Thermal processing and acidification: an undergraduate laboratory exercise

Procesamiento térmico y acidificación: un ejercicio de laboratorio de grado

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Keywords

Academic laboratory; thermal processing; acidification; non-linear regression; modeling.

Abstract

Food engineering programs should provide students with practical activities related to the analysis of unit operations, within the context of food processing. Both effectiveness and efficiency should be evaluated, so students can recognize the importance of achieving an objective and quantifying resources employed to achieve the goal. We describe an undergraduate-level food engineering laboratory exercise divided in two sections. Both use carrot cylinders in vinegar solutions, packed in glass jars with metal lids, thermally processed in boiling water. In the first section (thermal processing) students collect time and temperature data during a heat penetration study. The experimental variable is the process time, and the response variable is the accumulated lethality (Tref = 93.3ºC, z = 8.9ºC). In the second section (acidification), students collect time and pH data during an acidification study. The experimental variables are the acetic acid concentration and the acidification time, and the response variable is the carrot cylinders' pH. In both sections, students perform basic statistical analyses, carrying out calculations related to non-linear regression, prediction intervals, inverse prediction, and model verification. This laboratory exercise can assist in illustrating concepts, techniques, measurements, calculations, model development, and statistical analyses related to thermal processing and acidification of food.

Palabras clave

Laboratorio universitario; proceso térmico; acidificación; regresión no-lineal; modelado.

Resumen

Los programas de ingeniería de alimentos deberían brindar a los estudiantes actividades prácticas relacionadas con el análisis de las operaciones unitarias, dentro de un contexto de procesamiento de alimentos. Se debe evaluar la eficacia y eficiencia de manera que los estudiantes puedan reconocer la importancia de lograr un objetivo y cuantificar los recursos empleados para lograr el objetivo. Describimos un ejercicio de laboratorio en ingeniería de alimentos a un nivel de grado dividido en dos secciones. Ambas utilizan cilindros de zanahorias en una solución de vinagre, envasados en frascos de vidrio con tapas metálicas, procesados térmicamente en agua en ebullición. En la primera sección (procesamiento térmico) los estudiantes toman los datos de tiempo y temperatura durante un estudio de penetración de calor. La variable experimental es el tiempo de proceso, y la variable respuesta es la letalidad acumulada (Tref = 93.3ºC, z = 8.9ºC). En la segunda sección (acidificación), los estudiantes toman los datos de tiempo y pH durante un estudio de acidificación. Las variables experimentales son la concentración de ácido acético y el tiempo de acidificación, y la variable respuesta es el pH de los cilindros de zanahoria. En ambas secciones, los estudiantes realizan análisis estadísticos básicos, determinando cálculos relacionados a regresiones no lineales, intervalos de predicción, predicción inversa, y verificación de modelos. Este ejercicio de laboratorio puede ayudar a ilustrar conceptos, técnicas, mediciones, cálculos, desarrollo de modelos y análisis estadísticos relacionados con procesamiento térmico y acidificación de alimentos.

Introduction

Legislation states that acid foods are foods that have a natural pH of 4.6 or below, and acidified foods are low-acid foods that have been treated with acid(s) or acid food(s) so as to attain a finished equilibrium pH of 4.6 or below [1],[2]. Some acidified products can be manufactured without heat treatments and are microbiologically stable indefinitely at ambient temperatures in unopened containers. The exceptional resistance to microbiological spoilage of these products is attributable to pH values usually below 4.0, typically high concentrations of acetic acid, and limited headspace and oxygen availability [3]. Nonetheless, most acid and acidified products require a thermal process in order to achieve commercial sterility. The severity of this heat treatment, however, is significantly less than necessary for processing low acid foods, because the product's pH will prevent the germination and growth of bacterial spores, and the thermal resistance of microorganisms will be reduced at lower pH [4]. Suggested F-values (Tref = 93.3ºC, z = 8.9ºC), which are dependent on the product's equilibrium pH (for foods with pH of less than 4.6), can be found in the literature [5]. These F-values can be used to design and evaluate a thermal process.

The manufacturing process of acidified foods must therefore include at least two critical control points: acidification and thermal process. The acidification step must be carried out to ensure that the product's established finished equilibrium pH is reached throughout the food in a timely manner. On the other hand, the thermal process must be properly delivered, so that the established lethality is achieved in the product's cold spot. All critical factors related to both unit operations must be accordingly identified, controlled, and properly recorded.

Several procedures for acidification are described in the literature and regulations, which can be used by food processors. Selection of specific techniques depends on the effects of process variables on pH reduction and sensory properties of foods. Variables that affect the acidification procedures can be categorized as dependent (such as tissue structure and pH variability) or independent from raw materials (such as concentration of acid solution, treatment time, and dimension and shape of food pieces) [6]. The processor must control acidification of foods, which should be monitored by measurements on both in-process samples and finished product [7]. Excessively lowering pH values (which could affect sensory and functional attributes of the foods) and long acidification times should be avoided.

The thermal process of acidified food aims to inactivate molds, yeasts, vegetative cells of bacteria, inactivate enzymes, and cook the product where necessary [4]. However, the thermal process must be properly designed and executed, so that excessive degradation of sensory attributes (such as texture and color) and nutritional and functional components is avoided. At the same time, the thermal process should be optimized in order to increase efficiency, in terms of reduction of energy consumption and process time.

The use of models in science can serve 3 goals: understanding (as a tool in applying the scientific method, which contributes to understanding changes occurring in the studied food), prediction (to quantify the future state of a food, based upon prior knowledge of the food and the applied process), and control (process conditions and food properties are set in order to achieve a desired outcome) [8]. Acidification and thermal processing are unit operations which can be used to illustrate the utility of models with regards to their goals. Therefore, we propose a 4 hour undergraduate-level laboratory exercise which aims to help illustrate concepts, techniques, measurements, calculations, model development, and statistical analyses related to thermal processing and acidification of food.

Methodology

Materials

Carrots were locally purchased and stored at 5ºC until used (less than 1 week). They were washed, peeled, and cut in 2 cm diameter and 8 cm length cylinders. 458 mL glass jars (height and diameter: 10 and 8 cm) provided with plastisol-lined metal lug caps (DISTINCOMER, Cartago, Costa Rica) were used. Vinegar solutions of varying concentrations (as dictated by the experimental setup) were prepared from a commercial white vinegar with 3% acidity expressed as acetic acid (MFS, ELIMAR Y NAZLEA, Alajuela, Costa Rica). Carrot cylinders in vinegar solutions, packed in glass jars, were thermally processed in a boiling water bath, using a Groen DN/TA-10 SP steam kettle (Dover Corporation, Groen Division, Elk Grove Village, IL, USA). Six 62 mm, needle type T thermocouples (Ecklund-Harrison Technologies Inc., Fort Myers, FL, USA), a CALPlex datalogger, and CALSoft 5 software (TechniCAL, Inc., New Orleans, LA, USA) were used for time and temperature measurements. The datalogger used in this study was calibrated by TechniCAL Inc. following internal standards. Thermocouples were assessed for correction needs using an ice-water bath (approximately 0°C) and boiling water (approximately 96°C) against calibrated thermometers to verify the accuracy of the temperature measurements and ensure proper instrument performance pH measurements were performed using an OAKTON pH 700 benchtop meter with temperature compensation, provided with an OAKTON 35811-71 electrode (Eutech Instruments Pte. Ltd., Ayer Rajah Crescent, Singapore). pH meter was calibrated using the manufacturer's procedure.

Experimental setup: thermal processing

Heat penetration studies were conducted. Each experimental run consisted of 5 glass jars. 9 carrot cylinders were weighed (225 ± 1 g) and placed in each jar. A 50% vinegar and 50% water solution (1.5% acetic acid) at 80ºC was added to each jar (160 ± 1 g), which were promptly capped. Beforehand, a needle thermocouple (connected to the datalogger) was fixed to each of the 5 jar's metal caps. The thermocouple was inserted through the center of the carrot cylinder located at the middle of the jar. The length of the thermocouple allowed the temperature to be measured at the geometric center of the middle carrot cylinder. This position was determined by preliminary trials to be the product's cold spot, which for heat penetration studies is the slowest heating food particle in the slowest heating location within the container [9]. All 5 jars were placed at the same time in boiling water in a steam kettle. The kettle's size and steam supply were enough that the water temperature apparently did not change (water was boiling before, during, and after immersion of jars). Each of the 5 jars was taken out of the boiling water at process times 6, 11, 16, 21, and 26 min, respectively. Process time 0 corresponds to the time when jars were immersed. Jars were left to passively cool at ambient temperature (25 ± 2 ºC) until they reached 60ºC. Temperature of each jar was recorded every 10 s. Recording started before jars were placed in boiling water and ended when jar temperature reached 60ºC. Process times were selected based on preliminary trials, so that their resulting accumulated lethalities ranged between approximately 1 and 20 min (Tref = 93.3ºC, z = 8.9ºC). Each experimental run was carried out 4 times (each corresponding to a replicate). Two model verification experiments were carried out. Each experiment consisted of independently processing 3 jars (replicates) at process times 16 or 24 min (both process times were randomly selected). Experimental conditions of verification experiments were equivalent to those previously described.

Experimental setup: acidification

Acidification studies were conducted. The same experimental setup described for the thermal processing section was followed, with the following changes: (1) each experimental run consisted of 6 glass jars; (2) experimental runs were carried out using 3 acetic acid concentrations: 0.75, 1.5 or 3%, which were prepared by mixing vinegar and water at varying proportions; (3) temperatures were not measured (thermocouples were not inserted in carrot cylinders); (4) all jars were taken out of the boiling water at process time 15 min. Immediately after thermal processing, one jar was opened, carrot cylinders were drained, weighed, and homogenized to a uniform paste with a maximum of 20 mL of distilled water per 100 g of product [1]. pH of the paste was determined at 25 ± 2 °C. The same procedure for assessing the product's pH was repeated on the remaining jars, but 30, 60, 90, 150, and 210 min after thermal processing. Each experimental run at each acetic acid concentration was carried out 3 times (each corresponding to a replicate). Three model verification experiments were carried out, one for each acetic acid concentration. Each experiment consisted of independently processing 3 jars (replicates) at process time 15 min and assessing the product's pH at 70 min 20 s, 55 min 50 s, and 48 min 30 s after thermal processing (times were randomly selected), for acetic acid concentrations 0.75, 1.5 and 3%, respectively. Experimental conditions of verification experiments were equivalent to those previously described.

Data analysis: thermal processing

From recorded time and temperature data, accumulated lethality (F) was calculated for each of the 5 evaluated process times based on equation 1, where t corresponds to time, T to temperature, T_{ref} to a reference temperature of 93.3°C, and z to a thermal resistance of 8.9°C [5]. Only temperatures equal to or above 60°C were used to calculate accumulated lethality [10].

$$F = \int_0^t 10^{\left(\frac{T-T_{ref}}{z}\right)} dt \qquad \text{Equation 1}$$

A 3-parameter exponential model (equation 2) was used to fit accumulated lethality (F) as a function of process time (t_p). Parameter a corresponds to the asymptote, b to the scale, and c to the growth rate.

$$F = a + be^{(c*t_p)} \qquad \text{Equation 2}$$

This model was selected using the Akaike Information Criterion corrected for small sample sizes (AICc). Among the candidate models evaluated, the model with the lowest AICc value was considered the best-supported model, as AICc balances goodness-of-fit against model complexity while accounting for small sample sizes. [11]. The model enables the calculation of accumulated lethality for any given process time and also allows determination of the required process time to achieve a specific accumulated lethality through inverse prediction (Equation 3).

$$t_p = \frac{\ln\left(\frac{F-a}{b}\right)}{c} \qquad \text{Equation 3}$$

Lower and upper prediction intervals (PI) were calculated for each process time evaluated. Once the point estimates were obtained, the same 3-parameter model previously used for the experimental data (Equation 2) was fitted in order to obtain equations for lower and upper prediction intervals within the evaluated time range.

For model verification experiments, accumulated lethality values were determined for each of the three jars at process times of 16 and 24 minutes to obtain the observed (experimental) data. The predicted values were calculated using Equation 2, substituting t_p with 16 or 24 minutes.

Data analysis: acidification

The previously described 3-parameter exponential model (Equation 2) was also used to fit the product's pH as a function of acidification time for each of the three acetic acid concentrations tested. This model allows not only the estimation of pH at any given acidification time but also the calculation of the acidification time required to reach a target pH value through inverse prediction. Additionally, lower and upper prediction intervals (PI) were calculated individually for the data obtained at each process time evaluated, and the same 3-parameter model (equation 2) was subsequently fitted in order to obtain lower and upper prediction intervals within the evaluated time range.

For model verification, pH values were measured for each of the three jars at acidification times of 70 min 20 s, 55 min 50 s, and 48 min 30 s, corresponding to acetic acid concentrations of 0.75%, 1.5%, and 3%, respectively. These measurements provided the experimental (observed) data, while predicted values were obtained from Equation 2 using the respective acidification times.

Statistical analyses and model fitting

Statistical analyses and model fitting were conducted using statistical software JMP 11 (SAS Institute, Cary, NC). Statistical tests were performed at 0.05 significance level.

Results and Discussion

Thermal processing

The data collected during the experiments enable undergraduate students to analyze the behavior of the product throughout the entire thermal process. Figure 1 illustrates heat penetration, variation of lethal rate, and lethality accumulation over the processing time. In Figure 1A, it can be observed that after the jars are removed from the boiling water (points 1 through 5 marked with arrows), the product temperature continues to increase for several minutes in most cases, leading to a continued increase in the accumulated lethality (Figure 1C), even after effectively ending the thermal process and during the cooling stage. This observation allows students to appreciate the significant influence of the passive cooling stage (conducted at ambient temperature) on the accumulated lethality and, consequently, on process effectiveness. In most cases, the cooling phase is not considered in lethality calculations and is instead treated as a security factor by the Process Authority. However, if cooling is not accelerated, issues such as the degradation of nutritional compounds or change of texture may occur [12].

Figure 1C shows that Jar 1, subjected to the shortest processing time, reached a total accumulated lethality value of less than 1 minute. Considering the product's pH, this lethality value may be insufficient to achieve commercial sterility, indicating that the applied thermal treatment was inadequate, given that for a pH of 4,1-4,2, a lethality of 2,5 min (Tref = 93.3ºC, z = 8.9ºC) is required [5]. Moreover, it can be observed that at the geometric center of the middle carrot cylinder, the maximum temperature attained was below 80 °C and this temperature was achieved only after the jar was removed from the boiling water. These results help students understand the underlying heat transfer mechanisms, particularly conduction within a solid food

matrix such as carrots [13]. The trends observed in Figure 1(A, B, C) were expected for every jar at different process time, where a long process time achieved higher values of temperature, lethal rate, and accumulated lethality [4].

It is important to remark that the pH of the product plays a critical role due to its direct effect on microbial inactivation. Low pH values decrease the thermal resistance of microorganisms because changes in intracellular pH can damage enzymes, proteins, and microbial DNA, leading to irreversible disruption of the cell membrane and thus reducing heat resistance [14]. In addition, spore-forming microorganisms such as Clostridium botulinum cannot germinate at pH values below 4.6, thereby limiting the possibility of producing the lethal toxin [15]. Therefore, thermal treatments must be designed according to the product's pH to ensure food safety and stability.

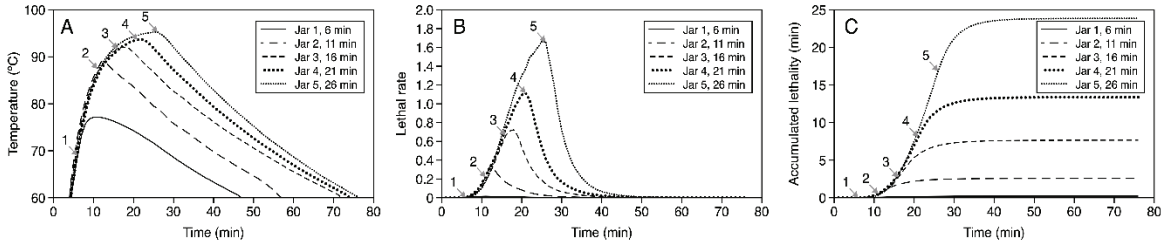


Figure 1. Temperature, lethal rate, and accumulated lethality as a function of process time.

The accumulated lethality as a function of total process time was modeled using the 3-parameter exponential model (Equation 2), as shown in Figure 2. Verification experiments are also included in this figure. Predicted values fall within the prediction intervals and importantly remain above the lower prediction interval.

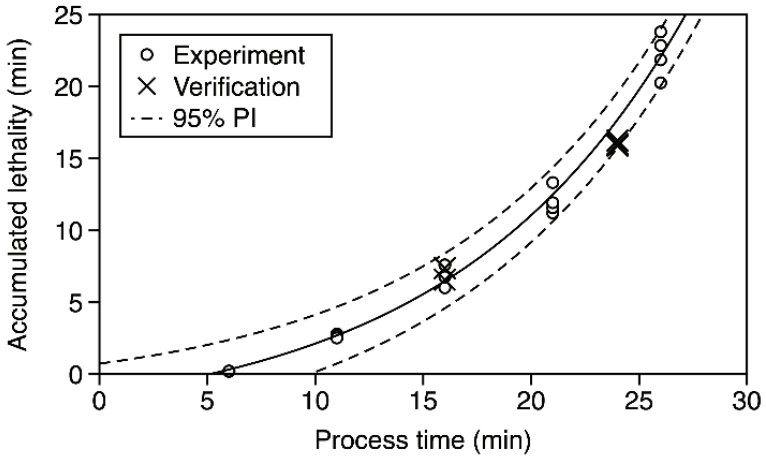


Figure 2. Accumulated lethality and values estimated from the validation experiments as a function of process time.

As expected, Figure 2 reveals that the accumulated lethality follows an exponential trend, indicating that small variations in process time can significantly impact the accumulated lethality.

Using the 3-parameter exponential model, students can predict the process time required to achieve a specific lethality value. For example, if a process must reach a lethality value of 6 minutes, students can use both the fitted model and the lower prediction interval (PI) equations (Table 1) to estimate the corresponding process time. In this case, the fitted model predicts a process time of 15.47 minutes, whereas the lower PI equation predicts 16.75 minutes.

Employing the lower prediction interval for inverse prediction acts as a security factor, since the accumulated lethality derived from this interval is lower than that obtained from the fitted model. Consequently, the process time estimated from the lower prediction interval is conservative, leading to an intentional overestimation of the thermal process. This approach also helps students understand and apply concepts such as nonlinear regression and inverse prediction in a practical, undergraduate laboratory context.

It is important to note that these equations must be developed for each specific food and thermal process, as the rate of heat penetration depends on the intrinsic characteristics of the food matrix and conditions of the process. Therefore, the corresponding equation can be established following the experimental procedure previously described.

Table 1. Fitted model and low predicted interval equations.

Model	Equation
Fitted model	$t = \frac{\ln((F + 3.67)/2.29)}{0.0931}$
Low PI	$t = \frac{\ln((F + 6.15)/3.00)}{0.0835}$

The values obtained during the model verification step showed error percentages of –9.9% and 9.3% for the 16 and 24 minutes processes, respectively, relative to the values predicted by the fitted model. The differences observed between the experimental lethality values and those predicted by the exponential model may be attributed to multiple factors that influence heat transfer in foods. These factors include the initial temperature of the product, the mass of both the product and the jar, the specific heat and thermal conductivity of the product, the geometry of the jar, the geometry of the carrot pieces, and the influence of natural convection during heating [16]. Although efforts were made to keep these variables constant, the geometry of the carrot pieces was the most difficult to control due to the manual handling required to shape each piece. This, in turn, directly affected the correct positioning of the thermocouple during each trial. Proper thermocouple placement within the carrot piece is essential for obtaining accurate heat penetration data, regardless of the processing temperature. Consequently, small variations in thermocouple position can significantly influence the magnitude of accumulated lethality for a given process.

Acidification

The second part of the experiment demonstrated the effects of acid concentration and process time [4]. These effects are illustrated in Figure 3, where, as expected, higher concentrations of acetic acid resulted in lower pH values. This behavior can be attributed to mass transfer phenomena, in which a greater number of acid molecules are available to diffuse from the acidulant into the carrot tissue. Other factors that could influence the acidification process such as temperature, mass ratio, and carrot cylinder dimensions [17], were carefully controlled to isolate the effect of acid concentration. Furthermore, the pH profiles presented in Figure 3 confirm the exponential trend over the acidification period, with the pH reaching a constant value after prolonged exposure of the solid food to the acidulant solution. This graphically illustrates the concept of equilibrium pH, which plays a critical role in food safety processes.

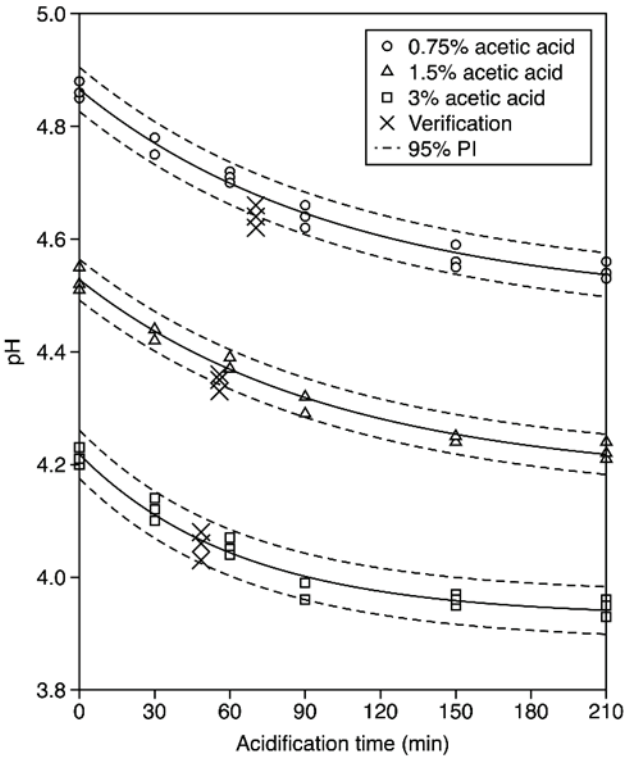


Figure 3. pH and values estimated from the validation experiments as a function of process time.

Following the same approach as described above, students can use the equations presented in Table 2 to predict different acidification times. For example, if the target pH is 4.3 at an acetic acid concentration of 1.5%, the fitted model predicts a process time of 41.9 minutes, while the high prediction interval yields 52.9 minutes. This outcome is desirable because a predicted value higher than that obtained from the fitted model provides an additional safety factor for processors. Although the difference of more than 10 minutes between both predictions could affect process efficiency, it is recommended to adopt the value from the upper prediction interval. This conservative approach ensures that the desired pH value is reached, preventing potential risks to product stability, if the food does not achieve the pH level for which the thermal process was designed. All corresponding equations for different acetic acid concentrations are presented in Table 2 .

Table 2. Fitted model and high predicted interval equations.

Model	Acid acetic (%)	Equation
Fitted model	0.75	$t = \frac{\ln(pH - 4.49)\ /0.38}{-0.0097}$
High PI		$t = \frac{\ln(pH - 4.54)\ /0.36}{-0.0011}$
Fitted model	1,5	$t = \frac{\ln(pH - 4.17)\ /0.35}{-0.01}$
High PI		$t = \frac{\ln(pH - 4.22)\ /0.34}{-0.012}$
Fitted model	3,0	$t = \frac{\ln(pH - 3.93)\ /0.29}{-0.016}$
High PI		$t = \frac{\ln(pH - 3.97)\ /0.29}{-0.018}$

The experimental values obtained in the model verification experiments showed an error percentage of 0.86, 0.46 and 0.24% for the concentrations of 0.75%, 1.5 % and 3% respectively, relative to the values predicted by the fitted model. The differences observed between the experimental data and the time values given by the fitted model are very similar. This result represents a great functionality of the model and a good experimental control of factors that can influence the acidification rate, such as the size of the pieces of carrots, the temperature of the acid solution, the moisture content of the food matrix and the stirring inside the jar [17].

The factors related to the food matrix and processing conditions are highly relevant, as they can significantly affect the acidification rate. For example, using a raw material with a high protein or lipid content may result in slower acidification rates, since proteins act as buffering agents and lipids can interfere with mass transfer because organic acids are generally distributed in an aqueous phase [18]. Nevertheless, other factors, such as food piece size, temperature, and even the level of agitation, may have an even greater influence on mass transfer phenomena. In the study conducted by Zeng et al. [19], acidified eggs treated with 2% acetic acid reached pH values below 4.0 after 1 hour at both 5 °C and 25 °C. These values are lower than those obtained in the present experiment after 1 hour, even though a higher acetic acid concentration (3%) was used with carrots as the food matrix. Similarly, in the experiment reported by Acosta et al. [17], pH measurements after 24 hours varied across different sections of the same food matrix (pickled eggs). These differences highlight that even small variations in the food matrix or experimental conditions can strongly affect the acidification rate and the resulting product pH. Therefore, specific experiments must be conducted for each product and process in order to ensure product stability.

By integrating both experiments and considering a hypothetical case in which a commercially sterile product with a target pH of 4.1 is desired using a 3% acetic acid concentration, students can apply the high prediction interval (PI) equation for 3% acetic acid (Table 2) to estimate the acidification time, obtaining a value of 44.57 minutes. Subsequently, they can use the lower

PI equation (Table 1) to calculate the process time required to achieve a lethality value of 2.5 minutes, which corresponds to 12.68 minutes [5]. In this way, students can design both the acidification and thermal processes to meet specific targets for the food matrix.

Conclusions

The integrated thermal processing and acidification experiments presented in this study provide undergraduate students with a comprehensive understanding of the fundamental principles governing heat transfer, microbial inactivation, and mass transfer in acidified foods. The thermal processing results highlight the relevance of passive cooling, the influence of heat penetration in solid foods, and the need to account for product pH when designing safe and effective thermal treatments. The application of a three-parameter exponential model, supported by prediction intervals, demonstrates how small variations in processing time and thermocouple positioning can significantly affect accumulated lethality, reinforcing the importance of precision in experimental procedures.

Similarly, the acidification experiments illustrate the impact of acid concentration on pH reduction kinetics, the exponential nature of pH decline, and the role of intrinsic and extrinsic factors in determining acidification rates. The fitted models and corresponding prediction intervals provide a practical framework for estimating process times required to reach target pH values, demonstrating how modeling tools can support decision making in food safety processes.

Finally, the integration of both experimental components enables students to design complete preservation strategies by combining acidification and thermal treatments for a specific food matrix. This approach not only strengthens their understanding of key concepts such as nonlinear regression, inverse prediction, accumulated lethality, and equilibrium pH, but also underscores the necessity of tailoring process parameters to the characteristics of each product. Overall, the laboratory experience provides an effective pedagogical tool for linking theoretical food science principles with practical applications in food safety and process design.

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Declaration on the Use of Artificial Intelligence (AI)

The authors declare that they used ChatGPT-4o for grammatical and stylistic editing. All AI-assisted results and information were thoroughly reviewed, verified, and validated by the authors, who assume full responsibility for the accuracy, coherence, and integrity of the published content.