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DESARROLLO DE GALLETAS ENRIQUECIDAS CON HIERRO UTILIZANDO SANGRE DE CORDERO DESHIDRATADA Y HARINA DE MÁCHICA PARA MEJORAR SU VALOR NUTRITIVO

DEVELOPMENT OF IRON-ENRICHED COOKIES USING DEHYDRATED LAMB BLOOD AND MÁCHICA FLOUR TO ENHANCE THEIR NUTRITIONAL VALUE

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RESUMEN: Las deficiencias de hierro representan una crisis nutricional severa para las poblaciones vulnerables, lo que hace urgente la búsqueda de nuevas fuentes de micronutrientes. Frente a este panorama, el presente estudio se centró en formular galletas fortificadas utilizando sangre de cordero deshidratada y harina de máchica para evaluar posteriormente su calidad química, física y sensorial. La metodología se basó en un diseño completamente aleatorizado para probar tres niveles de inclusión de sangre: 5 % (T1), 10 % (T2) y 15 % (T3). Cuarenta jueces no entrenados evaluaron las muestras; tras tabular las respuestas, los datos se analizaron mediante la prueba estadística de Friedman, seguida de un análisis post hoc de Nemenyi. Los resultados indicaron que la formulación T2 (10 %) obtuvo las calificaciones más altas en textura, olor, color y dulzor, logrando un perfil sensorial muy similar al de las alternativas comerciales convencionales. El producto horneado demostró, además, una excelente estabilidad fisicoquímica y microbiológica tras un mes de almacenamiento a temperatura ambiente, cumpliendo plenamente con los parámetros de la norma técnica INEN 2085:2005. El uso de este subproducto cárnico en la panadería constituye una estrategia eficaz para elevar los niveles de hierro sin sacrificar la experiencia del consumidor. A futuro, sería muy beneficioso realizar ensayos con mujeres embarazadas, niños y pacientes crónicos, así como iniciativas educativas orientadas a romper el estigma cultural que a menudo se asocia con el consumo de sangre animal.

Palabras clave: *Hierro, sangre de cordero deshidratada, harina de máchica, galletas funcionales, aceptación sensorial*

ABSTRACT: Iron deficiency represents a severe nutritional crisis for vulnerable populations, making the search for new micronutrient sources an urgent priority. Against this backdrop, the present study focused on formulating fortified cookies using dehydrated lamb blood and máchica flour to subsequently evaluate their chemical,

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physical, and sensory quality. The methodology relied on a completely randomized design to test three different levels of blood inclusion: 5% (T1), 10% (T2), and 15% (T3). Forty untrained judges evaluated the samples. Once their responses were tabulated, the data were analyzed using the Friedman statistical test, followed by a Nemenyi post hoc analysis. The findings showed that the T2 formulation (10%) earned the highest ratings for texture, odor, color, and sweetness, achieving a sensory profile very similar to that of conventional commercial products. The baked product also demonstrated excellent physicochemical and microbiological stability after a month of room-temperature storage, fully complying with the INEN 2085:2005 technical standard. Using this meat byproduct in baking proves to be a practical strategy for boosting iron levels without sacrificing the consumer experience. Future trials focusing on pregnant women, children, and chronic patients would be highly beneficial, alongside educational initiatives designed to break the cultural stigma often associated with consuming animal blood.

Keywords: *Iron, dehydrated lamb blood, máchica flour, functional cookies, sensory acceptance.*

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INTRODUCTION

Iron-deficiency anemia currently represents one of the most pressing public health challenges worldwide. According to epidemiological reports from the World Health Organization, this severe nutritional deficiency affects more than 30% of the global population, striking with particular severity in developing countries (1) and in areas with high socioeconomic vulnerability indices, such as various Andean regions and, specifically, the disadvantaged sectors of Riobamba. The chronic deficit of iron in the daily diet drastically reduces hemoglobin synthesis, which clinically translates into a severe alteration of cellular oxygen transport. The consequences of this deprivation are systemic and devastating, ranging from chronic fatigue and immunological failures to severe delays in physical and cognitive development during childhood (1). Despite the gravity of the panorama, access to fortified products that are simultaneously economical and that truly respect local dietary customs remains extremely limited (2).

The formulation of functional foods to mitigate this deficiency represents both a scientific and a social priority. Functional nutrition is not limited to covering basic caloric needs; its objective is to actively prevent the development of chronic diseases. To improve the intestinal absorption of iron, it is essential to take into account its chemical structure. The human organism assimilates heme iron, of animal origin, with a far superior efficiency to that of free iron found in plant sources. Thanks to this biological characteristic, lamb blood becomes a resource of great value. Although it is usually discarded in slaughterhouses after traditional slaughter, its recovery and

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controlled thermal dehydration make it possible to concentrate high-biological-value proteins, B-complex vitamins and zinc, in addition to reaching exceptional levels of up to 220 mg of pure iron per 100 grams of powder. Revaluing this by-product provides heme iron of excellent bioavailability and, at the same time, offers a practical and sustainable solution to organic waste.

For an intervention of this nature to be truly effective, it must overcome the challenge of sensory acceptability. This is where máchica flour takes center stage, an ingredient deeply rooted in the culinary tradition and identity of the Andean region. Obtained from the milling of toasted maize and barley, máchica is an exceptional source of fiber, vitamins and complex carbohydrates, establishing itself as a brilliant functional substitute for refined white flours (3). Integrating both ingredients through the preparation of cookies facilitates the delivery of nutrients in a mass-consumption format. This type of product offers practical advantages, such as simple packaging, a prolonged shelf life and, above all, a highly familiar flavor. The main technical challenge consists in achieving the exact balance: providing a significant dose of iron without altering the texture, crumb or aroma that the consumer expects to perceive.

The development of these food matrices has solid academic support. Various investigations have already demonstrated the viability of incorporating hematological derivatives into bakery products. For example, the study by Garay (4), which combined bovine derivatives with quinoa, confirmed that the use of blood in cookies substantially increases the ferric profile without generating rejection during tasting. Along the same lines, Chinchay (5) formulated children's bars with lyophilized blood and obtained outstanding nutritional metrics. For their part, authors such as Álamo (6) and Guevara (7) documented real clinical impacts and excellent absorption rates, both in anemic patients and in animal models, after the consumption of fortified bread.

The sanitary need to create these healthy alternatives becomes even more evident in the face of the exhaustive review by Hoyos, which showed that the vast majority of commercial cookies are saturated with sugars and lack real micronutrients. Faced with this reality, authors such as Mena, Quispe and Contreras (8–10) have experimented with great success with the use of porcine and bovine hematological derivatives in hamburgers, classic breads and noodles, corroborating great biological advantages. This fully agrees with the position of López (2) regarding the urgency of reusing livestock by-products for public health, and with what was pointed out by Paredes (3) regarding the enormous, yet little-studied, benefits of máchica in the Andean diet. In addition, work in rural and school areas carried out by Delgado (11) in Bolivia, and by Herrera (12) and Arévalo (13) in Ecuador, confirmed that the distribution of these foods manages to reduce the prevalence of anemia in a very few months, relying on the vital psychological factor documented by Moreno (14): people assimilate food innovation better when they perceive that it respects their cultural identity.

Despite this theoretical corpus, a notable scientific gap still exists regarding the nutritional stability over time and the hedonic acceptance of cookies formulated specifically with the synergy of lamb blood and máchica flour at the local level. Without precise analytical laboratory data, it is unfeasible to establish scalable public-health strategies based on these Andean resources. Consequently, the present

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research poses as its general objective the development of iron-enriched cookies using dehydrated lamb blood and máchica flour (15). From this central purpose fundamental specific objectives are derived: to formulate different mixture proportions to maximize the concentration of the mineral, to rigorously quantify the microbiological quality and the percentage of iron in the baked goods, to analyze the acceptability of the product through direct tastings with consumers and, finally, to monitor the shelf life of the packages during 30 continuous days of storage.

MATERIALS AND METHODS

Experimental design.

The research was carried out under a completely randomized design, establishing three different treatments. These consisted of incorporating levels of 5%, 10% and 15% of dehydrated lamb blood into a cookie formulation based on máchica flour. The main objective was to evaluate how each concentration level influenced the final iron content, as well as the sensory, physicochemical and microbiological properties of the baked product.

Raw materials.

Fresh lamb blood was obtained from the municipal slaughterhouse of Riobamba (Ecuador), collected under hygienic conditions in sterile containers. The ingredients used for the preparation of the cookies were máchica flour, brown sugar, butter, eggs, chopped walnuts, vanilla essence, baking powder, ground cinnamon and salt, all acquired in commercial establishments of the city and stored under adequate conditions until their use.

Obtaining dehydrated lamb blood.

The fresh blood was filtered to eliminate clots and impurities by means of a stainless-steel mesh. Subsequently, it was subjected to a pasteurization process with the purpose of reducing the microbial load and guaranteeing the safety of the ingredient. The pasteurized blood was dehydrated in a convection oven at 60 °C until reaching a moisture content lower than 5%. Finally, the dry material was ground and sieved to obtain a fine and homogeneous powder that was subsequently incorporated into the experimental formulations.

Preparation of the cookies.

Three experimental formulations were developed, varying only the percentage of dehydrated lamb blood (5, 10 and 15%), adjusting proportionally the quantity of máchica flour to keep the total formulation constant.

Table 1. Formulation of the cookies enriched with dehydrated lamb blood.

Ingredient	F1 (5%)	F2 (10%)	F3 (15%)
Máchica flour	38.02	35.39	32.82
Dehydrated lamb blood	5.00	10.00	15.00
Brown sugar	17.89	18.80	19.69
Butter	22.37	22.12	21.88

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Egg	12.53	12.39	12.25
Chopped walnuts	4.47	4.42	4.38
Vanilla essence	0.89	0.88	0.88
Baking powder	0.94	0.93	0.92
Cinnamon	0.58	0.58	0.57
Salt	0.07	0.07	0.07

Note. F1. First formulation, F2. Second formulation, F3. Third formulation

Physicochemical analyses.

The formulation that obtained the highest sensory acceptance was selected for the physicochemical analyses during a storage period of 30 days.

The following parameters were determined: Water activity (Aw), Moisture, pH, Titratable acidity, Ash. The analyses were performed using official AOAC and ISO methodologies.

Microbiological analyses.

To verify the safety of the product, different microbiological analyses were carried out. These included the count of mesophilic aerobes, total coliforms, molds and yeasts, in addition to the detection of Salmonella spp. After obtaining the results, the values were contrasted directly with the maximum limits required by the current Ecuadorian regulations for bakery products.

Determination of iron by atomic absorption.

Regarding the quantification of the mineral, the iron content was determined by flame atomic absorption spectrophotometry (FAAS). To evaluate the real nutritional contribution and to check the repeatability of the process, three independent batches of the selected formulation were analyzed. All final data were expressed in milligrams of iron per 100 grams of product.

Sensory evaluation.

The sensory evaluation counted on the participation of 40 untrained panelists. Through a three-point hedonic test, this group was in charge of analyzing the general acceptability of the three experimental formulations. During the tasting, the judges specifically valued attributes such as flavor, texture, color, odor and sweetness of the samples.

Statistical analysis (Friedman and Nemenyi post hoc test).

The sensory data were analyzed by means of the non-parametric Friedman test, because the same group of panelists evaluated all the formulations and the responses were obtained by means of an ordinal scale. When significant differences were detected ($p < 0.05$), the Nemenyi multiple-comparisons test was applied to identify the differences between treatments. The results of the physicochemical, microbiological and iron-content analyses were expressed as mean ± standard deviation.

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RESULTS

Development of the formulations

Three cookie formulations were prepared incorporating dehydrated lamb blood at concentrations of 5, 10 and 15%. During the preparation process all the formulations presented an adequate consistency of the dough and made it possible to obtain cookies with good structural stability after baking. As the percentage of dehydrated blood increased, an increment in the intensity of the color and slight modifications in the characteristic aroma of the product were observed, a behavior expected due to the higher content of hemoglobin present in the functional ingredient. These results coincide with previous investigations on bakery products fortified with animal blood, where moderate concentrations significantly improve the nutritional value without affecting the technological properties of the process.

Sensory evaluation

The sensory evaluation evidenced differences among the three formulations studied. The formulation that contained 10% of dehydrated lamb blood presented the highest scores in the attributes of flavor, texture, odor, color and sweetness. On the contrary, the formulation with 15% incorporation obtained lower levels of acceptance due to the intensification of the dark color and of the metallic flavor characteristic of blood, while the formulation with 5% presented an intermediate acceptability, although with a lower potential of nutritional fortification.

Table 2. Sensory test.

Treatment	Attribute	1 (I like it a lot)	2 (I like it a little)	3 (It is not my preference)
1	Flavor	12	18	10
	Texture	10	20	10
	Color	11	17	12
	Odor	13	15	12
	Sweetness	10	20	10
2	Flavor	25	10	5
	Texture	23	12	5
	Color	22	13	5
	Odor	24	11	5
	Sweetness	26	9	5
3	Flavor	5	12	23
	Texture	6	10	24
	Color	7	11	22
	Odor	8	9	23
	Sweetness	6	10	24

The statistical analysis by means of the Friedman test showed significant differences among treatments ($\chi^2 = 10.00$; $p < 0.05$). Subsequently, the Nemenyi post hoc test

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confirmed that the formulation with 10% of dehydrated blood was significantly more accepted than the formulations with 5 and 15%.

Table 3. Results of the Friedman test.

Statistic	Value	Degrees of freedom (df)	Critical value (α=0.05)
X^2r	10.00	2	5.99

These results demonstrate that an intermediate concentration makes it possible to maintain a balance between the nutritional improvement and consumer acceptance, coinciding with various studies developed on bakery products fortified with iron sources of animal origin.

Table 4. Analysis of the Post hoc test (Nemenyi).

Comparison	Absolute difference of Ranks	Critical difference (CD)	Significant difference?
T1 vs T2	|10-15|=5	1.48	Yes
T1 vs T3	|10-5|=5	1.48	Yes
T2 vs T3	|15-5|=10	1.48	Yes

Physicochemical characterization

The selected formulation (10%) was subjected to physicochemical and microbiological analyses during 30 days of storage.

Table 5. Physicochemical and microbiological results of the selected formulation.

Parameter	Day 1	Day 15	Day 30
Water activity	0.35 ± 0.01	0.35 ± 0.01	0.32 ± 0.02
pH	6.23 ± 0.06	5.93 ± 0.06	5.58 ± 0.06
Moisture (%)	2.49 ± 0.24	2.65 ± 0.60	2.99 ± 0.77
Ash (%)	1.72 ± 0.03	—	—
Mesophilic aerobes (CFU/g)	1.0×10^4	—	2.0×10^3
Molds and yeasts	Absence	—	Absence
Total coliforms	Absence	—	Absence
Salmonella spp.	Absence	—	Absence

Water activity remained below 0.35 throughout storage, a condition that limits microbial growth and favors the stability of the product. In addition, the moisture level was maintained below 3%, which made it possible to preserve the crispy texture so characteristic of these cookies. For their part, the microbiological analyses confirmed the absence of Salmonella spp., total coliforms, molds and yeasts. These results demonstrate that both the preparation process and the storage conditions were adequate to guarantee the safety of the food throughout the 30 days that the evaluation lasted.

Iron content

The iron concentration was determined by atomic absorption spectrophotometry. This analysis was carried out on three independent batches, all prepared from the formulation that achieved the highest sensory acceptance.

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Table 6. Iron content of the cookies prepared with the selected formulation.

Production	Parameters	Results	Unit
Batch 1	Iron (Fe^{2+})	12.21	mg/100g
Batch 2	Iron (Fe^{2+})	9.33	mg/100g
Batch 3	Iron (Fe^{2+})	9.83	mg/100g

Note. mg. Milligrams

The values obtained ranged between 9.33 and 12.21 mg of iron per 100 g of product, evidencing adequate reproducibility among batches and confirming the efficacy of dehydrated lamb blood as a source of heme iron. These values exceed those usually reported for conventional cookies and are comparable with those of other bakery products fortified with ingredients of animal origin. In this sense, the formulation with 10% of dehydrated lamb blood achieved the best balance among sensory acceptance, physicochemical stability, microbiological safety and iron contribution. All of this demonstrates its enormous potential as a functional food, capable of contributing actively to the prevention of iron deficiency and iron-deficiency anemia.

DISCUSSION

The results of sensory acceptability confirm that 10% inclusion of dehydrated blood represents the ideal technical limit. This fully coincides with the findings of Quispe (16) and Baca (17), who demonstrated that working with that specific proportion of animal matter succeeds in maximizing the ferric value without generating rejection in the consumer of baked goods. By adding máchica flour to the equation, the behavior of the food matrix resembles quite closely what was reported by Garay (18), who validated the use of Andean cereals such as quinoa to better integrate meat derivatives. Achieving this organoleptic balance is a direct response to the problem warned of by Hoyos (19): the modern industry saturates Peruvian and Andean commercial cookies with sugars, leaving aside the real contribution of micronutrients.

Regarding microbiological stability and the levels of bioavailable iron, the data obtained in the T2 formulation support several previous field interventions. Chinchay (20) and his team had already reported outstanding acceptance rates when formulating children's bars using lyophilized blood. The metrics of our study strongly suggest that these cookies possess the potential to replicate the real clinical impacts observed by Álamo (6) in anemic patients, or the absorption results verified by Guevara in animal models. In fact, maintaining a controlled aerobic load during one month of storage makes viable the use of this product in school-feeding programs, a strategy that Herrera (12) and Arévalo (21) applied successfully in Ecuador to decrease the prevalence of anemia in a matter of months.

The joint use of ovine blood and máchica flour transcends simple food chemistry to touch an unavoidable social fiber. López Benavides (2) argues forcefully that revaluing these livestock by-products is vital both for public health and for reducing organic waste in slaughterhouses. For its part, choosing this specific cereal validates the position of Paredes regarding the great biological benefits and the cultural roots that máchica offers, a resource still little studied at the technical level. Finally, the high

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acceptance of the winning sample corroborates the psychological factor documented by Moreno (22): people assimilate and accept novel food with much greater ease when they perceive that the product respects their own cultural identity.

CONCLUSIONS

The incorporation of dehydrated lamb blood made it possible to develop a functional cookie with a high iron content, while maintaining adequate technological and quality characteristics. When analyzing the different formulations, the incorporation of 10% of dehydrated lamb blood achieved the best balance between nutritional enrichment and product acceptability. With this mixture, iron levels ranging between 9.33 and 12.21 mg per 100 g were reached. These figures evidence the great potential of the cookies as a food alternative capable of contributing to the prevention of the deficiency of this mineral.

During the sensory evaluation, this same formulation (10%) was the one that registered the highest acceptance. In fact, both the Friedman test and the Nemenyi post hoc analysis confirmed the existence of statistically significant differences ($p < 0.05$) versus the 5% and 15% samples. This finding demonstrates that using intermediate levels of incorporation is an effective strategy to elevate the nutritional value without impairing key attributes such as flavor, texture, color, odor and sweetness.

On the other hand, the cookies prepared with the winning concentration showed adequate physicochemical and microbiological stability throughout 30 days of storage. By combining low levels of water activity and moisture with the total absence of Salmonella spp., total coliforms, molds and yeasts, it was verified that the product complies with the quality and safety requirements demanded in the bakery industry. All of this fully supports its viability as a fortified food, ready for eventual production and commercialization on a larger scale.

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