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Optimization Of The Debittering Process In Lupinus Mutabilis: Implications For Safety And Functional Quality

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Abstract
Andean lupin (Lupinus mutabilis) offers strong potential as a source of flours and protein isolates, but its utilization requires a technically consistent debittering process that ensures sensory quality and safety. This article presents and evaluates an integrated protocol centered on four critical nodes: seed selection, hydration, cooking, and leaching. Selection homogenizes the lot and reduces variability; hydration defines imbibition kinetics and the point beyond which extending soaking ceases to be useful; cooking functions as a sanitary barrier and preconditions the matrix; and leaching, through scheduled water changes, completes alkaloid attenuation and stabilizes the system. The objective is to establish reproducible operating windows that link yield, microbiological safety, and technological suitability for direct consumption or further transformation. The analysis of results is prioritized over simple description: mass trajectories are tracked throughout the process, control indicators are monitored, and stages are compared, incorporating models that capture between-lot variability and multicriteria tools to guide plant decisions. The findings confirm consistent input lots, hydration that defines a clear operating point, cooking that acts as a critical control point and promotes diffusion, and leaching with scheduled changes that achieves debittering and stability without compromising the grain's suitability as an ingredient. The proposed sequence establishes plant-transferable parameters that balance efficiency, safety, and functionality, consolidating a debittering protocol geared toward the industrialization of Andean lupin.
Keywords: *Lupinus mutabilis; debittering; hydration; leaching; industrial scaling.*

1) INTRODUCTION

Andean lupin (Lupinus mutabilis Sweet) combines an uncommon high protein content with strong agroecological adaptability, but its food use and industrialization depend on post-harvest processes that reduce quinolizidine alkaloids and ensure safety without compromising yield or technological functionality. Accumulated evidence consistently describes that the bitter nature and variability of these alkaloids, together with the seed coat structure, condition the acceptance and stability of the final product (1,2,3). Within this framework, analyzing the issue as a process sequence is more productive than approaching it through isolated measurements: homogeneous seed selection, controlled hydration kinetics, cooking as a sanitary barrier, and efficient leaching form an interdependent chain that collectively determines the quality and performance of debittered lupin (4,5).

In the first node, selection aimed at physical purity reduces initial heterogeneity (grain size, cuticle thickness, color), variables that modulate water diffusion and alkaloid removal in subsequent stages (6,7). With a more uniform raw material, hydration progresses toward an operational equilibrium in which mass and volume increase and color becomes clearer due to microstructural changes, preparing the matrix for cooking and subsequent leaching (8,9). The technological literature describes strategies to intensify this phase, such as ultrasound treatments, used to shorten processing times without compromising performance; however, their application requires balancing cost, thermal effects, and the stability of sensitive compounds (10,11).

The third node, controlled cooking, integrates two critical functions: it facilitates the diffusion and removal of alkaloids by softening the matrix and acts as a lethal intervention on the microbiota, achieving substantial reductions of coliforms and the absence of Escherichia coli when adequate time/temperature parameters are respected (12,13). This sanitary effect is necessary but not sufficient, since re-exposure to water during debittering can reintroduce a residual microbial load if hygienic controls fail; hence the role of the fourth node, leaching with programmed water replacements and controlled aqueous management which optimizes alkaloid reduction and limits microbiological risks (14,15,16). Comparative reports show that beyond bitterness, the process modifies tocopherols, carotenoids, and phenolic compounds; therefore, the operational design must ensure safety without losing sight of the ingredient's functional quality (17,7).

This study adopts an experimental process-oriented approach that prioritizes measurement over description: Performance is quantified across key stages (dry → cooked → dehulled), microbiological behavior is evaluated under current sanitary criteria, and bromatological readings are verified at equivalent process points. Hydration is monitored over a 12–48 hour window to identify the operational equilibrium; cooking is standardized under homogeneous conditions, and leaching is conducted through regular water changes at a defined grain-to-water ratio consistent with the Andean adaptation of the so-called "Cusco method" and its technological transfer manuals (18). This logic aims to provide comparable and transferable parameters to support the standardization of Lupinus mutabilis flour and protein isolate production, aligning artisanal practices with contemporary requirements of safety, quality, and scalability (19,20).

2) MATERIALS AND METHODS

A comparative evaluation of the debittering flow of Andean lupin (Lupinus mutabilis) was carried out, centered on four critical nodes—selection, hydration, cooking, and leaching—with the aim of measuring how each stage affects mass yield (dry → cooked → debittered) and, complementarily, verifying bromatological quality and microbiological safety under national regulatory frameworks. The raw material entered after a physical purity test (≈1000 seeds, fraction separation and weighing) with an acceptance criterion of ≥98% pure seed to minimize initial variability and ensure a homogeneous technological response in subsequent stages. The operational protocol was structured as an Andean adaptation of the "Cusco method," with control of temperature, time, and water changes to favor alkaloid removal and sanitary stability of the process.

Hydration was carried out in potable water at 40 °C with a grain:water ratio of 1:3 (w/v), recording mass and volume at 0, 12, 24, 36, and 48 h to characterize mass-gain kinetics and identify the operational equilibrium ≈24 h (measurements were continued up to 48 h for comparison). After this phase, cooking was performed at 100 °C for 50 min with an approximate 1:1.5 (weight:volume) ratio, considering it a sanitary barrier and a facilitator of subsequent leaching. Leaching was conducted with water changes every 6 h and a lupin:water ratio of 1:1.5 (v/v), recording at each change the grain weight, soluble solids in the wash water, and pH. Monitoring continued until stabilization (≈48–60 h), defined by a weight variation ≤1% between consecutive intervals. Traceability of weighings/measurements was preserved at all stages to calculate yields relative to the initial dry weight.

As interpretive support for each stage, bromatological determinations were performed (protein—INEN-ISO 20483, moisture—INEN 1235, fiber—INEN 522, fat—INEN 523, ash—INEN 520, and potentiometric pH) as well as microbiological analyses (total coliforms—NTE INEN 1529-7 and *E. coli*—NTE INEN 1529-8), which were compared against NTE INEN 616 and NTE INEN 2390 for debittered lupin.

The design was completely randomized with $n = 3$ replicates per stage. Comparisons among hydrated, cooked, and leached samples were carried out using one-way ANOVA and Tukey HSD ($\alpha = 0.05$), reporting means, SD, 95% CI, and partial η^2. Time series (hydration 0–48 h; leaching every 6 h) were analyzed as repeated measures; when sphericity was violated, the Greenhouse–Geisser correction was applied, and when variability among batches justified it, a mixed linear model was used with batch as a random effect, reporting β, 95% CI, and marginal/conditional R^2. Pearson correlations (r)—with Spearman verification (ρ) when applicable—evaluated key associations: leaching time vs. pH and vs. soluble solids (expected to be negative), and the relationship between mass gain at 24 h and the initial slope of solids decline. Coliform counts were transformed as $\log_{10}(x+1)$ and also expressed as log reductions between post-cooking and post-leaching; when assumptions were not met (Shapiro–Wilk; Levene), Welch/Games-Howell or nonparametric tests (Friedman/Wilcoxon) were applied. All statistical decisions followed the same analytical logic used in the study's results, explicitly stating assumptions and robustness criteria.

3) RESULTS AND DISCUSSION

Seed Selection

The selection process allowed the inclusion of a highly homogeneous input: the average usable yield was 98.68% ($n = 3$), with a CV = 0.33% and a 95% confidence interval (CI95%) of 97.88–99.49%. All three replicates exceeded the operational threshold of ≥ 98% pure seed, ensuring a comparable baseline for hydration and minimizing bias in leaching and overall yield. This homogeneity reduces variability in water absorption and in the kinetics of alkaloid removal—conditions recognized as decisive for an efficient and scalable debittering process (1,7,6,18).

The analytical reading of Table 1 shows: (i) consistent selected mass (300 g across all three replicates), (ii) minimal variation in discarded material (3–5 g), and (iii) sustained compliance with the purity threshold. From an inferential perspective, the mean usable percentage exceeds 98%, and all replicates are ≥ 98.36%, establishing input control suitable for valid comparisons of hydration trajectories (12–48 h) and pH/soluble solids declines during leaching. This standardization is critical because the morphological and compositional diversity of *chocho andino* (*Lupinus mutabilis*) can lead to heterogeneous process responses if seed purity and grain integrity are not properly controlled (1,6,21). In summary, the selection stage anchors reproducibility and enhances technological transfer by reducing uncertainty prior to the four critical nodes of the debittering process.

Hydration (0–48 h)

The hydration kinetics show a rapid absorption phase between 0 and 24 hours, followed by a plateau between 24 and 48 hours. At 24 hours, the average weight increase reached 125.47% with SD = 0.26, rising slightly to 128.30% at 36 hours and 129.24% at 48 hours (SD = 0.25 in both cases), indicating operational water equilibrium around 24 hours under 40 °C and a 1:3 grain-to-water ratio.

This behavior is supported by the repeated-measures ANOVA from the study itself, which detected no significant differences between 24, 36, and 48 hours. The stabilization of means and the consistent dispersion (SD ≈ 0.25) reinforce the conclusion of a plateau after 24 hours (see Table 2).

The jump from 0→24 h accounts for nearly all mass gain; the relative changes from 24→36 h (+2.83%) and 36→48 h (+0.94%) are minor and statistically indistinguishable, confirming saturation of the porous matrix and the reduction of the osmotic gradient as physical limits of the process. The low variability among replicates (SD ≈ 0.25 from 24 h onward) suggests a reproducible behavior that facilitates industrial transfer while saving time, water, and energy by setting 24 h as the operational cutoff point.

Stabilization at 24 h aligns with studies reporting water equilibrium in *Lupinus mutabilis* under standard soaking conditions (22) and with the sigmoidal shape of the absorption kinetics described in systems without technological assistance, where the initial rapid phase is followed by a slowdown as pores become saturated (10). These patterns also fit within the process framework that integrates selection and hydration as prerequisites for efficient leaching in debittering (1,7). Overall, setting 24 hours as the optimal soaking time maximizes efficiency without compromising the structure or subsequent performance of the process.

Cooking

Under standardized cooking conditions, the final cooked weight averaged 697.67 g and the residual water 226.67 ml, with a modest mean percentage increase of 3.553%. Variability among replicates was limited: 35 g in final weight and 120 ml in residual water, while the increase ranged between 1.783–5.882% (Table 3). This magnitude suggests that, after achieving water equilibrium during hydration, cooking adds only marginal water retention and primarily functions as structural preconditioning for subsequent leaching, rather than as a mass-gain stage. The observed dispersion aligns with subtle differences in prior hydration and grain microstructure—factors that influence both water retention and diffusive mobility during debittering (7,6,1).

The modest increase (%) confirms that the thermal stage mainly contributes to:

i) softening cell walls and colloidal matrices, promoting alkaloid diffusivity during later leaching, and ii) acting as a highly effective sanitary barrier. Given the water equilibrium reached after approximately 24 hours of hydration, cooking adds marginal retention and reduces heterogeneity among grains, conditions that tend to accelerate washing kinetics without compromising yield (1,7). Likewise, thermal treatments in legumes are consistent with significant microbial reductions, reinforcing the safety of the product stream prior to debittering (13,12). The variation in residual water and percentage increase suggests that uniform prior hydration is the key operational determinant for minimizing fluctuations and maintaining net yield (6,22,10).

Maintaining time/temperature and the 1:1.5 ratio within narrow tolerances stabilizes yield per stage and improves scalability to industrial operations. In terms of process economics, controlling variability during cooking reduces uncertainty about mass losses or gains and prepares a more predictable setting for controlled leaching.

Leaching

Leaching with water replacements every 6 hours (water-to-lupin ratio 1:1.5) exhibited biphasic kinetics: an early rehydration peak at 6 hours followed by a gradual loss of mass until stabilization around 48–60 hours. The maximum weight occurred at 6 hours (818.67 g; +17.34% relative to time 0), stabilizing around 742–748 g by the end of the process (48–60 h). The most pronounced inter-replacement variations occurred between 36–42 h (−2.19% and −2.13%), after which oscillations were minimal (≤ 0.62%). This trajectory is consistent with an initial imbibition pulse (swelling of starch–protein matrices) followed by a diffusion-controlled stage governed by decreasing solute (alkaloids, sugars, salts) and water gradients, tending toward quasi-equilibrium after approximately 8–10 water exchanges (see Figure 1).

Mass stabilization between 48–60 hours coincided with a final pH of 5.43, compatible with the attenuation of quinolizidine alkaloids to sensory-acceptable levels in debittered seeds. This pH is typical of soaked and cooked grains and, together with the reduction of bitter solids through washing, supports the suitability of the product for direct consumption or as an input for immediate transformation (flour or isolate), provided that subsequent drying is managed when applicable.

In terms of safety, the stepwise scheme confirmed the complementary role of cooking and leaching. After hydration, total coliforms (3.2×10^3 cfu/g) and *E. coli* (3.3×10^2 cfu/g) were detected; cooking eliminated both indicators; by the end of leaching, low coliform counts (30 cfu/g) reappeared, with *E. coli* remaining undetectable, a pattern attributable to environmental recontamination and/or water used for replacement, underscoring the need for safe water and hygienic handling during exchanges and draining.

From the literature, the early imbibition peak and subsequent plateau align with the restructuring of walls and matrices during prolonged soaking (23), while prior cooking promotes later diffusion through partial gelatinization and microstructural opening (24,14). Successive washes reduce alkaloids and phenolic compounds, with possible losses of certain antioxidants, a well-documented trade-off in *Lupinus mutabilis* (7,25). Microbiologically, the combination of thermal treatment and leaching effectively enhances safety, although the washing stage requires strict water and surface quality control to prevent reintroduction of indicators (13,26).

Overall, the 6-hour exchange interval and 1:1.5 water-to-lupin ratio achieved efficient debittering with predictable kinetics and a stable final state (mass and pH), supporting industrial transfer and the use of *chocho* as an ingredient for flours and isolates without compromising safety, provided good practices are maintained during washing and timely drying when flour processing is intended. The numerical and microbiological evidence from the batch validates this protocol.

Overall Process Yield (Selection → Hydration → Cooking → Leaching)

The usable yield after selection was high (98.68%), confirming the efficiency of screening in ensuring homogeneous batches and minimizing initial losses; this input control determines the accuracy of subsequent mass and water retention estimates.

Hydration accounted for most of the mass increase: under controlled conditions (1:3 water: grain, 40 °C), 24 hours produced an average grain weight increase of 124.56%. Variation among replicates was narrow (122.67–126.67%), indicating a stable and replicable process. This behavior agrees with the literature, which places water equilibrium around 24 hours, even when measurements up to 48 hours show marginal plateaus (125.47–129.24%).

Mechanistically, the initial rapid phase corresponds to water diffusion into intercellular spaces and the starch matrix; extending soaking time adds no operational benefit when the goal is to maximize yield and efficiency (22,10).

Cooking produced an additional moderate increase (+3.56% compared with the hydrated weight), attributed to partial starch gelatinization and water retention in the protein network. Controlled leaching—with water replacements every 6 hours and a 1:1.5 water-to-lupin ratio—added +6.31% over the cooked weight and resulted in a final average weight of 741.67 g (Table 4). Overall, the mass water trajectory shows that hydration governs yield, while cooking and leaching fine-tune water equilibrium and facilitate debittering.

These trends are consistent with gravity/drainage stabilization described for cooked legumes (7,27) and with the role of cooking in texture and digestibility (Cortés et al., 2020), whereas sustained leaching supports alkaloid reduction and the grain's technological suitability (25).

Hydration accounts for 85–90% of the accumulated yield, so controlling it (time, temperature, water:grain ratio) is the main determinant of productivity. Cooking adds mass in a limited but significant way for debittering engineering by modifying microstructure and facilitating subsequent diffusion/draining. Although leaching contributes little to mass, it is critical for quality because it combines the adjustment of retained water with alkaloid reduction; its biphasic curve (peak at 6 h and stabilization at 48–60 h) confirms that frequent replacement accelerates solute removal and homogenizes the batch, with diminishing returns beyond 48 hours.

Comparatively, studies on *Lupinus* report the same pattern: rapid absorption and subsequent plateaus during soaking (22), small mass gains from cooking due to physicochemical changes (24), and a leaching stage where gravity and capillary diffusion govern stabilization (7,27).

For industrial transfer, the control point is to set hydration at 24 h, 40 °C, and 1:3, and to maintain leaching with replacements every 6 h (1:1.5) up to ≥48 h when the goal is to balance yield and debittering. Extending to 60 h enhances stability without significant mass changes but strengthens alkaloid control and batch standardization for subsequent use (flour and protein isolate), consistent with techno-functional findings reported for Andean lupin (24,25,28).

Microbiological Verification and Bromatological Profile

Health and compositional validation of the process focused on two complementary aspects: i) microbiological indicators by stage (hydration → cooking → debittering), and ii) the bromatological profile of the debittered

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product. Experimental data show total elimination of coliforms and *E. coli* after cooking (100 °C, 50 min), with a residual reappearance of total coliforms at the end of debittering (30 CFU/g) and a final slightly acidic pH (5.43), consistent with legume matrices subjected to prolonged soaking and washing (Table 5). The microbiological sequence confirms that cooking operates as a critical control point: it reduced coliforms from 120 CFU/g to undetectable levels, with *E. coli* remaining absent throughout. At the end of leaching, 30 CFU/g of coliforms reappeared, attributable to post-thermal recontamination from contact with water or the environment during replacements. This pattern is expected: heat is the most effective intervention to eliminate accompanying flora in legumes (12), while the wet washing stage requires microbiologically safe water and good practices to prevent reintroduction (26,13). In magnitude, leaching achieved a partial reduction compared to the hydrated stage (~0.60 log; 120→30 CFU/g), a relevant but smaller effect than that of the thermal stage, confirming cooking as the primary sanitary barrier and underscoring the need to standardize hygienic procedures during water changes.
The final pH of 5.43 aligns with ranges reported for *Lupinus mutabilis* after cooking and debittering (≈5.2–5.6), a condition that does not favor the growth of common pathogens and contributes to microbiological stability in protein-rich plant matrices (25,17). This acidic environment, combined with thermal reduction, lowers health risks in direct consumption and facilitates the product's use as an ingredient for flours or isolates, provided that drying or refrigeration is properly managed if immediate processing is not performed.
Compositionally, the debittered product contained 14.66% protein, 69.12% moisture, 2.19% fiber, 2.37% fat, and 3.90% ash. The protein content is consistent with literature documenting the functional stability of the protein fraction in *chocho* after soaking/washing, despite expected aqueous dilutions (7). The high moisture content explains the palatability of the fresh product but shortens shelf life without dehydration; literature warns of antioxidant losses during drying, a technological trade-off that must be managed depending on the intended use (17). The fiber and low fat content reinforce the ingredient's health potential, suitable for functional formulations and as a base for protein isolates.
Methodologically, the tests were conducted under NTE INEN standards for bromatology (protein, moisture, fiber, fat, ash) and under NTE INEN 1529-1/7/8 for microbiology, aligned with NTE INEN 616:2015 and NTE INEN 2390:2004 parameters, ensuring traceability and comparability with other studies and with quality requirements for debittered grain.
Overall, the thermal barrier ensures safety, and leaching complements microbial control while defining sensory and functional quality; the pH and composition of the debittered product provide a robust platform for direct consumption or industrialization as flour or isolate, with drying adjustments when appropriate.

4) CONCLUSIONS

The sequence of selection–hydration–cooking–leaching proved to be a reproducible protocol for obtaining debittered *chocho* suitable for consumption or transformation into flour and protein isolate. Input control (≥98% pure seed) reduced response variability in wet stages and aligns with the morpho-compositional heterogeneity reported for *Lupinus mutabilis*, where grain size and integrity determine water diffusion and alkaloid removal (6,1). At 40 °C and 1:3 (grain:water), hydration reached operational equilibrium at 24 h, with marginal gains between 24–48 h. This stabilization agrees with typical lupin soaking kinetics—an initial rapid phase followed by a plateau—and supports establishing 24 h as the cutoff point for water and energy efficiency without compromising subsequent stages (10,22,1).
Cooking (100 °C, 50 min; 1:1.5) produced modest mass increases but was decisive as a sanitary barrier and structural preconditioning step that promoted subsequent diffusion. Evidence on thermal treatments in legumes supports substantial reductions in hygienic indicators and improved matrix accessibility without yield loss (13,12). Within this framework, cooking should be managed as a critical control point in the process flow.
Leaching with 6-hour replacements and a 1:1.5 (water:chocho) ratio showed a biphasic trajectory—an early imbibition pulse followed by a diffusion-driven decline toward stabilization at 48–60 h—consistent with optimized debittering processes for *L. mutabilis* (14,15). The final pH (~5.4) and low residual coliform counts, with *E. coli* undetectable, indicate adequate alkaloid reduction and low sanitary risk, provided that replacements are carried out with safe water and proper hygiene (25,26,13).
The bromatological profile of the fresh debittered product retained protein levels suitable for use as an ingredient (≈15%), with high moisture content requiring drying or refrigeration depending on the purpose. This outcome aligns with reports on protein fraction stability and the functional trade-offs associated with washing and drying in Andean lupin, relevant when producing flour or protein isolates (7,17,20).

Operationally, overall yield was governed by hydration; cooking and leaching acted as fine-tuning steps ensuring safety and debittering without significant losses. For industrial transfer, the sequence 24 h/40 °C/1:3 → 100 °C/50 min/1:1.5 → 6 h replacements/1:1.5 provides directly scalable parameters for pilot or industrial batches, supported by technological literature and the consistency of the study's data (1,14,7).

Further lines of research remain open to strengthen industrial adoption: (i) instrumental quantification of residual alkaloids by HPLC to establish sensory and regulatory quality thresholds; (ii) assessment of water and energy costs of the replacement regime (multi-objective optimization); and (iii) characterization of the techno-functional properties of the debittered product and its isolates in target matrices (e.g., composite flours and meat formulations), where the balance between compound extraction, yield, and functionality determines economic feasibility (19,28,20).

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Annex: Tables and Figures

Table 1. *Seed selection: weight, waste, and usable yield (n = 3).*

Test	InitialWeight(g)	Waste (g)	Weight after selection (g)	Usable(%)
1	303,00	3,00	300,00	99,01
2	305,00	5,00	300,00	98,36
3	304,00	4,00	300,00	98,68
Mean	304,00	4,00	300,00	98,68

Acceptance criterion applied: ≥98% pure seed; combined inert material and other seeds ≤2%.

Table 2. *Hydration of chocho (40 °C; 1:3 grain:water). Average weight, increase (%), and SD (n per time = 3).*

Time (h)	Average weight (kg)	Average increase (%)	SD
0	1,06	—	—
12	1,74	64,15	0,16
24	2,39	125,47	0,26
36	2,42	128,30	0,25
48	2,43	129,24	0,25

Table 3. *Cooking of chocho: mass, water, and yield.*

Test	Initial Weight (g)	Water used (ml)	Final cooked weight (g)	Residual water (ml)	Increase (%)
1	680,00	1 020,00	720,00	300,00	5,882
2	668,00	1 002,00	688,00	200,00	2,994
3	673,00	1 010,00	685,00	180,00	1,783
Mean	673,67	1 010,67	697,67	226,67	3,553
Max–Mín	—	—	35,00	120,00	4,099

Figure 1. *Mass kinetics during leaching with replacements every 6 h.*

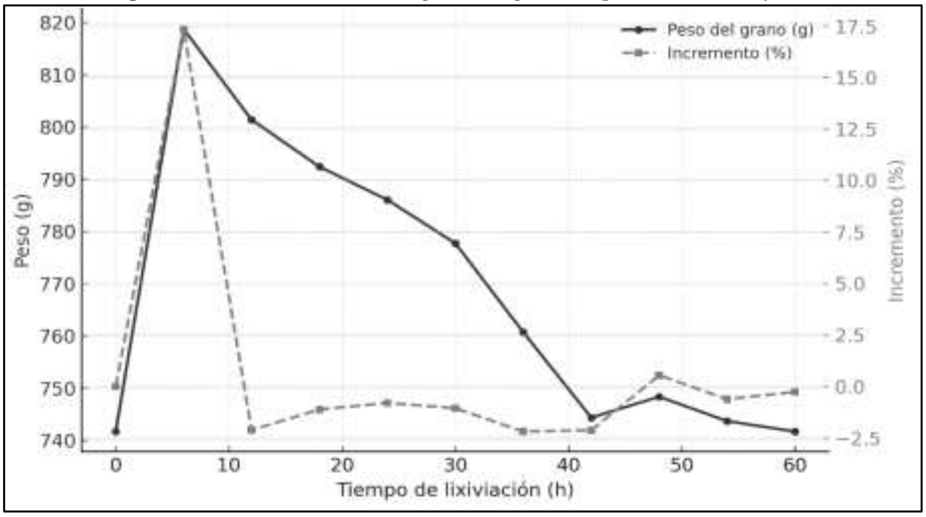

Table 4. *Evolution of weight and yield by stage (intermediate values estimated from observed percentage increases in the study).*

Stage	Process conditions	Expected weight (g)	Stage increase (%)	Cumulative from selection (%)
Selection	Screening and removal of impurities	300,00	—	0,00

Hydration (24 h)	1:3 water:grain; 40 °C	673,68	+124,56	+124,56
Cooking	Controlled heating	697,66	+3,56	+132,55
Leaching (60 h)	Water replacements every 6 h; 1:1,5 water:chocho	741,67	+6,31	≈ +147,2

Notes: hydration, cooking, and leaching increases according to experimental results; final weight from direct measurement.

Table 5. *Microbiological indicators by stage and bromatological profile of debittered chocho.*

Indicator	**Hydrated**	**Cooked**	**Debittered**
Total coliforms (CFU/g)	120	Absent	30
Escherichia coli (CFU/g)	Absent	Absent	Absent
pH (potentiometric)	–	–	5,43
Protein (%)	–	–	14,66
Moisture (%)	–	–	69,12
Crude fiber(%)	–	–	2,19
Total fat (%)	–	–	2,37
Ash (%)	–	–	3,90