Summer studentship report

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Project title: Investigating the chemical composition of lanolin waste to improve the production of sustainable natural fibre materials

Abstract

The Natural Fibre Company were experiencing intermittent manufacturing difficulties downstream of the scouring process of raw wool. A thicker, paste-like grease deposit formed at the point of processing difficulty. The chemical composition of different raw wool lanolin extracts were compared against a sample of problematic grease using high temperature gas chromatography coupled with mass spectrometry. The wool extracts contained varying distributions of fatty acids, cholesterol and cholesteryl derivatives and wax esters. The problematic deposit had a markedly different composition. Major components were tentatively identified as psi-cholesterol, pentadecenol, palmitic and oleic acid, and a homologous series with mass spectra consistent with glycol ethers. The identification of chemicals predominantly with high interfacial properties was indicative of a potential issue with the surfactants used in the scouring process. The high inorganic content indicated the presence of potassium salts derived from the suint, warranting further investigation of the inorganic fraction using ICP-OES or MS.

1. Introduction

Natural fibre (NF) and NF composites are sustainable materials, affording a low-cost, biodegradable and renewable option for various manufacturing sectors including the fabrics and electronics industries and biopolymer production. The Natural Fibre Company (NFC), a local southwest small to medium enterprise (SME), are capitalising on these emerging markets by focusing on the development of new production techniques. Raw wool accepted by the NFC, is scoured to remove excess grease (lanolin and suint). Being a natural animal product, 'grease' is considered a very broad term and the chemical composition of the company's major waste product is poorly understood. The company experienced intermittent processing difficulties during the processing of raw wool. Despite oil levels being comparatively low during these events, the grease seen at the point of processing difficulty has a different consistency (thicker, paste-like; Figure 1).

Figure 1: Photographs of (a) problematic grease sample and (b. sheep, c. alpaca, d. goat and e. sheep) examples of raw wool obtained from the Natural Fibre Company.

Wool is mostly protein (keratin) (1) with external wool lipids (lanolin) and minor internal wool lipids (cholesterols and free fatty acids) (2). The surface of raw wool is comprised of chemical constituents of the wool wax and suint excretions, produced naturally from the sebaceous and eccrine glands in the skin (3-5). Wool wax contains lipidic compound with a high molecular mass such as alcohols, diols, sterols, fatty acids, hydroxy acids, stearyl and aliphatic esters such as lanolin (6). Lanolin is reported to consist of sterols, fatty acids and fatty alcohols in a complex mixture with a varying composition (7), whilst 20% of suint consists of a mixture of potassium salts of fatty acids (between valeric and palmitic acid) and other organic compounds (such as lanaurin, urea, lactic, hippuric, and succinic acids and nitrogen containing compounds of unknown composition) constitute the rest (8). Raw wool also contains many naturally occurring environmental compounds due to dirt caught in the natural grease such as particulates, plant, soil, and faecal matter. Furthermore, raw wool received at a mill might also contain xenobiotic chemical impurities such as organophosphates or synthetic pyrethroids from sheep dips,(9) and benzene derivatives, naphtha, titanium dioxide, paraffin and hydrocarbon waxes from sheep markers (10, 11).

1.1 Aims and objectives

The aim of this BMSS summer studentship was to characterise and compare the organic content of unscoured raw wool processed by the NFC with the 'paste-like' grease deposit hindering the production of NF materials. The first objective was to develop a method for the facile preparation of organic extracts from the raw wool samples. The second objective was to perform a broad chemical classification of all the wool extracts and waste material using Fourier Transform Infrared spectroscopy (FTIR) and high temperature gas chromatography with flame ionisation detection (HTGC-FID). The third objective was to conduct a semi-targeted analysis using HTGC coupled with time-of-flight mass spectrometry (HTGC-TOF-MS) of chemical groups with interfacial properties and known contributors to deposition formation. In evaluating the data for diagnostic differences between the problematic substance and raw wool extracts, the chemical characterisation herein increased the company's understanding of their major waste product.

2. Methods

Visually obvious impurities, such as soil clumps and grass strands, were removed from wool samples prior to extraction. Method development was performed to evaluate six different extraction protocols

(sections 2.1-2.5). The organic solvents used for extractions: dichloromethane (DCM), propan-2-ol (IPA), toluene and cyclohexane, were HPLC grade. Water was high purity (Elga Maxima, 18.2 MΩ). An internal standard was prepared as described in section 2.6. For each extraction procedure the extracts were evaporated to dryness under nitrogen blowdown at 60 °C before reconstitution to 10 mg mL^{-1} and 1 mg mL^{-1}. The 10 mg mL^{-1} solutions were analysed using FTIR spectroscopy (section 2.7).Results from the FTIR analysis were used to decide whether samples required derivatisation prior to GC analysis based on the presence/absence of bands characteristic of polar functional groups. The extracted grease samples were analysed using HTGC-FID (section 2.8) and HTGC-TOF-MS (section 2.9) at a concentration of 1 mg mL^{-1}.

2.1 Soxhlet Bligh and Dyer

The Soxhlet Bligh and Dyer extraction method described by Manirakiza et al.(12) using a ratio of 2:1 DCM/methanol. Briefly, 5.03 g of wool was weighed into a Soxhlet thimble. Each Soxhlet cycle took approximately 20 minutes, and 10 cycles were completed.

2.2 Soxhlet Modified Bligh and Dyer

The Soxhlet Modified Bligh and Dyer extraction method described by Manirakiza et al.(12) was followed using a ratio of 11:8:10 water/IPA/cyclohexane. Briefly, 5.04 g of wool was weighed into a Soxhlet thimble. Each Soxhlet cycle took approximately 45 minutes, and 4 cycles were completed. This extraction caused solvent to be sucked up and bubble at the joints of the reflux condenser and vapour sprayed out of the top of the apparatus. Some of the (desired) top layer was pipetted off after centrifugation and the rest of the mixture was separated using a separating funnel.

2.3 Sonication Bligh and Dyer

A Bligh and Dyer sonication method was performed. Briefly, 4.96 g wool was weighed into a beaker. A ratio of 2:1 DCM/methanol was added to the beaker and the solution was sonicated for 20 minutes. The sample was centrifuged as it looked “muddy”, and the cleaner bottom layer was extracted.

2.4 Sonication Bligh and Dyer solvent extraction with a phosphate buffer

A Bligh and Dyer solvent extraction with a Phosphate Buffer described in Kates (13) was followed using a ratio of 2:1:0.8 methanol/DCM/phosphate buffer. Briefly: masses of 1.10 g and 0.15 g wool were weighed out and then the extraction method in Kates (13) was followed but using sonication for 5 minute intervals. The extraction was repeated three times and each time the extract was poured into an extract vial. The solvent ratio in the extract vial was adjusted to 1:1:0.8 methanol/DCM/phosphate buffer and was centrifuged before the top aqueous layer was pipetted off and discarded. The extract was washed with 5 mL of water twice and the top layer was discarded.

2.5 Toluene only extraction and cyclohexane only extraction

A toluene only extraction was performed. Briefly: a mass of 0.68 g of wool was weighed out and 2 mL of toluene was added before 5 minutes of sonication to extract the organic substances. The extract was transferred to a smaller vial and the extraction was repeated. The extraction was performed three times in total. A cyclohexane only extraction was performed using the same procedure as for the toluene only extraction. The mass of wool weighed out was 0.52 g and cyclohexane was the solvent used.

2.6 Internal standard

Deuterated triacontane ($C_{30}D_{62}$) was used as the internal standard (IS). The internal standard was prepared by diluting 7 mg of $C_{30}D_{62}$ to 1.6 mg mL^{-1} in cyclohexane. An 80 µL aliquot of IS solution was added per 0.5 g of wool prior to extraction

2.7 FTIR analysis

FTIR spectroscopy was carried out using a Bruker Alpha instrument with OPUS 6.5 software. The crystal was cleaned before each background measurement was taken. Two drops of each extract solution were

carefully dispensed onto the crystal using a glass Pasteur pipette and the solvent allowed to evaporate prior to recording the spectrum. Spectra were recorded with a resolution of 4 cm^{-1} and 32 scans were taken.

2.8 HTGC-FID analysis

HTGC-FID was carried out using an Agilent 6890 GC system fitted with a cool-on-column inlet (0.5 µL manual injection, +3 °C track oven mode), the FID detector was at 435 °C and an Agilent VF-5ht Ultimetal column (15 m x 0.25 mm x 0.1 µm, constant flow mode, He carrier gas 1 mL min^{-1}) was used. The oven was programmed from 40 – 400 °C at 10 °C min^{-1}, with a 10 minute hold.

2.9 HTGC-TOF-MS analysis

HTGC-TOF-MS analysis was carried out using a BenchTOF-dx™ reflectron time-of-flight mass spectrometer (Almsco International, Llantrisant, UK) interfaced with an Agilent 6890 gas chromatograph (similar set up to the HTGC-FID) via an in-line Siltite™ mini-union and HT-deactivated silica tubing (nom. 2 m × 0.18 mm id; Phenomenex, Macclesfield, UK). The general operating conditions were: helium carrier gas (constant flow mode, 2.5 mL min^{-1}); oven programmed from 40 to 380 °C at 20 °C min^{-1}, 2 min hold; transfer line and ion source at 350 °C; mass spectrometer in EI mode (70 eV and 15 eV) recording mass range *m*/*z* 50–550. The GC was controlled through Agilent MSD Chemstation and the MS through ProtoTOF™ software. Data processing software included dx-Connect™ and TargetView™ with library matching via NIST MS Search. Prior to operation, air/water background, were performed using auto-routines (ProtoTOF™ software).

3. Results and Discussion

The results from extraction method development are summarised in Table 1.

Table 1. Results from the extraction methods

Extraction method	Mass of wool(g)	Mass of extract (g)	Percentage grease (%)
Soxhlet Bligh and Dyer (DCM:MeOH; 2:1; 144 mL total)	5.03	0.6000	11.9
Soxhlet Modified Bligh and Dyer (water:IPA:cyclohexane; 11:8:10; 145 mL total)	5.04	0.4700	9.3
Sonication Bligh and Dyer (DCM:MeOH; 2:1; 120 mL total)	4.96	0.1800	3.6
Bligh and Dyer solvent extraction with a phosphate buffer using sonication (MeOH:DCM:buffer; 6:6:4.8 mL; 16.8 mL total)	0.15	0.0031	2.0
	1.10	0.0080	0.7
Toluene only (6 mL)	0.68	0.0209	3.1
Cyclohexane only (6 mL)	0.52	0.0158	3.0

HTGC-FID analysis was performed on the sample extracts. There were no large variations observed between the chromatograms obtained using HTGC-FID for the different wool extraction techniques (Figure 2), so a representative wool sample extract was selected for HTGC-TOF-MS analysis (wool from an alpaca). A wool sample extract and the grease sample extracts were chosen for analysis using HTGC-TOF-MS after examining the chromatograms obtained using HTGC- FID. Although Soxhlet

extraction was an exhaustive method, resulting in greater extraction quantities of 9-12% (Table 1), the procedure was very time consuming and used a large volume of solvent. Despite extracting a lower total mass of organic extract, sonication was quicker, used considerably less solvent, and showed a similar extraction selectivity evident in the same distribution of peaks observed in the HTGC chromatograms (Figure 2). The wool extracts from different animals (sheep, alpaca, goat) produced distinguishable chromatograms (Figure 3).

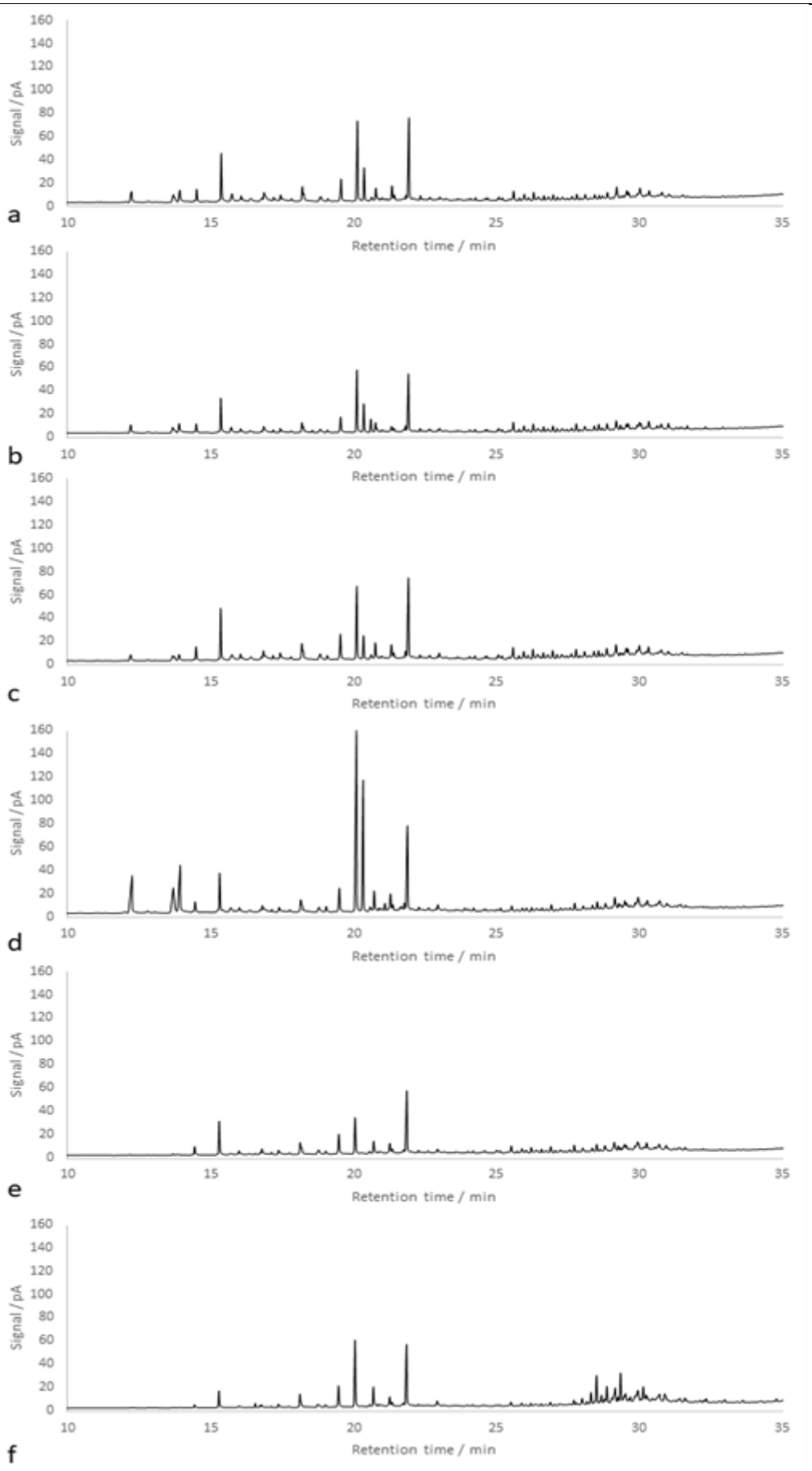

Figure 2: Method development HTGC-FID chromatograms of wool extracts using (a) Bligh and Dyer Soxhlet, (b) modified Bligh and Dyer Soxhlet, (c) Bligh and Dyer sonication, (d) Bligh and Dyer with phosphate buffer sonication, (e) toluene sonication and (f) cyclohexane sonication, extraction.

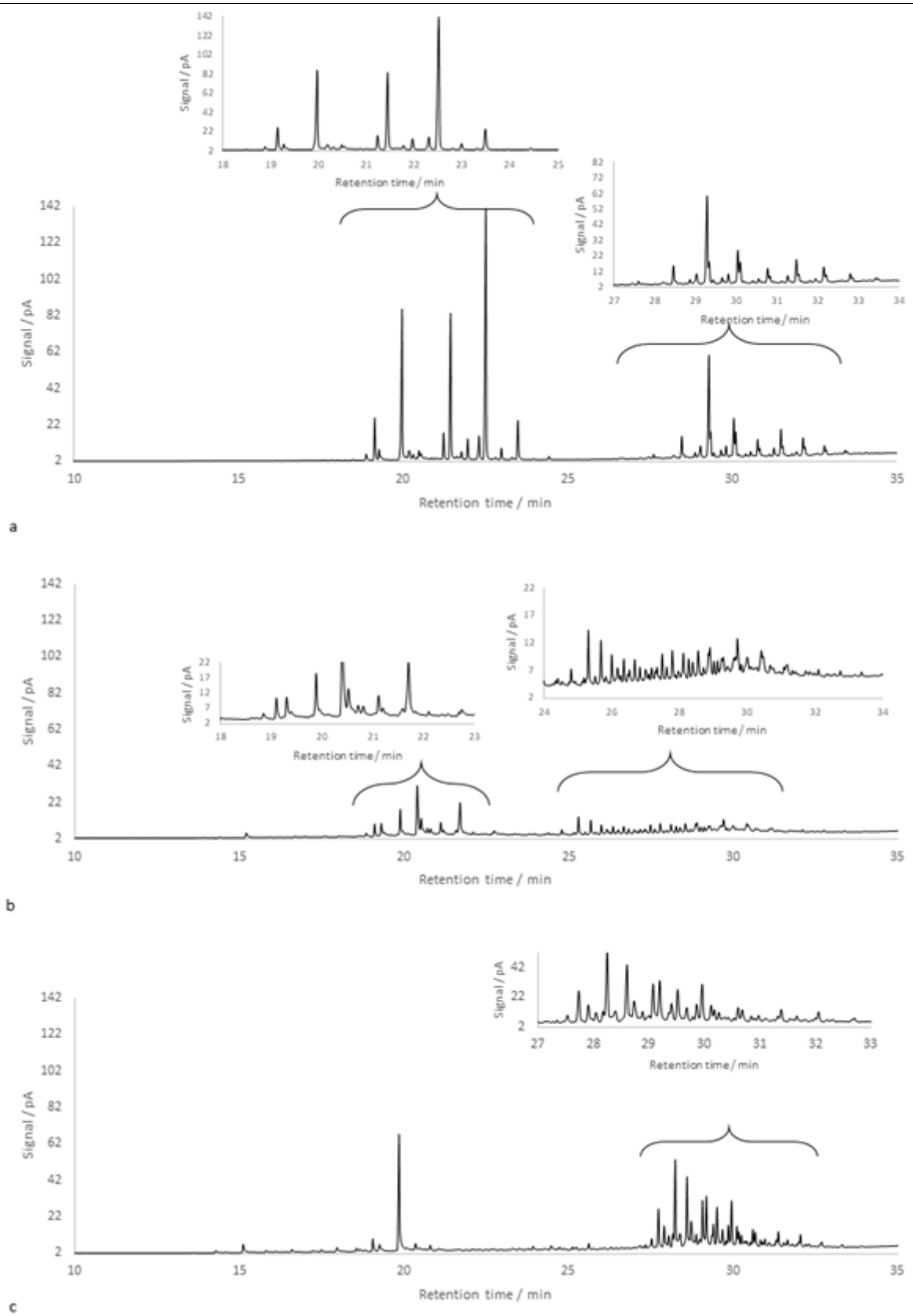

Figure 3: HTGC-FID chromatograms of the wool extracts from the different species: (a) alpaca, (b) mohair, and (c) sheep.

The HTGC-TOF-MS chromatogram for the grease deposit is presented in Figure 4a. The grease contained a cholesterol derivative tentatively assigned as *psi*-cholesterol ($C_{27}H_{46}O$) based on comparison with the NIST mass spectral library match. The mass spectrum showed a molecular ion at *m*/*z* 386 (Figure 4f) and a loss of *m*/*z* 18 consistent with a hydroxyl group loss. The structure of the cholesterol derivative *psi*-cholesterol (Figure 4f) has a different position of the double bond and alcohol group compared to cholesterol. Fatty acids including dodecanoic acid (Figure 4b), palmitic acid (Figure 4d) and oleic acid (Figure 4e) were also identified. A long-chain alcohol was tentatively assigned as pentadecenol (Figure 4c) with the molecular ion at *m*/*z* 226. Unknown I (Figure 4g) with a base ion at *m*/*z* 74 was observed along with unknowns II and III (Figure 4 h and i) which appeared to belong to a homologous series of compounds with a characteristic base ion at *m*/*z* 59. In each case molecular ions were not readily identified. The mass spectrum of Unknown I was similar to 2-(hexylamino)ethanol (Figure 4j), a type of amino alcohol which are frequently used as surfactants and corrosion inhibitors. Unknowns II and III were thought to contain either carboxylic acid, alcohol, amide or ether groups based on the fragment ions observed in the mass spectra (Figure 4i and h). A potential candidate for this class of compounds are (propylene) glycol ethers, with the mass spectra displaying prominent peaks at *m*/*z* 59, *m/z* 103 and *m/z* 117 consistent with the formation of $C_3H_7O^+$, $C_5H_{11}O_2^+$ or $C_4H_7O_3^+$, and $C_6H_{13}O_2^+$ (Figure 4k). Glycol ethers are key components in non-ionic surfactants (surface-active agents) and have been previously reported in solvents extracts from wool (14).

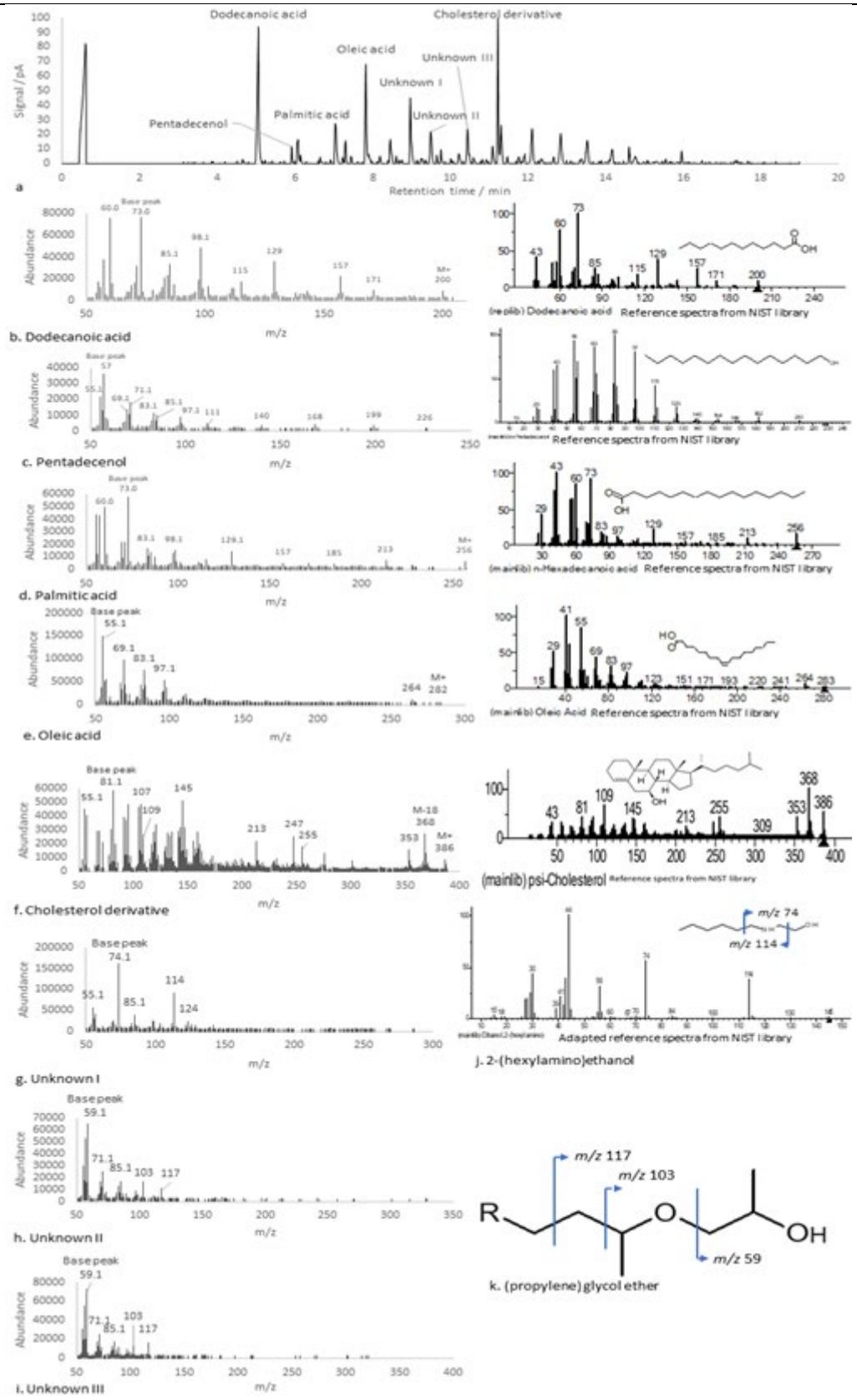

Figure 4: HTGC-TOF-MS total ion chromatogram (a), and selected mass spectra (b-i) from the analysis of the grease deposit.

The HTGC-TOF-MS total ion chromatogram for the wool sample extract is presented in Figure 5a. The wool extract contained cholesteryl derivatives with a putative molecular ion at *m*/*z* 388 (mass spectrum Figure 5f) for *epi*-coprostanol. This could be confirmed by co-injection of an authentic standard. Mass spectra of similar compounds were observed (Figures 5e and 5g) but here the putative molecular ions were at *m*/*z* 370, indicating cholestenes. The internal standard

(Figure 5b) and alcohols were also identified. One of the alcohols had a molecular ion peak at *m/z* 466 and was thought to be 1-dotriacontanol ($C_{32}H_{66}O$) (mass spectrum Figure 5c) and the other had a very weak molecular ion peak at *m*/*z* 494 and was thought to be 1-tetratriacontanol ($C_{34}H_{70}O$) (mass spectrum Figure 5d).

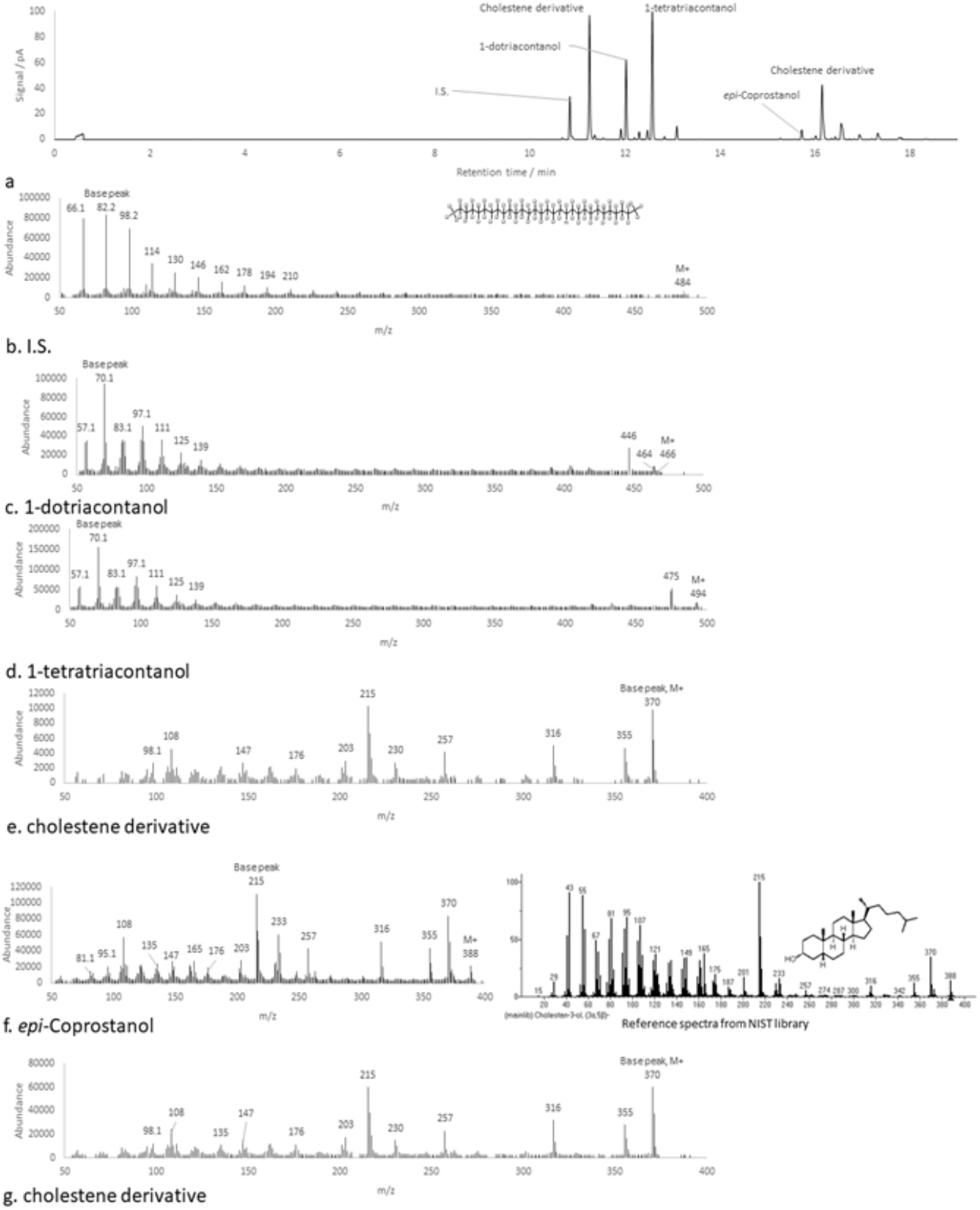

Figure 5: HTGC-TOF-MS total ion chromatogram (a), and selected mass spectra (b-g) from the analysis of the wool extract.

A comparison of a wool extract and the grease deposit extract is presented in Figure 6. There was not much overlap between chromatograms of the problematic grease deposit and the wool sample.

The method of using sonication in cyclohexane was chosen for its efficiency and that most of peaks in the HTGC-FID method development chromatograms were present in all the extractions, as shown in Figure 2. Use of 'softer' ionisation techniques e.g. chemical ionisation mass spectrometry (CI MS) (6), electrospray or atmospheric pressure chemical ionisation, could be performed to provide confirmation of the molecular weight of the larger molecules which fragmented when they were analysed by electron impact mass spectrometry (EI-MS). A common characteristic ion was found in the cholesterol derivatives at m/z 368 (6). Standards of the identified compounds could be analysed (separately or co-injected) to check and confirm the identities made herein. HTGC was more effective than standard GC would have been in characterising the samples, as some of the species only eluted at temperatures higher than routinely employed (i.e. >300ºC).

The results of the organic extracts isolated from the unscoured wool samples reported herein were consistent with Straovoytova and Sitati (1), who analysed grease extracts from wool from a textile mill and found the major constituent to be waxes, cholesterol, fatty acids and alcohols. The chemical composition of the problematic grease sample was markedly different to the raw wool extracts, containing several unknown compounds and homologous series tentatively assigned as glycol ethers. The chemicals identified in the problematic grease sample therefore predominantly reflect chemical classes with high interfacial properties, potentially indicative of an issue with the surfactants used in the scouring process. This was also consistent with the high percent of insoluble material in the problematic grease post-extraction with cyclohexane which may indicate a high inorganic content such as ionic salts and surface-active agents. The inorganic material could also derive from the suint (natural grease) known to contain potassium salts (14) and warrants further investigation of the inorganic fraction using ICP-OES or MS.

4. Conclusions

The wool extracts bore little resemblance to the extract from the problematic grease deposit. The identification of chemicals predominantly with high interfacial properties was indicative of a potential issue with the surfactants used in the scouring process. The high inorganic content indicated the presence of potassium salts derived from the suint or surfactants and additives used in the scouring or downstream processing of wool, warranting further investigation of the inorganic fraction using ICP-OES or MS.

5. Skills gained

Knowledge and practice of procedures used in organic research, such as how to clean pipettes to minimise contamination, the procedure for the dilution of greases and more experience of working in a laboratory was gained in this studentship. FTIR, GC-FID, GC-MS, HTGC-FID and HTGC-TOF-MS analysis and data interpretation were performed, and a greater understanding was acquired. An understanding of how to optimise substances for identification by GC, such as the transesterification of triglycerides was obtained. Confidence in working independently in the

laboratory and in the use a range of GC instrumentation was developed. The techniques, experience and guidance received in the studentship will be invaluable for both finishing my studies and in the career I hope to pursue afterwards.

6. Acknowledgements

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7. References

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