Produce surface treatments based on bacteriophages and bacteriocin-producing cultures to consistently reduce 2-log of *Listeria monocytogenes* on leafy greens and pre-cut fruit and vegetables

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Summary

The aim of this project is to test commercially available post-process treatments to identify intervention strategies able to provide a consistent 2-log reduction of *Listeria monocytogenes* (Lm) on the surface of leafy greens and pre-cut fruit and vegetables. A risk categorization has been performed to provide the produce industry with information about which commodities are more susceptible and therefore require the application of a post-process treatment. Promising results against Lm have already been obtained for different commercial treatments tested under lab-scale experiments. However, experience shows that results obtained in the laboratory are difficult to extrapolate to the industry. This project also focuses on the validation of control strategies in commercial processing plants. The impact of the selected post-process treatments on the quality aspects during shelf-life is being examined.

Objectives

1. Risk prioritization analysis of critical fresh products based on the ability of Lm growth in different commodities at different temperatures; search for commercially available produce surface treatments that meet regulatory requirements for use on food.
2. Establishment of the efficacy of commercially available post-process treatments against Lm and Listeria spp. by lab-scale trials mimicking commercial conditions.
3. Evaluation of the impact of the selected post-process treatments on the organoleptic quality and shelf-life of selected fresh produce.
4. Validation of selected post-process treatments in commercial fresh produce facilities and establishment of operational standards. Collaboration with two IPs, Flensted (http://www.flensted.dk/) from Denmark and Florette (http://www.florette.es/) from Spain, will allow validation of the most efficient treatment/s under industrial conditions.

Methods

The experimental design is illustrated in **Figure 1**. After the washing and rinsing steps, the product was divided into three batches: untreated product as control, lactic acid bacteria–treated product, and bacteriophage-treated product. Four post-process treatments–SafePro® (CHR Hansen, DK), Phageguard Listex™ (Micreos Food Safety B.V.), HOLDBAC® (IFF, France) and ListShield™ (Intralityx, US)—were tested at CEBAS-CSIC (Spain) and at ISI Food Protection Aps (Denmark). After treatment application, the product was stored at 7°C and 85% RH in perforated bags for 15 days. During storage, samples were taken at different sampling points, including initially (0 d) and after 1, 5, 12 and 15 days of storage. At each sampling point, Lm, bacteriophage and bacteriocin-producing bacteria levels were enumerated.

Results to Date

In general, all the commodities treated with the post-process treatments showed lower Lm counts when compared with the untreated product. Differences between post-process treatments were observed, with the highest reduction achieved when fresh commodities were treated with Listex (**Figure 2**). For each post-process treatment, no differences in Lm counts were observed among storage days when results from all the commodities were represented together (**Figure 3**). Listex was the most promising post-process treatment in reducing Lm, showing significant reductions of about 2 log units at 5 and 10 days of storage, except for baby spinach (**Figure 4**).

Benefits to the Industry

The main objective of this project is the elaboration of a user-friendly guideline including specific operational standards that indicate step-by-step the conditions required to apply these treatments to particular commodities successfully. This guideline will include the most appropriate treatments for different scenarios under industrial conditions. The specific outcomes are: i) Identification of the most susceptible commodities where the application of produce surface treatments is necessary as an additional control point; ii) Selection of effective treatments and operational standards that assure the efficacy of the selected treatments; iii) Validation of operational standards in commercial processing lines to guarantee the feasibility of the treatments via experimentation; iv) A user-friendly guideline for producers through the CPS website including evidenced-based standards for the application of the selected treatments.

Figure 1. Scheme of the steps followed for the inoculation and post-process treatment application carried out for different commodities.

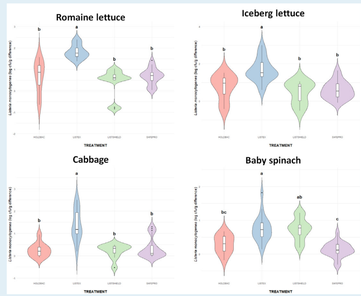

Figure 2. Differences in *Listeria monocytogenes* counts in each commodity (romaine lettuce, iceberg lettuce, cabbage and baby spinach) treated with a post-process treatment (Holdbac, Listex, Listshield and Safepro) or without the post-process treatment considering all the data through the shelf-life, including initially (0 d) and after 1, 5, 12 and 15 days. Values are the mean (n=15).

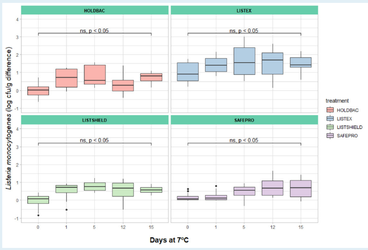

Figure 3. Differences in *Listeria monocytogenes* counts between each post-process treatment (Holdbac, Listex, Listshield and Safepro) and the control without the post-process treatment considering data from all the commodities at different storage days. Values are the mean (n=12). ns: non-significant.

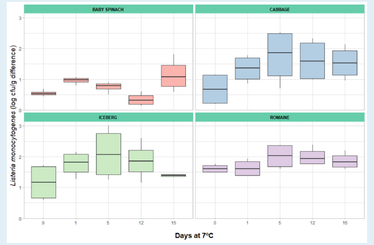

Figure 4. Differences in *Listeria monocytogenes* counts between post-process treatment with Listex and the control without the post-process treatment in each commodity at different storage days. Values are the mean (n= 3).