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Kinetic Modeling of the Growth of *Saccharomyces cerevisiae* in a Black Chuño (Freeze-Dried Potato)-Based Medium

Modelado cinético del crecimiento de *Saccharomyces cerevisiae* en un medio a base de chuño negro

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Abstract

Predictive microbiology enables the anticipation of microbial population behavior through mathematical models, a key tool in the development of fermented and functional foods. Black chuño, a traditional freeze-dried potato from the Andean region of Peru and Bolivia, is a substrate of cultural and nutritional value whose potential as a culture medium remains underexplored. The objective of this study was to evaluate the growth kinetics of Saccharomyces cerevisiae in a black chuño-based medium. Lyophilized strains were activated in a 2% sucrose solution and inoculated into the medium at 37±1 °C, with constant agitation for 7 hours. Microbial growth was measured by direct counting in a Neubauer chamber, expressed in cfu/ml and transformed to a logarithmic scale; the data were fitted to nonlinear models: Gompertz, Logistic, Log-logistic, and Weibull. Growth was sigmoidal, with differences in the latency and maximum rate parameters. The log-logistic model showed the best fit, with biomass reaching a maximum of 0.663 log cfu/ml at hour 7. The study confirms the viability of black chuño as a culture medium and highlights the usefulness of mathematical models in microbial kinetics.

Keywords: Microbial kinetics, sigmoidal growth, mathematical models.

Resumen

La microbiología predictiva permite anticipar el comportamiento de las poblaciones microbianas mediante modelos matemáticos, herramienta clave en el desarrollo de alimentos fermentados y funcionales. El chuño negro, papa liofilizada tradicional de la región andina de Perú y Bolivia, es un sustrato de valor cultural y nutricional cuyo potencial como medio de cultivo aún ha sido poco explorado. El objetivo de este estudio fue evaluar la cinética de crecimiento de Saccharomyces cerevisiae en un medio a base de chuño negro. Las cepas liofilizadas fueron activadas en una solución de sacarosa al 2% e inoculadas en el medio a 37±1 °C, con agitación constante durante 7 horas. El crecimiento microbiano se midió mediante conteo directo en cámara de Neubauer, expresado en UFC/ml y transformado a escala logarítmica; los datos se ajustaron a modelos no lineales: Gompertz, Logístico, Log-logístico y Weibull. El crecimiento fue sigmoidal, con diferencias en los parámetros de latencia y velocidad máxima. El modelo Log-logístico mostró el mejor ajuste, alcanzando la biomasa un máximo de 0,663 log UFC/ml en la hora 7. El estudio confirma la viabilidad del chuño negro como medio de cultivo.

Palabras clave: Cinética microbiana, crecimiento sigmoidal, modelos matemáticos.

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Introduction

Predictive microbiology is a key discipline in the field of food microbiology, as it allows us to anticipate the evolution of microbial populations over time. To achieve this, mathematical models are used to understand the dynamic behavior of microbial growth and how this impacts the study variables, substrate, and substrate formation (Olszewska-Widdrat et. al., 2024). These models can be refined, reparameterized, with fine-tuning of the parameters from a numerical/statistical point of view (Ferrer et. al., 2009; Baranyi et. al., 2024). One of the models used in this type of work is the Gompertz model, which is nonlinear in nature. These types of methods involve a higher level of complexity than linear approaches, especially in aspects such as the mathematical formulation of the model, the appropriate choice of initial values for its parameters and the implementation of the resolution algorithm in a computational environment (Guo, X, & Wang, J., 2024). This model and its modified versions are widely used in nonlinear kinetics, both to model microbial and tumor

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growth, drugs in the ascending phase and in inactivation, elimination, and heat treatment decays (Wang, J., & Guo, X., 2024; Gil et.al., 2006). The Logistic, Log-logistic and Weibull models are also applied to microbial growth or other sigmoid processes (such as in food, biotechnology or pharmacokinetics).

Black chuño, so called by the locals, is freeze-dried black potato, which is a traditional food, native to the Andean region of Peru and Bolivia, made in the winter season where it is subjected to freezing, thawing and sun drying, eliminating all humidity. This product is considered as part of the national sovereignty of Peru and Bolivia because it is prepared for times of famine (Peñarrieta et.al., 2011).

Fermented foods are made through desired microbial growth and enzymatic conversions of food components. Fermentation can enhance nutritional value and provide health benefits to consumers (Samtiya et.al., 2024). Probiotic cultures such as Lactobacillus, Limosilactobacillus, yeasts such as Saccharomyces boulardii, *Saccharomyces cerevisiae* are used for the preparation of these ferments (Chan et.al., 2021). The incorporation of *Saccharomyces cerevisiae* as a starter for fermentation in black chuño can allow quantifying the presence of beneficial microorganisms, which will provide valuable information about the additional nutritional potential of this traditional product. In addition, it could support the promotion of this food as part of a balanced diet, thus contributing to the digestive health of the communities that consume it. Understanding how probiotics behave in black chuño can open new opportunities for the development of functional foods based on traditional products.

In studies related to the microbiological kinetics of probiotics, research is observed that addresses different aspects of this field. Thus, Rodriguez and Chambi (2019) focus on the microbial growth curve of Saccharomyces boulardii in two varieties of white chuño, Chaska and Negra, using mathematical models. This study involves the application of the Gompertz and Logistic equations in four treatments with mixtures of distilled water and peptone saline solution, followed by sterilization and inoculation of Saccharomyces boulardii at different temperature and agitation conditions. Biomass, pH, °Brix, and acidity measurements were performed to evaluate growth kinetics and changes in physicochemical properties. Likewise, Colque et. al. (2023) focuses on characterizing and modeling microbial growth in a non-dairy probiotic beverage based on tarwi vegetable extract, using Saccharomyces boulardii as an inoculum, which is based on the formulation of treatments with different concentrations of sucrose and inoculum, followed by inoculation at specific temperatures and monitoring of biomass, °Brix, density, pH, and acidity. From these, microbial growth curves and kinetic parameters are constructed using the Gompertz model, and the relationships between microbial growth and the physicochemical properties of the product are analyzed. On the other hand, some research works focus on the survival of probiotics, as is the case of the study of six strains of Lactobacillus rhamnosus probiotics during storage in a synthetic peach medium and commercial peach jam at different temperatures. Changes in viable cell counts, pH values, sugar content, and color parameters are recorded. The probiotic strains show better performance in the jam compared to the synthetic medium, with counts above 7 Log cfu/g during 78 days of storage at 5 °C. It is observed that the variability in survival depends on the strain and the medium used (Randazzo, 2013). Based on the above, the objective of the present investigation was to evaluate the microbial kinetics of *Saccharomyces cerevisiae* in black chuño.

Materials and Methods

Collection and physicochemical analysis

The black chuño was collected in one of the markets of the city of Juliaca, in sterile polyethylene bags, this was transported to the facilities of the food science research center (CICAL) of the professional school of Food Industry Engineering to carry out the corresponding tests.

Yeast strain and growth conditions

The experiments were carried out using lyophilized strains of *Saccharomyces cerevisiae*. The culture medium consisted of a combination of chuño and peptone water, which was distributed in 250 ml flasks. These flasks were sterilized in a STURDY autoclave at 121 ºC for 15 minutes, thus ensuring the elimination of contaminants and the asepsis of the medium.

Then, *Saccharomyces cerevisiae* was activated in a 2% sucrose solution. 1 ml of the strain suspension was taken to inoculate the medium, carrying out the incubation at 37 ± 1 ºC, with continuous agitation at 20 RPM, controlled by a BS-11 water bath. During the incubation period (from 1 to 7 hours), periodic counts were performed every hour, removing a flask for analysis (Chambi A. & Torres A., 2021)

Microbial growth and mathematical modeling

For the measurements of biomass increase, a LW SCIENTIFIC monocular microscope was used together with a Neubauer chamber with a precision of 0.100 to 0.00025 mm^2. The results obtained were expressed in colony forming units per milliliter (cfu/ml), which were subsequently transformed to a logarithmic scale to facilitate kinetic modeling (Chambi A. & Torres A., 2021). This kinetic analysis was carried out using the Gauss-Newton algorithm, implemented in the Minitab version 19 software, using the following equations:

Gompertz Model

$$N = N_0 + C \cdot \exp(-\exp(-B \cdot (t - M))) \qquad (1)$$

Where: N is the number of microorganisms at time t, No > 0 is the number of microorganisms at the initial moment, the parameter C: common logarithm of the difference between the initial and final population in the stationary phase, the parameter B represents the slope of the curve and describes the growth rate and M time in which the growth rate is of greater magnitude. (Castro et al., 2008)

Logistic Model

$$N = \frac{D}{1 + \exp(B - M \cdot t)} \qquad (2)$$

Modified Logistic Model

$$N = C + \frac{D - C}{1 + \exp\left(B \cdot \ln\left(\frac{t}{M}\right)\right)} \qquad (3)$$

Weibull Model

$$N = C + (D - C)/\exp\left(-B \cdot t^M\right) \qquad (4)$$

Yeast Count and Viability

10.0 g of the sample were weighed in a sterile Petri dish and then transferred to an Erlenmeyer flask containing 90.0 ml of a 0.1% peptone water solution. To mix well, the sample was homogenized in a sterile blender: first at low speed for 10 seconds and then at normal speed for 30 seconds. This mixture was the primary dilution. From the resulting suspension, 1.0 ml was taken and transferred to a test tube containing 9.0 ml of peptone water solution. After shaking, the process was repeated as many times as necessary to obtain the required dilutions. A sterile pipette was used for each of the dilutions, thus ensuring the cleanliness of the procedure. (Camacho, A. et.al., 2009).

Results

Microbial Kinetics

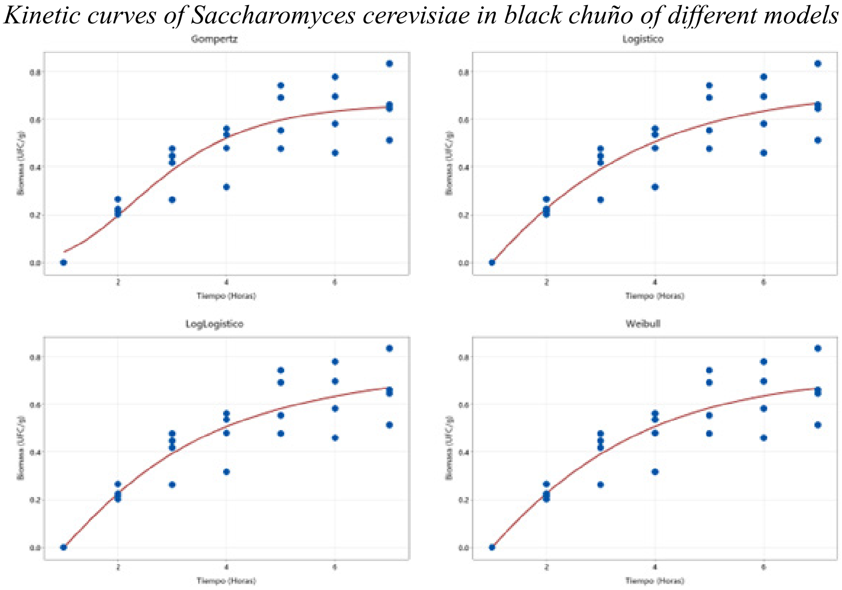
Data on time in hours and biomass in natural logarithm (LN) were recorded. At hour 1, an average biomass of 0.000 was observed with a standard deviation and variance of 0.000. At hour 2, the average biomass increased to 0.227, with a standard deviation of 0.027 and a variance of 0.001. In the third hour, the average biomass reached 0.402, presenting a standard deviation of 0.095 and a variance of 0.009. At hour 4, an average biomass of 0.473 was recorded, with a standard deviation of 0.110 and a variance of 0.012. For hour 5, the average biomass was 0.616, with a standard deviation of 0.122 and a variance of 0.015. At hour 6, the average biomass increased to 0.629, with a standard deviation of 0.139 and a variance of 0.019. Finally, at hour 7, an average biomass of 0.663 was observed, with a standard deviation of 0.132 and a variance of 0.017 (Table 1).

Table 1
Cell growth on a logarithmic scale of Saccharomyces cerevisiae in black chuño.

Time	Biomass (LN)		
(Hours)	Mean	Standard Deviation	Variance
1	0.000	0.000	0.000
2	0.227	0.027	0.001
3	0.402	0.095	0.009
4	0.473	0.110	0.012
5	0.616	0.122	0.015
6	0.629	0.139	0.019
7	0.663	0.132	0.017

Note: All means are expressed as mean ± SD (n=4)

Figure 1
Kinetic curves of Saccharomyces cerevisiae in black chuño of different models



Note: All means are expressed as mean ± SD (n=4)

In the presented graph, the fitted curves of four growth models (Gompertz, Logistic, Log-logistic, and Weibull) were observed as a function of time (hours) to describe the biomass in CFU/g. The experimental data, represented by black dots, were visually compared with the curves of the different models. It was evidenced that all models followed a sigmoidal behavior, characteristic of microbiological growth. However, small variations in the fit were noted between the models. The Log-logistic model showed a closer fit to the observed data, while the Gompertz model showed a slight underestimation in the initial hours. This suggests that, although all models adequately describe growth, there are differences in the precision with which they capture the dynamics of biomass in certain phases of growth.

It was observed that the Gompertz model presented a C value of 0.80 and a B value of 1.80, along with an upper asymptote M of 0.67. On the other hand, the logistic model exhibited a C of 2.13 and a B of 0.72, with a D value of -0.41, suggesting a negative relationship in growth. In the case of the modified logistic model, C and B values of 1.70 and 3.47 were recorded, respectively, in addition to an M of 3.42 and a D of 0.80, which indicates greater flexibility in the data fitting. Finally, the Weibull model was characterized by a C of 0.30, a B of 0.72, an M of 0.03, and a D of 0.68, reflecting a specific behavior in the growth rate over time (Table 2).

Table 2

Kinetic parameters of different models applied to Saccharomyces cerevisiae in black chuño

Model	Parameters						
	C	B	M	D	$\mu_{máx}$ (h^{-1})	λ (h)	G (h)
Gompertz	0.80	1.80	0.67	-	4.12	0.81	0.17
Logistic	2.13	0.72	-	-0.41	20.27	0.05	0.03
Modified Logistic	1.70	3.47	3.42	0.80	24.26	0.72	0.03
Weibull	0.30	0.72	0.03	0.68	0.20	52.45	3.45

Model fitting

In the analysis of the evaluated kinetic models (Table 3), different performances were observed in the applied statistical criteria. The R-squared presented close values between the models, highlighting the Logistic model with a value of 0.8606, followed closely by the Modified Logistic (0.8604) and the Weibull model (0.8600), while the Gompertz model obtained a slightly lower value of 0.851. Regarding the number of iterations required for convergence, the Logistic model showed greater efficiency by reaching the solution in 8 iterations, while the other models needed 11 iterations. The Sum of Squared Errors (SSE) was lower and equivalent for the Logistic, Modified Logistic, and Weibull models, with a value of 0.230, while the Gompertz model presented a slightly higher value of 0.244. The degrees of freedom for the error were 24 for all models, except in the case of Gompertz, where 25 degrees of freedom were obtained. Finally, both the mean squared error (0.009) and the standard deviation (0.09) were identical in all evaluated models.

Table 3

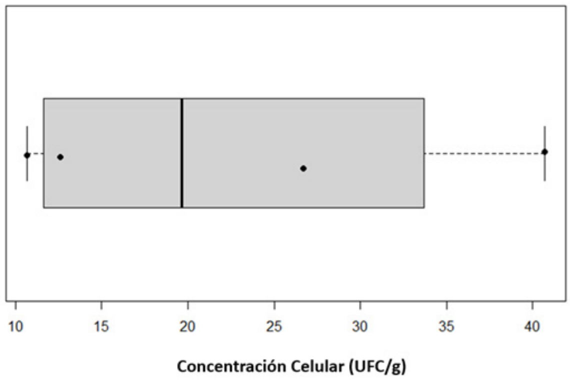
Statistical analysis of kinetic models

Criteria	Kinetic Models			
	Gompertz	Logistic	Modified Logistic	Weibull
R–squared	0.851	0.8606	0.8604	0.8600
Iterations	11	8	11	11
Sum of Squared Errors (SSE)	0.244	0.230	0.230	0.230
Degrees of freedom for error	25	24	24	24
Mean squared error	0.009	0.009	0.009	0.009
Standard deviation	0.09	0.09	0.09	0.09

Cell viability

A boxplot is presented showing the distribution of cell concentration (Figure 2), expressed in colony-forming units per gram (CFU/g). It is observed that the interquartile range (IQR) covers values between approximately 15 and 35 CFU/g, which indicates the central dispersion of the data. These whiskers represent the range of the data within 1.5 times the IQR from the lower and upper quartiles. The median, indicated by the black line inside the box, is close to the value of 20 CFU/g. The extreme values and their range allow us to infer the variability of cell concentration in the analyzed sample.

Figure 3
Boxplot for cell concentration



Note: All means are expressed as mean ± SD (n=4)

Discussions

The present study evaluated the microbial kinetics of *Saccharomyces cerevisiae* in black chuño using non-linear mathematical models (Gompertz, Logistic, Log-logistic, and Weibull), observing a sigmoidal behavior characteristic of microbial growth. The results obtained align with previous research, such as that of Rodríguez and Chambi (2019), who studied the growth of Saccharomyces boulardii in tunta of Chaska and Negra varieties, using similar models and demonstrating their usefulness in describing the different phases of microbiological growth. As in our study, they observed that microbial growth followed a sigmoidal curve, and the Gompertz and Logistic models were useful in capturing this dynamic.

The importance of evaluating the growth of yeasts such as *Saccharomyces cerevisiae* in traditional foods lies in the probiotic and functional potential of these microorganisms. Ramos et al. (2018) identified the presence of lactic acid bacteria (LAB) in the production of tunta, highlighting their role in inhibiting pathogenic microorganisms and their ability to improve the microbiological safety of the product. Although our study did not focus on LAB, the results suggest that black chuño may serve as a suitable substrate for the proliferation of beneficial microorganisms such as *Saccharomyces cerevisiae*, expanding its potential in the production of functional fermented foods.

In comparison with similar studies, such as those of Colque et al. (2023), who modeled the growth of Saccharomyces boulardii in probiotic beverages based on tarwi, it is confirmed that the growth and viability of yeasts depend largely on the conditions of the medium and the treatments applied. In our case, the use of black chuño combined with peptone water provided a favorable medium for the development of *Saccharomyces cerevisiae*, evidenced by the significant increase in biomass from the second hour of incubation. The application of the Log-logistic model in our study showed a superior fit, which coincides with previous observations where this model efficiently captures the exponential and stationary phases of microbial growth.

On the other hand, Randazzo (2013) reported the survival of probiotic strains in synthetic and natural media, observing that microbial stability and viability depend on the type of medium and storage conditions. Our results suggest that black chuño, being a food with particular characteristics derived from its preparation process, could act as a protective medium for *Saccharomyces cerevisiae*, allowing it to maintain its viability during incubation periods. However, further studies are required to evaluate the stability of the microorganism under prolonged storage conditions and its behavior in the presence of other components of black chuño.

Finally, the cultural and nutritional importance of black chuño, as mentioned by Ramos et al. (2018), lies in its traditional production and its association with Andean communities. The incorporation of beneficial yeasts such as *Saccharomyces cerevisiae* in this food not only highlights its functional value, but also opens opportunities for the development of innovative fermented products, which could contribute to food security and the preservation of Andean culinary heritage. In addition, the application of mathematical models in this type of study provides robust tools to optimize and predict microbial behavior in traditional foods, facilitating their incorporation into industrial processes.

Conclusion

The results obtained in the present study demonstrated that *Saccharomyces cerevisiae* has an adequate growth capacity in media based on black chuño, evidenced by the significant increase in biomass during the incubation period. The adjustment of the data to mathematical models allowed us to precisely describe the microbial kinetics, with the Log-logistic model presenting the best fit to the experimental data. These findings highlight the potential of black chuño as a viable medium for the development of beneficial microorganisms, which could have applications in the production of probiotic and functional foods. Additionally, the use of mathematical models provides important tools to optimize the study and prediction of microbial growth in traditional food systems. Additional studies are recommended to evaluate the stability and viability of *Saccharomyces cerevisiae* under prolonged storage conditions, as well as to explore its potential impact on the nutritional and functional properties of black chuño.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

NAH: Original draft writing and editing, research, validation. ADCR: Data curation. Reviewer and technical advisor. AMTJ: Original draft writing and editing. All authors reviewed the final version of the manuscript.

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