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Modeling the effects of process conditions on the accumulated lethality values of thermally processed pickled carrots

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Shelf-stable pickled products are thermally processed to ensure safety and stability. Carrots packed in glass jars and processed in a boiling water bath were chosen to construct models to predict accumulated lethality values given process conditions and heating times. Mixed models with a natural logarithmic transformation of accumulated lethality as response showed that the effect of blanching prior to filling did not significantly impact the response ($P > 0.05$), while the effects of ln process time, jar size, carrots to brine ratio, carrot spear diameter, brine temperature, and concentration of sucrose in the brine and interactions among these variables significantly affected the response ($P < 0.001$), as evaluated in different experimental designs. The residual unexplained replicate-to-replicate variability of all constructed models was always <3% and every trial was conducted in triplicate. Process authorities can use these models to establish processing guidelines or evaluate current processes for production of shelf-stable pickled carrots or similar foods (as demonstrated by the validation experiments) with pH values from <3.9 to 4.4. This study also demonstrated that it is feasible to use this experimental setup to evaluate the impact of changes in processing conditions on the accumulated lethality values reached through thermal processing of similar foods. Overall, these results contribute to the establishment of science-based processing guidelines that will ensure production of safe and stable products with optimized heating times to enhance quality parameters.

1. Introduction

According to US federal regulations (CFR Title 21, Chapter 1, Subchapter B, Part 114), a pickled product is an acidified food (a low-acid food to which acid is added) whose water activity is above 0.85 and has a finished equilibrium pH of 4.6 or below (GPO, 2013). The low pH allows acid and acidified foods to receive only a mild thermal processing to achieve shelf-stability (Tucker & Featherstone, 2011, chap. 5), since it has long been accepted that spores of *Clostridium botulinum* do not germinate and grow (and consequently produce toxin) at or below pH 4.6. Therefore, shelf-stable foods of pH <4.6 are not required to be processed to inactivate *C. botulinum* spores (Anderson et al., 2011), but the thermal treatment is designed to kill the less heat resistant molds, yeasts, vegetative cells of bacteria, to inactivate enzymes and cook the product (if necessary) (Tucker & Featherstone, 2011, chap. 5).

Thermal processing guidelines for these food products are often available from trade organizations or research associations such as the Grocery Manufacturers Association (http://www.gmaonline.org/), Pickle Packers International (http://www.ilovepickles.org/) and Campden BRI (http://www.campdenbri.co.uk/). However, given the lower public health concerns associated with acid and acidified foods, and the less severe thermal processes applied to these products (when compared to low-acid foods), research regarding the effect of process conditions on the lethality values obtained during thermal processing of acid and acidified foods is limited. Furthermore, there has been commensurably less attention given to producing, collecting and organizing the microbial control literature applicable to acid and acidified foods (Pflug, 2003, chap. 17.A).

It should also be considered that while the thermal process must achieve the target lethality (that all the regions of the product are processed at a high enough temperature for a long enough time), the action of heat also destroys nutrients and affects the texture of the product. Hence these undesirable effects need to be minimized (Awuah, Ramaswamy, & Economides, 2007; Banga, Balsa-Canto,

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Moles, & Alonso, 2003) while simultaneously reducing energy consumption, time, and other valuable resources.

Mixed models will be used in this study to establish processing guidelines for production of shelf-stable pickled foods, as well as to evaluate current processing conditions of similar products. Carrots were selected as the model food based on their high relative firmness and the tissue's homogeneity when compared to similar vegetables, as well as the flexibility to achieve the targeted dimensions in the pieces. This study will use the measure of accumulated lethality (F value) as the response variable as it is the best indicator to assess the safety and stability of thermally processed, shelf-stable foods.

The purpose of this study was to evaluate and model the effects of several process conditions on the accumulated lethality values of thermally processed pickled vegetables —using carrots as a model food— as well as to recommend general processing guidelines for production of shelf-stable pickled foods, based on the results obtained from the evaluation of the process conditions.

2. Materials and methods

2.1. Experimental setup

Carrots were purchased from a local supermarket and were stored at 4 °C for two weeks or less. Before being thermally processed, they were warmed in running water (until the inner temperature reached 25 °C), washed and peeled. Carrots were cut according to the experimental design (varying diameter and length), and if required by the experiment, were blanched (immersed in boiling water for 5 min, cooled in an ice and water mix for 2 min, immersed in water until the inner temperature reached 25 °C, drained and towel-dried). Carrot spears were weighed and placed in 237 or 473 mL (8 or 16 fl oz) Mason jars (Jarden Home Brands, Daleville, IN) (height × inner diameter: 9.8 × 6.0 and 12.1 × 7.6 cm for the 237 and 473 mL jars, respectively). A brine consisting of 50% distilled water and 50% distilled white vinegar with 5% acidity (Wegmans Food Markets, Inc., Rochester, NY) was weighed and added to each jar. The ratio of solids to liquid and the temperature of the brine were dictated by the experimental design.

Needle, type T thermocouples (Ecklund-Harrison Technologies Inc., Fort Myers, FL) were fixed to the jars' metal lids, and inserted through the center of the carrot piece located in the middle of the jar. The depth of the temperature probe was dictated by the experimental design. In order to pre-heat the exterior of the glass jars, filled jars were placed in a wire basket (7 or 5 jars, if 237 or 473 mL jars were used, respectively) and placed in a water bath at 49 ± 1 °C for 1 min. Afterwards, the basket and jars were placed in boiling water in an electric kettle (model TDB/6-10, Groen, Jackson, MS) and the time it took for the water to return to a boil was recorded. The temperatures were recorded every 10 s using a CALPlex temperature logger and CALSoft32 thermal processing software (TechniCAL, Inc., New Orleans, LA) as well as a Fluke Hydra Series II data acquisition unit and Hydra Logger version 3.0 (Fluke Corporation, Everett, WA) for preliminary tests, while a CALPlex temperature logger and CALSoft5 thermal processing software (TechniCAL, Inc., New Orleans, LA) were used for the rest of the experiments. Jars were taken out of the kettle when their corresponding thermocouples measured temperatures of 60.0, 65.6, 71.1, 76.7, 82.2, 87.8 and 93.3 °C (140–200 °F at 10 °F intervals) for the 237 mL jars, and 60.0, 68.3, 76.7, 85.0, 93.3 °C (140–200 °F at 15 °F intervals) for the 473 mL jars and left to cool at room temperature (24 ± 2 °C) until temperatures inside the jars reached 60 °C. Times were recorded when individual jars were removed from the kettle.

2.2. Data analysis

For each experiment, mixed models were constructed using a natural logarithmic transformation of accumulated lethality as response. Based on time and temperature data, accumulated lethality (*F*) was calculated according to the following equation (Awuah et al., 2007):

$$F = \int_0^{t_f} 10^{\left(\frac{T - T_{ref}}{z}\right)} dt$$

where T corresponds to the temperature, T_{ref} to the reference temperature (93.3 °C), z to the thermal resistance (8.9 °C), and t to the time. Only temperatures above 60 °C were used for the calculation of the accumulated lethality values.

The fixed main effects tested were the natural logarithmic transformation of process time (corresponding to the time between the return of the water to a boil and the time when jars were removed from the kettle) and the process conditions, in addition to the interactions between the process conditions. A random effect of replicate nested within the process conditions was added. Every combination of processing conditions (trial) was replicated three times, each replicate corresponding to a separate trial consisting of 5 or 7 jars, depending on their size. The order of the trials was randomized within experiments. Significant terms of the model were selected through backwards elimination. Terms were considered significant at $P < 0.001$. Analyses were performed using the statistical software JMP Pro 9.0.2 (SAS Institute Inc., Cary, NC).

2.3. Preliminary experiments

The effects of five process conditions were evaluated: (a) jar size (237 or 473 mL), (b) carrots to brine ratio (65:35 or 35:65), (c) carrot spear diameter (17 or 20% length of spear), (d) brine temperature (25 or 75 °C) and (e) blanching or not blanching prior to filling. Table 1 shows the detailed experimental conditions for the combinations of the first three variables. The last two variables did not make the rest of the conditions differ, so more detail was deemed unnecessary. Thermocouples used during these experiments were selected according to preliminary coldspot-location trials, which determine experimentally the slowest heating location within the container. Being a

Table 1
Experimental conditions for the combinations of three variables evaluated during the pickled carrot thermal processing trials.

Jar size (mL)	Carrots to brine ratio	Carrot spear diameter (% length of spear)
237 (Length of carrot spears: 7 cm)	65:35 (130 g of carrots and 70 g of brine)	17 (Center piece: 9 g, rest of pieces between 5 and 10 g)
		20 (Center piece: 12 g, rest of pieces between 15 and 25 g)
	35:65 (70 g of carrots and 130 g of brine)	17 (Center piece: 9 g, rest of pieces between 5 and 10 g)
		20 (Center piece: 12 g, rest of pieces between 5 and 15 g)
473 (Length of carrot spears: 9 cm)	65:35 (270 g of carrots and 145 g of brine)	17 (Center piece: 19 g, rest of pieces between 10 and 20 g)
		20 (Center piece: 25 g, rest of pieces between 30 and 50 g)
	35:65 (145 g of carrots and 270 g of brine)	17 (Center piece: 19 g, rest of pieces between 10 and 20 g)
		20 (Center piece: 25 g, rest of pieces between 10 and 30 g)

non-homogeneous food, the slowest heating food particle in the slowest heating location would be considered to be the coldspot (IFTPS, 2014). Therefore, thermocouple length varied according to jar size and carrots to brine ratio: 54 mm (237 mL/65:35), 72 mm (237 mL/35:65) and 65 mm (473 mL/65:35).

Four independent experiments were run (Table 2), and three 2^3 factorial designs were constructed by combining experiments B, C and D with experiment A, respectively. Therefore, each one of the three factorials tested the effect of brine temperature and the effect of blanching or not blanching prior to filling, in addition to the effect of jar size (first factorial), carrots to brine ratio (second) and carrot spear diameter (third), and their interactions. In order to overcome the issue that parts of the three factorial designs were run at different moments, the effect of order of the trial within the experiment was tested. This was achieved by adding a fixed and continuous effect of order to the model, which was nested within the different process factors tested in each of the three factorials (jar size, carrots to brine ratio and carrot spear diameter). The reasoning behind this setup is that if the effect of order is not significant within experiments, the effect would also be negligible between experiments. Therefore, the approach of combining four experiments to create three factorial designs is valid.

2.4. Effect of process conditions

The effects of four process conditions were evaluated: (a) jar size (237 or 473 mL), (b) carrots to brine ratio (65:35 or 35:65), (c) carrot spear diameter (17 or 20% length of spear) and (d) brine temperature (25 or 75 °C). The experimental conditions were identical to those used for the preliminary tests (Table 1). Thermocouples used during this test were selected according to preliminary coldspot-location trials. Therefore, thermocouple length varied according to jar size and carrots to brine ratio: 54 mm (237 mL/65:35), 72 mm (237 mL/35:65), 65 mm (473 mL/65:35) and 81 mm (473 mL/35:65). A 2^4 factorial design was constructed from two separate experiments differing by carrots to brine ratio (65:35 versus 35:65). The issue of segmenting the full factorial in two experiments was approached similarly to the preliminary tests in that the model included a fixed and continuous effect of order of the trial within the experiment, which was nested within the carrots to brine ratio factor.

Table 2
Process conditions evaluated in the four preliminary experiments used to test their effects on the accumulated lethality values of thermally processed pickled carrots.

Experiment	Jar size (mL)	Carrots to brine ratio	Carrot spear diameter (% length of spear)	Brine temperature (°C)	Blanching or not prior to filling
A	237	65:35	20	25	Blanched
					Unblanched
				75	Blanched
					Unblanched
B	473	65:35	20	25	Blanched
					Unblanched
				75	Blanched
					Unblanched
C	237	35:65	20	25	Blanched
					Unblanched
				75	Blanched
					Unblanched
D	237	65:35	17	25	Blanched
					Unblanched
				75	Blanched
					Unblanched

2.5. Validation experiments

Four validation experiments were carried out. Each experiment consisted of 7 or 5 jars per trial (if 237 or 473 mL jars were used, respectively), and was replicated three times (three independent trials). The first two experiments correspond to the following process conditions: jar size 237 mL, carrots to brine ratio 65:35, carrot spear diameter 17% length of spear, brine temperature 25 °C, process time 7.5 min. They were carried out using carrots (experiment 1) and green beans (experiment 2). Green beans were previously blanched: immersed in boiling water for 5 min, cooled in an ice and water mix for 2 min, immersed in water at 25 °C for 2 min, drained and towel-dried. The other two experiments correspond to process conditions: jar size 473 mL, carrots to brine ratio 65:35, carrot spear diameter 20% length of spear, brine temperature 75 °C, process time 11 min. They were carried out using carrots (experiment 3) and cucumbers (experiment 4). All process conditions were randomly selected for the validation trials. The lengths and diameters of carrots, green beans and cucumbers correspond to those indicated in Table 1 (green beans and cucumbers were prepared as close as possible to the indicated conditions). Trials were run according to conditions mentioned in Section 2.1, with the exception that the wire basket and all jars were taken out of the kettle when the chosen process time was achieved. The natural logarithmic transformation of the accumulated lethality value achieved by each jar was calculated, which corresponds to the observed values. A model with the natural logarithmic transformation of the accumulated lethality value as the response and trial as a random effect assessed significant differences between trials within the same experiment.

The predicted values of the natural logarithmic transformation of the accumulated lethality values at the tested conditions of the validation experiments were obtained from the model built from the experiment described in Section 2.4, after the non-significant terms were eliminated. 95% confidence intervals were calculated for the observed and predicted values.

2.6. Effect of position of thermocouple

A 2^4 factorial design was constructed which tested the effects of: (a) jar size (237 or 473 mL), (b) carrot spear diameter (17 or 20% length of spear), (c) brine temperature (25 or 75 °C) and (d) position of thermocouple (coldspot or middle of the carrot piece —non coldspot—).

Carrots to brine ratio was fixed at 35:65, and the experimental conditions were identical to those used for the preliminary tests (Table 1). Thermocouples used during this test were selected according to preliminary coldspot-location trials, as well as according to the length of the carrot spears. Therefore, thermocouple length varied according to jar size and position of thermocouple: 54 mm (237 mL/middle of carrot), 72 mm (237 mL/coldspot), 65 mm (473 mL/middle of carrot) and 81 mm (473 mL/coldspot). The factorial design was constructed from two separate experiments differing by the position of the thermocouple (coldspot versus middle of the carrot piece). The issue of segmenting the full factorial in two experiments was approached in a way that was similar to the preliminary tests in that the model included a fixed and continuous effect of order of the trial within the experiment, which was nested within the position of thermocouple factor.

2.7. Effect of concentration of sucrose in the brine

The experimental conditions selected to test the effect of concentration of sucrose in the brine correspond to: jar size 237 mL, carrots to brine ratio 65:35, carrot spear diameter 17% length of spear and brine temperature 75 °C (details shown in Table 1). The thermocouples used during this test correspond to 54 mm, and were chosen according to preliminary coldspot-location trials. The effect of a natural logarithmic transformation of concentration of sucrose (expressed as °Brix) was added to the model as a continuous fixed effect, in addition to its interaction with the natural logarithmic transformation of process time. Tested concentrations of sucrose correspond to 20, 24, 30, 35, 40, 50 and 60 °Brix, which comprise typical concentrations used for pickling vegetables. However, it must be noted that since concentrations increased during the heating of the brine due to the loss of water, the values that were used in the model correspond to those that were measured immediately before placing the lids on the jars. °Brix were measured using a digital Abbe refractometer (Leica Inc., Buffalo, NY). The volume of the brine was kept constant at 70 mL and thus its mass increased with increasing concentration of sucrose. The random effect of replicate was not nested within any process condition.

3. Results and discussion

3.1. Preliminary experiments

The fixed and continuous effect of order of the trial within the experiment, nested within the different process factors tested in each of the three preliminary factorial designs of jar size, carrots to brine ratio and carrot spear diameter did not present a significant effect ($P = 0.9051$, 0.6820 and 0.9215, respectively). Therefore, the method of combining four experiments to create three factorial designs was deemed valid. Table 3 shows the results from the three preliminary models used to evaluate the effects of the five process conditions on the natural logarithmic transformation of accumulated lethality. Neither the main effect of blanching or not blanching prior to filling nor its interactions with the other factors had a significant effect on the response variable ($P > 0.001$). Although blanching can result in undesirable softening of vegetable tissues (Reyes de Corcuera, Cavalieri, & Powers, 2004) due to loss of turgor pressure and occluded air, thermal degradation of middle lamella pectins and other cell wall polysaccharides, and starch gelatinization (Stanley, Bourne, Stone, & Wismer, 1995), it has been reported that low-temperature blanching can actually improve texture and increase firmness of thermally processed vegetables such as carrots and green beans (Lin & Schyvens, 1995; Vu et al., 2004). There are numerous studies in the literature that have attempted to explain thermal texture degradation in carrots (Peng, Tang, Barrett, Sablani, & Powers, 2014; Smout, Sila, Vu, Van Loey, & Hendrickx, 2005). While no measurements of firmness or any other texture property of the vegetable pieces were conducted in this experiment, the non-significant effect of blanching or not blanching prior to filling on the accumulated lethality values indicated that textural properties of the raw and blanched carrots were similar enough to result in comparable heat transfer properties. Therefore, this process condition was not evaluated in the following trials, and carrots were left unblanched. Since the effects of jar size, carrots to brine ratio, carrot spear diameter and brine temperature significantly affected the response variable ($P < 0.001$), they were all included in the following experiment, as part of a full factorial design.

Table 3
Results from the models for natural logarithmic transformation of accumulated lethality, according to the three 2^3 preliminary factorial designs. Dummy variables coded 0 and 1 were used for nominal factors.

Factorial	Amount of residual variance explained by the model	Parameter estimates			
		Term	Estimate	Standard error	$P > \|t\|$
Jar size	98.82%	Intercept	−4.92	0.07	<0.0001
		ln process time	2.81	0.03	<0.0001
		Jar size (473 mL = 0)	−0.30	0.06	<0.0001
		Blanching (Unblanched = 0)	0.01	0.06	0.8583
		Brine temperature (25 °C = 0)	−0.23	0.06	0.0009
Carrots to brine ratio	98.08%	Intercept	−3.24	0.07	<0.0001
		ln process time	2.65	0.03	<0.0001
		Carrots to brine ratio (65:35 = 0)	−1.31	0.08	<0.0001
		Blanching (Unblanched = 0)	−0.11	0.05	0.0533
		Brine temperature (25 °C = 0)	−0.90	0.08	<0.0001
		Carrots to brine ratio*Brine temperature	0.6	0.1	<0.0001
Carrot spear diameter	98.82%	Intercept	−4.28	0.06	<0.0001
		ln process time	2.82	0.03	<0.0001
		Carrot spear diameter (20% = 0)	−0.61	0.05	<0.0001
		Blanching (Unblanched = 0)	0.02	0.05	0.7786
		Brine temperature (25 °C = 0)	−0.32	0.05	<0.0001

3.2. Effect of process conditions

The fixed and continuous effect of order of the trial within the experiment, nested within the carrots to brine ratio factor did not present a significant effect ($P = 0.5693$). As with the preliminary trials, the method of combining two experiments to create a 2^4 factorial design was deemed valid. Table 4 shows the results from the model that was used to evaluate the effects of four process conditions on the natural logarithmic transformation of accumulated lethality. The amount of residual variance explained by the model is 98.38%. As expected, longer processing times produced higher accumulated lethality values, following a power trend. Also, as expected, smaller jars (237 mL), lower carrots to brine ratios (35:65), smaller carrot spear diameters (17% length of spear), and higher brine temperatures (75 °C) all increased the measured accumulated lethality. The reasons behind these higher accumulated lethality values mainly correspond to differences in initial temperature of the product and jar (affected by the brine's

Table 4
Results from the model for natural logarithmic transformation of accumulated lethality, according to a 2^4 factorial design. Dummy variables coded 0 and 1 were used for nominal factors.

Term	Estimate	Standard error	$P > \|t\|$
Intercept	−4.99	0.06	<0.0001
ln process time	2.54	0.02	<0.0001
Jar size (237 mL = 0)	0.54	0.07	<0.0001
Carrots to brine ratio (35:65 = 0)	0.69	0.07	<0.0001
Jar size*Carrots to brine ratio	−0.38	0.08	<0.0001
Carrot spear diameter (17% = 0)	0.73	0.05	<0.0001
Jar size*Carrot spear diameter	−0.30	0.06	<0.0001
Carrots to brine ratio*Carrot spear diameter	−0.47	0.06	<0.0001
Brine temperature (75 °C = 0)	0.51	0.06	<0.0001
Jar size*Brine temperature	−0.32	0.09	0.0005
Carrots to brine ratio*Brine temperature	−0.44	0.09	<0.0001
Jar size*Carrots to brine ratio*Brine temperature	0.7	0.1	<0.0001

temperature); mass of the jar and product (affected by the jar's size); density, specific heat, and thermal conductivity of the product (affected by the carrots to brine ratio); radius and volume of the jar and carrot pieces (affected by both the jar's size and diameter of the carrot spears); and influence of the natural convection process of heat transfer (affected by the carrots to brine ratio). The effect of these properties on heat flow in thermally processed packaged foods has been described in the literature (Holdsworth & Simpson, 2007, chap. 2).

Table 5 shows the least squares means for the combinations of terms included in the three significant interactions from the model (one three-way and two two-way interactions). Least squares means are values predicted by the model for levels of a categorical effect where the other model factors are set to neutral values (the sample mean for ln process time, and the average of the coefficients for the nominal effects not involved in the corresponding interaction). Because least squares means are predictions at fixed (neutral) values of the other factors, comparisons are able to be made (SAS Institute Inc., 2014). A post hoc multiple comparison with a Tukey correction was used to compare the combinations in this study. Results are presented in Table 5. For example, in the case of the three-way interaction, the lowest lethality value (0.40 min) corresponds to the combination of big jars (473 mL), high carrots to brine ratios (65:35), and low brine temperatures (25 °C), while the highest lethality (1.04 min) corresponds to the combination of the opposite values of the nominal terms. In general, intermediate lethality values from other combinations of the terms included in this interaction are not significantly different from each other (Tukey's test, $P > 0.05$).

Table 5
Least squares means for the combinations of nominal factors included in the three significant interactions from the model for natural logarithmic transformation of accumulated lethality, according to a 2^4 factorial design.

Interaction	Least squares mean ± Standard error[a]
Jar size*Carrots to brine ratio*Brine temperature	
Jar size 237 mL/Carrots to brine ratio 35:65/ Brine temperature 75 °C	0.03 ± 0.04[A]
Jar size 237 mL/Carrots to brine ratio 65:35/ Brine temperature 75 °C	−0.33 ± 0.04[B]
Jar size 473 mL/Carrots to brine ratio 35:65/ Brine temperature 75 °C	−0.38 ± 0.04 [BC]
Jar size 473 mL/Carrots to brine ratio 65:35/ Brine temperature 75 °C	−0.40 ± 0.05 [BC]
Jar size 237 mL/Carrots to brine ratio 35:65/ Brine temperature 25 °C	−0.45 ± 0.04 [BC]
Jar size 473 mL/Carrots to brine ratio 35:65/ Brine temperature 25 °C	−0.45 ± 0.04 [BC]
Jar size 237 mL/Carrots to brine ratio 65:35/ Brine temperature 25 °C	−0.52 ± 0.04[C]
Jar size 473 mL/Carrots to brine ratio 65:35/ Brine temperature 25 °C	−0.91 ± 0.04[D]
Carrots to brine ratio*Carrot spear diameter	
Carrots to brine ratio 65:35/Carrot spear diameter 17%	−0.25 ± 0.03[A]
Carrots to brine ratio 35:65/Carrot spear diameter 17%	−0.26 ± 0.03 [AB]
Carrots to brine ratio 35:65/Carrot spear diameter 20%	−0.37 ± 0.03[B]
Carrots to brine ratio 65:35/Carrot spear diameter 20%	−0.83 ± 0.03[C]
Jar size*Carrot spear diameter	
Jar size 237 mL/Carrot spear diameter 17%	−0.22 ± 0.03[A]
Jar size 473 mL/Carrot spear diameter 17%	−0.29 ± 0.03[A]
Jar size 237 mL/Carrot spear diameter 20%	−0.41 ± 0.03[B]
Jar size 473 mL/Carrot spear diameter 20%	−0.78 ± 0.03[C]

[a] Values in the same interaction section not sharing a common superscript letter represent significantly different values ($P < 0.05$) based on post hoc multiple comparisons with a Tukey correction.

These results can be used by food processors and process authorities to establish processing guidelines for production of shelf-stable pickled foods with pH values from <3.9 to 4.4, corresponding to accumulated lethality values between 0.1 and 10 min (reference temperature 93.3 °C, thermal resistance 8.9 °C) (Pflug, 2003, chap. 17.A). As an example, carrots packed in brine with an equilibrium pH between 4.2 and 4.3 would require a $F_{93.3\,°C}$ (z of 8.9 °C) of 5 min (Pflug, 2003, chap. 17.A). Therefore, if such product is packed in 237 mL jars, with a 65:35 carrots to brine ratio, the carrot spear diameter is 20% the length of the spear, and the brine's temperature is 75 °C, it should be processed in a water bath for at least 9.2 min (solving the equation below from the model shown in Table 4, using inverse prediction to determine ln process time).

$$\begin{aligned} \ln 5 = & -4.99 + 2.54(\ln \textit{Process Time}) + 0.54(0) + 0.69(1) \\ & - 0.38(0\times 1) + 0.73(1) - 0.30(0\times 1) - 0.47(1\times 1) \\ & + 0.51(0) - 0.32(0\times 0) - 0.44(1\times 0) + 0.7(0\times 1\times 0) \end{aligned}$$

The model can also be used to evaluate current processing conditions of similar products. Although results are limited to carrots and to the tested ranges of levels of process conditions, the model could be applied to vegetables with similar physical properties within the tested ranges of levels. Alternatively, the relatively simple methodology of this experiment as well as the low amount of residual variance unexplained by the model show that this methodology is valid and can be used in the future to further evaluate the impact of various processing conditions on the accumulated lethality values achieved during thermal processing.

3.3. Validation experiments

For validation experiments carried out with carrots and cucumbers, 0% of the total variance was explained by differences among trials. For the experiment using green beans, differences among trials explained 23% of the total variance. Nonetheless, no significant differences were found between trials ($P > 0.001$), and individual measured values of natural logarithmic transformation of accumulated lethality were averaged. Fig. 1 presents results from the four validation experiments that were conducted, including the predicted and observed accumulated

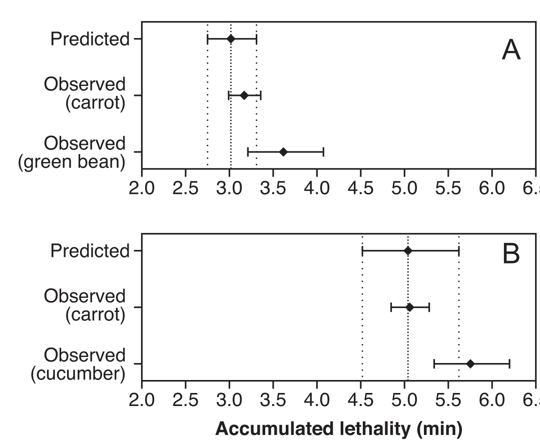

Fig. 1. Average accumulated lethality values estimated from the validation experiments. Error bars represent 95% confidence intervals ($n = 3$). Process conditions (jar size, carrots to brine ratio, carrot spear diameter, brine temperature, process time): (A) 237 mL, 65:35, 17% length of spear, 25 °C, 7.5 min (B) 473 mL, 65:35, 20% length of spear, 75 °C, 11 min.

lethality values (averages from three trials) as well as their respective 95% confidence intervals. Overall, the model seems to under predict the accumulated lethality values, but the measured observed values in carrots generally agree with the respective predicted values (averages from observed values fall within the predicted values' 95% confidence intervals). From the food safety and stability standpoint, this under-prediction is preferable compared to an over-prediction. Results showed larger differences between observed and predicted accumulated lethality values in green beans and cucumbers. This is likely caused by structural differences of the vegetable pieces. The effect is more marked in green beans, which in turn are a more heterogeneous product, hence the wider confidence interval shown in Fig. 1A. Also, the larger disparities of green beans and cucumbers can be due to differences in their thermal properties when compared to carrot pieces (mainly density, specific heat, and thermal conductivity of the product).

3.4. Effect of position of thermocouple

The fixed and continuous effect of order of the trial within the experiment, nested within the position of thermocouple factor did not present a significant effect ($P = 0.7646$). Table 6 shows the results from the model used to evaluate the effect of the position of the temperature probe in the carrot piece located in the middle of the jar, as well as three other process conditions on the natural logarithmic transformation of accumulated lethality. The amount of residual variance explained by the model is 98.42%. The carrots to brine ratio of 35:65 was fixed in this test so that there was a higher amount of liquid in the jars and therefore the effect of natural convection during heat transfer increased. It was expected that trials with thermocouples positioned in the coldspot of the container (lower section of the carrot spear) would heat slower than those with the thermocouple in the middle of the carrot piece. Thus it was anticipated that the coldspot trials would present overall lower lethalities than non-coldspot trials. Analysis of the model's coefficient for the effect of position of thermocouple (Table 6) confirmed this, showing a significant difference in accumulated lethality of 1.6 min when coldspot trials were compared to non-coldspot trials.

In order to evaluate the relative relevance of the four main effects included in this experimental design, likelihood ratios were used to compare reduced or restricted models with the full or unrestricted model. Four reduced models were tested, each without one of the four main effects and their corresponding interactions. The full model corresponds to the one shown in Table 6, which includes all significant terms (main effects of jar size, carrot spear diameter, brine temperature, and position of thermocouple, and their significant interactions).

When compared to the full model, the likelihood ratios showed that the model without the effect of the thermocouple position had a worse fit than the models without the effects of jar size, brine temperature, and carrot spear diameter. Furthermore, the least squares means were analyzed to assess the impact of the four main effects or the combination of effects in interactions on the response variable. In terms of difference in values of natural logarithmic transformation of accumulated lethality, the overall difference between coldspot trials and non-coldspot trials was higher than any other comparison of main effects or combination of factors within interactions. The only exception was within two combinations: smaller jars (237 mL), higher brine temperatures (75 °C) and any carrot spear diameter (17 or 20% length of spear), compared to larger jars (473 mL), lower brine temperatures (25 °C) and larger carrot spear diameters (20% length of spear).

These results show that even in low-temperature processes applied to pickled foods, the correct position of the temperature probe for data collection is vital for accurate measurements (Larousse & Brown, 1997, chap. 12). Furthermore, even small variations on its location can significantly affect the magnitude of the accumulated lethality values collected. This factor alone could have a larger impact than the variation of other process conditions.

3.5. Effect of concentration of sucrose in the brine

Table 7 shows the results from evaluating the effect of a natural logarithmic transformation of concentration of sucrose in the brine (expressed as °Brix) on the natural logarithmic transformation of accumulated lethality. The amount of residual variance explained by the final model is 97.61%. As in the previous experiments, the effect of process time on the resulting accumulated lethality follows a power trend. At the average ln sucrose concentration, longer processing times produce higher accumulated lethality values and the positive trend becomes stronger with an increase in ln sucrose concentration, as indicated by the interaction term. Inversely, the effect of concentration of sucrose in the brine on the resulting accumulated lethality also followed a power trend. However, this time higher sucrose concentrations produced lower accumulated lethality values at the average ln process time, and the interaction term indicates that this negative trend diminishes with an increase in ln process time. The observed effect of the concentration of sucrose on the accumulated lethality values is most likely due to changes in the mass of the product. The volume of the brine was kept constant at 70 mL, so higher concentrations of sucrose cause higher mass in the jars. Also, the addition of sucrose to the brine leads to variations in brine density, viscosity, thermal conductivity, and specific heat, which can affect the natural convection process of heat transfer (density variations due to changes in temperature being the driving force for the liquid motion) (Datta & Teixeira, 1988; Earle, 2004). Although natural convection tends to push the slowest heating region or coldspot to the bottom of the container (Ghani, Farid, Chen, & Richards, 1999), varying concentrations of

Table 6
Results from the model for natural logarithmic transformation of accumulated lethality, according to a 2^4 factorial design which includes the effect of position of thermocouple. Dummy variables coded 0 and 1 were used for nominal factors.

Term	Estimate	Standard error	$P > \|t\|$
Intercept	−4.26	0.06	<0.0001
ln process time	2.44	0.02	<0.0001
Jar size (237 mL = 0)	0.43	0.06	<0.0001
Position of thermocouple (middle of the carrot piece = 0)	0.49	0.03	<0.0001
Carrot spear diameter (17% = 0)	0.38	0.06	<0.0001
Jar size*Carrot spear diameter	−0.55	0.08	<0.0001
Brine temperature (75 °C = 0)	0.26	0.06	<0.0001
Jar size*Brine temperature	−0.03	0.08	0.6938
Carrot spear diameter*Brine temperature	−0.29	0.09	0.0014
Jar size*Carrot spear diameter*Brine temperature	0.5	0.1	0.0001

Table 7
Results from the model used to test the effect of concentration of sucrose (20–60 °Brix) in the brine on the natural logarithmic transformation of accumulated lethality. Both ln process time and ln sucrose concentration (°Brix) were centered.

Term	Estimate	Standard error	$P > \|t\|$
Intercept	5.6	0.3	<0.0001
ln process time	2.66	0.04	<0.0001
ln °Brix	−3.35	0.09	<0.0001
(ln process time − 2.31)*(ln °Brix − 3.60)	1.2	0.1	<0.0001

sucrose in the brine could possibly affect the location of the coldspot. Preliminary trials confirmed that the coldspot remained in the same lower location in the container, regardless of the sucrose concentration.

4. Conclusions

This study demonstrated that it is feasible to use mixed models to evaluate and predict the effects of process conditions (jar size, carrots to brine ratio, carrot spear diameter, brine temperature, blanching or not blanching prior to filling, and concentration of sucrose in the brine) on the accumulated lethality values of thermally processed pickled carrots. It is expected that this experimental setup can be applied to further evaluate the impact of variations in processing conditions on the accumulated lethality values reached through thermal processing of similar foods. Food processors and process authorities can use the results obtained from the models to establish processing guidelines and evaluate current processes for production of shelf-stable pickled foods. As demonstrated through validation trials, this can be applied to carrots or other similar products with pH values from <3.9 to 4.4. It was confirmed that the correct position of the temperature probe for data collection is essential for precise measurements, even in low-temperature processes such as those applied to pickled vegetables.

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