Vegetable snacks added with probiotic microorganisms

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Abstract

Introduction. The incorporation of probiotics into vegetable-based matrices demonstrated promising potential for developing functional snacks with significant health benefits.

Materials and methods. A 3×3×4 factorial experimental design was used, which results in 36 experimental combinations (3 microorganisms × 3 plant matrices × 4 process steps). Three replicates were carried out for each experimental combination, generating 108 observations.

Results and discussion. The incorporation of probiotics in plant matrices showed promising potential for developing functional snacks with significant health benefits. The factorial design revealed that the plant matrix (carrot, sweet potato and chayote) and processing stage significantly influenced probiotic viability, with carrot showing the highest colony forming unit (CFU) levels and better sensory acceptability. While lactic acid bacteria *Lactobacillus acidophilus*, *L. bulgaricus* and yeasts *Saccharomyces cerevisiae* exhibited viability throughout processing, matrix properties and dehydration-storage stages influenced CFU counts. For example, in carrot, average *L. acidophilus* counts of 8.0×10^6 CFU/100 g were observed after dehydration, while in sweet potato counts were 5.5×10^6 CFU/100 g at the same stage. During storage, the viability of *S. cerevisiae* in carrot remained relatively high, with an average of 7.5×10^6 CFU/100 g at the end of the period evaluated, demonstrating good stability. Texture was the most influential attribute in the sensory analysis, with significant differences between samples. The results demonstrate the technical and scientific potential of developing functional vegetable snacks through the successful incorporation of viable probiotic microorganisms without compromising consumer sensory acceptability. The carrot matrix was found to be particularly effective in maintaining probiotic viability, especially in combination with S. cerevisiae during storage, reaching average levels of 8.33×10^6 CFU/100 g. Although dehydration and storage generated a reduction in viability and affected texture, the results support the viability of these products as an innovative alternative within the functional food sector.

Conclusions. This study establishes a sound scientific basis for the production of innovative functional snacks that combine significant microbiological viability with favourable consumer sensory acceptability and promotes the use of agro-industrial raw materials, contributing to a healthier and more sustainable diet.

Introduction

The development of functional food products has experienced remarkable growth in recent years, driven by a convergence between advances in food biotechnology and a growing consumer awareness of the relationship between diet and health. This phenomenon has been particularly visible in the area of functional foods, defined as those that not only provide essential nutrients, but also provide additional benefits that improve quality of life and contribute to the prevention of chronic diseases (Essa et al., 2023; Cencic et al., 2010; Ivanov et al., 2021; Tsykhanovska et al., 2023). In this context, probiotics have emerged as a central pillar in the formulation of such products, due to their ability to modulate the intestinal microbiota and strengthen the gastrointestinal barrier, which has direct implications on immunological, metabolic and neurological health (Cui et al., 2018).

Among the most studied probiotics, *Lactobacillus acidophilus* stands out for its ability to effectively colonize the intestinal tract and contribute to microbial balance. Several studies have shown that its regular consumption can reduce the incidence of gastrointestinal infections, improve lactose digestion and modulate inflammatory responses at the systemic level. These mechanisms are explained, in part, by the production of antimicrobial compounds such as bacteriocins and organic acids, which inhibit intestinal pathogens, in addition to their interaction with immune cells through molecular patterns associated with beneficial microorganisms (Nuñez et al., 2024).

On the other hand, the formulation of vegetable-based snacks has gained significant relevance due to their exceptional nutritional profile and their potential to act as ideal matrices for the incorporation of probiotics. Vegetables such as carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*) and chayote (*Sechium edule*) are not only rich in dietary fibre, vitamins and antioxidants, but also present a matrix structure suitable for protecting probiotic microorganisms during processing and storage. Dietary fibre, in particular, can act as a natural prebiotic, favouring the survival of beneficial bacteria and increasing their efficacy after consumption (Rajagukguk et al., 2022).

The development of products that integrate *Lactobacillus acidophilus* in vegetable matrices poses important technical and scientific challenges. One of the critical aspects is the viability of the probiotic during the thermal processing and dehydration stages, processes necessary for snacking. Recent research has explored strategies such as microencapsulation and the use of natural cryoprotectants, which have shown promising results in protecting probiotics from adverse conditions. In addition, it is essential to evaluate probiotic stability during storage, as factors such as water activity (Aw), acidity and temperature play a crucial role in microbial survival (Taco and García-Godos, 2021).

From the consumer's point of view, the success of functional snacks depends not only on their nutritional value and health benefits, but also on their sensory properties, such as texture, flavour and aroma. Acceptance studies have shown that the incorporation of probiotics can slightly modify these characteristics, which makes rigorous sensory testing essential to optimize formulations and ensure high market acceptability. In this sense, the design of functional products must balance the consumer's sensory perception with the functional benefits offered by probiotics (Payne et al., 2024).

Therefore, the incorporation of *Lactobacillus acidophilus* in vegetable snacks represents an innovative opportunity to respond to current market demands for functional foods. However, the success of these products requires a comprehensive approach ranging from the selection of suitable plant matrices and processing techniques to the scientific validation of their functional and sensory benefits. This approach will not only contribute to expanding the possibilities in the field of functional foods, but will also reinforce the role of agribusiness in promoting global health and wellness (Zhan et al., 2023).

The present research aims to incorporate *Lactobacillus acidophilus* in snacks made from vegetable matrices such as carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*) and chayote (*Sechium edule*), in order to evaluate the viability and stability of the probiotic during the processing and storage stages (Álvarez and Lamas, 2021). It is proposed that these matrices, due to their composition rich in dietary fiber, antioxidants and carbohydrates, may act as natural protective substrates that favour the survival of the microorganism under adverse conditions, such as heat treatment or dehydration.

The central hypothesis of the study was established that it is possible to maintain the viability of *Lactobacillus acidophilus* above the minimum recommended limits (10^6-10^7 CFU/100 g) in final products, thus guaranteeing its functional properties and health benefits. In turn, the incorporation process was not expected to significantly alter keys sensory characteristics of the product, such as taste, texture and appearance, key parameters for consumer acceptance. To validate this hypothesis, the microbial stability, the behaviour of the probiotic during storage and its influence on the sensory attributes of the final snack were analysed in detail, which was allow determining its market potential as an innovative functional food (Latif et al., 2023).

The approach of the present research is experimental, based on a quantitative and descriptive research design that allows the controlled manipulation of independent variables to analyse their effect on the dependent variables. The experimental methodology facilitated the identification and quantification of cause-effect relationships between the selected factors - microorganism, plant matrix and process stage - and the responses of the evaluated system, such as probiotic viability, sensory properties and consumer acceptance (Oniszczuk et al., 2021). This approach ensures methodological rigor through the application of standardized procedures, the repetition of experiments and the use of robust statistical analysis to validate the results obtained. The quantitative nature of the study allowed objective and measurable data to be obtained, while its descriptive nature made it possible to interpret and detail the characteristics and behaviors observed in the different stages of the experimental process. Likewise, this design constituted an ideal tool to generate scientific evidence to support the formulation of functional products, contributing to the development of innovative alternatives in the agro-industrial and nutritional sector (Ilango and Antony, 2021).

The research was applied and experimental, focused on the development and evaluation of a functional food product that integrates probiotic microorganisms in vegetable matrices. Its purpose was to analyse, in a systematic and rigorous manner, the behavior of the product in terms of microbiological viability during the different stages of the production and storage process, as well as to determine the sensory properties - flavor, texture, appearance and aroma- and their impact on consumer acceptance and preference. This approach combined the generation of practical knowledge with the scientific validation of the factors involved, ensuring reproducible and applicable results in the functional food industry (Kruk et al., 2024).

Thus, the specific objectives of this study focused on evaluating the stability and viability of *Lactobacillus acidophilus* in vegetable snacks during different storage stages, analysing keys sensory properties – flavor, texture, appearance and aroma - using standardized sensory evaluation methods, and determining consumer acceptance and preference using hedonic and preference tests.

Materials and methods

Materials

Vegetable ingredients from local carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*) and chayote (*Sechium edule*) crops available in the region were selected for their nutritional value and availability.

The sample included three vegetable matrices (carrot, sweet potato and chayote) that were subjected to different stages of the snack production process: liquid impregnation, impregnated matrix, dehydrated and stored. Three probiotic microorganisms were evaluated: Lactobacillus acidophilus, Lactobacillus bulgaricus and Saccharomyces cerevisiae, applied in factorial combinations. The selection of these factors responded to their relevance in obtaining functional foods with high microbial viability and optimal sensory properties (Li et al., 2020).

Experimental design

A 3x3x4 factorial experimental design was used, which results in a total 36 experimental combinations (3 microorganisms × 3 plant matrices × 4 process steps). Three replicates were carried out for each experimental combination, generating 108 observations. The factors evaluated were microorganisms, plant matrices and process steps, while the dependent variables were microbial viability, sensory properties and consumer acceptance (Bernal-Castro et al., 2019), where experimental factors used were; probiotic microorganisms: *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Saccharomyces cerevisiae*; vegetable matrices: carrot, sweet potato and chayote and process stages: from initial impregnation to storage.

Variables evaluated

The research adopted an empirical approach, based on the observation and systematic measurement of the dependent variables by means of a 3×3×4 factorial experimental design. This design allowed simultaneous evaluation of the effect of three independent factors - type of probiotic microorganism (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Saccharomyces cerevisiae*), vegetable matrix (carrot, sweet potato and chayote) and stage of processing (liquid impregnation, impregnated, dehydrated and stored matrix) - on the dependent variables, which included microbiological viability, sensory properties and consumer acceptance of the product (Managa et al., 2021).

To determine microbiological viability, colony forming unit (CFU) count tests were performed using standard microbiological methods on specific selective means, ensuring accuracy and reproducibility of results. Sensory evaluation was included taste, texture, appearance and aroma tests by a panel of trained judges, using validated hedonic scales to measure the perceived organoleptic quality of the snacks. Finally, acceptance and preference surveys were conducted on a representative sample of consumers, applying descriptive and inferential statistical techniques to identify acceptance trends and correlate results with sensory and microbiological attributes (de Souza et al., 2024).

The combination of these empirical and analytical methods ensured a comprehensive evaluation of product performance at different stages, providing robust and reliable information for the development of functional snacks with stable probiotics and high market acceptance (Muñoz-Pabon et al., 2024).

Microbiological analysis

Measurement of colony forming units for the average probiotic concentration expressed in CFU/100 g.

The viability of *Lactobacillus acidophilus* was evaluated at each critical stage of the snack production process (impregnation, dehydration and storage) by counting colony-forming units. The plate dilution technique was applied using specific culture means such as MRS agar, which favour the selective growth of probiotic bacteria. Plates was incubated at optimum temperature 37 °C for a period of 24 to 48 hours to ensure accurate recovery and quantification of the probiotic, in order to determine its viability and stability in the different plant matrices (Tixicuro et al., 2021).

Sensory evaluation

A sensory analysis of the final product was carried out by a panel of trained tasters, selected according to criteria of sensitivity and previous experience in organoleptic testing. Critical attributes such as flavor, texture, appearance and aroma were evaluated using a hedonic scale of 1 to 5 (where 1 is "very unpleasant" and 5 is "very pleasant"). This analysis was allowing the identification of possible sensory variations derived from the probiotic incorporation process and production stages, ensuring a final product that meets sensory acceptability standards (Cosme et al., 2022).

Acceptance surveys

Product preference and acceptance were measured through structured surveys applied to a representative sample of potential consumers. A Likert scale of 1 to 5 was used to evaluate parameters of overall liking, willingness to purchase, and perception of the product in terms of quality and health benefits. The surveys were provided quantitative data to identify acceptance trends and correlate them with sensory and microbiological characteristics, facilitating decision making in the process of improving and positioning the product in the market (Drake et al., 2023). These techniques, combined with a rigorous statistical analysis, were guarantee reliable and scientifically supported results, contributing to the development of a functional snack with high nutritional and probiotic value that meets the expectations of the modern consumer (Zhu et al., 2024).

Statistical analysis

The data collected were analysed by ANOVA (analysis of variance) to determine the impact of the experimental factors on the dependent responses. Post hoc tests, such as Tukey, were applied to compare means and detect significant differences between treatments. In addition, scatter plots and boxplots were used to visually interpret the trends and variability of the data, using Minitab 21 and Phyton statistical software for data analysis.

Results and discussion

Analysis of Variance for general factorial regression

Microorganisms. (1: *Lactobacillus acidophilus*, 2: *Lactobacillus bulgaricus*, 3: *Sacchromyces cerevisiae*); Matrix; Stage, by means of analysis of variance (ANOVA), in which different sources of variation were evaluated in a model with three factors (microorganisms, matrix and stage) and their interactions.

Model global. The F-value of 14.34 with a p-value of 0.000 suggests that the model as a whole is significantly different from zero, i.e., at least one of the sources of variation has a significant effect.

Main effects. The microorganisms did not have a significant effect (p-value = 0.180), since the F-value (1.76) is not high enough to reject the null hypothesis. While in the matrix, they had a very significant effect (p-value = 0.000) on the dependent variable, with an F-value of 202.16, indicating a significant difference between the levels of the matrix. On stage, they also had a significant effect (p-value = 0.000), with an F-value of 18.27, indicating that the different stages affect the dependent variable (Boev et al., 2024).

Interactions of two terms. The interaction Microorganisms × Matrix, was not significant (p-value = 0.992), indicating that there is no significant interaction between these two factors. The following interaction. Microorganisms × Stage, is also not significant (p-value = 1.000), suggesting that there is no relevant interaction between microorganisms and stage. Finally, the interaction Matrix × Stage, is significant (p-value = 0.000), with an F-value of 6.38, suggesting that the interaction between matrix and stage significantly affects the dependent variable (Gutiérrez-Tlahque et al., 2024).

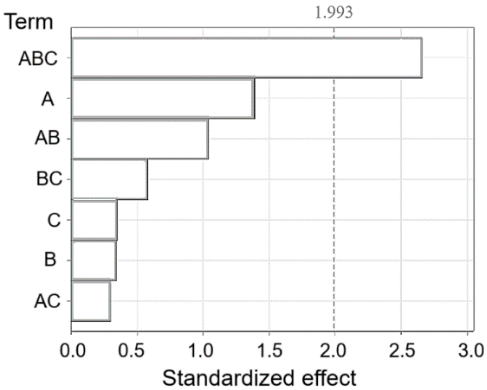
Three-term interactions. The interaction Microorganisms × Matrix × Stage is not significant (p-value = 1.000), which implies that the combination of the three factors does not have a significant effect on the dependent variable. With respect to Error, the variability explained by this is small, with a sum of squares of 20.012 and a mean squares value of 0.2780. Therefore, the model is significant, but the most important effects come from the matrix, the stage and the interaction between matrix and stage. Microorganisms have no relevant impact by themselves or in interaction with other factors (Table 1).

Table 1

Analysis of Variance for general factorial regression, Average probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage

Source	GL	SC Ajust.	MC Ajust.	Valor F	Valor p
Model	35	139.498	3.986	14.34	0.000
Linear	7	128.593	18.371	66.09	0.000
Microorganisms	2	0.976	0.488	1.76	0.180
Matrix	2	112.380	56.190	202.16	0.000
Stage	3	15.238	5.079	18.27	0.000
2-term interactions	16	10.759	0.672	2.42	0.006
Microorganisms×Matrix	4	0.075	0.019	0.07	0.992
Microorganisms×Stage	6	0.041	0.007	0.02	1.000
Matrix×Stage	6	10.643	1.774	6.38	0.000
3-term interactions	12	0.146	0.012	0.04	1.000
Microorganisms×Matrix×Stage	12	0.146	0.012	0.04	1.000
Error	72	20.012	0.278		
Total	107	159.511			

Using a Pareto diagram, significant effects on the average probiotic concentration expressed in CFU/100 g were identified ($\alpha = 0.05$). Factor B (Matrix) had the greatest impact, exceeding the critical value (1.993) with the most significant effect. Factor C (Stage) and the BC interaction were also significant, indicating their relevant influence on the response. In contrast, Factor A (Microorganisms) and the interactions AB, AC and ABC did not exceed the critical value, so they are not considered significant. These results highlight the importance of prioritizing the study and control of factors B, C and their interaction BC to optimize the response (Figure 1).



Factor	Name
A	Microorganisms
B	Matrix
C	Stage

Figure 1. Pareto plot of standardized effects for the general factorial regression, average probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage

The residual plots assessed the validity of the model assumptions and the quality of the fit. The normal probability plot showed that the residuals follow a reasonably normal pattern, with slight deviations in the tails. The residuals vs. fits show no clear patterns, suggesting that the model does not omit important terms or have fit problems. The histogram indicates that the residuals have a symmetric distribution centered at zero, supporting normality. The residuals vs. order of observation show no significant trends, although a dip is observed at the end, possibly related to outliers. In general, the assumptions of normality and random distribution are acceptable, with minor deviations (Figure 2).

Main effects

The main effects graph evaluated the impact of the factors on the response (average in CFU/100 g). The factor Microorganisms (A) did not show a significant effect, since its line is practically horizontal. Matrix (B) showed a notable difference between levels: level 1 had a higher mean (~8), while level 2 was lower (~6), confirming its significant impact. Stage (C) showed a clear downward trend, with a decrease in the mean response from level 1 to 4, indicating a significant effect. In summary, the most relevant factors are Matrix (B) and Stage (C), where level 1 in both factors generates the highest response. In contrast, the factor Microorganisms (A) has no significant impact (Figure 3).

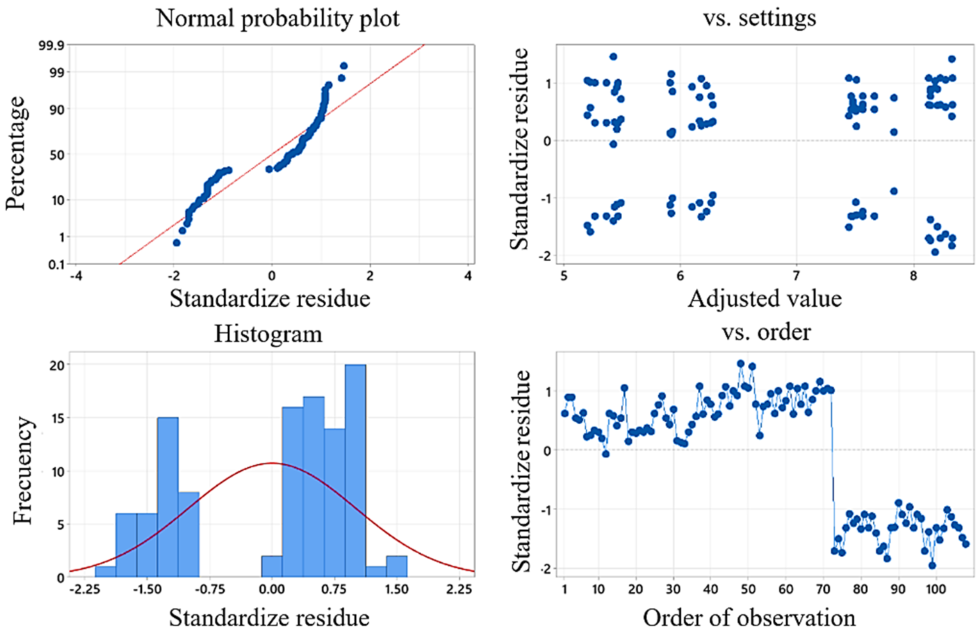


Figure 2. Graphs of average residues of probiotic concentration expressed in CFU/100 g for vegetable snacks (carrot, sweet potato, chayote), with addition of probiotic microorganisms

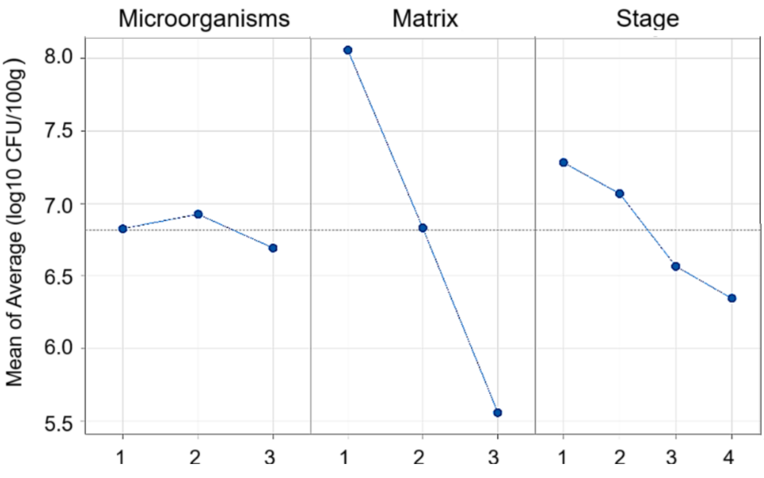


Figure 3. Main effect plot for average probiotic concentration expressed in CFU/100 g

Figure 4 shows the interactions between factors and their effect on the response (log10 CFU/100 g). No significant interactions were observed for combinations including Microorganisms (A), as the lines are parallel in the Matrix × Microorganisms, Stage × Microorganisms and Microorganisms × Stage panels. In the case of Matrix × Stage, the lines are not parallel: in Matrix 1, the response decreases markedly as one progresses through Stages (1 to 4), whereas in Matrix 2, the decrease is less pronounced. This confirms a significant interaction between Matrix and Stage, in line with previous results of the Pareto analysis. In summary, the most relevant interaction is between Matrix and Stage, while no significant interactions are detected for combinations with Microorganisms.

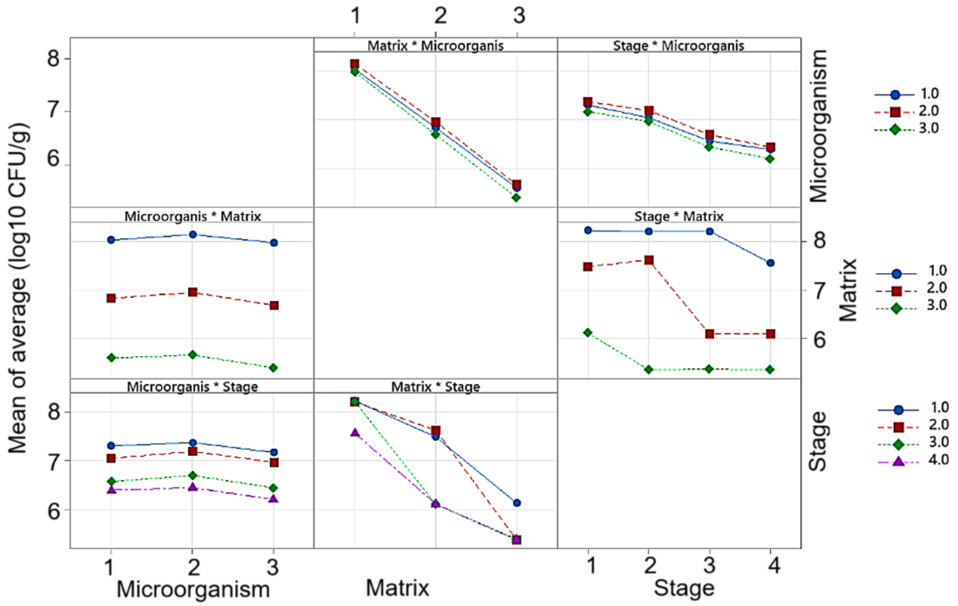


Figure 4. Interaction for average probiotic concentration expressed in CFU/100 g

The surface graph shows how the Matrix and the Microorganisms influence the average probiotic concentration expressed in CFU/100 g. Level 1 of the Matrix generates a higher response, while level 2 reduces it considerably (Figure 5). As for the Microorganisms, their impact is limited, which is in agreement with previous analyses where they were not significant. Although Microorganisms do not have a significant direct effect, specific combinations with the Matrix generate peaks in the response, possibly due to non-linear effects. In summary, the Matrix is the most influential factor in the response, while the Microorganisms have a minor impact and are limited to particular combinations.

Figure 6, a contour plot, shows the interaction between Matrix and Microorganisms on the average probiotic concentration expressed in CFU/100 g. Matrix 1 is associated with the highest response areas (CFU/100 g between 7 and 8.5), regardless of the level of Microorganisms. In contrast, Matrix 2 generates lower responses (CFU/100 g between 5 and 6), with little variation between Microorganism levels. This confirms that Matrix 1 has a greater influence on the response, while Microorganisms have a lesser impact.

Using a response optimization graph, Figure 7, in relation to the identified optimum, the graph shows the optimum conditions to maximize the response (Average CFU/100 g concentration). The maximum value reached (y) with 8.3333. The Desirability (D) with 0.8622, indicating that the solution is highly desirable but not perfect, and the optimum factors such as Microorganisms-Level 3, Matrix-Level 1 and Stage-Level 4 are conditions that optimize the average CFU/100 g concentration, reaching a high value. The lowest level of the Matrix and the highest level of Microorganisms and Stage contribute significantly to maximize the response.

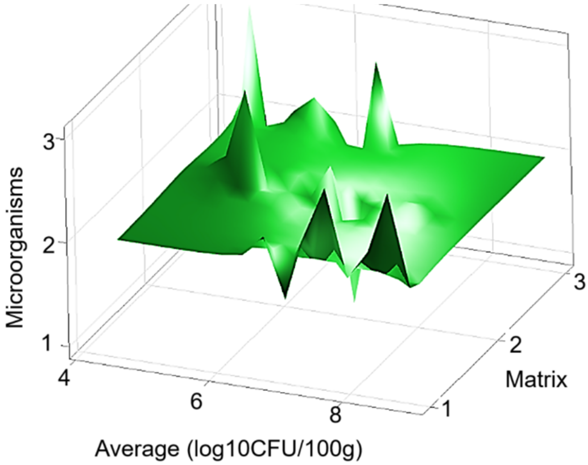


Figure 5. Surface plot Microorganisms vs. Matrix; average probiotic concentration expressed in CFU/g

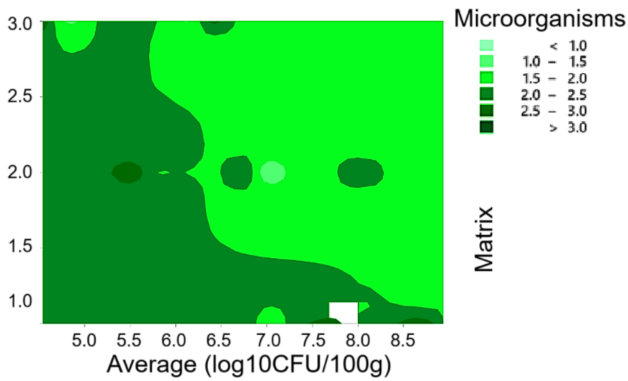


Figure 6. Contour plot of Microorganisms vs. Matrix; Average probiotic concentration expressed in CFU/100 g

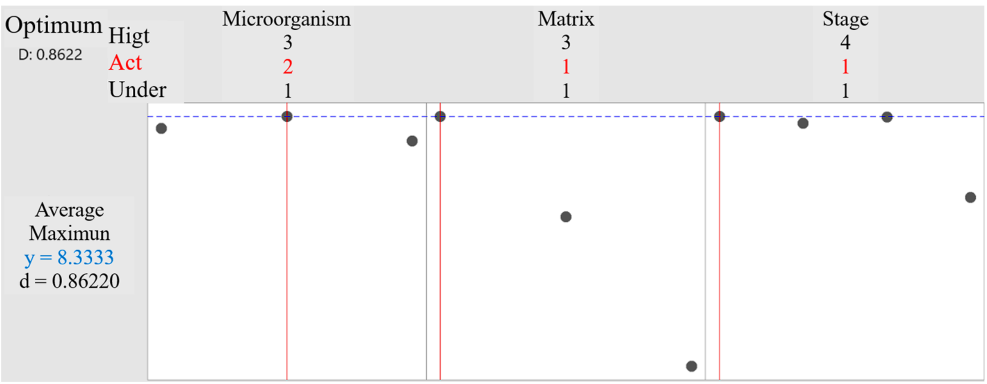


Figure 7. Response optimization graph for Average probiotic concentration expressed in CFU/100 g

Sensory evaluation

In this sense, the analysis of variance (ANOVA), with respect to Flavor, the value of p = 0.2068 (> 0.05), did not find significant differences between the samples with respect to the flavor attribute, since the changes in the samples do not significantly affect this attribute. Regarding Texture, the value of p = 0.0399 (< 0.05), indicates significant differences between samples with respect to texture, being the only sensory attribute where the samples present statistically significant differences, which could be relevant for the evaluation of the product. As for appearance, the p-value = 0.3945 (> 0.05), reveals no significant differences between the samples in relation to appearance, i.e., the samples are similar in appearance. Finally, the Aroma, presents a value of p = 0.3935 (> 0.05), where there were also no significant differences in terms of aroma, the samples do not differ significantly in terms of aroma (Table 2).

Table 2

Analysis of Variance sensory attributes of the average probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage

Sensory contribution	*p-value*	Result
Flavor	0.2068	Not significant
Texture	0.0399	Significant
Appearance	0.3945	Not significant
Aroma	0.3935	Not significant

The interpretation of Tukey's test for flavor reveals that no significant differences were found between the combinations of samples (Sweet Potato, Chayote and Carrot), therefore, all samples have a similar flavor. Regarding texture, a significant difference was identified between sweet potato and carrot (p = 0.0327), where carrot presented a different texture compared to sweet potato. There were no significant differences between the combinations Sweet Potato and Chayote or Chayote and Carrot, since Carrot differed significantly from Sweet Potato in texture, but not from Chayote. Regarding Appearance, no significant differences were found between any of the sample combinations, which have a similar appearance. On Aroma, there were also no significant differences between sample combinations, all samples are similar in aroma. From the Tukey tests, only significant differences were detected in texture between sweet potato and carrot. In the attributes of flavor, appearance and aroma, no significant differences were observed between samples. This reinforces that texture is the main sensory attribute that discriminates between samples (Table 3).

Observations by sensitive property, for carrot, Flavor (blue), has fairly high ratings (medians close to 4-5), with little dispersion. This indicates consistent acceptability. On Texture (orange), ratings vary widely, and there are low outliers, which could reflect a greater polarization in the perception of texture. On Appearance (green), similar to flavor, but with slightly lower acceptance (median around 3). Aroma (red), low overall acceptance (median close to 2), with a more moderate dispersion.

As for sweet potato, Flavor (blue) has moderate acceptance (median between 3-4), with less dispersion. Texture (orange), with high scores (median above 4), indicating that it was the most highly valued aspect of the sweet potato. In Appearance (green), there is lower acceptance compared to flavor and texture, with some dispersion. On Aroma (red), moderate median (~3), but more dispersion than in the other properties.

Table 3

Tukey's test results for Mean probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage.

Contribution	Comparison	Mean Difference	*p-value*	Significant
Flavor	Sweet Potato vs. Chayote	-1.75	0.1981	No
	Sweet Potato vs. Carrot	-0.50	0.8546	No
	Chayote vs. Carrot	1.25	0.4065	No
Texture	Sweet Potato vs. Chayote	-1.25	0.3844	No
	Sweet Potato vs. Carrot	-2.75	0.0327	Si
	Chayote vs. Carrot	-1.50	0.2675	No
Appearance	Sweet Potato vs. Chayote	-0.25	0.9729	No
	Sweet Potato vs. Carrot	1.25	0.5275	No
	Chayote vs. Carrot	1.50	0.4091	No
Aroma	Sweet Potato vs. Chayote	0.75	0.6784	No
	Sweet Potato vs. Carrot	1.25	0.3669	No
	Chayote vs. Carrot	0.50	0.8380	No

The distribution of ratings by sample and sensory property (Flavor, Texture, Appearance and Aroma) for three samples: Carrot, Sweet Potato and Chayote (Figure 8). The bars in the graph represent the median and interquartile range (IQR) for each sensory property per sample. Points outside the bars represent outliers, indicating individual responses that were significantly different from the others. Each color represents a different sensory property.

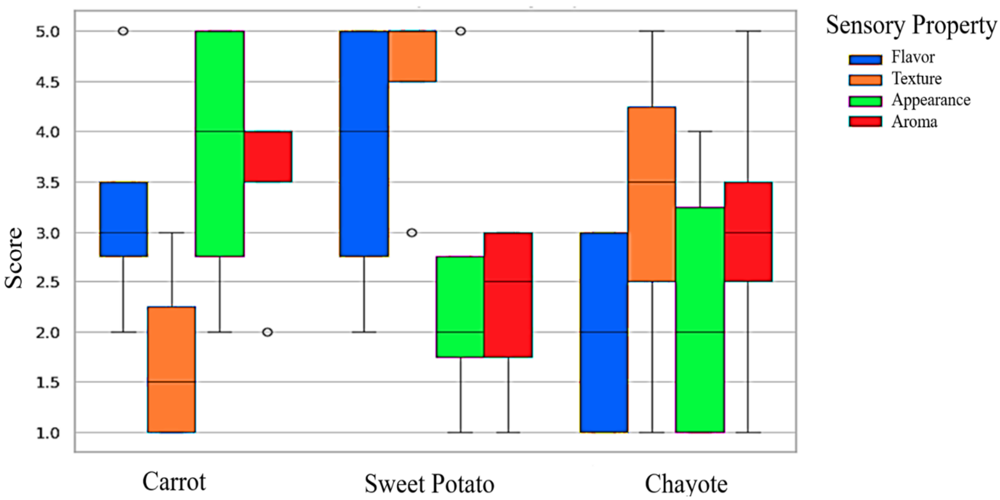


Figure 8. Graph of grade distribution by sample and sensory property such as flavor, texture, appearance

In the case of Chayote, Flavor (blue), presented ratings similar to sweet potato (median ~3.5), although more dispersed. For Texture (orange), intermediate acceptance (~3.5), but less dispersion than in flavor. Appearance (green), similar to flavor, but with slightly lower values and greater dispersion. Finally, in Aroma (red), consistent acceptance (median ~3), with less dispersion than in other properties.

Comparing the samples, we can infer that, in Flavor, Carrot obtained the highest scores, followed by Sweet Potato and then Chayote. In Texture, Sweet Potato stands out as the best evaluated, while Carrot has high variability (and outliers). In Appearance, the ratings are similar among the three samples, but somewhat lower compared to flavor and texture. On Aroma, all samples have a moderate to low rating (medians close to 2-3), being the least outstanding sensory property overall. Summarizing, Carrot stands out for flavor, but has a split perception in texture. Sweet Potato is highly valued for its texture, while its flavor and aroma are moderate. Chayote has a consistent evaluation in all properties, but does not excel in any, and aroma is the least accepted attribute overall for all three samples, with consistently low ratings (Boubezari et al., 2022).

Acceptance surveys

An analysis of variance (ANOVA) was applied to determine if there are significant differences between the samples based on the attribute ratings. Table 4. Where it is observed that the sum of squares (sum_sq) indicates the variability in the ratings explained by the "Sample" factor (52.92) versus the residual variability (9.0). Degrees of freedom (df) are 11 degrees for the "Sample" factor and 36 for the residual error. The F-statistic value is 19.24, suggesting a significant difference between the groups. The PR (>F), i.e. the p-value is extremely low (6.90×10^{-12}), much less than 0.05. This indicates that the differences between the samples are statistically significant. In sum, the average scores differ significantly between the different samples evaluated. This result reinforces the idea that the sensory properties and the specific characteristics of each sample have an important impact on the perception of the evaluators (Sipos et al., 2021).

Table 4

ANOVA test results for acceptance surveys of probiotic-impregnated samples

ANOVA	sum_sq	df	F	PR(>F)
C (Sample)	52.916667	11.0	19.242424	6.895967^{-12}
Residual	9.000000	36.0	NaN	NaN

The results of ANOVA for this case, because of the length of the results, summarize the main findings clearly and concisely presented below. Therefore, dehydrated and stored samples (regardless of species) tend to differ significantly compared to liquid and impregnated samples. This could be related to the impact of treatments on key sensory properties such as texture, aroma and appearance. Consequently, these results show that the treatments applied to the different samples (liquid, impregnated, dehydrated and stored) significantly affect the sensory perception of the evaluators. In particular, the stored and dehydrated treatments seem to be the most influential in generating differences. The impregnation technique appears to be less disruptive compared to the dehydrated and stored treatments (Kręcisz et al., 2021).

Distribution of attributes by sample

Figure 9 shows the distribution of the values of the sensory attributes evaluated for each sample according to the treatment applied (liquid, impregnated, dehydrated, and stored). The key points are highlighted below.

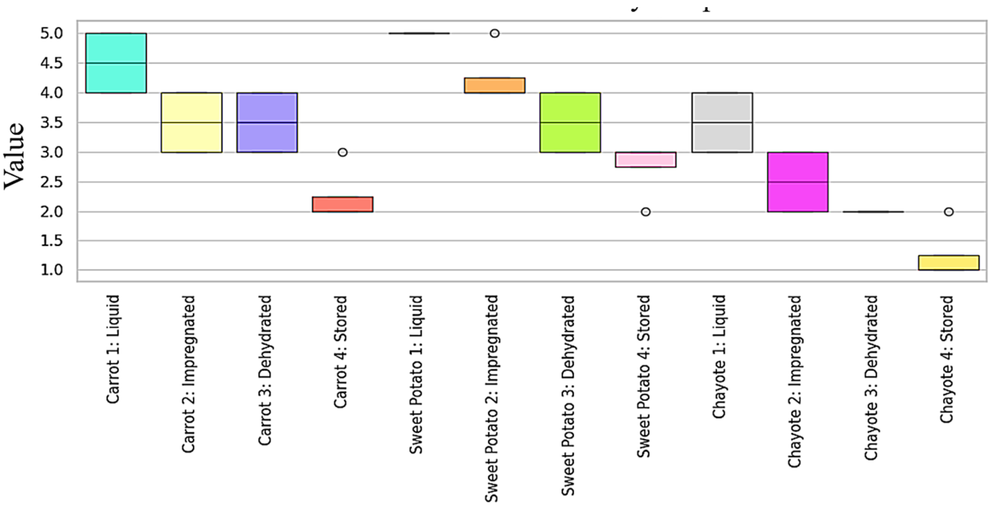


Figure 9. Boxplot for the distribution of the values of the sensory attributes evaluated for each sample as a function of the treatment applied (liquid, impregnated, dehydrated, stored)

The visual graph reinforces the statistical findings; the liquid treatments perform significantly better in sensory terms than the dehydrated or stored samples, which is consistent with the p-values obtained in the tests. The graph therefore supports the conclusion that liquid treatments are the most favored sensorially, while stored and dehydrated treatments have a negative impact on sensory ratings, regardless of the species evaluated. In addition, the lower dispersion in liquid samples could be related to a greater uniformity in sensory perception among evaluators.

The liquid samples (Carrot 1, Sweet Potato 1, Chayote 1) tend to have higher values in the sensory evaluation, with medians close to 4 or higher. This suggests that this treatment was more sensorially acceptable. Dehydrated and stored samples show lower values, with more dispersed medians and, in some cases, less than 3. This indicates that these treatments had a negative impact on sensory properties.

The stored samples show greater variability (longer box lengths), indicating more pronounced differences in raters' perceptions. Liquid and impregnated samples show lower dispersion, reflecting a more consistent evaluation among participants. There are some points outside the boxes (outliers), which could represent individual opinions significantly different from the majority. This suggests that some evaluators perceived certain samples very differently, particularly in treatments such as the stored simples.

Within the same species (carrot, sweet potato or chayote), dehydration and storage treatments tend to generate a greater drop in sensory scores. For example, Sweet Potato 4 (Stored) and Chayote 4 (Stored) have considerably lower values compared to the liquid versions of the same species.

Conclusions

1. On microbiological viability, the plant matrix and processing stage were critical factors that significantly influenced the microbiological viability of the probiotics. Carrot stood out as the best matrix for retaining high levels of probiotic viability, reaching an average of 8,333 CFU/100 g at the storage stage. This finding reinforces the importance of selecting matrices with protective properties for probiotics. As for the impact of the processing steps, these dehydration and storage steps generated a significant reduction in microbiological viability and sensory properties, particularly in texture and aroma. This underlines the need to optimize these processes to minimize probiotic viability losses and preserve consumer acceptance.
2. The sensory properties, among the attributes evaluated (flavor, texture, appearance and aroma), texture was the only one that showed significant differences among the samples. Carrot and sweet potato stood out for their overall sensory acceptability, while chayote showed a consistent but less outstanding evaluation. Consumer acceptance, in relation to the samples treated in the initial stages (impregnation liquid and impregnation) presented the highest values of sensory acceptance. However, acceptance decreased in the final stages (dehydration and storage), indicating a negative effect of the thermal and preservation processes on the organoleptic characteristics of the product.
3. Regarding industrial relevance, this study demonstrated that vegetable snacks can be effective matrices for probiotic vehicles, provided that processing steps are optimized to maximize microbiological viability and sensory properties. This has a positive impact on the development of innovative functional products that meet both market needs and consumer expectations. The results obtained support the development of functional vegetable snacks with high probiotic potential, offering an innovative and healthy nutritional solution. The application of these findings could significantly contribute to the advancement of the agri-food industry, promoting consumer health and innovation in functional products.

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