Journal of Food Research; Vol. 12, No. 4; 2023
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Effect of Stinging Nettle Leaf Flour Substitution on the Quality Characteristics of Fermented Corn Complementary Foods

Mary Nkongho Tanyitiku[1,2] & Igor Casimir Njombissie Petcheu[3]

[1] Department of Wine, Food and Molecular Biosciences, Lincoln University, Lincoln, New Zealand

[2] Women Network for Biodiversity and Food Science, Yaounde, Cameroon

[3] Global Mapping and Environmental Monitoring, Yaounde, Cameroon

Correspondence: Mary Nkongho Tanyitiku, RFH Building Room 043, Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand. Tel: 64-22-123-6924. E-mail: mary.tanyitiku@lincolnuni.ac.nz

Received: August 16, 2023 Accepted: September 25, 2023 Online Published: September 27, 2023

doi:10.5539/jfr.v12n4p31 URL: https://doi.org/10.5539/jfr.v12n4p31

Abstract

Proper complementary feeding is required to provide essential nutrients to growing infants. However, most families in developing countries are severely food insecure, leading to constant child malnutrition. This study developed fermented corn complementary foods (pap) supplemented with abundantly available and affordable stinging nettles**.** Stinging nettle leaf flour was incorporated into pap at 5%, 10%, 15% and 20% and evaluated in relation to nutritional, functional, and sensory properties. Soybeans were used to compare the nutritional and health benefits of nettles in infant nutrition. A gradual incorporation of nettle leaf flour increased ($p<0.05$) the ash, protein, and dietary fibre content of pap from 0.72%, 3.48% and 2.87% to 9.46%, 18.98% and 4.56% respectively. Likewise, nettle-enriched pap contained higher carotenoids (4.99mg/100g), vitamin C (48.76mg/100g), calcium (176.49mg/100g), phosphorus (35.21mg/100g), potassium (210.54mg/100g), and iron (284.55mg/100g) than soybean-enriched pap: 0.96, 4.40, 50.99, 29.29, 204.78 and 64.02mg/100g respectively. While total phenolic content and antioxidant activity index increased ($p<0.05$) with increasing addition of nettle leaf flour from 1.23mgGAE/g and 0.15 to 125.45mgGAE/g and 2.01 respectively, the metabolic glycaemic response decreased ($p<0.05$) from 68.53% to 35.60%. In addition, all functional qualities were within acceptable limits for complementary feeding. Nursing mothers rated the overall acceptability between 7.14 (5% nettle-enriched pap) and 6.23 (20% nettle-enriched pap), and 12 of these 20 mothers accepted to feed their babies with stinging nettle leaf flour. Our findings indicated that stinging nettle leaves are nutritionally important for improving low-cost complementary feeding and thus could contribute to the combat of infant malnutrition in rural communities.

Keywords: malnutrition, fermented corn, complementary foods, pap, stinging nettle, nettle-enriched pap, soybean-enriched pap

1. Introduction

Stinging nettle (*Urtica dioica* L.) is an abundant, nutritious, and underused perennial herb native to temperate and tropical Asia, Europe, northern America, and Africa (Devkota et al., 2022; Pant & Sundriyal, 2016; Rutto et al., 2013; Tarasevičienė et al., 2023). Indeed, Adhikari et al. (2015) demonstrated that its leaves are nutritionally richer than wheat and barley containing higher crude protein (33.8%), fibre (9.1%), ash (16.2%), carotenoids (3497µg/g) and phenolic compounds (129mg Gallic acid equivalent/g). Although nettles are commonly considered as weeds in cultivated fields or mainly in traditional medicine (Devkota et al., 2022; Jaiswal & Lee, 2022), their use as food has been emphasised in previous research, such as in the production of egg pasta (Marchetti et al., 2018), homemade noodles (Alemayehu et al., 2016), biscuits (Palikhe, 2012) and bread (Đurović et al., 2020; Maietti et al., 2021; Wójcik et al., 2021). Specifically, its incorporation into complementary foods remains under-researched.

Proper complementary feeding (providing 25-50% protein, copper, and riboflavin, 50-75% thiamine, calcium, and manganese, and 75-100% phosphorus, zinc, and iron, among others), is essential to improve infant development, morbidity, and mortality (Abeshu et al., 2016; Benoist, 1999; Oladiran & Emmambux, 2020;

PAHO, 2003). In sub-Saharan Africa, complementary foods are prepared mainly using less nutritive local staples: cereals (rice, corn, sorghum, millet), roots or tubers (cassava, yam, potato) (Benoist, 1999; Oladiran & Emmambux, 2020; Treche, 1999). For example, pap, a popular thin gruel prepared locally from fermented corn seeds was found less suitable for complementary feeding given it is bulky, highly starchy and lack several essential nutrients, such as, lysine and tryptophan (Oladiran & Emmambux, 2020; Treche, 1999). Though improved nutritional guidelines have recommended the combination of corn with legumes and pulses (Codex Alimentarius, 2013), most rural families are severely food insecure, leading to constant infant malnutrition (The Lancet Planetary, 2020; UNICEF, (2021, December 12)). At present, malnutrition is the single largest killer of children under five years of age and stunting (too short for age) affects about 29 million children annually (Tanyitiku & Njombissie Petcheu, 2022; UNICEF, (2021, December 12)).

Consequently, there is an urgent need to develop low-cost high protein complementary foods using abundantly available and affordable nutritious plants such as stinging nettle. This study investigated the possibility of developing nutrient-rich fermented corn complementary foods supplemented with stinging nettle leaves. To achieve this objective:

Four pap formulations containing 5%, 10%, 15% and 20% nettle leaf flour were prepared, and the nutritional (proximate analysis, total phenolic content, antioxidant activity, the predicted in vitro digestion and metabolic glycaemic response), functional, and sensory characteristics were examined.

In addition, soybeans, an essential source of food and a suitable ingredient in complementary foods (Codex Alimentarius, 2013; WHO, 2009), were used to compare the nutritional and health benefits of stinging nettle.

2. Method

2.1 Plant Materials

Three plant materials; yellow corn (*Zea mays* L.), soybeans (*Glycine max*) and stinging nettles (*Urtica dioica* L.) were used. Yellow corn and soybeans seeds were obtained from the Institute of Agricultural Research for Development (IRAD), Yaounde, Cameroon. Stinging nettle leaves were harvested from three selected farms in Buea, located southwest of Cameroon, precisely at latitudes $4^0$12′N and longitudes $9^0$12′E. Buea is made up of tropical rainforest and stinging nettles are highly noticeable due to their stinging effects encountered on footpaths and arable land (Tanyitiku et al., 2022) (Note 1), especially during the rainy seasons (March to October) (Field observation, June 07-10, 2022). The consumption of nettles as food is unknown in the region.

2.2 Preparation of Complementary Foods

2.2.1 Processing of Stinging Nettle Leaves and Soybean into Flour

Stinging nettle leaf flour was obtained using a freeze drying technique as described by Chakravartula et al. (2021). Compared with other methods such as blanching, and convectional drying, freeze drying was found suitable due to its ability to retain the plant pigments as well as its magnesium, phenolic, and antioxidant contents (Chakravartula et al., 2021). Fresh stinging nettle leaves were harvested locally and randomly among 4-week-old leafy vegetables (Note 2) in September 2022. The leaves were placed in sterile polythene bags, transported to the laboratory in ice packs, and processed within one hour. Here, the harvested leaves were gently washed with distilled water, drained, and crushed using an immersion blender (Breville, Australia). The crushed leaves were frozen at -80^0C and freeze-dried at -48 to -55^0C for 28 h in a domestic freeze-dryer (Harvest Right, USA). It was then milled and sieved using an ASTM E11 400 µm stainless steel to obtained nettle leaf flour.

Toasted soybean flour was processed as described by Krička et al. (2003). 1.5kg of soybeans were sorted, washed, and soaked (1part of the seeds in 10 parts of deionised water) in a 2 litre Sistema container (Sistema NZ) for 24 h. The soaked seeds were manually dehulled to reduce and/or eliminate fibre and antinutrients (Codex Alimentarius, 2013). It was then oven-dried at 55-60^0C for 24 h and toasted at 135^0C for 15 min in a fan-assisted 60cm pyrolytic oven (Miele, Germany). The toasted seeds were cooled for 30 min, milled, and sieved to obtain toasted soybeans flour.

2.2.2 Pap Preparation

Pap was prepared as described by Baningo and Akpapunam (1999) with slight modification of the natural fermentation time. 1.5kg of yellow corn was manually sorted for soil and splits. Seeds were soaked as for soybeans above, drained and re-soaked to ferment at room temperature for another 48 h. Subsequently, the fermented seeds were drained and ground into a paste using a tabletop stone wet grinder (Ultra Grind^+ Gold, USA). The paste was pressed through a 0.5 µm stainless steel sieve to remove any coarse particles and then squeezed using a clean porcelain cloth to obtain a thicker cake (pap). It was then oven dried at 55-60^0C for 24 h, milled, and sieved using an ASTM E11 400 µm sieve.

To prepare the different complementary foods, pap (initially 350g) was weighed and gradually replaced with nettle leaf flour or soybean flour at 5%, 10%, 15% and 20%. For example, considering nettle-enriched pap: 5% nettle-enriched flour contained 332.5g of pap + 17.5g of nettle leaf flour; 10% nettle-enriched pap contained 315g of pap + 35g of nettle leaf flour; 15% nettle-enriched pap contained 297.5 of pap + 52.5g of nettle leaf flour and 20% nettle-enriched flour contained 280g of pap + 70g of nettle leaf flour. Each complementary food was mixed for 3 min using an electric mixer (Breville, Australia), set at speed 1, and stored at 4^0C in labelled airtight polyethylene bags for further analyses. 100% fermented corn flour (pap) served as a control in all experiments.

2.3 Chemical Analysis

2.3.1 Sample Extraction

Sample extraction was carried out according to Chakravartula et al. (2021) with slight modifications. 2g of each formulation was added to a 100ml volumetric flask containing 40ml of methanol/water (95:5v/v). In dark conditions, it was incubated in a shaker incubator (ThermoScientific, NZ) at 25^0C for 24 h and then filtered using a Whatman No. 4 filter paper. 5ml of each filtered solution was then centrifuged at 8000 g for 5 min and the supernatant (methanolic extract) was obtained for the determination of total phenolic content and antioxidant activity, described in 2.3.6 and 2.3.7 below.

2.3.2 Proximate Composition

The moisture, ash, crude protein, fat and total dietary fibre were determined according to AOAC (2000). Moisture content was measured by drying 3g of each sample at 105^0C for 24 h in a hot-air oven (Stanhope-Seta, UK). Ash content was obtained by calcinating 10g of each sample in a muffle furnace (Stanhope-Seta, UK) at 550^0C for 6 h. Calcinated samples were then used to determine the mineral content of each formulation. Total crude fat was extracted from 1.5g of the sample in a Soxhlet extractor (ThermoFisher Scientific, USA) using petroleum ether as solvent. Crude protein was estimated by multiplying the crude nitrogen content by a factor of 6.25, that is, % crude protein = % Nitrogen × 6.25. Crude nitrogen was obtained using the Kjeldahl method. Total dietary fibre was measured using Megazyme enzymes (Megazyme International Ireland Ltd., Wicklow, Ireland). The caloric values were obtained using the Atwater general factor system by multiplying the crude protein, fat, and carbohydrate values by their physiological fuel values of 4, 9, and 4 respectively. Carbohydrate content was obtained by difference, that is, % carbohydrate = 100 - % protein + %fat + %crude fibre + %ash + %moisture.

2.3.3 Mineral Composition

Na, K, Ca, Fe, Zn, P and Mg was measured using the atomic absorption spectrophotometry according to AOAC (1997) method 985.35. 5ml of 6N HCl solution was added to 5g of each calcinated sample mentioned above. It was dried on a hot plate followed by the addition of 7ml of 3N HCL solution. Distilled water was added dropwise to dilute the solution to the 50ml mark on the flask. Mg, Ca, and Fe were determined using air/acetylene as a source of flame energy for atomisation. A flame photometer was used to estimate Na and K concentrations at 589 nm and 767 nm, respectively. P was determined using the ammonium molybdovanadate colorimetric method. Each mineral content was obtained by comparing its concentration with known standards and then expressed in mg/100g of each formulation.

2.3.4 Total Carotenoids

Total carotenoids were measured spectrophotometrically according to Lichtenthaler and Buschmann (2001). Each formulation (1g) was mixed with 200mg of $MgCO_3$, 3ml of 100% acetone, and centrifuge at 8000g for 3 min. The absorbance of the clear supernatant was measured at 470 nm and expressed in mg/100g of each formulation.

2.3.5 Vitamin C

Vitamin C was measured as described by Rahman et al. (2006). In a coupling reaction involving 2,4-dinitrophenyl hydrazine (DNPH) dye and vitamin C, 10g of each sample were thoroughly mixed in 50 ml of 5% metaphosphoric acid/10% acetic acid solution. The solution was diluted to 100 ml in a volumetric flask and then filtered using a Whatman No. 4 filter paper. Few drops of bromine and 10% Thiourea solution (2,4-Dinitrophenyl-hydrazine solution and 85% Sulphuric acid) were added and the absorbance was read and expressed in mg/100g of each formulation.

2.3.6 Total Phenolic Content (TPC)

TPC was measured by colorimetry using Folin-Ciocalteau reagent (FC) as described by Kupina et al. (2018) with slight modifications. 1ml of each methanolic extract was mixed with 1ml of Folin & Ciocalteu's phenol reagent and allowed to sit for 6 min. To neutralise the mixture, 3.0ml of sodium carbonate (20% Na_2CO_3) solution was added followed by 15ml of water after 15 min. It was then heated on a heating block for 120 min, and the precipitate formed was removed by centrifugation at 4000 g for 5 min. TPC was determined by measuring the absorbance of each sample at 765 nm using gallic acid, ranging from 40-200mg/l gallic acid as standard. Results were expressed as mg of gallic acid equivalents (GAE) per gram of each formulation.

2.3.7 Antioxidant Activity (AA)

AA was estimated using a DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay according to Khare et al. (2012). 2ml of each 10mg/ml DPPH solution was mixed with 2ml of each methanolic extract and stored in the dark for 30 min. The free radical scavenging activity was measured spectrophotometrically at 520 nm using vitamin C as a standard. The AA of each extract was calculated using the following equation.

$$Antioxidant\ activity\ (\%) = 1 - \frac{Absorbance(sample)}{Absorbance(control)} x100$$

The concentration required to obtain a 50% antioxidant capacity termed IC_{50}, was determined from the graph of antioxidant capacity (%) against extract concentration (mg/g). Additionally, the antioxidant activity index (AAI) was calculated based on Scherer & Godoy (2009).

$$AAI = \frac{Final\ extract\ concentration(mg/g)}{IC50\ value(mg/g} x100$$

2.4 Assessing the Functional Properties of Each Formulation

Three function properties: bulk density, water absorption capacity (WAC), and swelling capacity were determined as described by Appiah et al. (2011). Dispersibility was measured based on Kulkarni et al. (1991). The bulk density (g/ml) was calculated by the ratio of the weight (g) of each flour to its volume (ml) when 50g of each sample was gently tapped to a constant volume in a 100ml graduated cylinder. WAC (g/g) was calculated as grams of water absorbed per gram of each sample. 1g of each sample was mixed with 10ml of distilled water in a preweighed tube. The paste-like suspension was vortexed for 2 min, allowed to stand at 28^0C for 30 min, and then centrifuged at 8000 g for 15 min. The clear supernatant was discarded and the centrifuge tube containing the paste was reweighed and recorded as the amount of water absorbed per gram of sample. The volume of water absorbed was multiplied by the density of the sample to convert into grams. For the swelling capacity, 1g of each sample was mixed with 10ml of distilled water and heated at 80^0C for 30 min in a shaking water bath (ThomasScientific, USA). The suspension was then centrifuged at 8000 g for 20 min, and the supernatant was decarded. The swelling capacity (g/g) was calculated as a ratio of the weight of the paste to the weight of each formulation. Finally, 10g of each formulation was vigorously stirred in distilled water placed in a 100ml measuring cylinder. The suspension was then allowed to settle for 3h. and dispersibility (%) was obtained by subtracting the volume of settled flour from 100.

2.5 Estimation of in vitro Digestion and Glycaemic Index (GI)

In vitro starch digestion and glycaemic index was estimated according to Goñi et al. (1997), slightly modified by Leoro et al. (2010), 50mg of each sample was boiled in 15ml of distilled water for 3 min, then 10ml of HCI-KCI buffer was added to adjust the pH to l.5. To initiate gastric digestion, 0.2ml of pepsin solution (1g of Pepsin in 10ml of HCI-KCI buffer) was added, incubated at 40^0C for 1 h in a shaker incubator (ThermoScientific, NZ) and completed to 25ml with Tris-Maleate buffer (pH=6.9). 5ml α-amylase in Tris-Maleate buffer (2.6 UI/ml) was then added and incubated at 37^0C in a shaker incubator (ThermoScientific, NZ). Subsequently, 1ml aliquots were taken at different times (0, 30, 60, 90, and 120 min) and placed in a tube at 100^0C. It was briefly vortex for 5 min to inactivate the enzyme and then refrigerated until the end of the incubation time. To hydrolyse the digested starch into glucose, 3ml of 0.4M sodium acetate buffer (pH=4.75) and 60µl of amyloglucosidase enzyme (3000 U/ml) was added at 60^0C. Glucose concentration was measured using the glucose oxidase-peroxidase (GOD-POD) enzymatic colorimetric kit (NZYTech, NZ). Hydrolysis curves were plotted and the areas under the hydrolysis curves (AUC, at 0-120 min) were calculated using a non-linear model described by Goñi et al. (1997). The rate of starch digestion was expressed as a percentage of total starch hydrolysed during these times (30, 60, 90, and 120 min). The Hydrolysis Index (HI) was calculated as the ratio of the AUC of each sample to the AUC of a reference sample (glucoses) and expressed as a percentage. Glycaemic index (GI) was then estimated according to Leoro et al. (2010) as:

$$GI = 39.71 + (0.549xHI)$$

2.6 Sensory Evaluation

Twenty-five (25) breastfeeding mothers (aged 25 to 38 years) were recruited during routine infant immunisation and semi-trained to serve as sensory panelists. Each formulation was coded (N5, N10, N15, N20, S5, S10, S15, and S20) and prepared according to the nutritional guidelines of the World Food Programme for processed foods aged 6-23 months (WFP, 2018). A 'rule of thumb' was used where 50g of each formula was cooked in 250ml of boiling water for 3 min. The cooked gruels, corresponding to 0.8kcal/g of energy (WFP, 2018), were allowed to cool and served warm to the individual panelists. A nine-point hedonic scale ranging from 1 = Dislike extremely to 9 = Like extremely was used and the gruels were rated for colour, taste, flavour, smoothness, and overall acceptability. In addition, breastfeeding mothers were served with 100% nettle leaf flour (coded N) and using yes or no responses, they were asked if they would feed their babies with the ingredient (N) if revealed to them.

2.7 Statistical Analysis

All experiments were carried out in triplicate and are expressed as mean ± standard deviation. Using the Statistical Package for Social Sciences (SPSS, Version 21), analysis of variance (ANOVA) was used to test the differences in means between samples, and Duncan's multiple range test was used for mean separation at the probability level of 5%.

3. Results and Discussion

3.1 Chemical Composition

Eight complementary food formulations: four nettle-enriched pap (5%, 10%, 15%, 20%), four soybean-enriched pap (5%, 10%, 15%, 20%) and a control (pap) were analysed and compared (Table 1).

Moisture content for nettle-enriched pap ranged from 2.89 to 3.55%. Apart from 15% nettle-enriched pap (2.89%) and 5% soybean-enriched pap (4.91%) that were significantly ($p<0.05$) different, the moisture contents were significantly ($p<0.05$) not different. All values were <10% indicating all flours could have a good shelf life and stability during long-term storage (Tanyitiku & Njombissie Petcheu, 2022).

Ash, protein, and dietary fibre of nettle-enriched pap significantly ($p<0.05$) increased from 0.72%, 3.48% and 2.87% to 9.46%, 18.98% and 4.56% respectively. The highest ash content (9.46%) was obtained with 20% nettle-enriched pap and was nearly nine times higher than the control (0.72%) and 5% soybean-enriched pap (0.45%). Maietti et al. (2021) reported a similar increase in the ash content from 1.28g/100g (white bread) to 1.75g/100g (nettle-enriched bread). Likewise, Krawęcka et al. (2021) recorded an increase from 1.00g/100g (semolina durum pasta) to 2.04g/100g (nettle-enriched pasta). Ash content is the residue obtained after complete calcination of all combustible organic matter in plants. This research results indicated that nettle-enriched pap could be richer in minerals than soybean-enriched pap. Protein content for nettle-enriched pap ranged from 14.78 to 18.98% and it was significantly ($p<0.05$) higher than the control (3.48%) and soybean-enriched pap (12.50 to 13.95%). Adhikari et al. (2015) reported a high protein content of 33.77% in 100% nettle leaf flour, which could explain why a significant ($p<0.05$) increase in protein content was observed when nettle leaf flour was gradually incorporated into pap. Furthermore, it was observed that the dietary fibre for nettle-enriched pap gradually increased from 2.45 (5% nettle-enriched pap) to 4.56% (20% nettle-enriched pap) as soybean-enriched pap decreased from 2.22 (5% soybean-enriched pap) to 1.88% (20% soybean-enriched pap). A similar increase (5.10 to 8.82g/100g) was reported when nettle flour was gradually added to durum wheat pasta (Krawęcka et al., 2021). Krawęcka et al. (2021) further highlighted that nettle leaves are very rich sources of fibre (that is, 43.22±6.16g/100g) – a fibre content that is hugely greater than our own previously analysed toasted soybean flour (crude fibre - 1%) (Tanyitiku & Njombissie Petcheu, 2022). However, all dietary fibre values were within acceptable limits (that is, ≤5g/100g) for complementary foods (Codex Alimentarius, 2013) and this value range could improve the digestive function of infants.

On the other hand, the fat, carbohydrate, and energy values of nettle-enriched pap significantly ($p<0.05$) decreased with increasing incorporation of nettle leaf flour (Table 1). Fat content ranged from 3.32 to 3.98% and was significantly ($p<0.05$) not different from the control (3.50%), but lower ($p<0.05$) than soybean-enriched pap (6.45 to 10.46%). Maietti et al. (2021) reported a similar lower fat content of 2.13g/100g in nettle-enriched bread. Fat is a source of energy and fatty acids are essential in the diets of growing infants. Its recommended intake in complementary foods increases from 0% at 6-8 months to 5-8% at 9-11 months, and then 15-20% at 12-23 months (Abeshu et al., 2016). The presence of fat in this research formulated diets could meet the energy needs of a growing breast-fed child with several proportions consumed per day. Carbohydrates are responsible for the bulk of calories in infant foods. In this research, its value significantly ($p<0.05$) decreased from the control (87.87%) and ranged from 71.45 (5% nettle-enriched pap) to 57.01% (20% nettle enriched pap) in nettle-enriched pap. Soybean-enriched pap contained higher ($p<0.05$) values ranging between 74.10 (5% soybean-enriched pap) and 70.02% (20% soybean-enriched pap). Krawęcka et al. (2021) reported a similar decreased (78.40 to 72.34g/100g) in nettle-enriched durum wheat pasta. Yellow corn seeds are rich sources (69.659 to 74.549%) of carbohydrates (Ullah et al., 2010), and this could have contributed to the high carbohydrate content in the nettle and soybean formulations. Likewise, energy values significantly ($p<0.05$) decreased from 373.18kcal/100g (5% nettle-enriched pap) to 333.87kcal/100g (20% nettle-enriched pap). These values were significantly ($p<0.05$) lower than the control (397.56kcal/100g) as well as soybean-enriched pap (405.32 to 422.88kcal/100g). Nonetheless, these energy values indicated that our nettle formulations could be suitable for complementary feeding especially in developing countries where the energy required from complementary foods is about 200kcal/day at 6-8 months, 300kcal/day at 9-11 months, and 550kcal/day at 12-23 months (PAHO, 2003).

The macroelements (sodium, potassium, calcium, magnesium, phosphorus) and microelements (zinc, and iron) significantly ($p<0.05$) increased with gradual incorporation of nettle leaf flour (Table 2). In fact, in 20% nettle-enriched pap, Ca (176.49mg/100g), Mg (154.57mg/100g), K (210.54mg/100g) and Fe (284.55mg/100g) were more than 100 times higher than the control of 13.78, 26.78, 15.23 and 10.50mg/100g respectively. Furthermore, P, Ca, K, and Fe of the nettle-enriched pap were significantly ($p<0.05$) higher at 35.21, 176.49, 210.54 and 284.55mg/100g than the soybean-enriched pap (29.29, 50.99, 204.78 and 64.02mg/g) respectively. Krawęcka et al. (2021) recorded a similar high calcium content ranging from 73.77 to 175.89mg/100g but a lower iron content (2.83 to 3.23mg/100g) in nettle-enriched durum wheat pasta. Specifically, 20% soybean-enriched pap was higher in Na (149.54mg/100g), Mg (349.86mg/100g) and Zn (26.23mg/100g) than 20% nettle-enriched pap: 102.54, 154.57 and 18.47mg/100g respectively. Maietti et al. (2021) reported a decrease in Zn content from 7.26 (white bread) to 7.00μg/g (nettle-enriched bread). These improved mineral content results strongly confirmed that the blending of cereals and legumes is nutritionally essential during complementary feeding (Codex Alimentarius, 2013).

Total carotenoids were significantly ($p<0.05$) higher in nettle-enriched pap (3.97 to 4.99mg/g) than in the control (0.18mg/g) and soybean-enriched pap (0.19 to 0.96mg/g). Apart from 15- and 20% nettle-enriched pap that were significantly ($p<0.05$) not different from each other, all formulations were significantly ($p<0.05$) different (Table 2). Krawęcka et al. (2021) recorded lower carotenoids values (2.52 and 13.35μg/g) in nettle-enriched durum wheat pasta. However, Adhikari et al. (2015) reported high total carotenoids values (3496.67μg/100g) in 100% nettle leaf flour, which might have contributed to carotenoids increase in nettle-enriched pap. An important carotenoid in nettle is β carotene which could function as a potent antioxidant and dietary factor in infant growth when nettle-enriched pap is consumed. Also, carotenoids are precursors of vitamin A. Vitamin A deficiency is a major public health problem that affects infant vision and the formation of healthy skin and hairs (UNICEF, (2021, December 12)). As such, the introduction of carotenoids-rich nettles in complementary feeding could help combat such public health concerns.

Finally, vitamin C in nettle-enriched pap ranged from 42.45 to 48.76mg/g and this was significantly ($p<0.05$) higher than the control (1.28mg/100g) and soybean-enriched pap (1.20 to 4.40mg/100g) (Table 2). Although 10- and 15% nettle-enriched pap were significantly ($p<0.05$) not different, 5- and 20% nettle-enriched pap were significantly ($p<0.05$) different. With a reference nutrient recommendation of 30mg/100g of vitamin C to be added to complementary foods (Codex Alimentarius, 2013), the presence of high vitamin C (42.45-48.76mg/100g) in nettle-enriched pap could play several roles, such as in improving iron absorption, and in the formation of infant collagen, bones, and teeth.

Table 1. Proximate composition of complementary foods

		Moisture (%)	**Ash (%)**	**Protein (%)**	**Fat (%)**	**Dietary fibre (%)**	**Carbohydrate (%)**	**Energy (kcal/100g)**
Pap (control)		3.89 ± 0.26^{a}	0.72 ± 0.04^{a}	3.48 ± 0.08^{a}	3.50 ± 0.55^{a}	2.07 ± 0.04^{a}	87.78 ± 0.02^{a}	397.56 ± 0.08^{a}
Nettle-enriched pap	5%	3.55 ± 0.22^{a}	5.68 ± 0.02^{b}	14.78 ± 0.84^{b}	3.32 ± 0.10^{a}	2.45 ± 0.02^{a}	71.45 ± 0.10^{b}	373.18 ± 0.08^{b}
	10%	3.45 ± 0.78^{a}	8.35 ± 0.08^{c}	15.02 ± 0.33^{bc}	3.85 ± 0.02^{a}	3.32 ± 0.54^{b}	67.08 ± 0.04^{c}	361.77 ± 0.55^{c}
	15%	2.89 ± 0.20^{b}	7.67 ± 1.26^{cd}	14.89 ± 0.88^{bc}	3.02 ± 0.22^{a}	3.89 ± 0.71^{b}	63.98 ± 0.41^{d}	355.61 ± 0.27^{d}
	20%	3.01 ± 0.11^{ab}	9.46 ± 1.01^{de}	18.98 ± 0.54^{d}	3.98 ± 0.40^{a}	4.56 ± 0.04^{c}	57.01 ± 0.08^{e}	333.87 ± 1.24^{e}
Soybean-enriched pap	5%	4.91 ± 0.25^{c}	0.45 ± 0.33^{a}	12.50 ± 0.45^{e}	6.45 ± 0.98^{cd}	2.22 ± 0.26^{a}	74.10 ± 0.22^{f}	405.32 ± 0.77^{fg}
	10%	3.97 ± 0.78^{ac}	1.32 ± 0.08^{f}	12.65 ± 0.56^{e}	6.89 ± 1.25^{cd}	2.01 ± 0.20^{a}	74.25 ± 0.18^{f}	404.10 ± 0.04^{hg}
	15%	3.22 ± 0.06^{a}	1.55 ± 0.63^{f}	15.89 ± 0.98^{c}	8.04 ± 1.20^{ef}	1.88 ± 0.02^{d}	68.88 ± 0.55^{g}	415.63 ± 0.19^{i}
	20%	4.01 ± 0.28^{ac}	1.87 ± 0.44^{f}	13.95 ± 0.79^{ef}	10.46 ± 0.68^{fg}	1.99 ± 0.24^{d}	70.02 ± 0.10^{h}	422.88 ± 0.58^{j}

Note: Values are expressed as mean ± standard deviation of three replications. Means with different superscripts within the same column are significantly different at $p < 0.05$.

Table 2. Carotenoids, vitamin C and some mineral composition of complementary foods

Component (mg/100g)		**Total carotenoids**	**Vitamin C**	**Sodium**	**Phosphorus**	**Calcium**	**Magnesium**	**Potassium**	**Zinc**	**Iron**
Pap (control)		0.18 ± 0.54^{a}	1.28 ± 0.65^{a}	22.35 ± 1.08^{ab}	16.21 ± 0.50^{a}	13.78 ± 0.88^{a}	26.78 ± 0.38^{a}	15.23 ± 0.01^{a}	2.45 ± 0.30^{a}	10.50 ± 0.53^{a}
Nettle-enriched pap	5%	3.97 ± 0.11^{b}	42.45 ± 0.27^{b}	23.46 ± 1.04^{ab}	28.54 ± 0.66^{bi}	160.55 ± 0.08^{b}	140.64 ± 0.51^{b}	196.24 ± 0.30^{b}	15.89 ± 0.22^{b}	271.24 ± 0.98^{b}
	10%	3.00 ± 0.24^{c}	45.54 ± 0.08^{cd}	44.21 ± 0.01^{c}	28.65 ± 0.72^{bi}	168.41 ± 0.64^{c}	142.23 ± 1.28^{c}	195.87 ± 0.45^{b}	15.97 ± 0.08^{b}	273.88 ± 0.30^{c}
	15%	4.23 ± 0.55^{df}	45.64 ± 0.64^{cd}	86.00 ± 0.11^{d}	30.23 ± 0.72^{cj}	172.47 ± 0.99^{d}	142.87 ± 0.53^{c}	206.89 ± 0.20^{c}	17.64 ± 0.40^{c}	279.79 ± 0.04^{d}
	20%	4.99 ± 0.021^{ef}	48.76 ± 0.42^{e}	102.54 ± 0.31^{e}	35.21 ± 0.01^{d}	176.49 ± 0.57^{e}	154.57 ± 0.47^{d}	210.54 ± 0.99^{d}	18.47 ± 0.01^{c}	284.55 ± 0.08^{e}
Soybean-enriched pap	5%	0.19 ± 0.08^{g}	1.20 ± 0.02^{fg}	114.24 ± 0.88^{gf}	18.25 ± 0.49^{ek}	43.12 ± 0.21^{f}	335.42 ± 0.02^{e}	168.57 ± 0.54^{e}	19.24 ± 0.32^{e}	59.45 ± 0.86^{f}
	10%	0.54 ± 0.04^{h}	1.59 ± 1.00^{gh}	115.23 ± 0.65^{hf}	19.41 ± 0.54^{fk}	44.01 ± 0.02^{g}	336.87 ± 0.08^{f}	176.01 ± 0.45^{f}	25.56 ± 0.15^{fd}	63.27 ± 0.72^{g}
	15%	0.87 ± 0.28^{i}	3.45 ± 0.48^{ik}	123.41 ± 0.51^{i}	24.00 ± 0.01^{g}	46.78 ± 0.45^{h}	338.54 ± 0.75^{g}	176.87 ± 0.64^{f}	26.78 ± 0.25^{gd}	63.89 ± 0.50^{g}
	20%	0.96 ± 0.57^{j}	4.40 ± 0.60^{jk}	149.54 ± 0.08^{j}	29.29 ± 0.47^{hj}	50.99 ± 0.24^{i}	349.86 ± 0.19^{h}	204.78 ± 0.25^{g}	26.23 ± 0.04^{g}	64.02 ± 0.37^{h}

Note: Values are expressed as mean ± standard deviation of three replications. Means with different superscripts within the same column are significantly different at $p < 0.05$.

3.2 Functional Composition

The results of the bulk density, swelling capacity, water absorption capacity and dispersibility of the complementary foods are presented in Table 3. Bulk density of nettle-enriched pap was within 0.51 to 0.53g/ml, the control was 0.52g/ml and soybean-enriched pap ranged from 0.50 to 0.53g/ml. The bulk density indicates the particle size of a flour and measures the weight per unit volume occupied by a given product. The smaller the particle size, the more surface area is available for water absorption (Kulkarni et al., 1991). Also, the higher the fibre content, the bulkier the product and the higher the protein content, the lesser the bulk density (Codex Alimentarius, 2013). This research nettle-enriched pap had a low bulk density that could be attributed to its high protein and low fibre contents, and this was found suitable for complementary foods (Appiah et al., 2011).

The water absorption capacity of nettle-enriched pap was significantly ($p<0.05$) different and was within the same range (1.12-1.54g/g) as the control (1.14g/g) and soybean-enriched pap (1.00-1.89g/g). Infant formulations with low water absorption are desirable for thin gruels formed during reconstitution or cooking. This differences in water absorption could be mainly due to slight increases in soluble sugars and hydrophilic proteins that enabled the binding of water and fats in the flours (Appiah et al., 2011; Tanyitiku & Njombissie Petcheu, 2022).

The swelling capacity was highest (0.96g/g) in 20% nettle-enriched pap and lowest (0.89g/g) in 10% nettle-enriched pap. 20% nettle-enriched pap and 5% soybean-enriched pap were not significantly ($p<0.05$) different and were higher than the control (0.94g/g). Swelling capacity is the ability of flours to absorb moisture from their surroundings, and this research varying results could be linked to the molecular structure of the formulation's starch granules.

Furthermore, the dispersibility of nettle-enriched pap was high between 72.13% (15% nettle-enriched pap) and 79.65% (5% nettle-enriched pap) and was not different ($p<0.05$) from the control (79.56%) and soybean-enriched pap (74.25 to 79.50%). Kulkarni et al. (1991) reported a similar acceptable dispersibility value of 63 to 79% in weaning food formulations produced from malted sorghum and sesame flour. Dispersibility indicates the ease of reconstitution when a flour is mixed with water (Tanyitiku & Njombissie Petcheu, 2022), thus, the results showed that nettle-enriched pap could be easily cooked into gruels

Table 3. Functional Properties of Complementary Foods

	Formulations	**Bulk density (g/ml)**	**Water absorption capacity (g/g)**	**Swelling capacity (g/g)**	**Dispersibility (%)**
Pap (control)		0.52 ± 0.10^{a}	1.14 ± 0.01^{a}	0.94 ± 0.00^{f}	79.56 ± 0.01^{a}
Pap enriched with nettle	5%	$079..52\pm0.31^{a}$	1.32 ± 0.02^{b}	0.97 ± 0.01^{a}	79.65 ± 0.08^{a}
	10%	0.51 ± 0.01^{b}	1.54 ± 0.02^{c}	0.89 ± 0.08^{b}	77.60 ± 0.01^{b}
	15%	0.51 ± 0.08^{b}	1.33 ± 0.01^{d}	0.92 ± 0.01^{c}	72.13 ± 0.08^{c}
	20%	0.53 ± 0.01^{c}	1.12 ± 0.08^{e}	0.96 ± 0.00^{d}	79.25 ± 0.02^{a}
Pap enriched with soybeans	5%	0.50 ± 0.52^{d}	1.56 ± 0.01^{f}	0.96 ± 0.02^{d}	75.12 ± 0.02^{d}
	10%	0.51 ± 0.11^{b}	1.89 ± 0.04^{g}	0.91 ± 0.08^{e}	79.50 ± 0.04^{a}
	15%	0.50 ± 0.60^{d}	1.00 ± 0.02^{h}	0.94 ± 0.04^{f}	78.36 ± 0.01^{e}
	20%	0.53 ± 0.04^{c}	1.12 ± 0.02^{e}	0.90 ± 0.02^{g}	74.25 ± 0.08^{f}

Values are mean ± standard deviation of triplicate. Means with different superscripts in the same column are significantly different ($p<0.05$)

3.3 Nettle leaf flour increased TPC and Antioxidant activity of pap

TPC values of nettle-enriched pap were significantly ($p<0.05$) different and increased from 51.78mgGAE/g to 125.45mg GAE/g (Table 4). These results were significantly ($p<0.05$) doubled compared to soybean-enriched pap: 27.94mgGAE/g (5% soybean-enriched pap) to 58.12mgGAE/g (20% soybean-enriched pap). In particular, 15% nettle-enriched pap (102.09mgGAE/g) and 20% nettle-enriched pap (125.45mgGAE/g) were 100 times higher than the control (1.23mgGAE/g). Kukrić et al. (2012) recorded a higher TPC value (208.37±4.39mgGAE/g) for nettles and further highlighted that environmental, climatic, or geographic factors may influence the quality and the quantity of phenolic components in nettles. This research TPC values indicated that stinging nettles harvested in the tropical rainforest belt (Cameroon) are good sources of phenolic compounds and could provide various biological functions, including antioxidant and antimicrobial activities in infants.

Furthermore, antioxidant activity of plants is strongly related to the presence of polyphenols and other secondary metabolites (Đurović et al., 2020). DPPH scavenging free radicals was expressed in IC_{50} values and antioxidant activity index (AAI) (Table 4). IC_{50} values for nettle-enriched pap were significantly ($p<0.05$) different and increased from 86.12mg/g (5% nettle-enriched pap) to 135.78mg/g (20% nettle-enriched pap). These values were significantly ($p<0.05$) higher than the control (2.80mg/g) and soybean-enriched pap (62.50 to 73.52mg/g). Specifically, 15- and 20% nettle-enriched pap were 100-fold higher than the control (2.80mg/g). Himalian and Singh (2021) demonstrated that stinging nettles have higher IC_{50} values (frozen nettles-2555.023mg/ml, fresh nettles-967440.9mg/ml) than fennel crush seeds (0.922mg/ml), fenugreek whole seeds (2.055mg/ml) and the peels of pomegranate (0.111mg/ml) papaya (2.159mg/ml). AAI ranged from 0.89 to 2.01 for nettle-enriched pap and were significantly ($p<0.05$) higher than the control (0.15) and soybean-enriched pap (0.15 to 0.20). Kukrić et al. (2012) recorded lower IC_{50} and AAI values of 31.38±0.102μg/g and 0.85±0.003 respectively. Unlike IC_{50} values that may vary depending on the initial concentration of DPPH, AAI remains unchanged (Scherer & Godoy, 2009). According to Kukrić et al. (2012) and Scherer & Godoy, (2009), antioxidants are classified as weak, when AAI < 0.5, moderate, when AAI between 0.5-1.0, strong, when AAI between 1.0-2.0, and very strong, when AAI >2. Therefore, this research AAI values indicated that the gradual incorporation of nettles into pap led to an increase from weak antioxidants (control, AAI=0.15) to moderate (5% nettle-enriched pap, AAI=0.89) to strong (10- and 15% nettle-enriched pap) and then to very strong antioxidants (20% nettle-enriched pap, AAI=2.01).

Table 4. The TPC values and antioxidant activity of complementary foods

		TPC (mgGAE/g)	**Antioxidant activity**	
			IC_{50} (mg/g)	**AAI**
Pap (control)		1.23 ± 0.02^{a}	2.80 ± 0.04^{a}	0.15 ± 0.01^{a}
Nettle-enriched pap	5%	51.78 ± 0.01^{b}	86.12 ± 0.04^{b}	0.89 ± 0.04^{b}
	10%	98.89 ± 0.02^{c}	98.54 ± 0.02^{c}	1.23 ± 0.08^{c}
	15%	102.09 ± 0.00^{d}	102.23 ± 0.01^{d}	1.87 ± 0.00^{d}
	20%	125.45 ± 0.04^{e}	135.78 ± 0.02^{e}	2.01 ± 0.02^{e}
Soybean-enriched pap	5%	27.94 ± 0.08^{f}	62.50 ± 0.08^{f}	0.15 ± 0.04^{f}
	10%	32.51 ± 0.00^{g}	73.41 ± 0.01^{g}	0.18 ± 0.01^{g}
	15%	45.68 ± 0.01^{h}	72.92 ± 0.02^{h}	0.20 ± 0.01^{h}
	20%	58.12 ± 0.02^{i}	73.52 ± 0.00^{g}	0.19 ± 0.04^{af}

Values are mean ± standard deviation of triplicate. Means with different superscripts in the same column are significantly different ($p<0.05$)

3.4 Nettle Leaf Flour Decreased Metabolic Glycaemic Response of Pap

The rate of starch hydrolysis during 120-min in vitro digestion of the complementary foods are presented in Figure 1. From the hydrolysis curves, nettle-enriched formulations had slightly lower hydrolysis values than the control and soybean-enriched pap. A sharp increase was observed during the first 30 minutes of digestion in all formulations. This slightly increased to 60 min and then slowly flattened at 120 min. Leoro et al. (2010) observed similar starch hydrolysis trends when organic passion fruit fibre was added to corn flour.

In Table 5, reducing the quantity of pap in each formulation following the gradual incorporation of nettle leaf flour or soybean flour significantly ($p<0.05$) decreased the hydrolysis of starch into sugars, and subsequently, the metabolic glycaemic response. For the nettle-enriched pap, the highest GI value (48.12) was obtained in 5% nettle-enriched pap and the GI of 20% nettle-enriched pap (35.60) was approximately 30-times lower than the control (68.53) and four-times lower than 20% soybean-enriched pap (40.24). Kraw ęcka et al. (2021) recorded lower GI values of 49.64, 49.38, and 49.31% when durum wheat pasta was enriched with 1, 2, and 3% stinging nettle leaves. Likewise, Leoro et al. (2010) reported GI values of 47.93 to 49.74% in passion fruit fibre-enriched corn flour extruded breakfast. Starch is the major constituent of many formulated complementary foods and should be provided in a digestible form (Codex Alimentarius, 2013). The glycaemic index (GI) describes the rate of absorption of blood glucose after the consumption of starchy foods and is considered low when it <55%. In this research, the high GI value of fermented corn flour (pap) could be related to the structure of its non-gelatinized starch which is commonly known to resist enzymatic digestion (Goñi et al., 1997; Leoro et al., 2010). On the other hand, a decrease in nettle- and soybean-enriched pap could be due to 1) added protein and dietary fibre that formed polymeric complexes leading to incomplete gelatinization and starch retrogradation (Leoro et al., 2010), or 2) the high phenolic compounds recorded in nettle-enriched pap that could have inhibited amylolytic enzymes and delayed glucose absorption, (Kraw ęcka et al., 2021). Above all, nettle-enriched pap could be classified as a 'low glycaemic index complementary food', which could provide essential nutritional and health benefits to a growing infant.

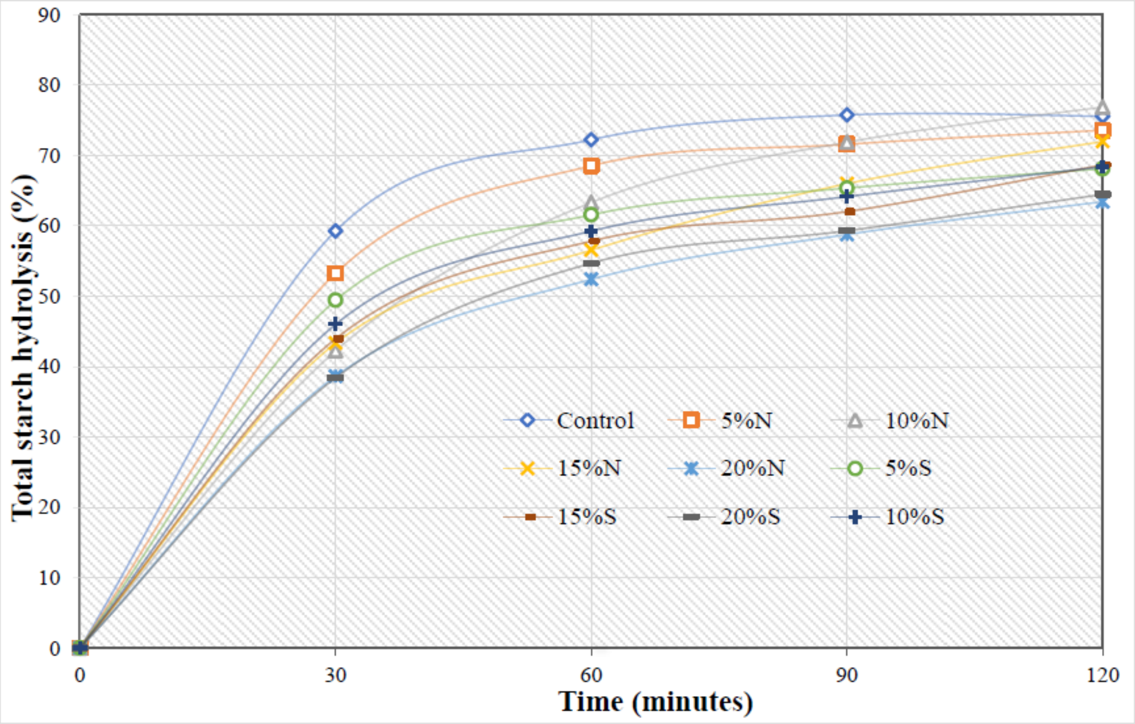

Figure 1. Starch hydrolysis of complementary foods during 120 min of in vitro digestion

Table 5. In vitro glycaemic response of complementary foods

		Glycaemic index (GI)
Pap (control)		68.53 ± 0.08^{a}
Nettle-enriched pap	5%	48.12 ± 0.02^{b}
	10%	44.36 ± 0.04^{c}
	15%	41.55 ± 0.08^{d}
	20%	35.60 ± 0.02^{e}
Soybean-enriched pap	5%	56.54 ± 0.02^{f}
	10%	53.61 ± 0.01^{g}
	15%	54.45 ± 0.08^{h}
	20%	40.24 ± 0.01^{i}

Values are mean ± standard deviation of triplicate. Means with different superscripts in the same column are significantly different ($p<0.05$).

3.5 Sensory Evaluation of Prepared Gruels

The results of the sensory analysis are presented in Table 6. Colour, smoothness, taste, flavour, and overall acceptability met the "targeted" sensory qualities of breastfeeding mothers. Colour for nettle-enriched pap was within 6.11 and 7.77. The scores for 10- and 15% nettle-enriched pap were higher than the control (7.02) and 100% nettle flour (4.52). Soybean-enriched pap was within 7.32 and 8.00 with no significant ($p<0.05$) difference between 5- and 10% soybean-enriched pap and 15- and 20% nettle-enriched pap. The colour was dependent on the ingredients used, that is, green for nettle-enriched pap and yellow for soybean-enriched pap. Smoothness for all formulations was within 7 and 8 except for 15% nettle -enriched pap that was lower (6.21). 100% nettle flour was rated lowest (4.70) for taste; otherwise, all formulations were within 6 and 8. In particular, 15% soybean-enriched pap was scored 8.54 for taste and this value was significantly ($p<0.05$) higher than the control (6.45) and all nettle-enriched formulations. Taste could be attributed to the presence of simple sugars arising from the fermentation of corn and the toasting of soybeans. Flavour for nettle-enriched pap was between 5.86 and 6.30 and was significantly ($p<0.05$) lower than the control (7.64) and soybean-enriched pap (7.20 and 8.55). Furthermore, toasting has been found to improve the taste and taste of foods through starch dextrination (Codex Alimentarius, 2013). In terms of overall acceptability, nettle-enriched pap ranged between 6.23 (20%

nettle-enriched pap) and 7.66 (15% nettle-enriched pap). These scores were within the control (7.12) but slightly lower than soybean-enriched pap (7.23 and 8.76). Throughout the sensory analysis, soybean-enriched pap was highly preferred over nettle-enriched pap. Couple with the fact that stinging nettle was entirely unknown to panelists, there was this tendency to generally accept familiar soybean products. However, 12 of the 20 breastfeeding mothers accepted that they could feed their babies with nettle leaf flour, thus indicating could be confidently used in complementary feeding following sensitisation of its nutritional and health benefits.

Table 6. Sensory evaluation of prepared gruels

Ingredient		Colour	Smoothness	Taste	Flavour	Overall acceptability
Pap (control)		7.02 ± 0.01^{a}	8.21 ± 0.01^{a}	6.45 ± 0.02^{a}	7.64 ± 0.02^{a}	7.12 ± 0.45^{a}
Nettle flour		4.52 ± 0.68^{b}	8.35 ± 0.30^{b}	4.70 ± 0.25^{b}	5.34 ± 0.08^{b}	4.50 ± 0.02^{b}
Nettle-enriched pap	N5	6.45 ± 0.71^{c}	7.12 ± 0.39^{c}	7.76 ± 0.12^{c}	6.21 ± 0.02^{c}	7.14 ± 0.01^{c}
	N10	7.77 ± 0.55^{d}	7.88 ± 0.66^{cf}	6.42 ± 0.04^{dh}	6.30 ± 0.41^{d}	7.38 ± 0.30^{c}
	N15	7.23 ± 0.08^{e}	6.21 ± 0.24^{d}	7.46 ± 0.01^{e}	5.86 ± 0.24^{b}	7.66 ± 0.08^{d}
	N20	6.11 ± 0.54^{f}	7.66 ± 0.67^{c}	7.33 ± 0.30^{f}	6.01 ± 0.04^{e}	6.23 ± 0.22^{e}
Soybean-enriched pap	S5	8.00 ± 0.98^{g}	8.12 ± 0.08^{d}	7.12 ± 0.04^{f}	7.25 ± 0.11^{a}	8.45 ± 0.02^{f}
	S10	8.12 ± 0.08^{g}	8.04 ± 0.15^{d}	6.89 ± 0.54^{gh}	7.20 ± 0.21^{a}	8.02 ± 0.02^{g}
	S15	7.81 ± 0.25^{d}	8.59 ± 0.10^{ef}	8.54 ± 0.21^{c}	8.55 ± 0.02^{f}	7.23 ± 0.10^{c}
	S20	7.32 ± 0.37^{d}	7.48 ± 0.02^{c}	7.98 ± 0.31^{c}	8.40 ± 0.01^{f}	8.76 ± 0.04^{h}

Note: N= nettle leaf flour, S= soybean flour. Values are mean ± standard deviation of triplicate. Means with different superscripts in the same column are significantly different ($p<0.05$).

4. Conclusion

In this study, stinging nettle leaf flour was gradually incorporated into fermented corn flour (pap) to improve its nutritional, functional, and sensory properties. In particular, the study observed a significant ($p<0.05$) increase in ash, protein, dietary fibre, total phenolic content, and antioxidants. The metabolic glycaemic response significantly decreased from 68.53% (pap) to 35.60 (20% nettle-enriched pap), indicating ‘low glycaemic index complementary foods’ could be produced when nettles are substituted in infant formulations. A consumption of such low-cost nutrient-rich nettle-enriched complementary foods could reduce and/or combat malnutrition especially in poorer developing countries where stinging nettle is growing abundantly as a ‘weed’. However, nutritional sensitization and awareness campaigns are required to initiate stinging nettle into complementary feeding in these communities. Further research is required to understand the health and food safety as well as the consumption limits of stinging nettles.

Acknowledgments

The authors wish to thank nursing mothers who voluntarily participated in this research.

Authors contributions

Dr. MNT and Dr. ICNP were responsible for the study design and revising. Dr MNT performed the laboratory work, analyzed the data, and drafted the manuscript. Dr ICNP revised the drafted manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Competing interests

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.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal’s policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned, externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

Open access

Copyrights

References

Abeshu, M., Lelisa, A., & Geleta, B. (2016). Complementary feeding: Review of recommendations, feeding practices, and adequacy of homemade complementary food preparations in developing countries - Lessons from Ethiopia. *Frontier Nutrition, 3*(41). https://doi.org/10.3389/fnut.2016.00041

Adhikari, B. M., Bajracharya, A., & Shrestha, A. K. (2015). Comparison of nutritional properties of stinging nettle (*Urtica dioica*) flour with wheat and barley flours. *Food Science & Nutrition, 4*(1), 119-124. https://doi.org/10.1002/fsn3.259

Alemayehu, D., Desse Haki, G., Ali, K., Berhanu, B., & Getahun, D. (2016). Proximate, mineral composition and sensory acceptability of home made noodles from stinging nettle (*Urtica simensis*) leaves and wheat flour blends. *2016*, 55-61.

AOAC. (1997). *Association of Official Analytical Chemists. Official Method Codex Type II Standard for Infant Formula Revised the First Action, AOAC, Rockville, Maryland.*

AOAC. (2000). *Official Methods of Analysis of AOAC International, Association of Official Analytical Chemists* (17th ed.). Vol. II. Washington, DC, USA.

Appiah, F., Asibuo, J., & Patrick, K. (2011). Physicochemical and functional properties of bean flours of three cowpea (*Vigna unguiculata* L. Walp) varieties in Ghana. *African Journal of Food Science, 52*(2), 100-104.

Baningo, E., & Akpapunam, M. (1999). Physico-chemical and nutritional evaluation of protein-enriched fermented maize flour *J. Manage. Technol, 1*(1), 30-36.

Benoist, B. (1999). Complementary feeding: a challenge to both children and mothers In M. Dop, D. Benbouzid, S. rèche, A. Verster, & F. Delpeuch (Eds.), *Complementary feeding of young children in Africa and the Middle East.* Geneva: World Health Organization.

Codex Alimentarius. (2013). *Guidelines on formulated complementary foods for older infants and young children CAC/GL 8-1991* (adopted 1991, revised 2013).

Devkota, H. P., Paudel, K. R., Khanal, S., Baral, A., Panth, N., Adhikari-Devkota, A., . . . Hansbro, P. M. (2022). Stinging Nettle (*Urtica dioica* L.): Nutritional Composition, Bioactive Compounds, and Food Functional Properties. *Molecules, 27*(16). https://doi.org/10.3390/molecules27165219

Đurović, S., Vujanović, M., Radojković, M., Filipović, J., Filipović, V., Gašić, U., ... Zeković, Z. (2020). The functional food production: Application of stinging nettle leaves and its extracts in the baking of a bread. *Food Chem, 312*, 126091-126091. https://doi.org/10.1016/j.foodchem.2019.126091

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition research (New York, N.Y.), 17*(3), 427-437. https://doi.org/10.1016/S0271-5317(97)00010-9

Himalian, R., & Singh, D. M. (2021). A Comparative account on Antioxidant activities, Total phenolic and Flavonoid contents of Punica granatum, Carica papaya, Foeniculum vulgare, Trigonella foenum-graecum, and *Urtica dioica*: An in vitro Evaluation. *Research Journal of Pharmacy and Technology, 15*, 1175-1183. https://doi.org/10.52711/0974-360X.2022.00197

Jaiswal, V., & Lee, H.-J. (2022). Antioxidant Activity of *Urtica dioica*: An important property contributing to multiple biological activities. *Antioxidants, 11*, 1-24. https://doi.org/10.3390/antiox11122494

Khare, V., Kushwaha, P., Verma, S., Gupta, A., Srivastava, S., & Rawat, A. (2012). Pharmacognostic evaluation and antioxidant activity of Urtica dioica L. *Chinese Medicine, 3*, 128-135. https://doi.org/10.4236/cm.2012.33021

Krawęcka, A., Sobota, A., Pankiewicz, U., Zielińska, E., & Zarzycki, P. (2021). Stinging Nettle (Urtica dioica L.) as a Functional Component in Durum Wheat Pasta Production: Impact on Chemical Composition, In Vitro Glycemic Index, and Quality Properties. *Molecules, 26*(22). https://doi.org/10.3390/molecules26226909

Krička, T., Jukić, Ž., Voća, N., Sigfild, N., Zanuškar, J., & Voća, S. (2003). Nutritional characteristics of soybean after thermal processing by toasting. *Acta Veterinaria (Beograd), 53*(2-3), 191-197. https://doi.org/10.2298/AVB0303191K

Kukrić, Z. Z., Topalić-Trivunović, L., Kukavica, B., Matoš, S. B., Pavičić, S. S., Boroja, M. M., & Savić, A. V. (2012). Characterization of antioxidant and antimicrobial activities of nettle leaves (Urtica dioica L.). *Acta Periodica Technologica, 43*, 257-272. https://doi.org/10.2298/APT1243257K

Kulkarni, K., Kulkarni, D., & Ingle, U. (1991). Sorghum malted and soya bean weaning food formulations: Preparation, functional properties and nutritive value. *Food nutrition bulletin, 12*, 322-327.

Kupina, S., Fields, C., Roman, M. C., & Brunelle, S. L. (2018). Determination of total phenolic content using the Folin-C assay: single-laboratory validation, First Action 2017.13. *J AOAC Int, 101*(5), 1466-1472. https://doi.org/10.5740/jaoacint.18-0031

Leoro, V. M., Clerici, M., Chang, Y., & Steel, C. (2010). Evaluation of the in vitro glycemic index of a fiber-rich extruded breakfast cereal produced with organic passion fruit fiber and corn flour. *Food Science and Technology (Campinas), 30*, 964-968. https://doi.org/10.1590/S0101-20612010000400019

Lichtenthaler, H., & Buschmann, C. (2001). *Chlorophylls and Carotenoids: measurement and characterization by UV-VIS spectroscopy.* https://doi.org/10.1002/0471142913.faf0403s01

Maietti, A., Tedeschi, P., Catani, M., Stevanin, C., Pasti, L., Cavazzini, A., & Marchetti, N. (2021). Nutrient Composition and Antioxidant Performances of Bread-Making Products Enriched with Stinging Nettle (Urtica dioica) Leaves. *Foods, 10*(5). https://doi.org/10.3390/foods10050938

Marchetti, N., Bonetti, G., Brandolini, V., Cavazzini, A., Maietti, A., Meca, G., & Mañes, J. (2018). Stinging nettle (Urtica dioica L.) as a functional food additive in egg pasta: Enrichment and bioaccessibility of Lutein and β -carotene. *Journal of Functional Foods, 47*, 547-553. https://doi.org/10.1016/j.jff.2018.05.062

Chakravartula, S. S. N., Moscetti, R., Farinon, B., Vinciguerra, V., Merendino, N., Bedini, G., ... Massantini, R. (2021). Stinging Nettles as Potential Food Additive: Effect of drying processes on the quality characteristics of leaf powders. *Foods, 10*. https://doi.org/10.3390/foods10061152

Oladiran, D., & Emmambux, M. (2020). Locally Available African Complementary Foods: Nutritional Limitations and Processing Technologies to Improve Nutritional Quality—A Review. *Food Reviews International, 38*, 1-31. https://doi.org/10.1080/87559129.2020.1762640

PAHO. (2003). *Guiding principles for complementary feeding of the breastfed child*. Pan American Health Organization, Washington, DC.

Palikhe, M. (2012). *Preparation of biscuit incorporating nettle powder and its quality evaluation* (B. Tech. Food). Purwanchal University, Nepal.

Pant, V., & Sundriyal, R. (2016). Nutritional and therapeutic efficacy of stinging nettle-A review *The Journal of Ethnobiology and Traditional Medicine, 126*, 1240-1254.

Rahman Khan, M., Rahman, M., Islam, M., & Begum, S. (2006). A Simple UV-spectrophotometric method for the determination of Vitamin C content in various fruits and vegetables at Sylhet Area in Bangaladesh. *Journal of Biological Sciences, 6*, 388-392. https://doi.org/10.3923/jbs.2006.388.392

Rutto, L., Xu, Y., Ramirez, E., & Brandt, M. (2013). Mineral properties and dietary value of raw and processed stinging nettle (*Urtica dioica* L.). *International Journal of food science & technology*. https://doi.org/10.1155/2013/857120

Scherer, R., & Godoy, H. (2009). Antioxidant Activity Index (AAI) by the 2,2-diphenyl-1 picrylhydrazyl method. *Food Chemistry, 112*(3). https://doi.org/10.1016/j.foodchem.2008.06.026

Tanyitiku, M. N., & Njombissie Petcheu, I. C. (2022). Formulation and nutritional analysis of processed sorghum, soybeans, and mango complementary foods. *Journal of Food Research, 11*(3), 11-21. https://doi.org/10.5539/jfr.v11n3p11

Tanyitiku, M. N., Graeme, N., Sullivan, J., Njombissie Petcheu, I. C., On, S. L. W. (2022). Snail meat consumption in Buea – Cameroon : exposures to foodborne pathogens through social practices assessed in 2019 and 2021. 80:256. https://doi.org/10.1186/s13690-022-01009-8

Tarasevičienė, Ž., Vitkauskaitė, M., Paulauskienė, A., & Černiauskienė, J. (2023). Wild stinging nettle (*Urtica dioica* L.) leaves and roots chemical composition and phenols extraction. *Plants, 12*(2). https://doi.org/10.3390/plants12020309

The Lancet Planetary, H. (2020). Food security in uncertain times. *The Lancet Planetary Health, 4*(6), 1-4. https://doi.org/10.1016/S2542-5196(20)30126-1

Treche, S. (1999). Production of flour for infants and young children: a review of experiences in Africa. In M.-C. Dop, O. Benbouzid, B. de Benoist, A. Verster, & F. Oelpeuch (Eds.), *Complementary feeding of young children In Mrica and the middle-East*. Geneva: World Health Organization.

Ullah, I., Ali, M., & Farooqi, A. (2010). Chemical and nutritional properties of some maize (*Zea mays* L.) varieties grown in NWFP, Pakistan. *Pakistan Journal of Nutrition, 9*, 1113. https://doi.org/10.3923/pjn.2010.1113.1117

UNICEF. (2021, December 12). *United Nations Children's Fund (UNICEF) Child Alert.* Sub-Saharan Africa: Growing up in crisis in a world of opportunities. Retrieved from https://www.unicef.org/media/96161/file/SubSaharan%20Africa%20%E2%80%93%20Growing%20up%20in%20crisis%20in%20a%20world%20of%20opportunities%20.pdf

WFP. (2018). *World Food Programme: Nutritional Guidance for Complementary Food: World Food Programme.*

WHO. (2009). *World Health Organization*. Infant and young child feeding: Model chapter for textbooks for medical students and allied health professionals. WHO Press, Geneva.

WHO. (June 9, 2021). *Infant and young child feeding.* Retrieved from https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding

Wójcik, M., Różyło, R., Łysiak, G., Kulig, R., & Cacak-Pietrzak, G. (2021). Textural and sensory properties of wheat bread fortified with nettle (*Urtica dioica* L.) produced by the scalded flour method. *Journal of Food Processing and Preservation, 45*(10).

Notes

Note 1. Usually, small areas of land used for vegetable cultivation destined for household consumption or for income earning when production is in excess of household needs.

Note 2. Namely, African nightshade (Solanum scabrum), amaranth (Amaranthus spp.) and jute mallow (Corchorus olitorius)