

Extrudates Based on Yellow Corn (*Zea mays*), Quinoa (*Chenopodium quinoa*) and Heme Iron as a Strategy to Combat Anemia

Extruidos a base de maíz amarillo (*Zea mays*), quinua (*Chenopodium quinoa*) y hierro hemínico como estrategia para combatir la anemia

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ABSTRACT

Fortifying foods with iron is a strategy for combating anemia, but it can alter the sensory and structural properties of extruded products. This study evaluated extruded formulations based on quinoa and corn fortified with heme iron (HI) and analyzed their sensory attributes, gelatinization index (GI), and microstructure, compared to a control sample without iron fortification. Significant differences were observed in aroma ($p = 0.0102$), color ($p < 0.001$), and appearance ($p < 0.001$) between the control sample (S0) and the fortified formulations, with S0 obtaining the highest sensory acceptance. However, texture ($p = 0.6763$) and flavor ($p = 0.8754$) did not show significant variations. The GI (94 ± 0.05) of the control sample was significantly higher ($p < 0.001$) than that of the fortified samples, indicating a combined effect of fortification and the composition of the cereal and pseudocereal on starch gelatinization. SEM micrographs revealed an amorphous and cohesive matrix, indicative of starch gelatinization during extrusion. Although exploratory, the results suggest that HI fortification can improve the nutritional profile of extrudates, while primarily affecting sensory acceptance in terms of aroma, color, and appearance, highlighting the need to adjust the formulation and extrusion to balance nutritional value and sensory acceptability.

Keywords: heme iron, extruded products, sensory evaluation, gelatinization index, microstructure analysis

RESUMEN

El enriquecimiento de los alimentos con hierro es una estrategia para combatir la anemia, pero puede alterar las propiedades sensoriales y estructurales de los productos extruidos. En este estudio, se evaluaron formulaciones extruidas a base de quinua y maíz enriquecidas con hierro hemínico (HE) y se analizaron sus atributos sensoriales, el índice de gelatinización (IG) y la microestructura, en comparación con una muestra de control sin fortificación. Se observaron diferencias significativas en el aroma ($p = 0.0102$), el color ($p < 0.001$) y el aspecto ($p < 0.001$) entre la muestra de control (M0) y las formulaciones fortificadas, siendo M0 la que obtuvo la mayor aceptación sensorial. Sin embargo, la textura ($p = 0.6763$) y el sabor ($p = 0.8754$) no mostraron variaciones significativas. El IG (94 ± 0.05) de la muestra de control fue significativamente mayor ($p < 0.001$) que el de las muestras enriquecidas, lo que muestra un efecto combinado del enriquecimiento y la composición del cereal y el pseudocereal sobre la gelatinización del almidón. Las micrografías SEM mostraron una matriz amorfa y cohesiva, indicativa de la gelatinización del almidón durante la extrusión. Aunque son exploratorios, los resultados sugieren que el enriquecimiento con HE podría mejorar el perfil nutricional de los extruidos, afectando principalmente la aceptación sensorial en términos de aroma, color y aspecto, lo que pone de relieve la necesidad de ajustar la formulación y la extrusión para equilibrar el valor nutricional y la aceptabilidad sensorial.

Palabras clave: hierro hemínico, extruidos, evaluación sensorial, índice de gelatinización, análisis de microestructura

Received: December 13th, 2024

Accepted: January 13th, 2026

Introduction

Anemia is one of the most common nutritional deficiencies worldwide [1] and significantly affects the child population, particularly in developing countries. It has a negative impact on children's cognitive and physical development, impairing their school performance and overall quality of life. In this context, the development of fortified and functional foods has emerged as an effective strategy to improve the intake of essential nutrients, especially iron, the deficiency of which can lead to adverse health outcomes and may limit the absorption of other nutrients [2], [3].

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The extrusion process has gained popularity in the food industry due to its ability to produce foods with specific characteristics, such as appealing texture, high nutritional value, and extended stability. This process allows for the combination and modification of raw materials to create innovative and enriched products that meet particular nutritional needs [4]. During extrusion, significant physical and chemical changes occur, including starch gelatinization, protein denaturation, and non-enzymatic browning reactions, which enhance flavor, texture, and nutrient availability, while increasing antioxidant properties and eliminating thermally sensitive compounds [5], [6]. This process combines water, pressure, heat, and mechanical forces [7], triggering molecular transformations and chemical reactions before the material is forced through an extrusion die, forming an expanded extrudate [8], [9] with a characteristic flavor, crunchy texture, and sterility [10], [11].

In this context, yellow corn and quinoa stand out as rich sources of complex carbohydrates, proteins, and essential micronutrients. Corn is one of the most widely used cereals in infant nutrition, accounting accounts for 82% of global starch production [12]. During extrusion, its structure undergoes changes that affect the final texture of the product. However, like other cereals, corn does not fully meet the nutritional requirements of consumers. Therefore, supplementation with other sources, such as quinoa, is necessary to improve its nutritional quality. Quinoa is known for its high protein content (13.8–16.5%) and balanced essential amino acid composition, which contributes to growth and the maintenance of metabolic functions. In addition, it provides 52%–69% carbohydrates and provides adequate levels of minerals such as calcium, magnesium, and potassium, thereby supporting a balanced diet [13], [14].

However, high temperatures during extrusion can reduce the content of unsaturated fatty acids; they also increase protein digestibility by deactivating antinutrients such as lectins and trypsin inhibitors, thus improving the nutritional quality of quinoa-based products [15]. In addition, extrusion facilitates the incorporation of additional functional ingredients such as proteins, lipids, and bioactive compounds that enhance the properties of the final product [16].

Despite the advantages of cereals in extrusion-based food production, they are deficient in certain essential micronutrients [17]. To overcome this, including heme iron (HI) sources, such as dehydrated blood, could enrich the final product, as HI exhibits higher bioavailability compared to plant-derived iron. The World Health Organization (WHO) and the CHOICE initiative recognize iron fortification as a cost-effective intervention to prevent iron deficiency in most developing countries [18].

Iron is critical for human growth and metabolism, playing an essential role in cellular respiration and oxygen transport [3]. Inadequate iron intake can compromise overall health and may interfere with the absorption of other essential nutrients [2]. Therefore, the combination of corn, quinoa,

and a source of heme iron through extrusion represents a potential strategy for developing a functional food aimed at combating childhood anemia.

The objective of this study was to develop an extruded food based on yellow corn, quinoa, and dehydrated poultry blood to improve iron levels in individuals with anemia. Through extrusion, the aim was to obtain a sensorially appealing product with high acceptability and an appropriate nutritional profile, thereby contributing to the reduction of anemia in vulnerable populations.

Methodology

Extrusion Process

Four mixtures (Table 1) were formulated using a white variety of quinoa (*Chenopodium quinoa*) from Juli and yellow corn (*Zea mays indentata*) purchased from a local market, and hemin iron (HI) derived from commercial dehydrated poultry blood as the source of fortification. All formulations were extruded under consistent operating conditions using a manually fed single-screw extruder (RBD Ing) equipped with a screw diameter of 25.4 mm, a length-to-diameter ratio of 20:1, a compression ratio of 1:1, and a 2.6 mm diameter die. The extruder had two heating zones—a feed zone operating at 55 ± 5 °C and a compression zone operating at 115 ± 5 °C—which operated constantly throughout the extrusion process. These variations corresponded to the operational tolerance of the equipment and do not represent differences in the experimental conditions between formulations. The mixtures were adjusted to a moisture content of $15 \pm 5\%$ and fed at 10 g/s. Each mixture was thoroughly blended to ensure homogeneous distribution of the components prior to extrusion. The level of HI included in each formulation was adjusted according to their respective nutritional objectives.

The formulations were defined considering technological and nutritional criteria. Corn served as the primary structural source of starch, enabling the formation of a continuous matrix during extrusion, while quinoa was incorporated as to enhance the formulation's nutritional value. The proportions of these two constituent elements were calibrated to maintain the extrudability of the system. HI was added at progressively increasing levels with the aim of evaluating its incorporation into the extruded matrix, using an unfortified formulation as a control.

Table 1. Formulation of extruded samples

Samples	Quinoa (%)	Corn (%)	Heme Iron (HI) (%)
S0	50	50	0
S1	45	50	5
S2	15	80	5
S3	10	80	10
S4	40	50	10

Source: Author

Physicochemical Analysis

The bulk density (BD) of the extrudates was determined according to Equation (1) [19]:

$$DE = \frac{4m}{\pi \times D^2 \times L} \quad (1)$$

Where m is the mass of an extrudate section of length L and diameter D after cooling. Twelve tests of density were performed on randomly selected samples.

The expansion index (EI) was calculated as the ratio of the extrudate diameter to the die diameter (2.6 mm) using 40 randomly selected samples, following the method described in [20].

For the water absorption index (WAI) and water solubility index (WSI), samples were evaluated in triplicate. The extrudates were ground to a particle size of 180–250 μm , and 2.5 g of each sample was mixed with distilled water. The mixtures were allowed to stand for 20 min and then centrifuged at 3000 rpm for 15 min. The supernatant was dried at 104 °C for 24 h. WAI was calculated as the ratio of the weight of the hydrated sample to the weight of the original dry sample, while WSI was expressed as the percentage of the dried supernatant weight relative to the initial dry sample weight.

Gelatinization Index

The degree of starch gelatinization (GI) was determined according to the methods described in [21], [22]. Ground samples (180 μm) were dispersed in 50 mL of 0.05 M KOH and centrifuged at 4000 rpm for 10 min. The supernatant was then treated with 0.05 M HCl and deionized water. Subsequently, iodine reagent was added, and the absorbance was measured at 600 nm using a spectrophotometer. The GI was calculated from the ratio of the absorbance of the sample to that of the control.

Microstructural Analysis

Micrographs of the extrudates were obtained using a scanning electron microscope (SEM, EM-30X, Korea) equipped with NanoStation 5.0 software. The samples were mounted on metal stubs using carbon tape and coated with a thin gold layer under high vacuum using a sputter coater. Observations were conducted at an accelerating voltage of 10.0 kV acceleration and magnifications of 5000 $\times$ and 7000 $\times$.

Sensory Analysis

Prior to sensory testing, a microbiological analysis was carried out to ensure product safety. Mesophilic aerobic bacteria, total coliforms, yeasts, and molds were quantified. All results complied with the limits established by Peruvian Ministerial Resolution No. 591-2008-MINSA

[23]. Subsequently, a hedonic sensory evaluation [24] was conducted with 15 untrained consumers over 18 years of age. Participants rated aroma, color, texture, flavor, appearance, and overall acceptability using a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely). The 9-point scale allows for a more accurate evaluation of the samples, effectively differentiating those perceived as highly pleasant from those perceived as highly unpleasant. Samples were presented on plastic trays labelled with four-digit numerical codes for evaluation. All participants provided informed consent, accepting the terms and conditions for the use of their data, after receiving detailed information about the study procedures. No side effects were reported.

Due to the characteristics of sensory evaluation and panelists, the results should be interpreted as indicative of preliminary consumer acceptance trends rather than definitive preference data.

Statistical Analysis

The data were analyzed using a completely randomized experimental design, with formulation (S0–S4) as the fixed factor. The physicochemical analyses were performed in triplicate, and results are expressed as mean $\pm$ standard deviation. A one-way analysis of variance (ANOVA) was applied to compare treatment means. When significant differences were identified, Tukey's *post hoc* test was used for multiple comparison at a significance level of $p < 0.05$. Statistical analysis of the data was performed using RStudio Desktop (version 2024.12.1+563) based on the R programming language.

Results and Discussion

Physicochemical Analysis

Figure 1 shows the effect of HI incorporation on the physicochemical properties of the extrudates. The expansion index (EI) decreased in iron-fortified samples (S1–S4) compared with S0 (3.89 ± 0.36), which exhibited a significantly higher EI ($p < 0.001$). Among the supplemented samples, S3 showed the highest EI (3.62 ± 0.21). These results indicate that formulations containing HI and a lower proportion of quinoa exhibit reduced EI, suggesting a combined effect of mineral fortification and cereal-pseudocereal composition on the expansive behavior of the extrudates. However, the relationship between HI concentration and EI was not strictly linear. Increasing HI from 5% to 10% resulted in a partial EI increase in some formulations (Figure 1A), such as S3, possibly due to interaction with the matrix. This behavior shows that, at moderate levels, EI could interact with the starch-rich matrix, promoting structural reorganization that allows partial expansion, while at lower concentrations its restrictive effect on bubble formation during extrusion would predominate. In addition, samples with higher corn content, such as M2 (15% quinoa) and S3 (10% quinoa), exhibited

higher EI values. This behavior can be attributed attributable to the higher carbohydrate content of corn, which promotes expansion, whereas quinoa, being rich in protein and fiber, limits this process by reducing the formation of air bubbles in the extrudate matrix [25], [26]. Even so, previous studies have shown that mixtures containing up to 50% quinoa can maintain high EI and low stiffness, while also providing additional nutritional benefits [27].

The water solubility index (WSI) reflects the proportion of soluble components generated during extrusion as a result of the breakdown of molecular bonds [28]. Sample S0 showed the highest WSI (26.42 ± 2.76), significantly higher than the rest ($p < 0.001$). No statistically significant differences were observed among S3 (23.09 ± 0.73), S4 (22.76 ± 0.67), and S1 (21.65 ± 0.52). Formulations with HI, together with a higher proportion of corn, generally exhibited lower WSI values than S0. However, when the HI content was increased from 5% to 10%, the WSI increased in some formulations (Figure 1B), which would indicate that higher iron concentrations could favor partial degradation of the matrix during extrusion, thereby increasing the soluble fraction. This behavior could indicate complex interactions between HI and the biopolymers of the extruded matrix. Similar trends have been reported for quinoa-based extrudates without HI supplementation [29], [30].

The water absorption index (WAI) reflects the water absorption capacity of starch, associated with the degree of gelatinization, a key factor for digestibility. Formulations with higher quinoa content (S1 and S2) showed higher WAI values. Likewise, formulations that included HI showed higher WAI values than the control sample; however, this behavior cannot be attributed exclusively to HI but rather to the combined effect of quinoa and corn in the matrix. This joint effect may be associated with modifications in the availability of hydrophilic groups, facilitating interactions with water and thus improving absorption capacity [31]. Sample S0 presented the lowest WAI (4.198 ± 0.69) of all formulations ($p < 0.001$), while samples with HI reached an average of approximately 5.5, with S3 being the sample with the lowest WAI (5.37 ± 0.19) in this group. The combination of high WAI and low WSI values suggest a greater degree of starch gelation [32].

Regarding bulk density (BD), sample S0 had the lowest value (0.053 ± 0.013), with no significant differences compared to formulations containing HI ($p = 0.544$). However, a tendency toward higher BD values was observed in the supplemented samples, consistent with lower EI values recorded in these formulations. In extrudates, lower radial expansion is usually associated with more compact structures and, therefore, higher BD [33]. The presence of HI, together with cereal and pseudocereal, may have contributed to greater matrix compaction, although without producing significant differences.

These changes suggest that the formulations with HI and variations in the proportion of quinoa and corn induced structural modifications in the extruded matrix, mainly of a physical and microstructural nature. These modifications likely contributed to the reduction in EI and the increase in BD, reflecting the formation of a more compact structure with limited air-cell development. Similarly, the decrease in WSI and the increase in WAI indicate alterations in the degree and form of starch gelatinization, possibly associated with reduced matrix rupture during extrusion. These observations are corroborated by SEM micrographs, which revealed a continuous amorphous matrix characterized by the loss of the original granular structure and lower porosity. This microstructural configuration may be attributed to the interference of HI in the expansion and reorganization of starch during the thermomechanical process.

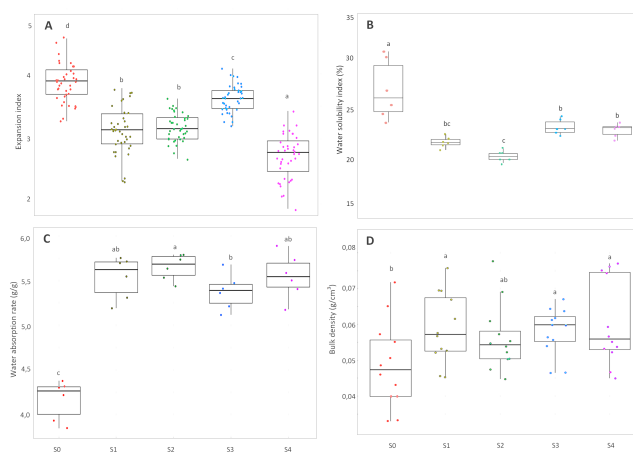


Figure 1. Physical parameters analyzed in extruded samples
Source: Authors

Sensory Analysis

Sensory evaluation of the developed products is essential when ingredient substitution is implemented in a formulation, as it allows to determine how changes affect both product structure and consumer taste perception [20]. Figure 2 presents the sensory scores for individual attributes and overall acceptability of the samples studied.

Regarding aroma, significant differences were observed among formulations ($p = 0.0102$). The control sample (S0) received the highest score (6.33), whereas samples containing HI (S1–S4) obtained lower scores (ranging from 5.06 to 5.53). These results suggests that consumers may have detected a change in the flavor of the HI samples, which influenced their preference.

For color (Figure 2B), S0 obtained the highest acceptance (6.8; $p < 0.001$) compared to the samples with HI. However, no significant differences were observed among the supplemented samples, although S1 (5.2) and S3 (5.7) were numerically superior. These results indicate that the incorporation of HI alters color perception, with

the control sample exhibiting lighter tonalities; however, increasing the fortification level did not produce additional perceptible changes in color acceptance. This behavior suggests that, although HI has an impact on the color of the product, its increase does not result in a progressive decline in color acceptance. This outcome is relevant from both technological and product acceptance perspectives. However, given the limited size of the sensory panel, these results should be interpreted as indicative rather than conclusive.

Texture scores did not differ significantly among samples ($p = 0.6763$), with all remaining values above 6 (like slightly) in the acceptance scale. This suggests that the texture, characterized by a porous and crunchy structure [34], did not have a significant impact on preferences, although the higher corn content in S2 and S3 may have contributed to a crunchier texture due to the greater starch proportion.

Similarly, taste ratings did not differ significantly among treatments ($p = 0.8754$). Neither HI inclusion (5% and 10%) nor variations in the proportion of quinoa and corn significantly affected this attribute. Although differences were not statistically significant, samples with higher quinoa content (S0 and S4) showed numerically higher scores, indicating a possible tendency toward having a slightly more appealing flavor profile.

Appearance showed significant differences among formulations ($p < 0.001$). Sample S0 obtained the highest score (6.6), followed by S3 (5.93), whereas S4 received the lowest score (3.67). The absence of HI in S0 may have contributed to improved visual acceptance, while higher fortification levels, particularly in S4, were associated with lower acceptance scores. This reduction may be related to more pronounced surface modifications of the extrudate, potentially linked to greater compaction of the matrix.

Finally, significant differences were observed between the samples in terms of overall acceptability ($p < 0.001$). S0 and S3 were the most preferred, with scores of 6.39 and 5.84, respectively, indicating that blend compositions influence overall sensory perception. Quinoa contributes positively to aroma and flavor, whereas corn improves texture. However, the addition of heme iron, especially at higher levels, influences color and appearance, possibly due to its low solubility and high reactivity. Heme compounds can catalyze lipid and protein oxidation during extrusion, potentially producing unpleasant odors and flavors that may be perceived by consumers, thus affecting the overall sensory quality of the product [1]. Notably, flavor and texture did not show significant variation between samples, suggesting that HI incorporation and variations in the quinoa–corn ratio did not substantially compromise these attributes.

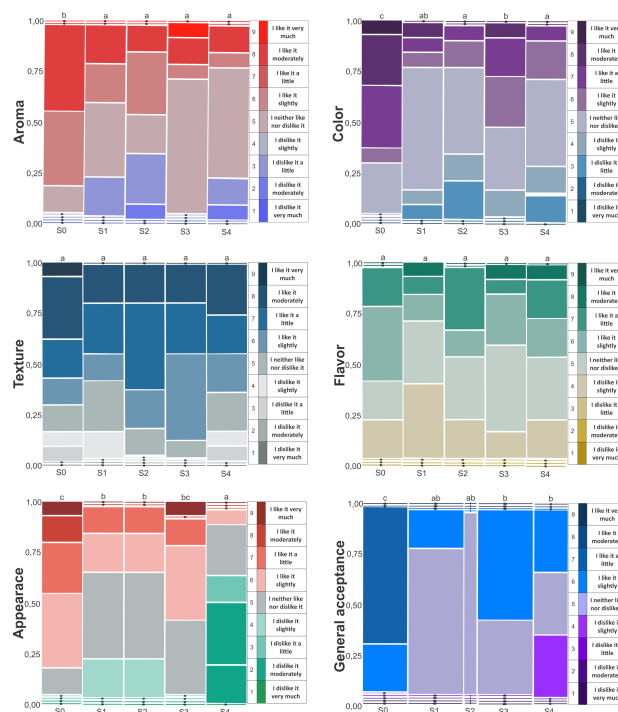


Figure 2. Sensory evaluation by attribute and overall acceptability of extruded samples

Source: Authors

Gelatinization Index

The Gelatinization Index (GI) was significantly higher in sample S0 (94 ± 0.05) compared to that of other formulations ($p < 0.001$). S0 reached the highest GI value, consistent with the Water Absorption Index (WAI), which increases as GI approaches its maximum, reflecting almost complete gelatinization. Among the samples with HI, S4 showed the highest value (81 ± 0.05), although there were no statistically significant differences compared to S1 (75 ± 0.06). Samples S2 and S3 exhibited lower GI values, suggesting that the inclusion of HI affects gelatinization, especially in formulations with lower quinoa content, given that quinoa has a lower gelatinization temperature than corn [35]. Samples S0 and S4, which achieved the highest GI values, contained balanced proportions of corn and quinoa, which may have favored starch gelatinization during extrusion (Figure 3).

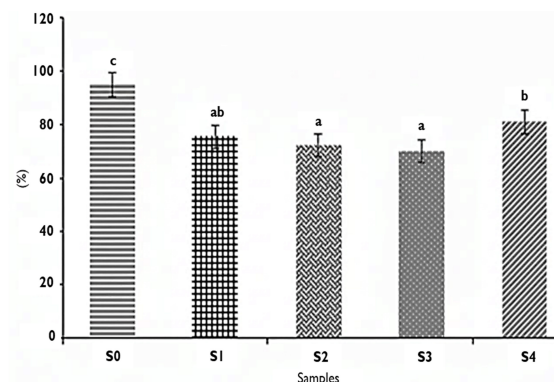


Figure 3. Gelatinization index

Source: Authors

Scanning Electron Microscopy (SEM) Images

Scanning Electron Microscopy (SEM) images revealed amorphous and cohesive structures with loss of granular appearance and large air spaces. The transition from a granular to an amorphous structure is typical in starch gelatinization, as reported in flours exposed to ultrasound, where protein or starch complexes undergo this transformation [36]. The micrographs of the treatments show that the granular structure was destroyed and modified during the extrusion process, suggesting that starch gelatinization in the samples was induced by the combination of heat, moisture, and shear inherent during extrusion.

Micrographs (Figure 4) show few regular particles in the rounded granule shape typical of corn and quinoa starch. Granules smaller than 5 μm , which are characteristic of both types of starch, were observed. According to the literature, corn starch granules range in size from 10 to 30 μm , while quinoa starch granules vary from 1 to 3 μm [37].

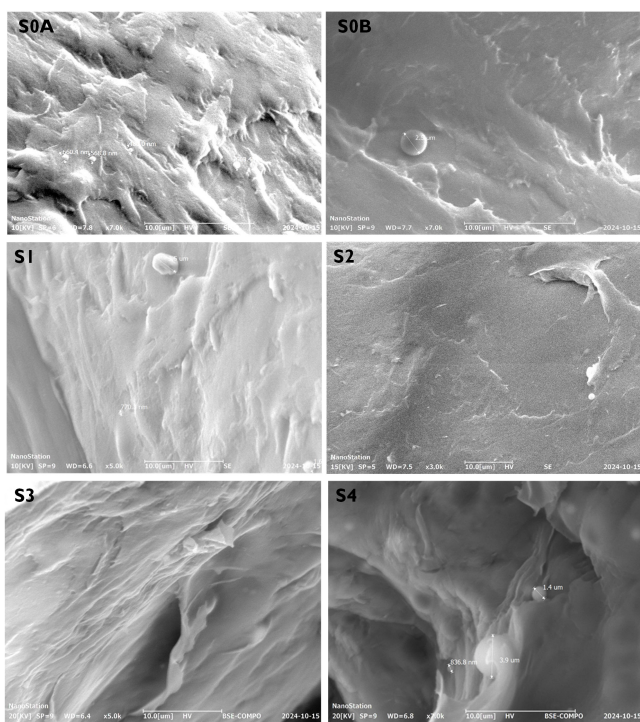


Figure 4. Representative scanning electron micrographs of extruded samples

Source: Authors

Conclusions

Formulations incorporating higher levels of HI together with a lower proportion of quinoa showed lower EI and GI values and higher BD, suggesting a combined effect of fortification and cereal and pseudocereal composition on the formation of the extruded matrix.

From a sensory perspective, preliminary results obtained from a small panel showed trends indicating that HI fortification did not lead to a progressive rejection of the product, particularly regarding flavor and texture. However, differences in color and appearance were evident compared to the control sample.

Formulations containing higher amounts of corn and balanced proportions of quinoa exhibited significantly higher GI values, which may be associated with greater efficiency in starch gelatinization during extrusion. The inclusion of HI in the mixtures showed a tendency to reduce GI, especially in formulations with lower quinoa content, indicating an interaction with the starch structure.

SEM analysis confirmed the transformation of starch particles from a granular to an amorphous and cohesive structure as a result of extrusion. This structural modification is consistent with the gelatinization observed in extruded products and indicates effective integration of the components within the starch matrix.

Despite variations in sensory acceptance, formulations with HI represent a viable strategy for the fortification of extruded products. Further research is required to determine the optimal amount of iron and isolate its individual effect in order to enhance the efficacy of this strategy, while adjusting the proportions of corn and quinoa to minimize negative effects on attributes such as color and appearance will increase the likelihood of consumer acceptance.

Acknowledgements

The authors are grateful to the Research Vice-Rectorate of the Private University of Tacna, Peru, for providing access to their research laboratories.

Conflicts of interest

The authors declare no conflicts of interest.

CRediT author statement

All authors: conceptualization, methodology, software, formal analysis, investigation, data curation, and writing (original draft, review, and editing).

Statement on artificial intelligence

The authors did not use IAG. The authors take full responsibility for the contents of this publication.

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