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Research Paper

Growth Potential of *Listeria monocytogenes* in Vegan Salami

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ABSTRACT

The increasing demand for plant-based products has raised concerns regarding the potential presence and growth of *Listeria monocytogenes* in ready-to-eat products such as plant-based deli slices. In this study, a challenge test was performed to evaluate the growth potential of *L. monocytogenes* in a vegan salami product. This particular product not only exhibited the highest pH value among the eight similar products available in the supermarket but also had a water activity of 0.96. Additionally, the vegan salami contained no chemical additives with antimicrobial activity, thus possibly representing a "worst case" product for enabling bacterial growth. The product samples were inoculated with two strains of *L. monocytogenes* and stored at 8 °C during their remaining shelf-life (approximately 30 days). The concentration of *L. monocytogenes* was measured at the beginning of the test, at three intermediate time points throughout, and at the end. Water activity and pH were also assessed during the challenge test, and noninoculated samples were analyzed for total bacterial counts (TBCs) and screened for the most prevalent bacterial species with MALDI-TOF-MS. A growth potential (δ) of 4.4 $\log_{10}$ cfu/g was observed for the vegan salami, i.e. *L. monocytogenes* levels increasing from an initial inoculation level of approximately 2.4 $\log_{10}$ to a maximum of 6.8 $\log_{10}$ cfu/g during the challenge test. The screening investigation revealed that species of the genera *Carnobacterium* and *Leuconostoc* were the most prevalent. To summarize, the findings from this study demonstrate that there are currently plant-based deli slices on the market that possess the potential to support the growth of *L. monocytogenes*.

Plant-based food products play an important role in the transition to a more sustainable food consumption system. In recent years, innovative plant-based meat substitutes have been designed to replicate the taste, texture, and nutritional profile of food of animal origin (He et al., 2020). A wide variety of plant-based substitutes are currently available in supermarkets, demonstrating their swift rise in popularity (Van Paepeghem et al., 2024). Deli slice meat analogs, e.g. vegan food products designed to mimic salami or ham, contain plant-based protein sources such as soy and wheat protein isolate, legumes such as peas and lupine, and grains such as rice and potatoes. Additional ingredients can include lipids and polysaccharides, spices, herbs, and natural dyes to imitate the taste, aroma, and color of meat (Flores & Piornos, 2021). Both vegetarian and vegan deli slices are subjected to similar processing steps that meat-based deli slices undergo, thus, there is a potential risk for *Listeria monocytogenes* contamination during e.g. slicing (Rivas et al., 2022). Since plant-based ready-to-eat (RTE) deli slices are frequently packaged in a modified atmosphere and have shelf-lives spanning several weeks, *L. monocytogenes* should be accordingly addressed as a food safety concern for these products (Roberts et al., 2020). To meet the consumer demand for food products that are free of chemical additives (Aschemann-Witzel et al., 2021), natural extracts with antimicrobial activity, such as beetroot concentrate, are often used in the formulation of plant-based deli slices. Beetroot contains colorants (betalains), antioxidants (betalains and phenolic compounds), and preservatives (nitrates) but research evaluating the antimicrobial activity of beetroot extract as an ingredient in food is scarce (Domínguez et al. 2020).

To the authors' knowledge, there are, at present, no reported cases of listeriosis linked to plant-based deli slices. On the other hand, findings of *L. monocytogenes* in plant-based deli slices have been reported within the database of the European Rapid Alert System for Food and Feed (RASFF) due to mandatory analyses performed by the producers in accordance with EU Regulation (EC) 2073/2005 (EC, 2005). The lack of data regarding the presence and proliferation of *L. monocytogenes* in plant-based RTE deli slices poses a serious challenge for both manufacturers and food safety authorities.

The main objective of this study was to determine the growth potential of *L. monocytogenes* in a product of plant-based deli slices

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by performing a challenge test. A secondary aim of this study was to screen for the most prevalent bacterial species in the examined product of plant-based deli slices.

Materials and methods

A challenge test was conducted to determine the growth potential (δ) of *L. monocytogenes* within a product of plant-based deli slices. This was based on the protocol described in the 'Technical guidance document on challenge tests and durability studies for assessing the shelf-life of ready-to-eat foods related to *L. monocytogenes*' of the EU Reference Laboratory for *L. monocytogenes* (EURL Lm, 2021).

Selection of product. A prestudy was carried out to identify a product with the potential for *L. monocytogenes* growth. The selection criteria focused on high pH and water activity (a_w) levels, combined with the suitability of both the product and packaging for inoculation purposes. Eight food items that were available in Swedish supermarkets in January 2024 were analyzed for pH and a_w, and the result values are presented in Table 1.

The food item "vegan salami" was selected because it exhibited the highest pH value among all the tested deli slices, along with an a_w of 0.96. Such characteristics indicated that the vegan salami presented the most favorable environment for *L. monocytogenes* growth of the tested products. Moreover, this product was suitable for a challenge test due to its homogenized structure (Fig. 1). The packaging of this product also had a headspace which enabled inoculation and measurement of gas composition. Details concerning the ingredients and storage recommendations, as displayed on the package of the selected product, are provided in Table 2.

Test points. The challenge test was performed following the Technical Guidance Document (EURL Lm, 2021). In brief, the test was repeated three times in independent trials, with each trial involving a new batch of vegan salami which were all sourced from the same manufacturer but produced on different days. Test units were inoculated with *L. monocytogenes* and subsequently analyzed for *L. monocytogenes* (quantitative method), pH, a_w, and headspace composition. Food control units (i.e. noninoculated products) were tested for the presence of *L. monocytogenes* (qualitative method), pH, a_w, headspace composition, and enumeration of total bacteria count (TBC). Control units were inoculated with the same volume as the test units but only with sterile saline solution. The control units were tested for pH, a_w, headspace composition, and enumeration of TBC. For further details, refer to Table 3.

Selection of bacterial strains. A mixture of at least two strains should be used in the challenge test to accommodate for variations in growth and the survival of different strains (EURL Lm, 2021). The strains 12MOB045LM and 12MOB048LM from the EURL Lm strain collection were selected for this study. Both strains have been used in various tests, including growth at low temperatures (8 °C), low pH (pH 5), and low a_w (0.95) (EURL Lm, 2013). The strain 12MOB045LM originates from meat. The decision to select a strain of meat origin was based on the assumption that plant-based deli slices may be produced in the same facility as meat-based deli slices. Given the likelihood that the same equipment is used for both products, there is a potential risk of cross-contamination between the two product types. Since the tested product (vegan salami) was predominantly comprised of rapeseed oil, potato, and pea protein, the aim was also to select a strain of plant-based origin. The available strains in the collection originated from either dairy, seafood, meat, or "other"; thus, the strain 12MOB048LM from the latter category was chosen although the origin was not further specified. The strains were obtained from the Swedish Food Agency (National Reference Laboratory for *L. monocytogenes*).

Table 1
The pH and water activity (a_w) values of each plant-based deli slice product available in Swedish supermarkets at the time of the prestudy

Product	pH	a_w
Vegan salami	6.01	0.96
Vegan slices (smoked)	5.84	0.96
Vegan slices (paprika)	5.63	0.97
Vegoslices (tomato & basil)	5.14	0.96
Vegoslices original	5.04	0.97
Vegoslices (paprika)	5.02	0.95
Vegan smoked flavor slices	4.85	0.97
Vegoslices salami	4.83	0.97

Preparation of the inoculum, inoculation, and storage. In accordance with the Technical Guidance Document, the two strains were adapted to cold storage conditions and prepared for an early stationary phase when pooled in equal quantities and inoculated in the test units (EURL Lm, 2021). Inoculation was performed on the same day that the product was obtained from the supermarket. The initial target for the inoculum was set at 100 cfu/g, within the range of 50–200 cfu/g for each test unit to comply with the method's detection limit. The inoculation of *L. monocytogenes* was carried out on the product within its packaging by using a needle and a septum (Septum white Ø15 mm, Dansensor®) in line with the Technical Guidance Document (EURL Lm, 2021). The septum was promptly covered by a second septum after inoculation to preserve the headspace composition within the package. A homogeneous distribution of *L. monocytogenes* inoculate was crucial; thus, a standardized procedure for both inoculation and sampling was tested prior to the challenge test. This was performed by inoculating products with a diluted dye to visualize the distribution of the inoculated volume. The test also included checking for any signs of leakage through the septum during inoculation to avoid any changes in the MAP conditions.

Inoculated test units were then stored in an incubator at 8 ± 1 °C to replicate the recommended conditions for consumer storage. The duration of the storage was adjusted accordingly to the remaining shelf-life of the product when it was available for purchase in the supermarket.

Sample preparation. The product's upper lid was cut and removed to open it. The product was then cut into pieces using a sterile scalpel, and the same technique was carried out for each sample (see Fig. 2). Subsequently, 10 g of the vegan salami was collected aseptically using a tweezer, placed in a stomacher bag along with 90 mL of 0.1% Peptone water solution tempered to 25 °C, and then homogenized in a stomacher (AES Laboratoire Easymix, bioMérieux, Craponne, France) for 60 s.

Microbiological analyses. Enumeration of *L. monocytogenes* in test units was conducted in accordance with ISO 11290-2:2017 (ISO, 2017a) immediately after inoculation (t − 0; in triplicate), at best before date (t − end), and at three intermediate time points during the challenge test, as outlined in the Technical Guidance Document (EURL Lm, 2021).

The food control units (noninoculated samples) were analyzed for the presence of *L. monocytogenes* in 25 g at the start of the challenge test, using the detection method specified in ISO 11290-1:2017 (ISO, 2017b). The concentration of background microbiota, i.e. total bacterial counts (TBCs), was also determined in the food control units at all time points during the challenge test. In addition, TBC was determined for the control units (inoculated with sterile saline solution) at t − 0 and t − end. A dilution series was performed after sample preparation using Dilucups (Dilucups® Elegance; LabRobot, Stenungsund, Sweden), and appropriate dilutions of each sample were applied to 3 M Petrifilms™ Aerobic Count Plate (3 M Petrifilm™, St. Paul, MN, USA) in accordance with the manufacturer's guidance. The Petrifilms were incubated at 30 °C for 72 ± 3 h. Following the incubation, all the colonies on Petrifilms were enumerated according to the interpretation guide. Bacterial counts were expressed as logarithmic values.

Bacterial identification was performed on colonies from food control samples representing all trials and the five time-points (t − 0 to

Figure 1. The selected product, vegan salami, with the top lid removed. Photo: Signe Magnussen.

t − end). The Petrifilms for TBC which displayed the highest dilution were selected to represent a subsample of the most common colonies for each sample. Colony material from five colonies was collected from each of these Petrifilms and spread separately onto bovine blood agar plates, which were incubated at 30 °C for 48 h. For analysis, colonies from the blood agar plates were collected using toothpicks and applied in duplicates onto metal 96-well plates customized for matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) (Bruker Daltonics GmbH, Karlsruhe, Germany). A 1 μL portion of HCCA matrix (α-cyano-4-hydroxycinnamic acid dissolved in Bruker© standard solvent containing acetonitrile, water, and trifluoroacetic acid) was then added to each well. The wells were left to dry out before the plates were placed in the MALDI apparatus. Bacterial organisms with a score of 1.7 or above were recorded.

pH, a_w, and headspace composition. Test and control units were assessed for a_w and pH at all time points during the challenge test, while the food control samples were tested at time points t − 0 and t − end (Table 3). The a_w was measured using an Aqualab CX-2 water activity meter (Aqualab, Pullman, United States). Each sample was then collected and placed in a sample cup and inserted into the AquaLab for measurement.

pH was measured using a pH/temperature measurement instrument (Testo 205, Testo SE & Co. KGaA, Germany), and a sample pH was measured by inserting the integrated probe into the top layers of the product. The headspace composition was assessed on all packages throughout the challenge test using Dansensor CheckPoint O_2/CO_2 (PBI Dansensor, Ringsted, Denmark).

Ocular assessment. An ocular assessment was performed on all samples by the same individual, who evaluated any changes in color, odor, or surface characteristics (e.g., slime formation). Additionally, the packages were inspected for signs of potential leakage or bloating.

Calculation of growth potential. The growth potential (δ) determination of *L. monocytogenes* was based on the Technical Guidance Document (EURL Lm, 2021). Thus, the growth potential within one batch was interpreted as 'the difference between the highest observed *L. monocytogenes* concentration during the challenge test and the mean of the initial observed concentration in three replicates at the start of the challenge test (day 0)'. As three batches were subjected to the challenge test, the maximal δ value among the three batches was considered the final and worst-case growth potential. According to the Technical Guidance Document (EURL Lm, 2021), a product is considered capable of supporting the growth of *L. monocytogenes* if $\delta \geq 0.50$ $\log_{10}$ cfu/g.

Table 2
Details on ingredients, nutritional values, storage instructions, length of shelf-life, and packaging conditions of the vegan salami selected for the challenge test

Ingredients	Water, rapeseed oil, potato protein (8% EU), pea protein (7% EU), thickeners (E407, E425, E461, E412), modified starch, crushed linseed, salt, natural food coloring (paprika extract, fenugreek extract, beetroot extract), flavorings, spices (paprika, parsnip), spice extract (paprika, black pepper), sugar, color (E172), barley malt extract
Nutritional values (per 100 g)	Energy 894 kJ / 216 kcal, protein 5 g, carbohydrates 5.5 g (of which sugars 3.5 g), fat 19 g (of which saturated fat 1.1 g), fiber 1.5 g, salt 1.8 g
Storage instructions	Refrigerate at or below +8 °C. Once opened, store in the refrigerator at the same temperature for up to 2 days
Shelf-life	Sealed product 42 days, open product 2 days
Packaging conditions[a]	Modified atmosphere − 80% N_2 and 20% CO_2

[a] Details provided by the manufacturer.

Results and discussion

Challenge test. The challenge test revealed that all tested batches of vegan salami supported the growth of *L. monocytogenes* (Table 4). Following inoculation of 2.4 $\log_{10}$ cfu/g in batch 1, the population of *L. monocytogenes* reached 6.8 $\log_{10}$ cfu/g at the end of the shelf-life, thus exhibiting a growth potential (δ) of 4.4 $\log_{10}$ cfu/g, which was the maximum value for this test. There was no detection of *L. monocytogenes* in the investigated batches prior to inoculation.

It is important to highlight that all batches were inoculated relatively late during their shelf-life of 42 days, i.e. 12 days after the production date for batch 1, 11 days for batch 2 and 16 days after the production date for batch 3. The vegan salami was produced in Germany and then transported to Sweden. It was subsequently stored in a Swedish warehouse after which it was distributed to supermarkets where it could finally be retrieved for this study. Despite the delayed inoculation, batch 3 exhibited a growth curve similar to batch 1 and reached 5.4 $\log_{10}$ cfu/g at the end of the test period. In batch 2, only a slight increase of *L. monocytogenes* was observed throughout the storage period with a growth potential (δ) of 0.65. If the inoculation had occurred within 2 days after the production (which is recommended in the Technical Guidance Document), even higher concentrations of *L. monocytogenes* possibly could have been observed during the challenge tests. It is important to note that, since only a single replicate was analyzed from t − 1 to t − end for each batch, no statistical conclusions can be drawn regarding differences between batches. However, despite a shorter test period than the shelf-life of 42 days, all batches exceeded the 0.5 $\log_{10}$ cfu/g threshold indicating their capacity to support growth of *L. monocytogenes.* This finding demonstrates that the tested product should fall under category 1.2 in the food safety criteria regarding RTE foods in Regulation (EC) 2073/2005 (EC, 2005).

The results of the present study contrast with the challenge tests performed on two types of plant-based deli sandwich slices in a Belgian study in which the maximum δ did not exceed 0.5 $\log_{10}$ cfu/g during their shelf lives of 36 and 45 days, respectively (Van Paepeghem et al. 2024). The pH and a_w values of the products in the Belgian study were within the range that supports the growth of *L. monocytogenes.*

Table 3
The number of food items required for the microbiological analysis (*L. monocytogenes* and TBC) and measurements of pH, a_w, and headspace composition during the challenge test of each batch. $t - 0$ and $t -$ end (best before date) represent the first and last day of the challenge test, respectively

Type of unit	Type of analysis	Number of units and time for analysis	
Test unit (inoculated food)	Enumeration of *L. monocytogenes* Measurement of pH, a_w and headspace composition	7	3 test units at $t - 0$, 3 intermediates, and 1 at $t -$ end 1 test unit at $t - 0$, 3 intermediates, and 1 at $t -$ end
Food control unit (noninoculated food)	Detection of *L. monocytogenes* Measurement of pH, a_w and headspace composition Enumeration of total bacteria count	5	1 test unit at $t - 0$ 1 test unit at $t - 0$ and 1 at $t -$ end 1 test unit at $t - 0$, 3 intermediates and 1 at $t -$ end
Control unit (food inoculated with NaCl)	Measurement of a_w, pH Headspace composition Enumeration of total bacteria count	5	1 test unit at $t - 0$, 3 intermediates and 1 at $t -$ end 1 test unit at $t - 0$ and 1 at $t -$ end 1 test unit at $t - 0$ and 1 at $t -$ end

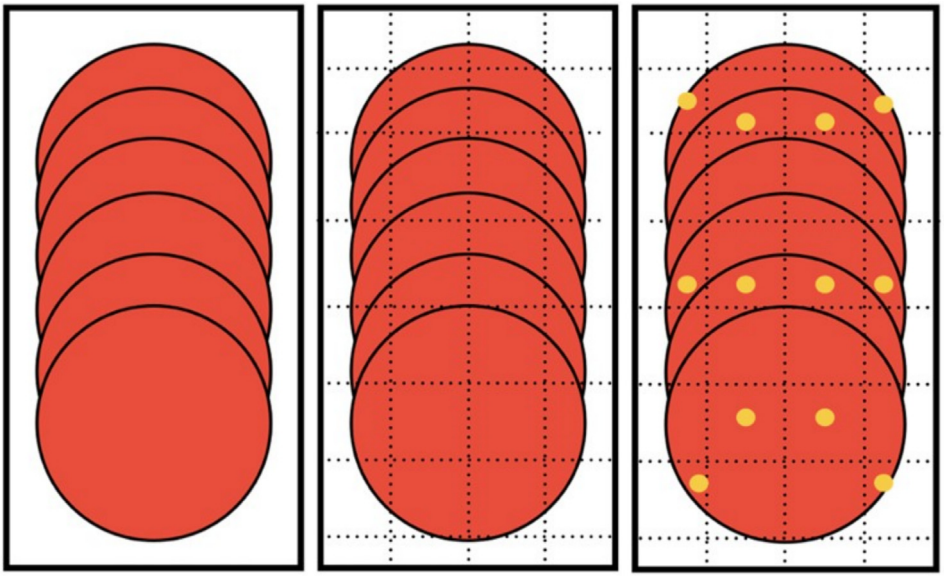

Figure 2. The illustration above shows the product (left), the method of slicing the product (middle), and the slices collected from the product indicated with yellow dots (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

However, it was speculated that growth was inhibited due to the inclusion of additives with antimicrobial activity (potassium and sodium acetate) in the tested products, suspected competitive growth with other bacteria such as LAB (Lactic acid bacteria), and the headspace composition with elevated CO_2 levels (Van Paepeghem et al. 2024). The tested product in the present study did not contain any of these preservatives possessing antimicrobial properties, which may have otherwise inhibited the growth of *L. monocytogenes*. This exclusion is likely due to the manufacturer's intention to meet consumer demand for food free from chemical additives (Aschemann-Witzel et al., 2021). The vegan salami contained beetroot concentrate, which exhibits antioxidant, coloring, and stabilizing properties. However, further research is required to assess its antibacterial effect in processed food products (Domínguez et al. 2020).

Total bacterial counts and screening for bacterial species. The noninoculated vegan salami (food control) displayed TBC counts ranging from 1.7 to 5.9 $\log_{10}$ cfu/g, which was similar to the TBC levels at the start and end of the test period with the samples that were inoculated with sterile saline solution (control units) (Table 5). In batch 1, *L. monocytogenes* exhibited a rapid growth, while maintaining TBC at approximately 3–4 $\log_{10}$ cfu/g. It is important to note that the TBC counts were derived from a different sample than the inoculated test unit and that only one replicate was analyzed at each time point; however, they serve as an indication of the background microflora for each tested batch, which may have inhibited *Listeria* growth. Regarding batch 2, an individual test value stood out ($t - 3$) with a TBC population of 5.9 $\log_{10}$ cfu/g. This was the same batch in which the growth of *L. monocytogenes* was almost completely inhibited which could suggest that a competitive microbiota was present.

To identify the most prevalent bacterial species in vegan salami, a screening investigation was performed, which also enabled an evaluation of potential correlations between certain bacteria and findings in the challenge test. In total, 103 pure-cultured isolates from vegan salami samples were analyzed with MALDI-TOF-MS, which included samples from all batches and time points of noninoculated samples (food control units). The isolates were recovered from the Petrifilms that displayed the most diluted volume from each sample, meaning that the species identified were likely to be among the most prevalent culturable bacteria within the tested product. Only 44 isolates were identified by MALDI-TOF-MS, which means that 59 isolates could not be recognized by the apparatus. This may be attributed to the fact that MALDI-TOF-MS was primarily developed for the identification of clinical isolates, and the database requires updating with reference spectra from microorganisms commonly associated with food (Pavlovic et al., 2013). The identified isolates represented nine bacterial species from the genera *Carnobacterium, Leuconostoc, Lactobacillus, Psychrobacter, Serratia, Acinetobacter,* and Acinetobacter, as well as a fungal species from the genera Debaryomyces (Table 6). *Leuconostoc*, *Lactobacillus,* and *Carnobacterium* are lactic acid bacteria (LAB) that have been associated with the spoilage of RTE meat products (Zagorec & Champomier-Vergès, 2017; Leisner et al., 2007). Bacterial species varied between batches with *Leuconostoc mesenteroides* and *Leuconostoc*

Table 4
Populations of *L. monocytogenes* and calculation of growth potential (δ) during the challenge test performed for vegan salami (values in $\log_{10}$ cfu/g)

Time-point	$t - 0$			$t - 1$ (15 days)	$t - 2$ (23 days)	$t - 3$ (33 days)	$t -$ end (42 days)	Batch δ	Maximal δ
Batch 1	12 days after production	2.49 2.54 2.17	2.40 ± 0.16[a]	3.12	3.55	5.15	6.83	6.83–2.40 = 4.43	4.43
Batch 2	11 days after production	2.58 2.61 2.59	2.59 ± 0.01[a]	2.77	3.03	2.9	3.24	3.24–2.59 = 0.65	
Batch 3	16 days after production[b]	2.63 2.38 2.00	2.34 ± 0.26[a]	–	3.31	3.86	5.44	5.44–2.34 = 3.10	

[a] Mean ± standard deviation
[b] Batch 3 was retrieved from the supermarket 16 days after its production date, resulting in a shortened test period with only 4 sampling points instead of 5 (i.e., no analysis at $t - 1$).

carnosum present only in batch 2. The dominating species at the end of the test ($t -$ end) for batch 2 was *Lactobacillus sakei*. Notably, species of LAB have been reported to show anti-*Listeria* activity (de Paula et al., 2015; Brillet et al., 2005), and they were the dominating species in the batch displaying the lowest growth potential (δ) of *L. monocytogenes*. This suggests that they may play a role in inhibiting *Listeria* growth within this product batch. The isolates identified from batch 1 exhibited a more diverse pattern, with seven species identified, among which *Carnobacterium divergens* was the most observed. Batch 3 yielded four species, with *Debaryomyces hansenii* identified at three different time points during the test (Table 6). *Debaryomyces hansenii* was also identified once in batch 1; it is recognized as a spoilage yeast but may also be included as part of a combined LAB-yeast starter culture in certain types of cheeses and dry-cured hams. However, the antagonistic effect of *D. hansenii* on the growth of *L. monocytogenes* has been limited (Alía et al., 2020; Goerges et al., 2006).

pH, a_w, and headspace composition. The physicochemical parameters, a_w and pH, were similar among the different sample units tested. The a_w for test units and control units was consistent with values of 0.956–0.969 and 0.959–0.971, respectively, throughout the entire duration of the challenge test (Table 5). The pH values for the different batches are shown in Table 5. All batches initially had a pH around 6.0. The control units exhibited a consistent decline in pH towards the end of the shelf-life period. The observed pH decrease may be attributed to the production of lactic acid by the microbiota present. As the test units were inoculated with *L. monocytogenes*, differences in microbiota dynamics between the control and test units may have influenced the presence of LAB and subsequent lactic acid production. Additional explanations for a decreasing pH during shelf life have also been reported, e.g. that CO_2 in the headspace composition reacts with water and generates carbonic acid, dissociating into hydrogen ions (Brown et al., 2018).

The headspace composition measurements during the challenge test are provided in Table 5. The O_2 values ranged between 0.0% and 1.9% which indicates no signs of leakage. The CO_2 values were relatively stable throughout the test for the test units in batch 1 and batch 3. Conversely, batch 2, despite having the same value at $t - 0$, had an increase of CO_2 during the later time points during the challenge test which reached concentrations of 40% and above. The CO_2 values also reached high levels for both the control units and food control units at the end of the test, ranging between 32.7 and 66.8 (Table 5). Since measurements were obtained from a single replicate at each time point for each batch (except at $t - 0$), the values should however be considered indicative. The gaseous environment within modified atmosphere packs is dynamic and directly influenced by factors such as microbial activity, packaging material permeability, product respiration, and the gas absorption by the food (Esmer et al., 2011). The increase in CO_2 levels that was observed during storage in batch 2 could be attributed to the consumption of available oxygen by microorganisms in the food item, along with the production of CO_2 as

Table 5
Results for the measurements of headspace composition (HC; O_2:CO_2 %), pH, a_w, and total bacterial count (TBC) populations ($\log_{10}$ cfu/g) for the inoculated test units, food control units, and control units (inoculated with sterile saline solution) during the challenge study

		Test units			Food control units				Control units			
		pH	a_w	HC	pH	a_w	HC	TBC	pH	a_w	HC	TBC
Batch 1	$t - 0$	6.08[a]	0.962[a]	0.6:13.2[a]	6.00	0.962	0.8:13.0	3.6	6.11	0.963	0.6:13.4	3.6
	$t - 1$	6.11	0.960	1.0:12.7				4.0	5.99	0.964		
	$t - 2$	6.12	0.967	1.2:12.4				3.0	5.26	0.965		
	$t - 3$	6.17	0.962	1.5:12.2				3.1	4.54	0.959		
	$t -$ end	4.46	0.966	1.9:11.1	4.46	0.972	0.0:66.8	2.8	4.48	0.971	0.0:60.5	4.2
Batch 2	$t - 0$	5.97[a]	0.964[a]	0.4:13.5[a]	6.04	0.964	0.1:29.3	2.6	6.02	0.964	0.0:26.1	2.6
	$t - 1$	6.06	0.956	0.9:14.3				4.2	6.03	0.962		
	$t - 2$	5.04	0.969	0.0:40.2				3.3	5.21	0.965		
	$t - 3$	4.66	0.965	0.0:51.8				5.9	4.63	0.967		
	$t -$ end	4.56	0.968	0.0:45.4	4.56	0.968	0.0:54.3	3.2	4.58	0.971	0.0:55.7	2.6
Batch 3	$t - 0$	6.04[a,b]	0.964[a,b]	0.9:16.1[a,b]	6.08	0.976	0.8:16.5	1.7[b]	6.08[b]	0.970[b]	0.5:23.1	1.7
	$t - 1$	–	–	–				–	–	–		
	$t - 2$	5.59	0.957	0.4:22.5				3.7	5.71	0.962		
	$t - 3$	5.4	0.967	0.0:17.2				3.5	4.8	0.969		
	$t -$ end	6.05	0.969	0.0:18.4	5.00	0.961	0.0:32.7	3.2	4.62	0.963	0.0:47.5	4.0

[a] Based on the mean of 3 samples as only one package was examined at the other time points.
[b] Batch 3 was retrieved from the supermarket 16 days after its production date, resulting in a shortened test period with only 4 sampling points instead of 5 (i.e., no analysis at $t - 1$).

Table 6
Number of isolates from the different batches identified as species of bacteria and yeast[a] in the screening investigation. The time point of isolation is indicated in parenthesis

	Batch 1	Batch 2	Batch 3	Total
Carnobacterium divergens	8 (t − 3)		2 (t − 3)	10
Leuconostoc mesenteroides		5 (t − 2), 4 (t − 3)		9
Psychrobacter maritimus	3 (t − 1); 2 (t − 2)			5
Lactobacillus sakei		4 (t − end)	1 (t − 2)	5
Debaryomyces hansenii[a]	1 (t − end)		1 (t − 2), 1 (t − 3), 1 (t − end)	4
Leuconostoc carnosum		1 (t − 2), 2 (t − 3)		3
Psychrobacter pulmonis	3 (t − 2)			3
Serratia proteamaculans	1 (t − 1), 1 (t − 2)		1 (t − 0)	3
Acinetobacter bereziniae	1 (t − 1)			1
Acinetobacter guillouiae	1 (t − 1)			1

a metabolic by-product by certain microflora, such as LAB (Esmer et al., 2011). Since no selective medium for LAB was employed in this study, differences in LAB counts between batches could not be assessed. However, the genus *Leuconostoc* was identified by MALDI-TOF-MS only in batch 2 at t − 2 and t − 3, where CO_2 concentrations exceeded 40%. Elevated CO_2 levels have been reported to promote the growth of lactic acid bacteria and inhibit *L. monocytogenes,* while MAP conditions with 3% O_2 and 5–20% CO_2 could stimulate *L. monocytogenes* growth (Francis & O'Beirne, 1997).

Ocular assessment. From a visual quality control perspective, no alterations in color or odor were observed in any of the examined products. However, during the later stages of the test period, a bloated appearance of some packages from batch 2 was observed. All packages from batch 1 and batch 3 maintained a normal appearance throughout the entire test period.

During the ocular assessment, no differences were observed between the inoculated samples with concentrations of up to 6.8 $\log_{10}$ cfu/g of *L. monocytogenes* and the food control samples that were not inoculated. This highlights the challenge posed by *L. monocytogenes*, as it does not cause any noticeable changes in odor or color. The concentrations of *L. monocytogenes* that were detected during the challenge test can lead to severe illness, particularly for individuals in risk groups (Chen et al., 2003).

The vegan salami was selected for the challenge test due to its high pH level and a_w, which were considered the most favorable for microbial growth, compared to the other plant-based deli slices. By purposefully selecting the product with the highest pH value, the test was aimed at assessing the product with the greatest potential risk of supporting microbial growth. When conducting a challenge test, it is important to note that the results are only applicable to the specific batches tested under the defined temperature and time conditions for this food item. Any alterations in the product's recipe, production process, or storage conditions would render the test results invalid for the tested product, thus necessitating the performance of a new challenge test. It should also be noted that the initial inoculation concentration employed in the challenge test was higher than what would typically be encountered in a production environment. Consequently, the final concentrations observed in this study may not accurately reflect realistic levels after contamination in a commercial production process.

In conclusion, the findings of this study indicate that the tested batches of vegan salami can support the growth of *L. monocytogenes*, resultingly placing them in food category 1.2 – "RTE foods capable of supporting the growth of *L. monocytogenes*" (EC, 2005). To comply with the current EU regulations, food producers must ensure the absence of *L. monocytogenes* in 25 g of the product before it is released from their control. Alternatively, the concentration of *L. monocytogenes* should not exceed 100 cfu/g during the specified shelf-life period. Further studies are required to provide a broader understanding of the growth potential and behavior of *L. monocytogenes* in plant-based deli slices.

CRediT authorship contribution statement

Signe Magnussen: Writing – original draft, Visualization, Investigation. **Emma Bergenkvist:** Writing – review & editing, Investigation. **Karin Söderqvist:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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