*Article*

# Oxidation Products of Ester-Based Oils with and without Antioxidants Identified by Stable Isotope Labelling and Mass Spectrometry

**Marcella Frauscher [1,2], Charlotte Besser [1], Günter Allmaier [2] and Nicole Dörr [1,*]**

[1] AC2T Research GmbH, Viktor-Kaplan-Straße 2C, 2700 Wiener Neustadt, Austria; marcella.frauscher@ac2t.at (M.F.); charlotte.besser@ac2t.at (C.B.)

[2] Institute of Chemical Technologies and Analytics, Vienna University of Technology, Getreidemarkt 9, 1060 Vienna, Austria; guenter.allmaier@tuwien.ac.at

[*] Correspondence: nicole.doerr@ac2t.at; Tel.: +43-2622-8160-0150

**Abstract:** As lubricants with a high thermo-oxidative stability such as synthetic esters are gaining more importance in the lubricant market, a detailed knowledge regarding their oxidative degradation behaviour is of high importance. In order to reveal their degradation products and processes, a novel approach combining artificial alteration, isotope labelling based on oxidation with $^{16}O_2$ and $^{18}O_2$, and mass spectrometry (MS), was applied to a bis(2-ethylhexyl) adipate base oil. The degradation products such as 2-ethylhexanol and its monoesters with short-chain fatty acids pinpointed the C–O ester bond as the site prone to oxidative attack, allowing the collection of information about the oxidation mechanisms. Furthermore, the influence of the antioxidant (AO) 4,4′-methylene-bis(2,6-di-*tert*-butylphenol) as an additive on the oxidation behaviour and resulting products was studied: blends containing AO showed a remarkably higher resistance against oxidation. However, similar degradation products were obtained after AO depletion and without AO. AO cleavage occurred at the carbon atom that bridges the phenols to give 2,6-di-*tert*-butyl-*p*-benzoquinone and 3,5-di-*tert*-butyl-4-hydroxybenzoic acid. By applying the isotope labelling approach, sites of preferential oxidative cleavage and hence differentiation of the origin of oxygen atoms—either from the atmosphere or from base oil components—can be unambiguously related in oxygen-containing base oils, as well as in blends with additives.

**Keywords:** oxidation; synthetic lubricant; ester base oil; antioxidant; thermo-oxidative stability; isotope labelling; mass spectrometry; degradation processes; lubricant chemistry; additive

## 1. Introduction

As a consequence of the trend towards machinery with an increasing performance, while scaling down in size, the appropriate selection of the lubricant quality, composition, and, hence, its performance profile, to fulfil the requirements of lubrication is becoming a more critical issue in the major fields of application. The base oil composition with regard to the application plays a crucial role in these considerations. Accordingly, group I base oils—among the five groups of base oils as defined by the American Petroleum Institute (API)—are in decline. In turn, mineral oil based group II and III base oils, which are more intensively refined petroleum crude oils, as well as fully synthetic polyalphaolefines (PAO) representing group IV, can be found in an increasing number of commercial lubricant formulations. Group V summarizes all of the other types of base oils such as silicones, phosphate esters, polyalkylene glycol (PAG) ethers, biolubes, and various synthetic esters [1,2]. With a global market size of USD 3.46 billion and a production amount of 652.2 thousand tons in 2015, the synthetic lubricants market represents a considerable economic factor [3].

In our paper, the focus is placed on ester-based oils according to their technical-economical potential and impact. Besides the growing market for environmentally friendly products, e.g., for hydraulic oils, an increasing demand from the heavy machinery industry is noticeable. Amongst the most important application fields, compressor oils have to be mentioned with a market share of 40.7% of 2013's global synthetic ester-based oil volume, followed by hydraulic fluids, engine oils, metalworking fluids, and other application fields including high temperature chain, aviation, and food lubricants.

The highly diverse fields of application of ester-based lubricants can be explained by their unique set of properties: they offer advantageous properties for high temperature and high stability applications, as well as a high flash point, low volatility, and good thermo-oxidative stability. Besides, they display an advantageous solubility for additives and an adequate inherent lubricity. Their low toxicity and good biodegradability are additional advantages [2,4]. Ester-based base oils can be distinguished in three main groups: (1) natural esters, including vegetable crops and animal fats; (2) synthetic oleochemical esters made by the reaction of alcohols and natural fatty acids; and (3) petrochemical esters derived from synthetic alcohols and acids [4]. Besides the above-mentioned general benefits of esters compared to high-quality hydrocarbons, they can exhibit a high thermal decomposition temperature of 275 °C according to ASTM D2879, a superior trade-off between a viscosity index (VI) of 124 and pour point of −68 °C, as exemplarily given for the bis(2-ethylhexyl) adipate ester [2].

Despite the widespread applications of esters at high temperatures and under harsh conditions, the knowledge about their thermo-oxidative stability behaviour still remains a critical issue. Nowadays, an increasingly important application field where the thermo-oxidative stability of esters is in focus is that of renewable biolubricants being employed as esters based on synthetic and vegetable oils, opening a wide field for eco-friendly and biodegradable lubricants [5]. Thereby, a knowledge of structure-stability relationships lays the foundations for the adjustment of thermo-oxidative stability based on the application-related modification of molecule geometry and targeted synthesis [6–9].

The thermo-oxidative stability of esters is highly linked to the hydrogen configuration of the β-carbon, especially of the alcohol part, the second carbon atom next to the functional group of the C–O bond in the alcohol (see Figure 1). Hydrogen atoms which bond to these β-carbons are prone to thermal decomposition, as a six-membered cyclic intermediate can be built and decomposes to acid and alkene. The replacement of these hydrogen atoms, e.g., to give a quaternary β-carbon, disables this mechanism and degradation products have to be generated via the thermodynamically less favorable free-radical pathway. Thus, the stability is increased with a higher degree of substitution on this carbon atom [2,10]. This strategy is exploited in polyol esters by replacing all of the hydrogen bonds to the β-carbon by alkyl groups in the alcohol moiety. Thus, the thermal decomposition of polyol esters occurs via a free-radical pathway and proceeds remarkably slower, resulting in a significantly higher stability [10–12].
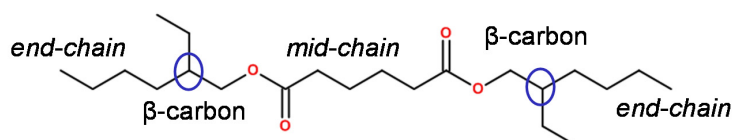


**Figure 1.** Diester bis(2-ethylhexyl) adipate with highlighted (blue circle) β-carbon atoms in the alcohol moiety (end chain).

The oxidative stability is increased with a decreasing mid-chain acid length, whereas the length of the end-chain alcohols shows less influence on the oxidative stability. However, branching of the alcoholic residue has a beneficial effect in terms of an improved oxidative stability, but is reduced by branched acid chains. While for the alcohol chain a complete H substitution at the β-carbons is particularly favourable, acid chain stability follows the order –CH$_3$ > –CH$_2$– > –CH– [2,13].

Beyond these considerations on the optimum base oil chemistries, an improvement of the thermo-oxidative performance with aminic and/or phenolic antioxidative additives is the most

common way to a achieve higher resistance against oxidation and a longer functional durability of lubricants [14,15]. Lubricant degradation follows the free-radical chain reaction, based on four steps: (1) initiation; (2) propagation; (3) branching; and (4) termination. Within this study, the focus was laid on sterically hindered phenols suitable for high temperature applications to decelerate oxidation.

In order to evaluate the resistance of a base oil against thermo-oxidative stress and, thus, the impact of an antioxidant, several methods are available—either standardised or customised—such as the thermo-oxidative stability test (TOST) (DIN 51587) [16], corrosion and oxidation stability test (DIN 51808) [17], or the rotating pressurised vessel oxidation test [18] (RPVOT, see Section 2.3). Subsequent to the accelerated alteration of lubricant samples in laboratory devices, appropriate analytical methods have to be applied to determine the degree of degradation by oxidation. On the one hand, parameters regarding the degree of degradation can be obtained via conventional analytical methods based, for example, on infrared (IR) spectroscopy and the neutralisation number (NN) (see Section 2.4.1). However, these analytical methods do not provide detailed information related to the structures of degradation products or the mechanistic aspects of the process. For this reason, more advanced instrumental analytical methods have to be applied that are able to provide structure identification capabilities. Capillary gas chromatography coupled with electron impact ionization mass spectrometry (GC–EI–MS) is considered suitable for this task (see Section 2.4.2). With this hyphenated technique, the identification and characterization of the degradation products formed can be achieved after the high resolution chromatographic separation of the oil sample [19–21].

Nevertheless, a clear assignment of the origin of the degradation products, as well as the mechanistic aspects from oxygen-containing base oils and additives by oxidation, cannot be solely provided by chromatographic and mass spectrometric methods. In previous research work, a novel approach combining artificial alteration, isotope labelling, and mass spectrometry was developed to elucidate and track oxidation reactions in oxygen-containing fuel components [22]. In detail, the approach enables the unambiguous attribution of oxygen atoms in the degradation products either to the original ester, i.e., the starting material, or to the reaction process with oxygen (from the gas phase), and particularly hints to the mechanism of the formation of the degradation products. In the current publication, the methodology developed in [22] has been applied to a diester and a lubricant model mixture composed of the diester and a sterically hindered phenolic antioxidant. To the best of our knowledge, this is the first publication that reports on the artificial alterations of base oil containing a phenolic antioxidant using $^{18}O_2$ labelling in combination with capillary GC–EI–MS.

## 2. Materials and Methods

### 2.1. Materials

As ester-based base oil component, bis(2-ethylhexyl) adipate (CAS 103-23-1, Sigma-Aldrich, St. Louis, MO, USA, structure is depicted in Figure 1) with a purity of 99% was applied. For the blends of antioxidant in base oil, 4,4′-methylene-bis(2,6-di-*tert*-butylphenol) (CAS 118-82-1, Sigma-Aldrich, St. Louis, MO, USA, structure is depicted in Figure 1) with a purity of 98% was applied at a concentration of 1 (*w/w*) %. Both chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA). $^{16}O_2$ and $^{18}O_2$ gases were used in purities of 99.998% and more than 99%, respectively. $^{16}O_2$ was obtained from AirLiquide (Paris, France) in conventional 50 L steel cylinders at 200 bar, and $^{18}O_2$ in 1.0 L steel cylinders at 2 bar from Sigma Aldrich (St. Louis, MO, USA).

### 2.2. Approach of Isotope Labelling

In a previous study, an approach of artificial alteration in $^{18}O_2$ atmosphere for producing isotope-labelled degradation products was developed and applied to the oxygen-containing model mixtures of fuel components [22]. Due to a noticeable lower abundance of oxygen isotope $^{18}O$ (0.22%) than $^{16}O$ (99.78%), a differentiation can be achieved between the oxygen atoms of the artificial $^{18}O_2$ atmosphere and those comprised in the respective sample by means of mass spectrometry (MS). Thus,

different isotopes were used to track the oxidation mechanism via the assignment of the site of the oxidative attack. Figure 2 shows the scheme of the isotope labelling approach. For the present work, the above-mentioned ester-based base oil component and a blend with 1 (*w/w*) % antioxidant were subjected to accelerated degradation, due to the modified artificial alteration method described in Section 2.3 under two different oxygen atmospheres—plain $^{18}O_2$ and standard $^{16}O_2$. Accordingly, the oxidation achieved with these gases yields the formation of degradation products either with or without $^{18}O$ isotope labelling.
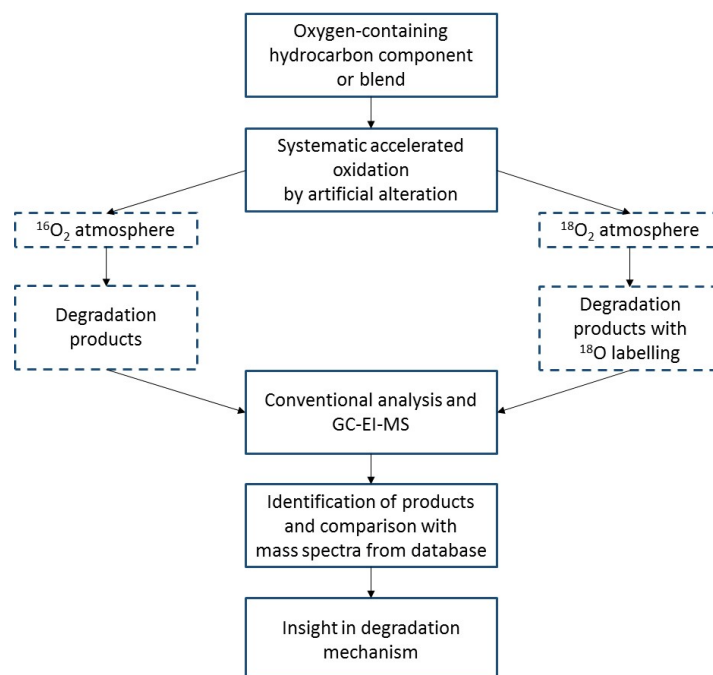


**Figure 2.** Scheme of the isotope-labelling approach.

Conventional analytical methods (see Section 2.4.1) revealed the general characteristics of the artificially altered samples compared to those which were fresh. A subsequent analysis with capillary GC–MS using the gas-phase ionization technique EI revealed, after comparison, the unambiguous origin of the oxygen atoms in the identified degradation products, as well as details related to the degradation mechanism [22].

*2.3. Artificial Alteration*

Most artificial alteration procedures of lubricants are performed within an open system. As a consequence, volatile compounds—both unreacted and reacted, i.e., newly formed—are removed by the gas flow and the elevated temperatures applied. Accordingly, they cannot react further, nor be investigated by subsequent fluid analysis.

In order to overcome this shortcoming, an artificial alteration procedure was utilised, based on standard ASTM D 2272 (Rotary Pressure Vessel Oxidation Test, RPVOT) [18]. In Figure 3, the respective alteration device is displayed.

For artificial alteration, 15 g of base oil—with and without antioxidant—is poured into a glass container and placed in the pressure vessel, which is closed tightly. The vessel is then filled with oxygen (either $^{16}O_2$ or $^{18}O_2$) until a pressure level of 1 or 2 bar, respectively, is reached at ambient temperature and heated to 150 °C in an oil bath. Over the entire duration of artificial alteration, the vessel is rotated by means of a rotary device in an inclined position to ensure a continuous circulation of the sample, as well as to improve the interaction with the oxygen gas-phase. The gas pressure is

monitored via a pressure sensor and recorded by a data processing computer. In contrast to ASTM D 2272, no copper wire catalyst or water addition was applied.
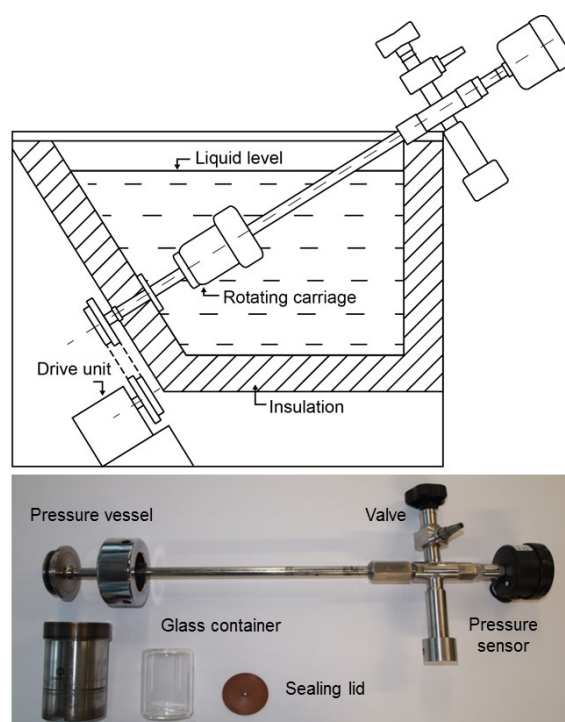


**Figure 3.** Schematic set-up of the RPVOT (rotating pressurised vessel oxidation test) device in the heating unit (**above**), RPVOT pressure vessel (**below**).

Tracking the oxygen pressure curve enables the monitoring of the alteration progress: stable pressure correlates to a stable sample condition, whereas a pressure drop (steep descent in the pressure curve) indicates increased oxygen consumption and, hence, the occurrence of oxidation reactions. By interrupting the experiment at selected points in time, an investigation of the samples with a defined condition is enabled, e.g., directly before the onset of oxidation or after the break down of pressure (see Figure 4).
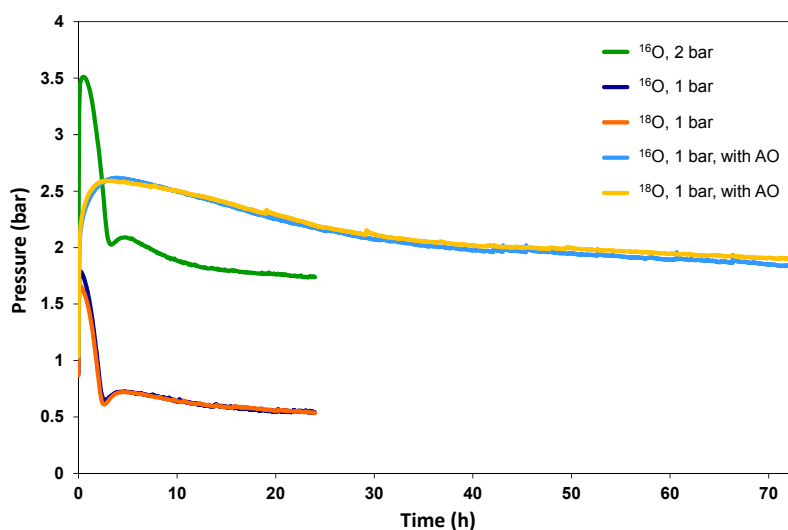


**Figure 4.** Progress of oxygen pressure during artificial alterations.

### 2.4. Analytical Approach

### 2.4.1. Conventional Analytical Methods

In [23], the neutralisation number (NN) and the water content of the conventional parameters were found to be the most important parameters involved in the degradation process during artificial alteration in the RPVOT device. According to these findings, the following conventional analytical parameters were chosen to characterise the fresh, as well as the altered, oil samples:

### Neutralisation Number (NN)

The NN was measured in accordance with standard DIN 51558 [24], by means of colour indicator titration. It is defined as the amount of potassium hydroxide required for the neutralisation of all of the acidic compounds in 1 g of sample aliquot.

### Water Content

The content of water in the oil samples was determined by Karl-Fischer titration, according to standard DIN 51777-2 [25]. The indirect method was applied by evaporating the water in the sample in an oven, inducing it in a measuring cell, and determining the concentration by coulometry.

### 2.4.2. Advanced Analytical Methods

In order to identify the degradation products, samples obtained from artificial alteration and in fresh condition were analysed with a capillary GC–EI–MS consisting of a TriPlus autosampler and a Trace capillary Ultra GC coupled to a TSQ Quantum XLS mass spectrometer, all of which were sourced from Thermo Scientific (Austin, TX, USA). Base components were applied as reference substances at various product concentrations: ester-based base oil component bis(2-ethylhexyl) adipate was injected with 0.02 (*w/w*) %, 4,4′-methylene-bis(2,6-di-*tert*-butylphenol) with 0.008 (*w/w*) %, and the blend of base oil component containing AO with 0.01 (*w/w*) %, which were all diluted in dichloromethane (DCM). The dilutions of all samples after artificial alteration were prepared with a concentration of 4 (*w/w*) % in DCM. The degradation products containing active hydrogen atoms such as acids were treated by silylation. The silylation reagent *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) was applied to enhance the volatility for GC–MS and to avoid the undesired adsorption of the polar degradation products on the column material. BSTFA was added in a 2:1 mass excess of (*w/w*) % to the sample and aliquots were kept at 70 °C for 1 h to complete the reaction.

Samples were subjected to GC–EI–MS by injection via a programmable temperature vaporization injector (PTV) with a volume of 1 μL and a constant injector temperature of 300 °C. Split was selected with a ratio of 1:25, and the carrier gas was helium and kept at a constant flow of 2 mL/min. To separate the sample prior to mass spectrometric analysis, a TG–17MS capillary column obtained from Thermo Fischer Scientific (Waltham, MA, USA) was used, consisting of a 50%-diphenyl/50%-dimethylpolysiloxane stationary phase with a length of 30 m, a 0.25 mm inner diameter, and a film thickness of 0.25 μm. An oven temperature program starting at 50 °C for 2 min with a ramp of 7 °C/min up to 300 °C was applied, and the end temperature was kept constant for 15 min. The separated compounds were transferred to the mass spectrometer via a transfer line thermostated at 280 °C and were subsequently ionized in an EI source at 70 eV. The source temperature was set to 200 °C, and the analyser scan range was between $m/z$ 40 and 650 for the full scan mode. Data acquisition and data processing were done with Xcalibur v2.0 software. For subsequent identification based on a full scan mass spectra comparison, the NIST library 2.0 (2011) with an update from the 2014 and reference substances was applied.

For the main degradation products, the calculated monoisotopic $m/z$ value was given, based on the sum formula and the respective amount of $^{16}O$ or $^{18}O$ atoms based on the mass spectrometric data, as well as the detected monoisotopic mass pattern, i.e., the molecular ion peak. For analytes without a clearly visible, i.e., abundant molecular ion peak with a relative intensity (RI) of more than 1%, the highest unambiguously detected $m/z$ value was reported.

## 3. Results and Discussion

### 3.1. General Degradation Behaviour Determined by Conventional Analytical Methods

In Figure 4, the oxygen pressure in the reaction vessel throughout the artificial alterations is displayed. The pressure curves of base oil without AO show an abrupt rise in the first few minutes, due to the adjustment to the elevated temperature. Almost immediately, a steep pressure drop is observed, induced by oxidation processes. After 2 to 3 h of alteration, the lower plateau of pressure is reached, characterised by a slow pressure decrease.

In the case of the oxidation of ester-based base oils with AO, the pressure rise in the first hours of alteration is far higher, approximately twice that of blends without AO, and lasts longer, i.e., up to 5 h of alteration. After reaching the pressure maximum, a slow decrease begins, resulting in a drawn-out lower plateau with hardly any change in pressure.

Generally, prolonged alteration durations until the beginning of the pressure decrease and the achievement of the stable lower pressure plateau designate the effectiveness of the applied AO. Thus, on the one hand, the start of the oxidative processes is retarded, and on the other hand, the base oil degradation itself is decelerated. Without the application of AO, oxidation begins immediately, whereas blends with AO only start to significantly deteriorate after approximately 5 h. The overall duration until reaching a stable degradation condition strongly depends on the utilisation of AO: around 72 h in the case of blends with AO, compared to around 24 h for ester oils without an AO dosage.

Moreover, it has to be stated that the initial oxygen pressure in the vessel seems to possess little influence on the alteration behaviour, as a comparison of the alteration with 1 and 2 bar of the originally applied oxygen shows (Figure 4, dark blue curve versus dark green curve). Both alterations exhibit an identical pressure behaviour. In a first approach, the same degradation mechanisms, and hence the same reactions, can be assumed under both conditions.

A comparison of the oxygen pressure curves of alterations with $^{16}O_2$ and $^{18}O_2$ suggests that the isotope-labelled atmosphere does not have an impact on either the degradation behaviour or on the degradation mechanisms, strongly indicated by the almost congruent curve shapes in both cases.

The visual appearances of the fresh, as well as the artificially altered, ester-based oil samples are shown in Figure 5. It was found that all altered samples exhibit a bright yellow colour compared to colourless fresh blends. The only exception is the sample altered with 2 bar oxygen pressure with a dark brown colouring and significant turbidity. The latter can be attributed to an elevated water content, which was measured to be approximately five times higher than in all other investigated oil samples (see Figure 6).
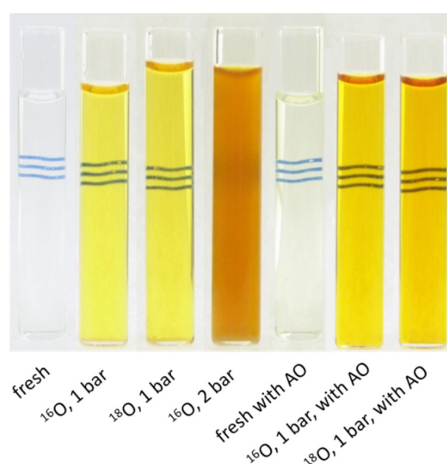


**Figure 5.** Visual appearances of ester-based oil samples with and without antioxidant (AO) before and after artificial alteration under the different oxygen atmospheres.
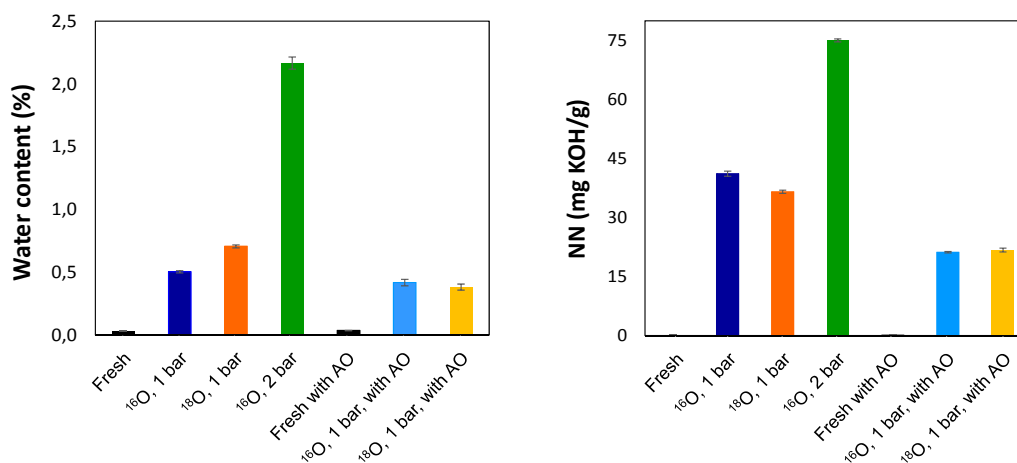
**Figure 6.** Water content (**left**) and neutralisation number NN (**right**) of oil samples before and after artificial alteration (under different oxygen atmospheres). The values for the fresh samples are <0.1 (*w/w*) % and <0.1 mg KOH/g.

Conventional condition monitoring was performed in terms of the NN and water content. Figure 6 compares the analytical data of the fresh oils with the respective results of the samples after artificial alteration. Fresh oil samples exhibited very low levels of water, as well as NN. On the contrary, a tremendous rise in both the water content and NN was detected in all samples after artificial alteration. Higher values for both parameters were found for oils without AO than for blends with AO, e.g., the NN for samples without AO was determined to be twice as high as the NN in samples with AO. The only outstanding exception is the oil sample after artificial alteration at a 2 bar oxygen pressure, which showed the highest values for both the NN and water content. In general, a good correlation was found between the detected water content and the NN in the oil samples after artificial alteration. This indicates an oxidative deterioration process producing acids, together with formation of water.

As a conclusion from the conventional oil analyses, it has to be stated that these methods provide valuable information on an oil sample which can be used to decide whether the oil has to be changed or can still be utilised in its respective application. Using these methods, equipment operators can rely on relatively rapid and cheap analyses as the basis for accurately timed counter-measures, e.g., in the case of an elevated acid concentration and/or a high water content, which may provoke corrosion. The most relevant shortcoming of conventional analysis is the lack of information on processes on the molecular level and of prognostic indications for counter-measures. Knowing these facts is essential to formulate adequate high performance oils operating at even harsher conditions or when applying them to completely new settings. Thus, conventional analytical tools have been extended by advanced analytical methods within this study to gain an insight into the degradation mechanisms and formation of degradation products, as well as the mode of action of the antioxidant.

### 3.2. Degradation Behaviour Identified by Advanced Analytical Methods

Figure 7 shows the total ion current (TIC) chromatograms of ester-based base oil samples without additives, altered at 150 °C and a pressure of 1 bar under $^{16}O_2$ (A) and $^{18}O_2$ (B) atmosphere, respectively. In accordance with the observations from the RPVOT pressure curves and conventional analyses, the same degradation products in a highly similar distribution were obtained for both alterations. Thus, no impact of the oxygen isotope on the type of degradation products formed was found, and a good reproducibility for the applied alteration method was demonstrated. The verification of these facts is essential for the successful application of the isotope labelling approach and the evaluation of the findings obtained.
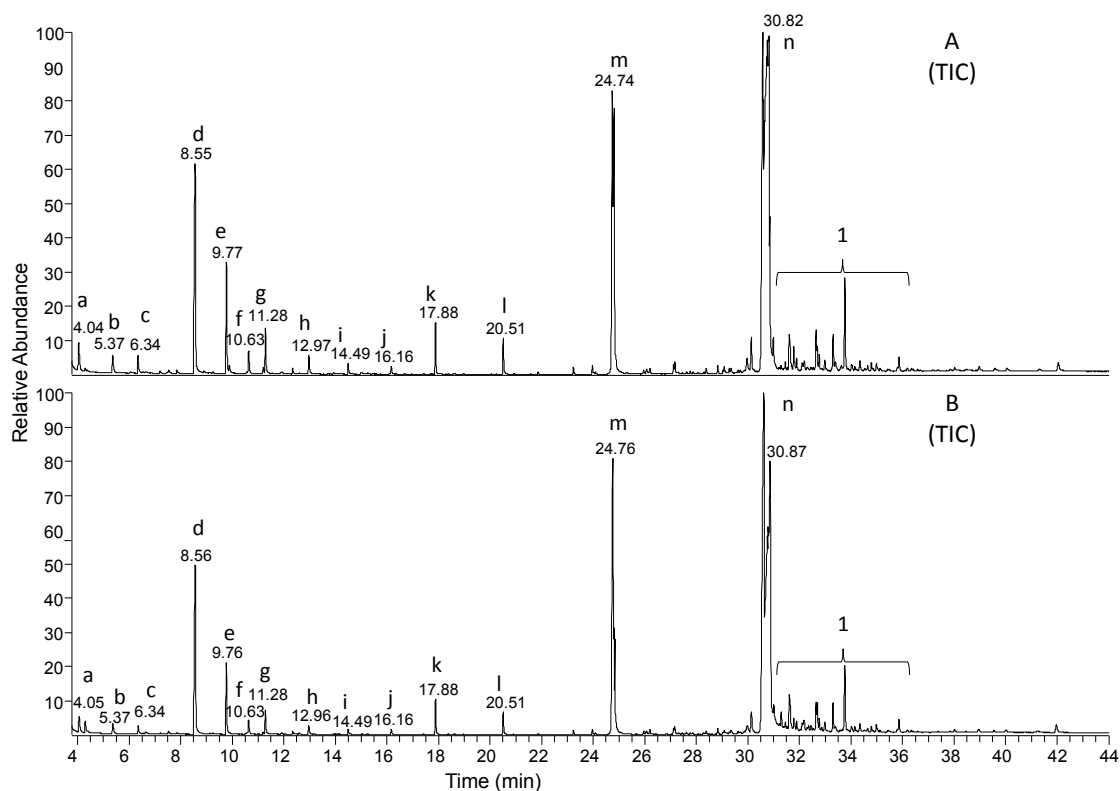
**Figure 7.** Total ion current (TIC) chromatograms of alteration products obtained under 1 bar $^{16}O_2$ (**A**) and $^{18}O_2$ (**B**) atmosphere without AO. Main products (a–m) and target molecule (n, starting material) are marked and given in Tables 1 and 2. Peaks under bracket with label 1 indicate oxidized molecules of ester-based base oil bis(2-ethylhexyl) adipate.

The target molecule (n), i.e., the starting molecule, and the main degradation products marked in Figure 7 are listed in Table 1 for $^{16}O_2$ atmosphere and Table 2 for $^{18}O_2$ atmosphere, respectively. Moreover, the retention time in minutes, calculated monoisotopic $m/z$ value of the molecular ion, detected monoisotopic mass of the molecular ion with a relative intensity in % (RI %), and the highest detected $m/z$ value are given. Besides linear carboxylic acids with short chain lengths such as propionic acid (a) and butanoic acid (b), detected as the trimethylsilyl (TMS) ester due to a prior sample silylation with BSTFA, hexanedioic acid TMS ester (k) referring to adipic acid in the base oil was found. 2-ethylhexyl moiety in the ester base oil was transformed into 2-ethylhexyl alcohol, detected as oxysilane (d), and 2-ethylhexanoic acid, detected as TMS ester (f). Esters of 2-ethylhexanol from formic acid up to pentanoic acid (e, g–j) were formed in distinct amounts, together with other esters (l, m) based on the alcohol moiety of the initial ester. Moreover, oxidation products with a ketone (c) structure were obtained after alteration, as well as a broad variety of oxidized molecules (peaks under bracket carrying the label 1) derived from the initial bis(2-ethylhexyl) adipate (n).

The benefit of using the isotope labelling approach to determine the degradation products related to the degradation mechanisms of the base oil is shown based on different molecules, either containing two (Figure 8), one (Figure 9 and Figure S1), or no (Figure S2) $^{18}O$ atoms. In Figure 8, the positive ion EI mass spectra of the degradation product 2-ethylhexanoic TMS ester (f) obtained under $^{16}O_2$ alteration atmosphere (A) and $^{18}O_2$ alteration atmosphere (B) are shown. When comparing mass spectrum A (top) and B (bottom), some particular, close-by ions clearly differ in their $m/z$ value, e.g., 128.9 (A) and 132.7 (B), 159.9 (A) and 163.9 (B), and 201.0 (A) and 204.9 (B). This shift of + 4 Dalton (Da) is obtained as the molecule contains two $^{18}O$ atoms when altered under $^{18}O_2$ atmosphere instead of $^{16}O_2$.

**Table 1.** Main degradation products of artificial alteration under $^{16}O_2$ atmosphere without AO (antioxidant). Molecular ions of analytes e–m were not detected due to very low RI. Analyte n is the original of ester-based base oil. Structures of main degradation products are displayed in Figure S4.

| $^{16}O_2$ | Analyte | Retention Time (min) | Calculated Monoisotopic $m/z$ Value of Molecular Ion | Detected Monoisotopic Molecular ion (RI %) | Highest Detected $m/z$ Value in the Mass Spectrum |
|---|---|---|---|---|---|
| a | Propionic acid TMS ester | 4.04 | 146.1 | 145.9 (0.6) | 130.9 |
| b | Butanoic acid TMS ester | 5.37 | 160.1 | 159.9 (1.0) | 144.9 |
| c | 3-heptanone | 6.34 | 114.2 | 114.0 (13.5) | 114.0 |
| d | (2-ethylhexyl)oxy TMS silane | 8.55 | 202.4 | 202.1 (0.8) | 187.0 |
| e | 2-ethylhexyl formic acid ester | 9.77 | 158.2 | - (<0.5) | 112.0 |
| f | 2-ethylhexanoic acid TMS ester | 10.63 | 216.2 | - (<0.5) | 201.0 |
| g | 2-ethylhexyl acetic acid ester | 11.28 | 172.3 | - (<0.5) | 124.8 |
| h | 2-ethylhexyl propionic acid ester | 12.97 | 186.3 | - (<0.5) | 156.9 |
| i | 2-ethylhexyl butanoic acid ester | 14.49 | 200.3 | - (<0.5) | 156.9 |
| j | 2-ethylhexyl pentanoic acid ester | 16.16 | 214.3 | - (<0.5) | 146.9 |
| k | Adipic acid TMS ester | 17.88 | 290.5 | - (<0.5) | 275.0 |
| l | 2-ethylhexyl 6-oxohexanoate | 20.51 | 242.4 | - (<0.5) | 139.8 |
| m | 2-ethylhexyl 2-oxobutyl adipate | 24.74 | 328.4 | - (<0.5) | 271.0 |
| n | *Bis(2-ethylhexyl) adipate* | 30.82 | 370.6 | 371.1 (1.7) | 371.1 |

**Table 2.** Main degradation products of artificial alteration under $^{18}O_2$ atmosphere without AO. Molecular ions of analytes e-m were not detected due to very low RI. Analyte n is the original of ester-based base oil. Structures of main degradation products are displayed in Figure S4.

| $^{18}O_2$ | Analyte | No. of $^{18}O_2$ Atoms | Retention Time (min) | Calculated Monoisotopic $m/z$ Value of Molecular Ion | Detected Monoisotopic Molecular Ion (RI %) | Highest Detected $m/z$ Value in the Mass Spectrum |
|---|---|---|---|---|---|---|
| a | Propionic acid TMS ester | 2 | 4.04 | 150.1 | 149.8 (<0.5) | 134.7 |
| b | Butanoic acid TMS ester | 2 | 5.37 | 164.1 | 163.8 (1.0) | 148.8 |
| c | 3-heptanone | 2 | 6.34 | 116.2 | 115.8 (12.9) | 115.8 |
| d | (2-ethylhexyl)oxy TMS silane | - | 8.55 | 202.4 | 201.9 (0.7) | 186.9 |
| e | 2-ethylhexyl formic acid ester | - | 9.77 | 158.2 | - (<0.5) | 111.9 |
| f | 2-ethylhexanoic acid TMS ester | 2 | 10.63 | 220.2 | - (<0.5) | 204.9 |
| g | 2-ethylhexyl acetic acid ester | 1 | 11.28 | 174.3 | - (<0.5) | 126.8 |
| h | 2-ethylhexyl propionic acid ester | 1 | 12.97 | 188.3 | - (<0.5) | 158.8 |
| i | 2-ethylhexyl butanoic acid ester | 1 | 14.49 | 202.3 | - (<0.5) | 158.8 |
| j | 2-ethylhexyl pentanoic acid ester | - | 16.16 | 214.3 | - (<0.5) | 148.8 |
| k | Adipic acid TMS ester | 2 | 17.88 | 294.5 | - (<0.5) | 278.9 |
| l | 2-ethylhexyl 6-oxohexanoate | 1 | 20.51 | 244.4 | - (<0.5) | 141.8 |
| m | 2-ethylhexyl 2-oxobutyl adipate | 1 | 24.74 | 330.4 | - (<0.5) | 271.0 |
| n | *Bis(2-ethylhexyl) adipate* | - | 30.82 | 370.6 | 371.0 (1.8) | 371.0 |

Considering the structure of the initial molecule in addition to this information, the most likely pathway of degradation of the ester and formation of the degradation product can be revealed: the 2-ethylhexyl moiety cleaves between the alcohol's C1 and O atom, which is followed by the oxidative attack of $O_2$ from the atmosphere. Thus, 2-ethylhexanoic acid is obtained, detected as a TMS ester via sample preparation with the BSTFA silylation reagent. Nevertheless, as the initial ester molecule is symmetric, this reaction can take place on both alcohol moieties of the adipate. In Figure 8, the simplified reaction steps are exemplarily shown on one side of the initial molecule.
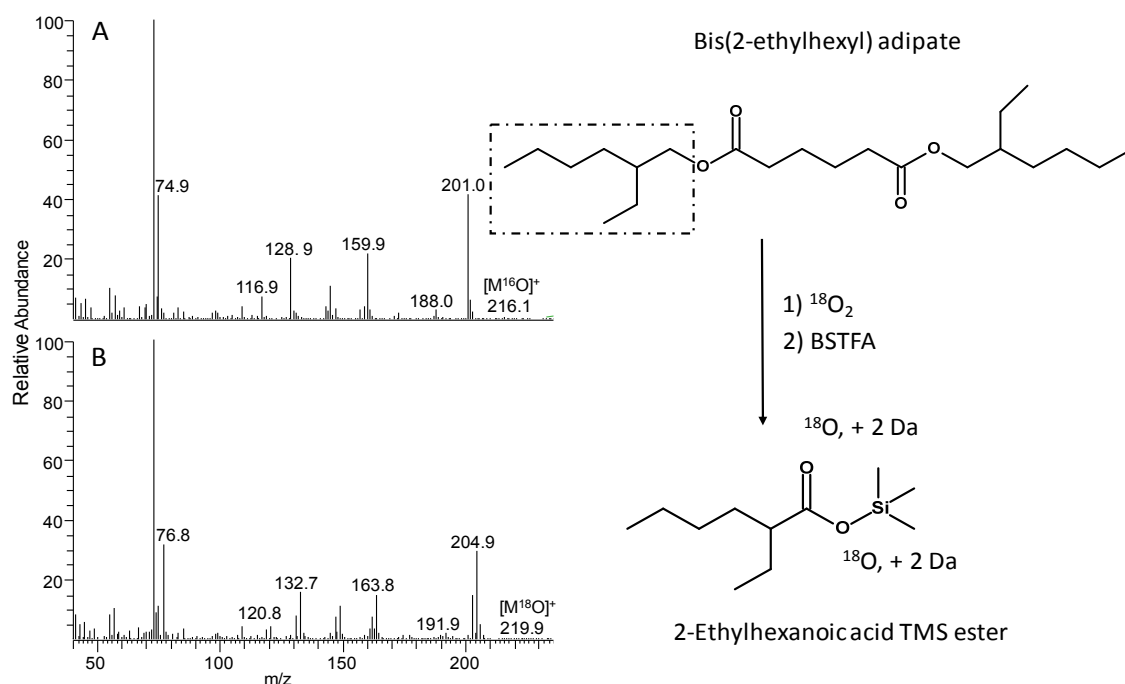


**Figure 8.** Positive ion mass spectra of 2-ethylhexanoic acid TMS ester (f) obtained after alteration under $^{16}O_2$ (**A**) and $^{18}O_2$ (**B**) atmosphere, respectively. Peaks of fragments where a change of the $m/z$ value due to isotope labelling occurred are labelled with the respective values. A simplified oxidation reaction is suggested for the respective molecule.

3-heptanone (c) is a degradation product containing only one isotope-labelled oxygen atom, as shown in Figure S1. When comparing the mass spectra obtained under $^{16}O_2$ and $^{18}O_2$ atmosphere, the presence of one $^{18}O$ atom is confirmed: besides fragments containing oxygen, i.e., $m/z$ 57.0 and 58.9, 72.0 and 73.9, 84.9, and 86.6, the molecular ion peak with a shift from $m/z$ 114.0 to 115.8 is also effortlessly visible. It can be assumed, that thermal decomposition takes place via the six-membered cyclic intermediate involving the hydrogen on the β-carbon, as reported in the literature (see Introduction). This results in an alkene counting eight C atoms and an (di)acid. With these fragments, further reactions can take place. Due to oxidation, evocated by the oxygen from the atmosphere, 3-heptanone is built, carrying one $^{18}O$ atom. However, when looking closely at the mass spectra of this molecule, one may also see low abundant fragments without isotope labelling such as 114.0, 84.9, and 72.0 (Figure S1B). This observation may lead to a second possible formation mechanism of 3-heptanone, without involving $^{18}O_2$. Hence, the application of the isotope labelling approach is not only feasible for explaining issues of interest, but also raises new ones.

Figure S2 in the supplement shows an example of a degradation product without any isotope-labelled oxygen, 2-ethylhexyl alcohol detected as (2-ethylhexyl)oxy TMS silane (d). Due to the fact that in none of the mass spectrometric fragments found in the mass spectrum of the compound formed under $^{18}O$ atmosphere a shift of $m/z$ values (+2 or 4 Da) is obtained, there is no hint of a participation of oxygen from the atmosphere in the formation of this product. Thus, by using the

isotope labelling approach, one can conclude that the molecule was formed by an unspecified cleavage of the C–O ester bond. Besides radical cleavage, the more likely mechanism can be hydrolysis of the ester bond. This mechanism requires conditions provided by proceeding alteration, in particular, an acid catalyst (see increased NN value for altered samples in Section 3.1) and a sufficient amount of water (see water formation for altered samples in Section 3.1). Accordingly, in the following passages, the term "cleavage of the C–O ester bond" refers to an unspecified mechanism of cleavage.

Besides the degradation products containing either $^{18}O$ or $^{16}O$ at the sites of the oxidative attack, molecules with a mix of isotopes are also possible. Figure 9 provides a 2-ethylhexyl acetic acid ester (g) as an example of this. Based on the shift in the molecular ion peak, as well as the highest detected shift of +2 Da, the presence of one $^{18}O$ atom can be confirmed. A comparison with the NIST library identifies the molecule as 2-ethylhexyl acetic acid ester, containing two oxygen atoms. The correlation of the fragment ions with the structure only gives a shift for these fragments, where either both oxygen atoms are involved or exclusively the C=O group, i.e., the fragment ions at *m/z* 43.1 and 45.0. This observation leads to the finding that the C=O group contains the $^{18}O$ atom. Most probably, cleavage of the C–O ester bond and the combination with a $CH_3CO$ moiety results in the identified ester. The $CH_3CO$ moiety is most possibly derived by the cleavage of the ethyl side chain of 2-ethylhexanol and subsequent oxidation.
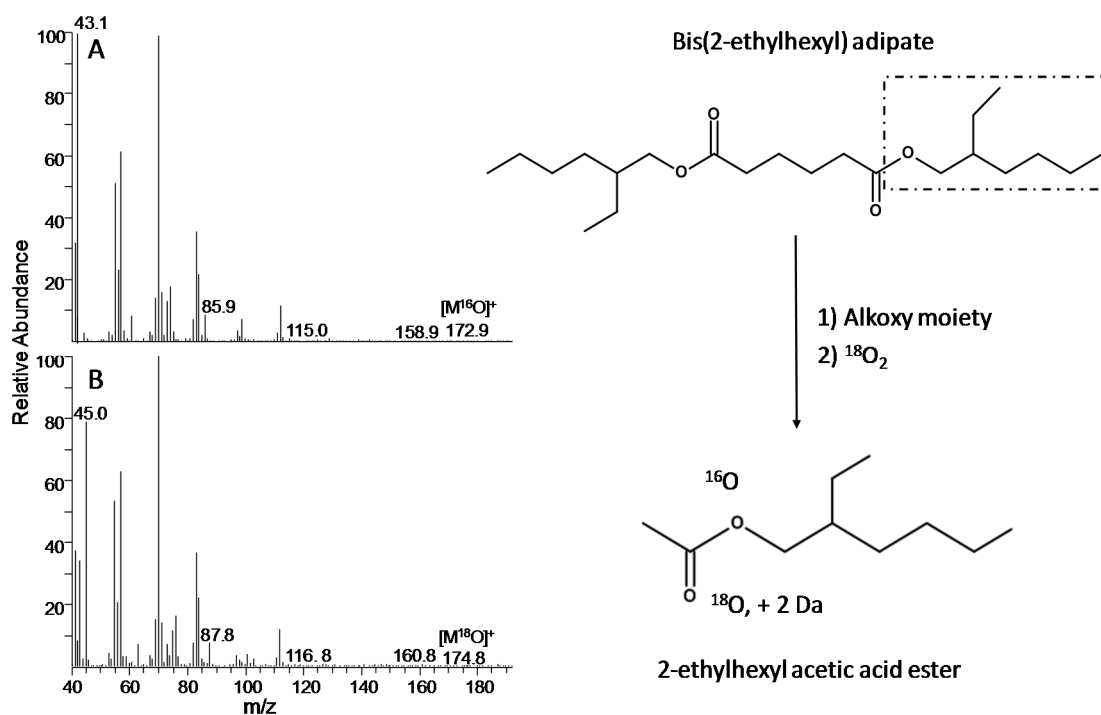


**Figure 9.** Positive ion mass spectra of 2-ethylhexyl acetic acid ester (g) obtained after alteration under $^{16}O_2$ (**A**) and $^{18}O_2$ (**B**) atmosphere, respectively. Peaks of fragments where a change of the *m/z* value due to isotope labelling occurred are labelled with the respective values. A simplified oxidation reaction is suggested for the respective molecule.

Summarizing the above described results of the neat base oil, relevant fragments of the oxidation mechanism could be identified: preferential sites of cleavage are between the C–O ester bond, resulting in products such as 2-ethylhexyl alcohol (d) and 2-ethylhexyl fatty acid esters with various acid chain lengths (e, g–j), and a bond cleavage between the O atom and C1 of the alcohol, providing 2-ethylhexanoic acid (f). Such a structure can be further oxidized to 3-heptanone (c) and, after a second cleavage step, to propionic and butanoic acid (a, b). For one of the main degradation products, adipic acid (k), the two $^{18}O$ atoms are present in each of the ester bonds (C–$^{18}O$), whereas $^{16}O$ was

found in the carbonyl groups (C=$^{16}$O) of the acid chain of the initial ester. Hence, a combination of oxidative cleavage of the C–O ester bond and subsequent acid formation occurs on both sides. Moreover, the highly abundant 2-ethylhexyl 2-oxobutyl adipate (m) results from bond dissociation and oxidation between C2 and C3 of the alcohol chain, whereas the rest of the initial molecule stays intact. The formation of short-chain fatty acids as found in esters (e, g–j) is assigned to the cleavage and oxidation of 2-ethylhexyl alcohol. In contrast to the expected oxidation mechanism, formic acid (in e) and pentanoic acid (in j) do not carry $^{18}$O. Furthermore, the oxidation mechanism of 2-ethylhexyl 6-oxohexanoate (l) could not be revealed.

　　　An alteration of the neat base oil was not only performed at 1 bar, but also at a pressure of 2 bar, to estimate the influence of pressure on the formation of degradation products. Figure S3 illustrates chromatograms of alteration under 1 bar $^{16}$O$_2$ atmosphere (A) and 2 bar $^{16}$O$_2$ atmosphere (B). For both alteration samples, the main products were marked and given in Table 1. Based on the degradation products obtained, it can be concluded that there is hardly any influence of the enhanced pressure on the structure of the degradation products formed, at least in the time frame and temperature regime applied. However, particular peaks show a noticeably lower abundance, e.g., (2-ethylhexyl)oxy TMS silane (d) at retention time $t_R$ = 8.55 min, and 2-ethylhexyl 2-oxobutyl adipate (m) at $t_R$ = 24.74 min, while some of them increased with increasing pressure, e.g., adipic acid bis(TMS) ester (k) at $t_R$ = 17.90 min. Therefore, an effect of pressure on the degradation mechanism and, consequently, on the quantities of the products formed can be assumed, whereas the quality of the degradation products is not influenced.

　　　In addition to the neat base oil, blends with 1 (*w/w*) % AO were subjected to artificial alteration. GC–MS analysis obtained from these samples altered under $^{16}$O$_2$ atmosphere and $^{18}$O$_2$ atmosphere, respectively, showed identical products and distribution. The most abundant degradation products in the artificially altered samples have already been given in Table 1. Additionally, the degradation products of AO under both atmospheres are listed in Table 3.

　　　Compared to alteration without AO (see Figure 10A), not all of the products were formed in similarly significant amounts and are therefore not marked as main degradation products. Exemplarily, short-chain fatty acids such as propionic acid (a) and butanoic acid (b), were detected as TMS esters in only minor amounts. Accordingly, 2-ethylhexyl fatty acid esters (e, g–j) with various acid chain lengths are generated in noticeably lower quantities. The lower amount of acidic degradation products of blends with AO is in good accordance with the results from NN analyses, which show values which are only half of the ones without AO. However, (2-ethylhexyl)oxy TMS silane (d) and adipic acid TMS ester (k) were formed in similar quantities. Regardless of the amounts of degradation products formed, qualitative analysis revealed almost completely the same list of degradation products for both the neat base oil and the blend containing AO.

　　　In addition to the ester degradation products discussed above, the degradation products of the AO were also detected: 2,6-di-*tert*-butyl-*p*-benzoquinone (o) and 3,5-di-*tert*-butyl-4-hydroxybenzoic acid TMS ester (p) were found in TIC and are shown in Figure 10B. Figure 11 shows the positive ion mass spectra of $^{16}$O$_2$ and $^{18}$O$_2$ atmosphere alteration of the AO degradation product 3,5-di-*tert*-butyl-4-hydroxybenzoic acid TMS ester. Due to a shift of +4 Da for the molecular ion from *m/z* 322.0 to 326.0, as well as for *m/z* 307.0 to 311.0, the oxygen atoms in the carboxyl acid group can be identified as $^{18}$O isotopes. Hence, one can conclude that the oxidation of the AO leads to a bond cleavage between the C bridge-atom and one of the phenols, and the C bridge-atom is oxidized to a carboxyl acid by oxygen from the atmosphere, detected as a TMS ester after silylation. Furthermore, the oxidation of the residual phenolic residue results in the second degradation product, detected as 2,6-di-*tert*-butyl-*p*-benzoquinone.

**Table 3.** Main AO degradation products of artificial alteration under $^{16}O_2$ and $^{18}O_2$ atmosphere.

| $^{16}O_2$ | Analyte | Retention Time (min) | Calculated Monoisotopic *m/z* Value of Molecular Ion | Detected Monoisotopic Molecular Ion (RI %) | Highest Detected *m/z* Value in the Mass Spectrum |
|---|---|---|---|---|---|
| o | 2,6-di-*tert*-butyl-*p*-benzoquinone | 18.11 | 220.3 | 220.0 (40.2) | 220.0 |
| p | 3,5-di-*tert*-butyl-4-hydroxybenzoic acid TMS ester | 25.50 | 322.20 | 322.0 (14.5) | 322.0 |
| $^{18}O_2$ | Analyte | Retention Time (min) | Calculated Monoisotopic *m/z* Value of Molecular Ion | Detected Monoisotopic Molecular Ion (RI %) | Highest Detected *m/z* Value in the Mass Spectrum |
| o | 2,6-di-*tert*-butyl-*p*-benzoquinone | 18.11 | 220.3 | 220.0 (38.3) | 220.0 |
| p | 3,5-di-*tert*-butyl-4-hydroxybenzoic acid TMS ester | 25.52 | 326.2 | 326.0 (12.8) | 326.0 |
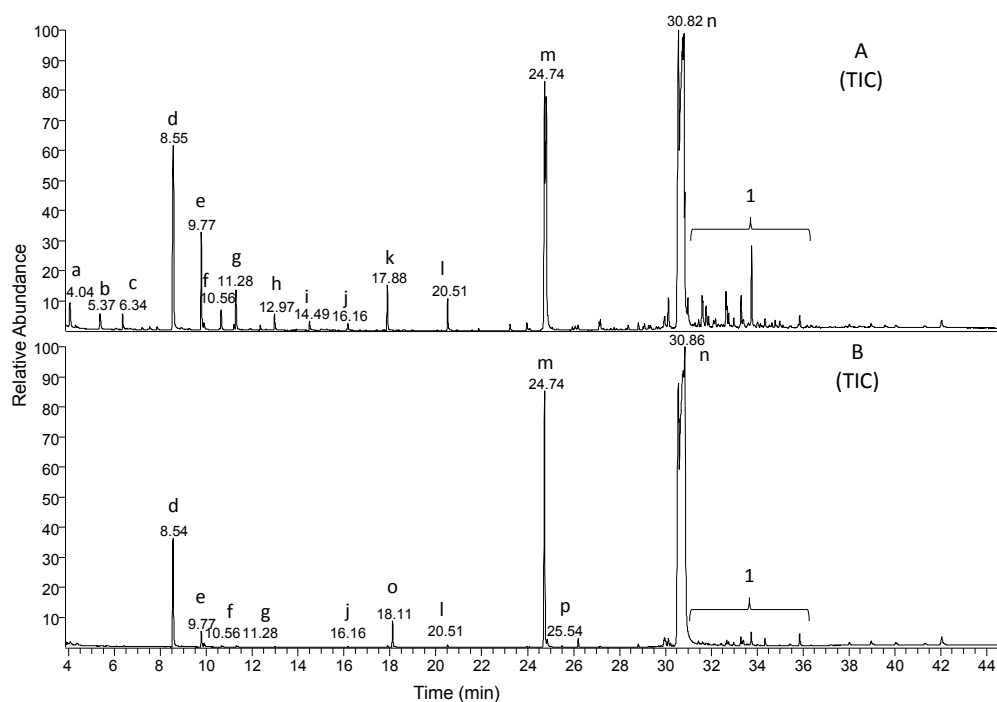
**Figure 10.** TIC chromatograms of alteration products obtained under 1 bar $^{16}O_2$ atmosphere without (**A**) and with 1 (*w/w*) % AO (**B**). Main products (a–m), AO degradation products (o, p), and target molecule (n, starting material) are marked and given in Tables 1–3. Peaks under bracket with label 1 indicates oxidized molecules of ester-based base oil bis(2-ethylhexyl) adipate.
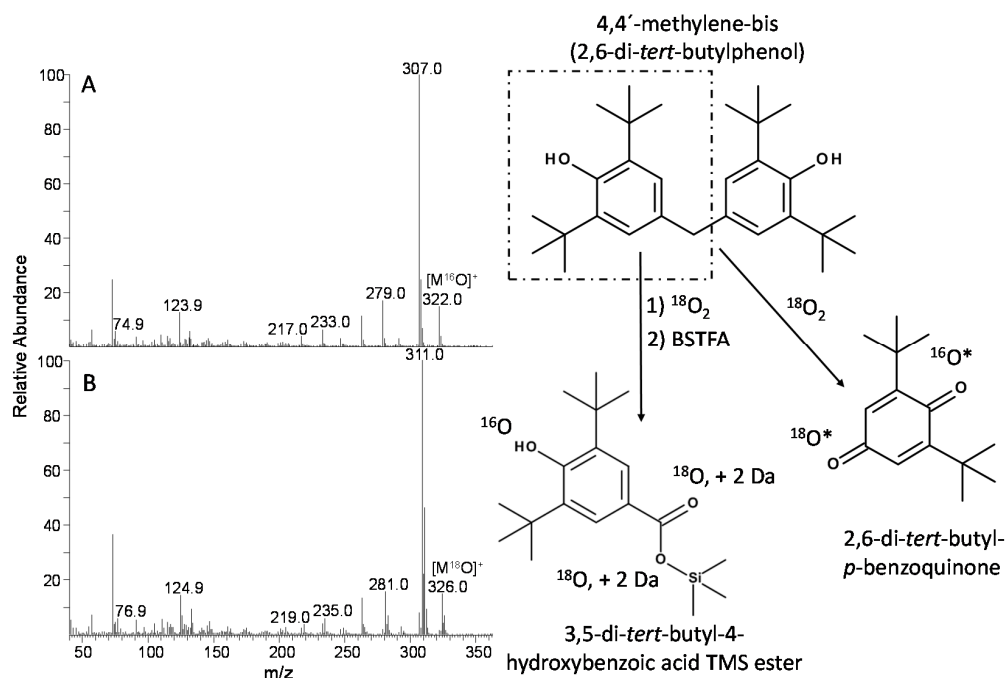


**Figure 11.** Positive ion mass spectra of 3,5-di-*tert*-butyl-4-hydroxybenzoic acid TMS ester (p) obtained after alteration under $^{16}O_2$ (**A**) and $^{18}O_2$ (**B**) atmosphere, respectively. Peaks of fragments where a change of the *m/z* value due to isotope labelling occurred are labelled with the respective values. A simplified oxidation reaction is suggested for the respective molecule. * signifies assignment of $^{16}O$ or $^{18}O$ atoms according to the most likely degradation pathway.

## 4. Conclusions

The unique usefulness of the isotope labelling approach based on mass spectrometry for the investigation of the thermo-oxidative stability of oxygen-containing lubricant components could be clearly demonstrated in this study. With a satisfactory reproducibility (in terms of the retention times and mass spectrometric peak pattern), neat diester bis(2-ethylhexyl) adipate, and a blend containing 1 (*w/w*) % antioxidant, 4,4′-methylene-bis(2,6-di-*tert*-butylphenol) was artificially altered in a closed vessel under $^{16}O_2$ and $^{18}O_2$ atmosphere, and subsequent high-performance capillary GC–EI–MS analysis was applied to identify the main degradation products containing varying numbers of $^{18}O$. Isotope labelling in combination with MS allowed a clear assignment of the location of the isotope-labelled oxygen in the degradation product, even in the case of a mix of $^{16}O$ and $^{18}O$.

The findings revealed the preferential bond cleavage sites in the diester molecule between the C–O ester bond and subsequent oxidative attack, resulting in 2-ethylhexyl fatty acid esters and 2-ethylhexyl alcohol. Furthermore, 2-ethylhexanoic acid is generated by the bond scission between the O atom and C1 of the alcohol, and is followed oxidation during alteration under the applied conditions. Moreover, the main degradation products were built after oxidative cleavage on one side or on both sides of the initial ester groups, to give 2-ethylhexyl 2-oxobutyl adipate and 2-ethylhexyl 6-oxohexanoate, respectively. 2-ethylhexyl 2-oxobutyl adipate was produced in distinct abundance, based on the oxidation between C2 and C3 of the alcohol chain. An increased pressure during the alteration seems to have no considerable impact on the quality of the formed degradation products, but the quantities vary, depending on the pressure level.

Base oil blends containing AO showed a far higher stability, resulting in an enhanced alteration duration until the end point. Although mainly the same degradation products were built, almost all of them showed a significantly lower abundance, in particular, short-chain fatty acids and esters thereof, as well as oxidized ester base oil molecules. The two main degradation products of the AO were obtained: 2,6-di-*tert*-butyl-*p*-benzoquinone and 3,5-di-*tert*-butyl-4-hydroxybenzoic acid.

In sum, the advanced analytical approach combining artificial alteration, isotope labelling, and mass spectrometry, proved to be an appropriate and powerful tool to unambiguously determine the degradation products of oxygen-containing lubricant components. Hence, the foundations have been laid for the elucidation of the underlying oxidation mechanisms.

Prospectively, the sampling of alteration aliquots at points of interest, e.g., before the pressure curve starts to drop, are planned. This opens up the way to develop a reliable prognostic tool (on the status of lubricant in certain devices for acting at an early stage) based on the early detection of certain quantities of degradation products long before NN or IR spectroscopy would indicate anything. GC–MS analysis shall reveal the time- and alteration state-dependent formation of major degradation products. For blends containing AO, the determination of the residual AO content at different alteration states is of high interest. For this purpose, various GC-MS techniques such as selected ion monitoring (SIM), multiple ion detection (MID), or low energy (collision induced dissociation) CID MS/MS will be applied. Moreover, this work showed that the conventional and advanced analytical data are in good accordance, e.g., lower NN values for samples containing less acidic compounds obtained with MS analysis. Based on the knowledge gained with such an advanced analysis, a correlation of the degradation products formed with conventional oil data such as NN and that obtainable by IR spectroscopy shall be achieved.

material) are marked and given in Tables 1 and 3. Peaks under bracket with label 1 indicate oxidized molecules of ester-based base oil bis(2-ethylhexyl) adipate. Figure S4: Structures of the main degradation products according to Tables 1 and 2.

**Author Contributions:** Marcella Frauscher established the experimental matrix and performed advanced analyses of the samples obtained by artificial alteration. Furthermore, she was responsible for the evaluation and interpretation of the derived data and main parts of the manuscript. Charlotte Besser carried out the conventional analysis, including the data evaluation, and wrote the results in this aspect and the experimental background. Nicole Dörr conducted the presented study and gave professional input. Günter Allmaier gave scientific support concerning mass spectrometry and revised the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. American Petroleum Institute (API), API 1509 2016, Appendix E. Available online: www.api.org (accessed on 5 December 2016).

2. Rudnick, L.E. *Synthetics, Mineral Oils, and Bio-Based Lubricants*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2014; pp. 47–74.

3. Global Market Insights Inc. Synthetic Lubricants Market Size to Exceed USD 5 Billion by 2023. Available online: www.gminsights.com (accessed on 5 December 2016).

4. Eastwood, J. Esters—The most versatile of base stock technologies. *Lube Mag.* **2015**, *129*, 32–37.

5. Nagendramma, P.; Kaul, S. Development of ecofriendly/biodegradable lubricants: An overview. *Renew. Sustain. Energy Rev.* **2012**, *16*, 764–774. [CrossRef]

6. Erhan, S.Z.; Sharma, B.K.; Perez, J.M. Oxidation and low temperature stability of vegetable oil-based lubricants. *Ind. Crop. Prod.* **2006**, *24*, 292–299. [CrossRef]

7. Salimon, J.; Salih, N.; Yousif, E. Improvement of pour point and oxidative stability of synthetic ester basestocks for biolubricant applications. *Arab. J. Chem.* **2012**, *5*, 193–200. [CrossRef]

8. Wu, Y.; Li, W.; Zhang, M.; Wang, X. Improvement of oxidative stability of trimethylolpropane trioleatelubricant. *Thermochim. Acta* **2013**, *569*, 112–118. [CrossRef]

9. Cavalcante, I.M.; de Rocha, N.R.C.; Maier, M.E.; Lima, A.P.D.; Neto, D.M.A.; Brito, D.H.A.; Petzhold, C.L.; Schanz, M.T.G.F.; Ricardo, N.M.P.S. Synthesis and characterization of new esters of oleic acid and glycerol analogues as potential lubricants. *Ind. Crop. Prod.* **2014**, *62*, 453–459. [CrossRef]

10. Housel, T. Synthetic esters: Engineered to perform. Available online: http://machinerylubrication.com/Read/29703/synthetic-esters-perform (accessed on 14 April 2017).

11. Gryglewicz, S.; Piechicki, W.; Gryglewicz, G. Preparation of polyol esters based on vegetable and animal fats. *Bioresour. Technol.* **2003**, *87*, 35–39. [CrossRef]

12. Bünemann, T.F.; Vank Aken, R.P. Synthetische Ester—-Wie kann man die Lebensdauer von biologisch schnell abbauenden Hydraulikflüssigkeiten verlängern. In *Tribology 2000—Plus, Proceedings of the 12th International Colloquium, Ostfildern, Germany, 11–13 January 2000*; Emerald Group Publishing Limited: Bingley, UK, 2000; pp. 109–110.

13. Moser, B.R.; Sharma, B.K.; Doll, K.M.; Erhan, S.Z. Diesters from Oleic Acid: Synthesis, Low Temperature Properties, and Oxidation Stability. *J. Am. Oil Chem. Soc.* **2007**, *84*, 675–680. [CrossRef]

14. Alias, N.H.; Yunus, R.; Idris, A.; Omar, R. Effects of additives on oxidation characteristics of palm oilbased trimethylolpropane ester in hydraulics applications. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 368–375. [CrossRef]

15. Chao, M.; Li, W.; Wang, X. Influence of antioxidant on the thermal–oxidative degradation behavior and oxidation stability of synthetic ester. *Thermochim. Acta* **2014**, *561*, 16–21. [CrossRef]

16. Deutsches Institut für Normung. *4263 Petroleum and Related Products—Determination of the Ageing Behaviour of Inhibited Oils and Fluids Using the TOST Test*; Deutsches Institut für Normung: Berlin, Germany, 2016.

17. Deutsches Institut für Normung. *51808 Testing of Lubricants—Determination of Oxidation Stability of Greases—Oxygen Method, Deutsches Institut für Normung (Draft)*; Deutsches Institut für Normung: Berlin, Germany, 2015.

18. ASTM International. *D 2272 Standard Test Method for Oxidation Stability of Steam Turbine Oils by Rotating Pressure Vessel*; ASTM International: West Conshohocken, PA, USA, 1998.

19. Santos, J.C.O.; Santos, I.M.G.; Souza, A.G. Thermal degradation process of synthetic lubricating oils: Part I—Spectroscopic study. *Petrol. Sci. Technol.* **2015**, *33*, 1238–1245. [CrossRef]

20. Keller, M.A.; Saba, C.S. Gas chromatographic monitoring of hydroxyl components in oxidized turbine engine lubricants. *Tribol. Trans.* **2003**, *46*, 576–579. [CrossRef]

21. Tripathi, A.K.; Vinu, R. Characterization of thermal stability of synthetic and semi-synthetic engine oils. *Lubricants* **2015**, *3*, 54–79. [CrossRef]

22. Frauscher, M.; Besser, C.; Dörr, N.; Allmaier, G. Unambiguous identification of degradation products related to elucidation of the degradation mechanism of oxygen containing fuel components by shared use of isotope labelling and mass spectrometry. *Anal. Chem.* **2017**. submitted.

23. Besser, C.; Pisarova, L.; Frauscher, M.; Hunger, H.; Dörr, N.; Litzow, U.; Orfaniotis, A. Oxidation products of biodiesel in diesel fuel generated by artificial alteration and identified by mass spectrometry. *Fuel* **2017**, submitted.

24. Deutsches Institut für Normung. *DIN 51558 Testing of Mineral Oils, Determination of Neutralization Number by Color-Indicator Titration*; Deutsches Institut für Normung: Berlin, Germany, 1979.

25. Deutsches Institut für Normung. *DIN 51777-2 Testing of Mineral Oil Hydrocarbons and Solvents, Determination of Water Content According to Karl Fischer; Indirect Method*; Deutsches Institut für Normung: Berlin, Germany, 1974.