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Development of a nutritious cereal-based instant porridge by the incorporation of protein-rich insect powder – An example from Zimbabwe

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ABSTRACT

Maize in Zimbabwe lacks essential nutrients like protein, iron, and zinc. This study explored alternative ingredients, including climate smart cereals used in porridge, to address this issue. A fortified porridge was developed by adding mopane worm powder, known for its high protein content. Consumer analysis in the UK and Zimbabwe confirmed acceptability of the fortified porridge. The study also assessed the nutritional quality by examining protein, iron, and zinc bioaccessibility after digestion. Results showed a significant increase in the porridge's nutritional profile. A 20 kg child consuming a 50 g portion of the fortified porridge had a potential 230 % increase in protein uptake and 164 % and 109 % increases in iron and zinc uptake, respectively. Acknowledging dietary diversity and use of local raw materials, the study concludes that food-to-food fortification offers a recommended and sustainable solution to address food security challenges in sub-Saharan African nations.

1. Introduction

Malnutrition, both undernutrition and micronutrient deficiencies is a global health concern, most prevalent in developing countries (INTERNATIONAL FOOD POLICY RESEARCH INSTITUTE, 2018). The triple burden of malnutrition, which includes simultaneously undernutrition, overweight/obesity and micronutrient deficiencies within a population (Prentice, 2023), is a major concern across sub–Saharan Africa. Globally it is estimated that 22 % of under 5 s are stunted (a child under 5 years, with length/height for age $<$ 2 SD below WHO child growth standard median), 6.7 % wasted (a child with low weight- for-height for age $<$ 2 SD below WHO child growth standard median) and 5.7 % overweight (a child who has a weight for height + 2 SD above WHO child growth standard median) (UNICEF, 2022). The ending of malnutrition in all its forms, is one of 17 Sustainability Development Goals set by the World Health Organisation (WHO) with a target date of 2025 (World Health Organization, 2021). Leading nutrition issues in Zimbabwe include stunting, micronutrient deficiencies of iron and zinc and an emerging problem of obesity and its associated complications (World Health Organization, 2021). Improving general childhood nutrition and micronutrient deficiencies could improve education, school performance and subsequent prospects (Jukes, 2005).

In Africa and Asia, malnutrition is intensified by a monotonous cereal-based diet (Ruzengwe, et al., 2022), up to 80 % of calorific intake

Abbreviations: AAS, Atomic Absorption Spectrometry; ANOVA, ANalysis Of VAriance; CATA, Check-All That Apply; FAMEs, Fatty Acid Methyl Esters; GC–MS, Gas Chromatography-Mass Spectrometry; HPLC, High-Performance Liquid Chromatography; ICP-MS, Inductively Coupled Plasma-Mass Spectrometry; IKS, Indigenous Knowledge Systems; LC-MS, Liquid Chromatography-Mass Spectrometry; MDL, Method Detection Limit; MRM, Multiple Reaction Monitoring; PUFA, Poly-Unsaturated Fatty Acid; RDI, Recommended Daily Intake; TDS, Temporal dominance of sensation; WHO, World Health Organisation.

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can come from cereal. Commonly consumed cereals in Zimbabwe include sorghum, millets and maize (Jiri, Mafongoya, & Chivenge, 2017), which lack essential micro-nutrients, including iron and zinc. Finger millet, rich in methionine is one of the most nutritious of the common cereals.

Strategies to overcome malnutrition and micronutrient deficiencies include food-to-food fortification (adding micronutrients and minerals to industrially processed food), supplementation (addition of micro-nutrients in the form of tablets, pills or powders) and biofortification (improving the nutritional qualities of food crops through agronomic practices, conventional plant breading or modern biotechnology) (Monroy-Gomez, et al., 2022).

In Zimbabwe from June 2017, it became mandatory for major local food manufacturers to fortify processed staple foods with micro-nutrients. The food vehicles targeted include sugar (vitamin A), cooking oil (Vitamin A & D), maize meal and wheat flour (A, B1, B2, B3, B6, B12, folic acid, iron and zinc) (Kairiza et al., 2020). In addition, the Government is promoting three biofortified crops; Nua 45 beans, orange fleshed sweet potato and orange maize. However, it is generally accepted that malnutrition and insecurities in food supply are best solved sustainably using Indigenous Knowledge Systems (IKS), understanding and utilising local knowledge of natural surroundings and skills developed within a community to solve a local issue.

The consumption of insects (Entomophagy) is practised in over 90 developing countries, it includes over 2100 species, which are consumed across all life stages (Huis, 2020). Insects as a food are high in protein and micronutrients, including Fe and Zn, easy to rear and have low impact to the environment (Jonas-Levi & Martinez, 2017). There is growing evidence that the incorporation of insects into foods increases the nutritional profile of the food (especially protein, iron and zinc) and are a potential strategy to reduce malnutrition in developing countries (Ruzengwe, et al., 2022). Insects have impressive nutrient profiles with the potential to deliver affordable high-quality protein, fat, vitamins and high amounts of iron and zinc. The protein content of insects varies between 25 and 75 % on a dry matter basis. Methionine and cystine are usually the first limiting amino acids in most insect species when fed to humans (Oonincx & Finke, 2020). The nutritional composition of the insects is also dependant on the processing methods applied. Further, protein digestibility of insects is generally high, however, if a larger proportion of amino acids is present in cuticular proteins complexed with chitin or is highly sclerotised protein digestibility is likely to be decreased (Ruzengwe, et al., 2022). Mopane worm (*Gonimbrasia belina*) is the caterpillar of the emperor moth, *Imbrasia belina*, which consumes the mopane tree leaves (Wessels, et al., 2006). The mopane worm is found in the woodlands across the savannah (~23,000 km^2) in South African countries (Baiyegunhi & Oppong, 2016). The are up to two harvests per annum, ~January and April, dependent on rainfall, with harvests lasting ~ 6 weeks (Thomas, 2013). Harvesting is predominantly the role of females and children, typically 25–50 kg per day can be harvested a day. Of an annual harvest 30–52 % were sold immediately, 14–25 % were a food source and 25–36 % were stored for off-season sale (Hope, Frost, Gardiner, & Ghazoul, 2009).

Maize is a staple food for Zimbabwe and is commonly consumed as a thin porridge for breakfast and as a thick porridge for lunch and supper. Food-to-food fortification of a staple food is more readily accepted (Darnton-Hill & Nalubola, 2002) (Olson, Gavin-Smith, Ferraboschi, & Kraemer, 2021).

Baobab fruit (*Adansonia Digitata*), from the baobab tree, common in hot, dry tropical regions across Africa. It is rich in ascorbic acid which can reduce phytic acid, an antinutrient of cereal grains, increasing bio-accessibility of both iron and zinc (Gabaza et al., 2018).

Extrusion is a food processing technique where soft ingredients are forced through a die at elevated temperature and pressure, followed by expansion and evaporation. For flours the functionality is changed, starches gelatinise, and proteins denature resulting in increased digestibility (Martínez et al., 2014). The extrusion process produces a precooked flour, that acts as a thickening and gelling agent, leading to an instant product with reduced in-home cooking times (Bryant, Kadan, Champagne, Vinyard, & Boykin, 2001).

The nutritional profile of food is insufficient alone to improve the nutritional outcomes for a population. Both the sensory experience of the consumer and bioaccessibility of food consumed are important. The sensory experience includes the texture, taste, smell, flavour and appearance attributes contributing to overall palatability and ultimately consumption of a food. Traditionally palatability was assessed by panellists accepting or rejecting foods and use of hedonistic scales. The use of technology through Check-All That Apply (CATA) and Temporal Dominance of Sensation (TDS) allow the application of statistical analysis to the collection of data, requiring fewer panellists and runs per panellists (Pineau, et al., 2009). TDS was developed in the LIRIS lab at the "Centre Européen des Sciences du Goût" in 1999 (Pineau, et al., 2009) and allows the continual identification of pre-selected attributes as the dominant attribute changes with time during the eating process and includes the post swallowing aftertaste as well.

Bioaccessibility can be assessed *in-vivo* or *in-vitro*, the development of the INFOGEST protocol has led to a harmonised method for the in-vitro digestion of foods. Following digestion, bioaccessibility can be assessed (Sousa, et al., 2023).

The aim of this study is to develop an instant mopane worm fortified porridge that has improved nutritional properties. This was achieved utilising local raw materials and knowledge, combined with nutritional analysis. Followed by an assessment of the bioaccessibility of key nutritional properties to demonstrate the benefit of a fortified porridge.

2. Materials and methods

2.1. Sample collection and preparation

Mopane worms were harvested from Gwanda district, Zimbabwe in April 2021 from three harvesting camps. Soon after collection, the mopane worms were manually degutted, washed with potable water, transferred to zip lock bags & transported to Chinhoyi University of Technology on flaked ice within 24 h and stored at −18 °C. Prior to processing, mopane worms were allowed to thaw at room temperature overnight, followed by boiling for 20 min and drying at 40 °C for 48 h. Mopane worm (boiled and dried) (for proximate analysis) were kept in zipped locked bags prior to shipment to Abertay University. Mopane worm (1 kg) were milled using a centrifugal mill fitted with a stainless-steel ring sieve with 0.50 mm trapezoid holes (Ultra Centrifugal Mill ZM 200, Retsch, Germany). Thereafter, samples were stored under refrigeration until use.

For nutritional analysis white maize, pearl millet and sorghum were purchased unprocessed from a Mbare farmers' market in Harare, Zimbabwe, prior to shipment to Abertay University. Grains (1 kg) were milled as above and were stored under refrigeration until use.

Pearl millet and sorghum (for consumer testing, following fortified porridge development) were purchased from Mbare farmers market in Harare, Zimbabwe. The pearl millet and sorghum were cleaned and extruded for 8 s above the pre gelatinization level using a twin-screw extruder (Buhler AG, Switzerland). Millets and sorghum were extruded at a maximum temperature of 175 °C, pressure 60 bars and at a feed rate of 700 kg/hr. Baobab powder was purchased from Mutoko district, Zimbabwe and was sieved using a screen mesh of 0.50 mm. Dried mopane worm and the grains were milled using a Buhler hammer mill fitted with a sieve with a screen mesh size of 1 mm.

Vanilla was purchased from Natural Vanilla Store UK (Australia) and

from Codchem (Harare, Zimbabwe), white sugar, chocolate and cream crackers were purchased from a UK supermarket (Lidl GC, Suriton). Goldstar white sugar and Lobels cream crackers were purchased from a local supermarket in Chinhoyi, Zimbabwe. All dry ingredients were stored in a dark, dry and cool place prior to use.

2.2. Proximate analysis

Moisture content was determined from milled flour (sub samples) by oven drying at 110 °C for 24 h. Ash content was determined by dry ashing at 400 °C for 4 h in a muffle furnace (AAF1100 Carbolite, UK). Fat content was extracted using Soxhlet with ~ 120 mL hexane (HPLC grade) as extraction solvent.

Protein content was determined by Kjeldahl method. Digestion was performed with a VELP DK6 heating digester, fitted with an SMS scrubber and JP recirculating water aspirator. Defatted samples (1 g) were digested for 40 mins at 300 °C followed by 90 mins at 420 °C with sulphuric acid with a copper catalyst (Kjtabs VCT). A VELP UDK 139 semi-automatic distillation unit steam distilled the resulting solutions, into an excess 2 % boric acid solution (Vreceiver TKN boric acid powder formula with indicators) and titrated against 0.2 N sulfuric acid solution.

Carbohydrate content was determined by difference using equation (1).

$$Carbohydrate(\%) = 100 - \sum Moisture, Ash, Fat, Protein(basedonaminoacidcontent) \quad (1)$$

Fe and Zn content were determined on the dry ash samples by AAS using the method of Murthy et al. (1971) with minor modification, residue dissolved in minimal warmed HNO_3 (PrimarPlus-Trace analysis grade), samples were transferred to a 50 mL volumetric flask and diluted to volume. Sample centrifuged (Hermle Z323 K, Wehingen, Germany) at 825 rpm for 5 min, to remove silica particles. Fe and Zn standards were prepared from metal at 1000 mg/L and 500 mg/L respectively in the minimum 1:1 HNO_3:H_2O, made to volume with water and the minimum 1:1 HCl:H_2O, made to volume with 1 % HCl (%v/v) respectively. Calibrations solutions were diluted from stocks 0–10 and 0–1 mg/L. Both calibrations were linear through the origin.

2.3. Phytic acid quantification

Phytate was quantified using the method of Haug & Lantzsch (1983) using variant a. following sample extraction in 0.2 M HCl, 0.5 mL is added to Ferric solution (200 mg/L in 0.2 M HCl) in a test tube. The tube was heated in boiling water for 30 min, cooled in ice water for 15 min. Varient A: Sample centrifuged for 30 mins. Supernatant (1 mL) is added to 2,2′-Bipyridine (10g/L) and thioglycollic acid (10 mL/L) solution in water (1.5 mL). The absorbance was recorded after 1 min at 519 nm using a spectrophotometer (Thermo Spectronic Genesys 10-S (Thermo-Fisher, Rochester)). The linear range for phytate quantification was 0–30 μg/mL.

2.4. Amino acid characterisation

Free amino acids were extracted from the mopane worm (100 mg) using 60:40, methanol:water (3 mL). Samples were shaken on an orbital shaker at 1000 rpm for 30 min followed by centrifuging (Hermle Z206 A, Wehingen, Germany) at 16,100 g for 15 mins at 4 °C. An aliquot was diluted by a factor of five and transferred to a HPLC vial for analysis.

Bound amino acids were released using both acidic and alkaline hydrolysis, as described by Ritvanen et al. (2020) with some modifications. Mopane worm (100 mg) was hydrolysed in hydrochloric acid (6 M) or sodium hydroxide (5 M) at 110 °C for 24 and 18 hrs respectively.

Table 1
Multiple reaction monitoring transitions, collision energy used and linear calibration range, MDL obtained for the amino acids.

Amino acid	Retention time (min)	MRM transition (*m/z*)	Collision energy (eV)	Linear range (μg/mL)	Method detection limit (μg/mL)
Alanine	4.53	90.15 → 44.10	14	0.0515–10.3	0.052
Arginine	9.53	174.85 → 70.1	24	0.245 – 9.8	0.245
Asparagine	6.10	132.85 → 74.05	18	0.0485 – 0.97	0.049
Aspartic acid	9.52	133.85 → 74.15	15	0.0485 – 3.88 1.94 – 9.7	0.049
Cysteine	7.47	240.75 → 151.90	14	0.051 – 1.02	0.051
Glutamic acid	8.05	147.8 → 84.1	18	0.5 – 10 0.05–1	0.050
Glutamine	5.97	146.85 → 84.05	19	0.108 – 4.32 0.054 – 0.81	0.054
Glycine	5.63	75.80 → 30.00	13	0.0485 – 3.88	0.049
Histidine	5.96	155.88 → 110.05	16	0.05—2	0.050
Isoleucine / Leucine	2.69	131.85 → 86.10	13	0.0535 – 10.7	0.054
Lysine	5.96	146.85 → 84.05	18	0.051 – 1.02	0.051
Methionine	3.14	149.85 → 104.10	13	0.048 – 0.96	0.048
Phenylalanine	2.51	165.85 → 120.10	15	0.765 – 10.2 0.102 – 1.02	0.102
Proline	5.43	116.13 → 70.09	22	0.062 – 12.4	0.062
Serine	5.97	106.15 → 60.1	14	0.97 – 9.7 0.0485 – 0.97	0.049
Threonine	5.83	119.85 → 74.00	13	0.0475 – 9.5	0.048
Tryptophan	2.41	204.85 → 146.00	18	0.51 – 10.2 0.102 – 1.02	0.102
Tyrosine	3.20	181.8 → 136.00	15	0.012 – 2.4	0.012
Valine	3.30	117.85 → 72.1	13	0.25 – 10 0.05–1	0.050

The sample was centrifuged (Hermle Z206 A, Wehingen, Germany) at 16,100 g for 15 mins at 4 °C. Aliquots of solution were neutralised with sodium hydroxide (5 M) and hydrochloric acid (6 M) respectively. Acid hydrolysed samples were diluted by a factor of 50 and alkali samples were diluted by a factor of 40.

2.4.1. Amino acid quantification by LC-MS

Amino acids (free, acid and alkali hydrolysed) in mopane worm were identified and quantified by LC-MS/MS. The system consisted of a LC20ADXR pump, SIL30AC auto sampler, CTO20A column oven, and a triple quadrupole mass spectrometer LCMS-8040 (Shimadzu Corporation, Japan). The chromatographic separation was performed on a Raptor Polar X column (100 mm x 2.1 mm, 2.7 μm, Restek, United States) fitted with a guard cartridge of the same stationary phase, maintained at 30 °C. Mobile phase A was water containing 0.5 % formic acid and mobile phase B was 9:1 acetonitrile:water. The gradient was 88 % B at 0.5 mL/min for 3.5 min, reducing to 30 % B over 5.5 min before re-equilibrating to initial conditions over 2 min. The MS was equipped with an electrospray ionisation (ESI) source and was operated in positive ionisation mode. A sample of 1 μL was injected in the column. The MS source conditions were spray voltage 4.5 kV, capillary temperature 200 °C, nitrogen was used as a nebuliser gas at a flow rate of 3 L/min and drying gas at 15 L/min. Data were collected with Lab solutions software (Shimadzu Corporation, Japan). Amino acids were quantified by external standard calibrations Retention time, Multiple Reaction Monitoring (MRM) transitions, collision energy, linear calibration range and Method Detection Limit (MDL) for each amino acid are reported in Table 1.

2.4.2. Protein conversion factor calculation

The protein conversion factor of mopane worm, K, was calculated based on the protein bound amino acid profile, using equation (2) as reported by Urbat et al. (2019).

$$K = \frac{1}{\sum_{k=1}^{n} \frac{a_k \bullet 14}{M_k - 18} \bullet q_k} \tag{2}$$

Where K is the Nitrogen to protein conversion factor, n is the number of amino acids, k is the amino acid, a_k is the number of nitrogen atoms in the amino acid (k), M_k is the molecular mass of the amino acid (k), q_k is the proportion of amino acid (k) in the total amino acid profile. The equation is based on the anhydrous mass of amino acids (k)(mass of water (18 g mol^{-1}) subtracted from amino acid molecular mass) as proteins are long chains containing up to 2000 amino acids.

Protein bound amino acids were calculated as per equation (3). Using this and the protein bound amino acid profiles the nitrogen to protein conversion factor for mopane worm was calculated.

$$Proteinboundaminoacids = \left(\sum Acidhydrolysisaminoacids + AlkalihydrolysisTryptophan\right) - \sum Freeaminoacids \tag{3}$$

2.5. Fatty acid profile quantification by GC–MS

The FAMES composition of samples was determined on the Soxhlet extracted fat samples. The method described by Christie (1989) was used with modifications as follows. Methanolic H_2SO_4 (1 %, 1 mL) was added to fat samples and incubated at 50 °C for 2 h. Samples were then washed with 7 % NaCl, FAMES were removed by hexane and washed with 2 % $NaHCO_3$ (1 mL) and dried with $MgSO_4$. Samples had 1,9-dichlorononane added as internal standard. External calibration was prepared using FAMEs (Fatty Acid Methyl Esters) 37 component mixture from Supelco.

The FAMEs composition was determined by GC–MS consisting of a GC (model GC.7820A) coupled with mass spectrometer, (model MS 7697A). Separation was performed on a capillary column DB-23 (30 m x 0.25 mm, 0.25 μm film thickness, Agilent Tech, USA). Sample (1 μL) was injected onto column using a split ratio of 50. The inlet temperature was 250 °C. The gas carrier was Helium with flow rate of 0.8 mL/min. The oven temperature was initially 50 °C, held for 1 min, with an initial ramp rate of 25 °C/min until 200 °C, held for 8 min, with a second ramp rate of 5 °C/min until 230 °C, held for 6 min. The MSD transfer line was at 250 °C. The mass spectrometer was set to scan in the range of *m/z* 50–550 with EI mode of ionization.

2.6. Fortified porridge development

A cross sectional survey carried out in Gwanda district of Zimbabwe (Manditsera, et al., 2022) informed the recipes used for both unfortified and mopane worm fortified porridge based on what is currently consumed in the district. The main ingredients in the traditional porridge are cereal grain flour including: maize, sorghum, finger millet; insects (in fortified porridges only), water (cold or boiling) and salt and/or sugar to taste (optional).

A gate approach was adopted for the new product development of a mopane fortified porridge (Cooper, 2003). The raw ingredients nutritional profile, recommended daily intake values (RDIs) and typical portion size for the age group were used to optimise porridge recipes. Pilot experiments were conducted to establish an acceptable porridge, including mopane worm at different ratios and additives to mask the addition, without impinging on the texture, taste and expected nutritional profile of the porridge. Inclusion of Mopane worm at a ratio of 1:3 the sample was deemed unpalatable through a small kitchen panel (n = 4). Mopane worm was incorporated at both a 1:4 and 1:3.5 ratio to the flour/baobab mix. The dry ingredients contained 1:20 baobab powder to flour (extruded pearl millet or extruded sorghum, to facilitate an instant porridge preparation), vanilla and sugar were added so the final porridge at 1 % and 2 % respectively. Boiled water was added at a 5:1 ratio to the porridge mix for preparing the final porridge for consumption. Vanila and sugar were added to the porridges for the TDS study but were excluded from the porridges for the INFOGEST digestion.

2.7. Temporal dominance sensation (TDS) consumer taste testing

2.7.1. Panellist selection for TDS analysis

The TDS testing was done as cross-cultural study in both the UK and Zimbabwe. For UK, a total of 20 panellists (9 females, 11 males) were recruited from within the Abertay University staff. The following inclusion criteria was used: interest in trying novel foods, aged between 20 and 60 years old, have good general health, fluent in English and used computers frequently. TDS analysis was conducted at Abertay University's Food Sensory Consumer labs (ISO8589:2007).

In Zimbabwe a total of 19 panellists (11 females, 8 males) were recruited, from within the Chinhoyi University of Technology staff and students., using the same inclusion criteria used in Abertay University. TDS analysis was conducted at Chinhoyi University of Technology's Food Science and Technology laboratory.

2.7.2. Porridge preparation for TDS taste testing

Porridge was prepared in the ratios as detailed in section 2.7. Porridge (90 g dry ingredients) were microwaved (Samsung Snack mate CM1069, 1100 W, South Korea) for 15 s on medium power (770 W), ensuring a cooked product and a core temperature above 78 °C. Porridges were aliquoted into ice cube portions and frozen (ensuring uniform product throughout TDS study).

Samples were defrosted and reheated at the point of use, using a microwave ensuring the core temperature was above 82 °C, the sample was mixed to ensure an even consistency (and allowed cooling) prior to consumption.

For Zimbabwean trial, samples of the porridges were prepared the same day the testing was done.

2.7.3. Sensory attribute generation for TDS

Feedback from a small preliminary consumer trial (including participants from both the UK and Zimbabwe, n = 6), independent panellists to the TDS trial were used to construct an attribute list for the porridges. Sensory attributes were discussed, resulting in a final list of 8 attributes. These were vanilla, sticky, gritty, smooth, bitter, sweet, earthy and sour. The order of the attributes was randomised during the trial to prevent positional bias.

2.7.4. TDS analysis

Ethical approval for TDS analysis granted through Abertay University Research Ethics Committee, reference: EMS3547, approval date 12/08/2021.

Panellists were explained the TDS process and the attributes described. Panellists were encouraged to ask questions and any instructions clarified.

Each panellist attended the sensory analysis three times, sampling six samples per session. Each session lasted 15–20 min with a 30 s break between samples. Samples were arranged in a randomised balanced design order generated by Compusense Software (Compusense, Ontario, Canada). Sample order was reordered for each analysis for the panellists.

Data collection used a similar protocol detailed by Wilkin et al. (2021), with the following amendments. For the UK panellists, panellists were seated in individual booths with a red-light illumination to mask differences between the mopane worm ratios and the porridge grain base. For Zimbabwe, the panellists were seated in individual booths with the omission of coloured light illumination.

All panellists tested each of the six porridges on three separate occasions, allowing a minimum of 1 h between sessions to prevent flavour fatigue. Panellists were instructed to place the porridge samples in their mouths and roll it around with their tongue, followed by pressing the start button on screen. Attributes were then presented to the panellists to select their perceived dominant attribute at any given time point. Panellists were asked to swallow at 10 s and to continue to record attributes for 30 s. Panellists were asked to rinse their mouth with water and a cream cracker in between samples.

2.7.5. TDS data analysis

The chance level (P_0), is the rate that an attribute can be selected by chance, its value is 1/p, where p is the number of attributes, in this case p is 8, making $P_0 = 0.125$. The significance level Ps is the minimum value an attribute should have to be considered significantly greater than the P_0. It is calculated as in equation (4), where Ps is the significance level, P_0 is the chance level, n is the product of replicates and no. of participants and 1.645 is the one-tailed normal z value for α = 5 % (Pineau, et al., 2009).

$$P_{s(\alpha=0.05)} = P_0 + 1.645\sqrt{\frac{P_0(1-P_0)}{n}} \quad (4)$$

The number of participants was determined as in equation (5), where P_0 is the probability of success, in our case n (product of replicates and participants) = 46, meaning a minimum of 16 participants were required to participate on three occasions (Pineau, et al., 2009). For the UK 20 panellists and in Zimbabwe 19 panellists completed the TDS analysis.

$$n = 5/P_0(1-P_0) \quad (5)$$

2.8. INFOGEST experiments of mopane fortified porridge

2.8.1. INFOGEST sample preparation

Reagents for INFOGEST digestion were prepared as detailed by Brodkorb et al. (2019) using pepsin from porcine gastric mucosa, pancreatin from porcine pancreas and bile extract porcine from Sigma (UK). Porridges were prepared as detailed in section 2.7 without the addition of sugar or vanilla. Porridge sample (5 g) was added to a 50 mL centrifuge tube, the protocol was followed to the end of the intestinal phase where the reaction was stopped with heat shock treatment, salivary amylase was excluded from the oral phase. Following heat shock treatment samples were centrifuged (Hermle Z323 K, Wehingen, Germany) at 17,000 g for 5 min, the supernatant and pellet were separated and both samples were frozen and freeze-dried. Unfortified porridges were digested as a control for comparison purposes.

2.8.2. INFOGEST sample protein content and digestibility

Samples (freeze dried supernatant and pellet, 0.2–1.7 g) were transferred to Kjeldahl digestion tubes and digested as described in section 2.3.

Digestibility was calculated using equation (6) reported by Sousa et al. (Sousa, et al., 2023)

$$invitrobiodigestibility(\%) = \frac{(F_s)}{(F_s + F_p)} x100 \quad (6)$$

Where:

F_s is supernatant from food.

F_p is pellet from food.

2.8.3. INFOGEST sample Fe & Zn content and bioaccessibility

Fe and Zn were quantified using ICP-MS. INFOGEST samples (supernatant and pellet freeze-dried) (50 mg) were digested in nitric acid (68–70 %, 5 mL) at 170 °C for 30 mins on a heat block, followed by dilution to 15 mL.

Samples were analysed on an Agilent 7500ce ICP-MS instrument with shield torch in hot plasma (no reaction gas) mode. Sample introduction used a MicroMist (Agilent) nebulizer and a pump speed of 0.3 revolutions per second. The argon carrier gas flow rate was 0.9 L/min. Acquisition was in spectrum mode with a 0.1 s integration time and 3 replicates.

The isotopes 56 Fe and 66 Zn were measured, calibration standards were prepared at concentrations of 0, 0.05, 0.1, 0.25, 0.5, 0.75 and 1 ppm using 1 % nitric acid. A 1 ppm solution of Yttrium (Y) was used as an internal standard. Fe and Zn digestibility was calculated using equation (6). Inductively coupled plasma mass spectrometry (ICP-MS) standards of Fe isotope 56 Fe and Zn 66 Zn certified reference materials were purchased from Fisher scientific Spex Certiprep standards and LGC reference standards respectively. Y certified internal standard was purchased from LGC reference standards.

2.9. Statistical analysis

IBM SPSS (version 28.0, Armonk, NY) was used to apply one-way Analysis of Variance (ANOVA), the Tukey Post Hoc test identified differences between groups at $p < 0.05$ confidence level. Outliers were identified using the Grubbs' test. All analysis was done in triplicate (n = 3).

Table 2
Proximate analysis of cereal grains and mopane worm.

	Ash %	Fat %	Moisture %	Protein %	Carbohydrate %	Overall std. dev.
Mopane worm	12.2 ± 0.8^{c}	21.4 ± 1.9^{e}	4.6 ± 0.1^{cd}	53.0 ± 4.3^{b}	8.8 ± 4.1^{a}	6.4
Baobab powder	5.2 ± 0.2^{b}	0.5 ± 0.1^{a}	8.9 ± 0.4^{d}	2.0 ± 0.1^{a}	83.4 ± 0.7^{c}	0.8
Pearl millet	2.7 ± 0.1^{a}	6.1 ± 0.5^{d}	6.1 ± 0.3^{cd}	8.6 ± 0.4^{b}	76.5 ± 0.4^{b}	0.8
Sorghum	3.2 ± 0.6^{a}	2.8 ± 0.7^{bc}	9.9 ± 0.6^{b}	8.8 ± 0.2^{b}	75.3 ± 0.5^{b}	1.3
White maize	3.4 ± 0.4^{a}	3.9 ± 0.3^{c}	10.1 ± 0.6^{a}	8.0 ± 0.1^{b}	74.4 ± 0.8^{b}	1.1

(Different letters in the same column indicate significant difference ($p < 0.05$). Results are expressed as mean ± SD for $n = 3$.

Table 3
Phytic acid content (mg/g) of cereal grains.

	Phytic acid (mg/g)
Finger Millet	12.2 ± 3.9^{b}
Pearl millet	5.8 ± 4.1^{a}
Sorghum	10.8 ± 2.0^{b}
White Maize	5.1 ± 3.1^{a}

(Different letters in the same column indicate significant difference ($p < 0.05$). Results are expressed as mean ± SD for $n = 9$ (3 UV readings of 3 independent preparations).

Table 4
Protein bound amino acid characterisation (g/100 g) and total amino acid content for Mopane worm.

Amino acid	Mopane Worm (Boiled and dried) (g/100 g dry weight)	WHO ideal protein (for essential amino acids) (Kwiri, Mujuru, & Gwala, 2020)	(% amino acid/ideal) x 100
Alanine (ala)	3.5 ± 0.3		
Arginine (arg)	6.0 ± 0.1		
Asparagine (asn)	0 ± 0		
Aspartic acid (asp)	6.6 ± 0.2		
Cysteine (cys)	0.63 ± 0.00	2.5 combined with met	126 combined with met
Glutamic acid (glu)	7.4 ± 0.3		
Glutamine (gln)	0.6 ± 0.1		
Glycine (gly)	0.1 ± 0.0		
Histidine (his)	0.9 ± 0.2	2.8	64
Isoleucine (Ile) / Leucine (leu)	3.3 ± 0.1	2.8/6.6	77
Lysine (lys)	0.6 ± 0.1	5.8	19
Methionine (met)	0.9 ± 0.0	2.5 combined with cys	126 combined with cys
Phenylalanine (ph)	5.8 ± 0.3	6.3 combined with tyr	33 combined with tyr
Proline (pro)	0 ± 0		
Serine (ser)	2.7 ± 0.2		
Threonine (thr)	2.1 ± 0.1	3.4	128
Tryptophan (trp)	0.5 ± 0.5	1.1	102
Tyrosine (tyr)	4.1 ± 0.3	6.3 combined with phe	33 combined with phe
Valine (val)	2.65 ± 0.34	3.5	157
Total	**48.0 ± 0.9**		

3. Results and discussion

3.1. Proximate composition of mopane worm and cereal grains

The proximate profile of cereal grains and mopane worm are reported in Table 2, for mopane worm a protein correction factor of 5.36 (calculated in section 2.5.2) was used to calculate the protein content of mopane worm. Mopane worm is found to have high protein and fat contents (Table 2), and a similar nutritional composition to that reported by Hobane (1994).

The ash, fat, moisture, protein and carbohydrate content of mopane worm was significantly different compared to the cereals and baobab powder. Therefore, food-to-food fortification of the cereal with mopane worm and baobab powder should lead to a significant change in the nutrition product compared to an unfortified porridge.

3.2. Phytic acid quantification

Table 3 shows that pearl millet and white maize contained significantly less phytic acid, than sorghum for the base porridge. Given the anti-nutrient properties of phytic acid (Petroski & Minich, 2020), higher levels of phytate reduce the bioaccessibility in the intestine of Zn and Fe by binding to the minerals. Therefore, a lower phytic acid content is beneficial to Zn and Fe bioaccessibility of porridges thus pearl millet was selected as choice of cereal grain to use due to a lower phytic content amongst the sampled climate smart cereal grains.

3.3. Amino acid profile characterisation

Amino acid characterisation for protein bound amino acids in mopane worm are reported in Table 4. Mopane worm is a source of all nine essential amino acids (National Research Council (US), 1989), which are not synthesized by the body and are therefore required to be obtained from dietary sources. The protein score of mopane worm is compared to the WHO ideal standard, mopane worm is a significant source of threonine, valine and methionine combined with cysteine.

Table 5
Fatty acids identified by FAMES analysis in raw ingredients following Soxhlet extraction, including ΣSFA (sum of the saturated fatty acids, ΣMUFA (sum of the monounsaturated fatty acids), ΣPUFA (sum of the poly saturated fatty acids).

	Fatty acids (% of total fatty acids)				
	Mopane worm	Baobab	Pearl millet	Sorghum	White maize
C6	0.1	0.0	0.0	0.0	0.0
C8	0.4	0.0	0.0	0.0	0.0
C11	0.2	0.0	0.0	0.0	0.0
C12	0.2	0.0	0.0	0.0	0.0
C13	0.1	0.0	0.0	0.0	0.0
C14	1.1	0.5	0.1	0.1	0.1
C15	0.3	0.2	0.1	0.0	0.0
C16	23.0	31.2	24.2	12.3	14.0
C17	0.9	0.8	0.1	0.2	0.1
C18	15.1	4.9	5.3	1.8	2.0
C20	0.0	1.0	1.1	0.3	0.0
C21	0.1	0.0	0.0	0.0	0.1
ΣSFA	**41.5**	**38.5**	**30.8**	**14.7**	**16.3**
C14:1	0.0	0.0	0.0	0.0	0.0
C15:1	0.4	0.0	0.0	0.0	0.0
C16:1	1.3	0.2	0.6	0.4	0.0
C17:1	4.3	0.0	0.0	0.1	0.0
C18:1 (Cis)	10.9	24.8	40.1	51.2	39.8
C20:1	0.0	0.0	0.5	0.5	0.0
ΣMUFA	**16.9**	**25.0**	**41.3**	**52.2**	**39.8**
C18:2	0.0	0.0	0.0	2.1	0.0
C18:2	8.7	15.0	23.6	28.7	42.9
C20:2	0.0	0.0	0.0	0.0	0.0
C18:3	32.9	21.5	4.3	2.4	1.0
ΣPUFA	**41.6**	**36.5**	**27.9**	**33.2**	**44.0**

Table 6
Nutritional content of control porridges and mopane worm fortified porridge based on a 50 g dry weight portion size. * Indicates 50 % recommended daily allowance (RDA) meet/exceeded (based on a 20 kg child).

	Protein (g)	Fe (mg)	Zn (mg)
White maize control	4.0	0.9	1.1
Sorghum control	4.4	2.0	2.4
Pearl millet control	4.3	10.1*	2.0
Sorghum (1:3.5 mopane worm fortified)	9.4*	2.7	3.4
Pearl millet (1:3.5 mopane worm fortified)	9.3*	8.7*	3.1
50 % RDA		5	4

For boiled and dried mopane worm a protein correction factor of 5.36 was determined. Using the mopane correction factor of 5.36 the protein content for boiled & dried mopane worm was 47.3 ± 3.1 respectively, considerably lower (~8%) than using the standard Jones' correction factor of 6.25. The calculated factor was higher than the 4.76 reported by Janssen et al (2017), for larvae from *Tenebrio molitor* (mealworm), Alphitobius diaperinus (lesser mealworm), and *Hermetia illucens* (black soldier fly) and lower than the 5.60 reported by Boulous et al. (2020) for *Tenebrio molitor* (mealworm), *Acheta domesticus* (house cricket) and *Locusta migratoria* (locust). Use of Jones' correction factor leads to an overestimation of the protein content. The differences in the conversion factors for different insect species necessitates the calculation of the new factor for mopane worm. Considering, mopane worm being a commonly consumed edible insect in Southern Africa, the protein content can be correctly estimated. In addition, the factor could be used to calculate protein content of closely related caterpillars in Lepidoptera order, like *Cirina forda* commonly consumed in West Africa.

3.4. Fatty acid profile quantification by GC–MS of mopane worm and grains

The FAMEs profile for the raw ingredients and mopane worm are reported in Table 5. Mopane worm is a good source of fat (21.4 %), of which about 41 % are saturated, and about 41 % are polyunsaturated fatty acids (PUFA), which are essential fatty acids, due to the requirement to obtain from dietary sources as humans cannot synthesize them.

3.5. Fortified porridge development

Based on the nutritional profile of the raw materials (section 3.1) and the results of a cross section survey carried out in Gwanda district of Zimbabwe (Manditsera, et al., 2022), two fortified porridges were developed, based on pearl millet and sorghum as base porridges. The nutritional profile of the control and fortified porridge are shown in Table 6. Mopane worm fortified pearl millet and sorghum porridges were able to meet the 50 % recommended protein, for a child weighing 20 kg, based on a 50 g dry basis portion. In addition, mopane worm fortified pearl millet fortified porridge was able to meet the requirements of iron, hence the formulation was selected for further analysis.

3.6. TDS consumer preference study

The TDS output for the six porridges, for the UK and Zimbabwe (Fig. 1) shows the control and inclusion of mopane worm at a ratios of 3.5 and 4 to 1 for both pearl millet and sorghum as a base porridge. For each porridge the P_0 (chance level, below this level attribute selection is attributed to probability) and P_s (significance level, above this level, the attribute selection is considered significant) are shown.

There were clear cultural differences between the TDS. For all the porridges the UK panellists' sensation to bitter and gritty was greater than Zimbabwe panellists. Additionally, the sensation was identified around 5 s earlier.

For Zimbabwe panellists, both controls had a dominant attribute of smooth, compared to earthy for pearl millet for UK panellists, indicating a cultural acceptance of the basic porridge. For Zimbabwe panellists the inclusion of mopane worm changed the dominant attribute to bitter and earthy.

For the UK panellists, earthy dominated all samples, except the sorghum control for which only a slight stickiness was dominant. For the samples fortified with mopane worm the earthy attribute is identified earlier than for the control by several seconds. For all mopane worm fortified porridges, a bitter sensation is identified at 15 s. The higher the inclusion rate of mopane worm the higher the dominance of earthy and bitter as attributes.

There are clear cultural differences identified by the TDS analysis between Zimbabwe and the UK, this is most pronounced for the control porridges. The porridge prepared is traditionally eaten in Zimbabwe but is not a known foodstuffs in the UK. This is identified by the smooth attribute being identified by Zimbabwe panellists whereas the dominant attribute for UK panellists is earthy.

The UK response to the pearl millet control porridge is earthy, this attribute is more significant and identified earlier with the inclusion of mopane worm (15 s for control, 10 s for mopane worm fortified porridge). As the mopane worm inclusion increases the dominance of both earthy and bitter attribute increases. For the UK panellists the sorghum control is blander, with sticky and later sweet being identified as slightly significant attributes. The blandness of the sorghum control leads to a reduction in the dominance of earthy and bitter attributes upon the inclusion of mopane worm compared to the pearl millet base.

For all panellists, the inclusion of vanilla and sugar mask the earthy and bitter attributes during the initial 15 s of the oral process, due to the size of the sucrose molecule, the sweetness is identified quickly and dissipates leading to the delay in the dominance of the bitter attribute.

TDS analysis is a useful tool allowing the measurement of consumer preferences in novel foods.

3.7. INFOGEST digestion and digestibility of fortified porridge

The bioaccessibility of protein and the micronutrients iron and zinc was determined for the mopane fortified porridge and the unfortified porridge as a comparison using the INFOGEST protocol. The % digestibility for protein and % bioaccessibility for Fe and Zn along with the available amount (in a 50 g dry weight portion), digested amount and the % increase uptake (fortified compared to unfortified) for protein, Fe and Zn are reported in Table 7.

The percentage protein digestibility for the fortified porridges is lower for both base grains, compared to the unfortified control, however, the difference in digestibility was not statistically significant. The protein digestibility was similar to that reported for mopane worm of 86 % by Kwiri et al. (2020). Additionally, it should be noted that when the protein content of the food serving is taken into account along with the percentage digestibility, the fortified porridge provides a good source of digestible protein, ~200 % compared to the unfortified porridge.

The uptake for iron increased for both base cereals, 112 and 164 % for sorghum and pearl millet respectively. For zinc the uptake was dependent on the base cereal, 83 % and 109 % for sorghum (a reduction) and pearl millet (an increase) respectively. This clearly demonstrates that the fortification of a porridge changes both the nutrients available and the bioaccessibility.

4. Conclusions

A fortified porridge has been developed based on the nutritional profile of the raw ingredients and nutritional guidelines for a healthy diet for a primary school aged child. The palatability of the fortified porridge was assessed by TDS, which demonstrated the inclusion of mopane worm (insect fortified), reduced the time to identify a dominant attribute. The base porridge is important to the overall sensation of the fortified product. The sorghum base, due to its blandness doesn't

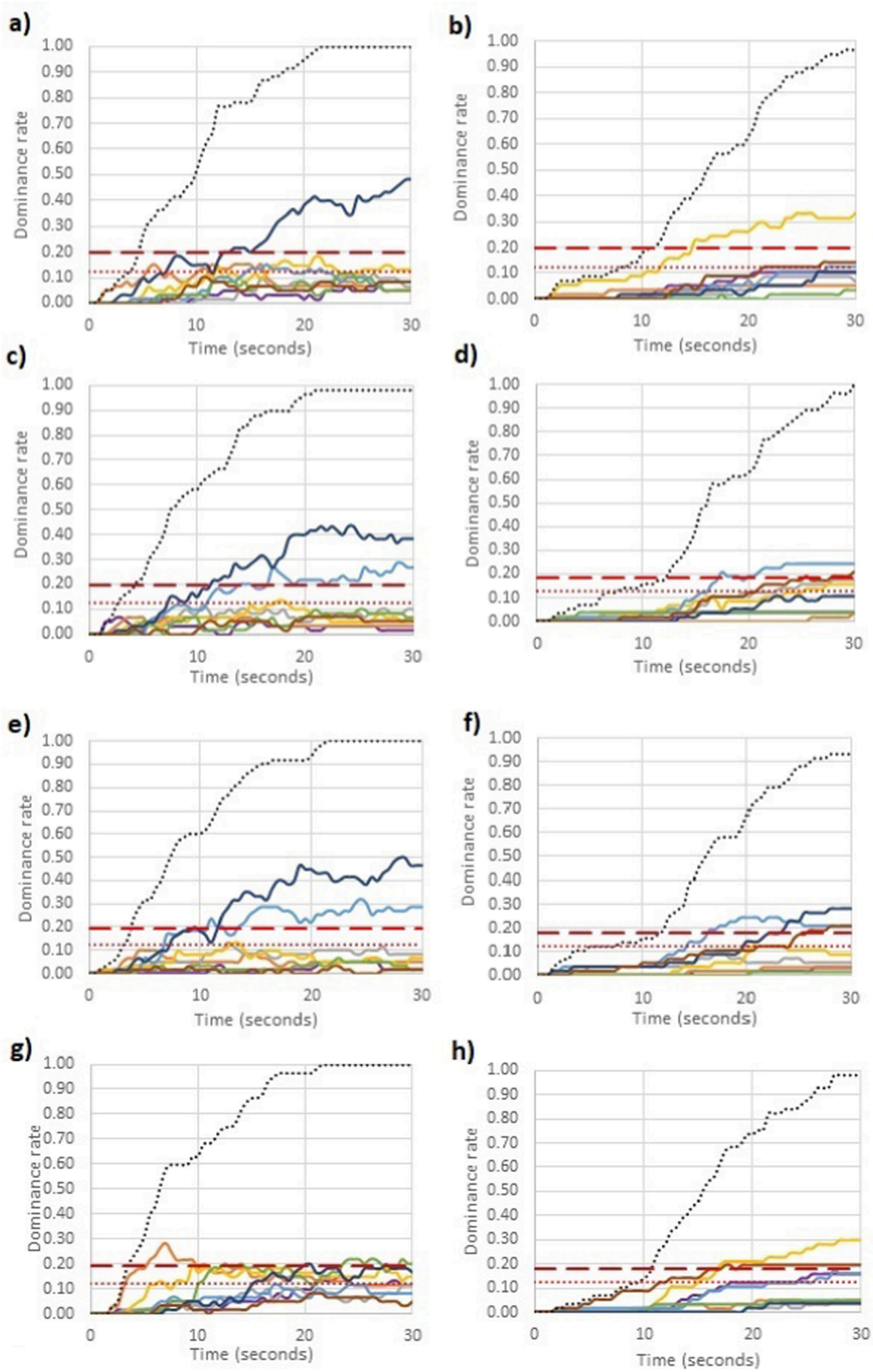

Fig. 1. TDS output for both UK and Zimbabwe consumers – (a) pearl millet control porridge UK, (b) pearl millet control porridge Zimbabwe, (c) 4:1 Mopane worm fortified pearl millet porridge UK, (d) 4:1 Mopane worm fortified pearl millet porridge Zimbabwe, (e) 3.5:1 Mopane worm fortified pearl millet porridge UK, (f) 3.5:1 Mopane worm fortified pearl millet porridge Zimbabwe, (g) sorghum control porridge UK, (h) sorghum control porridge Zimbabwe, (i) 4:1 Mopane worm sorghum porridge UK, (j) 4:1 Mopane worm fortified sorghum porridge Zimbabwe, (k) 3.5:1 Mopane worm fortified sorghum porridge UK), (l), 3.5:1 Mopane worm fortified sorghum porridge Zimbabwe. The P0 line indicates the rate an attribute can be selected by chance, Ps is a level of significance, below this the attribute is considered insignificant.

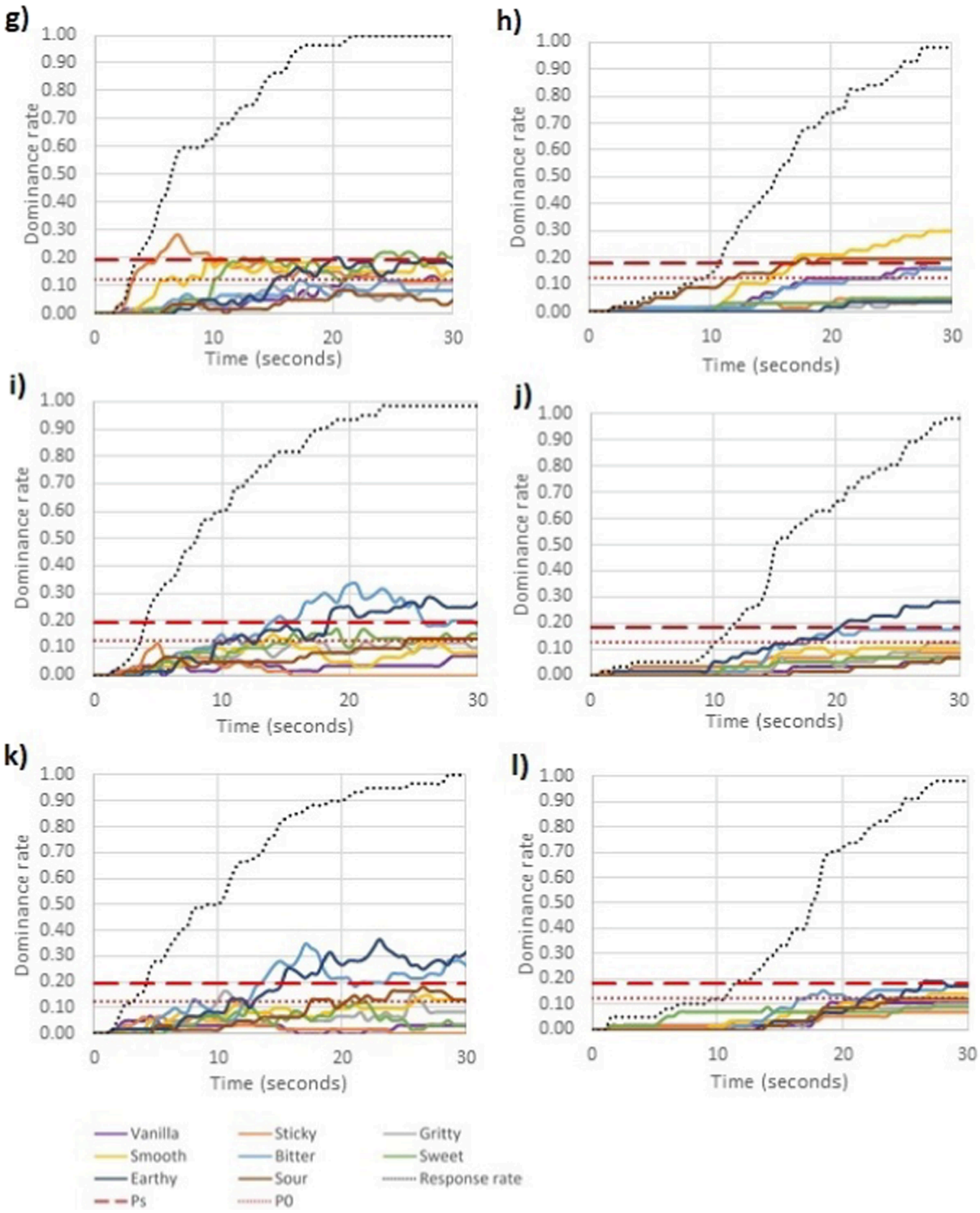

Fig. 1. (*continued*).

enhance the bitterness from the mopane worm for a high inclusion ratio.

Recommendations for further research include the assessment of the fortified porridge in a controlled feeding trial, establishing if the theoretical benefits of food-to-food fortification demonstrated here, are transferable to a population. The controlled feeding trial would additionally assess compliance, and therefore participant acceptability of the fortified porridge.

The INFOGEST digestion and subsequent assessment of bioaccessibility for Fe and Zn and digestibility of protein demonstrates the benefit of fortification of a base porridge. The nutritional content of the food is enhanced by fortification and bioaccessibility is not detrimentally reduced, due to the food-to-food fortification. The inclusion of mopane worm into porridge as a fortified food can provide a higher protein meal, contributing to reducing the triple burden of malnutrition.

Additionally, the nitrogen to protein correction factor has been calculated for mopane worm and is determined to be 5.36. This factor is considerably lower than the standard Jones' correction factor of 6.25, leading to an overestimation of the protein content of mopane worm.

Table 7
% Protein digestibility for mopane worm fortified and unfortified porridges, protein available from a 50 g dry weight serving and the protein digested.

	White maize control	Sorghum control	Pearl millet control	Sorghum (3.5 mopane worm fortified)	Pearl millet (3.5 mopane worm fortified)
% Protein Digestibility	59.4 ± 4.5	62.6 ± 0.7	62.9 ± 2.3	52.6 ± 0.6	66.2 ± 2.4
Protein available (g)	4.0	4.4	4.3	9.4	9.3
Protein digested (g)	2.4	2.7	2.7	5.0	6.2
% increase uptake				181 %	230 %
% Fe Digestibility	5.2 ± 0.8	4.8 ± 1.2	4.0 ± 1.4	5.8 ± 0.8	5.2 ± 0.6
Fe available (mg)	0.9	2.0	10.1	2.7	8.9
Fe digested (mg)	0.1	0.1	0.4	0.5	0.2
% increase uptake				112 %	164 %
% Zn Digestibility	3.9 ± 0.5	6.6 ± 5.5	4.9 ± 0.6	3.9 ± 1.7	3.5 ± 0.4
Zn available (mg)	1.1	2.4	2.0	3.4	3.1
Zn digested (mg)	0.	0.2	0.1	0.1	0.1
% increase uptake				83 %	109 %

Different letters in the same column indicate significant difference ($p < 0.05$). Results are expressed as mean ± SD for $n = 3$.

The current method overestimates the protein content of the Mopane worm by 8 % and we recommend that the lower protein correction factor be used going forward to provide more accurate nutritional profiles. This finding may have implications for government and NGO methodologies and policies relating to sources of sustainable nutrition in the relevant regions.

It is noted that this study covers only one seasons production for the raw materials (cereal grains, mopane worm and baobab), negating any seasonal variation or nutritional differences observed between "good" and "bad" years. A further study would consider multiple seasons for the ingredients, and regional variability in the insect nutritional properties.

5. Ethical statement for journal of functional food

Hereby, I Alberto Fiore consciously assure that for the manuscript "Development of nutritious mopane worm porridge for Urban and rural communities" the following is fulfilled:

(1) This material is the authors' own original work, which has not been previously published elsewhere.

(2) The paper is not currently being considered for publication elsewhere.

(3) The paper reflects the authors' own research and analysis in a truthful and complete manner.

(4) The paper properly credits the meaningful contributions of co-authors and co-researchers.

(5) The results are appropriately placed in the context of prior and existing research.

(6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.

(7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

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CRediT authorship contribution statement

Moira Ledbetter: . **Jonathon Desmond Wilkin:** . **Juliet Mubaiwa:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Faith Angeline Manditsera:** . **Lesley Macheka:** . **Faith Matiza Ruzengwe:** . **Obert Nobert Madimutsa:** . **Prosper Chopera:** . **Tonderayi Mathew Matsungo:** . **Sarah C Cottin:** Writing – review & editing, Resources. **Edryd Stephens:** . **Viren Ranawana:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Alberto Fiore:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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