Inactivation of Listeria innocua in Nisin-Treated Salmon (Oncorhynchus keta) and Sturgeon (Acipenser transmontanus) Caviar Heated by Radio Frequency

M. AL-HOLY, 1 * J. RUTTER, 2 M. LIN, 2 D.-H. KANG, 2 AND B. RASCO 2

1Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, Hashemite University, Zarqa-Jordan; and 2Department of Food Science and Human Nutrition, Box 646376, Washington State University, Pullman, Washington 99164-6376, USA

ABSTRACT

Recent regulatory concerns about the presence of the pathogen Listeria monocytogenes in ready-to-eat aquatic foods such as caviar has prompted the development of postpackaging pasteurization processes. However, caviar is heat labile, and conventional pasteurization processes affect the texture, color, and flavor of these foods negatively. In this study, chum salmon (Oncorhynchus keta, 2.5% total salt) caviar or ikura and sturgeon (Acipenser transmontanus, 3.5% total salt) caviar were inoculated with three strains of Listeria innocua in stationary phase at a level of more than 10^7 CFU/g. L. innocua strains were used because they exhibit an equivalent response to L. monocytogenes for many physicochemical processing treatments, including heat treatment. The products were treated by immersion in 500 IU/ml nisin solution and heat processed (an 8-D process without nisin or a 4-D process with 500 IU/ml nisin) in a newly developed radio frequency (RF; 27 MHz) heating method at 60, 63, and 65°C. RF heating along with nisin acted synergistically to inactivate L. innocua cells and total mesophilic microorganisms. In the RF-nisin treatment at 65°C, no surviving L. innocua microbes were recovered in sturgeon caviar or ikura. The come-up times in the RF-heated product were significantly lower compared with the water bath–heated caviar at all treatment temperatures. The visual quality of the caviar products treated by RF with or without nisin was comparable to the untreated control.

Refrigerated storage is currently the only available means to preserve and extend the shelf life of caviar as a ready-to-eat product. Yet, refrigeration alone cannot assure a pathogen-free product with a long shelf life. Dielectric heating is a term used to describe thermal processes that involve microwave (MW; 915 and 2,450 MHz) and radio frequency (RF; 27 MHz) heating. MW and RF pasteurization and sterilization processes have the advantage of being rapid and have shorter come-up times (37, 39). Industrial MW pasteurization and sterilization systems have been reported (7, 16, 20, 28, 34, 37). Yet, MW heating has the disadvantage of shallow heat penetration, which could result in survival of foodborne pathogens at certain locations in foods (35). This obstacle can be overcome by RF heating because RF has a greater penetration depth in food systems compared with MW (3). Also, the use of circulating hot water could boost energy efficiency in RF pasteurization (40). RF heating could provide an alternative to conventional thermal processing for heat-labile products. Dielectric heating processes have the advantage of shortening process time and often yield higher quality products than those produced by conventional thermal processing methods (14). An RF-based sterilization heating process for low-acid foods such as macaroni and cheese has been developed. In addition to the shorter heating time, minor changes in product quality have been observed in the RF-treated product compared with the retort-sterilized product (40). Pasteurized salmon caviar becomes soft and pale, at 71°C, immature eggs lose their shape and eggs appear dull; at 72°C, the egg yolk is completely coagulated and salmon caviar is transformed into a chewy mass (36). Hence, caviar is a product that could benefit from RF processing.

Of primary concern is the microbe Listeria monocytogenes, which is widely distributed in nature (8) and has been implicated in several fatal outbreaks of foodborne illness (11, 27, 32). This microorganism is difficult to control because it can be reintroduced into the processing environment and pose a risk for postprocessing contamination (9, 38). Listeria spp. were recovered from about 15% of ready-to-eat food samples in Portugal (15) and at rates of up to 60% from ready-to-eat fish products (18). Combining both a thermal process with other inhibitory factors might be the best strategy for heat-labile high-value foods. First introduced by Leistner (23), the concept of hurdle technology is widely accepted, particularly for minimally processed and heat-sensitive foods. The simultaneous effect of different preservative factors can be additive or synergistic (24). Proper evaluation is necessary, however, because certain combined treatments can be antagonistic (e.g., nisin and salt) (4). Nisin is a heat-stable bacteriocin produced by certain strains of Lactococcus lactis. Many studies have examined the antilisterial activity of nisin (5, 30, 33). It is primarily active against gram-positive bacteria, including Clostridium, Bacillus, and Staphylococcus spp. (31).
No studies have evaluated the combined effect of RF dielectric heating with antimicrobial nisin on the inhibition of foodborne pathogens or their surrogates. The objective of this work was to develop an RF (27 MHz) pasteurization process for sturgeon and salmon caviar products (60 to 65°C) inoculated with a cocktail of *Listeria innocua* strains. Another objective was to explore the effect of nisin as an additional antilisterial hurdle in combination with less severe RF processing on the inhibition of *L. innocua* and growth of mesophilic microbes in salmon and sturgeon caviars.

**MATERIALS AND METHODS**

**Preparation of nisin solution.** Nisin powder of 650 IU/mg (2.5% nisin content) activity was purchased (ICN Biomedicals Inc., Aurora, Ohio). A stock solution of nisin (10,000 IU/ml) was prepared by dissolving an appropriate amount of nisin in 0.02 N HCl. The solution was heated at 80°C for 7 min and kept at −20°C until use. Dilutions of nisin were prepared from the stock solution in 4 or 3% NaCl solutions for sturgeon and salmon caviars, respectively, for the preparation of 500 IU/ml nisin at the time of the experiment. Preliminary experiments elucidated that 500 IU/ml of nisin is the minimum solution strength necessary to impart a marked decrease in the *L. monocytogenes* population in caviar.

**Bacterial strains and culture media.** A three-strain mixture of *L. innocua* was used: *L. innocua* ATCC 51742, *L. innocua* ATCC 33090, and *L. innocua* ATCC 33091. Presumptive *Listeria* sp. colonies were streaked for purity from brain heart infusion (BHI; Difco Laboratories, Becton Dickinson, Sparks, Md.) agar slants on *Listeria PALCAM* medium base (Difco) supplemented with Bacto PALCAM antimicrobial selective supplement (Difco). A loopful of organisms from the selective medium was transferred into 50 ml of BHI broth in 250-ml screw-capped dilution bottles and incubated at 37°C for 24 h. Stationary-phase cells were used for the thermal inactivation experiments because they are the most thermally resistant phase (25). *Listeria* cells were harvested by centrifugation at 4,000 × g for 20 min and washed three times with 0.1% peptone water. The final pellet was resuspended in 0.1% peptone water, corresponding to approximately 10⁶ CFU/ml.

The *L. innocua* survivors were enumerated by diluting each heat-treated sample with 9.0 ml of sterile 0.1% peptone. The homogeneous dilution was 10-fold serially diluted in 0.1% peptone and then plated by the overlay method (22) to determine the number of survivors. This overlay method was designed specifically to improve the recovery of heat-injured cells. Tryptic soy agar (TSA; Difco) pour plates were incubated at 37°C for 24 h. Stationary-phase cells were used for the thermal inactivation experiments because they are the most thermally resistant phase (25). *Listeria* cells were harvested by centrifugation at 4,000 × g for 20 min and washed three times with 0.1% peptone water. The final pellet was resuspended in 0.1% peptone water, corresponding to approximately 10⁶ CFU/ml.

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**Thermal inactivation kinetics of *L. innocua* ATCC 51742 in sturgeon caviar.** For thermal inactivation experiments, thawed sturgeon caviar (*Acipeps transmontanus*) roe (3.5% salt) was transferred aseptically to a 100-ml sterile beaker and inoculated with *L. innocua* (~10⁶ CFU/g) mixed vigorously with a sterile spatula to obtain a uniform cell distribution. One gram of the inoculated caviar was placed inside sterile glass culture tubes (thermal death time [TDT] tubes, 10 mm outside diameter by 75 mm long). The filled tubes were kept in an ice bath at 0.0 ± 0.2°C for 30 min. Thermal inactivation was performed with the use of a circulating water bath (Digi-Bath, Laboratory Devices Inc., Holiston, Mass.). The temperature of the water bath was controlled at 60.0, 63.0, and 65.0 ± 0.2°C. The TDT tubes were submerged completely into the water bath for the specified treatment temperature. The come-up time was defined as the time to bring the material at the coldest point of the TDT tube to the specified treatment temperature after the tubes were submerged in the water bath.

An inoculated sample (1.00 ± 0.02 g) not exposed to heat served as a control. A sample was removed immediately after the come-up time was reached; this time was designated time zero. TDT tubes were removed from the water bath at intervals of 1.5, 0.5, and 0.33 min at 60.0, 63.0, and 65.0°C, respectively. After removal, the samples were immersed promptly into an ice bath at 0.0 ± 0.2°C.

The temperature of the inoculated TDT tubes was monitored by a subminiature type T thermocouple (Barnatt 115, model 600-1020, Barrington, Ill.) placed in the center of the caviar in the TDT tube. The temperature was recorded during the course of the experiment by a data acquisition system (Analog Connection ACPC, Strawberry Tree Inc., Computer Instrumentation and Controls, Sunnyvale, Calif.) and process control software.

The log number of survivors at each temperature were plotted against time. The best fit line was extrapolated, and the D-values were determined. The z-values were determined by plotting the calculated log D-values against the corresponding temperatures. Each single number is an average of at least three replicate experiments.

**Product treatments.** Sturgeon roe containing 3.5% salt (provided by Stolt Sea Farms LLC, Elvira, Calif.) and salmon (*Oncorhynchus keta,* Southern Southeast Regional Association, Ketchikan, Alaska) caviar containing 2.5% salt were used in this study. Thawed sturgeon and salmon caviars were added to a mixture of *L. innocua* strains in sterile petri plates (150 by 25 mm) (Becton Dickinson, Franklin Lakes, N.J.) at room temperature and allowed to air-blot under a laminar-flow biosafety hood for 30 min to allow *Listeria* cells to bind tenaciously to the caviar. A final 10⁶ to 10⁷ CFU/g of caviar was expected. This number is many-fold higher than the level of *L. monocytogenes* normally found in food. About 120 g of *Listeria*-inoculated caviar were submerged in 250 ml of 500 IU/ml nisin solutions for 40 s at room temperature under laminar flow. A short dwell time was used because this product is so fragile that large product losses would occur if a longer treatment time was used. Thereafter, 30 g each of sturgeon and salmon caviars were transferred to carbonated polyvinyl chloride (CPVC) tubes (11 cm long by 1.8 cm internal diameter, ASTM D2846, FlowGuard Gold, Thompson, Huntsville, Ala.). Three holes (5-mm diameter) were punched in one of the tubes. The holes were almost 2.4 cm apart from each other and from the edges. Fiber optic temperature sensors (UMI, FISO Technologies Inc., Quebec, Canada) were used to measure time-temperature at three different locations in the tube and in the circulating water during RF heating at 27 MHz. Three sensors were used because it is difficult to localize the coldest point inside a food package during dielectric heating. These sensors have the advantage of not interfering with the RF field, with a probe diameter of 0.8 mm, allowing short response time (0.05 to 0.2 s in most foods). Additionally, the accuracy of these sensors is comparable to thermocouples in a normal heating medium (13). Rubber stoppers were used to install the fiber optic sensors in the caviar tube and to prevent contact between circulating water and the product. The sensors were placed at the longitudinal centers of the tube (right
TABLE 1. Thermal inactivation kinetics of L. innocua ATCC 51742 in salmon and sturgeon caviars

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>D-value (min)</th>
<th>z-value (°C)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>3.55 ± 1.11</td>
<td>2.97 ± 1.12</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>0.85 ± 0.13</td>
<td>0.77 ± 0.13</td>
<td>5.3 (5.7) 0.99</td>
</tr>
<tr>
<td>65</td>
<td>0.41 ± 0.07</td>
<td>0.40 ± 0.01</td>
<td>1.12</td>
</tr>
<tr>
<td>Sturgeon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>5.60 ± 0.83</td>
<td>4.2</td>
<td>0.99</td>
</tr>
<tr>
<td>63</td>
<td>0.88 ± 0.14</td>
<td>0.77 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>0.37 ± 0.02</td>
<td>0.40 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Means ± standard deviations of three measurements.
* D-values for L. innocua ATCC 51742 in salmon caviar were determined in glass (outside parentheses) and aluminum (in parentheses) thermal death time (TDT) tubes (2). D-values obtained in the aluminum TDT tubes were used to calculate the thermal processing time for salmon caviar.
* D-values for L. innocua ATCC 51742 in sturgeon caviar were determined in glass TDT tubes only.

edge, center, left edge), and data were acquired by computer. Because salmon and sturgeon caviars can undergo serious quality changes if treated above 70.0°C, three different treatment temperatures (60.0, 63.0, and 65.0°C) were used. Different processing times for sturgeon and salmon caviars were selected on the basis of the thermal inactivation kinetics obtained for L. innocua ATCC 51742 in salmon and sturgeon caviars (Table 1). RF heating equivalent to an 8-D process without nisin or a 4-D process with 500 IU/ml nisin dipping was applied.

Figure 1 shows the RF heating system with the water circulation used in this study. A pilot-scale 6-kW, 27.12-MHz RF system (Proctor Strayfield, Proctor and Schwartz Co. Ltd., Wokingham, UK) with plate applicators was used. During RF processing, hot distilled water from a water bath (Digi-Bath) was circulated at a temperature matching the target temperature of the heated food. Circulating water helped to distribute heat more uniformly. Water circulation also reduced the fringe effect at the interface between the side of the food package and the air.

**Determination of come-up times.** Three fiber optic sensors were used to monitor the temperature in the aforementioned CPVC tubes containing caviar. The tubes were heated with either an RF heating system (Fig. 1) or the conventional water bath system. The come-up time was defined as the time to bring the material at the coldest point in the TDT tubes to the specified treatment temperature after the tubes had been heated by the RF system or submerged in the water bath at the designated temperatures (60.0, 63.0, and 65.0°C).

**Color measurement.** The color of sturgeon and salmon caviar treated by RF with or without nisin and control (caviar not treated with RF) was measured by a Minolta colorimeter (Minolta, Spectrophotometer CM-2002, Minolta Camera Co., Ltd., Osaka, Japan). L*, a*, and b* values were recorded, where L* is lightness or darkness ranging from 100 (perfect white) to 0 (black), +a* is red, −a* is green, +b* is yellow, and −b* is blue (12). The colorimeter was calibrated with a white tile before measurement. The caviar samples were placed in small petri dishes, and the measurements were carried out in triplicate.

**Statistical analysis.** Each number is an average of at least three replicate experiments, and standard deviations were determined. Data were analyzed with a computer software package (SAS Institute, Cary, N.C.) by analysis of variance and Fisher’s least significant difference test.

**RESULTS AND DISCUSSION**

The thermal inactivation kinetics of *Listeria* spp. must be well understood before pasteurization protocols to improve product safety can be developed (2). The relatively high heat resistance of *Listeria* spp. and their ability to grow at refrigeration temperatures, ubiquitous occurrence, and current zero tolerance in U.S. Food and Drug Administration–regulated foods make control of this organism vitally important in refrigerated ready-to-eat foods.

*Listeria monocytogenes* is a psychrotrophic microorganism, yet it exhibits more heat resistance than most non–spore-forming pathogenic microorganisms (1). Besides temperature, many variables affect the heat sensitivity of a microorganism. These factors include the physiological growth phase, with stationary phase being the most thermally resistant (25); composition and pH of the growth medium; water activity; and presence of solutes (1).

Table 1 shows the thermal inactivation kinetics of *L. innocua*, a closely related nonpathogenic microorganism to...
FIGURE 2. Thermal death time curve of L. innocua ATCC 51742 in sturgeon caviar (z-value was calculated as \(-1/\text{slope of the TDT curve}\)).

*L. monocytogenes* (19). First-order thermal inactivation kinetics for *L. innocua* ATCC 51742 in sturgeon caviar was observed. The *D*-value at 63°C was in the range for *L. monocytogenes*, at 62.8°C (0.58 to 1.22 min) reported by Brown (6). Additionally, *D*-values were comparable to those reported by Mazzotta (26) for *L. monocytogenes* in surimi-based imitation crabmeat at 62°C (2.1 min) and at 66°C (0.4 min). However, these *D*-values are higher than that for *L. monocytogenes* in plain egg yolk heated by a submerged-vial technique, which was 0.44 min at 64.4°C (29). The *z*-value for *L. innocua* in sturgeon caviar was 4.2°C (Fig. 2), which is lower than the *z*-values obtained for salmon caviar (Table 1).

Figure 3 shows a comparison of the time–temperature profiles at the potentially coldest spot from the fiber optic temperature sensors in sturgeon caviar heated by the RF and conventional water bath methods. Clearly, the RF heating system exhibited much more rapid heating and shorter come-up times (e.g., ~13 min for sturgeon caviar) compared with the water bath method (~19 min). Because water circulation in the RF system was stopped, water temperature declined sharply (Fig. 3) while the product continued to be heated by RF energy. Table 2 also compares the come-up times for salmon and sturgeon caviars heated at 60.0, 63.0, and 65.0°C with the RF system compared with the water bath method. These results agree with those obtained by Wang et al. (40) for heating macaroni and cheese with RF compared with a conventional retort. Shorter process times and better product quality with the use of RF processing were obtained by Wang et al. (40).

Figure 4 shows the temperature history of salmon caviar heated by an RF system at 65°C. A clear nonuniformity in the temperature progress of the product was observed. Nonetheless, it took just 9.2 min for the coldest point to reach the required temperature, which was almost half of the come-up time needed to bring the temperature up to 65.0°C in the water bath. The potentially coldest spot was localized at sensor position 2, which represents the geometric center of the CPVC tube. The sharp decline in all the curves is a result of cessation of water circulation and immediate immersion of the product in an ice bath.

The thermal inactivation kinetics obtained in this and an earlier study (2) were used to develop an RF-based thermal pasteurization process. Before conducting the RF treatment, preliminary experiments were carried out to deter-

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**TABLE 2.** Comparison of come-up times for thermally processed salmon and sturgeon caviar by the radio frequency and water bath methods

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Come-up time (min) a</th>
<th>Radio frequency</th>
<th>Water bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>13.32 ± 0.12 b</td>
<td>17.47 ± 0.35 A</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>10.09 ± 2.00 b</td>
<td>18.46 ± 1.53 A</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>9.18 ± 2.55 b</td>
<td>18.94 ± 0.25 A</td>
<td></td>
</tr>
<tr>
<td>Sturgeon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>13.06 ± 1.26 b</td>
<td>17.63 ± 0.18 A</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>13.30 ± 0.98 b</td>
<td>18.28 ± 0.25 A</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>13.10 ± 0.40 b</td>
<td>18.57 ± 0.79 A</td>
<td></td>
</tr>
</tbody>
</table>

*Data represent means ± standard deviations of two experiments. Means with different letters in the same row are significantly different (*P* < 0.05).
mine a suitable concentration of nisin to use in the RF thermal treatment. A nisin concentration of 500 IU/ml was used for the treatment of salmon and sturgeon caviar before RF heating. Treatment of salmon caviar with 500 IU/ml nisin resulted in an almost 1.2-log reduction in \textit{L. innocua} and a 1.8-log reduction in the total mesophilic microorganism population.

Figure 5 shows the effect of RF heating at 60.0, 63.0, and 65.0°C without (Fig. 5A) or with (Fig. 5B) 500 IU nisin per ml on the inactivation of three strains of \textit{L. innocua} and total mesophilic microorganisms in salmon caviar. Even though the processing time was shortened by half when nisin was used with RF treatment, no significant differences \((P > 0.05)\) were observed at 60.0 and 63.0°C in the nisin-treated and untreated salmon caviar. Interestingly, a total elimination of \textit{Listeria} cells was observed at 65.0°C with nisin treatment (~6.5-log reduction), which was significantly lower \((P < 0.05)\) compared with RF treatment at 65.0°C alone. Treatment at 65.0°C alone resulted in a reduction of only ~3 log units in \textit{L. innocua} in the RF-treated salmon caviar. This result suggests a synergistic interaction between nisin and RF heating against \textit{L. innocua}. Likewise, a similar inhibition pattern was observed for total mesophilic microorganisms. No significant differences in the mesophile counts were noticed at 60.0 and 63.0°C with and without nisin treatment despite the processing time differences.

Inhibition of \textit{L. innocua} via RF heating alone or with nisin in sturgeon caviar is shown in Fig. 6. Nisin treatment of sturgeon caviar at 500 IU/ml resulted in an almost 1.5-log reduction in populations of \textit{L. innocua} and total mesophilic microbes. A highly significant \((P < 0.05)\) and

![Figure 5](http://meridian.allenpress.com/jfp/article-pdf/67/9/1848/1675270/0362-028x-67_9_1848.pdf)

**Figure 5.** Survivors of \textit{L. innocua} (■) and total mesophiles (■) in salmon caviar treated at 60, 63, and 65°C by radio frequency processing without (A) or with (B) nisin (500 IU/ml). In the nisin-untreated samples, RF heating was equivalent to an 8-D process. In the nisin-treated samples, RF heating was equivalent to a 4-D process.

![Figure 6](http://meridian.allenpress.com/jfp/article-pdf/67/9/1848/1675270/0362-028x-67_9_1848.pdf)

**Figure 6.** Survivors of \textit{L. innocua} (■) and total mesophiles (■) in sturgeon caviar treated at 60, 63, and 65°C by radio frequency processing without (A) or with (B) nisin (500 IU/ml). In the nisin-untreated samples, RF heating was equivalent to an 8-D process. In the nisin-treated samples, RF heating was equivalent to a 4-D process.
greater reduction of *L. innocua* and total mesophiles was observed in the RF–nisin treatment compared with RF heating alone at all temperatures. An almost 7-log reduction in the *L. innocua* population was observed in sturgeon caviar heated by RF at 60°C with nisin compared with a <4-log reduction with RF heating alone. Similarly, a nearly 4.5-log reduction by an RF–nisin combination at 63°C was observed compared with a 2-log reduction at 63°C with RF alone. Similar patterns of inhibition were also seen for total mesophiles. *L. innocua* and total mesophilic microorganisms were completely eliminated in sturgeon caviar treated by RF heating at 65°C with 500 IU/ml nisin. In this treatment, no injured *Listeria* cells were recovered by the overlay method, suggesting that such a treatment could improve the safety of heat-labile caviar products and provide further evidence of synergism between RF heating and nisin.

Time–temperature history at the coldest point for a conventional thermal process is generally predictable for solid and liquid foods. For example, for a conduction-heated (solid) food, it is usually the geometric center. In MW heating, even for a solid food, the coldest point is less straightforward and more sophisticated to predict and can change during the heating process, depending on a number of food and oven factors (10). This possibly could explain the less pronounced antilisterial action of some RF heating in which high counts of *L. innocua* were recovered.

Two mechanisms are proposed for inactivation of microorganisms by MW and possibly RF heating. The first states that microwaves inactivate microorganisms entirely by heat through mechanisms comparable to other biophysical processes induced by heat, such as denaturation of enzymes, proteins, nucleic acids, or other vital components, as well as disruption of membranes (17). However, four predominant theories have been used to explain nonthermal inactivation by microwaves or “cold pasteurization”: selective heating, electroproportion, cell membrane rupture, and magnetic field coupling (21).

Color measurements (L*, a*, and b*) of the control and RF-heated salmon and sturgeon caviar with or without nisin are shown in Table 3. No significant differences (*P* > 0.05) in the L*, a*, and b* values were detected between the control and the RF-treated salmon caviar with or without nisin, except in the case of salmon caviar treated by RF at 65.0°C, at which temperature significant differences in the L*, a*, and b* values were detected. For sturgeon caviar treated by RF heating, the visual quality of the product did not change at the different temperatures in the presence or absence of nisin. There were slight significant changes (*P* < 0.05) in the L* values between the control and the RF-heated product with nisin at 60.0°C (Table 3). Generally, the visual quality of the caviar was acceptable. No serious quality changes were observed.

This study demonstrated that mild RF heating in conjunction with nisin treatment resulted in reductions in the viability of *L. innocua* and total mesophiles. This is significant because caviar is extremely heat sensitive and there is zero tolerance for *L. monocytogenes* in seafood products. Commercial adoption of a combined application of nisin with mild RF heating at 65.0°C has the potential to improve the safety and to save the shelf life of perishable salmon and sturgeon caviar. Color measurement and informal sensory evaluation indicate that product quality is maintained with the use of this combined treatment.

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