1 Original Research Article

FORMULATION AND EVALUATION OF READY-TO-USE THERAPEUTIC FOODS USING LOCALLY AVAILABLE INGREDIENTS IN BAUCHI, NIGERIA

4 Highlights:

- Globally, severe acute malnutrition (SAM) is reported to affect 19 million children 0 5 years of age, and is associated with 1 to 2 million preventable child deaths every
 year
- 60-90% of children with SAM without medical complications can be treated without being
 admitted to health facilities using Ready-to-use Therapeutic Food (RUTF).
- RUTF formulated using locally available ingredients are acceptable, comparable to
 existing RUTF, and meet the WHO recommended minimum nutrient requirements for
 RUTF

13 **Running Title**

14 FORMULATION AND EVALUATION OF READY-TO-USE THERAPEUTIC15 FOODS

16 Abstract

17

a) A known weight of sample was placed in Kjeldahl flask and about 200 milligram ofcatalyst mixture was added.

b) 10.0cm² of concentrated sulphuric acid was added to the content of the flask. It was heated
gently for few minutes until frothing ceased. The heat was increased to digest for 3 hours. It
was allowed to cool and made to a known volume with distilled water (100cm³).

c) 10.0cm³ aliquot of the dilute solution of the digest was distilled by pipetting the
volume into distillation chamber of micro Kjeldhal distillation apparatus. 10.0cm³ of 40%
sodium hydroxide solution and steam distil was added into 10.0cm³ of 2% boric acid
containing mixed indicator (note colour from red- Globally, severe acute malnutrition (SAM)
is reported to affect 19 million children 0-5 years of age, and is associated with 1 to 2 million

28 preventable child deaths every year. 60-90% of children with SAM without medical 29 complications can be treated without being admitted to health facilities using Ready-to-use 30 Therapeutic Food (RUTF). Shipping costs, delays & donor fatigue lead to periodical 31 unavailability of RUTF in Nigeria, undermining its effectiveness in combating malnutrition. 32 This study produced and carried out proximate and sensory evaluation of three samples of 33 RUTF (AOB, BOC and PCO). The energy content (523kcal) of PCO, AOB (555kcal) and 34 BOC (573kcal) were comparable to the recommendation of 520-550 kcal by the WHO. The 35 fat contents (45.11g and 43.04g) of BOC and AOB respectively were higher, while that of 36 PCO (32.14g) was within the recommendation of 45-60% from fat. The protein contents of 37 AOB, BOC and PCO (22.7g, 24.11g and 21.70g respectively) were higher than the 38 recommendation of 10-12% of energy. The ash contents (3.5g and 4.38g) of AOB and BOC 39 were similar to that of Plumpy'Nut. BOC was the most acceptable in terms of flavor, color 40 and consistency. There was no significant difference in flavor and color (p=0.175 and 0.471 41 respectively) but there were significant differences in consistency and taste (p=0.025 and 42 0.008 respectively) between the samples.

43 Introduction

44 Malnutrition is the most common nutritional disorder in developing countries and it 45 remains one of the most common causes of morbidity and mortality among children worldwide¹. Severe acute malnutrition is defined by a very low weight-for-height (below -3 z 46 47 scores of the median WHO growth standards) and a mid-upper arm circumference less than 115 mm, with or without nutritional oedema $^{2-4}$. Globally, 52 million children under five years 48 of age – one in twelve children in this age group –suffer from acute malnutrition⁵. Globally, 49 50 severe acute malnutrition (SAM) is reported to affect 19 million children 0-5 years of age, and is associated with 1 to 2 million preventable child deaths every year^{6, 7}. 51

52 In Nigeria, there has been a 97.7% increase in the prevalence of SAM over 10 years 53 (from 4.4% in 2003 to 8.7% in 2013). The highest spikes in SAM prevalence within this 54 period have been documented in the country's north-eastern and north-western regions 55 respectively (1.2% to 9.3% - an increase of about 775%; and 3.8% to 15.3%, an increase of 402.6%)⁸. An acutely malnourished child under 5 years is 20 times at higher risk of dying 56 than a well-nourished child⁹. Acute malnutrition inhibits children's physiological and mental 57 58 development, has life-long implications for their health, and heavily mortgages the 59 opportunities available to future generations¹⁰.

60 Ready-to-use therapeutic foods (RUTF) are energy-dense, micronutrient enhanced pastes used in therapeutic feeding¹¹. Ready-to-use therapeutic food (RUTF) has greatly 61 improved the recovery rate of children with severe acute malnutrition (SAM) in sub-Saharan 62 Africa¹². 60-90% of children with SAM without medical complications can be treated 63 without being admitted to health facilities using Ready-to-use Therapeutic Food (RUTF)¹³. 64 Shipping costs, delays & donor fatigue lead to periodical unavailability of RUTF in Nigeria, 65 66 undermining its effectiveness in combating malnutrition. Peanut milk-based ready-to-use therapeutic food (P-RUTF) which is used in community-based treatment of SAM is 67 expensive¹⁴. Lack of locally produced RUTF is a key challenge in the sustainable treatment 68 of SAM in non-emergency contexts¹⁵. RUTFs can be made with local ingredients to fit local 69 taste preferences¹⁶. Substituting soy for much of the milk in RUTF might reduce its cost 70 and/or increase its availability¹⁷. It is against this background that this study was designed to 71 72 formulate and evaluate RUTF using locally available ingredients in different ratios, in order 73 to meet the recommended nutrient composition for RUTFs while achieving products that are 74 culturally acceptable.

75 MATERIALS AND METHODS

76 Eight cereal, legume and oils mixtures were formulated and evaluated. In particular, 77 efforts were made to combine the various cereal, legume and oil seed mixtures to maximize 78 the protein quality, attempting to offset any essential amino acid deficiencies in one 79 ingredient by combining it with another ingredient that was high in that particular amino acid 80 (18). After evaluating the proximate composition, sensory qualities, cost of production and 81 overall acceptability of the 8 RUTF formulations, 5 were eliminated based on the parameters 82 listed above, and only the most acceptable and cost-effective formulations which satisfied the 83 nutrient recommendations were further evaluated.

The three samples of RUTF (AOB, BOC and PCO) were produced using the following ingredients: powdered milk, sugar, peanut paste, vegetable oil, soybean flour, crayfish powder, guinea corn flour, rice flour, cashew nut paste, acha flour, dried date palm powder and a vitamin-mineral mix in different combinations as presented in table 1.

The grains, powdered milk, sugar, crayfish, date palm and vegetable oil were purchased from Muda Lawal market and Central Market, Bauchi.

90 The vitamin-mineral mix was purchased from

91 **Production of the grain flours**

Acha flour and guinea corn flour were produced thus: grains were sorted, washed, fermented, drained, dried and toasted until very crisp and golden brown, cooled and drymilled into flour. Acha was fermented for 12 hours and dried at 60° C, while guinea corn was fermented for 24 hours and dried at 70° C.

Soybean flour was produced by sorting, washing, fermenting for 12 hours,
decortication, boiling for 20 minutes, cooling, drying at 70°C, toasting, cooling and drymilling into flour.

99 Date palm powder was produced by sorting, washing, drying and dry-milling into100 powder.

Peanut paste and cashew nut paste were produced by sorting, washing, air-drying,toasting and milling into a paste.

103 Crayfish powder was produced by sorting, washing, drying, slight toasting and104 milling into powder.

105 Table 1 shows the ingredient composition of the RUTF formulations

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- 107

Table 1: Ingredient composition of the RUTF formulations

Sample AOB		Sample BOC		Sample PCO	
Ingredient	%	Ingredient %		Ingredient	%
Rice flour	18	Acha flour	18	Guinea corn flour	18
Peanut paste	27	Cashewnut paste	25	Peanut paste	27
Soya bean flour	18	Soya bean flour	18	Soybean flour	18
Date powder	14	Sugar	14	Sugar	15
Milk	12	Milk	14	Milk	10
Vegetable oil	10	Vegetable oil	10	Vegetable oil	12
Multimix	0.07	Multimix	0.07	Multimix	0.07
		Crayfish powder	1		

- 109 An electric blender was used for mixing the different products. It was continuously mixed
- 110 until a fine consistency was achieved. The products did not contain lumps and water was not
- added during mixing. The locally produced RUTF was in a paste form.

112 Determination of the proximate composition of the RUTF

- 113 The protein content of the three samples AOB, BOC PCO was determined using 114 microkjedahl method. Also, the fat content of the samples were also analysed using soxhlet 115 method. The crude fiber, ash, and moisture content of the samples were determined.
- 116 <u>Procedure for moisture determination</u>
- 117 a) The samples were mixed thoroughly.
- d) The water content was determined by weighing 2.5g of each sample into a silica dish,which had been previously weighed.
- 120 c) The dish containing the sample was placed inside a hot air oven for 24hours at $60-70^{\circ}$ C
- 121 (drying at high temperature may result in losses of heat liable or volatile components).
- d) It was finally dried to a constant weight and allowed to cool for ten minutes in a desiccatorbefore weighing.
- 124 % moisture = $W1-W2 \times 100$ 125 W1
- 126 W_1 = Weight of biological material before drying
- 127 W_2 = Weight of biological material after drying
- 128 <u>Nitrogen determination by micro kjeldahl method (crude protein)</u>

129 The nitrogen of protein and other compounds were converted to ammonium sulphate by acid

- 130 digestion with boiling sulphuric acid.
- 131 green). It was titrated with standard 0.01N or 0.2N hydrochloric acid to grey end point.
- 132 % N = $(a-b) \ge 0.01 \ge 14.0057 \ge 100$

133	dxe			
134	a = titre value for the sample			
135	b = titre value for the blank			
136	c = Volume to which digest is made up with distilled water			
137	d = Aliquot taken for distillation			
138	c = Weight of dried sample (mg)			
139	To convert to % crude protein, multiply by necessary conversion factor (6.25)			
140	Ash determination			
141	The residue was charred from the moisture determination in a the muffle furnace between			
142	500° - 600° C until the ash turned grey or nearly white. It was cooled and weighed for 12hours			
143	(19).			
144	Fat determination (ether-extract)			
145	a) Ether-extract (oil) a soxhlet extractor with reflux condenser and a small flask which has			
146	been previously dried in the oven and weighed was fitted up.			
147	b) 2gm of the sample was weighed and transferred to a fat-free extraction thimble, it was			
148	plugged lightly with cotton wool, and the thimble was placed in the extractor.			
1.40	$(D, D, C) = 0^{3}$ of motor large other (D, D, C) $(0, 0)^{0}$ consistent dial into the flack contribution			
149	c) About 150cm^3 of petroleum ether (B.P. $60-80^{\circ}\text{C}$) was also added into the flask until it			
150	siphons over once. More ether was added until the barrel of the 100ml extractor was half full,			
151	the condenser was replaced.			
152	d)It was ensured that the joints were tight and placed on the electro thermal heating mantle.			
153	e) The source of heat was adjusted which enabled the ether to boil gently. It was left to			
154	siphon over for 8 hours.			
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- 155 f) It was watched until the ether was just short of siphoning over, the flask was detached and
- siphon the contents of the barrel of the extractor into the ether stock bottle.
- 157 g) It was drained well and the thimble was removed and dried in an oven. The condenser was
- replaced and the ether was continuously distilled until it was practically dry.
- h)The flask was detached (which contained the oil), the exterior was cleaned and dried in anoven until a constant weight was gotten.
- 161 j) The extracted residue was kept for fiber determination.

162Ether extracts= $\frac{\text{Weight of oil}}{\text{Weight of biological material}} x$ 100163Weight of biological material

164 <u>Crude fibre determination</u>

a) Mix 500ml glacial acetic acid, 450ml water and 50ml concentrated Nitric acid were mixed.

b) 20g trichoracetic acid was dissolved in this mixture

c) 1g of deffated material was weighed into a 250ml conical flask. 100ml of the TCA
mixture was added into the flask. It was refluxed for exactly 40 minutes, counting from the
time heating commenced.

d) A 3 feet long air condenser or a water-jacketed condenser was used to prevent loss ofliquid.

- e) The flask was disconnected and allowed to cool, it was filtered through a 15cm No.4Wathman filter paper previously dried and weighed.
- 174 f) It was washed 10 times with hot distilled water and once with industrial methylated spirit.
- 175 The filter paper containing the residue was dried in an oven at 105° C overnight.
- 176 g) It was transferred to a desiccator and weighed after cooling. An ashing crucible was
- 177 weighed and the weight of the crucible plus the filter paper containing the fiber was taken.
- h) Ashing was done overnight at 500° C, it was cooled and weighed. The percentage crude
- 179 fibre was calculated (AOAC, 2006).
- 180 Sensory evaluation

- 181 The three products were evaluated using a 5 point hedonic scale based on colour, flavor,
- taste, consistency, and general acceptability.
- 183 Fifty panelists were chosen from Federal Polytechnic Bauchi out of which 25 were mothers
- and 25 were children. The panelists were shared into sub groups, to assess the products that
- 185 were served to them, rinsing their mouths after each sample.
- 186 The results were analysed using ANOVA

187 **RESULTS**

Table 2: NUTRIENT COMPOSITION OF SAMPLE AOB, BOC AND PCO

189	Nutrient	AOB	BOC	РСО	PLUMPY'NUT
190	Energy (kcal)	555.0	573.0	523.0	530.0
191	Protein (g)	22.7	24.11	21.70	14.5
192	Carbohydrate (g)	19.67	17.83	36.73	43.0
193	Fat (g)	43.04	45.11	32.14	33.5
194	Ash (g)	3.5	4.38	2.92	4.0
195	Moisture (g)	2.73	0.63	0.59	<5.0

¹⁹⁶

Table 2 shows the nutrient content of the three samples. Sample BOC had the highest energy content of 573kcal while sample PCO had the lowest energy value of 523kcal. Sample PCO had the highest protein content of 24.11kcal while Plumpy nut had the lowest of 14.5. Fat was highest in sample BOC as 45.11kcal and lowest in sample PCO as 32.14. Ash was highest in sample BOC as 4.39 and lowest in sample PCO as 2.92 Sample AOB had the highest moisture content of 2.73 and was lowest in sample PCO as 0.59.

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206 Table 3: PERCENTAGE CONTRIBUTION TO ENERGY OF THE MACRO 207 NUTRIENTS

208 CHO (%) Nutrients Fat (%) Protein (%) 209 AOB 69.69 14.1 16 210 BOC 70.55 12.45 17 PCO 211 55.4 28.1 16.6

212

Table 3 shows the percentage contribution to energy of the macro nutrients. Sample BOC had

the highest percentage contribution to energy from fat, while sample PCO had the lowest

contribution from fat. Sample PCO had the highest contribution from carbohydrate, while

216 BOC had the lowest.

217 Table 4: SENSORY EVALUATION SCORES OF RUTF

218	Sample	Flavour	colour	consistency	Taste
219	AOB	3.50a±1.09	3.56a±1.01	3.28±1.01	3.48a±1.03
220	BOC	3.88a±0.91	3.76a±1.20	3.84b±1.01	3.88ac±0.92
221	РСО	3.68a±1.02	3.50a±1.02	3.52ab±1.03	3.24bc±1.12

Any two means not followed by the same letter on the same column are significantly different (p<0.005) using multiple comparison of 50 panelists.

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Table 4 shows the sensory evaluation scores of RUTF Table 4 indicates that sample BOC was generally accepted and had the highest acceptability in terms of flavor, colour, consistency and taste when compared with sample AOB and PCO. There were no significant differences in flavor and colour, but significant difference in consistency and taste were observed.

231 DISCUSSION

232 In the current study, the energy values of the three samples of locally produced RUTF were found to be comparable to the imported RUTF (Plump'y nut), and in conformity with 233 the recommendations, which indicate that the energy content of RUTF should not be below 234 235 500 kcal per 100g. The energy values of RUTF produced in the current study (555, 573 and 236 523 Kcals respectively) are also comparable to those of alternative RUTF formulations 237 produced in Malawi, having energy contents of 551, 567 and 512 Kcal respectively (18). 238 However, the energy contents of RUTF in the present study (including sample PCO with 239 10% milk) are higher than those indicated by Oakley et al (2010), of 2000KJ (478.0 Kcal) for 240 RUTF containing 10% milk. The lower energy content indicated by Oakley et al may be a 241 possible explanation for their RUTF being less effective in the treatment of SAM. (17). The 242 three samples (AOB, BOC, PCO) in the current study are therefore energy dense and suitable 243 for feeding to young children and other vulnerable individuals

244 According to the recommendations, Protein should contribute 10- 12% of the energy 245 value of RUTF (21). Compared with the standard, the imported RUTF and the locally 246 produced RUTF were similar in protein content. Samples AOB, BOC and PCO had protein 247 contents of 16%, 17%, 16.6% respectively, which were higher than that of Plump'y nut 248 (10.9%). The legumes used in producing the RUTF were roasted prior to milling into flour, 249 to reduce anti-nutritional factors such as phytate. This is because the content of phytate in 250 foods has a strong negative effect on bioavailability of important minerals, and food 251 processing methods that reduce the phytate content of foods should be promoted, especially 252 for children with SAM (21).

In addition, the legumes used in the formulation of the locally produced RUTF contributed to most of the protein content of the product. For instance, the average crude protein (CP) content of soybean is 38% with a rich and balanced amino acid profile, (rich in the amino acids lysine, tryptophan, threonine, isoleucine, and valine which are deficient in cereal grains) (22).

The fat contents of the three locally produced RUTF (69.9%, 70.55%, and 55.4% for sample

AOB, BOC and PCO respectively), are comparable to that of Plump'y nut (56.6%).

According to WHO standard, fat should contribute 40-60% energy value. Poly unsaturated

oils are used in the production of RUTF to provide essential fatty acids. Fat is very essential

262 in the formulation of RUTF, this is so because Severe Acute Malnutrition (SAM) leads to

severe wasting and loss of subcutaneous fat (23). Fat in addition to protein helps in tissue

264 regeneration, protection, and normal functioning of the immune cells which prevent children

from suffering from childhood diseases such as diarrhea and pneumonia.

The moisture content of the three samples were lower than that of Plump'y Nut. This will allow locally produced RUTF to be safely stored at ambient tropical conditions for 3-4 months.

269 Another major concern is the economics and sustainability of any wide use of RUTF 270 to prevent malnutrition or even to treat mild malnutrition. The products are expensive. At 271 around 35 US cents a package, a full two-month treatment with the imported RUTF costs 272 around between USD 45 -53 per carton (without shipping costs) (24). Many parents of 273 children with severe acute malnutrition cannot afford this. The costs so far have been borne 274 primarily by United Nations agencies and by international non-governmental organizations 275 such as MSF. The findings from this study indicate that the cost of production of locally 276 formulated RUTF is relatively cheaper when compared to the commercially produced RUTF. 277 The cost of production of sample AOB was \Re 79.19 per packet (24.6 cents per packet, USD 278 36.9 per carton of 150 packets of 100g). The cost of sample BOC was ¥103.86 per packet 279 (35 US cents per packet, USD 48.4 per carton) sample PCO was the cheapest to produce 280 ¥54.78 per packet (17 cents per packet, USD 25.5 per carton). The cost of sample BOC was 281 higher than the two other samples because cashew nut which is more expensive, was used to 282 substitute groundnuts in order to provide an alternative with a lower risk of aflatoxin 283 contamination.

284 RUTF contains 25% milk powder, an expensive ingredient that is not readily 285 available worldwide. But substituting locally produced soy, which is also high in protein 286 content, for milk powder reduces the cost of Fortified Spreads without significantly changing 287 the macronutrient content (25). Recent studies demonstrate the potential of new RUTF, 288 produced from locally available grains and legumes. Other ingredients such as rice, acha, 289 soya bean, guinea corn, crayfish, groundnuts, cashew nuts are locally grown in Nigeria 290 especially in the Northern region where the prevalence of malnutrition is very high. Even 291 with the importation of RUTF by UNICEF and other international agencies, the number of 292 malnourished children is still very high in Africa and Nigeria in particular. Local production 293 of RUTF in the regions with high burden of malnutrition will contribute immensely to the

reduction of severe acute malnutrition. Some of the ingredients used in this study constitutethe staple foods of the people living in northern Nigeria.

296 CONCLUSION

297 RUTF formulated using locally available ingredients are acceptable and meet the298 WHO recommended minimum nutrient requirements for RUTF.

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