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Nutritional value and acceptability of heme iron microparticles-fortified bread

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ABSTRACT

Iron deficiency anemia is a significant global health challenge, prompting the exploration of novel iron fortification strategies. This study investigated pectin microparticles coated with bovine blood as a heme iron source to fortify bread. Four treatments were prepared: control, ferrous fumarate fortified, and bread with 15% and 25% microparticles. The physicochemical characteristics (including iron content and peroxide values), microbial counts, and overall acceptability were analyzed. Results showed that adding microparticles significantly increased the iron content of the bread (7.77 ± 0.05 mg/100 g). Peroxide values remained below 3.43 meq O₂/kg extracted fat, and mold counts were within acceptable limits (<10 CFU/g) for all treatments. A 7-point hedonic scale acceptability test in school-aged children (8–12 years) revealed no significant differences among treatments. Therefore, incorporating microparticles containing bovine blood-derived iron in bread offers a promising solution for producing iron-enriched food, providing a potential strategy for improving the health of school-age children.

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1. Introduction

Iron deficiency anemia is one of the most pressing public health issues globally. According to the estimations of the World Health Organization (WHO), around 30% of the population (more than 1.8 billion people) are affected by this condition, with women and children being the most vulnerable (Safiri et al., 2021). In the 2022 Demographic and Family Health Survey performed by the National Institute of Statistics and Informatics (INEI) of Peru, it was reported that 33.6% of children under 5 years old in the country suffered from anemia (Instituto Nacional de Estadística e Informática, 2023). These data indicate that 4 out of 10 children present with this deficiency. Loreto is one of the most affected departments in the country, with 55.5% of children under 3 years old and 32.9% of women suffering from some type of anemia.

One of the most common strategies to combat iron deficiency is through the fortification of staple foods (Preedy et al., 2013). Previous studies have demonstrated that flour-based products such as bread are a suitable vehicle for iron as they are highly consumed around the world (Hansen et al., 2005). To date, elemental iron forms are the most commonly used fortificants; nevertheless, they present limitations such as low bioavailability and reactivity with other components, which can cause unpleasant sensory changes to the consumers (Pastore & Muizniece-Brasava, 2016). In this context, the search for alternative iron sources with high bioavailability that can be incorporated into mass-consumed products while maintaining their stability and acceptability is of great interest (Olson et al., 2021).

According to literature, there are two types of dietary iron: heme iron from animal sources with high bioavailability (25–30%), and non-heme iron found in vegetables and inorganic salts with a more variable bioavailability (1–10%) (Skolmowska & Głąbska, 2019). Therefore, it is advisable for the population to include animal-based foods in their diet; however, meat products are expensive and may not be accessible to everyone, especially in low-income populations (Mantadakis et al., 2020). In this context, blood, a byproduct of the meat industry, has attracted attention due to its high bioavailable iron content (Quintero-Gutiérrez et al., 2008). Thus, it can play a significant role in providing a more affordable and accessible source of heme iron (Lynch et al., 2017). Furthermore, the use of this underutilized resource in a circular economy approach could contribute to the reduction of waste and the development of a more sustainable food system (Ramirez et al., 2021).

There are various reports of the use of bovine blood in the fortification of food matrices, such as milk (García et al., 2022), chocolate (Pastore & Muizniece-Brasava, 2016), cookies (Garay Barrios, 2018), bread (Hoppe et al., 2013), etc. However, blood, as well as other iron sources, are susceptible to oxidation, which can affect both the shelf life, nutritional, and sensory properties of the fortified product (Shubham et al., 2020). Due to this, the direct incorporation of bovine blood into food products presents challenges in maintaining safety and sensory acceptability. To overcome these limitations, microencapsulation

technology offers a promising solution. This technology effectively incorporates the iron source in a protective matrix, preventing undesired interactions with other food components and preserving its bioavailability and sensory attributes (Alemán et al., 2016). Among the different microencapsulation techniques, ionic gelation has emerged as a highly suitable method for producing microparticles with a high iron load (Rengifo Silvano et al., 2022). This is attributed to the porous nature of the microparticles, which facilitates electrostatic interactions between the charged polymers and proteins (Silverio et al., 2018). The porous structure is particularly relevant when using bovine blood as the iron source, as it allows for the co-encapsulation of heme iron with valuable proteins, further enhancing the nutritional value of the fortified food product.

Given the above, this study aimed to investigate the feasibility of enriching bread with heme iron by incorporating pectin microparticles coated with bovine blood. The study evaluated the impact of this fortification strategy on the bread's nutritional composition, shelf-life stability, and sensory acceptability.

2. Materials and methods

2.1. Materials

Pectin (PEC) of low methoxylation GENU® (CP Kelco Brasil S. A., Brazil), alginate (ALG) (high molar mass, high content of guluronic acid, Manugel DMB, FCM Biopolymer, Brazil). Bovine blood, sourced from the municipal slaughterhouse of Punchana, Iquitos-Peru authorized by SENASA, Peru's National Agricultural Safety Service. The blood was collected under safe and aseptic conditions. Ferrous fumarate (GRANOTEC, Lima, Peru). Calcium chloride anhydrous, hydrochloric acid, chloroform, and sodium hydroxide from Merck Peruana S.A., Peru.

2.2. Microparticles production

Pectin microparticles were produced by ionic gelation, following the methodology of Rengifo Silvano et al. (2022), with modifications. Briefly, a 2% (w/w) pectin solution was sprayed into 500 mL of a 2% (w/v) CaCl₂ solution (pH 4.0) at room temperature (25°C). The spraying setup consisted of an atomizing nozzle (1 mm diameter) coupled with a peristaltic pump (VELP SCIENTIFICA, SP311, Usmate, Italy) operating at an atomization speed of 555 mL/hour and an air compressor (SCHULZ S.A., MSV 6, Joinville, Brazil) delivering 0.250 kgf/cm² air pressure. The nozzle was positioned 12 cm above the CaCl₂ solution, which was continuously stirred at 500 rpm throughout the process. The resulting microparticles were then hardened by an additional 30 min of stirring and washed using a stainless-steel mesh sieve (75 µm pore size) to remove excess CaCl₂.

For heme iron incorporation, 55.56 mL of bovine blood was diluted to 100 mL with distilled water, filtered, and adjusted to pH 4.0. Then, 25 g of moist microparticles (moisture = 92.57 ± 0.68) were added to the bovine blood solution and stirred for 30 min. Subsequently, the microparticles were washed with abundant distilled water (pH 4.0) to remove residues.

A second coating was applied by adding 25 g of the bovine blood-coated microparticles to 100 mL of a 0.25% (w/v) alginate solution (pH 4.0) and stirring for 30 minutes. The resulting microparticles were washed with distilled water (pH 4.0), freeze-dried, and stored in sealed glass containers at -18°C.

2.3. Microparticles characterization

The microparticles were characterized regarding their moisture (air-drying method 950.46), ash (method 920.153), protein (Kjeldahl method 945.10, N × 6.25) following the methodology of the Association of Official Analytical Chemistry (AOAC, 2012). The total iron content was determined using Atomic Absorption Spectrophotometry after dry ashing (COVENIN 1409[6.1.2], 1979); results were expressed as mg/100 g.

Furthermore, the morphology and mean size of the microparticles were determined using an optical microscope (ZEISS – Primo Start, U.S.A.) with image capture through a digital camera controlled by Microcal Origin Pro 9.1 software (Microcal Software, Inc., MA, U.S.A.).

2.4. Bread production

Bread production was conducted at the "Oriental" bakery in Iquitos, Peru. A soft dough formula, similar to those used for commercial sandwich breads, was employed. The specific ingredient ratios are detailed in Table 1. Using an electric stand mixer (ERRE4M, Tilt head spiral dough mixer 5 kg professional, Vedelago, Italy), the dry ingredients, including the microparticles and ascorbic acid (added to enhance oxidative stability, Osuna et al., 2018), and water were mixed at 180 rpm for 10 min until dough formation, followed by the incorporation of vegetable shortening and an additional 8 min of mixing. The dough was then scaled to 50 g each, molded into round shapes, and placed in greased baking pans. Proofing proceeded for 60 min at room temperature (~35°C) before baking at 180°C for 25 min in an electric modular deck oven (Loguidice, Modus LFM, Arcole, Italy). Finally, the samples were cooled at room temperature (~35°C) and packaged in polyethylene bags.

Table 1. Bread formulation.

Ingredients	Treatments			
	T1	T2	T3	T4
Wheat flour	59	58.78	57.46	56.58
Sugar	4.72	4.72	4.72	4.72
Butter	3.54	3.54	3.54	3.54
Salt	1.18	1.18	1.18	1.18
Enzymes	0.59	0.59	0.59	0.59
Water (3°C)	29.5	29.5	29.5	29.5
MBBA*	0	0	1.33	2.21
FF** (mg)	0	0.01	0	0
AA*** (mg)	0	0.21	0.21	0.21
Gluten	0.59	0.59	0.59	0.59
Yeast	0.88	0.88	0.88	0.88
Total	100	100	100	100

*MBBA= Microparticles coated with bovine blood and alginate FF = Ferrous Fumarate. **AA = Ascorbic Acid T1 = Control. T2 = Bread with Ferrous Fumarate. T3 = Bread with Microparticles 15%. T4 = Bread with Microparticles 25%.

2.5. Bread physicochemical characterization

The proximate composition of the bread samples (moisture, ash, protein, total fiber) was determined following the methodology of the Association of Official Analytical Chemistry (AOAC, 2012). Iron content was determined by atomic absorption spectrophotometry after dry ashing CONVENIN 1409[6.1.2], (1979); results were expressed as mg/100 g. Lipid content was analyzed using the Bligh-Dyer method (Bligh & Dyer, 1959; De Souza Viana et al., 2018).

2.6. Bread stability under storage conditions

To assess the product's stability, microbiological quality, and peroxide values using the standard IDF method (74A:1991) were determined. The analyses were conducted on days 0, 5, and 10 of storage at room temperature (~31°C).

The microbiological tests were carried out at the Microbiology Laboratory of the Faculty of Food Industry at the National University of the Peruvian Amazon, located within the university's pilot plant. These tests adhered to the guidelines established by the Ministry of Health of Peru in the *Sanitary Standard Establishing Microbiological Criteria for Health Quality and Safety of Food and Beverages for Human Consumption* (No. 071) (Ministerio de Salud, 2008). Specifically, Chapter VIII of this standard mandates the determination of molds in baked products, performed according to the AOAC 997.02 (Schumacher et al., 2023).

2.7. Bread acceptability

2.7.1. Participants

A total of 101 children (8–12 years old, 54 males and 47 females) randomly recruited from the I.E.P. "Ruy Guzman Hidalgo" (San Juan, Iquitos, Peru) participated in this study. Their parents provided written informed consent, which included information about the study's purpose, procedures, and risks/benefits. This study was approved by the ethics committee of the National University of the Peruvian Amazon (PI-059-14/12/22-CIEI-UNAP).

2.7.2. Procedure

The evaluation of the bread acceptability was performed according to the methodology described by Rocha et al. (2021). Considering the potential cognitive demands of a full sensory profile for children, we focused solely on overall liking to minimize participant burden and ensure reliable data. A 7-point hedonic facial scale ranging from 1 ("very bad") to 7 ("very good") was used, as this scale provides sufficient discrimination among the different bread samples (Swaney-Stueve et al., 2018). A classroom at the elementary school was set up with individual tasting booths. For each sample evaluation, children were provided with a piece of bread (approximately 10 g) on randomly coded plastic plates under normal lighting. Samples were presented monadically following a Williams Latin square design, and children rinsed their mouths with water between samples. The test was conducted under the supervision of researchers and teachers.

2.8. Statistical analysis

The results are presented as mean \pm standard deviation (SD). Statistical analyses were performed using SAS software (version 9.0, Cary, NC, U.S.A.). To determine the effects of independent variables on the dependent variables, Analysis of Variance (ANOVA) was conducted for all data. For the acceptability data specifically, the normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variances was verified using Levene's test. In the analysis of acceptability, consumers and the order of sample presentation were treated as random factors, while bread formulation was considered as a fixed factor. When significant differences were found, Tukey's multiple-comparison procedure was applied for post-hoc analysis. For all tests, the significance level was set at $p < .05$.

3. Results and discussion

3.1. Microparticles production

Figure 1, shows images of the microparticles at different stages of production. Initially, the uncoated microparticles (UM) appeared white (Figure 1a). Following the coating process with bovine blood, the microparticles (MBB) acquired a distinct dark brown coloration (Figure 1b), which could be due to the oxidation of the blood. This result is consistent with observations by Valenzuela et al. (2014) and Churio et al. (2018). The color change could be attributed to the presence of heme pigment (Sakata, 2000). Subsequently, MBB coated with sodium alginate (MBBA) did not present significant changes in their coloration, indicating that the alginate layer is relatively transparent (Figure 1c).

3.2. Microparticles characterization

Table 2 shows the results of the physicochemical characterization of the microparticles at different stages of production. As expected, uncoated microparticles contained no protein or iron, exhibiting a moisture content of $92.57 \pm 0.68\%$ and a size of $180.04 \pm 0.04 \mu\text{m}$. After coating with the bovine blood solution, the moisture content decreased to $77.34 \pm 0.22\%$, and the size decreased to $170.56 \pm 1.09 \mu\text{m}$. Concurrently, the protein and iron levels increased significantly to $73.02 \pm 0.01\%$ and $144.2 \pm 0.05 \text{ Fe}/100 \text{ g}$, respectively.

Our results are similar to those of a recent study by Ligarda et al. (2022), who reported total iron values ranging from 132 to 205 mg/100 g for microparticles produced by spray drying using tara gum and potato starch as encapsulating agents with spray-dried erythrocytes from guinea pig blood. The higher iron content in their study (132 to 205 mg/100 g) could be attributed to several factors, including the different source material (guinea pig blood), the spray drying encapsulation method, the choice of wall materials, and the low amounts of encapsulant used. However, our results, using bovine blood and ionic gelation, achieved a higher iron content than other studies employing bovine blood as the heme source. Valenzuela et al. (2014) reported an iron content of 86.72 mg/100 g when using ionic gelation and alginate as a wall material. This difference may be due to the lower adsorption

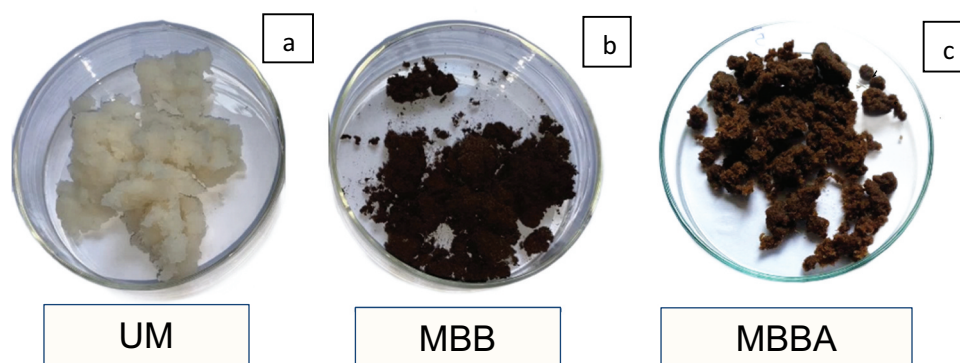


Figure 1. Photographs of microparticles: (a) Uncoated microparticles (UM), (b) Microparticles coated with bovine blood (MBB), and (c) Microparticles coated with bovine blood and alginate (MBBA). Images were captured using a camera under natural lighting on a plain white background.

Table 2. Physicochemical characterization of microparticles.

	Moisture	Ash	Lipid (%)	Protein	Iron (mg/100 g)	Mean size (μm)
UM	92.57 \pm 0.68 ^a	1.94 \pm 0.01 ^a	0.97 \pm 0.03 ^c	n.d.	n.d.	180.04 \pm 0.61 ^a
MBB	73.15 \pm 0.23 ^c	0.51 \pm 0.03 ^b	1.61 \pm 0.04 ^a	76.45 \pm 0.02 ^a	150.77 \pm 0.01 ^a	159.78 \pm 0.5 ^c
MBBA	77.34 \pm 0.22 ^b	0.88 \pm 0.34 ^b	1.38 \pm 0.09 ^b	73.02 \pm 0.01 ^b	144.2 \pm 0.05 ^b	170.56 \pm 1.9 ^b

Mean \pm statistical deviation ($n = 3$). n.d.= No data. UM = Uncoated Microparticles obtained by ionic gelation. MBB = Microparticles coated with bovine blood. MBBA = Microparticles coated with bovine blood and alginate. Note: Different letters in the same column represent significant difference ($p < .05$) between the means determined using the Tukey test.

capacity of alginate compared to pectin, which has a more disorganized structure and greater potential for adsorption (Rengifo Silvano et al., 2022). In another similar study, even when employing spray drying, Churio and Valenzuela (2018), achieved only 28–77 mg/100 g with maltodextrin as the encapsulating agent. This variation probably results from the combined effect of the different encapsulation methods and wall material. Therefore, our study demonstrates that whole bovine blood, combined with pectin and ionic gelation, can yield iron-enriched microparticles with higher iron content than previously reported for bovine blood-derived SDE, offering a promising system for oral iron delivery.

3.3. Bread production

Figure 2 shows images of cross-sections of the various bread treatments studied. Both the control sample (T1) and the bread containing ferrous fumarate (T2) presented a light color, suggesting that adding ferrous fumarate did not significantly alter the bread's color. However, when producing bread with different concentrations of MBBA, a pattern can be observed, whereas as the concentration of microparticles increases, the bread acquires a brown color that intensifies to dark brown (T3 and T4). The uniform coloration across samples suggests effective dispersion of microparticles during mixing, a characteristic supported by microencapsulation studies demonstrating homogeneous distribution of bioactive compounds in baked products (Alvim et al., 2016). Despite color variations, all samples maintained consistent crumb structure with uniform aeration patterns, indicating that microparticle incorporation did not compromise dough gas retention or baking expansion properties (Ashraf et al., 2022).

3.4. Bread characterization

Table 3 presents the proximal composition of the different bread treatments produced in this study. As expected, the control bread and the bread with ferrous fumarate showed no significant differences ($p > .05$) in their physicochemical composition, except for the iron content. The addition of ferrous fumarate powder led to an increase in iron content from 3.71 to 6.93 mg/100 g.

However, the breads with microparticles coated with bovine blood (MBBA) exhibited a distinct trend. As the concentration of microparticles increased, significant differences ($p < .05$) were observed in moisture, lipid, protein, and iron content. This could be due to the microparticles themselves contributing to the overall macronutrient composition. The treatment with the highest addition of microparticles (T4) showed a doubling of iron content and a 1 g increase in protein compared to the control (T1), highlighting the potential of this fortification strategy. This suggests that the microparticles effectively delivered the nutrients of interest to the breads, even after the baking process, which could potentially lead to nutrient loss (Aghalari et al., 2022).

The highest iron content (7.77 \pm 0.05 mg/100 g) was reported in the bread with MBBA 25% (T4). This represents a substantial increase compared to the ferrous fumarate fortified bread (T2), demonstrating the superior efficacy of microencapsulation for iron delivery. According to Food and Nutrition Board (2001) and the Technical Standard – Therapeutic and Preventive Management of Anemia in Children, Adolescents, Pregnant Women, and Postpartum Women (MINSAs, 2017), the iron requirements for children aged between 6 months to 8 years and for those aged 9–13 years are 11 mg/day and 8 mg/day, respectively. Thus, approximately two portions of MBBA 25% bread (equivalent to 100 g) would cover approximately 71% of the recommended daily intake of iron for children aged 6 months to

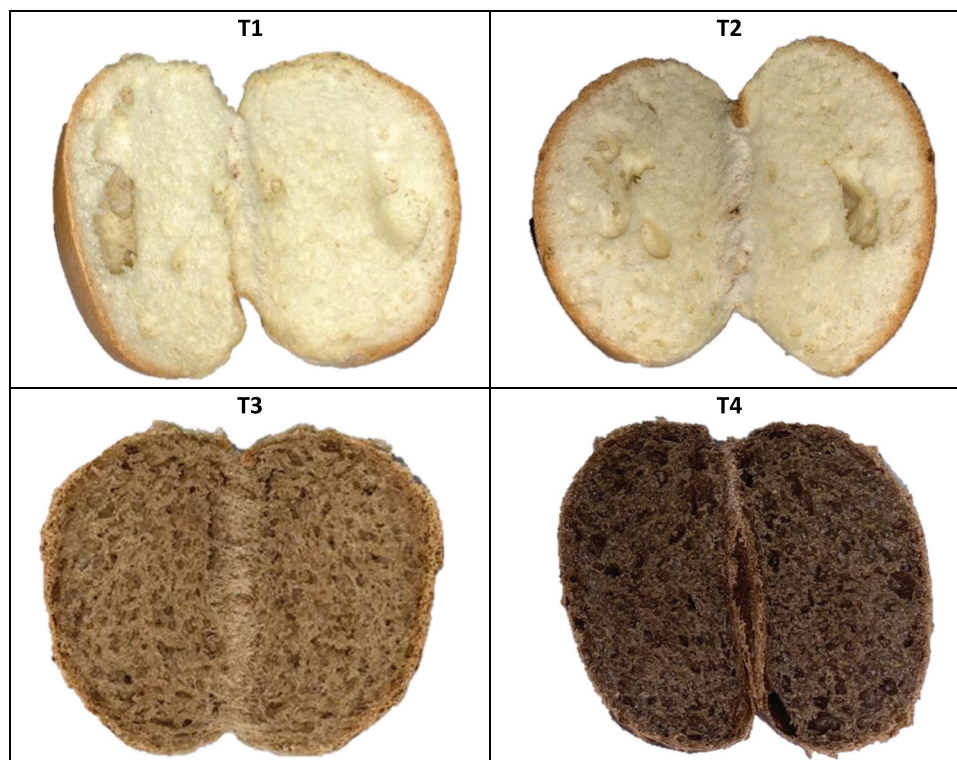


Figure 2. Images of transversal cuts of the iron fortified breads. T1 = Control (no iron fortification); T2 = Bread fortified with ferrous Fumarate; T3 = Bread fortified with 15% iron microparticles; T4 = Bread fortified with 25% iron microparticles.

Table 3. Physicochemical characterization of the bread.

Treatments	Moisture	Ashes (%)	Lipids	Protein	Fiber (%)	Iron mg/100gr
T1	26.22 ± 0.58 ^b	1.69 ± 0.18 ^a	6.50 ± 0.25 ^b	8.85 ± 0.02 ^b	0.35 ± 0.01 ^a	3.71 ± 0.02 ^d
T2	26.32 ± 0.97 ^b	1.77 ± 0.08 ^a	6.70 ± 0.33 ^b	8.92 ± 0.03 ^b	0.34 ± 0.01 ^{ab}	6.49 ± 0.11 ^c
T3	30.11 ± 1.92 ^a	1.61 ± 0.07 ^a	6.89 ± 0.11 ^{ab}	9.78 ± 0.01 ^a	0.32 ± 0.01 ^b	7.43 ± 0.05 ^b
T4	32.84 ± 0.55 ^a	1.67 ± 0.01 ^a	7.62 ± 0.22 ^a	9.94 ± 0.12 ^a	0.29 ± 0.02 ^c	7.77 ± 0.05 ^a

Mean ± standard deviation ($n = 3$). T1 = Control. T2 = Bread with Ferrous Fumarate. T3 = Bread with Microparticles 15%. T4 = Bread with Microparticles 25%. Different letters in the same column indicate significant differences ($p < 0.05$) according to the ANOVA. Means sharing the same letter are not significantly different ($p < 0.05$) according to Tukey's test.

8 years (11 mg/day) and 97% for children aged 9–13 years (8 mg/day), respectively. The fortified bread offers a promising approach to combating iron deficiency among these vulnerable age groups. Our results demonstrate that the microparticles act as a vehicle for iron loading, providing a valuable source of heme iron to enhance food nutrition. According to Carpenter and Mahoney (2009), heme iron is the least consumed form of iron by anemic populations in developing countries due to the limited access to animal products; in this sense, the bread produced in this study is a good alternative for at-risk populations. Additionally, Conrad and Umbreit (2002) reported that heme iron enhances non-heme iron absorption. Because wheat flour is mandatorily fortified with iron salts in Peru, the heme iron in this fortified bread could further improve iron bioavailability through this synergistic effect.

3.5. Stability under storage

Figure 3 shows the results of peroxide values, a key indicator of primary oxidation, in various bread treatments over a 10-day period. As expected, the control bread exhibited the lowest peroxide values, indicating minimal oxidation. The bread

fortified with ferrous fumarate only showed a significant increase ($p < 0,05$) in peroxide levels compared to the control. This observation is consistent with previous studies, which reported that iron fortification using ferrous fumarate, a more stable iron compound compared to ferrous sulfate, resulted in minimal changes to the properties of food vehicles due to its low solubility in water (Rebellato et al., 2017).

Likewise, the treatment with the highest percentage of microparticles exhibited high peroxide values. However, these values remained within the permissible limits for safe consumption (5 mg/kg) (MINSA, 2011). Despite the high iron content of the breads, the low levels of peroxides in the bread could be attributed to the microencapsulation process and the second layer added using a sodium alginate solution, which possibly acted as a physical barrier between the iron adhered to the surface of the microparticles and the other components of the bread. Similar results have been found by Cengiz et al. (2019), who in their study formulated alginate particles loaded with iron and evaluated their stability in water/oil emulsions. The authors found high levels of lipid oxidation in microparticles, however when an extra layer of alginate was added, lipid oxidation was considerably inhibited.

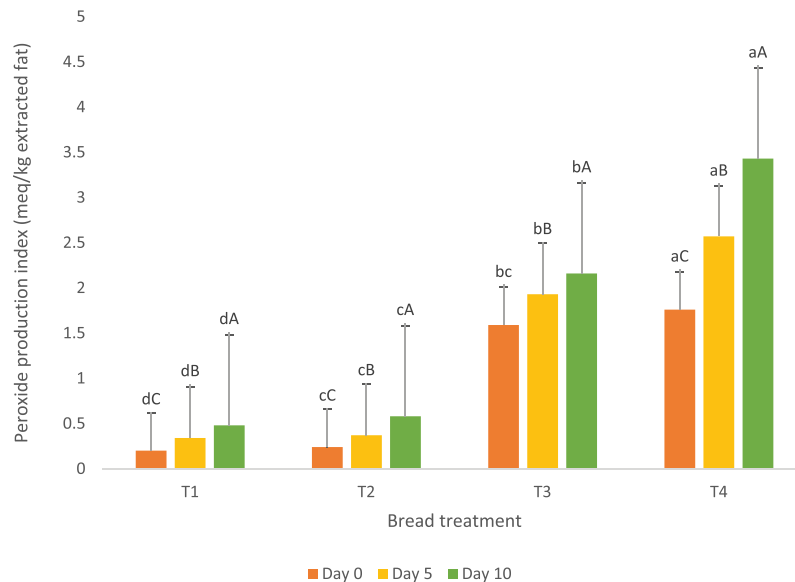


Figure 3. Peroxide index values of iron-fortified bread samples over the storage period. T1 = Control (no iron fortification); T2 = Bread fortified with Ferrous Fumarate; T3 = Bread fortified with 15% iron microparticles; T4 = Bread fortified with 25% iron Microparticles; Error bars represent standard deviations from the mean ($n = 3$). Different lowercase letters between treatments within each storage day indicate significant differences ($p < .05$). Different uppercase letters for the same treatment between storage days represent significant differences ($p < .05$), as determined by Tukey's test.

In the present study, ascorbic acid was incorporated into the formulation to enhance iron absorption. While ascorbic acid is susceptible to degradation at high temperatures, such as those used in baking bread (Kaźmierczak-Barańska et al., 2020), its potential antioxidant properties likely remained active enough to mitigate peroxide formation, resulting in the observed low peroxide values (Figure 3).

On the other hand, according to Health Technical Standard No. 071 (MINSAs, 2011) the microbiological indicators for bread include determining the presence of mold, where all the treatments presented <10 CFU/gr, which are in the permitted range.

3.6. Acceptability

The results for the overall acceptability of the bread are shown in Table 4. While the control bread received the highest score, no significant differences ($p < .05$) were found between the treatments and the control. Additionally, all acceptability scores ranged from 5.64 to 5.92 (out of 7), demonstrating good overall acceptability across all treatments. The positive response from school-age children shows their willingness to consume the fortified bread, supporting its potential as a vehicle for iron fortification, especially given bread's widespread consumption. These results suggest that the incorporation of heme iron microparticles, even at the highest concentration, did not negatively impact the overall acceptability of the bread. The lack of significant difference also indicates that the color variation among the breads, likely caused

by the added microparticles, did not deter children's acceptance. This is a crucial finding, as color changes in fortified foods can sometimes affect consumer perception (Hutchings, 2011).

Likewise, it is crucial to emphasize the methodology employed to assess acceptability in this study. A 7-point facial hedonic scale was found to be particularly effective in evaluating children's preferences. Chen et al. (1996) systematically evaluated age-appropriate hedonic scales in children aged 36–71 months and demonstrated that children aged 60–71 months (5–6 years) could reliably use a 7-point facial hedonic scale to express their food preferences. This research established that the 7-point scale is both valid and reliable for children at this developmental stage, providing sufficient discrimination without introducing confusion or fatigue. Similarly, Phu and Hang (2017) validated the application of the 7-point facial hedonic scale in a large sample of children aged 4–13 years, confirming that children across these age groups were able to use the scale effectively to rate their preferences for different milk products. The study's results showed consistent and meaningful responses, further supporting the appropriateness of the 7-point scale for sensory evaluation in children.

Furthermore, according to Guinard (2000), the use of hedonic scales in sensory evaluation minimizes confusion and enhances the accuracy of responses. Thus, the method used in the present study is consistent with extensive research on children's sensory perceptions (Jilani et al., 2019; Zeinstra et al., 2007), highlighting the significant influence of visual cues on their food preferences.

Table 4. Overall acceptability of bread.

Treatments	T1	T2	T3	T4
Score	5.92 ± 0.91 ^a	5.74 ± 1.06 ^a	5.61 ± 1.21 ^a	5.64 ± 1.22 ^a

Average ± statistical deviation ($n = 3$). T1 = Control. T2 = Bread with Ferrous Fumarate. T3 = Bread with Microparticles 15%. T4 = Bread with Microparticles 25%. Note: Different letters in the same column indicate significant differences ($p < 0.05$) according to ANOVA. Means sharing the same letter are not significantly different ($p < 0.05$) according to Tukey's test

On the other hand, despite the varying ages of the participants (8–12 years), statistically comparable scores across the four grade levels were observed. As Swaney-Stueve et al. (2018) reported, children in that age correspond to Piaget's "concrete operational stage", a developmental period characterized by an increasing capacity for understanding complex ideas. This developmental similarity justifies grouping them into a single evaluation group.

4. Conclusions

This study demonstrates the potential of bovine blood-derived heme iron microparticles as a strategy for fortifying bread. The microencapsulation process, combined with an alginate coating and ascorbic acid, effectively incorporated heme iron into the bread matrix while maintaining acceptable peroxide values during a 10-day storage period. Importantly, the fortification did not negatively impact the overall acceptability of the bread among school-age children. This suggests that such fortified bread could be a viable vehicle for increasing iron intake, particularly in vulnerable populations where access to diverse, iron-rich foods may be limited. In such settings, fortified staple foods like bread can play a crucial role in improving iron status. Further research, including longer-term stability studies and assessments of iron bioavailability and impact on iron status in at-risk populations, is needed to confirm the effectiveness of this approach in combating iron deficiency.

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Author contributions

CRedit: **Estefany Rengifo**: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft; **Rafael Vela-Paredes**: Methodology, Validation, Writing – review & editing; **Juan D. Rios-Mera**: Formal analysis, Methodology, Writing – review & editing; **Felix Cabrera**: Methodology, Resources; **Erick Saldaña**: Formal analysis; **Wilson Guerra**: Project administration, Supervision; **Thony Arce**: Writing – review & editing; **Julia Vásquez**: Writing – review & editing; **Jessy Vásquez**: Writing – review & editing; **Fernando Tello Célis**: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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No potential conflict of interest was reported by the author(s).

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