EFFECTS OF LOW-DOSE GAMMA IRRADIATION ON THE BACTERIAL MICROFLORA OF FRESHLY PICKED CRABMEAT

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ABSTRACT

Low-dose gamma irradiation is used to reduce or eliminate spoilage and pathogenic microorganisms that may contaminate seafoods during processing. Pathogenic bacteria that have been targeted for reduction or elimination in freshly picked blue crabmeat include *V. parahaemolyticus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli* and other aerobic spoilage organisms.

Microbiological analyses were performed to enumerate the surviving organisms in freshly picked crabmeat following treatment with 0, 0.125, 0.25, 0.5, 0.75, 1.0, 1.5 and 2 kGy of gamma irradiation. Both non-selective and selective media were used for enumeration of spoilage and various pathogens on day 0, 7 and 14 of ice storage.

The results indicate that low-dose gamma irradiation significantly reduced all microorganisms in crabmeat. The D-values, calculated from linear regression analysis for specific bacterial genera varied accordingly to each individual *L. monocytogenes* had the highest D-value of 0.59 kGy and *Vibrio parahaemolyticus* had the lowest of level of radiosensitivity among the microorganisms analyzed. *S. aureus* had the D-value of 0.16 kGy. Gamma irradiation doses equal
or greater than 1 kGy was found to significantly reduce the growth and multiplication of the bacteria. Therefore, it was concluded that 1 kGy gamma irradiation was sufficient to reduce the risk of food borne pathogen in freshly picked crabmeat, and at the same time, extend shelf life.

Introduction

Seafood products, including crabmeat, are highly perishable and may lose much of their value prior to complete spoilage and production of an off flavor. The high value of crabmeat and its susceptibility to spoilage have promoted investigations into the microbial flora and organisms responsible for spoilage (Conkey, and Chai, 1991). The environment has a great influence on the bacterial flora of freshly caught crabs. This study is focused on the natural microbial flora of the Gulf Coast blue crab meat.

The meat of freshly caught blue crabs should contain very few bacteria. However, during processing including peeling and picking, bacteria that are on the body surface or in the intestine can be introduced into the crabmeat itself and cause contamination.

The objective of this study was to isolate and enumerate the presence of four species, *E. coli*, *L. monocytogenes*, *S. aureus* and *V. parahaemolyticus* if present in the natural microbial flora of fresh raw crabmeat and to determine the effect of low dose gamma irradiation on those specific microorganisms.

Materials and Methods

Preparation of samples

Raw, unblanched crabs were purchased from local seafood markets every month from March to June, 1994. Those fresh crabs were then hand picked and sealed in 24 sterile plastic containers. The crabmeat samples which were mixed with hemolymph and intestine were placed in an ice chest filled with ice.

Irradiation

The crabmeat samples were transferred on ice to the Nuclear Science Center, LSU, and 21 samples were placed into a water tight
diving bell and lowered into the water for irradiation from a $^{60}$Co source which emitted 0.017 kGy per minute. The rest of the samples were labeled “control” and kept in the cooler. For a comparable length of time for the irradiation, one hour of exposure would equal 1 kGy. The diving bell was pulled up when desired exposure time and dose were reached. Four samples were removed from the diving bell, labeled and then placed in the cooler with ice. The diving bell was again lowered into the source for further exposure until all the samples were removed. The irradiated crabmeat samples were then transferred back to the Department of Food Science where the microbiological analyses were performed immediately. The unused samples were kept on ice and stored in the walk-in refrigerator in the Department of Food Science. The temperature of the ice stored crabmeat was 2°C. Every microbiological analysis procedure was repeated three times in order to calculate the average.

Preparation for Microbiological Evaluation

Aseptically weigh 10 g of crabmeat into a sterile plastic bag. Add 90 ml of sterile 0.1 % peptone dilution fluid with 8.5 % of NaCl, blend for 90 seconds with a stomacher (Lab-Blender 400, Tekmar Co., Cincinnati, OH). This represents the $10^{-1}$ dilution. Make further sequential dilutions using peptone dilution fluid.

Standard Plate Count

Procedures

1. Pipet 0.1 mL from the sequence of dilutions onto pregelled Plate Count Agar (Difco Laboratories, Detroit, MI) using the spread plate method.

2. When the surface of the plate count agar becomes dry, invert the plates and incubate at 35°C for 48 hours.

3. Count all plates which contained 25 to 250 colonies and calculate the total plate count per gram crabmeat.

4. Repeat the same procedures after 7 and 14 days, respectively.
Results and Discussions

The results of different irradiation doses and storage times effects on the standard plate count of both irradiated and unirradiated crabmeat are listed in Table 1 The numbers listed in the table are Log$_{10}$ Colony Forming Unit aerobic or Total Plate Count per gram (CFU/g) of crabmeat.

Table 1. The Log$_{10}$ numbers of the surviving microorganisms on Plate Count Agar

<table>
<thead>
<tr>
<th>Day</th>
<th>Control kGy</th>
<th>0.125 kGy</th>
<th>0.25 kGy</th>
<th>0.5 kGy</th>
<th>0.75 kGy</th>
<th>1.0 kGy</th>
<th>1.5 kGy</th>
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<td>4.3</td>
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<td>7.2</td>
<td>6.4</td>
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<td>6.8</td>
<td>5.8</td>
<td>4.1</td>
<td>3.9</td>
</tr>
</tbody>
</table>

By using the linear regression analysis, the D value, which is -1/slope, for the standard plate count is 0.42 kGy. This D value means that 1 kGy of gamma irradiation is capable of reducing 2.4 log number of the microorganisms in the crabmeat. The log of the numbers of CFU of irradiated samples remained stable or decreased in the first week and went up after one week of storage on ice. The log number of CFU control sample increased rapidly and slowed down after one week. The survival curves for standard plate counts are shown in Figures 1 and Figure 2.

Vibrio parahaemolyticus

Procedures

1. Pipet 0.1 mL from the sequence of dilutions onto pregelled thiosulfate citrate bile salt sucrose (TCBS) agar (Difco Laboratories, Detroit, MI) using the spread plate method.

2. When the surface of the TCBS agar becomes dry, invert the plates and incubate at 35°C for 48 hours.

3. Count all plates which contain 25 to 250 typical *V. parahaemolyticus* colonies. Typical *V. parahaemolyticus* colonies appear round, opaque green or bluish on TCBS agar.
Fig. 1 Survival Curve for Standard Plate Count

Fig. 2 Survival Curves of Standard Plate Count of Control and Irradiated Crabmeat during Ice Storage
4. Differentiate *V. parahaemolyticus* and *V. vulnificus* by halophilism and ONPG tests.

5. Repeat the same procedures after 7 and 14 days, respectively.

Results and Discussions

*V. parahaemolyticus* is very sensitive to gamma irradiation. The D value is only 0.05 kGy. They were totally eliminated by 0.5 kGy of gamma irradiation. The results of gamma irradiation on *V. parahaemolyticus* counts are shown in Table 2.

Table 2. The effect of gamma irradiation and 2°C ice storage on *V. parahaemolyticus*

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>0.125 kGy</th>
<th>0.25 kGy</th>
<th>0.5 kGy</th>
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The numbers are expressed in $\log_{10}$ CFU/g; NC: Not Countable, the number of CFU from the maximum dilution < 25 in one plate.

The D value and effect of ice storage on survival numbers of *V. parahaemolyticus* are shown in Figure 3, and Figure 4.

The numbers of *V. parahaemolyticus* kept decreasing during ice storage, indicating that low temperature (2°C) is inhibitory to this organisms.

*Listeria monocytogenes*

McBride Listeria medium was employed to detect and enumerate *Listeria monocytogenes*. 
**Fig. 3** Survival Curve of *Vibrio parahaemolyticus*

**Fig. 4** Survival Curves of *V. parahaemolyticus* in Control and Irradiated Crabmeat During Ice Storage
Procedures

0.1 mL of each dilution prepared in aerobic plate count was spread evenly onto three plates of McBride Listeria medium. The plates were then inverted and incubated at 35°C for 48 hours.

Typical *L. monocytogenes* colonies are round translucent, slightly raised bluish-gray colonies with fine textured surface that varies from 0.3 to 1.5 mm in diameter and have a narrow zone of beta-hemolysis. API Listeria, a rapid method for the identification of Listeria was employed to identify the suspected colonies.

Results and Discussions

The results showed that *L. monocytogenes* was very resistant to gamma irradiation. With a D value of 0.59 kGy, a large portion of the original microbial population survived the irradiation process. *L. monocytogenes* can survive and grow during the ice storage. Obviously, these organisms are capable of self-repair to damage by ionizing radiation. The results of the effect of gamma irradiation and ice storage on *L. monocytogenes* are shown in Table 3.

Figure 5 shows the reduction of *L. monocytogenes* by gamma irradiation and Figure 6 shows the relationship between ice storage time and log number of survival colony forming units.

Table 3. The effect of gamma irradiation and ice storage on *L. monocytogenes*.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
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<th>0.25 kGy</th>
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<th>0.75 kGy</th>
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<td>6.4</td>
<td>5.8</td>
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*Staphylococcus aureus* (Coagulase +)

Procedures
Fig. 5 Survival Curve of *Listeria monocytogenes*

Fig. 6 Effect of Ice Storage and Irradiation on *L. monocytogenes*
Sequential dilutions were made by the same procedures as in the standard plate count. For each dilution to be plated, a 0.1 mL of sample suspension was aseptically distributed onto three plates of Baird-Parker agar surface and was spread evenly by using sterile hockey sticks. After the inoculum was absorbed by the medium, the plates were inverted and incubated at 35° C for 48 hours.

The plates containing 25 - 250 colonies with the typical appearance of *S. aureus* were counted. Colonies of *S. aureus* were circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by an opaque zone and frequently with an outer clear zone (Bennet, 1984).

Colonies with a negative coagulase test reaction were subtracted from the initial colony count.

Results and Discussions

*S. aureus* was quite sensitive to gamma irradiation. The D value of this organism was found to be 0.16 kGy. The large initial population of this organism indicated that the raw crabmeat was contaminated probably by improper handling and hand picking. However, due to its irradiation sensitivity, the total population was eliminated by 0.75 kGy of gamma irradiation. During ice storage, the log number of CFU/g of untreated sample increased and the log number of CFU/g of irradiated sample decreased, indicating that low dose gamma irradiation is very effective in destroying or injuring *S. aureus*. The results of the effect of gamma irradiation and storage time on the survival of *S. aureus* are given in Table 4, Figure 7 and 8.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
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<th>0.25 kGy</th>
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NC: Not countable, the number of CFU from the maximum dilution < 25 in one plate.
Fig. 3.7 Survival Curve of Staphylococcus aureus

Fig. 8 The Effect of Ice storage and Irradiation on the Survival of S. aureus
**E. coli**

**Procedures**

5-tubes MPN technique was used to estimate the number of *E. coli* as well as the coliform number. 10 grams of fresh crabmeat was mixed with 90 mL of lauryl sulfate tryptose (LST) broth. Two lower sequential dilutions were also prepared. The tubes were then incubated at 35°C for 48 hours. The tubes with gas production were observed and recorded. The positive LST tubes showing gas were subcultured into EC (E Coli) broth and incubated in a 45.5°C hot water bath for 48 hours. The EC broth tubes showing gas production were subcultured onto the surface of McConkey agar and incubated at 35°C for 24 hours. The typical *E. coli* colony appeared bright red on the McConkey agar. The typical *E. coli* colonies were subcultured onto the non-selective plate count agar and the organisms from the colonies on the plate count agar were confirmed by API 20E rapid identification technique. The 5-tubes MPN table was used to estimate the MPN/g of both coliform group and *E. coli* in the crabmeat.

**Results and Discussions**

The results indicated that the numbers of *E. coli* as well as other coliform group member were very low in the crabmeat. For the control sample, the number of the coliform group and *E. coli* are 4 MPN/g and 2 MPN/g respectively. The coliform and *E. coli* were both eliminated after irradiation and did not appear after the ice storage. The results of the MPN analysis of coliforms and *E. coli* listed in Table 5

<table>
<thead>
<tr>
<th>Day</th>
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<th>0.25 kGy</th>
<th>0.5 kGy</th>
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Numbers in MPN/g
Conclusion

In this study, low dose gamma irradiation was found to be very effective on the reduction of targeted microorganisms except for *L. monocytogenes*, which required higher dose to achieve radurization. For the irradiation sensitive species like *S. aureus* and *V. parahaemolyticus*, 0.5 kGy of gamma irradiation was sufficient to eliminate them from the crabmeat. However, for *L. monocytogenes*, which can survive the low dose irradiation and multiply rapidly later during ice storage, the irradiation dose had to be higher in order to control this organism for a longer time. The numbers of *E. coli* and coliform are very low in crabmeat.

REFERENCES


EFFECT OF SPLIT DOSE APPLICATION ON THE RADIOSENSITIVITY OF LISTERIA MONOCYTOGENES

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INTRODUCTION

The ability of Listeria monocytogenes to survive and proliferate at refrigeration temperatures in a variety of food products, and its potential to survive minimal thermal processing (Doyle, 1988), have caused food safety concerns among food scientists and technologists. Due to the ubiquity of this genus, Listeria spp. have been shown to contaminate a variety of food products including red meats, seafood and dairy products either from harvest or during processing (Donnelly, 1994). The United States Food and Drug Administration (USFDA) currently mandates a zero tolerance level for Listeria in food products (USFDA 1994) due to the potential severity of infection in immunocompromised individuals due to liver disease, immune disorders, age, and/ or pregnancy.

One method suggested as a possible solution to reducing the risk of this bacteria has been irradiation processing. In minimally processed foods such as fresh seafood, dairy, and sous vide products, low dose irradiation might be used to reduce this risk while preserving the fresh quality of these products. Although L. monocytogenes has proven to be among the more radiation resistant vegetative bacteria, if present in small numbers, below 10⁴ CFU/g/ml, it has been shown to be sensitive to doses of 2 kGY (Andrews and Grodner, 1992; Huhtanen et al., 1989). In contrast, higher concentrations of the bacterium have been shown to resist 2 kGy of gamma radiation and recover with logarithmic growth (Andrews and Grodner, 1992; Juneau, 1989; Patterson et al., 1993).

In general, the radiosensitivity of bacteria varies depending on many different factors including medium in which the irradiation occurs (Urbain, 1986), temperature at time of irradiation (Josephson and Peterson, 1982), initial cell concentration (Kelner, 1955), and many other environmental factors such as oxygen level, pH, and water activity (Urbain, 1986; Josephson and Peterson, 1982). Traditionally, irradiation processing of food products has focused on single dose application to a
processed and packaged product. A method of radiation application used extensively to treat cancerous tumor cells has been to administer radiation doses in fractions over a period of time. This method was developed as a method to reduce the “good” tissue damage while effectively denaturing the activity of deoxyribonucleic acid of the tumor cells. It is not known whether use of fractionated doses will alter the overall sensitivity of bacterial cells. The purpose of this research was to determine if differences in the radiation sensitivity of a common food pathogen, *Listeria monocytogenes*, could occur following a split versus single dose gamma irradiation application.

**MATERIALS AND METHODS**

**Test Organism**

*Listeria monocytogenes* strain Scott A was obtained from the Louisiana State University Food Microbiology Culture Collection (August 1991). The bacterium was maintained on tryptic soy agar (TSA) at 4°C with quarterly subculturing. Following each subculture, the bacterium was tested (API Listeria 10-300, System for identification of *Listeria*) for typical physiologic and biochemical attributes to ensure consistency in growth and identification (Bille et al., 1992).

**Preparation of Inoculum**

*Listeria monocytogenes* strain Scott A was grown in tryptic soy broth (TSB-9 ml in a 20 x 125 mm pyrex screw cap tube) at 35°C for 18 h to achieve stationary phase cells at a concentration of $10^9$ CFU/ml broth. For a lesser cell concentrations of $10^6$ CFU/ml, dilutions were performed using 9 ml TSB to achieve the desired concentration. Cell dilutions were held at room temperature (20°C) and immediately irradiated.

**Irradiation**

*Listeria monocytogenes* samples stored in 20 x 125 mm screw cap tubes were maintained at constant temperature (20°C) until exposure to a 1.25 MeV cobalt-60 radiation source in the Nuclear Science Center at Louisiana State University. For the control samples, single dose, the radiation absorbed doses ranged from 0-5 kGy. For split dose irradiation application, cultures were irradiated with equally split doses of gamma radiation (0.0-0.0 kGy; 0.25-0.25 kGy; 0.5-0.5 kGy; 1.0-1.0 kGy; 1.5-1.5 kGy; 2.0-2.0 kGy; and 2.5-2.5 kGy) to achieve the same total dose as the controls (0-5 kGy). The “Time Between Fractions” (TBF) varied from 0 in the controls to 0.25, 0.50, 1.0, and 2.0 hours (h) in the test cultures. Test cultures were maintained at initial temperatures during intervals between dose application. Surviving cells were enumerated by serial dilution and spread plated onto TSA as described according to standard plating methods recommended by the USFDA (BAM, 1987). Initially negative cultures were incubated at 35°C for one week and examined for growth by visual observance of turbidity followed by plating on TSA. This procedure was to allow for the possibility of repair and recovery of injured cells exposed to sublethal radiation doses and for enumeration of very low numbers of survivors. The procedure schematic is presented in Table 1.
Statistical Analysis

Irradiation D-Values (the irradiation required to eliminate 90% of the cell population [D_{10}] in kGy) were calculated \([D_{10} = -\log_{10}\text{slope}]\) from the linear portion of irradiation survival curves using standard linear regression analysis (LRA) using Statistical Analysis System (SAS, 1987). Mean differences were compared using Tukey’s Pairwise Comparison (SAS, 1987).

The second method used to calculate irradiation D-Values was based on the reduction of the initial cell population to zero. This method, to be termed total dose, TD, used the beginning population in logs and the approximate amount of irradiation required to have no culturable survivors:

\[
\text{Irradiation D-Value} = \frac{\text{dose in kGy}}{\log \text{population}}
\]

This method was used to account for survival curves that exhibited a shoulder, tail, or two linear portions (Huhtanen et al., 1989) that might skew regression analysis. All experiments were performed three separate times (three replicates) with double plating of duplicate samples for each replicate.

RESULTS AND DISCUSSION

Irradiation of food products for the purpose of reducing spoilage bacteria or reducing the risk of pathogenic microorganisms has traditionally been applied in a single dose to achieve a targeted irradiation exposure. However, some foodborne pathogenic bacteria, like \textit{Listeria monocytogenes}, are resistant to federally approved low doses (<3 kGy), when present in numbers exceeding \(10^4\) CFU/g. When irradiation is applied in doses above 2 kGy, changes in the sensory quality may occur in some products including seafood products (Andrews and Grodner, 1992).

Alteration of the radiosensitivity of \textit{L. monocytogenes} was attempted by using split dose application of gamma radiation. This method of application is feasible in a commercial cobalt-60 facility, by simply shielding the product from the source for a desired time. When comparing the single versus split dose irradiation application to \textit{L. monocytogenes} in a nutrient broth, two levels of cell concentration were selected, \(10^6\) and \(10^9\) CFU/ml. Cell cultures of \textit{L. monocytogenes} at or less than \(10^3\) CFU/ml have proved to be sensitive to irradiation doses of 2 kGy with D-Value below 0.50 kGy (Andrews and Grodner, 1992). As many as 1 million Listeria/gram of food product may be present with no apparent spoilage detected by the usual sensory means (Marshall, 1993). The higher cell concentrations used in these experiments provided sufficient data points to construct irradiation survival curves in an attempt to predict the behavior of this bacterium.
Table 1. Procedures schematic for effect of split dose irradiation on radiosensitivity

Listeria monocytogenes, Strain Scott A (LSU)

- Tryptic Soy Broth - 18 h
  (Dilution to log 6, or 9 CFU/ml)

- Storage (20°C)

- Irradiated - 0-5 kGy
  (1.25 MeV gamma - Cobalt 60)

- Split Dose
  (0-2 h)

- Enumerate Survivors
  (Neg. cultures held 7 days & S/C for recovery)

- D-Value Calculations
  Linear Regression Analysis (LRA)
  (SAS, 1987)
  Total Dose Method (TD)
Temperature is an important factor to be considered in relationship to the food product of concern. In preparing, packaging and shipping food products, temperatures may fluctuate during handling. In this discussion, a single temperature of irradiation (20°C) will be considered.

The use of 20°C, as an irradiation processing temperature, is commercially applicable to dry spices, fruits and vegetables. The generation time for *L. monocytogenes* in TSB at 20°C was approximately 1.39 h (Andrews, 1994). Generation time is an important factor to consider in determining the ability of *L. monocytogenes* to recover from sublethal irradiation. This was especially a concern during these experiments since the available irradiation source was low with an emission rate of 18 Gy/min, compared with commercial irradiation facilities emitting 100's Gy/min.

Results of split dose irradiation of *L. monocytogenes* at 20°C with an initial cell concentration 10⁹ CFU/ml TSB are presented in Figure 1a. When comparing the control versus the composite of split dose, the survival curves appear to be quite similar. However, in calculating the D-Values using linear regression analysis, there were statistical differences (p <0.05) in the slopes of the 1 and 2 h time between fractional doses (TBF) when compared with the control and the two shorter TBF. By regrouping the survival plots, according to regression calculated D-Values, differences in the slopes become clearer. Figure 1 b groups the survival curves for the control (single dose) and samples with the 0.25 and 0.50 h TBF. The slopes of all three curves were similar with no statistical differences among regression or total dose calculated D-Values (Table 2). Figure 1c compares the survival plots of control sample with the 1 and 2 h TBF. In these plots, it is clear that the split applications were successful in reducing the total dose necessary to reduce the bacterial population to zero.

A 1 kGy reduction in total dose increased the slope of regression with a significant drop in D-Values from 0.50 kGy for the control to 0.42 and 0.41 kGy in the 1 and 2 TBF samples, respectively. Similar results were obtained using total dose calculation, Table 2. When the numbers of surviving bacteria were presented in a cumulative fashion for the control and each of the split dose conditions (Figure 2), it was apparent that the total numbers of bacteria recovered were less with the 1 and 2 h TBF than with the control or 2 shorter TBF (0.25 and 0.50 h). Noticeable differences occurred beginning with the 2 kGy total dose. At 2 kGy *Listeria*, when present at 10³ CFU/ml, has been shown to be highly sensitive and unable to recover (Andrews and Grodner, 1992). However, Patterson et al. (1993) reported that 10⁴ CFU/g *L. monocytogenes* was able to recover from a dose of 2.5 kGy with exponential growth after 2.5 days storage at 15°C. It appeared on cumulative plots, that at the 2 kGy level the radiosensitivity of *Listeria*, may have been most affected by using split dose application. One possibility for an increase in sensitivity with the 1 and 2 h TBF was that this time frame allowed for the bacterium to become actively involved in the repair and recovery of radiation damage. Subsequently, there may have occurred a lessening or depletion of enzymatic activity which might otherwise block the ionization (hydroxyl radical) or toxic products
(hydrogen peroxide) produced from the indirect effects of irradiation. Another explanation was that the bacterium was attempting to regenerate and thus more likely susceptible to irradiation damage. Bacteria have been shown to be most susceptible to irradiation damage during growth phase (Urbain, 1986; Josephson and Peterson, 1982; Kelner et al., 1955). In fact, all cells exhibit an inverse relationship between resistance and level of metabolic activity (Johns and Cunningham, 1983).

Results of the lower initial cell concentration (10^6 CFU/ml) at the same temperature (20°C) were similar to those just described in the previous paragraph. Figure 3a shows the plot of all split doses at this temperature and cell concentration. As indicated by the plot and subsequent regression analysis, D-Values for the control, 0.25, and 0.50 h TBF were the same 0.58 kGy (Figure 3b). D-Values for the 1 and 2 h TBF plots, 0.42 and 0.41 kGy, respectively, (Figure 3c), under the same conditions, were significantly (p < 0.05) lower than that of the control (0.58 kGy). Total dose D-Value calculations gave similar results, Table 2. The similarity of the results implied that survival plots were linear throughout with no significant shoulders or tails. Cumulative plots of survivors, Figure 4, indicated that, at 10^6 CFU/ml, the radiosensitivity of this bacterium was most susceptible to changes in dose application at the 1 and 2 kGy level. Again, this may have been related to the bacterium's level of metabolic activity.

Table 2. Comparison of D-Values for Listeria monocytogenes at 20°C

<table>
<thead>
<tr>
<th>TBF&lt;sub&gt;a&lt;/sub&gt; hr</th>
<th>LRA&lt;sub&gt;b&lt;/sub&gt; D&lt;sub&gt;10&lt;/sub&gt;</th>
<th>TD&lt;sub&gt;c&lt;/sub&gt; D&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁹ CFU/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.50&lt;sub&gt;d&lt;/sub&gt;</td>
<td>0.51&lt;sub&gt;d&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.25</td>
<td>0.51&lt;sub&gt;d&lt;/sub&gt;</td>
<td>0.53&lt;sub&gt;d&lt;/sub&gt;</td>
</tr>
<tr>
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<td>0.50&lt;sub&gt;d&lt;/sub&gt;</td>
<td>0.46&lt;sub&gt;de&lt;/sub&gt;</td>
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<td>1.00</td>
<td>0.42&lt;sub&gt;e&lt;/sub&gt;</td>
<td>0.43&lt;sub&gt;e&lt;/sub&gt;</td>
</tr>
<tr>
<td>2.00</td>
<td>0.41&lt;sub&gt;e&lt;/sub&gt;</td>
<td>0.43&lt;sub&gt;e&lt;/sub&gt;</td>
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<td>10⁶ CFU/ml</td>
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<td></td>
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<td>0.58&lt;sub&gt;f&lt;/sub&gt;</td>
<td>0.55&lt;sub&gt;h&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.25</td>
<td>0.58&lt;sub&gt;f&lt;/sub&gt;</td>
<td>0.50&lt;sub&gt;h&lt;/sub&gt;</td>
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<tr>
<td>0.50</td>
<td>0.58&lt;sub&gt;f&lt;/sub&gt;</td>
<td>0.50&lt;sub&gt;h&lt;/sub&gt;</td>
</tr>
<tr>
<td>1.00</td>
<td>0.42&lt;sub&gt;g&lt;/sub&gt;</td>
<td>0.45&lt;sub&gt;j&lt;/sub&gt;</td>
</tr>
<tr>
<td>2.00</td>
<td>0.41&lt;sub&gt;g&lt;/sub&gt;</td>
<td>0.41&lt;sub&gt;j&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> TBF = time between fraction of equally split doses.

<sup>b</sup> LRA = linear regression analysis r² >0.95

<sup>c</sup> TD = total dose/bacterial counts

Statistical differences (p < 0.05) occurred between

d<sub>i</sub> and e<sub>i</sub>, f<sub>i</sub> and g<sub>i</sub>, h<sub>j</sub> and j
CONCLUSION

Statistical differences occurred in the radioresistance of *L. monocytogenes* with a split dose of 1 and 2 h TBF. From an irradiation processors point of view, it is important to know that in the event of a brief shut down (up to 2 hours) of an ongoing process, little adjustment (if any) in exposure time would be necessary to complete the process as prescribed. There are situations in an irradiation facility when the radiation source is shielded and process temporarily halted. As long as the product remains in the plant, it is legal to resume and complete the process as prescribed (Everett, 1994). It is not legal, however, to apply a dose, ship a product out of the plant, and return the product to the plant for further processing.

Better understanding of the mechanisms involved in bacterial resistance to radiation exposure need to be explored. Biochemical activities, enzyme and growth metabolites, that may have occurred in these or similar experiments should be tested. This type of testing may be possible when using a higher output from the radiation source and under a more controlled environment such as self contained machine sources available today and in the future.

REFERENCES


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List of Figures

Fig. 1a  Survival plots for 10⁹ CFU/ml L. monocytogenes all times between fractions (TBF).

Fig. 1b  Survival plots for 10⁹ CFU/ml L. monocytogenes 0.00, 0.25, and 0.50h TBF.

Fig. 1c  Survival plots for 10⁹ CFU/ml L. monocytogenes 0.00, 1.0, and 2.0h TBF.

Fig. 2  Cumulative survival plots 10⁹ CFU/ml L. monocytogenes all time between fractions.

Fig. 3a  Survival plots for 10⁶ CFU/ml L. monocytogenes all times between fractions (TBF).

Fig. 3b  Survival plots for 10⁶ CFU/ml L. monocytogenes 0.00, 0.25, and 0.50h TBF.

Fig. 3c  Survival plots for 10⁶ CFU/ml L. monocytogenes 0.00, 1.0, and 2.0h TBF.

Fig. 4  Cumulative survival plots 10⁶ CFU/ml L. monocytogenes all time between fractions.
TBF/hours (time between split dose fractions)

D = D-value (linear reg. analysis)

Q hr

D = 0.50 ± 0.5 hr

D = 0.51 ± 0.5 hr

D = 0.50 ± 0.5 hr

D = 0.42 ± 0.5 hr

D = 0.41 ± 0.5 hr

Log (10) CFU/ml TSB

Total Dose/kGy

TBF/hr (time between split dose fractions)

D = D-Value (linear reg. analysis)

- 0.0 hr D=0.50
- 0.25 hr D=0.51
- 0.5 hr D=0.50
- 1.0 hr D=0.42
- 2.0 hr D=0.41
Total Dose ($kGy$)

- 0.0hr $D=0.50$
- 1.0hr $D=0.42$
- 2.0hr $D=0.41$

TBF/hr (time between split dose fractions)

$D = D$-Value (linear reg. analysis)

Log (10) CFU/ml TSB

Cumulative Log (10) CFU/ml TSB

- control
- 0.5 kGy
- 1.0 kGy
- 2.0 kGy
- 3.0 kGy
- 4.0 kGy
- 5.0 kGy

Time Between Split Dose Fractions/h
AUTOMATED OHMIC THAWING OF SHRIMP BLOCKS

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INTRODUCTION

The total U.S. shrimp catch and imports in 1993 were 133 million kg, and 273 million kg, respectively (Current Fishery Statistics, 1994). Shrimp constituted 37% of total edible imports in 1993. Seventy percent of shrimp processed in Florida is imported, mostly in the form of rectangular frozen blocks (Miyajima, 1993). These need to be thawed before further processing and handling.

The conventional process to thaw shrimp blocks is to place them into large warm water vats. Water is a good heat transfer medium (Singh and Heldman, 1993), and immersion in water reduces thaw drip loss in shrimp (James and Bailey, 1984). Henderson (1993) reported 1 - 2% increase in the moisture content of shrimp thawed in water. However, there are problems with this method. Warm water carries heat to the surface of the block by convection, and then heat is transferred by conduction from the surface to the center. This is a slow process. Since thermal conductivity of food is greater in the frozen than in the non-frozen state, and the heat capacity of food is greater in the non-frozen than in the frozen state (Karel and Fennema, 1975), the thawed layer of food on the surface of the block acts as a thermal insulator; thus, the thaw times are long. For 2.3 kg shrimp blocks (30 cm x 22 cm x 5 cm) the thawing time is on the order of 1.5 to 2 hours. The possibility of microbial growth is high for the outer layers exposed to the warm water for long periods of time. Another disadvantage is the loss of some soluble proteins during thawing. This reduces the nutrient quality of the shrimp (Peplow, 1975), and increases the load in the wastewater generated. The most important disadvantage is environmental. Gulf Coast shrimp processors use an estimated 3.4 billion L water annually (Bough and Perkins, 1977). In locations where availability of fresh water is limited, this is an impediment to the operations and growth of the plants. Wastewater generated by the Gulf Coast shrimp industries is estimated to contain 4.5 million kg/year dissolved and suspended solids (Bough and Perkins, 1977). Large shrimp processing plants pay up to $75,000/month on sewer costs (Miyajima, 1993). Therefore, they must find an alternative method to thaw frozen shrimp blocks.

When an electrical current passes through food, its resistance converts electrical energy to heat. This is called ohmic heating, and it is being seriously considered as a method to heat foods (Sastry, 1992). This method can also be used to thaw frozen foods by placing them between two...
electrodes and applying an alternating current. The advantages of this process are (1) there is no water used in thawing and no wastewater generated, (2) thawing can be relatively uniform due to volume heating, (3) the process can be easy to control. Previous research conducted in our lab (Henderson, 1993) pointed to a potential problem with ohmic thawing. As the frozen product thaws, current passes through the thawed portion of the block more readily, since frozen shrimp has 100 times lower electrical conductivity than thawed shrimp (Luzuriaga et al., 1994). With the current flowing through the thawed portion of the block, shrimp in that portion may cook, while the rest of the block is still frozen. This is called runaway heating, or the formation of hot spots. If this problem is solved, a new method to thaw foods would be available. In this study, an automated prototype ohmic thawing unit to eliminate the runaway heating was designed, built and tested. The specific objectives were: (1) To design a prototype portable ohmic thawing unit, with surface temperature sensing and computer automated capabilities, that can thaw two shrimp blocks at the same time. (2) To conduct extensive testing of the operation of the unit to assure the elimination of runaway heating, and to develop guidelines and suggestions for scale-up. (3) To compare the quality attributes (moisture content, microbial loads, and taste) of ohmically and conventionally thawed shrimp in a processing plant environment.

MATERIALS AND METHODS

Previous work showed that hot spots occur at both sides of the blocks simultaneously. Our objective was to automatically shut the current off when a point on the surface starts to heat. We decided to have four electrically isolated quadrants per electrode, so that when power to a quadrant is shut off the others will still continue to heat the rest of the block. The electrodes (24.13 x 30.48 cm x 1 cm) consisted of 304 stainless steel. Each quadrant has 32 thermistors covering the surface uniformly; the maximum distance from any point to a sensor was less than 1.91 cm (3/4”). The thermistors resided in holes drilled into the plates. The thermistor leads from each electrode quadrant were fed to an electronic circuit board designed by MicroTherm, Inc., Gainesville, FL. Temperatures were compared to an adjustable set point (above the melting point of shrimp and well below cooking temperatures). If the temperature of any of the 32 thermistors is greater than the set point, a microprocessor automatically turns off the power relay to that quadrant. A time delay is allowed for heat to dissipate. Then power to the quadrant is turned on by the computer. This is a positive method to control overheating regardless of size, moisture content, salinity etc. of different shrimp blocks.

At the beginning of the operation relatively high voltages are needed since the resistance of the block is high (Henderson, 1993). As thawing continues, the temperature of the block increases and resistance decreases. Therefore, reduction of voltage is necessary to keep the current at a given level and prevent overheating. The control of current was automated by a stepper motor and circuit board (designed by Dr. S.Yeralan, Ind. Eng. Dept.) connected to a transformer (602OCT-25, Staco Energy, Dayton, OH). The system monitored the current and voltage of each quadrant continuously. Since the control “intelligence” is not concentrated at the computer but distributed to several boards, the flexibility reliability, and speed of the operation is increased. A mobile cart was built to transport the prototype device to Singleton Seafood in Tampa, FL for in-plant testing.
A computer program was developed in Turbo Basic to supervise the operation. The logical algorithm of the program is as follows:
1. Turn on the heating relays to all four quadrants of an electrode.
2. Setup a fast loop where the amps, currents, temperature alarm status, and relay on/off status is read, the watt-hrs calculated and accumulated for each quadrant, and for the total shrimp block.
3. At predetermined intervals, these values are shown on the computer screen, and printed to an output file.
4. The time interval in which a quadrant is turned off due to a temperature alarm is monitored. When the alarm for that quadrant is off, additional time is allowed for the temperature to equilibrate before the relay is turned on again.
5. The maximum amp flowing through the system is kept constant by the stepper motor. Amperage levels are set by the operator.
6. The end point of the process is reached when the total accumulated watt-hrs reaches the heat necessary to thaw that particular block.

**Experiments**

Frozen rectangular shrimp blocks supplied by Singleton Seafood, Tampa, FL were transported to the University of Florida in Gainesville on dry ice, and placed into a freezer at -20°C. Four different kinds of shrimp were thawed using the ohmic system: (1) Yolita Brand, white shrimp, butterflied, tail-on, peeled and deveined, 41-50 count to the lb; (2) Moon Star Brand, pink shrimp, round, peeled and undeveined, 91-120 count; (3) Marbella Brand, pink shrimp, round, peeled and undeveined, 130-150 count; and (4) white shrimp, butterfly, tail-on, peeled and deveined, higher count than Yolita Brand.

Seven thawing experiments with two blocks thawing simultaneously were conducted in the lab. Before placing between the electrodes, each block was measured and weighed. The weight information was entered into the computer program. During the thawing process, the set amperage was recorded whenever it was changed. After thawing was complete, the shrimp and thaw drip were collected separately and weighed. Thawing times at different conditions were compared, and the proper functioning of the unit was tested.

**In-Plant Testing**

The mobile unit was transported to Singleton Seafood for two one-week periods. Conventional thawing experiments were conducted in the steam injected thawing tanks with 2,888 L capacity. Only similar types of shrimp were thawed at the same time. Each block was weighed and measured. The time necessary for the blocks to thaw was recorded. Then, samples for microbiology and moisture analysis were taken. Sanitary bags (Fischer Scientific, Pittsburgh, PA) were used to collect shrimp and water samples.

**Energy Input Calculations**

The actual energy input \( Q_{\text{actual}} \) was calculated in the program based on:

\[
Q_{\text{actual}} = P \Delta t = VI \Delta t
\]
where \( V \) = voltage (V), \( I \) = current (A), \( P \) = power (Watts), \( t \) = time (hrs), and \( E \) = energy (KJ). The theoretical energy, \( Q_{\text{theor}} \), needed to thaw the shrimp block was calculated as:

\[
Q_{\text{theor}} = m \left( C_{P_{\text{below}}} (T_{fp} - T_{\text{initial}}) + m \lambda + m C_{P_{\text{above}}} (T_{\text{final}} - T_{fp}) \right)_{\text{ice}} + m \left( C_{P_{\text{below}}} (T_{fp} - T_{\text{initial}}) + m \lambda + m C_{P_{\text{above}}} (T_{\text{final}} - T_{fp}) \right)_{\text{shrimp}}
\]

(2)

where: 
- \( m \) = mass of the shrimp, kg
- \( C_{P_{\text{below}}} \) = heat capacity of the product below freezing, KJ/kg°C
- \( C_{P_{\text{above}}} \) = heat capacity of the product above freezing, KJ/kg°C
- \( T_{fp} \) = freezing point of the product, °C
- \( T_{\text{initial}} \) = initial temperature of the block, °C
- \( \lambda \) = latent heat of freezing, KJ/kg
- \( T_{\text{final}} \) = final temperature after thawing the block, °C

The theoretical energy calculated was compared to the actual amount of electricity used to thaw the blocks by defining the coefficient of performance (COP) which is the percentage of total energy that came from the electricity to thaw the shrimp blocks.:

\[
\frac{Q_{\text{actual}}}{Q_{\text{theor}}} \times 100 = \text{COP}
\]

(3)

### Quality Attribute Comparison

**Microbiology**

Culture tubes were filled with 3.6 ml of Butterfield’s buffer (Speck, 1976). Twenty-five g shrimp and 225 ml Butterfield’s buffer were homogenized. Shrimp samples were serially diluted to \( 10^{-6} \) and water samples to \( 10^{-8} \). One-tenth ml of each dilution was plated on four aerobic plate-count agar (DIFCO Labs, Detroit, MI) plates. The incubation time was 48 hours at 25°C. The plates were evaluated by counting visible colonies, and averages taken.

**Moisture Content Determination**

Several shrimp were peeled, and diced in a food processor. A 10 g sample was weighed in the moisture balance (MB200, Ohaus Corporation, Florham Park, NJ). After 25-30 minutes the reading stabilized, and moisture values were recorded. A t-test was used to determine whether the moisture contents between immersion thawed shrimp and ohmic thawed shrimp were significantly different at the 5% level.

**Sensory Comparison**

Thawed shrimp from water immersion and ohmic methods were steamed in Singleton’s Stem Counterflow Oven at 104°C. The smaller shrimp (71-90 count) were cooked for 120 secs, and the larger shrimp (31-40 count) for 165 secs. They were then iced and transported to Gainesville, FL. A triangle test was given to untrained panelists who were asked to select the odd sample by considering sensory attributes of appearance, smell, texture and taste. They were given 2 consecutive tests. Statistical significance of difference was evaluated at \( \alpha = 5\% \), (Meilgaard et al., 1991).
RESULTS AND DISCUSSION

The computer program saved voltage, current, and cumulative watt-hrs vs time data. From these, plots were generated to determine a given shrimp block's thawing characteristics. With two ohmic units running simultaneously, eight quadrants were analyzed during a single experiment. One of the eight quadrants determined the voltage level applied to the entire ohmic system. This quadrant was the "limiting quadrant" and indicated the area in the shrimp block with the greatest conductivity. This limiting quadrant was determined by observing from the graphics which quadrant first allowed current at the given voltage level. During the initial period of thawing, the block has a high resistance, and a high level of voltage is necessary to pass the set current. During the later stages the temperature of the block is close to the freezing point, portions of it are partially thawed, and the resistance is lower. The level of voltage necessary to pass the set current is low. In this period, the operation is controlled by the surface temperature, indicated by frequent LED and relay off/on sequences. In all experiments, the temperature of the shrimp was below the set point temperature (12.7°C) at all times. This was also confirmed at the end of each experiment by touching the thawed shrimp. In all cases, the ohmically thawed shrimp was colder than the water immersion thawed shrimp.

Lab Test Analysis

Table 1 summarizes the results of the lab experiments. The thickness, net weight, time required to thaw, electrical energy used, and the C.O.P. for each experiment is shown.

<table>
<thead>
<tr>
<th>Test</th>
<th>Thick. cm</th>
<th>Net. Wt. g</th>
<th>Shrimp / water</th>
<th>Thaw time min</th>
<th>Calc. Watt-Hr</th>
<th>Theor. Watt-Hr</th>
<th>COP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1:1</td>
<td>5.4</td>
<td>2027</td>
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<td>124</td>
<td>57.5</td>
<td>305.85</td>
<td>18.8</td>
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<td>1918</td>
<td>2.07</td>
<td>124</td>
<td>55.8</td>
<td>307.42</td>
<td>18.2</td>
</tr>
<tr>
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<td>3.81</td>
<td>1950</td>
<td>3.76</td>
<td>190</td>
<td>34.1</td>
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<td>Set 4:1</td>
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<td>119</td>
<td>32</td>
<td>239.8</td>
<td>13.3</td>
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</table>

In plant Comparison Tests

The results of the lab tests showed that the ohmic system worked as designed, with the capability of thawing two blocks simultaneously. Since the approximate thawing time of similar by immersion thawing was reported in previous experiments, the lab test results proved that the ohmic units could thaw shrimp blocks in a time period competitive with water immersion, without the incidence of hot spots. Thus, the major obstacle of ohmic thawing has been solved, and the ohmic
unit could be used with confidence to thaw shrimp blocks and be competitive, if not better, than the conventional method. The next step was to use the unit in a plant environment, and to compare quality attributes of shrimp thawed with both methods. Table 2 summarizes the results of the in-plant experiments. The thickness, net weight, time required to thaw for the ohmic and control experiments, the electrical energy used, and the C.O.P. for the ohmic experiments are shown.

**Table 2. Operational Results from In-Plant Tests.**

<table>
<thead>
<tr>
<th>Test</th>
<th>Thick. cm</th>
<th>Net. Wt. g</th>
<th>Shrimp/water</th>
<th>Shrimp Thaw time min</th>
<th>Calc. Watt-Hr</th>
<th>Theor. Watt-Hr</th>
<th>COP</th>
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<td>2.5</td>
<td>45</td>
<td>13.2</td>
<td>293.5</td>
<td>4.5</td>
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<tr>
<td>2</td>
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<td>1930</td>
<td>2.17</td>
<td>45</td>
<td>81.2</td>
<td>304.4</td>
<td>26.7</td>
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<td>Control :</td>
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<td>1784</td>
<td>1.47</td>
<td>50</td>
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<td>1867</td>
<td>1.49</td>
<td>75</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Single 2</td>
<td>5.4</td>
<td>1914</td>
<td>5.01</td>
<td>85</td>
<td>20.2</td>
<td>240.9</td>
<td>8.4</td>
</tr>
<tr>
<td>Control :</td>
<td>5.4</td>
<td>1860</td>
<td>4.04</td>
<td>75</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>5.72</td>
<td>1890</td>
<td>3.03</td>
<td>75</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Single 3:</td>
<td>1</td>
<td>6.99</td>
<td>2066</td>
<td>3.93</td>
<td>121</td>
<td>13</td>
<td>273.0</td>
</tr>
<tr>
<td>2</td>
<td>7.62</td>
<td>2150</td>
<td>4.37</td>
<td>121</td>
<td>12.6</td>
<td>278.1</td>
<td>4.5</td>
</tr>
<tr>
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<td>7.62 - 8.89</td>
<td>1993</td>
<td>2.86</td>
<td>40</td>
<td>---</td>
<td>---</td>
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<tr>
<td>2</td>
<td>7.62 - 8.89</td>
<td>2089</td>
<td>3.01</td>
<td>40</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Single 4</td>
<td>6 - 7.5</td>
<td>1807</td>
<td>2.23</td>
<td>77</td>
<td>32.3</td>
<td>287.4</td>
<td>11.2</td>
</tr>
<tr>
<td>Control :</td>
<td>5.8 - 7.1</td>
<td>1714</td>
<td>1.52</td>
<td>71</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>5.8 - 6.0</td>
<td>1896</td>
<td>2.24</td>
<td>71</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Single 5</td>
<td>6.7</td>
<td>1855</td>
<td>1.92</td>
<td>170</td>
<td>81.6</td>
<td>305.9</td>
<td>26.7</td>
</tr>
<tr>
<td>Control :</td>
<td>6.7</td>
<td>1880</td>
<td>1.99</td>
<td>130</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>5.7</td>
<td>1851</td>
<td>2.08</td>
<td>130</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Single 6</td>
<td>5.72 - 6.99</td>
<td>1486</td>
<td>1.18</td>
<td>104</td>
<td>101.9</td>
<td>303.3</td>
<td>33.6</td>
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<td>1812</td>
<td>1.81</td>
<td>75</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>6.03</td>
<td>1794</td>
<td>1.89</td>
<td>75</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Note: The control samples were the shrimp thawed in the immersion tanks.

Table 2 shows the shrimp blocks used in the ohmic system and in the immersion were similar in thickness, net weight, and shrimp/water ratio within each experiment. Generally, the immersion thaw method was faster than the ohmic thaw method. However, optimization of the ohmic process should reduce the time. One noticeable difference between the two methods is the temperature of the shrimp after thawing. The temperature of the shrimp from the immersion method was much warmer than the shrimp from the ohmic thawed shrimp. The temperature of the water in the immersion tank was 28°C so the shrimp from this method was near 28°C. The shrimp from the ohmic unit never reached above 12.7°C. Typical voltage, current, and cumulative watt-hrs of quadrants vs. time are shown in Figure 1.

**Microbiology Comparison**

The shrimp and water from both methods of thawing were compared for aerobic microbial plate counts. Table 3 gives the results of this study. The microbial count of the ohmic thawed shrimp and water were on the same order of magnitude as that of the immersion thawed shrimp and water.
Figure 1. Typical volt, amps, and cumulative watt-hrs vs time profiles.
Table 3. Microbial Comparison Results.

<table>
<thead>
<tr>
<th>Thaw Method</th>
<th>Shrimp (per gram)</th>
<th>Water (per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (n = 3)</td>
<td>1.35 x 10^5</td>
<td>2.13 x 10^5</td>
</tr>
<tr>
<td>Ohmic (n = 4)</td>
<td>2.75 x 10^5</td>
<td>5.45 x 10^4</td>
</tr>
<tr>
<td>Immersion (n = 5)</td>
<td>3.34 x 10^5</td>
<td>6.63 x 10^4</td>
</tr>
</tbody>
</table>

Moisture Content Comparison

The moisture content of large shrimp showed no significant difference (5% level) between the two methods (Table 4). Small shrimp, however, showed significance differences. This can be explained by the larger surface-to-volume ratio of small shrimp, and is consistent with the findings of Henderson (1993). However, any difference in moisture content of the two thaw methods would disappear during storage in ice water prior to processing, as confirmed by Henderson (1993).

Table 4. Moisture Content Comparison Results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Immersion</th>
<th>Std. Dev.</th>
<th>ohmic</th>
<th>Std. Dev.</th>
<th>Diff. (@ 5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink peeled, 100-130 count</td>
<td>81.6%</td>
<td>0.79</td>
<td>80.4 %</td>
<td>0.63</td>
<td>No</td>
</tr>
<tr>
<td>Yellow, peeled, tail-on 91-00 count</td>
<td>83.2 %</td>
<td>0.4</td>
<td>82 %</td>
<td>0.57</td>
<td>No</td>
</tr>
<tr>
<td>Brown, tail-on 150-200 count</td>
<td>83.1 %</td>
<td>0.48</td>
<td>80.9 %</td>
<td>0.76</td>
<td>Yes</td>
</tr>
<tr>
<td>Pink, peeled 150-200 count</td>
<td>83.4 %</td>
<td>1.28</td>
<td>80.1 %</td>
<td>1.61</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Sensory Test Comparison

Table 5 shows the results of sensory comparison tests. For 54 panelists taking the test, at least 25 panel members need to answer correctly to report a significant difference at the 5% level (Meilgard et al., 1991). There was no significant difference (5%) between the two shrimp samples in either test. Many of the panelists who answered correctly said that they guessed. Trained panelists at Singleton thought the ohmic thawed shrimp had better texture than the immersion thawed shrimp.

Table 5. Taste Test Results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Correct Responses</th>
<th>Incorrect Responses</th>
<th>Significance (at P &lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>71-90 count</td>
<td>21</td>
<td>34</td>
<td>No</td>
</tr>
<tr>
<td>(n=55)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40 count</td>
<td>15</td>
<td>38</td>
<td>No</td>
</tr>
<tr>
<td>(n=53)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FUTURE WORK

The ohmic system performed to its design specifications. These results should encourage further work for improvement of the ohmic thawing unit towards scale-up and commercial operation. These are:

1. The thermistor-based surface temperature monitoring method should be replaced by a fiber optics-based method. When a liquid crystal that changes its color at the desired set point temperature is coated to the tip of a plastic fiber, it can detect temperatures much safer, since there is complete isolation of the circuit boards from the high voltage and current of the electrodes. This method is also much less expensive than using thermistors.

2. The current system operates in batch mode. Industrial applications require continuous operation. A system of rollers can be designed that would act both as the electrodes, and as the transport mechanism for the blocks on a conveyor system.

3. The inherent disadvantage of the current system is that it supplies the same voltage to all 8 quadrants, bound by the “limiting quadrant”. The high conductivity of the limiting quadrant causes that all other quadrants have lower amps passing through them. Since heat generated is proportional to the square of the current, they thaw slower. A system that can supply different levels of voltage to different quadrants would solve this problem. Ideally, each quadrant would have its own voltage, set current and control system.

4. A positive method of monitoring the electricity used, and therefore predicting the end-point of thawing would be to attach a watt-meter to the unit. This is quite inexpensive, and should be the first modification to the current system.

5. Optimization of the set current profile during thawing depending on shrimp species, size, shell on/off, shrimp to water ratio in the block, etc. This will also build the database of knowledge necessary to apply ohmic thawing effectively to commercial operations.

6. There are reports in the literature that coating of the electrodes may minimize or prevent electrolysis at the electrode in the high voltage and high current density operations. This would extend the life of the electrode, and minimize the deleterious effects of the interaction of gases evoking at the electrode (oxygen, chlorine, etc.) with the food.

REFERENCES


Using Edible Film to Improve Smoked Fish Quality

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University of Georgia
Athens, Georgia 30602-76 10

INTRODUCTION

Edible film is defined as thin layers of material which can be eaten by the consumer and provide a barrier to moisture, oxygen, oil and solute movement for the food. It can improve mechanical-handling properties, impart added structural integrity to foods, and retain volatile flavor compounds. It is sometime used to carry food additives. Edible film has been used for many years. The earliest recorded use was in 12th and 13th centuries in China. later in 16th century, “Larding” was used in England. Preservation of meats and other foodstuffs by coating with gelatin films was proposed in late 19th century. In 1930’s, hot-melt paraffin waxes became commercially available for citrus fruits. In 1950’s, carnauba wax oil-in-water emulsions were developed for coating fresh fruits and vegetables.

Smoked fish is a ready-to-eat product; however, this value added seafood product has a short shelf life. Rainbow trout is a high valued commodity in seafood markets in the US. In recent years, many small rainbow trout farmers have begun to produce smoke product. Due to several commercial smoked salmon products have been recently recalled for the contamination of Listeria monocytogenes, eliminating this pathogen becomes a timely task. In order to insure a high quality and safe smoked fish, this study was designed to evaluate edible film incorporated with an antimicrobial agent to reduce psychrotrophic bacteria population as well as eliminate the contamination of L. monocytogenes on rainbow trout.

MATERIALS AND METHODS

Smoked Rainbow Trout

Smoked rainbow trout fillets were purchased from a commercial trout farm in north Georgia. Each fillet was cut into 5X5 cm pieces.
Edible Films

Five percent mixture of 3:1 of hydroxy propyl methyl cellulose (I-IPMC) and methyl cellulose (MC) was suspended in 30 ml 95% ethanol. Seventy ml distilled water was added while stirring. Potassium sorbate was added at 0.3% level. After 20 min mixing, the solution was allowed to rest for 30 min to remove entrapped air. Ten ml of solution was then poured on a 20X20 cm glass plate and dried at 45-47°C for 7 hrs.

Inoculation and Packaging

For inoculated samples, fillets were inoculated with 1 ml of $10^4$ CFU/ml of mixed strain of Listeria monocytogenes (Scott A, LCDC, V-7, and Brie) (Huang et al., 1993). Each fillet section will be wrapped either with or without film before packaging. The packaging method included overwrapped and vacuum skin packaging. All samples stored at 4 and 10°C for 24 and 15 days, respectively and sampled at 3-day intervals.

Microbiological Analysis

Samples were massaged for 1 min with 25 ml sterile Butterfield’s phosphate buffer. Appropriate serial dilutions were plated onto the following agars by spread plate methods. Listeria Selective Medium (Oxid, Basingstoke, UK) agar was used to enumerate the L. monocytogenes population. Plate were incubated at 35°C for 48 hrs. The psychrotrophic population was determined on plate count agar (Difco, Detroit, MI) incubated at 20°C for 72 hrs. The aerobic and anaerobic population were incubated at 32°C for 48 hrs (Speck, 1984).

Data Analysis

Analysis of variance was performed on the data by means of PC SAS (SAS, 1987). Duncan’s multiple range test was used to determine any significant differences among F-values at different packaging treatment and microbial populations on smoked fillets with and without edible film wrapping.

RESULTS

Effect of Edible Films on Microbial Populations of Smoked Trout

Aerobic, anaerobic and psychrotrophic populations of vacuum skin packaged smoked rainbow trout stored at 4°C were shown in Fig. 1.1, 1.2 and 1.3. Statistically significant difference ($p<0.05$) between fillets wrapped with and without edible films was observed. Edible films reduced microbial populations on fillets by over 1 log. No significant difference on pH values was also found (Fig. 1.4).
**FIG-1.1 AEROBIC COUNT OF SMOKED TROUT (4°C)**

![Aerobic Count Graph](image)

**FIG-1.2 ANAEROBIC COUNT OF SMOKED TROUT (4°C)**

![Anaerobic Count Graph](image)
FIG-1.3 PSYCHROTROPHIC COUNT OF SMOKED TROUT (4°C)

FIG-1.4 pH PROFILE OF SMOKED TROUT (4°C)
FIG-1.5 AEROBIC COUNT OF SMOKED TROUT (10°C)

FIG-1.6 ANAEROBIC COUNT OF SMOKED TROUT (10°C)
FIG-1.7 PSYCHROTROPHIC COUNT OF SMOKED TROUT (10°C)

LOG CFU / Sq cm

DAYS (AT 10°C)

FIG-1.8 pH PROFILE OF SMOKED TROUT (10°C)

<table>
<thead>
<tr>
<th>DAYS (AT 10°C)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>FILM (VSP)</td>
<td>6.06</td>
<td>5.97</td>
<td>5.99</td>
<td>5.93</td>
<td>5.98</td>
<td>5.98</td>
</tr>
<tr>
<td>VSP</td>
<td>6.06</td>
<td>5.95</td>
<td>5.94</td>
<td>5.90</td>
<td>5.93</td>
<td>6.13</td>
</tr>
</tbody>
</table>
FIG-2.1 PSYCHROTROPHIC COUNT OF SMOKED TROUT (4°C)

LOG CFU/ Sq cm

FIG-2.2 *L. monocytogenes* COUNT OF SMOKED TROUT (4°C)

LOG CFU/ Sq cm
FIG-2.3 PSYCHROTROPHIC COUNT OF SMOKED TROUT (10°C)

FIG-2.4 *L. monocytogenes* COUNT OF SMOKED TROUT (10°C)
For samples stored at 10°C, no significant difference on microbial population was observed (Fig. 1.5, 1.6 and 1.7). No significant difference on pH values was found (Fig. 1.8).

Effect of Edible Film on Growth of L. monocytogenes on Smoked Trout

Smoked trout wrapped with edible before vacuum skin packaging showed a significant lower Listeria count throughout the entire storage period at 4°C (Fig. 2.2). However, no significant difference was found when samples stored at 10°C (Fig. 2.4). Similar results for psychrotrophic population at both temperatures were observed (Fig. 2.1 and 2.3).

In summary, edible film incorporated with potassium sorbate had potential antimicrobial effect on growth of psychrotrophic bacteria and L. monocytogenes. However, the concentration of antimicrobial agent need to be study to further eliminate the Listeria contamination.

REFERENCES

