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The origin of off-odours in packaged rucola (*Eruca sativa*)

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Abstract

Rucola (*Eruca sativa*) was decontaminated and then reinoculated with selected microorganisms. The produce was then stored in three different atmospheres and at two temperatures. The accumulation of off-odours in the packaging headspace was analysed. A dozen compounds were detected by olfactometry but only dimethyl sulphide and dimethyl disulphide were considered to have a strong or moderate intensity. Thus, they were identified as the substances causing an unpleasant smell inside the bags. Inoculation with microorganisms resulted in higher production of off-odours. Samples inoculated with Pseudomonadaceae&Xanthamonadaceae were particularly potent in producing the two sulphides. The off-odour problem was much more prominent in samples that were kept in a packaging material that did not allow gas exchange resulting in oxygen levels below 1%. Higher levels of sulphides were detected at 8 °C than at 4 °C.

Keywords: Rucola; Packaging; Off-odour; Microorganisms; Sulphides

1. Introduction

Rucola (*Eruca sativa*) is a green leafy vegetable that has become increasingly popular during the past decade. The product is generally stored refrigerated and has a typical shelf life of 1–2 weeks.

Two major processes affect the quality of fresh produce during storage: first, physiological processes as a result of the respiration that takes place in fruits and vegetables after harvest and, second, microbiological processes resulting in metabolite production. A majority of the research in the area has focussed on the physiological processes and little is known about the contribution of the microbiological processes to the quality of different types of produce (Ragaert et al., 2006a, 2006b).

For several reasons, rucola is very susceptible to microbial attacks. It can easily become contaminated during growth due to the edible parts' proximity to the soil. Furthermore, microorganisms that have found their way to the rucola surface are difficult to eliminate because of the possibility that microorganisms may reside in the stomata. The large ratio between surface area and weight also aggravates the problem. Finally, rucola is sensitive to mechanical damage and any such injury liberates nutrients from the cells for microorganisms to thrive on. In past years there have been many outbreaks related to consumption of contaminated rucola (Farber et al., 2003).

Fresh rucola has a characteristic pungent flavour that is thought to be related to the presence of glucosinolates and their breakdown products, e.g. isothiocyanates (Bennett et al., 2002; Bennett, Rosa, Mellon, & Kroon, 2006; Bones & Rossiter, 2006; Jirovetz, Smith, & Buchbauer, 2002; Miyazawa, Maehara, & Kurose, 2002). More than 70 volatiles have been identified in the headspace of fresh rucola and approximately 20 of these contribute to the aroma of the leaves (Miyazawa et al., 2002). The flavour has been described as sharp, spicy, pungent and peppery. The aroma of rucola changes with time during consumption. When the leaves are initially crushed they give off an aroma reminiscent of burnt rubber but this decreases rather quickly and a cut grass aroma, originating from hexanal, hexenals, hexanols and their acetates, begins to dominate (Jirovetz et al.,

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2002). As long as the leaves are intact, the aroma that is generated is not very potent.

The respiration rate of many types of produce can be retarded by storing them in a modified atmosphere (Day, 2001; Rooney, 2000; Zagory, 1997). The deterioration processes in the product are simultaneously slowed down as the respiration decreases and thereby shelf life can be prolonged. In order to prevent anaerobic conditions inside a package, as a result of the product's oxygen consumption, it is necessary to use permeable packages so that oxygen can be transferred from the surrounding air into the package. By matching the respiration of the product with the transmission characteristics of the packaging material, it is possible to obtain a desired atmosphere inside the package. The ideal gas composition varies a great deal between different fruits and vegetables. Recommendations for rucola storage have not been found in the literature, but one study reported beneficial effects on the quality from storage in modified atmospheres (Chennell, Tomkins, & Franz, 1999).

One drawback of storing produce in modified atmospheres is that such treatment can make the metabolism follow an alternative path. This can change the production of volatile substances and, as a result, off-odours can be produced (Edelenbos, Kjeldsen, & Christensen, 2003; Lee, Arul, Lencki, & Castaigne, 1995; Pelayo, Ebeler, & Kader, 2003).

A problem that has been identified in rucola storage is the development of objectionable odours inside packages. These off-odours are released upon opening of the bags and can result in reduced consumer acceptance. The origins of the off-odours are not known.

The objective of the reported work was to find out if the off-odours produced during storage could be attributed to the metabolism of the product, the microbial activity or a combination of both. Another aim was to study the effect of the packaging atmosphere on the development of off-odours.

2. Materials and methods

2.1. General

The presented work was divided into two parts.

2.1.1. Preliminary study

2.1.1.1. Materials. Rucola was supplied in commercial packages by Salico (Helsingborg, Sweden). The samples were then transported to SIK (Gothenburg, Sweden) under refrigerated conditions and were stored for two weeks at 4 and 8 °C.

2.1.1.2. Collection of volatiles. Volatile compounds were collected from the samples by withdrawing 200 ml from the headspace in the packages by a syringe and trapping the volatiles on a Tenax trap (Tenax 60–80 mesh, 150 mg) connected to the needle inserted into the packages. Separate traps were prepared for analysis by gas chromatography–mass spectrometry and gas chromatography–olfactometry. Each analysis was performed in duplicate on days 0, 7 and 14.

2.1.1.3. Gas chromatography–mass spectrometry. The adsorbent traps were transferred to an ATD 400 automatic injector (Perkin-Elmer, Norwalk, CT, USA) where the volatiles were desorbed for 5 min at 250 °C, and subsequently injected into a gas chromatography–mass spectrometry (GC–MS) system. The gas chromatograph used was a ThermoQuest Trace GC 2000 (ThermoQuest CE Instruments, Milan, Italy) equipped with a 30 m $*$ 0.32 mm capillary column with a 1.0 μm thick film of DB-5MS (J&W Scientific Inc., Folsom, CA, USA). The mass spectrometer used was an Automass Solo (ThermoQuest). The initial temperature of the GC oven was 25 °C and it was kept at that temperature for 5 min. Subsequently, the temperature was raised by 4 °C/min until a final temperature of 125 °C was reached, then by 50 °C/min until the final temperature of 220 °C was reached, which was held for 10 min. Helium was used as a carrier gas at a flow rate of 3.0 ml/min. The identifcation and integration of the GC peaks were carried out using computer software Xcalibur™ (Thermoquest). The compounds were identified on the basis of their mass spectra.

2.1.1.4. Gas chromatography–olfactometry. In order to identify and characterize the aroma compounds of the greatest importance to the odour, samples were evaluated using GC–olfactometry (GC–O). The collection and desorption of volatiles were performed as above. However, the effluent from the capillary column was split 1:1 between a flame ionization detector (FID) and a sniffer-port. In the latter, the column effluent was mixed with humidified air in order to facilitate sensory evaluation. Each sample was evaluated by two trained assessors during 30 min sniffing sessions. The signal from the FID was split, so that the signal could be registered by the computer software Xcalibur™ (Thermoquest) and a printer. The assessors were instructed to describe the odour of each substance detected, as well as the intensity, on a scale from one to five, with five being the maximum, and to make a note beside the GC peak on the paper from the printer. The resulting aromagram was used to select the impact compounds of the odour inside the rucola packages.

2.1.1.5. Identification of microorganisms. The aerobic and anaerobic microbiological flora of rucola, stored at 4 °C or 8 °C, were analysed at days 0 and 7 using five different media (TSA, MRS, TGE, VRG and DRBC). A total of 18 colonies were randomly picked and identified using the biochemical-based API system (50CHB, 50CHL, API20, API20NE, API20E, APIID32I). Nine isolates were chosen to be used for inoculation in the main study. The selection was based on whether the microorganisms grew during the incubation and if they were known to produce

off-odours. The identified isolates to be used were grouped into three groups, Enterobacteriaceae, Pseudomonadaceae&Xanthomonadaceae and yeast, each containing three isolates.

2.1.2. Main study

2.1.2.1. General. After doing the initial experiments, the main study was performed. In the main experiment, rucola was treated in different ways (chemical decontamination, chemical decontamination followed by antibiotics treatment, and chemical decontamination followed by inoculation with microorganisms) in order to obtain the desired microbial composition at the start of the study. Untreated rucola was used as a reference. The products were then packaged in three different packaging materials and stored for two weeks at 4 and 8 °C. Duplicate packagings were prepared for each combination of treatment and storage condition.

2.1.2.2. Materials. Rucola was harvested at Stamgården (Tygelsjö, Sweden). It was immediately transported to Salico (Helsingborg, Sweden) where it was kept at 4 °C overnight. The produce was then transported to SIK (Gothenburg, Sweden) under refrigerated conditions the day after harvest.

Three different packaging materials were used in the experiments, called A, B and C. They were supplied by Amcor Flexibles (Ledbury, England). The bags were made of antimist-coated oriented polypropylene of 35 μm thickness. The dimensions were 200 by 150 mm. Two of the materials, B and C, were laser-treated in order to obtain small holes, thereby facilitating increased gas exchange with the atmosphere. The oxygen permeation rates through the bags are presented in Table 1.

2.1.2.3. Decontamination. Rucola was decontaminated in order to reduce the natural occurring flora on the rucola prior to inoculation. A method using chlorite and citric acid was used to reduce the natural flora. This method consisted of washing 270 g rucola in 4 l of washing solution containing 0.5 g/l of sodium chlorite (Sigma-Aldrich, Stockholm, Sweden) and 10 g/l of citric acid (Merck, Darmstadt, Germany) at 21 °C and pH 2.3. The rucola was gently stirred during the 15 min wash. Thereafter, the product was placed in 4 l of sterile peptone water for 5 min. In some cases, this treatment was followed by immersion in a mix of antibiotics for 5 min in order to suppress growth during the two week storage. The antibiotics mix consisted of 0.02% Pimaricin (Sigma-Aldrich, Stockholm, Sweden) and chloramphenicol SR78 (Oxoid, Sollentuna, Sweden). Subsequently, the rucola was dried for 60 min at room temperature in a biosafety cabinet.

2.1.2.4. Packaging, inoculation and storage. Rucola was carefully put into packages with the aid of tweezers, 25 g going into each bag. The packages were then heat-sealed. Each package was inoculated with 1 ml of the chosen mix of microorganisms by inserting a syringe through a rubber septum. The three groups of microorganisms were inoculated separately. The Enterobacteriaceae group contained two strains of *Enterobacter cloacae*, and one new species identified as belonging to the *Pantoea/Erwinia* region. The Pseudomonadaceae&Xanthamonadaceae group contained *Stenotrophomonas maltophilia*, *Flavimonas oryzihabitans* and *Chryseobacterium indologenes* (Bergey's Taxonomic Outline, 2004), and the yeast group contained three isolates, all identified as *Cryptococcus laurentii*. The concentrations of inoculations were 6.7×10^7 colony forming units (cfu)/ml of Enterobacteriaceae, 2.9×10^7 cfu/ml of Pseudomonadaceae&Xanthamonadaceae and 5.7×10^6 cfu/ml of yeast. The samples were kept in the dark for 14 days at 4 and 8 °C, respectively.

2.1.2.5. Collection of volatiles. The collection of volatiles was performed in an identical manner to that in the preliminary study described above.

2.1.2.6. Gas chromatography–mass spectrometry. The analysis of volatiles was performed in an identical manner to that in the preliminary study described above. Quantification of DMS and DMDS was undertaken by performing GC–MS on the pure compounds in question.

2.1.2.7. Microbiological analysis. The viable cell numbers in the rucola, before and after decontamination, inoculation and storage, were determined by mixing 25 g rucola with 225 ml sterile peptone water. The mixture was mechanically homogenised for 3 min in a stomacher. The total aerobic count, the number from the Enterobacteriacae group, the Pseudomonadaceae&Xanthamonadaceae group and the yeast group were analysed using 3M™ Petrifilm™ Enterobacteriacae count (Raisio, Gothenburg, Sweden), TSA (Oxoid, Sollentuna, Sweden) and OGYE respectively. OGYE was mixed from glucose (Sigma, Stockholm, Sweden), yeast extract (Nordic Biolabs, Täby, Sweden), agar (Nordic Biolabs, Täby, Sweden) and oxytetracyclin 73A (Oxoid, Sollentuna, Sweden).

2.1.2.8. Atmosphere analysis. The concentration of oxygen and carbon dioxide inside the packages were monitored using a CheckMate II gas analyser (PBI Dansensor, Ringsted, Denmark). Analyses were performed by inserting the test probe through a rubber septum attached to the outside of the packaging. The instrument was calibrated towards air. Measurements were performed on days 0, 1, 2, 3, 5, 7, 10 and 14. The analyses were carried out in duplicate.

2.1.2.9. Odour analysis. The odour that had accumulated in the bags was analysed by four persons with experience of similar studies. Each person sniffed the samples immediately after opening the bags. They were instructed to make a note of any objectionable odours and also describe the odour with their own words.

3. Results and discussion

3.1. Preliminary study

3.1.1. General

In the preliminary study, rucola was investigated in order to identify different types of microorganisms that could potentially cause off-odours in the samples and also to determine the volatile substances that were most important for the odour of the headspace in packages containing the produce. The samples were stored in commercial packages made of one type of perforated material. The initial atmospheric composition was air, and on day 14 it was 17% O_2 + 5% CO_2 and 13% O_2 + 9% CO_2 at 4 and 8 °C, respectively.

3.1.2. Identification of microorganisms

The naturally occurring flora on rucola were dominated by species from the families Enterobacteriacae, Pseudomonadaceae, Xanthamonadaceae and yeast. They were all isolated from agar plates, representing concentrations of 5–6 log cfu per gramme. The rucola also contained 4 log cfu/g of sporeforming *Bacillus* sp. The results of the identification of 15 isolates from the natural flora on rucola are displayed in Table 2.

3.1.3. Identification of odorous volatiles

The results from the olfactometry analyses showed that a dozen compounds were present at such a level that they could be detected by sniffing, but only two of these were described as having a moderate or strong odour intensity. Several other substances were identified by mass spectrometry but they were not detectable by olfactometry. Considerably higher amounts of the volatiles were found in samples stored for 14 days than in the fresh samples. A higher storage temperature also resulted in higher levels of the substances. The two most potent odorants were dimethyl sulphide (DMS) and dimethyl disulphide (DMDS). These two sulphides were described by the sniffers as reminiscent of onion, cabbage and sewer, and it is highly probable that the foul smell could be attributed to the presence of DMS and DMDS.

Table 1
Oxygen permeation rates through the three different bags used in the experiments

Packaging material	Oxygen permeation rate (cm^3 O_2/bag/day/atm)
A	25
B	450
C	1040

Table 2
Microorganisms isolated from rucola stored for 7 days at 4 °C and 8 °C. Isolates were picked from a variety of media and identified using the API system

Identified microorganisms	API system used for identification	Media used for isolation
Bacillus pumilus, *B. megaterium*, *B. subtilis*, *B. licheniformis*	50CHB	TSA
Lactobacillus pentosus, *Lactococcus lactis*	50CHL	MRS
Enterobacter cloacae, *Serratia* sp., *Pantoea* sp.	API20	VRG
Stenotrophomonas maltophilia, *Flavimonas oryzihabitans*, *Chryseobacterium indologenes*	API20NE	TGE
Pantoea sp., *Enterobacter cloacae*	API20E	TGE
Cryptococcus laurentii	–	DRBC

3.2. Main study

3.2.1. General

In order to obtain a microbial flora with a dominance of selected microorgansisms, the samples had to undergo treatment prior to packaging. Ideally, the samples should be sterilised and then inoculated with the desired microbial flora. However, it was not possible to eliminate all microorganisms in the samples without causing damage to the product itself. Consequently, the efficiency of the decontamination had to be weighed against the effects on the rucola. Several decontamination methods were tried out. These included treatment with radiation, heat and different chemical agents. The treatments and their effects on the microbial count are summarised in Table 3.

As expected, the heat treatment was most effective and reduced the microbial count by 4.5 log units. However, in addition to killing off microorganisms, it resulted in irreparable damage of the rucola tissue and was no viable alternative. Of the other treatments, immersion in 0.4% chlorine at pH 7.1 was the preferred choice, as it resulted in the largest reduction of the microbial count by 2.0 log units, and there were no indications that it affected the product negatively as regards its appearance and volatile composition. Consequently, this method – which has also been reported to be effective by Inatsu, Bari, Kawasaki, Isshiki, and Kawamoto (2005) – was selected for the further experiments.

The aim of the main study was to find reasons for the development of objectionable odours inside rucola packages. Therefore, the experiments were designed to establish

Table 3
The effect of different decontamination methods on the total microbial count

Treatment	Log unit reduction
UVC radiation 1000 W for 15 min	1.0
UVC radiation 1500 W for 10 min	1.0
Immersion in 72 °C water for 2 min	4.5
Immersion in 0.4% chlorine at pH 7.1 for 2 min	2.0
Immersion in 0.4% chlorine at pH 13 for 2 min	0.5
Immersion in 0.05% sodium chlorite + 1.0% citric acid at pH 2.3 for 15 min	1.5

correlations between microbial activity, storage conditions, volatile production and the smelling experience. These factors are interconnected; e.g. the choices of packaging material and storage temperature influence the atmospheric composition, which itself is of great importance for the microbial activity and this, in turn, affects the aroma profile and, consequently, the perceived odour. Thus, the most appropriate way to present the results is to do it separately for each investigated parameter, followed by a general discussion as below.

3.2.2. Atmospheric composition

The three packaging materials that were used in the study exhibited different transmission characteristics, (see Table 1). Material A had not been perforated and, in this case, the transport of oxygen and carbon dioxide took place through the material, as opposed to materials B and C, where the permeation mainly occurred through the manufactured holes in the polymeric film. As a result of the different permeabilities, the atmospheres developed inside the bags differed greatly. The oxygen levels in packages made of material A were rapidly reduced. The storage temperature and the type of treatment also affected the rate at which the gas altered its composition. The oxygen decrease, (and the corresponding production of carbon dioxide), was more rapid at the higher temperature and in the samples that had been inoculated with microorganisms. This was an indication that oxygen consumption could not only be attributed to the produce itself but also to the present microorganisms. After 5 days of storage, all samples packaged in material A contained less than 1% of oxygen and 13–15% of carbon dioxide. While the O_2 levels remained below 1% during the remaining storage period, the CO_2 concentration steadily dropped and, in the final measurement on day 14, the bags contained 7–9% carbon dioxide.

The observations that the modification of the atmosphere was more rapid at higher temperature and for inoculated samples were also made for materials B and C. However, because of the perforations in these materials, the gas exchange was far greater and as a result the atmospheric conditions reached an equilibrium within a matter of 2–3 days. The equilibrium levels at the two storage temperatures were slightly different. The atmospheric composition in material B at 4 °C was 15–17% O_2 + 5–7% CO_2 and, at 8 °C, 11–14% O_2 + 7–9% CO_2, while the corresponding figures for material C were 19–20% O_2 + 1–2% CO_2 at 4 °C, and 18–19% O_2 + 2–3% CO_2 at 8 °C. Thus, material B resulted in atmospheres very similar to those observed in the commercial packages that were studied in the preliminary investigation. There were no indications that the type of treatment of rucola prior to packaging, i.e. the microbial activity, affected the composition of the atmosphere at equilibrium.

Unfortunately, leakage was observed in some of the packages made of material A. This was clearly indicated by a rapid increase of the oxygen levels during storage. As a result, the samples with leaks were discarded from the evaluations.

3.2.3. Microbial activity

The rucola used in this study initially contained 6.7 log colony forming units per gramme. The aim of the decontamination/inoculation procedures was to generate samples with different microbial flora with regard to the composition and the respective levels of selected groups of microorganisms. This was successfully achieved, as can be seen in Table 4. The chlorination treatment reduced the total microbial count by 1.4 log units and the antibiotics-treatment decreased the levels by a further log-unit. The number of colony forming units of the selected microbial groups, i.e. Enterobacteriacae, Pseudomonadaceae&Xanthamonadaceae and yeasts, were similarly significantly reduced. The inoculation procedures that followed resulted in each selected microorganism group being dominant in the flora at the start of the study.

Considerable growth of microorganisms occurred during the storage period. The results for rucola subjected to the different treatments stored in the different materials for 14 days at 4 and 8 °C, respectively, are displayed in Tables 5–7.

The results for samples kept in material C (high oxygen permeability) did not differ significantly from those kept in material B (medium oxygen permeability). However, rucola stored in the material with the lowest oxygen permeability, i.e. material A, had significantly lower microbial counts than had rucola stored in materials B and C. In general, there were 1–2 log units fewer colony forming units/

Table 4
Microbial counts (log cfu/g) on rucola at day 0

Sample	Total aerobic bacteria	Enterobacteriacae	Pseudomonadaceae& Xanthomonadaceae	Yeasts
Untreated	6.7 ± 0.1	4.6 ± 0.1	5.4 ± 0.3	5.0 ± 0.1
Chlorinated	5.3 ± 0.2	3.4 ± 0.2	3.8 ± 0.2	<2.0
Chlorinated and antibiotics-treated	4.3 ± 0.1	<2.0	2.5 ± 0.1	<2.0
Chlorinated and inoculated with Enterobacteriacae	6.3 ± 0.1	6.3 ± 0.1	4.4 ± 0.3	2.2 ± 0.1
Chlorinated and inoculated with Pseudomonadaceae&Xanthomonadaceae	5.9 ± 0.1	4.4 ± 0.2	5.8 ± 0.1	<2.0
Chlorinated and inoculated with yeasts	4.8 ± 0.3	3.2 ± 0.3	3.9 ± 0.3	5.3 ± 0.0

Table 5
Microbial counts (log cfu/g) in samples stored in material A for 14 days

Treatment	Storage temperature (°C)	Total aerobic bacteria	Enterobacteriacae	Pseudomonadaceae& Xanthomonadaceae	Yeasts
Untreated	4	7.3 ± 0.1	5.9 ± 0.2	6.3 ± 0.1	5.2 ± 0.3
Chlorinated and inoculated with Enterobacteriacae	4	6.8 ± 0.0	6.9 ± 0.1	6.0 ± 0.0	<3.0
Chlorinated and inoculated with yeasts	4	5.7 ± 0.2	5.3 ± 0.2	4.9 ± 0.6	5.0 ± 0.3
Untreated	8	7.6 ± 0.1	7.8 ± 0.2	6.5 ± 0.1	4.8 ± 0.2
Chlorinated and inoculated with Enterobacteriacae	8	8.3 ± 0.1	8.9 ± 0.2	7.3 ± 0.2	<3.0
Chlorinated and inoculated with yeasts	8	7.1 ± 0.1	7.1 ± 0.1	5.5 ± 0.1	5.2 ± 0.2

Due to leakage the samples that were chlorinated, antibiotics-treated and inoculated with Pseudomonadaceae&Xanthomonadaceae and stored in material A at both temperatures had to be eliminated from the study.

Table 6
Microbial counts (log cfu/g) in samples stored in material B for 14 days

Treatment	Storage temperature (°C)	Total aerobic bacteria	Enterobacteriacae	Pseudomonadaceae& Xanthomonadaceae	Yeasts
Untreated	4	7.9 ± 0.0	6.3 ± 0.1	6.8 ± 0.2	5.4 ± 0.0
Chlorinated	4	7.6 ± 0.3	5.6 ± 0.3	5.9 ± 0.3	4.8 ± 0.2
Chlorinated and antibiotics-treated	4	8.0 ± 0.1	5.6 ± 0.3	5.5 ± 0.2	<2.0
Chlorinated and inoculated with Enterobacteriacae	4	8.3 ± 0.4	6.9 ± 0.4	7.1 ± 0.5	4.6 ± 0.3
Chlorinated and inoculated with Pseudomonadaceae&Xanthomonadaceae	4	8.3 ± 0.3	7.1 ± 0.3	8.2 ± 0.3	4.7 ± 0.4
Chlorinated and inoculated with yeasts	4	8.0 ± 0.2	5.4 ± 0.3	6.3 ± 0.3	6.5 ± 0.2
Untreated	8	8.1 ± 0.2	6.7 ± 0.3	6.7 ± 0.2	5.6 ± 0.2
Chlorinated	8	7.8 ± 0.1	6.5 ± 0.1	6.2 ± 0.3	4.9 ± 0.1
Chlorinated and antibiotics-treated	8	8.3 ± 0.1	7.0 ± 0.3	7.2 ± 0.3	2.4 ± 0.3
Chlorinated and inoculated with Enterobacteriacae	8	8.3 ± 0.4	8.2 ± 0.2	6.7 ± 0.3	4.5 ± 0.3
Chlorinated and inoculated with Pseudomonadaceae&Xanthomonadaceae	8	9.4 ± 0.2	7.9 ± 0.4	9.2 ± 0.1	4.7 ± 0.4
Chlorinated and inoculated with yeasts	8	8.6 ± 0.2	7.6 ± 0.1	7.3 ± 0.0	6.6 ± 0.1

Table 7
Microbial counts (log cfu/g) in samples stored in material C for 14 days

Treatment	Storage temperature (°C)	Total aerobic bacteria	Enterobacteriacae	Pseudomonadaceae& Xanthomonadaceae	Yeasts
Untreated	4	7.8 ± 0.1	6.0 ± 0.3	6.5 ± 0.2	5.5 ± 0.2
Chlorinated	4	8.3 ± 0.1	6.6 ± 0.4	6.4 ± 0.3	5.0 ± 0.4
Chlorinated and antibiotics-treated	4	7.9 ± 0.6	5.8 ± 0.5	6.5 ± 0.5	3.0 ± 0.7
Chlorinated and inoculated with Enterobacteriacae	4	8.3 ± 0.1	7.3 ± 0.2	7.2 ± 0.3	4.5 ± 0.0
Chlorinated and inoculated with Pseudomonadaceae&Xanthomonadaceae	4	8.8 ± 0.0	6.6 ± 0.3	8.8 ± 0.0	4.6 ± 0.3
Chlorinated and inoculated with yeasts	4	8.3 ± 0.1	5.3 ± 0.3	6.6 ± 0.1	6.7 ± 0.1
Untreated	8	8.6 ± 0.1	7.0 ± 0.2	7.4 ± 0.1	6.0 ± 0.0
Chlorinated	8	8.1 ± 0.3	7.2 ± 0.1	6.9 ± 0.2	5.1 ± 0.1
Chlorinated and antibiotics-treated	8	8.3 ± 0.1	6.6 ± 0.2	7.3 ± 0.0	4.2 ± 0.2
Chlorinated and inoculated with Enterobacteriacae	8	8.3 ± 0.3	8.3 ± 0.2	6.7 ± 0.6	4.5 ± 0.0
Chlorinated and inoculated with Pseudomonadaceae&Xanthomonadaceae	8	9.0 ± 0.1	7.6 ± 0.1	8.9 ± 0.2	4.6 ± 0.3
Chlorinated and inoculated with yeasts	8	8.7 ± 0.2	6.9 ± 0.2	6.9 ± 0.4	6.6 ± 0.2

gramme of sample in the rucola stored in material A as regards total count, Pseudomonadaceae&Xanthamonadaceae and yeasts. For Enterobacteriacae, on the other hand, no difference could be observed between samples stored in materials with varying oxygen transmission characteristics. As expected, microbial growth was significantly higher at 8 °C than at 4 °C; this effect was more pronounced for samples kept in material A than for those stored in B and C.

The objective to obtain samples where the inoculated microorganism continued to dominate throughout the entire storage period was successfully accomplished with a few exceptions. In rucola inoculated with yeasts and stored at 8 °C, the yeasts were outnumbered by Enterobacteriacae and Pseudomonadaceae&Xanthamonadaceae in all materials. Apart from this, the only occasion where the aim was not reached was the sample inoculated by Enterobacteriacae kept in material B at 4 °C, where Enterobacteriacae appeared at slightly lower levels than did Pseudomonadaceae&Xanthamonadaceae. While the decontamination procedures significantly reduced the initial microbial concentrations, they did not prevent growth of the natural background flora during storage. The microbial counts in chlorinated and antibiotics-treated samples on day 14 were not significantly different from those observed in the untreated rucola, which may be explained by the possible maximum count on the rucola being reached. One exception was that yeasts were found in 2–3 log units lower amounts in antibiotics-treated samples than in untreated and chlorinated rucola.

3.2.4. Volatiles

The investigation of volatiles focussed on the two selected compounds that had been identified as having potential to cause off-odours in the olfactometry analyses, i.e. dimethyl sulphide and dimethyl disulphide.

The headspace in the packagings was analysed 2 h after sealing the bags in order to obtain an initial value. Neither of the two compounds was found in detectable amounts at the starting point. However, after 14 days of storage, the analytes were present at levels that were possible to quantitate in all samples. The results are presented in Tables 8–10.

Considerably higher amounts of the two sulphides had accumulated when the oxygen access was limited, i.e. in material A. This was found for uninoculated and inoculated samples.

In inoculated samples, the sulphide levels were higher in material C than in material B at 4 °C. By contrast, the sulphide levels were in general lower in material C than in material B for two of the three inoculated samples kept at 8 °C, with samples inoculated with Enterobacteriacae being the exception.

The effect of temperature differed between the samples. The concentration of DMS was generally much higher at 8 °C than at 4 °C in all three materials. The same observation was made for DMDS in materials B and C but not in A, where the DMDS levels were similar at the two temperatures.

The samples that were inoculated with microorganisms after decontamination produced larger amounts of the two sulphides than did naturally contaminated rucola. The difference in sulphide levels between chlorinated and inoculated samples was much larger at 8 °C than at 4 °C. Rucola inoculated with Pseudomonadaceae&Xanthamonadaceae generated the highest levels of DMS.

3.2.5. Odour

Objectionable odours were not detected in any of the samples that were chlorinated or antibiotics-treated, regardless of packaging material and storage temperature.

Table 8
Concentrations of dimethyl sulphide and dimethyl disulphide (ng/l) in the packaging headspace of samples stored in material A for 14 days

Treatment	Storage temperature (°C)	Dimethyl sulfide	Dimethyl disulphide
Untreated	4	981 ± 342	171 ± 42
Chlorinated and inoculated with Enterobacteriacae	4	1557 ± 275	924 ± 210
Chlorinated and inoculated with yeasts	4	1240 ± 338	721 ± 155
Untreated	8	2570 ± 178	193 ± 33
Chlorinated and inoculated with Enterobacteriacae	8	3928 ± 688	609 ± 100
Chlorinated and inoculated with yeasts	8	2653 ± 218	711 ± 36

Due to leakage, the samples that were chlorinated, antibiotics-treated and inoculated with Pseudomonadaceae&Xanthomonadaceae and stored in material A at both temperatures had to be eliminated from the study.

Table 9
Concentrations of dimethyl sulphide and dimethyl disulphide (ng/l) in the packaging headspace of samples stored in material B for 14 days

Treatment	Storage temperature (°C)	Dimethyl sulfide	Dimethyl disulphide
Untreated	4	49 ± 7	7 ± 4
Chlorinated	4	63 ± 22	10 ± 5
Chlorinated and antibiotics-treated	4	27 ± 11	5 ± 2
Chlorinated and inoculated with Enterobacteriacae	4	61 ± 11	12 ± 4
Chlorinated and inoculated with Pseudomonadaceae& Xanthomonadaceae	4	126 ± 56	19 ± 8
Chlorinated and inoculated with yeasts	4	90 ± 16	24 ± 5
Untreated	8	70 ± 14	23 ± 1
Chlorinated	8	68 ± 18	35 ± 2
Chlorinated and antibiotics-treated	8	247 ± 40	35 ± 8
Chlorinated and inoculated with Enterobacteriacae	8	536 ± 81	138 ± 25
Chlorinated and inoculated with Pseudomonadaceae& Xanthomonadaceae	8	2887 ± 396	442 ± 84
Chlorinated and inoculated with yeasts	8	1073 ± 144	208 ± 3

Table 10
Concentrations of dimethyl sulphide and dimethyl disulphide (ng/l) in the packaging headspace of samples stored in material C for 14 days

Treatment	Storage temperature (°C)	Dimethyl sulfide	Dimethyl disulphide
Untreated	4	59 ± 13	10 ± 4
Chlorinated	4	56 ± 1	2 ± 1
Chlorinated and antibiotics-treated	4	90 ± 41	43 ± 1
Chlorinated and inoculated with Enterobacteriacae	4	144 ± 38	66 ± 36
Chlorinated and inoculated with Pseudomonadaceae&Xanthomonadaceae	4	430 ± 50	164 ± 14
Chlorinated and inoculated with yeasts	4	257 ± 103	21 ± 8
Untreated	8	1489 ± 99	756 ± 126
Chlorinated	8	72 ± 31	2 ± 2
Chlorinated and antibiotics-treated	8	241 ± 58	35 ± 8
Chlorinated and inoculated with Enterobacteriacae	8	842 ± 180	154 ± 35
Chlorinated and inoculated with Pseudomonadaceae&Xanthomonadaceae	8	1469 ± 270	165 ± 18
Chlorinated and inoculated with yeasts	8	212 ± 36	134 ± 13

Untreated samples kept in material B at both temperatures and in material C at 4 °C were also regarded as having an acceptable odour. However, untreated samples stored under anaerobic conditions, i.e. in material A, and those kept in material C at 8 °C had an odour that was considered to be foul and reminiscent of rubber, grass and decaying leaves.

Samples inoculated with Enterobacteriacae kept in material B at both temperatures and in material C at 4 °C had an odour described as pungent, bitter and slightly acidic but not objectionable. The sample kept in material A at both temperatures and in material C at 8 °C exhibited an unpleasant smell, described as unfresh, sewer and wet dog.

Rucola that had been treated with yeasts had an odour giving a bitter impression and with a grassy and acidic note. Again, samples kept in material A and in material C at 8 °C were considered to be unacceptable. The odours of these samples were described by words such as wet hay, wet wool and cat.

All samples that were treated with Pseudomonadaceae&Xanthamonadaceae, at both temperatures, had a strong and foul smell that was reminiscent of urine, fur, manure, sewer, stables, and wet wool. The intensity of the odour was higher in samples stored at 8 °C than in those kept at 4 °C.

3.3. General discussion

The aim of the work was to determine the origin of off-odours in rucola packages. These could possibly originate from the product itself, from microbial growth on the produce, or from a combination of both. In order to distinguish between odours produced by rucola and the microorganisms it was desirable to obtain samples that were sterile and completely void of microbial activity. However, this was not possible to do. The reasons were that the decontamination methods that could be used without destroying the sample tissue, i.e. not heat, were not potent enough to kill all microorganisms. Irradiation was not efficient as it was not possible for the beams to reach all surfaces. Immersion in solutions of various chemical agents was more successful but it was also proved to be insufficient for creating sterility, probably since large proportions of the microorganisms were inaccessible, e.g. in stomata.

This meant that it was not possible to prepare samples containing only selected types of microorganisms. Instead, many types of microorganisms – of which some came from the natural flora and consequently their identity was unknown – were present in all samples. Thus, already from day 0, there was a background of flora that caused disturbance.

Furthermore, most microorganisms present in the samples have different growth characteristics, i.e. some prefer anaerobic conditions while others benefit from higher oxygen levels, and others have different sensitivities to elevated carbon dioxide levels. The temperature might also affect the microbial growth as certain microorganisms are more likely to become dominant at 4 °C and others at 8 °C.

As a result of these difficulties it was impossible to identify exactly which microorganisms were present at what levels in the samples, even if quantification of Enterobacteriacae, Pseudomonadaceae&Xanthamonadaceae and yeasts, as well as the total microbial count, could be achieved. In other words, it was possible that other types of microorganisms than the ones that were added could have affected the volatile production and thereby the odour of the rucola. However, with these things in mind, it was still possible to make some interesting observations.

There was a strong correlation between the perceived odour and the sulphide levels in the samples. The samples that were considered to have the most objectionable smell were, unsurprisingly, also the ones with the highest levels of DMS and DMDS.

Out of the three microorganism mixes that were used to inoculate the rucola, the Pseudomonadaceae&Xanthamonadaceae mix was the one creating the worst smell, at least where a comparison between all mixes could made, i.e. in materials B and C. There were no results for samples contaminated with Pseudomonadaceae&Xanthamonadaceae kept in material A since these bags started to leak.

Many microorganisms, including several Gram-negative bacteria, i.e. Pseudomonadaceae and Enterobacteriaceae, have been reported to produce volatile sulphur compounds on a number of different media (Dainty, Edwards, & Hibbard, 1984; Edwards, Dainty, & Hibbard, 1987; Intarapichet & Bailey, 1993; Labows, McGinley, Webster, & Leyden, 1980; Schöller, Molin, & Wilkins, 1997; Taylor & Kiene, 1987; Wang, Smith, & Spanel, 2004). Substances such as DMS and DMDS can be the end-products from breakdown of sulphur-containing materials. Rucola contains high amounts of sulphurous glucosinolates and it is probable that the microorganisms used such compounds for their growth and thereby produced the observed off-odours.

Very high levels of the two sulphides were detected in the samples kept in material A, i.e. under oxygen-restricted conditions. Pseudomonadaceae, Xanthamonadaceae and yeasts do not normally grow well in oxygen-depleted environments. Enterobacteriacae on the other hand, are facultative anaerobes, i.e. they can grow under both aerobic and anaerobic conditions. However, the results indicated that there was some growth not only of Enterobacteriacae but also of Pseudomonadaceae&Xanthamonadaceae in the rucola kept in material A. There were still a few percent oxygen left in the bags after one day of storage and Pseudomonadaceae&Xanthamonadaceae can have a very rapid growth even at low temperatures which could explain this observation.

Nevertheless, the high sulphide levels produced in samples stored in material A could not be strictly correlated with the microbial counts since these were lower than in corresponding samples stored in the presence of oxygen. A possible explanation could be that the product, i.e. the rucola itself, suffered from the oxygen depletion and as a result it degraded while producing DMS and DMDS. Fresh produce requires certain amounts of oxygen to be able to respire after harvest and, if there is no oxygen available, the metabolism can follow different pathways that often generate undesirable components. Another factor that might play a part is the microbial flora that remained even after decontamination. The identities of these microorganisms were unknown and it is possible that they could include certain species that have a large potential to grow and produce off-odours under anaerobic conditions.

The odour threshold values of the two compounds in question have been reported to be 6 ng/l for dimethyl sulphide and 48 ng/l for dimethyl disulphide (Devos, Patte, Rouault, Laffort, & Van Gemert, 1990). The detected concentrations of DMS in the present study were well above the odour detection level in all samples. The DMDS levels exceeded the threshold values in most samples but were below the detection level in all samples stored in material B at 4 °C, and also in the uninoculated samples kept in materials B and C at both storage temperatures. Hence, DMS, and in many cases also DMDS, were found in such high amounts that they would contribute to an off-odour detectable by the human nose.

With existing techniques it is virtually impossible to remove all microorganisms from rucola without destroying the produce. Thus, the off-odour problem stemming from microbial activity can not be eliminated. However, efforts can be made to minimise the production of undesirable volatiles. First of all, it is advisable to use a sanitising method that kills off as large a proportion of the microorganisms as possible, without negatively affecting any quality parameters of the product. Furthermore, it is recommended to maintain the temperature as low as possible, but above freezing point, during storage, and also to keep the rucola in a modified atmosphere. Further research is needed in order to determine the ideal gas composition but a decrease of oxygen and an increase of carbon dioxide levels, compared to air, seems favourable. However, it is important that the packaging material allows gas exchange with the surrounding atmosphere to prevent oxygen depletion, since too low oxygen levels cause an increase in the production of sulphides.

4. Conclusions

A dozen volatiles were detected by olfactometry in the headspace of the rucola packages. However, only two of these, dimethyl sulphide and dimethyl disulphide, had a moderate or strong intensity.

The levels of the sulphides detected in the packages were well above the odour thresholds of the two substances.

The highest sulphide levels were found in samples stored in the packaging material with such a low oxygen permeation rate that the oxygen levels were below 1%.

Inoculation with microorganisms caused an increase in the production of off-odours in the samples. Of the three microorganism mixtures that were studied, the Pseudomonadaceae&Xanthomonadaceae group appeared to be most potent in producing DMS and DMDS.

The off-odour problems were more severe at 8 °C than at 4 °C.

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