1. Final Summary

a. Overall Goal
The goal of this project was to develop and evaluate intervention foods that could be easily distributed among, and consumed by malnourished and vulnerable people.

b. Significant Technical Achievements-Host Countries and US
The project consisted of six objectives as listed in Section 3 below. Of these, three were technical in nature. They were (1) to formulate and produce at pilot (multi-kilogram) scale novel, peanut-based Ready-to-Use Therapeutic Foods (RUTFs); (2) to analyze these products for safety, nutritional, chemical, physical, and sensory properties; and (3) to conduct small scale testing in human subjects for acceptability and nutritional efficacy. These objectives were collectively addressed in three locations: in the U.S. at the University of Georgia (UGA); in Ghana at the University of Ghana-Legon (UGL); and in Uganda at Makerere University (MU). Initial collaboration in Mali was suspended due to insufficient funding.

1.a. Formulation
The starting point for the project was to develop novel Ready-to-Use Therapeutic Foods (RUTFs) that extended their applications from children to pregnant women and women of childbearing age, including those with HIV-AIDS. These foods were designed to be (a) based on locally available, plant-derived ingredients to avoid costly imported milk, (b) designed by a computer program that minimized cost while optimizing nutrient content, (c) have an easy-to-swallow, semi-solid, drinkable consistency which necessitated the use of hydrolytic enzymes (amylases, and proteases) to partially digest starch and protein, (d) use accessible, appropriate processing technologies, (e) contain an aflatoxin-binding mineral to scavenge any toxin that might accompany ingredients as well as that might be in other dietary components, (f) contain a vitamin-mineral supplement to ensure sufficient levels of micronutrients, and (g) contain or be presented along with probiotic bacterial cultures to counteract diarrhea and intestinal pathogens.

Two approaches to formulation were used. In the US/UGA and Uganda/MU, Creative Formulation Concepts® software (Concepts 4, Level 2), was used to design products. Human populations from among those most vulnerable to malnutrition with specific requirements were selected. Based on their requirements, the desired nutrient profile of the formula was specified. In this work, only a few macronutrients were considered for optimization, since all nutritional requirements cannot be met from a limited number of staple ingredients. It was assumed that addition of vitamin/mineral premixes would be necessary for complete formulations. Appropriate ingredients were selected and their nutrient profiles and costs entered. Other restrictions, such as the maximum fat or minimum level of essential amino acids, were imposed. Based on this information, the computer produced least-cost formulation that met the investigator's goals. (Alternatively, if insufficient ingredients or unrealistic requirements are specified, the program returns a "not feasible" result.) Adjustments of any of these inputs allow the development of alternative formulations.
The Uganda/MU team chose children 6 mo-5 yr or age and living with HIV/AIDS as the target population. Dietary Reference Intakes of healthy 6mo-1 yr and 1-3yr children adjusted for needs of HIV/AIDS children dictated the specified macronutrient profile matching milk-based F100. Ten potential commodities/ingredients, based on their importance in Uganda and their nutrient profiles, were chosen - peanuts, amaranth, orange fleshed sweet potatoes, cowpeas, sesame, corn, millet, beans, sorghum and bananas. Nutrient Data Bases provided approximate nutrient profiles of the ingredients. Multiple preliminary formulas were developed, and final formulations are shown in Table UGA122-1.

The UGA team selected women in the third trimester of pregnancy as their target group. The nutrient requirements of pregnant women set by the Food and Agriculture Organization and the Institute of Medicine/US National Academy of Sciences (Dietary Reference Intakes) were used as references to develop the RUTFs. The ingredients selected included peanuts, rice, cowpeas, millet, sorghum, cassava, corn, yams, sesame, wheat, and fonio. A number of formulas were generated before focusing on the final ones shown in Table UGA122-1. These resulted from restricting fat (≤20%), energy (≥452 kCal/100g), and essential amino acid content to the profile for the 1-3 yr child since specific requirements for pregnant women were not available.

The UGL team chose women of child-bearing age (18-45 yrs) and used a simplex design to systematically generate ten formula compositions in which peanut comprised 20-60%; cowpea, 20-50% and rice 20-50%. A number of analyses were conducted on these formulas as reported below. A single final formulation was developed for human intervention.

<table>
<thead>
<tr>
<th>Table UGA122-1</th>
<th>Ingredient Composition and Cost of Formulated RUTFs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
<td><strong>Ingredient g/100g</strong></td>
</tr>
<tr>
<td>UGA-A</td>
<td>Peanut 38.4, Cowpea 22.2, Millet 18.4, Rice 7.0, Barley 14.0, Beans 14.0, Sesame 0.490</td>
</tr>
<tr>
<td>UGA-B</td>
<td>Peanut 39.0, Cowpea 21.8, Millet 11.2, Rice 14.0, Barley 14.0, Beans 0.500</td>
</tr>
<tr>
<td>UGA-C</td>
<td>Peanut 39.5, Cowpea 21.4, Millet 4.4, Rice 21.0, Barley 14.0, Beans 0.500</td>
</tr>
<tr>
<td>UGA-D</td>
<td>Peanut 38.2, Cowpea 21.9, Millet 20.9, Rice 5.0, Barley 14.0, Beans 0.490</td>
</tr>
<tr>
<td>UGA-E</td>
<td>Peanut 38.5, Cowpea 21.2, Millet 16.3, Rice 10.0, Barley 14.0, Beans 0.500</td>
</tr>
<tr>
<td>UGA-F</td>
<td>Peanut 38.8, Cowpea 20.5, Millet 11.8, Rice 15.0, Barley 14.0, Beans 0.500</td>
</tr>
<tr>
<td>UGL</td>
<td>Peanut 32.0, Cowpea 35.0, Millet 33.0, ND</td>
</tr>
<tr>
<td>MU-A</td>
<td>Peanut 50.0, Cowpea 7.5, Millet 25.5, Barley 10.0, Beans 7.0, Sesame 1.012</td>
</tr>
<tr>
<td>MU-B</td>
<td>Peanut 50.0, Cowpea 10.0, Millet 7.5, Barley 25.0, Beans 7.0, Sesame 1.043</td>
</tr>
<tr>
<td>MU-C</td>
<td>Peanut 40.0, Cowpea 7.5, Millet 35.0, Barley 11.0, Beans 7.0, Sesame 1.066</td>
</tr>
</tbody>
</table>

**1.b. Processing and Production**

The formulations shown in Table UGA122-1 were used to produce significant quantities of products in University pilot plants. A number of similar processing steps were employed in the three locations. Following procurement, some raw materials required pre-processing. This included decortication of cowpea and blanching of peanut; boiling decorticated cowpea and beans, and boiling or steaming rice and barley. Cooking starchy legumes and cereals resulted in thick pastes due to starch gelatinization. This necessitated use of amylases to hydrolyze and liquefy the starches to produce semi-liquid products. In Ghana and Uganda, malted (sprouted) millet and amaranth were first used for this purpose (UGL later used commercial amylase), while at UGA, the *Aspergillus oryzae*, solid-state fermented rice and barley (koji) were used. These enzyme sources were analyzed for activity by standard methods. After amylase digestion, bromelain was used to partially digest and make more available the proteins in all the formulations. These
processing steps resulted in semi-liquid, drinkable formulas. At UGA and MU, they were sealed in glass containers and retorted to produce a 'sterile' product. At UGL, drum drying was used, but had insufficient capacity for large scale production. Ultimately liquid formulas were pasteurized for short term holding, and ultimately produced on an as-needed basis. The strategy for the UGA formulas was to deliver them in reusable bottles with probiotic cultures and vitamin/mineral mix loaded into edible capsules and sealed into polyethylene soda straws attached. Assays of the five-culture probiotic mixture and the vitamin/mineral mix in these containers and under realistic conditions indicated they were sufficiently stable to make this a feasible approach.

2. Analysis

Nutritional-chemical, physical, and sensory properties of the final formulations were determined by standard methods. Macronutrient composition is shown in Table UGA122-2. Reliance on plant sources necessitated a higher level of protein in these formulations than in those containing milk powder (F100, PlumpyNut). Computed essential amino acid profiles met targets with lysine being the limiting EAA. However, analysis of the UGA formulas showed significant loss of lysine during retorting. If retorting is used for microbiological safety and stability, additional lysine would have to be added. These formulas were also much lower in fat and energy than peanut paste/milk formulas. This is appropriate for malnourished adults, where excess fat is undesirable, but would require consumption of a greater amount of product in the case of children.

<table>
<thead>
<tr>
<th>FORMULA</th>
<th>UGA-A</th>
<th>UGA-B</th>
<th>UGA-C</th>
<th>UGA-D</th>
<th>UGA-E</th>
<th>UGA-F</th>
<th>UGL-F¹</th>
<th>MU-A</th>
<th>MU-B</th>
<th>MU-C</th>
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<tbody>
<tr>
<td>COMPOSITION, (g/100g dry matter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Protein</td>
<td>19.5</td>
<td>19.3</td>
<td>18.5</td>
<td>19.8</td>
<td>19.6</td>
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<td>22.9</td>
<td>21.3</td>
<td>22.6</td>
<td>22.4</td>
</tr>
<tr>
<td>Fat</td>
<td>19.3</td>
<td>19.1</td>
<td>19.3</td>
<td>19.8</td>
<td>19.8</td>
<td>19.6</td>
<td>15.3</td>
<td>27.6</td>
<td>24.1</td>
<td>24.1</td>
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<tr>
<td>Ash</td>
<td>3.0</td>
<td>2.8</td>
<td>2.8</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.7</td>
<td>3.5</td>
<td>3.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Fiber</td>
<td>6.9</td>
<td>6.4</td>
<td>6.5</td>
<td>6.1</td>
<td>7.2</td>
<td>7.9</td>
<td>ND²</td>
<td>12.2</td>
<td>12.7</td>
<td>13.2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>51.3</td>
<td>52.4</td>
<td>53.1</td>
<td>51.3</td>
<td>50.5</td>
<td>50.1</td>
<td>59.2</td>
<td>35.4</td>
<td>37.4</td>
<td>36.7</td>
</tr>
<tr>
<td>Energy (kCal)</td>
<td>456.0</td>
<td>458.0</td>
<td>461.0</td>
<td>455.0</td>
<td>454.0</td>
<td>451.0</td>
<td>464.5</td>
<td>475.2</td>
<td>457.2</td>
<td>453.1</td>
</tr>
</tbody>
</table>

¹Final Formulation used in human subjects trial
²Carbohydrate value includes fiber

Liquid formulas ranged from 17-20% solids. Compared to milk-based F100 which must be consumed at 1,800 ml/day to meet nutrient requirements of the malnourished child, the MU formulations must be consumed at 1,600-1,800 ml/day. For women in the third trimester of pregnancy, UGA formulas must be consumed at about 2,700 ml/day to meet energy and lysine requirements. The intake of UGL-F required to meet daily requirements was ~3,000 ml/day.

As with normal consumers, malnourished people expect the sensory quality of food to be acceptable, and therefore, the sensory quality of the RUTFs was measured. In Ghana, ten preliminary formulas were compared and the results shown in Figure UGA122-1. The clear area at the bottom of the figure indicates the optimum proportions of the three different ingredients with overall acceptabilities in the range of 7 on a 1-9 point scale. The final formula developed by UGL fell within this range. In Uganda, a panel of 60 untrained mothers of young children evaluated the three formulas and found overall acceptabilities of 3.4-3.5 on a 5 point scale. At UGA, formulas with three levels of koji (amylase) and 0 and 1% protease were evaluated for
sweetness, bitterness, beany/pea flavor, thickness, and grainy/grittiness by 11 trained women at 15-49 yr. Sweetness increased with amylase activity in rice but not barley formulas, while bitterness increased with bromelain levels. These results are consistent with increasing sugar content with amylase activity and increasing bitter peptide content, especially in the milder flavored rice formulas. Thickness decreased with koji/amylase levels as starch was degraded, but increased with bromelain, probably due to unraveling native proteins by limited proteolysis.

**Figure UGA122-1. Sensory results for UGL Formulas**

Physical properties, including color and viscosity were determined. Color of the products ranged from near white for UGL pasteurized formula to tan for retorted UGA products. Viscosities were consistent with a thick, but drinkable consistency (400-700 cp for UGL formulas).

### 3. Small-Scale Human Intervention Study

The UGL formula described above was made in large quantities for this study. It contained a vitamin/mineral premix, but no probiotic supplement and no HSCAS clay. Two hundred and twenty (220) women were recruited after the objective of the study has been explained to them and also met the inclusion criteria. They were then randomized into control and experimental groups, 110 each. Some of the participants withdrew leaving 134; 73 were in the control group and 61 in the treatment group. The first feeding for 6 weeks involved the 61 women while the second 6 weeks of feeding involved 52 women, 3 times a week. The feeding ranged from 20-25 times for the first 6 weeks of feeding and 18-21 times for the second 6 weeks of feeding. At baseline, 6 weeks and 12 weeks after feeding, venous blood was drawn for biomarker assessment and anthropometry measures were taken. The ready to use supplementary peanut based food has the potential to improve the nutrition status of women of child bearing age. The data (Table UGA122-3) shows some improvement over the control groups but we are not able to report significant changes partly due to the short term duration of feeding as well as the three times a week of feeding. To be able to see the full potential of the RUSF the feeding time should be extended to 6 months, and the consumers fed every other day.
c. Significant Issues/Challenges.

The most significant challenges in the project were experienced by HC scientists who sometimes had difficulties in obtaining needed materials from international sources, and, especially in Ghana, with the scale of producing sufficient materials for a large scale human feeding study. Because of equipment limitations, formula was produced several times per week during the feeding study, pasteurized, and kept cold during distribution.

d. Capacity development.

Capacity development included visits by US team members and the presentation of seminars in the HC locations. Formulation software was provided to host country colleagues/institutions with concomitant training in its use. HC PIs visited the US and attended IFT symposia.

e. Human Capacity/training.

Table UGA122-4 summaries the number of students who have been trained under the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Gender</th>
<th>Country</th>
<th>Degree</th>
<th>Completion Date</th>
<th>Training Location</th>
<th>Significant Employment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asante, Linda</td>
<td>F</td>
<td>Ghana</td>
<td>M.Phil Food Sci.</td>
<td>June-09</td>
<td>University of Ghana</td>
<td>Nestle, Ghana</td>
</tr>
<tr>
<td>Anim-Fofie, Matilda</td>
<td>F</td>
<td>Ghana</td>
<td>M.Phil Nutrition</td>
<td>March-12</td>
<td>University of Ghana</td>
<td>Nestle, Ghana</td>
</tr>
<tr>
<td>Amaditor, Patrick</td>
<td>M</td>
<td>Ghana</td>
<td>M.Phil Food Sci.</td>
<td>December-10</td>
<td>University of Ghana</td>
<td></td>
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<tr>
<td>Agemefie, Isaac</td>
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<td>Ghana</td>
<td>M.Phil Nutrition</td>
<td>June-13</td>
<td>University of Ghana</td>
<td></td>
</tr>
<tr>
<td>Kra, Agartha</td>
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<td>Ghana</td>
<td>M.Phil Food Sci.</td>
<td>June-13</td>
<td>University of Ghana</td>
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<tr>
<td>Dua-Barning, Joyce</td>
<td>F</td>
<td>Ghana</td>
<td>B.Sc Food Sci</td>
<td>(2010)</td>
<td>University of Ghana</td>
<td>MS in Sweden</td>
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<tr>
<td>Tsigbe, Jocelyn</td>
<td>M</td>
<td>Ghana</td>
<td>B.Sc Food Sci</td>
<td>(2010)</td>
<td>University of Ghana</td>
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<tr>
<td>Bechman, Allison</td>
<td>F</td>
<td>USA</td>
<td>PhD.</td>
<td>May-13</td>
<td>University of Georgia</td>
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<tr>
<td>Klu, Yaa</td>
<td>F</td>
<td>Ghana</td>
<td>Ph.D.</td>
<td>December-13</td>
<td>University of Georgia</td>
<td></td>
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<tr>
<td>Nabuuma, Deborah</td>
<td>F</td>
<td>Uganda</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

f. Key workshops/short-term training. No workshops with entrepreneurs or stakeholders were held. Women receiving nutritional intervention in Ghana received training.
g. Publications

Peer reviewed journal articles


Bechman, A, R. D. Phillips, and J. Chen. Feasibility of utilizing least-cost computer software in developing ready-to-use therapeutic foods (RUTFs) for malnourished pregnant women in Mali. (Ready for submission).


Posters


Klu, Y., J. H. Williams, R. D. Phillips, and J. Chen. 2013. Survival of Lactobacillus rhamnosus GG in peanut butter and peanut spread as influenced by storage conditions. One Health Symposium. 21-23 March. The University of Georgia Center for Continuing Education and Conference Center, Athens GA.


2. **Final Interpretation**

   a. **Importance of Technical Achievements**
      
      i. **Host Country**
      
      The concept of systematic development of nutritious formulas for nutritional intervention among at-risk and malnourished populations in both West Africa (Ghana) and East Africa (Uganda) was fully developed. This provided HC scientists with the skills and background information to extend the accomplishments of this project into the future and to address very specific nutritional needs of local populations using largely local ingredients, with particular emphasis on peanut. The unique contribution of peanut in providing good quality protein along with high energy density by healthy fats was emphasized. Previous research at UGA demonstrating the generation of bioactive peptides during the digestion of peanut protein as well as the high content of arginine in peanut proteins helped promote peanut as a health-benefitting commodity. The concept of multi-purpose, multi-focused intervention foods that also provide defense against both mycotoxins in the local diet and pathogenic bacteria that cause diarrhea, a leading cause of morbidity and mortality in developing countries broadened the perspective of host countries scientists. It is important to emphasize that the Host Country scientists involved in this project were themselves capable, well trained, and competent and that they made very significant contributions to the work, both in carrying out research under sometimes difficult circumstances, and in the initial planning and conceptualization of the project.

      
      ii. **US.**
      
      The very extensive research on multi-functional dietary intervention products provided a foundation to continue research in this area in the future. The concept of providing nutrition along with probiotic and prebiotic components in products designed for specific populations will be extended to, e.g. elderly American populations, taking full advantage of the health-promoting properties of peanut if additional funding becomes available. This project has provided background information for attracting funding from other peanut industry and commodity commission sources for these purposes.

   b. **Importance of physical and human capacity development**
      
      i. **Host Country**
      
      A sizable number of graduate and undergraduate students were trained in Ghana and Uganda as part of this project. These young scientists are the most important product of the project. They will become part of the academic, governmental, and industrial human infrastructure in the future. Provision of formulation software to HC institutions (including IER, where the project had to be suspended) provided a valuable tool to continue this research in the future. Funding was not sufficient to provide additional equipment in the HC. A very great need in developing countries is for infrastructure, including trained technical personnel and parts, for maintaining scientific equipment. That is beyond the scope of this project and others like it until the importance of such developments are recognized. It is discouraging to see relatively new
equipment no longer being used because of failures of components that in the US would be repaired quickly.

**ii. US.**
The training of US graduate students remains an important mission of USAID sponsored research projects. In the current project, one US and one Ghanaian student have been trained to the PhD level. The Ghanaian student will return to her country and contribute as will those trained in country, having the advantages provided by a U.S. university education. The U.S. student has gained, in addition to technical knowledge, a wider, deeper understanding of the needs of developing countries. Whatever future employment she undertakes, this understanding will broaden her perspective and contribute to a globalized world view.

c. **Heritage left from workshops and short-term training**
Not applicable.

d. **Heritage left in publications**
Many if not most publications from this work are in the process of being reviewed prior to being submitted. When complete, they will form a foundation for expanding the concept and practice of developing systematically formulated intervention foods for specific populations anywhere in the world.

3. **Final Summary of Accomplishments by Objective**

1. **Formulate and produce at pilot scale peanut-based RUTFs**
The proposed RUTFs will be nutrient dense but with a liquid consistency for ease of swallowing. This objective comprises the core research activity of the proposed project. It is, in turn, comprised of two major components, formulation, and production of RUTFs
A significant number of RUTFs were systematically designed and produced at the lab and pilot scale in three locations – The U.S, Ghana, and Uganda. These addressed the nutritional needs of three different populations – young children with HIV-AIDS (Uganda), women of child-bearing age (Ghana) and pregnant women in the third trimester (U.S. targeting Mali).

2. **Analyze safety, nutritional, chemical, physical and sensory properties of formulas**
The final formulas were analyzed for chemical-nutritional composition, physical, and sensory quality. They met the nutritional, physical, and sensory goals of the project.

3. **Small scale testing of formulas in human populations for acceptability and nutritional efficacy**
An ambitious human study among women of child-bearing age was undertaken in Ghana. The study, which met IRB guidelines and approvals, examined a number of physical and biometric indicators among the test and control groups. Although subjects thrived on the formula, limitations on the production of sufficient formula compromised the ability of the study to achieve statistically significant improvement in most indicators.

4. **Training entrepreneurs to produce RUTF formulas on commercial scale**
This goal was not addressed in the present study.
5. Training graduate students and publishing findings.
A number of students were trained and their work forms the basis of several publications.

6. Capacity development of participant scientists and institutions.
Both the U.S. and the HC institutions benefitted from this project in expansion of knowledge and research skills.