Valorization Of Guava Residues: Evaluation of Substrates and Nutritional Potential in Seed Germination

Valorización De Residuos De Guayaba: Evaluación De Sustratos Y Potencial Nutricional En La Germinación De Semillas

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Recibido: 16 de junio 2023
Aceptado: 28 de julio 2024

Abstract
Guava seeds, a byproduct of guava (*Psidium guajava* L.) industrialization, stand out for their high protein content compared to the pulp, which can be further enhanced through germination. In this study, two substrates were evaluated: cotton (CN) and a mixture of peat moss, vermiculite, and perlite (PVP). Under controlled conditions at 30 °C for 30 days, parameters such as sprout height (h), root/shoot ratio, germination speed index (GSI), germination percentage (GP), biomass weight (P), and protein content (PC) were measured. The average height of the sprouts was 5.57 ± 0.15 cm in AL and 5.03 ± 0.15 cm in PVP. The root/shoot ratio varied, with values of 0.52 ± 0.43 for AL and 0.44 ± 0.11 for PVP. GSI was higher in AL with 2.5 ± 0.87 seeds/day, compared to PVP with 1.3 ± 0.4 seeds/day. However, the GP % at the end of the evaluation period was similar in both substrates, at 40 % and 39 % for AL and PVP, respectively. The protein content of the sprouts at the end of the evaluation showed no significant differences (10.39 % ± 0.34 and 11.06 % ± 1.57 for PVP and AL, respectively). Both substrates proved suitable for germination, and the increase in protein content suggests the potential use of these byproducts in both animal and human nutrition.

Keywords: Seed, Guava, Sprouts, Germination, Germination media, Protein.

Resumen
Las semillas de guayaba, como subproducto de la industrialización de la guayaba (*Psidium guajava* L.), se destacan por su elevado contenido proteico frente a la pulpa, siendo este susceptible a incrementarse mediante la germinación. En el presente trabajo, se evaluaron dos sustratos: algodón (AL) y una mezcla de turba, vermiculita y perlita (PVP). En condiciones controladas a 30 °C durante 30 días, se midieron parámetros como altura de los germinados (h), relación raíz/tallo, índice de velocidad de germinación (IVG), porcentaje de germinación (PG), peso de la biomasa (P) y contenido de proteínas (CP). La altura media de los brotes fue de 5.57 ± 0.15 cm en AL y 5.03 ± 0.15 cm en PVP. La relación raíz/tallo varió, siendo 0.52 ± 0.43 para AL y 0.44 ± 0.11 para PVP. El IVG fue mayor para medio AL con 2.5 ± 0.87 semillas/día, mientras que el medio PVP fue de 1.3 ± 0.4 semillas/día. Sin embargo, el % PG al final del tiempo evaluado fue similar en ambos medios con 40 % y 39 % para AL y PVP, respectivamente. El contenido de proteínas de los germinados al final de la evaluación no presento diferencias significativas (10.39 % ± 0.34 y 11.06 % ± 1.57 para PVP y AL, respectivamente). Ambos sustratos resultaron adecuados para la germinación, y el aumento en el contenido proteico sugiere el potencial uso de estos subproductos en la alimentación, tanto animal como humana.

Palabras clave: Semilla, Guayaba, Germinados, Sustrato, Proteína.

INTRODUCTION

Guava (*Psidium guajava* L.), a tropical fruit native to Mexico (Gutiérrez et al., 2008; Vial et al., 2022), is grown throughout South America, Europe, Africa, and Asia (Nivia et al., 2007). Worldwide, its production is around 2,075,000 tons. The main producers are Pakistan, Brazil, India, Mexico, and Egypt, with shares of 22 %, 17 %, 16 %, 15 % and 12 %, respectively. According to 2020 data, Mexico ranks third in the world in production, with an average annual volume of 302,000 tons. India, China, Indonesia, and Pakistan are also important guava producers (Kaur & Ghosh, 2023). Guava is a source of antioxidant compounds, ascorbic acid (vitamin C), phenolic compounds and carotenoids, as well as carbohydrates, dietary fiber and minerals (potassium, calcium, and phosphorus) (Gutiérrez et al., 2008; Kaur & Ghosh, 2023; M. Kumar et al., 2022).

Guava is a raw material for a wide variety of foods such as juices, nectars, sweets, and desserts. After processing a series of residues composed of seeds, peel and pulp are generated, which can represent up to 30 % of the weight of the processed fruit (Gill, 2016; Kaur & Ghosh, 2023; Lima et al., 2019). The final disposal of these wastes is a challenge for the industry, the management and final disposal increase the production cost and can cause serious environmental problems due to microbial decomposition and leachate production (Torres-Leon et al., 2018). Few investigations focus on the use or obtaining of products from guava seeds (Nivia et al., 2007), so it is necessary to develop alternatives that strengthen the production chain and propose possible uses for these agro-industrial residues (Restrepo et al., 2010).

The seed represents between 10-12 % of the weight of fresh fruit (Serna-Cock et al., 2013), it is a material with a high nutritional value (Silva-Vega et al., 2017), significant amounts of bioactive compounds such as ascorbic acid (87.44 mg/100 g), total carotenoids (1.25 mg/100 g), insoluble dietary fiber (63.55 g/100 g) (Uchôa-thomaz et al., 2014) and oils, phenols, tannins, vitamins and lecithins (Irshad et al., 2020). Fiber and protein are found in a greater proportion in the seeds than in the pulp, the crude fiber present in the pulp is between 2.8-5.5 % and in the seed between 65-70 %, and the protein present in the pulp is between 0.9-1.0 % while the seed is between 5-10 % (Irshad et al., 2020). Proteins from guava pulp are more digestible than those from soybeans (Silva-Vega et al., 2017), they are rich in glycine, with antimicrobial activity, they are characterized by having a low molecular weight and a three-dimensional structure like antimicrobial peptides from other families; capable of inhibiting the development of bacteria that cause gastrointestinal diseases (Pelegrini & Franco, 2011).

The germination is a very simple, inexpensive, environmental-friendly and safe way to cultivate the germinated seeds and sprouts within a short time (Gan et al., 2017). Seed germination is a low-cost technique that significantly reduces antinutrients, making the consumption of sprouts a nutritionally advantageous option compared to grains or seeds. During this process, enzymes like phytases break down phytic acid, enhancing the absorption of essential minerals. The reduction of enzymatic inhibitors not only improves digestibility, facilitating nutrient assimilation, but also preserves essential nutrients. This approach not only minimizes the presence of antinutrients but also promotes a healthier and more efficient nutritional intake. (Chávez García et al., 2023; Gupta et al., 2015).

Many studies have shown that seed germination induces physiological changes, and the resulting sprouts have a greater number of bioactive compounds. This type of food is increasingly popular, and its consumption is associated with multiple health benefits (Liu et al., 2019). Silva-Vega et al., (2017) have reported that the protein content increases when germinating guava seeds from 8.78 % to 30.5 %. Due to its high vegetable protein content and the relative ease of germination, guava seeds have been considered as a potential food alternative for ruminants (Silva-Vega et al., 2017). However, more focused research is needed to characterize sprouts for their use in human and animal nutrition.

Seed germination is influenced by several factors, mainly temperature and substrate, which can be controlled to improve germination percentage, uniformity, and germination time (Tuan et al., 2019). However, when the guava seeds germinate poorly and unevenly, requiring more time for seedling emergence (Brijwal & Kumar, 2013), this is attributed to the seed dormancy generated by the hard layer and the impermeability to water and gases. Studies on the germination of guava seeds focus on the effects of pretreatments to break the dormancy of the seed and increase the percentage of germination, as well as the ideal conditions for germination. Different pre-treatments (physical and chemical), germination substrate (cotton, filter paper, absorbent paper, sand, vermiculite, among others) and temperatures (15 to 30 ° C, have been evaluated, with the range between 20-30 ° C being those higher germination percentages (Alves et al., 2015; Kumar et al., 2012; Serratos Tejeda, 2012).

The evaluation of guava seed germination and its characterization is essential due to the lack of updated information. Previous studies have revealed valuable data, including the positive impact of passage through the digestive tract of black howler monkeys on germination and the usefulness of guava seeds in ruminal nutrition (Serratos Tejeda, 2012; Silva-Vega et al., 2017). These findings are fundamental for the conservation and sustainable use of the species, as well as to optimize agricultural production. The economic and nutritional importance of guava underlines the need for updated information on its germination, opening opportunities to improve the quality of human and animal food. Research in these aspects not only enriches scientific knowledge but also has direct and positive impacts on food, agriculture, and the environment.

The objective of this study was to evaluate the effect of cotton, and different substrates mix (peat moss, vermiculite, and perlite) on the germination of guava seeds (*Psidium guajava* L.) under controlled conditions of humidity and temperature. It also explored whether germination increased the nutritional value of the seeds compared to ungerminated seeds. The potential applications of these findings in animal and human nutrition were discussed, along with the potential of germination as a method for valorizing agro-industrial byproducts.

MATERIALS AND METHODS

Raw material

The seeds were obtained from guava fruits (*Psidium guajava* L.) of the "media china" variety. The fruits were acquired at the municipal market in the city of Saltillo, originating from crops in the municipality of Calvillo, state of Aguascalientes (Mexico), and harvested in December 2018. Fruits of light-yellow color, in a state of ripeness suitable for consumption according to the standards set in the NMX-FF-040-SCFI-2002 norm, were selected. Subsequently, the fruits were washed and immersed in a solution of sodium hypochlorite (50 ppm) for 5 minutes. Once cleaned and disinfected, the fruits were cut in half, and the seeds were manually extracted using a spoon. Following this, the seeds were separated from the rest of the fruit, washed with water using a fine mesh sieve, and left to dry on trays at 30 ºC, following the methodology proposed by Alves et al., (2015) (Alves et al., 2015; NMX-FF-040-SCFI, 2002).

Pretreatment

Seeds were submerged in sterile distilled water for 24 hours prior to planting (Núñez-Gastélum et al., 2023). According to the procedure outlined by Silva-Vega et al., (2017), the seeds were then placed in rectangular aluminum trays (185 x 135 x 30 mm), with 100 seeds deposited in each tray (Silva-Vega et al., 2017).

Evaluation of germination substrate

Two germination substrates were evaluated: cotton (CN) (6 grams per tray) and a mixture of peat moss (PVP) consisting of peat moss (60 %), vermiculite (20 %), and perlite (20 %) (20 grams of bed + 5 grams on the seeds). Germination substrates were maintained under consistent humidity and temperature conditions (30 ± 3 °C and 80 % relative humidity) in a bioclimatic chamber (Artificial Climate Chamber - ECOSHEL), as shown in Figure 1.

Figure 1. Evaluating Germination Media for Guava Seeds

Sampling and germination evaluation procedures

Samples were taken at 0, 5, 10, 15, 20, 25, and 30 days. All determinations were conducted in triplicate, and trays were randomly selected at each interval. Germination was considered when the seed exhibited a primary root emission greater than 2 mm in length (Alves et al., 2015; Ramirez et al., 2017).

Measurement and evaluation of growth parameters and germination indices

For root length, the measurement considered the distance from the base of the stem to the apex of the root. Stem length was measured from the base to the apex of the seedling. Additionally, the root/stem ratio was calculated as the relationship between these two lengths (Ramirez et al., 2017).

The Germination Speed Index (GSI), germination percentage (GP) and biomass weight (PB) were calculated using the formulas and methodology outlined by Alves et al. (2015) (Alves et al., 2015). The GSI was determined at the same time as the germination percentages. It was calculated by dividing the germination percentage by the number of days the seeds were in the germinator using Equation 1.

Eq 1. GSI = (G1/N1) + (G2/N2) + (Gn/Nn)

Where: GSI is germination speed index; G1 is the percentage of seeds germinated at time 1; N1 is the time (days) since the experimental units were placed in the trays.

Figure 2. Guava seed germination cycle

Determination of protein content

The protein content and weight of seeds (germinated and non-germinated) were determined in triplicate at times of 0, 5, 10, 15, 20, 25 and 30 days. The sample was taken from the 100 seeds sown in each tray; the samples were ground in an Analytical Mill electric trademark Cole Colemer. Protein content was determined by the "Kjeldahl Method" (AOAC, 1999) and a factor of 6.25 was used for the conversion of nitrogen to protein.

Statistical analysis

All determinations were performed in triplicate, and results were expressed as the mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's test with a significance level of 5 %, using INFOSTAT software version 2018 for Windows (Córdoba, Argentina). A factorial treatment with 2 factors was employed, where factor 1 was the type of culture substrate with two levels (CN and PVP), and the second factor was the follow-up time with seven levels (0, 5, 10, 15, 20, 25, 30 days). The response variables included germination percentage (GP), protein content, and biomass weight (W).

RESULTS AND DISCUSSION

Height of the sprouts

The germination process typically begins between 5 and 7 days after sowing, characterized by the rupture of the seed's outer coat and the emergence of an initial root (Núñez-Gastélum et al., 2023). Figure 2 depicts the various stages of development of guava seed sprouts. By the end of the evaluation period, the sprouts had exceeded 5 cm in height.

Table 1 presents the germination indicators for the evaluated substrate. The sprouts in the CN substrate exhibited an average height of 5.57 ± 0.15 cm, while those in the PVP substrate measured 5.03 ± 0.15 cm. Both values surpassed those reported by El-Deeb et al., (2024), who reported the height of the *Psidium cattleianum* Sabine sprouts, for 28 days, between 1.53 cm and 3.56 cm (El-Deeb et al., 2024). Like Méndez et al., (2009), who documented germination heights of 2.43 cm and 2.68 cm at 37 and 47 days, respectively (Méndez et al., 2009). No statistically significant differences were observed in the parameters of height and root/stem ratio of the sprouts. The ratios were 0.52 ± 0.43 and 0.44 ± 0.11 for CN and PVP, respectively, values similar to those reported by Ramírez et al. (2017) for guava seeds (0.59) (Ramirez et al., 2017).

Table 1. Germination indicators in cotton and peat moss media

Germination media	Cotton (CN)	Peat moss (PVP)
Height (cm)	5.57 ± 0.15[a]	5.03 ± 0.50[a]
Root / Stem	0.52 ± 0.43[a]	0.44 ± 0.11[a]
Germination Speed Index (Seeds / day)	2.50 ± 2.42	1.40 ± 3.60

The variation in GSI values in the present study may also be attributed to the exposure of seeds to the substrate and temperature. In the PVP substrate, seeds were covered by a thin layer of germination substrate, reducing the impact of temperature on the seed coat. Temperature directly influences water absorption and the metabolic reactions necessary for germination (Alves et al., 2015).

Germination percentage (GP)

The germination of guava seeds, including both the initiation and the percentage of seeds that germinate (GP), is influenced by several factors. These factors include temperature, the type of substrate the seeds are placed on, and any pretreatment processes the seeds receive (Serratos Tejeda, 2012). Figure 3a shows the germination percentage (GP). The results suggest that initially, the CN substrate promoted higher sprout growth compared to the PVP substrate. After 5 days of starting the evaluation, the CN substrate showed a significant advantage with 20 % GP compared to 6 % for PVP. However, at the end of the evaluation period, no significant differences were observed between substrates in terms of GP. Furthermore, it was observed that the CN substrate maintained higher GP levels throughout the study period, reaching its maximum of 40 % on day 15 of evaluation. In comparison, the PVP substrate reached its maximum of 39 % on day 30. This suggests that the CN substrate may provide more favorable conditions for initial germination, but in the long term, both substrates may be equally effective in promoting germination.

Although few studies reporting the GP of guava seeds, the reviewed studies show differences in germination times and percentages, mainly due to the conditions of the experiment, such as temperature, substrate, and pretreatment to which the seeds are subjected before germination. Quintero et al., (1999) noted germination starting between 14 and 45 days (Quintero et al., 1999). Méndez et al., (2009) reported germination onset at 13 days post-sowing in different substrates, with a maximum GP of 78.75 % achieved after 27 days (Méndez et al., 2009). Alves et al., (2015) reported an average germination time of 17 days at an alternative temperature range of 20-30 °C (Alves et al., 2015). Ramírez et al., (2017) observed guava seed germination starting on day 7, with a germination percentage (GP) ranging from 1.0 to 5.2 % after pretreatment and specific conditions. By the end of the 30-day evaluation period, GP reached values between 91.4 % and 100 % (Ramirez et al., 2017).

The germination percentages obtained in the present study are similar to those reported El-Deeb et al., (2024), evaluated different sterilization treatments and culture media to determine their effect on the germination and development of seeds of *Psidium cattleianum* Sabine, during 28 days and obtained germination percentages between 4.76 % and 63.46 %, corresponding to the treatments T1 (10 % Clorox® commercial bleaching/15 min) and T4 (15 % Clorox® commercial bleaching, soaking in 15 % HCl/24 h, then 10 % H_2O_2/48 h), respectively (El-Deeb et al., 2024). On the other hand, Gentil et al., (2018), evaluated the effect of temperature on the germination percentage of *Psidium friedrichsthalianum* (O. Berg) seeds using a paper towel as germination substrate, for 90 days. Germination of *P. friedrichsthalianum* seeds was greater than 80 % at the temperatures of 15, 20, and 25 ºC (Gentil et al., 2018).

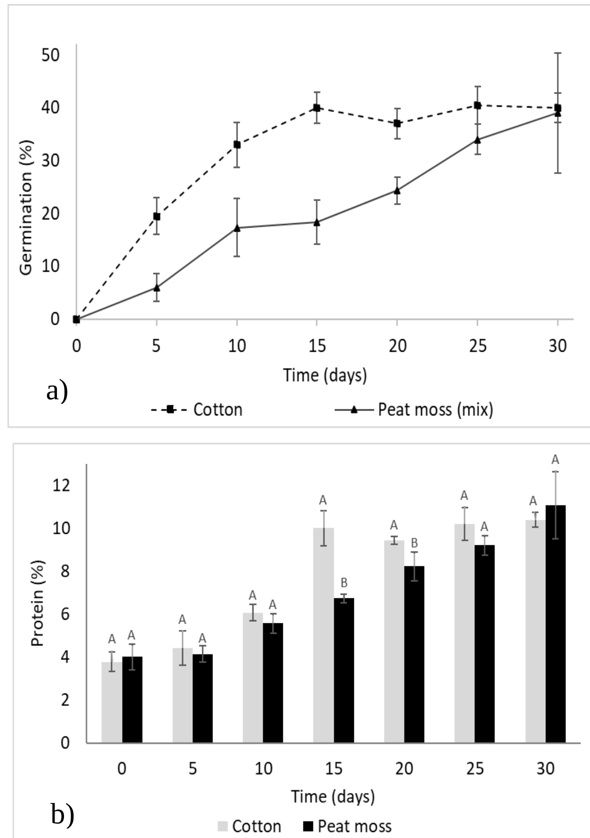

Figure 3. a) Germination percentage of guava seeds. b) Percentage of protein in guava seed sprouts.

Serratos (2012) documented germination values of 5.87 % for seeds directly obtained from the fruit (untreated), and 95.5 % for guava seeds recovered from feces. The passage of seeds through the digestive tract of monkeys acts as a chemical scarification process, weakening the seed coat with gastric juices. This facilitates water penetration and induces germination (Serratos Tejeda, 2012). Méndez et al., (2009) evaluated various germination substrates including sand, soil, bagasse, sand + soil, sand + bagasse, soil + bagasse, and sand + soil + bagasse. They observed the highest germination percentage in the sand substrate, occurring 15, 16, and 17 days after planting with percentages of 12.5 %, 30 %, and 47.08 %, respectively. However, after 27 days, germination reached its peak at 78.75 % (Méndez et al., 2009).

The variation in germination initiation is directly associated with the Germination Speed Index (GSI). Low germination percentages are attributed to seed dormancy caused by the hardness of the seed coat, which restricts water and gas exchange (Rocas et al., 2002). Additionally, the presence of tannins in guava plants can inhibit seed germination, leading to poor, uneven, and delayed germination (Butt et al., 2013). Pre-treating seeds to facilitate seed coat breakage and interaction with the medium accelerates germ development and emergence compared to untreated seeds. High germination percentages are linked to temperature fluctuations, which can cause small cracks in the seed coat due to expansion and contraction of the integument, facilitating water penetration (Bewley & Black, 1982). Alves et al., (2015) studied the impact of temperature on the germination of guava seeds without pre-treatment. They found that germination percentages at 30 °C ranged from 1 % to 33 %. Alternating temperatures between 20 °C and 30 °C resulted in germination percentages between 94 % and 100 %. They used paper rolls and sand as germination substrates, achieving germination within 23 days (Alves et al., 2015). For their part, Pereira and Andrade (1994) not only evaluated the effect of temperature but also the germination substrate for untreated guava seeds. They found that at 30 °C, the germination percentage was 33.6 %. When temperatures varied between 20-30 °C and 15-35 °C, germination percentages increased to 55.9 % and 53.6 %, respectively. There were no statistically significant differences among the different germination substrates evaluated (vermiculite, filter paper, paper towel). The total evaluation period was 46 days (Pereira & Andrade, 1994). However, some research reports different germination percentages for the same temperature, due to variations in light intensity and water holding capacity provided by the germination substrate (Alves et al., 2015).

Determination of protein content

The observation that guava seeds shed their covering upon germination, thereby reducing their weight, aligns with biological processes known for many seeds. This shedding allows for easier penetration of water and nutrients essential for growth. However, the lack of a direct relationship between seed weight and protein percentage suggests that variations in weight observed during the experiment, particularly with the peat moss substrate, are likely influenced by substrate adherence rather than changes in protein content. Initial substrate adherence can temporarily increase seed weight, affecting early measurements.

The protein percentage did not show statistically significant differences ($P \leq 0.05$) across the evaluated germination times and substrates (Figure 3b). The highest protein content was observed in the peat moss substrate at 20 days (11.06 % ± 1.32), showing a positive correlation with the germination percentage at the same time point. In contrast, the cotton substrate at 15 days had a protein content of 10.38 % ± 1.48 but did not show a positive correlation with germination percentage, which reached its peak at 30 days with 39 %. This discrepancy may be due to the higher standard deviation observed for the cotton substrate at 30 days. The variation in determining protein content can be attributed to the inclusion of all germination material in the analysis, regardless of its germination status. During periods of lower germination percentages, such as at the onset of germination trials, the concentration of ungerminated seeds can skew the average protein percentage towards values typical for untreated seeds. This phenomenon underscores the importance of accounting for the germination state when analyzing seed composition.

In time 0, the protein content of guava seeds was 6.59 %, consistent with values reported in the literature 8.78 % (Silva-Vega et al., 2017), 11.19 % (Uchôa-thomaz et al., 2014) and 7.71 % (Serna-Cock et al., 2013). Literature corroborates the wide range of protein percentages observed in guava seeds. For example, Silva-Vega et al., (2017) reported significantly higher protein values of 30.46 % for guava seed sprouts, which indicates the potential nutritional benefits of germinated guava seeds in animal nutrition contexts (Silva-Vega et al., 2017). This contrasts with the initial protein content of untreated guava seeds, suggesting a significant biochemical transformation during germination that enhances nutritional value.

CONCLUSIONS

The study found that guava seed germination did not significantly differ in terms of germination percentage, sprout weight, and protein percentage between cotton and the mixture of peat moss, vermiculite, and perlite substrates. However, the cotton substrate showed higher values for germination indicators such as height, root/shoot ratio, and germination rate throughout the evaluation period. These results suggest that cotton may provide more favorable conditions for guava seed germination and growth. Further research is needed to understand the underlying mechanisms and long-term effects of different substrates on guava seed germination.

The germination substrate is not a significant factor for the germination speed index (GSI). One of the most crucial factors, as reported, is the pretreatment of seeds before germination. These pretreatments facilitate the breaking of the seed coat, allowing the exchange of substances between the seed and the substrate, thereby promoting the germination process.

Seed germination is an accessible, economical, environmentally friendly, and safe way to cultivate nutrient-rich foods. Further research is needed to establish optimal conditions for guava seed germination and to increase their protein content, which can enhance the utilization of this byproduct and potentially integrate it into both human and animal diets.

ACKNOWLEDGMENTS

The authors thank the National Council of Science and Technology (CONACYT) of Mexico and the postgraduate degree in Food Science and Technology of the Autonomous University of Coahuila. This financing was made by granting a postgraduate scholarship to the student Jorge Eduardo Angulo López, with scholarship number/CVU 945769 for the completion of their master's studies.

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