CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title
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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Principal Investigator
Ana Allende
CEBAS-CSIC, Campus de Espinardo
Murcia, E-30100, Spain
T: +34-968-396-200 Ext. 6377
E: aallende@cebas.csic.es

Co-Principal Investigators
Anne Elsser-Gravesen
ISI FOOD PROTECTION ApS
Agro Food Park 13
DK-8200, Aarhus N, Denmark
E: aeg@isifoodprotection.com

Maria I. Gil (Mabel)
CEBAS-CSIC, Campus de Espinardo
Spain
T: +34-968-396-200 Ext. 6315
E: migil@cebas.csic.es

Objectives

1. *Risk prioritization analysis of critical fresh produce commodities based on the ability of Listeria monocytogenes (Lm) to grow at different temperatures in different fresh commodities, and 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.*

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FINAL REPORT

Abstract
Produce decontamination practices represent a useful tool in further reducing the number of pathogenic microorganisms when used as part of a fully integrated control program. Nowadays, the fresh produce industry is still searching for effective control strategies to reduce, control and/or eliminate the risk of listeriosis in manufactured/ processed and packed produce. The aim of this project was to evaluate implementation of commercial post-process treatments to control *Listeria monocytogenes* (Lm) growth in leafy greens and fresh-cut fruits and vegetables. Two post-process treatments based on bacteriophages, including ListShield (Intralytix, US) and PhageGuard Listex (PhageGuard, US), and two bacteriocin-producing cultures, SafePro® (CHR Hansen, DK) and HOLDBAC® Listeria (DowDuPont, US), were tested in five different fresh fruits and vegetables. The first objective was focused on a risk prioritization analysis of the most relevant commodities, considering that the growth kinetics of Lm in fresh produce is strongly commodity-dependent. More than 800 data points obtained from a systematic review were used for performing the meta-analysis. Additionally, the Gamma Concept was validated to determine its suitability to predict the Lm behavior in a specific commodity, avoiding the extra resource expenditure of performing challenge tests. The results of the meta-analysis concluded that the most relevant fresh products that support the highest growth of Lm are leafy greens and melon. Data showed that the Gamma Concept is a very valuable tool to predict Lm behavior in leafy greens. The screening of the post-processed treatments in the five commodities showed that Listex was the most promising treatment to inhibit growth of Lm, as it consistently reduced growth of Lm by about 2 log units during the shelf-life of the product. The main Lm reduction by Listex was observed during the first 24 h of storage, and the efficacy did not increase along product shelf life. On the other hand, significant differences in the efficacy of Listex were observed among the different types of products. The results obtained showed that Listex can be safely applied to fresh product, as the sensory analysis did not show any significant difference between the untreated control and the treated product. The validation and verification activities associated with *Listeria* spp. and Lm preventive controls were possible thanks to collaboration with two industry cooperators, (Flensted (http://www.flensted.dk/) in Denmark and Florette (http://www.florette.es/) in Spain. Validation of the selected treatments included the monitorization in these commercial processing lines of the fine-tune critical parameters previously identified including application equipment adjustment, confirmation of application doses and quality impact on the product. The validation trials determined that the application of Listex at the optimum concentration is feasible under industrial conditions. It was confirmed that the quality of the product was not affected by the application of the treatment. It could be concluded that Listex is an efficient post-process treatment that could be safely applied to leafy greens as products that support high Lm growth. The use of bacteriophages is a promising option for the control of Lm in fresh produce.

Background
Experience tells us that the control of *Listeria monocytogenes* (Lm) in fresh produce requires a multiple hurdle approach including prevention in the field, avoidance of cross-contamination during processing, but also, and probably only for specific cases, the use of post-process treatments that effectively reduce the load of Lm throughout shelf-life, when present in raw materials. The combination of hurdles at critical points would be able to control Lm but also to improve sensory quality and even prolong the shelf-life of fruits and vegetables (Mogren et al., 2018). Produce decontamination practices represent a useful tool in further reducing the number of pathogenic microorganisms when used as part of a fully integrated control program (EFSA, 2016). These can also play an important role in preventing Lm growth, which remains a principal control element

(Buchanan et al., 2017). Several biological decontamination treatments, such as bacteriophages and bacterial protective cultures, meet regulatory requirements for use on several food products (Truchado et al., 2020).

Bai et al. (2016) defined bacteriophages as the next-generation biocontrol agents due to the potential of single phage and phage cocktail treatments to control various foodborne pathogens, constituting an alternative for conventional food preservatives. This is the case of the bacteriophage products ListShield™ from Intralytix (Baltimore, US) and PhageGuard Listex™ (PhageGuard, US). Both ListShield™ and PhageGuard Listex™ have been approved by the U.S. FDA for Lm control on food. Specifically, in fresh produce, 2-log reductions in the level of Lm have been reported in different fresh-cut fruits such as melon and pear stored at 10 °C (2.5–4.0 log pfu/g) (Oliveira et al., 2014). Previous results obtained in our research groups showed that PhageGuard Listex™ treatment reduced Lm in fresh-cut endive by 2.5 log, regardless of the point of treatment application (conveyor belt or centrifuge) (Truchado et al., 2020). Other studies have shown similar results. A bacteriophage treatment (10^8 pfu/ml) reduced Lm levels on carrots (2.02–2.88 log cfu/ml) after 6 days of refrigerated storage (Oladunjoye et al., 2017). The use of phages was also studied to inactivate Lm on melons (Leverentz et al., 2003). These authors obtained reductions of about 2.0–4.6 log cfu per sample. On fresh-cut spinach stored under modified atmosphere packaging, a bacteriophage cocktail reduced Lm populations compared to the uninoculated control, by 3.24 and 1.95 log cfu/g after 10 and 14 days at 10 °C, respectively (Boyacioglu et al., 2016). In general, the application of 6.0–8.0 log pfu/g or ml phage concentration significantly ($p < 0.05$) reduced Lm populations on inoculated fresh produce. However, most of the studies have been done based on lab-scale experiments and thus, results are difficult to extrapolate to the industrial conditions.

Another post-process principle that potentially can effectively reduce Lm is the use of bacteriocin-producing (Bac+) strains of lactic acid bacteria (LAB) that are used singly or in combination as protective cultures. These commercial Bac+ strains of LAB species are normally present in food products. Industrial cultures like SafePro® (Chr. Hansen Holding, Hoersholm, Denmark) and HOLDBAC® Listeria (DowDuPont, US) have been developed for Lm control. Previous studies have demonstrated the antimicrobial activity of commercial LAB solutions to inhibit the presence of foodborne pathogens on fresh produce. Gragg and Brashears (2010) reported that Bovamine® Meat Cultures inhibited *E. coli* O157:H7 by 1.25 log units on baby spinach stored at 7 °C for 12 days. Brown et al. (2011) observed that the application of commercial LAB, LactiGuard, decreased the levels of *E. coli* O157:H7 by 1.4 log units compared to the control after 9 days at 4 °C. A similar observation was made by Cálix-Lara et al. (2014), who reported that the application of LactiGuard to spinach reduced the levels of *E. coli* O157:H7 and *Salmonella* populations by 1.6 and 1.9 log cfu/g, respectively, after 12 days of storage at 7 °C. However, little is known about the capacity of bacteriocin-producing strains to control Lm growth when tested on fresh fruits and vegetables.

In general, the efficacy of these post-process treatments in reducing the Lm load has been mostly investigated in foods of animal origin, particularly on ready-to-eat (RTE) meat and cheese products. Previous studies have shown very promising results, but optimization of the use of these treatments should be done under commercial conditions on specific fresh products to get insight into their real potential. To our knowledge, the proof of concept on the influence of microorganism type, mode of application, doses, and storage temperature on the listericidal activity of these commercial treatments in reducing Lm loads on different fresh produce and formats has not been proven yet under commercial conditions. Consequently, there is an urgent need for the evaluation of commercially available post-process treatments based on bacteriophages and bacteriocin-producing cultures able to reduce Lm by 2 log units on different products such as leafy greens and pre-cut fruits and vegetables.

This project evaluated the suitability of commercial post-process treatments to control Lm growth by inactivating Lm cells and inhibiting their growth in selected fruits and vegetables that support Lm growth as well as the impact of these treatments on their organoleptic quality over the shelf-life. To achieve this goal, the activities included in this project were divided into four objectives:

1) Risk prioritization analysis of most relevant fresh produce commodities based on their ability to support growth of Lm.
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.

Research Methods and Results

Objective 1

A systematic review was conducted to retrieve the most relevant information to risk prioritize fresh fruits and vegetables based on their ability to support Lm growth, expressed as Lm exponential growth rate (EGR), in different commodities. A general search was conducted in Web of ScienceTM Core Collection (1986–January 2022) to retrieve review papers, research papers, book sections and books summarizing information about the growth of Lm on fresh produce. The systematic review was conducted following the recommendations previously described (Pautasso, 2003; Marik et al., 2020). **Table 1** includes the selected strings used for the search. The records were screened for information about the growth of Lm in fresh fruits and vegetables in three screening steps: (1) titles, (2) abstracts and (3) full-text documents to further identify records to be excluded based on criteria related to report and study characteristics considering whether the record contains quantitative data about Lm behavior (growth/survival or inactivation) on fresh produce. The studies focused on *Listeria* spp. were excluded. Selected full-text documents were screened to extract the relevant information (growth, survival or inactivation of Lm) to determine the EGR of Lm.

$$EGR(T) = EGR\ (5°C)\ \times \left(\frac{T - T_{min}}{5 - T_{min}}\right)^2\ if\ T\ <\ T_{min} \rightarrow EGR(T) = 0$$

Quantitative behavior data was extracted from texts, tables and graphics (Web Plot digitizer (Ankit Rohatgi, San Francisco, CA; https://apps.automeris.io/wpd/)) using pre-defined tables in an Excel file (Microsoft Corp., Redmond, WA). In cases where the EGR was not reported, DMFit software (https://nmr.cemhti.cnrs-orleans.fr/dmfit/) was used. The Lm behavior (log cfu/g or log reductions) reported by selected studies was qualitatively classified into inactivation, survival, growth according to the following statements:

- Inactivation: reduction of Lm higher than 1 log.
- Survival: reduction or increase of Lm lower or equal to 1 log.
- Growth: increase of Lm higher than 1 log.

Besides the Lm behavior in fresh fruits and vegetables, other relevant parameters were collected when available: pH, water activity (a_w), temperature (T^a), microbial strain (and conditions for preparing the inoculation culture), and analysis (method and media, time and storage conditions including packaging). The variability of $EGR_{5°C}$ of Lm was assumed to be log-normally distributed and the mean, standard deviation, and truncated maximum rate (mean + 2 standard deviations) were considered.

A further evaluation of the Lm behavior in fruits and vegetables was performed using a predictive modelling approach based on the Gamma Concept**.** Cardinal models such as the **Gamma Concept** ($\boldsymbol{\gamma}$) are used for the prediction of microorganism growth in food products, in which relevant environmental factors such as T^a, pH, and a_w are introduced as individual terms with microbe-dependent parameters, and the effect of foodstuffs on the growth rates of these species are described with food- and microbe-dependent parameters (Pinon et al., 2004). Based on this secondary model, if $\boldsymbol{\gamma}$ <0, the model predicts no growth of Lm; If 1 ≥ $\boldsymbol{\gamma}$ >0, the model predicts growth of Lm. The Gamma Concept was integrated into the following model:

$$\sqrt{\mu_{max}} = \sqrt{\mu_{opt} \cdot \prod \gamma_{x_i}(X_i) \cdot \xi}$$

Where,

μ_{max} is the Lm growth rate retrieved from the selected studies or estimated (DMFIT);

μ_{opt} is the Lm optimum growth rate when a_w, pH, T^a or other factors do not have an impact; and $\prod \gamma_{x_i}(X_i) \cdot \xi$ is the gamma value ($\boldsymbol{\gamma}$), which quantifies the impact of different factors (T^a, pH, a_w) in the Lm growth.

In general, μ_{opt} is higher when the $\boldsymbol{\gamma}$ value is lower and/or when μ_{max} is higher. When the μ_{opt} is high it means that other factors, different than those included in the $\boldsymbol{\gamma}$ value such as type of commodity, inoculation method, inoculum size, storage conditions, have an impact on the Lm growth.

The selection of the commodities more relevant to be tested in *Objective 2* was done based on the results obtained from the meta-analysis as well as the discussions that took place among the project research members, including industrial partners. Therefore, the risk prioritization took into account not only the results from the meta-analysis, but also other relevant parameters such as the economic relevance of the commodities, the volumes produced for each commodity, as well as the expert knowledge of the stakeholders.

Objective 2

Four commercially available post-process treatments were applied to the selected commodities. Two were based on bacteriophages, including ListShield™ from Intralytix (Baltimore, US) and PhageGuard Listex™ (Micreos Food Safety B.V., NL), and two contained protective cultures, namely SafePro® (Chr. Hansen Holding, Hoersholm, Denmark) and HOLDBAC® Listeria (DowDuPont, US). Working solutions of PhageGuard Listex™ and SafePro® (Chr. Hansen Holding, Hoersholm, Denmark) were prepared as described by Truchado et al. (2020). In the case of ListShield™ and HOLDBAC®, working solutions were prepared following the manufacturers' recommendations. The target concentration was, in all the cases, about 10^6–10^7 pfu/g or cfu/g of the product. The aim was to mimic, as much as possible, real conditions used by the industry, including the format of the products, the post-process application of treatments, and the storage conditions. **Figure 1** describes the experimental setup used for conducting the trials.

Experiments were performed with four different leafy greens (including baby spinach, chopped romaine lettuce, chopped iceberg lettuce, and chopped cabbage) and melon chunks. Fresh, whole fruits and vegetables were obtained from local suppliers and kept refrigerated (7 °C) for a maximum of 24 h. The raw material was tested to confirm the absence of Lm before processing. Leafy greens were processed following industry practices, which included the removal of external leaves and core, and cutting if needed (**Figure 1**, step 1). Romaine and iceberg lettuce heads were cut in 3 cm pieces, cabbage in 3 mm strips, and in the case of baby spinach, the entire leaves were used. The melons were peeled and cut into 3 cm chunks.

Inoculation was performed following the description included in Truchado et al. (2020). Briefly, a six-strain Lm cocktail was applied in a final concentration of 10^4 cfu/mL. The final concentration of the inoculum was confirmed by plating duplicate serial suspension dilutions on OCLA agar (Scharlau, Barcelona, Spain) followed by incubation at 37 °C for 24 h. About 1 kg of each product was placed in a tray inside the inoculation chamber to contain aerosols formed during the spray inoculation of the cocktail (**Figure 1**, step 2). The product was allowed to dry inside the inoculation cabinet for at least 12–24 h. The inoculation and the drying steps were performed in a cold room at 4 °C. After drying, the product was washed in 10 liters of chlorinated water (5 ppm of free chlorine) for 1 min and centrifuged for 1 min (**Figure 1**, step 3). Then, the product was divided into two batches of 500 g each (untreated and treated batches).

The batch designated as treated was sprayed for 30 seconds with the post-process solutions. Application of the treatments was performed following the recommendations of the suppliers by using the Spraying System CO® device (AUTOJET model 1550+ spray system with a lab-scale tank and a J-series nozzle) (**Figure 1**, step 4). The treated product was then divided and packed in 25 g product bags. Individual bags (230 mm × 280 mm) were made of oriented polypropylene (OPP) film of 35 µm thickness (Amcor Flexibles, Bristol, UK) showing O_2 permeance of 2.629 E12 mol/m^2 s Pa, CO_2 permeance of 9.84 E12 mol/m^2 s Pa and H_2O permeance of 5.408 E6 mol/m^2 s Pa. A total of 32 bags were prepared for each product and treatment, including control and treated product, to allow the analysis of 4 replicates per sampling day. All bags were thermally sealed (Magneta 421, Audion Elektro BV, Netherlands) (**Figure 1**, step 5). Samples were taken after 0, 1, 5, 12, and 15 days of storage at 7 °C.

Samples were processed for enumeration of Lm, phages and bacteriocin-producing bacteria following the same protocol as described by Truchado et al. (2020) with some modifications. In this case, Half Fraser broth was used with samples for homogenization in the stomacher, and the OCLA plates were incubated at 27 °C for 48 h.

The trials performed for each product and post-process treatment were made at least in duplicate and in some cases even in triplicate, always separated in time. Each sampling point corresponds to 8–12 different data points per product and treatment. The data generated were analyzed using a non-parametric test. Based on the nature of the experiments and the final adjustment of the data, the selected approach was a mixed model. The statistical analysis was performed using the R software (R Core Team, 2021) as a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

Among the four commercially available post-process treatments, the most effective one inhibiting Lm growth during storage was selected to progress with *Objectives 3* and *4*.

Objective 3

This objective focused on evaluating the impact of the most effective post-process treatment on the sensory quality of the selected commodities. In this case, the selected fresh fruits and vegetables were subjected to the same processing steps as previously described in *Objective 2*, without the inoculation of Lm cocktail. Briefly, the product was processed, spray treated (tap water as control and treated product with the post-process treatments), packaged (25 g per bag) and stored at 7 °C for 15 days. Cabbage and baby spinach were packaged in a passive modified atmosphere (MAP) while iceberg and romaine lettuce were flushed with N_2 to reduce the initial concentration of O_2 to a range between 0–3%. An industrial-scale vertical packaging machine (ETNA HT 280; Ulma Packaging) was used.

The organoleptic attributes included in the analysis were browning, texture, flavor, spoilage, and general visual quality of the fresh-cut produce (control and treated). The evaluation of the product was done initially (day 0) and after 5, 12 and 15 days of storage by a six-member expert panel. Coded (3 digits) samples were presented individually to the trained evaluators (judges) to make independent evaluations. Browning, off-flavor and odor, texture and spoilage were scored on a continuous scale with a range of 0–10 (0= absence; 10= completely damaged). The overall visual aspect was scored on a continuous 0–10 scale (0= extremely unpleasant; 10=extremely pleasant). At every sampling point, photographs of two replicates of the same product were taken to objectively measure color changes. Photographs were taken using a Canon EOS (70D) (Canon Lens: EF-S18-55mm f/3,5-5.6 IS) camera with a shutter speed of 1/50 sec II and an aperture of f / 5,6 ISO: 100. A RGB spectrum (SpyderCheckr™ v1.3, Datacolour, Electrical & Electronic Manufacturing, Lawrenceville, NJ, USA) was used to calibrate the reference color chart. Photographs were processed (background removing and format conversion) with Adobe® Photoshop® 2020 (V 21.1.0, Adobe Photoshop CS, 2004, Berkeley, CA, USA). ImageJ (v 1.53 K, Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA) (URL https://imagej.nih.gov/ij/, 1997-2018) was used to convert RGB image to Lab stack and obtain the image values in the CIE L* a* b* color scale. In detail, L* indicates the lightness from black (0 value) to white (100 value), a* the redness (+) or greenness (-), and b* the yellowness (+) or blueness (-). The color was measured on the surface of two portions of product for each replicate. The instrument was calibrated with a white plate as standard reference (L* = 97.55, a* = 1.32, b* = 1.41). The a* and b* color parameters recorded were used to calculate the hue angle (h°) using the following formula: h° = arctg(b*/a*).

Changes in O_2 and CO_2 concentrations in the headspace of the bags were monitored each sampling day using a gas chromatograph (Shimadzu GC-14, Kyoto, Japan) equipped with a thermal conductivity detector (TCD). The gas was drawn from the bags using a septum attached to the bags and a calibrated syringe. All the experiments were performed twice. Data obtained from test 1 and test 2 were analyzed separately because the initial quality of the product was different, which could influence the results obtained. Due to the low quality of the product in test 2, samples from iceberg and baby spinach were not evaluated at day 15, as the products were rejected. Kruskal-Wallis test was used to determine differences between treatments for gas composition and image data during shelf life. Mann–Whitney–Wilcoxon (WMW) test was used to compare the paired samples for the sensory panel data.

Objective 4

The validation of the selected treatment in an industrial setting was performed in two processing plants: the Torre-Pacheco processing plant from Florette Iberica (Murcia, Spain) and in Flensted (Ansager, Denmark). The industrial validation aimed to apply the optimized conditions of the most effective post-process treatment selected at the lab-scale trials, to demonstrate its efficacy under industrial settings. In these trials, the processing line selected was the one processing shredded iceberg, which was running under the conditions established by both industrial partners. The main objective of the validation trials was to demonstrate that the target concentration of the active microorganisms needed to inhibit the growth of Lm could be achieved under industrial conditions by adding a sufficiently low amount of water to the product to prevent any deterioration from excess water on the product. The target concentration of the selected post-process treatment was 10^6–10^7 pfu/g.

The conditions used for the sprayer system were the same as those previously applied under the lab-scale conditions. For the application of the treatment in the processing plant, a prototype was built in an arc design above the selected conveyor belt, with several nozzles to cover the conveyor width (**Figures 2 and 3**). The prototype consisted of a tank with the treatment solution connected to a suction pump which feeds the nozzles that applied the treatment to the produce. The nozzles were installed using a metallic structure placed above the conveyor belt that allowed adjustment of the height of the nozzles. The treatment was applied to the product at a flow rate of 0.0033 ml/s. The same prototype was used in the two industrial settings. The post-process treatment was applied after washing and drying, just before packaging (**Figures 2 and 3**). In Spain, the prototype was placed above the vibration conveyor belt after the visual inspection control point before weighing and packaging the product. The post-process treatment was applied for approximately 15 min. A total of 40 bags (500 g each) for each type of treated and untreated product were sampled and transported to the lab (30 miles) for further analysis. After the treatments were applied, the processing lines were cleaned and disinfected to eliminate any residual of the treatments. The tests in Denmark were organized and run in a similar way. Two trials were performed in each industrial setting.

Microbiological tests were performed during the shelf-life at 0,1, 5, 9 and 15 days. However, the length of the shelf-life was adjusted depending on the quality of the products. Three out of four trials were cancelled after 9 days of storage because of the deterioration of the product, while in the other assay, a shelf-life of 15 days was reached. This fact is not surprising because shredded iceberg in 500-g bags is for food service with a shelf-life of 6 days maximum. Bacteriophage enumeration was performed as described in *Objective 2*. Enumeration of *Listeria* spp. and Lm in iceberg lettuce was based on the ISO-11290-1 method, with slight modifications. For enrichment, 25 g of iceberg lettuce were mixed with 225 ml of Half Fraser broth and 1% of pyruvate and incubated 48 hours at 30 °C. Then 1 ml of the homogenate was transferred to 9 ml of Fraser broth and incubated at 37 °C for 24 h. Potentially positive colonies for Lm were confirmed by conventional polymerase chain reaction (PCR) using a PCR System (Applied Biosystems® thermal cycler). Strains were tested by PCR with specific primers to confirm the presence of virulence *hly* and *iap* genes.

Outcomes and Accomplishments

Objective 1

The systematic literature bounded search in WOS's Core Collection database retrieved 778 articles. After duplicate removal, the remaining 743 articles were screened at the title and abstract levels. A total of 122 papers were potentially identified as relevant for the present study and were included in the full text reading. After full text screening, several studies were removed for different reasons: **1)** included fresh mixed products (n=6), **2)** lack of data (n=18), **3)** inoculated with mixed species (n=3), **4)** focused only on *L. innocua* (n=3), **5)** included unclear graphics that did not allow the data extraction (n=2), and **6)** control data was not reflected (n= 2). In the end, 105 papers were selected for inclusion in the present study and used for data extraction. **Table 2** summarizes the statistic parameters of the data extracted used for the meta-analysis. This final dataset includes 809 points of Lm primary growth rate, including those directly reported by the papers (186 data points) as well as those estimated using DMFit based on growth data obtained directly either from texts or tables (123 data points) or extracted from graphs (500 data points). Data from 57 different types of commodities were included, and classified in three general categories: **1)** leafy greens and herbs (arugula, basil, Brussel sprouts, cabbage, cilantro, endive, garlic, iceberg lettuce, lamb's lettuce, romaine lettuce, other lettuce types (green leaf lettuce & butterhead lettuce), parsley, perilla leaves and spinach); **2)** other vegetables (artichoke, asparagus, beetroot, broccoli, carrots, cauliflower, celeriac, celery, cherry tomato, cucumber, eggplant, mushroom, onion, peppers, radish, rutabaga, sprouts, squash, tomato, turnips and zucchini); and **3)** fruits (apple, avocado, blackberry, blueberry, cactus-pear fruit, cantaloupe, coconut, kiwi, lemon, mandarin, mango, other melons (Canary, honeydew and Galia), orange, papaya, pear, persimmon, pineapple, pitaya, raspberry, strawberry, sweet cherry and watermelon. Among the selected papers, 53 papers did not report any information about the packaging conditions maintained during storage, which made it impossible to assign a specific gas composition within the bags during storage. On the other hand, 36 studies reported data on the effect of the atmospheres on the Lm behavior. The other remaining

16 papers, reported the packaging conditions but they did not focus on the impact of the packaging on the Lm behavior.

Figures 4–6 show scatter plots of Lm EGR retrieved from the extracted data at different storage temperatures from studies performed in leafy greens, other vegetables, and fruits, respectively. **Figure 7** shows a box-plot summary of the Lm EGR at 5 °C for all types of commodities. Based on the results obtained, it was observed that leafy greens supported the growth of Lm well, followed by several fruits such as melons. Taking into account the low-temperature ranges at which most of the leafy greens are stored, data obtained at 5 °C was considered as the most relevant. Based on the results, it could be concluded that Lm showed higher EGR in apple, avocado, cabbage, melon, celery, iceberg lettuce, pear, pepper, romaine lettuce and spinach (**Figure 7**). Apart from the Lm EGR values, other relevant factors were considered for the selection of the most relevant commodities. Several considerations were taken between the research groups and the industrial partners of the project and different factors were identified as relevant for the selection of the most relevant commodities. The selected factors included the economical relevance of the commodity, the suitability of each commodity for the application of the treatment, and the preparation and consumption patterns and consumer habits. The result of the comparison of the obtained EGR values for the different commodities as well as the consideration of other relevant factors, led to the following initial selection of commodities: **romaine, iceberg, cabbage, baby spinach and melon**. The inclusion of melon as a relevant commodity was not only due to the EGR retrieved by the search but also because of the number of times that this commodity has been involved in foodborne outbreaks linked to Lm.

Major Outcome 1

The meta-analysis of Lm growth in fresh fruits and vegetables was fed with more than 800 data points from 105 research papers. Results obtained show that leafy greens and melon support the growth of Lm better than other produce such as cucumber, asparagus, pears and apples. **The meta-analysis allowed the ranking of the ability of fruits and vegetables to support the Lm growth with the aim of identifying when the use of a post-process treatments is most relevant**.

The dataset obtained after the data extraction was used to determine the most relevant parameters affecting the survival and growth of Lm in different commodities. First, the **Gamma Concept** was validated for the prediction of the behavior of Lm in different commodities. This was possible for the normalization of the Lm EGR data obtained from the different studies performed under different conditions of pH, a_w and T^a, to the optimal Lm growth conditions. Results showed that 636 out of 809 predictions made by the Gamma Concept were correct. Based on the model, 552 cases corresponded to cases where Lm growth was predicted and observed, and 84 were associated with cases where Lm inactivation was predicted and also observed. However, there were 173 predictions made by the Gamma model, that were not aligned with the observed data. In this case, 84 cases predicted Lm inactivation, while the observation concluded growth of Lm, and the opposite case was observed in 89 cases. This means that in about 80% of the cases, the Gamma Concept model gave the right prediction of the Lm behavior considering all types of fresh produce. In the 20% of the cases where the model did not provide a good prediction, it could be due to the impact of other factors that could modify the behavior of Lm, such as the gas composition of the atmosphere in the package, the type of processing applied to the product, or because other specific characteristics of the commodities do not allow the prediction of the Lm behavior. However, it is relevant to highlight that half of the wrong predictions made by the model can be classified as “fail-safe” predictions, which means that the model predicted growth but the observations showed no growth. In the cases of the “fail-dangerous” predictions, (the model predicted no growth but the observations showed growth of Lm), all of them were associated with different types of fruits such as coconut and soft fruits. None of the “fail-dangerous” predictions were associated with leafy greens. Therefore, in the case of leafy greens, it was observed that all the predictions given by the Gamma Concept gave a good prediction of the Lm behavior (>90% of the cases) or were “fail-safe” predictions. The intrinsic characteristics of the different fruits, mainly due to the changes in pH and a_w during maturation along with shelf-life, could have an impact on the capacity of prediction of the Gamma Concept.

Therefore, the Gamma Concept represents a very useful tool to predict the behavior of Lm in some commodities during storage. It can be used to determine where (in which commodities) the use of post-process treatments to control Lm growth is more relevant.

Major Outcome 2

Challenge tests are very resource demanding and difficult to accomplish under industrial conditions. The Gamma Concept has been used for Lm growth prediction in fruits and vegetables, and results were compared with the reported observations. **In the case of leafy greens, the predictions made by the Gamma Concept were in line with the data obtained from experimental studies**. Therefore, **this model can be used to predict the Lm behavior in different types of leafy greens, reducing the need to perform more demanding trials for these commodities**.

Objective 2

The efficacy of four selected commercially available post-process treatments was evaluated in five different commodities, including iceberg, romaine, baby spinach, cabbage and melon. **Figure 8** shows the log reductions (Log (N_0/N)) observed between control and treated samples for each of the treatments in the selected leafy greens. The statistical model (**Table 3**) confirms the observed trend, that significant differences ($p<0.05$) were observed when comparing the *Lm* counts of the untreated control versus the product treated with Listex™, ListShield™, Holdbac® and SafePro®. Listex™ was the most effective treatment for the reduction of Lm levels on the surface of leafy greens. The confidence intervals obtained for each of the post-process treatments show very clearly the highest efficacy of the Listex™ treatment when compared to the rest of the treatments. The shape of the violin plots in **Figure 8** represents the variability among the different samples. In general, HOLDBAC® and ListShield™ show the greatest variability in all the leafy greens, while SafePro® shows the lowest variability followed by Listex™. **Figure 9** shows that only the Listex™ treatment was able to consistently reach a significant difference in the Lm inactivation when compared to the other treatments, as it was the only one above the threshold. The results showed that Listex™ consistently reduced the levels of Lm by approximately 2 log during shelf-life. In the case of melon, the experiments were repeated several times. However, several problems such as the difficulties in the application of the inoculum and the solution treatments to the melon cubes, as well as quality maintenance after washing during shelf-life did not allow for consistent results. Although three separate trials were performed, only data from one of these trials were used. **Table 4** shows the reductions observed by SafePro® and Listex™ in melon cubes. The results obtained confirmed the highest efficacy of Listex™ against Lm, similarly to the previous results on leafy greens.

Major Outcome 3

Among all the commercially available post-process treatments tested in fruits and vegetables, **Listex was the treatment capable of consistently reducing Lm by about 2 log during product shelf-life when compared to the untreated product**. Therefore, Listex was the treatment selected for further analyses.

Statistical differences were not observed among the different storage days for the selected treatments when data from all types of leafy greens were combined (**Figure 10)**. When looking at the Listex™ data on its own, no significant differences were observed between day 1 and the remaining storage days (**Figure 11**). However, significant differences were found for the Lm inactivation efficacy of Listex™ among the different types of leafy greens (**Figure 12**, **Table 5**). Listex™ showed the highest efficacy against Lm in iceberg, romaine and cabbage, and the lowest efficacy in baby spinach (**Figure 12**).

Major Outcome 4

The Lm reductions were mostly observed during the first 24 h after the post-process treatment. **Listex can be considered as a potential processing aid, as in most of the cases no further reductions were observed during storage**.

Objective 3

The impact of Listex™ on the sensory quality of the products was evaluated by a sensory panel as well as using objective parameters such as image analyses. Results did not show any significant differences between untreated and treated samples (**Table 6**). As illustrated in **Figures 13–17**, the same trends were observed for all the selected commodities, with no differences between treated and untreated samples for all the sensory attributes evaluated by the trained panel in both tests. The box plots are comparatively short in most of the sampling days, indicating the high level of agreement for all the sensory attributes compared with the data at the end of the storage that suggested quite different opinions about the visual appearance and browning for iceberg, and texture for cabbage and romaine. Additionally, in the case of the changes in the gas composition and color evolution, no significant differences were observed between untreated and treated samples (**Table 6**).

Major Outcome 5

The post-process treatment Listex™ does not cause any detrimental impact on the sensory quality of the selected commodities over the shelf-life. **Therefore, this treatment can be a potential application to reduce Lm growth in the products from the commercial operations.**

Objective 4

Validation trials in industrial settings were a key step to confirm that the optimal conditions selected under the lab-scale conditions could be reproduced in a commercial processing line. Two major goals were established for the validation trials: first, to reach the optimal concentration of Listex™ (10^6–10^7 pfu/g) needed to control Lm in case of its presence in the product, and second, to confirm that the sensory quality of the product was not affected by the treatment processed and applied under industrial conditions. Two trials were performed at each location (Spain and Denmark) to obtain robust data for the validation of the treatments. **Figure 18** shows the levels of bacteriophages reached after the post-process application as well as during the shelf-life of the product.

Major Outcome 6

The validation trials demonstrated that it is possible to reach the optimal concentration of Listex needed to consistently reduce the load of Lm by 2 log in iceberg lettuce. **The data obtained in lab-scale trials for the application of the Listex treatment was validated in industrial settings**.

Table 7 shows the differences between untreated control and the Listex™ treated iceberg samples on the changes in sensory quality during shelf-life. Data obtained showed no significant differences between them when applied in industrial settings.

During shelf-life, counts of putative Listeria spp./Lm were detected in the OCLA plates. These colonies were found in both untreated control and treated products. However, none of the isolated colonies were confirmed by PCR as Lm. Therefore, conclusions about the efficacy of the treatment in naturally present Lm could not be made.

Major Outcome 7

The selected treatment, Listex, did not cause any detrimental effect on the quality of iceberg lettuce when applied in an industrial setting. **Listex can be safely applied in commercial processing plants without causing any detrimental impact on the quality of the product.**

Summary of Findings and Recommendations

- Leafy greens support the growth of Lm better than other vegetables, such as cucumber and asparagus, as well as some fruits. A screening to determine the capacity of different commodities to support the growth of Lm before a post-process treatment was implemented.
- For leafy greens, predictions made by the Gamma Concept were in agreement with the observations obtained in the challenge tests. Therefore, this model can be used to predict the Lm behavior in different types of leafy greens, avoiding the need to perform more demanding trials for these commodities.
- Listex™ was the treatment capable of consistently reducing about Lm by 2 log during the product shelf-life when compared to the untreated product. Therefore, Listex™ was the treatment selected for further analyses.
- The selected post-process treatment, Listex™, can be considered as a processing aid, as in most of the cases no further reductions than the ones observed initially after 24 h were observed during storage.
- The post-process treatment Listex™ does not cause any detrimental impact on the sensory quality of the selected commodities or reduce shelf-life. Therefore, this treatment can be recommended to be applied in commercial facilities to control Lm growth in fresh produce.
- The validation trials demonstrated that it is possible to reach the optimal concentration of Listex™ needed to consistently reduce the load of Lm by 2 log in iceberg lettuce. The data obtained in lab-scale trials for the application of the Listex™ treatment was validated in industrial settings.
- The selected treatment, Listex™, did not cause any detrimental effect on the quality of iceberg lettuce when applied in an industrial setting. Listex™ can be safely applied in commercial processing plants without causing any quality impact on the product.

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APPENDICES

Publications and Presentations

Publications in preparation

Gómez-Galindo, M., Serra Castelló, C., Férez Rubio, J.A., Truchado, P., Gil, M.I., Bover, S., Allende, A. 2022. Meta-analysis and Biometrics of the Rates of *Listeria monocytogenes* in Fresh Produce under Commercial Distribution and Storage Conditions. International Journal of Food Microbiology. *In preparation*.

Gómez-Galindo, M., Truchado, P., Volpi, M., Férez Rubio, J.A., Gil, M.I., Elsser-Gravesen, A., Allende, A. 2022. Identification of suitable commercial post-process treatments to reduce growth of *Listeria monocytogenes* by at least 2 logs on the surface of fresh fruit and vegetables. Food Microbiology. *In preparation*.

Gómez-Galindo, M., Truchado, P., Volpi, M., Allende, A., Elsser-Gravesen, A., Gil, M.I. 2022. Industrial validation of the efficacy of Listex™ as a post-process treatment to control *Listeria monocytogenes* in leafy greens. International Journal of Food Microbiology. *In preparation*.

Presentation

Gómez-Galindo, M., Volpi, M., Gil, M.I., Truchado, P., Elsser-Gravesen, A., Allende, A. 2021. Uso de tratamientos comerciales de bacteriófagos y cultivos protectores para reducir el riesgo asociado a Listeria monocytogenes en productos vegetales. XXVIII Congreso Nacional de Microbiología, 28 de Junio al 2 de Julio de 2021. Oral Presentation.

Budget Summary

This project was awarded a total of $281,404 in grant funds, of which $115,840 was a subaward to Co-PI Anne Elsser-Gravesen at ISI FOOD PROTECTION ApS. Most of the project expenditures were for salaries, followed by supplies. The grant funds were all expended and were adequate to fully implement all the objectives of this project.

Tables 1–7 and Figures 1–18 (see below)

Table 1. Search strategies for the literature review on Lm growth on different fresh fruits and vegetables.

Set	TI (Title), AB (Abstract) , AK (Abstract Keywords)
#1	"lettuce" or "cabbage" or "cauliflower" or "spinach" or "endive" or "celery" or "carrot" or "melon" or "apple" or "pepper" or "onion" or "mushroom*" or "cucumber" or "raw" OR "minimally processed" OR "shredded" OR "sliced" OR "whole" or "dic*" OR "fresh*" OR "produce" OR "vegetable*" OR "fruit*" OR "leafy green*" OR "leaves" OR "ready-to-eat" OR "RTE" OR "salad*" OR "fresh*" OR "produce"
	AND
#2	"Listeria monocytogenes" OR "listeria spp" OR "microb*" OR "L. monocytogenes" OR "listeriosis" OR "foodborne"
	AND
#3	OR ("behavi*" OR "surviv*" OR "fate" OR "inhibit*" OR "inactivat*" OR "proliferat*" OR "reduct*" OR "grow*"
Set	AND
#4	"stor*" OR "ºC" OR (("time*" OR "period") OR "incubation") OR "day*" or "challenge test*")
Set	NOT AB, TI, AK
#5	"meat" OR "fish" OR "cheese" OR "milk" or "ice cream" or "dairy" OR "sausage*" OR "salmon" OR "turkey" OR "beef")
Set	EXCLUDED
#6	“Transplantation”, “Surgery”, “Pediatrics”, “Ophthalmology”, “Oncology”, “Obstetrics Gynecology”, “Medical Laboratory Technology”, “Marine Freshwater Biology” , “History”, “Entomology”, “Electrochemistry”, “Crystallography” , “Research Experimental Medicine”, “Radiology Nuclear Medicine Medical Imaging”, “Polymer Science” , “Geriatrics Gerontology”, “Geography”, “Forestry” , “Demography”, “Water Resources”, “Information science library science”, “Education Educational Research”, “Dermatology”, “Cardiovascular system cardiology”, “Biophysics”, “Biodiversity conservation”, “Automation control systems”, “Fisheries “,“Acoustics”, “Microscopy”, “General internal medicine”, “Hematology”, “Gastroenterology hepatology”, “Pathology”, “Psychology”, “Veterinary Sciences”, “Genetics hereditary”.

Table 2. Summary of the studies included in the meta-analysis.

Category	Product	Type of inoculum	Storage temperatures	References
LEAFY GREENS	Arugula	Sprinkled (6) / Dip (3) / NR (1)	4-8	Lokerse et al., 2016; Ziegler et al., 2019; Culliney and Schmalenberger, 2020; Ramos et al., 2020
	Basil	Spot (1)	10	Salazar et al., 2020
	Brussel sprouts	Dip (2)	7	Jacxsens et al., 1999
	Cabbage	Dip (18)/ Spot (7)/ Sprinkled (4)/ NR (2)	4-35	Beuchat et al., 1986; C. Ells et al., 2010; Lokerse et al., 2016; Manios et al., 2013; Ongeng et al., 2007; Wang et al., 2013; Yoon et al., 2014; Ziegler et al., 2019
	Cilantro	Spot (1)	10	Salazar et al., 2020
	Endive	Dip (50)/ Sprinkled (3)/ NR (3)	3-20	Aytac and Gorris, 1994; Bennik et al., 1996; Carlin and Nguyen-The, 1994; Carlin et al, 1995; Carlin et al., 1996; Jacxsens et al., 1999; Lokerse et al., 2016; Niemira et al., 2005
	Garlic	Spot (1)	10	Salazar et al., 2020
	Iceberg lettuce	Dip (21)/ Spot (28)/ Sprinkled (61)/ NR (1)	1-35	Beuchat and Doyle (1995); Carrasco et al., 2008; Delaquis et al., 2002; Francis and O' Beirne, 1997; Francis and O' Beirne, 2001; Francis and O'Beirne, 2001b; Francis and O'Beirne, 2005; Hellström et al., 2006; Jacxsens et al., 1999; Li et al., 2002; Lokerse et al., 2016; O'Beirne et al., 2015; Ramos et al., 2020; Tian et al., 2012; Yuk et al., 2006; Koseki and Isobe, 2005; Szabo et al., 2003; Trias et al. (2008); Tucci et al., 2019; Yin et al., 2018; Ziegler et al., 2019; Ding et al., 2010; McManamon et al., 2017; Culliney and Schmalenberger, 2020; Dong et al., 2021
	Lamb lettuce	Dip (1)/ Sprinkled (2)	5-10	Carlin and Nguyen-The, 1994; Ziegler et al., 2019
	Lettuce	Dip (4)	4-10	Carlin and Nguyen-The, 1994; Rodgers et al., 2004; Wang et al., 2021
	Parsley	Dip (3)/ Sprinkled (2) / NR (1)	4-8	Lokerse et al., 2016; Ziegler et al., 2019; Ramos et al., 2020
	Perilla leaves	Spot (2)	4-15	Tian et al., 2012
	Romaine lettuce	Dip (12)/ Spot (3)	4-25	Manios et al., 2013; Oliveira et al., 2010; Tian et al., 2012
	Spinach	Dip (3) / Sprinkled (16) / NR (1)	3-36	Lokerse et al., 2016; Omac et al., 2015; Omac et al., 2018; Söderqvist et al., 2017, Culliney and Schmalenberger, 2020; Ramios et al., 2020

Category	Product	Type of inoculum	Storage temperatures	References
OTHER VEGETABLES	Artichoke	Dip (1)	4	Sanz et al., 2003
	Asparagus	Dip (11)/ Spot (5)	2-22	Berrang et al. 1989; Castillejo Rodríguez et al., 2000; Molinos et al., 2005
	Beetroot	Sprinkled (2)	5-8	Ziegler et al., 2019
	Broccoli	Dip (8)/ Spot (7)	4-23	Berrang et al. 1989; Pinton et al., 2020; Girbal et al., 2021
	Butternut squash	Sprinkled (2)	4-10	Farber et al., 1998
	Carrot	Dip (2)/ Sprinkled (6)/ Spot (4)/ NR (1)	4-10	Dhokane et al., 2006; Farber et al., 1998; Jacxsens et al., 1999; Lokerse et al., 2016; Ziegler et al., 2019; Girbal et al., 2021
	Cauliflower	Dip (8)/ Spot (7)	2-35	Berrang et al. 1989; Pinton et al., 2020; Girbal et al., 2021
	Celeriac	Sprinkled (2)	5-8	Ziegler et al., 2019
	Celery	Dip (3)/ Spot (21)/ NR (1)	4-25	Vandamm et al., 2013; Kaminski et al., 2014; Lokerse et al., 2016; Sahu et al., 2017
	Cherry tomato	Dip (7) / Sprinkled (8) / Spot (2)	7-10	Beuchat and Brackett, 1991; Kim, Lee and Yoon, 2021
	Cucumber	Dip (1)/ Spot (15)/Sprinkled (4)/ NR (4)	2-37	Bardsley et al., 2019; Dhokane et al., 2006; Jacxsens et al., 2002; Lokerse et al., 2016; Salazar et al., 2017; Szewczuk et al., 2016
	Eggplant	Dip (1)	10	Salazar et al., 2020
	Mushroom	Dip (3)/ Spot (6)	4-25	Gonzalez-Fandos et al., 2001; Leong et al., 2013; Salazar et al., 2017; Yuk et al., 2007
	Onion	Spot (7)/ Sprinkled (10)/ NR (1)	4-25	Farber et al., 1998; Lieberman et al., 2019; Lokerse et al., 2016; Salazar et al., 2017
	Peppers	Spot (21)/ NR (1)	5-25	Han et al., 2001; Lokerse et al., 2016; Salazar et al., 2017; Salazar et al., 2020
	Radish	Sprinkled (2)/ NR (1)	5-8	Lokerse et al., 2016; Ziegler et al., 2019
	Rutabaga	Sprinkled (7)	4-10	Farber et al., 1998; Francis and O' Beirne, 2001; Francis and O'Beirne, 2001b
	Sprouts	Dip (8)/ Spot (2)/ Sprinkled (13)	3-22	Aytac and Gorris, 1994; Bennik et al., 1999; Francis and O' Beirne, 2001; Francis and O'Beirne, 2001b; Lee et al., 2002; Molinos et al., 2005; Thomas et al., 1999; Tian et al., 2012
	Tomato	Spot (5)	2-35	Girbal et al., 2021

Category	Product	Type of inoculum	Storage temperatures	References
	Turnip	Sprinkled (8)	4-10	Brierley et al., 2020
	Zucchini	NR (3)	4-37	Szewczuk et al., 2016
FRUITS	Apple	Dip (4)/ Spot (27)/ Spread (1)/ NR (1)	3-25	Alegre et al., 2011; Conway et al., 2000; Kim, Lee & Yoon, 2021; Leverentz et al., 2003; Leverentz et al., 2006; Lokerse et al., 2016; Macarisin et al., 2019; Martinez, Ferguson & Datta, 2020; Naqash et al., 2020; Rodgers et al., 2004; Salazar et al., 2016; Trias et al., 2008
	Avocado	Dip (1)/ Spot (9)/ Sprinkled (1)	5-25	Iturriaga et al., 2002; Salazar et al., 2017; Salazar et al., 2020
	Blackberry	Spot (7)	2-35	Molinos et al., 2008; Girbal et al., 2021
	Blueberry	Spot (8)	4-12	Concha-Meyer et al, 2014
	Cactus-Pear fruit	Sprinkled (8)	4-20	Corbo et al., 2005
	Cantaloupe	Dip (8)/Spot (53)/ Spread (1)	4-25	Huang et al., 2015; Nyarko et al., 2016a; Nyarko et al., 2016b; Salazar et al., 2017; Rodgers et al., 2004; Martinez et al, 2016; Ukuku & Fett, 2002; Ukuku et al., 2016; Jang et al., 2021; Collu et al., 2021; Martinez, Ferguson & Datta, 2020; Zhang et al., 2020
	Coconut	Spot (4)/ Spread (16)	2-12	Sinigaglia et al., 2006; Collu et al., 2021
	Grapes	Spot (2)	10-15	Kim, Lee & Yoon, 2021
	Kiwi	Spot (4)	6-22	Molinos et al. (2008); Jang et al., 2021
	Lemon	Spot (5)	2-35	Girbal et al., 2021
	Mandarin	Spot (5)	2-35	Girbal et al., 2021
	Mango	Spot (4)/ NR (6)	4-25	Feng et al., 2015; Penteado et al (2014); Lokerse et al., 2016; Luciano et al., 2022
	Melon	Spot (22)/ NR (6)	4-35	Leverentz et al., 2003; Leverentz et al., 2004; Penteado and Leitao, 2004; Molinos et al. 2008; Lokerse et al., 2016; Scolforo et al., 2016; Collu et al., 2021; Luciano et al., 2022
	Nectarine	Spot (4)	4	De Jesus et al., 2020
	Orange	Spot (1)	10	Jang et al., 2021
	Papaya	Spot (7)/ NR (3)	4-30	Feng et al., 2015; Penteado and Leitao (2004); Luciano et al., 2022

Category	Product	Type of inoculum	Storage temperatures	References
	Peach	Dip (8)	4	De Jesus et al., 2020
	Pear	Dip (13)/ Spot (3)	4-22	Colás-Medà et al., 2015; Colás-Medà et al., 2016; Molinos et al., 2008
	Persimmon	Spot (12)	10-30	Uchima et al., 2008
	Pineapple	Spot (6)/ NR (4)	4-25	Feng et al., 2015; Lokerse et al., 2016; Kim, Lee & Yoon, 2021; Collu et al., 2021
	Pitaya	NR (3)	5-25	Feng et al., 2015
	Raspberry	Spot (10)	2-35	Molinos et al., 2008; Siro et al., 2006; Girbal et al., 2021
	Strawberry	Dip (1)/ Spot (6)/ NR (17)	4-24	Flessa et al., 2005; Lokerse et al., 2016; Molinos et al., 2008; Rodgers et al., 2004; Siro et al., 2006
	Sweet cherry	Spot (5)	2-35	Girbal et al., 2021
	Watermelon	Spot (7)	6-30	Molinos et al., 2008; Penteado and Leitao, 2004; Jang et al., 2021

Table 3. Differences found in the efficacy of Lm reduction among the different post-process treatments including ListShield™, PhageGuard Listex™, SafePro® and HOLDBAC® Listeria when all the types of fresh produce and all the sampling days were considered together (significance level at $p < 0.05$).

Comparison	Estimate	Std. Error	z value	Pr(>|z|)
LISTEX - HOLDBAC	0.695780	0.136463	5.098667	0.000002
LISTSHIELD - HOLDBAC	0.325052	0.136463	2.381979	0.080401
SAFEPRO - HOLDBAC	0.070830	0.136463	0.519042	0.954552
LISTSHIELD - LISTEX	-0.370728	0.136463	-2.716688	0.033460
SAFEPRO - LISTEX	-0.624950	0.136463	-4.579625	0.000021
SAFEPRO - LISTSHIELD	-0.254222	0.136463	-1.862937	0.244206

Table 4. *Listeria monocytogenes* reductions (log (N_0/N)) (log CFU/g) in melon after the application of two post-process treatments (SafePro® and Listex™). Values are the mean of n= 5.

Treatment	**Control**	**SafePro**	**Listex**
Before treatment	4.1 ± 0.0	2.5 ± 0.0	4.0 ± 0.0
After treatment (1h)	2.8 ± 0.0	1.3 ± 0.2	0.8 ± 0.2

Table 5. Differences found in the efficacy of Lm reduction when Listex™ was applied to two different fresh products (significance level at $p < 0.05$).

Comparison	Estimate	Std. Error	z value	Pr(>|z|)
CABBAGE - BABY SPINACH	0.634946	0.143265	4.431968	0.000054
ICEBERG - BABY SPINACH	0.909033	0.143265	6.345116	0.000000
ROMAINE - BABY SPINACH	1.076600	0.143265	7.514747	0.000000
ICEBERG - CABBAGE	0.274087	0.143265	1.913148	0.222408
ROMAINE - CABBAGE	0.441654	0.143265	3.082779	0.011045
ROMAINE - ICEBERG	0.167567	0.143265	1.169631	0.646076

Table 6. Differences found in sensory quality, gas composition in the bag headspace (O_2 and CO_2 levels) and image analyses between untreated control and the post-process Listex-treated iceberg samples (significance level at p< 0.05).

Comparison	TEST	p-value
LISTEX – CONTROL O2 T1	KRUSKAL	0.3507
LISTEX – CONTROL CO2 T1	KRUSKAL	0.8509
LISTEX – CONTROL O2 T2	KRUSKAL	0.8206
LISTEX – CONTROL CO2 T2	KRUSKAL	0.7728
LISTEX – CONTROL H T1	KRUSKAL	0.7259
LISTEX – CONTROL L T1	KRUSKAL	0.9507
LISTEX – CONTROL H T2	KRUSKAL	0.8570
LISTEX – CONTROL L T2	KRUSKAL	0.8058
LISTEX – CONTROL flavor T1	KRUSKAL	0.9927
LISTEX – CONTROL texture T1	KRUSKAL	0.8524
LISTEX – CONTROL browning T1	KRUSKAL	0.5755
LISTEX – CONTROL spoilage T1	KRUSKAL	0.7426
LISTEX – CONTROL visual appearance T1	KRUSKAL	0.8485
LISTEX – CONTROL flavor T2	KRUSKAL	0.8601
LISTEX – CONTROL texture T2	KRUSKAL	0.6831
LISTEX – CONTROL browning T2	KRUSKAL	0.9822
LISTEX – CONTROL spoilage T2	KRUSKAL	0.6445
LISTEX – CONTROL visual appearance T2	KRUSKAL	0.8279

Table 7. Differences found in sensory quality, gas composition in the bag headspace (O_2 and CO_2 levels), and image analyses between untreated control and the post-process Listex-treated iceberg samples in the validation trials performed in Spain (significance level at $p < 0.05$).

Comparison	TEST	p-value
LISTEX – CONTROL O2 T1	KRUSKAL	0.6033
LISTEX – CONTROL CO2 T1	KRUSKAL	0.5253
LISTEX – CONTROL O2 T2	KRUSKAL	0.4975
LISTEX – CONTROL CO2 T2	KRUSKAL	0.0648
LISTEX – CONTROL H T1	KRUSKAL	0.8206
LISTEX – CONTROL L T1	KRUSKAL	0.4963
LISTEX – CONTROL H T2	KRUSKAL	0.0265
LISTEX – CONTROL L T2	KRUSKAL	0.0010
LISTEX – CONTROL flavor T1	KRUSKAL	0.9229
LISTEX – CONTROL texture T1	KRUSKAL	0.9743
LISTEX – CONTROL browning T1	KRUSKAL	0.9577
LISTEX – CONTROL spoilage T1	KRUSKAL	0.8945
LISTEX – CONTROL visual appearance T1	KRUSKAL	0.8606
LISTEX – CONTROL flavor T2	KRUSKAL	1.0000
LISTEX – CONTROL texture T2	KRUSKAL	0.9871
LISTEX – CONTROL browning T2	KRUSKAL	0.8597
LISTEX – CONTROL spoilage T2	KRUSKAL	0.8597
LISTEX – CONTROL visual appearance T2	KRUSKAL	0.8598

Figure 1. Scheme of the experimental design used for the screening of the selected post-process treatments.

Figure 2. Validation study performed in Spain.

Figure 3. Validation study performed in Denmark.

Figure 4. Scatter plot representing the exponential growth rate (EGR) at different storage temperatures. Data points retrieved from the selected studies performed in various leafy greens.

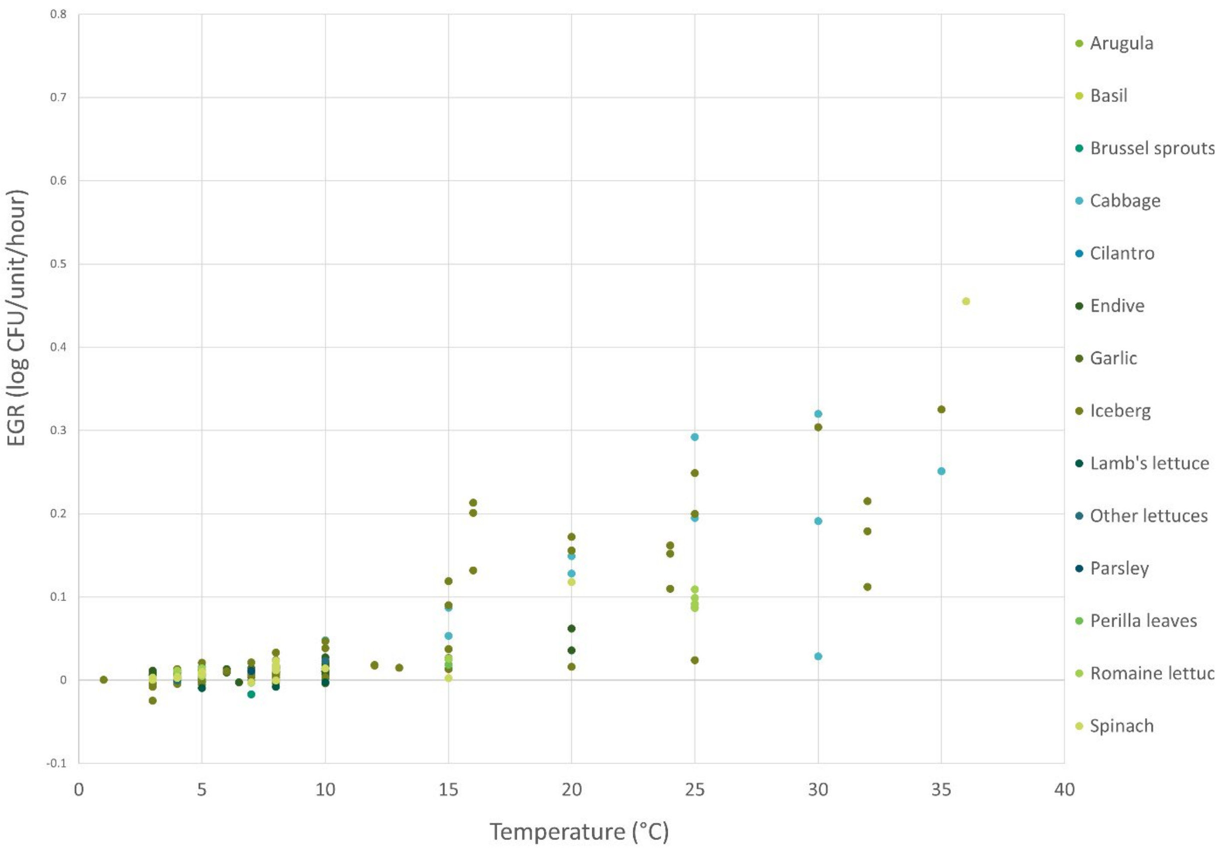

Figure 5. Scatter plot representing the exponential growth rate (EGR) at different storage temperatures. Data points retrieved from the selected studies performed in various vegetables.

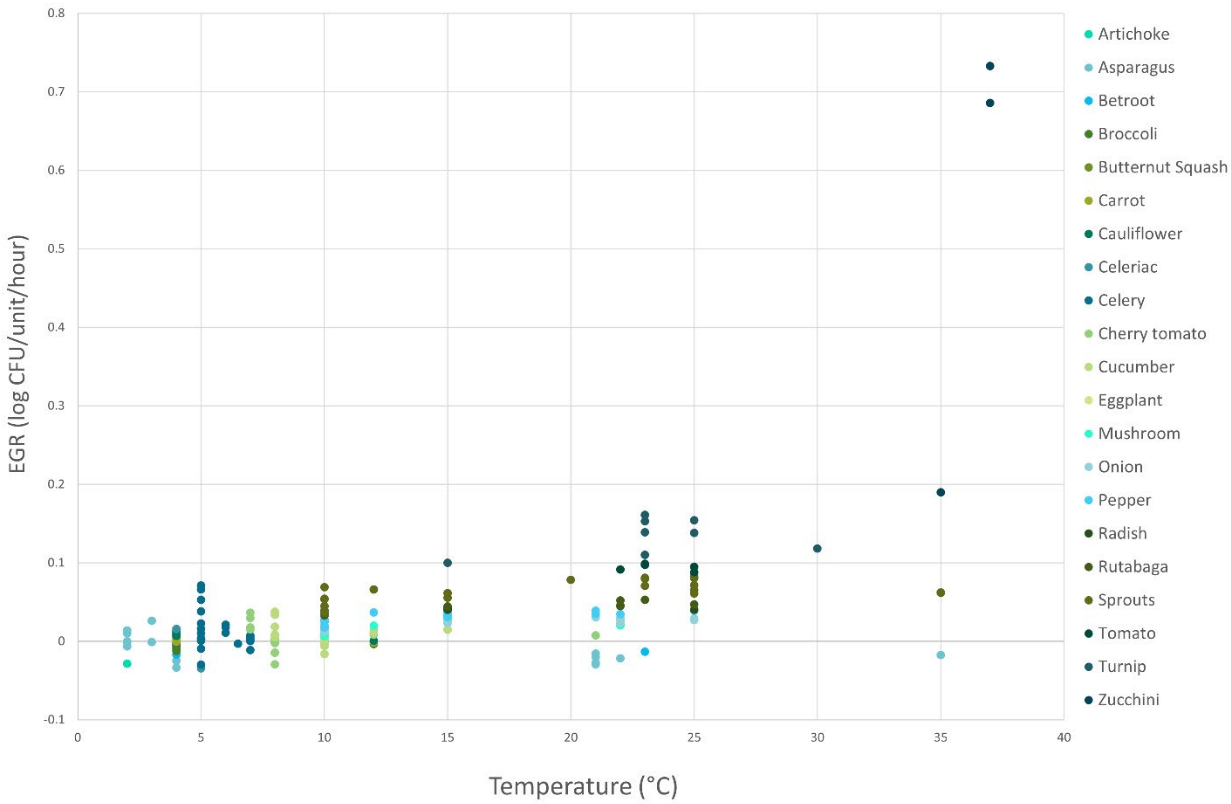

Figure 6. Scatter plot representing the exponential growth rate (EGR) at different storage temperatures. Data points retrieved from the selected studies performed in various fruits.

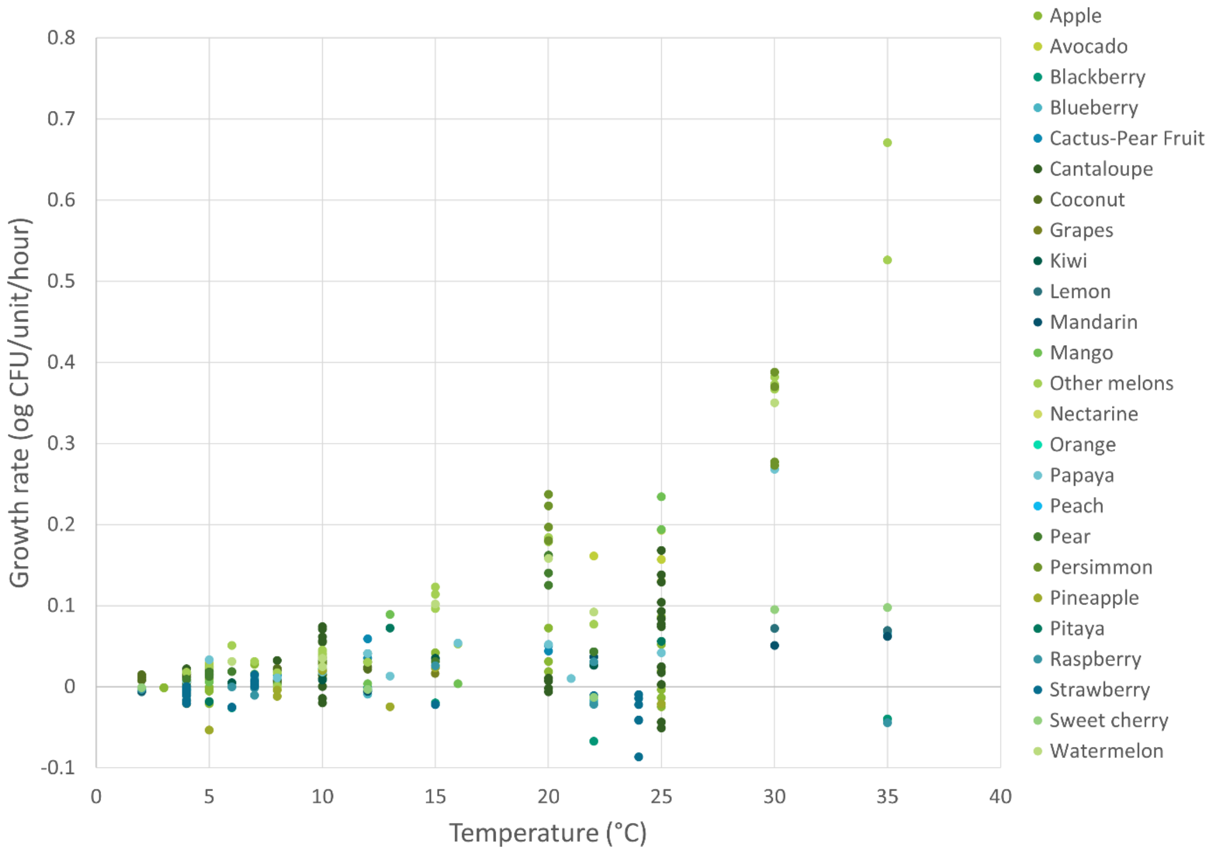

Figure 7. Box plot representing the data distribution of the exponential growth rate (EGR) predicted at 5 °C. Data points retrieved from selected studies performed in different fruits and vegetables. Box plots represent the interquartile interval, where 50% of the data from is the median (middle quartile) and the lower and upper quartiles (25 and 75% of the scores, respectively).

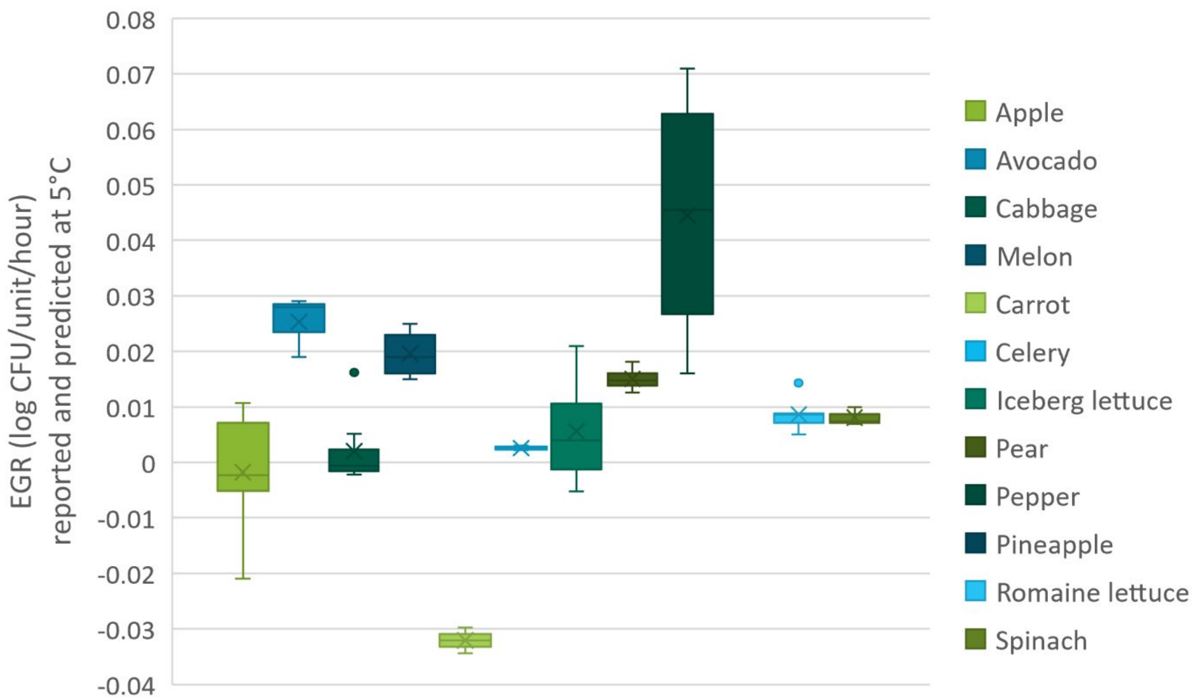

Figure 8. Violin plots representing the distribution of the Lm reductions (log (N_0/N) obtained for each of the post-process treatments (Holdbac® Listeria, Listex™, ListShield™, and SafePro®) when all the commodities (baby spinach, iceberg, romaine and cabbage) and days of storage (0, 1, 5, 12 and 15 days) are considered together. Each violin represents a minimum of 80 data points (n=80). Different letters represent significant differences among treatments for each commodity. Significance level at p< 0.05.

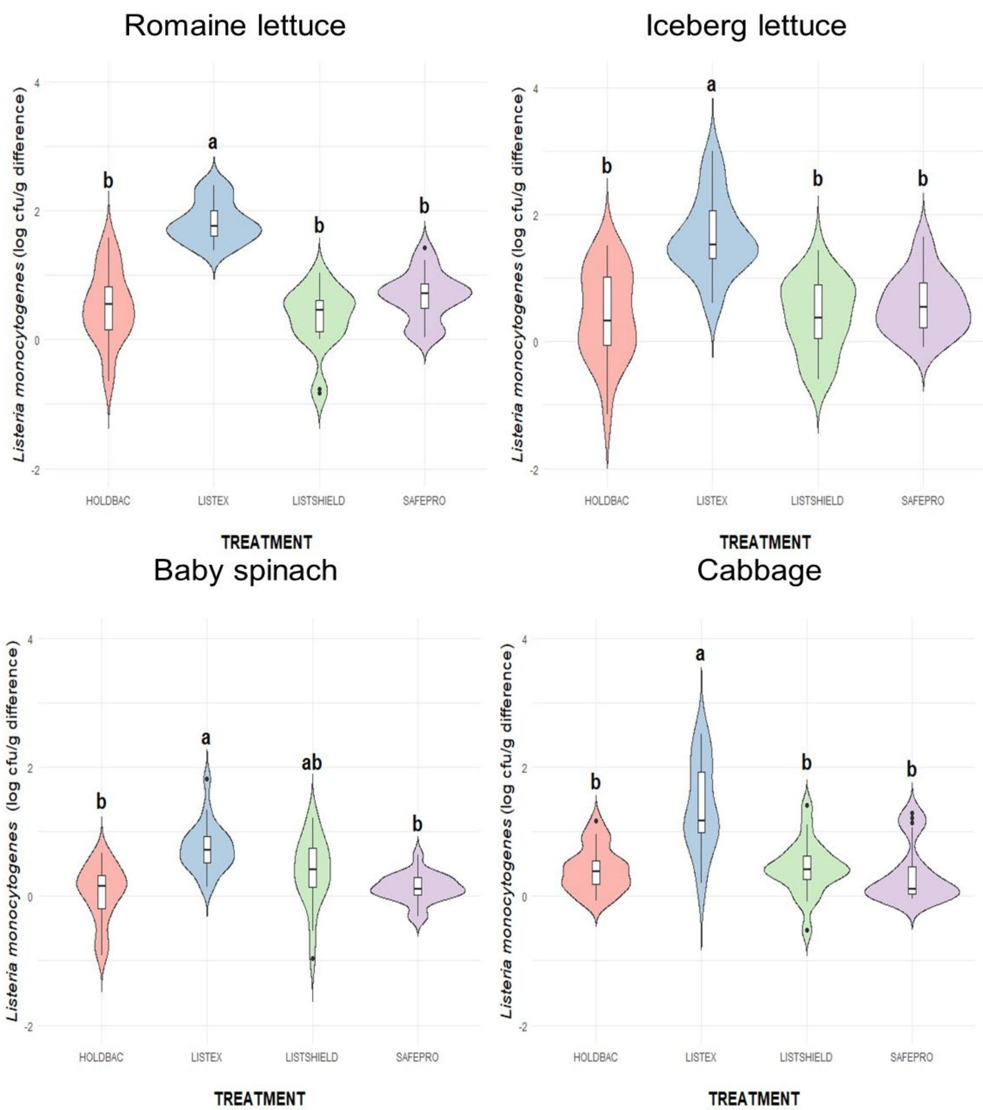

Figure 9. Estimated confidence interval plot of the pair comparison between the Lm reductions (log (N_0/N) obtained between two different post-process treatments (ListShield™, Listex™, SafePro® and Holdbac® Listeria) when all the commodities (baby spinach, iceberg, romaine and cabbage) and days of storage (0, 1, 5, 12 and 15 days) are considered together. Significance level at $p < 0.05$. Pairwise comparisons are statistically significant when the confidence level does not cross over the zero value.

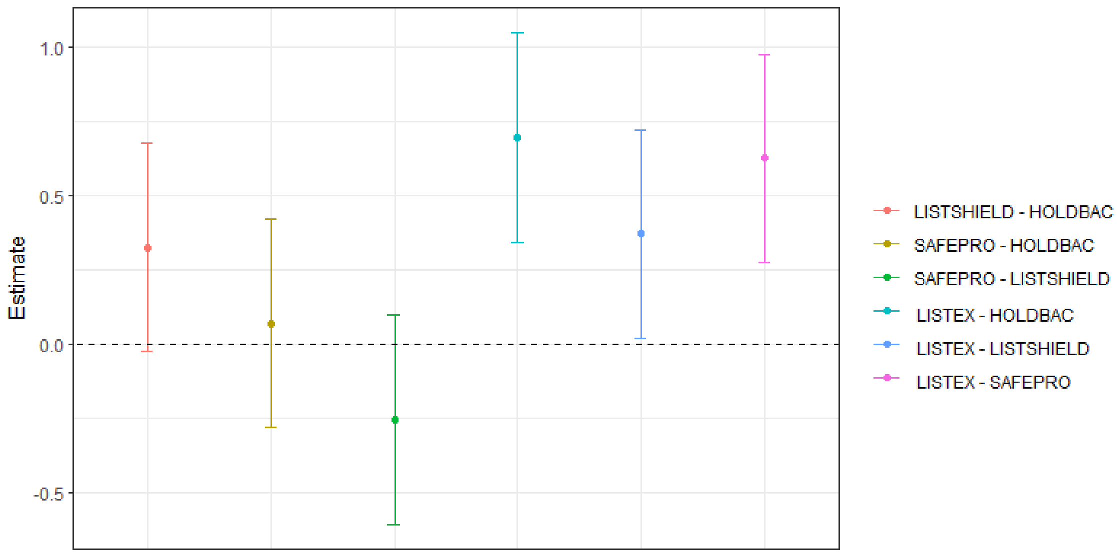

Figure 10. Box plots representing the distribution of the Lm reductions (log (N_0/N) obtained for each of the post-process treatments (Holdbac® Listeria, Listex™, ListShield™, and SafePro®) at different sampling days (0, 1, 5, and 12 and 15 days) when all the commodities (baby spinach, iceberg, romaine and cabbage) are considered together. Each box plot represents a minimum of 16 data points (n=16). Significance level at p< 0.05. ns: not significant. Box plots represent the interquartile interval, where 50% of the data from is the median (middle quartile) and the lower and upper quartiles (25 and 75% of the scores, respectively).

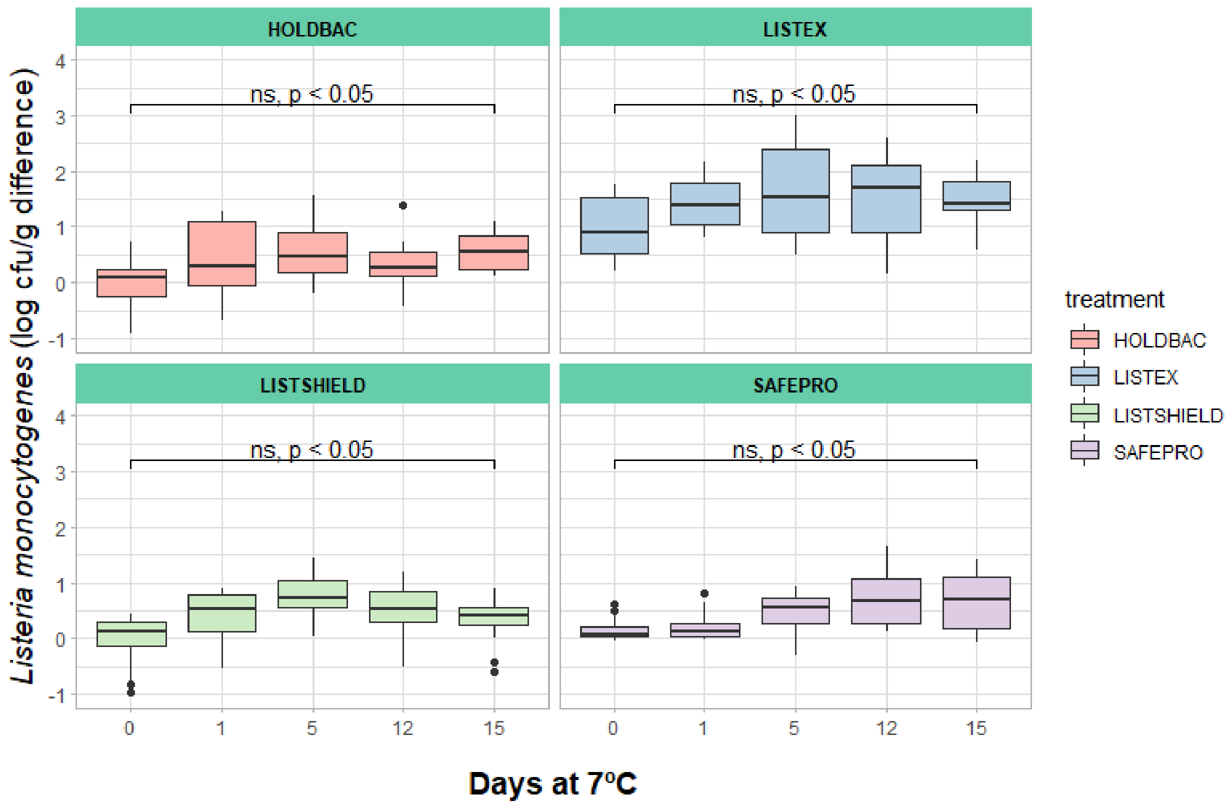

Figure 11. Box plots representing the distribution of the Lm reductions (log (N_0/N) obtained for Listex™ at different sampling days (0, 1, 5, 12 and 15 days) for each of the commodities (baby spinach, iceberg, romaine and cabbage). Each box plot represents a minimum of 8 data points (n=8). Significance level at $p < 0.05$. ns: not significant. Box plots represent the interquartile interval, where 50% of the data from is the median (middle quartile) and the lower and upper quartiles (25 and 75% of the scores, respectively).

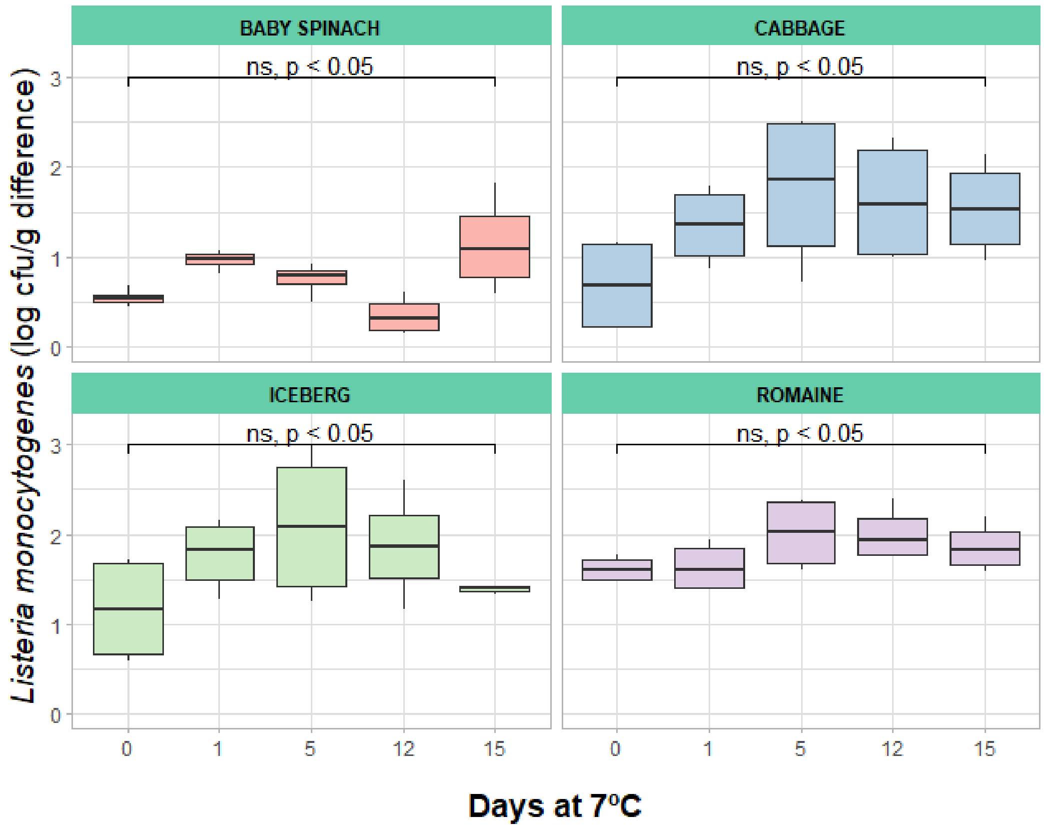

Figure 12. Violin plots representing the distribution of the efficacy of Listex™ (Lm reduction (log (N_0/N)) for each commodity when all the sampling days (days 0, 1, 5, 12 and 15) are considered together. Each violin represents a minimum of 20 data points (n=20). Different letters represent significant differences among commodities. Significance level at $p < 0.05$.

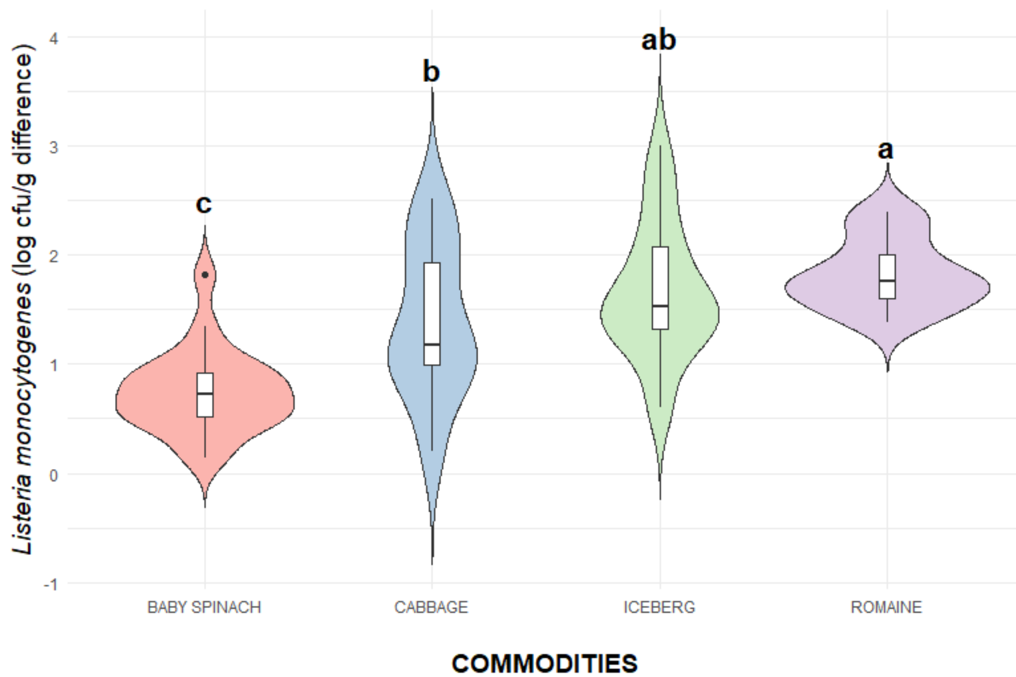

Figure 13. Changes in visual appearance between control and post-process Listex™ treatment of baby spinach, and fresh-cut cabbage, iceberg, and romaine over 15 days of storage at 7 °C. Box plots represent the interquartile interval, where 50 % of the data from Test 1 and Test 2 (n= 5) is the median (middle quartile) and the lower and upper quartiles (25 and 75 % of the scores, respectively).

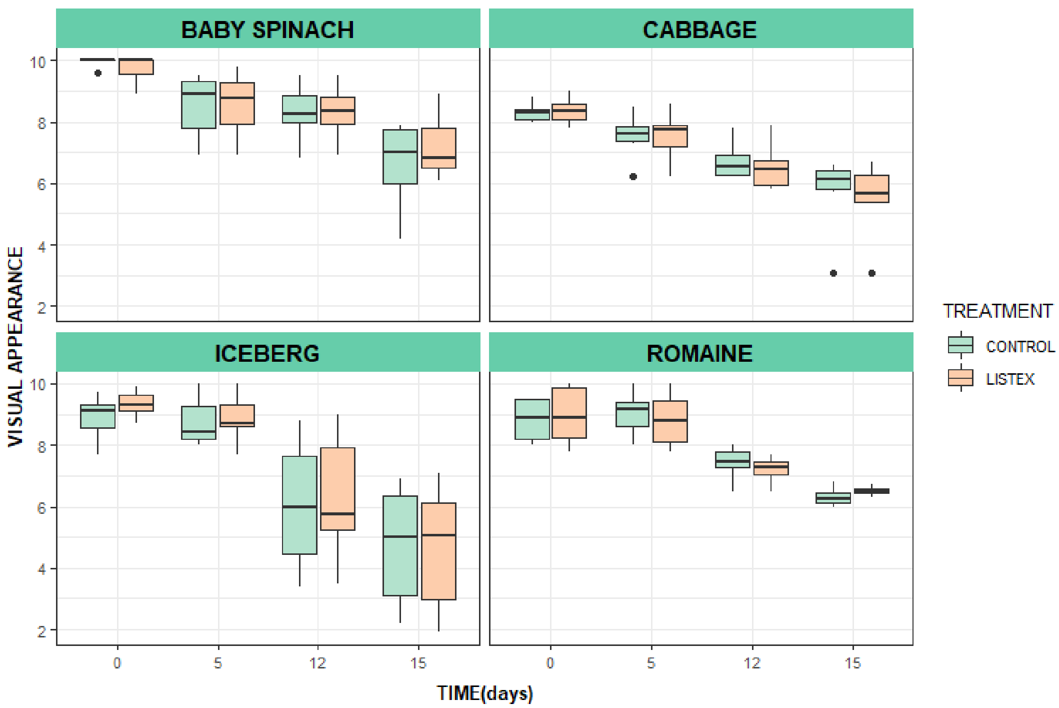

Figure 14. Changes in flavor between control and post-process Listex™ treatment of baby spinach, and fresh-cut cabbage, iceberg, and romaine over 15 days of storage at 7 °C. Box plots represent the interquartile interval, where 50% of the data from Test 1 and Test 2 (n= 5) is the median (middle quartile) and the lower and upper quartiles (25 and 75% of the scores, respectively.

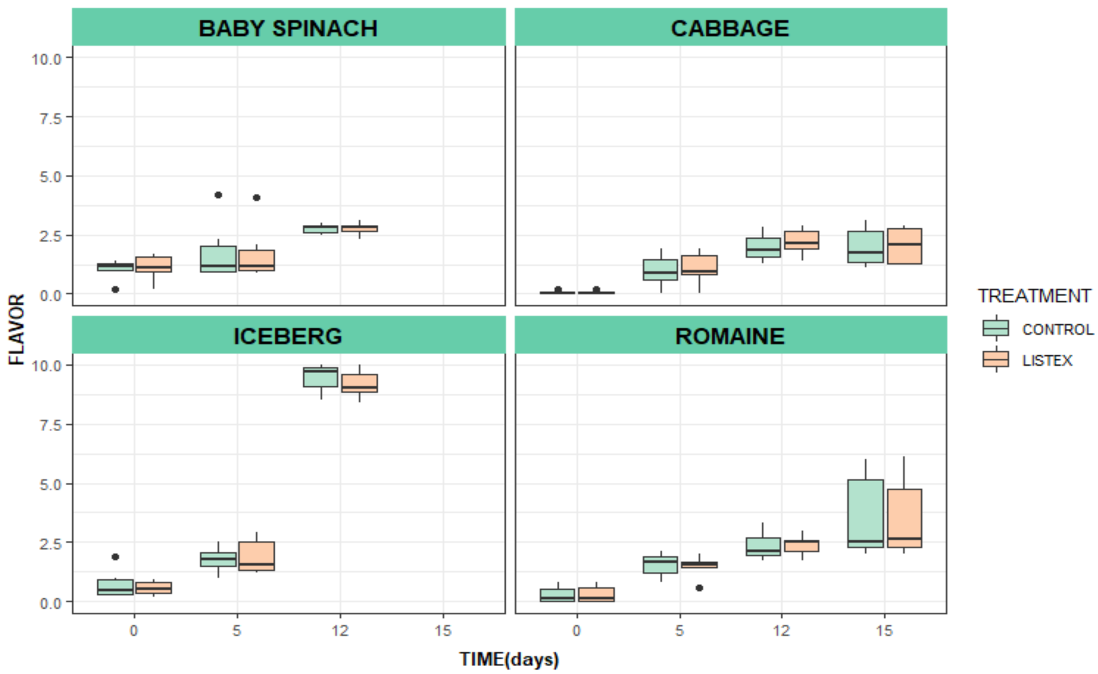

Figure 15. Changes in texture between control and post-process Listex™ treatment of baby spinach, and fresh-cut cabbage, iceberg, and romaine over 15 days of storage at 7 °C. Box plots represent the interquartile interval, where 50% of the data from Test 1 and Test 2 (n= 5) is the median (middle quartile) and the lower and upper quartiles (25 and 75% of the scores, respectively).

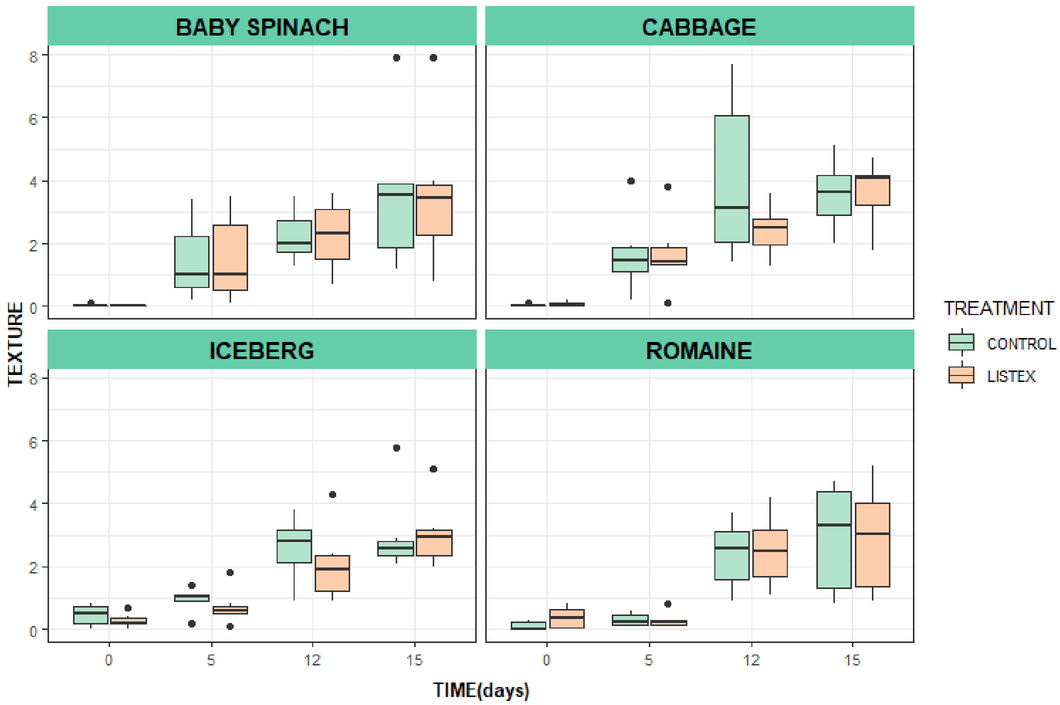

Figure 16. Changes in browning between control and post-process Listex™ treatment of baby spinach, and fresh-cut cabbage, iceberg, and romaine over 15 days of storage at 7 °C. Box plots represent the interquartile interval, where 50% of the data from Test 1 and Test 2 (n= 5) is the median (middle quartile) and the lower and upper quartiles (25 and 75% of the scores, respectively).

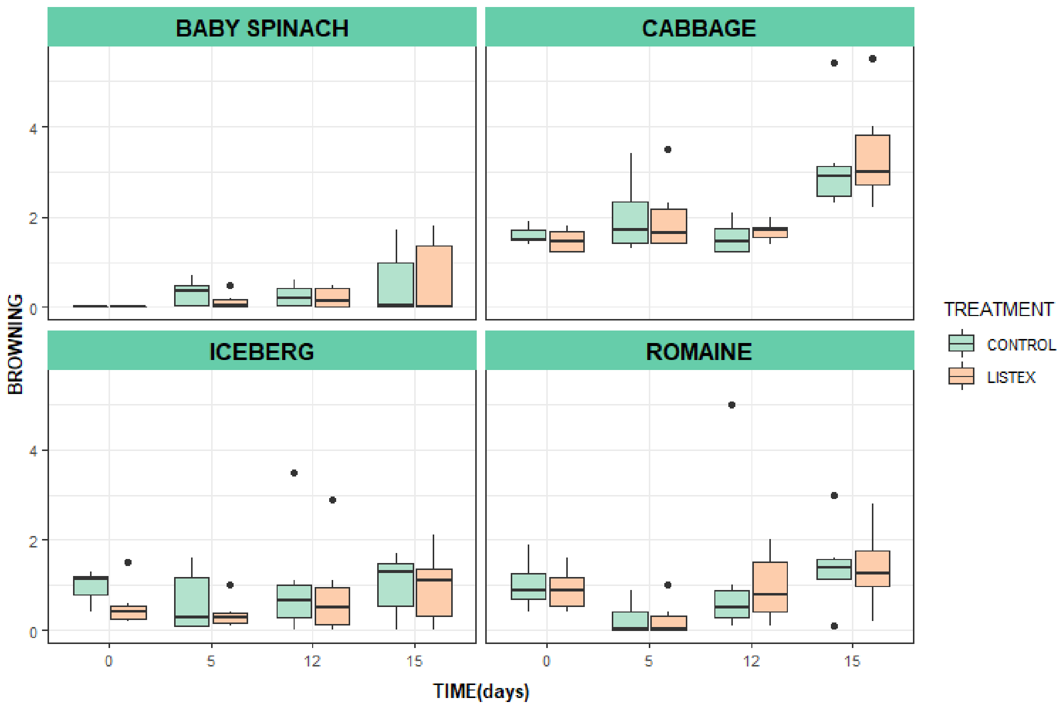

Figure 17. Changes in the degree of spoilage between control and post-process Listex™ treatment of baby spinach, and fresh-cut cabbage, iceberg, and romaine over 15 days of storage at 7 °C. Box plots represent the interquartile interval, where 50% of the data from Test 1 and Test 2 (n= 5) is the median (middle quartile) and the lower and upper quartiles (25 and 75% of the scores, respectively).

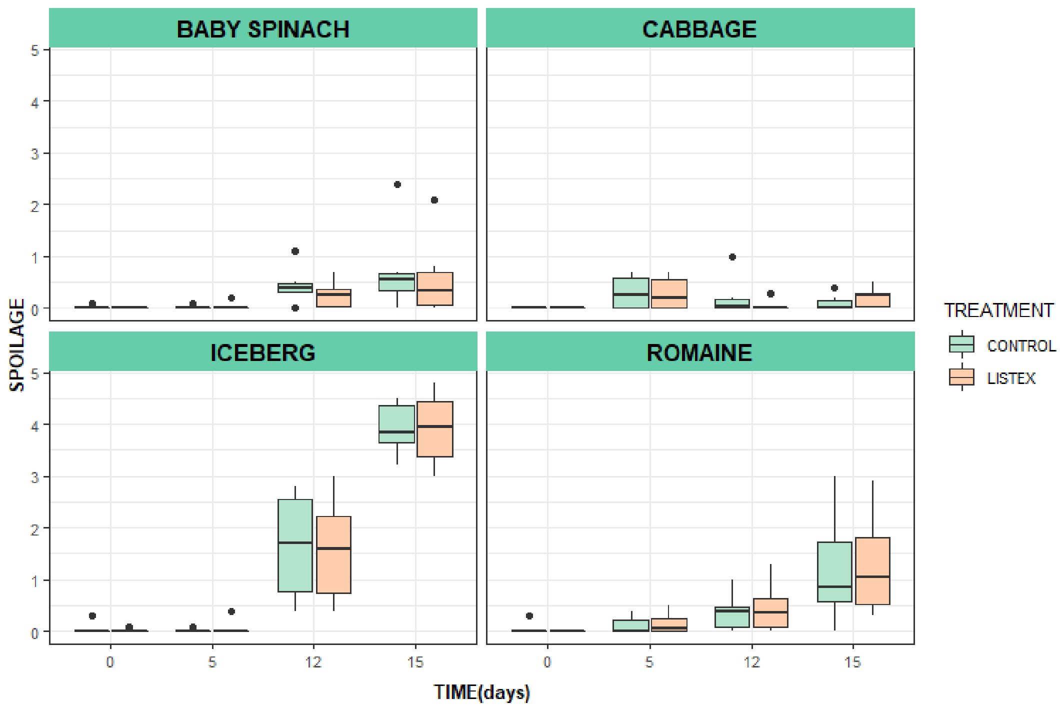

Figure 18. Counts of bacteriophages recovered from shredded iceberg after processing and during the shelf-life of the product at 7 °C in the validation tests performed at industrial scale. Tests A and B were performed in Spain and Tests C and D were performed in Denmark.