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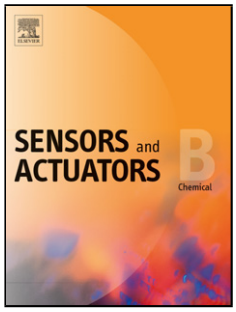

Application of O_2 sensor technology to monitor performance of industrial beef samples packaged on three different vacuum packaging machines

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Application of O_2 sensor technology to monitor performance of industrial beef samples packaged on three different vacuum packaging machines

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Highlights

- O_2 sensing is used to assess packager performance at meat processing plant;
- Residual O_2 in vacuum packed meat is measured non-destructively with a handheld reader;
- Rapid detection of microbial spoilage in meat by GreenLight® respirometric assays
- Residual O_2 and sensory characteristics of meat are correlated with packager performance

Abstract

Two optical oxygen sensor systems we applied to monitor the evolution of meat spoilage in vacuum packaged (VP) samples of raw beef meat, which were produced at

a large meat processing factory in Ireland under standard industrial settings on three different vacuum packaging machines. After packaging, the samples were put on storage at +1 °C for 90 days, while analysing their quality parameters. Residual oxygen levels and their time profiles, were measured non-destructively with disposable O_2 sensors placed in each pack and interrogated with a handheld reader Optech Platinum (Mocon). Total aerobic viable counts (TVC) were measured by destructive sampling on a sensor based platform Greenlight (Agilent). Furthermore, sensory attributes of meat samples were analysed by internal sensory testing panel. The two commercial oxygen sensor-based systems allowed us to assess the performance of the three VP machines and correlate it with quality and safety characteristics of packaged meat products. They provide convenient and versatile tools for simple, rapid and *in situ* assessment of various packaged products and packaging systems.

Keywords: Optical oxygen sensors; residual oxygen levels; vacuum packaged meat; non-destructive assessment; rapid microbial test; food safety and QC/QA; Optech® and GreenLight® systems

1. Introduction

Fresh raw meat represents a large segment of packaged food products manufactured commercially on a large scale, with different types of meat, sizes and forms (e.g. small cuts, steaks, portions, larger chunks and pieces, minced and diced meat) and packaging conditions. To ensure high quality and safety of the final product, prevent its degradation and extend shelf life, vacuum packaging (VP) is routinely used. By

reducing the exposure of raw meat to oxygen, VP helps to preserve the natural flavour of the product, its taste and aesthetic appearance, reduce microbial growth and other spoilage-related degradative processes. Also colour is an important visual quality of raw meat, which is used by consumers at the point of sale. Oxygen-dependent meat browning leads to significant rejection of the product by consumers [1–4].

Residual O_2 in VP meat is a sensitive indicator of package integrity or accidental damage, quality of the packaging materials and processes used, storage and transportation conditions that the product was exposed to. Therefore, non-destructive checks of residual O_2 levels in meat packs at various stages of the process are necessary [5]. Monitoring of O_2 levels within packages, such as VP meat products, enables to pick up faults in packaging, identify damaged packs and promptly repackage them before they leave the factory or are even removed from the production line. Accurate quantification of O_2 levels in food packages is useful for the assessment of product shelf-life and its quality and safety parameters [6].

Phosphorescence based O_2 sensor systems, such as those developed by Mocon, Presens, Oxysense and some other companies [5], provide efficient and accurate means to quantify O_2 levels in a host of product samples, including packaged food products. Unlike conventional destructive methods, this sensor technology allows non-destructive, real-time, quantitative and repetitive measurements of residual O_2 levels in packaged food samples using small disposable sensors placed inside packs [7,8]. Efforts are being made to make such sensors more affordable (currently cost a few dollars each), more accurate and calibration-free, and to produce them on a large scale, so that they can be incorporated ultimately in every food pack and used *in situ* at the food production site, if and when necessary [9,10].

So far, a number of small and medium scale laboratory and industrial trials with home-made and commercial O_2 sensors have been carried out using various foods, including: meat and plant-based foods such as deli meat [11], beef [12], raw beef and chicken [13], cheese [14] and lettuce [15], bread [16], convenience foods [6], industrial cheese production [17].

In addition to the non-destructive measurement of residual O_2 levels in packaged food products, the solid-state and liquid phosphorescent O_2 sensors have been used for rapid microbiological testing of food samples and enumeration of live bacteria (total viable counts, TVC) by oxygen respirometry [18]. This micro-method includes destructive sampling and subsequent monitoring of crude food homogenates for aerobic respiration and enumeration of TVC. This test was validated with meat samples as an alternative to conventional agar plating method [19], and later certified by AOAC [20].

In this study, we monitored by means of commercial O_2 sensor systems the evolution of spoilage in VP raw beef cuts, which were produced at a meat processing factory using three different VP machines, during their storage at +1 °C for 90 days. We analysed the following quality parameters of these samples: i) residual oxygen measured non-destructively with disposable O_2 sensors placed in each pack and interrogated with a handheld reader Optech™ Platinum (Mocon); ii) TVC measured by destructive sampling on a sensor-based Greenlight™ platform (Agilent); iii) sensory attributes of meat analysed by sensory testing panel. So far, very few studies have been performed with commercial sensor systems to assess *in situ* the quality of VP meat produced under large-scale industrial settings. Our study provides valuable findings regarding the quality of raw beef cuts during storage.

2. Materials and Methods

2.1. Materials

The factory-calibrated disposable O_2 sensor stickers and handheld reader Optech^{TM} O_2 Platinum and were purchased from Mocon (Minneapolis, USA). The sensors were previously validated in food packaging applications [5, 15, 17] and were used as supplied, following manufacturer's instructions. GreenLight^{TM} O_2 probe was obtained from Agilent Technologies Ireland (Cork, Ireland). Standard 96-well plates (sterile, clear polystyrene, flat bottom with lids) were from Sarstedt (Ireland). Sterile stomacher bags and Stomacher Lab System Model 400 were from Colworth (UK). Buffered Peptone Water (BPW) was made up using peptone (10.0 g/L), NaCl (5.0 g/L), Na_2HPO_4 (3.5 g/L), KH_2PO_4 (1.5 g/L), adjusted to pH 7.2 ± 0.2 with NaOH, sterilized by autoclaving. Plate count agar (PCA) was from Oxoid (Basingstoke, UK).

2.2. Preparation and testing of packaged meat samples

Processing and packaging of fresh beef meat samples (48 h after slaughtering, stored at 0 °C) was carried out directly on the processing line of one of the primary Irish meat processing factories, using its packaging equipment, packaging materials and process settings. Three different VP machines, designated here as M, O, and F (full details cannot be disclosed for confidentiality reasons), were used and compared. Following routine procedures for meat packaging in the production plant, chuck boneless beef was divided into two halves and each piece was cut into ten equal pieces, approximately 200g each. Half of the pieces were randomly selected and packed on the VP machine M, the other half – on the VP machine O. Finally, a shin piece was cut into six pieces (2.5 kg each) which were packed on the VP machine F. One O_2 sensor sticker was attached to the inner surface of each vacuum packaging

pouch CLEAR-TITE 50 (PE&EVA blend / PVdC / PE&EVA blend, gauge 50 μ, OTR 19 cc/m^2/24hr/bar) supplied by Bemis Swansea, (UK). Then meat cuts were placed in these pouches and sealed on a VP machine. The three sets of VP meat samples thus generated were stored in the dark at +1 °C. All packed samples were analysed immediately after preparation (day 0) and then on days 30, 60 and 90 using a number of quality readouts.

2.3. Measurement of residual O_2 levels in VP meats

Residual O_2 levels were measured non-destructively through the packaging film - in all the sealed packs on days 0, 30, 60, 90. For that, Optech^{TM} O_2 Platinum reader was brought in optical contact (0-10 mm distance) with the active area of the sensor sticker sealed inside the pack, and reading was initiated with a push-button. The instrument measured sensor optical signal along with sample temperature (using a built-in infrared T-probe), and its software (Mocon) performed automatic calculations and correction for temperature variation, then displayed the O_2 reading in the software and also saved it into a log file. Typically, it took 2-3 sec to read each sensor.

2.4. Measurement of microbial counts (TVCs) in the meat samples

On days 0, 30, 60 and 90, one meat sample from each group (packagers M, O, F) was taken, opened and subjected to destructive testing to quantify their microbial load on a GreenLight® platform [19]. In accordance with ISO 4833:2013 standard [21], beef samples (10 g) were aseptically taken from each pack using sterile forceps and scalpels, added to 90 ml of sterile BPW and homogenized in a stomacher. A dry Greenlight® probe (Agilent) was reconstituted with 1 mL of BPW, diluted to 10 mL with sterile BPW, and then dispensed in 100 μL aliquots in sample wells of a 96-well

plate. Subsequently, 100 μL of the meat homogenate were added to assay wells, followed by the addition of 100 μL of mineral oil which sealed the samples from ambient O_2. Negative control (pure PBW) and positive controls (*E. coli* stock in PBW, ≈ 10^8CFU/ml) were also prepared using the same multiwell plate. Then each plate was measured kinetically at 30°C on a fluorescent plate reader FLUOstar Omega (BMG Labtech, Germany), using excitation and emission wavelengths 380nm and 650 nm. O_2 probe signal was measured in each well every 15 min for 10-12 hr. From these readings, MARS® software of the instrument plotted time profiles of the phosphorescent signal and determined the onset time of the signal for each sample well. This onset time was then used to calculate the microbial load, log(CFU/g), for each VP meat sample, using a pre-determined calibration [19]. All samples were analysed in triplicate.

On day 0, when bacterial load in the meat samples was below the limit of detection for the GreenLight™ test (10^2 -10^3 cfu/g [19]), TVCs were determined using a standard agar plate counting technique, in accordance with ISO 4833-1:2013 [21]. All homogenates were diluted ten-fold in PBW and 0.1 ml of each dilution was inoculated on duplicated plates of solidified agar (PCA), followed by 48 h incubation at 30 °C and counting of the colonies.

2.5. Sensory analysis

Four trained panellists, 30-40 years of age, selected on the basis of commitment and motivation, were recruited from the University College Cork, Ireland. To eliminate any bias, each panellist was presented randomly chosen meat samples from each of the packaging conditions, and asked to evaluate on the following descriptors: overall odour (meat-like odour), aesthetic appearance (visual colour immediately after opening), off-odour. Sensory attributes and ranking scales are reported in Table 1.

Sample presentation to individual panellists in tasting sessions was also conducted randomly.

2.6. Data analysis and statistics

Residual O_2 levels and TVC data values were analysed in the MiniTab 19 software (Minitab, LLC) using all pairwise multiple comparison procedures (Tukey *post hoc* test) with a 0.05 significance level. Spearman correlation test was performed on sensory evaluation data, using a significance level of 0.05.

3. Results and Discussion

In these experiments, commercial solid-state O_2 sensors were placed in samples of VP meat and used to assess both the performance of the vacuum packaging process and the quality of meat products produced on a large-scale industrial meat processing line. Three different VP systems were employed within the meat processing plant, namely: the old (M) and new (F) conventional VP machines and a custom-built rotary VP system (O). One O_2 sensor sticker was attached to the inner side of a standard VP bag, then a meat cut was added to it and sealed on a VP machine. One such VP meat sample, sealed with an O_2 sensor sticker in it, is shown in Fig. 1. All samples produced were held in a refrigerated storage room maintained at +1^{o}C.

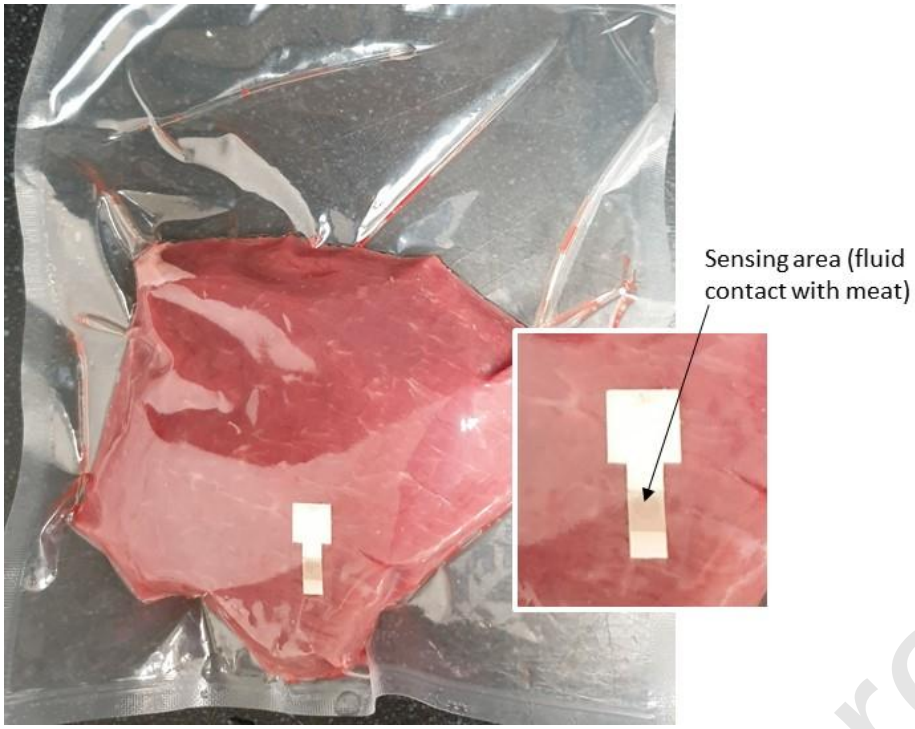

Figure 1. Photograph of a VP beef meat sample with an O_2 sensor sticker attached to the inner surface of the vacuum pouch.

On storage days 0, 30, 60 and 90 all treatment samples were assessed for the following parameters: residual O_2 content, microbial load/TVC, aesthetic appearance and shelf-life stability. At each time point, residual O_2 content was measured non-destructively in the chill room for all the treatment samples. ~~Subsequently, one sample for each treatment was taken for testing purposes.~~

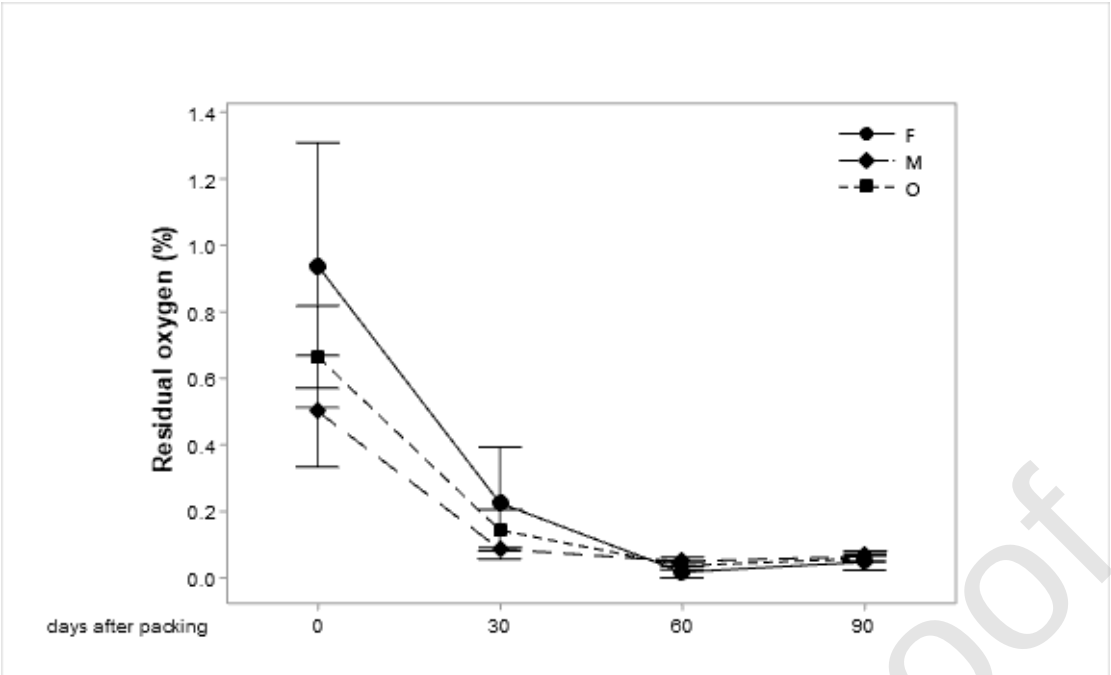

Figure 2. Residual oxygen levels (% O_2) in VP beef samples produced on three different industrial VP machines (F, M, O) during 90 days of storage at 1°C. Mean O_2 values and standard errors are shown.

As shown in Figure 2, the samples packed in machine F had significantly higher O_2 levels on day 0: 0.94% ± 0.37 (mean ± standard error of mean) O_2 vs 0.67 % ± 0.15 O_2 for packager O, and 0.5% ± 0.17 for machine M. The high initial levels of O_2 in F packs decreased significantly ($p < 0.05$) over the first 30-60 days of storage down to about 0.05% ± 0.02 (packager F), which we attributed to O_2 consumption by the product. From day 30 of the study onwards, all O_2 levels remained practically constant ($p < 0.05$), and all stayed below 0.23% ± 0.17 (recorded for packager F at 30 days), thus indicating the absence of package leaks or O_2 ingress through the package. Before microbiological analysis, samples were examined for any off-odour and visual colour changes and the results are summarised in Table 1. As samples were gradually taken from storage for destructive sampling they were examined for any off-odour and colour changes. The results indicated that all VP meat samples showed a progressive change in colour from bright purple-red at day 0 to brown on day 90.

Visual colour and off-odour scoring changed over time (positive correlation with storage time with $r > 0.75$, $p < 0.05$), indicating that storage time affected significantly the colour perception and the appearance of off-odour as the storage time increased. As off-odours increased, meat like odour vanished over time ($r = -0.412$, $p < 0.05$), for all the packing machines used. No correlation was found between visual colour or off odour and any of the packaging machines ($p > 0.05$). F samples showed the fastest increase in off odour, with definite off-notes noted on day 60 and strong off-notes on day 90. Overall, odour decreased for all samples from day 30 onwards. No meat like odour was smelled in F samples on day 90, where strong off-notes prevailed.

Table 1. Changes in sensory attributes of beef samples packaged on VP machines O, M, and F, upon their storage at +1°C. Data represent medians (n=12) and numbers in brackets represent ranking scores.

	Packing machine	Overall Odour (Meat like odour)	Off odour	Visual colour
day 0	O	Slight (3)	None (1)	Bright purple-red (1)
	M	Slight (3)	None (1)	Bright purple-red (1)
	F	Slight (3)	None (1)	Bright purple-red (1)
day 30	O	Mild (4)	Trace (2)	Dull purple-red (2)
	M	Mild (4)	Trace (2)	Dull purple-red (2)
	F	Slight (3)	Trace (2)	Slightly brownish-red (3)
day 60	O	Mild (4)	Slight (3)	Slightly brownish-red (3)
	M	Mild (4)	Mild (4)	Slightly brownish-red (3)
	F	Slight (3)	Definite (6)	Moderately brownish-red (4)
day 90	O	Trace (2)	Definite (6)	Moderately brownish-red (4)
	M	Trace (2)	Definite (6)	Moderately brownish-red (4)
	F	None (1)	Strong (7)	Brown (5)

Microbiological testing of the meat samples from the different treatments using a Greenlight® system revealed that TVC levels for meat packed in machines M and O stayed below 10^5 CFU/g for the whole period (see Fig. 3). The initial TVC count at day 0 was 2.88 ± 0.02 LogCFU/g for samples packed on machine F, 2.02 ± 0.15 LogCFU/g for machine O, and 1.87 ± 0.02 LogCFU/g for machine M.

Samples from machine F showed significantly higher ($p<0.05$) microbial load of 6.84, 6.83, 4.94 Log CFU/g on days 30, 60, 90, respectively, than those observed from samples derived from machines M and O. Lower TVC values were obtained on day 30 and 60 for packagers M and O. On day 30, samples packed on machine O reached a microbial load of 4.56 Log CFU/g, and 4.89 log CFU/g for machine M. On day 60 the samples packed on machines M and O showed similar TVC values of 4.71 and 4.67 Log CFU/g, which on day 90 reached the values of 4.19 and 3.43 LogCFU/g for machines M and O, respectively. The differences in TVC values between samples packed on machines M and O were not significant.

The data obtained with the GreenLight™ tests were recorded in 12 hours as threshold time, whereas the data on conventional TVC count obtained by plate counting (day 0) were obtained after 48 hours. This results suggest that GreenLight™ test is a faster alternative to conventional TVC count as previously reported by Hempel *et al*. [16], allowing to get LogCFU/g counts 4 times faster than conventional methods.

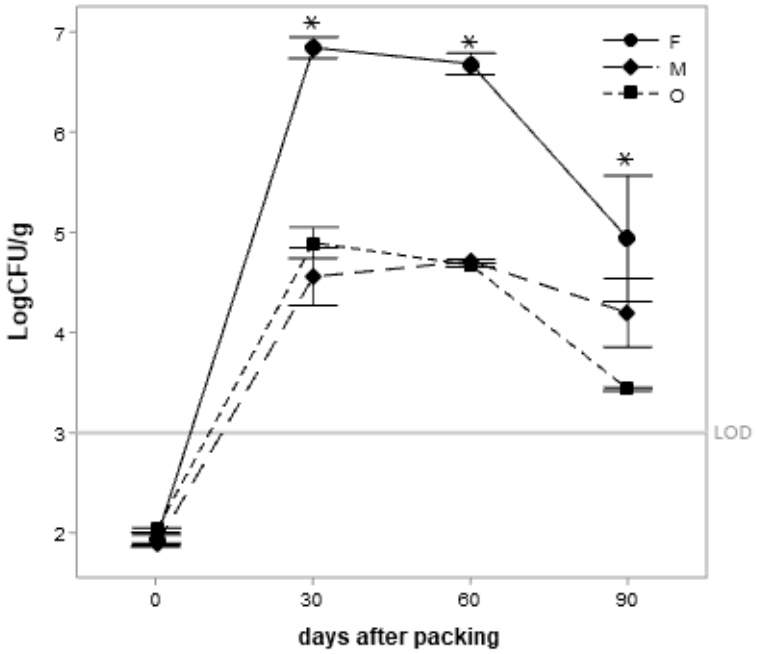

Figure 3. Profiles of total aerobic viable counts (TVC) in beef samples packaged on VP machines F, M, O, during 90 days of storage at +1°C. Mean values for 6 samples and standard errors are shown. Asterisks indicate significant differences according to ANOVA and Tuckey *post-hoc* test, for the different VP systems on each sampling day. LOD line indicates detection limit for the Greenlight® test. LogCFU/g on day 0 were measured by conventional ISO method.

In summary, in this 90-day study we assessed the in-situ performances of the three different VP machines by monitoring the residual oxygen levels, microbial contamination and sensory attributes of VP meat samples produced at a meat processing plant under standard industrial settings, and then stored for 90 days under retail settings. The samples packed on machine F exhibited the least quality and machine performance than the other two batches packaged on machines M and O. Significantly higher microbial load and strong off-odour, indicated that the quality of the beef decrease over the time. These data are in accordance with published studies on the effect of vacuum packaging on beef meat [22,23]. Fast results on O_2 levels and microbial loads were obtained *in situ*, in the meat processing plant and in less than 12 hours from sampling. Figure 4 shows that time to result for Greenlight™ TVC test

ranged from 3.33 hours ± 0.22 (day 30, packager F) to maximum 11.17 hours ± 0.22 (day 90, packager O). This is a lot faster than for conventional ISO 4833-1:2013 TVC test (time to result - 48 h).

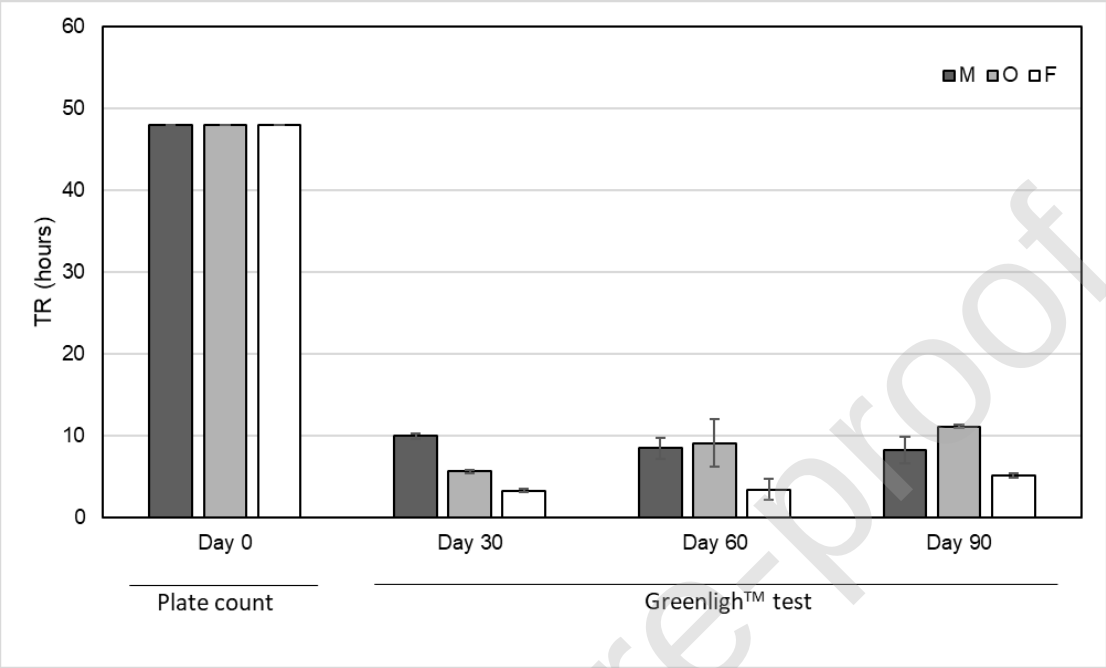

Figure 4. Time to results (TR) in beef meat samples packaged on VP machines F, M, O, during 90 days of storage at +1°C, using conventional method (plate count) and Greenlight™ test. Mean values for 6 samples and standard errors are shown.

Furthermore, the results obtained using GreenLight™ tests allowed us to determine which of the VP machines installed in the plant was the most efficient. It is also worth noting that for all the vacuum packagers used, none of the packaged meat samples showed abnormal levels of residual O_2 or variations over shelf life storage and handling of these samples, which otherwise would indicate a faulty packaging or accidental package damage.

4. Conclusions

The commercial phosphorescence based O_2 sensors read in a contactless, non-destructive, quantitative manner with a handheld reader Optech® were applied to assess the quality of vacuum-packaged raw beef products, including small cuts (200 g) and larger chucks of meat (~2.5 kg) prepared on three different packaging machines installed on a large Irish meat processing plant.

The monitoring of the oxygen levels was fast and easy, and required a handheld reader connected to a laptop via USB cable and an unskilled operator. The sensor trial did not interfere with the normal working activities of the meat processing plant. Destructive lab-based tests which require minimal sample processing and manipulation were also carried out, including the rapid microbial test GreenLight™ and product sensory evaluation. The O_2 sensor system gave accurate non-destructive data on the residual oxygen levels within the packaging, which can be correlated with product susceptibility to deterioration and microbial growth. The setup for microbial testing, which requires standard stomacher, fluorescence plate reader and disposables, is also easy to implement in a small lab. Thus, Optech® and GreenLight® systems provide convenient, portable and versatile tools for rapid, high-throughput assessment of various packaged samples and packaging processes. The fast product analysis by the methods proposed int his study can provide competitive advantages to producers of meat products, providing reduced time and cost of analyses. Furthermore, the possibility to detect *in situ* and in few seconds/hours any O_2 leakage or high TVC levels, provides important quality and hygiene indicators and allows consumers and retailers to benefit from fresher and safer meat products and their longer shelf life. Studies are in progress to optimize the various handling steps, rendering the methods more applicable for a higher sample throughput, lower microbial loads, and *in situ*

use. Furthermore, the presented method for total viable microflora counting can be adjusted for the detection of other bacteria in raw meat.

Declaration of Interest Statement

The authors declare no competing interests in relation to this manuscript.

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Author Biographies

Dr. Caroline A. Kelly received the BSc. Degree in Forensic and Environmental Chemistry from Dublin Institute of Technology in 2012. She then did her PhD project at the University College Cork which was completed in 2016. Her research has been focused on the design and fabrication of solid-state oxygen sensors, with particular emphasis on their use in food and packaging applications.

Dr. Elisa Santovito received her BSc, MSc and PhD degrees in biotechnology, plant virology and food protection from the University of Bari, Italy. She then worked as a research assistant and postdoctoral researcher in a number of research institutions, including the Italian Institute of Sciences of Food Production, Instituto Nacional de Engenharia Biomédica (INEB), Portugal, ProBioVegan SRLs start-up company. She is currently employed as postdoctoral researcher at the University College Cork, leading a project on development of optochemical sensor systems and their application in food packaging and quality assurance of packaged food products,

Dr. Malco Cruz-Romero is research support officer at the Food Packaging Group, School of Food & Nutritional Sciences, UCC. He has a BSc in Food Industries (National Agricultural University "La Molina", Lima, Peru in 1992), a degree of engineer in Food Industries (National Agricultural University "La Molina", Lima, Peru, 1995), MSc in Food Technology (University College Cork, 2002) and a PhD in Food Technology (University College Cork, 2006). Research expertise of Dr. Cruz-Romero includes nanotechnology, development of smart packaging systems, application of optochemical oxygen sensors, development/use of bio-based plastics as alternatives to petroleum-based plastics, use of the hurdle approach (including high pressure processing) to improve the microbiological quality and safety of food products and the application of novel preservation technologies.

Prof. Joe P. Kerry graduated from University College Galway in 1986. He received his PhD in Microbiology at University College Galway in 1995. Afterwards, Prof. Kerry joined University College Cork where he is currently Professor in the School of Food and Nutritional Sciences, Head of the food packaging group. Research expertise of Prof. Kerry includes use and manipulation of modified atmosphere packaging systems for use with foods, use of extrusion technology for the manufacture of food products and packaging materials, development of optochemical sensors and applications of smart packaging technology within the area of food packaging.

Prof. Dmitri B. Papkovsky graduated from the Chemistry Department of Moscow State University in 1982 and received his PhD in 1986 from the Institute of Biochemistry, Russian Academy of Science, Moscow. In 1997 he joined Biochemistry Department of University College Cork, where he is currently Professor at the School of Biochemistry and Cell Biology, Head of Biophysics and Bioanalysis Lab. Research interests of Prof. Papkovsky include quenched-luminescence oxygen sensing and its applications, phosphorescence based probes and (bio)analytical techniques.