The Effects of High Hydrostatic Pressure on the Color, Texture and Microbiology of Selected Pork Organ Meats

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Abstract

Pork organ meats (Liver, lung, kidney and heart) were processed at either 0, 400 or 600 MPa using high Hydrostatic Pressure Processing (HPP) for 4 minutes. Color and texture were measured before and after processing. All of the HPP processed samples were lighter (Increasing L* value) than the homologous control samples, with the liver and heart samples showing significant differences between the 400 and 600 MPa processes. HPP processed samples were less red (Decreasing a* value) and more yellow (increasing b* value) than the control samples, with the exception of lung tissue. HPP processed samples trended towards increasing peak force with increasing pressure, although there was considerable variability in the results both within and between samples. The organ meats were inoculated with a mixed culture of non-Typhoidal Salmonella. HPP reduced the populations by approximately 2 log10 at 400 MPa and 4 log10 at 600 MPa. Risk modeling indicated that 400 MPa would not reliably reduce a hypothetical population of non-Typhoidal Salmonella to less than 1 cell in an 85 g serving with a hypothetical population of 50 cells/gram, although 600 MPa would achieve this level of reduction for liver, lung and kidney.

Keywords: Color; High pressure Processing; Organ meat; Pork; Salmonella; Texture

Introduction

Organ meats, also known as variety meats, including hearts, livers, kidneys and lungs are a valuable part of the processed hog. However, relatively little has been published in the scientific literature in regard to shelf life and the incidence of specific foodborne pathogens. Since the consumption of organ meats in the United States is very low [1], for North America producers the largest potential market for these products is in the export market [2]. In the United States, lungs are not considered edible, but are used to manufacture heparin or in pet foods [3]. Although there is limited data on the subject, variety meats of all production species are usually considered microbiologically inferior to muscle meats [4]. This may be attributable to deficiencies in the cooling of the variety meats, and not necessarily attributable to poor handling practices during slaughter [5-7]. Most of the variety meats that enter international trade are frozen, with relatively little refrigerated product being exported. Freezing extends the shelf life of the organ meats, but may be less than desirable to the consumers in the importing country.

There is limited data on the incidence of foodborne pathogens in variety meats. Data from wholesale markets in Germany [8] indicated that approximately 64% of pork variety meats were contaminated with salmonellae. Zerby, et al. [9] found that approximately 15% of pork variety meats offered for sale were contaminated with salmonellae. However, both of these reports are dated, and the one survey from North America [9,10] was conducted prior to the full implementation of HACCP. It would be reasonable to assume that the incidence of salmonellae in pork variety meats has declined since that time, as has the incidence of salmonellae on pork carcasses. The conventional approach to interventions on fresh meat products has involved the application of washes or chemical rinses. Several authors have demonstrated the effectiveness of these procedures [9,11-13]. Although these interventions are effective on surface contaminants, they are limited by the willingness of the importing country to accept them. In addition, during the slaughter process, some microbial contaminants may become internalized in these meats, and would not be affected by external washes.

High Hydrostatic Pressure Processing (HPP) is currently used as a post packaging intervention in ready-to-eat meats [14]. High pressure processing subject’s foods to very high pressures, typically 400 to 600 MPa, or approximately 58,000 to 87,000 psi [15-17]. High pressure coagulates proteins, including those which are essential to microbial cell functions, and the rapid depressur-
ization may result in additional structural damage to the cells. In general, Gram negative bacteria, including non-Typhoidal *salmonella* and *Escherichia coli*, are more sensitive to high pressure processing than Gram positive bacteria, such as *Listeria or Clostridium* [18]. HPP advantages include its’ ability to destroy bacteria, both spoilage and pathogens, in internal surfaces, and that it does not require labeling.

The objective of this study was to determine the effects of high hydrostatic pressure processing on the physical and microbiological properties of selected pork organ meats.

**Materials and Methods**

**Organ Meats**

Lungs, liver, kidneys and hearts were obtained from Federal-ly-inspected market weight hogs immediately after slaughter. The organ meat samples were frozen until needed.

**Sample Preparation**

Samples of the lungs, livers, kidneys and hearts were cut into pieces (2cm x 2cm x 1cm) and inoculated with *Salmonella enterica* from the ATCC MP-15 panel, and included *Salmonella enterica* subsp. enterica serovar Choleraesuis ATCC 13312, serovar Enteretidis ATCC 4931, serovar Newport ATCC 6962 and serovar Typhimurium ATCC 700720. Individual cultures were grown to late logarithmic/early stationary phase in trypticase soy broth at 37°C. The individual cultures combined and serially diluted 1:10 in buffered peptone water. The samples inoculated by immersion for 3 min, allowed to drain for approximately 1 min, and then placed into vacuum bags and vacuum packaged. Once vacuum packaged, the outside of the packages were sanitized with 70% ethanol, and the vacuum packaging process and sanitizing procedure were repeated two more times, such that all samples were triple bagged. The vacuum packaged samples chilled to less than 5°C and stored for approximately 24 - 36 hours prior to processing, to allow for environmental adaptation of the inoculum. All of the samples had initial populations of non-Typhoidal *Salmonella* of > 5.5 log<sub>10</sub> colony forming units/cm<sup>2</sup> (cfu/cm<sup>2</sup>). Non-inoculated samples were packaged in the same manner and used for the color and texture measurements.

High Pressure Processing: The refrigerated samples (<5°C) were processed in an Avure/Flow Pressure Systems QFB 35L-600 at ambient temperature (ca. 22°C). The samples were processed at either10-5 MPa (Atmospheric pressure, control), 400 MPa for 4 minutes or 600 MPa for 4 minutes. Although the temperatures after processing were not determined, the samples were still cool to the touch and estimated to be <8°C. The samples were transported at less than 5°C to the laboratory.

**Color**

Triplicate samples, with 2 measures per sample, were analyzed using a HunterLab Labscan XE, using the CIE L*, a*, and b* scale. Briefly, L* measures darkness to lightness, with higher numbers indicating a lighter product. The a* values measure the red to green spectrum, with higher numbers indicating greater redness. Finally, the b* values measure the blue to yellow spectrum, with higher numbers indicating a sample which is more yellow. The analysis conditions were MI illuminant D75 (Daylight at 7500K), an Observer Angle of 10° C and a 12.7 mm (½ inch) aperture.

**Microbiological Sample Analysis**

The samples were evaluated for microbiological populations of non-Typhoidal *Salmonella*. The samples were homogenized in buffered peptone water for 2 minutes in a Tekmar 400 stom-acher, and then serially diluted as necessary in buffered peptone water. The samples were surface plated using the Thin Agar Layer method [19,20], with Xylose Lysine Desoxycholate Agar (XLD) as the selective medium and Trypticase Soy Agar (TSA) as the non-selective medium. The plates were incubated at 37°C for 48 hours, and the populations determined by standard plate counting methods.

**Statistical Analysis**

Each experiment was independently replicated three times. The microbiological populations were converted to cfu/cm<sup>2</sup>. When no detectable populations were present, the minimum detection limit for the assay (1 log<sub>10</sub> cfu/cm<sup>2</sup>) was used for statistical analysis. All measurements (Color, texture, microbiology) were analyzed using WINKS SDA ver. 6.0.93 Professional Edition (Texasoft, www.texasoft.com/), with pressure and organ meat type as fixed variables. Unless otherwise stated, statistical decisions were made at P = 0.05.

**Estimated Surviving Populations**

Surviving populations were estimated using @Risk 7.0 (Pal-isade Corporation, Ithaca NY). Since there was no available data to estimate an initial population, a hypothetical initial population was estimated using a triangle distribution of log<sub>10</sub> 0.1, 1.0 and 1.7 cfu/g (Approximately 1, 10 and 50 cells/gram). The reductions for each organ type and processing pressure were estimated using triangle distributions, with the mean reduction and the mean reduction + 2 standard deviations as parameters. Each simulation was performed with 5 replications of 1000 iterations each. The estimated surviving populations were calculated for an 85 g serv-ing [21], using both the maximum value reported by the simulation and the maximum value reported for 95% of the simulations.

**Results**

**Color**

The color of the high pressure processed samples became signifi- cantly lighter (Increasing L* value; P<0.05) with increasing pres-
sure, in comparison to the control samples (Table 1). For the liver and heart samples, there were differences (P<0.01) observed between the 400 and 600 MPa processes. The effect of HPP processing on the a* (Green - red) values was less consistent (Table 2) than on the L* values. The HPP processed heart samples were less statistically (P<0.001) red (Lower a* value) than the homologous untreated samples. However, there was no consistent effect of pressure on the liver samples, and no statistical difference noted for either the kidney or lung samples. HPP processing resulted in an increase in the b* values (More yellow), with significant increases observed for all 4 organ tissues (Table 3). The increase in b* values was generally associated with increasing pressure, with statistical differences (P<0.001) observed between 400 and 600 MPa for both liver and lung samples.

<table>
<thead>
<tr>
<th>MPa</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>26.28a (2.14)</td>
<td>16.31a (4.02)</td>
<td>34.50a (1.63)</td>
<td>34.72a (2.87)</td>
</tr>
<tr>
<td>400</td>
<td>35.55b (1.42)</td>
<td>21.20b (3.48)</td>
<td>42.92b (0.44)</td>
<td>50.25b (2.62)</td>
</tr>
<tr>
<td>600</td>
<td>45.38c (2.65)</td>
<td>24.82c (3.79)</td>
<td>46.28c (2.14)</td>
<td>54.15c (4.21)</td>
</tr>
</tbody>
</table>

1Mean (Standard deviation); Means within columns with different superscripts are significantly different (P<0.05)

Table 1: L* values of High hydrostatic pressure treated pork organ meats.

<table>
<thead>
<tr>
<th>MPa</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.13a (0.72)</td>
<td>8.31a (4.37)</td>
<td>11.48a (1.06)</td>
<td>8.55a (1.16)</td>
</tr>
<tr>
<td>400</td>
<td>11.10b (0.92)</td>
<td>9.33b (2.38)</td>
<td>8.11b (0.67)</td>
<td>6.98b (1.05)</td>
</tr>
<tr>
<td>600</td>
<td>9.93b (0.97)</td>
<td>12.16b (2.13)</td>
<td>8.12b (0.45)</td>
<td>6.93b (1.5)</td>
</tr>
</tbody>
</table>

1Mean (Standard deviation); Means within columns with different superscripts are significantly different (P<0.05)

Table 2: a* values of High hydrostatic pressure treated pork organ meats.

<table>
<thead>
<tr>
<th>MPa</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.72a (1.12)</td>
<td>1.39a (2.05)</td>
<td>10.56a (1.326)</td>
<td>11.48a (2.47)</td>
</tr>
<tr>
<td>400</td>
<td>14.55b (0.82)</td>
<td>2.78b (0.96)</td>
<td>15.51b (0.32)</td>
<td>17.73b (1.14)</td>
</tr>
<tr>
<td>600</td>
<td>16.90c (1.08)</td>
<td>5.66c (0.95)</td>
<td>15.57c (1.93)</td>
<td>18.90c (1.34)</td>
</tr>
</tbody>
</table>

1Mean (Standard deviation); Means within columns with different superscripts are significantly different (P<0.05)

Table 3: b* values of High hydrostatic pressure treated pork organ meats.

Texture

For all of the organ meat samples, the trend was increasing peak force with increasing HPP pressure (Table 4). For the lung and heart samples, there were statistical differences noted between the 600 MPa process and the Control and 400 MPa processes (P<0.05). Considerable variability was noted both within and between samples in each replication and process, which contributed to the lack of statistical differentiation of the processes for liver and kidney samples.
Microbiology

The populations of the inoculated non-Typhoidal *Salmonella* bacteria are shown in (Figure 1). The average reduction in population of the inoculated *Salmonella* was approximately $\log_{10} 2$ after high pressure processing at 400 MPa. The average $\log_{10}$ reduction in population was approximately 4.5 after high pressure processing at 600 MPa (Table 5). The population reduction was significantly different ($P<0.05$) between 400 and 600 MPa. Although there were numerical differences in the population reductions between the different organ meats, there was no statistical difference ($P=0.25$) in the observed population reductions.

![Figure 1: Populations of Inoculated *Salmonella enterica* on high hydrostatic pressure processed pork organ meats.](image)

### Table 4: Peak Force in Grams (texture).

<table>
<thead>
<tr>
<th>MPa</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1076.31$^1$ (444.83)</td>
<td>23.85$^a$ (5.22)</td>
<td>428.71$^a$ (118.11)</td>
<td>482.45 (161.74)</td>
</tr>
<tr>
<td>400</td>
<td>942.76 (391.77)</td>
<td>33.54$^a$ (2.15)</td>
<td>514.96$^a$ (120.17)</td>
<td>639.12 (382.7)</td>
</tr>
<tr>
<td>600</td>
<td>1184.63 (332.75)</td>
<td>144.77$^b$ (56.08)</td>
<td>874.67$^b$ (323.91)</td>
<td>1075.39 (126.41)</td>
</tr>
</tbody>
</table>

$^1$Mean (standard deviation); Means within columns with different superscripts are significantly different ($P<0.05$)

### Table 5: $\log_{10}$ reductions in the populations of inoculated *Salmonella enterica* on high hydrostatic pressure processed pork organ meats.

<table>
<thead>
<tr>
<th>MPa</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>400</td>
<td>3.7$^{a}$ (0.57)</td>
<td>2.1$^a$ (0.45)</td>
<td>2.4$^a$ (0.67)</td>
<td>2.2$^a$ (0.85)</td>
</tr>
<tr>
<td>600</td>
<td>4.6$^b$ (0.35)</td>
<td>4.4$^b$ (0.56)</td>
<td>3.6$^b$ (0.95)</td>
<td>4.5$^b$ (0.49)</td>
</tr>
</tbody>
</table>

$^1$Mean $\log_{10}$ reduction (standard deviation) = $\log_{10}$ control population - $\log_{10}$ HPP processed; Means within columns with different superscripts are significantly different ($P<0.05$)

Modelling

Based on the data determined in this study, the surviving populations in an 85 g serving size [21] were estimated based on a hypothetical initial population of 1 to 50 cells per gram, and variation within each organ and pressure based on the mean reduction plus/minus 2 standard deviations (Table 6). It was evident from the data that 400 MPa would not reliably result in an 85 g serving size which would be free of non-Typhoidal *Salmonella*, with the maximum estimated populations in heart, lung and kidney of 1 to approximately 2 cells per gram (62 cfu/85 g, 114 cfu/85 g and 164 cfu/85 g, respectively). Ninety-five percent of the time these three organ meats would have contained populations of non-Typhoidal *Salmonella* at greater than 1 cell in 25 g.
### Table 6: Estimated surviving populations in an 85 g serving of organ meats processed with HPP, based on a hypothetical initial population of 1 to 50 cfu/g.

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Percentage</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>100%(^1)</td>
<td>6.8(^2)</td>
<td>114.7</td>
<td>62.3</td>
<td>164.1</td>
</tr>
<tr>
<td></td>
<td>95%</td>
<td>1.2</td>
<td>22.6</td>
<td>16</td>
<td>30.2</td>
</tr>
<tr>
<td>600</td>
<td>100%</td>
<td>0.2</td>
<td>0.5</td>
<td>9.5</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>95%</td>
<td>0.1</td>
<td>0.1</td>
<td>1.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

\(^1\)100% = the maximum value predicted based on 5 replications of 1000 iterations; 95% = the maximum value predicted 95% of the time based on 5 replications of 1000 iterations 
\(^2\)Estimated surviving cells in 85 g of HPP processed product

### Discussion

High Pressure Processing resulted in organ meats which were lighter and with less red color (With the exception of lung tissue). Previously published research with HPP processed intact pork muscle meat reported similar results [22]. In a review of high pressure processing of meat and poultry products Simonin, et al. [23] reported an increase in L* values for many different types of meat. This increase in lightness has been variously attributed to protein coagulation or heme group release [23].

The changes in color were noticeable in side-by-side comparisons of the treated and un-treated samples. However, if the untreated were not available for comparative purposes, the processed samples with their lighter color were not objectionable, and the change in color may not have been noticeable to a consumer. It is difficult to know, without consumer evaluations of the pressure treated products, at what point the color would become unacceptable.

High pressure processing also resulted in an increase in peak force, indicating what would be perceived as an increase in the toughening of the product. This was expected and is consistent with other published results [23]. However, it is important to remember that the products in the experiments presented here were raw, and an increase in peak force would occur with cooking to an internal temperature of 70°C. Further evaluation of the products after cooking would be necessary to determine if the increase in peak force in the raw products effected the texture after cooking.

HPP resulted in significant reductions in the populations of non-Typhoidal *Salmonella*, with approximately a 2 log\(_{10}\) reduction at 400 MPa and a 4 log\(_{10}\) reduction at 600 MPa. In contrast to these data Yuste, et al. [25] reported a 7 to 8 log\(_{10}\) reduction of *Salmonella* in chicken sausage at 500 MPa for 10 min, but conducted the processing at 50°C. Escriu and MorMut [26] and Garriga, et al. [27] reported 3 to 4 and 6 to 8 log\(_{10}\) reductions, respectively after high pressure processing at 400 MPa and 17°C or 20°C. Garriga, et al. processed for 10 min, while Escriu and MorMut processed their samples for only 2 min, which may partially explain the observed differences.

The primary difference in the results of the experiments presented here was that the inoculated bacteria were allowed to adapt to the cold environment for at least 24 hours before processing, and were still cold after processing. The temperature of the samples and process generally impacts the lethality of high pressure processing, with higher temperatures resulting in greater lethality [23]. However, higher temperatures may have a detrimental effect on the quality of the products, and as with any process there must be a balance between food safety and food quality. Based on the modelling results, the estimated surviving populations suggest that 600 MPa would be a better choice than 400 MPa, strictly from the point of view of reducing *Salmonella*.

Future research will focus on optimizing processing parameters to achieve a maximum reduction in bacterial populations with minimal changes in color and texture. Additional data will be needed to determine the initial populations of non-Typhoidal *S. enterica* on organ meats, so that a more accurate assessment of the degree of inactivation which would be needed on a commercial basis may be determined. It is known that the prevalence of non-Typhoidal *S. enterica* in organ meats is fairly high. However, the actual population, in cfu’s/gram, is unknown. This population is vital in establishing minimum commercial processing parameters for microbial reduction.

Two other aspects of this would include the properties of the organ meats after preparation and cooking, and consumer panels to evaluate the products both before and after cooking to determine the acceptability. A process which increases the safety of a food and yet results in unacceptable changes from a consumer perspective will not be commercially viable. These consumer panels would need to be conducted with individuals who routinely purchase and prepare organ meats, as they would be most familiar with the desirable product characteristics.

### Conclusions

High Pressure Processing was shown to be effective in con-
trolling non-Typhoidal Salmonella in organ meats. This improvement in safety did not come without noticeable changes in both the color and texture of the raw products. As the pressure increased, the organ meats became noticeably tougher (Peak force increased) and lighter (L* value increased). Further experiments are needed to optimize the processing parameters necessary to achieve the best microbial population reductions with minimal changes to the texture and color. It is not known to what degree consumers would find these changes acceptable, however the improvement in the food safety of the products might be sufficient to encourage acceptance.

Author Contributions

Author Niebuhr prepared the cultures and performed the inoculation of the samples. Author Larson conducted the colorimetric and texture analyses. Author Dickson developed the experimental design and conducted the statistical analyses of the data.

Funding

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Conflicts of Interest

The authors declare no conflict of interest.

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1. USDA-ARS (2014) Food Patterns Equivalent Data Base. Table 1e. Protein Foods: Mean Amounts of Food Patterns Ounce Equivalents Consumed per Individual, by Gender and Age, in the United States 2013-2014.


