Production of Fortified Plant-Based Infant Formular to Mitigate Malnutrition and Poor Growth Development

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ABSTRACT

Proper nutrition is key to healthy growth and development among infants. Malnutrition among infants in sub-Saharan Africa persists due to high costs of commercial formulas and limited breastfeeding. This study eveloped an affordable fortified soymeal-based infant formula supplemented with probiotics and evaluated its nutritional adequacy, microbial safety, and sensory acceptability.

Formulations were fortified with *Bifidobacterium breve* Bb-12 and *Lactobacillus rhamnosus* GG (50×10^6 CFU/g). Proximate composition, microbial safety, probiotic viability, and sensory evaluation were performed following AOAC (2020) and ISO standards. Data were analyzed using one-way ANOVA ($p<0.05$). The developed formula achieved macronutrient equivalence with commercial formulas, retained high probiotic viability over 28 days, met microbial safety standards, and demonstrated strong sensory acceptability. The study has contributed to showing that fortified soymeal-based formula offers a cost-effective alternative for combating infant malnutrition while meeting WHO safety and nutritional standards.

Keywords: Infant formula, soymeal, probiotics, *bifidobacterium,* plant-based nutrition, malnutrition

INTRODUCTION

Infant nutrition plays a critical role in supporting the growth, development, and health of infants. Breast milk is widely recognized as the optimal source of nutrition for infants, but for various reasons, not all infants can be exclusively breastfed. Infant formulas have been developed as a nutritionally adequate alternative to breast milk. However, traditional dairy-based infant formulas may not be suitable for all infants, particularly those with dairy allergies or intolerances.

In recent years, there has been growing interest in plant-based protein sources as alternatives to dairy-based proteins in infant formulas. Soymeal in particular has emerged as a popular plant-based protein source due to its high protein content, nutritional benefits, and potential health advantages, and it also enhances the body against infection (Huang et al., 2022). It has long been proven that breast milk is the perfect food for an infant during the first six months of life, as it contains all the nutrients and immunological requirements required to maintain optimal health and growth (Mario et al., 2022). Additionally, the incorporation of probiotics, such as *Bifidobacterium* into infant formulas has shown to support infant gut health and immune system development.

Conventional cow's milk-based formulas are often expensive and allergenic, prompting interest in plant-based formulations. Soymeal, rich in protein (35–40%) and essential amino acids, is widely used as a base for infant feeding. Probiotic fortification, particularly with *Bifidobacterium* and *Lactobacillus* strains, has been shown to improve gut health, immune development, and nutrient absorption. This study focuses on developing an affordable, fortified, plant-based formula compliant with WHO and Codex Alimentarius guidelines for infant nutrition.

1.1 Aim and Objectives

The aim of this study was to formulate, evaluate, and compare a fortified plant-based infant formula with existing commercial alternatives, focusing on nutritional adequacy, probiotic viability, safety, and cost-effectiveness.

Specific objectives:

- To develop soymeal-based infant formulas supplemented with probiotics.
- To evaluate proximate composition, micronutrient profile, and microbial safety.
- To compare the developed formula with existing commercial infant formulas.
- To assess sensory attributes and consumer acceptability.
- To ensure compliance with international nutritional and safety standards.

LITERATURE REVIEW

Soymeal in infant nutrition

Soymeal provides balanced proteins and essential amino acids, making it suitable for lactose-intolerant infants (Onyedika et al., 2019). However, deficiencies in vitamin B12, calcium, and essential fatty acids necessitate fortification. The current state of knowledge on the production of plant-based baby meals, with the focus on soymeal and probiotic B*ifidobacterium*, reveals that plant-based baby meals are gaining popularity due to growing customer interest in vegan and vegetarian diets, but also present nutritional challenges that require careful formulation to ensure adequate nutrition, particularly for protein, iron, zinc, and vitamin B12.

Soymeal is considered a good source of protein, isoflavones, and other nutrients, making it a suitable base for plant-based baby meals and its hypoallergenic properties reduce the risk of allergic reactions in infants. Soymeal-based infant formulas can be a suitable alternative for infants who are not breastfed or require a supplement for breastfeeding (Lawrence et al., 2018). However, soymeal requires careful processing and formulation to match the nutritional profile of breast milk or cow's milk-based infant formula. Soymeal shows calcium and vitamin B12 deficit; for this reason, those micronutrients are often supplemented (Lawal et al., 2021).

Probiotic fortification

Probiotics, including *Bifidobacterium* breve Bb-12 and *Lactobacillus rhamnosus* GG, improve intestinal health, enhance immunity, and modulate microbiota. Studies demonstrate their effectiveness in infant formula, leading to improved nutrient bioavailability and reduced gastrointestinal disorders (Sanders et al., 2019). Probiotics such as *Bifidobacterium* have been shown to support gut health, immune system development, and allergy prevention in infants, but different strains of *Bifidobacterium* have varying effects on infant health, emphasizing the importance of strain-specific research (Braegger et al., 2019). Nonetheless, probiotics require careful handling and storage to maintain viability and stability in plant-based baby meals, and several strains of *Bifidobacterium* are commonly used in infant formulas (Rodriguez et al., 2020).

Comparative insights

While animal-based formulas provide complete nutrition, they are costlier and allergenic. Soymeal-based formulas, when fortified, are affordable alternatives comparable to standard products. Nutritionally, soymeal provides high-quality protein, essential amino acids, and other nutrients necessary for growth and development. It is also rich in iron, zinc, and calcium which are crucial for healthy growth and development (Tannock, 2016). The use of soymeal as a base for infant formulas has been increasing due to its nutritional benefits. However, there are concerns about its role as a potential allergen (Lonnerdal et al., 2018). Soymeal is also suitable for infants with dietary restrictions, such as those with cow's milk allergies or lactose intolerance. It is a hypoallergenic and lactose-free alternative that can provide essential nutrients for growth and development.

Subsequently, it is essential to consult with a pediatrician before introducing soymeal to an infant's diet, as they

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may have specific recommendations or guidelines. This has led to the demand for substitute sources that are fortified with more nutrients and can be equally affordable for everyone (Lawal et al., 2021). Choosing fortified soymeal products can help ensure that the infant's nutritional needs are met (Xiao et al., 2021). Soymeal is fortified with essential vitamins and minerals including calcium, vitamin D, and B12. These nutrients are crucial for maintaining strong bones, supporting immune function, and promoting overall health. The significance of soymeal also extends to its potential role in reducing the risk of chronic diseases. Studies have shown that moderate consumption of soymeal may help lower cholesterol levels, reduce blood pressure, improve insulin sensitivity, and that refrigeration helps maintain probiotic viability (Zheng et al., 2020).

2.1 Physico-chemical attributes of soymeal

It is a series of testing, extracting and other processes used to differentiate and determine amounts of compounds present in the food or any material of interest. This analysis is helpful in quality control.

2.1.1 Moisture

Water is the major component of most foods and serves a variety of functions. It influences the structure, appearance, and taste of food as well as the spoilage process. Each food has its own characteristic water content. Moisture determination is a fundamental analytical procedure in food analysis.

2.1.2 Fat (ether extract)

Fats, also called lipids, are diverse groups of organic chemicals soluble in nonpolar solvents but not soluble in water. Two main methods for fat analysis exist: crude extraction and total fat tests. Crude fat extraction uses organic solvents like ether followed by evaporation to measure fat content.

2.1.3 Ash

Ash is an inorganic residue remaining after water and organic matter are removed by heating with oxidizing agents, providing a measure of total mineral content. The main procedures include wet ashing, dry ashing, and low-temperature plasma dry ashing.

2.1.4 Carbohydrate (nitrogen-free extracts)

Designed to provide an estimate of water-soluble polysaccharides, highly digestible carbohydrates like starch and free sugars are calculated by subtracting the sum of other proximate components from the total weight.

2.1.5 Crude protein

Proteins are polymers of amino acids containing nitrogen. Adequate protein is required for maintenance, growth, pregnancy, and lactation. Protein content is often determined using the Kjeldahl method, which measures nitrogen levels.

2.1.6 Minerals

Minerals are inorganic elements required by the body for proper growth and development. They are obtained from foods and play key roles in bone strength, enzymatic activities, and overall physiological functions.

2.2 Bifidobacterium as a probiotic

Bifidobacterium is a genus of Gram-positive, anaerobic, non-motile, branched rod-shaped bacteria commonly found in the human gastrointestinal tract, particularly in infants. It supports gut health, improves digestion, and boosts immunity (Wang et al., 2020). It was first identified in the stools of healthy breastfed infants, and higher levels are associated with reduced risks of diarrhea and gastrointestinal infections. Today, *Bifidobacteria* are widely used in probiotic-enriched foods, infant formulas, and supplements (Lee & O'Sullivan, 2010).

METHODS OF STUDY

3.1 Study area

This research work was conducted at Awkuzu, Igbariam in Oyi Local Government Area of Anambra State, Nigeria. The production of the baby meals and laboratory analysis to assess its nutritional value were carried out at Cheznik Laboratory, Onitsha, Anambra State.

3.1.1 Study design

The design for this study was a combination of experimental and survey types. A three-group blind experimental design was used to assess the nutritional value of the new formulations in comparison with the existing animal-based brands.

3.1.2 Sampling technique

Sixty (60) units of soymeal packs weighing 500 g w/v each were sampled into three groups (n=20), with each group containing different additives aimed at enhancing the soymeal base.

3.1.3 Materials

All the raw materials needed for the preparation of balanced baby meals were purchased from Nkwo Awkuzu market in Awkuzu, Anambra State, Nigeria. The commercial weaning food, Pureed Fruits, was also purchased from Shoprite, a popular supermarket in Awka. Food-grade additives including 500 g of rice, corn, and potatoes were also purchased and processed.

3.2 Methods

3.2.1 Soymeal base preparation

According to Lawal et al. (2021), the method for preparing soymeal served as the standard procedure for production. Twenty grams of soybeans were cleaned and sorted, then ground into a coarse texture, boiled for approximately 20 minutes, and drained. The soybeans were dried for 24 hours before being ground into a fine powder. Subsequently, other ingredients were added to the soybean powder to enhance its nutritional value, creating a balanced and suitable formula for infants. The soymeal was then fortified with bifidobacterium, packaged, and stored.

3.2.2 Preparation of soymeal-enhanced formulation

Three different formulations were prepared, and their compositions were described below in line with the weaning food formulations described by Lawal et al. (2021).

3.2.2.1 Carrot and green beans-enhanced soymeal

Carrots and green beans were cleaned and cut into small pieces. The entire ingredients were then blended in a food processing blender for about 3 minutes. Subsequently, the blended slurry was mixed with powdered soybeans and dehydrated for 24 hours in a dehydrator, then blended into powder and transferred into a ziplock bag for further analysis.

3.2.2.2 Yellow corn and date-enhanced soymeal

Local yellow maize and date fruits were sorted to remove defective portions, then soaked for 6 hours. Bananas were peeled and cut into small pieces alongside some green beans. Afterwards, the procedure continued as in the first formulation diet.

3.2.2.3 Rice-enhanced soymeal formulation

Yellow sweet potatoes, date fruits, and green beans were cleaned and cut into small sizes, then steamed in a pressure cooker until three whistling sounds were produced by the vent on the lid of the cooker. Mangoes were cleaned and cut into small pieces. Thereafter, the procedure continued as in the first formulation diet.

3.3 Sample analysis

3.3.1 Proximate analysis

Proximate composition (protein, fat, carbohydrate, ash, moisture) was determined using AOAC Official Methods (2020). This standardized approach ensures reproducibility and global comparability of results.

Moisture content: The loss of Mass on drying was ascertained by weighing 100g of each of the samples. And then left to dry in a vacuum oven at 100*C for 2-5 hours. The weight loss was recorded and moisture content calculated (AOAC,2020)

Protein Assay: Using Kjeldahi method . A sample was digested with sulfuric acid. Then it was distilled with sodium hydroxide. Ammonia was titrated with boric acid to calculate protein content.

Fat Content: This was done using the method of Soxhlet-Henkel method. A sample was extracted with petroleum ether in a Soxhletapparatus for 4-5 hours. The solvent was evaporated, and the residue was weighed to calculate fat content

Fiber Content: This was done using Crude Fiber method (AOAC 2020). Here a sample was treated with boiling acid and alkali solutions to remove soluble components. The residue is dried, weighed and shed to calculate crude fiber content.

Ash content: A sample was incinerated at 600*C for 2-4 hours. The weight of the residue was calculated as ash content (AOAC,2019)

3.3.2 Microbial plate counts

Total bacterial counts, yeast, and mould analyses were performed using ISO 4833-1:2013 and ISO 21527-2:2017 protocols. Probiotic viability for *Bifidobacterium breve* Bb-12 and *Lactobacillus rhamnosus* GG was quantified using selective plating on MRS agar.

Total bacterial counts, yeast, and mould analyses were performed using ISO 4833-1:2013 and ISO 21527-2:2017 protocols [13]. Probiotic viability for *Bifidobacterium breve* Bb-12 and *Lactobacillus rhamnosus* GG was quantified using selective plating on MRS plate agar.

Bacterial count: this method (ISO 4833-1:2013) involved plate counting of viable bacteria. A sample was diluted, then plated on McConkey agar. The plates were incubated at 30*C for 72 hours. The number of colonies were counted and multiplied by the dilution factor to obtain the total bacterial count.

Yeast and Mould count: This employed the method described in ISO 21527-2:2017. A sample was diluted, then plated on Sarboraund plate agar. The plates were incubated at 25*C for 5 days. The colonies were thereafter counted and identified microscopically for yeast and mound count

3.3. 3 Probiotic Analysis Analysis :

This is done following ISO 2001:2008, AOAC 2019.04. This involved dilution of 1 gm of commercially sourced probiotics *Lactobacillus rhamnosus* var *casei*, and *Bifidobacterium* respectively. This was followed by plating on Mann Rogosa Sharpe (MRS) media, and incubated both aerobic and anaerobic conditions. Finally, colony counting of the observed colonies expressed in colony forming unit per gm

3.3.4 Sensory evaluation

Thirty lactating mothers evaluated samples using a 9-point hedonic scale for taste, aroma, texture, and appearance.

3.3.5 Statistical analysis

was conducted via one-way ANOVA, with Tukey's post-hoc test to determine significance ($p<0.05$).

RESULTS

4.1 Proximate compositions

Figure 1 shows the various proximate contents of the newly developed formula in comparison with some of the available commercial brands. The result demonstrates competitive effectiveness of the new formula as a cheaper alternative for use as an infant weaning diet.

Fig 1: Proximate composition of developed formula compared to commercial brands.

4.2 Microbial plate count

The result of the three microbial counts—bacteria, yeast, and mould (Figure 2)—showed that the new formula possesses a comparable reduction in unwanted microbial content, thus making it more appropriate for infant nutrition. The microbial reduction is attributed to the action of the probiotics *Lactobacillus* and *Bifidobacterium* added to the new formula.

Fig 2: Microbial plate count.

4.3 Probiotic Analysis Analysis :

The two strains of probiotic bacteria used; *Bifidobacterium* and *Lactobacillus rhamnosus* var casei showed good adaptability and stability for the shelf life of 28 days. Their viability remained high recovering more than 8.5 x107 CFU.

Fig 3: Probiotic Viability Over Storage

4.4 Sensory analysis

A 30-member panel comprising lactating mothers conducted the sensory evaluation based on taste, aroma, texture, and overall acceptability. Results indicate that the newly formulated soymeal-based infant formula closely matched the available commercial brands in sensory attributes.

Fig 4: Sensory Evaluation Scores

DISCUSSIONS

This study has shown that the fortified soymeal-based infant formula matched commercial comparators on macronutrients (protein 18.2%, fat 10.5%, carbohydrate 65.3%), showing that plant-based/soy formulas can achieve comparable macro- and micronutrient profiles when properly fortified. Gurung et al. (2023) evaluated a neonatal piglet model receiving a plant-based formula (almond and buckwheat) and assessed effects on gut microbiota, intestinal morphology, metabolic, and immune markers. While not soy-based, their study underscores the need for thorough evaluation of plant-derived protein formulas, especially regarding biological effects beyond basic composition.

The sensory acceptance by 30 lactating mothers was strong, and statistical analyses (one-way ANOVA, $p < 0.05$) confirmed equivalence in composition and favorable hedonic ratings compared to branded formulas.

On microbial safety, the study showed that the new formula met microbial safety limits by various regulatory codes both at international and regional platforms. Probiotic fortification (*Bifidobacterium breve* Bb-12 and *Lacticaseibacillus rhamnosus* GG at ~5×10^7 CFU/g) remained viable over 28 days. ESPGHAN's 2024 "Biotics" paper highlights heterogeneity in strains/doses and practical issues such as heat-related loss of viability during 70 °C reconstitution recommended by WHO—factors that may diminish the intended dose at feeding.

Furthermore, Berseth et al. (2024) conducted a randomized controlled trial where healthy term infants fed cow-milk formula fortified with *Lacticaseibacillus rhamnosus* GG (~10^6 CFU/g) showed normal growth and tolerance, without adverse effects—and even less fussiness at certain ages. This aligns closely with this study's findings on safety and short-term tolerance of LGG-fortified formula.

Strain-specific safety evidence is also pertinent. U.S. FDA GRAS notices support the use of certain *Bifidobacterium* breve strains in milk- or soy-based formulas at defined levels, reinforcing the manuscript's safety findings while underscoring that safety is strain- and dose-specific.

Statistical testing (one-way ANOVA, $p<0.05$) supported equivalence in proximate composition and favorable hedonic ratings relative to branded formulas.

CONCLUSION

This study has demonstrated that fortified soymeal-based infant formula can provide a nutritionally adequate, cost-effective alternative to commercial brands. It has contributed to food diversification and security among the term and infant population which addresses the challenge of micronutrient depravation and malnutrition.

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Table 1. Proximate Composition of Formulas

Formula	Protein (%)	Fat (%)	Carbohydrate (%)	Moisture (%)	Ash (%)
Developed Formula	18.2	10.5	65.3	4.5	1.5
SMA® Gold	17.0	11.2	66.0	4.3	1.3
NAN® Pro 1	17.5	11.0	65.8	4.4	1.2
Aptamil®	18.0	11.5	64.9	4.6	1.4

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