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The effect of ozone treatment on the microbial contamination of pork meat measured by detecting the emissions using PTR-MS and by enumeration of microorganisms

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Abstract

In this paper, we report the results of treating commercial samples of pork meat with ozone in order to determine whether such treatment reduces microbial growth and hence extends the shelf lifetime of such products. The technique of Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) was used to study volatile emissions with the signal detected at mass 63 (assumed to be a measure for dimethylsulphide) being used as a diagnostic of bacterial activity. Such a signal was found to strongly increase with time for an untreated meat sample whereas ozone-treated meat samples showed much reduced emissions—suggesting that the microbial activity had been greatly suppressed by ozone treatment. An independent analysis, however, revealed that microbial counts were very high, independent of the treatment.

Keywords: Ozone; PTR-MS; Microbial spoilage; Meat; Pork; Emissions; Volatiles; Shelf-life

1. Introduction

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 [1]. In order to preserve food, it is necessary for pathogens to be destroyed or inactivated and non-pathogenic microorganisms and enzymes responsible for food spoilage need to be eliminated or at least reduced [2]. Several techniques for extending 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 food, the kind of microorganisms, national regulations and, most important, public demands (which require unaltered taste, aroma, colour and vitamin content with no chemical residues after treatment). The treatment of the food using ozone gas meets all of these requirements quite well.

Ozone is a strong oxidant that kills many microorganisms without leaving any toxic by-products or residues [3,4]. Furthermore, O_3 enhances the taste of most fresh perishable food (e.g., fruit) by oxidizing pesticides and neutralizing ammonia and ethylene gases produced by ripening or decay. The reduction of ethylene gas increases shelf life and reduces shrinkage [3]. Ozone has been used for many years in the water industry as an alternative to chlorine to treat pathogens such as bacteria and algae [4]—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

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as extensively as it might since ozone must be manufactured on-site and until recently ozone generators were bulky and expensive [5]. 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, even at the level of individual supermarkets. A significant reduction in the aerobic plate count on beef has been reported after using ozonized water (0.5% ozone) [6] or ozone gas (2.3 ppm) [7]. The use of ozone in ground beef production process can be effective for reducing microbial pathogens with minimal effects on colour or odour characteristics [8]. Ozone gas penetration through packaging material and its effectiveness in controlling sporulation has also been evaluated on oranges finding a sporulation inhibition that was clearly related to ozone gas exposure [9].

To date, there have been only a few studies to quantify the ozone concentrations needed to ameliorate microbial spoilage. To fill this gap in this knowledge, the aim of this study was to investigate the influence of ozone on microbial spoilage using the novel technique of PTR-MS to analyse VOC emissions derived from microbial spoilage. It has been shown recently that the emission of some specific VOCs are characteristic of bacterial activity [10] 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. Two different doses of ozone at two different times were used in two sets of measurements. Pork has been chosen for the measurements since the decay behaviour of meat (pork, beef and poultry) has already been studied by PTR-MS in detail [10,11]. The microbial contamination was determined at the end of the experiment by the microbiological standard technique (enumeration of bacteria and yeasts).

2. Experimental

2.1. Sample preparation and treatments

Two experiments 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) and three (second set) days after purchase.

2.1.1. Experiment 1 and 2 (first set)

Three pieces of about the same shape (approximately 35 mm × 50 mm × 10 mm), weight and consistency were cut out of a single cutlet for experiments 1 and 2, respectively. Each sample was placed into a glass flask (volume V = 300 ml) with a metal cover containing two gas inlets through which gas could be passed over the meat sample. One sample was treated with a high ozone dose (1000 ppm), the second-one with a low ozone dose (100 ppm) and the third with oxygen to see the effect of oxidation. Each treatment took 10 min, after another 10 min, the vials were flushed with oxygen to remove the remaining ozone and stored under identical conditions in a cabinet at room temperature. The samples were stored at room temperature to accelerate the spoilage; however, the temperature was not constant during the analysis period.

2.1.2. Experiment 3 (second set)

Twelve pieces of about the same shape described above were cut out of two single cutlets from the identical package. Two of them were immediately frozen at −20 °C for microbiological analysis, see below. Each of the remaining 10 samples was put into a glass flask as described above and covered by the metal lid. Two were then treated with a high ozone dose (1000 ppm), two with a low ozone dose (100 ppm), four just with oxygen and two samples remained untreated. Two of the oxygen-treated pieces were exposed to a high ozone dose (1000 ppm) after 42 h. Each treatment lasted 10 min and afterwards the samples were flushed with synthetic air. The meat samples were then covered with a breathable transparent film and stored in an oven at 25 °C. The glass flasks were covered with the metal lid to connect to the PTR-MS for measuring their emissions. After each measurement the transparent film was renewed.

2.1.3. PTR-MS measurements

Measurements of the emissions from the meat samples were made 30 min after first treatment, allowing the system to reach equilibrium after flushing and this time was set at t = 0. The emissions from the differently treated samples were regularly measured over for 47 h in experiment 1, for 30 h in experiment 2 and for 46 h in experiment 3. In addition in experiment 2 after 30 h of measurement, the oxygen-treated pork sample was exposed to a high ozone dose (1000 ppm) and its emissions were monitored on-line until t = 100 h. In experiment 3, the emissions of the oxygen plus ozone-treated pieces were measured at time t = 44 h and t = 49 h, after their ozone exposure to better see the ozone's effect. Moreover, the samples of experiment 3 were transferred to sterile 400-ml plastic bags (BagFilter© P, Interscience) and frozen at −20 °C at the end of the measurements (46 and 49 h, respectively) for microbiological analysis.

2.2. Analysis of VOCs

A PTR-MS system was used for analyzing the VOCs. The system allows an on-line measurement of trace components with concentrations as low as a few parts per trillion in volume (pptv). The method is based on ionizing reactions of H_3O^+ ions with the VOCs allowing the latter to be detected by non-dissociative proton transfer. Most of the common VOCs react with H_3O^+, whereas the other major

components present in clean air do not react. The generation of the primary H_3O^+ and the chemical ionization of the VOCs are individually controlled and spatially and temporally separated processes. One important consequence is that approximate absolute headspace concentrations can be calculated without calibration or use of standards [12]. Another advantage of PTR-MS is that the samples containing the volatile compounds do not need any preparation (presampling, pre-concentration or sample dehydration) before being admitted to the PTR-MS. Thus, some of the problems inherent to sampling in alternative methods used so far (e.g., gas-chromatography) are avoided, the food itself is not disturbed and the measured mass spectral profiles closely reflect genuine headspace distributions [12]. The PTR-MS system and measuring procedure have been described in detail in refs. [13,14].

One of the inlets in the metal cover at the glass flask was connected to the PTR-MS for measuring the VOCs emitted by the pork samples. Pork's headspace air was then drawn at 12 ml min^{-1} through a heated teflon transfer line into the PTR-MS system for on-line analysis. The mass spectrometric data were 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). Instrument background concentrations of the VOCs were detected directly before the meat measurements and subtracted from the obtained emissions.

2.3. Microbiological analysis

The meat pieces were thawed in the plastic storage bags at room temperature (20 °C). After adding a sterile solution consisting of 0.85% NaCl and 0.1% peptone (Oxoid), in order to obtain a 10-fold dilution, the meat was homogenized in a stomacher (BagMixer© W, Interscience) for 4 min at room temperature. Decimal dilutions in 0.85% NaCl/0.1% peptone were prepared and 1 or 0.1 ml samples of appropriate dilutions were poured (PC, VRBD, MRS) or spread (SB, GSP, SAB) on the following media to determine microbial counts: Total viable aerobic counts were enumerated on Plate Count agar (PC, Merck) incubated at 30 °C for 48 h. The number of *Pseudomonas* sp. was determined on *Pseudomonas* selective agar according to Kielwein (GSP, Merck) supplemented with 100,000 IU penicillin G (Calbiochem) and incubated at 30 °C for 72 h; positive oxidase reaction was confirmed by using oxidase test strips (Bactident, Merck). Lactic acid bacteria were enumerated on *Lactobacillus* agar according to De Man, Rogosa and Sharpe (MRS, Merck) incubated at 30 °C for 72 h under microaerophilic conditions. The number of Enterobacteriaceae was determined on Violet-Red Bile Dextrose agar according to Mossel (VRBD, Merck) incubated at 37 °C for 24 h. *Enterococcus* spp. counts were determined on Slanetz and Bartley agar (SB, Oxoid) incubated at 37 °C for 48 h; positive latex agglutination reaction was confirmed by using streptococcal latex grouping reagent D (Oxoid). The number of yeasts was counted on Saboraud-Dextrose Agar (SAB, Oxoid) supplemented with penicillin and streptomycin (each 60 mg/l) after 72 h of incubation at 25 °C.

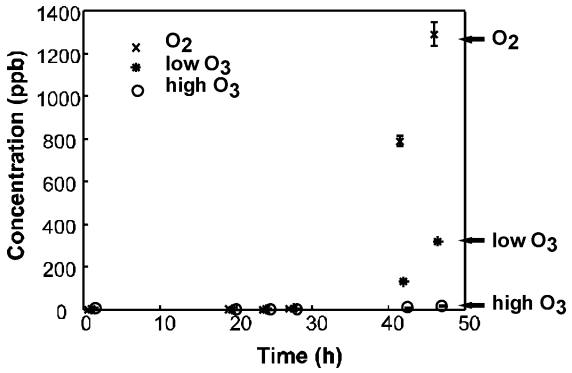

Fig. 1. Concentrations of a typical spoiling compound of meat at mass 63 as a function of time emitted by pork samples that were treated for 10 min with oxygen, a low ozone dose (100 ppm) and a high ozone dose (1000 ppm), respectively prior to the first measurement at time t = 0 and then stored at 25 °C.

3. Results and discussion

3.1. Volatiles

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 [10].

3.1.1. VOC emissions in experiments 1 and 2

Fig. 1 shows the results of the experiment 1. After a certain time lag the DMS signal detected from the oxygen-treated sample 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 = 0–30 h) of experiment 2 (see Fig. 2). However, the oxygen-treated sample was exposed to a high dose of ozone at t = 30 h and the DMS concentration was found to strongly decrease (Fig. 2), indeed, it took about 9 h until the initial concentration was reached again.

Comparing Figs. 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 experiments 1 and 2 were similar before the exposure to ozone at t = 30 h. In experiment 1, signals from the non-ozone-treated sample reached a concentration of 1.3×10^3 ppb at the end of the measurements (t = 46 h, Fig. 1), whereas in experiment 2, the DMS concentration of the ozone-treated sample was only 90 ppb at t = 46 h. The online monitoring in experiment 2 was concluded at t = 100 h (not shown in Fig. 2). The highest DMS signal

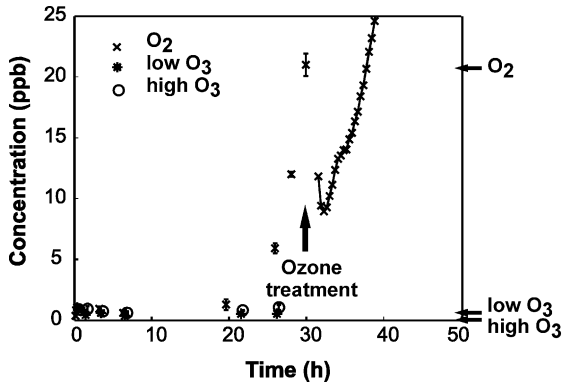

Fig. 2. Concentrations detected at mass 63 emitted by pork samples that were treated for 10 min with oxygen, a low ozone dose (100 ppm) and a high ozone dose (1000 ppm), respectively prior to the first measurement at time t = 0 and then stored at 25 °C. After 30 h of measurement the oxygen-treated meat sample was exposed to a high ozone dose (1000 ppm) for 10 min and its emissions were monitored on-line.

(with a concentration of 300 ppb) of ozone-treated meat was reached at t = 68 h and stayed constant for 6 h and was much lower than the highest measured DMS signals from the non ozone-treated samples in Fig. 1.

3.1.2. VOC emissions in experiment 3

The trends seen in the first two experiments were confirmed by the results of experiment 3, shown in Fig. 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 h 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.

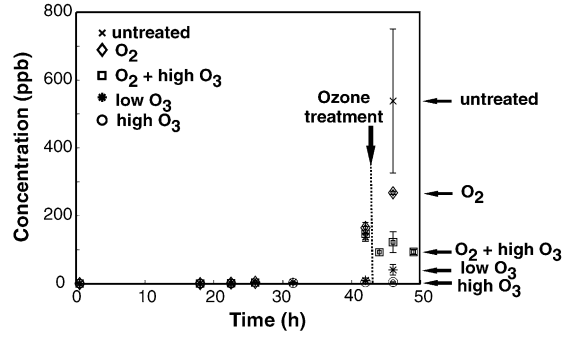

Fig. 3. Concentrations detected at mass 63 (mean values of two analyses (experiment 3) ± standard deviation) emitted by pork samples that were treated for 10 min with a high ozone dose (1000 ppm, labelled 'high O_3'), a low ozone dose (100 ppm, labelled 'low O_3'), with oxygen (labelled 'O_2'), respectively prior to the first measurement at time t = 0. Two samples remained untreated (labelled 'untreated'). Two of the oxygen-treated pieces were exposed to a high ozone dose (1000 ppm) after 42 h (labelled 'O_2 + high O_3'). The samples were incubated at 25 °C during the experiment.

3.2. Microbiological analysis (experiment 3)

The initial numbers of bacteria and yeasts were 4×10^3 cfu/g and 5×10^2 cfu/g meat, respectively. Fig. 4 shows the counts of various bacterial groups and yeasts after 10 min of treatment with oxygen, low or high ozone doses, and subsequent incubation for 46–49 h at 25 °C. After this incubation time, untreated meat was highly contaminated and contained 9×10^{10} total aerobic bacteria/g meat (pseudomonads dominated) and 2×10^8 yeasts/g meat. Higher fungi were not detected. Oxygen treatment had generally no or only a weakly stimulating effect on microbial counts (7×10^{10} total aerobic bacteria and 3×10^8 yeasts/g meat). A similar result was obtained for pseudomonads, lactic acid bacteria and enterococci when meat was treated with oxygen at the beginning, and then stored for 42 h at 25 °C before a high ozone dose was applied, while numbers of enterobacteria and yeasts were slightly decreased. Obviously, ozone treatment did not reduce effectively the number of microorganisms that had multiplied over the long incubation period. Neutralization of the positive effect of oxygen and of the inhibiting effect of ozone on microbial counts is also a possible explanation. Low-ozone-treatment followed by 46 h of incubation at 25 °C led to a decrease of all bacterial groups except lactic acid bacteria (3×10^{10} total aerobic bacteria and 2×10^8 yeasts/g meat), while an increase in microbial counts (with the exception of yeasts and enterococci) was noted when high ozone doses were applied (4×10^{10} total aerobic bacteria and 6×10^7 yeasts/g meat). Generally, microbial counts were very high, independent of the treatment, which might be primarily attributed to the long incubation time under favourable conditions (25 °C, non-sterile environment) for microbial reproduction. The standard deviation of two independent analyses is typical for the plate count technique.

3.3. Comparison of the results: VOC emissions versus microbiological analysis

The meat's VOC emissions were strongly influenced by the ozone exposure. The concentrations of many volatiles showed a large increase in non-treated samples over the measurement time (up to 100 h), whereas the ozone-treated samples were found to emit much less. This trend is illustrated in Fig. 5 for the DMS signals in experiment 3, detected at mass 63. In an earlier study, it has been shown that the concentration at mass 63 is statistically significant strongly correlated to the aerobic counts, to the counts of *Pseudomonas* spp., *Enterobacteriaceae* and *Enterococcus* spp. [10]. Therefore, similar trends were expected to be observed in the microbiological analysis for these bacterial groups. The large increase of the microbial counts during the measurement time is in contrast to these expectations and suggests that the bacteria were just strongly inhibited in their physiological activities by the ozone (and therefore, the emissions were reduced) but not totally killed. Despite their reduced activity, microorganisms surviving the treatment might have been able to grow

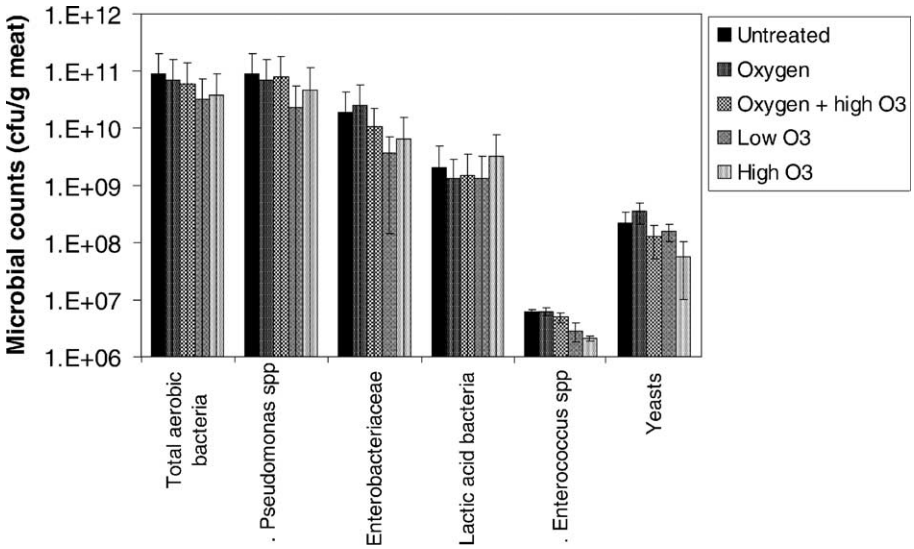

Fig. 4. Bacterial and yeast counts in meat after 10 min of various treatments and subsequent incubation for 46 h (49 h for the oxygen- plus ozone-treated samples) at 25 °C (mean values of two analyses (experiment 3) ± standard deviation).

and reproduce on meat that, however, offers ideal conditions for their growth. The incubation temperature of 25 °C was additionally favourable for microbial meat spoilage. This could be checked by determining the remaining microbial counts directly after the ozone treatment. The ozone dose may have to be increased to effectively reduce the microbial contamination. Oxidants like ozone cause irreversible damage to the fatty acids in the cell membrane and to cellular proteins of the microorganisms [15] that seems to be in contrast to the results obtained in this study. Another possible reason for the high microbial counts of the ozone-treated samples is the long incubation period after the treatment (46 and 49 h) under non-sterile conditions. However, this should have led to a large increase in the VOC emissions. Therefore, further studies are needed with a larger number of samples to allow accuracy in the microbial count rate to be obtained. It may then be possible to monitor microbial spoilage to optimize the parameters for the ozone exposure of meat for the extension of its shelf-life.

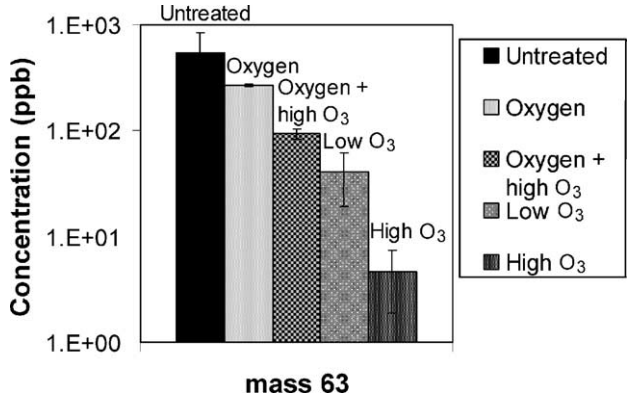

Fig. 5. Concentrations detected at mass 63 (mean values of two analyses (experiment 3) ± standard deviation) at the end of the experiment (t = 49 h for the oxygen- plus ozone-treated samples, t = 46 h for all other samples) before freezing them for the microbiological analysis (results shown in Fig. 4).

4. Conclusion

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. Even treatment with high ozone doses did not result in a sufficient reduction of microbial counts. Further studies are needed to optimize the use of ozone in order to reduce microbial spoilage of meat.

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References

[1] http://www.zentox.com/ozfood.pdf.
[2] http://ip.cals.cornell.edu/courses/intag402/readings/processingtech.pdf.
[3] http://www.appliedozone.com.

[4] http://www.ecosensors.com/pg4_1applozonetnp_101.html.
[5] http://aggie-horticulture.tamu.edu/extension/newsletters/foodproc/dec01/rrart1.html.
[6] B.M. Gorman, J.B. Morgan, G.C. Smith, J. Food Protect. 58 (9) (1995) 984.
[7] J.O. Reagan, G.R. Acuff, D.R. Buege, M.R. Buyck, J.S. Dickson, C.L. Kastner, J.L. Morgan, R. Nickelson II, G.C. Smith, J.N. Sofos, J. Food Protect. 59 (7) (1996) 751.
[8] M.R. Stivarius, F.W. Pohlman, K.S. McElyea, J.K. Apple, Meat Sci. 60 (2001) 299.
[9] L. Palou, J.L. Smilanick, C.H. Crisosto, M. Mansour, P. Plaza, Crop Protection 22 (2003) 1131.
[10] D. Mayr, R. Margesin, E. Klingsbichel, E. Hartungen, D. Jenewein, F. Schinner, T.D. Märk, Appl. Environ. Microbiol. 69 (2003) 4697.
[11] D. Mayr, R. Margesin, F. Schinner, T.D. Märk, Int. J. Mass. Spectrom. 223/224 (2003) 229.
[12] C. Yeretzian, A. Jordan, R. Badoud, W. Lindinger, Eur. Food Res. Technol. 214 (2002) 92.
[13] W. Lindinger, A. Hansel, A. Jordan, Int. J. Mass Spectrom. Ion Processes 173 (1998) 191.
[14] A. Hansel, A. Jordan, R. Holzinger, P. Prazeller, W. Vogel, W. Lindinger, Int. J. Mass Spectrom. Ion Processes 149/150 (1995) 609.
[15] E. Luck, M. Jager, Antimicrobial Food Additives, second ed., Springer-Verlag, New York, 1998, p. 42.