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Research Paper

Formulation and evaluation of complementary weaning food prepared from single and combined sprouted/fermented local red sorghum (*S. bicolor*) variety blended with cowpea (*Vigina unguiculata*) and groundnut (*Arachid hypogea*)

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Abstract. The study investigated the use of locally sourced flours of sprouted and combined sprouted/fermented sorghum, cowpea and groundnut which were blended in the ratio of 70:20:10 (W/W) to produce weaning food rich in energy and protein. The results showed significant differences (P < 0.05) between the processed formulated, unprocessed and the commercial weaning diet "Cerelac" (Nestle, Nigeria) in terms of protein, minerals and energy content respectively. The combined sprouted/fermented formulated diet showed significant (P < 0.05) higher values when compared with the unsprouted and unfermented sorghum flour. The animal feeding experiment further correlates with the *in vitro* results by recording significantly (P < 0.05) higher values of PER (Protein Efficiency Ratio), AD (Apparent Protein Digestibility), and BV (Biological Value) and NPU (Net Protein Utilization) for the processed formulated diet compared to the unfortified diets. Comparing the test diet (combined sprouted/fermented and fortified) sample favourably with the standards FAO/WHO values, it can be concluded that the test diet has adequate protein quality that can be been used in alleviating protein energy malnutrition (PEM) in infant especially during the weaning period.

Keywords: Protein-energy malnutrition combined sprouted/fermented, protein efficiency ratio, net protein utilization, apparent digestibility, biological value.

INTRODUCTION

In many developing countries such as Nigeria, malnutrition is a common dietary problem that is said to be endemic (Mbaeyi and Onweluzo, 2010). This is characterized by micronutrient-deficiency and protein-energy malnutrition. Animal protein products are quite expensive and beyond the reach of low-income family, thus such protein source cannot be afforded. As a result, dietary diversification has been employed as a solution to malnutrition challenges (Hensen et al., 1989). Various workers reported the possible ways of enriching our locally prepared cereal diets with indigenous plant legumes (Nkama et al., 1995; Malleshi, 1998). This involves the use of commonly available cereals and legumes in various proportions to meet dietary need of the target people with a view to complementing essential amino acids.

The commonly available cereal grains and legumes include maize, millet, sorghum soyabean, cowpea and groundnut, respectively (Elyas et al., 2002; Echendu et al., 2004; Gupta and Sehgal, 2001).

The need for high quality protein foods to feed a child at weaning age is necessary to help alleviate protein energy malnutrition by employing simple processing. Thus there is the need to introduce ones indigenous foods prepared by employing simple physical extraction methods such as soaking, dehulling alongside other advanced food processing technology process such as sprouting and fermentation. In many developing countries, like Nigeria, traditional weaning foods are prepared mainly from cereals like maize and sorghum (Elemo et al., 2011), which are usually poor in protein quantity and quality, this coupled with the high cost and viscous nature of commercially available complementary foods are the major constraints in providing children with adequate nutrients. It is therefore desirable to study ways and means of developing less costly but equally nutritious complementary foods that may be within the reach of the wider population, by using locally available staple cereals or/legumes and simple or adaptable processing technologies. Several work has been done on the formulation and development of nutritious complementary foods from locally and readily available raw materials using sprouting and fermentation technologies, which are simple traditional processing methods that have been reported to be effective in reducing bulk or viscosity of gruels (Elemo et al., 2011; Hensen et al., 1989; Hibberd et al., 2003). Such reports involving the use of cereals blended with legumes notably: pigeon pea, soya beans have achieved remarkable success (Gernah et al., 2011; Hensen et al., 1989; Hibberd et al., 2003). However, information on the combined effect of sprouting and fermentation on sorghum cowpea and groundnut food formulations is inadequate.

Sorghum, cowpea and groundnut used in this study have been shown to adequately complement one another to produce food blend, in addition to the combined sprouting and fermentation of the sorghum, thus improved the availability and quality of their proteins as well as reduce anti-nutritional factors that may affect utilization of their nutrient. The objectives of this study was, therefore, to evaluate the nutritional composition and protein quality of food formulations by combined sprouting and fermentation techniques and subsequent fortification with cowpea and groundnut.

MATERIALS AND METHODS

Source of materials: About 500 g of sorghum red variety and groundnut (200 g) were obtained from Maiduguri Monday market. Cerelac (a maize-soya bean based infant food made by Nestle Foods, Nigeria Plc, Lagos) was purchased from a local super market in Maiduguri, Borno State Nigeria. Wistar albino rats at weaning age of (21 days old) were obtained at the Department of Biochemistry Animal Breeding Unit, University of Maiduguri, Nigeria. All chemicals used for analysis were of Analar grade (British Drug House chemical poole England), and a protein free diet cassava.

Preliminary treatment

All the grains and legumes were manually cleaned, by removing the ones that are mouldy or broken.

Preparation of sprouted sorghum flour

The grains were sprouted as described by (Kulkarni at al. 1991). About 500 g of the sorghum sample was soaked in a plastic bucket containing 300 ml of distilled water and steeped for one hour at room temperature (28 ± 2°C). The steep water was discarded by decantation and the steep grains were germinated for seventy-two hours by spreading on a clean grease free tray pan and thereafter, it was sundried for two-three days by putting it in a sterilized tray pan. The sorghum grains was then milled using a disc attrition mill (Hunt No. 2A Premier Mill Hunt and Co, UK) to an average particle size of less than 0.3 mm. The milled grains were then sieved through a fine mesh (0.5 mm) to obtain the sorghum four.

Preparation of fermented sorghum flour

Fermentation was carried out using the method described by Ariahu et al. (1991). About 500 g of sprouted sorghum grains was soaked in water, about three times its weight by volume for seventy-two hours. The fermented grains were washed thoroughly and sundried for three days. The dried fermented sorghum grains was milled and sieved through a 0.5 µm mesh screen to obtain the sorghum flour.

Preparation of cowpea

About 200 g of cowpea seeds was cleaned, washed and then soaked in water for twenty minutes. The seeds were dehulled, washed to remove the husk, after which it was dried to a constant weight. The cowpea seeds was roasted and then milled into fine powdered flour as shown in Figure 1.

Preparation of groundnut

Two hundred grams of groundnut seeds were roasted for thirty minutes, on cooling the skin were removed by rubbing between palms. All seeds were roasted to golden brown colour.

Formulation of sprouted and fermented sorghum with cowpea and groundnut

Sorghum, cowpea and groundnut were blended in a ratio of 70:20:10 as shown in Figure 1.

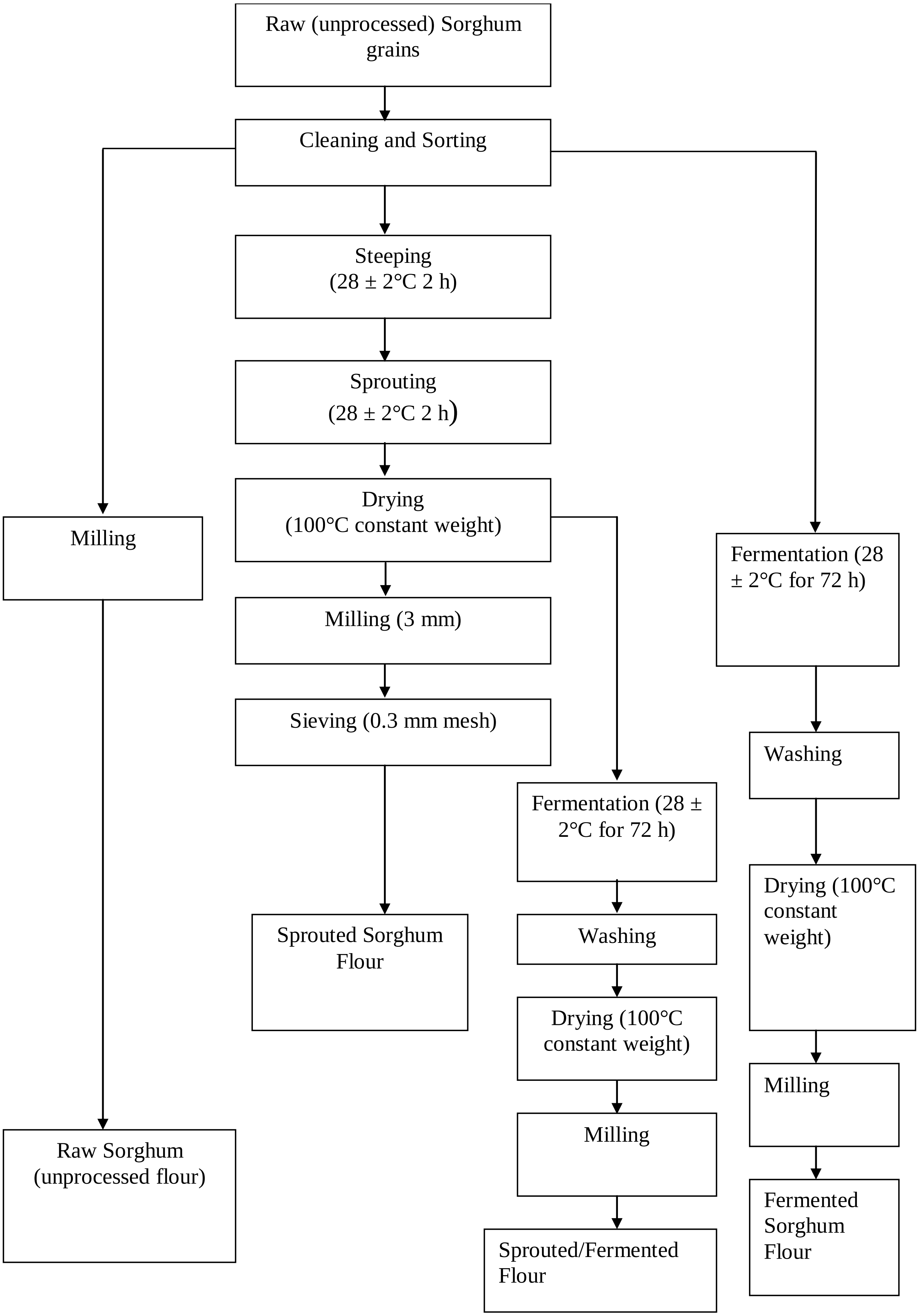

Figure 1. Flow chart for production of different sorghum flours. Source: (Kulkarni et al., 1991).

Table 1. Ingredient rations of designed food formulations.

	RCR	SCR	FCR	SFCR	SFCRF
Sorghum	100	70	70	70	70
Cowpea	-	-	-	-	20
Groundnut	-	-	-	-	10
Cerelac	100	100	100	100	100

Keys: RCR, SCR, FCR, SFCR and SFCRF. Source: (Kulkarni *et al*., 1991).

Food products formulation

The four different foods were formulated from sorghum, cowpea and groundnut. The four formulations were: raw (unprocessed) sorghum (RCR), sprouted sorghum (SCR), fermented sorghum (FCR) and sprouted/fermented fortified with cowpea and groundnut (SFCRF). SFCR was blended in a ration of 70:20: 10 as shown in Figure 1. These were packaged in a polyethylene bags and stored in a plastic containers with air tight lids and kept before the commencement of the work (Table 1).

Determination of protein quality

Protein quality indices were determined using standard methods. The nitrogen content of the faeces and urine was determined by the standard, micro Khjedahl method (AOAC, 2000). From the values of mean daily feed intake and mean weekly weight gain obtained, protein efficiency ratio (PER), Net Protein Utilization (NPU) were estimated by the method of Pellet et al. (1980). Apparent digestibility (AD) and biological value (BV) were calculated by using approved formulae.

Animal feeding experiment

For nitrogen determination studies, six groups of rats each weighing about 45 to 50 kg were housed in a well ventilated room and allow access to feed and water *ad libitum*. Each group of six replicates was fed a different weighed experimental and control diet for thirty five days including the period of acclimatization. A weighed diet (100 g) was given daily and unconsumed diet was collected and body weight gain/lost were recorded weekly throughout the period of the study.

The feeding regime lasted for twenty eight days within which faeces and urine samples were collected on a daily basis and later pooled together, the volume of urine and faeces were recorded and used for the determination of nitrogen by micro Khjedahl method. Another group of rats of the same weight and ages were fed on nitrogen free diet to calculate for nitrogen content, apparent protein digestibility (APD), net protein utilization (NPU) and biological value (BV) were assessed by using the following formulas: described by Pellet et al. (1980).

$$\text{Biological Value (BV)} = \frac{(\text{Nintake} - \text{FN} - \text{UN})}{\text{Intake}} \times 100$$

$$\text{Apparent Protein Digestibiliy (APD)} = \frac{(\text{Nintake} - \text{FN})}{\text{Nintake}} \times 100$$

$$\text{Net Protein Utilization (NPU)} = \frac{[\text{Nintake} - \text{FN} - \text{UN}]}{\text{Nintake}} \times 100$$

or $$\frac{\text{BV} \times \text{TPD}}{100}$$

Where:
BV = Biological value,
FN = Faecal nitrogen,
UN = Urinary nitrogen.

These equations were used because correction was not made for obligatory losses; therefore, the calculation gave the apparent BV, digestibility and NPU (Pellet et al., 1980).

RESULTS

Table 2 presents the results on the effect of combined processing techniques of sprouted/fermented sorghum and unfortified sorghum blends on chemical composition compared with commercial weaning food, standard set by SON (1988) and RDA. The chemical composition of RCR, SFCR and SFCRF weaning foods compared to standard and RDA, are shown in Table 2: the moisture content RCR was 6.50%; SFCR was 8.0%; and SFCRF was 9.1%, respectively. There were significant differences ($P < 0.05$) in the protein content of RCR, SFCR and SFCRF (6.50, 9.38 and 14.2%, respectively). The protein content of RCR and SFCR did not meet the RDA of protein required for infants 0 to 12 months and 1 to 3 years old. While the protein content of SFCRF met a RDA of protein required infant 0 to 1 year these findings were similar to those reported by Elemo (2011) for germinated sorghum and steamed cooked cowpea weaning foods. RCR had fat content of 4.52%, SFCR 4.23% and SFCRF had 6.33%, which did not meet the RDA for fat for both young and older infant

Table 2. Effects of combined processing techniques of sprouted fermented fortified and unfortified sorghum flour blends on chemical composition compared with commercially weaning foods and standard set by SON (1998).

Chemical composition (%)	RCR	SFCR	SFCRF	SON (1988)	Commercial weaning food cerelac	% RDA 7-12 months 1-3 yrs
Moisture	6.50 ± 0.12	8.48 ± 0.09	9.67 ± 0.02	5-10 max	4.0	-
Protein	6.97 ± 0.13	9.38 ± 0.32	14.2 ± 1.20	14-17 (mm)	16.0	8.30 – 8.6
Fats	4.51 ± 0.06	4.23 ± 0.15	4.33 ± 0.08	10 max	9.0	9.10 ND
Ash	3.65 ± 0.09	2.32 ± 0.41	2.43 ± 0.16	10 (max)	ND	-
fiber	4.06 ± 0.17	2.43 ± 0.23	3.17 ± 0.18	5 (max)	5.0	5.75
Carbohydrates	81.4 ± 0.78	71.1 ± 0.45	75.1 ± 2.64	—	63.7	28.79 and 39.40
Energy value (kcal/100 g)	361.6	352.0	396.1	350 – 400	400	-

Key: RCR = Raw (unprocessed) Chakalari Red, RCR = Fermented Chakalari Red SCR = Sprouted SFCR = Sprouted / Fermented.

Table 3. Apparent digestibility, biological value and net protein utilization (NPU) of formulated diets made from sorghum cowpea and groundnut.

Diets	N excreted Faecal	Urinary N	Apparent digestibility (%)	Biological value (BV) (%)	Net protein utilization (NPU) (%)
RCR	0.21 ± 0.03^{a}	0.20 ± 0.03^{a}	70.3 ± 0.68^{a}	75.3 ± 0.36^{a}	94.0 ± 0.33^{a}
FCR	0.29 ± 0.01^{c}	0.38 ± 0.01^{a}	76.0 ± 0.57^{cb}	82.0 ± 0.86^{c}	90.3 ± 0.33^{a}
SCR	0.01 ± 0.003^{a}	0.10 ± 0.01^{b}	82.6 ± 0.67^{e}	90.6 ± 0.88^{ac}	95.0 ± 0.55^{b}
SFCR	0.3 ± 0.03^{a}	0.40 ± 0.01^{a}	89.0 ± 0.87^{g}	93.6 ± 0.68^{e}	94.6 ± 0.33^{a}
SFCRF	0.46 ± 0.04^{eh}	0.33 ± 0.02^{a}	92.6 ± 0.33^{i}	95.6 ± 0.33^{a}	95.0 ± 0.60^{cb}
Cerelac	0.50 ± 0.03^{g}	0.34 ± 0.04^{da}	98.6 ± 0.33^{k}	99.0 ± 0.93^{g}	98.3 ± 0.3^{a}

Means in the same column followed by different letters differ significantly according to Duncan New Multiple Range test. Key: RCR = Raw (unprocessed) Chakalari Red, RCR = Fermented Chakalari Red SCR = Sprouted SFCR = Sprouted / Fermented.

1 to 3 years. The ash content of RCR, SFCR and SFCRF were 4.40, 2.32 and 3.17%, respectively while the carbohydrate contents were 60.1% for RCR, 60.5% for SFCR and 73.0% for SFCRF, respectively and provided a RDA for both 7 to 12 months and 1 to 3 years groups. The energy value of RCR (365.8 kcal/100 g), SFCR (361.1 kcal/100 g) and SFCRF (396.1 kcal/100 g), compared favorably well with the standard set by SON (1988) and that of the commercial weaning food 'Cerelac'.

Figure 2 presents the results of the *in vitro* protein digestibility of the raw, fermented, sprouted, combined sprouted fermented unfortified and fortified blends from red sorghum variety. The IVPD at 6 h of digestibility are significantly ($p < 0.05$) higher indicating that it is time dependent. A significant increase was also observed in both the combined sprouted/fermented fortified and unfortified samples, that is, SFCR and SFCRF. The sprouted/fermented fortified blend had the higher digestibility values of 90.2% at 1 h and 98.7% at 6 h followed by sprouted/fermented unfortified blends with the digestibility values of 87.9% at one hour and 96.1% at six hours; while the raw (unprocessed) sample had the least values of digestibility. The IVPD values were within the range of those reported by Sanda et al. (2012), who recorded similar increases in digestibility of processed sorghum flour from five local varieties.

Table 3 shows the results of apparent Biological Value, Net Protein Utilization and Apparent Digestibility of the processed fortified and unfortified prepared from red sorghum flour. There were no significant difference in the Biological Value (BV), Net Protein Utilization (NPU), and Apparent Digestibility (AD) of the entire test diets ($P < 0.05$) expect for the reference diet and formulated weaning foods. Group RCR and FCR are similar in all respect and also had lower biological value (BV), Net Protein Utilization (NPU) and Apparent Digestibility (AD) than the other test diets. The results of the BV for reference diet was (100%) followed by SFCRF (98%). The close BV for the test diet to Cerelac, a commercially prepared weaning food indicates adequate complementation of amino acids in the test diet supplemented with cowpea and groundnut. Apparent Digestibility of the formulated diet from Red sorghum flour ranged from 91.3 to 98.6% with SFCRF and Cerelac recording the higher AD.

DISCUSSION

Chemical composition

Chemical composition of the combined, processing effects of sprouted/fermented fortified and unfortified

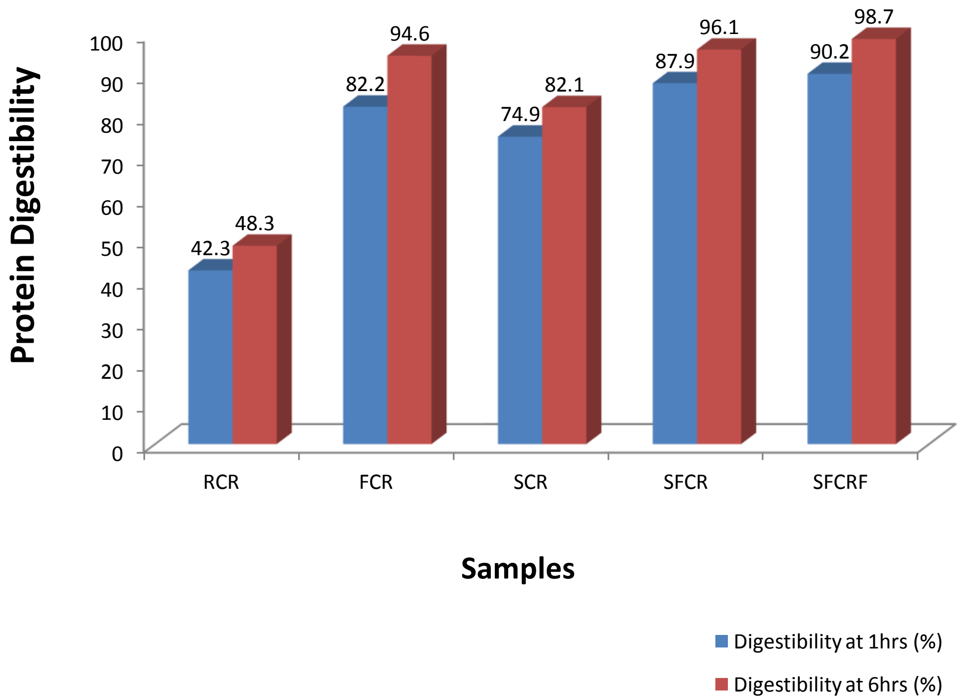

Figure 2. *In vitro* protein digestibility of different processed sorghum (*S. bicolor)* flour from red sorghum.

sorghum blends. The improvement in the protein content of the SFCRF formulated weaning food blend may be due to the combined processing effects, that is, sprouting and fermentation and further fortification of grain legumes. This improved the protein content of the formulated blends. This is in consistent with earlier work reported by Mbaeyi and Onweluzo (2010), which reported significant increase in protein content of sprouted sorghum-pigeon pea composite blend.

An increase in carbohydrate content was noticed in the SFCR and SFCRF formulated weaning food blend prepared from sorghum flour which compared favorably with the RDA of infants (0 to 1 year) and commercial weaning foods cerelac and this is in agreement with the reports of Modu et al. (2005) and Nkama and Gbeny, (2001) in the production of Ogi form six pearl millet varieties. In terms of fat contents the weaning food blend prepared from combined sprouted/fermented sorghum, cowpea and groundnut compared favorably well with the recommended dietary allowance (RDA) of infants (0 to 1 year) and commercial weaning food cerelac$^{(R)}$.

The increase in fat content may be due to increase in activity of lipolytic enzymes in the fermentation medium which hydrolysis fat to glycerol and fatty acids. The free fatty acids used by the fermenting organisms in the synthesis of new lipids (Gernah et al., 2011).

The energy value of RCR, SFCR and SFCRF compared favorably well with the standard set by SON (1988) and that of the commercial weaning foods ceralac$^{(R)}$.

In vitro protein digestibility

Improvement in the protein digestibility of the weaning food blend prepared from sprouted and fermented fortified sample could be due to the activities of proteolytic enzymes during fermentation, which degrades protein into simple protein, polypetides and amino acids, thus enhancing digestibility of the food samples. This result agreed with the observations of some numerous workers (Hibberd et al., 2003), who reported that enzymic processing techniques improved protein digestibility due to reduction in contents of antinutritional factors during the process of fermentation of sorghum grains. Elyas et al. (2002) and supplementation of grain legumes cowpea and groundnut may also be partly responsible for the increase in protein digestibility of the weaning food blends.

Nitrogen balance studies

There were no significant ($P < 0.05$) differences in the Biological Value (BV), Net Protein Utilization (NPU) and Apparent Digestibility (AD) of all the diets expect for the reference and formulated weaning food blend. The higher BV value for the group of fed the reference diet cerelac$^{(R)}$ was (100%) and that of the formulated diet SFCRF (98%). The close BV for the test diet to cerelac$^{(R)}$ indicates adequate complementation of amino acids in the test diet supplemented with cowpea and groundnut.

This is in consistent with the work reported by Modu et al. (2010). For Apparent Digestibility and NPU, the formulated weaning food recorded the highest value (97%) close to the reference diet ceralac (98%). The close proximity of the formulated weaning food blends to the reference diet (cerelac) could be due to complementation with cowpea and groundnut. This also suggested that the blend (SFCRF) to be more superior to the rest of the blends. The sprouted/fermented sample without fortification was the second best.

CONCLUSION

The results have shown that the formulated weaning food prepared from sorghum, cowpea and groundnut, had protein content which conformed to the FAO/WHO recommended value of 10%. The results of the nitrogen balance study further revealed that these formulated weaning food blends was nutritionally comparable with the reference diet (cerelac$^{(R)}$), indicating that the underutilized crops used in this study which are available in tropical countries could be used in producing best weaning foods that promote the combat of malnutrition.

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