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PREPARATION OF COOKIES ENRICHED WITH EDIBLE MUSHROOM FLOUR (*Suillus luteus*) AND BOVINE BLOOD MEAL

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ABSTRACT

Iron deficiency anemia continues to represent a significant public health challenge, particularly in developing countries, due to its negative impact on physical development, cognitive performance, and overall quality of life. This situation underscores the importance of developing innovative, nutritionally enhanced food products that are both effective in improving iron intake and acceptable to consumers. Bakery products, such as cookies, offer an attractive vehicle for nutritional fortification because of their wide consumption, long shelf life, and ease of distribution. Within this framework, the present study aimed to develop functional cookies enriched with edible mushroom flour (*Suillus luteus*) and bovine blood flour, to improve their nutritional profile—especially protein and iron content—while preserving appropriate sensory characteristics. To support the formulation process, the raw materials were first subjected to proximate chemical analysis to determine their moisture, protein, fat, fiber, ash and carbohydrate contents. Based on these results, four experimental cookie formulations were prepared using wheat flour as the main structural base, mushroom flour as a source of bioactive compounds, and bovine blood flour as a fortifying ingredient rich in heme iron and essential amino acids. All formulations contained a fixed proportion of 95% wheat flour and 5% mushroom flour, while the concentration of bovine blood flour varied as follows: F0 (0%), F1 (4%), F2 (5%), and F3 (6%). The cookies were produced under standardized technological conditions, allowing for consistent evaluation of their nutritional, physicochemical, microbiological and sensory properties. The results demonstrated that increasing the level of bovine blood flour significantly enhanced the nutritional value of the cookies. Among the formulations tested, F3 exhibited the most favorable balance between nutritional improvement and consumer acceptability, achieving the highest sensory evaluation scores. This optimized formulation showed a protein content of 17.76% and an iron concentration of 16.44 mg/100 g, levels that meet and exceed the recommended daily iron intake. Microbiological analyses confirmed the safety and hygienic quality of the product, with microbial counts below established limits. Overall, these findings indicate that cookies enriched with edible mushroom flour and bovine blood flour constitute a promising functional food, combining animal and fungal protein sources to support nutritional improvement, food innovation, and sustainable dietary strategies aimed at addressing iron deficiency anemia.

Key words: Heme iron, edible mushrooms, fortified cookies, anemia, hemoglobin

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RÉSUMÉ

L'anémie ferriprive continue de représenter un défi majeur pour la santé publique, en particulier dans les pays en développement, en raison de son impact négatif sur le développement physique, les performances cognitives et la qualité de vie en général. Cette situation souligne l'importance de développer des produits alimentaires innovants et enrichis sur le plan nutritionnel, qui soient à la fois efficaces pour améliorer l'apport en fer et acceptables pour les consommateurs. Les produits de boulangerie, tels que les biscuits, constituent un vecteur intéressant pour l'enrichissement nutritionnel en raison de leur large consommation, de leur longue durée de conservation et de leur facilité de distribution. Dans ce cadre, la présente étude visait à développer des biscuits fonctionnels enrichis en farine de champignons comestibles (*Suillus luteus*) et en farine de sang bovin, dans le but d'améliorer leur profil nutritionnel, en particulier leur teneur en protéines et en fer, tout en préservant leurs caractéristiques sensorielles appropriées. Pour faciliter le processus de formulation, les matières premières ont d'abord été soumises à une analyse chimique approximative afin de déterminer leur teneur en humidité, en protéines, en graisses, en fibres, en cendres et en glucides. Sur la base de ces résultats, quatre formulations expérimentales de biscuits ont été préparées en utilisant de la farine de blé comme base structurelle principale, de la farine de champignons comme source de composés bioactifs et de la farine de sang bovin comme ingrédient enrichissant riche en fer héminique et en acides aminés essentiels. Toutes les formulations contenaient une proportion fixe de 95 % de farine de blé et 5 % de farine de champignons, tandis que la concentration en farine de sang bovin variait comme suit: F0 (0 %), F1 (4 %), F2 (5 %) et F3 (6 %). Les biscuits ont été produits dans des conditions technologiques standardisées, ce qui a permis une évaluation cohérente de leurs propriétés nutritionnelles, physicochimiques, microbiologiques et sensorielles. Les résultats ont démontré que l'augmentation de la teneur en farine de sang bovin améliorait considérablement la valeur nutritionnelle des biscuits. Parmi les formulations testées, la F3 présentait l'équilibre le plus favorable entre l'amélioration nutritionnelle et l'acceptabilité par les consommateurs, obtenant les scores d'évaluation sensorielle les plus élevés. Cette formulation optimisée présentait une teneur en protéines de 17,76 % et une concentration en fer de 16,44 mg/100 g, des niveaux qui satisfont et dépassent l'apport quotidien recommandé en fer. Les analyses microbiologiques ont confirmé la sécurité et la qualité hygiénique du produit, avec des counts microbiens inférieurs aux limites établies. Dans l'ensemble, ces résultats indiquent que les biscuits enrichis en farine de champignons comestibles et en farine de sang bovin constituent un aliment fonctionnel prometteur, combinant des sources de protéines animales et fongiques pour soutenir l'amélioration nutritionnelle, l'innovation alimentaire et les stratégies alimentaires durables visant à lutter contre l'anémie ferriprive.

Mots clés: Fer héminique, champignons comestibles, biscuits enrichis, anémie, hémoglobine



INTRODUCTION

One of the persistent public health challenges in Peru is anemia [1,2]. Anemia results in low academic performance [3, 4] due to fatigue, physical impairment and impaired growth [5]. It also constitutes a major cause of infant, maternal and perinatal mortality. At the national level, anemia affects 42.8% of children, while chronic malnutrition reaches 12.2% [1]. In the department of Lambayeque, Peru 36.2% of children aged 5 to 35 months suffer from anemia.

A significant proportion of anemia cases can be mitigated through the consumption of iron-rich foods. Heme iron substantially increases hemoglobin levels, as its absorption exceeds 50% and does not require cofactors [5]. Bovine blood meal is a rich source of heme iron and lysine, one of the most essential amino acids for human development. Lysine supplementation improves growth parameters in children and adolescents by increasing height, weight, and body mass index [7].

Bovine blood is largely utilized for its high iron content, which adds value to this by-product. It is commonly incorporated into foods such as blood sausages and other meat fillings. Additionally, bovine blood meal can be incorporated into bakery products such as cookies, breads, cakes and other flour-based goods [8].

Another ingredient used in this study is edible mushrooms, valued for their high nutritional contribution and antioxidant activity. They are considered non-timber forest products [9]. Edible mushrooms are characterized by a high protein content (20.32%) [9]. They also contain carbohydrates such as mannitol, glycogen, arabitol, sorbitol, pentose, hemicellulose, and reducing sugars (glucose and levulose); minerals including calcium, chlorine, phosphorus, potassium, and sodium; trace elements such as manganese, zinc, copper, rubidium, bromine, silver, titanium, lithium, and vanadium; as well as iron at levels of 131.07 mg/100 g [11], which act as essential biocatalysts in small amounts. Furthermore, edible mushrooms provide important vitamins including A, D, C and B-complex [12].

In the rural community of Marayhuaca, district of Incahuasi, *Suillus luteus* mushrooms began to be processed industrially in 2015 to produce flour for the local Chiclayo market. However, only 10% of this production is directed towards cookies, pastries, breadmaking, pasta and gastronomy. This food source is particularly suitable for consumers seeking healthier diets, and the market increasingly favors its use given its promising functional properties [13].

Based on this context, the present study aimed to develop cookies enriched with edible mushroom flour and bovine blood meal. This formulation offers a comparative advantage over conventional cookies by incorporating bovine blood meal as a

source of heme iron, the essential component for increasing hemoglobin levels and thereby reducing the prevalence of iron-deficiency anemia among children.

The objectives of this research were: (i) to characterize edible mushrooms (*Suillus luteus*) used for the preparation of fortified cookies, (ii) to determine the optimal processing parameters for cookie elaboration, (iii) to evaluate the physicochemical properties of the cookies, and (iv) to assess the sensory quality and microbiological safety of the most suitable formulation enriched with edible mushroom flour and bovine blood meal.

MATERIALS AND METHODS

Raw materials and supplies

The edible mushroom flour (*Suillus luteus*) was sourced from the village of Marayhuaca, district of Incahuasi, province of Ferreñafe. Bovine blood meal was obtained from the company “Orginor Natural,” located in Lima, Peru. Additional ingredients included: pastry wheat flour “Anita Extra” (unprepared), brown sugar “Cartavio,” baking powder “Royal,” margarine “Danessa,” cocoa powder “Winter’s,” vanilla essence “Universal,” chocolate chips “Negusa,” sodium bicarbonate and marine table salt.

Processing and laboratory equipment and materials

The equipment used for processing included: an analytical balance with a precision of 0.0001 g (KERN); a stainless-steel dough mixer (NOVA brand) with a 50 kg capacity and dimensions of 1.3 m height, 2 m length, and 0.9 m width; and an electric oven (NOVA brand) with a capacity for 36 trays, a tempered glass viewing door, a maximum operating temperature of 280°C, approximate weight of 100 kg, and a three-phase 3 hp drive motor.

The quality control laboratory employed the following instruments: electric oven, handheld digital refractometer, digital pH meter, electronic balances, Soxhlet extraction apparatus and a micro-Kjeldahl system.

The materials used for processing included: stainless steel trays and containers, plastic measuring jugs, stainless steel bowls, small stainless-steel ladles, circular stainless-steel molds, parchment paper, and high-density polyethylene bags for packaging the cookies.

Analytical methods – Proximate analysis

Moisture determination (AOAC 925.10)

A complete analysis was performed on the four experimental formulations (F0, F1, F2, and F3). Moisture content was determined by weighing approximately 5.000 g $\pm$ 0.01 of ground sample in pre-weighed porcelain crucibles. The crucibles were placed in a drying oven at 105 $\pm$ 2°C for 4 h (or until constant weight). The procedure involved drying, cooling in a desiccator, and re-weighing, which was repeated until the difference between successive weighings was less than 0.005 g.

Ash determination (AOAC 942.05)

Two grams of dried sample were weighed into pre-tared porcelain crucibles. The samples were incinerated in a muffle furnace at 550 $\pm$ 25°C for 4 h (until the residue was whitish-yellow and free of carbonized organic matter). The crucibles were then cooled in a desiccator and reweighed.

Total protein (Kjeldahl method — AOAC 2001.11)

One gram of dried sample was weighed and digested with 10 mL of concentrated H₂SO₄ using copper sulfate as a catalyst until a clear solution was obtained. After digestion, distilled water was added, and the released ammonium was distilled into a 0.1 N HCl solution and titrated with standard acid. The nitrogen-to-protein conversion factor of 6.25 was applied (Protein = N $\times$ 6.25).

Total fat (Soxhlet extraction — AOAC 922.06)

Five grams of dried sample were placed in an extraction thimble. Lipid extraction was carried out with hexane in a Soxhlet apparatus for approximately 8 h, until the solvent became clear. The solvent was recovered by distillation, and the lipid residue was dried and weighed.

Crude fiber (Gravimetric acid-base method — AOAC 962.09)

Two grams of dried sample were digested sequentially with diluted sulfuric acid (1.25%) and sodium hydroxide (1.25%) under defined time and temperature conditions. The residue was filtered, washed, dried, and incinerated to correct for ash content.

Carbohydrates

Total carbohydrate content was estimated indirectly by difference from other proximate components:

$$\% \text{Carbohydrate} = 100 - (\% \text{Moisture} + \% \text{Protein} + \% \text{Total Fat} + \% \text{Crude Fiber} + \% \text{Ash})$$

Energy value (kcal/100 g)

The theoretical calculation was performed using Atwater conversion factors:

$$\text{Energy value (kcal/100 g)} = (4 \times \% \text{Protein}) + (9 \times \% \text{Fat}) + (4 \times \% \text{Carbohydrates})$$

pH and titratable acidity

Ten grams of sample were homogenized with 90 mL of distilled water (1:10 ratio) and stirred for 5 min. The pH was measured using a digital pH meter calibrated with pH 4.00 and 7.00 buffers (two-point calibration) at $25 \pm 2^\circ\text{C}$.

Microbiological analysis

This analysis was conducted by the certified external laboratory Microservilab E.I.R.L., in compliance with the microbiological requirements established in the Peruvian Technical Standard (Norma técnica Peruana) NTP 206.001.2016: Bakery, Pastry, and Confectionery Cookies Requirements [14, 15]. The following methods were applied: determination of *Escherichia coli* (ICMSF method), determination of *Salmonella* spp. (ICMSF method), determination of molds (ICMSF method), determination of *Staphylococcus aureus* (ICMSF method), and determination of *Clostridium perfringens* (ICMSF method).

Flowchart of the production process

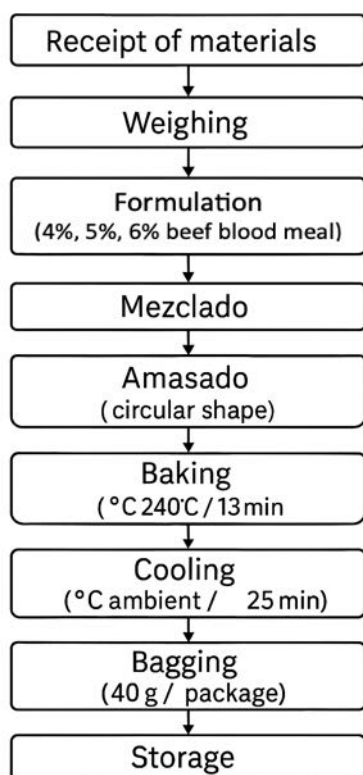


Figure 1: Flow diagram for the production of cookies enriched with edible mushroom flour (*Suillus luteus*) and bovine blood meal

Sensory analysis

The sensory analysis was conducted by 30 semi-trained panelists who assessed flavour, odour, colour, texture, and appearance. Sensory analysis was performed using a 7-point hedonic scale survey with 30 semi-trained panelists; The hedonic scale had the following scores: 1 = I dislike it extremely; 2 = I dislike it very much; 3 = I dislike it slightly; 4 = I neither like nor dislike it; 5 = I like it a little; 6 = I like it very much; 7 = I like it extremely. The results were subjected to ANOVA and Tukey's test.

RESULTS AND DISCUSSION

The proximate and physicochemical composition of the raw materials used in the elaboration of the cookies enriched with edible mushroom flour (*Suillus luteus*) and bovine blood meal are shown in Tables 1 and 2. The mushroom flour presented moisture (6.65%), fat (0.90%), ash (6.10%), fiber (7.25%), protein (31.92%), carbohydrates (47.18%), caloric value (325.04 kcal), pH (6.80), iron (13.09 mg/100 g), and acidity (0.045%). The bovine blood meal showed moisture (8.40%), fat (1.20%), ash (2.80%), fiber (0.50%), protein (85.75%), carbohydrates (1.35%), caloric value (359.92 kcal), pH (6.70), acidity (0.073%), and iron (18.74 mg/100 g).

These results confirm that both ingredients are suitable as fortifying agents in bakery formulations according to NTP 205.064.2015 – Flour for Human Consumption [16]. Based on the established standards, the maximum allowable acidity is 0.18%. Both raw materials exhibited acidity levels below this limit, ensuring their suitability for safe human consumption. Additionally, UNIT Standard N°534-82 – Chemical Composition of Blood Meal specifies maximum limits of 10% moisture, 6% ash, and 4% fiber, and a minimum of 78% protein [19,20]. The analyzed bovine blood meal fully met these requirements, indicating its quality and suitability for food formulation. These findings are consistent with previous research that has demonstrated the potential of animal and fungal ingredients as effective fortifying agents in bakery products, especially when used in controlled proportions [17, 18].

All formulations met the requirements of the Peruvian Technical Standard NTP 206.001:2016 for bakery, pastry, and confectionery products [14], which specifies a maximum allowable moisture content of 12%. Likewise, the Sanitary Regulation RM N°1020-2010/ [21] specifies maximum limits of 3% for ash and 0.10% for acidity; all formulations complied with these values, presenting ash between 1.5–1.7% and acidity between 0.064–0.073%.

The formulations presented moisture between 8.6–8.9%, carbohydrates between 53.8–54.2%, protein from 15.8–17.76%, fat from 15.4–16.6%, fiber around 1.0%, and caloric value between 440–447 kcal/100 g. These results indicate that the addition of mushroom and blood flours did not adversely affect the cookie's texture

or stability. Furthermore, according to the Qali Warma nutritional standards [20], the minimum protein requirement for cookies is 8.5%. All experimental formulations exceeded this requirement, with formulation F3 showing the highest protein content (17.76%).

Formulation F3 (95% wheat flour, 5% *Suillus luteus* flour, and 6% bovine blood meal) showed the best nutritional profile with the following values: moisture 8.90%, carbohydrates 54.24%, protein 17.76%, fat 16.60%, fiber 1.00%, ash 1.50%, acidity 0.073%, caloric value 447.40 kcal, pH 6.60, and iron 16.44 mg/100 g. The control formulation (F0) presented 11.89 mg/100 g of iron, while F3 reached 16.04 mg/100 g, demonstrating a marked increase proportional to the blood meal percentage.

According to Amaya and Chung [23], cookies fortified with 25% bovine blood flour provided 27.53 mg of iron/100 g. However, that high fortification level negatively affected sensory properties. In contrast, the 6% inclusion level used in the present study achieved a balance between nutritional improvement and acceptability, evidencing that moderate fortification is sufficient to enhance iron content without compromising sensory quality.

Regarding iron enrichment, formulation F3 reached a concentration of 16.44 mg of iron/100 g, confirming that bovine blood meal is an efficient source of heme iron, characterized by its high bioavailability. Several studies have reported that heme iron from animal byproducts exhibits greater intestinal absorption compared to non-heme iron of plant origin, making it a strategic ingredient for the development of fortified foods [24].

Comparatively, Chirinos and Santa María [22] reported that *Suillus luteus* flour presented 7.15% moisture, 53.91% carbohydrates, 25.02% protein, 7.14% total fat and 6.78% ash. The current findings showed improved nutritional values, particularly in protein and mineral content, supporting its potential use in fortified foods. Other studies, by Bravo and Mondragón [12] and Zhao *et al.* [10], have reported similar increases in protein and fiber with mushroom-based enrichment, though with lower iron levels than those obtained in this research.

Considering daily nutritional recommendations, Ministerial Resolution N°028-2015/MINSA [20] establishes that children and adolescents aged 1–18 years require 7–15 mg of iron per day. Therefore, consuming three 40 g packages of the fortified cookies (each providing approximately 6.4 mg of iron) would cover or exceed the daily iron requirement, offering a practical dietary strategy to combat anemia.



Figure 2: Four types of cookies prepared with edible mushrooms and bovine blood meal

The sensory analysis was conducted by 30 semi-trained panelists (children, adolescents, and adults), following the methodology described by Deng and Ma [27]. Using the attributes of flavor, odor, color, texture, and appearance were evaluated. Statistical analysis (ANOVA and Tukey test, $\alpha = 0.05$) revealed significant differences among formulations for flavor ($p = 0.000019$), odor ($p = 0.0000039$), and texture ($p = 0.000$), while no significant differences were found for color ($p = 0.556$) or appearance ($p = 0.400$). Formulation F3 obtained the highest mean scores for flavor (6.5), odor (6.4), and color (6.3), corresponding to “like very much,” while formulation F2 scored slightly higher in texture (6.2). These results confirm that moderate inclusion levels of *Suillus luteus* and bovine blood meal maintain high sensory acceptability. In Figure 2, we observe the results of all preparations F0, F1, F2, F3 for each attribute.

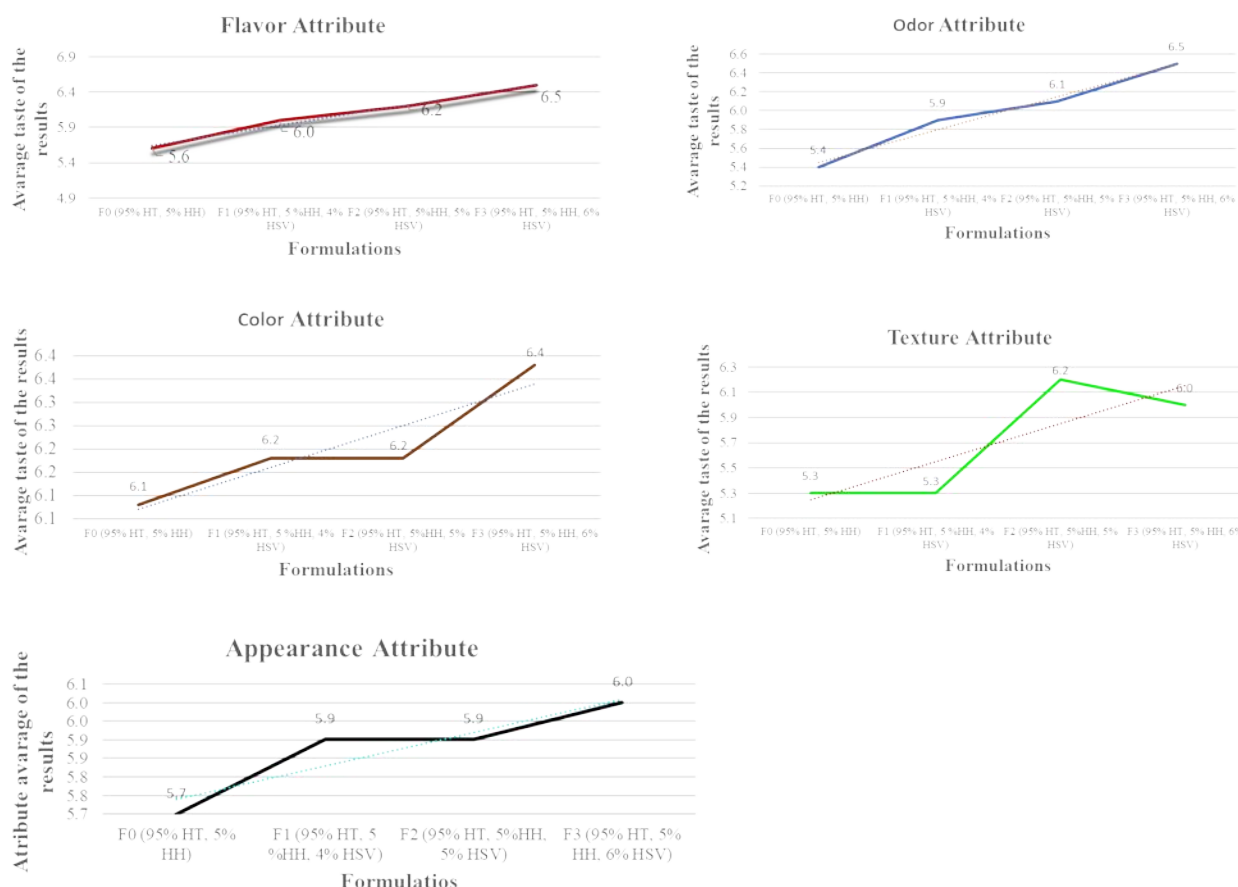


Figure 3: Graph of means for the attribute, Flavor, Odor, Color Texture and Appearance

F0 = 95% wheat flour, 5% edible mushroom flour. F1 = 95% wheat flour, 5% edible mushroom flour, 4% beef blood meal relative to the mixture. F2 = 95% wheat flour, 5% edible mushroom flour, 5% beef blood meal relative to the mixture. F3 = 95% wheat flour, 5% edible mushroom flour, 6% beef blood meal relative to the mixture.

The microbiological analysis performed on the most accepted formulation (F3) demonstrated compliance with the Peruvian Technical Standard NTP 206.001.2016 [14], showing *Escherichia coli* <10 CFU/g, molds <10 CFU/g, *Staphylococcus aureus* <10 CFU/g, and absence of *Salmonella* spp. and *Clostridium perfringens* in 25 g. These values are well within the permissible microbiological limits defined by the standard: molds ($\leq 10^2$ CFU/g), *E. coli* (≤ 3 CFU/g), *S. aureus* (≤ 10 CFU/g), and *Salmonella* spp. (absence/25 g).

Overall, the combined use of 6% bovine blood meal and 5% edible mushroom flour (*Suillus luteus*) significantly improved the nutritional value of cookies, particularly in

protein and iron content, while maintaining compliance with all national technical and sanitary standards [14-16, 21]. Moreover, the sensory and microbiological analyses confirmed that the final product was both safe and acceptable for human consumption, reinforcing its potential application as a functional food aimed at preventing iron deficiency anemia and enhancing nutritional quality in the population [22, 23].

Taken together, when comparing the results obtained with the available scientific evidence, this research not only confirms previous findings, but also expands existing knowledge by demonstrating that moderate fortification with bovine blood meal and edible mushroom meal allows the development of a nutritionally improved, safe and sensorially acceptable functional food, with potential application in dietary strategies aimed at preventing iron deficiency.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The formulation of cookies enriched with edible mushroom flour (*Suillus luteus*) and bovine blood flour improved protein and iron content, with formulation F3 (95% wheat flour, 5% mushroom flour, and 6% bovine blood flour) showing the best balance between nutritional value and sensory acceptability. Physicochemical, microbiological, and sensory results confirmed that moderate levels of fortification are sufficient to increase available iron without affecting product quality or safety, highlighting its potential as a functional food. In this context, it is recommended to evaluate the effects of regular consumption of this product in vulnerable populations through nutritional intervention studies, as well as to conduct further research on production scaling and its possible incorporation into food programs to contribute to the prevention of iron deficiency anemia.

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AUTHORS' CONTRIBUTIONS

E.B.N. and E.E.A.S. wrote the idea of study and wrote first draft of the manuscript, M.L.U, D.M.H.A. and J. B. C. wrote the last version of the manuscript, E.B.N.



analysed and interpreted the data. All the researchers approved the final version to be published.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

Table 1: Proximate and physicochemical composition of edible mushroom flour (*Suillus luteus*)

Parameters (%)	Edible Mushroom Flour
Moisture (%)	6,65
Carbohydrates (%)	47,18
Protein (%)	31,92
Total fat (%)	0,90
Crude Fiber (%)	7,25
Ash (%)	6,10
Acidity (%)	0,045
Caloric Value (kcal)	325,04
pH	6,80
Iron (mg)/100g	13,09

Table 2: Proximate and physicochemical composition of bovine blood meal

Parameters (%)	Harina de sangre vacuno
Moisture (%)	8,40
Carbohydrates (%)	1,35
Protein (%)	85,75
Total fat (%)	1,20
Crude Fiber (%)	0,50
Ash (%)	2,80
Acidity (%)	0,07
Caloric Value (kcal)	359,92
Ph	6,70
Iron (mg)/100g	18,74

Table 3: Proximate and physicochemical composition of the four formulations of enriched cookies

Parameters (%)	Formulations			
	F0 (95% HT; 5% HH; 0% HSV)	F1 (95% HT; 5% HH; 4% HSV)	F2 (95% HT; 5% HH; 5% HSV)	F3 (95% HT; 5% HH; 6% HSV)
Moisture (%)	8,60	8,50	8,85	8,90
Carbohydrates (%)	54,40	53,98	54,43	54,24
Protein (%)	17,50	17,67	17,72	17,76
Total fat (%)	16,70	16,90	16,50	16,60
Crude Fiber (%)	1,25	1,25	1,00	1,00
Ash (%)	1,55	1,70	1,50	1,50
Acidity (%)	0,064	0,064	0,073	0,073
Caloric Value (kcal)	447,92	447,92	447,00	447,40
pH	6,80	6,80	6,70	6,60
Iron (mg)/100g	11,89	13,39	14,64	16,04

(Using wheat flour (HT), mushroom flour (HH), and bovine blood meal (HSV))

Table 4: Microbiological analysis of the winning formulation (F3)

Analysis	F3
Escherichia coli count	<10 CFU/g
Salmonella spp. detection	Absence/25 g
Mold count	<10 CFU/g
Staphylococcus aureus count	<10 CFU/g
Clostridium perfringens detection	Absence/25 g

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