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ORIGINAL ARTICLE

Physicochemical and bioactive aspects of germination of lima bean seeds[1]

Aspectos físico-químicos e bioativos da germinação de sementes de feijão-lima

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HIGHLIGHTS:

Germination between 72 and 96 hours provides sprouts with greater bioactive compounds content.
Among the varieties, the protein content did not present a common period.
It is possible to modulate the nutritional and functional composition of lima beans by controlling the germination period.

ABSTRACT: Controlled germination is a simple low-cost procedure to improve the nutritional, functional and sensory quality of seeds intended for food. Germination conditions directly influence the final composition of the sprout, being mainly a function of the process time. Thus, the aim was to examine the effects of germination periods on the nutritional properties of lima bean seeds of the varieties ‘Orelha-de-Vó Preta’, ‘Raio-de-Sol’, ‘Branca’, and ‘Raio-de-Sol Grande’. The water content, proteins, starch, total and reducing sugars, total phenolic compounds, tannins, flavonoids, and anthocyanins were evaluated at times 0, 24, 48, 72, and 96 hours. The data was processed in a completely randomized design and arranged in a factorial scheme (4 × 5), with three replicates. The germination process increased the protein content and reduced the starch content, promoting a partial increase in reducing sugars and total sugars. The total phenolic compounds, tannins, flavonoids, and anthocyanins increased with the process time, reaching absolute maximum values between 72 and 96 hours. The variety ‘Orelha-de-Vó Preta’ presented the highest values of total phenolic compounds and anthocyanins; the flavonoid content was higher in ‘Orelha-de-Vó Preta’ and ‘Raio-de-Sol Grande’, while the variety ‘Branca’ presented the highest of total tannins content. Therefore, it is concluded that germination has proven to be a process capable of enhancing the nutritional and functional composition of lima beans.

Key words: *Phaseolus lunatus*, sprouts, phenolic compounds, tannins

RESUMO: A germinação controlada é um procedimento simples e de baixo custo que busca melhorar a qualidade nutricional, funcional e sensorial de sementes destinadas à alimentação. As condições de germinação influenciam diretamente na composição final do broto, sendo função principalmente do tempo de processo. Assim, objetivou-se examinar os efeitos de períodos de germinação nas propriedades nutricionais de sementes de feijão lima das variedades ‘Orelha-de-Vó Preta’, ‘Raio-de-Sol’, ‘Branca’ e ‘Raio-de-Sol Grande’. Foram avaliados os teores de água, proteínas, amido, açúcares totais e redutores, compostos fenólicos totais, taninos, flavonoides e antocianinas nos tempos 0, 24, 48, 72 e 96 horas. Os dados foram processados em delineamento inteiramente casualizado e dispostos em esquema fatorial (4 × 5), com três repetições. O processo de germinação aumentou o teor de proteína e reduziu o teor de amido; promovendo o aumento parcial dos açúcares redutores e dos açúcares totais. Os compostos fenólicos totais, taninos, flavonóides e antocianinas aumentaram com o tempo de processo, atingindo valores máximos absolutos entre 72 e 96 horas. A variedade ‘Orelha-de-Vó Preta’ apresentou os maiores valores de compostos fenólicos totais e antocianinas; o teor de flavonóides foi maior na ‘Orelha-de-Vó Preta’ e no ‘Raio-de-Sol Grande’, enquanto a variedade ‘Branca’ apresentou o maior teor de taninos totais. Portanto, conclui-se que a germinação demonstrou ser um processo capaz de aprimorar a composição nutricional e funcional do feijão lima.

Palavras-chave: *Phaseolus lunatus*, brotos, compostos fenólicos, taninos

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Introduction

Lima bean (*Phaseolus lunatus*) is the second most important crop of the *Phaseolus* genus, and has many genotypes that vary in seed morphology, color, and size (Costa et al., 2017; Sousa et al., 2018). In Brazil, the Northeast region is responsible for 99% of the production of *Phaseolus* grains, which are consumed green, ripe or dry (IBGE, 2019).

Germination is a process that begins with the absorption of water by the seeds, promoting the reactivation of enzymes that initiate the degradation of macromolecules such as carbohydrates, proteins and lipids, to generate the necessary components for cell division and, consequently, seedling growth (Rasera & Castro, 2020). During this process, biochemical changes occur that enhance the nutritional and functional quality of the seeds, including improvements in protein digestibility, increased bioavailability of minerals and vitamins, reduction of antinutrients (such as proteolytic inhibitors, lectins, phytates, and tannins), and synthesis and accumulation of bioactive compounds (Chu et al., 2020).

Germination, therefore, depends on humidity, temperature, light, germination period, species and its varieties. These factors influence the nutritional and bioactive content, motivating studies on their effects on various grains and seeds such as: fava (*Vicia faba* L.) (Mekky et al., 2020), field lupine (*Lupinus albus*), chickpeas (*Cicer arietinum* L.), lentils (*Lens culinaris* Medikus), and fenugreek (*Trigonella foenum-graecum* L.) (Saleh et al., 2019). The studies seek to increase the potential for use of grains, by inducing the synthesis of bioactive principles or changes in composition that increase or reduce certain nutrients, according to nutritional or market demands. Modified grains can provide raw materials necessary for the development of functional ingredients, enriching foods, and provide economic alternatives for the agroindustry.

Despite the wide variety of seeds already evaluated, little information is available on the changes in the composition of lima beans (*Phaseolus lunatus*) caused by controlled germination. Thus, this study aimed to evaluate the effect of five germination periods on the physicochemical properties and bioactive compounds of lima bean seeds from varieties ‘Orelha-de-Vó Preta’, ‘Raio-de-Sol’, ‘Branca’, and ‘Raio-de-Sol Grande’.

Material and Methods

Four cultivars of lima bean were used ‘Orelha-de-Vó Preta’, ‘Raio-de-Sol’, ‘Branca’, and ‘Raio-de-Sol Grande’ (Figure 1), all of which were purchased from local vendors in the municipality of Campina Grande city (7° 13’ 50” S and 35° 52’ 52” W, altitude of 550 m), Paraíba state, Brazil. The seeds were selected by choosing those that were whole, free of perforations, and of uniform size. They were then stored in kraft paper bags at room temperature for 30 days, until they were used.

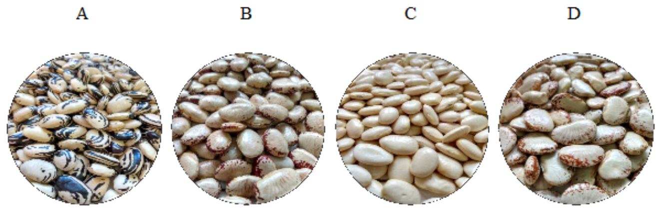


Figure 1. Seeds of *Phaseolus lunatus* ‘Orelha-de-Vó Preta’(A), ‘Raio-de-Sol’ (B), ‘Branca’ (C), and ‘Raio-de Sol Grande’ (D)

Samples of 100 g of each variety were sanitized by immersing them in distilled water at a 1:10 ratio, containing 7% (w/v) of sodium hypochlorite at room temperature (28 ± 1 °C) for 5 min. Immediately after sanitization, the seeds were washed with distilled water, drained and placed in trays at room temperature to eliminate surface water.

The seeds were germinated following the recommendations of the Rules for Seed Analysis (BRASIL, 2009), using Germitest paper. Fifty-six seeds were distributed per sheet, for each variety, kept in BOD-type (Q315M25, Quimis, Brazil) chambers at 25 °C and in the dark. The samples were irrigated every 24 hours, with approximately 25 mL of distilled water being applied to each Germitest paper. After germination, the samples were removed from the paper and crushed in a domestic blender for further characterization.

The analyses were performed in triplicate at 0, 24, 48, 72, and 96 hours. Water content was analyzed by direct drying in an oven at 105 °C (IAL, 2008); crude protein content was determined using the Kjeldahl method (IAL, 2008); starch content was analyzed using a method based on acid hydrolysis of starch and titration with Fehling’s solution (IAL, 2008); total sugars were determined by the anthrone method (Yemm & Willis, 1954); and reducing sugars were measured using the 3,5-dinitrosalicylic acid method (Miller et al., 1959).

The content of total phenolic compounds was determined using the method described by Waterhouse (2006), with the Folin-Ciocalteau reagent. Tannins were quantified using the methodology described by Goldstein & Swain (1963), using the tannic acid curve as a standard. Total anthocyanins and flavonoids were evaluated according to the methodology described by Francis (1982).

The data were processed using Assistat software version 7.7 Beta (Silva & Azevedo, 2016) in a completely randomized design and arranged in a 4 × 5 factorial scheme (varieties x germination periods), with three replicates. For the qualitative factor (varieties), the means were compared using the Tukey’s test ($p \leq 0.05$). To evaluate the effect of the germination period, regression analysis was applied, and the chosen model was the one that showed significance and best represented the data behavior. Pearson’s correlation coefficients (r) were calculated between all variables using JASP software version 0.17.1 (University of Amsterdam).

Results and Discussion

The Table 1 presents a summary of the variance analyses for moisture content, crude protein, starch, total and reducing sugars, as well as for the bioactive compounds in lima bean seeds, across five different germination periods. It is observed that there was an interaction effect between the factors for all the studied variables, with a significance level of 1%, indicating a statistical difference between the treatments.

Table 1. Analysis of variance for moisture content, crude protein, starch, total and reducing sugars, and bioactive compounds in lima bean seeds germinated over different periods

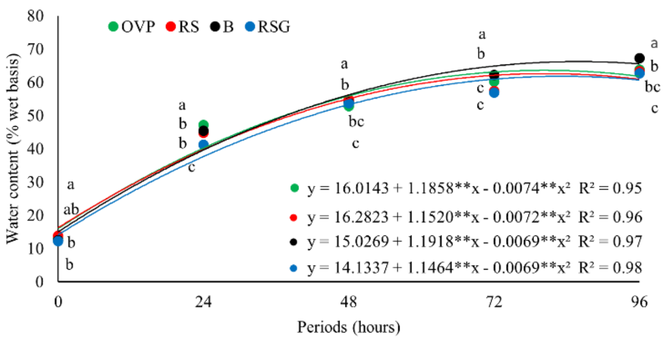
Source of variation	DF	Mean squares				
		Water content	Crude protein	Stach	Total sugars	
V	3	22.69**	2.62^{ns}	245.29**	7.20**	
GP	4	4979.32**	35.69**	186.60**	5.37**	
V x GP	12	7.81**	7.15**	10.27**	2.79**	
Error		0.16	1.42	0.43	0.01	
CV		0.85	5.22	1.30	0.30	
		Reducing sugars	TPC	Total tannins	Flavonoids	Anthocyanins
V	3	0.56**	14654.12**	3703.28**	1834.27**	552.65**
GP	4	2.23**	130969.81**	49976.36**	255817.67**	43790.08**
V x GP	12	0.24**	13367.97**	5493.91**	2319.71**	952.42**
Error		0.01	1.38	1.41	0.27	0.06
CV		0.50	0.14	0.16	0.35	0.40

(**) - Significant at $p \leq 0.01$ and (ns) - Not significant by F- test. DF - Degrees of freedom; V – Varieties ('Orelha-de-Vó Preta', 'Raio-de-Sol', 'Branca' and 'Raio-de-Sol Grande'); GP - Germination periods (0, 24, 48, 72 and 96 hours); TPC - Total phenolic compounds; CV - Coefficient of variation

The water contents of lima bean varieties at different germination periods are represented in Figure 2, where it is noted that the model that best fit the data was the second-degree polynomial. It is noted that most of the water absorption occurs between 0 and 24 hours, with the difference between these two periods corresponding to 24.01% (OVP), 23.50% (RS), 24.63% (RSG), and 23.54% (B). According to Nonogaki & Nonogaki (2017), this intense water absorption during the initial phase occurs due to the lower water potential of the cells, which triggers the onset of metabolic and cellular activities in the seeds. Overall, the water content increased with the germination period, with the varieties OVP, RS, B, and RSG reaching peaks of 63.51% (80 hours), 62.36% (80 hours), 66.48% (83 hours), and 61.75% (83 hours), respectively.

For crude protein content (Figure 3A), the linear model provided the best fit for the experimental data of all varieties, with OVP and RS represented by the equations $y=22.0029+0.0264x$ ($R^2=0.25$) and $y=21.1358+0.0395x$ ($R^2=0.34$). Between 0 and 96 hours of the process, increases of 2.53% for the OVP variety, 3.79% for the RS, 4.70% for B, and 5.49% for RSG were observed.

Overall, there was an increase in protein content throughout the germination process, although this varied between periods. The increase in protein content may be associated with the growth of metabolic activity in the seeds, which promotes the activation of enzymes and protein synthesis through numerous reactions, or it may also be related to variations in composition after the degradation of some of the seed constituents (Zhang et al., 2015; Xu et al., 2019). A similar pattern was identified by



The vertical bars represent the standard deviation. ** - Significant at $p \leq 0.01$ by the F test. OVP – 'Orelha-de-Vó Preta'; RS – 'Raio-de-Sol'; B – 'Branca'; RSG – 'Raio-de-Sol Grande'

Figure 2. Water content of lima bean varieties at different germination periods

Xu et al. (2019) during the germination process of chickpeas, lentils, and yellow peas; after six days, the authors observed a 10.6% increase in the protein content of the samples.

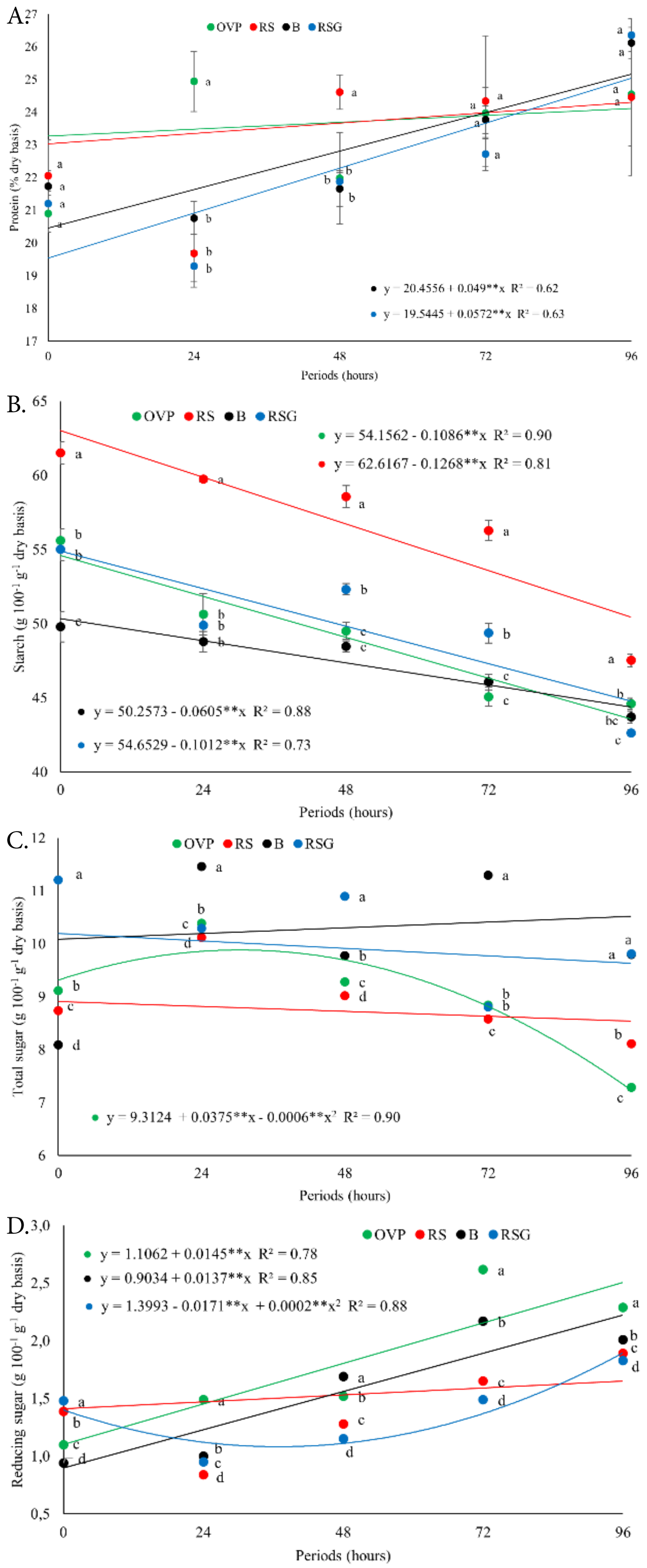
The starch content (Figure 3B) decreased linearly with the increase in the germination period; it is observed that the varieties 'Raio-de-Sol', 'Orelha-de-Vó Preta', and 'Raio-de-Sol Grande' showed the largest reductions, with values of 19.44, 19.25, and 17.78%. In contrast, the variety 'Branca' had a decrease of 11.56%, the smallest among the varieties.

This reduction, during germination, results from the breakdown of starch by α-amylase, the main enzyme responsible for the degradation that will convert starch into oligosaccharides or monosaccharides, which will be used in seed respiration and in the synthesis of new cellular constituents (Cho & Lim, 2016; Xu et al., 2019; Liu et al., 2022). The reduction of starch with the germination process was also observed by Rosa-Millán et al. (2019) when evaluating black beans (*Phaseolus vulgaris*) variety 'San Luis'; they identified a decrease of 22.51% at the end of the process.

Figure 3C graphically shows the behavior of total sugars, in which can be noted that the experimental data for the RS and RSG varieties were described by linear equations, with $y=11.0635-0.0179x$ ($R^2=0.51$) and $y=9.47-0.0116x$ ($R^2=0.35$), respectively, indicating linear reductions of 15.53% for the 'Raio-de-Sol' and 11.76% for the 'Raio-de-Sol Grande' between the beginning and the end of the germination process. The OVP and B varieties, in turn, were better described by second-degree polynomial equations, with B represented by the equation $y=8.4984+0.0913x-0.0008x^2$ ($R^2=0.55$). The maximum values were obtained at 31 hours (9.8983 g $100g^{-1}$) for OVP and at 57 hours (11.1033 g $100g^{-1}$) for B, while the minimum values were identified at 96 hours (7.38 g $100g^{-1}$) and at 0 hours (8.50 g $100g^{-1}$), respectively.

According to Cho & Lim (2016), changes during germination can diversify depending on the seed variety and germination conditions, which justifies the difference in behavior between the varieties. The decrease in sugar content of seeds during germination is related to the production of energy necessary for metabolic activity (Zhang et al., 2015). In millet, buckwheat, and peas, Yang et al. (2021) reported a reduction in total sugars for 1 or 2 days followed by a trend of increase or stabilization.

The maximum value for reducing sugars in the RSG variety (Figure 3D) was 1.60 g $100g^{-1}$, obtained at 96 hours. However, the varieties OVP, B, and RS showed linear increases of 125.84,



The vertical bars represent the standard deviation. ** - Significant at $p \leq 0.01$ by the F test. Lowercase letters differentiate the varieties using the Tukey test ($p \leq 0.05$). OVP – 'Orelha-de-Vó Preta'; RS – 'Raio-de-Sol'; B – 'Branca'; RSG – 'Raio-de-Sol Grande'

Figure 3. Crude protein (A), starch (B), total sugars (C), and reducing sugars (D) of lima bean varieties at different germination periods

145.58, and 68.65%, respectively. The experimental data for RS was best described by the equation y=1.0493+0.0075x, with a coefficient of determination (R^2) of 0.51. A similar behavior was identified by Kalita et al. (2017), who observed a significant increase in reducing sugars during rice germination. This increase is the result of the rise in enzymatic activity, which leads to the hydrolysis of complex carbohydrates (such as starch) into simple sugars through the activation of α-amylase (Kalita et al., 2017). According to Zhang et al. (2015), the increase in reducing sugars can improve the digestibility and taste of the samples.

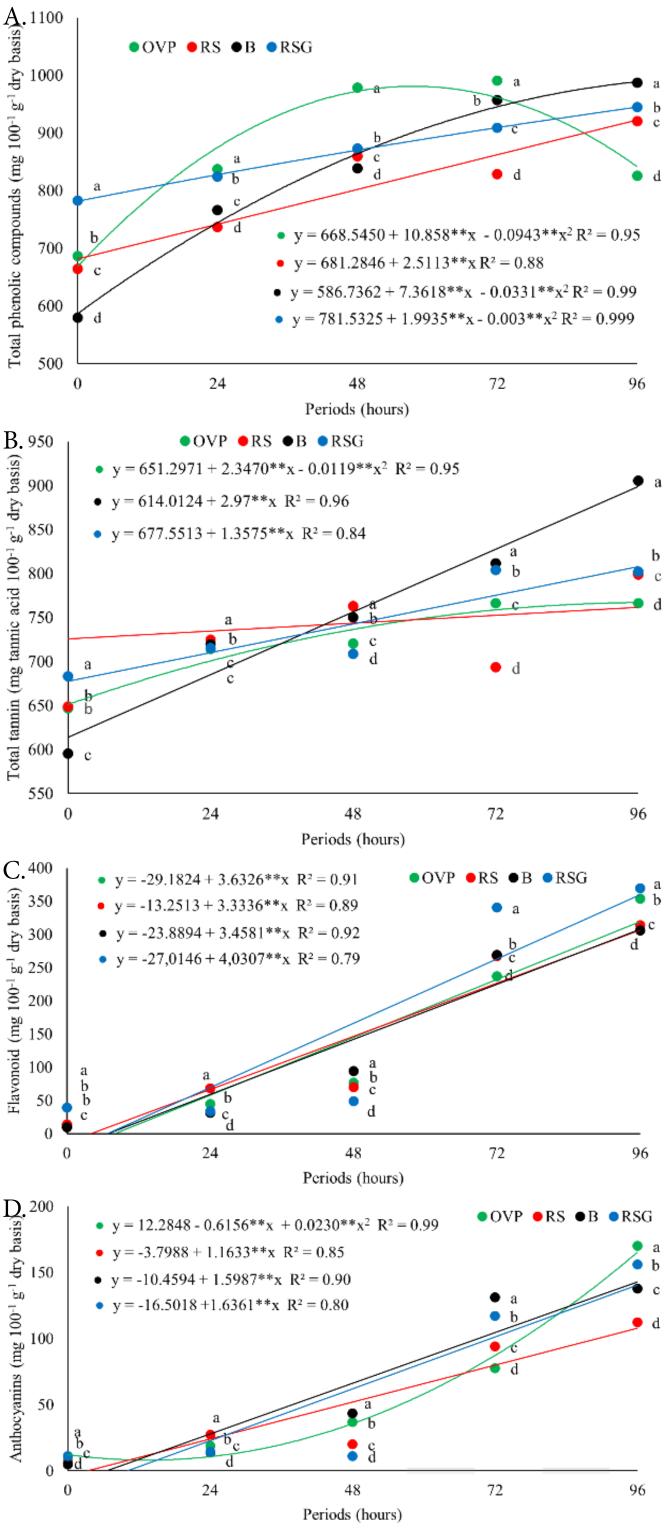
Based on the analysis of qualitative factors (varieties) within the quantitative factors (germination period), it can be stated that although the varieties exhibited different behaviors at each germination period, the results obtained for proteins (Figure 3A) at 24, 72, and 96 hours did not show statistically significant differences between them. Hung et al. (2020) found protein levels close to those of lima beans; they evaluated germinated mung beans (*Vigna radiata* L.) in the dark and under light over 96 hours, identifying average protein values ranging from 26.4 to 31.9%.

According to RDC n° 54 (BRAZIL, 2012), a food product can only be labeled as "high in protein" if it contains at least 12 g of protein per 100 g of the product. Resolution n° 265 (BRAZIL, 2005) specifies that the Recommended Daily Intake (RDI) for protein is 9 to 34 g for infants and children and 50 g for adults. Based on the results found, sprouted lima beans have a high protein content, and consuming 100 g of these beans is sufficient to meet the daily needs of children and infants, while 200 to 250 g is adequate to meet the daily needs of adults.

Regarding starch (Figure 3B), it is observed that in all periods, the 'Raio-de-Sol' variety statistically differs from the others, showing the highest starch contents, ranging from 42.63 g $100g^{-1}$ (at 96 hours) to 55.02 g $100g^{-1}$ (at 0 hours). Meanwhile, the 'Raio-de-Sol Grande' variety shows intermediate values between 0 and 72 hours. The other samples exhibit distinct behaviors at each period. Total and reducing sugars did not exhibit a clear behavioral pattern among the varieties; however, the OVP and B varieties stood out by showing the highest averages for reducing sugars between 24 and 96 hours.

Figure 4A graphically represents the total phenolic compounds of lima bean varieties at different germination periods. It can be observed that the second-degree polynomial model provided the best fit for the experimental data of OVP, B, and RSG, with maximum values of 980.91, 988.42, and 905.79 mg $100g^{-1}$, respectively, identified at 96 hours of the process, except for OVP, which reached its maximum at 59 hours. The RS variety, in turn, showed a linear increase of 35.39%.

In general, the longer the germination period, the higher the content of phenolic compounds. This behavior was also reported by Wei et al. (2022) when evaluating germinated fava beans (Snowdrop cultivar). In the fresh sample, an average value of 42.48 mg $100g^{-1}$ was identified, and after 72 hours, the authors found 44.50 mg $100g^{-1}$. The increase in phenolic compounds may be related to their release from cell walls during germination or to the result of the biosynthesis and bioaccumulation of phenolic acids, as a defense mechanism in conditions of environmental stress (Randhir et al., 2004). This behavior may also be related to the degradation of starch, which corresponds to a component used in respiration and consequent growth during germination, resulting in the loss of dry matter and, therefore, increasing the



The vertical bars represent the standard deviation. ** - Significant at $p \leq 0.01$ the F test. Lowercase letters differentiate the varieties using the Tukey test ($p \leq 0.05$). OVP – 'Orelha-de-Vó Preta'; RS – 'Raio-de-Sol'; B – 'Branca'; RSG – 'Raio-de-Sol Grande'

Figure 4. Total phenolic compounds (A), total tannins(B), flavonoids (C), and anthocyans (D) of lima bean varieties at different germination periods

content of total phenolics and flavonoids compounds (Hung et al., 2020). These compounds have antioxidant capacity, potentially preventing the formation of free radicals and consequently reducing cardiovascular diseases, type 2 diabetes, and cholesterol (Liu et al., 2022).

In Figure 4B, it can be observed that the content of total tannins tends to increase as the germination period is extended. It is also noted that all experimental data were best represented by linear models, except for OVP, which was described by a second-degree polynomial equation. The RS variety was described by the equation y=671.9720+1.1202x, with a determination coefficient (R^2) of 0.53. OVP reached its maximum value of 766.94 mg $100g^{-1}$ at 96 hours, while B, RS, and RSG exhibited increases of 46.44, 16, and 19.23%, respectively.

The increase in tannin content with germination was also verified by Zhang et al. (2015); the authors observed a 300% increase in the content of condensed tannins at the end of 72 hours of wheat seed germination. In contrast, Chinma et al. (2021) reported that germination promotes the reduction of tannin content in legumes. However, the inverse behavior observed in this work demonstrates that the influence of germination varies with the species, variety and conditions in which the procedure is performed.

Tannins are water-soluble phenolic compounds, which generally have greater antioxidant activity than flavonoids (Santos et al., 2021). According to Bezerril et al. (2021), tannins may or may not have beneficial characteristics in foods, depending on their structure and association with phenolic compounds. The effects of tannins on human health are still questionable because although they bind to some components and reduce their bioavailability, they also have a strong antioxidant action.

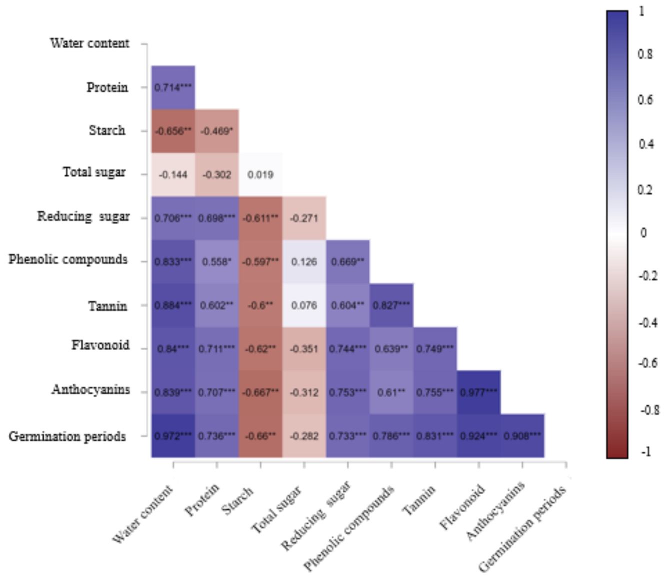
Figure 4C graphically represents the behavior of flavonoids, indicating a linear increase in these constituents as the germination period extends. The varieties OVP, RS, B, and RSG showed growth rates of 3.63, 3.33, 3.46, and 4.03 mg $100g^{-1}$ per hour, respectively, reaching maximum values of 319.5472, 306.7743, 308.0882, and 359.9326 mg $100g^{-1}$, in that order, at 96 hours, respectively. Increases in flavonoids were also verified by Saleh et al. (2019) during a six-day germination period after evaluating the husk, cotyledon and radicle of fava beans (*Vicia faba* L.), lupine (*Lupinus albus*), chickpeas (*Cicer arietinum* L.), lentils (*Lens culimaris*), fenugreek (*Trigonella foenum*), and common bean (*Phaseolus vulgaris*).

Phenolic acids, flavonoids and tannins are important substances in plant growth because during growth the amino acid phenylalanine is transformed into trans-cinnamic acid through the catalysis of the enzyme phenylalanine ammonia lyase (PAL). Thus, phenolic components such as p-coumaric, caffeic and ferulic acid are synthesized and can be translated into flavonoids, tannins and other compounds (Shahidi & Yeo, 2016).

In Figure 4D, a trend of increasing anthocyanins with the prolongation of the germination period is observed. Additionally, it is noted that the second-degree polynomial model provided the best fit for the OVP variety, with a maximum value reached at 96 hours corresponding to 165.16 mg $100g^{-1}$. However, the varieties RS, B, and RSG were better described by linear models, showing growth rates of 1.16, 1.60, and 1.64 mg $100g^{-1}$ per hour, and maximum values of 107.88, 143.02, and 140.56 mg $100g^{-1}$, respectively.

Based on the analysis of qualitative factors within the quantitative ones, it can be stated that each variety exhibited distinct behavior. Between 24 and 72 hours, the OVP variety stood out in phenolic compounds, reaching the highest values, while the B variety had the highest average at the end of the process (96 hours). For tannins, between 72 and 96 hours, the B variety, followed by RSG, showed the highest results. For flavonoids, RSG not only stood out at the beginning of the experiment but also remained prominent at the end, during the 96-hour period. On the other hand, for anthocyanins, the OVP variety excelled at 96 hours of germination, followed by RSG.

To evaluate the interrelationship between the variables in the germinated lima bean seeds, Pearson's correlation analysis was applied (Figure 5). It is observed that germination period showed positive, significant and very strong correlation (r between 0.908-0.972) with water content, flavonoids and anthocyanins, meanwhile, the correlation was positive, significant and strong (r between 0.733-0.831) with proteins, reducing sugars, total phenolic compounds and total tannins. Germination period was still negatively, significantly and moderately correlated (r - 0.66) with starch, while with reducing sugars the correlation was negative and insignificant (r - 0.282). Therefore, it is evident that the content of proteins and bioactive compounds are directly related to germination period, the longer the period, the higher the content of these compounds, corroborating the results previously exposed. There is also a strong correlation between total phenolic compounds and total tannins, as well as between flavonoids and anthocyanins.



Significance of Pearson's correlation - * p < 0.05; ** p < 0.01; *** p < 0.001

Figura 5. Heat map representing the Pearson correlation between all variables

Conclusions

1. Germination proved to be a process capable of altering the composition of lima beans, allowing for the modulation of their nutritional and functional composition through control of the germination time.

2. The 96-hours germination period was considered the most suitable for obtaining a product with higher content of proteins, tannins, flavonoids, and anthocyanins.

3. To maximize the phenolic compound content in the OVP variety, a germination period of 59 hours is recommended, while for the other varieties, a 96-hours period is ideal.

4. The application of a germination process adds value to lima bean seeds, maximizing their nutritional and functional benefits and expanding the possibilities for utilizing this material, whether through direct consumption or incorporation as a raw material in a product.

Contribution of authors: Investigation, Amadeu, L. T. S. and Silva, E. T. V; validation and supervision, Queiroz, A. J. M. and Figueiredo, R. M. F; data curation, Moura, H. V. and Carvalho, R. O.; formal analysis, Paiva, Y. F. and Ferreira, J. P. L.; writing – original draft, Amadeu, L. T. S.; writing – review and editing, Santos, F. S.

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