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Estimation of Toxin (β-ODAP) Contents in Fresh, Stored, and Germinating Lathyrus Sativus Seeds

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Abstract

Lathyrus sativus (Grass Pea) remains an indispensable pulse crop due to its rich nutritional profile and climate adaptability; however, its dietary utility is severely limited by the neurotoxin β-N-oxalyl-α,β-diaminopropionic acid (β-ODAP). This study systematically evaluated the impact of long-term ambient storage, seed hydration (soaking), and multi-stage germination regimes on the dynamic degradation of β-ODAP and moisture retention. Fresh untreated seeds (baseline) recorded an initial moisture level of 12.20±0.30% alongside a mean toxin concentration of 0.80±0.04% dry weight. Prolonged dry storage for 12 months demonstrated negligible detoxification capacity, achieving a minor reduction of only 3.8%. In contrast, biological processing significantly enhanced toxin elimination. Imbibition via 12-hour soaking increased seed moisture to 49.50±1.10% and facilitated a 12.5% toxin loss through passive leaching. Subsequent germination further accelerated enzymatic degradation, systematically reducing β-ODAP levels to 0.50±0.03% (37.5% reduction) at 24 hours, 0.35±0.02% (56.3% reduction) at 48 hours, and reaching a minimum of 0.20±0.02% at 72 hours. Ultimately, the maximum relative neurotoxin reduction of 75.0% was accomplished during Stage III sprouting (72 hours), corresponding to a peak moisture accumulation of 72.00±1.90%. The findings establish that controlled germination is a highly sustainable, low-cost domestic protocol to effectively detoxify *L. sativus* seeds before dietary consumption.

Keywords: *Lathyrus sativus*, β-ODAP, Neurolathyrism, Detoxification, Germination Kinetics, Seed Hydration, Biological Processing.

1. Introduction

1.1 Background and Agronomic Importance

Lathyrus sativus L., commonly designated as Grass Pea, chickling pea, or Khesari pulse, represents one of the most ancient and durable legume crops known to human agriculture (Campbell,1997). Belonging to the family Fabaceae, *L. sativus* is extensively cultivated across South Asia, Sub-Saharan Africa, and the Mediterranean basin. The crop is renowned for its remarkable agronomic resilience, demonstrating an exceptional capacity to thrive under extreme environmental stressors including severe drought, extensive flooding, soil salinity, and nutrient-depleted marginal lands where nearly all other food crops fail. Nutritionally, Grass Pea seeds possess a high crude protein content ranging between 26% and 32%, alongside essential minerals, dietary fiber, and complex carbohydrates, making them a crucial dietary lifeline for low-income populations during agricultural famines and socio-economic crises.

Despite its immense nutritional and agricultural benefits, the food use of *L. sativus* is severely restricted due to the biosynthesis of endogenous anti-nutritional compounds, most notably β-N-oxalyl-α,β-diaminopropionic acid (β-ODAP). β-ODAP is a non-protein amino acid that acts as a structural analog to glutamate, functioning as a potent agonist at the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors within the central nervous system (Spencer et al. , 1986).

Prolonged, exclusive dietary reliance on *L. sativus* seeds—typically accounting for more than 30% of daily caloric intake over a period of 2 to 4 months—leads to neurolathyrism. Neurolathyrism is an irreversible neurological disorder characterized by metabolic excitotoxicity, oxidative damage to anterior horn motor neurons, mitochondrial dysfunction, and the progressive degeneration of upper motor neurons in the spinal cord. Clinical manifestations range from mild spasticity and muscle weakness in the lower limbs to severe irreversible paraplegia, necessitating crutches or lifelong mobility assistance. Consequently, mitigating β-ODAP toxicity remains a paramount global food safety mandate.

1.3 Rationale and Study Objectives

Industrial detoxification mechanisms—such as solvent extraction, chemical hydrolysis, or genetic modification—often prove unfeasible, expensive, or inaccessible to rural populations in developing countries where *L. sativus* is predominantly consumed. Therefore, optimizing accessible, household-level processing techniques is vital. While soaking and thermal cooking partially decrease water-soluble toxins, biological processing through germination (sprouting) triggers endogenous enzymatic degradation pathways capable of catabolizing β-ODAP into non-toxic metabolites (Lambein et al., 2019).

This investigation was conducted to systematically quantify and evaluate:

- The effect of long-term ambient storage (12 months) on β-ODAP stability and moisture dynamics.
- The hydration kinetics and dry biomass changes during soaking and progressive 72-hour germination stages.
- The precise relative toxin reduction percentage achieved across each developmental stage to establish a safe domestic processing model.

2. Materials and Methods

2.1 Seed Selection and Treatment Categorization

High-quality, mature, healthy *Lathyrus sativus* seeds were acquired for the experimental treatments. Seeds were thoroughly cleaned to remove foreign matter, dust, broken kernels, and botanical debris. The sample set was divided into six experimental conditions:

- **Fresh Raw Seeds (Baseline Control):** Freshly harvested seeds evaluated immediately without processing (0 hours).
- **Stored Seeds (12 Months):** Seeds stored in sealed containers under ambient room conditions (25±2°C) for 12 months (0 hours processing).
- **Soaked Seeds:** Fresh seeds submerged in distilled water at a 1:5 (w/v) seed-to-water ratio for 12 hours at room temperature.
- **Sprouting Seeds (Stage I):** Soaked seeds placed on moistened filter papers in dark germinating chambers for 24 hours.
- **Sprouting Seeds (Stage II):** Germination extended under humid conditions for 48 hours.
- **Sprouting Seeds (Stage III):** Germination extended to full sprout development at 72 hours.

The soaking and germination procedures were adapted from previously reported methods for *L.sativus*

seed processing (Girma & Korbu,2012).

2.2 Determination of Seed Weight and Moisture Kinetics

For each treatment stage, initial sample weight (W1) was fixed at exactly 10.00 g. Following treatment execution, samples were dried in a hot-air forced-convection oven at 105±2°C until reaching constant weight to establish the final dry biomass weight (W2 in g). Moisture Content (%) was computed using the standard gravimetric formula:

Moisture Content (%) = ((W1 - W2) / W1) * 100

The moisture content was determined according to the standard methods of AOAC International (2019).

2.3 Extraction and Quantification of β-ODAP

The quantitative estimation of β-ODAP content was performed using spectrophotometric assays based on the Rao spectrophotometric method. Seed tissues were ground into fine powders, extracted with 60% ethanol, and hydrolyzed with sodium hydroxide. The reaction product formed a colored complex with OPT (o-phthalaldehyde), and absorbance was read at 420 nm against standard calibration curves(Rao,1964). Mean β-ODAP concentrations were recorded as percentage dry weight (% Dry Weight).

2.4 Calculation of Relative Toxin Reduction

The Relative Toxin Reduction percentage (%) was calculated in relation to the baseline concentration determined in Fresh Raw Seeds (0.80% dry weight) following the approach described by Kuo et al.(1995).

Relative Toxin Reduction (%) = ((Mean β-ODAP Baseline - Mean β-ODAP Sample) / Mean β-ODAP Baseline) * 100

2.5 Statistical Analysis

All experiments were performed in triplicate (n=3). Results are reported as Mean ± Standard Deviation (SD). Data were subjected to One-Way Analysis of Variance (ANOVA), and treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at a significance threshold of $p < 0.05$ (Steel & Torrie,1980).

3. Results

The quantitative parameters recorded across all treatment stages—including duration, initial wet weight (W1), final dry weight (W2), moisture percentage, mean β-ODAP concentrations, and relative toxin reduction—are presented in Table 1.

Table 1: Effect of Processing and Germination Duration on Seed Moisture and β-ODAP Levels

Seed State / Stage	Duration (Hours)	Initial Weight, W1 (g)	Final Dry Weight, W2 (g)	Moisture Content (%)	Mean β-ODAP Content (% Dry Weight)	Relative Toxin Reduction (%)
Fresh Raw Seeds	0	10.00	8.78	12.20±0.30	0.80±0.04	0.0% (Baseline)
Stored Seeds (12	0	10.00	8.90	11.00±0.40	0.77±0.05	3.8%

Seed State / Stage	Duration (Hours)	Initial Weight, W1 (g)	Final Dry Weight, W2 (g)	Moisture Content (%)	Mean β-ODAP Content (% Dry Weight)	Relative Toxin Reduction (%)
Months)						
Soaked Seeds	12	10.00	5.05	49.50±1.10	0.70±0.03	12.5%
Sprouting Seeds (Stage I)	24	10.00	4.25	57.50±1.60	0.50±0.03	37.5%
Sprouting Seeds (Stage II)	48	10.00	3.45	65.50±1.70	0.35±0.02	56.3%
Sprouting Seeds (Stage III)	72	10.00	2.80	72.00±1.90	0.20±0.02	75.0%

Note: All values represent Mean ± Standard Deviation (SD) of triplicate determinations (n=3). Relative reduction calculated against baseline fresh raw seed concentration (0.80%).

3.1 Dry Biomass and Moisture Dynamics

- Fresh raw control seeds registered a final dry weight (W2) of 8.78 g, establishing an initial moisture level of 12.20±0.30%.
- Stored seeds after 12 months showed a slightly higher dry weight (8.90 g), reflecting minor dehydration down to 11.00±0.40% moisture.
- Imbibition during soaking rapidly increased seed moisture to 49.50±1.10% (W2=5.05 g).
- During progressive sprouting, seed moisture continuously expanded to 57.50±1.60% at Stage I (24 h), 65.50±1.70% at Stage II (48 h), and peaked at 72.00±1.90% at Stage III (72 h). Simultaneously, final dry weight (W2) steadily declined from 4.25 g to 2.80 g, signaling metabolic dry matter loss during seedling development.

3.2 β-ODAP Degradation and Relative Toxin Reduction

- Stored seeds exhibited an insignificant reduction in toxin level (0.77±0.05%), achieving only 3.8% relative reduction.
- Soaking for 12 hours decreased β-ODAP to 0.70±0.03%, corresponding to a 12.5% reduction.
- Germination caused a dramatic, time-dependent breakdown of the toxin. Mean β-ODAP concentrations fell to 0.50±0.03% (37.5% reduction) at 24 hours, 0.35±0.02% (56.3% reduction) at 48 hours, and reached an absolute low of 0.20±0.02% (75.0% reduction) at 72 hours.

4. Discussion

4.1 Toxicity Persistence in Stored Seeds

The empirical data obtained from 12-month ambient stored seeds (0.77±0.05% β-ODAP; 3.8% reduction) demonstrates that prolonged dry storage is ineffective as a standalone detoxification strategy. β-ODAP is a highly stable, low-molecular-weight non-protein amino acid that resists thermal degradation and oxidative decay under low-moisture dry conditions (11.00±0.40%). In the absence of cellular hydration, metabolic enzymes remain dormant, preventing enzymatic hydrolysis. This confirms that rural farming communities cannot rely on extended post-harvest storage to render *L. sativus* safe for human consumption (Tarade et al.,2007).

4.2 Mechanics of Hydration and Passive Leaching during Soaking

The 12-hour soaking treatment induced a substantial increase in seed moisture content from 12.20% to 49.50%, accompanied by a 12.5% reduction in β-ODAP. The mechanism driving toxin reduction during soaking is primarily physical passive leaching. Because β-ODAP is a water-soluble free amino acid, rapid cellular hydration disrupts seed coat permeability, enabling solute diffusion along a concentration gradient into the surrounding soaking water. However, passive leaching alone leaves a substantial residue of neurotoxin (0.70±0.03%), indicating that soaking must serve as a preparatory step rather than a final treatment (Kuo et al.,1995) .

4.3 Enzymatic Catabolism and Kinetic Relationship during Germination

Progressive germination (24 to 72 hours) proved to be the most potent driver of β-ODAP degradation, achieving 37.5%, 56.3%, and 75.0% relative reductions across Stages I, II, and III, respectively.

This accelerated degradation is governed by two complementary biological mechanisms:

1. **Enzymatic Catabolism:** Imbibition triggers the activation of endogenous aminotransferases, oxalyl-CoA synthetases, and specialized oxidases. During seed germination, nitrogen stored within non-protein amino acids like β-ODAP is mobilized to support embryonic axis growth and sprout synthesis. β-ODAP is metabolically degraded into non-toxic compounds such as diaminopropionic acid (DAP) and oxalyl derivatives.
2. **Biomass Respiration and Conversion:** As embryonic growth accelerates, seed reserves are consumed via cellular respiration, as evidenced by the progressive drop in final dry weight (W2) from 8.78 g to 2.80 g.

A direct kinetic correlation is observed: as moisture content rises from 57.50% (Stage I) to 72.00% (Stage III), β-ODAP concentrations continuously decay. At Stage III (72 hours), the neurotoxin level drops to 0.20±0.02% dry weight. Concentrations below 0.20% are widely recognized by international food safety organizations as safe threshold limits for human consumption without risking neurolathyrism.

5. Conclusion

This research evaluated the kinetic relationship between seed hydration, dry biomass loss, and neurotoxin degradation in *Lathyrus sativus* seeds across ambient storage, soaking, and multi-stage germination regimes.

Key conclusions derived from the study include:

- **Storage Inefficacy:** Ambient dry storage for 12 months produces negligible toxin reduction (3.8%), proving that dry seeds retain their neurotoxic potential.
- **Soaking Dynamics:** A 12-hour soak facilitates seed hydration to 49.50% moisture and achieves a 12.5% toxin loss via passive solute leaching.

- **Germination Efficiency:** Extending germination to 72 hours (Stage III) maximizes endogenous enzymatic degradation, reducing β-ODAP from 0.80% to a safe baseline level of 0.20%, representing a **75.0% relative toxin reduction**.

Recommendation:

Implementing a 12-hour soak followed by a 72-hour germination protocol is strongly recommended as an accessible, chemical-free, highly effective domestic processing method to eliminate neurolathyrism risks and enhance the nutritional security of *Lathyrus sativus.*

References

1. Rao, S. L. N., Malathi, K., & Sarma, P. S. (1964). An active neurotoxin from the seeds of *Lathyrus sativus*. *Biochemistry*, 3(3), 432-436.
2. Campbell, C. G. (1997). *Grass pea.*Lathyrus Sativus L. Promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome
3. Lambein, F., Travella, S., Kuo, Y. H., Van Montagu, M., & Heijde, M. (2019). Grass pea (*Lathyrus sativus* L.): orphan crop, multidimensional food and health security tool. *Phytochemistry Reviews*, 18(5), 1323-1354.
4. Urga, K., Fite, A., & Kebede, B. (2005). Effect of processing on β-ODAP content of *Lathyrus sativus* seeds. *African Journal of Food, Agriculture, Nutrition and Development*, 5(1), 1-12.
5. Fikre, A., van Moorhem, M., Ahmed, S., Lambein, F., & Gheysen, G. (2011). Studies on β-ODAP degradation in germinating *Lathyrus sativus* seeds under varying environmental conditions. *Food and Chemical Toxicology*, 49(3), 587-593.
6. Girma & Korbu, (2012). Effect of soaking, germination and cooking on anti-nutritional factors of grass pea (*Lathyrus sativus* L.) seeds. *African Journal of Food, Agriculture, Nutrition and Development*, 12(5), 6512-6525.