Trypsin inhibitor inhibits the activity of pancreatic proteolytic enzymes, mainly trypsin and chymotrypsin. High levels may consequently result in child growth faltering (Michael H Golden, 2009). Phytates and condensed tannins complex minerals and trace elements (Gibson et al., 2010). Condensed tannins also influence protein digestion through forming complexes with larger quantities of proteins (Knuckles, Kuzmicky, & Betschart, 1985). Presently, there are no prescribed limits for anti-nutrients; therefore, the principle is to reduce their presence as much as possible. Traditional processing methods like germination, soaking and fermentation have the capacity to reduce anti-nutrients and provide a cheaper solution at household level (Hotz & Gibson, 2007).

4.4 Aflatoxins

While aflatoxin contamination in METU-2, met the 5ppb maximum limits set for therapeutic diets by WHO, contamination levels in plumpynut were slightly above this limit. Groundnuts and cereals are highly affected with aflatoxins (Kaaya & Warren, 2005). Aflatoxin intake is associated with growth retardation (Khlangwiset, Shephard, & Wu, 2011; Smith, Stoltzfus, & Prendergast, 2012). Evidence from West Africa shows a dose-response relationship between serum aflatoxin and stunting (Gong et al., 2004). One way growth retardation is mediated is gut inflammation (Smith et al., 2012). Additionally, Aflatoxins being immune suppressing, they further expose acute malnourished children to incidences of infectious diseases. Aflatoxin contamination should therefore be kept as low as possible in therapeutic dietary formulations.

5. Conclusion

METU-2 has a proximate and fatty acid profile which is not only comparable to plumpy-nut, but also meets the

WHO recommendations for SAM diets. However, it is lacking in important zinc, potassium, and vitamin A. Present anti-nutrients further limit the bioavailability of zinc. Despite a few gaps, METU-2 provides a nutritional profile which can cure SAM and further studies could ensue its efficacy.

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