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Optical Methods to Determine the Gas Atmosphere in Various Modified Atmosphere Packages: Applications and Correlation in Meat Spoilage †

Jasmin Dold [1,*], Clarissa Hollmann[1], Caroline Kehr[1] and Horst-Christian Langowski [2]

1 Chair of Brewing and Beverage Technology; Technical University of Munich; Weihenstephaner Steig 20; D-85354 Freising, Germany
2 TUM School of Life Sciences, Technical University of Munich, Alte Akademie 8, D-85354 Freising, Germany
* Correspondence: jasmin.dold@tum.de; Tel.: +498161 71-5633
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Abstract: The use of non-invasive optical measurement systems for the quality evaluation of packed food is becoming more important for the reduction of food waste and quality improvement. In this study, the gas atmosphere of packed poultry was monitored using optical measurement systems based on fluorescence quenching for oxygen determination and mid-infrared (MIR) laser spectroscopy for the detection of carbon dioxide. Over 15 days of storage, the gas atmosphere was evaluated continuously, and total viable count and a simultaneous optical and olfactory sensory evaluation was performed in simultaneously by a trained sensory panel. The results revealed that irregular storage conditions could be detected, while microbiological growth under regular conditions does not lead to a significant change in the headspace atmosphere.

Keywords: meat spoilage; poultry; non-destructive; fluorescence quenching; infrared; spectroscopy; modified atmosphere packing; quality control; shelf life prediction; sensory

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1. Introduction

Non-destructive measurement systems for the quality evaluation of packed food are becoming increasingly important because the quality standards and amount of packed food is increasing. However, sustainability and reduction in food waste is gaining importance. In Europe, approximately 88 million tons of food is wasted annually [1], a high proportion of which is meat or meat products, which is often due to expired shelf-life or use-by date.

Optical measurement systems, which include fluorescence quenching and infrared technology, are studied well for the determination of food quality [2,3]. However, many of them are extremely product-specific or involve very elaborate conversion factors. As observed from previous studies, the amount of O_2 decreases characteristically and that of CO_2 increases upon spoilage of poultry or beef in high O_2 modified atmosphere packaging (MAP) due to the respiration of spoilage microorganisms [4–6]. Often, this spoilage is accompanied by the formation of volatile organic compounds (VOCs), which affect sensory perception [7].

This study combines the previously described topics. High O_2 packed poultry was monitored using novel non-destructive measurement devices with simultaneous control of total viable count (TVC) and sensory acceptability over a storage period of 15 days at different temperatures. Afterward, the correlations between the parameters and suitability for shelf life prediction were evaluated.

2. Materials and Methods

2.1 Optical Measurement Systems

2.1.1 Fluorescence Quenching to Detect O_2

For the non-destructive determination of O_2, a fluorescence-based measurement system and associated sensor spots (PreSens Precision Sensing GmbH, Regensburg, Germany) were used. The measurement device works via fiber optics (λ_{ex} = 505 nm and λ_{em} = 650 nm). To integrate the sensor spots into the lid film, a sensor spot was placed on the inside of the lid film that faced upward (PP/PA/PP/PA, 100 µm, allvac Folien GmbH, Waltenhofen, Germany) and then covered with a PP film (56 µm, Huhtamaki Flexible Packaging Germany GmbH & Co. KG, Ronsberg, Germany) and sealed with a ring-shaped sealing tool at 155°C. Before sealing, a two-point calibration of the sensor spots in the relevant measuring range (0% and 60% O_2) was carried out.

2.1.2 MIR Spectroscopy to Detect CO_2

The non-destructive measurement of CO_2 was carried out with a measurement system based on MIR spectroscopy (KNESTEL Technologie und Elektronik GmbH; Hopferbach, Germany). Three different wavelengths were used: λ_1 = 4.26 µm, λ_2 = 4.45 µm, and λ_3 = 4.27 µm. The laser beam was pointed at 45° through the corner of the packaging. A two-point calibration with 0% and 40% CO_2 was carried out on the empty reference trays.

2.2 Sample Preparation

A total of 400 g of fresh chicken strips (Donautal Geflügelspezialitäten, Bogen, Germany) were weighed into transparent polypropylene trays (ES-Plastic GmbH, Hutthurm, Germany) and sealed with a semiautomatic traysealer (T250, MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany) under a modified gas atmosphere (70% O_2/30% CO_2 or 80% O_2/20% CO_2). For each atmosphere, six samples with integrated sensor material were used as the lid film. In addition, 44 samples were prepared for each gas atmosphere without integrated sensor materials for sensory and microbiological evaluation. Samples were stored at 4°C and 10°C. Furthermore, three empty trays were prepared for each temperature and gas combination with sealed-in sensor spots to monitor the concentrations of O_2 and CO_2 without product influence during storage.

2.3 Non-destructive Gas Determination

The gas atmosphere of the prepared trays was monitored for 15 days (except for day 3 for filled trays and days 3, 5, 6, 12, and 13 for the empty trays) via the non-destructive measurement devices.

2.4 Microbiological Analysis

TVC was determined for each temperature and gas composition on days 0, 1, 4, 6, 8, 11, 13, and 15 in duplicate. A total of 70 g of chicken strips was weighed into a sample bag (VWR International, Darmstadt, Germany) and homogenized for 120 sec with 50 ml Ringer's solution (Merck KGaA, Darmstadt, Germany) in a stomacher (LabBlender400, Gemini BV, Apeldoorn, Netherlands). A dilution series was prepared with Ringer's solution using 1 ml of the filtrate and 100 µl of the chosen dilutions were later spread onto the brain heart infusion agar (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). After incubating the plates aerobically at 30°C for 3 days, the colony-forming units per gram sample (CFU/g) were calculated.

2.5 Sensory Evaluation

For sensory evaluation, the samples were investigated by a previously trained panel (n = 15; 5 f, 10 m, average age 29 years) on days 0, 1, 4, 6, and 8 (4°C and 10°C) and on days 11 and 14 for the samples stored at 4°C. The intensity of previously specified attributes

was evaluated visually and olfactorily on an analog scale ranging from 0 to 100 (0 = not perceptible/fresh; 100 = strong perceptible/rotten). For the evaluation, a sample was defined as no longer acceptable when the average value of the orthonasal or visual impression was ≥50.

2.6 Statistical Analysis

Statistical analysis was performed using MS Excel. To calculate significance, a two-sample t-test was performed.

3. Results

3.1 Development of Gas Concentration in Empty and Filled Trays

For the empty trays, almost no changes in the gas content were detected. The amount of O_2 and CO_2 increased and decreased slightly, respectively. In addition, the optical measurement method for O_2 deviated from the real values at the first two to three measurement points. This was because the sensor spots were sealed into the lid film under atmospheric conditions and the higher O_2 concentration in the MAP had to permeate into the spot area first.

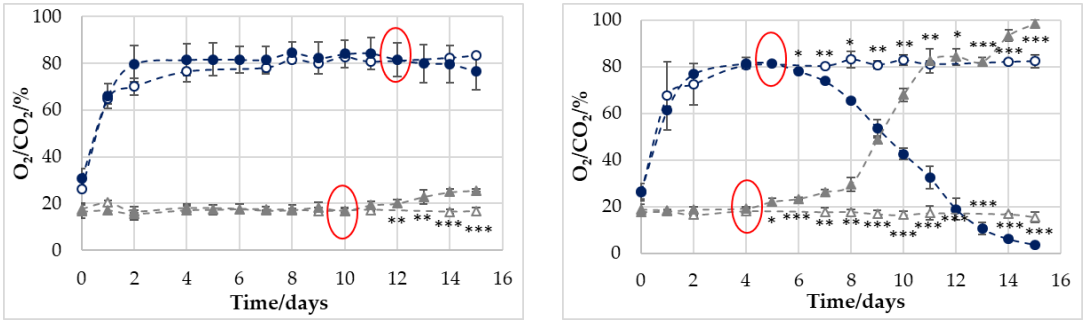


Figure 1. Development of O_2 (○/●) and CO_2 (△/▲) under different storage conditions over 15 days in trays with (●/▲) and without poultry (○/△): (**a**) 80% O_2/20% CO_2 4°C (**b**) 80% O_2/20% CO_2 10°C. Indices indicate a significant difference between the curves with and without poultry:* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. The red circles mark the point when the curve of the respective gas concentration in the filled trays intersects that for the empty trays (cross-over), which indicated a microbiologically induced change in the headspace atmosphere.

By comparing the empty and filled trays, the influence of the product was determined. For the 80/20 4°C samples (Fig. 1 (a)), a significant deviation was noted between the filled and empty trays for CO_2 measurement from days 12 to 15. However, O_2 did not show any statistical significance. The cross-over was on day 12 and then the O_2 content of the filled package decreased steadily until day 15. In addition, the cross-over was noticeable for the 70/30 4°C samples (Fig. S1(a)), but only from day 13. For CO_2 content, however, no change in the headspace atmosphere was observed for filled and empty trays. Samples that were stored at 10°C in 80/20 (Fig. 1 (b)) MAP showed the earliest deviation from the empty trays, with a significant change at day 5 for CO_2 and day 6 for O_2. Afterward, a fast increase in the amount of CO_2 (approximately 100%) on day 15 and a decrease in O_2 was visible. The 70/30 MAP at 10°C (Fig. S1 (b)) showed a similar but slower trend.

3.2 Microbiological Analysis

All samples had a similar starting value of approximately 10^4 CFU/g (Table 1). The samples that were stored at 10°C showed faster growth and reached the defined critical value of 10^7 CFU/g [8] after 3 (80/20) or 4 days (70/30). The samples that were stored at 4°C reached the value after 6 (80/20) or 7 (70/30) days. At the end of storage, all the samples were well above the critical limit. The highest value of $>10^{10}$ CFU/g was reached by the

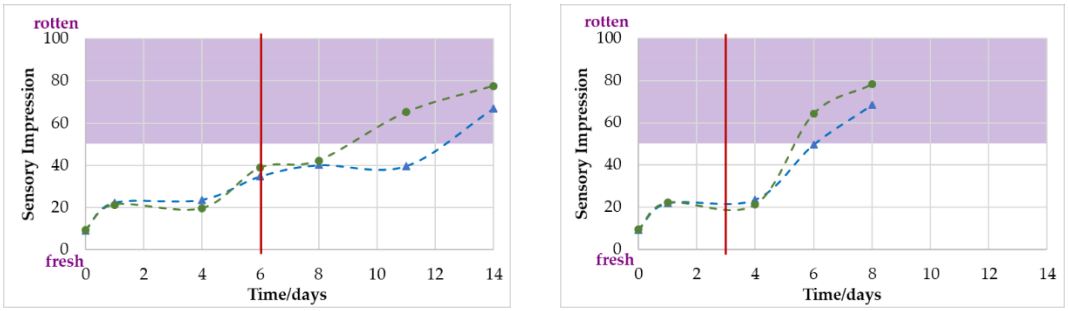
sample packed with 80% O_2 and 20% CO_2, which was stored at 10°C. However, the samples stored at 4°C reached at the final values of >10^9 CFU/g.

3.3 Sensory Evaluation

The results of the sensory evaluation of the samples are shown in Fig. 2 (80/20) and Fig. S2 (70/30). The microbiologically critical values (section 3.2) are marked by a red line. The sensory impression, which was visual and orthonasal, remained acceptable for all the samples when the microbiological limit was exceeded. In addition, the poultry stored at 4°C was first classified with an unacceptable orthonasal sensory impression of >50% on testing day 11 (80/20) or 14 (70/30) for the olfactory evaluation, whereas the samples at 10°C had reached that point on day 6 (80/20) or day 8 (70/30).

Table 1. TVC for the poultry on days 0 and 15 for each storage condition (n = 4) and the day the critical limit of 10^7 $CFUg^{-1}$ was reached (end of shelf life).

	Day 0	Day 15	Shelf life expired
80/20 4°C	$1.36x10^4$ $CFUg^{-1}$	$4.00x10^9$ $CFUg^{-1}$	Day 6
70/30 4°C	$1.27x10^4$ $CFUg^{-1}$	$4.29x10^9$ $CFUg^{-1}$	Day 7
80/20 10°C	$1.36x10^4$ $CFUg^{-1}$	$2.19x10^{10}$ $CFUg^{-1}$	Day 3
70/30 10°C	$1.27x10^4$ $CFUg^{-1}$	$4.47x10^9$ $CFUg^{-1}$	Day 4



(**a**) (**b**)

Figure 2. Visual (▲) and orthonasal (●) impression of poultry under different storage conditions over 8 or 14 days: (**a**) 80% O_2/20% CO_2 4°C and (**b**) 80% O_2/20% CO_2 10°C. The red line marks the point when microbiological limit value was achieved. The purple area indicates the previous defined sensory limit of 50 scores.

4. Discussion

4.1 Correlation of Results

Table 2 gives an overview of the tested parameters and indicates some possible correlations. Yellow describes a possible association between the cross-over and microbiological spoilage. Orange indicates a correlation between the cross-over and olfactory spoilage. Green indicates a correlation between the gas change in the headspace and olfactory spoilage.

Table 2. Possible correlations between the tested parameters. P ≥ 0.05 represents the first day where the difference between empty and filled trays was significant with P ≥ 0.05, after the cross-over was reached. "Microbiologically spoiled" indicates a TVC of 10^7 $CFUg^{-1}$ and "olfactory spoiled" shows the panel classification of the sensory panel with >50 scores. A description of the color correlations can be found in the previous text.

	4°C		10°C	
	80/20	**70/30**	**80/20**	**70/30**
Cross-over O_2	12	13	5	5
P ≥ 0.05 O_2	-	-	6	7
Cross-over CO_2	10	-	4	4
P ≥ 0.05 CO_2	12	-	5	6
Microbiologically spoiled	6	7	3	4

Olfactory spoiled	11	14	6	8

4.1.1 Correlation between O_2 and CO_2 Concentrations and Microbial Spoilage

As described previously, some studies have been carried out that indicated a correlation between O_2 respiration with microbiological spoilage, even for chicken meat [4,6]. However, this study could not completely confirm these findings. For all samples, no significant change in the gas concentration was noted when the limit of 10^7 CFU/g was reached. Microbial spoilage might be visible considering the previously described cross-over point for CO_2 detection; however, it is only for the samples that were stored at 10°C. This point was reached either on the day (70/30) or 1 day after (80/20) the critical value of 10^7 CFU/g was reached. A significant change occurred in the gas atmosphere for the samples stored at 10°C, and for CO_2 detection in addition for the 80/20 4°C samples; however, the TVC was already $\geq 10^9$ CFU/g. For the samples stored at 10°C, a very strong change occurred for both gases in the gas atmosphere after 5 or 6 days, and at the end, the gas atmosphere completely changed in the headspace. Nevertheless, the total microbiological growth was not very different compared with the 4°C samples after 15 days. This was a strong indication that the type of microorganisms, and not necessarily the quantity, was crucial for O_2 consumption. This was confirmed in a study wherein beef was inoculated with different meat-spoiling bacteria. Samples contaminated with *Brochothrix thermospacta* showed significant O_2 consumption while samples contaminated with *Carnobacterium divergens* and *Carnobacterium maltaromaticum* showed no consumption at all, even at microbial populations of $\geq 10^8/cm^2$ [5]. This shows that it is mainly the microbiota that determines O_2 consumption and CO_2 production, which makes correlation, for example, for shelf-life prediction, difficult.

4.1.2 Correlation of O_2/CO_2 with Sensory Evaluation

For all samples, it was observed that the achievment of the defined shelf life did not correlate with the visual and olfactory impression of the panel. However, there appeared to be a correlation between the gas development and sensory acceptance in some cases. For the poultry that was stored at 4°C at initial gas concentrations of 80% O_2 and 20% CO_2, a significant change in CO_2 was observed on day 12, and the cross-over happened 2 days earlier. This agreed with the sensory evaluation on day 11 when the panel classified the sample as not acceptable for the first time. The 4°C 70/30 sample had its cross-over with O_2 on day 13 and the first classification as olfactory spoiled on day 14. However, the classification on day 11 was slightly below the 50 scores limit, which is why a prediction via gas determination is rather unlikely. For the samples stored at 10°C, a very good fit between the gas concentration and sensory acceptance was observed. All samples showed their first significant CO_2 change before olfactory spoilage. In addition, O_2 detection was in accordance with the results that were obtained by the sensory panel. The cross-over gives a strong indication of the sensory spoilage for the 10°C samples.

4.2 Influence of the Microbiom

These results allowed some conclusions to be drawn about the type of spoilage microorganisms, with some limitations. Franke et al. (2017) showed that chicken breasts packed at high O_2 and stored at 4°C were mainly populated with *B. thermospacta,* and develop *Carnobacteria* sp. and *Pseudomonas* sp., mainly under MAP with lower CO_2 concentrations (≤15%) [9]. Because *Pseudomonas* sp. are responsible for the formation of VOC's [7], this agreed well with the earlier sensory spoilage of the 80/20 sample, compared with that of the 70/30 sample. However, O_2 was hardly respired at this point, which could be because of the small population of *B. thermospacta* or due to the lack of heme, compared with beef or because of the low temperature [4,5]. The influence of storage temperature was visible, especially with respect to sensory evaluation and gas development. In addi-

tion, this was also observed by Höll et al. (2016). They had a mixed microbiota at the beginning of the storage. Later, at 4°C, a mixture of *B. thermospacta*, *Pseudomonas* sp., and *Carnobacteria* sp. grew, and at 10°C, the microbiota mainly consisted of *Pseudomonas* sp. and *Serratia* sp. at the end of storage period. After 4–8 days, *B. thermospacta* was present, which probably favored O_2 consumption. Then, VOC forming *Pseudomonas* sp. could grow [6]. That effect was probably the same as observed in this study.

5. Conclusions

This study demonstrated that non-destructive measurement systems can monitor the gas atmosphere. The systems, however, cannot be used to predict the shelf-life of high O_2 packed poultry stored under regular conditions. For premature microbial spoilage, for example, due to contamination or an interruption in the cold chain, especially the CO_2 detection might be useful because a significant deviation was measurable before sensory spoilage. In further research, a correlation with the concentration of volatile emissions (e.g. 2,3-butanedione) will be further elaborated and the influence of the heme concentration will be clarified by experiments with beef. Another possible application for the technologies might be the detection of leakages in packages or process control for MAP production lines.

Supplementary Materials: Figures S1 and S2 are available online at www.mdpi.com/xxx/s1

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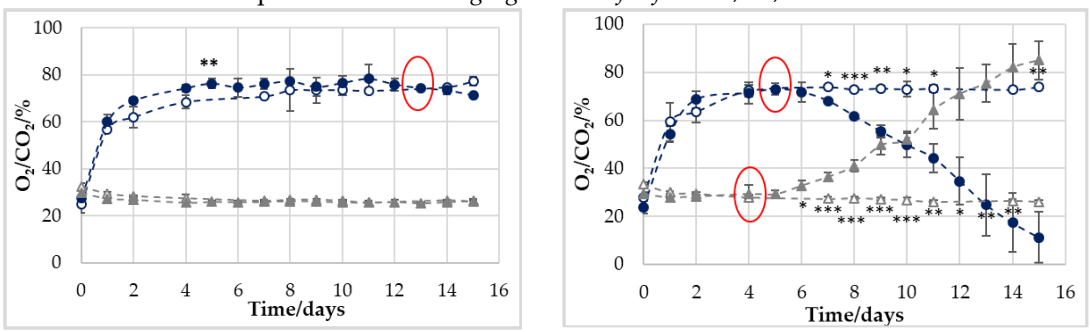

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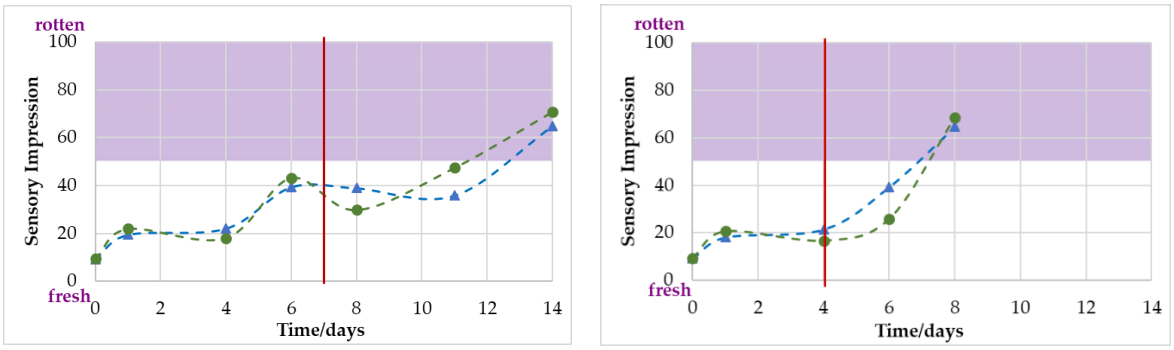

Figure S2. Visual (▲) and orthonasal (●) impression of poultry under different storage conditions over 8 or 14 days: (**a**) 70% O_2/30% CO_2 4°C and (**b**) 70% O_2/30% CO_2 10°C. The red line marks the point when microbiological limit value was achieved. The purple area indicates the previous defined sensory limit of 50 scores.