

## Application of Box-Behnken design with response surface methodology in the optimization of thermally processed “*lechon paksiw de leyte*”

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### Abstract

*Lechon* is a widely known delicacy that is always present in a Filipino home at every event and celebration. The Tacloban City Litson Industry Association (TACLIYA) is a group of micro-entrepreneurs producing “*lechon*” (roasted pig), that has an average daily excess production of 30 kg and surges up to 200 kg during off-peak season. Retort technology was employed in an optimization study using the box-Behnken design of the response surface methodology. Response surface regression results revealed that the variables and levels used had linear and quadratic effects on the sensory parameters evaluated which produced significant differences in the acceptability ratings. The optimum combination was obtained with the highest possible acceptability as the boundary at  $\geq 6.5$  on the 9-point Hedonic scale. The optimum product was commercially sterile with a *F<sub>o</sub>* value of 5 mins and an estimated shelf life of 1.5 years.

## 1. Introduction

The term *Lechon* is Spanish of origin and can be translated to “pig” in English. However, it means more than that in the Philippine context. Eckhardt (2007) pointed out that “*lechon*” refers to the cooking technique; cows and goats are also spit-roasted, but pig is so ubiquitous that the term has come to imply pork by default”, therefore, equating the term to the dish itself. The dish can be described as a slow-roasted whole pig on a bamboo spit characterized by its tender and juicy meat while having crispy caramelized orange-brown skin (Tee, 2023). It can be readily observed that *lechon* is of great significance to the Filipino culture as evidenced by its wide popularity in the local food scene, especially during huge occasions. Naturally, this continuous demand led to more vendors opening up shops along the road for more visibility all year long. As previously surveyed, in Leyte alone, there are hundreds of *lechon* vendors, and even more numerous in the entire Philippines.

Due to the ever-soaring prices of the commodity, selling in retail has become a usual practice among vendors like the group of micro-entrepreneurs or “*lechoneros*” who are collectively known as the Tacloban City Litson Industry Association (TACLIYA). A surplus of around 30 to 200 kg is reached per day leading to wasted meat. In addition, the time and

temperature abuse of this type may pose a great risk to the quality and more importantly, the safety of the unsold *lechon*.

In this study, a reworking technique using thermal processing and acidification is being focused on to valorize surplus meat from retailed *lechon*. Shelf-life extension is achieved by rendering the product commercially sterile or free of any viable microorganism and enzyme activity during storage (Lee, 2017). Retort technology has been used in making shelf-stable products such as Chettinad style goat meat curry (Rajkumar *et al.*, 2010), buffalo meat blocks (Devadason *et al.*, 2014), ready-to-eat pork curry (Girish *et al.*, 2018), ready-to-eat pepper chicken (Nalini *et al.*, 2018), and marinated Samgyetang (Kim *et al.*, 2020).

A Box-Behnken design (BBD) following the response surface methodology (RSM) was utilized in the optimization of “*Lechon Paksiw de Leyte*.” RSM is a mathematical and statistical method widely used to model and analyze various processes where the desired response is influenced by various variables and the goal is to optimize the response. BBD was used as a widely used design to establish cause-and-effect relationships between factors and responses without highest and lowest-level combinations and is not necessary to run trials in extreme conditions (Szpisják-Gulyás *et al.*, 2023).

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## 2. Materials and methods

### 2.1 Raw materials and product preparation

The delivered unsold 'lechon' samples from different association members were primarily washed with 2% lactic acid to remove surface microbial load, cut to desired sizes, and portioned to 202 × 306 2-piece tin cans and retortable pouches at 70±5 g and 50±5 g, respectively. The sauce was then hot-filled, sealed, and thermally processed in a Vertical Water Immersion Retort.

### 2.2 Box-Behnken experimental design

To optimize the product formulation, a Box-Behnken Experimental Design with three factors and three levels presented in Table 1 was employed with a total of 15 treatments for experimental combinations (Montgomery, 2017), in determining its effects on sensory acceptability (appearance, aroma, taste, texture, and general) and the product pH. Variables were identified from the preliminary experiments of Patindol et al. (2022).

A second-order model of full quadratic polynomial equations (Equation 1) was used in predicting the effects of the factors and their interactions on the different responses (Verma and Kumar, 2019; Tan et al., 2012; Machado et al., 2020; Alhajabdalla et al., 2021; Song et al., 2021; Thuy et al., 2023):

$$Y = C_0 + C_1X_1 + C_{11}X_1^2 + C_2X_2 + C_{22}X_2^2 + C_3X_3 + C_{33}X_3^2 + C_{12}X_1X_2 + C_{13}X_1X_3 + C_{23}X_2X_3 \quad (1)$$

where Y is the response;  $C_0$  is the intercept of the model;  $X_1$ ,  $X_2$ ,  $X_3$  are the independent variables with coded values (-1, 0, +1); while  $(C_1, C_2, C_3)$ ,  $(C_{11}, C_{22}, C_{33})$ , and  $(C_{12}, C_{13}, C_{23})$  are regression coefficients representing linear, quadratic, and interaction effects, respectively.

### 2.3 pH determination

The equilibrium pH of the different treatments was

determined using a calibrated bench-top pH meter (Thermo Scientific™ Orion Star™ A112 and/or Hanna Instruments HI5521). Calibration was performed using two buffer solutions and a minimum slope of 92%. Three readings were taken and the mean of the three values was recorded as the pH of the product.

### 2.4 Commercial sterility testing

Commercial Sterility Testing was conducted based on Bacteriological Analytical Manual (BAM) for low-acid foods (Landry et al., 2001). Samples were first incubated at 30°C for ten days, then undiluted 1 – 2 g of each sample were inoculated to prepared Cooked Meat Medium and Bromcresol Purple Dextrose Broth and incubated at 35°C and 55°C with positive and negative controls.

### 2.5 Hedonic analysis

The different commercially sterile treatments were subjected to sensory evaluation using a 9-point Hedonic scale labeled from "dislike extremely" to "like extremely" (Fu and Labuza, 1997). An Incomplete Block Design (IBD) Type I set plan of  $t = 15$ ,  $k = 3$ ,  $r = 7$ ,  $b = 35$ ,  $\lambda = 1$ ,  $E = 0.71$ , as laid out by Cochran and Cox (1957) was used during the presentation of the treatments. A total of seven observations per treatment with two runs from 35 recruited, screened, selected, and trained panelists using the methods of ISO 8586-1:1993 and ISO 22935-1 IDF99-1:2009.

### 2.6 Validation of the optimum formulation

After determining the optimum levels of the variables, another trial was run to validate the optimized formulation using a 9-point Hedonic test comparing the observed and predicted sensory acceptability using a student t-test at  $p \leq 0.05$ .

Table 1. Variables used in Box-Behnken design for the optimization of parameters.

Variables	Level		
	(-1) Low	Central (0)	High (+1)
Independent Variables			
Sugar, $X_1$ (g)	75	150	300
Vinegar, $X_2$ (g)	100	200	300
Meat Size, $X_3$ (cm)	1	2	3
Independent Variables			
Appearance Acceptability, $Y_1$			
Aroma Acceptability, $Y_2$			
Taste Acceptability, $Y_3$			
Texture Acceptability, $Y_4$			
General Acceptability, $Y_5$			
pH, $Y_6$			

## 2.7 Data analysis

The response surface regression (RSREG) analysis was processed using StatSoft STATISTICA 6.0 to obtain the regression coefficients, contour and surface plots, and model equations. In addition, the fitness polynomial model equations were evaluated through coefficients of determination ( $R^2$ ).

## 2.8 Thermal validation

The heat distribution trials were done using “dummy products” in starch solution. A heat penetration test, on the other hand, was conducted at 121.1°C and 116°C as the reference and processing temperatures, respectively. Since the product was low-acid, the target microorganism was *Clostridium botulinum* with a death rate or z-value of 10°C, the process calculation used was  $F_0 = 5.0$  mins, using Ball’s Formula Method using the Valsuite Pro ver. 6.2.2.0 software (Ellab A/S, Trollesmindealle 25, DK-3400 Hillerød, Denmark).

## 2.9 Accelerated shelf-life testing

Accelerated Shelf-Life Testing (ASLT) employed the methods of Calligaris *et al.* (2019)

was conducted using test temperatures of 35°C, 45°C, and 55°C. The target longevity was one (1) year for the product in cans and six months in pouch. Evaluations or tests performed were pH determination, commercial sterility testing, and sensory evaluation employing a focus group discussion (FGD) on perceptible changes in the products through time.

A mathematical model using the Arrhenius equation, allowed the estimation of the shelf-life of the products at 30°C using first-order reaction kinetics at elevated conditions, plotted by reporting the changes of the natural logarithm of pH (ln pH) as a function of the storage time ( $1/T - 1/T_{ref}$ , measured in K).

$$k = k_{ref} \exp \left[ -\frac{E_a}{R} \left( \frac{1}{T^*} - \frac{1}{T_{ref}} \right) \right] \quad (2)$$

Where  $k_{ref}$  and  $E_a$  were substituted to the corresponding estimates and  $T^*$  was the temperature at which to predict shelf-life at 30°C using the formula:

$$SL = \frac{\ln I_0 - I_{lim}}{k_{ref} \exp \left[ -\frac{E_a}{R} \left( \frac{1}{T^*} - \frac{1}{T_{ref}} \right) \right]} \quad (3)$$

Where  $I_0$  was the experimental value of  $I$  at time zero (0) or the initial pH of the product, and  $I_{lim}$  was the limit of the pH value for low-acid food, which is 4.6.

## 3. Results and discussion

### 3.1 Product pH and commercial sterility

The pH values ranged from 4.63 - 5.31 with a mean pH value of 4.91, indicating that the samples are Low-Acid foods with the target microorganism *Clostridium botulinum*. Moreover, no observed bubbling or gas production was exhibited with the anaerobic tubes at different incubation temperatures and no color change and growth were observed with aerobic tubes; indicating commercial sterility of the products produced.

### 3.2 Effect of the variables on sensory acceptability and pH

In terms of the significant influences of the variables (Tables 2 and 3), Meat Size was with high significance at  $p < 0.001$  except the texture acceptability. As expected, sugar was significant for the taste response. However, with the regression coefficient results, the appearance of the meat size variable was the only significant with a positive linear coefficient, suggesting a bigger size of meat is preferred. Other attributes were not significant even though their ANOVA results were, indicating a joint effect of variables. Upon looking at their linear interactions, texture, and general acceptability responses were significant at  $p < 0.05$  for ANOVA and parameter estimates for the linear interaction of vinegar and meat size. This shows the high interaction of these variables, which can be more depicted with their 3D response surface plots in Figure 1.

With regards to the effect of the variables on the product pH, all of which significantly influenced its acidity as also gleaned in their linear negative coefficients indicating decreased pH values as the levels of the variables are increased. The acetic acid had a direct effect on the acidity of the product, as also stated in the study of Jones *et al.* (2016) where the addition of vinegar significantly decreased the pH of *Biltong*, which is a traditional salted/dried meat product in South Africa. Increasing meat size increases meat water holding capacity (WHC), which is affected as pH approaches the isoelectric point, thereby affecting meat quality and

Table 2. Summary of the ANOVA F ratios for all sensory parameters.

Regression	Appearance	Aroma	Taste	Texture	General	pH
(1) Sugar L+Q	0.089	0.791	4.802**	1.241	1.366	149.957***
(2) Vinegar L+Q	0.334	0.885	0.837	0.150	0.291	519.186***
(3) Meat Size L+Q	3.213*	5.904**	6.920**	1.254	6.430**	14.845***
Error	518.98	368.00	457.39	443.73	367.31	0.8119
Total SS	537.53	401.28	514.02	466.20	396.49	6.2044

\*significant at  $P < 0.05$ , \*\*significant at  $P < 0.01$ , \*\*\*significant at  $P < 0.001$ .

Table 3. Summary of the regression coefficients for all sensory parameters.

Regression	Appearance	Aroma	Taste	Texture	General	pH
Intercept	3.650846	3.218045	3.598997	5.757519	3.065006	6.149759
(1) Sugar L	-0.000715	0.002306	0.011830	-0.012769	0.008144	-0.004251**
Sugar Q	0.000003	-0.000006	-0.000007	0.000029	-0.000012	0.000008**
(2) Vinegar L	0.007299	0.014474	0.002782	0.003703	0.010796	-0.005627**
Vinegar Q	-0.000011	-0.000025	-0.000006	0.000011	-0.000012	0.000009**
(3) Meat Size L	2.410401*	1.550752	1.077068	1.483083	1.573935	-0.184035**
Meat Size Q	-0.360119	-0.142857	-0.044643	-0.107143	-0.029762	0.040417**
1L by 2L	0.000009	0.000006	-0.000008	0.000005	0.000006	0.000000
1L by 3L	-0.000827	0.000175	-0.001779	0.000100	-0.001479	0.000147*
2L by 3L	-0.002679	-0.002857	-0.000357	-0.004286*	-0.003750*	0.000075

\*significant at  $P < 0.05$ , \*\*significant at  $P < 0.01$ , \*\*\*significant at  $P < 0.001$ .

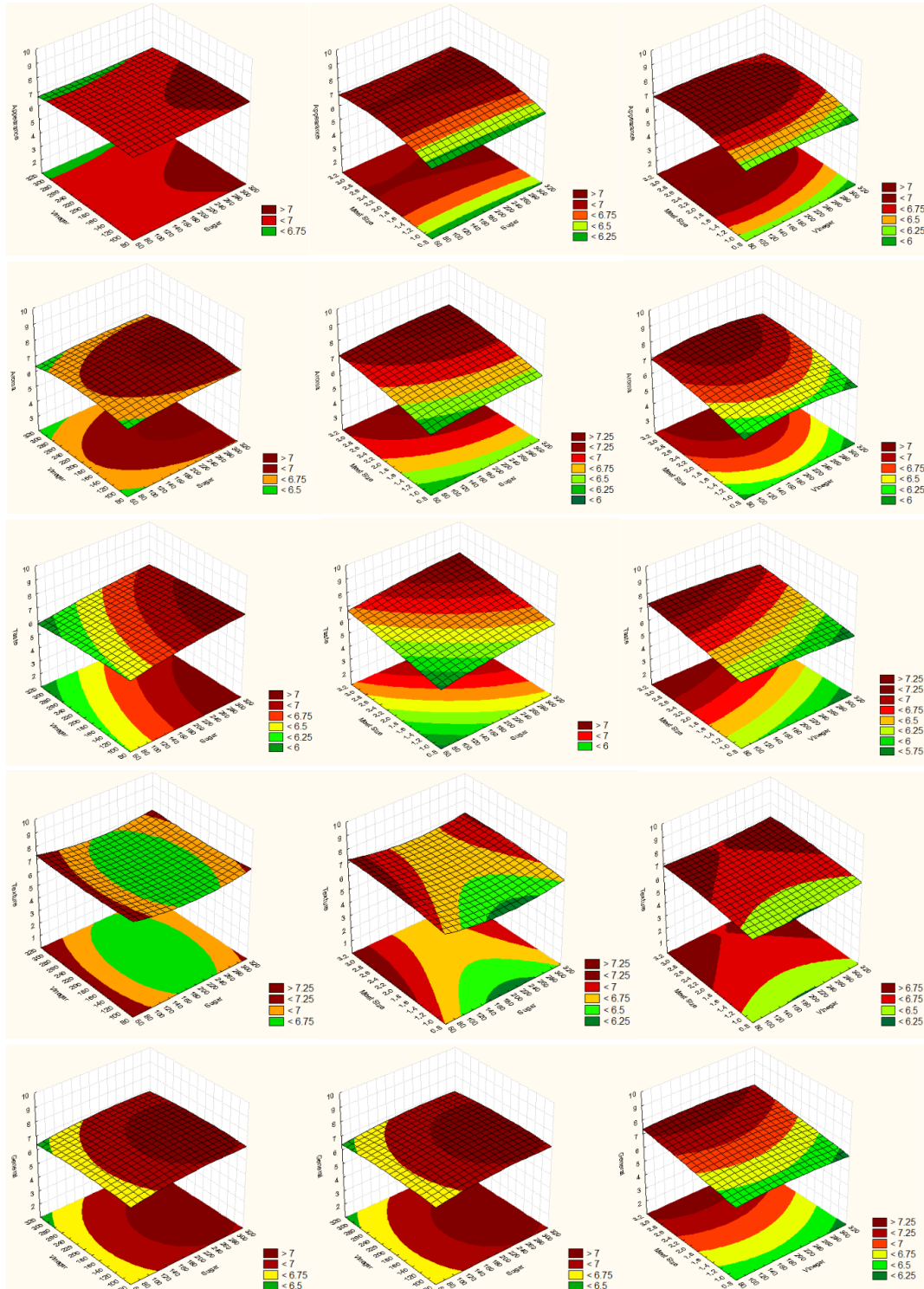


Figure 1. Surface plots depicting the effects of the dependent variables to (a) appearance, (b) aroma, (c) taste, (d) texture, and (e) general acceptability.

acceptability (Kim *et al.*, 2020).

### 3.3 Model regression and validation

The second-order polynomial regression model for all dependent variables,  $Y_1$  to  $Y_6$  was generated with the following equations:

$$\begin{aligned}
 Y_1 &= 3.650846 - 0.000715X_1 + 0.000003X_1^2 + 0.007299X_2 - 0.000011X_2^2 + 2.410401X_3 \\
 &\quad - 0.360119X_3^2 + 0.000009X_1X_2 - 0.000827X_1X_3 - 0.002679X_2X_3 \\
 Y_2 &= 3.218045 + 0.002306X_1 - 0.000006X_1^2 + 0.014474X_2 - 0.000025X_2^2 + 1.550752X_3 \\
 &\quad - 0.142857X_3^2 + 0.000006X_1X_2 + 0.000175X_1X_3 - 0.002857X_2X_3 \\
 Y_3 &= 3.598997 + (0.011830X_1 - 0.000007X_1^2 + 0.002782X_2 - 0.000006X_2^2 + 1.077068X_3 \\
 &\quad - 0.044643X_3^2 - 0.000008X_1X_2 - 0.001779X_1X_3 - 0.000357X_2X_3 \\
 Y_4 &= 5.757519 - 0.012769X_1 + 0.000029X_1^2 + 0.003703X_2 + 0.000011X_2^2 + 1.483083X_3 \\
 &\quad - 0.107143X_3^2 + 0.000005X_1X_2 + 0.0001X_1X_3 - 0.004286X_2X_3 \\
 Y_5 &= 3.065006 + 0.008144X_1 - 0.000012X_1^2 + 0.010796X_2 - 0.000012X_2^2 + 1.573935X_3 \\
 &\quad - 0.029762X_3^2 + 0.000006X_1X_2 - 0.001479X_1X_3 - 0.00375X_2X_3 \\
 Y_6 &= 6.149759 - 0.004251X_1 + 0.000008X_1^2 - 0.005627X_2 + 0.000009X_2^2 - 0.184035X_3 \\
 &\quad + 0.040417X_3^2 + 0.00000X_1X_2 + 0.000147X_1X_3 + 0.000075X_2X_3
 \end{aligned}$$

Observed responses from the Box-Behnken data were substituted to the equations and correlated to the predicted values as presented in Figure 2, which evaluated the validation of the model. The coefficient of determination ( $R^2$ ) were 0.58, 0.81, 0.87, 0.76, 0.75, and 0.87 for appearance, aroma, taste, texture, general acceptability, and pH, respectively. The suitability of the model requires  $R^2$  to be higher than 0.8 for greater variation of responses to be accounted for (Suherma, *et al.*, 2021; Giang and Tan, 2022), however,  $R^2$  values of  $\geq 0.6$  can be regarded as a valid model (Tan *et al.*, 2010;

Domingo *et al.*, 2019).

### 3.4 Response optimization and verification

The quality or desirability of a product or process cannot be simplified to the goodness of a single attribute, so in optimizing a product with multiple responses different strategies in selecting an optimal operating combination of the design factors should be employed (Myers *et al.*, 2016). In the *Lechon Paksiw de Leyte*, the approach used in locating the best acceptable combination is the superimposing contour plots. Overlaying responses to determine overall optimum conditions have been carried out several times through RSM across different fields (Mishra *et al.*, 2008; Kumar *et al.*, 2016; Hadisoewignyo *et al.*, 2022; Alsadi *et al.*, 2022; Alanazi *et al.*, 2023).

At constant (a) 200 g sugar, (b) 150 g vinegar, and (c) 2 cm meat size, which were identified as the cut-off point for all the sensory responses at a sensory acceptability rating of  $\geq 6.5$ , the limiting response was the texture acceptability of the products which was greatly affected by the joint effect or interaction of vinegar and meat size variables. With this consideration, the optimum conditions were 260 g of sugar, 290 g of vinegar, and 2.8 cm meat size.

The chosen values were verified by running another sensory acceptability test and the observed data were compared with the predicted responses using a t-test. Results of insignificant differences at  $p > 0.05$  were ascertained except for appearance acceptability, which denoted valid estimation of the optimum conditions and acceptability scores, as also reported by the study of Domingo *et al.* (2019) and ways enumerated by Liu (2011) on model validation metrics to provide a quantitative measure that characterizes the agreement between predictions and observations.

### 3.5 Calculated process schedule

With an equilibrium pH of 4.90, the product was categorized as low-acid food and *Clostridium botulinum* as the target microorganism at a z value of 10°C. The calculated processing schedule at a reference temperature processing temperature of 240.8°F (116.0°C) and 250.0°F (121.1°C), respectively were: a) 35 mins and 4.20 s at 45.0°C; b) 37 mins and 25.20 s at 35.0°C; c) 39 mins and 31.20 s at 25.0°C; and d) 41 mins and 24.60 s at 15.0°C. The  $F_0$  value was 5 mins to achieve commercial sterility, which was lower than most studies on retorted meat products such as studies of Rajkumar *et al.*, 2010 with a  $F_0$  value of 12.1 for retorted Chettinad style goat meat curry; Devadason *et al.* (2014) with  $F_0$  value of 12.13 for retorted buffalo meat blocks; while Girish *et al.* (2018) with a  $F_0$  value of 11.81 for shelf-

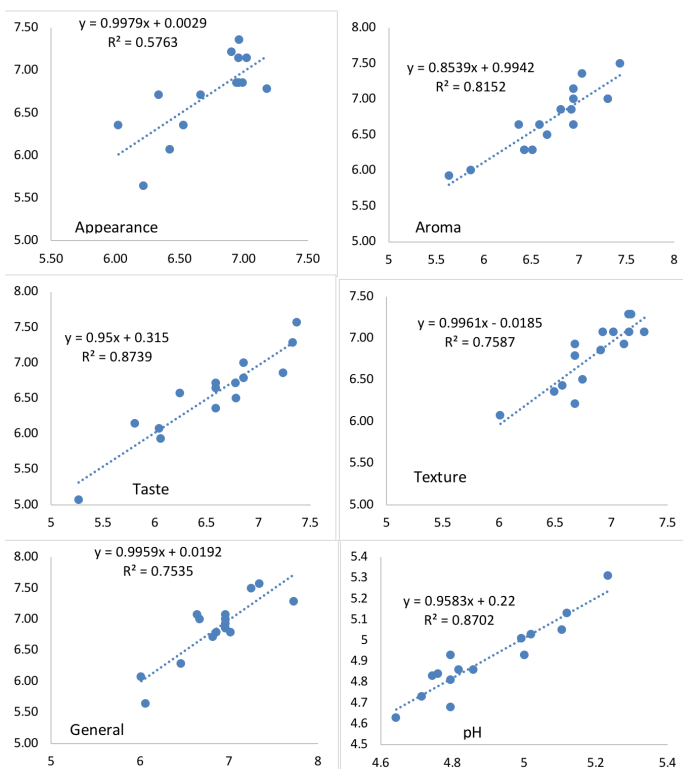


Figure 2. Correlation between observed and predicted data for all sensory parameters and pH.

stable pork curry; Nalini *et al.* (2018) with a *Fo* value of 7.2 for RTE pepper chicken; and Kim *et al.* (2020) with a *Fo* value of 8 for retorted and marinated Samgyetang.

### 3.6 Estimation of shelf-life

Based on the pH values as well as the commercial sterility test results, shorter longevity was depicted on packaged products in a pouch. The End-of-Shelf-life (ESOL) was established when the Cooked Meat Medium exhibited bubbling or gas formation, indicative of microbial positive growth. The shelf life plots (Figure 3) indicate the estimated shelf-life of the products as 448 days and 337 days, for cans and pouches, respectively. These values are lower than those reported by Lee, 2017, where canned products can be kept for 2 – 3 years, while 1.5 – 2 years for retort pouches at room temperature. Conversely, lower shelf life was depicted in the studies of Devadason *et al.* (2014) and Nalini *et al.* (2018) for meats processed in retort pouches which were acceptable for 90 days, while Girish *et al.* (2018) reported keeping the quality of 6 months.

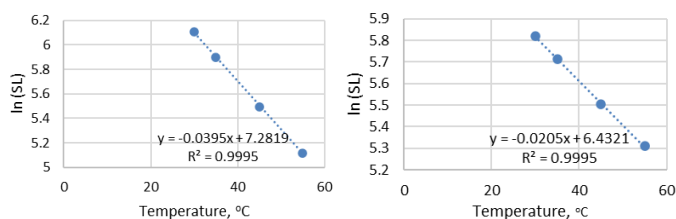


Figure 3. Shelf-life plot (left) can, (right) pouch.

## 4. Conclusion

Though considered waste, unsold *lechon* can be reprocessed using retort technology and is acceptable when packaged with convenient materials such as tin cans and flexible pouches. Additionally, processing at elevated temperatures and pressurized equipment increases products keeping quality to (6) months to one (1) year.

### Conflict of interest

The authors declare no conflict of interest.

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