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MODELING THE GROWTH OF SPOILAGE BACTERIA IN COSTEÑO CHEESE SUBJECTED TO THERMOSONICATION

MODELADO DEL CRECIMIENTO DE BACTERIAS ALTERANTES EN QUESO COSTEÑO SOMETIDO A TERMOSONICACIÓN

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ABSTRACT

The effect of thermosonication at three temperatures on the growth of spoilage bacteria in Costeño cheese was investigated. Bacterial counts were fitted to primary models such as Gompertz, Huang, and Buchanan. Polynomial equations were used to describe the effect of thermosonication on the specific growth rate (µ). The mean square error (MSE), bias factor (Bf), and accuracy factor (Af) were used to evaluate the performance of predictive models. The most severe treatment applied in this study was thermosonicated at 40 kHz at 60°C, which led to an increased latency phase (λ) and a decreased µ of the spoilage bacteria analyzed. The µ values obtained from the Gompertz and Buchanan models were employed to construct polynomial equations. These secondary models had bias factors and accuracy factors close to one, indicating that the polynomial models were able to describe microbial growth in cheese. These results could likely contribute to initiating the application of thermosonication to extend the shelf-life of Costeño cheese

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Keywords: polynomial equations, predictive microbiology, primary models, thermosonication.

RESUMEN

Se investigó el efecto de la termosonación a tres temperaturas en el crecimiento de bacterias alterantes en el queso Costeño. Se ajustaron recuentos bacterianos a modelos primarios como Gompertz, Huang y Buchanan. Se utilizaron ecuaciones polinómicas para describir el efecto de la termosonación en la tasa de crecimiento específico (µ). El error cuadrático medio (ECM), el factor de sesgo (Bf) y el factor de precisión (Af) se emplearon para evaluar el rendimiento de los modelos predictivos. El tratamiento más severo aplicado en este estudio fue la termosonación a 40 kHz a 60°C, lo que resultó en una fase de latencia aumentada (λ) y una disminución de µ de las bacterias alterantes analizadas. Los valores de µ obtenidos de los modelos de Gompertz y Buchanan se utilizaron para construir ecuaciones polinómicas. Estos modelos secundarios tenían factores de sesgo y factores de precisión cercanos a uno, lo que indica que los modelos polinómicos fueron capaces de describir el crecimiento microbiano en el queso. Estos resultados podrían contribuir a iniciar la aplicación de la termosonación para prolongar la vida útil del queso Costeño.

Keywords: Ecuación polynomial, microbiologia predictiva, modelos primaries, termosonicación.

INTRODUCTION

In recent decades, there has been a growing demand for processed food products that maintain their original sensory and nutritional characteristics. This demand has driven researchers to explore alternative technologies, such as Ohmic heating, ionizing radiation, high hydrostatic pressure, electromagnetic fields, and ultrasound technology (Shen Cai et al., 2021). Ultrasound, defined as sound waves with frequencies that exceed the human ear's hearing limit, typically produces compression and expansion cycles, generating cavitation phenomena responsible for antimicrobial effects (Huang et al., 2017). From a cost perspective, ultrasound technology is relatively affordable compared to other equipment, making it a cost-efficient and environmentally friendly option. High-power ultrasound can be combined with heat (thermosonication) to enhance microbial inactivation and retain the nutritional attributes of food products (Jalilzadeh et al., 2018).

Costeño cheese (CC) is a dairy product primarily marketed in the Caribbean region. This product is traditionally manufactured through enzymatic coagulation without any thermal treatment, necessitating immediate consumption (Gutiérrez et al., 2017). Due to its pH and high moisture content, CC is susceptible to microbial contamination, making it essential to study its microbiological parameters to determine its shelf-life. Predictive microbiology focuses on understanding the impact of intrinsic and extrinsic factors, such as temperature, pH, and water activity (Aw), on the growth/inactivation of foodborne pathogens. It can also be applied to predict the microbiological shelf-life of foods (Cayre et al., 2005). Predictive modeling offers a rapid and cost-effective means to obtain reliable estimates of bacterial growth and survival. Numerous researchers have employed this approach to predict growth parameters, including the logarithmic rise in microbial population, specific growth rate (μ), and latency phase (λ) of the microorganism growth curve.

Predictive models have proven invaluable in describing the behavior of microorganisms during the food manufacturing process and optimizing food production (Possas et al., 2017). Sigmoidal equations are frequently used to describe microbial growth, offering an excellent fit for microbial growth curves. Statistical indices like the mean square error (MSE), bias factor (Bf), and accuracy factor (Af) have been effective in assessing the model's quality of fit (Geitenes et al., 2013; Slongo et al., 2009). Predictive microbiology frequently employs both primary and secondary models. Primary models describe microbial cell fluctuations over time, while secondary models elucidate the responses of growth parameters to changes in environmental conditions (Panikov, 2023).

The use of predictive microbiology to model microbial growth in costeño cheese processed through thermo-sonication could be pivotal in assessing the technology's effects on the food product's shelf life. However, limited research has employed predictive models for this purpose. Consequently, this study aims to model the impact of ultrasound combined with heat treatment on spoilage bacteria in costeño cheese.

MATERIALS AND METHODS

Inoculum preparation

Costeño cheese (CC) was manufactured according to the methodology developed by Acevedo et al., (2014). CC samples were cut into slices. Next, slice cheeses were placed into sterile plastic bags and subjected to thermos-sonication using a Labscient Model KSL5120-5 ultrasonic processor (Frequency 40 kHz, high power 120 w, Germany) attached with a sonotrode Model Ezodo with a precision: ± 1,5 dB (94 dB ref @ 1 kHz). The temperature range varied from 30 to 60°C. The specific growth rate (µ) was used as a variable response. A non-treated sample (not thermo-sonicated) was used as a control.

Determination of growth

At predetermined time intervals, 11 g of samples of cheese were diluted in peptone water until 10-3. Cells were enumerated by the plate count method using agar standard plate count (SPC). The effect of thermosonication was observed by plotting the growth of Log CFU versus time. In order to ensure reproducible results, each experiment was conducted at least three times in duplicates.

Growth curve fitting

Growth curves were constructed by plotting the logarithm of the number of microorganisms versus time at the different temperatures (30, 40, 50 and 60 °C). The Huang, Gompertz and Buchanan models were employed to fit the growth of spoilage bacteria in CC and obtain the growth parameters used as a response variable.

For growth curve fitting, the Gompertz model was used:

$$\left[Y = y_0 + (y_{max} - y_0) * exp\left\{-exp\left[\frac{\mu_{max}^{e}}{y_{max}-y_0}(\lambda - t) + 1\right]\right\}\right] \quad [1]$$

Where y0, ymax, and y(t) are the natural logarithm of bacterial concentration at initial, maximum, and time t; μmax is the growth rate, and λ is the duration of the latency phase.

The Huang model was the following:

$$\left[Y(t) = y_0 + y_{max} - In\{e^{y_0} + [e^{y_{max}} - e^{y_0}]\, e^{-\mu max B(t)}\}\right]$$

$$\left[B(t) = t + \frac{1}{\alpha} In \frac{1+e^{-\alpha(t-\lambda)}}{1+e^{\alpha\lambda}}\right] \qquad [2]$$

Where y0, ymax and y(t) are the natural logarithms of the bacterial concentration at initial, maximum, at time t; μmax is the growth rate, and λ is the latency phase. The latency phase coefficient is α 4.

The following equation defines the Buchanan model:

$$[y = y_0, si\ t < \lambda]$$

$$[y = Yy_0 + k(t-\lambda), si\ \lambda \leq t < t_{max}]$$

$$[y = y_{max,}\pi si\ t \geq t_{max}] \qquad [3]$$

Where t_{max} is the time at which y is equivalent to y_{max}

Development of the secondary model

The polynomial model for describing μmax as a function of sonication temperature was evaluated. The equation was as follows:

$$In(x) = a + b * T + c * T^2 \qquad [4]$$

In x is the natural logarithm of the μ_{max}, a, b and c are adjustment factors and T is the temperature (° C).

Reliability Assessment of the Survival Kinetics Model for *L. delbrueckii*

The mathematical validation of the constructed polynomial model was carried out by calculating bias factor (Bf), accuracy factor (Af), and mean square error (MSE) (Abou-zeid et al., 2009; Ross, 1996; González et al., 2020). Af was employed to assess the variation range of the predicted values, while Bf was used to identify differences between the predicted and measured values. The specific formulas for Af, Bf, and MSE are provided in equations (5), (6), and (7), respectively

$$\left[A_f = 10^{\left(\Sigma\left|{\log\mu\, predictive}/{\log\mu\, observed}\right|/n\right)}\right] \qquad [5]$$

$$\left[Bf = 10^{\left(\Sigma\, Log\left(\frac{\mu\, observed}{\mu\, predictive}\right)/n\right)}\right] \qquad [6]$$

$$\left[MSE = \frac{\Sigma(obser-predic)^2}{n}\right] \qquad [7]$$

Where, n corresponds to the number of observations, the variables obs and pred are the observed and predicted values respectively

Data analysis

All microbial curves were carried out in triplicate to obtain growth parameters. The software GraphPad PRISM was used for curve fitting. Differences between growth parameters were calculated through analysis normal of variance (ANOVA- one way) employing the SPSS software.

RESULTS AND DISCUSSION

Microbial Growth Curves - Primary Modeling

Growth curves of spoilage bacteria in CC subjected to thermosonication at different temperatures (30–60°C) were obtained as described in the Materials and Methods section. These curves were adjusted to primary predictive models such as Gompertz, Huang, and Buchanan to determine growth parameters: initial cell population (Y0), maximum population (Ymax), latency phase (λ), and specific growth rate (µ). These parameters are crucial for optimizing microbial fermentation processes. The λ and exponential phases are particularly significant for food microbiologists because food spoilage often occurs before bacteria reach the stationary phase (Cubero et al., 2019).

Y0 is a parameter that can be controlled by adjusting the number of microorganisms incorporated into a food product (González et al., 2020). However, in this case, Y0 corresponds to spoilage bacteria naturally present in CCC, leading to significant differences between models and the analyzed temperatures. Table 1 illustrates the growth parameters, revealing statistical differences between models. Notably, as the temperature increases, Y0 values are modified, particularly evident for the Gompertz and Buchanan models. In contrast, no statistical differences ($p>0.05$) were observed between control samples (without thermosonication) (1.03 log CFU) and those treated at 30, 40, 50, and 60°C when the Huang model was employed.

λ values represent the time microorganisms take to adapt to a new culture medium. Therefore, this parameter is useful for reducing the fermentation time in various fermented foods. In a fermentation process, the primary objective is to enhance microbial growth and decrease the fermentation time. Hence, the absence or low values of λ are desirable. However, it's worth noting the negative values reported in Table 1 for spoilage bacteria grown at 30°C and modeled using the Gompertz and Buchanan models (-12,443 min and -123,642 min, respectively). These values indicate that both models were unsuitable for modeling microbial growth with low values or an absence of λ.

Conversely, when Huang's model was applied to the CFU vs. time curve, an increase in λ values was observed. For example, among the treated samples, the lowest λ value (8,163 min) was found in cheese samples subjected to 30°C, while the highest value (125.58 min) was observed in samples treated at 60°C. This demonstrates that temperature plays a significant role in bacterial spoilage, with λ being temperature-dependent. Nevertheless, it is essential to consider that ultrasound can damage cell walls and cytoplasmic membranes, causing disruption (Wu et al., 2015), and can affect intracellular components (Kon et al., 2005). A combination of heat treatment and ultrasound can be more effective than heat or ultrasonic treatment alone (Mani et al., 2022). Gao et al., (2014) found that Enterobacter aerogenes suspensions are more sensitive to ultrasonication during the exponential growth phase than in the stationary phase.

Ymax values ranged from 7.528 to 7.262 (log CFU), indicating only minor significant differences ($p<0.05$) between the Gompertz, Huang, and Buchanan models. Furthermore, changes in temperature from 30 to 60°C under ultrasound treatment did not appear to affect this parameter, in line with findings from Antunes-Rohling et al., (2019), who reported that temperature had no significant effect ($p > 0.05$) on Ymax values for the microbial groups studied. However, it's important to note that these Ymax values were achieved at different times. Ymax represents the maximum concentration of bacteria reached at the end of the logarithmic growth phase and can be crucial for developing functional foods containing probiotic bacteria (Tripathi & Giri, 2014; Palabiyik et al., 2018). A safety limit of 10^7 CFU/g has been suggested for certain food products (Ruiz-Capillas et al., 2007).

The specific growth rate (μ) is commonly influenced by nutrient availability, oxygen levels, and metabolite production, resulting in environmental stress (Jeanson et al., 2015; Skandamis & Jeanson, 2015). The lowest μ values were obtained in cheese samples sonicated at 30°C and modeled using the Gompertz (0.012 min^{-1}), Huang (0.012 min^{-1}), and Buchanan (0.011 min^{-1}) models, while the highest values were observed in control samples (0.029 to 0.037 min^{-1}), followed by samples sonicated at 30°C (0.021 to 0.025 min^{-1}). These results indicate that no significant differences ($p>0.05$) were noted between the models used. In general, μ was affected by the thermos-sonication treatment, with similar values ($p>0.05$) observed among the primary models, although these models are not directly comparable with each other.

Table 1. Growth parameters of spoilage bacteria at different temperatures grown on Costeño cheese

Treatment	Parameters	Gompertz	Huang	Buchanan
Control	Y_0 (log CFU)	0,631[a]	1,03[b]	0,528[a]
	λ (min)	-0,447[a]	0,892[b]	-20,366[c]
	Y_{max} (log CFU)	7,464[a]	7,399[a]	7,354[a]
	$\mu(min^{-1})$	0,037[a]	0,031[b]	0,029[b]
30 °C	Y_0 (log CFU)	0,963[a]	1,04[a]	1,04[a]
	λ (min)	-12,443[a]	8,163[b]	-123,642[c]
	Y_{max} (log CFU)	7,45[a]	7,353[a]	7,262[a]
	$\mu(min^{-1})$	0,025[a]	0,022[a]	0,021[a]
40 °C	Y_0 (log CFU)	0,46[a]	1,03[b]	-0,612[c]
	λ (min)	38,99[a]	23,6[b]	21,5[b]
	Y_{max} (log CFU)	7,509[a]	7,409[a]	7,445[a]
	$\mu(min^{-1})$	0,017[a]	0,015[a]	0,014[a]
50 °C	Y_0 (log CFU)	0,611[a]	1,03[b]	1,03[b]
	λ (min)	22,344[a]	31,509[b]	18,836[c]
	Y_{max} (log CFU)	7,506[a]	7,474[a]	7,438[a]
	$\mu(min^{-1})$	0,018[a]	0,016[a]	0,016[b]
60 °C	Y_0 (log CFU)	0,808[a]	1,052[b]	1,052[b]
	λ (min)	128,384[a]	125,58[b]	123,257[b]
	Y_{max} (log CFU)	7,528[a]	7,417[a]	7,54[a]
	$\mu(min^{-1})$	0,012[a]	0,012[a]	0,011[a]

It must be highlighted that the elucidation of growth parameters of nonspecific microbial groups is always a complicated task since microorganisms represent the sum of growth curves of a mix of different bacteria. The microorganisms reported herein likely belongs to aerobic mesophilic, whose growth has been used as indicators of the microbiological quality of foods.

Influence of Temperature: Secondary Modeling

Secondary models (polynomial equations) were constructed by plotting the μ values estimated from primary models versus temperature changes; therefore, three polynomial equations were built. It should be mentioned that μ values were transformed into natural logarithms (Ln) to provide a reasonably good fit for the relationship concerning μ and temperature. Figure 1 depicts the influence of temperature on μ values for the spoilage bacteria studied. As observed in Figure 1, an increase in temperature resulted in lower μ values for spoilage bacteria. However, Gompertz and Buchanan models gave negative values for λ and Y0 parameters, indicating a poor fit of these models, and accordingly, these parameters were excluded from the rest of the study.

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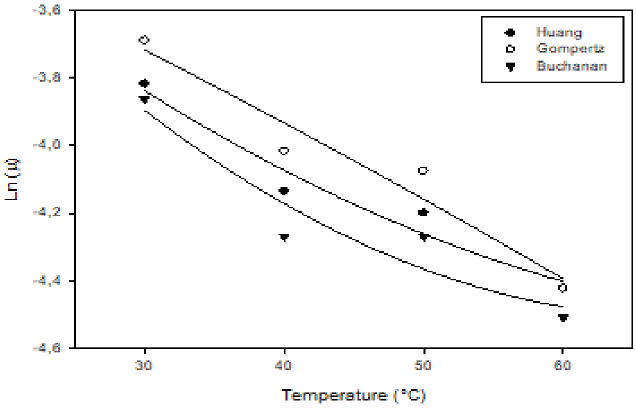

Figure 1. Effect of temperature on maximum growth rates obtained from primary models.

Three polynomial equations were chosen to describe the relationship between the natural logarithm of µ and temperature. All equations provided an excellent fit. The goodness of fit was very high ($R^2 > 0.93$) for all polynomial equations, as can be observed graphically in Figure 1. Likewise, to verify the accuracy of each polynomial equation, the experimentally determined values were compared with those predicted by the models.

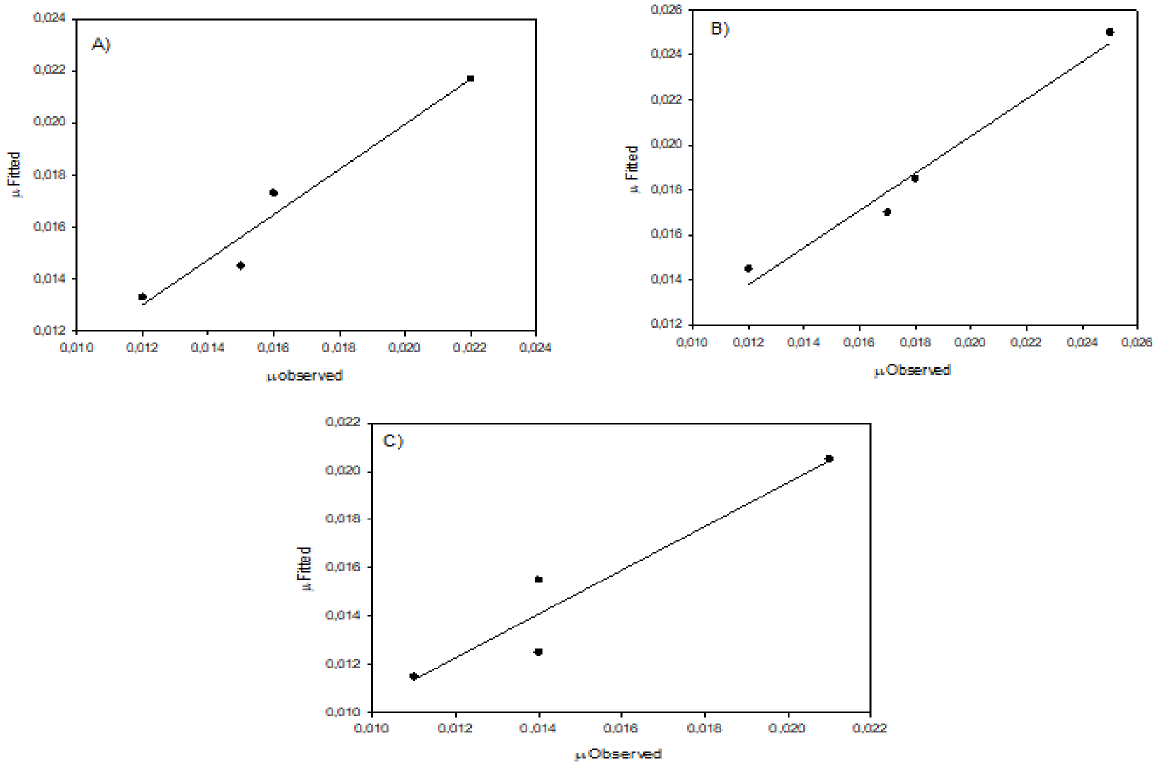

Figure 2. Observed and Fitted number of µ values employing polynomial equation

A: *ln (µ) = 8E-06x² - 0,001x + 0,0445;* ***B:*** *ln (µ) = 5E-06x² - 0,0008x + 0,0445, and* ***C:*** *ln (µ) = 1E-05x² - 0,0012x + 0,0475*

The results can be seen in Table 2, which includes the accuracy factor (Af), bias factor (Bf), and mean square error (MSE), which have been successfully used for polynomial model validations (López et al., 2006; González et al., 2020). Af represents the sum of absolute differences between predictions and observations, while Bf is a factor employed to determine if the model over- or under-predicts microbial growth (Chen et al., 2020). An ideal agreement should have values of one for Af and Bf, respectively, and MSE values close to zero.

Table 2. Mathematical validation of the secondary model to describe the behavior of spoilage bacteria grown on Costeño cheese

Polynomial model	Af	Bf	MSE
Huang ln (μ) = 8E-06x^2 - 0,001x + 0,0445	1,033	1,033	0,001
Gompertz ln (μ) = 5E-06x^2 - 0,0008x + 0,0445	1,055	1,055	0,001
Buchanan ln (μ) = 1E-05x^2 - 0,0012x + 0,0475	1,002	1,002	0,001

When μ data obtained from the Huang model were used to develop the polynomial model (ln(μ) = 8E-06x^2 - 0.001x + 0.0445), an Af value of 1.033, a Bf value of 1.033, and an MSE of 0.001 were calculated. Similar results were obtained when μ values were derived from the Gompertz model, with values of 1.055, 1.055, and 0.001 for Af, Bf, and MSE, respectively. When the polynomial equation (ln(μ) = 1E-05x^2 - 0.0012x + 0.047) was constructed from μ values calculated from the Buchanan model, the validation values were as follows: Af (1.002), Bf (1.002), and MSE (0.001). These results align with those reported by Slongo et al. (2009), who found similar values for MSE, Bf, and Af when studying lactic bacteria in pressurized and non-pressurized (control) vacuum-packaged ham stored at 8 °C.

A Bf factor of less than 1.0 indicates that the model is fail-safe. Therefore, the number of spoilage bacteria indicated in this work may be slightly overestimated due to their Bf values (>1). In accordance with the validation process, these equations had better MSE values than those reported by Antunes-Rohling et al., (2019), who used the Ratkowsky and Inverse Ratkowsky models to describe the relationship between μ, λ, and storage temperature. Similarly, Kalschne et al. (2014) published similar findings when modeling the behavior of lactic acid bacteria in ham containing nisin, with values of Af and Bf close to 1 using the modified Gompertz predictive model proposed by Zwietering et al. (1991) (Salakkam et al., 2023) However, their MSE values were higher than those reported in the current work. Chen et al., (2020) calculated Af values ranging from 1.4 to 4.0 for Listeria monocytogenes growing in various types of seafood.

In summary, the data presented in this work provide an insight into the evolution of spoilage bacteria in CC subjected to ultrasound at different temperatures. The polynomial equations developed here enable the prediction of spoilage bacteria in this dairy product when treated with ultrasound at different temperatures (ranging from 30 to 60°C), which can be valuable for the dairy industry.

CONCLUSIONS

This paper offers insights into the behavior of spoilage bacteria in Costeño cheese subjected to thermosonication. Temperature emerged as the most significant factor affecting the growth parameters derived from the primary models. The most rigorous treatment applied in this study involved ultrasonication at 40 kHz and 60°C, resulting in an increase in λ and a decrease in μ. These findings highlight the efficacy of ultrasound technology combined with heat treatment at 60°C for controlling spoilage microorganisms in Costeño cheese. Based on the data obtained, three polynomial equations were developed. Although the Gompertz and Buchanan models yielded negative values for λ and Y0, the μ values were employed to construct polynomial equations, facilitating the prediction of spoilage bacteria. Notably, these polynomial models exhibited bias and accuracy factors close to one, indicating their ability to accurately describe microbial growth in cheese. Further research is warranted to evaluate the impact of these treatments on the sensory, physical, and chemical properties of the cheese.

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