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Functional and sensory properties of iron and folic acid fortified NABE-3 bean and silver fish composite instant flour

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Abstract
Despite the need for Iron and Folic acid (IFA) during critical stages of child development and gestation, there is observed persistence in deficiencies despite several interventions. This situation motivated a food-based approach using IFA-fortified NARO Bean-3 (NABE-3 bean) and silverfish. IFA fortified composite flours with NABE-3 (BF): silver fish flour (SFF) proportions as 100% BF, 90% BF: 10% SFF, 80% BF: 20% SFF, and 70% BF: 30% SFF were developed. Functional, pasting, and sensory properties were assessed using standard methods. Increasing silverfish proportions in the composite flours was associated with decreased water absorption properties of the composite flours indicating reduced reconstitution abilities; reduced final viscosity but increased bulk density implying a high density of nutrients at lower viscosity and volume; and increased peak time indicating increased cooking energy requirements. The compressibility and Hausner ratios of the flours were high and not significantly different, indicating low flowability of the bean flour and its composites with silver fish. Increasing silverfish proportions reduced sensory score from liked moderately to indifferent on a 9-point hedonic scale due to colour darkening, and increased intensity of fishy aroma and flavour. The 90% BF: 10% SFF and 80% BF: 20% SFF composites were more acceptable than the 70% BF: 30% SFF. However, all the composites were more acceptable than the control 100% BF: 0% SFF. Incorporation of silver fish into NABE bean flour generally leads to increased sensory acceptability and nutrient density but reduced functionality.

Keywords Sensory properties · NARO Bean · Functional properties · Iron deficiency · Folic acid · Fortification

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1 Introduction

Inadequate diets are a key driver for malnutrition, a major development challenge in developing countries. Malnutrition commonly manifests in macro and micro-nutrient deficiencies, especially of energy and essential nutrients such as proteins, iron, iodine, vitamin A, and zinc [1]. Iron and Folic acid deficiency especially among children and women of childbearing age is also prevalent in developing countries of Africa but it is often underestimated due to methodological limitations associated with collecting data about its prevalence [2]. Deficiencies of iron and folic acid (IFA) are common among children and women of reproductive age, largely due to high consumption of low micronutrient (iron and folate) plant-based foods, since high biological value nutrient sources, especially from animal-based foods, tend to be inaccessible for the majority of the population due to high cost [3]. The dominance of starchy staples alongside limited animal-source foods supply is responsible for the region's high micronutrient deficiencies [4].

Addressing micronutrient intakes through compositing with a micro-nutrient-rich food is an example of a food-2-food fortification [5]. Composite flour (CF) is a growing technology that aims to increase the nutritional composition of flour [6]. Compositing sorghum and orange-fleshed sweet potato flour increased vitamin A and protein content intake [7]. Similarly, a soup processed by compositing amaranth and vegetables such as mushroom flour was shown to contribute to 25% of the adolescent RDA requirements for carbohydrates, protein, dietary fiber, vitamin A, and iron [8]. Fortification programs have prioritised foods that have a high consumption and distribution. Hence, bio-fortified crops such as commonly consumed beans (*Phaseolus vulgaris*) are a viable option since they are a staple food and currently the major source of iron in Uganda and other countries of Sub-Saharan Africa [9].

Fortification vehicles have been expanded to include biofortification through breeding [10] such as the biofortification of beans [11]. The Uganda National Crops Resources Research Institute (NaCRRI) at Namulonge released NARO Beans (NABE), i.e., NABE 1, NABE 2, NABE 3, and NABE 5C bean hybrids that, compared to the local varieties, have: a higher yield; higher amounts of bio-available iron; shorter cooking times; and are liked by the farmers for their commercial viability [12]. In addition, silverfish (*Rastronebola argentea*), locally known as *Mukene* in Uganda, is one of the most consumed types of fish particularly among the rural poor [13]. It is very nutritious with high protein (47.9–58.8%) and micronutrient content, especially iron (8.18–12.57 mg/100 g) on a dry matter basis [14]. Therefore, blending locally available food commodities such as bio-fortified NABE-3 beans and silverfish which are rich in macro and micronutrients, especially iron could mitigate micro-nutrient deficiencies. On the other hand, fortifying flours is promoted in Uganda as a multi-sectorial response to micronutrient deficiencies in women and children, emphasising vitamins such as folic acid and minerals such as iron [15]. Besides nutrient enhancement, fortification through the compositing of foods and the use of premix can affect the functional [16] and sensory properties of food [17].

Functional properties such as particle size, water absorption capacity, and oil absorption capacity, can directly influence the usability and quality of derivable products. For example, these properties determine whether the blends would be useful in either bakery products where hydration to improve handling is desired, and therefore in which case high water absorption capacity is advantageous; or inground meat, doughnuts, and pancakes where increased oil absorption properties are of prime importance [18]. These changes lead to variations in structure and conformation, which affect the sensory properties such as texture [19]. Functional characteristics are required to evaluate and predict how the product components especially nutrients such as proteins, fat, fibre and carbohydrates, may behave and interact in composites to impact the physical behaviour and consequently, the sensory characteristics of the composite [20].

Meeting consumer needs and sensory attributes is key to the development and adoption of new products. It is used to assess the characteristics of the product (appearance, odour/aroma, texture/consistency and flavour/taste), which define the quality of the product, and the expectations of consumers and their reactions to the product, the latter being assessed by affective (hedonic) sensory testing methods [21]. Sensory evaluation therefore plays an important role during the planning and formulation-reformulation stages that lead to full production [22]. For sensory evaluation, affective and descriptive tests are used to determine consumers' expressions of their preference for or dislike of the attributes of a product, while descriptive sensory analysis on the other hand provides information about product differences and sensory drivers of liking of a product based on organoleptic characteristics among other functions [23].

This study intended to develop an iron and folic acid-fortified functional and sensorially acceptable composite flour from silverfish and iron bio-fortified NABE-3 bean. This is because fortification through compositing and/or the addition of fortificants has been used to address micronutrient deficiencies with minimal risk to health and a high public health benefit [24]. It is normally applied to widely and/or highly consumed foods. Because of a centralised and formalised sector, as well as an efficient distribution system, wheat flour has been fortified with iron for reducing anaemia and improving the iron status of populations in many countries [25]. Folic acid fortification of cereal and cereal products has been associated with decreased prevalence of neural tube defects and reduction in anemia, blood serum homocysteine, and the risk of developing cardiovascular diseases [26]. The objective of the study was therefore to assess the sensory acceptability and functional properties of the bean-silver fish composite for suitability for utilization by the target population.

2 Materials and methods

2.1 Materials

The NABE-3 bean varieties were purchased from the National Crops Resources Research Institute (NaCCRI) at Namulonge (0.5232° N, 32.6158° E). Fresh silver fish was procured from Katosi landing site in Mukono district (0.15278° N 32.80139° E). Powdered onions, tomatoes and garlic (Tropical Heat Limited, Nairobi, Kenya); and muchuzi mix (Unilever Kenya Ltd, Nairobi, Kenya) were sourced from Mega Standard Supermarket in Kampala (0.31134° N, 32.57891° E), Uganda.

2.2 Methods

2.2.1 Preparation of the iron and folate-fortified composite flour and sauce

The NABE-3 beans were sorted to remove unwanted (contaminating) material, washed with portable water until clean, soaked for 12 h, dried (50 °C for 24 h) to 12% moisture content in a convectional air dyer (JW-1350ED, Jin-woo Electronics, South Korea), and roller milled (THGCR—100, SO Sejin, South Korea) into coarse grits. Fresh silver fish was procured from Katosi landing site in Mukono district, placed on ice in a clean disinfected cooler box, and transported to the National Agricultural Research Organisation (NARO) Food Bioscience laboratory at Kawanda within 8 h of collection for standard handling and management under controlled conditions. The silverfish were sorted to remove debris, washed three times using potable water, placed in a clean disinfected oven tray, and convection air dried (JW-1350ED, Jin woo Electronics, South Korea) at 55 °C for 48 h to constant weight. Thereafter, the silverfish was beheaded to tone down the fishy aroma, roasted (150 °C for 15 min), and roller milled (THGCR-100, So Sejin, South Korea) into 2.5 mm grits. The dried bean and roasted silverfish grits were respectively mixed in the four ratios (100:0, 90:10, 80:20, and 70:30) of 10 kg portions each. Dry powdered onions, tomatoes, garlic, and muchuzi mix (Unilever Kenya Ltd, Nairobi, Kenya) were added to each portion, and extruded (150 °C, main screw speed of 1450 rpm for 10 min) (THEX-100, SO Sejin, South Korea). The NABE-3 bean-silver fish composite flour was processed by extrusion cooking as shown in Fig. 1.

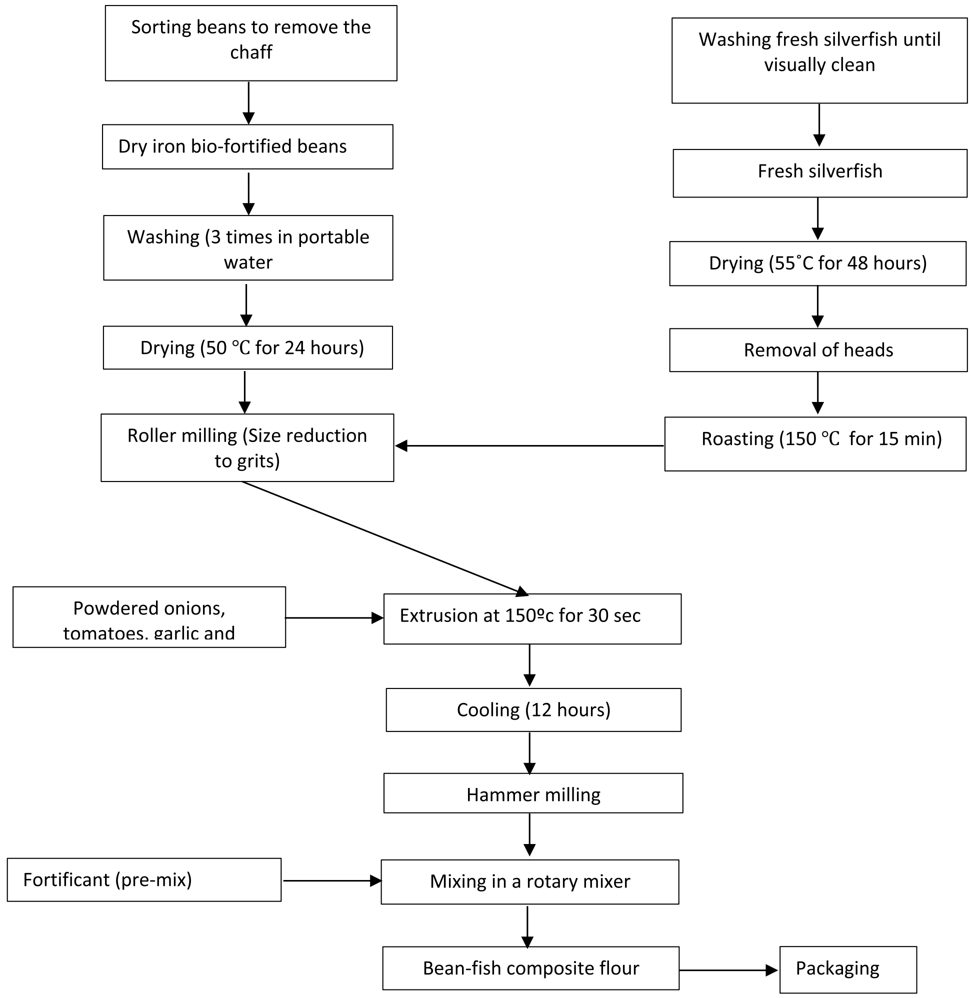
After cooling and milling (SFSP60X3545, Hengshiu Electrical Motors, PRC) the bean—silver fish composite into flour, the iron and folic acid premix (Hexagon Nutrition PVT Ltd, Chennai, India) was applied at a rate of 119.8 g/100 kg and thoroughly mixed in a rotary mixer (THRM, SO Sejin, South Korea). The composite flours were primarily packaged in low-density polyethylene (LDPE) bags (10 × 10 cm, L × W; 0.2 mm thick) that were inserted in labeled cardboard paper bags as secondary packages.

2.3 Functional properties and colour of the composite flour

2.3.1 Water holding capacity

The water holding capacity of samples was determined as described by Mesías and Morales [27]. Flour (2 g) was weighed into a dry pre-weighed 50 mL centrifuge tube to which 15 mL of water was added. The mixture was vigorously vortexed for 1 min, held at room temperature for 30 min, and centrifuged (Megafuge 8, Thermo Fisher Scientific, Waltham, Massachusetts) at 1400*g* for 15 min. The non–absorbed water was discharged and the tube was weighed. Water holding Capacity (g/g) according to the weight of the sample was calculated using Eq. 1.

Fig. 1 Flow diagram for the production of iron bio-fortified beans-silver fish composite flour



$$\mathrm{WAC(g/g)} = \frac{\text{Weight of tube with sample and water retained (g)} - \text{Weight of tube with sample (g)}}{\text{Weight of sample (g)}}. \quad (1)$$

2.3.2 Oil absorption capacity

This was determined as described by Mesías & Morales [27]. Flour (1 g) was mixed with 10 mL of refined, blended, and fortified vegetable oil (ROKI Mukwano Vegetable oil) in a pre-weighed 20 mL centrifuge tube. The slurry was stirred for 1 min for complete dispersion of the sample in the oil. The dispersion was allowed to stand at ambient temperature (~ 29 °C) for 30 min and then centrifuged at 1400*g* for 15 min. The clear supernatant was decanted and discarded. The adhering drops of oil in the centrifuge tube were removed with cotton wool, the tube was weighed, and the oil absorption capacity (OAC) was calculated using Eq. 2.

$$\mathrm{OAC(g/g)} = \frac{\text{Weight of tube with sample and oil retained (g)} - \text{Weight of tube with sample(g)}}{\text{Weight of sample (g)}}. \quad (2)$$

2.3.3 Swelling power and solubility index

Swelling power and solubility index of the flours were determined according to Mesías and Morales [27]. Flour (2 g) was placed in a pre-weighed centrifuge tube to which 25 mL of water was added and heated in a water bath (WB-T 13, FMH electronics, Gauteng, South Africa) maintained at 90 °C for 30 min and thereafter cooled by placing the tubes in cold water (− 15 °C). The suspension was centrifuged at 1400*g* for 10 min and the supernatant was collected in a pre-weighed dry aluminium dish. The contents of the dish were evaporated at 105 °C for 24 h and the weight was noted for calculation of solubility index (Eq. 3). Similarly, the weight of wet sediment from the centrifugation was noted to determine the swelling power (Eq. 4).

$$\text{Solubility}(\%) = \frac{\text{Weight of disolved solids in supernatant (g)} \times 100}{\text{Weight of sample (g)}}. \tag{3}$$

$$\text{Swelling Power}(\%) = \frac{\text{Weight of the sediment paste (g)} \times 100}{\text{Weight of sample (g)} \times (100 - \%\text{ solubility})}. \tag{4}$$

2.3.4 Bulk and tapped density

The determination of the bulk and tapped density was carried out according to Banki et al. [28]. The flour sample (10 g) was placed into a clean and dry graduated 100 mL measuring cylinder and its volume was noted. The measuring cylinder was manually tapped on a work bench until no further decrease in the volume of flour was observed. The final volume of the flour after tapping was noted and recorded. The bulk and tapped density were calculated using Eqs. 5 & 6 below.

$$\text{Bulk density}(\text{g/mL}) = \frac{\text{Weight of the sample (g)}}{\text{Volume of sample (mL)}} \tag{5}$$

$$\text{Tapped density}(\text{g/mL}) = \frac{\text{Weight of the sample (g)}}{\text{Volume of sample after tapping (mL)}}. \tag{6}$$

2.3.5 Hausner ratio and the compressibility index

The Hausner ratio and compressibility ratio were computed according to Bala et al. [29] as follows.

$$\text{Hauser Ratio} = \frac{\text{Tapped density}}{\text{bulk density}} \tag{7}$$

$$\text{Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} * 100 \tag{8}$$

2.3.6 Pasting properties

The pasting properties of the bean-fish composite flours were determined using a Rapid Visco Analyzer RVA-4500 (Perten Instruments, Australia) [30]. Briefly, composite flour slurries (3 g sample, 25 mL distilled water) were equilibrated at 50 °C for 1 min, heated at a rate of 6 °C/min to 95 °C, held at 95 °C for 5 min, cooled at a rate of 6 °C/min to 50 °C, and held at 50 °C for 2 min. The spindle speed was 960 rpm for the first 10 s (to disperse the sample) and then 160 rpm for the remaining 23 min. Analysis was done in duplicate.

2.4 Colour measurement

The physical colour parameters L*, a*, b* for NABE-3 bean-fish composite flours were measured using a Hunter Labscan colorimeter (Hunter Associates Laboratory, Inc, VA, USA) [31]. The L* values measure lightness (0–100), a* values measure redness when positive and b* measures yellowness when negative. The measurements were taken in duplicate for each sample.

2.5 Preparation of instant bean flour-silverfish sauce for sensory evaluation

The three NABE-3 beans flour (BF) and silverfish flour (SFF) composite (90% BF: 10% SFF; 80% BF: 20% SFF; 70% BF: 30% SFF) and control (100%BF) were reconstituted into a sauce and evaluated for sensory acceptability. Reconstitution of the fortified composite flour into the sauce was achieved by dissolving 1 part (50 g) of the flour in 6 parts of hot water (about 300 mL), followed by proper mixing to avoid the formation of lumps.

2.5.1 Affective sensory testing

The composite and control sauces were blind coded and served to the general consumer community alongside rice and boiled green bananas commonly known as *matooke*. The consumers were informed in advance in writing about the product, its ingredients and the importance of the study in the process of securing their consent to participate. This information was provided to members at the entrance of the sensory testing area in both English and the local language (Luganda) [Supplementary Information (S1)]. It was assumed that after reading the pinned information, consumers joining the sensory testing session were duly informed and therefore fully consented to testing the product. Evaluations were performed under fluorescent light and ambient temperature. A total of 100 consumers were targeted. They were served with blind coded samples, an evaluation ballot, and drinking water to rinse their mouth in between tasting of the sauces. The samples were assessed for flavour, aroma, taste, colour, appearance, mouthfeel and overall-acceptability on a 9-point hedonic scale [1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like or dislike (indifferent), 6 = Like slightly, 7 = Like moderately, 8 = Like very much, 9 = Like extremely] [22].

2.5.2 Descriptive analysis

Following procedures elaborated by Ruiz-Capillas and Herranz [22], quantitative descriptive analysis of instant NABE-3 bean-silverfish composite sauces was conducted using a 10 member trained panel of assessors who were accustomed to the description of the specific food items. Before the descriptive sessions, panellists were briefed about the purpose and objective of the study. Similar to affective testing, descriptive sensory evaluations were also performed under fluorescent light at ambient temperature, and assessors were provided with drinking water to rinse their mouths in between tasting the samples. The panel was joined by the product production expert to discuss and through consensus, agree on the critical sensory attributes that represent and define the quality of the product. A parameter was regarded as critical if changes in this parameter would significantly negatively influence the sensory acceptability of the product. Thereafter, the panel generated descriptors for the identified attributes and developed a standardised vocabulary to describe the differences among samples. Consensus building was moderated by the panel leader who as much as possible ensured that product samples were uniformly prepared and availed to the panellists.

2.6 Ethical considerations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the research and ethics committee (REC) of the Makerere University School of Heath Science (MAKSHREC-2020-8). Informed consent was obtained from all individual participants included in the study during sensory evaluation studies. The silver fishe used in this study was harvested from the lake for use as food and feed at both subsistence and commercial scale, and therefore, use of silver fish in research does not require ethical clearance.

2.7 Data analysis

Following a normality check, the ANOVA technique was used to determine the mean difference due to addition of silver fish to the NABE-3 bean flour based on the functional, pasting and colour properties of the NABE-3 bean-silver fish composite flours, and the sensory properties of the sauces. Quantitative data obtained in triplicate was analysed using XLSTAT version 2019.2.2.59614 and Statistical Package for Social Scientists (SPSS) Version 22. Means were separated using Tukey option at a significance level of $p = 0.05$. Qualitative sensory data was presented as described by the trained panel.

3 Results and discussion

3.1 Functional properties of the composite flours

The inclusion of silverfish in bean flour had a significant influence on all the functional properties (swelling capacity, solubility index, bulk density, tapped density, water absorption capacity, and oil absorption capacity) of the composite flours, except on compressibility and the Hausner ratio (Table 1).

3.1.1 Swelling power and solubility index

Swelling power and solubility index measure the ability of starch to imbibe water and swell, and reflect the extent of associative forces within the granules [32]. The swelling power of the composite flour increased with an increase in the proportion of silverfish flour (Table 1) which was unexpected since the proportion of the swelling starch reduces as the silverfish proportion increases. However, though silverfish lacks carbohydrates, it contains substantial amounts of protein (47–59%) [14] which have hydrophilic groups that after interaction with starch molecules allow water entrance into the granules, thus leading to increased swelling power [32]. Often, the solubility index is linked to the amount of amylose and amylopectin that leaches out during the swelling process [33] which in this case decreased as the proportion of the silverfish in the flour increased. However, the proportion of hydrophilic proteins increased as the fish inclusion levels increased, possibly contributing to the increased solubility index.

3.1.2 Water absorption capacity (WAC)

The WAC of flour represents the ability of the starch granules to associate with water (reconstitute) under limited water conditions [34]. The WAC reduced with an increase in the proportion of silverfish flour in the source. This is possible because the proportion of starch molecules that are responsible for the reconstitution reduced with reducing proportions of bean flour as the proportion of the silverfish flour increased. Higher WAC values are associated with better reconstitution abilities of powders [8], which implies that sauces with higher ratios of silverfish will have reduced reconstitution abilities.

3.1.3 Oil absorption capacity (OAC)

This is the difference in the flour weight before and after oil absorption. It is attributed to the physical entrapment of oil within proteins and non-covalent bonds such as hydrophobic, electrostatic, and hydrogen bonding, and results from the binding of the hydrocarbon chains of oil to the non-polar side chains of amino acids [35]. In this study, the OAC of the composite flours significantly reduced with increased substitution levels of fish flour, contrary to the increase in OAC that would be expected with increased fish content, and thus protein content. The protein content, the form of the non-polar arrangement of the amino acids, as well as the type of bonding between the oil and the starch and proteins, are determinant factors that increase or decrease the OAC of the extrudates [36]. It is therefore possible that during extrusion cooking, the proteins in the fish increasingly bind with the starch and flour components to form particles that don't allow the protein side chains to interact with the oil thus reducing the OAC.

Table 1 Functional properties of the extruded bean-silver fish composite flours

Sample	Functional properties							
	Water absorption capacity (g/g)	Oil absorption capacity(g/g)	Solubility (%)	Swelling power (g/g)	Bulky density (g/cm^3)	Tapped density (g/cm^3)	hausner ratio	Compressibility (%)
NABE 3 + Spices	2.19 ± 0.22^{b}	0.49 ± 0.06^{a}	7.23 ± 0.06^{a}	2.00 ± 0.19^{a}	0.44 ± 0.01^{a}	0.63 ± 0.00^{a}	1.45 ± 0.59^{a}	30.08 ± 1.16^{a}
90:10	1.55 ± 0.16^{a}	0.41 ± 0.10^{a}	6.24 ± 0.40^{b}	2.05 ± 0.12^{a}	0.44 ± 0.02^{a}	0.63 ± 0.00^{a}	1.43 ± 0.00^{a}	30.04 ± 2.40^{a}
80:20	1.76 ± 0.02^{a}	0.19 ± 0.06^{b}	10.42 ± 0.20^{c}	2.79 ± 0.20^{b}	0.49 ± 0.02^{b}	0.70 ± 0.02^{b}	1.43 ± 0.02^{a}	31.14 ± 2.85^{a}
70:30	1.45 ± 0.04^{a}	0.03 ± 0.08^{c}	11.94 ± 0.46^{d}	3.76 ± 0.20^{c}	0.50 ± 0.00^{b}	0.72 ± 0.00^{b}	1.43 ± 0.46^{a}	30.00 ± 0.19^{a}

Figures in a column having different letters as superscripts are significantly ($p \leq 0.05$) different from each other

3.1.4 Bulk and tapped density

Bulk density (g/cm^3) of the flour is the density measured without the influence of any compression while tapped density is the bulk density attained after mechanically tapping a container containing the powder sample [20]. The composite flours with higher percentages (20% and 30%) of fish flour inclusion levels had significantly higher ($p < 0.001$) bulk and tapped densities than those with zero and lower (10%) fish flour inclusion levels. This implies that the interactions between the flour and fish particles during extrusion resulted in heavier composite flour particles, and thus a high bulk density and consequently tapped density. This is because bulk density is dependent on particle size among other parameters [20] and so is the tapped density that is derived from bulk density. Given that density is inversely related to volume, high-density flours therefore occupy less volume relative to their mass. A high mass per unit volume implies higher quantities of nutrients packed in a reduced volume of the product. Reduced volume is also associated with reduced paste thickness which makes such flour thinner and easy for ingestion by children and convalescents [29].

3.1.5 Compressibility index and Hausner ratio

These parameters indicate the flow behaviour of the flour and its ability to settle [29]. For the composite flours, the compressibility index remains ¬ 30% for all the flours and is unaffected by the level of inclusion of the fish flour. The average compressibility index (30–31) and Hausner ratio (1.4–1.5) are associated with poor flow properties [37]. Given that the compressibility and Hausner ratios don't depend on the proportions of fish flour added, other functional properties such as the ease of reconstitution and sensory parameters influence the choice of the composite.

3.2 Pasting properties of the composite flour

Pasting properties refer to physicochemical properties relating to the ability of an item to act in a paste-like manner, resulting from the application of heat in the presence of water, which affects texture, digestibility, and end use of the food product [38]. The pasting properties of the NABE-3 beans and their composites with 10, 20, and 30% silverfish flour is presented in Table 2.

Unlike pasting temperature, the peak viscosity, trough viscosity, breakdown viscosity, final viscosity, set back viscosity, and peak time were significantly affected ($p < 0.05$) by the inclusion of silverfish flour. Peak viscosity indicates the water-holding capacity of the starch or mixture [39]. The variation of peak viscosity is often associated with the swelling power of starch and the rate of disruption of the starch granules [40]. The inclusion of 10% silverfish flour halved the peak viscosity from 175.5 cP to 88 cP, and further increments in the proportions of silverfish flour resulted in a further, though less drastic decrease than the former. This implies that the inclusion of silverfish flour contributes to a reduced rate of water holding capacity and associated swelling of the starch granules. Beyond swelling, the starch granules breakdown leading to a decrease in paste viscosity, termed trough viscosity [41]. Given that the inclusion of silverfish flour reduces the swelling rate and hence the disruption rate, the trough viscosity also reduced proportionately to the reduction in peak viscosity. However, the percentage inclusion of fish flour didn't lead to further reductions in trough viscosity. The same observation was made for pasting temperature.

Given the reduced rate of disintegration of the starch granules due to the inclusion of silverfish, (a) the breakdown viscosity also decreased with increasing levels of fish flour, and the re-association (retrogradation/re-ordering) of the

Table 2 Pasting characteristics of composite flours

Sample	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	Pt (min.)	PT (°C)
NABE 3 + Spices (control)	175.5 ± 3.5^a	70.5 ± 2.1^a	112.5 ± 2.1^a	135.5 ± 3.5^a	86.5 ± 3.5^a	3.5 ± 0.1^c	51.5 ± 0.4^a
90:10	88.0 ± 1.4^b	39.0 ± 1.4^b	49.0 ± 0.0^b	89.0 ± 0.7^b	37.5 ± 2.1^c	3.1 ± 0.0^d	50.2 ± 0.0^b
80:20	67.0 ± 1.4^c	39.0 ± 1.4^b	31.0 ± 2.8^c	85.5 ± 0.7^c	50.0 ± 2.8^b	6.2 ± 0.0^b	$50.8 \pm 0.1a^b$
70:30	61.5 ± 0.7^c	40.0 ± 0.0^b	21.5 ± 0.7^d	84.5 ± 0.7^c	$45.0 \pm 0.0b^c$	7.1 ± 0.1^a	50.2 ± 0.1^b

PV Peak Viscosity, *TV* Trough Viscosity, *BD* Breakdown Viscosity, *FV* Final Viscosity, SB =Setback Viscosity, *Pt* Peak time, *PT* Pasting Temperature. Results shown are for duplicate analyses. Figures in a column having different letters as superscripts are significantly ($p \leq 0.05$) different from each other

Table 3 CIE L*, a*, b* color values for NARO-3 bean/fish composite flours

Sample	L*	a*	b*
NABE 3 + Spices (control)	68.42 ± 0.03[a]	8.81 ± 0.03[a]	28.90 ± 0.01[a]
90:10	64.14 ± 0.9[b]	8.43 ± 0.08[b]	28.14 ± 0.21[b]
80:20	61.67 ± 0.20[c]	7.71 ± 0.05[c]	27.82 ± 0.09[b]
70:30	58.32 ± 0.01[d]	6.88 ± 0.02[d]	25.62 ± 0.01[c]

Composite flours contained tomato, onion and garlic powders. Analysis was done in Duplicate. Figures in a column having different letters as superscripts are significantly ($p \leq 0.05$) different from each other

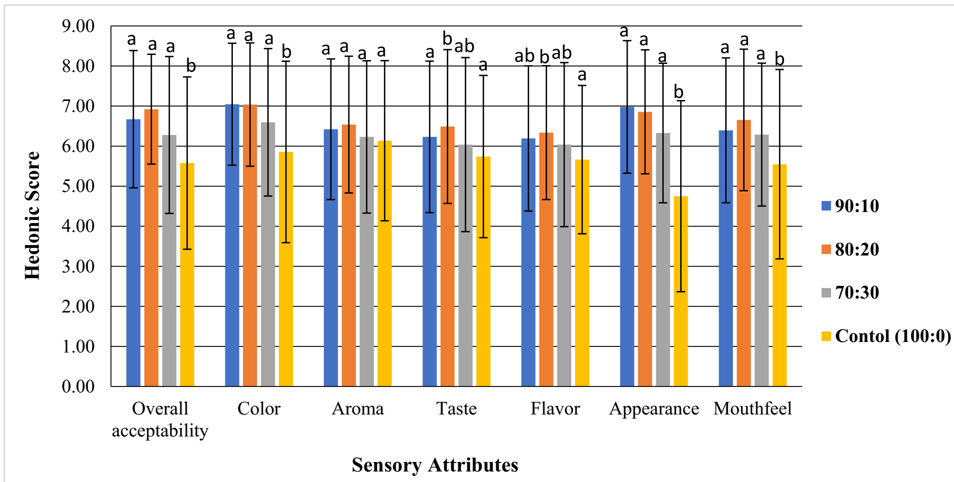


Fig. 2 Sensory acceptability of NABE-3 extruded beans with 10 (90:10), 20 (80:20) and 30 (70:30) % of the bean flour substituted with silver fish flour

starch granules was also limited, resulting in reduced setback viscosity. The final viscosity, i.e., the ability of the material to form a viscous paste/gel after cooking [39], especially for the NABE-3-silver fish composites was comparable to the peak viscosity, and for increased levels of silverfish, even higher than the peak viscosity. It corresponded to the storage viscosity when the sauce was left to cool at room temperature. Peak time, i.e., the time to reach peak viscosity, increased with an increased proportion of silverfish flour, hence reduced amounts and thus the capacity of the starch granules to gelatinize. This indicates utilisation of more energy to bring about gelatinization [42] which might imply increased cooking times and associated energy costs.

3.3 Colour

CIE L*a*b* colour space is the most frequently used colour space for colour measurement of foods due to its uniform colour distribution and its perception of colour being close to that of the human eye [43]. The L*a* and b* values of the NABE-3-silver fish composite flours are presented in Table 3.

The L* value corresponds to the degree of lightness, i.e., 100 being a white object and 0 being a black object. The degree of lightness decreased as the proportion of silverfish increased from 0 (control) to 30% (70:30). The a* value represents the green–red axis while the b* values indicated the blue-yellow axis, with positive values being red and yellow respectively. It was observed that the degree of yellowness and redness of the samples reduced as the level of fish inclusion increased, though remaining positive. The tendency of the flour colour to reduce in lightness, yellowness, and redness results in a product being darker in colour, which is consistent with the trained panel observations of the reconstituted sauces during descriptive analyses. For example: (i) For flours, the panel observed an increase in brownness from a relatively lighter toasted soy flour colour to a coconut seed brown colour and finally, the ginger skin brown colour as the proportion of fish flour increased. (ii) For sauces reconstituted from the composite flours, the panel observed a shift from medium roasted ground nut paste (lighter in colour) to progressively darker shades of brown, i.e., Nuttela chocolate brown for 90:10, highly roasted groundnut paste for 80:20 and a mixture of peanut (with skin) and simsim butter colour for 70:30. According to the hedonic scores, consumers had a preference for light coloured shades of brown, i.e., the control sample and 90:10 had higher scores than 80:20 and 70:30 NABE 3-silver fish composites.

3.4 Sensory properties

3.4.1 Sensory acceptability of extruded bio-fortified bean-silver fish flour sauce

The sensory acceptability of the sauces reconstituted from NABE-3 bean-silver fish composite flours is shown in Fig. 2.

It is observed that the overall sensory acceptability of the 90:10 bean source was 6.7, which can be interpreted as liked moderately, on a 9-point hedonic scale.

Overall acceptability wasn't significantly ($p > 0.05$) different from that of other combinations (80:20 and 70:30). The extruded beans without silver fish flour, i.e., the control with 100% bean flour had the least overall acceptability score, which was on average 5.6 (thus consumers liking the product slightly). With exception of the taste attribute in which the 80:20 sauce scored significantly ($p < 0.05$) higher (and thus better) than that of 90:10, acceptability of all other attributes did not differ significantly ($p > 0.05$) for the composite flours. Similarly, their acceptability averages range between 6 and 7, indicating that the consumers liked them slightly or moderately. Without basing on the statistics and for all the attributes, the acceptability scores of the 70:30 composite flour were always lower than those of 80:20 and 90:10. On the other hand, besides the absence of a consistent pattern with regard to scores for 80:20 and 90:10 samples, there was also no statistically significant difference among the scores for all the attributes between these two composite flours. This implies that both flours 80:20 and 90:10 were equally acceptable to the consumers.

3.4.2 Qualitative descriptive analysis of the composite flours

The trained panel agreed on the following terms to describe the colour and aroma of the flour products as indicated in Table 4.

Colour and aroma were regarded as the most important attributes of composite flours. This is because, besides price, these were the immediate attributes the consumer would use to judge the quality of the product during purchase. As the proportion of fish increased in the sample, the fishy aroma masked the beany aroma to finally become dominant in the 70:30 sample. The 90:10 sample had a balanced aroma between fish and beans, while 80:20 and 70:30 had a fishy and salty aroma. All the samples had a brown colour of different shades as elaborated in Table 4.

Upon reconstitution of the composite flour samples, it was observed that the control sample had a burnt bean aroma, flavour, and taste. This is most likely the reason why it had the lowest overall acceptability and the lowest scores for all the other attributes (Fig. 1). The burnt aroma most likely resulted from exposing the beans to high extrusion temperatures.

Like observations about the aroma of the flour samples, the reconstituted 70:30 sample had a predominantly silver fish flavour and taste. The beany aroma dominated the control and 90:10 sauces, while a bean-fish aroma and fish aroma dominated the 80:20 and 70:30 samples respectively. Also, as the proportion of fish increased and thus dominated flavour and taste, the beany taste and flavour could not be recognised by the panelists. Although changes in the processing operation might lead to a change in the undesirable burnt aroma, masking this aroma with another ingredient is a possibility, as already exemplified by the fish.

4 Conclusion

Increasing proportions of silverfish led to increased bulk density signaling increased nutrient density but might be relatively difficult to reconstitute due to reduced water absorption (reconstitution) capacity. The increased peak time of the composite flours as the level of silverfish proportions increased signaled increased energy requirements during preparation. Due to the reductions in oil absorption capacity with increasing silverfish proportions, supplementation of the flour with oil-based pre-mixes may become increasingly difficult to sustain as the levels of silverfish increase in the composite.

Table 4 Qualitative Descriptive Analysis of selected sensory attributes of the extruded bean-fish flour and sauce

Sensory Parameter	Composites			
	NABE 3 + spices (control)	90:10	80:20	70:30
Flour				
Aroma	Boiled Soup of dried Nambaale Beans	Mild bean-fish	Mild fishy	Sprat fish (Nkejje)
Colour	Toasted soy flour brown OR 100% durum sata lucia pasta brown	Toasted soy flour brown	Coconut shell brown	Whole ginger powder brown
Sauce				
Aroma	Extremely Burnt boiled bean	Burnt Boiled dry beans	Mild boiled bean-silver fish	Mild bean-silver fish
Colour	Medium Roasted groundnut paste (Kipooli)	Nuttella chocolate	Highly Roasted groundnut paste (kipooli)	Peanut (with skin) butter simsim mixture
Flavour	Burnt beens flavour	Burnt beans flavour	Burnt silverfish	Mukene flavor
Taste	Burnt-like beans	Burnt-like been	Silverfish-bean taste	Mukene taste
Mouthfeel	Smooth	Slightly particulate	Slightly particulate	Gritty/particulate

In terms of sensory acceptability, most consumers preferred lighter brown composites which had lower proportions of fish silver-flour, i.e., 90% BF: 10% SFF and 80% BF: 20% SFF than darker coloured composites with higher proportions of silver fish (70% BF: 30% SFF). In effect, both the 90:10 and 80:20 composite flours were acceptable to the same degree by the consumers. Going forward, future studies on nutrient enhancement, especially on the 80:20 and 90:10 composites, should be further considered and refined for use. Beyond these ratios, technological applications to increase reconstitution (functionality) and sensory acceptability should be explored.

Author contributions G.S: conceptualization, methodology, investigation, writing—original draft preparation. S. H: investigation. K.N: writing—reviewing and editing. E.G.M: investigation. D. M: investigation. P. R: writing- reviewing and editing. H.K: writing- reviewing and editing. L.W: writing—reviewing and editing, project administration, funding acquisition. J.J.M: writing—reviewing and editing, project administration, funding acquisition.

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Data availability Raw data is available upon request from the authors.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate This study and its protocols involving human participants were approved by the Makerere University School of Health Science Research and Ethics Committee (REC) following the 1964 Helsinki Declaration. The REC number is MAKSHREC-2020-8.

Informed consent Informed consent was obtained from all individual participants included in the study.

Competing interests The authors declare no competing interests.

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