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Development and Characterization of Fortified Ready-to-Eat Banana Porridge Flour: A Multi-Age Nutritional Intervention

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ABSTRACT

One-fifth of banana production is wasted due to failure to meet market ripening standards. This study aimed to develop ready-to-eat (RTE) green banana porridge flour and fortify it with moringa leaf powder (RTEM), hemp powder (RTEH), soybean powder (RTES), and pea powder (RTEP) to formulate nutritionally enhanced porridges for older infants, children, and adults, while assessing the structural and microbial safety of the RTE banana porridge product and the nutritional value of the porridge formulated. Green banana flour (GBF) was produced through sequential washing, peeling, slicing, oven-drying, milling, and sieving of unripe banana fruit. The GBF was cooked into porridge, oven-dried, and milled again to create dried RTE banana porridge flour. This flour was divided into two portions: one for fortification studies and another for storage stability testing under +4°C: 65% RH, +18°C: 58% RH, +30°C: 20% RH, and packaging materials over 90 days. Four protein-rich fortificants were prepared: moringa leaf powder (through leaf collection, cleaning, oven-drying, grinding, and sieving), hemp seed powder (via cleaning, dehulling, grinding, cold-pressing, and milling), soybean powder (by cleaning, boiling, soaking, oven-drying, and milling), and pea protein powder (by selecting, rinsing, soaking, drying, milling, sieving, and packing). All formulations were standardized to contain 14% protein on a dry weight basis, designed to provide 25% of the recommended daily protein intake for the target populations. Total bacterial and coliform counts of freshly prepared RTE porridge ranged from 22 ± 0.4 to 818 ± 2.8 cfu/mL and 18 ± 0.6 to 200 ± 0.5 cfu/mL, respectively, indicating safety for human consumption. GBF showed moisture content of 6.3/100 g, protein 4.23/100 g, total carbohydrate 69.15/100 g, and fiber 14.50/100 g, while RTE flour had moisture 3.4/100 g, protein 4.08/100 g, total carbohydrate 72.67/100 g, and fiber 14.57/100 g. Fortification increased protein content from 4.08 to ~14.00 g/100 g, while total starch (TS) decreased from 71.72 to 62.19 g/100 g. RTEP was identified as the superior formulation based on overall assessment.

1 | Introduction

Protein-energy malnutrition (PEM) and micronutrient deficiencies are persistent public health issues in many developing countries [1, 2]. These conditions affect an estimated 170 million children under 5 years of age, with prevalence rates of 23.2% for PEM, 40% for Vitamin A deficiency [3], and 72.4% for iron deficiency [4]. In Nigeria, PEM affects 37% of children under 5 years [5]. Children aged 6 to 23 months are particularly vulnerable, as this is a critical period for physiological development, and inadequate feeding practices can lead to lasting developmental problems.

After 6 months, breast milk alone may not meet a child's nutritional needs, necessitating the introduction of complementary foods (CFs) [6]. CFs are any foods besides breast milk, given in liquid or semi-liquid form to supplement a child's diet [7]. In developing countries, CF often consists of porridge made from ready-to-eat (RTE) flours, which are pre-processed and safe for consumption without additional cooking. However, homemade flours from cereals and tubers typically lack sufficient proteins, vitamins, minerals, and essential amino acids [8]. Additionally, porridge made from these homemade flours tend to have a lower energy density [9]. While some imported porridge options on the market offer good nutritional quality, their high cost makes them inaccessible for much of the population, particularly those with low or no income [10].

To address this issue, the World Health Organization (WHO) suggests creating CF with enhanced nutritional value using locally sourced ingredients, which can help reduce the reliance on costly imports [7, 11]. The WHO recommends that these formulated CF should provide 400 kcal, comprising 68% sugars (with 81% as free sugars), 13% proteins (minimum 7% digestibility), and 7% lipids [12]. Additionally, they should contain 5% fiber, 7 mg of iron, 350 μg of vitamin A per 100 g of flour [12], and viscosity between 1 and 3 Pa·s [11].

Given that a single food source rarely provides all these nutrients, many researchers have developed flour blends that combine tubers and cereals for energy [13], legumes for proteins and fats [14], and fruits and vegetables for vitamins and minerals [15, 16]. In many developing countries, including Nigeria, porridge flours are commonly made from cereals [17], although they can also be produced from starchy sources with high energy density, such as bananas [18].

The banana (Musa cavendish) is a crucial perennial starchy fruit crop, playing a significant role in food and nutrition security for many Africans [19]. Nigeria stands out as the top banana producer in Africa, boasting an annual production of 8,019,203 tonnes [20]. Bananas are rich in fiber, vitamins (A and C), minerals (potassium and magnesium), and beneficial phytochemicals [21, 22]. Green banana flour (GBF) contains a substantial amount of starch (61.3–76.5 g/100 g dry weight) and is high in fiber (6.3–15.5 g/100 g dry weight) [23]. These qualities make bananas a healthy, carbohydrate-rich food, aligning with the increasing consumer demand for minimally processed, plant-based starchy foods [24]. GBF's neutral flavor and aroma make it an excellent addition to composite flours, readily absorbing the flavors of the other ingredients [24]. However, a significant portion, ~20%, of harvested bananas is wasted due to not meeting market ripening standards, a consequence of their climacteric ripening behavior [25]. To address this waste problem, research has demonstrated that processing green bananas into shelf-stable, RTE products, such as porridge for infants, children, and adults, offers a viable solution [26–31]. The porridge will enable consistent utilization of green banana fruit without requiring ripening, thereby reducing banana waste. Porridge is traditionally used for infant and children's feeding; it can also serve as a nutritious food option for adults [32]. Developing RTE porridge using green bananas offers a promising approach to utilize unripe bananas and create a new product for the broader consumer market. Therefore, breakfast, often hailed as the most important meal of the day, is a crucial kick-start to our mornings [33]. However, due to hectic lifestyles, many skip breakfast or opt for RTE instant foods. The benefit of RTE breakfast food is not only that it saves time, but it also eliminates the need for food preparation. These advantages, together with the appealing flavor and crispiness of the products, contribute to their popularity [34]. Therefore, RTE breakfast foods are processed grain-based products designed for direct consumption without additional cooking [35]. Their shelf stability, light weight, and ease of storage and transport make them advantageous. Typically derived from corn, wheat, oats, or rice, RTE foods are often enhanced with flavorings and fortifying ingredients [36]. Current literature on porridge has little information on developing a single porridge product that can serve three distinct age groups (older infants, children, and adults) at the same time and being fortified with nutrient-dense ingredients such as moringa leaf powder, soy powder, pea powder, and hemp powder. These fortificants provide valuable sources of essential amino acids, unsaturated fatty acids, and minerals, thereby potentially enhancing the porridge's overall nutritional profile [37]. These fortificants were chosen based on their protein content, availability, cost-effectiveness, and their color differences. Moringa leaf powder contains 25%–30% protein, 15%–20% fiber, 7%–10% fat, and is rich in vitamins A, C, E and minerals. Soy protein isolate comprises 85%–90% protein with balanced essential amino acids and isoflavones. Hemp protein powder consists of 30%–50% protein, 10%–15% omega-rich fat, and 20%–30% fiber. Pea protein isolate contains 80%–85% protein, high in branched-chain amino acids but limited in methionine. This study aimed to develop a RTE banana porridge flour fortified with four complementary plant-based ingredients (moringa leaf powder, hemp seed powder, soybean powder, and pea powder). The objectives were to formulate nutritionally enhanced banana porridge products suitable for different age groups (older infants, children, and adults), ensure all formulations meet 25% of daily protein requirements, and identify the optimal formulation.

2 | Materials and Methods

2.1 | Materials and Reagents

Green bananas (Musa cavendish) at the ripening stage 1, along with four different plant-based protein sources (*Moringa oleifera* leaf powder, hemp powder, soy powder, and pea powder), were used in this study. The green bananas were obtained from Leeds City Kirkgate Market, Leeds, UK. Hemp powder, soy powder, and pea powder were purchased from Buywholefoodsonline.co.uk, while *Moringa oleifera* leaf powder was purchased from Morrison (Leeds). These four fortificants were selected based on their composition, color, and aroma. The DNS assay, NaOH, HNO_3, TCA, and potassium sodium tartrate-4-hydrate were obtained from Sigma Aldrich and were of analytical grade.

2.2 | Methods

2.2.1 | GBF

Green bananas (Musa cavendish) at the ripening stage 1 were processed using minimal processing methods with some modification described by Vatanasuchart et al. [38]. Seven fingers of green banana were washed with tap water at 20 °C, cleaned with a clean hand towel, and weighed. The bananas were peeled with

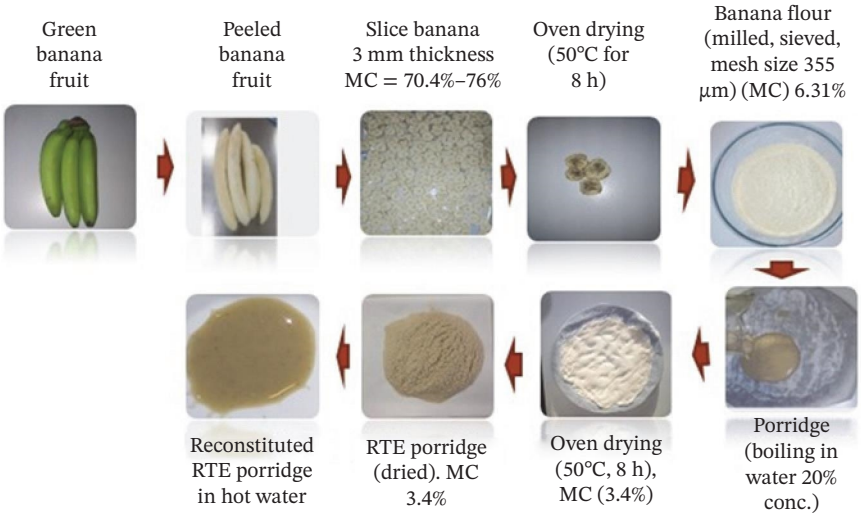

FIGURE 1 | Flowchart for the production and reconstitution of ready-to-eat (RTE) porridge from green bananas.

a stainless-steel knife, weighed, and cut into 3 mm thick slices without using any chemicals to reduce enzymatic browning. They were then immediately dried into chips in an air-hot Memmert laboratory oven at 50°C for 8 h, with an air velocity of 1.0 m/s. After drying, the banana chips were milled with a blender (Kenwood Attract) and sieved (355-μm mesh sieve) as GBF, which was then weighed and stored in a cellophane bag and kept in a freezer (−20 °C) for product development and further analysis (Figure 1).

2.2.2 | RTE Porridge

To develop the RTE porridge, GBF was cooked into a porridge. Specifically, 2000 ± 0.1 mg of GBF was added to preheated potable water (85 °C) at a concentration of 20% (w/v). This mixture was continuously stirred with a wooden spoon on an electric heating mantle set at 85 °C for 20 min. Banana starch gelatinizes between 57 and 80 °C, with peak gelatinization at 71–77 °C and completion by 80–92 °C, depending on variety. Heating at 75–85 °C achieves near-complete gelatinization, eliminating crystalline structure and producing a smooth, uniform porridge with improved digestibility. The resulting cooked banana porridge was then poured into a stainless-steel tray lined with aluminium foil and oven-dried at 50 °C for 8 h. After cooling to room temperature, the dried material was milled with a blender for 10 min, resulting in the RTE porridge (dried) flour (Figure 1).

2.2.3 | Moringa Leaf Powder

Moringa leaves were hand-picked to allow only the selection of good quality leaves. The selected leaves were washed under running tap water to remove dirt and subsequently drained in a plastic sieve. The washed leaves were then oven-dried at 50°C for 5 h. Following drying, the leaves were ground into a coarse powder using a grinder. The coarse powder was pressed with a pressing machine to extract the oil. The defatted residue was then milled to reduce particle size and sieved to obtain a fine moringa leaf powder. The resulting powder was stored in air-tight containers prior to analysis.

2.2.3.1 | Hemp Seed Powder.

Hemp seeds were cleaned and dried to remove impurities such as stems and leaves, and to facilitate the release of oil from the cotyledons. The hulls were cracked using a hammer mill, after which the hearts were separated and the cotyledons milled into hemp flour. This flour was then subjected to cold pressing to extract the oil, yielding hemp flour cake as a byproduct. The cake was subsequently milled into flour and sieved through a 355-μm mesh sieve to produce hemp protein powder, which was stored in air-tight containers until analysis.

2.2.3.2 | Soybean Powder.

Soybean seeds (500 g) were cleaned and sorted to remove stones and dirt, then washed in cold water to remove impurities. The cleaned 500 g seeds were boiled for 30 min, after which the water was decanted. The boiled beans were soaked with cold fresh water for 48 h, with the water being changed every 6 h to ensure freshness. The soaked beans were dried at 50°C for 48 h until completely dried and the seed coat removed. The dried beans were then milled into flour using a blender and sieved through a 355 μm sieve to obtain a uniform particle size. The resulting flour was stored in suitable containers for future analysis.

2.2.3.3 | Pea Powder.

Yellow split pea seeds were selected for their high protein content. The seeds were thoroughly rinsed with cold water to remove any dirt and debris. Following rinsing, 500 g of seeds were soaked overnight (8–12 h) in cold water to facilitate rehydration and reduce phytic acid content, which can inhibit mineral absorption. After soaking, the seeds were oven-dried at 50°C until a constant weight was achieved. The dried seeds were then milled into flour using a blender and sieved through a 355 μm sieve to obtain a uniform particle size. The resulting flour was stored in appropriate containers for subsequent analysis.

2.2.4 | Packaging and Storage of RTE Porridge

The milled RTE porridge (dried) flour was divided into two portions. The first portion was packaged in three different materials

for storage stability testing: glass bottles (100 mL laboratory borosilicate bottles with caps from Getty), plastic bags (polythene with gusseted bottoms allowing them to stand upright), and paper bags. Each package contained 80 g ± 1 mg of the flour. These packaged samples were then stored under three different environmental conditions: +4°C with 65% relative humidity (RH), +18°C with 58% RH, and +30°C with 20% RH for a total duration of 90 days. Samples were collected for analysis at four time points: 0, 30, 60, and 90 days. At each sampling time, 0.5 g of RTE porridge flour was measured in triplicate from each packaging type (glass, paper, and polythene) at each storage temperature (4°C, 18°C, and 30°C), resulting in nine treatment combinations per time point. The second portion of the milled RTE porridge (dried) flour was packaged in a cellophane bag and stored in a freezer at −20 °C for future use. Both portions were characterized for proximate composition and microbial analysis.

2.2.5 | Microbial Safety Analysis

The aerobic total plate count and coliform count were evaluated by McCleary and Monaghan [39]. Briefly, 0.5 g of RTE porridge powder was homogenized in 50 mL of Milli-Q water in a 50 mL tube and incubated for 30 min in a shaking water bath set at 37 °C. Serial dilution up to 10^{-5} was made and plated onto sterile agar plates. Twenty-five molten nutrient agar (25 mL) for total plate count or MacConkey agar for coliform count was poured into sterile labeled plates and allowed to set. Then, 1 mL of each serial dilution (10 mg/mL) was pipetted into the same corresponding labeled petri dishes. Plates were incubated for 48 h at 37 °C and colonies were counted. To ensure product safety, further confirmatory test with *Escherichia coli* was done with eosin methylene blue agar. A sterile loop was used to collect individual colonies from the MacConkey agar plate and streak the new, poured eosin plate, and incubate as described before. At the end of the incubation period, viable cells were counted and expressed as a colony-forming unit per milliliter of the sample (cfu/ml) according to Muhammad et al. [40]. Color is used to identify the presence of *E. coli*.

$$\mathrm{CFU/ml} = \text{No of colonies/Vol(ml)} * \text{Total dilution factor.}$$

Calculation formula

$$\mathrm{CFU/ml} = \sum c/v(n1 + 0.1 * n2)d,$$

$\sum c$ is the sum of colonies (mesophilic bacteria per/mL) counted on the dishes from two successive dilutions (one must contain at least 15 colonies). v is the volume of inoculum added to each dish in mL. $n1$ is the number of dishes of dilution 1. $n2$ is the number of dishes of dilution 2. d is the dilution factor of the 1st dilution.

2.2.6 | Morphology Assessment

2.2.6.1 | Cytochemical Staining of Starch and Cellulose.

Approximately 20 mg of banana flour was mixed with distilled water in a small beaker to form a suspension. Using a clean pipette, aliquots of the sample mixture were collected, and one drop of the suspension was placed on a clean microscope slide. A drop of iodine solution was then added to the sample, and a coverslip was carefully placed over it. Excess solution was gently removed using tissue paper. Additional slides were prepared following a similar procedure. One drop of the suspension was placed on a clean slide, followed by one drop of Calcofluor white and one drop of 10% NaOH. The reagents were mixed on the slide before applying the coverslip. All prepared slides were then observed under a light microscope for the iodine-stained samples and under a UV microscope for the Calcofluor white.

2.2.7 | Fortification

The fortification method used in this study was adapted from the US government's Fortified Blended Foods (FBFs) program, introduced into the Food for Peace initiative in 1960 to provide protein-rich, micronutrient-dense foods for vulnerable populations, particularly children in developing countries [40]. This approach was combined with protein requirement guidelines from [41]. The recommended dietary allowance (RDA) for protein varies by age and sex: older infants (6–11 months) require 11 g/day, children (1–3 years) require 13 g/day, adult women (>18 years) require 46 g/day, and adult men (>18 years) require 56 g/day [41]. Rather than developing separate formulations for each demographic group, we designed a single fortified product capable of meeting 25% of the daily protein requirements for all target populations—older infants, children, adult women, and adult men—in one standardized serving. The traditional portion sizes for dried porridge vary by group: older infants (6–11 months) consume 25 g, children (1–3 years) consume 30 g, adult women (>18 years) consume 75 g, and adult men (>18 years) consume 85 g per serving. However, for this study, we standardized the serving size to 100 g of RTE banana porridge to simplify formulation while ensuring adequate nutrition across all groups. The preformed dried RTE banana porridge flour produced was used as the basic matrix and mixed with moringa powder, hemp powder, soy powder, and pea powder individually as flours to be taken as fortifying ingredients, to formulate the fortified dried RTE banana porridge flours. Four porridge flours were formulated based on the composition of the different ingredients. The compositing data is based on 100 g of mixed dried RTE banana porridge flours with fortificants, meeting 25% target of protein-to-protein requirement for an older infant, a child, adult female, and adult male, which is equivalent to roughly 14 g of protein per 100 g of mixture.

RTE Porridge calculations/formula with fortificants to meet the target consumers.

RTE porridge contribution per portion size for older infant (6−11 months) is about 25 g, a child (1−3 years) is about 30 g, adult female is about 75 g, and adult male is 85 g = X.

RTE banana porridge protein contribution is 4.88 g of protein in 100 g of RTE porridge = A.

Protein requirement allowance for different age groups: Older infant is 11 g/day, child 1–3 years is 13 g/day, adult female > 18 years is 46 g/day and adult male > 18 years is 56 g/day = B.

Protein content of the fortificants: Moringa is 38.7 g, soy is 90 g, hemp is 50 g, and pea is 78 g = C.

The quantity of the protein in the fortificant = D.

Using the formula above to calculate

1. Quantity of protein in all the portion sizes = X/100 * A.

2. Percentage protein contribution to protein requirement for each group: Amount of protein found in each group portion size, divided by the protein requirement for each group, and then multiplied by 100 = X /protein req * 100.
3. 25% target of protein-to-protein requirement: multiply 25 by the protein requirement of each group and divide by 100 = 25 * protein req. for each group/100.
4. Fortificants' protein contribution: The difference between the target and the RTE banana protein contribution = Target – RTE protein contribution.
5. The quantity of the fortificants needed to give the quantity of the protein estimated: Quantity of the protein in that fortificant multiplied by 100 and divided by the protein content of the fortificants = D/C *100.

Since the target is a single fortified banana porridge formulation to cover all consumer groups, fortification was based on a 100 g serving size. Portions can then be adjusted from this base formulation for any specific group targeted.

Applying the calculation above, these are the percentage portion sizes of each plant-based protein source: moringa 23.56 g, soya 10.10 g, hemp 18.24 g, and pea 11.69 g (Table 1 and Appendix Tables A–C).

2.2.8 | Reconstitution of RTE Banana Porridge

The reconstitution method for the porridge sample was adapted with some modifications from [42]. To prepare the porridge, 20 g of dried RTE banana flour was mixed with 100 mL of preheated boiled water at either 85 or 100 °C (20% w/v ratio). The mixture was immediately covered and allowed to stand for 5 min. After the resting period, the mixture was uncovered and stirred continuously for 2–3 min without any additional cooking. The control porridge was prepared using the same procedure: the dried RTE banana flour was mixed with hot water, covered for 5 min, stirred, and served without further cooking. The reconstituted banana porridge samples were then characterized for reconstitution properties, morphology, and carbohydrate digestibility.

2.2.9 | Chemical Composition/Nutrient

2.2.9.1 | Proximate Composition. The moisture, ash, and fat contents of samples were determined using the method described in [39]. Protein content was analyzed by first measuring nitrogen (N) using inductively coupled plasma optical emission spectroscopy (ICP-OES), then converting the nitrogen values to protein by applying a conversion factor of 6.25.

2.2.9.1.1. Moisture. About 2 g of porridge premix was weighed into dry aluminum pans, placed in a forced-air oven at 105°C for 12 h, and then cooled to room temperature in glass desiccator. The weight of the dried samples was recorded to determine the moisture content as g/100 g.

2.2.9.1.2. Ash. About 3 g portion of porridge was added to preweighed empty crucibles with covers and placed in a muffle furnace overnight at 550°C. The samples were allowed to cool in the furnace and then transferred to glass desiccators to complete cooling at room temperature. Crucibles plus ash were weighed and ash content calculated as g/100 g.

2.2.9.1.3. Crude Fat. Crude fat was extracted from 1 g of porridge in a microwave reaction system, Multiwave, model 3000 (Anton Paar, Inc., Ashland, NC, USA) using 10 mL each of acetone and hexane. The operating conditions of the extractor were: temperature ramp increased 5°C/min, 110°C final temperature; held for 10 min at 110°C; and cooled to 40°C. The extracts were filtered through glass wool into a preweighed flask, and solvent was evaporated using a rotary evaporator. Flasks containing crude fat were dried at 100°C for 10 min, cooled in a desiccator, and weighed to determine total crude fat as g/100 g.

2.2.9.1.4. Protein. The protocol was adapted with modifications from [43]. Approximately 600 mg of RTE banana porridge and fortified RTE banana porridge with varying percentages of moringa, hemp, soya, and pea powders were weighed separately in glass test tubes. To each tube, 3 mL of 69% HNO_3 (Hiperpur; Panreac, Spain) and 2 mL of deionized water (Milli-Q; Merck, Spain) were added. The mixtures were digested using microwave treatment (Milestone Ultra Wave, Italy) at 240°C and 40 bar for 40 min at 1500 W. After digestion, the samples were diluted to a final volume of 50 mL with Milli-Q water. Carbon and nitrogen were analyzed using ICP-OES, which involves first converting a liquid sample into an aerosol and then injecting it into an argon plasma to excite and ionize the elements. When these excited atoms return to their ground state, they emit light at specific wavelengths unique to each element. An optical system separates this light, and a detector measures the intensity of each wavelength to identify and quantify the carbon, nitrogen, and other elements present in the sample. The intensity of the light is proportional to the concentration of each element in the original

TABLE 1 | Protein fortification to achieve 25% of the recommended intake for target consumers.

Nutrient	Quantity of RTE used (g)	Quantity of fortificant used (g)	Protein content (g/100 g) of fortified
RTE only	100	—	4.88
RTE + moringa	76.44	23.56	13.53
RTE + hemp	81.76	18.24	13.60
RTE + soya	89.90	10.10	13.80
RTE + pea	83.31	11.69	13.73
25% target protein cont.	—	—	14.00

Note: Data is expressed as mean with SD of $n = 3$. The data represents the quantity of RTE to be used with fortificant and the protein content of the fortified product. Total formulation $n = 9$.

sample, but we are interested in carbon and nitrogen. The quantity of nitrogen is multiplied by 6.23 to convert it to protein.

2.2.9.2 | Carbohydrate Determination and Digestion.

2.2.9.2.1. Reducing Sugar Determination Using DNS Assay. The detection of reducing sugar was performed by the DNS assay according to [44, 45] with some modifications. Briefly, 5 g of DNS (Sigma–Aldrich) was dissolved in 250 mL of preheated (80°C) distilled water and cool to room temperature. Then 100 ML of 2 N NaOH plus 150 g of potassium sodium tartrate-4-hydrate (Sigma–Aldrich) were added to the solution and mixed using magnetic stirrer and volume made up to 500 mL with distilled water as DNS solution. Serial dilutions of 0.1–2 mg Ml^{-1} were made from a stock solution of glucose or maltose standard of concentration 2 mg/mL. 1 mL of DNS solution was added to a separate test tube containing 2mlL of sample and standard. The mixture was shaken using a vortex and placed in a boiling water bath(Grant SBB 14) for 15 min to facilitate the reaction of the reducing sugar with DNS reagent. After cooling, 9 mL of distilled water was added to the solution in the tube and 200 μL of each solution was pipetted into a 96-well plate, Absorbance was read at 50 nm using a multimode plate reader (SPARK TECAN). A blank was made by replacing the sample/standard with distilled. Absorbance of a blank was subtracted from the sample and standard readings. A standard curve was used to calculate reducing sugar values in samples.

2.2.9.2.2. Free Sugar Determination. Free sugars were extracted from mixing 250 mg of GBF with 5 mL of water, vortexing for 5 min, and centrifuging 40 ng for 10 rpm for 10 min at 20°C for 4000 rpm. The supernatant was analyzed for reducing sugar using the DNS assay

2.2.9.2.3. Starch Determination. RDS, SDS, and RS (total starch) were determined enzymatically using a commercial resistant starch (RS) assay kit (rapid format) from Megazyme (Bray, Ireland) according to the updated method by McCleary et al. [46]. Two enzymes used in the kit were pancreatic α-amylase (PAA) and amylo-glucosidase (AMG), and have been purified, standardized, and stabilized [47]. Digestion was performed using 0.4 units of PAA and 0.17 units of AMG with stirring at pH 6, 37 °C for 4 h, simulating in vivo conditions in the human small intestine. About 100 mg of sample was added to 3.5 mL of 50 mM maleate buffer at pH 6 in a tube, and mixed by vortexing for 5 s at room temperature. The tube placed in a shaking water bath(200 strokes/min) set at 37 °C for 5 min to equilibrate and 0.4 units of PAA and 0.17 units of AMG were added. The digestion mixture was incubated for 20 , 120, and 240 min to measure RDS,SDS and RS. The reaction was terminated by adding 20 mL of 50 mM acetic acid, mixed vigorously by vortexing, centrifuging at 4000 rpm for 10 min. The supernatants collected contained the soluble products of digestion at different times. Then, 0.33 U of AMG was added to each of the supernatants and incubated at 50°C for 30 min to hydrolyze soluble digestion products to glucose.

The reducing sugar in the supernatants was analyzed using DNS colorimetric assay. The pellet from starch digestion containing non-digested RS was mixed with 2 mL of 1.7 NaOH and stirred continuously for 20 min in ice water bath placed over a magnetic stirrer. Then, 8 mL of 1.0 M sodium acetate buffer (pH 3.8) was added, followed by 0.33 U of AMG. The mixture was incubated at 50 °C for 30 min with intermittent shaking. The digestion mixture was transferred into a 100 mL flask and topped up with water, then centrifuged as previously described. The supernatant was analyzed for reducing sugar content using the DNS assay.

The following calculations were performed:

- Total starch = TS (DS240 min − FS + RS).
- Available carbohydrate = DS240 min + FS.
- Total carbohydrate = Total starch + FS.

Where Ds20 = RDS, Ds120 = SDS and starch that did not digest after 240 min of digestion is RS.

Carbohydrate digestibility of fortified reconstituted RTE porridge was evaluated using the same method as total starch (TS), but calculated as a percentage of TS. Therefore, to obtain digestibility, this formula can be used.

$$\text{Digestibility} = \frac{\text{DS}}{\text{TS}} \times 100,$$

where DS is the digestible starch. TS is the total starch.

2.2.9.2.4. Fiber. Total fiber was evaluated by weighing out the residue from the RS determination method. The residue was filtered through an A4 filter paper (Whatman). The filter paper was dried, cooled in a desiccator, and weighed. Fiber is calculated by subtracting the filter paper's weight after drying the residue. The difference in weight is the amount of the total fiber. The fiber value did not include the RS. The gross energy value was estimated by multiplying the crude protein, fat, and carbohydrate by their 1 g equivalent to kcal (4, 9, and 4).

2.2.9.2.5. Mineral Determination. Minerals (F, K, Mg, Mn, P, and Zn) were evaluated by inductively coupled plasma mass spectroscopy (ICP-MS) using the analysis parameters by Otero-Romaní et al. [48]. Analysis was performed on a PerkinElmer Optima 4600 DV ICP analyser (Waltham, United States). The running parameters were set as follow: plasma flow 15 L/min, auxiliary flow 0.2 L/min, nebulizer flow 0.8 L/min. power 1300, reading distance 15 mm, reading position radial (K) and axial (Mg, Mn, Zn, Fe, and P), integration time 5–10 s and number of replications 3.

2.2.10 | Statistical Analysis

All digestions and analyses were done in triplicate, except for morphological results. Data are means with standard deviations. A statistical package (SPSS version 12.0 for Windows) was used for data analysis, including one-way analysis of variance (ANOVA) at 95% confidence levels.

3 | Results and Discussion

3.1 | Effect of Processing on the Proximate Composition of GBF and RTE Flour

Table 2 presents the impact of cooking and drying on the proximate composition of GBF and RTE banana porridge flour. The moisture content of GBF (6.31 ± 0.0 g/100 g) was significantly higher than that of RTE dried porridge (3.4 ± 0.0 g/100 g). These

TABLE 2 | Proximate composition of green banana flour (GBF) and ready-to-eat banana porridge (RTE).

Compound	GBF (amount ± SD (g/100 g))	RTE (amount ± SD (g/100 g))
Moisture	6.31 ± 0.13^{a}	3.4 ± 0.0^{b}
Protein	4.23 ± 0.05^{a}	4.08 ± 0.1^{a}
Ash	3.13 ± 0.06^{a}	3.13 ± 0.4^{a}
Fat	0.007 ± 0.01^{a}	0.007 ± 0.5^{a}
Total carbohydrate	69.15 ± 0.05^{b}	72.67 ± 0.5^{a}
Free sugar	1.84 ± 0.1^{a}	18.00 ± 0.01^{a}
Total starch	67.28 ± 6.4^{a}	65.64 ± 0.01^{b}
Digestible starch (DS)	12.11 ± 1.45^{b}	34.57 ± 0.01^{a}
Resistant starch	55.11 ± 5.41^{a}	31.07 ± 0.01^{b}
Dietary fiber (DF)	14.50 ± 0.8^{a}	14.57 ± 0.4^{a}

Note: The data is expressed as mean of SD ($n = 3$). Different letters within the row indicate significant differences ($p < 0.05$) determined by Tukey test following one-way ANOVA. Total formulations $n = 9$. Fiber excludes resistant starch.

moisture values fall within the range (6–13 g/100 g) reported by Muhammad et al. [49]. The low moisture content observed in the product is favorable for storage stability, as moisture levels above 13% can promote microbial growth and spoilage [49]. During cooking, starch gelatinization occurs when starch is heated with sufficient water. Starch granules absorb water and swell irreversibly as intermolecular hydrogen bonds in the crystalline structure break down. The starch molecules unfold and become hydrated, transitioning to a disordered state, while soluble amylose leaches out, forming a viscous gel. This process significantly increases digestibility by making starch more accessible to digestive enzymes, resulting in rapid glucose release and a high glycemic response. However, gelatinized starch products are highly susceptible to staling, resulting in reduced shelf life [50]. Again, cooking of GBF induces protein denaturation and aggregation, reducing solubility and apparent digestibility, while simultaneously restructuring dietary fiber through RS transformation and polysaccharide solubilization. These changes reflect molecular rearrangements rather than true nutrient loss, and they strongly influence the nutritional, functional, and physiological properties of the cooked product [51]. As a result, no significant differences were observed in lipid, carbohydrate, protein, ash, free sugar, and fiber contents between GBF and RTE flour. The high carbohydrate content in both forms supports their potential use as staple foods for meeting energy requirements. The stability of ash and protein contents during cooking can be attributed to minimal nutrient leaching, since the cooking water was not discarded despite the water-soluble nature of minerals and proteins [36]. Similar findings were reported for pre-cooked plantain flour by Ovando-Martinez et al. [52]. The proximate composition of the developed RTE green banana porridge was compared with that of porridge produced from composite maize-wheat flour, as well as porridge formulated from blends of yellow maize, pumpkin seeds, soybeans, and carrots in varying proportions [51, 52]. The results showed that the proximate composition of the three porridges formulated was comparable in terms of their moisture, protein, ash, fiber, and carbohydrate. Their results were different but not significantly different.

The effect of processing GBF into RTE porridge on carbohydrate digestibility was thoroughly investigated. Table 3 presents the impact of different processing stages (GBF, freshly cooked porridge, RTE porridge, and reconstituted porridge) on carbohydrate composition. Significant differences ($p < 0.05$) were observed in TS, digestible starch (DS), RS, sugar content, available carbohydrate (AV.CHO), and total carbohydrate (TOT) across the processing stages. However, fiber content remained unchanged throughout processing.

At the raw GBF stage, DS content was low during 20 , 120 , and 240 min of digestion (5.8, 7.0, and 12.1 g, respectively), while RS content was high (55.1 g). This low digestibility can be attributed to several factors. First, the crystalline structure of raw starch limits its accessibility to digestive enzymes [37]. Second, starch entrapped within cell wall material (RS1) remains physically inaccessible to enzymatic breakdown [51]. Third, GBF contains larger starch granules, which are inherently less digestible than smaller granules due to their reduced surface area available for enzyme contact [52].

The digestion pattern showed distinct phases. During the initial 20 min, the digestion rate was rapid, yet overall digestibility remained low. Digestibility increased progressively from 20 to 120 min, indicating the presence of slowly digestible starch. By 240 min, digestion was essentially complete. The health implications of varying digestible and resistant starch fractions are significant, particularly in relation to glycaemic response, insulin sensitivity, and gut health. High-digestible starch diets tend to promote rapid glucose absorption and higher glycaemic loads, contributing to insulin resistance and an elevated risk of diabetes over time. Resistant starch consumption generally improves fasting glucose, insulin sensitivity, and some cardiometabolic markers in clinical and animal models, especially with sufficiently high doses and longer interventions. RS-induced fermentation and changes in gut microbiota/SCFA production underpin benefits for gut health, potentially reducing chronic inflammation and improving metabolic regulation [53].

TABLE 3 | The effect of processing stages on the carbohydrate content of green banana flour (GBF) and porridge at various processing stages (g/100 g dry weight).

Treatment	DS_{20}	DS_{120}	DS_{240}	RS	TS	Free sugar	AV.CHO	TOT.CHO	Fiber
Raw GBF	5.8 ± 0.10^{d}	7.06 ± 0.03^{d}	12.11 ± 1.4^{a}	55.11 ± 5.4^{a}	67.22 ± 6.4^{b}	1.8 ± 0.01^{a}	14.00 ± 1.4^{a}	69.06 ± 6.4^{a}	14.50 ± 9.8
Freshly cooked porridge	11.59 ± 0.37^{a}	51.10 ± 0.06^{a}	62.69 ± 2.6^{a}	16.06 ± 5.2^{d}	78.75 ± 3.4^{a}	1.99 ± 0.3^{c}	64.68 ± 2.6^{a}	$80.74 \pm .3.4^{a}$	15.80 ± 0.2^{a}
Dried porridge	8.07 ± 1.55^{b}	26.5 ± 0.15^{c}	34.57 ± 1.1^{c}	31.07 ± 1.6^{b}	65.64 ± 1.1^{b}	7.03 ± 0.3^{a}	41.6 ± 1.1^{c}	72.67 ± 2.7^{b}	17.27 ± 0.9^{b}
Reconstituted porridge	10.02 ± 1.2^{b}	40.05 ± 1.0^{b}	50.07 ± 1.8^{b}	17.60 ± 1.2^{c}	67.67 ± 1.8^{b}	6.77 ± 0.2^{b}	56.84 ± 1.8^{b}	74.44 ± 2.7^{b}	$14.90 \pm 9\text{-}5$

Note: Data are means with SD of $n = 3$. Different letters within a column indicate significant differences ($p < 0.05$), determined by the Tukey test following one-way ANOVA. Total formulations $n = 9$.
Abbreviations: AV.CHO, available carbohydrate (DS + sugar); DS, digestible starch; DS (20–240) min, digestible starch at 20, 120, and 240 min; RS, resistant starch; TOT.CHO, total carbohydrate (TS + sugar); TS, total starch.

Cooking induces starch gelatinization through heat, resulting in a decrease in resistant starch from 55.11 to 16.06 g and a corresponding increase in digestible starch at 240 min from 12.11 to 62.68 g. This transformation occurs because gelatinization disrupts the crystalline structure of starch granules, thereby enhancing enzyme accessibility [54].

The significant difference observed in TS during cooking may be attributed to the analytical method employed. The enzymatic approach used in this study offers the advantage of separately quantifying the three main starch fractions: rapidly digestible starch (RDS), slowly digestible starch (SDS), and RS. Considering the limitations of current analytical methods, particularly enzyme specificity in starch digestion assays, alternative approaches are needed. The INFOGEST simulated digestion model or complementary techniques such as NMR and in vivo digestibility studies can provide more detailed mechanistic and physiological insights. During the drying process, the RTE porridge exhibited a decrease in DS at 240 min (34.57 g) and a concurrent increase in RS (31.07 g) compared to fresh porridge RS (16.06 g). This shift can be attributed to starch retrogradation, a process in which gelatinized starch molecules reassociate and recrystallize during cooling and drying [55]. During this stage When gelatinized starch cools, its disordered chains reassociate into ordered structures through recrystallization (staling). This occurs in two phases: amylose recrystallization (hours to days), where linear chains rapidly form double helices creating a gel network, and amylopectin recrystallization (days), where branched molecules slowly rearrange into crystalline regions. As hydrogen bonds tighten the starch network, syneresis expels water, producing a drier, harder texture.

This type of resistant starch formed during drying is classified as RS3 (retrograded starch). During the reconstitution stage, DS at 240 min increased to 50.07 g, while RS decreased to 17.60 g. Notably, the DS and RS values during reconstitution were intermediate between those of the dried and freshly cooked stages, indicating that retrogradation can be partially reversed by reconstituting at 85°C. However, the DS at 240 min for reconstituted porridge (50.07 g) remained lower than that of freshly cooked porridge (62.68 g), suggesting that retrogradation reversal is incomplete. Overall, these results clearly demonstrate how processing stages significantly affect starch digestibility. The transformation follows a pattern: raw GBF exhibits high RS due to native starch structure, cooking reduces RS through gelatinization, drying increases RS through retrogradation (RS3 formation), and reconstitution partially reverses this retrogradation. Additionally, free sugar content increased progressively from GBF to the reconstituted samples. This increase can be attributed to two mechanisms: (1) thermal degradation of starch into simple sugars during cooking and (2) the activity of endogenous amylolytic enzymes present in GBF, which remain active during the early stages of cooking [56]. It is important to note that the food matrix plays a crucial role in the digestibility and bioavailability of nutrients, including enzymes. The presence of antinutritional factors can significantly impact enzyme accessibility and digestibility. The food matrix influences the release, mass transfer, accessibility, digestibility, and stability of food compounds. Antinutritional factors, such as insoluble fiber, tannins, β-glucosidase, and phytates, can reduce the digestibility of proteins, carbohydrates, and amino acids, leading to reduced enzyme accessibility

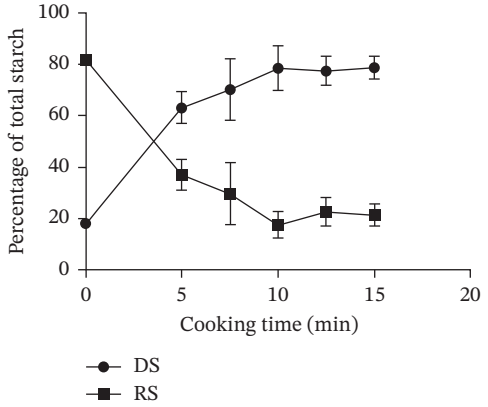

FIGURE 2 | The effect of cooking time on digestible starch (DS) and resistant starch (RS) in green banana flour (GBF).

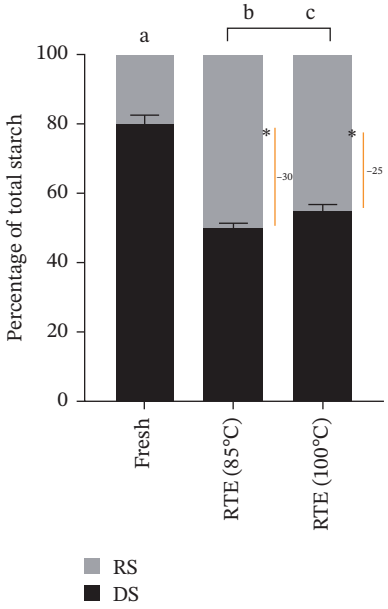

FIGURE 3 | The effect of reconstitution temperature on the digestibility of ready-to-eat (RTE) banana porridge. The digestibility at two reconstitution temperatures (85°C and 100°C) applied for 5 min, was determined and compared with freshly cooked porridge.

[57]. In vitro digestibility methods offer valuable insights into protein digestibility and nutrient bioavailability, but their relevance to in vivo outcomes is limited by an inability to fully replicate complex human digestive processes. These limitations create discrepancies between in vitro and in vivo results, underscoring the need for further validation and refinement of in vitro models to improve their accuracy and applicability across different foods [57].

The effect of cooking time on DS and RS in GBF was evaluated. Figure 2 presents the relationship between cooking time and starch digestibility in fresh GBF. The results demonstrated that starch digestion increased rapidly during the initial cooking period. At 5 min of cooking, DS increased dramatically from 18% to 65.9%, with a corresponding significant decrease in RS from 81% to 34.1% ($p < 0.05$). This trend continued as cooking time extended to 10 min, reaching 81.1% DS and 17.7% RS. These findings align with [54]. Who reported that cooking GBF for 5 min produces the highest amylose leaching at temperatures between 50°C and 65°C, with reduced leaching occurring during extended cooking periods of 15–25 min. Notably, starch digestibility plateaued between 7.5 and 15 min of cooking, showing no significant changes ($p < 0.05$) during this period. Based on these results, optimal cooking of GBF is achieved at 10 min. All measurements were calculated as percentages of TS.

The effect of reconstitution temperature on the digestibility of RTE banana porridge was evaluated. Figure 3 presents the digestibility results for two reconstitution temperatures (85°C and 100°C) applied for 5 min, compared with freshly cooked porridge as a reference. Compared to fresh porridge, RTE products exhibited significantly reduced digestibility when reconstituted with water. Specifically, digestibility decreased by 30% at 85°C and 25% at 100°C reconstitution temperatures, relative to the fresh porridge baseline. However, no statistically significant difference ($p > 0.05$) was observed between the two reconstitution temperatures themselves. These findings are consistent with [58], who demonstrated that higher reconstitution temperatures result in smaller reductions in starch digestibility. This phenomenon can be attributed to greater disruption of retrograded starch structures at elevated temperatures, thereby enhancing enzyme accessibility. While both reconstitution temperatures yielded lower digestibility than fresh porridge, the higher temperature (100°C) provided marginally better preservation of digestible starch content, though this difference was not statistically significant.

It is good to understand the relevance of in vitro digestibility to in vivo outcomes. The in vitro digestibility methods can provide insights into protein digestibility and nutrient bioavailability, which are crucial for understanding how dietary proteins are utilized in the human body. However, there are discrepancies between in vitro and in vivo results due to the limitations of in vitro models, such as the inability to replicate the complex physiological processes of digestion and absorption in the human digestive tract. These discrepancies highlight the need for further validation and refinement of in vitro methods to ensure their accuracy and applicability across various foods and substrates [59].

3.2 | Microbial Count

Table 4 presents the microbial quality of RTE porridge over 90 days of storage under various packaging and environmental conditions. During the first 30 days of storage, no microbial growth was detected for total plate count or total coliform count under all experimental conditions. This microbial stability can be attributed to the low moisture content of the RTE banana porridge (3.4 g/100 g), which resulted in reduced water activity that inhibited microbial proliferation. Initial plate counting experiments confirmed the microbial safety of the product at the beginning of the storage period. Beyond 30 days, microbial growth became detectable and increased progressively with storage duration and temperature. The total bacterial count ranged from 22 ± 0.4 CFU/mL (glass packaging, 60 days, +4°C, 65% RH) to 818 ± 28.2 CFU/mL (paper packaging, 90 days, +4°C, 65% RH). Similarly, coliform counts ranged from 18 ± 0.6 CFU/mL (glass packaging, 60 days, +4°C, 65% RH) to 200 ± 0.0 CFU/mL (paper packaging, 90 days, 30°C, 20% RH). Microbial loads increased

TABLE 4 | Total plate and coliform count of RTE banana porridge stored up to 90 days in three different types of packaging materials at three environmental conditions.

		Plate count (cfu/mL)			Coliform count (cfu/mL)		
Treatment	**Storage time (days)**	**Glass**	**Paper**	**Polythene**	**Glass**	**Paper**	**Polythene**
+4°C: 65% RH	0	0	0	0	0	0	0
	30	0	0	0	0	0	0
	60	$22 \pm 0..4^{a}$	45 ± 1.5^{a}	27 ± 0.5^{a}	18 ± 0.6^{a}	33 ± 0.5^{a}	30 ± 0.5^{a}
	90	29 ± 0.8^{b}	61 ± 3.8^{b}	36 ± 0.8^{b}	39 ± 1.2^{b}	69 ± 1.4^{b}	57 ± 1.1^{b}
+18°C: 58% RH	0	0	0	0	0	0	0
	30	0	0	0	0	0	0
	60	190 ± 5.8^{a}	215 ± 10.4^{a}	210 ± 10.1^{a}	106 ± 3.4^{a}	116 ± 5.6^{a}	110 ± 5.6^{a}
	90	390 ± 19.4^{b}	610 ± 29.4^{b}	460 ± 19.2^{b}	150 ± 7.3^{b}	180 ± 15.2^{b}	160 ± 8.3^{b}
+30°C: 20% RH	0	0	0	0	0	0	0
	30	0	0	0	0	0	0
	60	110 ± 2.9^{a}	200 ± 15.6^{a}	172 ± 6.8^{a}	45 ± 1^{a}	55 ± 2.5^{a}	50 ± 6.6^{a}
	90	200 ± 8.1^{a}	818 ± 28.2^{a}	396 ± 19.3^{a}	130 ± 0.8^{b}	200 ± 0.5^{b}	190 ± 1.2

Note: Data are mean with SD of $n = 3$. Different letters within a column indicate significant differences ($p < 0.05$) determined following one-way ANOVA. ICMSF [60] thresholds for bacterial count in ready-to-eat foods: acceptable range = (0–1000), tolerable range = (10,000–100,000), not acceptable is from 106 and above. Total formulation $n = 9$.
Abbreviations: CFU, colony-forming units; RH, relative humidity.

significantly with increases in both temperature and storage duration [59]. This microbial proliferation is likely attributable to packaging permeability to oxygen and moisture, which facilitated moisture absorption by the samples. The moisture content of RTE banana porridge increased from an initial 3.4% to 4.8%, 5.0%, and 14.2% at 30, 60, and 90 days of storage, respectively. Paper packaging, which exhibited the highest air and moisture permeability, consequently showed the highest microbial counts. These findings are consistent with [61], who reported increased microbial loads in banana flour with extended storage periods.

Environmental conditions (temperature and relative humidity) significantly affected the microbial load in this experiment. Storage of GBF at +4°C with 65% relative humidity produced the lowest microbial counts, while storage at +30°C with 20% relative humidity resulted in the highest microbial counts. The higher temperature of 30°C approaches the optimum growth temperature for mesophilic microorganisms. Again, similar results regarding the influence of storage conditions and packaging on the microbial load of fortified wheat flour were reported by Hemery et al. [62]. The total plate count for RTE foods should be 0–1000 CFU/mL to be acceptable for human consumption, while 10,000–100,000 CFU/mL represents a tolerable level. Counts exceeding 1,000,000 CFU/mL are unacceptable by the International Commission for Microbiological Specifications for Foods [60]. Based on these criteria, all RTE banana porridges produced were within the acceptable limit for human consumption, including those stored at the highest temperatures (30°C, 20% RH). Additionally, gram-negative bacteria, including coliforms, should not exceed 10,000 CFU/g in RTE foods according to ICMSF [60]. Coliform counts between 100 and 1000 CFU/g are considered borderline, while values below 100 CFU/g are satisfactory (www.foodstandards.gov.au). Based on these standards, all stored and analyzed RTE banana porridges were within acceptable limits. The results demonstrated that RTE banana porridges showed no microbial contamination under any storage conditions during the first 30 days. The subsequent contamination by gram-positive and gram-negative bacteria could be attributed to opening the packaging materials during sample collection for microbial testing and re-storage for another 30 days. Contamination could also result from the permeability of moist air through packaging materials, promoting microbial growth. Opening packaging materials could be minimized by using individual small packages designed for single use; however, the current approach better represents typical consumer handling practices.

Importantly, *E. coli* was not detected in any RTE banana porridge samples throughout the storage period, as confirmed by color testing with Eosin agar. The presence of coliforms in food, regardless of concentration, indicates contamination due to poor hygienic practices [63]. The absence of microbial contamination from day 0 to 30 demonstrates that RTE banana porridge was produced under hygienic conditions. However, the detection of coliforms after day 30 likely resulted from environmental exposure during package opening for sampling at the 30-day interval.

Moisture content changes during processing and storage further explain the observed microbial patterns. Initially, the moisture content of GBF was 6.31%, which decreased to 3.48% upon processing into RTE porridge due to drying. During subsequent storage, moisture content increased progressively: from 3.48% to 4.89% at 30 days, from 4.89% to 4.95% at 60 days, and from 4.95% to 14.02% at 90 days. This progressive moisture absorption occurred across all packaging materials, though the rate varied depending on packaging permeability. The substantial moisture

increase by 90 days (reaching 14.02%) created favorable conditions for microbial growth, as moisture levels above 13% are known to support microbial proliferation.

Based on previous research, paper packaging is expected to be more permeable to air than glass and polyethylene, while polyethylene would be more permeable than glass. The results showed that only at 90 days did the moisture content of RTE porridge exceed 13% (the moisture threshold for microorganism survival in stored samples). This suggests that contamination could only occur in this product after 90 days of storage, assuming packages were not opened for periodic sampling every 30 days. In summary, the microbial ecology of dry GBF during storage is primarily shaped by spore-forming bacteria (*Bacillus* spp., e.g., *B. subtilis*, *B. licheniformis*, *B. cereus*, which remain viable but inactive under low water activity) and opportunistic molds and yeasts when moisture increases. Packaging permeability to moisture and oxygen is a central factor: permeable materials allow moisture ingress and oxygen diffusion that elevate water activity, facilitating microbial growth and quality deterioration, while hermetic or barrier packaging restricts these transfers, preserving low aw and limiting microbial proliferation. Research on flour storage underscores the importance of packaging technology in extending shelf life and maintaining safety in dry flour products. However, if there is post-packaging contamination, it will pose real risks even for low-moisture foods like GBF. Effective minimization requires a holistic food safety approach that includes hygienic handling, choice of appropriate food-grade packaging, careful sealing and inspection, controlled storage, and comprehensive management systems. Drawing from food safety literature, these strategies collectively reduce the risk of contamination, protect public health, and maintain product quality over storage and distribution [64].

3.3 | Physical

3.3.1 | Morphology Assessment

3.3.1.1 | Light and UV Microscopy of Banana Samples.

Figure 4A–H presents microscopic observations of banana samples at different processing stages, revealing structural changes that influence starch digestibility. Figure 4A is the milled raw GBF, containing intact starch granules stained with iodine, most of which appeared as free granules. However, some granules were encased within cell wall material (stained with calcofluor white) (Figure 4B). The free intact granules are characteristic of RS1 (physically inaccessible starch), while starch granules entrapped within cell wall material represent RS2 (resistant granule starch). The presence of cell walls creates a physical barrier that prevents digestive amylase enzymes from accessing the starch granules, thereby reducing the digestibility of the flour [65]. During cooking, starch granules absorb water and swell, undergoing gelatinization, a structural transformation that increases the water absorption capacity of the flour. The cooked porridge formed a starch–cell wall polymer gel during the cooking process (Figure 4C,D), though some intact starch granules remained visible. This results in forming the paste and affecting the mouthfeel, due to the different viscosity formed. Gelatinization disrupts the crystalline structure of starch granules through heat-induced phase transitions, including the melting of ordered starch regions and subsequent swelling [65]. This structural disruption enhances alpha-amylase accessibility to starch molecules compared to the tightly packed crystalline structure of raw starch, thereby significantly improving starch digestibility [66]. Through gelatinization, alpha amylases gain enhanced access to starch molecules compared to raw starch, thereby improving starch digestibility. During drying, the starch granules appeared

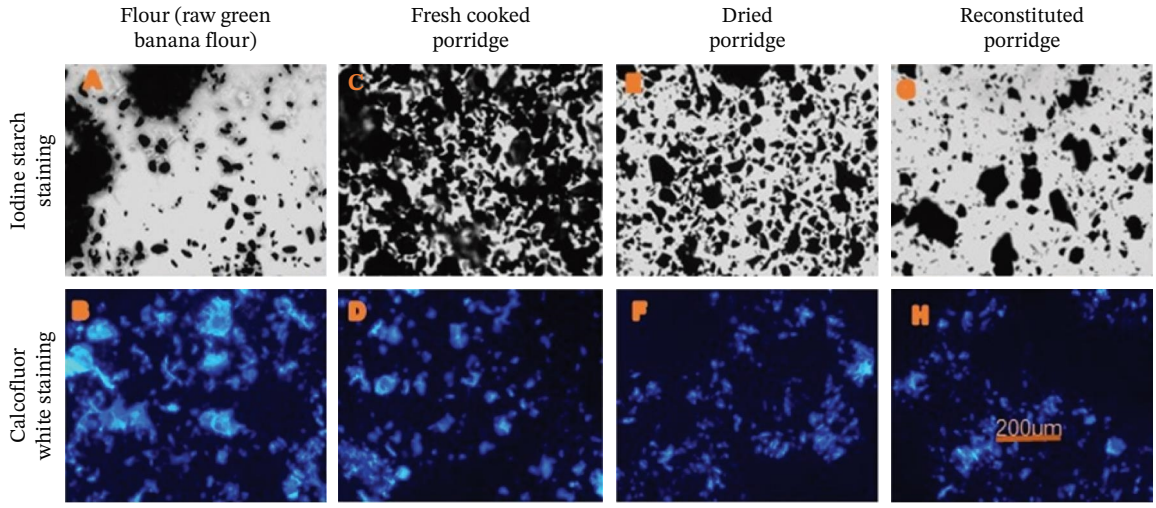

FIGURE 4 | (A–H) Light and UV microscopy images of four different products produced during the developmental stages of RTE banana porridge. Figure A = Raw green flour stained with iodine showing the presence of starch. (B) = Raw green flour stained with Calcofluor white, showing the presence of the cell wall. (C) = Flesh-cooked porridge stained with iodine showing the presence of starch. (D) = Flesh-cooked porridge stained with Calcofluor white, showing the presence of a cell wall. (E) Dried porridge stained with iodine, showing the presence of starch. (F) Dried porridge stained with Calcofluor white, showing the presence of cell wall. (G) Reconstituted porridge stained with iodine, showing the presence of starch. (H) Reconstituted porridge stained with, Calcofluor white, showing the presence of cell wall. Magnification is ×4. Scale bar = 200 μm.

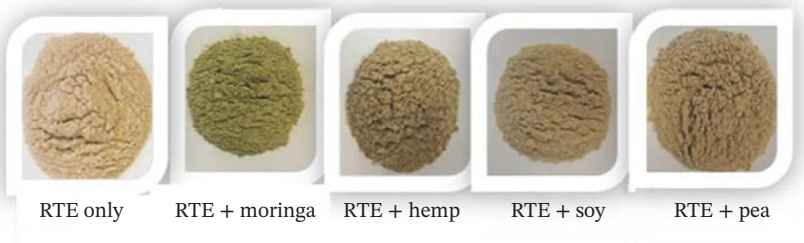

FIGURE 5 | Visual characteristics of fortified ready-to-eat banana porridge products prepared by incorporating various fortificants into the dried formulation prior to reconstitution.

to shrink and develop angular, irregular structures, likely representing clusters of dehydrated starch (Figure 4E), and Figure 4F shows evidence of dry cell wall. Retrogradation is expected to have occurred during this stage, potentially influencing the digestibility of the starch. When the sample was later reconstituted, the granules did not regain the fully swollen morphology observed in the original material. This incomplete restoration reflects the inherent characteristics of starch retrogradation (a process involving the rapid recrystallization of amylose molecules (Figure 4G), followed by the gradual formation of a gel network and increased crystallinity in processed starch (Figure 4H). These structural changes contribute to the well-known staling phenomenon in products such as bread and cakes, which is primarily driven by amylopectin retrogradation [66]. I suggest that for future work, integrating quantitative image analysis or more advanced microscopy (e.g., SEM, confocal) will be good for greater mechanistic insight.

3.4 | Fortification and Formulation

GBF is low in protein and serves mainly as an energy-rich base, while pea, soybean, hemp, and moringa flours significantly enhance protein content and amino acid balance. Fortification improves nutritional quality but must be carefully balanced to avoid reduced digestibility, sensory changes, and antinutritional effects. Optimal formulations maintain banana flour as the primary matrix and incorporate plant proteins at moderate levels to achieve both nutritional adequacy and consumer acceptability. Five different formulations were developed based on the ingredient combinations shown in Table 1, and subsequently analyzed for protein content, digestible starch, and protein digestibility. Figure 5 presents the visual appearance of the fortified RTE porridge products, while Figure 6 presents the percentage starch digestibility of the fortified RTE porridge products.

Analysis of the formulations (Table 1) identified optimal combinations that achieved the target protein requirement of 25% of daily needs, equivalent to 14 g protein per 100 g of the mixed product. The most effective formulations comprised:

76.44% RTE flour with 23.56% moringa flour (RTEM).

81.76% RTE flour with 18.24% hemp flour (RTEH).

89.90% RTE flour with 10.10% soy flour (RTES).

88.31% RTE flour with 11.69% pea flour (RTEP).

These formulations were selected based on their ability to deliver 14 g of protein per 100 g of prepared porridge, corresponding to ~25% of the recommended daily protein intake for older infants, children, and adults, in accordance with established RDA guidelines.

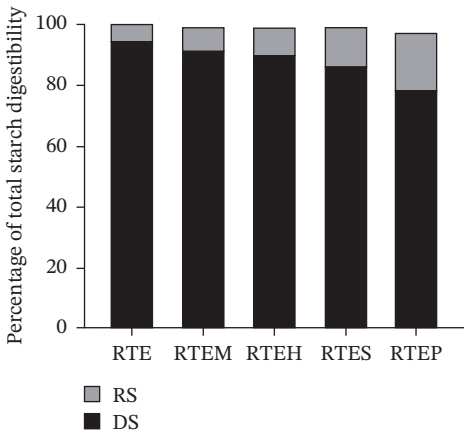

FIGURE 6 | Starch digestibility bar chart for 100 mg reconstituted RTE banana porridge (RTE) and RTE mixed with different fortificants, hydrolyzed by 0.4 U of pancreatic α-amylase and 0.17 U of amyl glucosidase and incubated for 4 h at 37°C in a water-shaking bath. Data expressed as mean with SD ($n = 6$) % of hydrolysis of total porridge starch. RTE, ready-to-eat banana porridge; RTEH, ready to eat banana porridge mixed with hemp; RTEM, ready-to-eat banana porridge mixed with moringa; RTEP, ready to eat banana porridge mixed with pea; RTES, ready to eat banana porridge mixed with soya.

Fortification with plant-based protein sources offers multiple nutritional benefits beyond protein enhancement. Previous studies have demonstrated that protein fortification strategies can substantially improve the nutritional profile of cereal-based food products [67]. In the context of RTE banana porridge, fortification with moringa, hemp, soy, and pea not only elevates protein content to meet dietary requirements but also enriches the product with essential micronutrients, including Fe, K, Mg, Mn, P, and Zn [68]. The visual characteristics of these fortified products also showed acceptable color and appearance despite the addition of protein-rich ingredients (Figure 7).

Protein serves as an essential macronutrient critical for growth and development in both children and adults. The results presented in Table 1 demonstrate a substantial increase in protein content, rising from 4.88 g per 100 g in the unfortified RTE dried porridge to ~14.00 g per 100 g in the fortified versions supplemented with moringa, soy, hemp, and pea flours. This represents nearly a threefold increase in protein content, successfully meeting the target of 25% of daily protein requirements.

FIGURE 7 | Visual presentation of non-mixed (RTE only) and mixed RTE banana porridge with different fortificants, which impart their colors on the reconstituted mixed porridge.

TABLE 5 | Nutrient composition of RTE banana porridge and RTE banana porridge mixed with moringa powder, soya powder, hemp powder and pea powder.

Nutrient	RTE	RTE banana porridge with moringa powder	RTE banana porridge with soya powder	RTE banana porridge with hemp powder	RTE banana porridge with pea powder
Protein (g)	4.88 ± 0.1^{b}	13.53 ± 0.1^{a}	13.60 ± 0.1^{a}	13.80 ± 0.1^{a}	13.73 ± 0.1^{a}
Ash (g)	3.13 ± 0.06^{c}	3.64 ± 0.5^{a}	3.21 ± 0.2^{b}	3.72 ± 0.4^{a}	3.70 ± 0.7^{a}
Fat (g)	0.007 ± 0.01^{c}	1.44 ± 0.15^{a}	0.38 ± 0.6^{b}	1.44 ± 1.2^{a}	0.66 ± 0.1^{b}
Total starch	77.67 ± 0.05^{b}	68.50 ± 0.1^{c}	76.00 ± 1.4^{b}	66.20 ± 1.1^{c}	81.16 ± 1.2^{a}
Fiber (g)	14.50 ± 0.02^{b}	17.47 ± 0.5^{a}	13.27 ± 0.5^{b}	15.50 ± 0.6^{b}	11.89 ± 0.2^{c}
Iron (g)	0.69 ± 0.01^{d}	8.52 ± 0.12^{a}	2.22 ± 1.2^{c}	5.78 ± 1.2^{b}	1.05 ± 0.5^{d}
Phosphorus (mg)	85.91 ± 0.01^{c}	131.43 ± 0.05^{b}	146.84 ± 0.5^{b}	150 ± 0.5^{a}	111.28 ± 0.5
Potassium (mg)	$104v.06 \pm 0.01^{a}$	1074.17 ± 3.5^{a}	1148 ± 0.60^{a}	850.39 ± 1.3^{b}	919 ± 0.4^{b}
Magnesium (mg)	99.40 ± 0.00^{d}	197.39 ± 1.5^{b}	115.28 ± 0.3^{c}	224.00 ± 0.4^{a}	97.60 ± 0.5^{d}
Manganese (mg)	1.73 ± 0.1^{b}	2.23 ± 0.1^{a}	1.75 ± 0.4^{b}	1.73 ± 0.5^{b}	2.03 ± 0.5^{a}
Zinc (mg)	0.57 ± 0.01^{c}	$0.86 \pm 0.0^{b,c}$	0.78 ± 1.2^{c}	2.76 ± 0.4^{a}	1.16 ± 0.1^{b}

Note: Data are expressed as mean with SD of $n = 3$. Different letters within a row indicate significant differences ($p < 0.05$) determined by Tukey test following one-way ANOVA. Total formulation $n = 9$.

Table 5 presents the comprehensive nutrient composition of RTE banana porridge and its fortified variants (RTE + moringa, hemp, soy, and pea). Statistical analysis revealed significant differences ($p \leq 0.05$) across all nutrients except protein. The absence of significant protein differences among the fortified formulations can be attributed to the target-based formulation approach employed in this study. Specifically, each formulation was designed to achieve the same protein target (14 g per 100 g), which required varying proportions of fortifying ingredients. Consequently, while the quantities of moringa, hemp, soy, and pea flours differed among formulations, the resulting protein content remained consistent across all fortified products.

Protein content ranged from 4.88 g per 100 g in the control to ~14.00 g per 100 g in the fortified samples. All four fortified variants—RTE + moringa, RTE + soy, RTE + hemp, and RTE + pea—achieved the target protein content of 14 g per 100 g, with values ranging from 13.53 g to 13.80 g.

Fat content varied considerably among formulations, ranging from 0.007 g to 1.44 g per 100 g. The highest fat content (1.44 g) was observed in both RTE + moringa and RTE + hemp formulations, with no significant difference between them ($p > 0.05$).

Ash content ranged from 3.13 to 3.72 g per 100 g, with the control RTE flour showing the lowest value (3.13 g). Total starch content exhibited the highest value in unfortified RTE porridge (77.67 g per 100 g) and the lowest in RTE + hemp porridge (66.20 g per 100 g). This reduction in starch content in fortified samples reflects the displacement of carbohydrate-rich banana flour by protein-rich fortificants, consistent with findings reported by Alamu et al. [69].

Dietary fiber content varied significantly among formulations ($p \leq 0.05$), ranging from 11.89 to 17.47 g per 100 g. RTE + moringa porridge contained the highest fiber content (17.47 g), followed by RTE + hemp (15.50 g), RTE + soy (13.27 g), and RTE + pea (11.27 g). The control RTE porridge exhibited the lowest fiber content (11.89 g).

Mineral content increased substantially across all tested minerals in the fortified samples compared to the control. RTE + moringa porridge demonstrated the highest concentrations of fiber, iron, potassium, and magnesium, with values of 17.47 g, 8.52 mg, 1074.17 mg, and 197.39 mg per 100 g, respectively.

The bioavailability of nutrients in fortified banana flour depends on matrix effects rather than nutrient content alone. Resistant starch (RS2/RS3), dietary fiber, and polyphenols in banana flour can limit nutrient release and mineral absorption through physical entrapment and chelation. Interactions with fortificants introduce phytates and polyphenols that further reduce iron

and zinc bioavailability, while protein–starch complexation may slow protein digestion. Despite these limitations, appropriate processing and regular consumption can allow fortified banana flour products to provide meaningful nutritional benefits [70].

3.5 | Effect of Fortification on the Carbohydrate Content of the Fortified Porridges

Table 6 presents the effect of fortification on the carbohydrate content of mixed RTE banana porridge after reconstitution with individual fortificants. The results revealed significant differences ($p \leq 0.05$) in DS, RS, TS, sugar, and fiber content across all samples. The carbohydrate analysis showed considerable variation among samples: Total starch: 47.45–81.10 g, digestible starch: 39.91–71.78 g, fiber: 13.16–17.76 g, and sugar: 1.2–3.6 g. The impact of fortification on the starch digestibility of non-fortified RTE banana porridge exhibited the highest digestible starch content (71.78 g/100 g), while all fortified variants showed reduced digestible starch levels. Conversely, the non-fortified porridge had the lowest resistant starch content (5.88 g/100 g). Among the fortified samples (moringa, hemp, soy, and pea), digestible starch values varied but showed no significant differences from each other ($p \leq 0.05$). Similarly, resistant starch values among the fortified porridges were not significantly different.

The mechanism of reduced starch digestibility is the decrease in digestible starch upon fortification, which can be attributed to the presence of bioactive compounds in the fortificants. These materials contain polyphenols, proteins, and dietary fiber, which inhibit digestive enzyme activity, particularly α-amylase, during in vitro starch digestion, and collectively, these mechanisms explain the reduced rates of starch hydrolysis and the attenuated glycaemic responses observed in fortified food systems [70]. This enzymatic inhibition effectively reduces the conversion of starch to digestible forms. Research has found that polyphenols decrease the overall digestibility of carbohydrates in the rat intestinal tract and inhibit α-amylase and α-glycosidase, hence controlling the glycaemic response of carbohydrates [71]. Research has shown that the postprandial blood glucose level of a diabetic person could be controlled by the inhibition of digestive enzymes (α-amylase and α-glucosidase) [72]. Finally, fortificants can be used as a tool to decrease rapidly digested starch and increase slowly digestible starch and the hydrolysis index of the cooked-to-optimum samples [73]. It could be said that fortification reduces the digestibility of the carbohydrate content of this product. The fortification strategy, involving the enrichment of banana flour with pea, soy, hemp, and moringa flours, is nutritionally justified, as it markedly enhances protein content, improves amino acid balance, and increases micronutrient density in an otherwise carbohydrate-dominant matrix. However, this approach simultaneously introduces antinutritional factors that may compromise nutrient bioavailability and physiological effectiveness. Key antinutritional components associated with the fortificants include phytates, polyphenols, oxalates, and protease inhibitors. Fortification altered the banana flour matrix by increasing dietary fiber, protein, and polyphenolic content, resulting in reduced starch and protein digestibility. This reduction in starch digestibility may be beneficial for glycaemic control. However, altered digestibility can influence overall physiological nutrient utilization. Consumer acceptability was a key limitation of the fortification strategy. Moringa leaf powder was rejected due to bitterness, while soybean flour raised allergenicity concerns and hemp flour contains tetrahydrocannabinol (THC).

3.6 | Selection of the Best Product

The rationale for best product selection was based on developing a single formulation with one fortificant suitable for all age groups in the study, ensuring consistency in nutritional composition and simplifying implementation across different developmental stages. Using the formulations outlined in Table 1 and guided by the results of protein digestibility (Figure 8), starch digestibility, and social and cultural acceptability, four fortified variants were selected and analyzed for their protein content, digestibility and social and cultural acceptability. All four formulations successfully achieved the target protein content of ~14 g per 100 g: RTEM (76.44% RTE + 23.56% moringa leaf powder), RTES (89.90% RTE + 10.10% soy flour), RTEH (81.76% RTE + 18.24% hemp flour), and RTEP (88.31% RTE + 11.69% pea flour). Although all formulations met the protein content target, RTEH (81.76% RTE banana porridge flour + 18.24% hemp flour) was identified as the highest formulation due to its superior protein digestibility of 81% but considering social and cultural acceptance the optimal product will be RTEP (88.31% RTE + 11.69% pea flour). This is because hemp powder contains THC. It is important to note that in starch digestibility, the RTE mixed porridge with pea had the least digestibility among the fortified porridges formed. Pea powder exhibits variable starch content depending on the degree of processing. In this study, the seed coat of pea was removed during processing, which reduced the

TABLE 6 | Effect of fortification on carbohydrate content of mixed RTE banana porridge after reconstitution with individual fortificants (g/100 g).

Formulation	DS	RS	TS	Fiber	Sugar
RTE	71.78 ± 0.01^{a}	5.88 ± 0.01^{a}	77.66 ± 0.0^{a}	14.90 ± 0.4^{d}	1.50 ± 0.3^{c}
RTE + moringa	62.19 ± 0.74^{b}	5.94 ± 0.67^{f}	68.13 ± 1.0^{b}	17.76 ± 1.5^{b}	3.6 ± 0.5^{a}
RTE + hemp	59.53 ± 0.97^{b}	6.67 ± 6.75^{d}	66.20 ± 1.1^{b}	15.64 ± 0.27^{c}	1.2 ± 0.27^{c}
RTE + soy	65.53 ± 2.85^{b}	10.47 ± 3.55^{b}	76.00 ± 1.1^{a}	13.59 ± 1.1^{e}	1.5 ± 1.73^{c}
RTE + pea	64.97 ± 0.15^{b}	16.13 ± 1.3^{a}	81.10 ± 1.2^{b}	13.1 ± 0.15^{c}	1.4 ± 1.19^{c}

Note: Data are expressed as mean with SD of $n = 3$. Different letters within a column indicate significant differences ($p < 0.05$) determined by Tukey test following one-way ANOVA. Total formulation $n = 9$.
Abbreviations: DS, digestible starch; RS, resistant starch; TS, total starch.

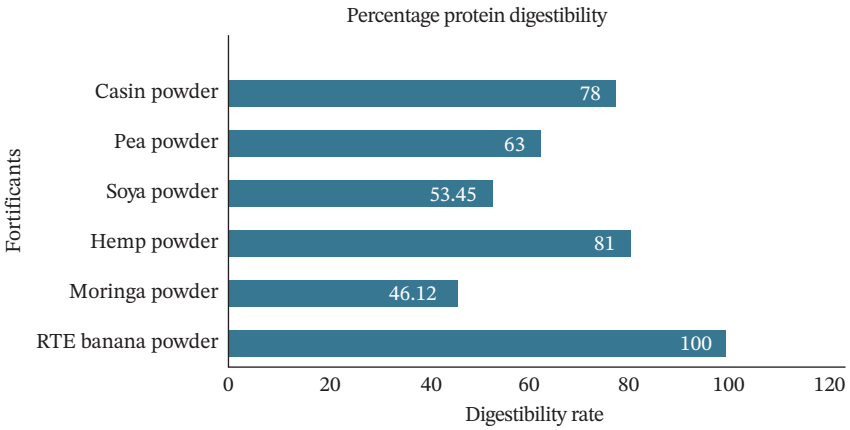

FIGURE 8 | Protein digestibility of the RTE banana porridge, fortificants, and control.

overall starch content. However, protein digestibility is a measure of how well your body can break down protein from food and absorb its amino acids for use in building and repairing tissues, making enzymes and hormones, and supporting overall health. The higher it is for a product, the better it is for a fortificant, for the product will be digestible. However, the remaining starch demonstrated high digestibility when adequately cooked. Therefore, considering the combined advantages of adequate protein content, favorable digestibility of the fortificant, and strong social and cultural acceptability, RTEP emerges as the most nutritionally effective fortification strategy for RTE banana porridge. The result of protein digestibility indicates that hemp protein ranks 81% digestibility, followed by pea with 63% and soya with 53.45%, and essential amino acid adequacy, 0.64, 0.76, and 1.03, respectively. The PDCAAS for hemp, peas, and soybeans are 0.52, 0.48, and 0.55, respectively. These differences are driven by the presence of limiting amino acids and antinutritional factors that reduce protein digestibility. Processing and blending strategies can improve overall protein quality and amino acid balance in fortified foods. (Appendix Table C). The trade-off between protein enrichment and digestibility in fortified GBF mirrors broader patterns observed in plant-based CFs: more protein does not automatically translate into more bioavailable or utilizable amino acids. Matrix effects, antinutritional factors, and sensory changes all this relationship. Literature supports that strategic processing (e.g., fermentation, heat treatment) and ingredient selection (balanced blends, reduction of antinutrients) are essential to maximize both protein content and digestibility while maintaining acceptability in target populations. The fortified GBF formulations are scalable using locally available bananas and legume(pea), in supporting smallholder supply chains. This is cost-effective, as plant-based fortificants are affordable and processing requires low-technology methods, though quality control and antinutrient reduction may increase costs. Social and cultural acceptability is generally high due to familiarity with banana-based porridges, but sensory changes from fortificants (bitterness, beany flavors) require careful formulation and consumer testing to ensure adoption. A clinical study should be conducted to establish nutrient bioavailability and metabolic effects, followed by longitudinal studies to demonstrate sustained health benefits and consumer acceptability; labeling should then be aligned strictly with the level of evidence obtained to validate nutritional and health claims and determine the product's potential for functional food designation.

4 | Conclusion

This study successfully developed and characterized RTE green banana porridge flour with enhanced nutritional value through protein fortification. Four of the five formulations, fortified with moringa, hemp, soy, and pea powders, achieved the target protein contribution of 25% of daily requirements, providing ~14 g of protein per 100 g of product. Beyond protein enhancement, these fortified formulations exhibited increased levels of other macronutrients and energy content, while producing porridges with favorable energy density and appropriate total solids for infant, older children, and adult feeding. Among the tested formulations, RTE banana porridge flour fortified with pea powder (RTEP) emerged as the optimal choice. The RTEP formulation (88.31% RTE flour: 11.69% hemp powder) demonstrated superior nutritional performance, combining the target protein content of 14 g per 100 g with good protein digestibility among all variants. This combination positions RTEH as a promising CF for addressing PEM in vulnerable populations. Storage stability studies revealed that the RTE porridge maintained microbiological safety for 30 days under all tested conditions, with moisture content and microbial growth remaining acceptable during this period. However, extended storage beyond 30 days resulted in progressive moisture absorption and increased microbial counts, particularly in paper packaging and at elevated temperatures.

Further research should focus on: (1) conducting long-term storage stability studies with unopened packages for extended durations (60–90 days) under various environmental conditions, (2) performing comprehensive sensory evaluation with target consumer groups to assess acceptability and preference, and (3) conducting in vivo digestibility and bioavailability studies to validate the nutritional efficacy of RTEH formulation. These investigations will provide critical data to inform commercialization strategies and market positioning decisions.

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Catherine Okafor: writing – original draft and experiments. **Idolo Ifie:** writing – review.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*)

The Appendix Tables (A and B) show results of RTE porridge with fortificants for meeting the target consumers and using 100 g of the product respectively. Appendix Table C shows the protein quality of the selected fortificants.