Development and quality evaluation of a soy-fortified Ghanaian weaning food

Nana Tekyiwa Annan and Wisdom Annorsey Plahar

Abstract

Appropriate process characteristics and blend formulations were developed for the preparation of a high protein-energy weaning food, FRI Weaner, using maize, soya beans, groundnut, and milk powder. Its quality was evaluated in terms of its nutritive value; physicochemical, functional, and sensory characteristics; content of antinutritional factors; and biochemical and haematological properties. The FRI Weaner had physical and sensory characteristics similar to those of a traditional Ghanaian cereal-based weaning food but was of superior nutritional quality. The protein content was 171%, with 10.6% fat and 67.8% carbohydrates. Calcium, iron, and phosphorus levels were also high. Animal studies indicated good growth and development in rats fed with the blend, with no adverse biochemical or haematological effects. The blend's protein efficiency ratio was 2.5. The blend can therefore be used as an ideal weaning food to improve the nutrition status of Ghanaian children and help solve problems associated with protein-energy malnutrition.

Introduction

The formulation and development of nutritious weaning foods from local and readily available raw materials has received a lot of attention in many developing countries. The widespread problem of infant malnutrition in the developing world has stimulated efforts in research, development, and extension by both local and international organizations. Legumes are largely replacing milk and other sources of animal protein, which are expensive and not readily available, as suitable substitutes for high-quality protein. Over 70% of dietary protein in developing countries is supplied by cereals that are relatively poor sources of protein [1]. The high lysine content of legumes improves the nutritional quality of cereals by complementing their limiting amino acids (sulphur-containing amino acids are limiting in legumes and relatively high in cereals, whereas lysine is limiting in cereals and high in legumes). Several blends using legumes singly or in combination have been evaluated [2-5].

A large variety of oilseeds and pulses, including cowpeas, groundnuts, pigeon peas, and melon seeds, grow well in Ghana, forming part of the traditional diets of many people. Soya beans have recently become popular in the West African subregion due to their high protein content and quality, and are being cultivated at a steadily increasing rate. Traditional food uses of soya beans are very limited, however, and efforts are being made to promote their incorporation in the people's diets.

Extensive research into the process characteristics, nutritional quality, and consumer acceptability of soya beans and soy-cereal blends is necessary if these items are to be used effectively to improve the nutrition status of the vulnerable group of the population. At the Food Research Institute, Ghana, soy flour produced on pilot scale is gaining popularity among middle-income mothers as a protein supplement for cereal-based weaning foods. From this product, a weaning food, code-named FRI Weaner (Food Research Institute Weaner), was formulated using maize, soy flour, groundnuts, and small amounts of milk powder. This study was undertaken to evaluate FRI Weaner for its nutritive value, content of antinutritional factors, biochemical and haematological indexes, physicochemical and functional characteristics, and consumer acceptability compared with locally available cereal-based traditional and commercial weaning foods.

Materials and methods

Weaning formulations

Local soya beans, purchased from the Ghana Food Distribution Corporation, Accra, were processed into full fat soy flour. The beans were soaked for 20 min uses, drained, and boiled in water for 30 minutes. They were then dried in a hot air oven at 65°C to a final moisture content of about 8%. The dried beans were dehulled in a disc attrition mill, winnowed, and milled into a smooth flour [6]. Maize and groundnuts, purchased at a local market in Accra, were roasted separately...
at 150°C until golden brown. The roasted maize was mixed with 5% dehulled groundnut and milled uniformly into a
smooth flour using a disc attrition mill.

Using a stepwise calculation procedure for blend formulation [7], FRI Weaner was formulated to contain 75% maize-
groundnut flour, 20% full-fat soy flour, and 5% full-fat milk powder. Maximum complementation of amino acids and
protein content of about 20% were targeted to satisfy the minimum protein content of 15% required under the Food and
Agriculture Organization (FAO)/World Health Organization (WHO) Codex Alimentarius standards [8]. The traditional Tom
Brown weaning food was prepared by roasting maize till golden brown and milling to a smooth flour. A popular
commercial cereal-based weaning food, Cerelac, was purchased and used for comparison.

Analytical procedures

Amylograph pasting viscosity measurements

The pasting properties of the samples were determined with a Brabender VISCO/amylo/GRAPH (Model VA-YE, C. W.
Brabender Instruments, Inc., South Hackensack, NJ, USA) equipped with a 700 cm/g sensitivity cartridge. A 10% slurry
(dry weight basis) of each flour was prepared with distilled water and heated uniformly (1.5°C/min) from 25°C to
95°C, held at 95°C for 15 minutes, and cooled at the same rate to 50°C [9]. The resulting amylograms provided
pasting temperatures, peak viscosities, viscosity at 95°C, stability, cooking times, and set-back viscosities.

Chemical composition

Samples of the weaning foods and raw ingredients were analyzed for moisture, protein, fat, and ash by standard
procedures [10]. Carbohydrate was determined by difference and energy by calculation. Iron, phosphorus, and calcium
were measured using standard methods [11].

Amino acid analysis

Selected amino acid composition of samples was determined by digestion under vacuum with 6 N hydrochloric acid in
sealed ampules at 110°C for 24 hours. The hydrolysates were analysed for amino acids on a Beckman 121 Automatic
Amino Acid Analyser [12]. Cystine was determined as cysteic acid by performic acid oxidation [13]. A colourimetric
technique [14] was used to determine tryptophan in extracts prepared by a method developed elsewhere [15].

Sensory evaluation

Acceptability scores of FRI Weaner and Tom Brown were determined by a 10-member trained panel using a 9-point
hedonic scale [16].

Determination of antinutritional factors

Trypsin inhibitor activity [17], phytate content of samples [18], and oxalate content [19] were determined by methods
described elsewhere [19].

Animal studies

Four-week-old weanling albino rats (Rattus norvegicus) obtained from the Department of Biological Sciences Animal
House, University of Science and Technology, Kumasi, Ghana, were divided into groups of four and acclimatized, after
which they were fed the test diet. The basic composition of the diet was vegetable oil (8%), vitamin premix (1%), mineral
premix (1%), cellulose (1%), test protein-casein (10%), sugar (7%), and cornstarch added to make up to 100%. Food
and water were given ad libitum, and the weights of the animals were recorded daily for four weeks. The feed intake was
recorded and the protein efficiency ratio (PER), feed efficiency ratio (FER: weight gained/g intake), and mean weight
gain were calculated.

The physical appearance of the animals was recorded. They were then killed, and blood for haematological analysis was
quickly drawn by jugular incision and put in ethylenediaminetetraacetate (EDTA) solution. Blood for serum protein and
enzyme determination was drawn into tubes and centrifuged for 30 minutes. The serum was stored in a refrigerator for
subsequent analysis.

Biochemical and haematological analyses

Aspartate transaminase (AST) substrate (0.5 ml) was warmed at 37°C for 5 minutes and incubated with 0.1 ml serum
for exactly 60 minutes. Dinitrophenyl hydrazine (DNPH, I ml) was added and allowed to react for 20 minutes. The
mixture was then reacted with 5 ml 0.4 M sodium hydroxide and allowed to stand for 10 minutes, and absorbance was
read at 520 nm against a blank [20]. For serum albumin, the bromocresol green (BCG) method [21] was used.
Samples of blood serum (0.02 ml) were mixed thoroughly with 4 ml buffered indicator (BCG) and read at 600 nm against
a blank of 0.02 ml distilled water with buffered indicator.

The haemoglobin content was measured with 0.02 ml blood samples diluted in 4 ml of Drabkin’s reagent. Absorbance
readings were taken after 10 minutes at 540 nm [22].
The packed cell volume was determined by the microhaematocrit method [23]. Two capillary tubes (length 75 mm, internal diameter 1.16 mm, wall thickness 0.20 mm) were filled with blood to between 5.5 and 6 cm of the total length. The dry ends of the tubes were sealed and centrifuged for 5 minutes at 12,000 x g. The volume occupied by the red cells was measured by a microhaematocrit reader and expressed as a percentage of the total blood in the tubes.

Blood samples (0.02 ml) were mixed with anticoagulant (sequestering) and diluted in 4 ml formal citrate solution. The diluted blood was placed in a counting chamber, and red cells were counted under a dry objective lens.

Blood samples (0.02 ml) were mixed with sequestering and diluted in 0.38 ml diluting fluid (1.5 ml glacial acetic acid, 0.5 ml malachite green, 98.0 ml water). The diluted blood was mounted in a counting chamber, and white blood cells were counted.

**Statistical analysis**

The statistical significance of the observed differences among the means of the experimental results was evaluated by analysis of variance followed by pairwise comparison of means [16].

**FIG. 1. Amylograph pasting characteristics of traditional and improved weaning foods**

**Results and discussion**

FRI Weaner was prepared to possess physical characteristics similar to those of a popular traditional roasted maize weaning food known as Tom Brown. Except for the initial pasting temperature, all the hot paste viscosity values were slightly lower in FRI Weaner (fig. 1). This is quite desirable, as it would facilitate a higher nutrient density on cooking the formulated product.

**Composition and acceptability of traditional and improved weaning foods**

The chemical composition of the raw ingredients used is given in table 1. Protein and fat contributions to the blend are mainly provided by soya beans, groundnuts, and milk powder, and the main source of carbohydrate is maize. The protein content of the formulated blend was almost double that of the traditional weaning food, and fat increased from 4.3% to 10.6% (table 2). According to the recommendations of the Protein Advisory Group (PAG) guidelines [24] for weaning foods, protein content should be at least 20% (on a dry weight basis), fat levels up to 10%, moisture 5% to 10%, and total ash not more than 5%. The results for the composition of FRI Weaner fall within the acceptable ranges of the recommendations given, but Tom Brown is well below the standards. Compared with Cerelac, FRI Weaner had higher protein and fat but slightly lower ash content. The increase in protein is attributable to the incorporation of soy flour in the blend, whereas the sharp increase in the fat content is due to both the full-fat soy flour and the groundnuts. Total energy, iron, calcium, and phosphorus levels were also significantly higher in FRI Weaner. The total ash content of 1.98% is an improvement on Tom Brown but is still less than 5%, which is the value for ash recommended by the PAG [24]. This suggests that fortification with minerals may be necessary.

**TABLE 1. Chemical composition of raw ingredients of FRI Weaner**

<table>
<thead>
<tr>
<th>Component</th>
<th>Ingredient</th>
<th>Maize</th>
<th>Soya beans</th>
<th>Groundnuts</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td></td>
<td>11.1</td>
<td>9.7</td>
<td>7.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Protein (%)</td>
<td></td>
<td>8.8</td>
<td>39.6</td>
<td>21.8</td>
<td>25.7</td>
</tr>
<tr>
<td>Fat (%)</td>
<td></td>
<td>3.9</td>
<td>19.5</td>
<td>47.8</td>
<td>29.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td></td>
<td>1.3</td>
<td>5.6</td>
<td>2.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td>75.1</td>
<td>25.6</td>
<td>20.9</td>
<td>36.6</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td></td>
<td>359</td>
<td>436</td>
<td>601</td>
<td>510</td>
</tr>
<tr>
<td>Iron (mg/100 g)</td>
<td></td>
<td>4.8</td>
<td>7.8</td>
<td>4.1</td>
<td>12</td>
</tr>
<tr>
<td>Phosphorus (mg/100g)</td>
<td></td>
<td>253</td>
<td>269</td>
<td>346</td>
<td>750</td>
</tr>
<tr>
<td>Calcium (mg/100 g)</td>
<td></td>
<td>45</td>
<td>204</td>
<td>83</td>
<td>930</td>
</tr>
</tbody>
</table>

a. Values are means of triplicate determination expressed on as is basis.

**TABLE 2. Composition and acceptability of traditional and improved weaning foods**

<table>
<thead>
<tr>
<th>Component</th>
<th>Weaning food</th>
</tr>
</thead>
</table>

Lysine, tryptophan, and the sulphur-containing amino acids are the essential amino acids of major importance in blend formulations with cereals and legumes. Due consideration is given to the right proportions of the blend components for maximum protein quality through the mutual complementation of the limiting amino acids. The proportions of ingredients effectively maximized the concentrations of all three important limiting amino acids in FRI Weaner, resulting in very high protein scores. Lysine increased by 100%, with 20% and 10% increases achieved in tryptophan and sulphur-containing amino acids, respectively. Sensory scores also indicated high acceptability values for FRI Weaner.

Concentration of antinutritional factors in weaning foods

Soya beans used in the preparation of the FRI Weaner had trypsin inhibitor activities of 20 to 25 mg pure trypsin inhibited per gram sample. The processing techniques, however, reduced this value to less than 3 mg/g sample, with the final blend product containing a safe level of 0.5 mg/g sample (table 3). This represents a decrease of 88% trypsin inhibitor activity. In experiments with rats, nutritional benefits measured by PER were maximum at 87% inactivation of trypsin inhibitor [25]. This observation is in agreement with the high PER (2.47) values obtained for rats fed FRI Weaner.

Oxalates, tannins, and phytates were also quite low in both the Tom Brown and the FRI Weaner. Oxalates in large amounts bind with calcium, forming calcium oxalate, which is insoluble and not absorbed by the body. They are therefore considered poisonous but harmless when present in small amounts, such as those observed in this study [26]. Dietary levels of phytates of 1% or more have been reported to interfere with mineral availability [27]. The levels of phytate in FRI Weaner and Tom Brown are low enough to avoid any detrimental effects. The lower level of phytates in FRI Weaner than in Tom Brown is attributable to higher amounts of crude fibre in Tom Brown, as evidenced by the latter's higher level of total carbohydrates. Foods with high fibre contain equally high amounts of phytate [28].

<table>
<thead>
<tr>
<th>Component</th>
<th>Traditional Tom Brown</th>
<th>FRI Weaner</th>
<th>Commercial Cerelac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>2.32</td>
<td>2.56</td>
<td>5.30</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>9.60</td>
<td>17.10</td>
<td>15.50</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.30</td>
<td>10.56</td>
<td>9.00</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.39</td>
<td>1.98</td>
<td>3.30</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>82.40</td>
<td>67.80</td>
<td>66.90</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>406.62</td>
<td>442.60</td>
<td>411.00</td>
</tr>
<tr>
<td>Iron (mg/100 g)</td>
<td>3.60</td>
<td>4.50</td>
<td>7.50</td>
</tr>
<tr>
<td>Phosphorus (mg/100g)</td>
<td>369.70</td>
<td>374.40</td>
<td>430.00</td>
</tr>
<tr>
<td>Calcium (mg/100 g)</td>
<td>139.15</td>
<td>253.50</td>
<td>530.00</td>
</tr>
<tr>
<td>Selected amino acids(g/100 g N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lysine</td>
<td>2.7</td>
<td>5.3</td>
<td>-</td>
</tr>
<tr>
<td>tryptophan</td>
<td>0.7</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>S-aa</td>
<td>3.5</td>
<td>3.8</td>
<td>-</td>
</tr>
<tr>
<td>Protein score (%)</td>
<td>47</td>
<td>91</td>
<td>-</td>
</tr>
<tr>
<td>Acceptability</td>
<td>7.5</td>
<td>8.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>

a. Values are means of triplicate determination.
b. Protein scores were based on FAO/WHO/UNU reference pattern using the most limiting amino acid 1341.
c. Based on a nine-point hedonic scale [16].
According to their physical appearance, the rats used in the animal studies showed ideal growth and development with the FRI Weaner. Rats fed the traditional weaning food had spiky fur and rough tails, whereas those on the FRI Weaner had neat fur and fine tails, similar to those fed the casein-control diet or Cerelac.

Mean weight gain, PER, and FER were more than 300% greater in rats fed FRI weaner than in rats fed the traditional weaning food (table 4). Test diets containing FRI Weaner, casein, or Cerelac all gave similar values for PER, FER, and mean weight gain in experimental rats. The PAG guideline recommends a PER of not less than 2.1 and preferably greater than 2.3 for weaning foods [24]. A similar recommendation is made by the general US Department of Agriculture guidelines for corn-based blends [29]. Thus, a diet based on 100% roasted maize (PER 0.88) does not meet the required standards of PER for weaning foods, whereas the FRI Weaner has a highly acceptable PER (2.47), comparable with that of casein (2.42).

The AST levels (table 4) for rats fed the different diets were too low in all cases to indicate any tissue impairment or damage. In many clinical disease states, serum enzymes become elevated by the breakdown of tissues. The extent of the enzyme changes in the serum depends on the severity of the damage. AST is a widely distributed intracellular enzyme whose escape into the circulating blood, in which its activity is usually low, is a sensitive index of tissue impairment.

The measurement of erythrocyte AST is also a sensitive indicator of long-term vitamin B6 status [30]. In B6 deficient persons, the activity of AST decreases in erythrocytes, leucocytes, and serum [30]. The higher levels of B6 vitamins in FRI Weaner as a result of supplementation with soya beans and milk may have contributed to the higher levels of AST in rats fed FRI weaner than in rats fed Tom Brown.

Serum albumin levels in rats given the test diets were lower in those given Tom Brown (4.06%) than in those given FRI Weaner (5.05%) and the other diets. Normal levels in human serum range from 4.4% to 5.3% [31]. Therefore, all test diets, with the exception of Tom Brown, which produced albumin levels slightly below normal values, are nutritionally adequate to maintain normal circulating levels of albumin. Most plasma proteins are resistant to severe energy deficiency and starvation [32]. Levels of albumin are well maintained in marasmic children and in cases of prolonged dietary food restriction.

### TABLE 4. Nutritional and biochemical data on test animals fed different weaning formulations

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mean weight gain (g)</th>
<th>PER</th>
<th>FER</th>
<th>AST (IU/L)</th>
<th>Serum albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional weaning food</td>
<td>12.30</td>
<td>0.88</td>
<td>0.08</td>
<td>2.30</td>
<td>4.07</td>
</tr>
<tr>
<td>FRI Weaner</td>
<td>34.39</td>
<td>2.47</td>
<td>0.25</td>
<td>5.68</td>
<td>4.50</td>
</tr>
<tr>
<td>Casein control diet</td>
<td>33.85</td>
<td>2.42</td>
<td>0.25</td>
<td>4.40</td>
<td>5.05</td>
</tr>
<tr>
<td>Cerelac</td>
<td>32.40</td>
<td>2.31</td>
<td>0.23</td>
<td>5.1 5</td>
<td>6.03</td>
</tr>
</tbody>
</table>

PER = protein efficiency ratio; FER = feed efficiency ratio; AST = aspartate transaminase.

### TABLE 5. Haematological data on test animals fed different weaning formulations

<table>
<thead>
<tr>
<th>Diet</th>
<th>WBC (x 109/L)</th>
<th>RBC (x 1012 /L)</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional weaning food</td>
<td>4.5</td>
<td>10.0</td>
<td>13.5</td>
<td>45.5</td>
</tr>
<tr>
<td>FRI Weaner</td>
<td>6.2</td>
<td>10.7</td>
<td>17.3</td>
<td>46.0</td>
</tr>
<tr>
<td>Casein control diet</td>
<td>7.9</td>
<td>9.7</td>
<td>14.4</td>
<td>43.3</td>
</tr>
<tr>
<td>Cerelac</td>
<td>6.4</td>
<td>10.0</td>
<td>17.4</td>
<td>45.3</td>
</tr>
</tbody>
</table>
Haematological data (table 5) showed similar effects of FRI Weaner and Cerelac on white blood cell count, red blood cell count, haemoglobin levels, and packed cell volume (PCV) of test animals. Normal white cell counts range from 5 to 9 x 10^9/L [30]. All the weaning foods studied produced white blood cell counts within the normal range, with the exception of Tom Brown, for which the white blood cell counts were slightly below the lower limit of the range. Starvation and debilitating conditions, such as nutritional disorders, particularly protein-energy malnutrition, cause a decrease in counts [30, 33]. This confirms that Tom Brown is inadequate for maintaining healthy nutrition status in weanling rats.

Advanced stages of iron deficiency are associated with a significant decrease in circulating haemoglobin. Subjects with levels below cut-off according to sex, age, or other physiological circumstances are considered anaemic. These cut-off levels range from 11 g/dl for infants and pregnant women to 13 g/dl in adult men [23]. All weaning foods, including Tom Brown, maintained haemoglobin levels above cutoff levels for humans, in particular for children.

Red blood cell counts average 5 x 10^12/L [31]. Levels in weanling rats were all above average after feeding with the weaning foods. The PCV levels were all above human cut-off levels and confirm, with the haemoglobin levels, that the weaning foods are adequate to prevent anaemic conditions over the period studied. The human cut-off levels ranged from 32% in children age 0.5 to 4 years to 40% in men above 15 years [23].

The PCV measures the ratio of the volume occupied by red cells to the volume of whole blood in a sample of capillary or venous blood [23]. It is a convenient and rapid measure of the degree of anaemia, and from a nutritional standpoint provides information comparable to the haemoglobin concentration.

Conclusion

Soya beans can be effectively used in traditional cereal based weaning foods as an acceptable protein supplement. The process parameters and formulations developed through this study successfully produced a high protein-energy weaning food with acceptable functional and sensory characteristics as well as excellent nutritional quality. The current Weanimix (weaning foods based on local legumes and cereals) promotional drive in Ghana by the National Nutrition Co-ordination Committee has achieved some degree of awareness among mothers with regard to the poor nutritive quality of traditional weaning foods and the need to incorporate legumes to enhance protein quality. The results of the present study demonstrated the beneficial effects of adding grain legumes to cereal foods. The technique can be easily adopted at both household and village levels to produce high protein-energy weaning food to help enhance the nutritional status of Ghanaians, especially children.

Acknowledgements

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References

Requirements for effective fortification in food-aid programmes will not be discussed, although the issue is very important and is currently receiving a lot of attention. In a start-up programme in Bolivia, involving a partnership among government, donors (US Agency for International Development and UNICEF), and a commercial firm, sustainability has yet to be assured, although there are currently plans for scaling up nationally by the private sector. For instance, a recent evaluation of the levels of vitamin A in fortified sugar has shown them to be quite low. Other vehicles include infant weaning foods, salt, sugar, rice, curry powder, fish sauce, soy
sauce, bakery products, beverages, biscuits and cookies, low-fat milk, chocolate milk, maize flour, margarine, and water [4,10]. Rice.