The Effects of Cold Pork Loins on the Pasteurization Temperature in Sous Vide Cooking

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ABSTRACT

Objective: Sous vide is a relatively new cooking method introduced in restaurants in British Columbia. Sous vide cooking involves placing vacuum sealed food inside a temperature controlled water bath or steam convection oven. Unlike conventional cooking, sous vide cooking involves cooking food at a lower temperature (usually < 65°C) with a longer cook time. The low temperature allows chefs to precisely control the changes within the food. Thus sous vide cooked dishes have consistent texture and color, with retained flavor, moistness and nutrients. With all the benefits, sous vide cooking does have some disadvantages. Lower cooking temperature may not be sufficient for bacterial count reduction, resulting in unsafe food. In addition, every validated sous vide menu requires chefs to precisely follow the cooking temperature and cook time. Any deviation can cause the food to not reach the required 6.5 log reduction in bacterial count. The purpose of this experiment was to determine the effect on the internal temperature of cooking-in-process pork loin packages when additional chilled pork loin packages with an internal temperature of 4°C are submerged into the water bath.

Methods: Two groups of pork loin packages with data loggers inside (SmartButton) at approximately 4°C were introduced into a 60°C water bath at different intervals. The first group (6 packages) was immersed inside the water bath at time = 0 minute, while the second group (6 packages) was immersed inside the water bath at time = 10 minutes. Both groups were taken out when they were cooked for 31 minutes (at time = 31 minutes and 41 minutes respectively). Water bath temperature was recorded using SPER Scientific 8000024 data logger. Temperature data for pork loin packages was used to calculate the mean lethality achieved by each group. One sample t-test and two sample t-test were used for statistical analysis.

Results: There was a more than 3 mean log lethality difference in group A and group B pork loins. Pork loins cooked sous vide style in group A achieved a mean lethality of 5.12 at 31 minutes (range 0.42 to 12.78) while group B pork loins achieved a mean lethality of 8.44 at 31 minutes (range 3.35 to 11.87). With the same cook time, group A had a statistically significantly lower mean lethality than group B pork loins with p value = 0.003. Although statistically inconclusive whether group A pork loins achieved a mean lethality of 6.5, group B pork loins did reach the recommended mean lethality of 6.5.

Conclusion: The result indicated when new cold pork loin packages at 4°C are introduced into a cooking-in-process sous vide water bath at 60°C, the lethality of the original pork loin packages in the bath will be lowered if the cook time remains unchanged. However, it is inconclusive on whether the original pork loin packages will reach 6.5 lethality recommended by BCCDC. The new pork loin packages will reach 6.5 lethality if the original cook time is used.
INTRODUCTION

Sous vide cooking has become increasingly popular among restaurants in British Columbia. However, as a relatively new cooking method, a full understanding of sous vide has not been established between government authorities and restaurants. In traditional cooking methods, high heat is usually applied to the food to achieve a reduction in bacteria load. Sous vide, however, uses low temperature, long time (LTLT) cooking method to cook the food inside hot water baths or steam ovens. This difference raises a concern about the safety of sous vide products, as the required log reduction of bacteria load may not be achieved. In addition, refrigerated and frozen food will have an impact on the sous vide water bath temperature when introduced into the bath, causing water bath temperature to decrease. This is particularly important to sous vide as an effect on water bath temperature may have an effect on the internal temperature of the cooking food. Thus a longer cooking time than normal may be required to achieve a similar log reduction in pathogen. In this research, the recovery time of the sous vide water bath temperature will be analyzed when chilled food is introduced. The result of this research can be used by “Guidelines for restaurant sous vide cooking safety in British Columbia” to establish a safer control on the required cooking time for sous vide products. This research topic was presented by Lorraine McIntyre from British Columbia Centre for Disease Control (BCCDC).

The purpose of this experiment was to determine the temperature changes and recovery time of a sous vide water bath when chilled pork loins with internal temperature of 4°C were submerged inside.

LITERATURE REVIEW

What is sous vide cooking?

Sous vide, which means “under vacuum” in French, is a relatively new cooking method introduced to restaurants in B.C. Sous vide cooking involves placing vacuum sealed food inside a temperature controlled hot water bath or steam oven, usually with a lower temperature (< 100 °C) and longer cooking times than traditional cooking methods (1). As such, sous vide is also called low temperature and long-time cooking (LTLT). The three types of food being cooked with the sous vide technique are meats, vegetables and eggs. Meat usually is cooked at a lower temperature than vegetable (70 °C vs 95 °C) (1). Eggs, however, are not cooked under vacuum compared to the others. Sous vide cooking is different from traditional cooking in two fundamental ways. First, foods are required to be vacuum sealed for sous vide cooking and second, sous vide cooking provides chefs with a way to cook with controlled temperature (2).

Advantages of sous vide cooking

There are several advantages with sous vide cooking compared to traditional cooking. In traditional cooking, high temperature is applied to the food during the cooking process. This causes the protein structure of the food to rapidly change. For a chef, it is very hard to detect the optimum time for traditional cooking as half a minute difference can make the same food taste differently. Sous vide cooking, however, allows chefs to precisely control the changes within the food. This is done by a lower cooking temperature which allows more flexibility on the cooking time, which leads to more consistent dishes (2). Moreover, even though sous vide cooking has a long cooking process, it is not labour intensive. This allows better use of labour and equipment in a centralized production environment (1).
The low temperature of sous vide cooking also has an effect on the texture and color of the meat, as sous vide cooked meat obtains a consistent and appealing texture and color (3). In addition, vacuum sealed packaging retains flavor, moistness and reduces nutritional loss of the meat, since nothing is leached during the cooking process. This result in a superior flavor and increased tenderness in sous vide cooked meat (4).

Equipment required for sous vide cooking

According to BCCDC’s sous vide guidelines, there are several pieces of equipment required for safe and consistent sous vide cooking. The list includes convection steam oven or sous vide machine, thermometer, sous vide packaging and vacuum pouches, vacuum-packager and cleaning & sanitation equipment. Moreover, processing equipment (oven and sous vide machine) should be evaluated for even heat distribution (4).

Procedures for sous vide cooking

The initial preparation of sous vide cooking involves vacuum packaging of foods. Before starting this process, foods are required to be chilled to avoid overcooking or uneven cooking, as air pressure decreases the boiling point of liquids (5). Chilled Foods marinated with various ingredients are put into sous vide safe vacuum pouches and vacuum sealed using a vacuum packager. It is recommended not to use any alcohol based marinades, as they can cause ballooning during the water bath process (2). After this, vacuumed food should be either cooked within 2 hours or chilled and stored under 3 °C within 2 hours, as this step is considered a critical control point in sous vide cooking (5).

Cooking involves fully submerging the vacuumed food in the temperature controlled hot water bath. If the vacuumed food is floating in the bath, it is a sign of excessive air inside the pouch which causes uneven heat transfer (5). The temperature of the water bath should be checked with a calibrated thermometer to ensure sous vide circulator is working properly. After the water bath, vacuumed food can undergo additional cooking steps such as searing or browning, or cooling and storing for future use. For cooling, it is recommended to cool the vacuumed food with a slush ice (6).

Sous vide temperature safety zone

In traditional cooking, an internal temperature of 74°C for a minimum of 15 seconds is required for safe food consumption (7). Sous vide, with a longer cooking time, requires a lower internal temperature to achieve pasteurization zone. Nevertheless, sous vide cooking temperature is recommended to be at minimum 55°C, except for poultry which require a minimum temperature of 60°C (5). Between 10°C to 55°C is considered to be the danger zone. While in the danger zone, bacteria is able to grow rapidly causing foodborne illness or spoilage of the food. Thus, it is extremely dangerous to have sous vide cooking temperature in the danger zone. In order to preserve public health, raw sous vide food and cooked sous vide food are recommended to be stored at temperature below 3°C (5).
Temperature, Time and Log Reduction

The safety of sous vide cooked food is a major concern for both chefs and regulators such as EHOs. In traditional cooking, the required cooking temperature of various food is well established and strictly regulated. For example, ground beef patties are required to be cooked to a minimum of 71°C to achieve safe consumption (7). Sous vide cooking, however, does not have a strict temperature for cooking. Instead, a temperature safety zone for sous vide is given (Figure 1). Any temperature within the tolerance zone or higher can be used for sous vide cooking with a specific time required to achieve pasteurization.

Furthermore, there are two components for sous vide cooking time, the come up time (CUT) and the actual cooking time. Come up time is the time required after vacuumed food is submerged in hot water bath until it reaches the same temperature as the bath (5). It can be tested by inserting a thermocouple in the meat’s coldest point (usually the geometric centre). There are several factors affecting CUT: heat transfer properties of food, packaging, heat medium, thermal technology applied and how the food is exposed to the heat (9). This variability in CUT causes an issue in standardizing strict sous vide cooking time and temperature criteria based on reducing bacteria counts in foods. It is recommended to reduce the bacteria loads by a minimum of 6.5 logs for most foods, and a minimum of 7 logs for poultry (5). Baldwin (2012) provided a detailed table for the time sufficient to pasteurize meat, fish or poultry in water bath from 55°C to 66°C with the thickness of meat from 5 mm to 70 mm. This is critical information for sous vide chefs who want to determine the pasteurization time and temperature. However, they are still required to measure and record the CUT for each sous vide menu. Major log reduction in bacteria load should be achieved by water bath cooking, as the searing step in sous vide was not conclusive in major log reduction in a study on sous vide duck breasts (10).

Water bath temperature depression with cold sous vide pouches

When chilled foods are added to a sous vide water bath, the temperature of the water bath will drop. It was noted by Li (2015) that there was a temperature fluctuation between 0.5°C to 1.5°C in her sous vide salmon experiment and she suggested that this was possibly due to chilled salmon. Vikraman (2011) also observed temperature reduction by 8-10°C when chilled food was added to
the water bath and it took 15-20 minutes before the water bath returned to its original temperature. As such, BCCDC recommends no chilled product to be added to an immersion circulator if it is undergoing a sous vide process already (5). However, the effects of chilled food on temperature of the water bath is not quantified.

Microbiological Concerns

Microbiological hazards are a particular concern for sous vide cooking. The use of low temperature long time (LTLT) cooking method does not provide a quick kill step. In addition, the tolerance zone for sous vide cooking (55-60°C) is within the range of the danger zone (4-60°C) provided by the Food Retail and Food Services Code (7). Thus, a long come up time or low cooking temperature can provide perfect environment for pathogen growth in sous vide products. Jorgensen et al. (2017) demonstrated that preparation by sous vide has the highest proportion of unsatisfactory microbiological quality in light cooked food (11). Three particular pathogen of interest are Clostridium perfringens, Bacillus cereus and Staphylococcus aureus (6). The three bacteria have two things in common: first they are either anaerobic or facultative anaerobic and second, they are all able to produce heat stable toxins in food (12)(13)(14)(6). Low oxygen or anaerobic environment is available inside the vacuum sealed food. When these bacteria are introduced to the food before the vacuum process, they are able to grow rapidly if temperature is not controlled, both during cooking and storing. Thus, all three bacteria are especially dangerous for sous vide cooking. Clostridium botulinum is an anaerobic, spore-forming bacteria that is ubiquitous in the environment (15). It is able to produce heat resistant botulinum toxin that causes foodborne botulism. Foodborne botulism is relatively rare foodborne illness, however, it is extremely dangerous as the toxin can cause serious harm or even death (15). C. botulinum is able to multiple inside the vacuum sealed package of sous vide product and multiply under the temperature danger zone.

BCCDC recommends a minimum temperature of 55°C in sous vide cooking (5). This is because Clostridium perfringens is only able to grow under 52.3°C, unlike most food pathogens that stop growing at 50°C (Baldwin, 2014). Proper food handling and storage is required to reduce the initial bacteria load. This is critical in controlling foodborne pathogenic bacteria. BCCDC recommends a storage temperature for sous vide products to be 3°C or lower and no products should be left above 3°C for more than two hours. (5). This is because sous vide products stored at 3°C showed negligible microbial growth over 5 weeks of storage (4).

Regulations and Guidelines

As a newly emerging cooking method, the only guideline available in Canada is the “Guidelines for restaurant sous vide cooking safety in British Columbia” published by the BCCDC. Although the guideline is not enforceable, it is recommended to have an understanding of this guideline for both sous vide chefs and Environmental Health Officers (EHOs). According to Chen et al, EHOs who read the sous vide guideline are more frequently checking the temperature and cooking period of sous vide prepared food (17). Regulation wise, sous vide cooking falls under Public Health Act. According to section (15) of Public Health Act, “a person shall not willingly cause a health hazard” (18). This allows EHOs to assess each sous vide menu individually and enforce the Act if the menu is deemed hazardous to the public.

EHO’s Responsibilities

In general, it is the EHO’s responsibility to eliminate, reduce or mitigate any health hazards related to sous vide cooking. Before starting, EHOs should have a solid understanding of sous vide cooking by reading BCCDC’s “Guidelines for restaurant sous vide cooking safety in British
Columbia”. During inspections, EHOs can cooperate with sous vide chefs to determine the safety of their products by checking records of come up time, cooking temperature, storage temperature, HACCP plan and sanitation plan. It is critical to ensure the temperature and time combination during cooking is able to achieve the recommended log reduction in bacterial load. In case violation occurs, EHOs should use progressive enforcement to correct the contravention.

**MATERIALS AND METHODS**

**ACR SmartButton**

ACR SmartButtons were used to monitor and record the internal temperatures of each pork loin package. TrendReader® was installed on a personal computer as the controlling software for SmartButtons. By following the ACR SmartButton Guideline, data collection interval was set to 1 minute and start time was set to 10 minutes before the experiment (19). SmartButtons were calibrated using ice water at 0°C. Any SmartButtons with an error range greater than ±1°C were discarded.

**Immersion Circulator**

The Anova Precision Cooker was used as the immersion circulator (20). It has a temperature range between 25°C to 99°C. Before the experiment, it was calibrated with a calibrated thermometer for temperature accuracy. The immersion circulator was clamped onto the short side of the water tank and the temperature was set to 60°C.

**Pork Loin Preparation**

All the pork loins were purchased from Costco. Packages of pork loins were taken out from the cooler, then cut and trimmed into a small portion that had an approximate thickness between 20 mm to 23 mm and a weight between 155 grams to 165 grams. A small incision (approximately 15 mm) was cut at the thickest part of each pork loin and a SmartButton was inserted inside.
Vacuum Packaging

Pork loin portions were packaged inside food grade vacuum seal bags. The FoodSaver FM5400 vacuum sealer was used to seal each package. After vacuum sealing, packages were visually inspected for any air bubbles as this can cause floating during sous vide immersion (5). Vacuum sealed pork loin packages were stored in a 4°C domestic fridge for at least 6 hours before the sous vide cooking process. This ensured all pork loin portions would be at approximately 4°C before the experiment.

Data Logging Thermometer

SPER Scientific 800024 data logger was used to monitor and record the water bath temperature with Type K thermocouple. They were used to observe the recovery time of the water bath when cold pork loin packages were introduced.

Before the experiment, the data logger was calibrated with boiling water bath to an error range of ± 0.1°C according to the operation manual (21). During the experiment, the thermocouple was placed at the center of the water bath recording the temperature at an interval of 1 second.
Experiment Procedure

The 18 liter immersion circulator water bath was heated to 60°C. The thermocouple for the data logging thermometer was taped to maintain a location at the center of the tank. At time = 0 minute, 6 packages of vacuum sealed pork loin (group A) at approximately 4°C were introduced into the same water bath.

At time = 10 minutes, another 6 packages of vacuum sealed pork loins (group B) at approximately 4°C were introduced into the water bath.

At time = 31 minutes, packages from group A were taken out.

At time = 41 minutes, packages from group B were taken out.

The control group for the experiment involved only introduced packages of first batch of packages (group A) into the 60°C water bath, with other settings remaining the same (without group B). The control was performed 5 times with 6 samples each time for a total of 30 samples. The control values in Group A differed from Group B by having a different starting water bath temperature. The experiment was performed 3 times, which includes 18 samples for group A and 18 samples for group B.

Temperature data from the data logger and SmartButtons was collected and analyzed. SmartButton data was input into AMI Process Lethality Spreadsheet to calculate the lethality for each package (22).

Inclusion and Exclusion

The meat used in this experiment includes any regular pork loins purchased from Costco but no other kinds of meat that are not pork loins, not from Costco, and not organic.
Statistical Analysis

Description of Data

In this experiment, numerical data temperature, time and lethality (log reduction in bacterial counts) was collected or calculated. The temperature data as a function of cook time for each pork loin package was input into American Meat Institution’s process lethality spreadsheet to calculate the lethality (22). The Z-value and D-value were based on 6.5 log reduction of *Salmonella* at 60°C for ham (23). Ham was used because it is the closest data available with similar protein composition as pork loin (24). Z-value is the number of degrees temperature is required to be increased to achieve a tenfold reduction in the D-value. D-value is the time required at a given setting (e.g. temperature), for a tenfold reduction in the number of organism.

Descriptive Statistics

Table 1: Summary of descriptive statistics on lethality for each sample group.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Count</td>
<td>30</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Mean Lethality</td>
<td>8.44</td>
<td>5.12</td>
<td>8.44</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>4.07</td>
<td>3.67</td>
<td>2.29</td>
</tr>
<tr>
<td>Median</td>
<td>8.21</td>
<td>5.24</td>
<td>9.10</td>
</tr>
<tr>
<td>Range</td>
<td>14.80</td>
<td>12.36</td>
<td>8.52</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.61</td>
<td>0.42</td>
<td>3.35</td>
</tr>
<tr>
<td>Maximum</td>
<td>15.41</td>
<td>12.78</td>
<td>11.87</td>
</tr>
</tbody>
</table>

Table 2: Summary of descriptive statistics for water bath temperature in Celsius.

<table>
<thead>
<tr>
<th></th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>59.15</td>
<td>59.21</td>
<td>59.01</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Median</td>
<td>59.50</td>
<td>59.60</td>
<td>59.40</td>
</tr>
<tr>
<td>Mode</td>
<td>59.60</td>
<td>59.60</td>
<td>59.40</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.61</td>
<td>0.50</td>
<td>0.60</td>
</tr>
<tr>
<td>Range</td>
<td>2.20</td>
<td>2.10</td>
<td>2.60</td>
</tr>
<tr>
<td>Minimum</td>
<td>57.70</td>
<td>57.80</td>
<td>57.30</td>
</tr>
<tr>
<td>Maximum</td>
<td>59.90</td>
<td>59.90</td>
<td>59.90</td>
</tr>
<tr>
<td>Count</td>
<td>2700.00</td>
<td>2700.00</td>
<td>2700.00</td>
</tr>
</tbody>
</table>
Figure 7: The sous vide water bath temperature over a period of 2700 seconds for experiment 1.

Figure 8: The sous vide water bath temperature over a period of 2700 seconds for experiment 2.
Statistical Package

NCSS12 was the statistical package used for analyzing data from this experiment (25).

Inferential Statistics

The lethality data acquired from this experiment was categorized into 3 groups: Control, Group A and Group B.

Group A Comparing to Lethality of 6.5

<table>
<thead>
<tr>
<th>Left-tailed One Sample t-test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_0$ Pork loins (group A) exposed to cold pouches of pork loins (group B) will reach equal to 6.5 log10 reduction for <em>Salmonella</em> (mean lethality) when cooked for a total time of 31 minutes in the same sous vide circulator. (Ho: mean Group A lethality =6.5)</td>
<td>$P = 0.065$</td>
</tr>
<tr>
<td>$H_a$ Pork loins (group A) exposed to cold pouches of pork loins (group B) will reach lower than 6.5 log10 reduction for <em>Salmonella</em> (mean lethality) when cooked for a total time of 31 minutes in the same sous vide circulator. (Ha: mean Group A lethality &lt;6.5)</td>
<td></td>
</tr>
</tbody>
</table>

Mean lethality of Group A was compared with a lethality of 6.5 by One-Sample T-Test. A one-tailed test was used because prior knowledge indicated Group A pork loins will have a lethality less than 6.5. Tests of assumptions could not reject normality, thus the parametric One-Sample T-Test was used to review the results. P value was equal to 0.065, so the null hypothesis could not be rejected (fail to reject null hypothesis). Therefore, the mean lethality of Group A is not statistically significantly lower than 6.5. Power was equal to 0.454, which was lower than the optimum 0.800. The p value was in between 0.05 to 0.09, which indicates potential beta error (26). By using $\beta = 1 - $ Power, beta error was 0.546. The beta error can be minimized by an increase in sample size.
Group B Comparing to Lethality of 6.5

| Ho | Pork loins (group B) immersed 10 minutes after pork loins (group A) will reach higher than or equal to 6.5 log10 reduction for *Salmonella* (mean lethality) when cooked for a total time of 31 minutes in the same sous vide circulator. | P = 0.999 |
| H_a | Pork loins (group B) immersed 10 minutes after pork loins (group A) will reach less than 6.5 log10 reduction for *Salmonella* (mean lethality) when cooked for a total time of 31 minutes in the same sous vide circulator. |  |

Mean lethality of Group B was compared with a lethality of 6.5 by One-Sample T-Test. A one-tailed test was used because prior knowledge indicated Group B pork loins will have a lethality less than 6.5. Tests of assumptions could not reject normality, thus the parametric One-Sample T-Test was used to review the results. P value was equal to 0.999, so the null hypothesis could not be rejected. Therefore, the mean lethality of Group B is not statistically significantly lower than 6.5. Power was equal to 0.800, which was lower than the optimum 0.800. The p value was not in the range for alpha or beta error.

Group A Comparing to Group B

| Ho | Pork loins (group A) exposed to cold pouches of pork loins (group B) will reach the same log10 reduction for *Salmonella* (mean lethality) as the pork loins (group B) when cooked for a total time of 31 min in the same sous vide circulator regardless of the temperature fluctuation. | P = 0.003 |
| H_a | Pork loins (group A) exposed to cold pouches of pork loins (group B) will not reach the same log10 reduction for *Salmonella* (mean lethality) as the pork loins (group B) when cooked for a total time of 31 min in the same sous vide circulator. |  |

Mean lethality of Group A was compared with mean lethality of Group B by Two-Sample T-Test. A two tailed test was used because there was no prior knowledge about the lethality of the two groups. Test of assumption could not reject normality but rejected equal variances, thus the parametric Aspin-Welch Unequal-Variance T-Test was used to analysis the results. The P value was equal to 0.003, so rejected the null
hypothesis. Therefore, there was a statistically significant difference between the mean lethality achieved by Group A pork loins and the mean lethality achieved by group B pork loins. Group A had statistically significantly lower lethality than Group B. Power was equal to 0.881, which was higher than the optimum 0.800. The p value was not in the range for alpha or beta error.

Group A Comparing to the Control

<table>
<thead>
<tr>
<th>Left-tailed Two Sample t-test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_0 ) Pork loins (group A) exposed to cold pouches of pork loins (group B) will reach higher than or equal to the log10 reduction for Salmonella (mean lethality) as the pork loins (control) when cooked for a total time of 31 min in the same sous vide circulator.</td>
<td>( P = 0.003 )</td>
</tr>
<tr>
<td>( H_a ) Pork loins (group A) exposed to cold pouches of pork loins (group B) will reach lower than the log10 reduction for Salmonella (mean lethality) as the pork loins (control) when cooked for a total time of 31 min in the same sous vide circulator.</td>
<td></td>
</tr>
</tbody>
</table>

Mean lethality of Group A was compared with mean lethality of the Control by Two-Sample T-Test. A one-tailed test was used because prior knowledge indicated Group A pork loins would have a lethality less than the lethality of the Control. Tests of assumptions could not reject normality and equal variance, thus the parametric Equal-Variance T-Test was used to review the results. P value was equal to 0.003, so null hypothesis was rejected. Therefore, the mean lethality of Group A is statistically significantly lower than the mean lethality of the Control. Power was equal to 0.887, which was higher than the optimum 0.800. The p value was not in the range for alpha or beta error.

Group B Comparing to the Control

<table>
<thead>
<tr>
<th>Left-tailed Two Sample t-test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_0 ) Pork loins (group B) immersed 10 minutes after pork loins (group A) will reach higher than or equal to the log10 reduction for Salmonella (mean lethality) as the pork loins (control) when cooked for a total time of 31 minutes in the same sous vide circulator.</td>
<td>( P = 0.500 )</td>
</tr>
<tr>
<td>( H_a ) Pork loins (group B) immersed 10 minutes after pork loins (group A) will reach lower than the log10 reduction for Salmonella (mean lethality) as the pork loins (control) when cooked for a total time of 31 minutes in the same sous vide circulator.</td>
<td></td>
</tr>
</tbody>
</table>

Mean lethality of Group B was compared with mean lethality of the Control by Two-Sample T-Test. A one-tailed test was used because prior knowledge indicated Group A pork loins will have a lethality less than the lethality of the Control. Tests of assumptions could not reject normality, but rejected equal variance, thus the parametric Aspin-Welch Unequal-Variance T-Test was used to review the results. P value was equal to 0.500, so null hypothesis could not be rejected. Therefore, the mean lethality of Group B is not statistically significantly lower than the mean lethality of the Control.
Power was equal to 0.050, which was lower than the optimum 0.800. The p value was not in the range for alpha or beta error.

**Interpretation**

The mean lethality of group A pork loins showed inconclusive result on whether they reached the recommended 6.5 lethality. Though the One-Sample T-Test rejected the $H_a$, the p value was 0.065 which was close to 0.050. Moreover, the mean lethality for Group A pork loins was 5.12 which was lower than 6.5. A larger sample may be required for a conclusive result. The mean lethality of Group A pork loins were statistically lower than Group B and the Control. This could be explained by the fact that Group A pork loins were at the bottom of the water tank, covered by group B pork loins on top. Group B might have shielded Group A from water bath circulation thus minimized the heat transfer.

The mean lethality of group B pork loin reached 6.5 lethality according to the statistical analysis. Thus the cooler starting water bath temperature did not statistically significantly affect the lethality of group B pork loins. However, group A pork loins had a mean lethality statistically significantly lower than the mean lethality of group B and the control. As such, adding cold pork products to a cooking-in-progress sous vide bath may increase the chance of causing a health hazard, which prohibited by the Public Health Act (18).

**DISCUSSION**

**The Mean Lethality Achieved**

Although not statistically significant, Group A pork loins that were subjected to cold loins added to the immersion circulator 10 minutes into the come-up-time (i.e. the Group B loins) resulted in many of the Group A pork loins failing to reach the recommended log reductions value of 6.5. The mean lethality value of Group A pork loins was actually 5.1. Although the temperature depression when the cold loins were added was less than 2C, it took another 10 minutes before the temperature fully recovered. Due a small sample size and variable factors such as positioning of the package, additional research is needed to determine the mean lethality achieved by placing pork loin packages into a 60°C water bath at time = 0 minutes. In contrast, pork loin packages that were introduced into a relatively cooler water bath (approximately 59°C) at time = 10 minutes (Group B) achieved a mean lethality of 6.5. This could be explained by the fact that Group B packages were on top of Group A packages in the water tank, which shielded the Group A from water bath circulation. Otherwise with a higher initial water bath temperature, Group A should have achieved a higher mean lethality than Group B. Without the knowledge on Anova Precision Cooker’s circulation pattern or the budget, this suggestion could not be confirm with another experiment. Another explanation that can be visualized in figures 7 to 8 is the length of time group A and B pork loins spend at higher temperatures. The come-up-time for group A pork loins is interrupted by addition of cold group B loins at the 10 minute mark. It took more than 20 minutes for the come-up-time to recover, and for group A pork loins to heat up. This resulted in group A loins spending less time at temperatures above 55°C where lethality occurs, and therefore had a lower overall log reduction than the group B. The author was unable to find published studies with similar experimental procedures to compare the mean lethality.

**Water Bath Temperature Depression**

The water bath temperature started at 60°C for all three experimental runs. After the introduction of Group A packages, the water bath temperature dropped to approximately 58°C. After 10 minutes, the water bath temperature raised up to approximately 59°C. After the introduction of Group B packages, the water bath temperature dropped back down to approximately 58°C. At approximately 23 minutes, the water bath temperature reached 59.6°C and eventually
plateaued at 59.9°C at approximately 40 minutes. The maximum water bath temperature decrease was approximately 2°C. Considering the water bath temperature directly affects the mean lethality achieved by both groups, the 2°C decrease was minimum and the temperature recovery time was relatively fast. This supports the result that Group B packages were able to achieve a mean lethality of 6.5 due to minimum fluctuation of water bath temperature in this study. Fluctuation of water bath temperatures occurred in other sous vide studies. In Vikraman’s study, a temperature fluctuation of 8-10°C was recorded with a 20 to 25 minutes recovery time (27). While in Li’s study, water bath temperature fluctuations were only 0.5-1.5°C (28). Although the result confirms with Li’s and not Vikraman’s study, all studies agreed on factors that affect water bath temperature and recovery time. These are: the amount of refrigerated meat added, the volume of water in the tank and the type of immersion circulator. With an increased refrigerated meat load, the water temperature for a sous vide bath with 18 liters of water and Anova Precision Cooker could have a more intense fluctuation than 2°C.

Public Health Significance

The result indicated it is not safe to add refrigerated meats into a sous vide water bath while it is cooking other meats. According to BCCDC, sous vide cooked meat (except for poultry) is recommended to be cooked to a lethality of 6.5 in order to ensure safety (5). Although Group B achieved recommended lethality of 6.5, it was statistically inconclusive on whether or not Group A achieved the lethality recommendation. Most group A loins (66.7%) failed to reach the minimum required log reduction of 6.5. Thus, Group A pork loins might contained sufficient number of pathogens [M1] that were able to cause an illness to people who consumed the pork loin. In a busy restaurant setting, two groups of sous vide meats in a water bath with different end time are prone to accident. It is possible for the operator to mix up the group and take out the wrong packages at the wrong time even with labelling. In this experiment, if Group B packages were to be cooked for 21 minutes, a mean lethality of only 2.84 would be achieved, with no single package reaching 6.5 lethality. To err on the side of caution, it is not recommended to add new refrigerated vacuum packaged meats into a cooking-in-progress sous vide water bath. This practice in a food services establishment can endanger the public with potentially unsafe food. According to the Public Health Act, a person should not create any public health hazard that endangers the public (18).

LIMITATIONS

The Number of Pork Loin Packages

Due to financial and time constraints, the number of pork loin packages used in each group were not optimum. For Group A and Group B, 30 samples for each should be used for a more confident statistical analysis. This was particularly a problem for Group A as the result was inconclusive due to beta error.

Lacking Access to Commercial Equipment

A domesticate refrigerator was used in this experiment. With uneven cooling and slow cooling rate, pork loin packages were not uniformly cooled to 4°C before the start of each experiment run. The initial temperature ranged from 3.5-7.5°C. This affected the accuracy of the experiment. In order to minimize this problem, a commercial grade cooler should be used to cool all packages. In addition, without access to a meat slicer, all pork loins were cut by hand. This increased the variability of thickness between each pork loin. This is a problem as the internal temperature of each pork loin was monitored by a SmartButton inserted into the center of the meat. The thickness variation of each pork loin slice affected the internal temperature elevation during sous vide cooking as a function of time. Thin
slices of pork loins would have a faster temperature elevation while thick slices would have a slower temperature elevation. With lethality calculation based on internal temperature and time, thickness would affect the lethality achieved by the meat.

**Smart Buttons Data Interval**

SmartButtons used to record the internal temperature of pork loins had a data interval of 1 minute. Due to this limitation, the number of temperature data used to calculate the lethality was restricted, which may have affected the accuracy of the results.

**Immersion Circulator**

Anova Precision Cooker was used in this experiment. It has an error range of ±0.1°C (20). However, in a restaurant setting, a different brand immersion circulator may be used. Due to the variability between each brand, the water bath recovery time can be different depending on the immersion circulator used. This can potentially correct or exacerbate the problem of refrigerated food on sous vide water bath.

**KNOWLEDGE TRANSLATION**

The knowledge obtained from this experiment can be incorporated into BCCDC’s “Guidelines for restaurant sous vide cooking safety in British Columbia”. Through their guidelines, operators should be recommending to not add refrigerated meats into a cooking-in-progress sous vide bath as it may increase the chance of causing a health hazard.

Through direct communication with public health units, the findings from this experiment can be translated into a potential “Sous Vide Inspection Checklists”. This is especially useful during a sous vide restaurant inspection which it can give guidance to EHOs on potential risky sous vide preparation procedures and behaviors of food handlers. EHOs should verify that operators are not adding refrigerated meats into a cooking-in-progress sous vide bath. This can be determined by asking questions towards operators or checking their time records on the start time for each sous vide packages.

**FUTURE RESEARCH**

- Sous vide research on the shielding effects by packages in a sous vide bath.
- The mapping of sous vide water tank heating efficiency at locations inside the tank.
- The effect of additional refrigerated sous vide meats on a cooking-in-progress sous vide tank, at a temperature of 55°C (similar experiment, with different meat type or cooking temperature).
- The effect of additional refrigerated sous vide meats on a cooking-in-progress sous vide convection oven.

**CONCLUSIONS**

The result indicated when new refrigerated pork loin packages (Group B) at approximately 4°C are introduced into a cooking-in-progress (Group A) sous vide water bath at 60°C, the lethality achieved by Group A packages in the bath will be lowered if the cook time remains unchanged. This is due to a combination of shielding effect by Group B packages on Group A and a lowered water bath temperature. However, it is inconclusive on whether Group A packages will reach 6.5 lethality recommended by BCCDC. The Group B packages will reach 6.5 lethality if the cook time remains unchanged.

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**COMPETING INTEREST**
The authors declare that they have no competing interests.

REFERENCES


10. Plain S, Heacock H, McIntyre L. Examining the Safety of Duck Breast Prepared the Sous Vide Method. 2015;(1). Available from: https://circuit.bcit.ca/repository/islandora/object/repository%3A410?solr_nav%5Bid%5D=31f84dcb96f58d6e6b64&s_lsr_nav%5Bpage%5D=0&solr _nav%5Boffset%5D=0


15. WHO. Botulism [Internet]. WHO. World Health Organization; 2017 [cited 2017 Oct 24]. Available from:


20. Anova. ANOVA PRECISION COOKER WI-FI USER MANUAL. 2014;

21. SPER SCIENTIFIC. 4 Channel Datalogging Thermometer 800024 Instruction Manual. 2017;


24. McIntyre L. Personal Communication. 2017;


28. Li RC, Heacock H, McIntyre L. Sous Vide Salmon Pasteurization Temperature. 2014;1–17. Available from: https://circuit.bcit.ca/repository/islandora/object/repository%3A64?solr_nav%5Bid%5D=dbf2318c13b888a5bbf0&sorl_nav%5Bpage%5D=0&solr_nav%5Boffset%5D=1