Influence of temperature and solute concentration during osmotic dehydration of apple (*Malus domestica*) cubes on the stability of probiotics

Influencia de la temperatura y concentración de solutos durante la deshidratación osmótica de cubos de manzana (*Malus domestica*) sobre la estabilidad de probióticos

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ABSTRACT

Currently, there is an upsurge in preference for the consumption of probiotic-rich foods. Besides their nutritive function, these compounds have demonstrated, in some instances, medicinal properties. The purpose of this study was to evaluate how temperature and sucrose concentration influenced the stability of probiotics (specifically *Saccharomyces boulardii)* during the osmotic dehydration of Granny Smith apple (*Malus domestica*) cubes. We prepared osmotic solutions with different sucrose concentrations (40, 50, and 60°Brix). We inoculated *S. boulardii* (250 mg each) into these solutions, followed by immersion of 1 cm-cubed apple cubes. We exposed these cubes to varying temperatures (37°C, 42°C, and 47°C) for a duration of 80 min. Various parameters were calculated, including the percentage of weight loss, percentage of solid gain, number of generations, and the doubling time. Results indicated that the apple cubes with more extreme dehydration were those treated at 50°Brix and 47°C exhibiting a weight loss of 40%. The treatment at 60°Brix and 42°C stood out, showing an increase of 350% of solid gain compared to other groups. Additionally, the highest number of generations of the strain occurred in the group treated at 50°Brix and 37°C, with a value of 9.32 ± 0.11 CFU/g and a doubling time of 7.50 ± 0.09 min. In conclusion, we deduced that under conditions of elevated temperatures and high solute concentrations, the *S. boulardii* strain might undergo inhibition and fail to develop adequately in the apple cubes subjected to osmotic dehydration.

Key words: *Saccharomyces boulardii***,** heat treatment, dehydrated fruit, cell viability.

RESUMEN

En la actualidad, hay un aumento en la preferencia por el consumo de alimentos ricos en probióticos. Estos compuestos, más allá de su función nutritiva, han demostrado en algunos casos poseer propiedades medicinales. En este sentido, el propósito de este estudio consistió en evaluar cómo la temperatura y la concentración de sacarosa influyen en la estabilidad de los probióticos (específicamente *Saccharomyces boulardii*) durante el proceso de deshidratación osmótica de cubos de manzana (*Malus domestica*) de la variedad Granny Smith. Para esto, se prepararon soluciones osmóticas con distintas concentraciones de sacarosa (40, 50 y 60°Brix). En estas soluciones se inoculó *S. boulardii* (250 mg cada una), seguido de la inmersión de cubos de manzana de 1 cm de lado. Estos cubos fueron expuestos a diferentes temperaturas (37, 42 y 47°C) durante un período de 80 min. Se procedió a calcular diversos parámetros, como el porcentaje de pérdida de peso, el porcentaje de ganancia de sólidos, el número de generaciones y el tiempo de duplicación. Los resultados indicaron que los cubos de manzana que experimentaron una mayor deshidratación correspondieron a los tratados a 50°Brix y 47°C, registrando una pérdida de peso del 40%. En cuanto al porcentaje de ganancia de sólidos, se destacó el tratamiento a 60°Brix y 42°C, alcanzando un aumento del 350% en comparación con los demás grupos. Además, el mayor número de generaciones de la cepa ocurrió en el grupo tratado a 50°Brix y 37 °C, con un valor de 9.32 ± 0.11 UFC/g y un tiempo de duplicación de 7.50 ± 0.09 min. Se concluyó que en condiciones de temperaturas elevadas y altas concentraciones de soluto, la cepa de *S. boulardii* podría sufrir inhibición y no desarrollarse adecuadamente en los cubos de manzana sometidos al proceso de deshidratación osmótica.

Palabras clave: *Saccharomyces boulardii*, tratamiento térmico, fruta deshidratada, viabilidad celular.

Introduction

Most foods that contain probiotics are derived from milk (Vinderola *et al*., 2017). This creates a big problem for some consumers since it involves the consumption of allergens and lactose. In addition, it implies the consumption of products of animal origin that are not suitable for a vegetarian or vegan diet and have a short shelf life (Makinen *et al*., 2016). Because of this the demand for non-dairy probiotic products has grown because of an increased incidence of dietary restrictions on dairy products for reasons including lactose intolerance, allergic reactions to milk proteins, and veganism (Kumar *et al*., 2015; Neffe-Skocińska *et al*., 2018).

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Probiotics are defined as live microorganisms that, when administered in sufficient amounts, confer a health benefit on the consumer (Hill *et al*., 2014). *Saccharomyces boulardii* is included among the microorganisms with probiotic effect. This microorganism can remain viable and active so that when ingested it can give pharmacodynamic effects similar to the physiological effects of normal intestinal flora (Peña, 2007; Zamora Vega *et al*., 2015); it also acts as a transporter, releasing enzymes, proteins, and trophic factors during its intestinal transit, improving the host's immune defenses, digestion, and nutrient absorption. So, this yeast is an important source for probiotic products (Mejía *et al*., 2016; Sen & Mansell, 2020). Its probiotic activity has been linked to multiple pathways, including enhancement of gut barrier function, competitive pathogen exclusion, antimicrobial peptide production, and immunomodulatory and nutritional effects (Zhang *et al*., 2021). This probiotic strain helps in maintaining the balance of intestinal flora by stimulating the production of lactic acid and acetic acid, lowering intestinal pH, and preventing the proliferation of pathogenic bacteria (Li, Xia *et al*., 2021; Li, Zhu *et al*., 2021).

Fruits and vegetables are an essential part of the human diet, since they are a source of bioactive compounds such as vitamins, minerals, phytosterols, dietary fiber, etc. Generally, fruits and vegetables are marketed fresh. However, their shelf life is limited by metabolic activity, and they are susceptible to mechanical damage and the presence of microorganisms that accelerate their senescence and death (Yousuf *et al*., 2018; Al-Tayyar *et al*., 2020). Osmotic dehydration is a technique used to reduce the water content and to include solutes in fruits and vegetables by immersion in an osmotic solution. This technique has been widely used in the food industry to produce dehydrated fruits with a longer shelf life and nutritional value (Yousuf *et al*., 2018).

The apple fruit matrix has been proven to be highly applicable for probiotics, possibly due to its high porosity and, therefore, the easy incorporation of probiotics. Espírito Santo *et al*. (2012) attribute this to the cellulose content in apples that is not digested and could serve as a protective matrix for probiotics through the intestinal tract (Kourkoutas *et al*., 2006). Rêgo *et al*. (2013) demonstrate the compliance of apple as a fruit matrix for probiotic survival over time. They studied hot-air-dried apple cubes with *Lactobacillus plantarum* incorporated during 65 d of storage and found a loss of viability of 1 log CFU/g.

Gupta and Garg (2009) suggest a dose of 5 billion colony forming units (CFU) for at least 5 d (5×10^9 CFU/d) to produce health benefits. Probiotics may be available in foods and dietary supplements (capsules, tablets, and powders). Also, to improve probiotic survival, the food should be dehydrated by lyophilization instead of hot air (Betoret *et al*., 2003; Rascón *et al*., 2018). Another methodology that is increasingly of interest in the impregnation of probiotics is osmotic dehydration. This consists of submerging the food in a hypertonic solution that contains microbial cells. The water migrates from the food to the solution, partially dehydrating it; and the solutes, including the probiotics, migrate towards the food (Rascón *et al*., 2018).

In a study carried out on plantain impregnated with *Lactobacillus rhamnosus,* the process was successful, maintaining levels of 10^7 CFU/g (Huerta-Vera *et al*., 2017). Using 50% w/w hypertonic sucrose solutions, Rascón *et al*. (2018) impregnated *L. rhamnosus* in banana slices by osmotic dehydration to subsequently freeze-dry them. The survival kinetics of the microorganism showed that its viability decreased significantly when water activity (aw) exceeded values of 0.327.

The purpose of this study was to evaluate how temperature and concentration influence the stability of probiotics (specifically *Saccharomyces boulardii)* during the osmotic dehydration of Granny Smith apple (*Malus domestica*) cubes.

Materials and methods

Raw material

Granny Smith apples were purchased at the local market in the province of San Román, department of Puno (Peru) and stored at 4°C. We washed them for 5 min in an aqueous solution of active chlorine at a concentration of 7500 mg L^{-1} and then cut them into cubes of 1 cm square. Freeze-dried strains of *S. boulardii* Hansen CBS 5926 from Mexico were inoculated into the solutions.

Maturity index

To determine the maturity index in apples, we measured °Brix and acidity. According to the AOAC (2005) method, we determined the acidity by preparing a 0.1N NaOH solution; then we weighed 10 g of apple pulp and added 50 ml of distilled water. The mixture was vigorously shaken for a few minutes, and we collected an aliquot of 25 ml of the solution. Once filtered, we added 3 drops of phenolphthalein. We titrated the solution with a standard 0.1 N NaOH solution until it reached the equivalence point by changing color to light pink. We used the volume spent to calculate the acidity percentage (Eq. 1). We determined Brix with an ATAGO range refractometer (0-40°Brix).

$$\%\text{Acidity} = \frac{(A \times B \times C) \times 100}{D} \quad (1)$$

where A represents the volume of NaOH spent, B signifies the normality of NaOH (0.097 meq ml^{-1}), C denotes the equivalent weight expressed in grams of the predominant acid in the fruits (citric acid 0.064 g meq^{-1}; malic acid 0.067 g meq^{-1}), and D stands for the weight in grams of the sample used.

The maturity index (MI) was determined with the following formula:

$$MI = \frac{°Brix}{Acidity} \quad (2)$$

Osmotic dehydration and inoculation of the strain

We prepared aqueous osmotic solutions with sucrose at different concentrations (40°Brix, 50°Brix, and 60°Brix). The apple cubes were immersed in 500 ml beakers with 200 ml of the prepared osmotic solution at three different temperatures (37°C, 42°C, and 47°C), and 250 mg of *S. boulardii* were immediately added. We applied treatments by shaking at 350 rpm. Each apple sample was removed every 10 min up to a total time of 80 min to conduct weight measurements on an OHAUS analytical balance and measure the soluble solids content in the liquid phase (Brix) using an ATAGO range refractometer (0-85°Brix). We conducted all the experiments in triplicate.

Weight loss and solid gain

During osmotic dehydration, we monitored mass transfer by the time variation in solid gains and weight loss (Della Rocca & Mascheroni, 2011; Wais, 2011).

Equation 3 was used to calculate weight loss.

$$WL\ (\%) = \left(\frac{M_o - M_f}{M_o}\right) \times 100 \quad (3)$$

where WL (%) is the percentage of weight loss, M_o represents the initial weight of the sample in grams, and M_f is the weight of the sample treated at time t.

The solid gains were calculated as indicated by Equation 4.

$$SG\ (\%) = \left(\frac{°B_f - °B_o}{°B_o}\right) \times 100 \quad (4)$$

where SG (%) were the solid gains percentages, $°B_f$ were the Brix degrees of the osmodehydrated sample and $°B_o$ were the Brix degrees of the fresh sample.

Microbiological kinetics

To assess the microbiological growth kinetics in the polynomial model, we employed several key statistical metrics. To gauge the model's fitness for survival, doubling time, and the number of generations, we utilized the coefficient of determination (R^2). We computed this coefficient using the following equation:

$$R_2 = 1 - \frac{SSE}{SST} \quad (5)$$

where SSE (Sum of Squared Errors) represented the sum of the squares of differences between actual observations and model predictions, and SST (Total Sum of Squares) was the sum of the squares of differences between actual observations and their mean.

Additionally, to evaluate the model's goodness of fit in terms of complexity, we employed the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). We calculated AIC using the following equation:

$$AIC = -2 \times \text{Log} - \text{likelihood} + 2k \quad (6)$$

where log-likelihood was the logarithmic likelihood function of the model and k was the number of estimated parameters in the model.

BIC was computed as follows:

$$BIC = -2 \times \text{Log} - \text{likelihood} + k \times \text{Log}(n) \quad (7)$$

where log-likelihood was the logarithmic likelihood function of the model, k was the number of estimated parameters, and n was the number of observations in the data.

We applied these statistical metrics in the study's methodology to assess the goodness of fit of the polynomial model and its ability to explain the observed data.

Survival, doubling time, and number of generations

To determine cell survival, we used a 10^{-1} dilution in 1% saline solution, and then Petri dishes were inoculated with 0.1 ml of Sabouraud Dextrose Agar and dispersed with a Drigalski spatula; we used a convection incubator at 27°C for 48 h; the readings were expressed in colony-forming units per gram (CFU/g).

To calculate the number of generations, we applied the following equation:

$$N_G = \frac{LogN_2 - LogN_1}{Log2} \quad (8)$$

where N_G was the number of generations, N_2 was the concentration of cells at time 2 and N_1 was the concentration of cells at time 1, expressed in colony-forming units per gram (CFU/g).

The doubling time was calculated with the following equation:

$$T_D = \frac{t_f - t_i}{N_G} \quad (9)$$

where T_D was the doubling time, t_f was the final incubation time, and t_i was the initial incubation time in minutes.

Results and discussion

Osmotic dehydration

In Figure 1, the treatments with the highest percentage of weight loss were the treatments 50°B47°C, followed by 50°B37°C. In contrast, the treatment 60°B42°C obtained an unfavorable result. All other treatments had a positive slope indicating that weight loss was continuous over time.

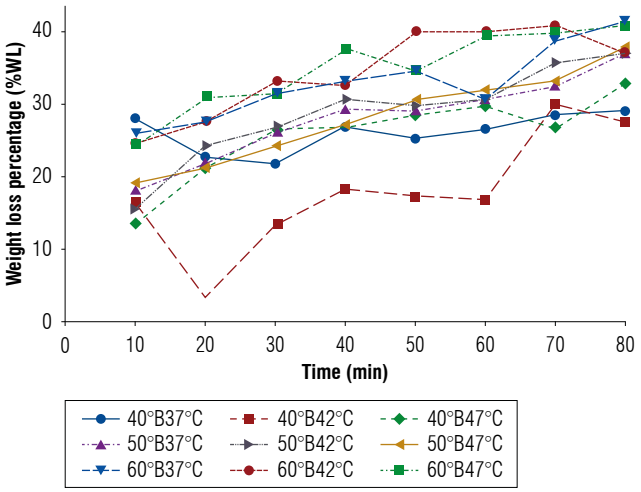

FIGURE 1. Weight loss percentage (%WL) as a function of time of apple cubes inoculated with *Saccharomyces boulardii* at different concentrations and temperatures.

The statistical analysis of the regression (Table 1) revealed that 50°B47°C was the treatment with the best fit with an R^2 of 0.98 in contrast to the others. Likewise, it showed the highest value in the R^2 adjusted (0.98) related to the typical error. The same treatment showed the smallest value of typical error (0.91). On the other hand, all the treatments were significant with a *P*-value less than 0.05. However, two had higher values, 40°B42°C with 0.21 and 50°B42°C with 4.60.

TABLE 1. Statistics of the linear regression of the percentage of weight loss in apple cubes inoculated with *Saccharomyces boulardii*

Treatments	Regression statistics				
	Determination coefficient (R^2)	R^2 adjusted	Typical error	MSE	Probability
40°B37°C	0.26	0.13	2.45	12.84	0.00
40°B42°C	0.61	0.54	5.56	291.84	0.21
40°B47°C	0.72	0.68	3.41	188.00	0.00
50°B37°C	0.93	0.92	1.65	239.76	0.00
50°B42°C	0.87	0.85	2.58	278.38	0.00
50°B47°C	0.98	0.98	0.91	289.51	4.60
60°B37°C	0.82	0.79	2.37	158.26	0.00
60°B42°C	0.75	0.70	3.22	188.75	0.00
60°B47°C	0.85	0.83	2.36	200.46	0.00

Note: In each treatment, B corresponds to degrees Brix in the medium followed by the treatment temperature. MSE – Mean Square Error.

Figure 2 shows that the gain of solids increased as the treatment time increased. This was probably due to the incorporation of soluble solids in the fruit. Also, the rate of gain of solids increased as the concentration increased.

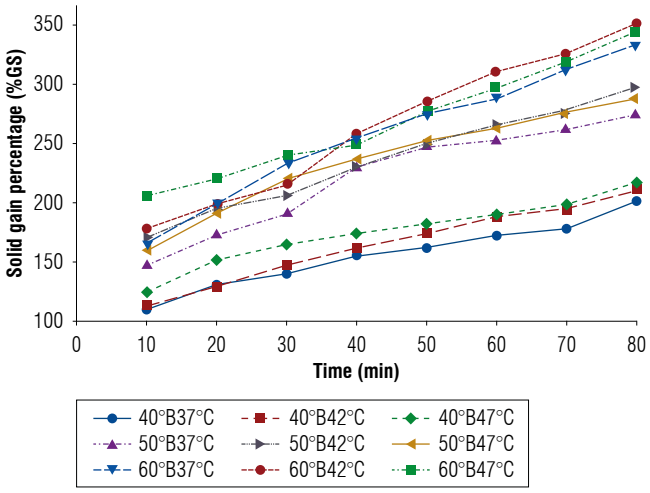

FIGURE 2. Solid gain percentage (%GS) as a function of time in apple cubes inoculated with *Saccharomyces boulardii* at different concentrations and temperatures.

Table 2 presents the results of the statistical regression analyzes, where all the treatments had a good adjustment (R^2>0.93) ranking between 0.94 and 0.99. The error 40°B42°C and 50°B42°C had the smallest values of all the treatments.

TABLE 2. Statistics of the linear regression of the percentage solid gain in apple cubes inoculated with *Saccharomyces boulardii.*

Treatments	Regression statistics				
	Determination coefficient (R^2)	R^2 adjusted	Typical error	MSE	Probability
40°B37°C	0.97	0.97	4.66	5562.16	1.23
40°B42°C	0.99	0.99	3.14	8353.05	1.33
40°B47°C	0.95	0.94	6.77	5503.31	4.04
50°B37°C	0.94	0.93	11.6	14276	5.08
50°B42°C	0.99	0.99	3.91	13438.68	3.82
50°B47°C	0.95	0.94	10.15	12804.23	1.07
60°B37°C	0.98	0.97	8.29	22189.70	3.60
60°B42°C	0.99	0.98	6.71	28141.88	1.27
60°B47°C	0.99	0.98	5.34	16432.79	1.04

Note: In each treatment, B corresponds to degrees Brix in the medium followed by the treatment temperature. MSE – Mean Square Error.

Microbiological kinetics

The microbiological kinetics of each of the treatments revealed intriguing findings. Specifically, *S. boulardii* experienced a significant increase in its development at a temperature of 42°C across all evaluated concentrations. This strongly suggested that this temperature represented the optimal condition for cellular reproduction of this microorganism. This discovery holds significant relevance as it provided valuable insights into the ideal conditions for the cultivation and stability of *S. boulardii* in osmotic dehydration. Furthermore, when analyzing the kinetic curves in 60°Brix media, we noted a distinct behavior. These curves exhibited significantly lower values compared to the other two graphs. This phenomenon could be associated with the influence of sugar concentration in the growth medium on the activity and growth of microorganisms. These results open new avenues of research to better understand how environmental variables, such as temperature and nutrient concentration, impact the growth kinetics of biotechnologically relevant microorganisms. This, in turn, could lead to improvements in the production and application of *S. boulardii* in apple cubes.

Survival of *Saccharomyces boulardii*

The survival of *S. boulardii* during osmotic dehydration exhibited divergent responses across the various treatments. In certain instances, such as in the case of 60°B47°C and 40°B47°C, we observed limited development with values of 1.75 and 1.72 CFU/g. Conversely, two treatments yielded the highest number of generations: 50°B37°C with 9.32 CFU/g and 60°B37°C with 8.08 CFU/g. Furthermore, these two treatments demonstrated the shortest doubling times, indicating a more rapid increase in biomass.

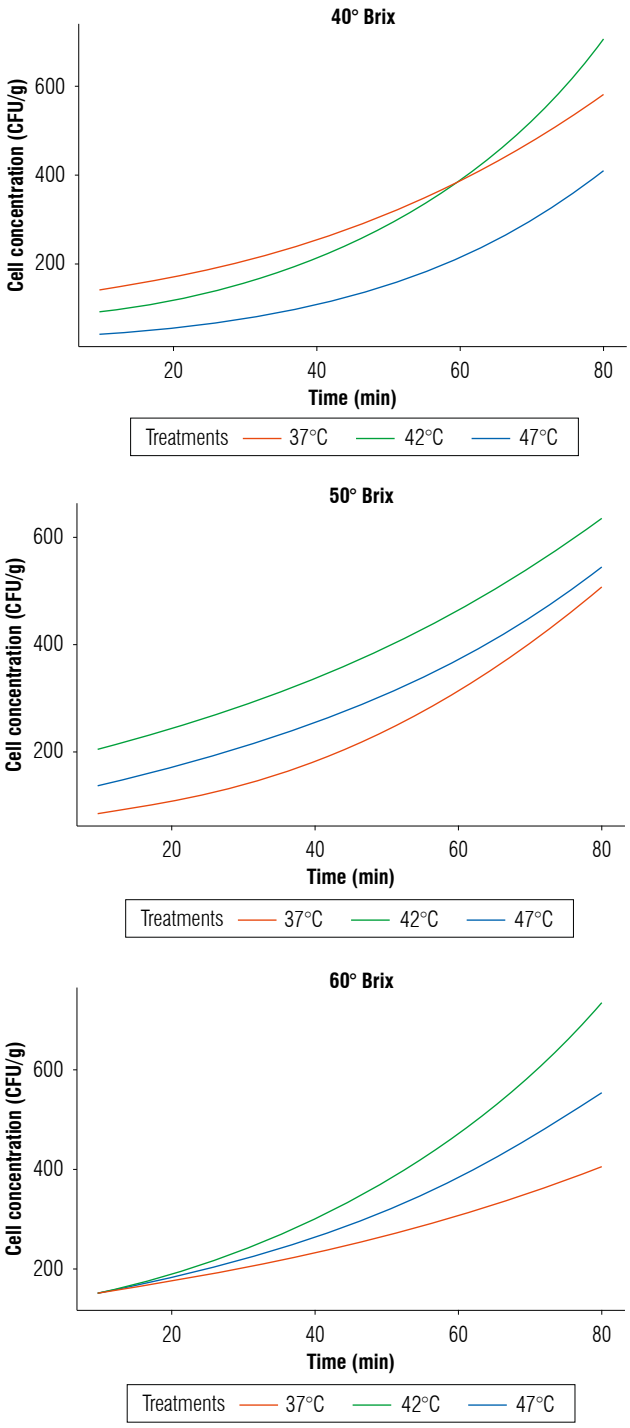

FIGURE 3. Microbiological kinetics of *Saccharomyces boulardii* in apple cubes at different concentrations of solids and temperatures.

Specifically, 50°B37°C had a doubling time of 7.50 min, whereas 60°B37°C had a doubling time of 8.69 min (Tab. 3). These findings underscored the significant influence of temperature and solute concentration conditions on the survival and growth of *S. boulardii* during osmotic dehydration. Treatments conducted at lower temperatures, such as 50°B37°C, facilitated greater bacterial growth and

more abundant biomass compared to treatments conducted at higher temperatures, such as 60°B47°C. These results emphasized the critical importance of judiciously selecting osmotic dehydration conditions to achieve desired outcomes in terms of microbial survival and growth.

TABLE 3. Viability of *S. boulardii* in apple cubes inoculated during osmotic dehydration at different concentrations and temperatures.

Treatments	Number of generations (CFU/g)			Doubling time (min)		
	$\bar{x}$	±	σ	$\bar{x}$	±	σ
40°B37°C	4.38	±	0.58	16.12	±	2.14
40°B42°C	2.40	±	0.01	29.14	±	0.17
40°B47°C	1.72	±	0.13	40.70	±	3.12
50°B37°C	9.32	±	0.11	7.50	±	0.09
50°B42°C	1.91	±	0.01	36.62	±	0.26
50°B47°C	2.15	±	0.38	33.05	±	5.86
60°B37°C	8.08	±	0.07	8.69	±	0.07
60°B42°C	2.33	±	0.34	30.31	±	4.49
60°B47°C	1.75	±	0.72	39.89	±	1.72

Note: All means are expressed as mean ± σ (n = 3). In each treatment, B corresponds to degrees Brix in the medium followed by the treatment temperature.

In the analysis of the response surface graph for the number of generations, increasing the total solids concentrations (°Brix) had a noticeable impact on biomass concentration. This impact varied with temperature. Initially, as we increased the solids concentration, the biomass concentration also increased, and this relationship held true up to a certain point. However, beyond that point, there was a decline in biomass concentration. This trend is clearly illustrated in the cont our graph, where the green region represents the range of conditions that result in the highest biomass concentrations.

Likewise, when examining the response surface for doubling time, at higher concentrations of solids and temperatures, the rate of cell doubling increased. The contour graph further corroborated this observation. It highlighted the blue area that signified those lower solid concentrations (specifically 40 and 45°Brix) lead to the shortest doubling times within the temperature range of 40°C to 47°C. This means that under certain conditions, cells doubled more rapidly. This could be crucial information for optimizing the process.

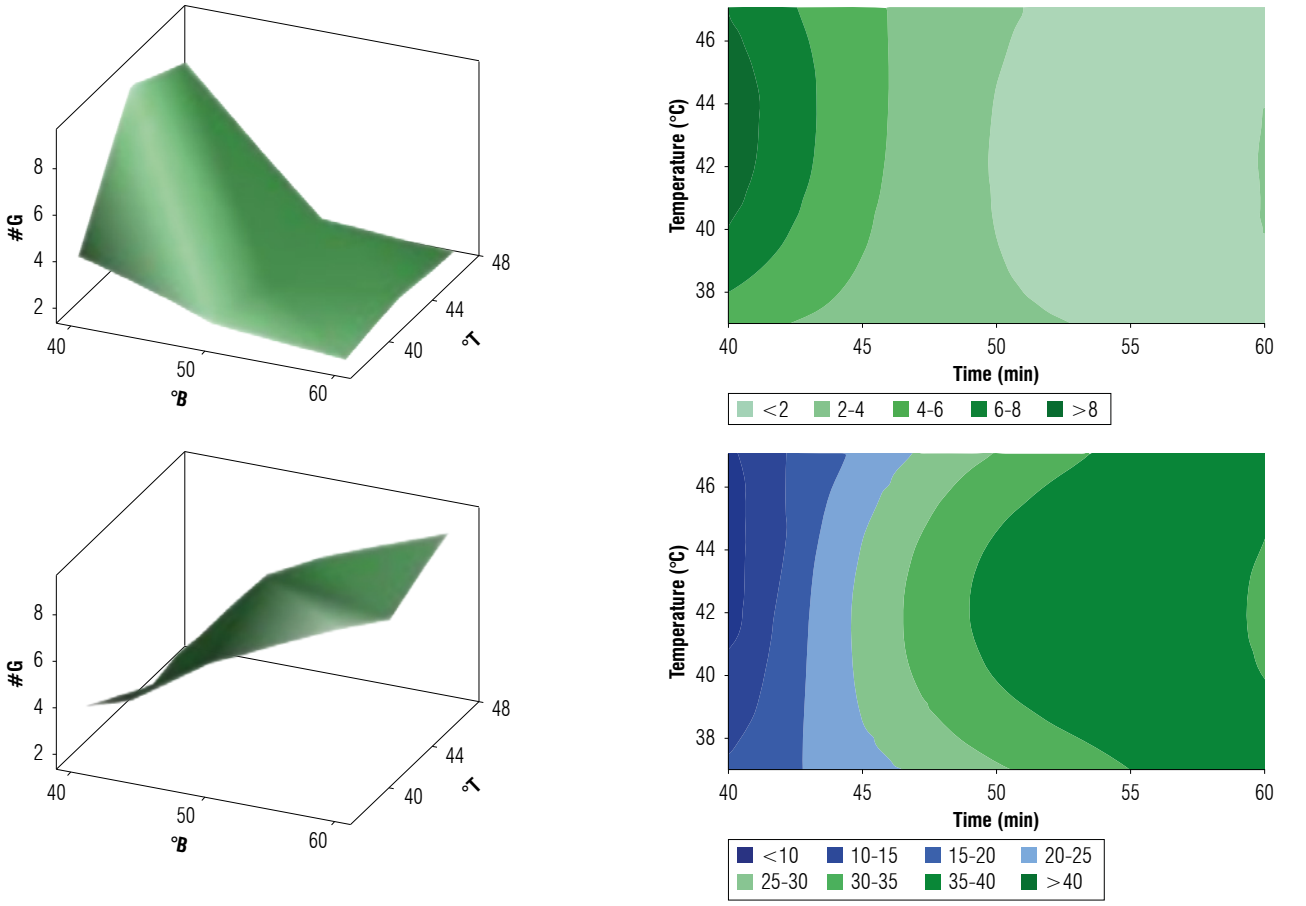

FIGURE 4. Response surface and contour plots for survival of *S. boulardii* in osmodehydrated apple cubes. #G is the number of generations expressed in CFU/g, °B is °Brix, T is the temperature in C° and TD is the doubling time in minutes.

Discussion

Cui *et al*. (2018) evaluate weight loss during osmotic dehydration of pears in solutions of sucrose and sodium chloride at different concentrations. Their results show that weight loss increases with the concentration of the solution and with dehydration time, agreeing with the data shown in Figure 1. This also agrees with the findings of Ayala-Aponte *et al*. (2010) in mangoes with sucrose solutions, where weight loss increased with the concentration of the solution and with dehydration time.

Parra-Palacios (2020) mentions that the increase in weight loss in pineapple slices shows that when the concentration and temperature increase there is a 19.50% weight loss.

Giraldo *et al*. (2005) point out that the weight loss in mango at different concentrations is due to the amount of water transferred from the mango to the osmotic medium that is greater than the quantity of soluble solids that migrate from the hypertonic medium. Likewise, Della Rocca and Mascheroni (2011) argue that temperature is one of the most significant variables since it will modify the kinetics in the osmotic dehydration process, hence weight loss. Weight loss is more affected than a gain in soluble solids; because of the use of high temperatures, the syrup solute cannot easily diffuse.

In Figure 2, the results of a gain of solids with respect to time are shown. It is possible to observe that weight loss increases as the treatment time increases. This is probably due to the incorporation of soluble solids in the fruit; and, also, the rate of solid gains increased as the concentration of the osmotic solution and the temperature increased. This agrees with what is found by Ochoa and Ayala (2009) who state that in slices of yacón an increase in solids is proportional to the concentration and temperature. Huerta-Vera (2021) reveals that at higher concentrations of the osmotic solution, the solid gain increased during osmotic dehydration in chayote slices, while an increase in the temperature of the osmotic medium also causes an increase in an increase in solids of chayote. Likewise, the results Arias *et al*. (2017) indicate that as the temperature of the osmotic solution increases, there is a greater alteration of the cellular structure, which favors the entry of solutes into the interior of the food. To limit the impregnation, it is convenient to use high solute concentrations and short osmotic dehydration times (Della Rocca & Mascheroni, 2011).

The survival of *S. boulardii* under osmotic dehydration conditions in the different treatments showed that under adverse conditions these could be inhibited, generating an increase in the biomass concentration (Tab. 3, Fig. 4). This is corroborated by Rascón *et al*. (2018) who affirm that the addition of *Lactobacillus rhamnosus* to banana slices causes an inhibition after 300 min, achieving a concentration of $9.40 \pm 0.23 \log_{10}$ CFU/ml. Likewise, Rodrigues *et al*. (2018) agree that a prolonged time for *Lactobacillus casei* in apples dried at 60°C generates a decrease in biomass, which shows that the treatments carried out at low temperatures (37°C and 42°C) do not exceed the adverse conditions for the normal development of the biomass.

Conclusions

In this study, microbiological growth kinetics were assessed under osmotic dehydration conditions. We observed that treatments 50°B47°C and 50°B37°C exhibited the highest weight loss and displayed an excellent fit in the regression analysis, with R^2 values of 0.983 and 0.980. Furthermore, they stood out for their low typical errors (0.912).

Regarding microbiological kinetics, 42°C represented the optimal temperature for the growth of *S. boulardii* across all evaluated concentrations. Additionally, sugar concentrations significantly influenced the kinetics, resulting in reduced growth at 60°Brix.

Survival of *S. boulardii* during osmotic dehydration varied among treatments, with 50°B37°C and 60°B37°C demonstrating a higher number of generations and shorter doubling times. These findings underscored the critical influence of temperature and solute concentrations on microbial survival and growth during osmotic dehydration.

In summary, this study furnished valuable insights into the optimal conditions for the growth and survival of *S. boulardii* during osmotic dehydration. These discoveries hold significant implications for the production and application of *S. boulardii* in apple cube processing and pave the way for further research into environmental variables affecting microbial kinetics.

Conflict of interest statement

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

Author's contributions

MYCC: writing of original draft, research, validation. ADCR: conceptualization, methodology, formal analysis, research, writing of original draft. All authors reviewed the final version of the manuscript.

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