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RESEARCH ARTICLE

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Applicability of oxygen scavengers for shelf life extension during illuminated storage of cured cooked meat products packaged under modified atmosphere in materials with high and low oxygen permeability

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The aim of this study was to evaluate the effect of illumination, packaging material and application of oxygen scavengers on the shelf life of two different cured cooked meat products in diverse retail storage conditions. For this purpose, ham luncheon sausage and pork liver pâté were packaged in high- and low-OTR (oxygen transmission rate) packages under modified atmosphere. Packages either did or did not contain a 50-ml O_2 capacity oxygen scavenger. Samples were stored at 7°C either in complete darkness or illuminated by fluorescent or LED lamps for the last 48 h of storage. Microbial quality of pâté was more crucial than that of ham sausage, partially due to a higher initial microbial load. Pâté was far more susceptible to discolouration and lipid oxidation than ham luncheon sausage. Illumination was a crucial aspect for discolouration of ham sausage, whereas pâté exhibited discolouration without illumination when packaged in low barrier packages. Hence, high barrier packages are indispensable for these types of products, especially pâté. Inclusion of an oxygen scavenger may lead to improved colour stability and lower lipid oxidation, provided that the packaging material had a sufficiently low OTR and some days of dark storage preceded illumination. Replacing a multilayered high-barrier packaging system with a lower barrier system with an added scavenger is not an interesting option as there is competition for oxygen absorption between the food product and the scavenger.

KEYWORDS
gas transmission rates, meat products, oxygen scavengers, packaging, shelf life

1 | INTRODUCTION

Packaged cured cooked meat products are sensitive to spoilage due to discolouration, lipid oxidation and microbial growth. The rate of spoilage is affected by multiple factors such as residual oxygen in the package headspace, product/headspace ratio, oxygen transmission of the packaging material, product composition and illumination conditions.[1–6] Visual appearance and especially the colour is of major importance, as this is the only product property by which a consumer's buying decision is influenced in a retail environment. Rejection of fresh meat by consumers due to early discolouration may lead to major revenue losses, although discolouration does not necessarily imply that the product is unfit for consumption.[3,7] The colour of cured cooked meats is due to denatured nitrosylmyoglobin or nitrosylmyochrome. Light-induced oxidation of this pigment leads to the formation of denatured metmyoglobin, which has pro-oxidative capacities and imparts a brown-grey hue to the product.[3–5,8]

Three aspects of illumination are of importance in the discolouration of cured meat products: the emitted wavelengths (spectral distribution), their spectral irradiance on the meat product and the exposure time. Nitrosylmyoglobine is particularly sensitive to wavelengths around 420, 545 and 575 nm.[3,4,9] Application of illumination sources focusing more on the red part of the visible spectrum, with less contributions of the aforementioned wavelengths, has been found to lead to less intense oxidation.[3] Photo-oxidation is a fast reaction: most colour of illuminated cured cooked meat products is lost during the first 6–24 h of illumination.[10–12] However, illumination is hard to completely eliminate in a retail environment. Overall visual appearance, which is strongly influenced by illumination, after all is a crucial factor in a consumer's buying decision.[13]

Influence of oxygen on the quality of meat products has been studied extensively.[3,5,10,11,14] Residual oxygen in the headspace after packaging should always be considered together with the O_2 permeability of the packaging material. Møller et al.[5] found the critical O_2 percentage for illuminated chilled storage of cured cooked ham to be between 0.1% and 0.5%. Similarly, Larsen et al.[10] found that 0.15% of O_2 was the highest acceptable amount of oxygen at the time of illumination to prevent discolouration of packaged cured cooked ham. Böhner et al.[3] found strongly increasing oxygen consumption and discolouration when increasing the oxygen content from 0.0% over 0.5% to 1.0% when exposing packaged cured boiled sausages to light.

Multiple precautions can be taken to achieve sufficiently low O_2 levels. Thorough flushing should be applied during modified atmosphere packaging to minimize residual oxygen. A packaging material with an adequately low permeability to oxygen should be used to avoid excessive O_2 ingress through permeation during storage that could result in deteriorative reactions. Last, active packaging in the form of the addition of an oxygen scavenger can be applied.[1,15,16]

Oxygen-scavenging modifications to packaging are the most commercially important category of active packaging.[16,17] Oxygen scavenging functionality can be added to a packaging material through the inclusion of sachets or labels or through incorporation into the plastic components of the package itself.[16,18] One of the most common types of oxygen scavenger uses iron powder which reacts with oxygen to form iron oxide, allowing the reduction of residual oxygen in MAP packages from an initial value typically ranging from 0.3% to 3%, to below 0.01%.[16,17,19] Oxygen scavengers have been studied for storage of packaged fresh meats,[20–23] providing better colour, odour and lipid stability, and providing some inhibition to aerobic spoilage organisms. Grini et al.[11] found superior colour stability during illuminated storage of sliced bologna sausage when oxygen scavengers were used.

The current study combines multiple techniques to preserve the quality of two cured cooked meat products, sliced ham luncheon sausage and sliced pork liver pâté. The products were packaged in two packaging types, one with high permeability to oxygen, one with low permeability to oxygen. The low barrier packaging concept has the benefit of a monomaterial tray, which allows for improved recyclability. This is important in light of the European Commission Plastics Strategy published recently, ordering that by 2030, all plastic packaging placed on the European market should be either recyclable or reusable.[24] The high-barrier concept is less easily recyclable due to the fully multilayered construction, but it is expected to reduce food losses as it protects the product from O_2 ingress more intensely. Both packages were used either with or without an oxygen scavenging sticker with a capacity of 50-ml O_2 applied to the inside of the lidding film. Three illumination conditions were used: dark storage, dark storage followed by illumination by fluorescent tubes at 1000 lux for the final 48 h of storage and dark storage followed by illumination by LED sources tailored for meat products for the final 48 h of storage. The main goal of the study was to evaluate whether a less complex, yet more permeable packaging system can be used (either with or without an additional oxygen scavenger) for meat products subjected to different types of illumination, considering multiple microbiological, chemical and organoleptic quality indices.

2 | MATERIALS AND METHODS

2.1 | Food products and packaging

Two meat products were selected for this test: ham sausage (21% fat content, 2.2% salt) and paté (28% fat content, 2% salt). They were provided sliced and packaged in 150 g trays, on the day of production by two different local producers. The day after reception of these samples, portions of 70 g were weighed aseptically in plastic trays and briefly stored in sterile stomacher bags and kept at 2°C before repackaging. No longer than 2 h after opening the original packages, the trays 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 s. Before sealing, the trays were flushed with a MAP gas mixture (CO_2 and N_2, Air Products, Brussels, Belgium). These mixtures consisted of 40% CO_2 and 60% N_2 for the ham sausage and 30% CO_2 and 70% N_2 for the pâté. The mixtures were prepared using a Witt MG18-3MSO gas mixer (Gasetechnik, Germany). Samples were repackaged one by one whereas the other samples were kept in a closed refrigerator. This was done to minimize exposure to light and elevated temperatures as much as possible during these steps.

Two different tray and lidding film combinations were used: a low O_2 barrier simple concept using a polypropylene (PP) tray with an oriented polyamide/polypropylene (OPA/PP) lidding film and a more complex high O_2 barrier concept using a PP/EVOH/PP tray with an OPA/EVOH/OPA/PP lidding film. The O_2 transmission rate (OTR) of the films was measured according to ASTM standard F1927 using a Mocon Ox-Tran 2/21 (Minneapolis, USA). A total of 99.9% pure O_2 was used as testing gas. OTR of the preformed trays was measured similarly, according to ASTM standard F1307 and using air (20.9% O_2) as testing gas. Measurement conditions and results are shown in Table 1, along with calculated oxygen permeability of the tray and film combination. The measurement conditions were chosen to reflect the real storage conditions.

The trays were used with and without the addition of an oxygen scavenger sticker (ATCO 50 ml O_2 capacity, Laboratoires Standa,

TABLE 1 Specifications of packaging concepts (film/tray combinations) used

Combination		Material and Supplier	Thickness, µm	Dimensions, mm	OTR	O_2 ingress (ml/day/package)
1	Film	OPA/PP (Bemis, France)	65	132 × 182	28.85 ± 0.32 ml[a] $O_2/m^2/d/atm$	0.13
	Tray	PP (ES-plastic, Germany)		132 × 182 × 25	2.09 ± 0.05 ml[b] O_2/tray/d/atm	
2	Film	OPA/EVOH/OPA/PP (Bemis, France)	65	132 × 182	6.57 ± 1.03 ml[a] $O_2/m^2/d/atm$	0.003
	Tray	PP/EVOH/PP (ES-plastic, Germany)		132 × 182 × 25	0.001 ± 0.001 ml[b] O_2/tray/d/atm	

Note. O_2 ingress was determined by follow-up of the gas concentrations in empty MAP packages stored at 7°C for 1 month.
[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.

Caen, France) on the inside of the lidding film. The stickers had a scavenging rate of approximately 1.5 ml O_2/h/sticker. The scavengers were iron powder-based and were activated by the presence of moisture within the package (water vapour).

This resulted in a total of four evaluated packaging concepts (high O_2 barrier package without scavenger, high O_2 barrier package with scavenger, low O_2 barrier package without scavenger, low O_2 barrier package with scavenger). It should be noted clearly that the slices did not have direct contact with the lidding film, so their whole surface was exposed to the headspace within the package. This choice was made to represent a worst-case scenario: in retail packages, typically contact between product and film is maximized to avoid exposure to headspace oxygen.

2.2 | Storage and sampling

The packaged food samples were stored up to 44 days on shelves in a dark cooling cell, in chilled conditions (7°C). Part of the samples were stored in darkness throughout storage, part were illuminated for the final 48 h before analysis, with either LED sources or fluorescent lamps. For example, a 'dark' sample may be analysed after 8 days of storage without illumination. The corresponding 'fluorescent' sample would be stored for 6 days in darkness and 2 days (48 h) in a cabinet with fluorescent illumination. This simulated a product being stored in a darkened retail storage, followed by exhibition in an illuminated retail cabinet. The 48-h illumination period was chosen to represent a realistic worst case scenario.[25] Moreover, most discolouration of meat products occurs within the first 24 h of illumination.[12,14] 'Cool white' fluorescent lamps were selected as this is the most commonly used illumination type by retailers.[3,26] LED lighting is gaining popularity, as it is more energy-efficient, has a longer lifetime when properly dimensioned and the spectral distribution can be tailored to certain products. For example, the LED sources used in this study have a higher emission in the red wavelength range of the visible spectrum, as to enhance the perceived colour of the illuminated meat and meanwhile emit less of the wavelengths corresponding to absorption maxima of photosensitizers inherent to meat products.[4]

The 'LED' illumination set-up consisted of two LED spots (Philips LED27S/827, 22.5 W, 2700 K) mounted in a cabinet, 50 cm above a plastic diffusor. The diffusor was used to improve the homogeneity of illumination from the two spot light sources across the shelf. The set-

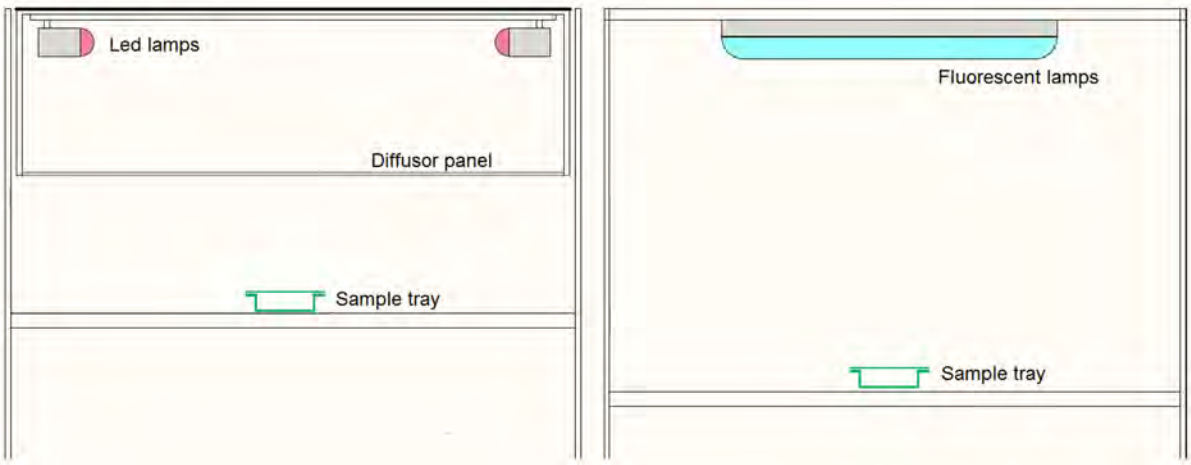

FIGURE 1 LED and fluorescent illumination setups

up is illustrated in Figure 1. The 'fluorescent' illumination set-up consisted of two fluorescent lamps (840 cool white, 36 W, 4000 K) suspended in a metal rack 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 spots. The side walls of both illumination setups consisted of white reflective panels to increase homogeneity of illumination.

The illuminance at sample position was measured using an UPRtek MF250N handheld spectrometer (UPRtek Europe, Aachen, Germany). The distance between the samples and the light source was adjusted to achieve 1000 lux at sample position, which is a typical illuminance value encountered in a retail environment.[5,25,27] Illuminance uniformity was checked to deviate no more than 50 lux from the setpoint. For the LED set-up, this was at a distance of 50 cm to the diffusor panel. For the fluorescent set-up, this was at 135 cm from the lamps. The relative spectral distribution of both light sources was measured using a Gigahertz-Optik BTS256-EF spectrometer (Gigahertz-Optik GmbH, Türkenfeld, Germany) and is presented in Figure 2.

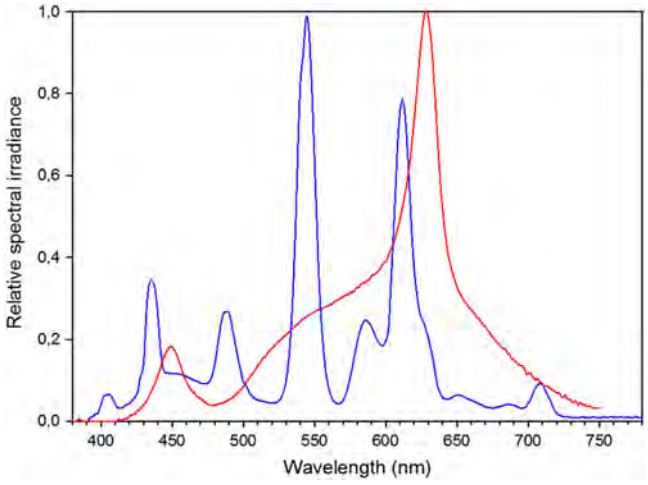

FIGURE 2 Relative spectral distribution of the fluorescent (blue) and LED (red) sources

The initial quality of the products was analysed in threefold immediately after repacking. On each sampling day, three non-illuminated samples, three LED illuminated samples and three fluorescent illuminated samples (i.e., nine packages in total) were analysed for each of the four packaging concepts. The full schematic set-up of the test is illustrated in Table 2.

2.3 | Analyses

2.3.1 | 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). Measurements were performed by piercing the top film through a septum sticker with a syringe connected to the Checkmate apparatus.

2.3.2 | Microbiological quality

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, UK) or deMann Rogosa Sharpe (MRS, Oxoid Ltd., Hampshire, UK) to 1 ml of the appropriate dilution in a petri dish. Total psychrotrophic count (PCA) and lactic acid bacteria (MRS) counts were evaluated after incubation at 22°C for 5 days.

TABLE 2 Schematic test set-up

Packaging	O_2 Scavenger	Illumination
Low O_2 barrier OPA/PP film PP tray	Scavenger	Dark
		Dark + final 48 h LED
		Dark + final 48 h fluorescent
	No scavenger	Dark
		Dark + final 48 h LED
		Dark + final 48 h fluorescent
High O_2 barrier OPA/EVOH/OPA/PP film PP/EVOH/PP tray	Scavenger	Dark
		Dark + final 48 h LED
		Dark + final 48 h fluorescent
	No scavenger	Dark
		Dark + final 48 h LED
		Dark + final 48 h fluorescent

2.3.3 | Colour

Colour stability of the top slice of each analysed package was followed up by measuring CIELAB L*a*b* colourimetric values by aid of a Konica Minolta CM2500-D handheld spectrometer (Konica Minolta Sensing Europe, Bremen, Germany), set at D65 Standard Illuminant and 10° Standard Observer. Data were collected by measuring the slice through a transparant plastic film. Ten measurements, spread across the surface of the slice, were taken and averaged to account for within-sample variability. Average L*a*b* values were rendered in CIELAB colour space.

2.3.4 | Lipid oxidation

Hexanal, a reaction product from the oxidation of linoleic acid, was followed up as a marker for the extent of lipid oxidation in the meats. It was determined using headspace solid-phase microextraction (HS-SPME) coupled with a gas chromatograph with mass spectrometer (GC-MS) using a stable isotope dilution assay.

Phosphate buffer was prepared by dissolving 8.95 g/L disodium hydrogen phosphate dodecahydrate (Chem-lab Analytical, Zedelgem, Belgium) and 3.04 g/L potassium dihydrogen phosphate (Chem-lab Analytical, Zedelgem, Belgium) in water and adjusting to pH 2.0 with ortophosphoric acid (Fischer Scientific, Merelbeke, Belgium). BHA antioxidant solution was prepared as 0.25 g butylated hydroxyanisole (Sigma-Aldrich, Overijse, Belgium) in 0.5 ml HPLC grade methanol (Fischer Scientific, Merelbeke, Belgium). The internal standard was prepared as 25 mg/ml hexanal-*d12* (CDN Isotopes, Quebec, Canada) in HPLC grade methanol further diluted to 37.5 µg/ml in water.

Samples were prepared by adding 1 g of meat product to a 20-ml glass headspace vial, also containing 4.0 ml of phosphate buffer, 5.0 µl of BHA solution and 15.0 µl of internal standard solution. A calibration standard solution was prepared by diluting a 0.815-mg/ml solution of hexanal (Sigma-Aldrich, Overijse, Belgium) in HPLC grade methanol further to 0.815 µg/ml in water. External calibration curves were created by standard addition, through adding 0–75.0–150.0–300.0–450.0–600.0 µl of this standard solution to headspace vials containing 0.5 g of meat, 5.0 µl of antioxidant solution, 15.0 µl of internal standard solution and 4 ml of buffer solution. The vials were sealed with PTFE/silicone caps (Agilent Technologies, Palo Alto, CA).

The HS-SPME process was performed automatically by a CTC-CombiPAL autosampler (Agilent Technologies, Palo Alto, CA). The samples were preincubated at 75°C for 5 min, while being agitated at 500 RPM. Extraction was done for 10 min with a DVB/CAR/PDMS *grey* fibre (Supelco, Bellefonte PA, US). The fibre was desorbed for 2 min at 250°C in the inlet. For chromatographic analysis, an Agilent 7890A GC with a 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA) and equipped with an Agilent J&W DB-624 60 m, 0.25 mm, 1.40 µm capillary column was used. Helium was used as carrier gas at a constant flow rate of 1.3 ml/min. The oven temperature was programmed as follows: initial temperature 50°C, hold time 5 min ramp up to 140°C at 4.5°C/min. The MSD conditions were the following: transfer line to MSD, 280°C; ionization energy, 70 eV; operating in selective ion mode (SIM). The selected ions monitored were *m/z* 44, *m/z* 46, *m/z* 56 and *m/z* 64. Quantifiers were *m/z* 56 (for hexanal) and *m/z* 64 (for hexanal-d_{12}).

2.4 | Statistics

Data were analyzed using R Statistics 3.5.[28] Significance of the main effects 'packaging type', 'illumination type' and 'time of storage' and the interaction effect were evaluated using analysis of variance

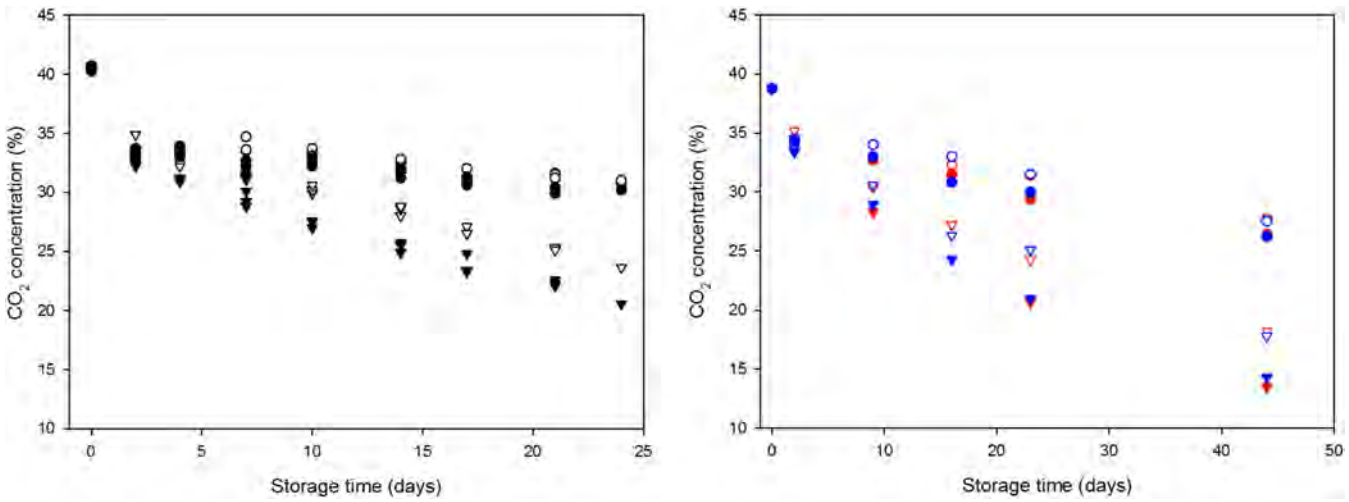

FIGURE 3 (left) CO_2 concentration in headspace of ham sausage stored in darkness continuously (black symbols) and (right) stored in darkness followed by LED illumination for the last 48 h (red symbols) and stored in darkness followed by fluorescent illumination for the last 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (◯) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger. Each symbol represents one measurement

(ANOVA) on univariate general linear models. The appropriateness of these models was checked using a histogram of the residuals. A main effect was interpreted as a global difference over all levels of the other factor. An interaction effect was interpreted as an evolving effect of packaging type over time. 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).

3 | RESULTS AND DISCUSSION

3.1 | Ham sausage

3.1.1 | Gas measurements

CO_2 concentrations measured throughout storage are shown in Figure 3. Initial CO_2 concentration within the ham sausage packages was just over 40%. Two days after packaging, the concentration had decreased to under 35% in all packages, mainly due to CO_2 dissolving in the product. This allowed for an antimicrobial effect of CO_2, as described by Devlieghere et al.[29] Throughout storage, CO_2 was further lost due to permeation through the packaging. As the low barrier packaging was more permeable, a higher CO_2 loss was observed. The concentration kept declining throughout storage, indicating that microbial growth was not significant enough to allow CO_2 to be built up within the package. CO_2 concentrations were lower when an O_2 scavenger was included in the package. This was especially evident in the low barrier packages, where a difference between 2% and 3% CO_2 was observed. In the high barrier packages, the difference amounted to only 0.5%–1%. This large difference was not as clearly present if CO_2 concentrations were followed up in MAP packages containing no product (data not shown).

O_2 concentrations in the low barrier package increased at a rate of approximately 0.11% O_2 per day (Figure 4). Apparently, aerobic microbial growth was not significant enough for microbial oxygen consumption to allow deviation from a linear increase due to permeation.[30] In the low barrier packages with oxygen scavengers, oxygen concentration started increasing between 17 and 21 days of storage (based on a one-sample *t* test with *p* value = 0.005 on day 21), indicating that, at that moment, the scavengers had exhausted its capacity due to the high permeability of the packages. O_2 concentrations within the high barrier packages remained below 0.5% throughout the majority of the test. In the high barrier packages with scavengers, 0.0% O_2 was maintained throughout the test.

3.1.2 | Microbiological quality

Initial total aerobic and lactic acid bacteria counts were below the quantification limit of 2.5 log CFU/g. They were however above the detection limit: indicative total aerobic counts of 1.3 and 2.4 log CFU/g and lactic acid bacteria counts of 1.3 log CFU/g were detected on fresh ham sausage samples. Throughout storage, most counts remained below the quantification limit and no trend of microbial growth could be detected in any of the packaging concepts. Only after 44 days, some packages exhibited counts up 5 log CFU/g, but this was already after the producer's proposed shelf life of 28 days. The lack of significant microbial growth corresponded with observations concerning the lack of microbial influence on measured CO_2 and O_2 concentrations (Figures 3 and 4).

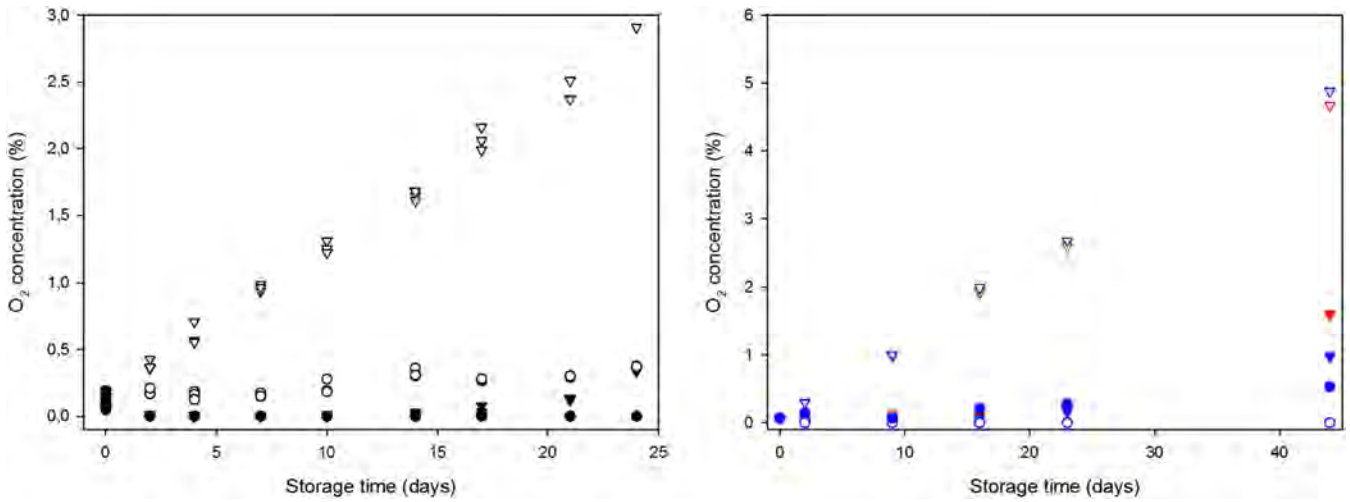

FIGURE 4 (left) O_2 concentration in headspace of packages with ham sausage stored in darkness continuously (black symbols) and (right) O_2 concentration in headspace of packages with ham sausage stored in darkness followed by LED illumination for the last 48 h (red symbols) and stored in darkness followed by fluorescent illumination for the last 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (◯) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

3.1.3 | Colour

Colour changes observed in all samples were mostly due to a loss of redness, which in CIELAB colour space corresponds with a decrease in a* value. Earlier publications also found this parameter to correlate the best with cured cooked meat colour changes.[2,4,31]

In order to evaluate the colour stability based on L*a*b* differences in relation to human visual perception, a threshold value for the acceptability of colour differences should be defined. However, little research with respect to acceptable colour differences of meat products can be found in literature. Hutchings[32] pointed out that, although for classic colour using industries like paint and textile very close tolerances exist between just noticeable and just acceptable colour differences, the situation is generally different for natural foods, for which a larger variation in acceptability may exist due to inherent colour variations.

Therefore, two psychophysical experiments were conducted, in which the optimal colour and the allowed colour deviation of ham sausage were determined, respectively. An experimental set-up was built by aid of which the colour of the ham sausage could be changed against an invariant background by use of a projector. Based on the projector calibration and on the measured spectral reflectance of the ham sausage, in the first experiment, 144 different hues were defined and presented to a panel of 15 naïve observers. Observers indicated their likeliness of buying the presented product on an 11-point scale, from which the optimal colour of the ham sausage was derived. Colour vision of all observers was first screened using the Farnsworth-Munsell 100 Hue Test. In a second stage, 15 further naïve observers, for whom colour vision was screened in a similar fashion as in the first test, were asked to indicate the maximum allowed colour deviation of the ham sausage using a staircase method, starting from the optimal colour as derived from the first test. Both tests were performed at an

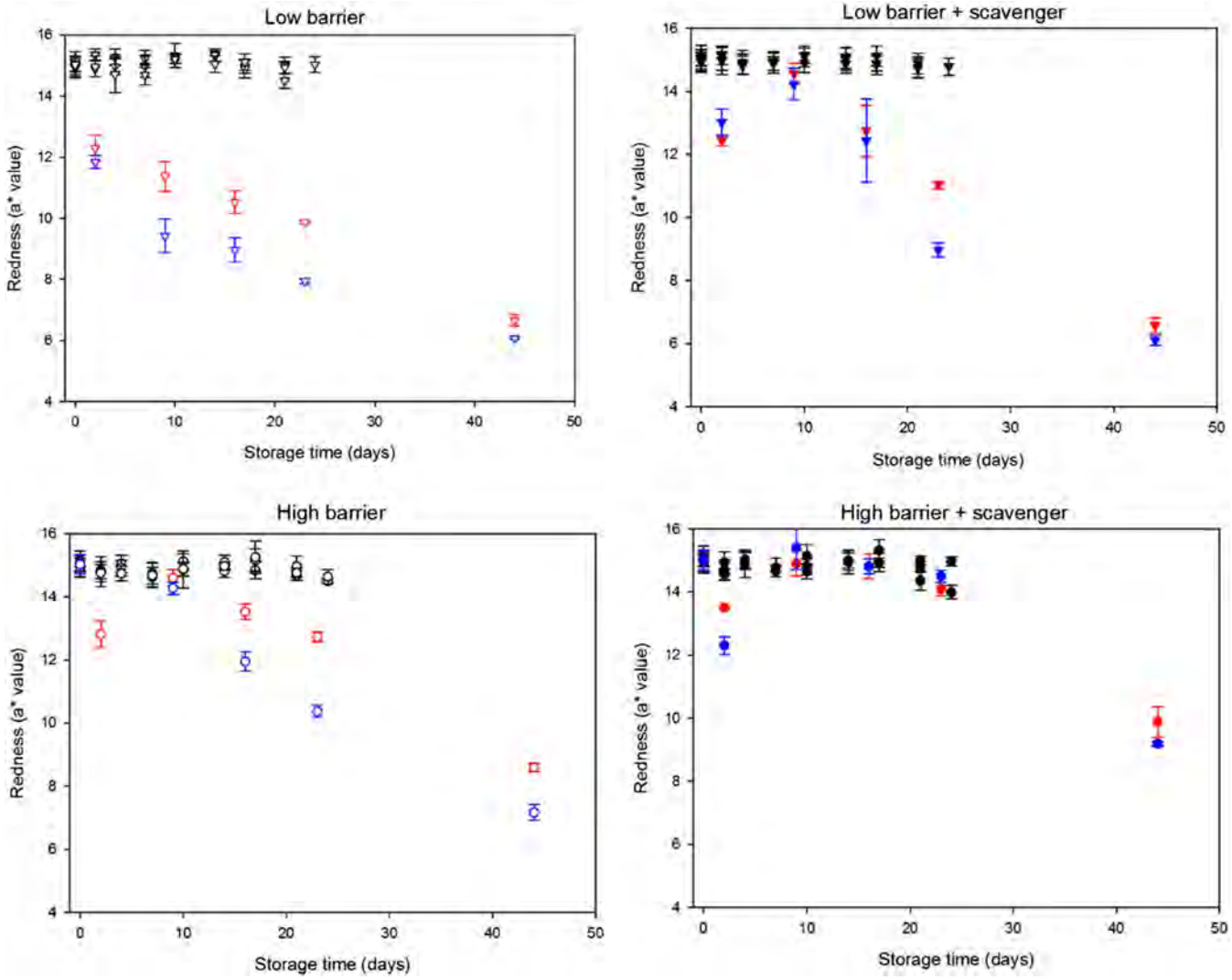

FIGURE 5 Tristimulus a* (redness) score of ham sausage stored in darkness continuously (black symbols), stored in darkness in combination with LED illumination for the last 48 h (red symbols) and stored in darkness in combination with fluorescent illumination for the last 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (◯) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

illuminance level of 1000 lux, corresponding to the illumination conditions during storage.

Results indicate that, on average, observers have a clear opinion on what the optimal intrinsic colour of ham sausage is (a* = 18.1/b* = 14.3). For half of the observers, the maximum acceptable colour difference is around 3.5 units. This value was taken as threshold for further evaluation of discolouration.

Regardless of packaging type, the colour of samples stored in darkness barely changed throughout storage confirming that the discolouration of cooked ham is a light-induced process. Illuminated samples stored in the low barrier packages showed a progressively increasing degree of discolouration as the storage time prior to the 48 h of illumination increased. This was related to the increasing amount of oxygen that had accumulated within the package due to permeation (Figure 4). The degree of discolouration in the high barrier packages was lower than in the low barrier packages but also increased as oxygen accumulated through permeation. In the packages with scavengers, the discolouration after 2 days of storage (48 h illumination without prior dark storage) was higher than after 9 days of storage (7 days of dark storage followed by 48 h of illumination). As the former samples were immediately placed under illumination, the scavenger had not been able to absorb all of the residual oxygen yet, leaving some oxygen to interact with the product and provoke light-induced discolouration. This shows that, when a scavenger would be

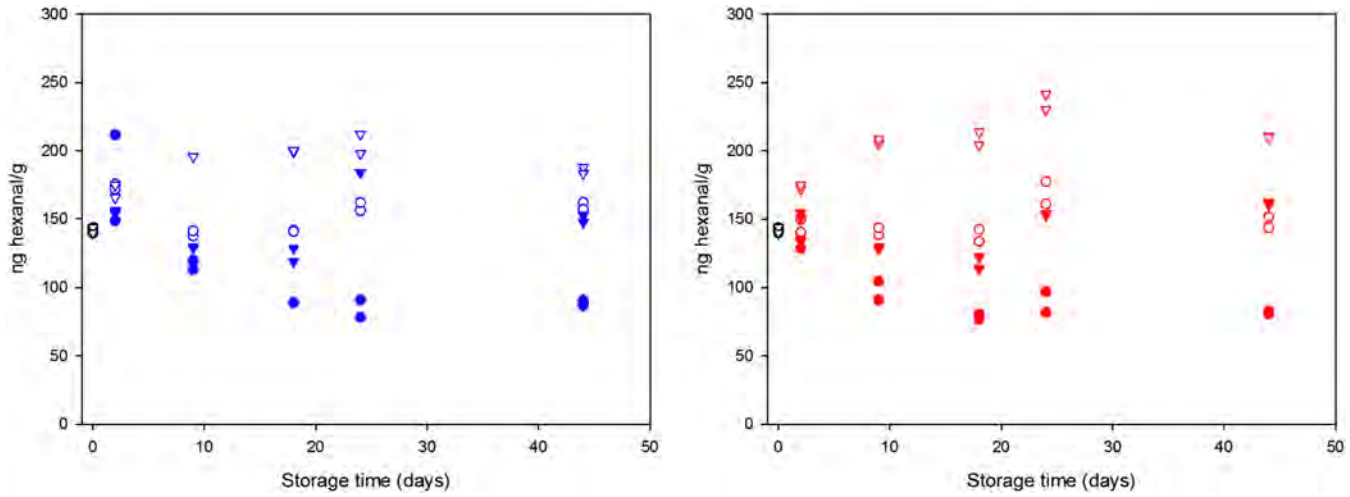

FIGURE 6 Hexanal concentration of ham sausage stored in darkness followed by LED illumination for 48 h (red symbols) and stored in darkness followed by fluorescent illumination for 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (◯) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

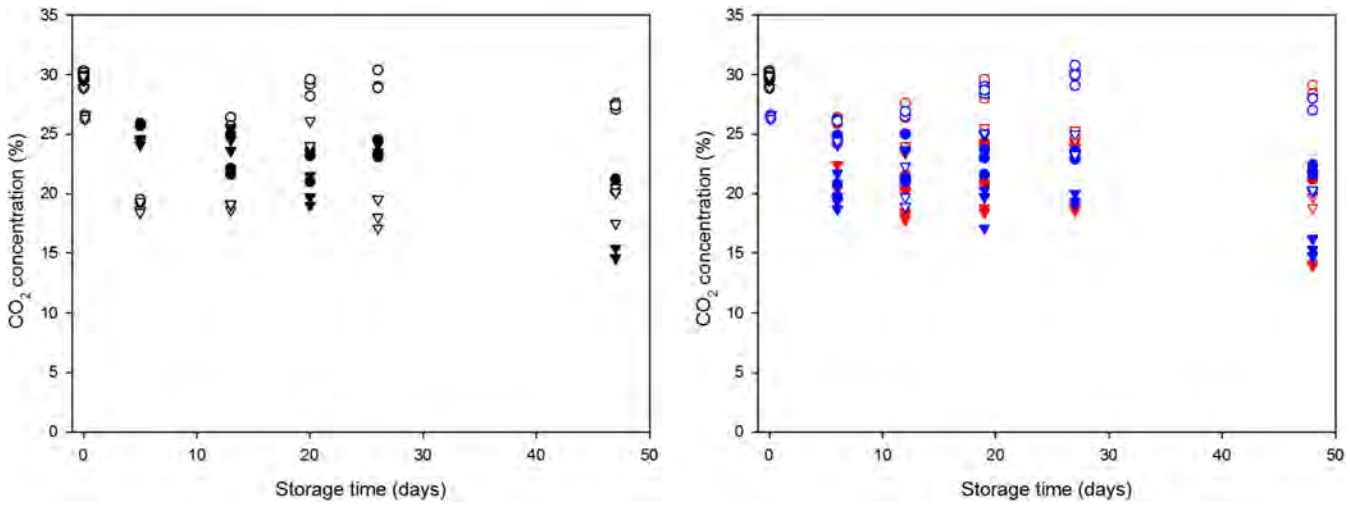

FIGURE 7 CO_2 concentration in headspace of pâté stored in darkness continuously (black symbols), stored in darkness and in LED illumination for 48 h (red symbols) and stored in darkness and in fluorescent illumination for 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (◯) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

applied, prior dark storage is crucial not to lose the beneficial effect it might have in preventing light- and oxygen-induced discolouration. The importance of dark storage prior to illumination is confirmed by findings from Andersen et al.,[13] Haile et al.[14] and Møller et al.[5]

For all samples stored in low barrier packages illuminated for 48 h, noticeable discolouration occurred (decrease of a* value > 3.5). In the high barrier with scavenger concept, discolouration was minimal except after 44 days of total storage, where strong discolouration occurred in all cases. This may be due to the inherent reducing capacity (presence of antioxidants) in the meat being depleted, leaving it more vulnerable to oxidative processes such as discolouration. Overall, it was also clear that fluorescent light was more detrimental to meat colour than the LED light (Figure 5), as a* values had decreased more in all cases when under fluorescent illumination. The improved colour stability under certain illumination types corresponds to findings from Böhner et al.[3,4] on cured boiled sausage and Steele et al.[33] on raw ground meats. However the latter also found that LED is not necessarily beneficial to lipid oxidation.

3.1.4 | Lipid oxidation

The type of illumination had no significant overall effect on the amount of hexanal measured (p = 0.27), but the packaging type did (p = 0.000). In high barrier packages with scavenger, some decrease was observed as compared to the initial measurements, as hexanal can be further converted to the corresponding carbonic (hexanoic) acid.[34] Only for samples stored in low barrier packages without scavengers, a clear increase in concentration was observed (Figure 6). Concentrations measured in low barrier packages with scavenger and high barrier packages without scavenger are not significantly different from each other on most analysis days. As for sensory quality, the samples stored in low barrier packages without scavenger had acquired a bland odour by the end of the experiment. Samples stored in high barrier packages with oxygen scavengers retained their fresh scent very well, even at the end of the storage period. The other samples did not retain the fresh scent, but also did not develop any clear off-odour.

Compared with the results for pâté in the following sections, hexanal formation in this product was rather limited. Similarly, Parra et al.[35] found hexanal of less crucial importance in monitoring the oxidative quality of packaged sliced ham. Møller et al.[5] found no difference in oxidative status after 27 days of illuminated chill storage of sliced cooked ham with 0.5% residual oxygen, despite apparent discolouration. Böhner et al.[3] found no changes in hexanal content during 23days of illuminated chill storage of cured boiled sausage.

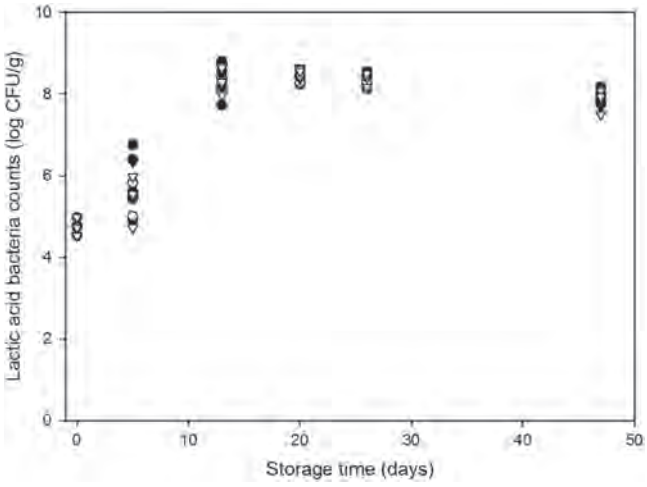

FIGURE 9 Lactic acid bacteria counts of pâté throughout dark storage. Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (◯) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

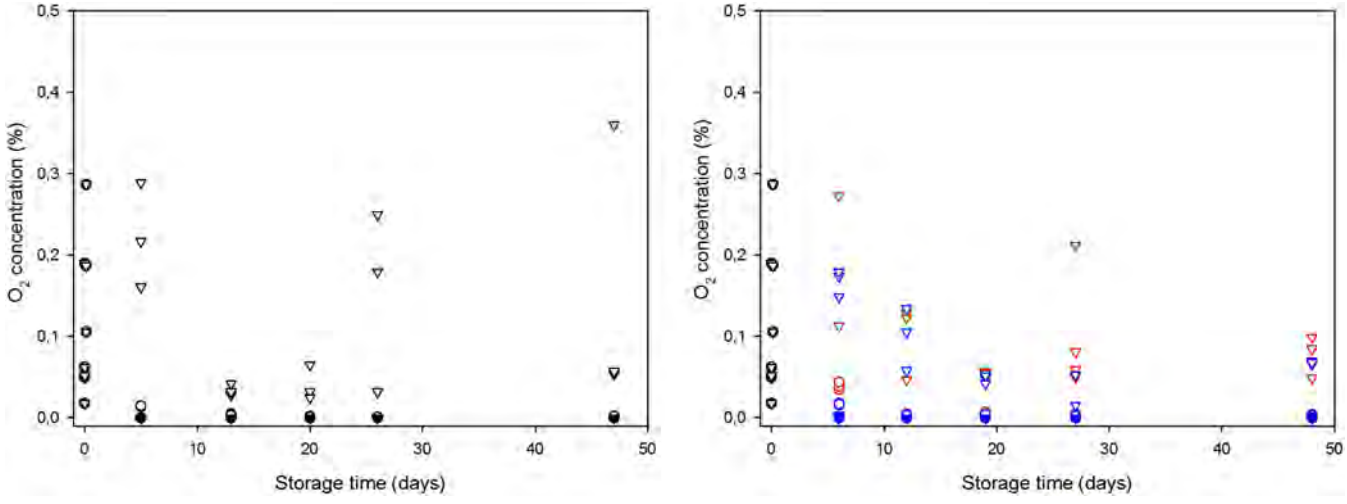

FIGURE 8 O_2 concentration in headspace of pâté stored in darkness continuously (black symbols), stored in darkness and in LED illumination for 48 h (red symbols) and stored in darkness and in fluorescent illumination for 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (◯) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

3.2 | Pâté

3.2.1 | Gas measurements

Similar to the ham sausage experiment, on the first analysis after packaging a decrease of CO_2 of about 5% was noticed due to dissolution in the product. Between 12 and 20 days of storage, CO_2 concentrations showed an increase. This was due to microbial outgrowth with accompanying microbial respiration (see section microbiological quality). Further throughout storage, the CO_2 concentrations were the result of permeation through the package and build-up of CO_2 through respiration (Figure 7).

Residual O_2 concentrations were between 0.0 and 0.3%. The concentrations remained below 0.5% throughout storage for all packaging concepts. All packages with scavengers remained at 0.0% throughout the test. Despite the same packaging concepts being used as in the ham sausage experiment described earlier, measured oxygen concentrations are far lower. This can be explained by significant microbial activity (see further section microbiological quality). As expected, O_2 concentrations are higher for low barrier packages than for high barrier packages (Figure 8).

3.2.2 | Microbiological quality

Initial counts of the paté were high (between 4 and 5 log CFU/g). Lactic acid bacteria made up the majority of the total microbial load (Figure 9). These results indicated a less sterile slicing and/or packaging environment at this producer in comparison with the producer of the ham sausage. The initial load was above the tolerance value for lactic acid bacteria of 3.5 log CFU/g, as proposed by De Loy-Hendrickx et al.[36] By the 13th day of storage, microbial growth

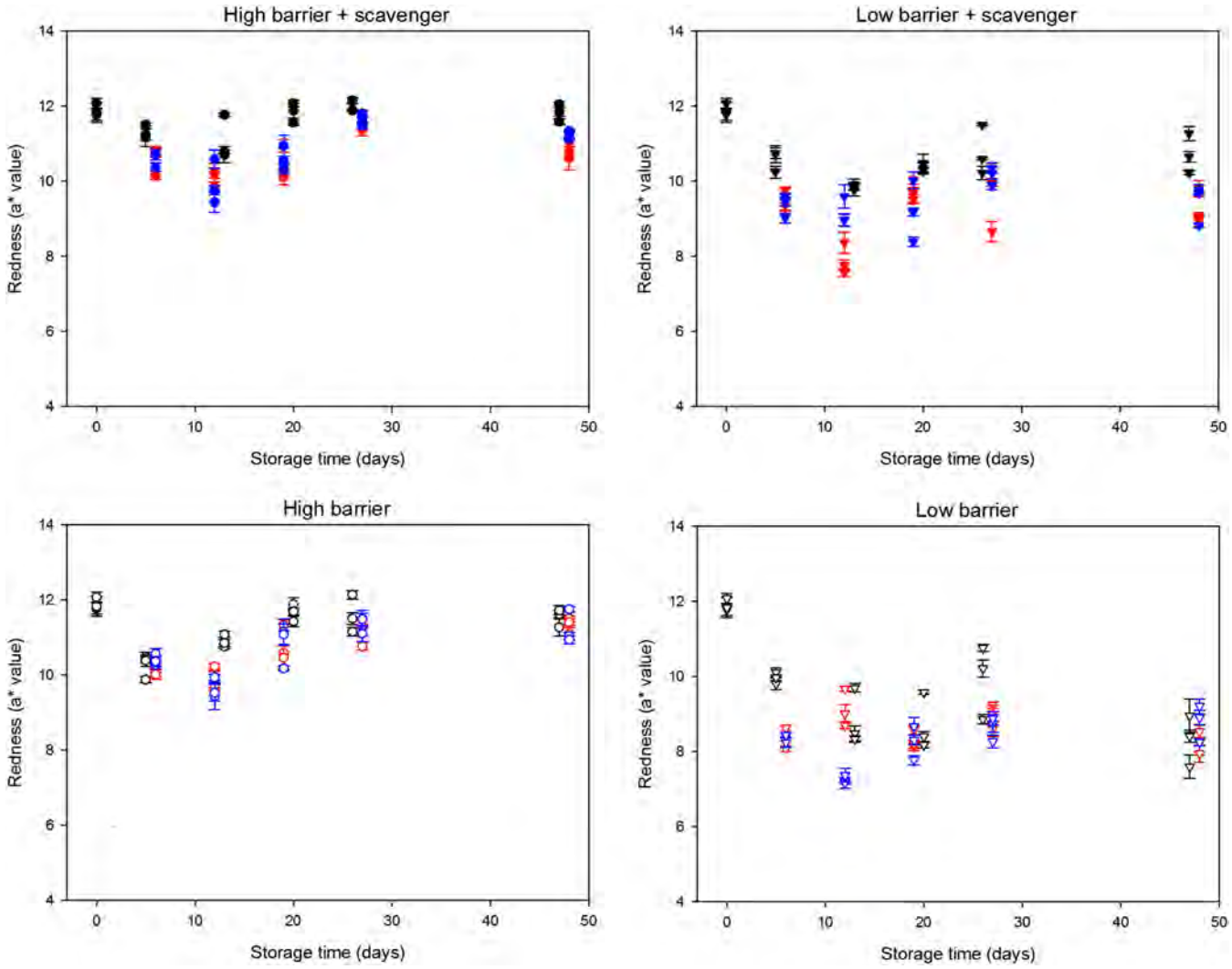

FIGURE 10 Tristimulus a* (redness) score of pâté stored in darkness continuously (black symbols), stored in darkness in combination with LED illumination for the last 48 h (red symbols) and stored in darkness in combination with fluorescent illumination for the last 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (○) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

within all packages had reached the stationary phase. The high initial microbial load and fast growth up to the stationary phase was reflected in the production of CO_2 (Figure 3) and consumption of O_2 (Figure 4). The type of packaging concept had no significant effect on the growth of lactic acid bacteria ($p = 0.057$).

3.2.3 | Colour

The discolouration of the pâté samples (Figure 10) differed rather strongly from that of the ham sausage. In this case, discolouration did occur when the samples were not illuminated although this was more pronounced when more oxygen was available (e.g., in the low barrier package without scavenger). Discolouration of the pâté was indeed more dependent on oxygen availability than it was on the presence of the combination of both oxygen and light. On the contrary, for the ham sausage, no discolouration occurred when the samples were stored in the dark, and discolouration was minimal in the high barrier concept with oxygen scavenger. Discolouration of the pâté was stronger in all cases before 20 days of storage, which was when bacterial growth had reached the stationary phase (Figure 9). As the microbial load reached its maximum level, competition for oxygen increased, resulting in less discolouration. The difference between LED and fluorescent illumination was found unsignificant ($p = 0.85$), as the discolouration was more dependent on oxygen availability than on the type or presence of illumination. The barrier properties of the packaging concept did have a significant effect ($p = 0.000$). Addition of a scavenger had no significant effect on discolouration of the pâté in high barrier packages ($p = 0.33$), but it did in low barrier packages ($p = 0.000$). The pâté seemed to have a very high affinity for oxygen, so when oxygen was able to permeate the package, the pâté competed strongly with the oxygen scavenger. Discolouration was seldom homogeneous,

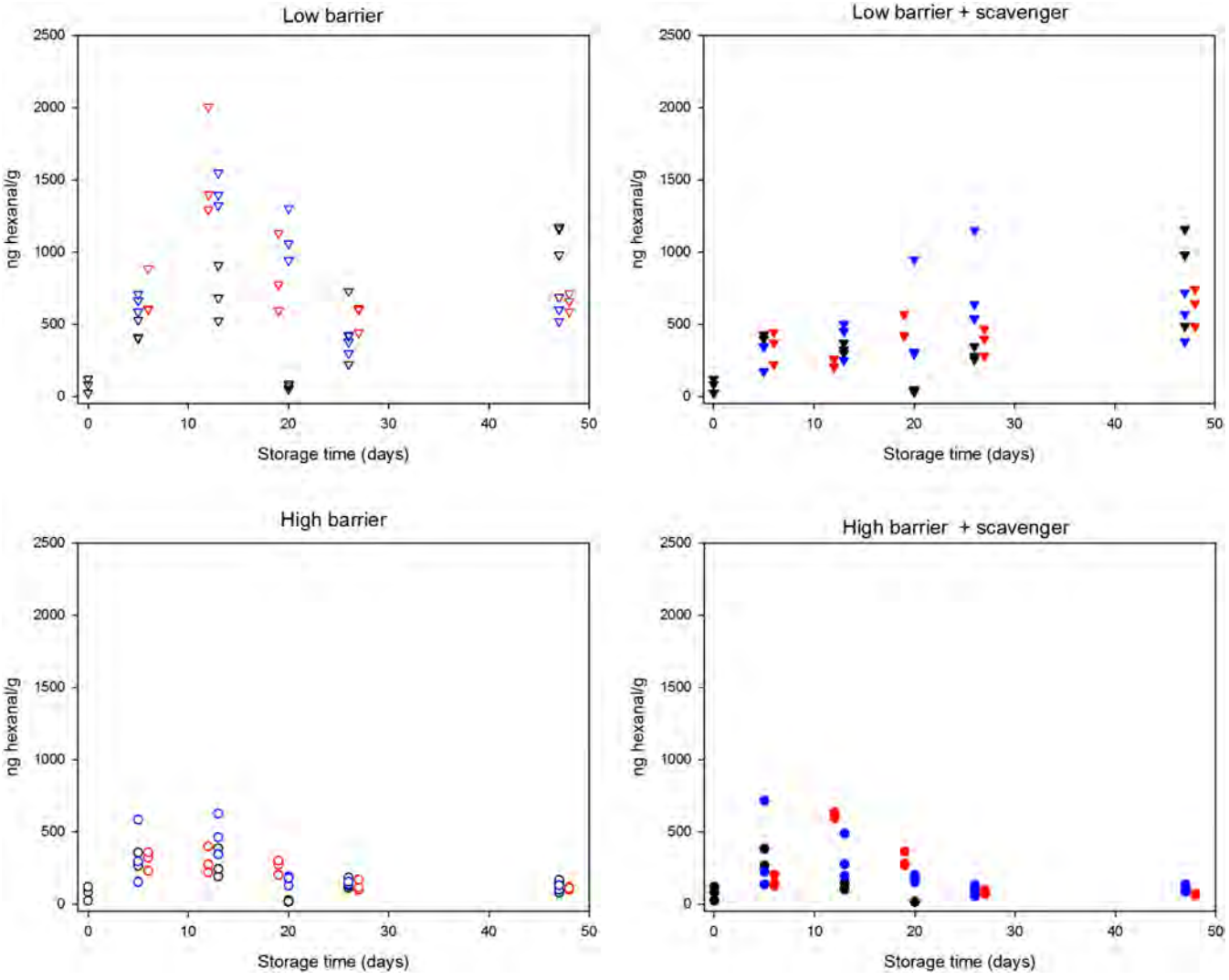

FIGURE 11 Hexanal concentrations in pâté stored in stored in darkness continuously (black symbols), stored in darkness followed by LED illumination for the last 48 h (red symbols) and stored in darkness followed by fluorescent illumination for the last 48 h (blue symbols). Packaging materials are a high barrier PP/EVOH/PP tray and OPA/EVOH/OPA/PP lidding film with (●) and without (○) oxygen scavenger, and a low barrier PP tray and OPA/PP lidding film with (▼) and without (▽) oxygen scavenger

but rather concentrated along the edge of the product and around the lard layer that surrounded the slices of pâté. Yet, as specified earlier, colour measurements were randomly distributed over the surface of the product, leading to less pronounced reported instrumental differences.

The increased sensitivity of pâté to oxidation as compared to ham sausage has been addressed by multiple authors and is mostly due to pâté containing a high concentration of nonhaem iron, which is an important pro-oxidant.[12,37–39] In this case, it is hypothesized that discolouration may only likely be avoided by allowing the top slice to be in contact with the film, preventing headspace oxygen to directly interact with the surface.

3.2.4 | Lipid oxidation

Hexanal concentrations measured in the pâté were up to 10 times higher than in the ham sausage illustrating how much more sensitive to oxidation this product is (Figure 11). Intrinsic variability was rather high with these measurements, so trends were hard to observe. Overall, the concentrations measured for the low barrier packages are the highest (p = 0.000). Addition of a scavenger had a significant effect on hexanal concentrations (p = 0.000).

Hexanal concentrations measured in the high barrier packages (with and without scavenger) followed a trend comparable to the colour evolution: as microbial load increased, competition for oxygen increased, so less oxidation occurred. In some cases, the amount of hexanal exceeded 1000 ng/g. These unexpectedly high measurements just exceeded the calibration range (0–960 ng/g), thus meaning that extrapolation was needed for quantification. There was no significant effect of the presence or absence of the scavengers in the high barrier packages on the hexanal concentrations (p = 0.83). Bland and warmed-over off-odours developed in most samples. For samples stored in low barrier packages, this was already clear after 1 week of storage. The off-odour was also already present in the low barrier packages with oxygen scavengers, but was more strongly developed by the second week of storage. Samples packaged in high barrier packages with and without scavengers did not develop very strong off-odours but also did not retain a clearly fresh smell. It should be noted here though that off-odours could also originate from the high microbial load of the pâté.

4 | CONCLUSIONS

A low O_2-barrier packaging concept was included in this test as it has improved recyclability over a more complex multilayer high O_2-barrier package. O_2 scavengers were included for both packaging concepts as an added protection against ingress of O_2. Yet, to minimize discolouration and lipid oxidation of cured cooked meat products, high O_2 barrier packaging material seems to be indispensable. For ham sausage, a high O_2 barrier in combination with an oxygen scavenger provided superior colour, oxidation and odour stability. For such products, oxygen scavengers may be a useful addition provided that the improved quality weighs up against the additional cost, and the consumer has no concerns over the modification of the packaging. In that sense, the scavenger may better be integrated in the package material rather than being visually present in the form of a sachet or sticker. Moreover, the environmental impact of the use of a scavenger has also to be considered. Furthermore, in the presence of oxygen, LED illumination proved to be less detrimental to the colour stability of the product than fluorescent illumination at the same intensity and duration.

For the more oxidation-sensitive pâté, the addition of a scavenger provided little additional benefits. In this case, other modifications to the packaging are required such as avoiding residual oxygen, reducing the gas/product ratio (however, this may compromise the MAP concept as too little CO_2 may be present in the reduced headspace) and making sure that the surface of the product is in direct contact with the film, so that less oxygen can directly interact with the surface.

Low barrier packages with oxygen scavenger and high barrier packages without scavenger performed highly similar overall. It should be noted that a scavenger does not completely eliminate all oxygen, but rather competes with the packaged product itself and its microbial flora for consumption of permeating oxygen. Therefore, for oxygen sensitive products, scavengers should be regarded as an addition to high barrier packages to eliminate residual oxygen, rather than an improvement of underperforming packaging material. Avoiding permeation of additional oxygen into the package atmosphere should be prioritized over scavenging oxygen from the package atmosphere as at that point, there is already competition for oxygen absorption between the scavenger and the packaged food product.

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