

Locally Developed Ready-To-Use Therapeutic-Food (RUTF) for Management of Malnutrition Using Animal Models

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Abstract

Context: The prevalence of protein energy malnutrition in developing countries has reduced globally. However stunting, wasting, and underweight are still unacceptably high. These high levels of protein energy malnutrition are a major cause of high infant and child morbidity, and mortality rates. Community-based therapy for acute childhood malnutrition has been successful in a variety of settings over the last five years. This study therefore sought to develop a ready-to-use- therapeutic food using foods that are locally available in Western Kenya.

Objective: The objectives of this study were to develop a ready-to-use-therapeutic-food using soybeans; assess consumer acceptability of the locally developed ready-to-use-therapeutic-food, test the effect of formulated products on malnourished animal models, and analyze the cost of local production of the ready-to-use-therapeutic-food.

Experimental design: Three formulations were developed using soybeans (*Glycine max* L. Merr), maize (*Zea mays*), peanut (*Arachis hypogaea*), sugar, vegetable oil and mineral mix. Proximate analyses for protein, fat, moisture, carbohydrate and energy were done according to standard AOAC International methods. Hedonic characterization on a 9-point scale was done to determine the liking for colour, flavour, texture, appearance and general acceptability. Preference ranking for consumer acceptability of three formulations was done by 50 University of Eldoret students; 27 female and 23 male students aged 21-30 years.

Results: soybeans are a cheaper alternative to dairy products for the production of an acceptable RUTF as recommended by UNICEF standards. This will go a long way in reducing the costs incurred by use of imported ready-to-use-therapeutic food.

Keywords: Protein energy; Malnutrition; Therapeutic food; Soybeans

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Introduction

According to the Food and Agriculture Organization [1], an estimated 12 million children below the age of 5 (five) years die annually with more than 50% of all these cases attributed to malnutrition. Malnutrition is also the leading cause and effect of poverty, ill-health [2] and permanent impairment of the physical and mental growth of those children who survive [3]. Child vulnerability to malnutrition is most severe at the complementary feeding stage [4] because their macro and micro-nutrient needs might not be sufficiently provided for in the complementary foods

[1]. As well, low nutrient densities in some of the complementary foods may enhance under-nutrition [4].

Protein nutrition is important for human health as its deficiency leads to a major public health problem of Protein and Energy Malnutrition (PEM) that faces most children in the developing world [5]. The affected infants and young children become most susceptible to its characteristic growth impairment because of their high energy and protein needs [6]. The United Nations International Children's Emergency Fund (UNICEF) [7], has indicated that the prevalence of PEM in developing countries is globally reducing. However, the levels of stunting, wasting and

underweight are still unacceptably high. In 2012, 162 million of children below the age of 5 (five) years were stunted globally with 36% of them in Africa. Additionally, the underweight rates were 15% (99 million) and wasting affected 8% (51 million) of which 17 million children were severely wasted [8]. These high levels of PEM are a major cause of high infant and child morbidity and mortality rates [1]. The cost of treating malnutrition is also high. For instance, in Zambia estimated community based treatment of Severe Acute Malnutrition (SAM) with hospital access cost US\$ 203 per case treated, US\$1766 per life saved and US\$ 53 per disability-adjusted life year gained [9]. Cost per disability-adjusted life year gained suggests that community bases treatment of SAM is cost effective compared with other priority health intervention.

RUTF has been shown to be very effective in the rehabilitation of malnourished children. It can be safely and easily produced in most settings worldwide [10]. Plumpy'Nut, commonly used for nutrition intervention in Kenya is imported from France. This increases the disease burden [11]. Carried out a rat bioassay where soybean flour was used for complementation. Adding 30% of soymeal resulted in an increase in protein, moisture, oil and ash contents but a reduction in carbohydrates.

Maize is a staple in Kenya and consumed in majority of households. Rats fed on maize meal neither gained nor lost significant weight while those fed on protein free diet lost significant weight (Figure 1). Low protein diets, a major cause of protein energy malnutrition, have been known to result in reduced food intake causing protein deficiency, emaciation, and death [12].

All the soy fortified flours had significantly higher protein content compared to their respective unfortified flours thus having a positive impact growth. Fortifying maize with soy made it superior to other fortified cereals (Figure 2).

Food formulations using locally available food materials have been proposed as a means of averting nutrient deficiency among young children in developing countries [13]. Consumer awareness is important in creating an environment to develop product familiarity and the ability to evaluate different alternatives available for satisfying desire. According to Mosha and Vicent [14], consumers prefer those foods with which they are familiar rather than those they are unfamiliar. These facts informed the need to develop a Ready to Use Therapeutic Food (RUTF) using maize and soybeans. The study was designed to formulate a low cost, nutrient dense ready-to-use-therapeutic food using locally available foods, perform a consumer acceptability test of the products and test its effect on rehabilitation of malnutrition using animal models.

Materials and Methods

Materials

Maize grains, soybean and peanuts were chosen as potential ingredients of the ready-to-eat complementary diet. Soybeans, peanuts, and the maize were bought from the local Municipal market in Eldoret town, Kenya. In addition, soya oil and icing sugar (Kenafric-Kenya) were purchased from the local supermarket in Eldoret.

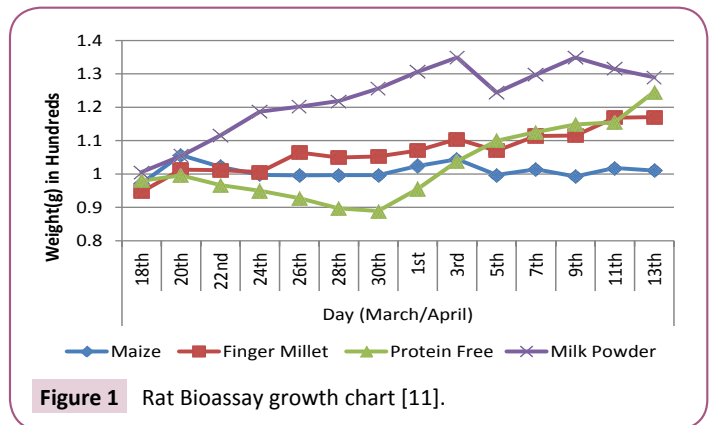


Figure 1 Rat Bioassay growth chart [11].

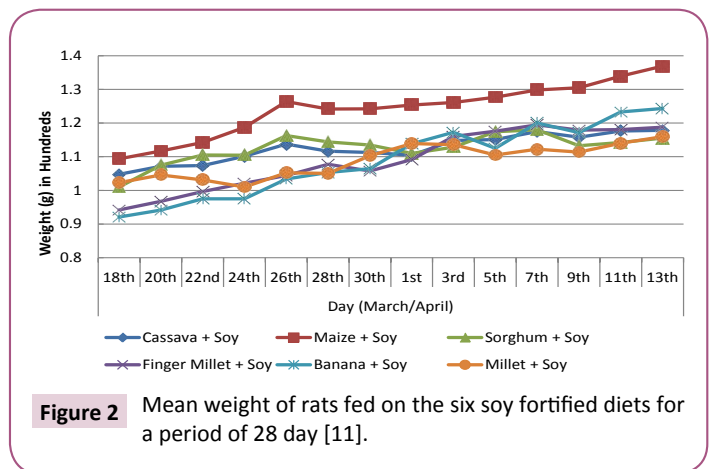


Figure 2 Mean weight of rats fed on the six soy fortified diets for a period of 28 day [11].

Experimental design

The consumer acceptability tests were carried out using central location consumer test which best suits the Completely Randomized Design (CRD) approach. Numeric codes were randomly assigned to the samples for blinding purposes and sample arrangements on the set-up trays were also randomized for each panelist. The panelists also came in at random to conduct the acceptability tests.

WHO/FAO/UNU [15] suggests that animal models, for example albino rats, should be used in testing such products. This is because the metabolic and digestive processes in the rats which are monogastric are similar to that of humans. Albino rats are laboratory rats of the species *Rattus norvegicus* which are bred and used for scientific research in psychology, medicine, nutrition and other fields. Complete randomized trial using 28 weanling rats in groups of seven was carried out for a period of 35 days. A group of seven rats was fed on 20 g/rat/day of a specific formulation. Thus group 1 were fed on product 1, group 2 on product 2, group 3 on product 3 and group 4 on Plumpy'Nut which was the control. The animals were housed individually in wire-bottomed cages to allow faecal matter to drop on a base tray. Growth and rehabilitation study lasted 28 days from i.e., day 7 to day 34 (18th March–14th April 2015). Before the start of the experiment, the weight of the rats was taken using an electronic balance (Gebr. Bosch PE 625, Germany) and repeated

on alternate days throughout the study. The rats were weighed on alternate days. The rats had exactly 12 hours of light and 12 hours of darkness in a day. Temperature was maintained at 21- 25°C while a humidifier was used to maintain the humidity between 40-70%.

Processing of ingredients

Maize grains, soybean and peanuts are locally available and commonly consumed in Western Kenya. The products were sorted, washed and immediately sun-dried after washing. To neutralize the anti-nutrients in the soybean, the grains were heat treated in a preheated oven at 120°C for 30 min. The cleaned ground nuts and maize grains were also roasted in the oven at 120°C for about 10 min to improve the flavour of the final product. All the grains were later milled separately using a commercial hammer mill (Powerline®, BM-35, Kirloskar, India) in Eldoret, fitted with a 2.0 mm opening screen.

Formulation of the ready-to-use therapeutic foods

The typical UNICEF recipe of RUTF (**Table 1**) guided the formulation. Formulation of flours was done according to the procedures of Briend et al. to prepare Ready-To-Use Therapeutic Foods (RUTF) based on the F100 nutrient profile to provide a similar nutrient profile in a form that needed no additional preparation consisting of roasted soybean flour, roasted maize flour, pounded peanuts, oil and icing sugar. Three different foods were formulated as indicated in **Table 2**. Each individual ingredient was weighed into a bowl and mixed using an electric mixer (Kenwood® Chef, KMC 200, Kenwood Co. Ltd., and UK) at medium speed for 2 min. The samples were then transferred into air tight plastic containers which were stored at ambient temperature until when required for proximate analyses and consumer evaluation

Table 1: Typical UNICEF recipe of RUTF.

Ingredients	Weight (%)
Full fat milk	30
Peanut	25
Vegetable oil	15
Sugar	28
Mineral mix	1.6

Table 2: Ingredients in the formulation of ready-to-use therapeutic foods.

Ingredients	Product 1 (%)	Product 2 (%)	Product 3 (%)
Soybean	30	55	30
Maize flour	0	28	28
Peanut	25	0	0
Soy oil	15	15	15
Sugar	28	0	25
Mineral mix	2	2	2
Total	100	100	100

Consumer acceptability of the ready-to-use therapeutic foods

The three formulated ready-to-use therapeutic foods were subjected to consumers to rate their degree of liking for appearance, smell, flavour and texture on a nine-point hedonic scale where 1=dislike extremely, 5=neither like nor dislike and 9=like extremely. The minimum value of 1 denoted not intense or not much and the maximum point of 9 denoted very intense or very much [16]. Adult evaluators instead of the target recipients who were children below the age of 5 years were involved because of their ability to objectively evaluate the sensory characteristics of the formulations. A sample of 50 consumers aged between 20 and 45 years comprising of 17 males and 33 females was obtained from the University of Eldoret through an advert and telephone calls. The advert contained information on all the ingredients used in preparing the ready-to-use products. Each panelist signed a consent form informing him/her of the nature of the samples they would evaluate before engaging in the sensory exercise. The consumer evaluations were conducted at the Foods Laboratory of the Department of Family and Consumer Sciences of the University of Eldoret with each session lasting about 45. Each panelist received a white tray containing the three samples in transparent glass bowls and a spoon for each sample and a glass of deionized water to cleanse the pallet before and in between tasting different samples (**Supplementary Figure 1**). Each sample was labeled with three digit blinding codes and the samples were also randomized for each panelist.

Results and Discussion

Proximate analysis

All the formulated products had a significantly protein content compared to 92 g sachet of Plumpy'Nut that is often used for nutrition intervention (**Table 3**). According to [11], fortification of cereals with 30% soy meal resulted in an increase in protein, moisture, oil, and ash (mineral) content. Ash content represents the minerals in food products [14]. Using soy-fortified therapeutic foods can alleviate hidden hunger or Micro-Nutrient Malnutrition (MNM) in children aged 1-2 years, a condition that is highly prevalent in developing countries [11] also found that maize: soy had a protein digestibility-corrected amino acid score (PDCAAS) of 70% compared to 53% in pure maize meal which translates into a 32.08% increment as a result of complementation [17]. Asserts that lesser protein of higher PDCAAS is better than higher quantity protein with lower PDCAAS at supporting growth.

The proximate analysis affirmed that soy is nutritionally superior to most of the foods used in Western Kenya. This is consistent with the values for the nutrient composition of soy [18]. This protein boost also made the products meet the minimum protein content threshold recommended for complementary foods which is 15 g/100 g [19]. A daily 100 g serving of the soy-fortified therapeutic foods can meet the daily protein needs (13 g) for a child aged 1-3 years [20]. Product 2 had the highest content of protein because of the peanut paste in it [21] found that a diet that contained yellow maize: soy: groundnuts in the ratio 60: 30: 10 exceeded the protein Recommended Dietary Allowance

(RDA) for the children aged 1-3 years. These studies confirm that legume-fortification is an effective strategy in treating and alleviating PEM.

Carbohydrate is the main source of energy in the body with an RDA for children aged 1-3 years at 95 g/day [20]. One 100 g serving of the soy-fortified diets would provide between 55.95% and 61.16% of these children's RDA. The developed products did not therefore meet the threshold for carbohydrate requirement in children aged 1-3 year (Table 3). The energy content of the products was slightly below what is required for an RUTF which is 530 Kcal/2218 KJ/100 g [22]. The energy RDA for children aged 1-3 years is about 4142.2 kJ/day [23]. A 100 g serving of the soy-fortified foods would provide 38.57–40.13% of the children's daily needs. If the children take at least 3 meals in a day, then their energy needs would be met. This conclusion was also reached by Negri et al. [24] who established that a single serving of the soy-fortified complementary foods would not meet the nutrient needs of children.

Consumer evaluation

Fifty consumers evaluated their liking of the three products for the sensory attributes of appearance, smell, flavour and texture. There was a significant difference in consumer acceptance of the different ready-to-use therapeutic formulations (Table 4). In terms of the appearance, product 2 was liked most and product 1 was liked the least in appearance. The aroma of product 1 was the most liked and that of product 2 was the least preferred. For the attribute of flavour, product 1 was again significantly different from the rest of the products. In consideration of the texture attribute, product 2 was significantly different from the rest of the varieties and was the most liked by the consumers.

The appearance of a food product is an important attribute in food choice and acceptance. In the present study, many of the consumers liked the appearance of product 2 and this might have been due to the influence of its superior physical characteristics of appealing product colour [25]. Earlier research by Obatolu and Osho [25] has examined consumer demand and noted that they focus on visible characteristics. The flavour and aroma are the main limiting factor affecting the product acceptability

by the consumer panel. Flavour and aroma of product 1 were rated highly followed by those of product 3. The acceptability of the products aroma and flavour might have probably been enhanced by addition of sugar [26]. Similar results have also been reported by Walker and Pavitt [27] who noted that the addition of sugar to complementary foods improved the flavour contents and encouraged the infants eat the food product. The texture attributes of product 2 was the most acceptable by many consumers in relation to product 1 and 3. This might have been influenced by the composition of the sample mixture that imparted a smoother texture to the product as compared to the rest of the products evaluated [27]. Texture attributes of ready-to-use complementary diets have been previously been studied in relation to consumer acceptance. For instance, found that consumers preferred complementary products that had a smoother texture [26]. Consumer acceptability of new products is vital in ensuring their success [28]. In general, all the products were well accepted by the consumers and this may have been due to the consumers' familiarity with the locally available ingredients that were used to formulate the products [14] established that developing new formulations of complementary foods using ingredients that are commonly used at homes results in higher acceptability of the products [29] also noted that consumers are more likely to accept foods that they are more familiar with even if it were prepared in different food method. Likewise, the consumer acceptability of all the three products might have also been influenced by the roasting and addition of oil that might have enhanced the aroma and flavour [27].

Effects of formulations on nutritional status of animal models

Each rat received 20 g/rat/day of a specific formulation per day. Water and food were available *ad libitum*. The data collected from this study was used in calculating the weight gained. There was significant reduction in weight of all rats during the first 7 days of the growth study (Figure 3). This is attributed to low protein diets which have been known to result in reduced food intake causing protein deficiency, emaciation, and death [12].

Protein in the body is needed for growth and development of body tissues [17]. Soy fortification resulted in an increase

Table 3: Proximate analysis per 100 g of the formulations.

	Ash (%)	Moisture (%)	Crude Protein (%)	CHO (%)	Crude Fiber (%)	Crude Fat (%)	Energy KJ
Product 1	5.27	4.98	14.47	11.64	9.32	19.05	1137.65
Product 2	7.74	7.65	15.43	39.75	9.51	18.00	1584.62
Product 3	4.24	6.80	12.30	23.67	8.65	19.21	1308.69
Plumpy'Nut 92 g sachet	4	5	14.5	43	-	33.5	2218

Table 4: Consumer perception (n=50) of sensory attributes for formulated ready-to-use therapeutic foods Values are means ± standard deviations. Values followed by the same letter superscripts in the same row are not significantly different at (p<0.05) as assessed by Fisher's Least Significant Difference; 1=Disliked extremely and 9=Liked extremely.

Parameters	Product 1	Product 2	Product 3
Appearance	6.50 ^c ± 1.21	7.94 ^a ± 1.43	6.90 ^b ± 1.64
Smell/Aroma	6.50 ^a ± 1.20	5.08 ^c ± 1.28	6.40 ^b ± 1.72
Flavour	6.88 ^a ± 1.34	5.06 ^c ± 1.64	6.20 ^b ± 1.69
Texture	6.10 ^b ± 1.76	6.58 ^a ± 1.90	5.82 ^c ± 1.80

in indispensable amino acids, isoleucine, leucine, lysine, tryptophan, threonine, and valine in maize [11]. Weight gain is an indicator of growth. The overall growth between the four groups was also very similar. This implies that all the formulations were statistically similar to the Plumpy’Nut considering weight gain as a parameter (Figure 3). They also indicated the ability to support growth and rehabilitation.

Cost of production of the formulations

In the RUTF formulations soybeans were used for protein provision instead of powdered milk. Maize flour was used to complement energy in the recipes. All the three formulations were found to be effective in rehabilitation of malnourished albino rats. Ingredients required for the production of these formulations are cheaper than ingredients used to produce Plumpy’Nut (Table 5) [30].

Conclusion

The formulations evaluated in the present research have been formulated based on the typical UNICEF recipe of RUTF using maize, soybeans, peanuts, sugar and vegetable oil which are locally available. These formulations were highly rated by the consumers due to their associated sweet flavour and aroma enhanced by roasting thus, have high potential of being adopted for human consumption in Western Kenya to treat severe acute

malnutrition. They were effective in reducing malnutrition and promoting growth of animal models and therefore might be effectively used in rehabilitating acutely undernourished children. They can also be used in supplemental feeding and other nutrition support programs. Finally, the costs of producing these options of RUTF are much lower than using milk and importing RUTF.

Recommendations

There is need to improve the energy density of the formulated products through further research. It is also essential to ascertain the shelf life of the formulated products. Clinical trials on malnourished children need to be carried out to extrapolate the findings of the study. Production and utilization of soybeans should be enhanced to make raw materials readily available. Cost comparison of different scales of production should also be determined.

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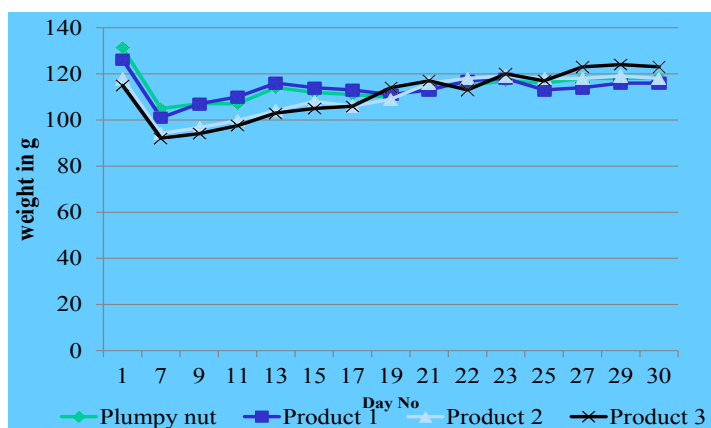


Figure 3 Mean weight of the rats for a period of 30 days.

Table 5: Comparison of the prices of ingredients used in RUTF formulations and Plumpy’Nut. Note: It is estimated that 100 KES equivalent to US\$1.

Plumpy’Nut			Locally produced RUTF		
Ingredient	Price per 100 g (KES)	Price per 100 g (US\$)	Ingredient	Price per 100 g (KES)	Price per 110 g (US\$)
Groundnuts	15	0.15	Groundnuts	15	0.15
Powdered milk	-	-	Soybeans	9	0.09
Safari land brand	115	1.15	-	-	-
Nido brand	933	9.33	-	-	-
Miksi brand	374	3.74	-	-	-
Kenya Highland brand	435	4.35	-	-	-
KCC brand	250	2.5	-	-	-
Cooking oil	15	0.15	Cooking oil	15	0.15
Sugar	15	0.15	Sugar	15	0.15
			Maize	7.5	0.075

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