SRYAHWA PUBLICATIONS

Research Journal of Food and Nutrition
ISSN: 2637-5583 | Volume 8, Issue 1, 2025
https://doi.org/10.22259/2637-5583.0801001

RESEARCH ARTICLE

Silver Cyprinid Fish-Enriched Snack for Pregnant Women's Nutrient Supplementation

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Received: 10 February 2025 Accepted: 25 Feberuary 2025 Published: 28 Feberuary 2025

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Abstract

Pregnant women in developing nations face significant micronutrient deficiencies, due to inadequate nutrition, insufficient nutrient supplementation, and increased nutritional requirements for fetal growth and development. A potential solution to this issue is the provision of nutrient-dense foods such as snack bars. This study used silver cyprinid, a small pelagic fish, as a novel ingredient in the formulation of fish-enriched bars to address the micronutrient needs of pregnant women. The fish was processed through ashing, steam-blanching, drying, extrusion, and roller-milling to produce roller-milled extruded fish. Fish-enriched bars were formulated by combining roller-milled fish with vegetable oil, salt, sugar, corn flour, water, and vanilla essence, with optional additions of caramelized sugar or honey. The bars were baked at 180°c for 10 minutes, cooled, and packaged for subsequent nutrient, microbial safety, and shelf stability assessment. The bars were nutritionally rich, containing 25g protein, 325mg calcium, 615mg phosphorus, 223mg Magnesium, 27mg iron, 11.6mg of zinc, 0.3mg copper, <0.05 g of Selenium, 276.71mg sodium and 895.45 mg potassium per 100g. Fish bars with honey were highly favored, with sensory scores ranging from 6.56±0.96 to 7.13±0.89 for texture, appearance, aroma, taste, and acceptability. The bars were safe with total aflatoxin of 1 mg/kg, log total plate count of 4.6cfu/g, and below detection limit for *total coliforms, salmonella, clostridium perfringens, Listeria monocytogenes, yeast, and mold*. Moreover, lipid peroxidation levels were in acceptable ranges after 6 months of storage. In conclusion, fish bars were wholesomely capable of meeting pregnant women's nutritional requirements but digestibility and clinical trial results need investigation.

Keywords: *Rastrineobola argentea*, Undernourishment, Fish-Enriched Bar, Peroxidation, Micronutrient Deficiencies, Roller Mill-Extrusion.

1. Introduction

In low- and middle-income countries (LMICs), maternal undernutrition is prevalent due to the inability of traditional diets to meet the nutritional requirements of pregnant women (Lee et al., 2013). Worldwide, more than 40% of expectant mothers are at risk for nutrient deficiencies, with 40.1% suffering from anemia, affecting 35.3 million pregnant women (IFPRI, 2020). Undernutrition among pregnant women is one of the leading causes of low birth weight (Beveridge et al., 2013; Masette, 2013). Infants born with low birth weight (LBW) (2500 g) are typically

Citation: Mary Namwanje, Agnes Nandutu Masawi, Margaret Masette, *et al*. Silver Cyprinid Fish-Enriched Snack for Pregnant Women's Nutrient Supplementation. Research Journal of Food and Nutrition. 2025;8(1):1-15.

considered at higher risk for early-onset iron deficiency (Berglund et al., 2020), growth retardation, impaired cognitive development, susceptibility to infections, developmental delays, increased morbidity, mortality during infancy and childhood, and non-communicable diseases (NCDs) in later life (Anil et al., 2020; Negrato & Gomes, 2013).

Despite progress, in 2020, approximately 14.7% (19.8 million) of infants worldwide were born with low birth weight (LBW), with the majority occurring in South Asia and Sub-Saharan Africa (UNICEF & WHO, 2023). The average annual risk reduction (AARR) for LBW has remained at 1% for over ten years (Blencowe et al., 2019), potentially hindering the achievement of the World Health Assembly's 2025 goal to decrease LBW prevalence by 30% (UNICEF & WHO, 2019). Therefore, the current AARR needs to be tripled to achieve the World Health Assembly target (UNICEF & WHO, 2019). While this is a global objective, progress varies among countries and regions. However, insufficient data from some nations, such as Uganda, impedes accurate progress tracking (UBOS & ICF, 2018; UBOS, 2019; UNICEF & WHO, 2023). Nonetheless, addressing the maternal undernutrition burden and reducing LBW incidence requires a holistic approach that tackles immediate (disease and malnutrition), underlying, and fundamental causes of LBW and maternal undernutrition during pregnancy (United Nations, 2023).

The high burden of LBW is biologically linked to the high rates of maternal malnutrition (Beveridge et al., 2013; IFPRI, 2015; Masette, 2013). Lower maternal body mass index (BMI) and shorter stature are linked to increased risks of various adverse pregnancy outcomes, including small for gestational age (SGA) and preterm birth, which are the two primary factors contributing to LBW (Kozuki et al., 2015; Madanijah et al., 2016; Rahman et al., 2015; UNICEF, 2023). In Uganda, 38% of expectant mothers suffer from anemia, with an additional 19% experiencing mild anemia and 10% of children born being underweight (UBOS & ICF, 2018). The typical Ugandan diet is predominantly based on staples, with plantains and roots or tubers (425–700 g/day) providing the majority of energy, followed by cereals. Only 11-13% of energy is derived from animal-based foods (25–60 g/day) (Harvey et al., 2010). These food items lack essential micronutrients, and the ongoing consumption of micronutrient-deficient foods by Ugandan women (Durairaj et al., 2019; GAIN & UNICEF, 2019), coupled with inadequate nutrient supplementation, frequent pregnancies, birth spacing of less than 24 months (UBOS & ICF, 2018), and poor household food and nutrition security (Atif et al., 2018), contribute to increased nutritional demand among pregnant women (Agbozo et al., 2020).

The World Health Organization's antenatal guidelines advocate for balanced energy and protein supplementation during pregnancy as a nutritional intervention in undernourished populations (WHO, 2016). This recommendation is backed by evidence showing that dietary supplementation during pregnancy enhances nutrient intake among expectant mothers and reduces the risk of stillbirth, small-for-gestational-age birth, while increasing birth weight (Lassi et al., 2019; Vanslambrouck et al., 2021; de Kok et al., 2022; Argaw et al., 2023; Ciulei et al., 2023; Gernand et al., 2023). Maltepe and Koren (2013) suggested nutritional snacking as a dietary approach for the preemptive treatment of pregnancy-related nausea and vomiting. Furthermore, utilizing bars as a snack and food matrix to deliver functional nutrients has shown positive outcomes due to increased acceptance and adherence among target groups (Nurdin et al., 2022). Snack bars encompass a variety of products often composed of cereals, pulses, fruits, nuts, and seeds, forming an ideal food format for delivering healthy nutrients, bioactive compounds, and dietary fiber to consumers. These bars can be prepared using ingredients such as soy products (Lobato et al., 2012), amaranth (Singh et al., 2022), mangoes (Lama et al., 2022), pulses (Rajagukguk et al., 2022), tempeh (Dzulhijjah et al., 2022), and whey proteins (Jabeen et al., 2021). During the production of snack bars, cereal-based raw materials can be fortified with animal food sources like milk (Gill & Meena, 2020) and fish (Sarika et al., 2020; Zulaikha et al., 2021). Additionally, snack bars can be formulated to address the nutritional and health needs of vulnerable individuals.

Small-sized fish are available, accessible, nutrient-dense, and affordable; hence, they are an excellent source of micronutrients that can enrich snack bars (Konyole et al., 2019). Uganda has several small-sized fishes, including *Rastrineobola argentea* (silver cyprinid), *Brycinus nurse* (ragogi), and *Engraulicypris bredoi* (muziri). Among these, silver cyprinid stands out, dominating the total catch and comprising the majority (96.97%) of small-sized fish (along with haplochromines) harvested from Lake Victoria, with widespread trade across East Africa (CAS, 2014).

Silver cyprinid is a nutrient-dense food, providing 406-606 kcal of energy, 54.9-79.4g of protein, 11.1-15.7g of fat, 1.27-4.87g of calcium, 0.83-2.83g of phosphorus, 5.9-45.5 mg of iron and 9.5-10.7 mg of zinc (Kigozi et al., 2020; Kubiriza et al., 2020), making it an ideal choice for fortifying snack bars. It's worth noting that the nutritional content of silver cyprinid can fluctuate based on seasonal changes, processing techniques, and preparation methods (Kubiriza et al., 2020).

Despite its nutritional value, silver cyprinid is often associated with lower-income populations (Gibson et al., 2020) and suffers from subpar processing and preservation practices (Kigozi et al., 2020; Namwanje et al., 2020; Wessels et al., 2023), which hinders its widespread use. The consumption of silver cyprinid among expectant mothers is particularly low, due to a lack of awareness regarding its nutritional benefits and a persistent preference for larger fish species, meat, and poultry. This study aims to address these issues by incorporating silver cyprinid into nutrient-dense food supplements, specifically fish-enriched bars (fish bars), to promote increased fish consumption among pregnant women through innovative products that provide essential micronutrients.

2. Materials and Methods

2.1 Raw Material Acquisition

Dried maize (*Zea mays*) was purchased from a local maize mill. Raw and ripe pawpaw (*Carica papaya*) fruits were purchased from a local food market. Freshly landed silver cyprinid fish were purchased from fishers at Kigungu landing site in Lake Victoria and transported in a cooler box to the Fisheries Training Institute Laboratory.

2.2 Preparation of the Raw Material

2.2.1 Pawpaw

The pawpaws were washed with potable running water, peeled, and chopped into smaller pieces. The raw and ripe pawpaw were mixed at a ratio of 1:1 and blended in Hoffman's Electronics super compact blender with a dry mill (HM-166, made in Germany) to form a mash mixture. The pawpaw mash was spread 1 mm thick onto the refractance window dryer at 95^{o}C for 40 min (Raghavi et al., 2018). The dried pawpaw flakes were cooled at room temperature for 5 min before packaging in sterile clean polythene bags for later use.

2.2.2 Maize Grits

Maize grains were roller-milled and water was added to increase the moisture content to 16% before extrusion.

2.2.3 Silver Cyprinid

In the laboratory, fresh silver cyprinid was washed with potable water, soaked in 7% (v/v) banana peel ash solution for 20 minutes, and allowed to drip to improve the aroma (Mohsin et al., 1999). The treated silver cyprinid was steam-blanched for 20 minutes to drip fats before sun drying on raised racks for 8 h.

2.2.4 Microbial Quality and Safety of Raw Materials

The microbial content and safety of the maize grits and dried silver cyprinid were determined before and after extrusion. Microbial quality was determined using total coliform (TC) and total plate count (TPC) determined using the pour plate techniques (ISO 4832:2006) and (ISO 4833:2013, respectively). Yeast and mold (Y&M) were determined using surface-spread techniques (ISO 21527 – 2:2009). After counting the colonies on petri dishes, colony-forming units per ml were computed as follows: (no. × dilution factor)/volume of culture plates. Colony-forming units per milliliter were then converted to colony-forming units per gram. The aflatoxin (microbial metabolite) content was determined according to USDA (2015).

2.2.5 Extrusion process

The recipes were extruded using an extruder (Jinan Eagle Food Machinery Co. Ltd. Model DP- 70- III, China) operated at a barrel temperature of 155^{o}c, a main motor speed of 1493 rpm, and a knife speed of 811 rpm. The feed moisture content in the extruder barrel was set at 16% (160 g/kg). The extrudates were dried in a convection oven at 105^{o}c for 3 min, cooled to room temperature, milled, and sieved through a 0.5 mm mesh. The extruded silver cyprinid-maize flour and extruded maize flour alone were stored in airtight sterile polythene bags at ambient temperature.

2.3 Formulation of fish-enriched bars

Three variants of the fish-enriched bars were prepared by combining different proportions of the roller-milled extruded silver cyprinid, refractance window dried paw paws, and extruded maize as shown in Table 1, depicting the experimental design of the variations for the preparation of snack bars.

Table 1. *Percentage contribution of ingredients in fish-enriched bar formulations*

Ingredients	WS1-None	WS2-Syrup	WH1-honey
Roller-milled silver cyprinid	38.68	38.68	38.68
Extruded maize	2.76	2.76	2.76
Vitamin A-fortified vegetable oil	5.16	5.16	5.16
Pawpaws	3.20	3.20	3.20
Salt	0.23	0.23	0.23
Sugar	6.91	6.91	6.91
Corn flour	4.01	4.01	4.01
Water	37.02	33.15	33.15
Caramelized sugar	0	3.87	0
Honey	0	0	3.87
Vanilla essence	1.80	1.80	1.80
Ginger	0.23	0.23	0.23

2.4 Mixing Ingredients

Water was boiled and then cooled to 60 ± 3˚C and the binding ingredient (corn starch) was added followed by continuous stirring of the mixture for 4 minutes. Other ingredients were mixed to form a paste made into rectangular shapes (1mm thick) with aluminium molds and placed on pre-greased baking trays. The rectangular bars were baked in a preheated oven at 180˚C for 10 minutes and then allowed to cool at room temperature before being packaged.

2.5 Nutrient Content, Bioavailability, and Sensory Perception of Fish-Enriched Bars

Moisture, ash, and fat contents were determined according to methods described in (AOAC, 2005). Crude protein was determined by the Kjeldahl method as described by Kirk & Sawyer (1991). Gross energy was determined by adiabatic bomb calorimetry (ISO 9831–1998: Gallenkamp auto-bomb, CABOO1.ABI.C (U.K) Ltd). Iron and zinc contents were determined using the atomic absorption spectrophotometric (Perkin Elmer 400 instrument) method following the procedure described by (Perkin-Elmer, 1996). Mineral bioavailability was computed using phytic acid content which was determined following methods described by Gao et al., (2007).

2.5.1 Sensory Evaluation of Fish-Enriched Bars

The fish-enriched bars were subjected to sensory analysis at the Food Biosciences Laboratory, National Agricultural Research Laboratories, Kawanda (Uganda). Sensory analysis was performed by sixteen trained panelists (9 females and 7 males) using a 9-point hedonic scale (Wichchukit & Mahony, 2014).

2.5.2 Shelf Life and Safety

The selected fish-enriched bars stored at ambient temperature were analyzed for lipid oxidation stability and safety within the first, second, fourth, and sixth months. The degree of lipid oxidation was assessed by assaying the free fatty acid value, peroxides (AOAC, 2005), and thiobarbituric acid according to (Osheba et al., 2010). Microbial quality was determined using total coliform and total plate count according to the pour plate techniques (ISO 4832:2006) and (ISO 4833:2013) respectively. Yeasts and molds were determined using surface spread techniques (ISO 21527 – 2:2009) as in section 2.2.3.

2.6 Statistical Data Analyses

Descriptive statistics were used to summarise data and graphs were used for visual presentation of data. Shapiro-Wilk test was used to check the normality of data before log transformation where necessary. One-way ANOVA tested for the differences in moisture, dry matter, fat, ash, zinc, iron, protein, energy, phytate, and ratios of phytate to iron, zinc, and zinc to iron between the different fish-enriched bars. One-way ANOVA was also used to test for the difference in free fatty acid value, thiobarbituric acid reactive substances, peroxide value, total coliforms, total plate count, and, yeasts and molds between the storage time (months). Tukey-HSD test identified formulae where the differences existed. Kruskal-Wallis test checked for differences in the preference of sensory attributes between fish-enriched bars. All statistical analyses were done in SPSS version 21 (IBM Corp. New York, USA).

3. Results

3.1 Microbial Quality and Safety of Raw Materials

The microbial and microbial metabolite contents of maize grits and dried silver cyprinid decreased with the extrusion of raw materials (table 2). With extrusion, there was a significant reduction in silver cyprinid's, TPC, TC, and aflatoxin contents from 5.24±0.93 to 4.05±0.09 log cfu/g, 2.53±0.67 to 0.57±1.04 log cfu/g, and 6.13 ± 1.54 to 0.00±0.00 µg/kg respectively. Maize grits' TC, Y&M, and aflatoxin contents significantly decreased from 1.70±0.25 to 0.32±0.12 log cfu/g, 3.23±0.87 to 3.03±0.36 log cfu/g, 8.32±0.21 to 0.00±0.00 µg/kg respectively (table 2).

Table 2. *Microbial quality and microbial metabolite content of raw materials*

Food material	Microbial quality	Before extrusion	After extrusion
Silver cyprinid	TPC (log cfu/g)	5.24±0.93^{b}	4.05±0.09^{a}
	TC(log cfu/g)	2.53±0.67^{b}	0.57±1.04^{a}
	Y&M(log cfu/g)	3.12 ±0.42^{a}	2.98±0.09^{a}
	Aflatoxin (µg/kg)	6.13 ± 1.54^{a}	0.00±0.00^{b}
Maize grits	TPC (log cfu/g)	4.67±0.32^{a}	4.21±0.08^{a}
	TC(log cfu/g)	1.70±0.25^{a}	0.32±0.12^{b}
	Y&M(log cfu/g)	3.23±0.87^{b}	3.03±0.36^{a}
	Aflatoxin (µg/kg)	8.32±0.21^{a}	0.00±0.00^{b}

Different superscripts (a, b) across the rows for before and after extrusion show significant differences ($p < 0.05$*) in the variable between the extrusion.*

3.2 Nutrient Content, Bioavailability, And Sensory Perception Of Fish-Enriched Bars

The moisture content of the fish-enriched bars varied between 7.18±0.36% and 13.25±0.04% but ash content ranged from 3.36±0.85 to 3.76±0.05%. Protein content ranged from 25.02±0.15 to 25.03±0.12% while fat content varied between 14.86±0.33 to 14.86±0.39%, iron varied between 26.97±0.04 to 27.01±0.04mg, and zinc varied between 11.7±0.01 to 11.8±0.00 mg, energy varied between 453.63±0.72 and 461.33±0.67 Kcal per 100g (Table 3).

Regardless of the formula, the molar ratios for phytate: iron were < 0.1, while those of phytate: zinc were < 0.3 and < 1 for zinc: iron (Table 3). The inhibiting effect of phytate on both zinc and iron absorption followed a dose-dependent response. The molar ratio of zinc: iron content of WS2 (Syrup) was significantly lower than the zinc: iron content of WH1-honey and WS1-none (Table 3).

Table 3. *Proximate composition, mineral bioavailability, and sensory attributes (mean± stdev) of fish-enriched bars for pregnant women*

Parameter	Variable	Fish-enriched bars for pregnant women			Standard
		WH1-honey	**WS2-Syrup**	**WS1-None**	
Proximate composition	Moisture (%)	13.25±0.04^{a}	7.6±0.06^{b}	7.18±0.36^{c}	<10$^{\#}$
	Ash (%)	3.36±0.85^{a}	3.6±0.45^{a}	3.76±0.05^{a}	
	Protein (%)	25.03±0.12^{a}	25.02±0.16^{a}	25.02±0.15^{a}	71*
	Fat (%)	14.86±0.33^{a}	14.86±0.39^{a}	14.86±0.38^{a}	ND
	Energy (Kcal)	461.33±0.67^{c}	456.07±0.63^{a}	453.63±0.72^{b}	
	Zinc (mg)	11.7±0.01^{a}	11.7±0.01^{a}	11.8±0.00^{a}	11*
	Iron (mg)	27.01±0.04^{a}	27.00±0.03^{a}	26.97±0.04^{a}	27*
Mineral bioavailability	Phytate: iron	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	1$^{\infty\pounds}$
	Phytate: zinc	0.06±0.00^{a}	0.06±0.00^{a}	0.05±0.00^{a}	15$^{\text{¥}}$
	Zinc: iron	0.41±0.03^{a}	0.34±0.01^{b}	0.40±0.01^{a}	
Sensory attributes	Appearance	6.81±0.98^{a}	6.42±0.86^{b}	6.31±1.40^{b}	
	Taste	6.75±1.39^{ab}	6.53±1.75^{cb}	6.13±2.25^{cd}	
	Texture	6.56±0.96^{ab}	6.67±1.26^{b}	6.56±1.86^{ab}	
	Aroma	7.13±0.89^{a}	6.56±1.88^{b}	6.56±1.67^{b}	
	Overall acceptability	7.13±0.81^{ab}	6.86±1.39^{bc}	6.56±1.59^{c}	

**RDA adopted from (Brown, 2011), US 908:2013, (Hallberg et al., 1989; Gibson et al., 2010;) (Morris & Ellis, 1989), (Siegenberg et al., 1991). ND- not defined*

The hedonic values of the fish-enriched bars' sensory perception for appearance, aroma, taste, texture, and general acceptability are shown in Table 2. The range of attributes scores by trained panelists were: 3.88 to 8.14 for taste, 4.91 to 7.79 for appearance, 4.68 to 8.02 for aroma, 4.70 to 7.93 for texture, and 4.97 to 7.94 for overall acceptability. The sensory properties of fish-enriched bars were significantly different ($p<0.05$) across formulations (Table 3).

The fish-enriched bar (WH1-honey) formula containing 38.7% roller milled silver cyprinid, 2.8% extruded maize, 5.2% oil, 3.2% pawpaws, 0.2% salt, 6.9% sugar, 4.0% corn flour, 37.0% water, 3.9% honey, 1.8% vanilla essence and 0.2% ginger scored highest by panel members for appearance (6.81±0.98), aroma (7.13±0.89), taste (6.75±1.39), sensory texture (6.56±0.96) and overall acceptability (7.13±0.81).

3.3 Nutritional Composition and Microbial Quality of the Selected Fish-Enriched Bars

Fish-enriched bars (WH$_1$-honey) had the most outstanding nutritional composition with high micro-mineral bioavailability, and sensory scores (Table 2). It was followed by WS$_2$-Syrup and WS$_1$-None in that descending order. Fish-enriched bars (WH$_1$-honey) were therefore selected for further nutritional composition and microbial analysis. Fish-enriched bars (WH$_1$-honey) had a high macro-and micro-nutrient content (Table 3). Moreover, the microbial content of the Fish-enriched bar (WH$_1$-honey) was low and acceptable for supplements meant for pregnant women (Table 3).

Table 4. *Nutrient composition and microbial content of the fish-enriched bar (WH$_1$-honey)*

	Parameters	Results	Standard	RDA*
[b] Chemical parameters	Moisture (%m/m)	10	<10	
	Protein	24.85		71
	Fat content	15.08		
	Ash content	5.8		
	Gross energy (Kcal/100g)	460.8		
	Phosphorus (mg/100g)	615	300-600 (N/A)	700
	Calcium (mg/100g)	325	300-600	1000
	Magnesium (mg/100g)	223	80-140	25
	Iron (mg/100g)	27	10-14	27
	Zinc (mg/100g)	11.6	11-14	11
	Copper (mg/100g)	0.3	1.4-1.8	1000
	Selenium (g/100g)	<0.05	20-40	60
	Sodium (mg/100g)	276.71	<290	1.5
	Potassium (mg/100g)	895.45	1110-1400	470*
[a] **Microbiology**	Total aflatoxin (mg/kg)	1	<5	
	Total coliforms	0	0	
	Salmonella spp. (25g)	Not detected	Negative	
	Clostridium perfringens (cfu/g)	<10	Negative	
	Yeasts and moulds (cfu/g)	<10	<1000	
	Listeria monocytogenes (25g)	Not detected	Negative	
	Total plate count (cfu/g)	42000	<100000	

US 908:2013, UG2021-0003, phosphorus excluded phytates, <10 cfu/g is equivalent in meaning to 'not detected in 1g', N/A means 'not available'

3.3.1 Oxidation of fish-enriched bar (WH$_1$-honey) over the 6 months of storage

Storage duration affected the lipid quality of fish-enriched bar (WH$_1$-honey) through oxidation (Figure 1).

The FFA concentration of fish-enriched bar (WH$_1$-honey) generally increased from 1.32 ± 0.06 to 2.14 ± 0.00 mgKOH/g over the storage duration. Peroxide value (PV) increased from 2.76 ± 0.50 to 9.20 ± 0.70 mEqO$_2$kg. TBARs content of fish-enriched bar (WH$_1$-honey) was about 2.38 ± 0.07 mgMDA/Kg immediately after processing but increased with storage time to 5.94 ± 0.27 mgMDA/Kg in 6 months of storage.

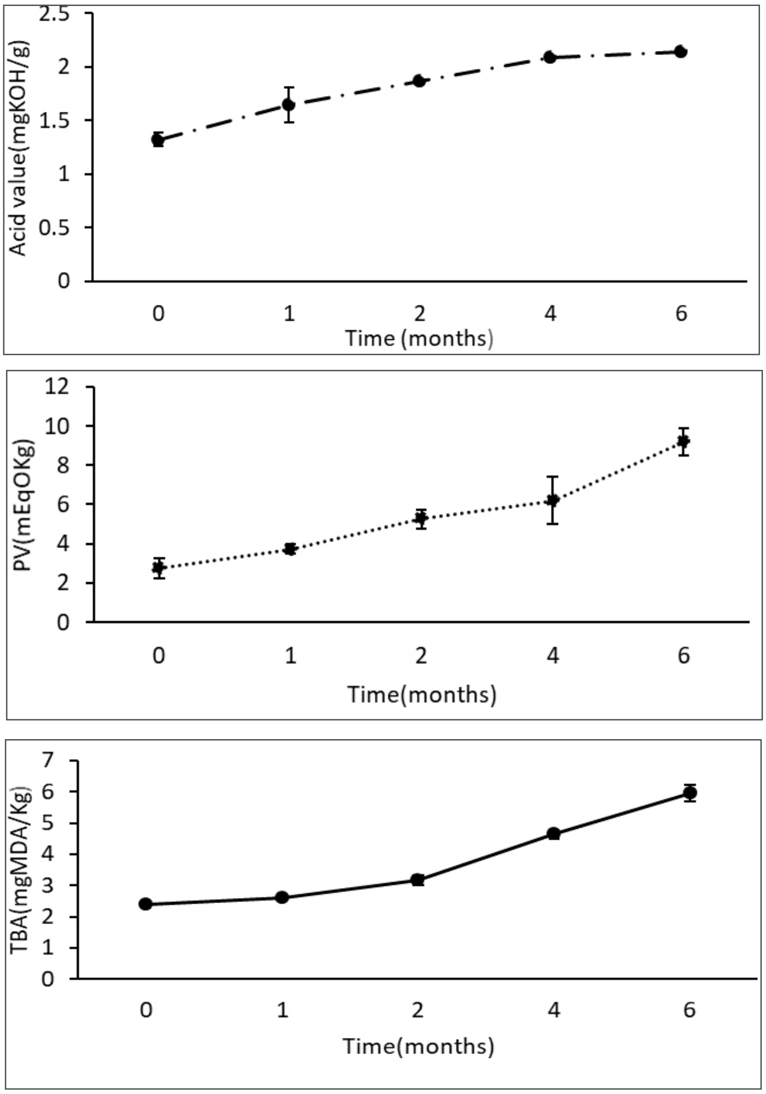

Figure 1. *Oxidation product levels of the fish-enriched bar (WH1-honey) during the 6 months of storage (Error bars represent standard deviation)*

3.3.2 Microbial Quality of Fish-Enriched (Wh_1-honey) Bar Over the 6 Months of Storage

During storage, TPC decreased from 4.06 ± 0.16 log cfu g^{-1} to 3.42 ± 0.03 log cfu g^{-1}, but TC was not detected and yeast and mold contents decreased from 3.34 ± 0.14 log cfu g^{-1} to 2.90 ± 0.26 log cfu g^{-1} (Figure 2).

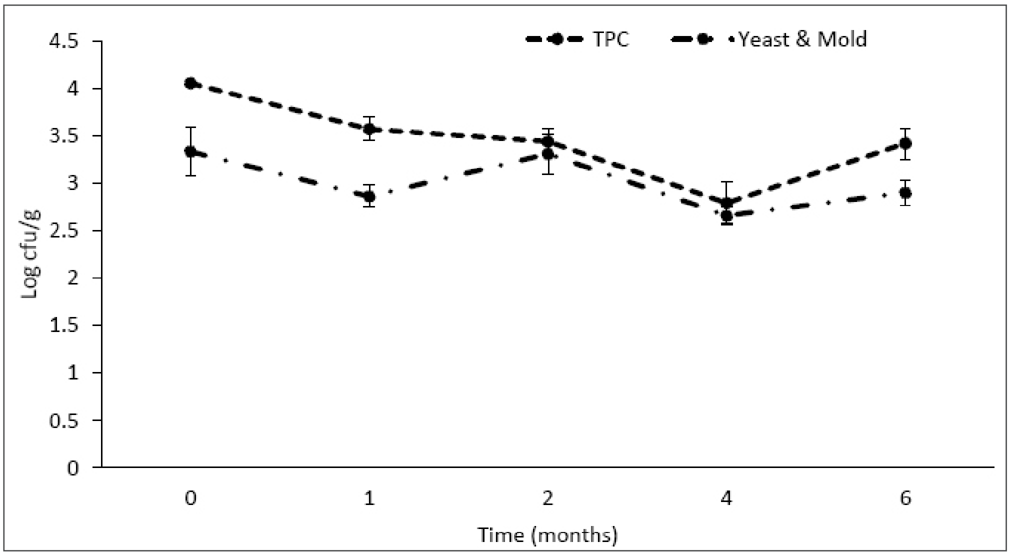

Figure 2. *Microbial quality of fish-enriched bar (WH_1-honey) over the 6 months of storage. (Error bars represent standard deviation)*

4. Discussion

4.1 Nutrient Composition of Fish-Enriched Bars

The moisture content of the fish-enriched bars was below the recommended level (12 %) for safe keeping of cereal grains and dried silver cyprinid powder (Codex Alimentarius, 1995; EAS, 2014). Likewise, it was below the optimum (15%) for microbial growth (Glucas, 1982; EAS, 2014). The moisture content in fish-enriched bars was within the range of snack bar moisture content (Farouk Abdel-salam et al., 2022; Singh et al., 2022). The observed low moisture content of extruded fish-enriched bars may be due to the high temperature of the extrusion cooking process up to 155°C for less than 5 minutes.

The bars formulated in this study were generally rich in proteins, and lipids high in iron and Zinc. Overall the energy content of the WHI bar was high. Assuming 100% bioavailability, 100g of fish-enriched bar (WH_1-honey), a day would meet all the iron requirements for a successful pregnancy (Brown, 2011). Therefore, fish-enriched bars can provide sufficient micronutrients to meet the daily needs for the growth and development of a fetus, maintenance of immunity, and prevention of iron-deficient anemia among pregnant women. Nonetheless, the high protein content of fish-enriched bars can be attributed to the high protein content of silver cyprinid as compared to other plant food materials. Thus, fish-enriched bars contained more protein than snack bars made from plant proteins (Pande & Deo, 2018; Dzulhijjah et al., 2022; Farouk Abdel-salam et al., 2022; Rajagukguk et al., 2022; Singh et al., 2022), whey-protein (Jabeen et al., 2021), or enriched with milk (Gir & Mridula, 2016) and fish (Hughes et al., 2012).

The fat content of fish-enriched bars was higher mean value than the standard value of 14% (UG, 2021-0003). With the addition of silver cyprinid the fat content of snack bars was higher than fat contents of tilapia hydrolysate by-product (Zulaikha et al., 2021), and fish oil enriched bars (Hughes et al., 2012) but lower than fat content of tilapia by-product enriched snack bars (Zulaikha et al., 2021). Low fat content is a typical characteristic of hydrolysates because during hydrolysis process, membrane cells assemble and form insoluble vesicles which result in the removal of membrane structured lipids (Kristinsson & Rasco, 2000).

Energy content of the fish enriched bars varied with variation in bar formulation. Fish-enriched bars had more energy, compared to snack bars made from legumes (Farouk Abdel-salam et al., 2022; Rajagukguk et al., 2022), tilapia hydrolysate by-products (Zulaikha et al., 2021), and sesame (Pande & Deo, 2018). The high energy content of fish-enriched bars can be attributed to the role of a combination of steam blanching and extrusion in producing energy dense foods (Ragaee et al., 2014; Delimont, Chanadang, et al., 2017; Delimont, Fiorentino, et al., 2017; Araro et al., 2020).

The ash content of fish-enriched bars was within the permissible limit of 15% for dry silver cyprinid products (EAS, 2014; UG2021-0003, 2021). Moreover, studies that utilized banana peel powder concentrations to improve the physico-chemical properties of fish products, indicated the ash (total ash and ash insoluble) content of silver cyprinid to be within acceptable limits (Zaini et al., 2019). Ash represents the mineral material in flour (AACC, 2000), and is affected primarily by the ash content of the raw materials from which it was milled and its milling extraction (Kent & Evers, 1994). Fish-enriched bars in this study contained higher ash when compared with snack bars made from plant proteins (Dzulhijjah et al., 2022; Farouk Abdel-salam et al., 2022; Rajagukguk et al., 2022) or enriched with milk (Gir & Mridula, 2016) and fish (Hughes et al., 2012; Zulaikha et al., 2021). However, the amaranth oat, and BPP snack bar prepared by Singh et al. (2022) had higher ash content as compared to the formulated fish-enriched bar. The high ash content of BPP enriched snack bars can be attributed to the high ash content of BPP as compared to small pelagic fish (Virkar et al., 2019; Zaini et al., 2022).

The fish-enriched bars contained sufficient zinc and iron to meet the recommended daily allowance for pregnant women as mentioned by Brown (2011). Hence, the fish enriched bars were classified as iron-rich according to the FDA standard of 5-35 mg of iron contribution per day. Fish-enriched bars in this study had higher iron content than fish enriched bars by Shirazi et al. (2012), as well as amaranth, oat and BPP snack bars of Singh et al. (2022). Likewise, fish enriched bars had higher iron and zinc content than legume bars reported in studies of Farouk Abdel-salam et al. (2022) and Rajagukguk et al. (2022).

The desirable molar ratios of phytate: minerals that is to say; phytic acid: iron (< 1), and phytic acid: zinc (<15) (Lazarte et al., 2015) were achieved by all fish-enriched bars. Therefore, all fish-enriched bars had highly bioavailable iron which could readily be absorbed and utilized by the human body. The lower the value of phytic acid: mineral ratio, the higher the bioavailability of these micro nutrients (Olivares et al., 2007; Gibson et al., 2010). Zinc did not affect the iron bioavailability since zinc to iron ratio was low (<20) in fish-enriched bars (Kondaiah et al., 2019). The high mineral bioavailability of fish-enriched bars is attributed to the improved ability of extrusion to reduce factors that inhibit absorption (Nkundabombi et al., 2016; Hackl et al., 2017; Penugonda et al., 2018).

4.2 Sensory Quality Perception of Fish-Enriched Bars

The sensory attributes of the fish-enriched bars were liked by the panelists and WH1-honey was more accepted, compared to the other formulations. Moreover, sensory scores of 6-7 have been previously considered good for snack bars (Aramouni & Abu-Ghoush, 2011; Hughes et al., 2012; Lama et al., 2022). The baking process develops flavor, aroma and colour due to the browning effect and the formation of colored complexes from Maillard reactions in the bar (Zulaikha et al., 2021; Singh et al., 2022). The sensory scores were similar to the sensory values obtained among fish oil fortified bars (Hughes et al., 2012), sesame enriched bars (Pande & Deo, 2018), potato extrudates bars (Gir & Mridula, 2016), and oat meal enriched bars (Singh et al., 2022).

4.3 Safety of Fish-Enriched Bars

The developed fish-enriched bars were generally safe for consumption by pregnant women.

4.3.1 Microbial Safety of the Fish-Enriched Bars (Wh$_1$-honey)

The microbial content of the fish-enriched bar (WH$_1$-honey) was low (*Salmonella* not detected, 0 cfu/g of total coliforms, <10 cfu/g yeasts and mould) and acceptable for fish bars that are to be consumed by pregnant women. Moreover, the total aflatoxin content of fish-enriched bar (WH$_1$-honey) was within acceptable limits for consumption.

Microbial load (total plate counts, yeast and moulds) decreased over the 6-month storage under ambient conditions. In agreement with the present findings, microbial content of dry silver cyprinid reduced over the 8 weeks of storage at ambient temperature (Namwanje et al., 2021). Since moisture content was below 15% the microbes could not thrive and thus their populations decreased over the 6 months in store. Earlier findings by Singh et al. (2022) and Jabeen et al. (2021) revealed a similar trend when snack bars were stored. Total plate counts, yeast and moulds, as well as total coliforms were within the acceptable limits of 5, 4 and 0 log cfu/g respectively, set by East Africa Community (EAS, 2014). The low yeast and moulds content in fish-enriched bar (WH$_1$-honey) could suggest that its consumers were not at risk of mycotoxin ingestion (Nnagbo et al., 2018) which could cause immunity suppression, decreased growth, malnutrition and mortality (Gong & Hall, 2008). Therefore, over the 6 months' storage duration, fish-enriched bar (WH$_1$-honey) stored at room temperature were microbiologically acceptable just like fish bars under cold storage (Sarika et al., 2020).

4.3.2 Lipid Oxidation of Fish-Enriched Bar (Wh$_1$-honey) over the 6 Months of Storage

Lipid oxidation products (free fatty acid value, peroxides value and TBARs) increased gradually over the 6 months of storage under ambient conditions (Figure 3). A similar increase in lipid oxidation products was observed when Sarika et al. (2020) kept fish bars under cold storage for up to 12 months or when fish granola bars were stored under varied conditions (Sarika et al., 2016). However, over the 10 weeks storage duration for fish-oil-enriched granola bars at 20^{o}C peroxide value increased above 5mEqO$_2$kg (Shirazi et al., 2012). The minimal oxidation in fish-enriched bars as opposed to fish oil enriched bars can be attributed to low levels of polyunsaturated fatty acids in bars as compared to fish oil. Lipid oxidation contents below 3 mgKOH/g, 111.0 µmolMDA/kg and 5 mEqO$_2$kg are considered food-grade quality (Codex, 2013, 2017). In the current study, lipid oxidation product values were within the requirement by the standard and thus fish-enriched bars (WH$_1$-honey) were fit for consumption by pregnant women.

5. Conclusion

Locally available food materials commonly used in Uganda (silver cyprinid, maize and pawpaws) were successfully used to formulate and develop a highly nutritious, 6 months' shelf stable fish-enriched bars. This product matches the requirements for supplementation of pregnant women and has the potential to contribute to the nutrient intake and overall health of pregnant women in Uganda and the world at large. However, further studies will be needed to examine the amino acid profile, mineral digestibility, nutritional effect of consumption of fish-enriched bar among pregnant women. Also, antioxidants could be employed to reduce or halt the rate of lipid oxidation.

Funding: This work was carried out with financial support from the Australian Center for International

Agricultural Research (ACIAR) and Canada's International Development Research Center (IDRC), Cultivate Africa's Future Fund Phase 2 (CultiAF-2), Project #109041.

Author Contributions: "Conceptualization, MN, ANM , MM, EB, GKK, JE, KST, NMS, RO; methodology, MN, ANM , MM, EB, GKK, KST, NMS; software, MN, GKK, KST, NMS.; validation, MN, GKK, KST, NMS; formal analysis MN, GKK, KST, NMS.; investigation, MN, GKK, KST, NMS.; resources, MN, ANM , MM, EB, GKK, JE, KST, NMS, RO; data curation, MN, GKK, KST, NMS.; writing—original draft preparation, MN, GKK, KST, NMS; writing—review and editing, MN, ANM, MM, EB, GKK, JE, KST, NMS; visualization, MN, GKK, KST, NMS.; supervision, ANM, MM, EB, project administration, JE, RO; funding acquisition, JE, RO.

Data Available: Data will be made available data available on reasonable request from the corresponding author

Conflicts of Interest: The authors declare no conflicts of interest. Funding sources were not involved in the study design, collection, analysis, interpretation of data, writing of the report, or submission of the article.

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