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## Research Note

# Efficacy of LISTEX P100 at Different Concentrations for Reduction of *Listeria monocytogenes* Inoculated in Sashimi

**S. MIGUÉIS,* C. SARAIVA, AND A. ESTEVES**

*School of Agrarian and Veterinary Sciences, Animal and Veterinary Research Centre, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal (ORCID: http://orcid.org/0000-0003-4521-2202 [S.M.])*

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## ABSTRACT

Sushi restaurants have become quite popular in Europe, with an increase in the consumption of the sashimi speciality. Pathogenic bacteria such as *Listeria monocytogenes, Staphylococcus aureus,* and *Bacillus cereus* have been reported in this kind of food. Controlling the presence and multiplication of *L. monocytogenes* is a challenge for food safety management systems owing to its ubiquitous presence and psychrotrophic growth. Bacteriophages have been used as pathogenic biocide agents for decades. The bacteriophage P100, present in LISTEX P100, was used in this study to understand the possibility of implementing a new critical control point for *L. monocytogenes* in sashimi preparation and sale. Different concentrations of LISTEX P100 were used for reduction of *L. monocytogenes* in inoculated samples at 3 and 22°C. The reduction in initial counts of 2 log CFU/g was effective in the first 24 h with the 8-log PFU/g inoculation. Promising results were obtained in assays with the 6-log CFU/g initial counts and the 8-log PFU/g inoculation, at 22°C, achieving a maximum reduction of 4.44 log CFU/g, compared with the control group. These results seem to confirm that bacteriophages can be an option in reducing the population of the *L. monocytogenes* pathogenic bacteria in sashimi, mainly in takeaway sales.

Key words: Bacteriophages; Hazard analysis and critical control point; *Listeria monocytogenes*; LISTEX P100; Sashimi

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Sushi restaurants have become quite popular in Europe. These kinds of restaurants provide the consumer with new gastronomic experiences, mainly for Occidental consumers who like to try unusual and unique flavors.

One different way to consume fish is eating a sliced raw finfish (sashimi) served with or without vegetables. This kind of speciality brings forth some food safety issues owing to the absence of any biocide treatment. Food safety is mainly granted by the implementation of good hygienic practices within a hazard analysis and critical control point (HACCP) system that was created to prevent microorganism contamination and growth from reception to consumption.

However, some authors have expressed concerns about sashimi's microbiological quality because pathogenic bacteria such as *Listeria monocytogenes, Staphylococcus aureus,* and *Bacillus cereus* have already been found in sashimi *(1, 22, 23)*.

*L. monocytogenes* is one of the bacterial species causing more concern in sashimi preparation. Its presence was detected in 24.6% of takeaway sashimi meals collected in northern Portugal, with 3.5% of them having above legal limits *(22)*. The high mortality rate of 15 to 40% *(9, 31),* mainly in risk groups such as children, pregnant women, the elderly, and immunosuppressed people, has also been contributing to this concern.

*L. monocytogenes* is a ubiquitous and psychrotrophic pathogenic bacterium that has the ability of grow above –1°C *(7, 8)*. Combined with the fact that it is impossible to establish a thermal biocide critical control point (CCP) in the HACCP plans of this food specialty, a concern for food safety arises. Currently, the implemented control measures established in the HACCP plans are aimed at preventing the microbial growth; however, with refrigerated storage at 3°C, these measures are insufficient in preventing growth. In addition, the European Food Safety Authority *(8)* states that this temperature is sufficient to ensure food safety for 11 days (<100 CFU/g) if the start count is equal to 1 CFU/g of *L. monocytogenes*. Another concern related to *L. monocytogenes* is the takeaway service option. When this service is used at an uncontrolled temperature, it can give way to rapid and uncontrolled growth of *L. monocytogenes*. Under ideal conditions and with normal fish pH (pH 6), *L. monocytogenes* can double in population in 1 h at room temperature (20 to 21°C) *(26)*.

Hence, it is clear that *L. monocytogenes* is a challenge to food safety of sashimi. Therefore, a new approach is needed to eliminate or reduce this hazard to acceptable levels in the preparation stage. Some alternative methodologies for the control of this pathogen may include the application of high pressure, the addition of chemical compounds, or the use of biological solutions such as bacteriophages. However, application of high pressure revealed undesirable quality changes in the fresh tuna *(27)*.

* Author for correspondence. Tel: 00351 259 350659; Fax: +351 259 350 482; E-mail: samuel.migueis@gmail.com.

The addition of chemical compounds does not always comply with the precautionary principle that states that consumers must be protected from unnecessary exposure to chemicals in food. The use of biological solutions such as bacteriophages seems to be the most promising choice due to its safety and ubiquitous characteristics of bacteriophages. Bacteriophages have been reported as the most abundant organisms on this planet *(29)*; they are found in vegetables *(16, 17),* dairy products *(3),* the human digestive tract *(24, 25, 28),* saliva *(2),* skin *(12),* and the respiratory tract *(36)*. Moreover, a high amount of bacteriophages is regularly ingested by animals and humans with neither risk occurrence nor side effects on nontarget cells *(32, 35)*.

Bacteriophages are specific obligatory parasites of bacterial cells that maintain an inert metabolic state until attacking a bacterium host. They can be divided in two major groups: virulent phages and temperate phages. Virulent bacteriophages instantaneously redirect the host's metabolism to produce new phage virions that are released upon cell death within minutes to hours after the initial infection event. Temperate phages act more imperceptibly and can replicate either by lysing the host cell or by establishing a commensal relationship with their host bacteria *(5)*.

Because of the lytic cycle, virulent bacteriophages are an excellent option in controlling pathogens in food *(20)*. This approach, using LISTEX P100 bacteriophage, was tested and found to be successful in reducing counts of *L. monocytogenes* in raw salmon fillet tissue *(33)* and in fresh channel catfish fillets *(34)*. Other successful studies were performed in fruit *(18)*; surface-ripened soft cheese *(4)*; hot dogs, cooked and sliced turkey breast meat, smoked salmon, mixed seafood, chocolate milk, mozzarella cheese brine, iceberg lettuce, and cabbage *(15)*; and sausage *(30)*.

European Food Safety Authority scientific opinion reports that no acquisition of resistant genes to therapeutic antibiotics by bacteriophage-mediated transduction in *Listeria* spp. has been described as of yet *(10)*.

To follow the guidelines of the scientific opinion of the European Food Safety Authority *(10)* for further studies about the efficacy of LISTEX P100 in naturally contaminated ready-to-eat foods, this study put in place a new possible solution for the reduction or elimination of *L. monocytogenes* in tuna sashimi, ensuring safety mainly in a takeaway sale, by testing the LISTEX P100 in tuna sashimi samples. The effectiveness of LISTEX P100 was tested in a hypothetical CCP at the preparation stage to reduce or eliminate *L. monocytogenes* serotype 1/2a by using different concentrations of LISTEX P100 in tuna sashimi stored at different temperatures.

## MATERIALS AND METHODS

**Bacteria and bacteriophages.** *L. monocytogenes* serotype 1/2a (ATCC BAA-679D-5) was used as a courtesy of the Portuguese Army's Laboratory of Bromatology and Biological Defense (Lisbon, Portugal) and LISTEX P100 phage, courtesy of Micreos (Amsterdam, The Netherlands).

The *L. monocytogenes* strain was preserved in tryptic soy broth (TSB) slants. A culture of the strain was prepared in 10-mL tubes containing TSB solution and left for 24 h at 37°C. The tubes were then subjected to centrifugation at 10,000 $\times$ *g* for 10 min and washed with sterile 0.9% NaCl. The centrifugation was done twice until the solution became clear.

The last pellet was suspended again to 1.2 optical density at 600 nm (model V-530 spectrophotometer, JASCO International Co., Ltd., Tokyo, Japan) to obtain a cell concentration of 8 log CFU/mL. This solution was used directly in *L. monocytogenes* (hereafter LM) 6 (6 log CFU/g) assays. For LM2 (2 log CFU/g) assays, a diluted decimal solution of 4 log CFU/mL was used.

The original LISTEX P100 stock solution, in buffered saline, had a concentration of 10.5 log PFU/mL. This concentration was confirmed using the direct plating plaque assay as described by Mazzocco et al. *(21),* using the ATCC BAA-679D-5 *L. monocytogenes* strain.

To perform LISTEX P100 (hereafter LTX) 8 (8 log PFU/g) and LTX5 (5 log PFU/g) assays, diluted solutions of 10 log and 7 log PFU/mL, respectively, were prepared using the dilution equation $C1 \times V1 = C2 \times V2$.

**Tuna samples.** Frozen tuna loins (*Thunnus albacares*), weighing about 2 to 3 kg, were acquired at the supermarket and transported in an ice bag to the laboratory within 10 min. Before inoculation, loins were kept at 2°C for 1 h. Then, slices of about 1 cm in thickness were aseptically cut. Next, with a square (2 by 2 cm) sterile metal tube, cuts were made in small pieces of 2 cm of edge with approximately 1 cm of height. The tuna pieces were put on a ventilated sterile 55-mm petri dish, which was previously identified.

The assay was done in quadruplicate. Each batch was submitted to two temperature groups: 3 and 22°C. Each temperature sample was then subdivided into two groups, with a different concentration of the *L. monocytogenes* inoculated: LM2 = 2 log CFU/g and LM6 = 6 log CFU/g. Each group inoculated with *L. monocytogenes,* LM2 and LM6, was treated with three different concentrations of LISTEX P100: LTX0 = 0 log PFU/g (control), LTX5 = 5 log PFU/g, and LTX8 = 8 log PFU/g. All groups of each batch were tested at 13 times: 0, 2, 4, 6, 12, 24, 36, 48, 72, 144, 216, 288, and 336 h.

**Contamination procedure.** Each piece was weighed before inoculation, showing an average of 4.10 g. The inoculated volume of *L. monocytogenes* (LM2 = 2 log CFU/g and LM6 = 6 log CFU/g) was calculated assuming a 1-to-1 ratio (grams and milliliters), using the following formula:

$$\text{Inoculation volume } (\mu\text{L}) = \left(\frac{\text{desired CFU or PFU/g} \times \text{sample weight (g)}}{\text{initial solution UFC or PFU/mL}}\right) \times 1,000$$

An average volume of 40.96 μL was inoculated by sample. The total volume of inoculation was divided by 5, and the *L. monocytogenes* solution was inoculated with a sterile micropipette in five different points on the upper surface of the cut, with four points on each edge and one point in the middle.

**Phage treatment.** After 30 min of *L. monocytogenes* inoculation, the calculated volume of LISTEX P100 for each treatment (LTX5 = 5 log PFU/g and LTX8 = 8 log PFU/mL) was added in the same locations of the *L. monocytogenes* inoculation, with 30 min of dry period before start of the different times considered above. The same formula used in the contamination procedure was used for this procedure. The multiplicity of infection for the LM6 assay was 0.1 and 100 for LTX5 and LTX8, respectively, and for the LM2 assay, it was $10^3$ and $10^6$ for LTX5 and LTX8, respectively.

TABLE 1. *Tuna sashimi 3°C test with two groups of* L. monocytogenes *concentrations: 2 log/g (LM2) and 6 log/g (LM6)*[a]

| | LM2 | | | | LM6 | | | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | LTX0 (untreated) | LTX5 | LTX8 | Significance | LTX0 (untreated) | LTX5 | LTX8 | Significance |
| 0 | 0.52 ± 0.90 AB | 1.38 ± 0.38 A | 0.00 ± 0.00 B | 0.033 | 6.18 ± 0.50 | 6.12 ± 0.27 | 5.61 ± 0.33 | 0.123 |
| 2 | 1.13 ± 1.60 | 1.18 ± 0.39 | 0.00 ± 0.00 | 0.147 | 6.49 ± 0.21 A | 6.07 ± 0.24 AB | 5.74 ± 0.11 B | 0.007 |
| 4 | 0.98 ± 1.39 | 0.89 ± 0.44 | 0.00 ± 0.00 | 0.209 | 6.44 ± 0.17 A | 5.95 ± 0.27 AB | 5.62 ± 0.26 B | 0.035 |
| 6 | 1.13 ± 1.60 | 0.84 ± 0.09 | 0.00 ± 0.00 | 0.195 | 6.40 ± 2.19 A | 6.04 ± 0.24 AB | 5.61 ± 0.35 B | 0.040 |
| 12 | 1.01 ± 1.49 | 1.15 ± 0.21 | 0.00 ± 0.00 | 0.174 | 6.68 ± 0.30 | 5.83 ± 0.53 | 5.72 ± 0.22 | 0.086 |
| 24 | 0.98 ± 1.39 | 0.98 ± 0.16 | 0.23 ± 0.22 | 0.362 | 6.51 ± 0.11 A | 6.05 ± 0.33 AB | 5.70 ± 0.15 B | 0.016 |
| 36 | 1.00 ± 1.41 | 1.14 ± 0.12 | 0.13 ± 0.18 | 0.369 | 6.35 ± 0.20 | 5.67 ± 0.62 | 5.44 ± 0.29 | 0.137 |
| 48 | 1.03 ± 1.45 | 0.94 ± 0.33 | 0.00 ± 0.00 | 0.240 | 6.45 ± 0.31 A | 5.92 ± 0.23 AB | 5.83 ± 0.03 B | 0.019 |
| 72 | 1.06 ± 1.49 | 0.44 ± 0.76 | 0.00 ± 0.00 | 0.436 | 6.60 ± 0.41 A | 5.96 ± 0.16 AB | 5.52 ± 0.40 B | 0.017 |
| 144 | 0.98 ± 1.39 | 0.72 ± 0.64 | 0.00 ± 0.00 | 0.368 | 7.60 ± 0.30 A | 5.86 ± 0.28 B | 5.61 ± 0.23 B | <0.001 |
| 216 | 1.38 ± 1.95 | 0.94 ± 0.19 | 0.33 ± 0.47 | 0.696 | 7.53 ± 0.57 | 6.51 ± 1.14 | 6.24 ± 1.21 | 0.437 |
| 288 | 1.77 ± 2.50 | 1.08 ± 0.92 | 0.00 ± 0.00 | 0.669 | 8.25 ± 0.32 | 6.74 ± 1.00 | 6.04 ± 1.68 | 0.257 |
| 336 | 3.33 ± 0.95 A | 0.85 ± 0.74 B | 0.00 ± 0.00 B | 0.020 | 8.25 ± 1.77 | 6.84 ± 0.67 | 6.36 ± 0.98 | 0.071 |

[a] Each group was inoculated with three levels of LISTEX P100 concentration: 0 PFU/mL (LTX0; untreated), $10^5$ PFU/mL (LTX5), and $5 \times 10^7$ PFU/mL (LTX8). For each *L. monocytogenes* concentration, means within a row with different letters differ significantly ($P < 0.05$).

**Monitoring bacterial counts.** Aseptically, each tuna sample was removed from the petri dish and put into a stomacher bag, adding the respective volume of 0.9% NaCl to a 1/25 dilution and then homogenizing for 2 min in a Stomacher lab blender (Worthing, West Sussex, UK). Ten milliliters of homogenization was centrifuged at 12,000 × *g* for 5 min. Supernatant was then removed, and the pellet was suspended in 10 mL of 0.9% NaCl solution. A serial 10-fold dilution was performed in the same solution, and 0.1 mL of each dilution was spread in PALCAM dishes (Biokar Diagnostics, Beauvais Cedex, France). Dishes were kept for 24 h at 37°C.

**Data analysis.** Univariate analysis of variance with Tukey's honestly significant difference post hoc test was performed to identify statistical differences between each treatment effect, LTX0, LTX5, and LTX8, at each particular time, for a significance level of 0.05. All statistical analysis was completed with SPSS Statistics version 23 (IBM Corp., Armonk, NY).

## RESULTS

Results of LISTEX P100 treatments against *L. monocytogenes,* inoculated at two concentrations in tuna samples over the storage time at 3 and 22°C, are presented in Tables 1 and 2, respectively.

At 3°C for LM2, significant differences were observed between LTX groups in two different storage times: 0 and 336 h. LISTEX P100 treatment with higher a concentration of phage (LTX8) was revealed to be more efficient against *L. monocytogenes*; the levels of this pathogen are mostly near zero, having only three storage times with counts above zero: 24, 36, and 216 h (Table 1).

At 3°C for LM6, *L. monocytogenes* counts at each time differed significantly until 144 h (Table 1), except at 12 h, showing the LTX8 treatment as the most effective in reducing about 1 log/g, slowing and attenuating the log phase.

TABLE 2. *Tuna sashimi 22°C test with 2 groups of* L. monocytogenes *concentrations: 2 log/g (LM2) and 6 log/g (LM6)*[a]

| | LM2 | | | | LM6 | | | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | LTX0 | LTX5 | LTX8 | Significance | LTX0 | LTX5 | LTX8 | Significance |
| 0 | 1.11 ± 0.96 AB | 1.38 ± 0.38 A | 0.00 ± 0.00 B | 0.004 | 6.13 ± 0.78 | 6.12 ± 0.27 | 5.61 ± 0.33 | 0.242 |
| 2 | 1.86 ± 0.14 A | 1.44 ± 0.62 A | 0.00 ± 0.00 B | 0.007 | 6.48 ± 0.40 A | 6.24 ± 0.30 AB | 5.58 ± 0.26 B | 0.017 |
| 4 | 2.16 ± 0.27 A | 1.36 ± 0.31 B | 0.00 ± 0.00 C | <0.001 | 6.58 ± 0.39 A | 6.53 ± 0.07 A | 5.97 ± 0.21 B | 0.010 |
| 6 | 2.63 ± 0.18 A | 1.68 ± 0.16 B | 0.00 ± 0.00 C | <0.001 | 7.01 ± 0.31 A | 6.79 ± 0.20 A | 5.82 ± 0.04 B | 0.001 |
| 12 | 3.02 ± 0.03 A | 2.64 ± 0.71 AB | 0.59 ± 0.46 B | 0.029 | 7.57 ± 0.34 A | 6.16 ± 1.16 AB | 4.37 ± 0.84 B | 0.031 |
| 24 | 4.33 ± 0.32 A | 3.90 ± 0.30 A | 1.30 ± 0.37 B | 0.001 | 8.44 ± 0.65 A | 4.51 ± 0.32 B | 4.00 ± 1.24 B | 0.004 |
| 36 | 6.97 ± 0.32 A | 5.57 ± 0.51 A | 3.85 ± 0.79 B | 0.003 | 8.91 ± 0.47 A | 4.47 ± 0.78 B | 4.68 ± 1.42 B | 0.009 |
| 48 | 7.51 ± 0.42 A | 6.11 ± 1.09 AB | 4.44 ± 0.66 B | 0.023 | 9.04 ± 0.40 A | 5.18 ± 1.05 B | 6.00 ± 0.85 B | 0.004 |
| 72 | 8.56 ± 0.23 A | 6.29 ± 0.67 B | 4.55 ± 0.32 C | <0.001 | 9.66 ± 0.15 A | 6.40 ± 0.97 B | 6.98 ± 0.33 B | 0.005 |
| 144 | 8.06 ± 0.13 A | 6.42 ± 0.88 AB | 5.17 ± 0.90 B | 0.022 | 8.75 ± 0.30 A | 6.70 ± 0.73 B | 6.99 ± 0.47 B | 0.018 |
| 216 | 7.90 ± 1.27 | 6.92 ± 0.40 | 5.65 ± 1.32 | 0.101 | 8.94 ± 0.26 A | 7.35 ± 0.43 B | 7.89 ± 0.09 B | 0.002 |
| 288 | 8.16 ± 0.71 A | 6.92 ± 0.86 AB | 5.31 ± 0.94 B | 0.022 | 8.32 ± 0.43 | 7.44 ± 0.35 | 7.30 ± 0.79 | 0.190 |
| 336 | 8.08 ± 0.65 | 7.00 ± 1.20 | 5.65 ± 1.32 | 0.146 | 8.00 ± 0.46 | 7.38 ± 0.46 | 6.89 ± 1.20 | 0.371 |

[a] Each group was inoculated with three levels of LISTEX P100 concentration: 0 PFU/mL (LTX0; untreated), $10^5$ PFU/mL (LTX5), and $5 \times 10^7$ PFU/mL (LTX8). For each *L. monocytogenes* concentration, means within a row with different letters differ significantly ($P < 0.05$).

Data obtained from the 22°C assay are presented in Table 2. At LM2, the most effective LISTEX P100 treatment was also LTX8, with a significant difference compared with the control group (LTX0) at all hours (Table 2). The decrease of *L. monocytogenes* counts was evident in the LM6 assay for the factor LTX8, with a maximum reduction of 4.44 log CFU/g at 24 h, compared with the control group. A similar effect was observed in the LTX5 treatment, 4.44 log CFU/g, however, with a delay in response, showing the greatest decrease at 36 h (Table 2).

## DISCUSSION

In the assay at 3°C, representing the highest temperature that must be used in sashimi restaurants to guarantee the food safety and quality of fresh fish during storage, no significant ($P < 0.05$) *L. monocytogenes* reductions were observed in the LISTEX P100 factor at LM2's initial contamination, except at 0 h ($P < 0.05$) and 336 h. Despite the absence of statistical significance, LTX8 treatment results show counts of *L. monocytogenes* near zero most times, suggesting a tendency of reducing this pathogen (also probably owing to limit of count technique [100 CFU/g]). These data are in agreement with the results presented in other studies at 4°C and with raw fish *(34, 35)*. This ability of bacteriophage to infect its host at refrigerated temperatures had also been reported by Greer and Dilts *(14)* in relation to pork adipose tissue. Also, adsorption rate is influenced by several factors that are unique to each combination of phage, host, and environment *(13, 15)*. One example was reported by Leverentz et al. *(18)* with apple slices. They noted population declines rapidly to nondetectable levels within 30 min after the application of bacteriophages. However, the ability of bacteriophages to start their lytic growth program at this stress temperature (4°C) is limited, with bacterial lysis only occurring when suitable temperature is promoted *(12)*.

For food safety management systems in sashimi restaurants, this bacteriophage could be used for the implementation of a new CCP in the cooling stage of fish to ensure a reduction of *L. monocytogenes,* if present, to residual levels. This CCP can be achieved in a step before refrigeration by immersing or spraying the tuna loins with a solution of bacteriophage concentration close to 8 log PFU/g of fish.

In the LM6 3°C assay, LTX8 treatment had significant ($P < 0.05$) effects in reducing *L. monocytogenes* counts during almost all of the storage times. The maximum reduction was obtained at 36 h, i.e., near 1 log CFU/g of difference to control LTX0.

At 22°C, with an initial *L. monocytogenes* LM2 concentration, the most effective treatment was obtained with LTX8, which ensured an absence of LM for 6 h and a compliance with legal values up to 24 h (<2 log CFU/g). This result is in line with the results presented by Soni and Nannapaneni *(33),* who revealed that 120 min is sufficient for a 1.4- to 2.3-log *L. monocytogenes* reduction; with Chibeu et al. *(6),* who reported a reduction of 1.4 log CFU/cm$^2$ in 2 days at 10°C; with Rossi et al. *(30),* who showed a reduction of *L. monocytogenes* levels in fresh Brazilian sausage at about 2.5 log; and with Leverentz et al. *(19),* who reported a reduction of *L. monocytogenes* in honeydew melons between 2.0 to 4.6 log units, relative to the control.

This result should be considered in context, the takeaway service of restaurants, because it can guarantee the elimination or reduction of an initial population of *L. monocytogenes* of 2 log for 6 to 24 h until consumption by the consumer, and taking into account that sashimi is kept at room temperature some hours after sale.

*L. monocytogenes* counts were significantly reduced in assay LM6 at 22°C with LTX8 treatment, with a maximum of 4.4 log CFU/g registered at hour 24, compared to the control group LTX0. Similar results were obtained by Fister et al. *(11),* who observed a decrease of 3 to 5 log CFU/g in high *L. monocytogenes* concentrations.

A worse result was obtained with LTX5 treatment, with some assays presenting no significant differences to the control group (Table 2).

The absence of eradication of *L. monocytogenes* in this assay, as was reported by Fister et al. *(11),* confirms that P100 bacteriophages are unable to eradicate high initial concentrations of *L. monocytogenes*.

The data obtained with this study brought about a new approach to introduce a potential CCP at the preparation stage of sashimi meals for takeaway sale, with the addition of an 8-log PFU/g concentration of LISTEX P100 in sashimi and an expiration date of 12 h until consumption as the critical limit for the CCP.

Also, good indicators were obtained at 3°C in the LM2 assay, with the concentration of 8 log PFU/g being effective in reducing *L. monocytogenes* to near zero counts during the first 24 h, thereby providing the possibility of a new preventive measure in the storage stage of sashimi.

Results obtained with this study open a new approach to food safety management systems for restaurants that sell sashimi, in particular for the takeaway system. The effectiveness of LISTEX P100 against the pathogenic *L. monocytogenes* in sashimi allows us to recommend the implementation of two new *L. monocytogenes* biocide CCPs: one CCP in the storage stage (3°C) and the other CCP in the preparation stage of sashimi for takeaway sales. The latter CCP can be essential in eliminating or reducing *L. monocytogenes* to an acceptable level in cases of abusive room temperature.

The set critical limit of 8 log PFU/g for those CCPs can now be achieved, thereby providing a new method for sashimi food safety, and perhaps even for sushi.

An assay, with more realistic conditions, is needed to understand the effectiveness of this CCP on the ground, such as testing the effectiveness of the P100 bacteriophage in wild *L. monocytogenes*. An assay to study the effectiveness of mixtures of bacteriophages in different pathogenic bacteria would also be of particular interest.

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