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RESEARCH ARTICLE

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Effect of packaging oxygen transmission rate on the shelf life of ready-to-heat foods susceptible to postcontamination during refrigerated and illuminated storage

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Since more and more pressure is exerted to reduce the use of plastic packaging materials, optimizing the use of food packaging is opportune. The aim of this study was to evaluate the combined effect of packaging materials, spanning a range of oxygen transmission rates (OTR), and retail illumination, on the microbial shelf life and safety of refrigerated ready-to-heat foods. Cooked potato slices were packaged in OPA/PP bags with a high OTR (28.85 $ccO_2/m^2/d$) and OPA-EVOH/PP bags with a low OTR (6.57 $ccO_2/m^2/d$). Cooked composite meals were packaged in tray and foil combinations, also spanning a range of OTR: PP trays (2.09 ccO_2/tray/d) with OPA/PP foils (28.85 $ccO_2/m^2/d$), PP trays with OPA-EVOH/PP (6.57 $ccO_2/m^2/d$) foils, and PET trays (0.07 ccO_2/tray/d) with PET top foil (32.86 $ccO_2/m^2/d$) . The packages were stored in a dark environment, or under fluorescent or LED light. Due to the rapid growth of lactic acid bacteria, the microbial shelf life of both food products was largely unaffected by the type of barrier. Illumination at 1000 lux for 12 hours per day led to temperature differences significantly affecting microbial growth. Based on the results, it could be concluded that re-evaluating packaging material choices for these foods may prove valuable, since the use of high-barrier multilayer packaging materials may be considered as a case of overpackaging.

KEYWORDS
dynamic oxygen concentration, packaging, potato slices, ready meals, shelf life

1 | INTRODUCTION

Modern lifestyles have led to an increased demand for convenient and easy-to-prepare food products.[1] Consumers' expectations for these products include high sensorial quality, nutritional value, and a traditional taste and appearance. Also minimal processing, minimal use of additives, and easy preparation are valued. Meanwhile, the producer aims to ensure the food's safety while maintaining quality as long as possible.[2-4] Packaged and refrigerated ready-to-heat products are a typical example of these convenience foods. They typically undergo a heating (pasteurization) step during production, yet remain susceptible for bacterial postcontamination in further processing steps.

The shelf life of these foods, which is the period during which safety and minimal loss of sensory attributes can be guaranteed, is typically dictated by microbial phenomena. Being an excellent substrate for microbial growth, microbial spoilage may occur quickly and lead to sensory defects (off-odors, off-tastes, acidification, visual defects).[3,5] On the other hand, food-borne pathogens may compromise the microbial safety. *Listeria monocytogenes* is an important food-borne human pathogen, which is an aerobic and facultative anaerobic organism, able to grow under refrigerated

conditions, and relatively heat resistant. Ubiquitous and persistent in food-processing environments, it may occur in food products due to postcontamination.[6-9] If the pathogen is not adequately inactivated by heating the food before consumption, this will pose a health risk to the consumer.[10]

To optimize the shelf life of packaged cooked and refrigerated ready-to-heat foods subject to postcontamination, modified atmosphere packaging (MAP) is often applied.[11] This technique allows for shelf life extension with a reduction in the need for preservatives. The main goal of this technique is to exclude atmospheric O_2, which is crucial to the growth of aerobic spoilage organisms, many pathogens, and oxidation phenomena.[12-14] This is achieved by introducing N_2 as a filler gas. CO_2 is often included in rather high concentrations for its effect on microbial growth.[15,16] The overall effect of MAP on microbial flora is an inhibition of aerobic bacteria and/or pathogens and a selection toward lactic acid bacteria (LAB), which are tolerant to these conditions.[17] *L. monocytogenes* is resistant to CO_2, provided that sufficient O_2 is present.[18]

However, the gas composition within a package's headspace does not remain constant. O_2 may be consumed due to bacterial respiration or oxidation processes. Part of the initial amount of CO_2 will dissolve in the product, while the concentration may increase again due to bacterial growth, which leads to bombing of the package.[16] Despite packaging has an indispensable role in maintaining the modified atmosphere, packaging materials always have a certain permeability toward gases.[18] This means that gases will permeate out of or into the package, depending on the partial pressure gradient and transmission properties of the materials.[19]

Gas transmission properties of packaging materials may be improved in two ways. Different materials can be combined into a multilayer material, adding up their properties leading to increased barrier properties and thinner materials. EVOH is a commonly used material in multilayer systems, as thin layers of this material provide excellent gas barrier properties.[20] EVOH is sensitive to moisture, which is why it is commonly embedded within two polymer layers with good moisture barrier properties. The multilayered materials are, however, difficult to recycle, putting a lot of pressure on them given the fact that the European Commission has decided that by 2030 all plastic packaging placed on the EU market should be recyclable or reusable Plastics Strategy).[21] Another option is to increase the thickness of a particular material, since the material permeability is inversely correlated to its thickness. This necessitates the use of more raw materials when compared with multilayer packaging, yet the recycling potential of these materials is higher.

It is however often unclear to food producers which gas permeability is actually adequate for a certain food product.[22] This may lead to so-called "over-packaging" as a matter of precaution. On the other hand, the product could be considered as "under-packaged" if spoilage occurs prematurely due to inadequate packaging.[23] This can be due to growth of aerobic spoilage or pathogenic bacteria by O_2 ingress and loss of CO_2,[24] or due to oxidation phenomena by O_2 ingress.

Illumination is an indispensable part of the life cycle of packaged food products, as an illuminated display allows the consumer to visually evaluate a product before purchase. Illumination can accelerate discoloration and/or oxidation of particularly sensitive products. The effect depends on the illumination time, and on the intensity and spectral distribution of the light.[25,26] Many retailers use fluorescent illumination. However, retailers tend to switch to more energy-efficient LED light sources, which may be beneficial to the quality of particular products.[27,28]

Most retail products are subjected to light exposure and transmission of gases through the package during storage. However, most studies consider products such as meat and cheese, as these are particularly sensitive to O_2 and light. Tsigarida and Nychas[24] evaluated the effect of temperature and packages possessing different gas transmission rate on microbial spoilage of meat fillets. The authors question the necessity of high-barrier packaging materials to control micro-organisms in MAP applications. Møller[29] evaluated the interaction between light and O_2 on sliced ham and defined a critical residual O_2 level between 0.1% and 0.5% to avoid discoloration of the products. Initial O_2 levels up to 0.5% were found not to affect microbial growth. However, the study uses only the initial O_2 level as a factor rather than the dynamic O_2 concentration measured during storage. Møller[30] underlined the importance of maintaining a low O_2 level for the color stability of cured ham, especially with strong illumination. Böhner[27] describes a prolongation of the shelf life of cured sausage with low initial O_2 levels and illuminated with LED lamps instead of fluorescent tubes. Mortensen[31] found strong effects of packages possessing various levels of gas and light permeability on the color and odor of cheeses. Vasquez-Caicedo[32] underlined the importance of minimizing headspace and O_2 availability on quality retention of illuminated mango purée. Similar studies that focus specifically on cooked refrigerated ready-to-heat foods subject to post-contamination are rare; this is likely because these products are not considered as especially sensitive to illumination and O_2. Nonetheless, research identifying possibilities for shelf life extension using improved packaging materials or possibilities to decrease use of packaging materials while maintaining shelf life may prove especially valuable.

In this study, the effect of packaging materials with different barrier properties and different illumination settings on the microbial quality and safety of two cooked refrigerated ready-to-heat food products susceptible to postcontamination is evaluated, the two products being cooked composite meals and cooked potato slices. The packaging materials were selected to cover a range of barrier properties (low to high O_2 transmission) with specific attention toward monolayer or less complex materials. The packaging materials were selected together with the food producers to validate the comparison with real cases. For each product, microbial spoilage was monitored during storage, and a challenge test with *L. monocytogenes* was performed in order to assess the impact on microbial safety. The two food products were selected together with a group of food and packaging producing companies as part of a broader research project on optimization of packaging materials for multiple food products.

2 | MATERIALS AND METHODS

2.1 | Food products

Two ready-to-cook food products were selected for this experiment. The first was precooked potato slices (approximately 5 mm thick and 5 cm in diameter). The second was a composite meal, a typical Flemish dish consisting of mashed potatoes and stewed chicken in béchamel sauce containing cooked mushroom slices, commonly called "vol-au-vent." Samples were delivered by local producers on the day of production. Potato slices were supplied in 450-g portions in six-layer PET/PE/PA/EVOH/PA/PE bags with a thickness of 54 μm. The composite meals were supplied in 450-g trays. The products were stored at 2°C between reception and further handling on the morning after reception.

2.2 | Packaging

Potato slices were weighed in 150-g portions in sterile bags under aseptic conditions in a safety cabinet (Herasafe HS12, Fisher Scientific, Merelbeke, Belgium). The bags were sealed using a Multivac C300 vacuum packaging machine (Multivac, Mechelen, Belgium). The dimensions, materials, and transmission properties of the bags are provided in Table 1. For the *Listeria monocytogenes* challenge tests, an additional high-barrier PET-SiOx bag was used, as *L. monocytogenes* growth is particularly dependent on O_2 availability.

After a vacuum step to 5 mbar, the chamber was flushed with the MAP gas mixture (80% CO_2, 20% N_2) until a pressure of 220 mbar after which the bags were sealed and the vacuum was compensated in the chamber. The gas mixture was created using a Witt MG18-3MSO gas mixer (Gasetechnik, Germany).

The composite meals were weighed in 250-g portions in trays, respecting the original ratio of chicken stew and mashed potatoes. The packages were sealed using a tray sealer (Decatechnic Meca 900, Herentals, Belgium) with a seal temperature of 180°C and a seal time of 1.6 seconds. Before sealing, the trays were flushed with a gas mixture (50% CO_2, 50% N_2, Air Products, Brussels, Belgium). Three different tray and foil combinations were used, as shown in Table 2. For the *L. monocytogenes* challenge tests, an additional high-barrier PP/EVOH/PP tray with OPA-EVOH/PP foil was used.

The O_2 transmission rate (OTR) of foils and bags was measured according to ASTM standard F1927 using a Mocon Ox-Tran 2/21 (Minneapolis, USA). For the foils and bags, 99.9% pure O_2 was used as testing gas. OTR of trays was measured in a same way, according to ASTM standard F1307. For the trays, air (20.9% O_2) was used as testing gas. Measurement conditions are shown in Tables 1 and 2, respectively. These conditions were chosen to reflect the real storage conditions.

2.3 | Storage and sampling

The packaged food samples were stored on shelves in chilled conditions (7°C). Three packages were analyzed before packaging and during the shelf life tests for each combination of illumination and packaging type on each sampling day. For the challenge tests, triplicate measurements were performed before the packaging process and at a proposed end of shelf life. The temperature inside the potato bags was registered each hour throughout storage using iButton temperature loggers (iButtonLink, Whitewater, USA). In this test, the potato slices were stored in the bag in a single layer with minimal overlap between slices. The loggers were stored in these bags as if one of the potato slices.

2.4 | Illumination

Samples were either illuminated with 12-hour dark–12-hour light cycles using a timer switch, or stored in the dark continuously. Non-illuminated samples were kept in a sealed corrugated cardboard box, ensuring no light could reach the samples. For the illuminated samples, two different lamp types were used, being LED spots and fluorescent tubes. The illuminance at sample location was measured using a UPRtek MF250N handheld spectrometer (UPRtek Europe, Aachen, Germany). The apparatus was placed on the shelves where food samples would be stored, measuring at points approximately 30 cm apart to evaluate the homogeneity of illuminance across the entire storage surface. The spectral distribution of the light sources was measured using a Gigahertz-Optik BTS256-EF spectrometer (Gigahertz-Optik GmbH, Türkenfeld, Germany).

The "LED" illumination setup consisted of two LED spots (Philips LED27S/827, 22.5 W) mounted in a cabinet above a plastic diffuser. The goal of this diffuser was to ensure homogeneity of illumination from the two spot light sources across the shelf. This setup is illustrated in Figure 1. The "fluorescent" illumination setup consisted of two fluorescent lamps (840 cool white, 36 W) suspended in a metal rack, approximately 100 cm above the shelves on which the samples were stored. No diffusor was needed for these lamps, as the lamps themselves spanned most of the width of the shelves as opposed to the LED point source.

TABLE 1 Specifications of bags used for potato slice packaging

	Material and Supplier	Thickness, μm	Dimensions, mm	OTR, cc $O_2/m^2/d/atm$
1	OPA/PP (Bemis, France)	65	250 × 300	28.85 ± 0.32[a]
2	OPA/EVOH/OPA/PP (Bemis, France)	65	250 × 300	6.57 ± 1.03[a]
3[b]	PET-SiOx (KMplast, UK)	52	250 × 300	0.18 ± 0.02[a]

[a]Measured at 23°C, 90%RH at outside, 50%RH at inside. Measured with 100% O_2 gas flow.
[b]This packaging was used only for the *L. monocytogenes* challenge test as a third alternative.

TABLE 2 Specifications of packaging concepts (foil/tray combinations) used for composite meal packaging. O_2 ingress was determined by follow-up of the gas concentrations in empty MAP packages stored together with the composite meals

Combination		Material and Supplier	Thickness (μm)	Dimensions, mm	OTR, cc $O_2/m^2/d/atm$	O_2 Ingress, mL/day/Package
1	Foil	OPA/PP (Bemis, France)	65	132 × 182	28.85 ± 0.32[a]	0.13
	Tray	PP (ES-plastic, Germany)		132 × 182 × 37	2.09 ± 0.05[b]	
2	Foil	PET (Petroplast, Germany)	40	132 × 182	32.86 ± 0.48[a]	0.02
	Tray	PET (Faerchplast, Germany)		132 × 182 × 36	0.07 ± 0.01[b]	
3	Foil	OPA/EVOH/OPA/PP (Bemis, France)	65	132 × 182	6.57 ± 1.03[a]	0.12
	Tray	PP (ES-plastic, Germany)		132 × 182 × 37	2.09 ± 0.05[b]	
4[c]	Foil	OPA/EVOH/OPA/PP (Bemis, France)	65	132 × 182	6.57 ± 1.03[a]	0.003
	Tray	PP/EVOH/PP (ES-plastic, Germany)		132 × 182 × 37	0.001 ± 0.001[b]	

[a]Measured at 23°C, 90%RH inside, 50%RH outside. Measured with 100% O_2 as test gas.
[b]Measured at 23°C, 90%RH inside, 50%RH outside. Measured with 21% O_2 as test gas.
[c]This packaging was used only for the *L. monocytogenes* challenge test as a fourth alternative.

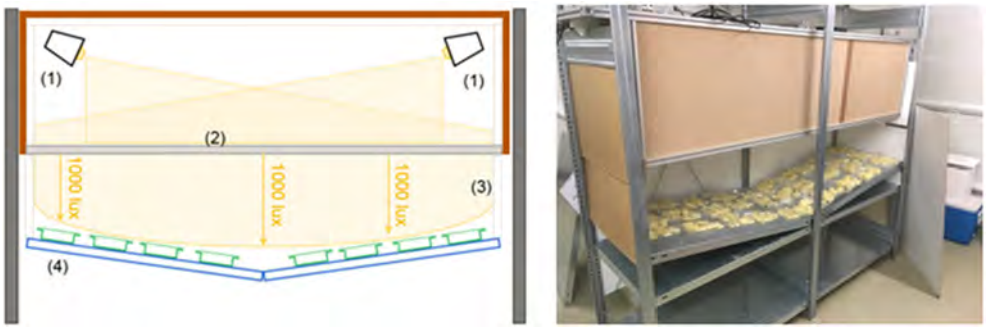

FIGURE 1 Scheme and picture of the illumination setup. A, LED lamps; B, plastic diffusor panel; C, white reflective MDF panels; D, declined storage shelf

The shelves in both setups were declined toward the middle, so that the samples in the middle of the shelf were slightly further from the lamp, as the illuminance on the shelves decreases with distance to the lamps. Also, the storage shelf was lined with white MDF panels, reflecting the light to increase homogeneity of illumination (Figure 1). Both of these modifications were added to ensure that the illuminance across the shelves was constant at approximately 1000 lux, which is a typical illuminance value in a retail environment.[12,28]

3 | ANALYSIS

3.1 | Gas measurement

The headspace gas composition (O_2, CO_2) of the packages was measured using a Checkmate 3 Headspace Gas Analyzer (Mocon Dansensor, Denmark).

3.2 | Microbiological parameters

Approximately 20 g of food sample was weighed in a sterile stomacher bag (Novolab, Geraardsbergen, Belgium), diluted 10 times with peptone physiological solution (PPS, 1 g/L peptone (Oxoid LTD, UK) and 8.5 g/L NaCl (Sigma-Aldrich, Belgium)), and homogenized with a stomacher (IUL masticator, L.E.D. lab solutions, Heusden-Zolder, Belgium). Further serial dilutions were prepared in PPS. Pour plates were prepared by adding Plate Count Agar (PCA, Oxoid Ltd. [Hampshire, United Kingdom]) or deMann Rogosa Sharpe (MRS, Oxoid Ltd. [Hampshire, United Kingdom]) to 1 mL of the appropriate dilution in a petri dish. Spread plates were made by spreading 0.1 mL of inoculum on a petri dish with YGC or ALOA medium (ALOA, Bio-Rad, Temse, Belgium). Total psychrotrophic count (PCA), lactic acid bacteria (MRS), and yeast and mold (YGC) counts were evaluated after incubation at 22°C for 5 days. ALOA plates were incubated for 1 to 2 days at 37°C.

3.3 | *L. monocytogenes* challenge test

Challenge tests were performed on both food products to determine the growth potential of *L. monocytogenes* in all of the packaging concepts. *L. monocytogenes* strains LFMFP-392 (isolated from liver pâté), LFMFP-394 (isolated from cheese), and LFMFP-491 (isolated from tuna salad) were obtained from the FMFP-UGent in-house collection. The strains were incubated for 24 hours at 37°C in TSB (Trypton Soy Broth, Oxoid LTD, UK). Next, 0.1 mL of this solution was diluted in 10-mL TSB and incubated again for

24 hours at 37°C. To perform a cold adaptation, 0.1 mL of the solution was diluted in 10-mL TSB and incubated for 4 days at 7°C to obtain cells at the end of the exponential phase, as described in the EU RL guidance for conducting shelf life studies with *L. monocytogenes*.[6] Subsequently, the three strains were mixed in equal amounts. The concentration of this mixed inoculum was determined on ALOA plates. The inoculum was diluted in PPS in order to inoculate the sample at a level of 100 CFU/g. Potato slices were inoculated by adding 0.150 mL of inoculum to 150 g of slices and mixing thoroughly. The composite meals were inoculated by thoroughly mixing 0.250 mL of inoculum through 250 g of sample. Samples were analyzed in threefold immediately after inoculation. Inoculated potato slices were stored for 14 days at 7°C; inoculated composite meals were stored for 7 days at 7°C. The growth potential of *L. monocytogenes* was calculated as the difference between the median count at the end of the shelf life and the median count immediately after inoculation.[6,33] In addition, maximal growth was calculated as the difference between the highest count observed at the end of the shelf life and the lowest count immediately after inoculation.

3.4 | pH measurement

The food products were homogenized, and the pH was measured directly on the samples using a SevenEasy pH probe (Mettler Toledo, Zaventem, Belgium).

3.5 | Color measurements

Color of potato slices was measured by performing three repeated measurements on three separate slices using a Konica Minolta CM2500-D (Konica Minolta Sensing Europe, Bremen, Germany) handheld color analyzer set at D65 Illuminant and 10° Standard Observer. Results were rendered in the CIELAB color space.

3.6 | Statistics

Data were analyzed using R Statistics 3.5.[34] Significance of the main effects "packaging type," "illumination type," and "time of storage" and the interaction effects "packaging type—time" and "illumination type—time" were evaluated using univariate general linear models. The results are shown in Tables 3 and 4. A main effect was interpreted as a global difference over all levels of the other factors. A time-based interaction effect was interpreted as an effect of the factor over only some of the time points evaluated. Assumption of normality was evaluated using model residuals histograms and Q-Q plots for all factor levels. Levene's test was used to check homogeneity of variances. Estimated marginal means were used to compare pairs of levels of one factor for all levels of the other two factors. Comparisons were based on Fischer's least significant difference test (LSD). *L. monocytogenes* counts were evaluated using IBM SPSS statistics 25 (IBM, Armonk, NY). Graphs were constructed using Sigmaplot 13 (Systat Software, San Jose, CA).

TABLE 3 Significance of effects of storage time (time), packaging type (package), and illumination type (light) on O_2 and CO_2 concentrations and microbial counts of potato slices. Significant effects are shown in bold

	Main Effects			Interaction Effects			
	Time	Package	Light	Time × package	Time × light	Package × light	Package × light × time
O_2 concentration	**0.00**	0.13	**0.02**	0.50	**0.00**	**0.02**	0.10
CO_2 concentration	**0.00**	**0.00**	0.07	0.05	0.05	0.08	0.76
Total aerobic counts	**0.00**	0.84	**0.00**	0.61	0.24	0.39	0.23
Lactic acid bacteria counts	**0.00**	0.10	**0.00**	**0.00**	**0.00**	0.81	**0.00**
Yeast and mold counts	**0.00**	**0.00**	0.68	0.44	0.93	0.46	**0.00**

TABLE 4 Significance of effects of storage time (time), packaging type (package), and illumination type (light) on O_2 and CO_2 concentrations and microbial counts of ready meals. Significant effects are shown in bold. * indicates that some heteroskedasticity was detected for the parameter

	Main Effects			Interaction Effects			
	Time	Package	Light	Time × package	Time × light	Package × light	Package × light × time
O_2 concentration*	**0.00**	**0.00**	0.08	**0.00**	0.13	0.34	**0.00**
CO_2 concentration	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.90	**0.02**
Total aerobic counts*	**0.00**	0.15	**0.00**	**0.04**	**0.00**	0.26	**0.02**
Lactic acid bacteria counts	**0.00**	0.95	**0.00**	0.25	**0.00**	0.16	0.05

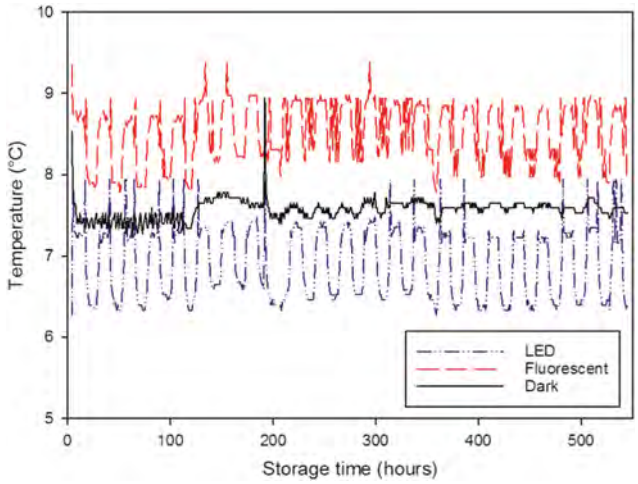

FIGURE 2 Temperature profile of packages stored in a dark environment (solid line), and under fluorescent (dashed line) or LED illumination (dashed and dotted line)

4 | RESULTS

4.1 | Potato slices

4.1.1 | Temperature inside bags

The small temperature fluctuations for the samples stored in the dark environment were due to latency during temperature regulation in the cooling chamber (Figure 2). For illuminated samples, stronger fluctuations appeared. The temperature cycled in parallel with the illumination, due to heat output of the lamps.[13] When the lamps were switched on, temperature increased over the course of a 3-hour period and remained at this increased level throughout the illuminated period. When the lamps were switched off again, the temperature decreased back to the initial temperature. The average temperature difference was 0.7 ± 0.3°C for fluorescent and 0.8 ± 0.3°C for LED illumination. These fluctuations brought about an overall temperature increase, likely to affect spoilage kinetics.[35] The average temperature of the potato slice samples stored in the dark was 7.6 ± 0.2°C. Samples stored in LED light had an average temperature of 6.9 ± 0.5°C, and samples stored in fluorescent light had an average temperature of 8.5 ± 0.4°C. A momentary temperature peak at about 180 hours of storage was due to a temporary power cut. The base temperature in the LED illumination cabinet was found to be lower than in the fluorescent illumination cabinet. This was likely due to the position in the cooling chamber, as the former cabinet was positioned alongside the wall neighboring another cooling chamber which is set at 4°C. The fluorescent illumination cabinet was positioned next to an outer wall, as was the rack where the nonilluminated samples were stored. The average temperatures in all three illumination conditions differed significantly from each other (P = .000 in all cases). The temperature was not separately followed up in the composite meal packages, but was expected to be similar, as the storage conditions were the same.

4.1.2 | Color

High color variability, both between and within slices, made it difficult to draw any conclusions from the color measurements on potato slices. However, upon visual evaluation of the samples, no color defects were noticed during storage in any of the experimental conditions considered.

4.1.3 | Headspace gas concentrations

The initially measured O_2 concentrations (Figure 3) were a result of the residual O_2 and the combined effect of ingress and O_2 released from the product itself. When microbial growth reached the exponential phase (Figure 4), O_2 consumption increased. This was clearly noticeable in the potato slices experiments, where the O_2 concentration rapidly decreased toward zero after the third day of storage. The storage time, as should be expected, had a significant effect on the measured O_2 concentrations (P = .000). Packaging did not affect the O_2 concentrations (P = .134) as a main effect, nor did the packaging type affect the change of O_2 over time (P = .505 for the "time" × "packaging" interaction). Illumination conditions had a significant main effect (P = .023). This effect is likely not due to the illumination itself, but rather to the temperature effect discussed above. During the *L. monocytogenes* challenge test, concentrations were followed up more frequently but without replications (results not shown). The effect of illumination was not significant during this test (P = .54).

The CO_2 content gradually decreased during the first days as 10% to 20% of CO_2 dissolved in the product.[16] The CO_2 concentration reached a minimum at day 2-3. After 4 days of storage, as microbial growth exceeds 6 log CFU/g (Figure 4), CO_2 concentrations started to increase, before stabilizing at just below 90% from Day 7 onward (Figure 3). The OPA-EVOH/PP bags led to higher overall CO_2 concentrations (P = .000) which may be due to higher barrier properties of the material. The effect of packaging type and illumination type on the changes during storage was not significant (P = .053 and P = .053). In the challenge test, both of these effects were significant (P = .000 and P = .000). The packaging effect is likely due to differences in permeation, while the illumination effect may be due to increased microbial CO_2 production due to the temperature rise effect.

4.1.4 | Microbial growth

As the products were packed under modified atmosphere, LAB were the predominantly specific spoilage organisms, determining the total aerobic counts as well during storage. Initial counts were below 4 log CFU/g (Figure 5). Exponential growth started immediately after repackaging, and the stationary phase was reached by the 10th day of storage. No global effect of packaging material on LAB counts was detected (P = .10). Packaging material did, however, affect the change throughout time (P value of time × packaging interaction = .001). Yet,

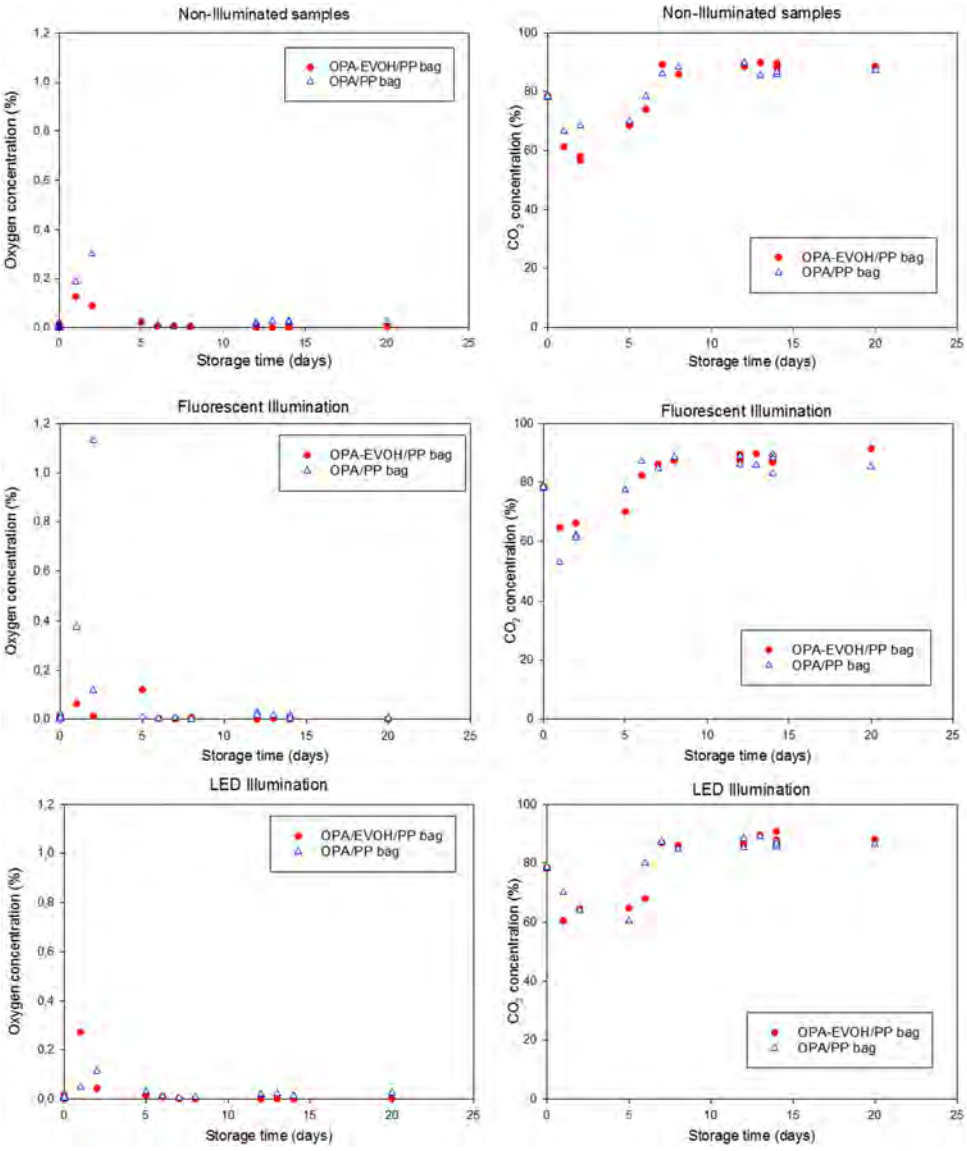

FIGURE 3 O_2 (left) and CO_2 (right) concentration profiles of OPA-EVOH/PP (OTR = 6.57 ± 1.03 $ccO_2/m^2/day/atm$) and OPA/PP (OTR = 28.85 ± 0.32 $ccO_2/m^2/day/atm$) bags containing potato slices, stored in darkness (average temperature = 7.6 ± 0.2°C), fluorescent illumination (average temperature = 8.5 ± 0.4°C), or LED illumination (average temperature = 6.9 ± 0.5°C). Each data point represents one measurement on one package

by comparing the individual contrasts, this effect could not be brought back to one of the packaging concepts leading to consistently higher counts than the other. A significant effect was not expected, as the measured O_2 concentrations were not significantly different between packages, and LAB are less affected by CO_2 concentrations. Illumination conditions had both a global (P = .000) and a time-based effect (P = .000) on LAB counts. The samples under fluorescent illumination had overall a higher count than the samples under LED illumination, in particular during the exponential growth phase. This corresponds with the illumination effect on CO_2 concentrations as discussed above.

After 3-week storage, the pH level of the potato slices decreased from 5.7 to 4.7-4.9. No significant global effect of the packaging material on the pH level of the potato slices was detected (P = .90). Packaging slightly affected the change throughout time (P = .035). However, no clear trend could be determined from the individual contrasts. Illumination type had a distinct effect on the pH level (P = .000) and its change throughout time (P = .001). Overall, samples in LED illumination showed less pronounced pH level decrease than the fluorescent illuminated samples.

Yeast and mold counts remained close to the detection limit during storage. Neither packaging (P = .44) nor illumination type (P = .93) affected the counts throughout time. No visual signs of mold growth were observed, as they were inhibited by the exclusion of O_2 due to MAP packaging, as well as other intrinsic factors of the products such as pH and water activity.[36]

4.1.5 | Microbial safety: *L. monocytogenes* growth potential

The main goals of *L. monocytogenes* challenge tests are to evaluate whether a food product supports the growth of the pathogen, and the extent to which it can grow. The latter dictates which tolerance should be applied on the contamination of the product immediately after production to ensure that the contamination level at the end of shelf life does not exceed 2 log CFU/g.

Three samples of potato slices were analyzed immediately after inoculation, revealing *L. monocytogenes* inoculation levels of 2.3, 2.6,

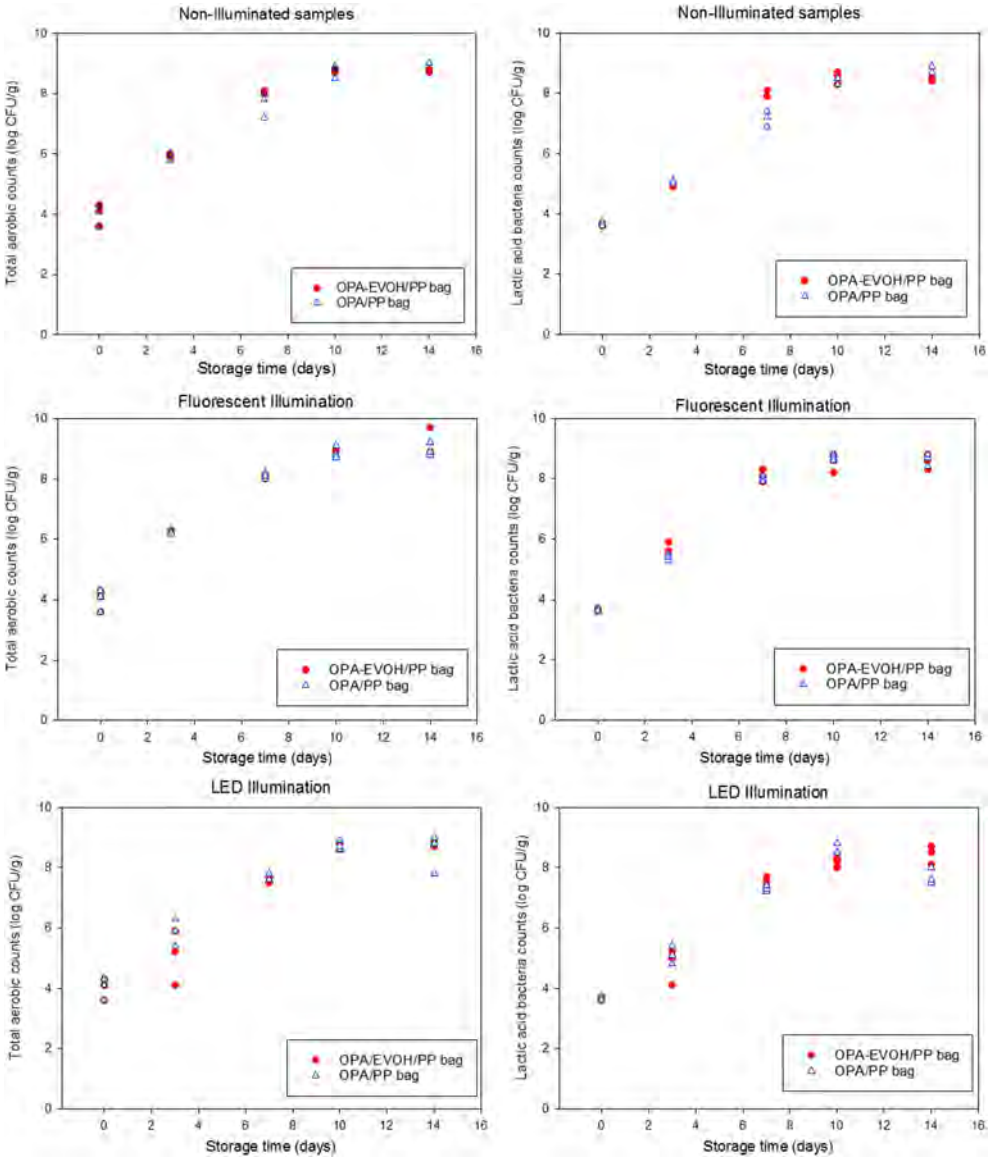

FIGURE 4 Total aerobic counts (left) and lactic acid bacteria counts (right) of the potato slices stored in OPA-EVOH/PP (OTR = 6.57 ± 1.03 $ccO_2/m^2/day/atm$) bags and OPA/PP (OTR = 28.85 ± 0.32 $ccO_2/m^2/day/atm$) bags, in darkness (average temperature = 7.6 ± 0.2°C), fluorescent illumination (average temperature = 8.5 ± 0.4°C) or LED illumination (average temperature = 6.9 ± 0.5°C). Each data point represents one measurement on one package

and 2.8 log CFU/g. The counts after 14 days of storage at 7°C and the resulting growth potentials and maximal growth are shown in Table 5. Based on these results, no clear effects of the packaging transmission properties and the illumination conditions was noticed. For the dark and fluorescent illuminated storage conditions, the differences between all growth potentials remain below 0.5 log and thus cannot be considered as significantly different. Even if the worst case is considered (maximum growth), microbial safety may be guaranteed in all cases after 14 days of storage at 7°C if the absence of *L. monocytogenes* in 1 g of sample is achieved.

TABLE 5 Counts of L. monocytogenes inoculated on potato slices, before and after 14 days storage at 7°C. O2 transmission rates of the packaging types decrease from left to right. A triplicate measurement on the product immediately after inoculation showed an initial contamination level of 2.3-2.6-2.8 log CFU/g. The growth potential was calculated as the difference of the median values at day 0 and day 14. The maximal growth was calculated as the difference between the highest value at day 14 and the lowest value at day 0

Packaging Type	OPA/PP Bag			OPA-EVOH/PP Bag			PET/SiOx Bag		
Illumination	**Dark**	**Fluorescent**	**LED**	**Dark**	**Fluorescent**	**LED**	**Dark**	**Fluorescent**	**LED**
Levels at day 14 (Log CFU/g)	3.0	< 2.0	3.2	3.6	3.0	2.7	2.9	3.4	<2.0
	2.9	3.2	3.3	2.8	3.2	<2.0	3.3	2.0	2.8
	2.7	3.7	2.8	2.0	3.3	2.0	3.0	2.7	2.6
Growth potential	0.3	0.6	0.6	0.2	0.6	−0.6	0.4	0.1	0.0
Maximal growth	0.7	1.4	1.0	1.3	1.0	0.4	1.0	1.1	0.5

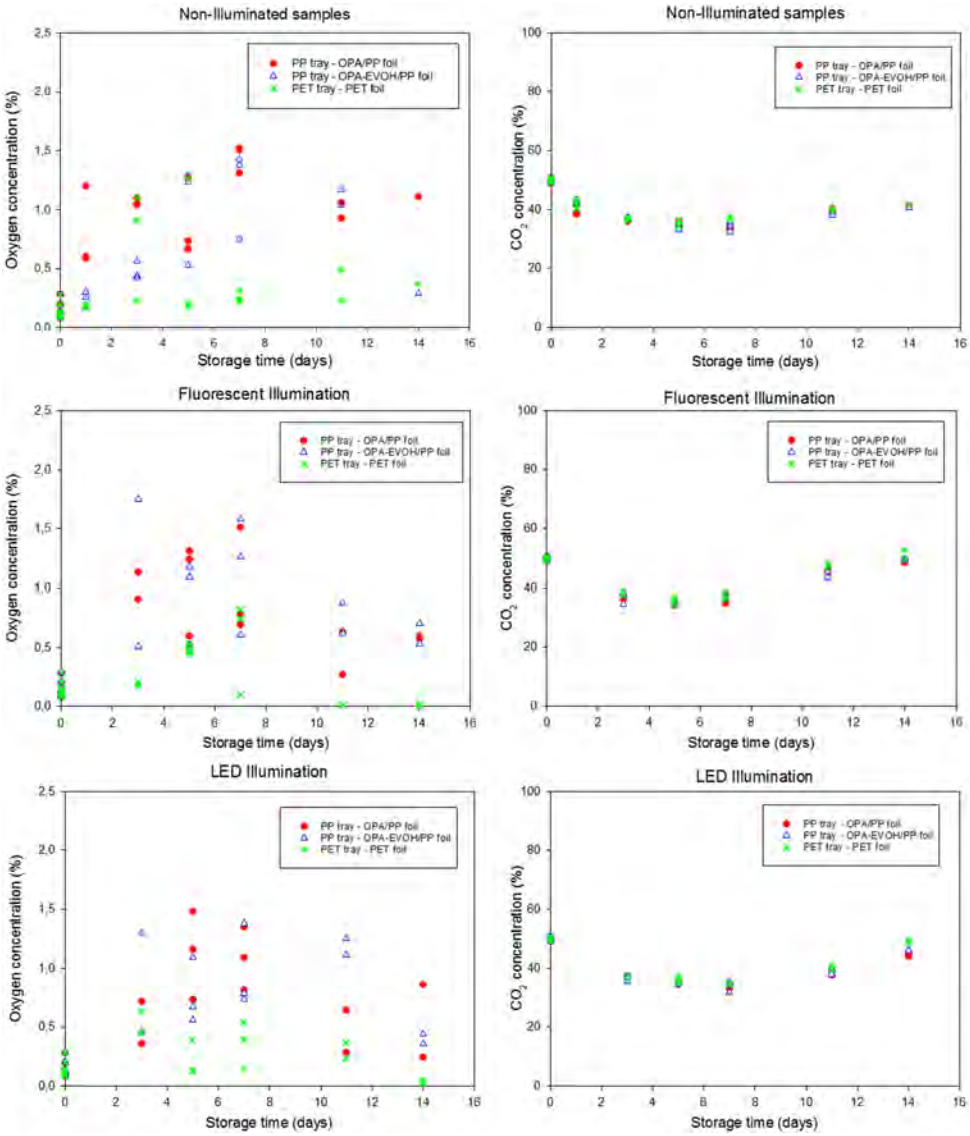

FIGURE 5 O_2 (left) and CO_2 (right) concentration profiles of composite meals stored in PP trays with OPA/PP foil, PP trays with OPA-EVOH/PP foils, and PET trays with PET foils. The samples were either stored in darkness, fluorescent illumination, or LED illumination. Each data point represents one measurement on one package

4.1.6 | Sensory properties

Samples were evaluated visually on each sampling day and smelled immediately after opening. No visual defects were noticed during storage. Some increase of acidity was noticed after 1 week of storage, yet no discernible difference between packaging or illumination conditions could be identified. Based on these observations, it is not likely that an average consumer would evaluate either of the studied conditions as less (or not) fit for consumption at a certain point of time.

4.2 | Composite meals

4.2.1 | Headspace gas concentrations

For the composite meal experiment (Figure 5), the trend in the O_2 concentration changes discussed above is less pronounced. Residual O_2 levels were higher than in the potato slice test, as the stew and mashed potatoes are more likely to contain dissolved O_2 or air pockets than the potato slices. Analysis of variance showed an overall effect of packaging concept (P = 0.000) and the over-time effect of packaging concept (P = 0.000). This is mainly due to the PET package outperforming the other two packaging concepts. Changing the top foil for PP packages from OPA/PP to OPA-EVOH/PP (the latter having a four times lower permeability to O_2) had very little effect on the overall transmission of the package, as most of the transmission seemed to occur through the tray rather than the foil. In this experiment, the illumination conditions had no significant global effect (P = .8) nor an effect over time (P = .13) on O_2 concentrations.

Considering CO_2 (Figure 5), both packaging and illumination conditions were found to be significant (P = .000). This difference is clear from Day 5 onward. The CO_2 concentrations for the fluorescent illuminated samples, in particular on Day 11 and Day 14, are notably higher than the nonilluminated samples. This points to an increased microbial activity, likely to be attributed to the temperature differences discussed above.

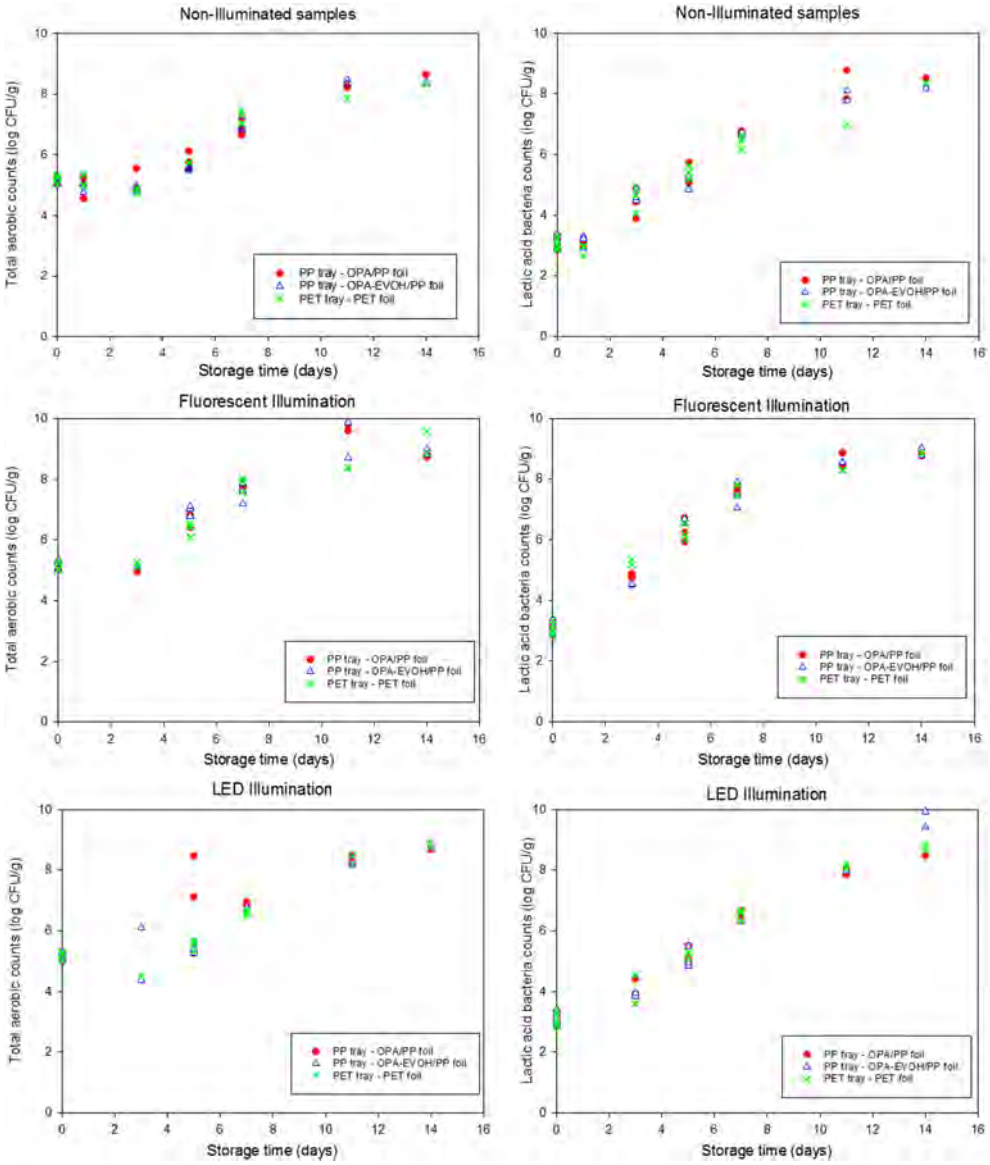

FIGURE 6 Total aerobic counts (left) and lactic acid bacteria counts (right) of composite meals stored in PP trays with OPA/PP foil, PP trays with OPA-EVOH/PP foils, and PET trays with PET foils. The samples were either stored in darkness, fluorescent illumination, or LED illumination. Each data point represents one measurement on one package

4.2.2 | Microbial growth

The composite meals showed an initial total aerobic count of 5.1 log CFU/g (Figure 6). In this case, initial LAB counts were found to be significantly lower at 3.1 log CFU/g (P = .124). LAB started to grow during the first day of storage, becoming the dominant species after 5 days (no more significant difference with total aerobic counts). The stationary phase was reached after 9 to 10 days of storage. Packaging type had no significant global effect on the LAB counts (P = .95), nor an over-time effect (P = .247). This is the same finding as for the potato slice experiments, although the gas concentrations measured were affected by packaging type in this case. Illumination did have an effect (P = .000) on storage Day 5 and 7, where the counts in fluorescent illumination are significantly higher than the others.

4.2.3 | Microbial safety: *L. monocytogenes* growth potential

Three samples of the composite meals were analyzed immediately after inoculation, revealing *L. monocytogenes* inoculation levels of 2.8, 3.1, and 3.2 log CFU/g. The counts after 7 days of storage at 7°C, and the resulting growth potentials and maximal growth are shown in Table 6.

None of the median growth potentials exceeded 3 log CFU/g. This means that an initial tolerance of absence in 10 g (< −1 log CFU/g) would imply microbial safety after 14 days of storage at 7°C. When considering the worst cases (maximal growth), some cases exceed 3 log CFU/g growth potential, requiring a stricter target value (absence in 25 g) to ensure safety. It is notable that this is the case for all the samples in fluorescent illumination. This is likely due to the temperature differences (Figure 2).

TABLE 6 Counts of L. monocytogenes inoculated on composite meals, before and after 7 days of storage at 7°C. O_2 transmission rates of the packaging types decrease from left to right. A triplicate measurement on the product immediately after inoculation showed an initial contamination level of 3.2-3.1-2.8 log CFU/g. The growth potential was calculated as the difference of the median values at day 0 and day 14. The maximal growth was calculated as the difference between the highest value at day 14 and the lowest value at day 0

Packaging Type	PP Tray			PP Tray			PET Tray			PP/EVOH/PP Tray		
	OPA/PP foil			OPA-EVOH/PP foil			PET foil			OPA-EVOH/PP foil		
Illumination	Dark	Fluorescent	LED	Dark	Fluorescent	LED	Dark	Fluorescent	LED	Dark	Fluorescent	LED
Levels at day 7 (Log CFU/g)	5.6	5.8	5.3	5.7	5.8	5.6	5.6	5.5	5.4	5.6	5.5	5.4
	5.4	6.2	5.4	5.7	6.7	5.5	5.8	6.2	5.6	5.7	5.8	5.3
	5.6	6.1	5.1	6.0	5.8	5.2	5.7	5.8	4.8	5.3	5.3	5.3
Growth potential	2.5	3.0	2.2	2.6	2.7	2.4	2.6	2.7	2.3	2.5	2.4	2.2
Maximal growth	2.8	3.4	2.6	3.2	3.9	2.8	3.0	3.4	2.8	2.9	3.0	2.6

Within each illumination condition, the difference between the growth potentials for the different packaging concepts remains below 0.5 log. This means that the difference between packaging concepts cannot be conventionally considered as significant.

4.2.4 | Sensory properties

As with the potato slices, the samples' visual appearance and smell immediately after opening the package was evaluated. Once more, an average consumer would have considered all samples to be fit for consumption. No visual or olfactory signs of spoilage were apparent.

5 | DISCUSSION

Microbial growth was indicated as the main index of failure for both studied food products. In the considered tests on both ready-to-heat foods, no consistent significant effects of the difference in OTR values on bacterial growth were detected, as evaluated by the total aerobic and LAB counts. Due to the highly specific nature of the products evaluated in the current study, comparisons can only be made to the general group of MAP packaged short shelf life food products. The limited effect of packaging type on microbial growth, as observed in the current study, is supported by findings by Peelman et al[37] who evaluated multiple bio-based and conventional packaging materials with a broad range of OTR on multiple MAP packaged products. The rapid growth of LAB in MAP conditions, becoming the dominant specific spoilage organisms early during storage, corresponds with similar studies: Pothakos et al[38] found LAB to dominate the psychrotrophic counts at the end of shelf life of six MAP-packaged ready-to-eat dishes, which ranged from 7.3 to 9.3 log CFU/g. Similar results were obtained by Spencer.[39] McAteer et al[40] used permeable (OTR 140 cc O_2/m^2/day) trays for chill storage of beef and mashed potatoes and found rapid spoilage due to growth of Streptococcus sp. and aerobic Pseudomonas sp., showing the benefits of MAP technology for this type of product.

No effect of packaging material choice on the growth of *L. monocytogenes* on the potato slices or the composite meals could be detected. The finding that the potato slices did not support the growth of *L. monocytogenes* corresponds with findings from Juneja,[10] who found no significant growth of *L. monocytogenes* on inoculated potato slices stored under refrigeration for up to 21 days. It should be noted though that in this research the slices were stored at 4°C. Composite meals did support the growth of *L. monocytogenes*, yet the packaging type again had no significant effect. The additional high-barrier packaging concept evaluated in both of the challenge tests did not hold any significant benefit over the lowest tested barrier packaging concepts. This shows that food safety should not necessarily be decisive when reconsidering packaging concepts for these foods. Respecting the cold chain and the initial quality tolerances will be of major importance in this case, rather than the packaging choice.

Food producers should not only carefully consider which packaging material to use for their product, but also how well a foil and tray are matched. The current results show no effect of changing the top foil for a PP tray from OPA/PP to OPA-EVOH/PP, despite the latter having a four times lower O_2 permeability. Based on the gas measurements, both of the concepts are outperformed by a monolayer PET tray and PET foil, the PET foil being even thinner than both the OPA/PP and OPA-EVOH/PP foils.

The products studied are not considered as particularly sensitive to illumination. The effects of illumination conditions identified in this manuscript were likely due to heat transfer from the lamps and heat sinks, as well as to overall temperature differences. Twelve hours of illumination per day throughout the whole storage period was a severe overestimation of the illumination to be expected in an actual retail environment, as Larsen[14] found that realistically, an average cumulative illumination time of 24 hours should be expected for retailed processed meat. Despite illumination times likely differing for different food products depending on the type of grocery store and rollover, this should make a better assumption for further tests. Eventual temperature increases due to heat transfer will also be less significant on average when respecting this.

Considering the sensory properties, the general visual appearance of the food product and the scent immediately after opening the

packaging was evaluated, just as a consumer would do before consuming the product. For both evaluated food products, no clear discernible difference between samples stored under different conditions were reported. Despite the significant microbial growth, it was hypothesized that an average consumer would not consider any of the food products stored under the reported conditions as not fit for consumption.

6 | CONCLUSIONS

As microbial growth, in particular that of LAB, is considered the main spoilage factor of the studied food products, raising or lowering the barrier properties of the packaging materials seems to have very limited effect. Superficial sensory evaluation, similar to what a consumer would do before purchasing or consuming the product, did in no case lead to a rejection of one of the products. It can be concluded that based on the range of transmission properties evaluated in this study, the considered products do not necessarily benefit from one of the applied high-barrier packaging materials to extend the shelf life. Both food products were delivered in packages consisting fully or partially of high-barrier multilayered materials. Monolayer packaging materials such as PET could be considered over multilayer packages as a more easy-to-recycle solution, provided a minimal protection and barrier capacity is met. Re-evaluating the selected packaging materials for food products that are not considered particularly sensitive to O_2-related spoilage phenomena may prove especially valuable from a sustainability point of view.

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