Article

Iron supplementation through partial replacement of wheat flour with chickpea (*Cicer arietinum*) and chicken liver powder: Formulation optimization via iron-content, sensory, and shelf-life assessments

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Abstract: Anemia is a global health condition affecting infants and pregnant women, and one of its most common causes is iron deficiency. Iron is an essential nutrient for children ages 6 and 11. In several countries, governments counter iron deficiency by promoting mineral fortification of widely consumed foods. In this study, we evaluated the acceptability of a flour formula composed of chickpea flour and chicken liver powder as iron sources, partially replacing wheat flour in a sweet bread (muffin) preparation. In the first part of our experiment, three formulations with variable shares of chickpea and chicken liver powder were presented to a children's taste panel to assess odor, color, texture, and product acceptability, identifying the most acceptable substitution formulation (consisting of 65% chickpea flour, 5% chicken liver, and 30% wheat flour). The control recipe consisted of 100% wheat flour. The second part of the experiment evaluated product durability following three storage dates after preparation (1 day, 8 days, and 15 days). The attributes of color, texture, and flavor degraded slightly over time, and odor varied markedly in disproportion with storage time. The selected formulation can serve as an iron supplement without affecting the hedonistic perception of the final product made with it.

Keywords: muffin; nutrition; supplement; chickpea; liver

1. Introduction

The World Health Organization [1] states that anemia affects approximately 48% of children, 38% of pregnant women, and 29% of non-pregnant women worldwide. One of the leading factors driving this blood iron deficiency is the low consumption of food sources of iron [1].

Latin American government bodies report that nutritional iron deficiencies are globally widespread, with approximately 800 million children and women being in a chronic state of iron deficiency; estimates suggest that 273.2 million children under five were anemic in 2011, and about half of them also iron deficient [2].

In countries such as Colombia, where malnutrition prevails, authorities seek to improve healthy eating by promoting the intake of foods with high nutritional content. This follows evidence that the consumption of lower-quality foods predominates over foods of animal origin [3]. Moreover, reports reveal that about 30% of children under two years fail to meet their iron needs [3]. The ENSIN [3] revealed that anemia occurs with a prevalence of 11.6 and 12.1 cases for every 100 school-age children aged 5 and 12 years, respectively [3]. Therefore, the national strategy for preventing and controlling micronutrient deficiencies in Colombia 2014–2021 deployed pertinent actions targeting children up to 12 years [4].

Several countries possess strategies to counter iron deficiency in their populations. For instance, Chile has enacted a food iron fortification plan where powdered milk, fortified with iron and vitamin C, is supplied to low-income families [5], and Cuba developed intervention programs with iron-fortified foods [6]. Recently, flour fortification has been adopted in Canada, the United States, and in 20 Latin American countries [7]. A study conducted in 2019 evaluated the impact of such micronutrient fortification programs, demonstrating measurable improvements and a decline in iron deficiency prevalence [1]. This finding supports further food fortification endeavors.

There are products in Central America and Panama, such as Incaparina, and in South America, such as Bienestarina in Colombia [8,9]. These products have been developed with a mixture of soy and other cereals, which are also supplied to the infant population, but none of them include chickpea.

Chicken liver is a food source rich in protein and nutrients, including iron, with beneficial effects on human health. Chicken liver nutrients can be concentrated in powders obtained via dehydration, which extends their shelf life. Chicken liver powder has been employed as an ingredient in formulations for the preparation of products such as sponge cakes, in which iron bioavailability was evaluated with favorable results [10–12].

On the other hand, chickpea (Cicer arietinum), a pulse that grows in several world regions, including India, North Africa, the Middle East, the Americas, and Australia, among others [13,14], has a well-studied nutritional value. Chickpea's nutritional value, which depends on plant maturity, variety, and environmental growth conditions, has increased its demand. Chickpeas have a protein content between 12% and 25%, and they are rich in essential amino acids but limited in methionine and cysteine; thus, chickpea consumption is advised in combination with cereals to balance the intake of essential amino acids. Chickpea also has a protein digestibility of up to 79.4% [15], making it an ideal option for people with limited access to animal protein, vegetarians, and vegans. Likewise, chickpeas provide around 5 mg/100 g of iron, 4.1 mg/100 g of zinc, 138 mg/100 g of magnesium, and 3 mg/100 g of calcium [16].

Since chickpeas can be transformed into flour and protein concentrates, they are considered a functional ingredient in food products such as desserts, custards, baked goods, milk imitation products, infant formulas, and meat products. Depending on the chickpea's maturity stage, different preparations across the globe have been developed, for instance, germinated seeds; Kollo, a snack from the pulse soaked for two days and toasted; Nifro, in which the chickpea is soaked for two days, cooked with enough water, and mixed with cereal (commonly wheat). Shiro is another chickpea-based flour prepared by soaking, sun-drying, and toasting pulses and used in multiple Ethiopian preparations, including baby food. In other cuisines, chickpeas are also used for stews, soups, creams, salads, cooked, roasted, salted, and fermented as an appetizer, sun-dried, and in flour for the preparation of atole and mondongo [12,14,17–19].

Thus far, the use of partially substituted wheat flour with chickpea flour and the addition of chicken liver in baked goods has not been documented. Therefore, in this study, we evaluated and compared the sensory properties, such as texture, color, flavor, and acceptability, of sweet bread (muffin) preparations made with flours composed of variable shares of chickpea flour and chicken liver as iron content enhancers and partial replacements of wheat flour.

2. Materials and methods

Raw materials: The components of the tested preparation were chickpeas, chicken liver, wheat flour, sugar, vegetable oil, vinegar, whole milk, baking powder, vanilla essence, salt, and eggs, purchased in a local market in the city of Bogota.

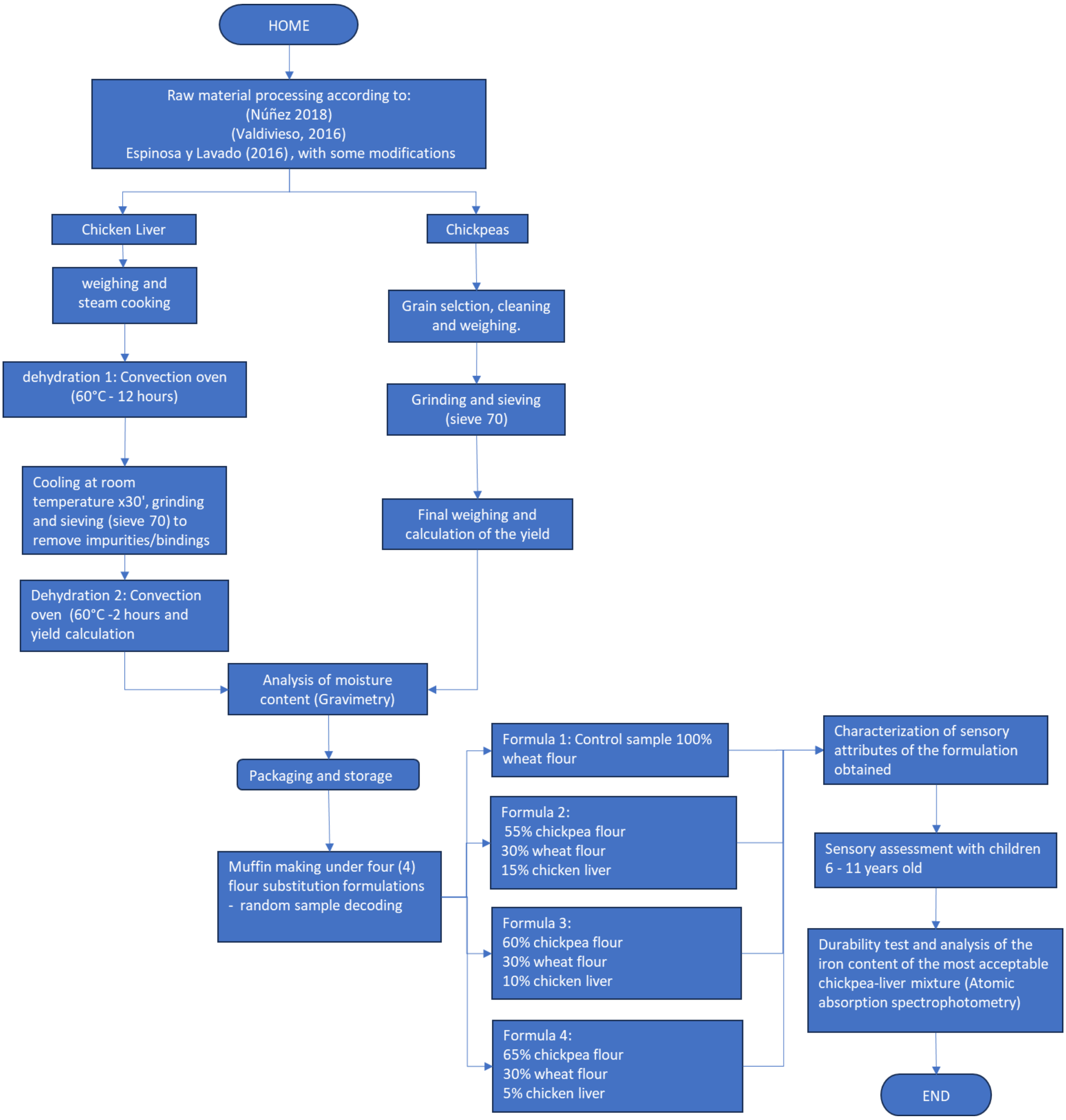


Figure 1. Chickpea and chicken liver processing and composition of test flour formulations.

Procedure: Chickpeas and chicken livers were processed to flour and powder, respectively, to construct three partially replaced wheat flour test formulations, following the steps described in **Figure 1**. These three test flour formulations and the 100% wheat flour, as a control, were subsequently used in a muffin recipe. The muffins obtained were sensory evaluated as indicated above.

2.1. Sensory evaluation

A total of four flour formulations were made, including the control formula. The main attribute evaluated was taste as an indicator of acceptability through a sensory panel.

Muffin taste assessments were performed at room temperature, and muffin formulations were each identified with unique three-digit random number codes and presented to 89 children from Bogotá-Colombia school years from first to fourth grade. All children were untrained panelists aged six to eleven years old, both sexes; each child was given a form explaining the test and how they should rate the product; distilled water was provided between formulas, which allows neutralization and taste bud cleansing.

The evaluation was carried out through a survey applying an affective facial hedonic scale model [20], with an ordered scale (ordinal variable) from one (1) to five (5) points, with five (5) being the highest level of acceptability. Scores were collated and analyzed statistically. Biosafety standards and preparation, cooling, packaging, storage, and transportation were applied throughout the assessments.

2.2. Shelf-life stability test

Finally, we evaluated the most acceptable formula from its shelf-life perspective, considering storage in polypropylene bags and conditions at room temperature. For this assessment, muffins were prepared at three different time points. The first muffin batch was made three weeks before their testing; the second batch was prepared eight days in advance, and the third batch 24 h in advance. All samples were stored in sealable polypropylene bags at room temperature. All products were monitored daily to determine the onset of signs alerting quality decay (color, odor, and appearance). Subsequently, these attributes were evaluated by 46 untrained adult panelists following the recommendations of Varela and Ares [21] of involving 20 to 50 naive consumers. The panelists completed the sensory analysis in a sensory testing laboratory. Distilled water was used to rinse the mouth between muffin samples during the evaluation. A methodology based on the identification of appropriate descriptors was applied to assess sensory properties. Using a five-point Likert scale (where 0 = unacceptable, 1 = bad, 2 = acceptable, 3 = good, 4 = very good, 5 = excellent quality), the attributes evaluated were color, smell, taste, flavor, texture, and the degree of overall acceptability.

2.3. Statistical analysis

For the sensory tests by children and adult panels, we considered an alpha of 5% and probabilistically determined the number of samples. Data were analyzed with an application developed on the MATLAB® platform. A non-parametric statistical inference ANOVA test was applied, following the assumptions of a normal distribution, randomness, and uniformity of variance (homoscedasticity), and considering that children and adults were untrained evaluators.

2.4. Moisture content

The loaves were weighed in a weighed pan and allowed to dry in an oven at 600 ℃ and the reduction in weight was recorded. The dry bread was ground in an electrical mill, and a sample was weighed in weighing glass and allowed to dry at 1050 ℃ for 3 h till constant weight. The total moisture was determined by the gravimetric method at 105° (AOAC 930.15 method).

2.5. Iron determination

Weighed samples (500 mg) were digested in nitric acid, and the digest was diluted for the determination of total iron using atomic absorption spectrometry (AAS) using standard AOAC methods (AOAC 985.35 Ed 21:2019 (Atomic Absorption Spectrophotometry)).

2.6. Ethical approval

The ethics committee at the Soydoy Foundation approved the protocol drafted for the sensory assessment by a children's panel. The fundamental aspects of the protocol included the physical delivery of the informed consent to parents/guardians of the children and the ingredients that made up the formulations, identifying possible allergens to avoid affecting the taster's health. Authorization was requested for image and data protection under Colombia's law 1581 of 2012.

3. Results and discussion

3.1. Yield analysis

The time and temperature of dehydration of chicken liver influenced the product's sensory characteristics. The most common problems correspond to color changes to green or blue and flavor [22]. A moisture loss was also evidenced, with a final yield of 20.6%. On the other hand, chickpea flour was processed with dry grain, yielding 87% (**Table 1**).

Table 1. Yield of raw materials processed.

	Chickpea	**Chicken liver**
Initial weight	1000 g	1712
Final weight	878 g	353
Yield	87%	20.6%

The values are an average of n = 3.

3.2. Moisture analysis

Moisture content in wheat flour is a quality factor that influences its deterioration. The Codex Alimentarius standard [23] was followed for flour moisture content, stating that a maximum of 15.5% m/m was acceptable. Gravimetric analyses revealed a moisture content of 12% m/m for the dehydrated chicken liver powder and 10.1% m/m for the chickpea flour.

3.3. Characterization of sensory attributes of the formulations obtained

Muffins made with the 100% wheat flour (control) sample (formula 1) were compared with those from the three constructed flour samples (formulas 2 to 4) for the sensory features: color, smell, taste, and texture, as shown in **Table 2**.

The most significant changes were related to sample color and smell, which varied with the concentration of chicken liver powder. However, the samples displayed the characteristic texture of the product developed, with evident variation in air cell sizes.

Table 2. Assessed attributes of the constructed flour formulations.

	1–675 Control	2–634	3–394	4–120
Product sensory feature				
Color: Estimated using Pantone color chart	Internal color #EBD18D, with caramelized crust #D6A15A	Internal color #E3B46E, with darker crust #78471C	Internal Color #BE974A, with crust #A3683B	Internal Color #F7D281, with crust #B47C3E
Texture: Estimated following ISO5492	Soft and chewy, granular, moist. Spongy and with small air cells.	Soft, chewy, granular, moist. medium air cells	Soft, chewy, granular, moist small air cells compact, compact mass	Soft, chewy, granular, moist. compact mass, small air cells.
Smell	Neutral smell without highlighting any of its ingredients	It has an intense metallic smell combined with a mild vanilla scent.	Nutty smell, characteristic of chickpea flour combined with the sweetness of sugar.	Sweet smell, no metallic Odor.
Taste	Sweet taste, low intensity	Sweet taste, with low intensity, leaving a slightly bitter aftertaste	Sweet taste that identifies a little more of the chickpea flour flavor, light metallic taste.	Sweet taste with low intensity, no detectable metallic taste with high acceptability compared to the control sample.

3.4. Sensory evaluation by children aged 6 to 11 years

Code-labeled muffin samples were randomized and presented to child panels for sensory assessment, as indicated in the methods section. The assumption of independence was met for the formulations evaluated. Taste was assessed using an ordered scale (ordinal variable) with a p-value of 0.05. The summary of taste differences between formulations is shown in **Figure 2**. A Fisher's multiple range test was also applied to determine the detail of the significant differences between formulations (**Figure 3**).

We focused our statistical interest on the relative localization of formulation median taste ratings, specifically determining which test formulation(s) differed significantly from the control formulation (100% wheat flour) and obtaining information on the test formulation(s) that optimize(s) the result. As shown in **Figure 2**, test formula 2 differed most significantly in interquartile range and median value from the control and the other two test formulas, and test formulation 4 (with 65%

chickpea flour content and 5% dehydrated chicken liver content) had the highest preference (i.e., it was as preferred as the control formula).

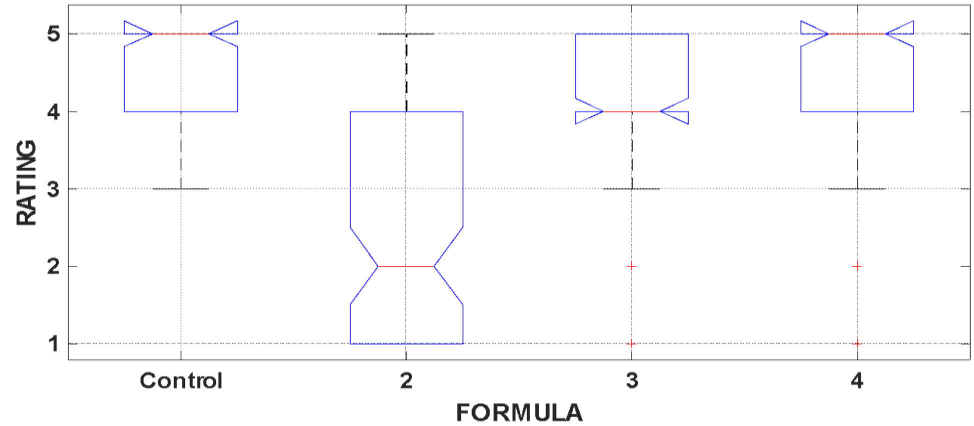
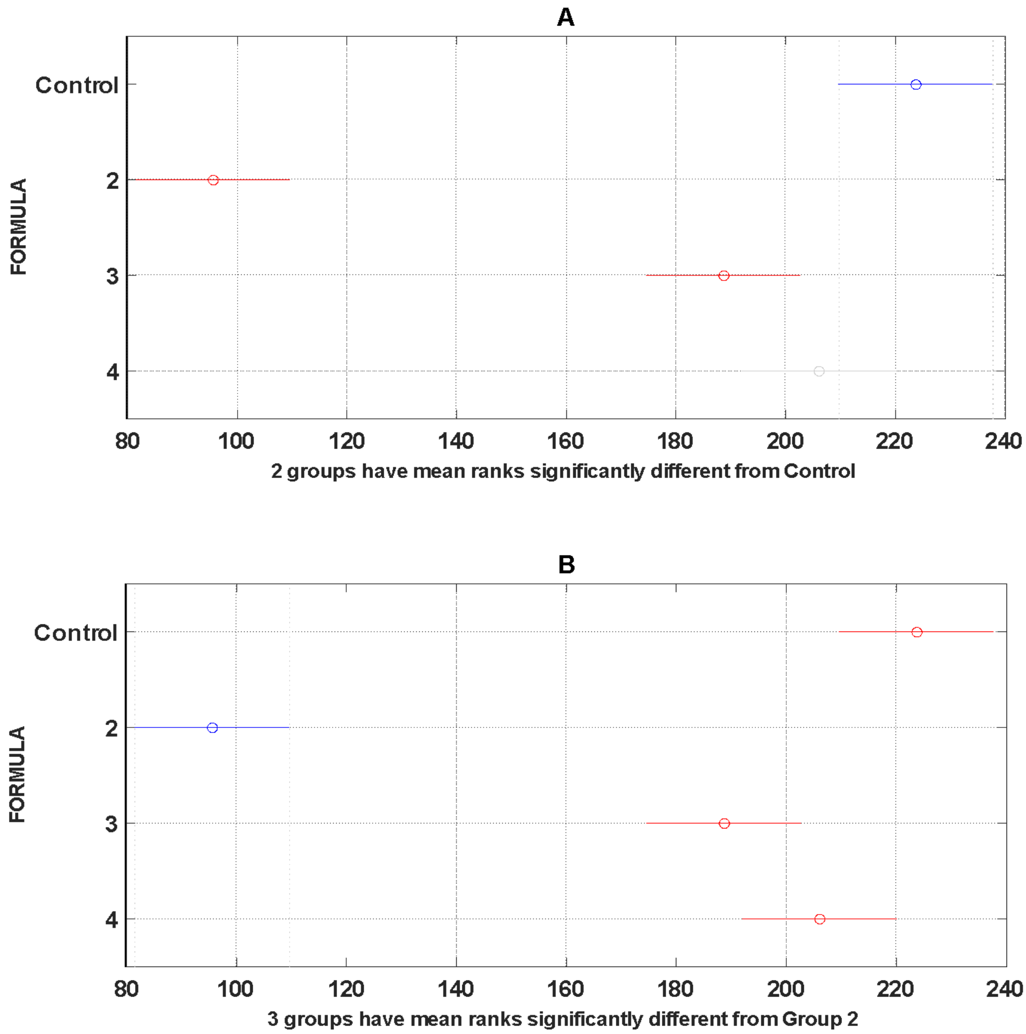
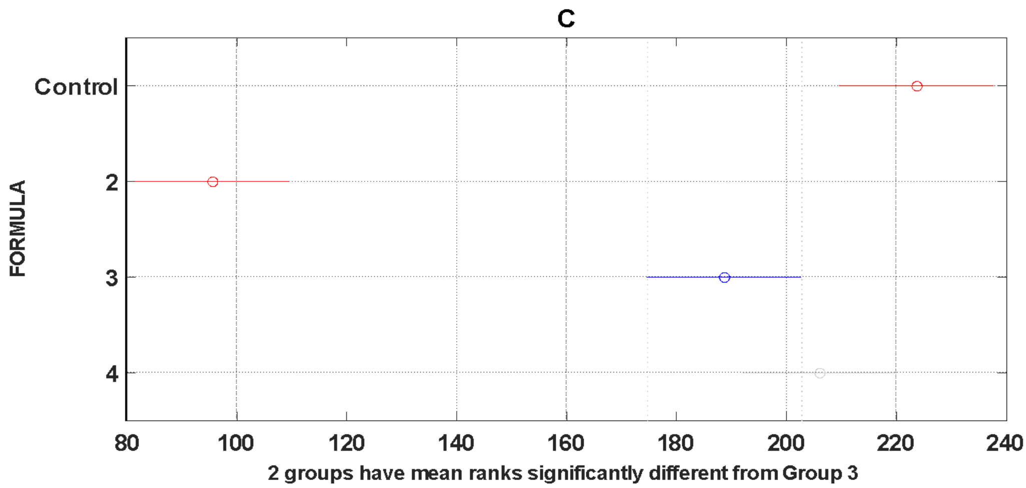


Figure 2. Evaluated attributes of the formulations.





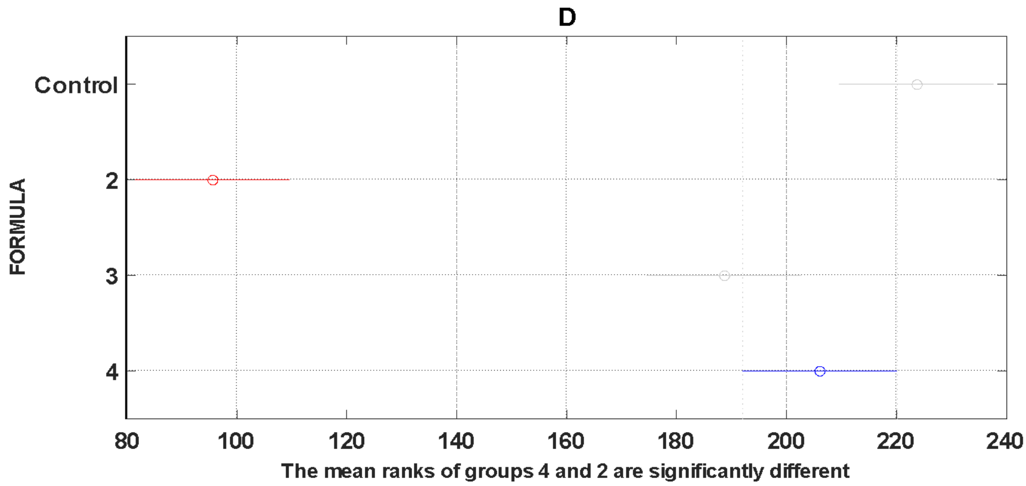


Figure 3. Multiple range comparisons among flour formulations—Fisher's test.

Note: For each panel in **Figure 3**, the reference formulation is shown in blue, and the compared flour formulations depicted in red differed significantly from the reference. Compared formulations in grey did not differ from the reference.

The measured moisture and iron contents of formulation 4, the one with the highest acceptance, are shown in **Table 3** and fall within the optimal ranges determined by local food regulations. The reference moisture value (≤ 15.5% m/m) was obtained from the Codex Alimentarius [23]. As for iron, the same normative poses that the reference value for minerals shall be the one established locally; Colombia's Ministry of Health's Decree 1944 of 1996 states that the minimum iron content in wheat flour must be 44 mg/1000 g (i.e., 4.4 mg/100 g).

Table 3. Moisture and iron content of the most acceptable flour formula.

Parameter	Methods used	Results	Units
Humidity	P-LF-008 Version 3 (Gravimetry—oven drying at 105 °C) Accredited	10.1	g/100 g
Iron (Fe)	AOAC 985.35 Ed 21:2019 (Atomic Absorption Spectrophotometry) Accredited	6.0	mg/100 g

3.5. Shelf-life stability test results

For the test flour formula with the highest acceptability, a 46-adult tasting panel (consisting of 60.9% women and 39.1% men over 18 years of age) conducted a sensory evaluation of muffins prepared at three different time points (day one, day eight, and day 15) before the assessment. The observed product acceptability shares, as per production date (i.e., storage period), are shown in **Figure 4**, revealing that fresher samples were the most accepted and that samples stored for 15 days were second in acceptability scores.

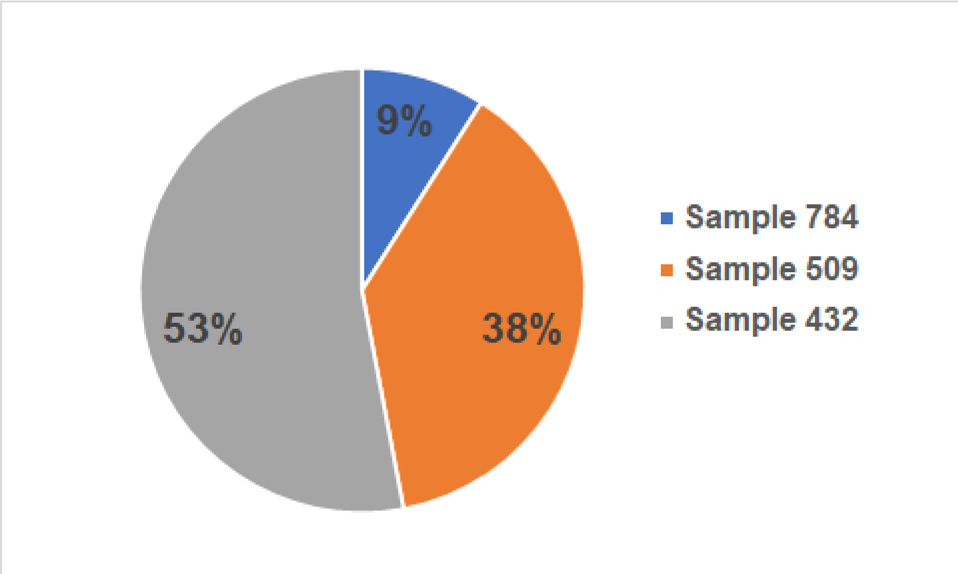


Figure 4. Overall acceptance of preparations as per storage time.

Note: Sample 509 (orange) was produced 15 days before sensory assessment, sample 784 (blue) was produced eight days before sensory assessment, and sample 432 (grey) was produced one day before sensory assessment.

An analysis of variance was performed to determine significant differences between tested products with three different storage times for a set of taste variables (i.e., durability attributes). Due to the number of samples (46), the assumptions of normality, randomness, and homoscedasticity are considered fulfilled; the results are presented in **Table 4**.

Table 4. ANOVA of durability attributes for samples prepared with flour formulation 4 at three different time points.

Attribute	Average			Standard Deviation			*p*-value	Comments (a = 5%)
	784	509	432	784	509	432		
Color	3.72	3.96	3.93	1.07	1.07	1.04	49.14%	No significant differences
Taste	3.61	3.74	3.85	1.04	1.08	1.03	55.33%	No significant differences
Smell	2.83	3.13	3.48	1.08	1.17	1.24	2.98%	No significant differences
Texture	3.57	3.63	3.96	1.07	1.10	1.03	17.37%	No significant differences
Acceptance	3.52	3.70	3.91	1.05	1.13	1.03	21.79%	No significant differences

Note: Sample 509 was produced 15 days before sensory assessment, sample 784 was produced eight days before sensory assessment, and sample 432 was produced one day before sensory assessment.

As seen in **Figure 5**, regardless of preparation (i.e., storage) time, all samples were equally accepted for color, taste, and texture, but sample 784's odor (prepared eight days in advance) experienced less acceptability. All products were overall well accepted throughout the 15 days of storage, although our data revealed signs of light storage time-dependent degradation. Odor was the sensory attribute with the most noticeable change with time among samples.

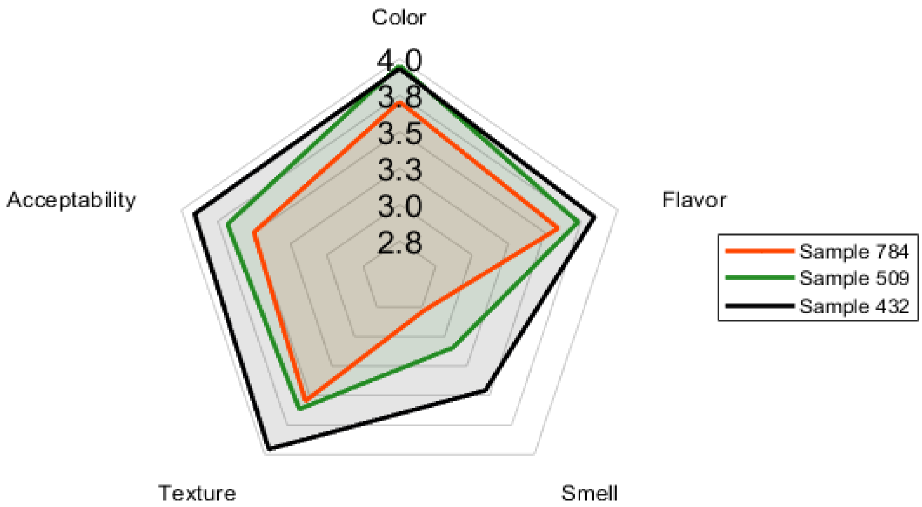


Figure 5. Attribute radar, from product durability assessments.

Note: Sample 509 (green) was produced 15 days before sensory assessment, sample 784 (red) was produced eight days before sensory assessment, and sample 432 (black) was produced one day before sensory assessment.

According to a study by Kiskini et al. [24], in a tested iron-fortified baked good, all sensory attributes were affected except for texture, which does not agree with the results obtained in our study. In contrast, in another study by Kiskini et al. [25], baked good pore size, odor, and color were significantly affected by the flour iron fortification process, leading to a darkening of the sample, a metallic taste, and a musty odor. Our current results agree with these previous observations.

However, Kiskini et al. [25] attribute changes in the texture of the crust of their baked samples, made with gluten-free ingredients, to the fortification process. We note that the absence of gluten in fortified flour poses a confounding factor. Thus, it is essential to evaluate the viscoelastic properties of baked goods from doughs with and without gluten in the context of fortification and how these factors affect end-product properties. We propose the study of this issue in greater depth and under specific instruments to improve the understanding of the properties of fortified baked goods.

4. Conclusions

The present work's approach revealed that chickpea flour and liver powder with yields of 87% and 21%, respectively, were possible.

Three muffin types were made with different flour formulations with variable contents of chickpea flour and chicken liver powder as iron sources. Following sensory evaluations, formulation 4 (consisting of 65% chickpea flour, 30% wheat flour, and 5% chicken liver powder) was accepted as the control formula (100% wheat flour) and had an iron content that exceeded the local minimum requirements and revealed favorable results from the durability test.

Author contributions: Conceptualization, LDMA and CGP; methodology, JJJC and MCR; software, CACH; validation, CACH, LDMA and CGP; formal analysis, LDMA; investigation, CGP, LDMA and MCR; data curation, CACH; writing—original draft preparation, LDMA; writing—review and editing, CGP, CACH; visualization, MCR; supervision, CGP; project administration, CGP; funding acquisition, LDMA and CGP. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest: The authors declare no conflict of interest.

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