INVESTIGATING OZONE TREATMENT AS A REMEDIAL ACTION FOR MICROBIAL SPOILAGE OF MEAT BY MEASURING VOLATILES USING PROTON-TRANSFER-REACTION MASS-SPECTROMETRY

D. Mayr[a,b], T. Mikoviny[a], J.D. Skalny, E. Hartungen[a], N. J. Mason[b], T.D. Märk[a,c]

[a] Institut für Ionenphysik , Leopold-Franzens-Universität, Technikerstr. 25, A-6020 Innsbruck, Austria
[b] Department of Physics and Astronomy, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK; d.mayr@open.ac.uk
[c] Department of Plasmaphysics, Comenius University, 84248 Bratislava, Slovak Republic

Background

An estimated 30% of fresh produce is lost by microbial spoilage from the time of harvest, through handling, storage, processing, transportation, shelving and delivery to the consumer (web1). To preserve food pathogens need to be destroyed or inactivated and the non-pathogenic microorganisms and enzymes responsible for food spoilage need to be eliminated or at least reduced (web2). Several techniques to extend food's shelf-life have been developed over the years for example heating, drying, irradiation and treatment with ozone. All these methods have their advantages, drawbacks and limitations depending on the type of the food, the kind of microorganisms, national regulations and the public demands (like unaltered taste, aroma, colour and vitamin content with no chemical residues after treatment). The use of ozone to treatment of the food using ozone gas meets these requirements quite well.

Ozone is a strong oxidant that kills many microbacterial organisms without leaving any toxic by-products or residues (web3, web4). Ozone has also been used for many years to treat pathogens such as bacteria and algae in water for applications such as drinking water supplies (web4) - ozone should therefore be a useful agent for the destruction of pathogens which are active in microbial spoilage of meat. Despite these advantages the use of ozone in the food industry has not been exploited as extensively since ozone must be manufactured on-site and until recently ozone generators were bulky and expensive (web5). However new developments in the design of small scale in situ ozone generators (using either UV lamps or electrical discharges) now make it practical to develop ozone treatment for food preservation on a commercial scale.

To date there have been only a few studies to quantify the ozone concentrations needed to ameliorate microbial spoilage. To fill the gap in this knowledge the aim of this study is to investigate the influence of ozone on microbial spoilage using the technique of PTR-MS to analyse VOC emissions derived from microbial spoilage in real-time.

RH Dainty's group has shown that it is possible to get information about the spoilage status by chemical measures e.g. analysis of VOCs. The products of microbial metabolism depend on the types of bacteria growing, the substrate on which they are growing and on the storage conditions (air, vacuum or modified atmosphere) (Dainty, 1996). This is confirmed by another study (Mayr *et al.*, 2003) that has shown that the emission of some specific VOCs are characteristic of microbacterial activity, for example emissions detected at mass 63 (dimethylsulphide and diethylsulphide) correlate with total number of aerobic bacteria and enterobacteriaceae respectively in air-packaged beef and ethanol is highly correlated with the bacterial counts of lactic acid bacteria in vacuum packed beef hence monitoring VOC emissions from the food provides a direct methodology for assessing bacterial activity. In contrast to technique of counting bacteria (requiring the incubation period of 1-3 days) detection of VOCs may be performed online and with rapid sampling rates. The use of a PTR-MS system provides an on-line measurement of VOCs with concentrations as low as a few parts per trillion in volume (pptv) and thus may detect bacterial activity when it is just beginning. Another advantage of PTR-MS is that the samples containing the volatile compounds do not need any preparation (pre-sampling, pre-concentration or sample dehydration) before being admitted to the PTR-MS. Thus some problems inherent to sampling in alternative methods used for VOC detection (e.g.: gas chromatography) are avoided, since the food itself is not disturbed and the measured mass spectral profiles closely reflect genuine headspace distributions (Yeretzian *et al.*, 2002). The PTR-MS system and measuring procedure has been described in detail in Refs. (Lindinger *et al.*, 1998) and (Hansel *et al.*, 1995).

Objectives

The purpose of the study was to investigate if ozone treatment affects microbial spoilage of pork.

Materials and methods

Two sets of measurements were performed six months apart. In each case retailed pork cutlets that were air packaged in an oxygen-permeable polyethylene film were bought in a supermarket in Innsbruck on the day when the respective measurements were started. Their expiry date was listed as two (first set of measurements, i.e. Experiment (Exp) 1 and 2) and three (second set, i.e. Exp 3) days after purchase.
Pieces of about the same shape (approx. 35x50x10mm), weight and consistency were cut out of a single cutlet for Exp 1-3, respectively. Each sample was placed into a glass flask (volume V=300ml) with a metal cover containing two gas inlets through which gas could be passed over the meat sample. The control samples were flushed with oxygen/air the others exposed to different concentrations of ozone (see table 1) for ten minutes. All the treated flasks were subsequently stored under identical conditions at room temperature. Measurements of the VOC emissions from the samples were then made at regular intervals over the period of several hours (see table 1). Headspace air was drawn through a heated teflon transfer line into the PTR-MS system for on-line VOC analysis. The mass spectrometric data being collected over a range of masses (m) with m/z=20–150 amu, where z is the charge of the measured ions (in our case z=1), different m being characteristic of different VOCs - in turn a monitor of different microbacterial processes.

Results and discussion

The effect of ozone treatment on the pork's decay behaviour was monitored through the observation of the concentration detected at mass 63 assumed to be dimethylsulphide (DMS) as this signal has been shown to have the largest correlation (up to 99%) with the bacterial contamination of meat (Mayr et al., 2003).
Figure 1 shows the results of the Exp 1-3. After a certain time lag the DMS signal detected from the oxygen treated sample in Exp 1 strongly increased with time whereas the low dose ozone treated sample showed only a slight increase, and the signal of the high dose treated pork piece remained almost constant. The same emission behaviour was found for the first part (t=0-30h) of Exp 2. However, the oxygen treated sample was exposed to a high dose of ozone at t=30h and the DMS concentration was found to strongly decrease, indeed, it took about 9 hours until the initial concentration was reached again. Comparing the results of Exp 1 and 2 one can see the strong influence of the additional ozone treatment on the emissions of the oxygen treated pork samples. The DMS concentrations of the both oxygen treated samples in Exp 1 and 2 were similar before the exposure to ozone at t=30 h. In Exp 1 signals from the non ozone treated sample reached a concentration of $1.3x10^3$ppb at the end of the measurements (t=46h, Fig. 1) whereas in Exp 2 the DMS concentration of the ozone treated sample was only 90ppb at t=46h. The online monitoring in Exp 2 was concluded at t=100h (not shown in Fig.1). The highest DMS signal (with a concentration of 300ppb) of ozone treated meat was reached at t=68h and stayed constant for six hours and was much lower than the highest measured DMS signals from the non ozone treated samples in Exp 1. The trends seen in the first two experiments were confirmed by the results of Exp 3. The DMS signal of the untreated and oxygen treated samples strongly increased with time – less strongly for the oxygen treated pieces. The oxygen treated samples were exposed to ozone after 42 hours with a subsequent decrease in the detected DMS signal and the concentrations remained low until the end of the experiment. The highest ozone exposure resulted in the detected DMS signal showing nearly no increase during the whole measurement time.
The microbiological analysis revealed that microbial counts were very high, independent of the treatment (Mayr *et al.,* submitted).

Conclusions

In the present work we have shown the strong effect of ozone exposure on pork cutlet's emissions, which have been found earlier to be highly correlated to the bacterial contamination, suggesting its usefulness as a remedial action for microbial spoilage to extend food shelf life. Even a later treatment with ozone strongly delayed the bacterial activity. The reduction of VOCs on one hand and the high microbial counts on the other hand indicate that the treatments applied in this study were effective to inhibit and thus reduce physiological activities, but are not necessary effective enough to produce a lethal effect on microorganisms present in meat. Further studies are needed to optimize the use of ozone in order to reduce microbial spoilage of meat.

References

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Web2: http://ip.cals.cornell.edu/courses/intag402/readings/processingtech.pdf

Web3: http://www.appliedozone.com

Web4: http://www.ecosensors.com/pg4_1applozonetnap_101.html

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		Treatment				
		untreated	O_2	O_2+high[a] O_3	low[b] O_3	high[a] O_3
Exp 1	no of samples	-	1	-	1	1
	analysis period (h)	-	47	-	47	47
Exp 2	no of samples	-	-	1	1	1
	analysis period (h)	-	-	100 (30)[c]	30	30
Exp 3	no of samples	2[d]	2[d]	2[d]	2[d]	2[d]
	analysis period (h)	46	46	49 (42)[c]	46	46

[a] 1000ppm [b] 100ppm [c] Ozone treatment after (x) hours [d] microbiological analyzed

Table 1: Samples were cut out of a single pork cutlet for Experiment (Exp) 1-3 respectively, treated in the different ways for 10 min and stored under identical conditions at room temperature. The emissions were regularly measured over the given time period. A microbiological analysis was performed for the samples of Exp 3 at the end of the analysis period.

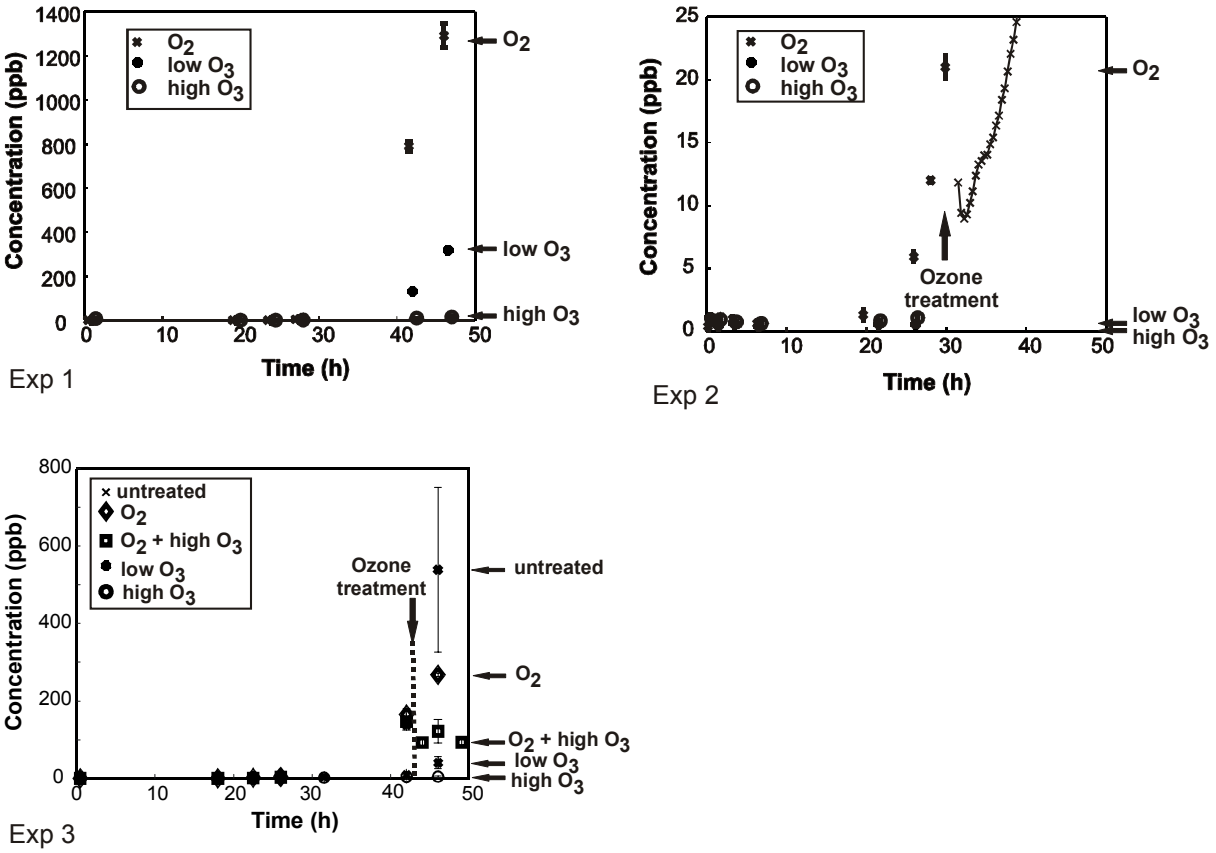

Fig. 1: Concentrations detected by PTR-MS at mass 63 as a function of time emitted by pork samples that were differently treated for 10 min prior to the first measurement at time t=0. These results suggest ozone significantly retards microbial spoilage.