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Stability Report
Kānuka oil

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1.0	Convert 12 month summary report to final format.	David Speed	16 Feb 2025
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1. Executive Summary

This report summarises investigations made into the stability of kānuka oil, which were commissioned by Hikurangi Bioactives Limited Partnership, and the Kānuka Charitable Trust. No robust data was previously available for this type of oil, and for the establishment of a viable kānuka oil industry in Aotearoa New Zealand, it is important to be able to set a sensible "best before" date, so that users can be assured of the continuing quality of materials.

This stability study has been carried out in parallel with other related work, which included validation and accreditation of a method for quantitative determination of the terpenoids α-pinene, p-cymene, and viridiflorol in kānuka oil. This validation has been assessed by IANZ, and analytical reports issued after the end of 2024 will be accredited and permitted to bear the IANZ logo confirming compliance with ISO17025.

This stability report presents results of stress testing of two kānuka oils, as well as results at 4, 8, 12 and 26 months of long-term storage under different conditions.

Initial data from stress testing experiments shows that both of the oils under investigation are resistant to most of the acute conditions assessed (acid, hydrogen peroxide, UV light, and temperature). Exposure to base did result in visible and measurable changes, however typical storage conditions (dark, under nitrogen) should be effective in excluding this risk. It is considered reasonable to expect that good stability will be observed under these conditions.

Long term storage of the oil has been carried out for 26 months, and additional samples remain in storage for future assessment if desired. The storage conditions include a range of assessments including elevated temperature (40 °C) which is considered an "accelerated" stability condition, since higher temperatures lead to an increase in reaction rates. In pharmaceutical science, data generated under accelerated conditions which shows no significant change may be used to extrapolate a longer retest period (up to twice, but not more than 12 months beyond the period covered by data under normal storage condiitons), and this approach will be applied for the current study if supported by the data (see ICH Q1E guideline).

After 26 months of storage, no significant changes in the major terpenoid components of kānuka oil were observed (i.e. α-pinene, p-cymene and viridiflorol) under most storage conditions. Peroxide value did change significantly in some storage conditions – indicating oxidation processes occurring, and GC-MS and LC-HRMS also revealed differences in the less abundant oil components.

Oils in contact with metals (aluminium, galvanised steel and stainless steel) with a high surface area to volume ratio were observed to increase in concentration of aluminium, zinc and iron; however a commercial oil stored long term in a large stainless keg showed no change in metal levels. Where metal storage containers are used, it is recommended that they are of stainless steel (e.g. type 304), of at least 5 L in volume and with a high-quality finish to minimise surface area.

Storage in plastic led to a range of changes in the measured parameters, and plastic containers are not considered suitable for storage of kānuka oils.

Oil stored in clear glass vials placed on a windowsill that receives no direct sunlight showed obvious colour changes; however most other properties were not significantly affected, suggesting that the colour change was due to very minor components. Oils stored in amber glass vials, in a dark room, showed good stability.

Changing the vial headspace from nitrogen to air had little effect overall, and this measure is not considered critical; however as a simple precaution it is still recommended for adoption.

Storage at elevated temperature also did not have a significant impact. Some increase in p-cymene is tentative evidence for degradative processes.

All oils at ambient or higher temperature showed a decrease in pH, the reason for this is not currently understood. After 26 months the change was 0.6 to 0.8 units, and even oil stored at -20 °C decreased in pH by 0.3-0.5 units. Depending on the intended purpose of the oil, this change may have some significance.

Overall however, the data acquired to date shows that no significant change occurs to the important characteristics of kānuka oil, when stored in glass containers away from light, under a nitrogen atmosphere, at ambient temperatures, for up to 26 months. No adverse impact was observed for oil stored in 5 L or larger stainless steel kegs. Very little impact was observed for oil stored at the accelerated storage condition of 40 °C in glass containers away from light with a nitrogen atmosphere, and up to **3 years** of stability at ambient temperature is therefore considered reasonable. This finding is made with the provisos that:

- Where particular components of kānuka oil are responsible for providing a function then these components (or their function) should be specifically investigated. (The data acquired to date may be suitable for performing this assessment.)
- Changes in pH were observed during long term storage, and this should be taken into account.

See section 7.3 for further discussion of results to date from the long-term storage study.

Figure 2 contains plots of the change in numerical parameters measured throughout the study for the sample oils stored in amber glass vials at ambient temperature, with nitrogen headspace. Linear trends have been plotted and extrapolated to 36 months. Changes in α-pinene, viridiflorol are projected to be insignificant after 36 months. P-Cymene increased in both oils, but is still projected to be within the observed range for kānuka oils after 36 months. Peroxide value data is noisy, but shows an increasing trend over time, indicating that oxidative processes are occurring. pH is decreasing consistently, but would be expected to reach an equilibrium level, although at what level this would occur is not established.

If the projected trends are correct, then changes in both pH and peroxide value could indicate changes that begin to have an impact on some applications of the oil after extended storage. The increasing trend in p-cymene content may be an outcome of the same processes causing pH and POV changes, since p-cymene is a known oxidation product of other terpenoids.

However, on the basis of current information, none of the changes projected at 36 months would be of a magnitude rendering the material inconsistent with kānuka oil. Mānuka essential oil available commercially is shipped with a stated best before date of at least 4 years (author's observation for at least three suppliers). This is a similar use period as suggested for a range of other essential oils (e.g. rosemary, tea tree, eucalyptus).

2. Glossary

LOD	Limit of detection
LOQ	Limit of quantitation
NA	Not applicable
POV	Peroxide value, a measure of the oxygen content as peroxides, which is commonly used as a measure of spoiling of oils.
RSD or %RSD	Relative standard deviation. The standard deviation expressed as a percentage of the mean. Replicates determined on the same day should have RSD ≤10%
% w/w	Percentage concentration on weight basis. A result of 1 % w/w means that 1 g of sample contains 0.01 g of the analyte.

3. Study preparation

3.1 Purpose

Hikurangi Bioactives Limited Partnership and the Kānuka Charitable Trust are investigating kānuka oil for a number of purposes, and they are aware that there is no publicly available data regarding the stability of this oil. Discussion with commercial kānuka oil producers suggests that the properties of kānuka oil are maintained for more than 4 years, and it is desired to obtain evidence to support this claim.

Two samples of kānuka oil were supplied to ASL Food and Environmental NZ to conduct a stability trial for kānuka oil.

3.2 Stress testing

Stress tests were carried out on each oil. These are designed to give a rapid indication of changes that might occur slowly under normal storage conditions. The conditions employed were:

- Hydrolysis (acid, 40 °C for 1 hour)
- Hydrolysis (base, 40 °C for 1 hour)
- Oxidation (peroxide, 40 °C for 1 hour)
- Temperature (60 °C for 1 hour)
- Photolytic (direct exposure to UV light source for 1 hour)

The results of testing on these samples have been used to make the following assessments:

- Are any changes noted as a result of the stress test? Changes noted under these extreme conditions will be checked for at future dates to see if they occur under typical storage conditions. Observation of such changes can then be related to the stress condition producing the effect.
- If a test does not reveal any changes as a result of the stress conditions, then it may be removed from the study since it is unlikely to reveal any useful information over time.
- If no changes are observed as a result of a stress test, then this lends support to the oil being stable to that condition.

3.3 Long term storage

Samples of the oils have been stored under a range of conditions, designed to represent a range of real-world circumstances. These include:

Table 1: Summary of conditions for long term storage assessment

Treatment	Vial type	Headspace	Conditions
A: Control conditions, designed to minimise any change	Amber glass	Nitrogen	-20 °C, dark
B: To assess exposure to metal components, e.g. storage drum	Amber glass, with aluminium and steel inserts	Nitrogen	Ambient, dark
C: Normal storage	Amber glass	Nitrogen	Ambient, dark
D: To assess need for inert atmosphere	Amber glass	Air	Ambient, dark
E: To assess impact of plastic storage	Polypropylene	Nitrogen	Ambient, dark
F: Accelerated storage condition	Amber glass	Nitrogen	40 °C, dark
G: Exposure to light	Clear glass	Nitrogen	Ambient, daylight

The initial weight of vials was recorded so that changes due to evaporative loss can be measured.

3.4 Key dates

Table 2: Key dates for study

T = 0 (set down of samples)	10 Jul 2023
Sample pull T = 4 months	24 Nov 2023
Sample pull T = 8 months	19 Mar 2024
Sample pull T = 12 months	09 Jul 2024
Sample pull T = 26 months	22 Sep 2025

4. Study parameters

4.1 Appearance

Changes in appearance of the oil, e.g. becoming cloudy, lighter or darker in colour, are indicative of chemical or microbial changes taking place, and provide a simple and direct assessment of quality.

4.2 pH

Provides a measure of the change in water soluble, acidic/basic components.

4.3 Conductivity

Provides a measure of the change in water soluble, conductive components

4.4 Terpenoid analysis (α-Pinene, p-cymene and viridiflorol)

α-Pinene is the main component of kānuka oil, and the other specified terpenoids are characteristic of this oil. Significant changes in the levels of these compounds would be considered critical for the quality of the oil.

4.5 GC-MS profile

In addition to the specified terpenoids above, kānuka oil contains many other volatile components which may contribute to its distinctive aroma. A GC-MS profile can be used to estimate the percentage contributions from these different components, and changes in the relative proportions would therefore be indicative of changes in the composition. In addition, appearance of new components in the profile would also indicate changes occurring over time. Storage of the GC-MS full scan data will allow investigation of the identities of changing components at a future date if significant targets are identified.

4.6 LC-HRMS profile

Kānuka oil also contains other, less volatile, components which may be more amenable to LC-MS analysis. In order to assess the widest range possible of components, an extract of the oils will be analysed by liquid chromatography with high resolution mass spectroscopy (LC-HRMS). Similarly to the GC-MS profile, the LC-HRMS chromatogram can be used to estimate the percentage contributions from different components, and measure changes occurring over time. It will also identify the appearance of new components, and the stored LC-HRMS full scan data will allow investigation of the identities of changing components at a future date if significant targets are identified.

4.7 Water content

Changes in water content in this study would most likely be due to deficient packaging, and this parameter has been included as a quality check on the effectiveness of the packaging used.

4.8 Peroxide value

A commonly used measure of degree of oxidation (i.e. degradation), an increase in this value would indicate that oxidising activity has occurred, which may have consequences for some intended uses.

4.9 Heavy metals

A change in heavy metal content would indicate that an interaction is occurring with the metal storage container. The drums used for oil storage are plastic lined, however the highly non-polar oil may permeate through some plastics, and defects in the plastic lining could lead to exposure of the oil. This test is being carried out to assess whether deliberate exposure to metals could result in uptake of metals, and whether such uptake could cause degradation to occur.

5. Methods

5.1 Stress testing

Portions of oil were treated as follows:

Table 3: Preparation of stress tested oils

Treatment	Volume of oil	Solution added	Conditions
1: Acid	15 mL	15 mL of 1M hydrochloric acid	40 °C water bath, 1 hour
2: Acid	2 mL	2 mL of 1M sulfuric acid	Room temp, 24 hours
3: Acid	2 mL	2 mL of 1M nitric acid	Room temp, 24 hours
4: Base	15 mL	15 mL of 1M sodium hydroxide	40 °C water bath, 1 hour
5: Base	2 mL	2 mL of 10% ammonia solution	Room temp, 24 hours
6: Base	2 mL	2 mL of 10% sodium hypochlorite	Room temp, 24 hours
7: Oxidation	15 mL	15 mL of 10% hydrogen peroxide	40 °C water bath, 1 hour
8: Oxidation	2 mL	2 mL of 1M potassium permanganate	Room temp, 24 hours
9: Temperature	15 mL	None	60 °C water bath, 1 hour
10: Photolysis	15 mL	None	UV lamp, lid off, 1 hour
11: Reference	15 mL	None	Lid off one hour, ambient

5.2 Long term storage

Table 4: Conditions used for long term stability assessment

Treatment	Storage location
A: Control conditions	Freezer
B: To assess exposure to metal	Basement vault, vial contains paper clip and Al foil [Note 1]
C: Normal storage	Basement vault

D: To assess need for inert atmosphere	Basement vault
E: To assess suitability of storage in plastic containers	Basement vault
F: Accelerated storage condition	40 °C incubator
G: Exposure to light	Windowsill in office

Note 1: A section of stainless steel was added to each of these vials on 07 Sep 2023

5.3 Appearance

The sample is viewed against a white paper background under natural lighting, and compared to the control sample of the starting oil (stored frozen).

5.4 pH

2 mL of oil and 2 mL of type 1 water are vortex mixed for 30 seconds, then allowed to settle. The lower aqueous layer is transferred to a clean vessel, and pH measured using a calibrated meter.

5.5 Conductivity

Using the aqueous extract prepared for pH measurement, conductivity will be measured using a calibrated meter.

5.6 Terpenoid analysis (α-Pinene, p-cymene and viridiflorol)

The oil is diluted in acetone containing a system monitoring compound and internal standard solution, and analysed by GC-MS, against a calibration curve. In order to assess any variation between analytical batches, the control oils (stored frozen) will be analysed on each occasion as a quality control.

Results are reported in % w/w.

5.7 GC-MS profile

The sample prepared for terpenoid analysis is also acquired in full scan mode.

5.8 HRMS profile

100 µL oil is added to 1 mL of 10% ACN in water and vortex mixed for 30 seconds. The layers are allowed to separate and the lower layer transferred to an HPLC vial for analysis by LC-HRMS.

5.9 Water content

Samples in 20 mL amber glass vials are shipped to Flinders-Cook (Auckland) for this test. A Karl Fischer titration is employed for determination of water content, which is reported in % w/w.

5.10 Peroxide value

Samples in 20 mL amber glass vials are shipped to Flinders-Cook (Auckland) and analysed according to their standard test procedure (compliant with AOCS Cd 8-53).

5.11 Heavy metals

2 mL portions of oil are digested and analysed by ICP-MS using Analytica's in-house method.

5.12 Equipment and materials

Table 5: Equipment list

Equipment ID	Description
EN21-0065	Hannah "Edge" pH meter
EN14-003	GC-MS Instrument TSQ
EN16-161	GC-MS Instrument MSD

EN21-0020	Conductivity meter
EN22-1033	LC-HRMS instrument, Thermo Fischer Exploris 480
EN21-1099	ICP-MS instrument, Perkin Elmer

Two bulk samples of kānuka oil were provided by the client for the study.

Table 6: Test materials

Analytica ID	Name
23-10912-1	Sample One, produced Mar-Apr 2023
23-10912-2	Sample Two, produced May 2022

6. Results

The following sections summarise results from the range of testing carried out.

Each sample vial was weighed when removed from storage, and before sampling for analysis, weights were within 99.9 to 100.8% of the starting weight, with the exception of samples stored in plastic, which decreased in mass to 97.1% after three months, to 92.8% after 12 months, and as low as 78.5% after 26 months.

NA = Not applicable (e.g. tests not carried out where the treatment used would interfere with the results, or where the test is not expected to give useful information for the sample storage conditions)

6.1 Sample One (23-10912-1):

	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-10912-1	T=0	Pale straw coloured, slightly viscous oil	3.41	136.6 (21.3)	52	1.7	2.6	0.14	8.3	1.1	<1	<0.1	<0.4
23-10912-1-1	Stress test: Acid	No change	NA	NA	56	1.7	2.7	NA	NA	NA	NA	NA	NA
23-10912-1-4	Stress test: Base	Cloudy colourless oil	NA	NA	55	1.8	2.5	NA	NA	NA	NA	NA	NA
23-10912-1-7	Stress test: oxidation	No change	3.48	98.5 (21.0)	55	1.8	2.5	NA	NA	NA	NA	NA	NA
23-10912-1-9	Stress test: Heat	No change	3.44	114.7 (21.0)	54	1.7	2.6	NA	10	NA	NA	NA	NA
23-10912-1-10	Stress test: UV	No change	3.31	107.6 (21.2)	55	1.9	2.3	NA	12	NA	NA	NA	NA
23-10912-1-11	Stress test: Reference/Air	No change	3.37	106.3 (21.3)	55	1.7	2.7	NA	NA	NA	NA	NA	NA

6.1.1 T = 4 months

23-20489-1	N_2 HS, -20 °C, amber glass	Pale straw coloured, slightly viscous oil	3.3	NA	60	1.9	2.9	NA	NA	NA	NA	NA	NA
23-20489-2	N_2 HS, ambient with metal contact, amber glass	Yellow brown oil	3.28	NA	60	2.1	2.9	NA	NA	2.2	13	<0.1	9.4
23-20489-3	N_2 HS, ambient, amber glass	Same as frozen sample	3.01	NA	59	1.9	3.0	0.13	31	NA	NA	NA	NA
23-20489-4	Air HS, ambient, amber glass	Same as frozen sample	3.06	NA	59	2.0	2.8	NA	24	NA	NA	NA	NA
23-20489-5	N_2 HS, ambient, plastic container	Same as frozen sample	2.95	NA	59	2.2	3.0	0.12	50	NA	NA	NA	NA
23-20489-6	N_2 HS, 40 °C, amber glass	Same as frozen sample	2.93	NA	58	2.3	3.0	NA	10	NA	NA	NA	NA
23-20489-44	N_2 HS, ambient, colourless glass on windowsill	Clear peach/sepia oil	2.97	NA	59	2.2	3.0	NA	11	NA	NA	NA	NA

6.1.2 T = 8 months

	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-20489-15	N_2HS, -20 °C, amber glass	Pale straw coloured, slightly viscous oil	3.19	NA	60	2.1	2.7	NA	NA	NA	NA	NA	NA
23-20489-19	N_2HS, ambient with metal contact, amber glass	Yellow brown oil	2.98	NA	NA	NA	NA	NA	NA	4.3	27.0	<0.1	2.4
23-20489-20	N_2HS, ambient, amber glass	Same as frozen sample	3.02	NA	57	2.2	2.4	0.13	17	NA	NA	NA	NA
NA	Air HS, ambient, amber glass	Not available (insufficient material to set down more of these samples)											
23-20489-28	N_2HS, ambient, plastic container	Same as frozen sample	2.78	NA	57	2.4	2.5	0.17	140	NA	NA	NA	NA
23-20489-36	N_2HS, 40 °C, amber glass	Similar to frozen sample but stronger yellow	2.84	NA	57	2.5	2.4	NA	7.3	NA	NA	NA	NA
23-20489-7	N_2HS, ambient, colourless glass on windowsill	Clear peach/sepia oil	2.74	NA	52	2.4	2.5	NA	8.1	NA	NA	NA	NA

6.1.3 T = 12 months

	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-20489-16	N_2 HS, -20 °C, amber glass	Light yellow oil	3.21	NA	55	1.6	2.4	NA	NA	NA	NA	NA	NA
23-20489-19	N_2 HS, ambient with metal contact, amber glass	Not available (insufficient material to set down more of these samples)											
23-20489-21	N_2 HS, ambient, amber glass	Light yellow oil	2.86	NA	54	2.0	2.5	0.13	16	NA	NA	NA	NA
NA	Air HS, ambient, amber glass	Not available (insufficient material to set down more of these samples)											
23-20489-29	N_2 HS, ambient, plastic container	Light yellow oil	2.69	NA	52	2.4	3.1	0.23	160	NA	NA	NA	NA
23-20489-37	N_2 HS, 40 °C, amber glass	Light to medium yellow oil	2.86	NA	55	2.0	2.6	NA	3.0	NA	NA	NA	NA
23-20489-45	N_2 HS, ambient, colourless glass on windowsill	Peach/sepia oil	2.80	NA	54	1.9	2.3	NA	2.9	NA	NA	NA	NA

6.1.4 T = 26 months

	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-20489-17	N_2 HS, -20 °C, amber glass	Light yellow oil	Light yellow oil	3.08	NA	59	2.4	2.5	0.14	25	NA	NA	NA
23-20489-19	N_2 HS, ambient with metal contact, amber glass	Not available (insufficient material to set down more of these samples)											
23-20489-22	N_2 HS, ambient, amber glass	Light yellow oil	2.83	NA	61	2.7	2.6	0.15	47	NA	NA	NA	NA
NA	Air HS, ambient, amber glass	Not available (insufficient material to set down more of these samples)											
23-20489-30	N_2 HS, ambient, plastic container	Light to medium yellow oil	2.72	NA	34	1.2	4.5	NA	NA	NA	NA	NA	NA
23-20489-38	N_2 HS, 40 °C, amber glass	Yellow oil	2.74	NA	58	3.2	2.6	0.094	19	NA	NA	NA	NA
23-20489-46	N_2 HS, ambient, colourless glass on windowsill	Light yellow oil	2.84	NA	61	3.0	2.5	NA	NA	NA	NA	NA	NA

6.2 Sample Two (23-10912-2):

	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-10912-2	T=0	Light yellow, slightly viscous oil	3.67	90.4 (21.5)	27	2.3	2.7	0.11	6.8	3.2	<1	<0.1	<0.4
23-10912-2-1	Stress test: Acid	No change	NA	NA	28	2.4	2.6	NA	NA	NA	NA	NA	NA
23-10912-2-4	Stress test: Base	Cloudy orange oil	NA	NA	33	2.8	3.3	NA	NA	NA	NA	NA	NA
23-10912-2-7	Stress test: oxidation	No change	3.33	119.4 (21.1)	29	2.5	2.6	NA	NA	NA	NA	NA	NA
23-10912-2-9	Stress test: Heat	No change	3.45	76.5 (21.0)	28	2.5	2.3	NA	11	NA	NA	NA	NA
23-10912-2-10	Stress test: UV	No change	3.53	73.2 (21.3)	27	2.3	2.8	NA	13	NA	NA	NA	NA
23-10912-2-11	Stress test: Reference/Air	No change	3.52	73.6 (21.0)	28	2.3	2.7	NA	NA	NA	NA	NA	NA

6.2.1 T = 4 months

23-20489-52	N_2 HS, -20 °C, amber glass	Light yellow, slightly viscous oil	3.44	NA	31	2.5	3.3	TBD	TBD	NA	NA	NA	NA
23-20489-56	N_2 HS, ambient with metal contact, amber glass	Reddish brown oil	4.17	NA	30	2.7	3.3	TBD	TBD	4.6	302	<0.1	74.5
23-20489-57	N_2 HS, ambient, amber glass	Same as frozen sample	3.19	NA	29	2.5	3.2	0.11	19	NA	NA	NA	NA
23-20489-93	Air HS, ambient, amber glass	Same as frozen sample	3.2	NA	30	2.5	3.2	TBD	15	NA	NA	NA	NA
23-20489-65	N_2 HS, ambient, plastic container	Same as frozen sample	3.14	NA	30	2.8	3.4	0.12	29	NA	NA	NA	NA
23-20489-73	N_2 HS, 40 °C, amber glass	Same as frozen sample	2.96	NA	29	2.8	3.3	TBD	10	NA	NA	NA	NA
23-20489-81	N_2 HS, ambient, colourless glass on windowsill	Light brown/yellow	3.02	NA	28	2.6	3.3	TBD	8.3	NA	NA	NA	NA

6.2.2 T = 8 months

	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-20489-8	N_2 HS, -20 °C, amber glass	Light yellow, slightly viscous oil	3.37	NA	30	2.4	3.2	NA	NA	NA	NA	NA	NA
23-20489-9	N_2 HS, ambient with metal contact, amber glass	Reddish brown oil	3.44	NA	NA	NA	NA	NA	NA	5.8	723	<0.1	34.6
23-20489-10	N_2 HS, ambient, amber glass	Same as frozen sample	3.02	NA	31	2.5	3.1	0.12	12	NA	NA	NA	NA
23-20489-11	Air HS, ambient, amber glass	Same as frozen sample	3.09	NA	31	2.6	3.2	NA	9.1	NA	NA	NA	NA
23-20489-12	N_2 HS, ambient, plastic container	Same as frozen sample	2.92	NA	30	2.8	3.3	0.12	29	NA	NA	NA	NA
23-20489-13	N_2 HS, 40 °C, amber glass	Same as frozen sample	2.88	NA	29	2.8	3.1	NA	4.3	NA	NA	NA	NA
23-20489-82	N_2 HS, ambient, colourless glass on windowsill	Light brown/yellow oil	2.89	NA	30	2.7	3.1	NA	3.7	NA	NA	NA	NA

6.2.3 T = 12 months

	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-20489-53	N_2 HS, -20 °C, amber glass	Light yellow oil	3.37	NA	28	2.1	3.3	NA	NA	NA	NA	NA	NA
23-20489-9	N_2 HS, ambient with metal contact, amber glass	Not available (insufficient material to set down more of these samples)											
23-20489-58	N_2 HS, ambient, amber glass	Light yellow oil	2.99	NA	28	2.3	3.3	0.12	9.2	NA	NA	NA	NA
23-20489-94	Air HS, ambient, amber glass	Light yellow oil	3.06	NA	28	2.2	3.3	NA	4.8	NA	NA	NA	NA
23-20489-66	N_2 HS, ambient, plastic container	Light yellow oil	2.90	NA	28	2.5	3.5	0.11	32	NA	NA	NA	NA
23-20489-74	N_2 HS, 40 °C, amber glass	Light to medium yellow oil	2.89	NA	28	2.3	3.3	NA	1.7	NA	NA	NA	NA
23-20489-14	N_2 HS, ambient, colourless glass on windowsill	Light brown-yellow oil	2.83	NA	28	2.3	3.5	NA	1.5	NA	NA	NA	NA

6.2.4 T = 26 months

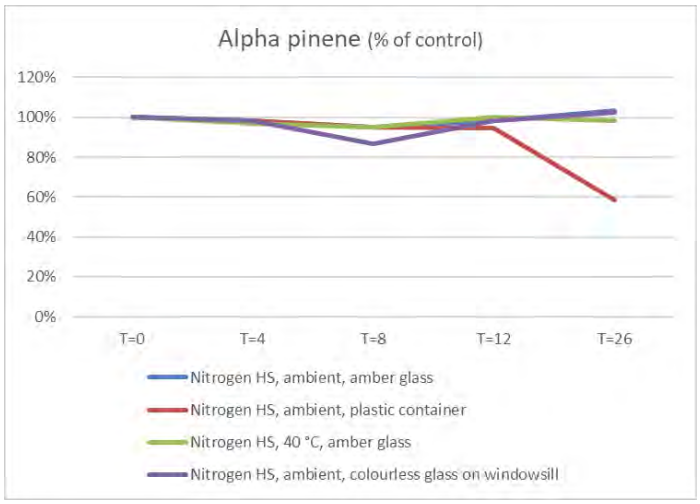
	Time/treatment	Appearance	pH	Conductivity	α-Pinene	p-Cymene	Viridiflorol	Water	Peroxide value	Al	Fe	Cr	Zn
ID	Units	NA	NA	µS/cm (temp in °C)	% w/w	% w/w	% w/w	% w/w	meq/kg	mg/L	mg/L	mg/L	mg/L
23-20489-54	Nitrogen HS, -20 °C, amber glass	Light yellow oil	3.14	NA	30	3.1	3.3	0.11	8.8	NA	NA	NA	NA
23-20489-9	Nitrogen HS, ambient with metal contact, amber glass	Not available (insufficient material to set down more of these samples)											
23-20489-59	Nitrogen HS, ambient, amber glass	Light yellow oil	2.90	NA	30	3.5	3.4	0.16	19	NA	NA	NA	NA
23-20489-95	Air HS, ambient, amber glass	Light yellow oil	2.97	NA	30	2.9	3.4	NA	NA	NA	NA	NA	NA
23-20489-67	Nitrogen HS, ambient, plastic container	Light yellow oil	2.94	NA	26	3.4	3.6	NA	NA	NA	NA	NA	NA
23-20489-75	Nitrogen HS, 40 °C, amber glass	Yellow oil	3.04	NA	28	3.7	3.4	0.11	9	NA	NA	NA	NA
23-20489-83	Nitrogen HS, ambient, colourless glass on windowsill	Light yellow oil	2.92	NA	29	3.3	3.5	NA	NA	NA	NA	NA	NA

6.3 Summary plots

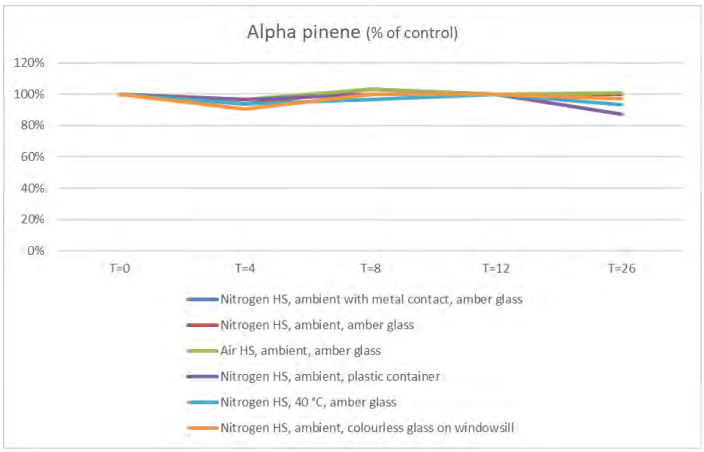
The numerical parameters measured throughout the study are summarised in the plots below.

Table 7: Plots of terpenoid results throughout study.

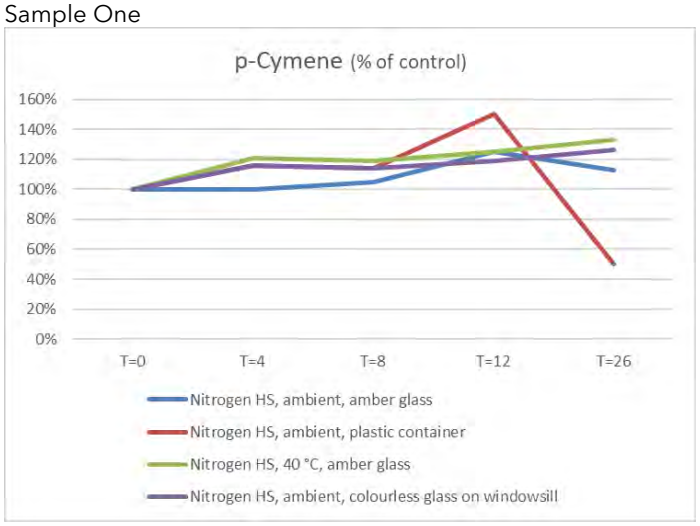
Sample One



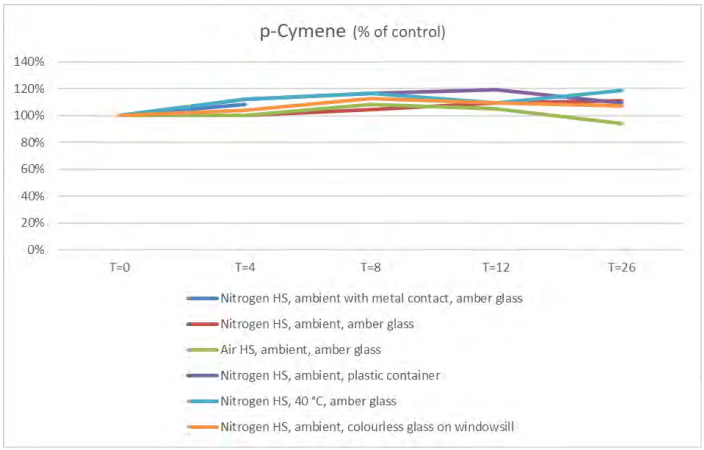
Sample Two



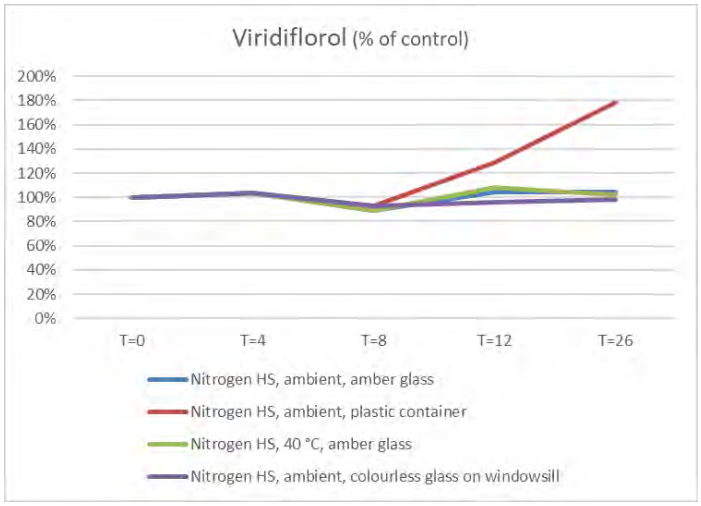
Sample One



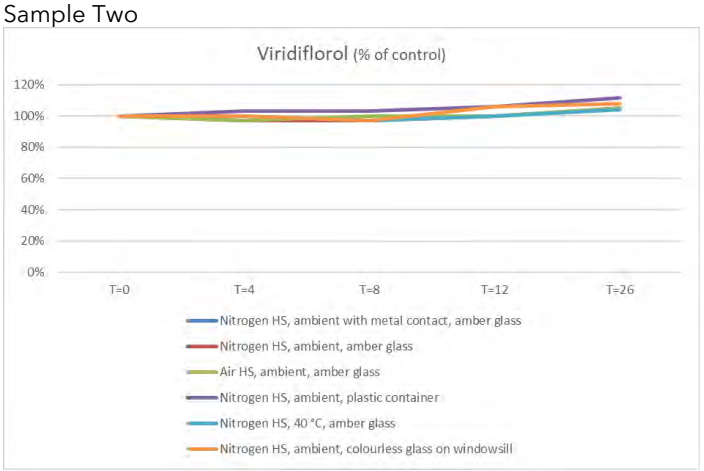
Sample Two



Sample One



Sample Two



Additional information about terpenoid concentrations is available from the quality control oil, which has been analysed periodically for more than two years while being stored in a refrigerator.

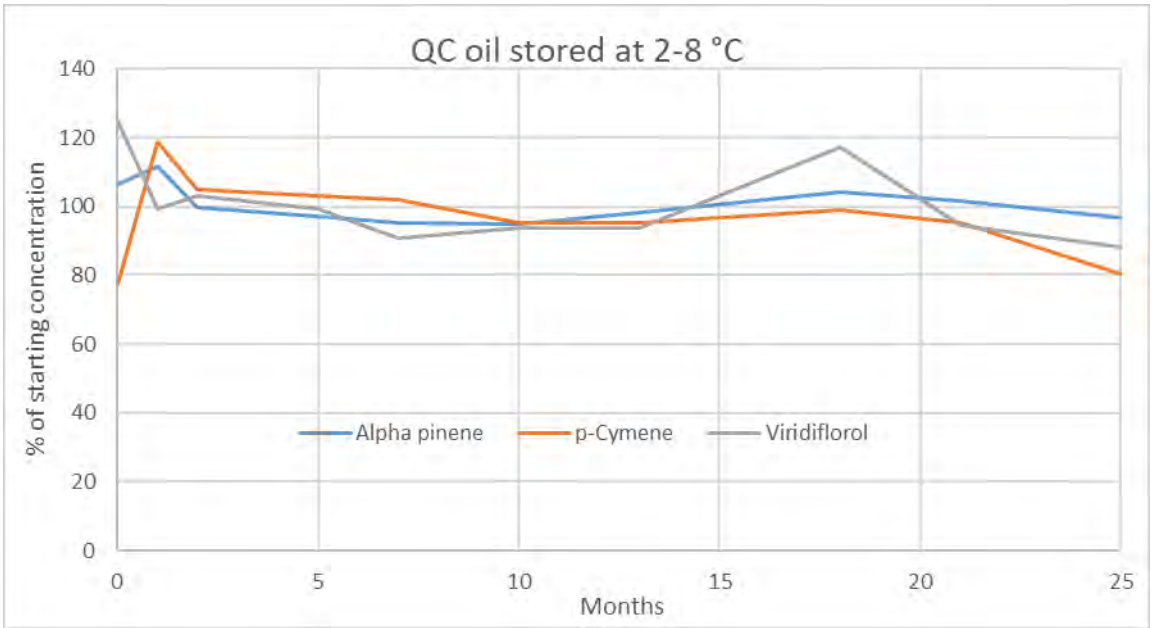
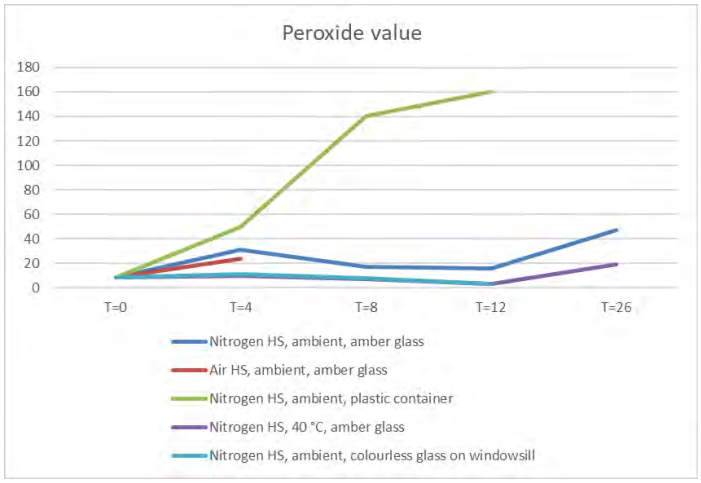


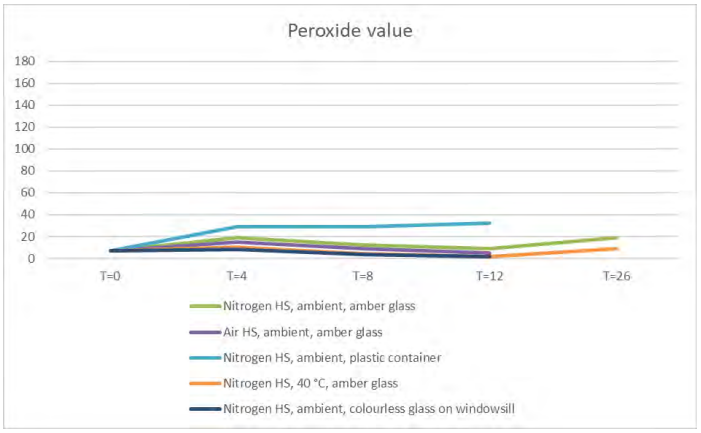
Figure 1: Terpenoid concentrations in oil stored in a fridge for over two years.

Table 8: Plots of peroxide value, pH, and moisture results throughout study.

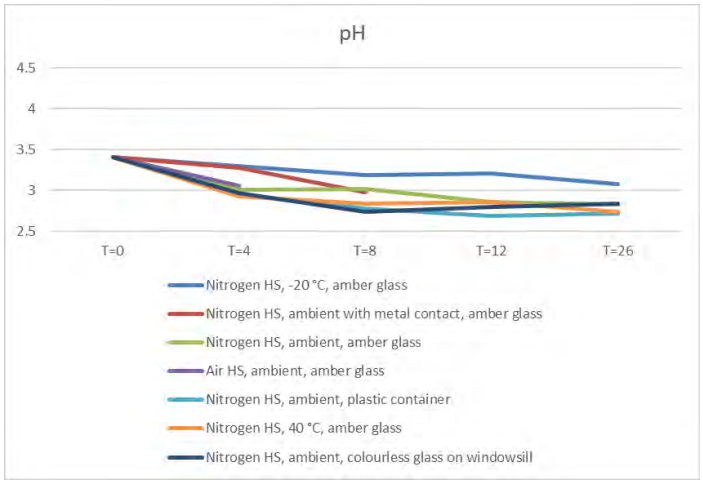
Sample One



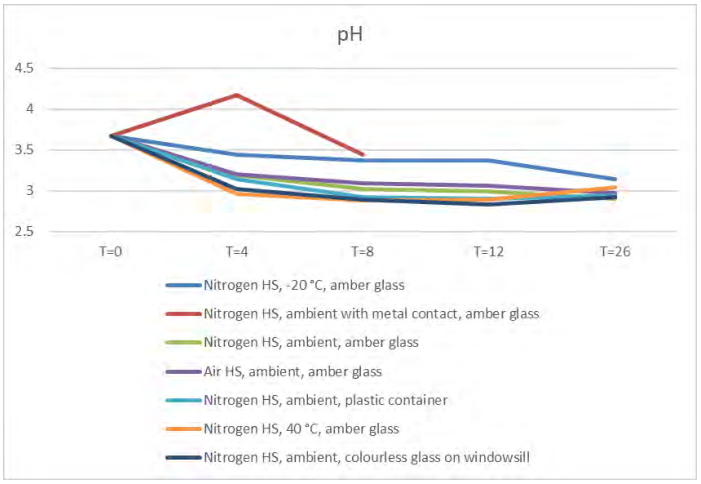
Sample Two



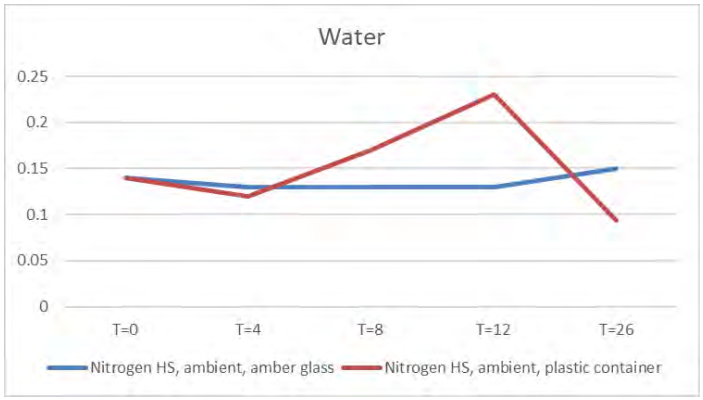
Sample One



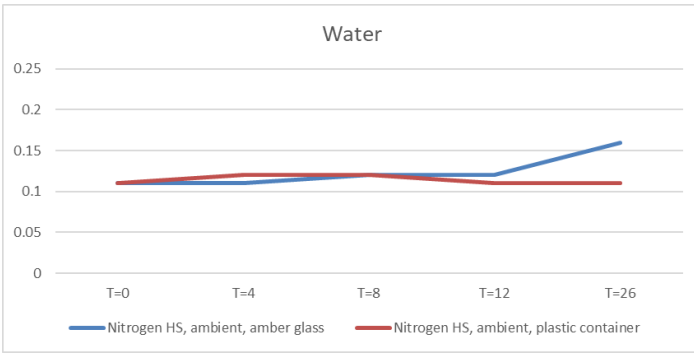
Sample Two



Sample One



Sample Two



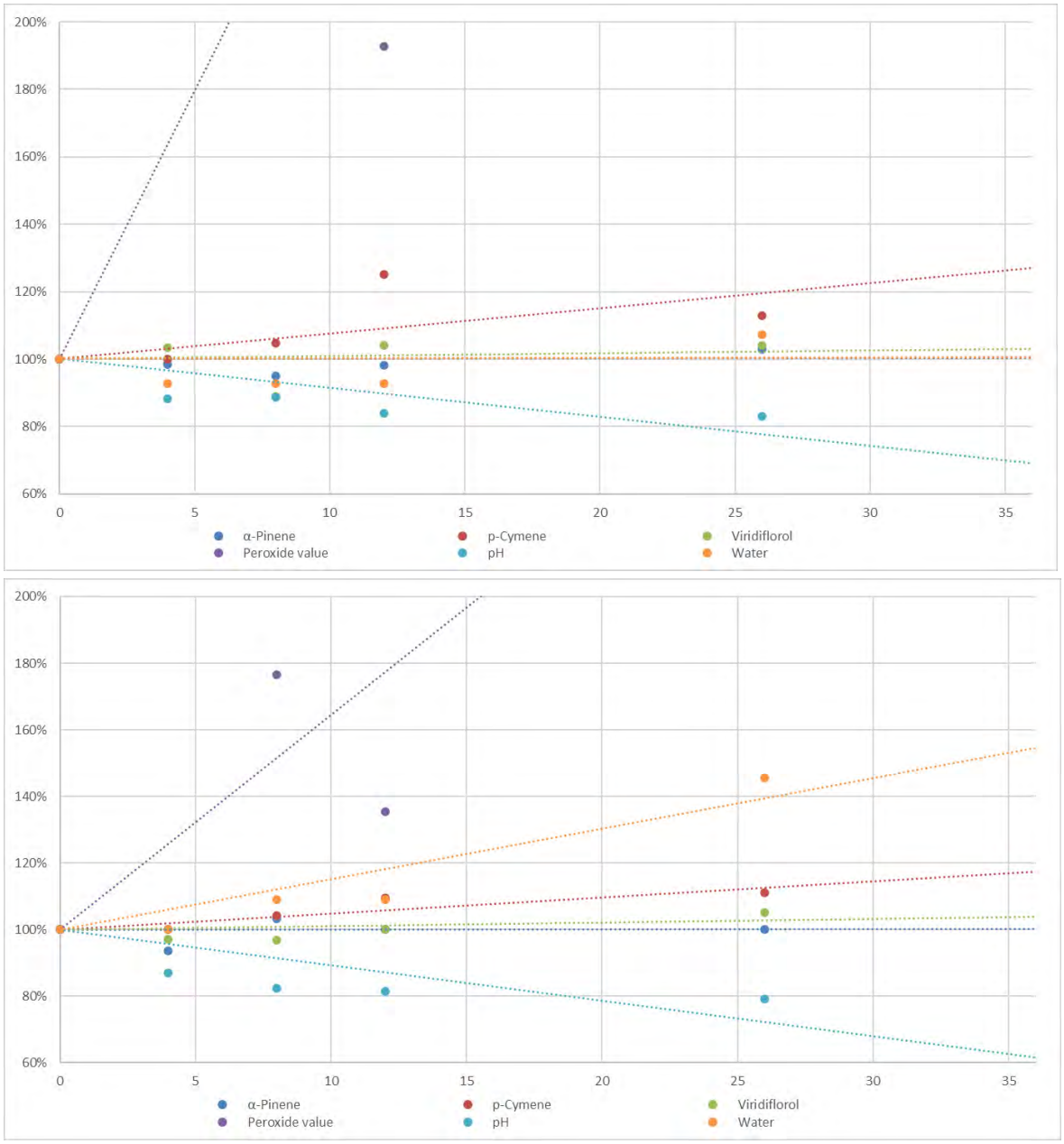


Figure 2: Comparison of all parameters for Sample One (top) and Sample Two (bottom), extrapolated to 36 months. 4 months peroxide value data has not been included since it was significantly out of trend, suggesting an issue with method performance on this occasion.

6.4 Photos of oil samples taken at 8 months

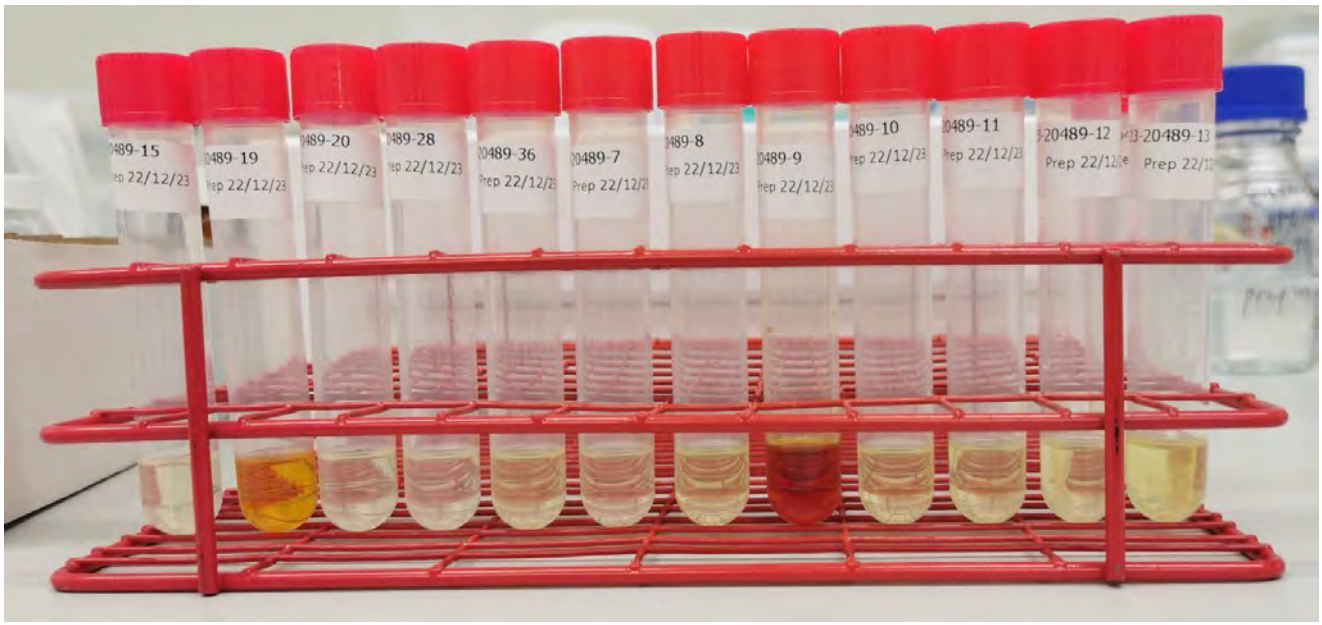


Figure 3: From left to right: Sample One, 15=Control/-20 °C, 19= metal contact, 20=ambient/N_2, 28=plastic, 36=40 °C, 7=clear glass/windowsill. Sample Two, 8=Control/-20 °C, 9= metal contact, 10=ambient/N_2, 11=ambient/air, 12=plastic, 13=40 °C

6.5 Photos of oil samples taken at 12 months

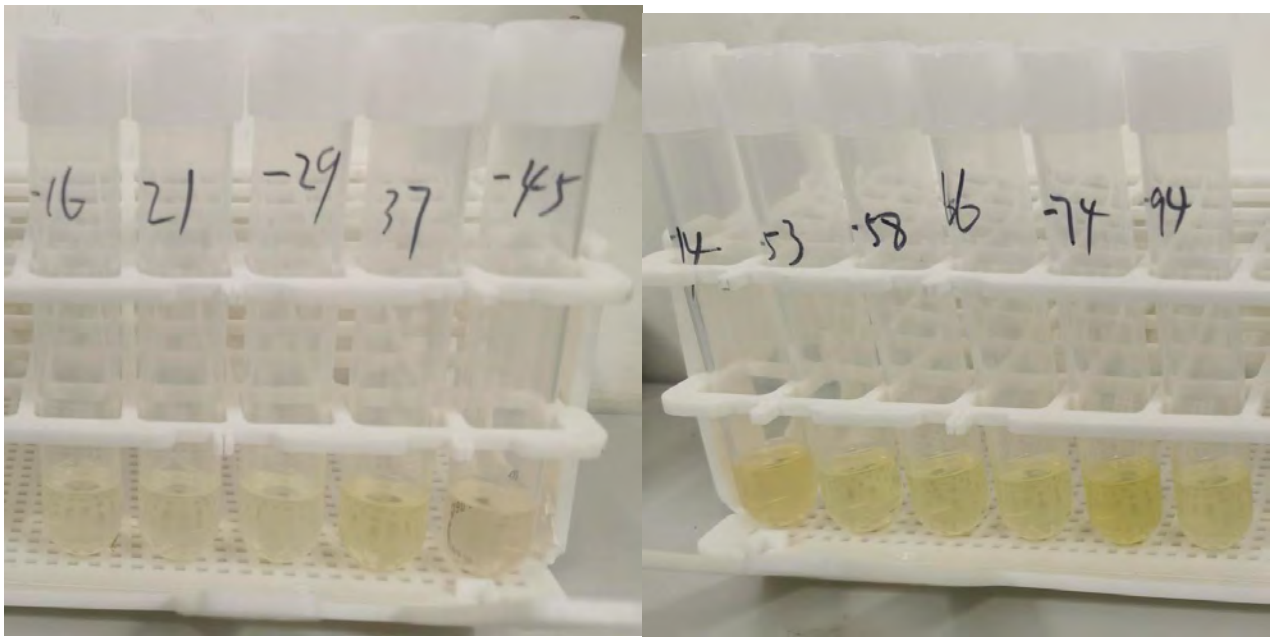


Figure 4: From left to right: Sample One: 16= Control/-20 °C, 21=ambient/N_2, 29=plastic, 37=40 °C, 45=windowsill. Sample Two: 14= windowsill, 53=Control/-20 °C, 58=ambient/N_2, 66=plastic, 74=40 °C, 94=ambient, air

6.6 Photos of oil samples taken at 26 months

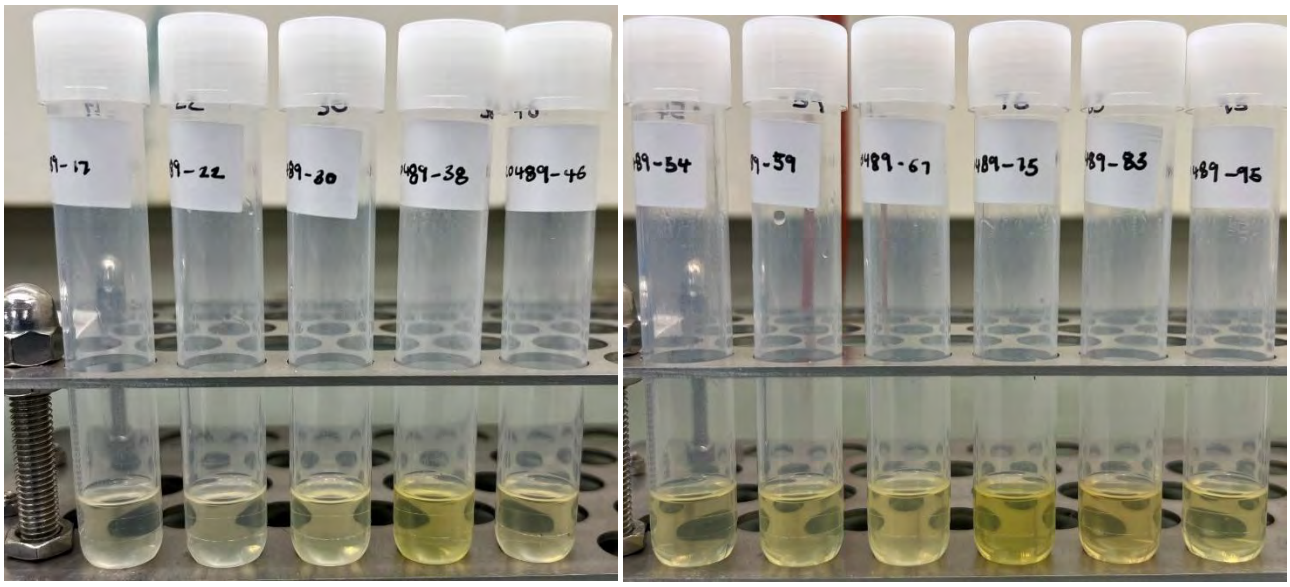


Figure 5: From left to right: Sample One: 17= Control/-20 °C, 22=ambient/N_2, 30=plastic, 38=40 °C, 46=windowsill. Sample Two: 54=Control/-20 °C, 59=ambient/N_2, 67=plastic, 75=40 °C, 83= windowsill 95=ambient, air

6.7 Additional results for metal contamination of stored bulk oil

Following the initial observation of elevated metal results in kānuka oils, additional samples were provided, from bulk amounts of oil, both stored in sealed 304 stainless steel containers, one 5 L and one 40 L. Subsamples in glass containers were available after storage for different lengths of time. Chromium comprises 18-20% of 304 SS and is a contaminant of concern, but was not detected. A small amount of aluminium was detected, concentrations were stable within experimental error.

Original ID	Storage	Sample ID	Receipt date	Zn (mg/L)	Cr (mg/L)	Fe (mg/L)	Al (mg/L)
JHEX000032	5 L container (304 SS)	23-10911-10	13/4/2023	<0.40	<0.10	<1.0	1.5
		24-11301-3	9/4/2024	<0.40	<0.10	<1.0	1.9
JHB000011	40 L drum (304 SS)	23-37318-1	11/12/2023	<0.40	<0.10	<1.0	1.3
		24-12311-2	11/4/2024	<0.40	<0.10	<1.0	1.4

6.8 Additional results for long term stored oil

A portion of oil (24-27660-1) stored for approximately 4.5 years (ambient, clear glass bottle in the dark) was supplied for inclusion in this study. The supplier noted that the oil appeared to have decreased in volume and become slightly more viscous.

Findings for this oil are tentative, since there was no measurement for the oil at time of manufacture, and the air-tightness of the storage container was not known. However, a fresh sample from the same supplier was analysed, which had much higher α-pinene, slightly higher p-cymene, and lower viridiflorol. These observations are not surprising, and are consistent with evaporation of the most volatile components (α-pinene, and to a lesser extent p-cymene), with a resulting increase in the less volatile components, e.g. viridiflorol.

Origin	Sample ID	Receipt date	α-Pinene	p-Cymene	Viridiflorol
4.5 year-old oil	24-27660-1	5/9/2024	34	0.68	1.1
Fresh oil (internet purchase)	24-23034-15	22/7/2024	67	0.82	0.60

A full scan GCMS chromatogram of the aged oil was also collected for comparison to other oils analysed throughout the study (see section 6.10).

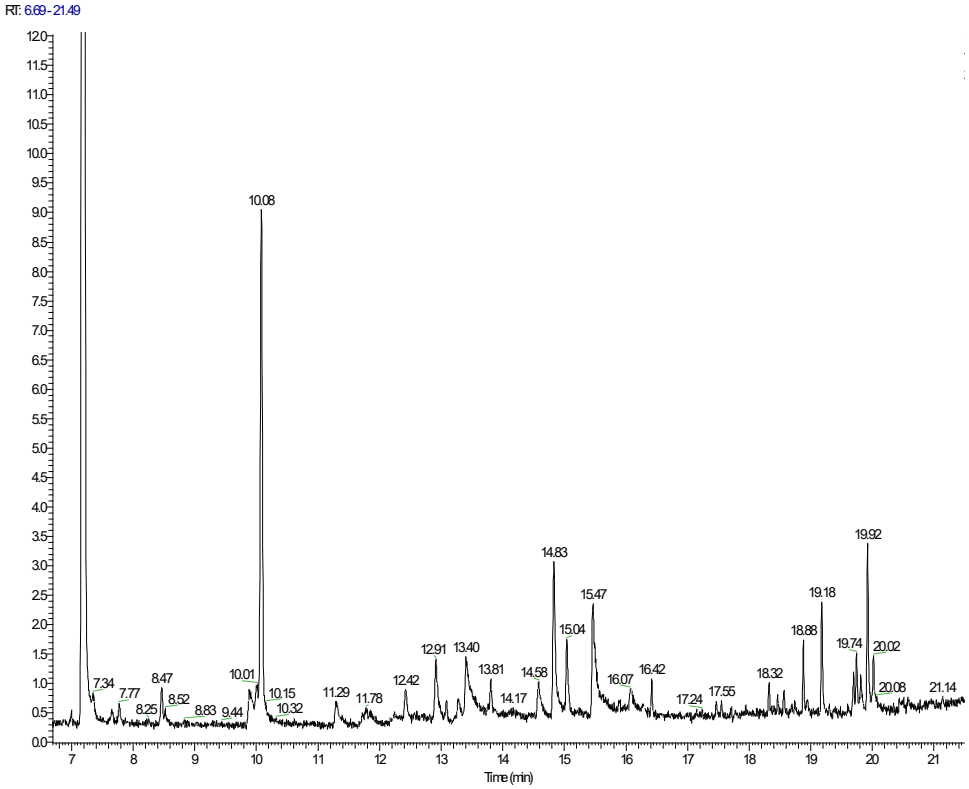


Figure 6: Full scan chromatograms for aged oil (24-27760-1)

Table 9: Major features in GCMS chromatogram for aged oil. Compound names proposed from m/z library searching are in parentheses.

Retention time	% area	Compound name
7.19	83.14	α-pinene
8.47	0.25	(β-pinene)
9.88	0.27	p-Cymene
10.08	6.89	(Eucalyptol)
12.91	0.56	
13.4	0.64	
14.83	1.89	
15.04	0.62	
15.47	1.66	
18.88	0.42	(Ledene)
19.18	0.88	(cis-Calamenene)
19.74	0.33	
19.92	1.22	(Globulol)

6.9 LC-HRMS results

Many changes were noted in stress tested oils when analysed by LC-HRMS, and 20 of the most significant features were selected for ongoing monitoring.

A summary of these features is given in the table below, showing the percentage difference (compared to the control oil) in each species for the different treatments. Values close to 100% (no change) have a white background, values with increasing divergence from 100% are shaded from pink through to red. Features that were not detected are represented by a hyphen.

6.9.1 Sample One (23-10912-1)

After 8 months the most significant changes were observed for samples stored in plastic and on a windowsill. Few of the same features were observed after 12 months, and not much information can be

drawn from those that were seen. It is possible that some compounds have decreased below the limit of detection, however this may also be due to slight changes e.g. in the efficiency of extraction.

One observation that can be drawn from the dataset is that none of the extreme changes seen in oxidative and basic treatments were present in the long term stored oils.

6.9.2 Sample Two (23-10912-2)

After 8 months moderate but very similar changes were observed in the ambient samples under nitrogen and air, while samples at 40 °C and on a windowsill had moderate changes, except for the 6.65 minute peak in the windowsill sample which was hugely increased (and which was nearly all eliminated in the other sample under similar conditions). Overall the plastic sample had the most changes.

Few of the features could be found after 12 months, and once again it is not clear whether this is indicative of loss of the compounds, or due to a shift in method/instrument performance. In common with sample one, none of the extreme changes seen in oxidative and basic treatments were present in the long term stored oils.

Table 10: Summary of HRMS results for Sample One

Rt (min)	Formula	Stress testing						8 months				12 months			
		23-10912-1-1 Acid	23-10912-1-4 Base	23-10912-1-7 Oxidation	23-10912-1-9 Heat	23-10912-1-10 UV	23-10912-1-11 Air/reference	23-20489-20 Ambient	23-20489-28 Plastic	23-20489-36 40 °C	23-20489-7 Clear glass, windowsill	23-20489-21 Ambient	23-20489-29 Plastic	23-20489-37 40 °C	23-20489-45 Clear glass, windowsill
9.57	C15 H22 O5	78	995	979	159	151	117	161	1187	211	35	-	-	-	-
8.42	C14 H20 O5	83	245	877	166	160	129	-	-	-	-	-	-	-	-
10.69	C19 H22 O5	100	377	2073	172	155	141	114	1732	71	24	-	-	-	-
9.56	C10 H14 O4	81	919	905	156	148	109	-	-	-	-	-	-	-	-
8.96	C15 H22 O4	112	1	95	96	99	107	-	-	-	-	25	-	22	39
18.84	C22 H43 N O	90	96	85	84	83	90	96	130	102	99	84	77	79	53
14.19	C15 H24	293	75	107	64	69	69	104	111	124	98	104	108	95	91
7.31	C13 H18 O5	-	-	13812	-	-	-	89	776	57	0	-	-	-	-
9.74	C15 H22 O5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13.94	C15 H24 O	-	-	-	-	-	-	0	310	256	182	-	-	-	-
9.6	C10 H16	102	101	88	100	94	97	100	92	96	94	-	-	-	-
7.67	C10 H16 O	78	103	79	88	104	105	101	100	98	99	-	-	-	-
7.42	C10 H16 O	98	110	102	109	103	103	-	-	-	-	-	-	-	-
13.4	C15 H22 O4	269	101	105	72	71	86	96	114	106	102	103	93	93	92
8.43	C10 H14 O4	-	-	41994	-	-	-	-	-	-	-	-	-	-	-
6.65	C14 H20 O5	130	-	60	123	122	134	87	15	94	2	-	-	-	-
7.86	C7 H8 O	107	102	99	100	105	99	101	105	116	3	-	-	-	-
8.26	C10 H12	94	100	97	103	97	93	111	146	121	127	-	-	-	-
10.35	C10 H16	106	109	97	100	102	98	-	-	-	-	-	-	-	-
9.6	C6 H8	106	98	94	98	98	97	99	92	97	95	-	-	-	-

Table 11: Summary of HRMS results for Sample Two

Rt (min)	Formula	Stress testing						8 months					12 months				
		23-10912-2-1 Acid	23-10912-2-4 Base	23-10912-2-7 Oxidation	23-10912-2-9 Heat	23-10912-2-10 UV	23-10912-2-11 Air/reference	23-20489-10 Ambient	23-20489-12 Plastic	23-20489-13 40 °C	23-20489-82 Clear glass, windowsill	23-20489-11 Ambient, air HS	23-20489-58 Ambient	23-20489-66 Plastic	23-20489-74 40 °C	23-20489-14 Clear glass, windowsill	23-20489-94 Ambient, air HS
9.57	C15 H22 O5	78	15	590	132	128	105	177	152	96	49	163	192	-	-	336	135
8.42	C14 H20 O5	60	15	971	168	156	114	-	-	-	-	-	-	8	19	12	-
10.69	C19 H22 O5	94	2	924	147	155	113	211	246	27	12	188	102	98	87	105	92
9.56	C10 H14 O4	71	10	710	125	117	95	-	-	-	-	-	-	-	-	-	-
8.96	C15 H22 O4	114	10	66	103	111	110	-	-	-	-	-	-	-	-	-	-
18.84	C22 H43 N O	86	81	79	91	77	77	106	106	102	117	130	-	-	-	-	-
14.19	C15 H24	73	96	69	85	82	82	98	102	77	93	98	-	-	-	-	-
7.31	C13 H18 O5	58	-	1863	165	147	108	366	712	96	46	291	-	-	-	-	-
9.74	C15 H22 O5	-	-	5761279	-	-	-	-	-	-	-	-	-	-	-	-	-
13.94	C15 H24 O	-	-	113	-	-	-	257	296	228	137	259	98	95	82	101	94
9.6	C10 H16	104	136	89	107	101	109	108	100	91	101	109	-	-	-	-	-
7.67	C10 H16 O	90	140	108	109	105	111	100	98	99	97	101	159	173	82	92	125
7.42	C10 H16 O	92	98	101	100	96	89	-	-	-	-	-	-	-	-	-	-
13.4	C15 H22 O4	104	21	59	117	115	96	99	103	68	65	90	-	-	-	-	-
8.43	C10 H14 O4	62	11	1491	167	158	109	-	-	-	-	-	-	-	-	-	-
6.65	C14 H20 O5	8389	-	293	9472	8937	14137	85	103	197	26861	93	0	8	19	12	0
7.86	C7 H8 O	96	115	96	96	102	93	102	93	97	75	102	-	-	-	-	-
8.26	C10 H12	99	137	97	108	104	108	112	124	127	108	105	-	-	-	-	-
10.35	C10 H16	92	112	95	98	96	94	-	-	-	-	-	-	-	-	-	-
9.6	C6 H8	109	122	78	105	103	102	107	100	90	99	108	-	-	-	-	-

6.10 GCMS scan results

Stress testing of kānuka oils gave rise to two new peaks in the GCMS chromatogram for the second oil, at 19.0 and 19.2 minutes (not observed for Sample One).

The same instrument as used for the initial full scan analysis (MSD) was not available for subsequent analyses (TSQ used instead), therefore retention times were different, and it was not possible to conclusively match retention times within the constraints of the study. Instead, a comparison between the control oil (stored at -20 °C) and each storage condition is made.

In general, the changes observed in the GCMS fingerprint for oils under different storage conditions were relatively small, and each oil retained the majority of its features.

Since these oils are prepared by steam distillation, the components are all volatile to some degree, and the lack of significant changes in GC-MS chromatograms (and targeted terpenoid analyses) shows that the major components have good stability.

6.10.1 Sample One (23-10912-1) @ 8 months

ID	Description	15.5 min peak	15.9 min peak	16.1 min peak
23-20489-15	Nitrogen HS, -20 °C, amber glass	Present	Present	Present
23-20489-20	Nitrogen HS, ambient, amber glass	Present	**Lost**	Present
23-20489-28	Nitrogen HS, ambient, plastic container	**Lost**	**Lost**	Present
23-20489-36	Nitrogen HS, 40 °C, amber glass	**Lost**	**Lost**	Present
23-20489-7	Nitrogen HS, ambient, colourless glass on windowsill	Present	**Lost**	Present

6.10.2 Sample One (23-10912-1) @ 12 months

Numerous changes were noted in plastic storage (loss of peaks at 10.91, 18.1, 19.3 minutes and appearance/enhancement of peaks at 16.43, 18.47, 21.15)

Storage on a windowsill in light led to the appearance or enhancement of peaks at 13.10, 16.43, 18.47, 20.01, and 21.15 minutes.

No changes were observed in the sample stored in glass at ambient temperature, and at 40 °C only small changes were seen (enhancement at 13.10 and 16.43 minutes)

6.10.3 Sample Two (23-10912-2) @ 8 months

ID	Description	16.6 min peak	17.8 min peak
23-20489-8	Nitrogen HS, -20 °C, amber glass	Present	Present
23-20489-10	Nitrogen HS, ambient, amber glass	**Lost**	**Lost**
23-20489-11	Air HS, ambient, amber glass	Present	Present
23-20489-12	Nitrogen HS, ambient, plastic container	Decreased	Decreased
23-20489-13	Nitrogen HS, 40 °C, amber glass	Present	Present
23-20489-82	Nitrogen HS, ambient, colourless glass on windowsill	Present	Present

6.10.4 Sample Two (23-10912-2) @ 12 months

The largest peak at 19.18 minutes increased in samples stored in plastic, and to a lesser extent stored at 40 °C.

No changes were observed in the sample stored in glass at ambient temperature, or on the windowsill. Ambient storage with an air atmosphere led to significant increases in peaks at 13.10 and 16.43 minutes.

7. Discussion

7.1 Oils for study

The two oils chosen for this study are very different in nature, sample 23-10921-1 (Sample One) has an alpha-pinene content over 50% by weight, typical of most kānuka oils analysed in the last year, whilst 23-10921-2 (Sample Two) has a much lower alpha-pinene content (approximately half), and therefore a relatively larger content of less volatile components. This oil has a stronger colour, and a different odour, with less of the strong pine component of the first oil.

Both oils were supplied in aluminium bottles (1 and 2 litre), and the initial metal analysis detected aluminium in both oils. A set of stability samples has been stored in contact with metals (aluminium, stainless steel and galvanised steel) as well as in clear glass, amber glass, and plastic, and changes will be monitored over time. For normal storage, avoiding metal containers (unless inert lined) is recommended.

7.2 Stress testing

The oils were subjected to a range of extreme conditions in order to investigate the sort of changes that might occur gradually in normal storage conditions over a long period of time.

Initial results suggested that most of these conditions did not result in any significant changes to the oil. HRMS data added at a later date revealed changes in the more polar constituents.

- Appearance was not affected, except for the base (high pH) treatment of both oils, which resulted in a cloudy precipitate, a bleaching of one oil, and an intensification of colour in the other.
- pH was not significantly affected by any treatment.
- Conductivity was slightly less in the treated samples than the T=0 sample, however the reference sample of each oil gave similar results to the treated oils. This parameter has not given any readily useful information, and will be removed from the study in future.
- Terpenoid content was not significantly affected by any of the treatments, except for the base treatment of the second oil – which resulted in an increase concentration of all three (approximately 20%). This indicates a significant reduction of some other component(s) has occurred, for example by hydrolysis – with movement of the products into the aqueous phase, resulting in a corresponding increase in the relative terpenoid concentration.
- Peroxide value increased in response to heat and UV treatment. This test was not carried out for acid, base, or peroxide treatments, which would have led to interference with the results.
- The GCMS profile of the oils shows that a similar range of components is present in both oils, although the relative concentrations are very different. For the first oil, the characteristic "fingerprint" profile is similar for all of the treatments. The second oil contains two peaks at 19.0 and 19.2 minutes that are not present at a significant level in the first oil. These two peaks were substantially reduced or completely removed in the base treatment. No other treatments caused any significant change for the second oil.
- HRMS profiles also showed that the two oils were very different, for instance Sample One contained a significant peak consistent with leptospermone, suggesting contamination with mānuka oil.
- The preparation of the HRMS samples tends to emphasise the more polar components of the oils, and the different stress treatments identified a number of very significant changes in these molecules.
- Some of the changes observed were similar for both oils, however some were very different, indicating that the degradation was dependent on more than one species in the oil – some components having a protective effect – or enhancing degradation. Overall, base and oxidation caused the biggest changes. The acidic treatment led to increases in 2 distinct species, while heat and UV treatments had relatively little impact.

The stress testing data shows that in general, kānuka oil is highly stable to a wide range of chemical treatments. Both oils were impacted by treatment with sodium hydroxide, and for one oil, the reduction of two volatile components was directly observed.

Polar components measured by LC-HRMS were affected by many treatments (acid, heat and UV causing the least change), and a number of compounds have been identified as useful markers of different types of degradation.

These initial observations suggested that both oils would be very stable in long term storage, especially in the absence of contaminants that are basic in nature – the acidic pH of aqueous extracts suggests that this is the case.

The principal component of both oils is α-pinene (illustrated below) which has a hydrocarbon structure, which can undergo a number of reactions, although exposure to particular reagents or catalysts is required for these to occur. Aerobic oxidation of α-pinene is known to occur, and therefore storage under nitrogen is a sensible precaution.

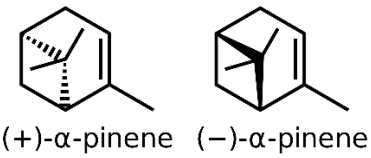


Figure 7: enantiomers of α-pinene

Pure α–pinene is supplied with a three-year expiry date (when stored at 2-8 °C, see appendix 2), and the stress testing investigation also suggests that it is the other components of kānuka oil which are more susceptible to change. Polar components of the second oil (with a lower α-pinene content and greater proportion of other species) were the most affected by stress treatments.

Taken together, these observations suggested that oils with higher α-pinene content would retain more of their original character after an extended time in storage, whilst oils with lower α-pinene content (and therefore a higher percentage of other components) would be more susceptible to change.

7.3 Long term storage results

Summary of observations for each parameter at up to 26 months storage.

α-Pinene	No significant trend was observed for all conditions, including 2 years @ 2-8 °C.
p-Cymene	Slight increases observed, ambient storage in glass performed best. Up to a 25% increase at 40 °C (but still within typical range for kānuka oil). Plastic was worst. For oil at 2-8 °C a decrease of up to 20% was seen over 2 years.
Viridiflorol	No significant trend, except in plastic (where it increased, possibly a result of the decrease in mass). For oil at 2-8 °C a decrease of up to 10% was seen over 2 years.
pH	Larger decreases noted for all oils compared to control oil at -20 °C. Ambient nitrogen was the next best, the largest change was seen for samples on windowsill, 40 °C storage was intermediate.
Peroxide value	Large change in plastic. Mixed results with air, but most other treatments stayed below 20 up to 26 months, except for a large increase for sample 1.
Water content	Increased in plastic for oil one, but not two. No change in glass vessel, until 12 months, but some increase after this time.
GCMS fingerprint	No significant change under ambient conditions with nitrogen headspace. Very minor changes at 40 °C, some other changes with different conditions but not dramatic.
LC-HRMS fingerprint/markers	Data not conclusive, but breakdown products observed from oxidation and base stress treatments were not seen in long term storage up to 12 months.
Sample mass	Losses in mass were observed in samples stored in plastic, but not glass.

Peroxide values of less than 20 are considered acceptable in the cosmetics industry (D. Wabner, in *The International Journal of Aromatherapy* 2002 vol 12 no 3), however the peroxide species are not stable themselves. An Australian Government report on tea tree oil (*p-Cymene and organic peroxides as indicators of oxidation in tea tree oil*, for the Rural Industries Research and Development Corporation, by Ian Southwell, September 2006) suggested that oxidised tea tree oils are always seen to be associated with high p-cymene contents and *sometimes* high peroxide values. The explanation proposed is that oxidation intermediates (e.g. peroxides) react with components of the oil, such as α-terpinene, γ-terpinene and terpinolene, resulting in the formation of p-cymene. Kānuka oil contains components (e.g. α-terpineol) that may undergo the same conversion, and therefore p-cymene could also be considered as a useful measure of kānuka oil oxidation.

p-Cymene concentrations in long-term stored samples shows small to moderate increases in p-cymene concentrations for samples stored in plastic, at elevated temperature, and exposed to light.

7.3.1 Summary of observations for each storage condition over the course of the study.

Nitrogen HS, ambient, amber glass	Good performance determined by most techniques, some decrease in pH. Peroxide value tending to increase.
Nitrogen HS, ambient, amber glass, in contact with metal	The oils are not stable in contact with metal – both oils became darker in colour, and the levels of aluminium, iron and zinc all increased, dramatically in some cases. Note that these stability samples represent an extreme case, since the ratio of metal surface area to oil volume is higher than would exist for oil stored in a drum, but the same change would still be expected to occur over time. 8 month results were higher than 4 months for aluminium and iron, but lower for zinc. We suspect that this is illustrative of the impact of

	surface area to volume ratio, with small changes having a marked effect. A comparison of bulk commercial oil stored in stainless steel kegs showed no change in metal levels, even after up to 1 year in storage.
Air HS, ambient, amber glass	Good performance determined by most techniques. Small changes observed by GCMS, and some decrease in pH.
Nitrogen HS, ambient, plastic container	Not suitable, changes in multiple parameters.
Nitrogen HS, 40 °C, amber glass	Good performance determined by most techniques, some increase in p-cymene and peroxide value (1 sample), and decrease in pH.
Nitrogen HS, ambient, colourless glass on windowsill	Colour changes in both oils plus changes in pH.

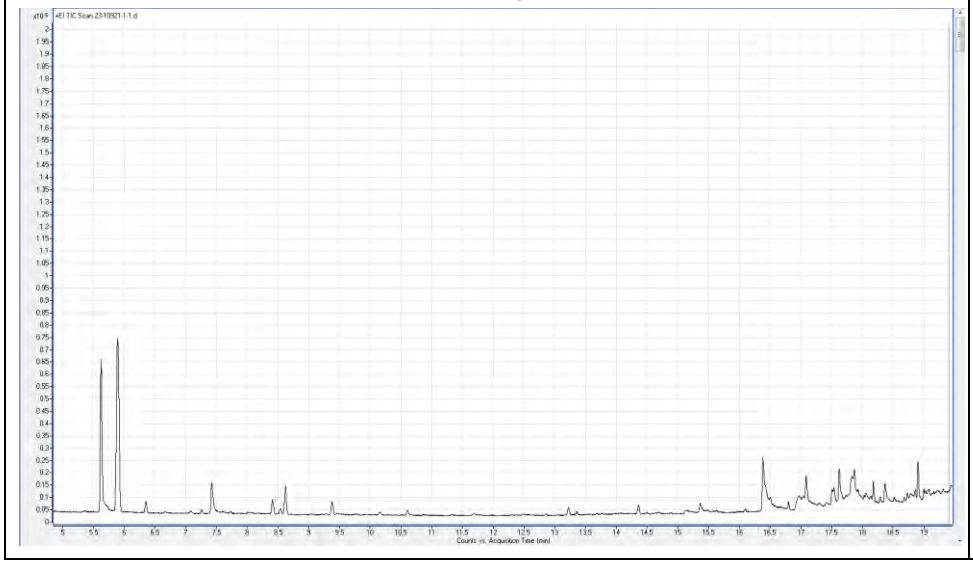
8. Appendices

8.1 Appendix 1: GC-MS chromatograms

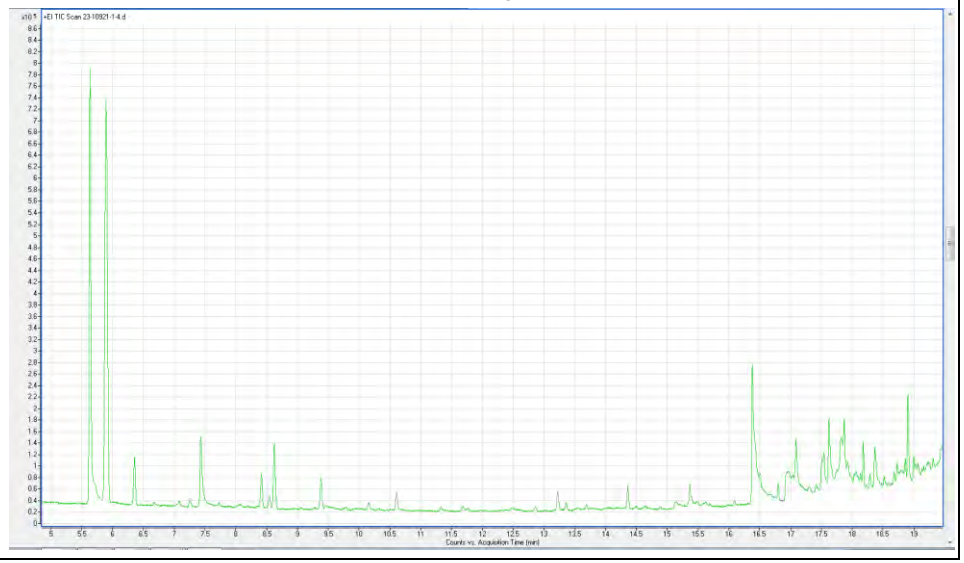
Chromatograms are shown in the figures below for stress testing, and long-term storage samples. Note that some variation in these results is observed from one occasion to the next, due to the presence of background materials resulting from other use of the instrument. The chromatograms are useful however for comparison of samples within each run.

8.1.1 23-10921-1: Sample One

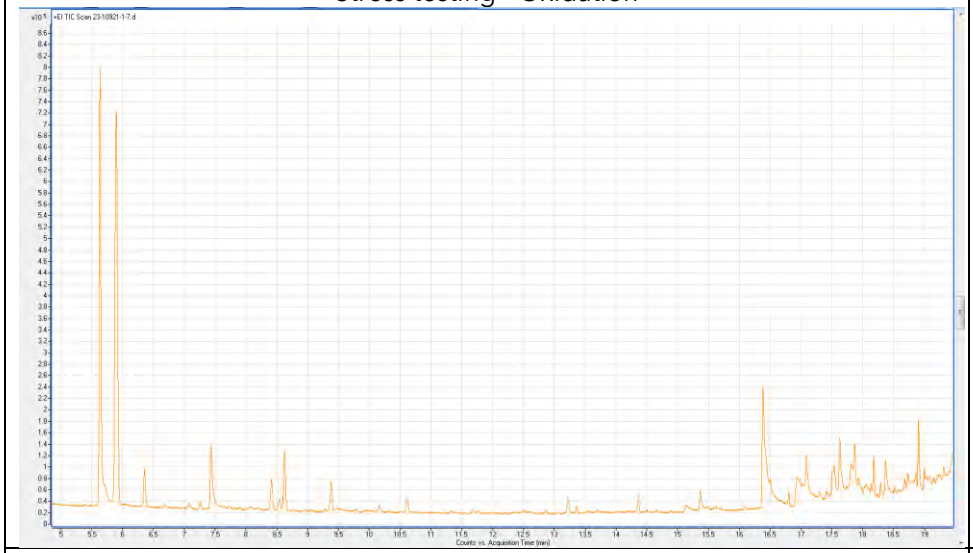
Stress testing - Acid



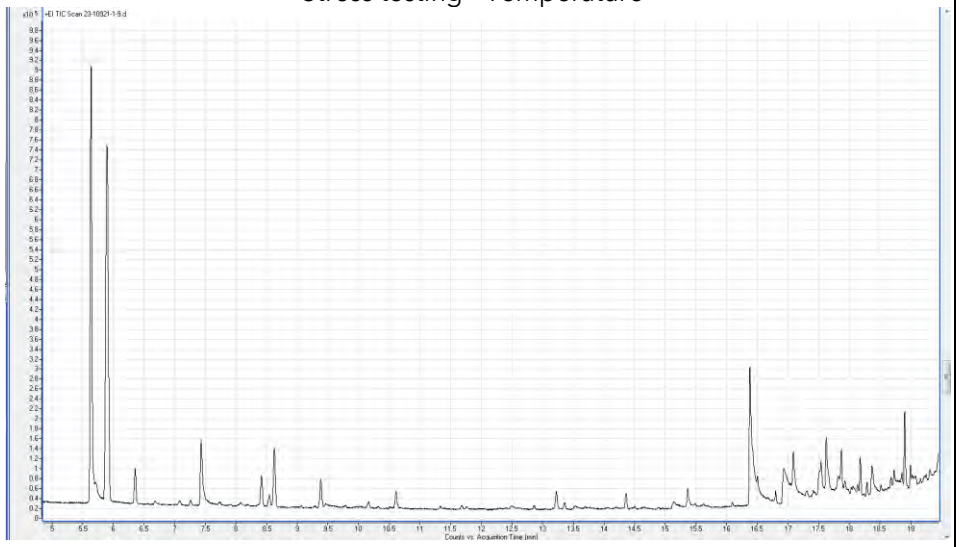
Stress testing - Base



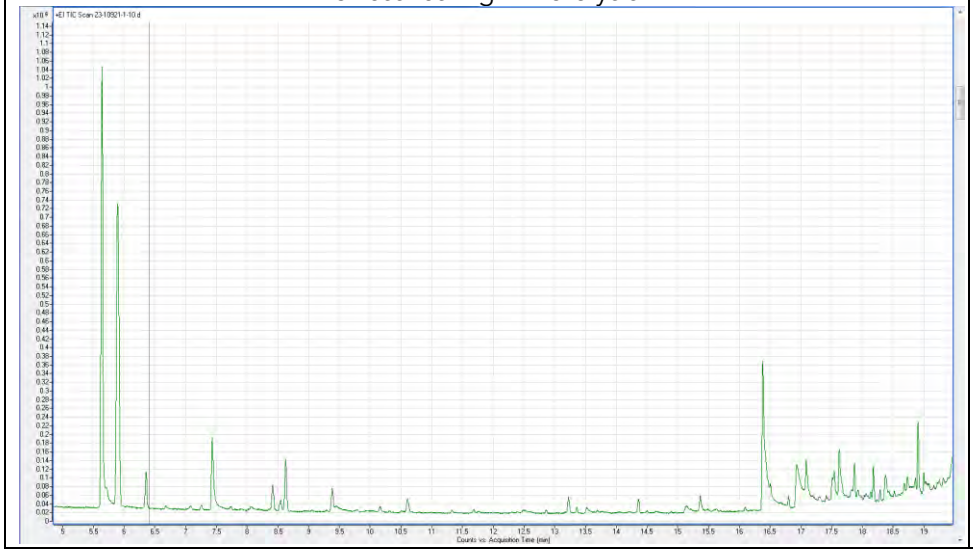
Stress testing - Oxidation



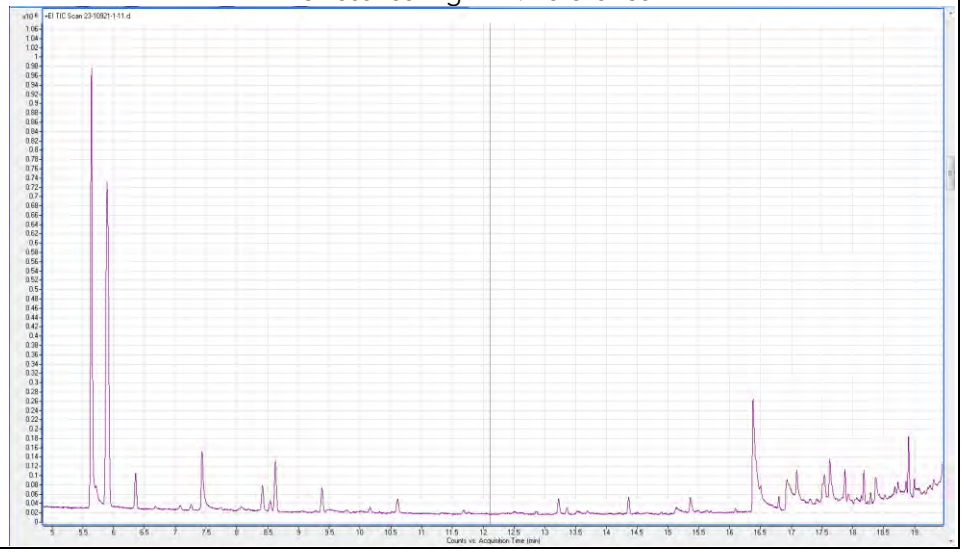
Stress testing - Temperature



Stress testing - Photolysis

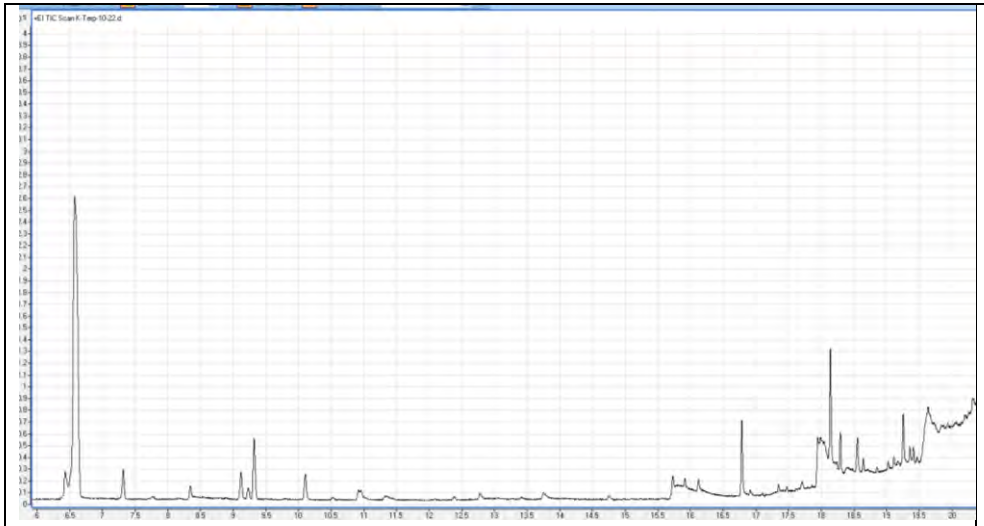


Stress testing - Air/Reference

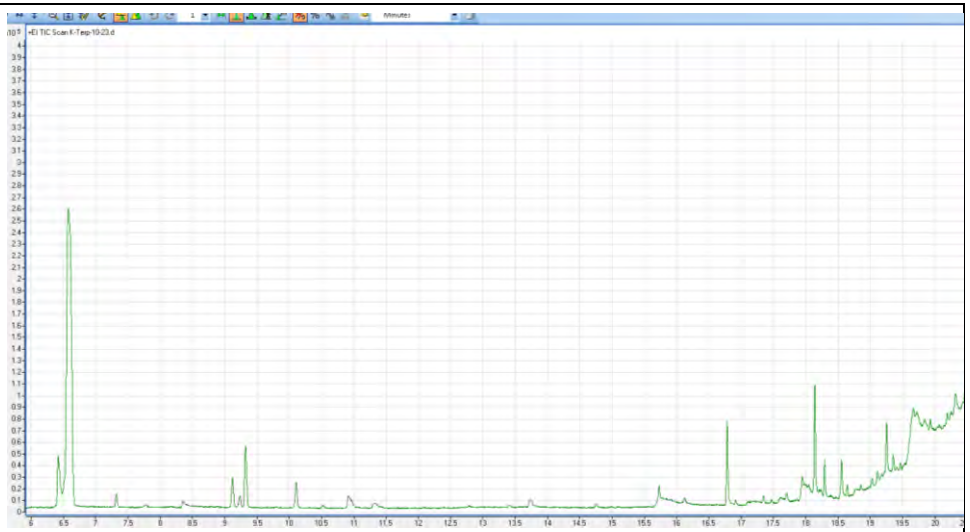


T = 8 months

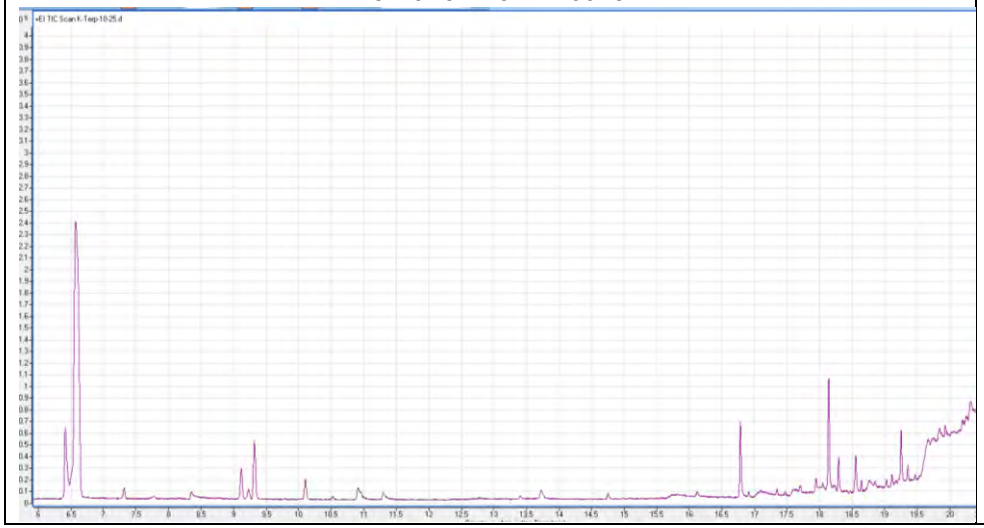
23-20489-15 Control	23-20489-20 Nitrogen, ambient

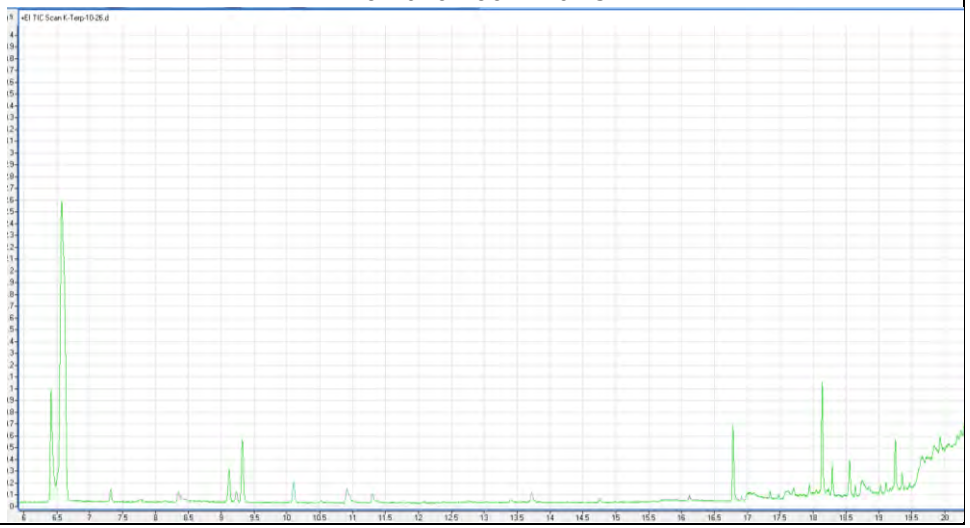


23-20489-28 Plastic

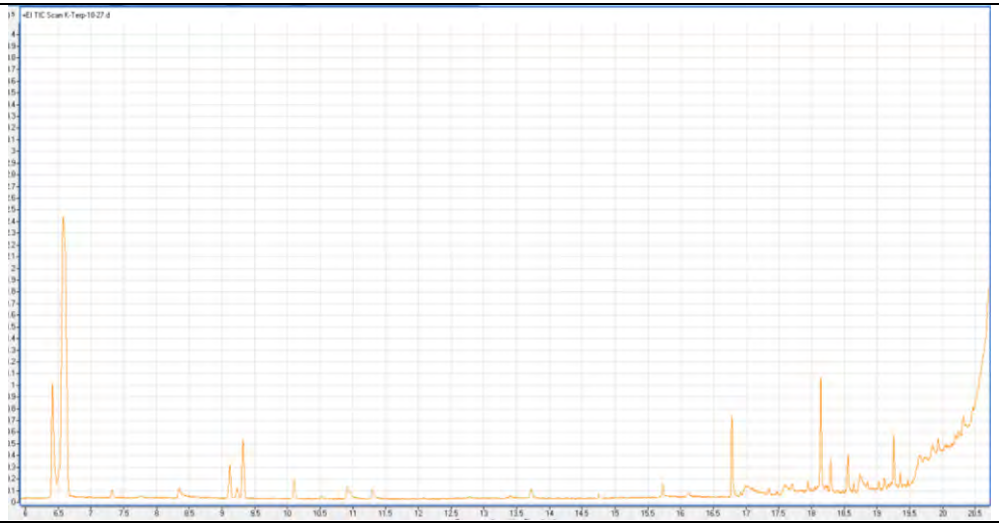


23-20489-36 40 °C



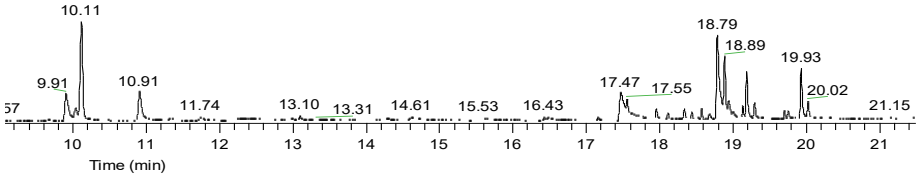


23-20489-7 Clear glass, windowsill	

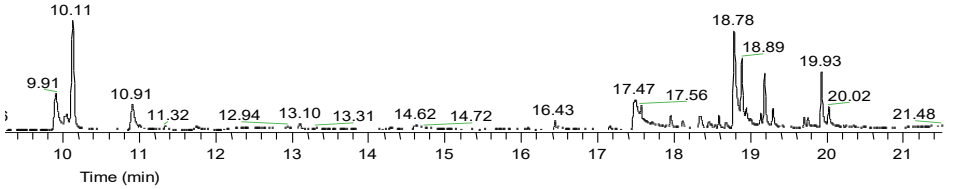


T = 12 months

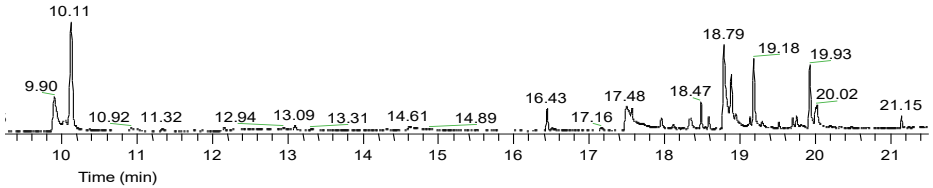
23-20489-16 (Control)



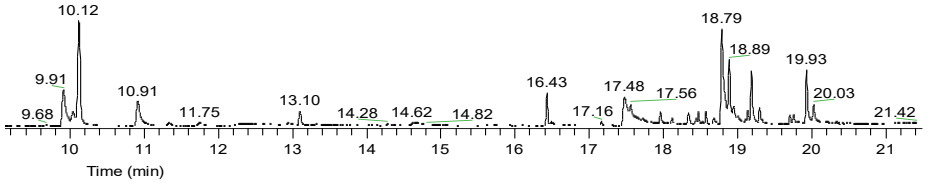
23-20489-21 (Nitrogen ambient)



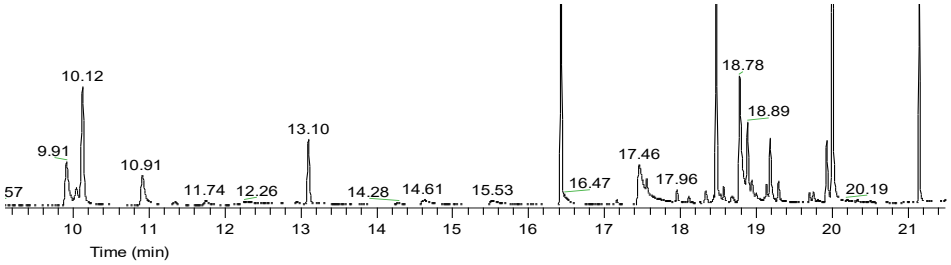
23-20489-29 (Plastic)



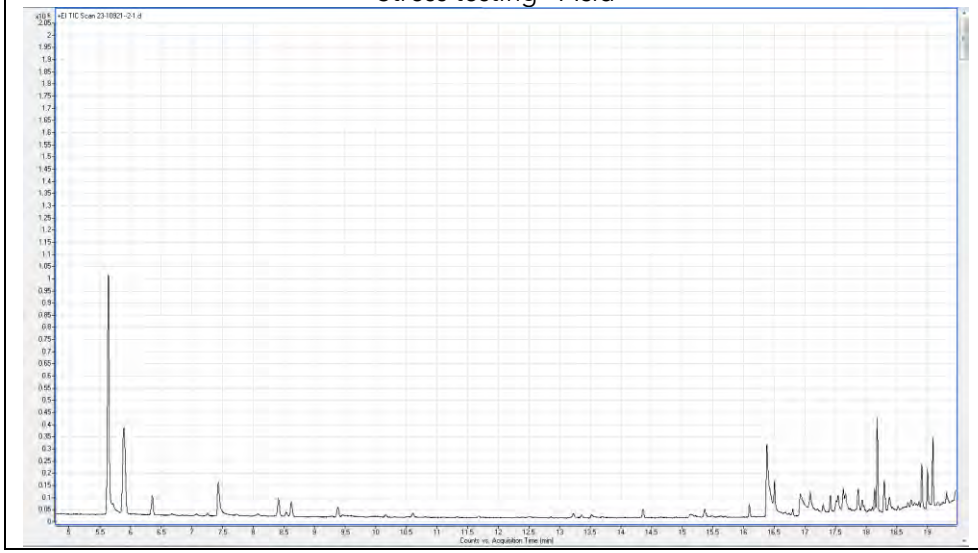
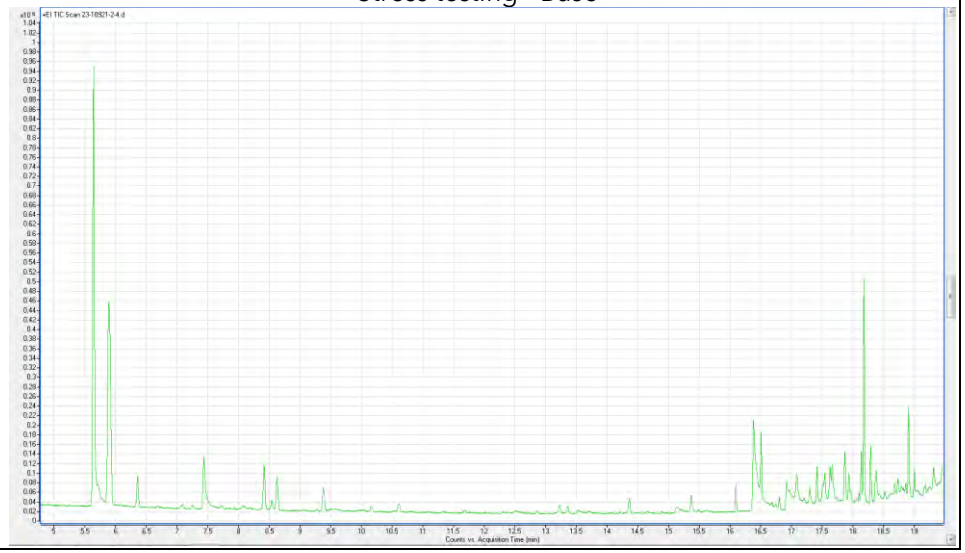
23-20489-37 (40 °C)



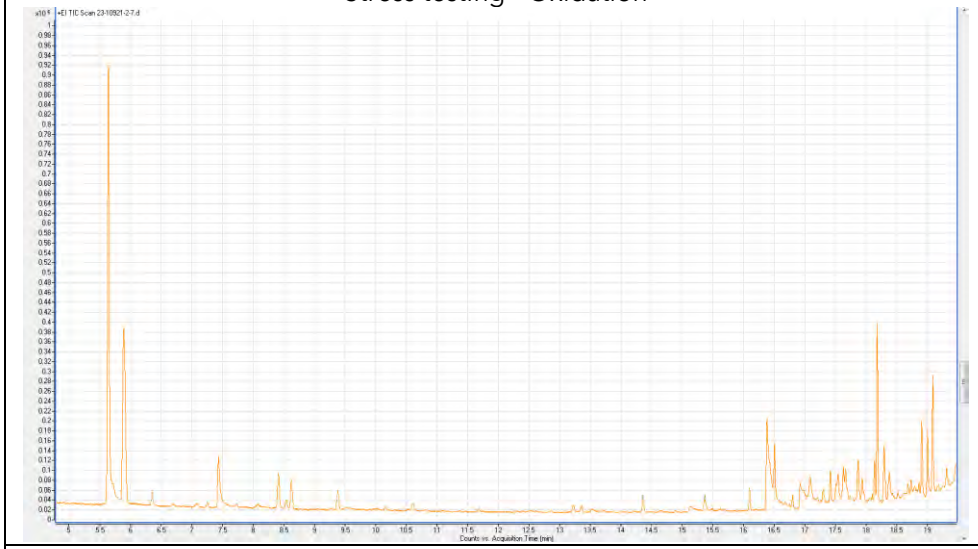
23-20489-45 (Clear glass/windowsill)



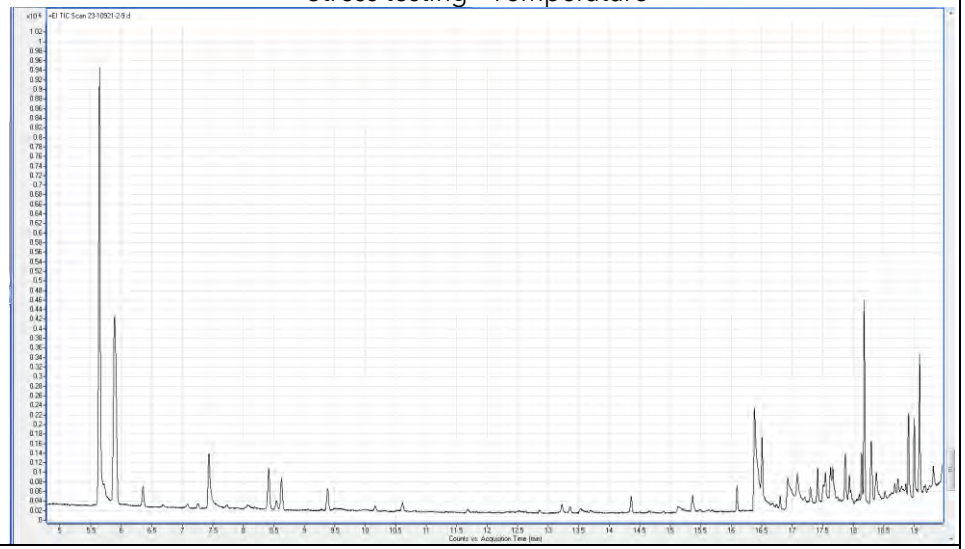
8.1.2 23-10921-2: Sample Two

Stress testing - Acid	Stress testing - Base
	

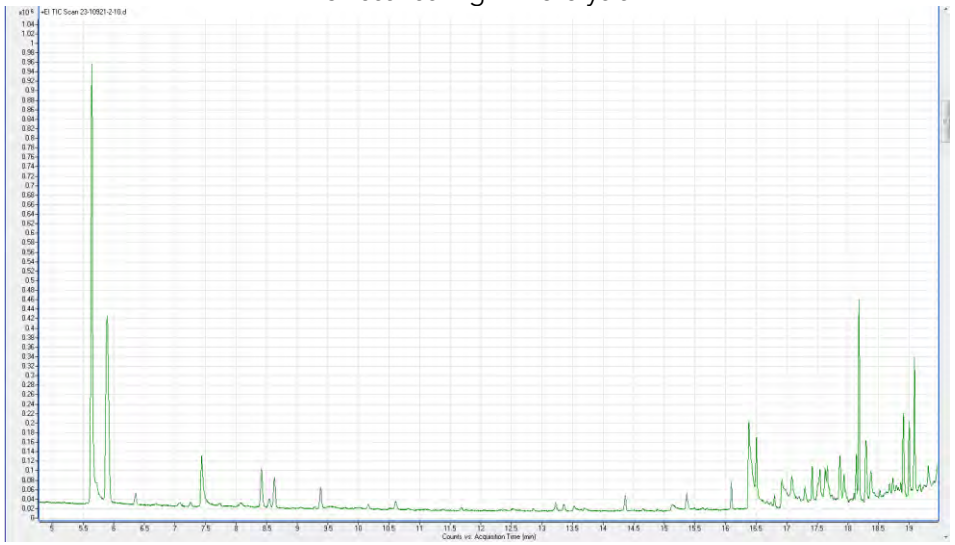
Stress testing - Oxidation



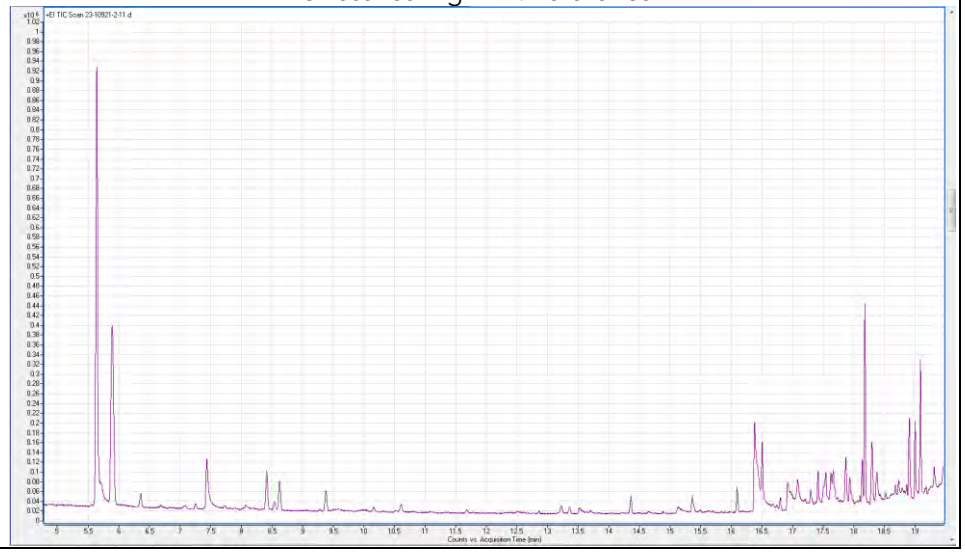
Stress testing - Temperature



Stress testing - Photolysis

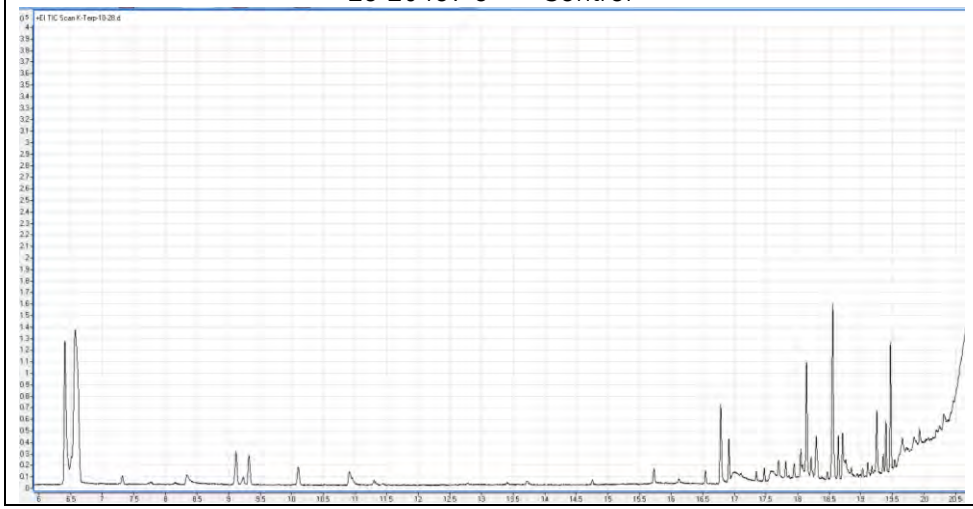


Stress testing - Air/Reference

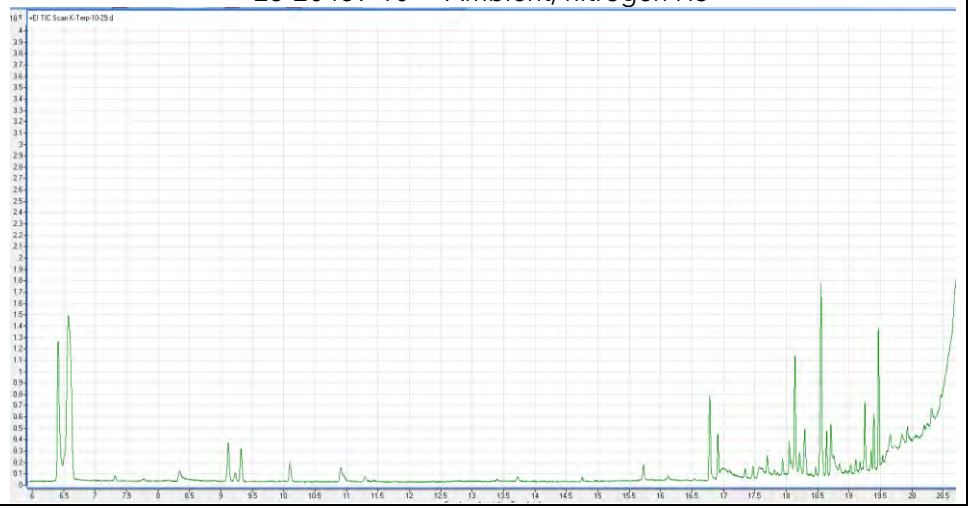


T = 8 months

23-20489-8 Control



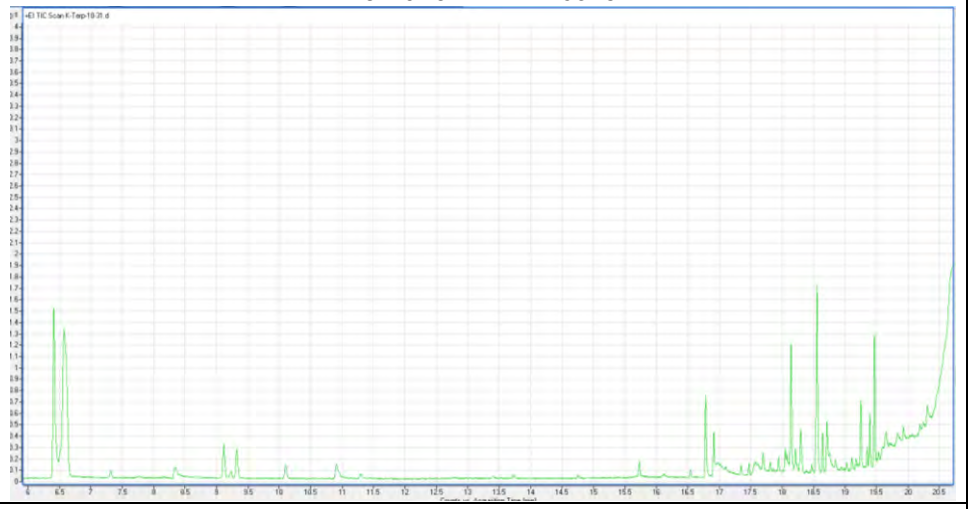
23-20489-10 Ambient, nitrogen HS



23-20489-11 Ambient, air HS

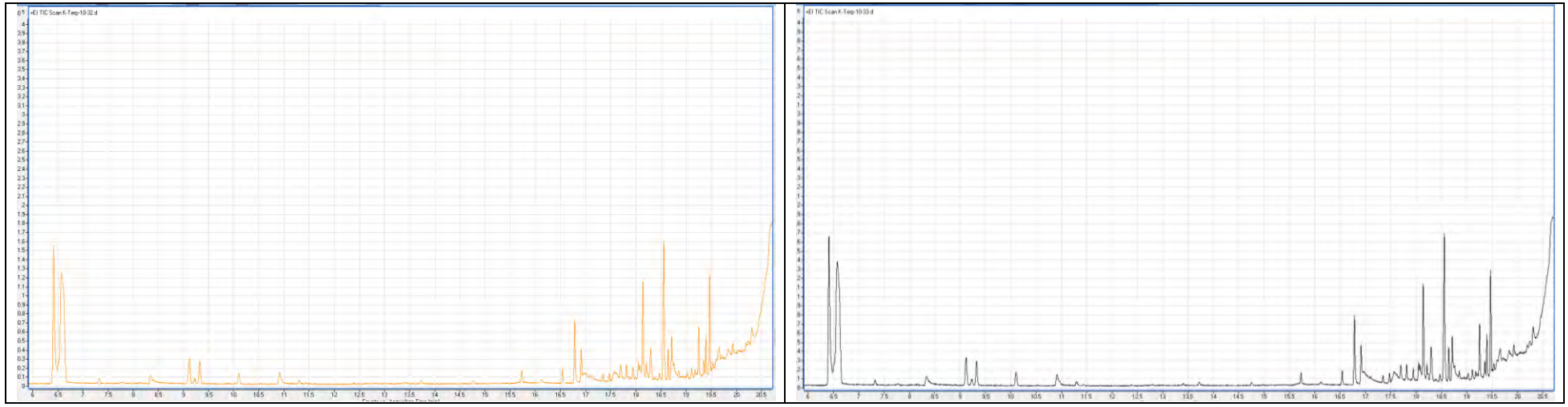


23-20489-12 Plastic



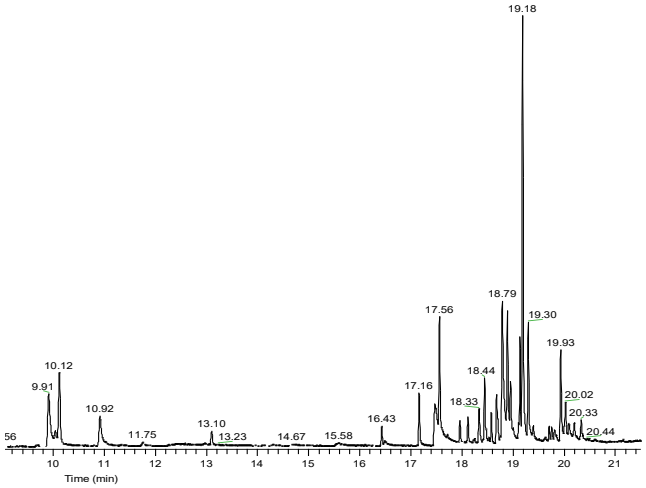
23-20489-13 40 °C

23-20489-82 Clear glass, windowsill

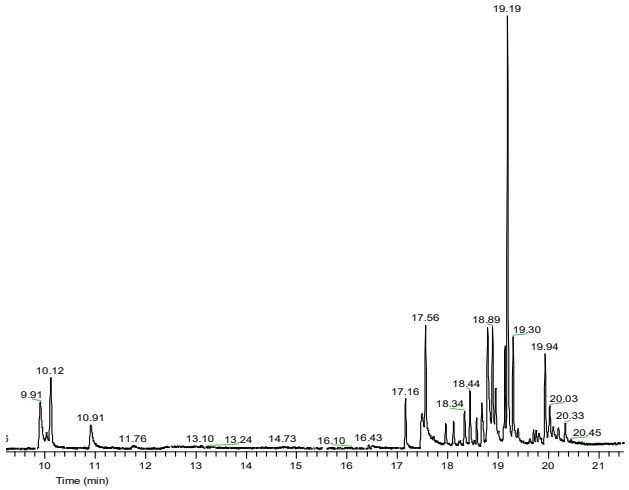


T = 12 months

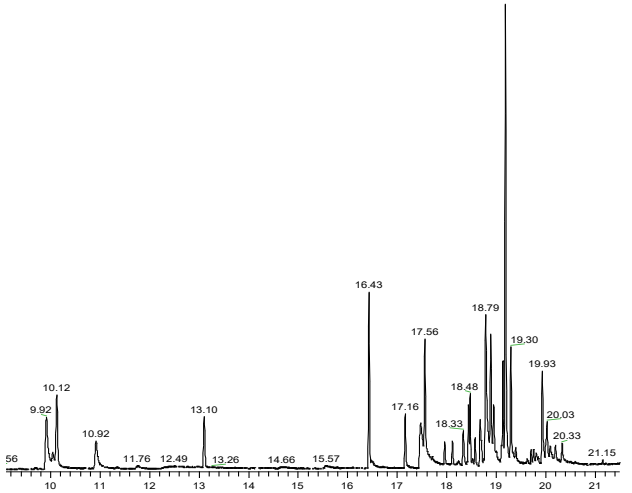
Control



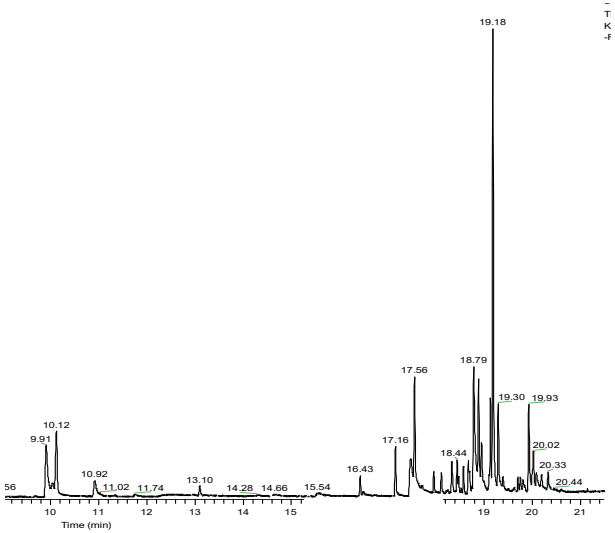
Nitrogen/ambient



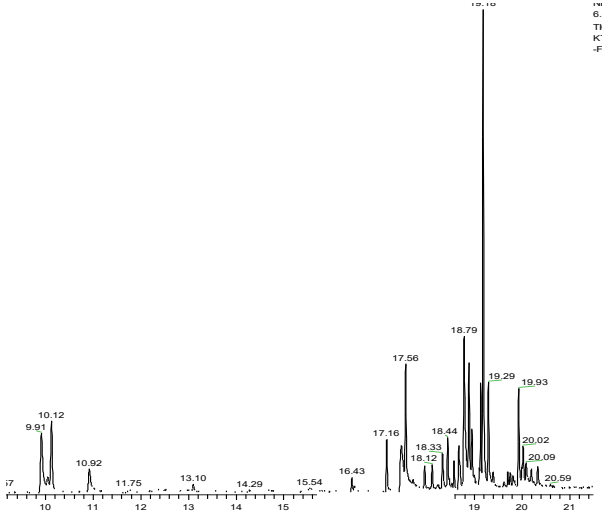
Air/ambient (appearance/enhancement of 13.1, 16.43)



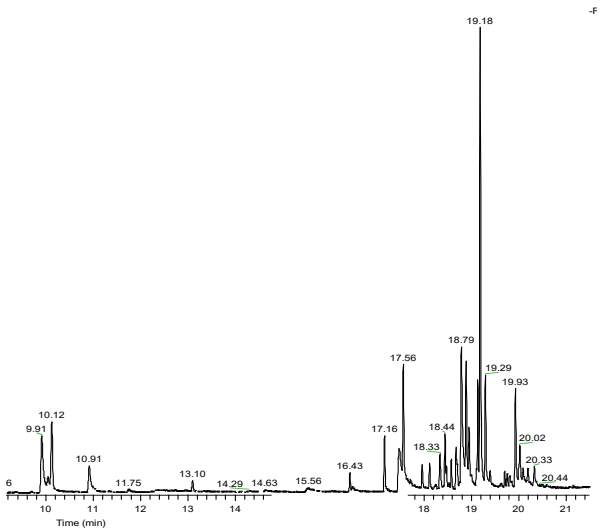
Plastic (appearance/enhancement of 19.18)



40 C(appearance/enhancement of 19.18)



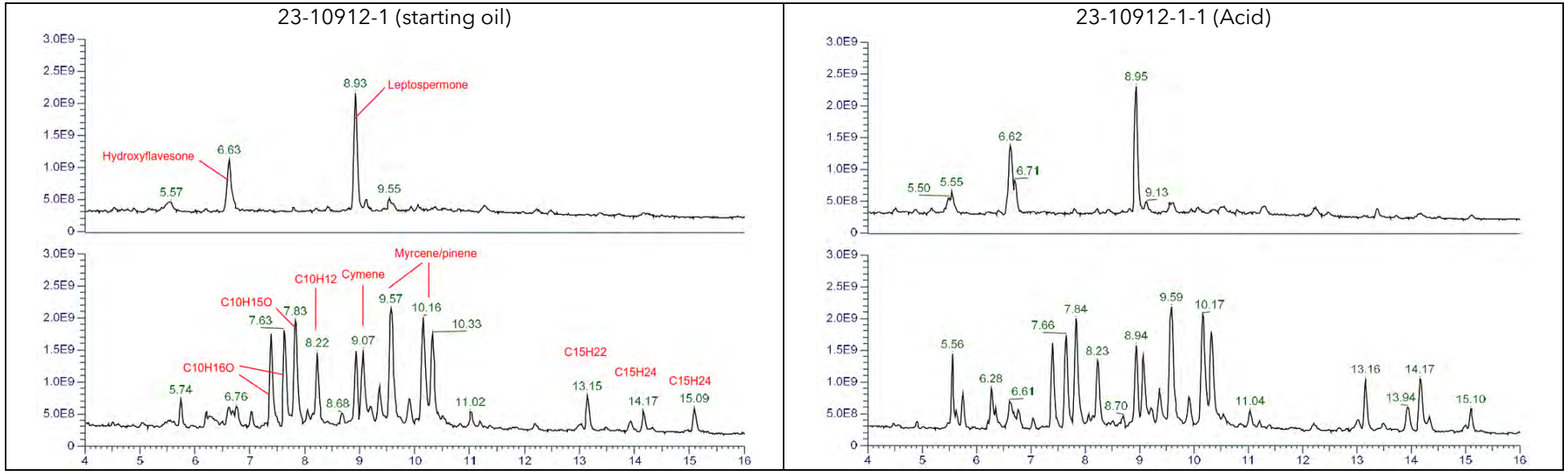
Windowsill (no change)



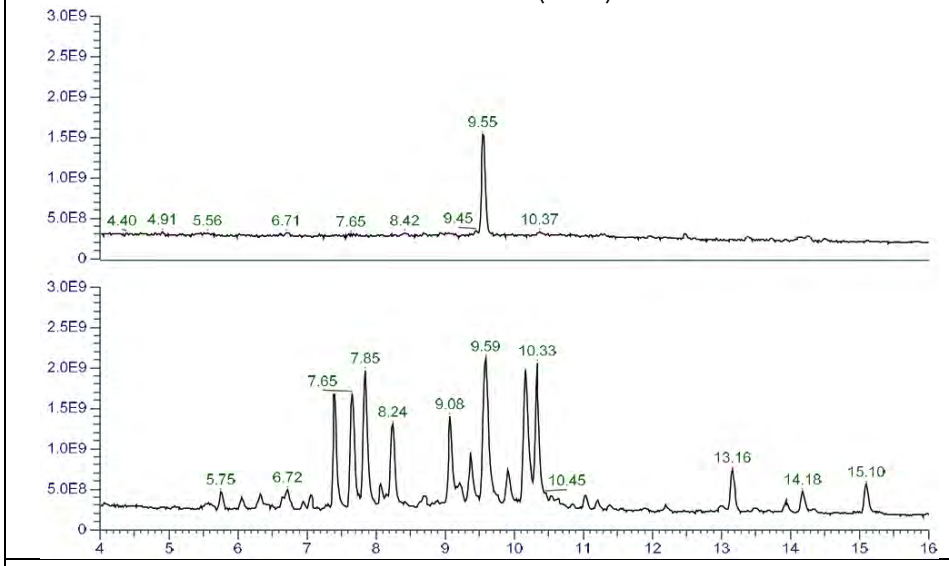
8.2 Appendix 2: LC-HRMS Analysis

Chromatograms are given in the figures below. The top trace is total ion current for negative ions, and the bottom is for positive ions. Intensity is on the vertical axis, and time in minutes on the horizontal axis. For the first chromatogram, names or molecular formulae have been indicated for major components.

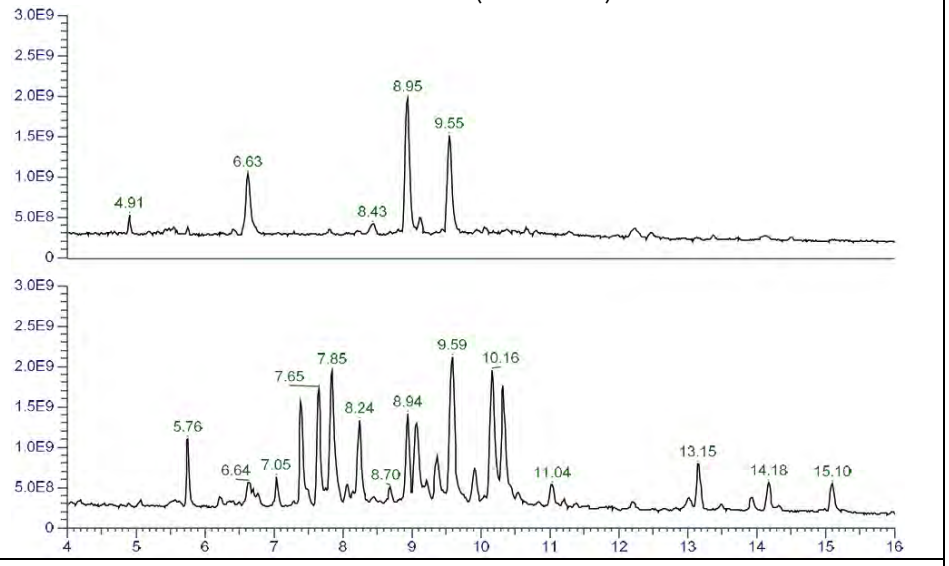
8.2.1 23-10921-1 Sample One, stress testing LC-HRMS chromatograms. Negative TIC on top, positive on bottom, with intensity (Y axis) vs time (minutes, X).



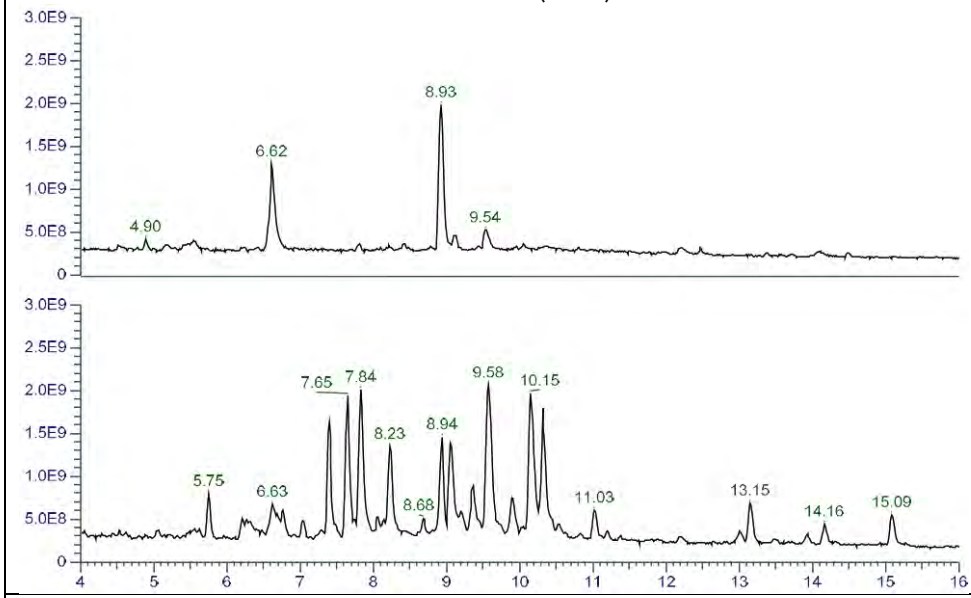
23-10912-1-4 (Base)



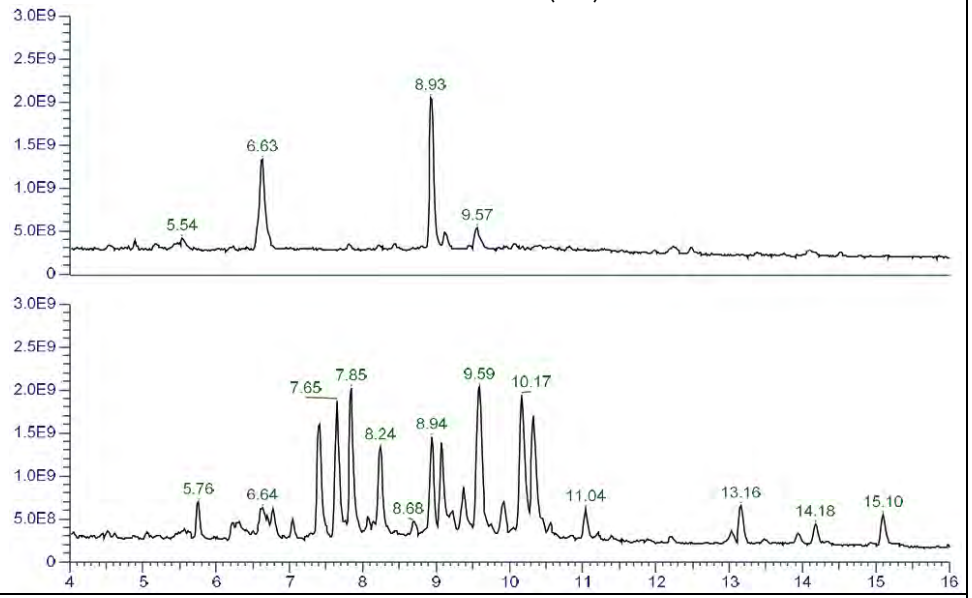
23-10912-1-7 (Oxidation)



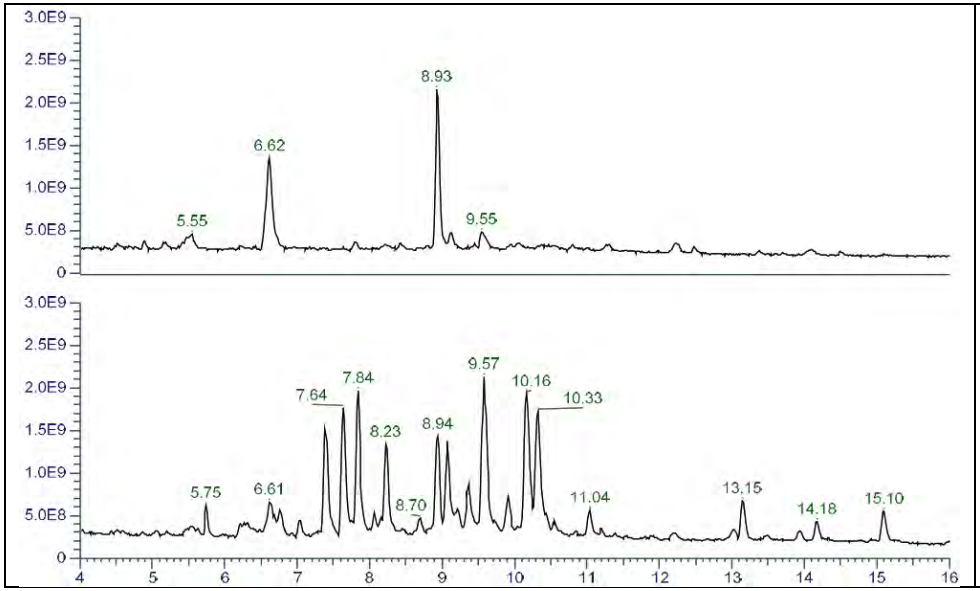
23-10912-1-9 (Heat)



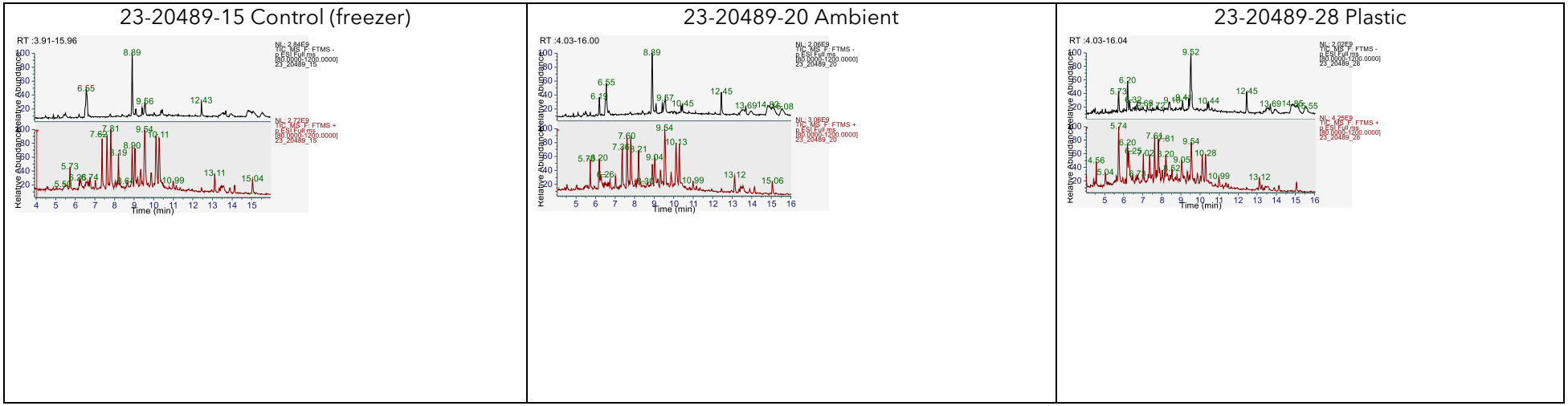
23-10912-1-10 (UV)

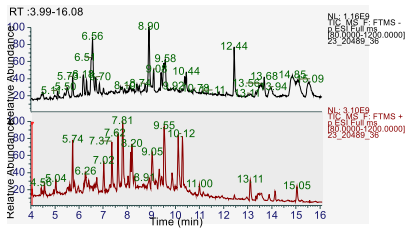
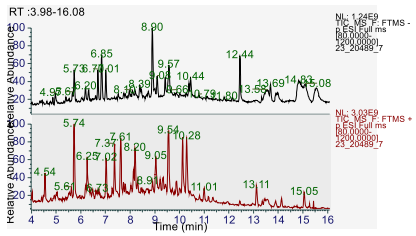


23-10912-1-11 (Air)

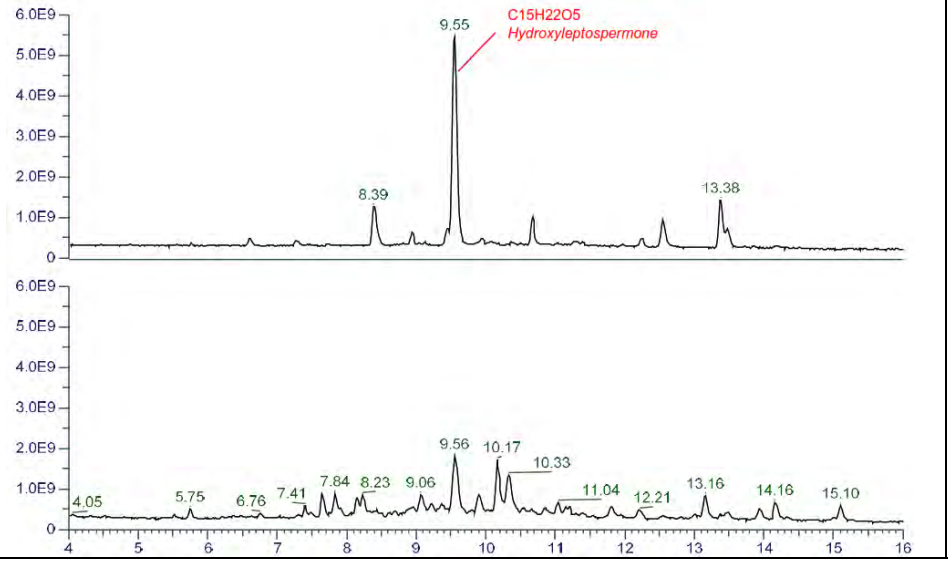
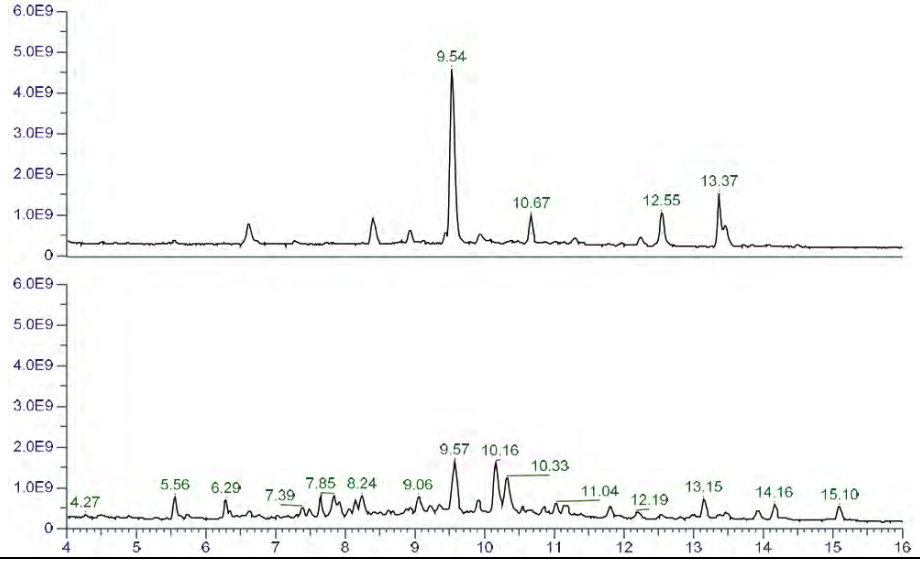


8.2.2 8 month LC-HRMS chromatograms

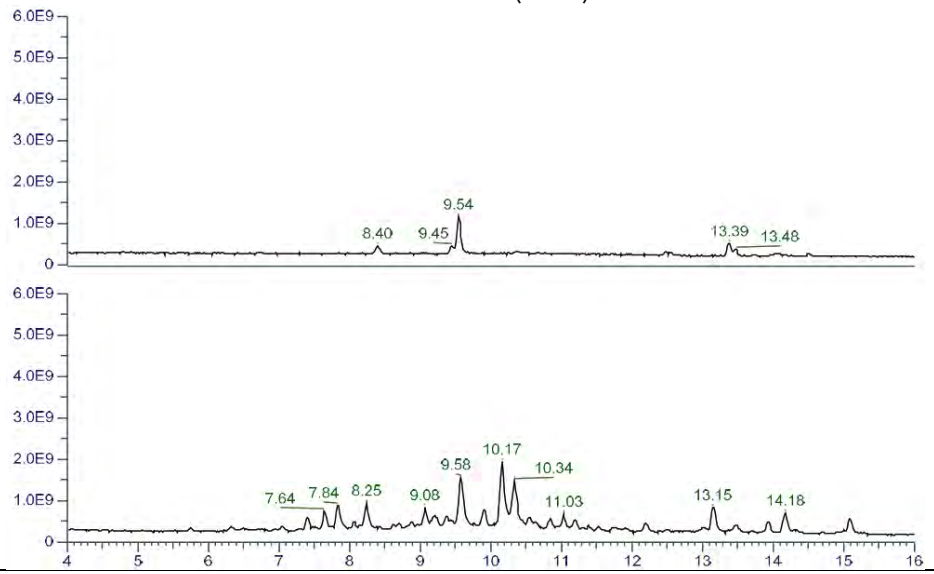


23-20489-36 40 °C	23-20489-7 Clear glass, windowsill	
		

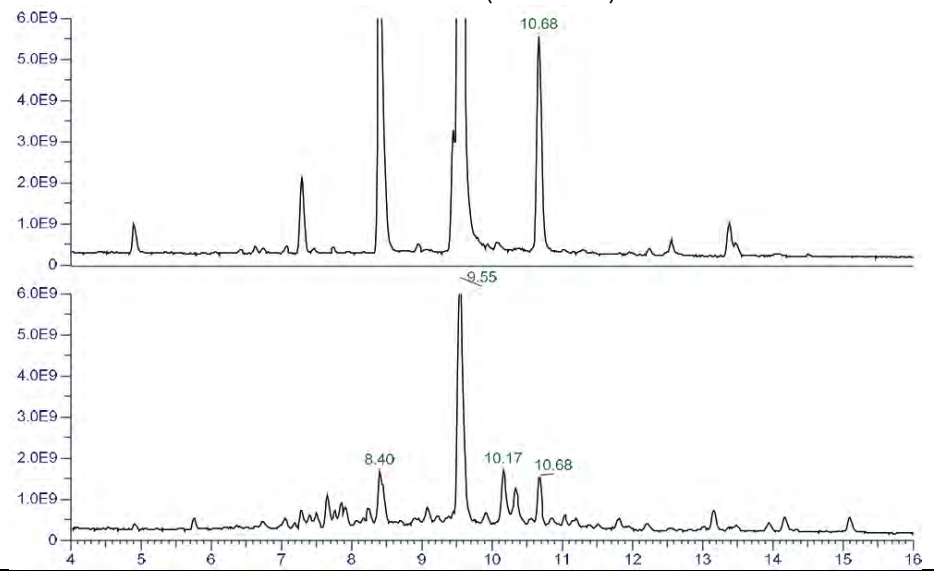
8.2.3 23-10921-2 Sample Two, stress testing LC-HRMS chromatograms. Negative TIC on top, positive on bottom, with intensity (Y axis) vs time (min, X axis).

23-10912-2 (starting oil)	23-10912-2-1 (acid)
	

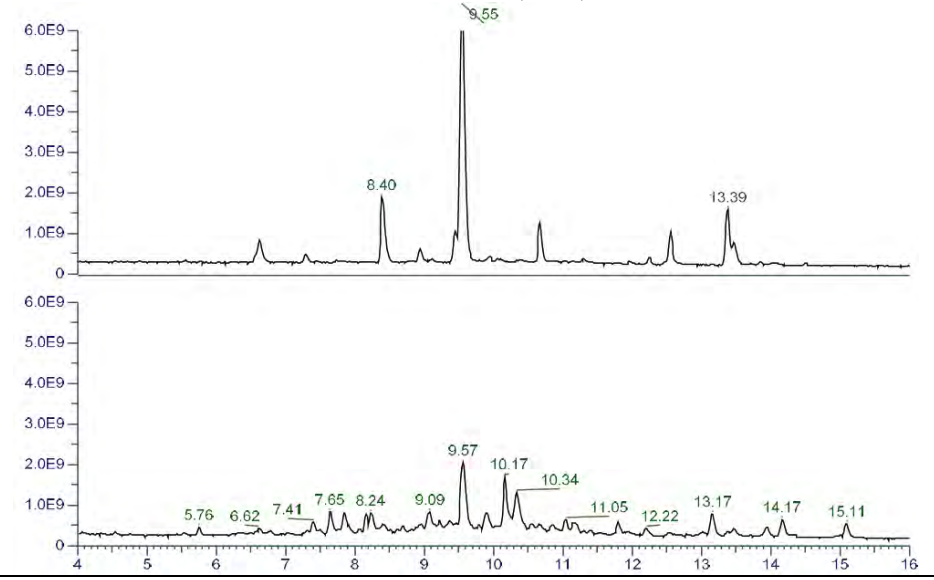
23-10912-2-4 (base)



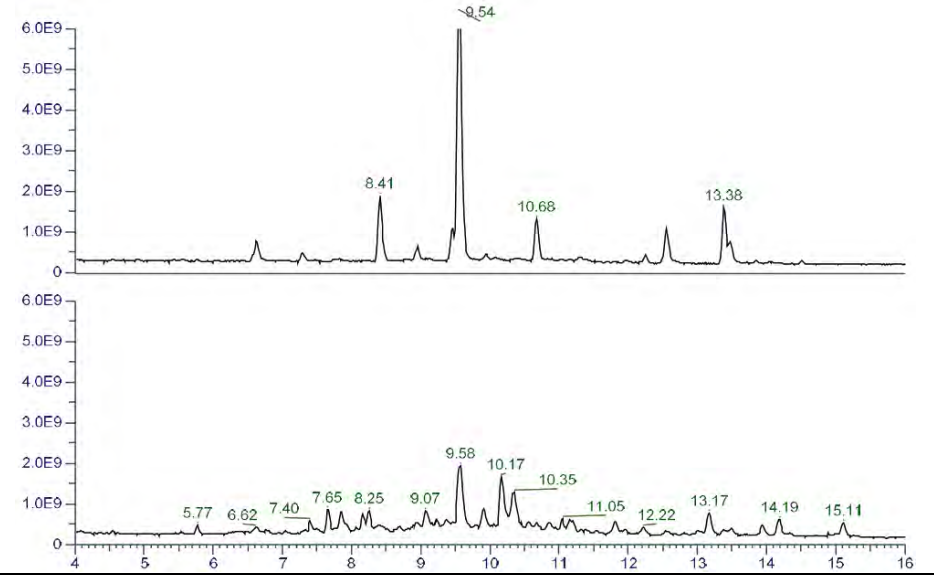
23-10912-2-7 (oxidation)



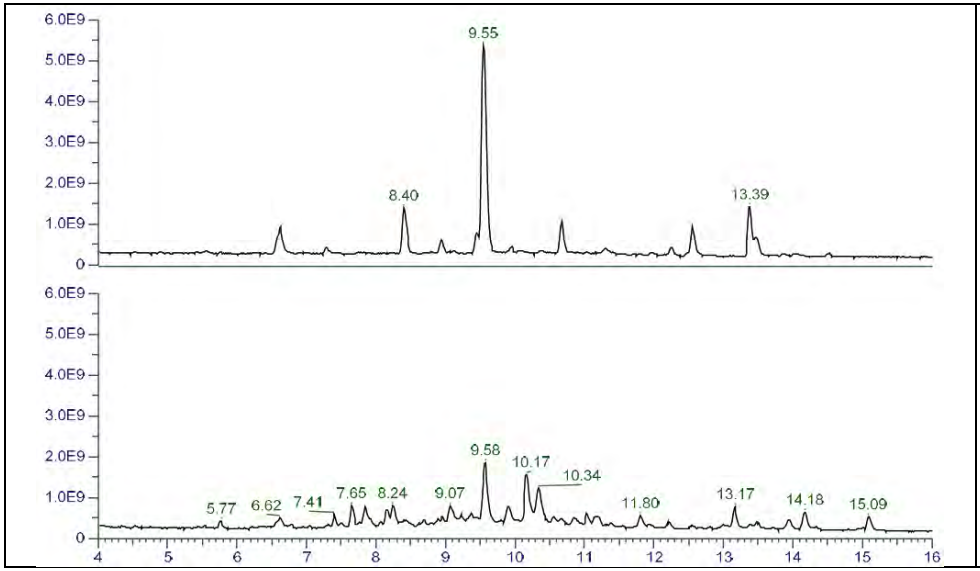
23-10912-2-9 (heat)



23-10912-2-10 (UV)

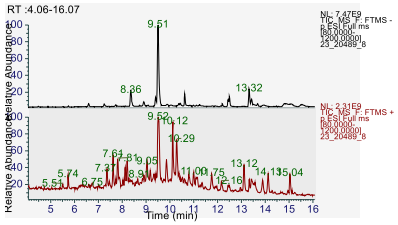


23-10912-2-11 (reference)

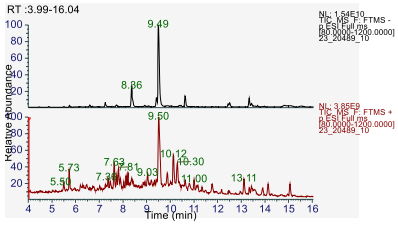


8 months

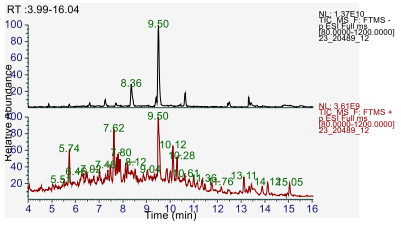
23-20489-8 Control (freezer)

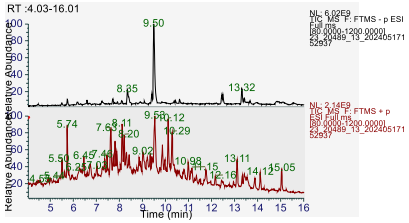
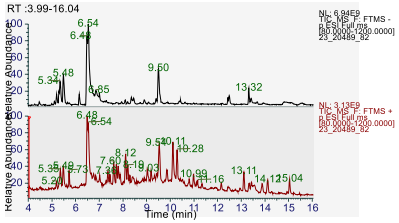
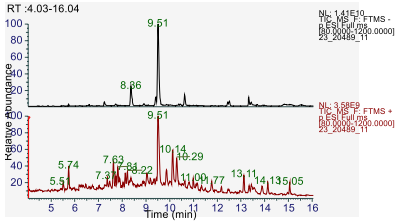


23-20489-10 Ambient



23-20489-12 Plastic



23-20489-13 40 °C	23-20489-82 Clear glass, windowsill	23-20489-11 Ambient, air HS
		

8.3 Appendix 3: Certificate of analysis for example of α-pinene reference standard

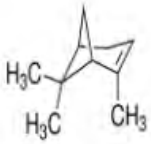
Supelco

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Website: www.sigmaaldrich.com
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Certificate of Analysis

Product Name:
(+)-α-Pinene - analytical standard

Product Number:	**80605**
Batch Number:	**BCCG6386**
Brand:	SIAL
CAS Number:	7785-70-8
Formula:	C10H16
Formula Weight:	136,23 g/mol
Storage Temperature:	Store at 2 - 8 °C
Quality Release Date:	01 OCT 2021
Expiration Date:	SEP 2024



Test	Specification	Result
Appearance (Color)	Colorless	Colorless
Appearance (Form)	Liquid	Liquid
Purity (GC)	≥ 98.5 %	99.1 %
Enantiomer Content (GC)	≥ 98.0 %	98.8 %
Specific Rotation (20/D) Neat	49.0 - 53.0 deg	50.2 deg
1H NMR Spectrum	Conforms to Structure	Conforms

Dr.Reinhold Schwenninger
Quality Assurance
Buchs,Switzerland CH

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