Summary for the final report

Development of a non-destructive optical detection method for the individualized evaluation of meat spoilage in protective gas packaging with CO_2 and oxygen (Individualised Evaluation of Meat Spoilage)

Modified atmosphere packaging (MAP) of fresh meat is one of the most important methods to extend the best before date and to present the product attractively to the consumer. Poultry meat is nowadays often packaged at high oxygen levels, as this inhibits pathogenic microorganisms such as *Campylobacter jejuni*.

It has been shown that the composition of the modified gas atmosphere changes due to the growth of microorganisms during storage, as oxygen (O_2) is respired to carbon dioxide (CO_2) - resulting in an inversion of the headspace atmosphere. The aim of the project was therefore to develop optical non-invasive measurement methods and to adapt these to food packaging in order to be able to evaluate meat spoilage individually. For this purpose, a correlation of the microbial growth and the development of the gas atmosphere had to be established. Two main measurement methods were researched for this purpose. Firstly, a sensor spot based on the principle of fluorescence quenching was integrated into a packaging system in order to determine the O_2 content in the headspace. Furthermore, a measurement method based on IR spectroscopy for CO_2 determination was developed. This should lead to a reduction of the avoidable amount of discarded and spoiled meat.

To integrate the sensor spots into the packaging system, a heat-sealing process was selected with which the sensor spots were integrated into the lid film of the meat packaging and simultaneously covered with a compatible and O_2-permeable film in the direction of the food. This simultaneously protects the sensor material from the product (since contamination can lead to inhibition of fluorescence) and makes the food conformity of the product probable. Through corresponding tests it could be shown that a sealing process has no significant influence on the measurement accuracy of the measurement method. Likewise, in comparison with destructive gas analyzers, it could be shown that precise results can be expected in real time if the gas atmosphere is inverted (O_2 decrease with simultaneous CO_2 increase). If there is a change of atmosphere and at the same time an increase of another gas component (e.g. entry of nitrogen in case of leakage), the results are not detectable in real time due to different permeation properties.

For CO_2 detection, an instrument was developed that measures the gas content by inserting the package into a rail system and passing the laser over the corner of the package at a 45° angle (see Figure 1). This approach offers the advantage that the measurement path is free of product residue, and the rail system provides a continuously consistent measurement path. The measurement takes place in the mid-infrared range at 4.26, 4.27 and 4.45µm, the latter being localized as a reference beam next to the CO_2 absorption band. The measurement allows very precise measurement results in real time - even for packaging perforations. When investigating possible influencing factors, it was found that in most cases measurements of different tray colors and film prints are possible, and even slight product residues have no significant influence on the measurement precision. A screening of various modified atmosphere packaged products in retail also showed that the application is suitable for a wide range of tray shapes and sizes.

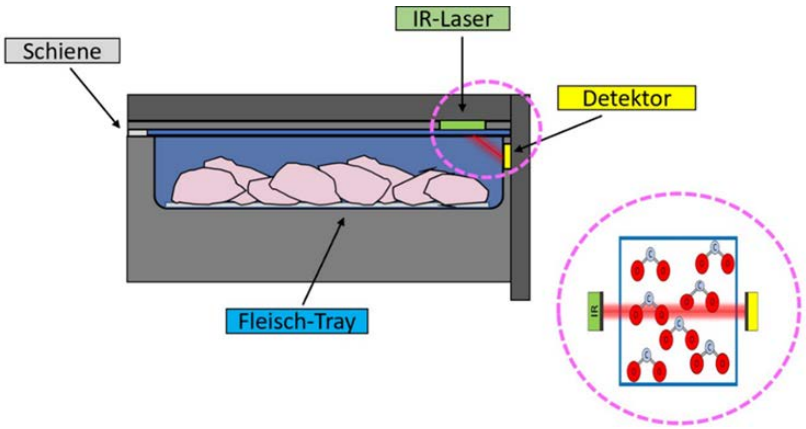

Figure 1: Schematic representation of the measuring device for non-invasive CO_2 determination in the MIR range.

For the microbiological work, 6-11 strains each of Gram-positive, meat spoilage key bacteria were first selected from the strain collection and characterized at the genomic, proteomic and physiological levels using RAPD, MADLI-TOF MS fingerprinting methods and anaerobic and aerobic growth curves. Furthermore, a cultivation medium for the selected bacteria was developed, which corresponds to the chemical composition of real beef and was used for the investigation of the protective gas influence using a special gassing system in high pressure glass bottles. Five key bacteria were identified as the main spoilage organisms (*B. thermosphacta* TMW2.2101, *C. divergens* TMW2.1577, *C. maltaromaticum* TMW2.1581, *L. gelidum* subsp. *gelidum* TMW2.1618 and *L. gelidum* subsp. *gasicomitatum* TMW2.1619), as they were able to grow at a gas atmosphere of 70% O_2/30%CO_2. Furthermore, marker genes were identified for rapid and easy prediction of the potential of meat spoilage bacteria.

In addition, the anaerobic growth of pseudomonads and their specific markers were characterized. Specific strains of meat spoilage *Pseudomonas* species were able to grow on anoxically packed meat. However, the extent of the contribution of these strains to the spoilage of the anoxically packaged product remains to be seen. Nevertheless, specific marker genes (e.g., arginine deiminase or ornithine carbamoyl transferase) could be identified here as well, allowing a quick and easy assessment of the potential of meat spoilage *Pseudomonas* species for their anaerobic growth/survival on meat. Finally, it is important to mention that *Pseudomonas* strains persisting on meat, just like actively growing strains, can also contribute significantly to product spoilage through their metabolic activity.

To characterize the oxygen consumption rate of each microorganism, the above mentioned measurement setup was used, and the five selected strains were introduced into the developed meat boullion, and incubated at room temperature, while controlling the O_2 content. The results are shown in Figure 2. When directly comparing the O_2 uptake rate of all five species, a 31-fold increased OUR/cell was observed for *B. thermosphacta* TMW2.2101 compared to the other species. In general, however, all microorganisms used were able to consume a significant amount of O_2 under these conditions.

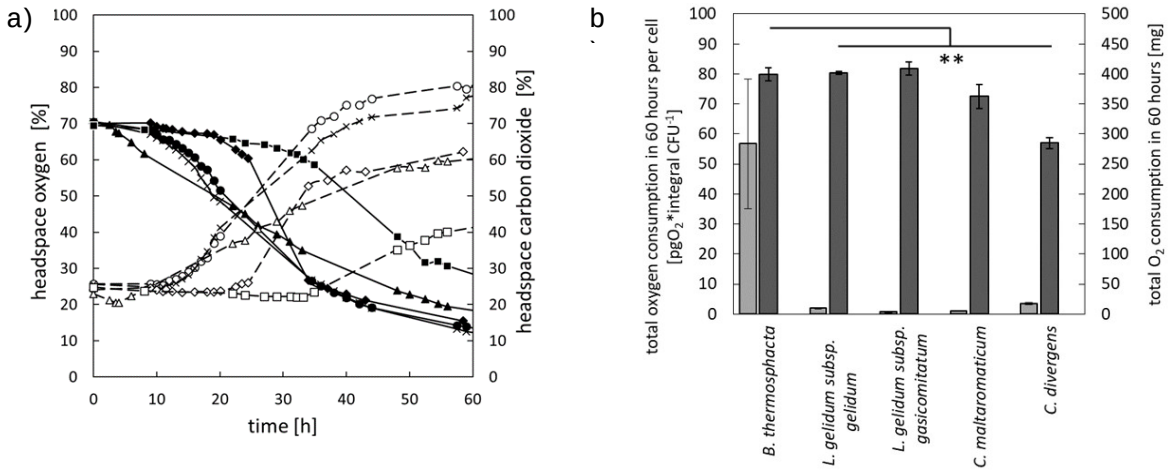

*Figure 2: a) Development of headspace atmosphere (30% CO_2 / 70% O_2) of glass bottles half-filled with MS medium inoculated with typical meat spoilage microorganisms. B. thermosphacta TMW2.2101 (◆), C. divergens TMW2.1577 (■), C. maltaromaticum TMW2.1581 (▲), L. gelidum subsp. gelidum TMW2.1618 (●) and L. gelidum subsp. gasicomitatum TMW2.1619 (x). Black symbols: O_2 content, White symbols: CO_2 content. b) Calculated total oxygen consumption in 60 h for all cells (■) (Secondary y-axis) or per individual cell (■) (Primary y-axis). * Significant differences between replicates with $p < 0.05$.*

In order to make a correlation of the results for application, experiments were carried out with meat samples (beef and chicken) in real packaging systems and the development of the gas concentration was determined with the developed non-invasive methods. First, the meat samples were selectively inoculated with the five selected bacterial strains, and both O_2 development and microorganism growth (total plate count) and sensory evaluation were assessed by an untrained panel at 4°C storage. Figure 3 shows the results of inoculated beef with *B.thermosphacta* und *L. gelidum* subsp. *gasicomitatum*.

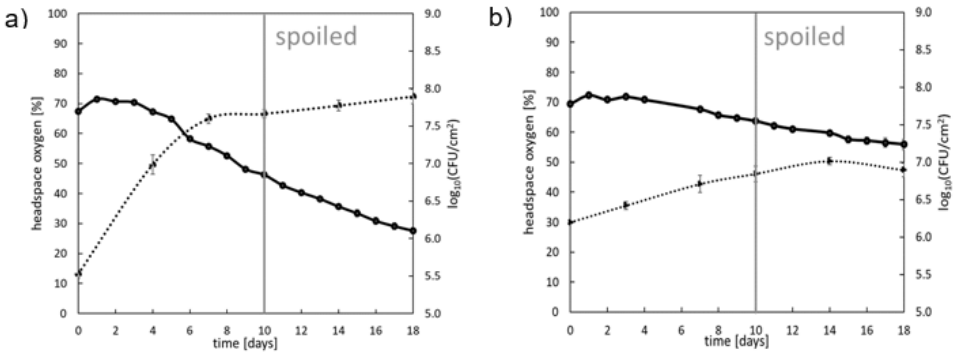

Figure 3: Development over time of the O_2 content in the headspace of the packages and the bacterial count of beef steak inoculated with a) B. thermosphacta TMW2.2101 b) L. gelidum subsp. gasicomitatum TMW2.1619. Black line: oxygen content in the headspace of the packages, dashed line: bacterial count per cm^2 of meat. Horizontal line: time of perceptible spoilage of the piece of meat, based on sensory evaluation by 10 test subjects.

A significant decrease in the O_2 content in the headspace of packages containing beef inoculated with one of the three species *B. thermosphacta* TMW2.2101, *L. gelidum* subsp. *gelidum* TMW2.1618 and *L. gelidum* subsp. *gasicomitatum* TMW2.1619 was observed. The decrease in O_2 content in the headspace of the beef packages was clearly evident before the onset of perceived spoilage. Also, the cell counts at the time of the first measurable O_2 decrease in the headspace were before those at the time of perceived spoilage. Therefore, a prediction of meat spoilage based on the O_2 content in the headspace of modified atmosphere packed beef can be considered promising. In contrast, no change in O_2 content in the headspace of packages inoculated with *C. divergens* TMW2.1577 or *C. maltaromaticum* TMW2.1581 chicken meat was detected throughout the storage period. Since chicken meat contains relatively little heme compared to beef meat, the lack of O_2 growth in packages of *C. divergens* TMW2.1577 and *C. maltaromaticum* TMW2.1581 was therefore explained as a consequence of dysfunctional respiratory activity.

However, the experiments shown could not yet be fully related to practice, since on the one hand the samples were artificially inoculated and thus did not reflect the microbiota of an actual package. Secondly, the meat:headspace ratio of 1:8 was clearly undersized. Third, the sensory panel was untrained.

For this reason, meat:headspace ratios of 1:3 were chosen for the final experiment with chicken stripes. The samples were left "natural" and the microbiota was not affected as a result. A pre-trained panel was used for sensory evaluation. In addition, both the O_2 content with the integrated sensor spots and the CO_2 content with the IR measuring device were determined. In order to include permeation processes, empty packages were stored for both storage temperatures (4°C and 10°C) (see Figure 4).

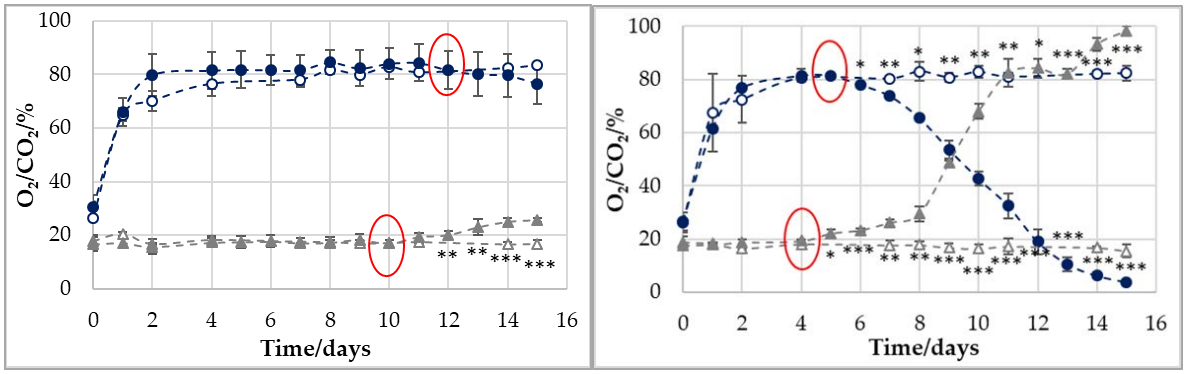

Figure 4: Development of O_2 (○/●) and CO_2 (△/▲) in the packaging headspace with (●/▲) and without poultry (○/△) at an initial gas concentration of 80% O_2/20%CO_2 stored at 4°C (left) and 10°C (right).The indices show a significant difference between the curves with and without meat (P < 0.05, ** P < 0.01, and *** P < 0.001).*

The red circles mark the point at which the curve of the respective gas concentration in the filled trays intersects with that of the empty trays ("cross-over"), indicating a microbiologically induced change in the headspace atmosphere. For the 4°C, this point is not reached until day 10 (CO_2) or day 12 (O_2). At 10°C, a significant change in the gas atmosphere occurs.

Figure 5 shows the sensory perception of the samples over the storage time of the trained panel and also indicates the reaching of the microbiological critical threshold. Here it can be seen that all samples were perceived as unacceptable by the panel only after reaching the critical limit. When looking at the gas development and the development of the sensory perception, it becomes clear that a better correlation is possible in these cases. For the gas development and the reaching of the critical limit value, no correlation or only limited correlation could be made for this type of meat.

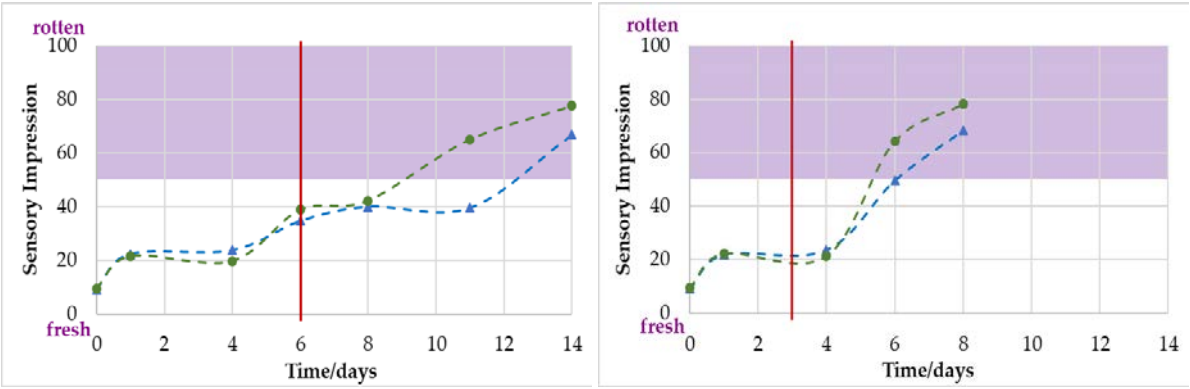

Figure 5: Development of the visual (▲) and olfactory (●) overall impression during the storage period (left: 4°C, right: 10°C). The red line marks the day on which the microbiological critical limit of 10^7 CFU/g was reached. The purple area marks the exceeding of the perception from "fresh" to "rotten", which was defined from a value of 50.

Table 1 shows possible correlations of these trials in more detail. Yellow describes a possible correlation between the cross-over and microbiological spoilage. Orange indicates a correlation between the cross-over and sensory changes. Green indicates a correlation between gas change in the headspace and sensory changes. Gray indicates a correlation between microbiological spoilage and sensory changes.

The results could again clearly underline that the developed non-invasive measurement methods are both well suited for non-invasive O_2 and CO_2 measurements. A correlation with the critical value of 10^7 CFU/g and the gas development in the headspace is not possible under these, real storage conditions for white chicken meat. However, the "cross-over" may be an indication of improper storage and thus of premature spoilage. Correlation of sensory attributes with the microbiological threshold was partially possible. Likewise, sensory perception could be related to gas change.

Table 1: Possible correlations of the tested parameters. P ≥ 0.05 represents the first day when 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 "sensory changes" indicates the panel rating of the sensory panel with: >50 points for overall olfactory impression ("olfactory spoiled") and > 20 points for the descriptors slime, color, buttery and cheesy.

		4°C		10°C	
		80/20	**70/30**	**80/20**	**70/30**
	Cross-over O_2	12	13	5	5
	Cross-over CO_2	10	-	4	4
	P ≥ 0.05 O_2	-	-	6	7
	P ≥ 0.05 CO_2	12	-	5	6
	Microbiologically spoiled	6	7	3	4
Sensory changes	Olfactory impression	11	14	6	8
	Slime	6	6	4	4
	Color („Gray“)	8	8	6	8
	Buttery	6	6	6	8
	Cheesy/Rancid	11	14	6	8

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