Anatomical location dependence of human decomposition products in clothing

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Abstract

The human decomposition process results in the formation of particular biological compounds, the chemistry of which provides valuable insight into the nature of a death. This paper reports the findings of a study of the decomposition process of clothed human remains at the Australian Facility for Taphonomic Experimental Research (AFTER). An investigation into how decomposition products appear in opposing anatomical regions, namely the anterior and posterior regions of the body, has been carried out. The chemistry of the lipid and protein components and their by-products formed in the first months of decomposition were examined using infrared spectroscopy. The study has demonstrated a clear difference in the pattern of formation of human decomposition products absorbed by textiles located in the anterior versus posterior regions. The time of appearance of established compounds at recognised stages of human decomposition varies notably depending on the anatomical location of the clothing.

1. Introduction

Following death, human remains undergo recognised decomposition processes involving the disintegration of soft tissue and resulting finally in skeletonised remains. The decomposition processes involve the release of lipid, protein and carbohydrate compounds and their resulting by-products at various times and under certain environmental conditions[1,2]. The identification of such products can provide valuable information about the nature of a death, including where and when human remains were placed in a specific location. Although forensic textile damage analysis is utilised to answer the 'how' question in criminal cases[3], an examination of the decomposition products in textiles can further assist investigations with respect to when and where a death has occurred. A number of studies have established that the migration of decomposition products can be monitored and enable biomarkers in the surrounding environment to be identified[4-6].

A previous investigation of the migration of human decomposition fluids into cotton clothing has demonstrated that decomposition products are collected by this absorbent fibre type and their identification can aid in an understanding of the decomposition process[6]. Lipids, proteins and the resulting products formed during the decomposition of human remains were able to be identified at the various stages of decomposition. The Collins et al.[7] study utilized infrared spectroscopy to monitor the changes to cotton samples collected during the first post-mortem months from the posterior region of human remains located in an outdoor setting. This approach enabled the various compounds to be simultaneously identified in a non-destructive manner with minimal sample preparation involved.

The aim of the current study was to investigate the collection of human decomposition products in clothing at different anatomical locations of the body. A comparison of the decomposition fluids migrating into cotton and polyester clothing items from the anterior and posterior regions of the body was carried out to determine if the compositions of the products are

the same in each region and appear in the same time frame. This is designed to provide insight into the influence of positioning of the body and how potential relocation of the remains may be determined based on the presence of recognised decomposition products in clothing.

For the study, a donated clothed human cadaver was placed in an outdoor human decomposition facility, the Australian Facility for Taphonomic Experimental Research (AFTER), location in the Sydney region of Australia. Cotton and polyester clothing exposed to decomposition fluids were examined using an attenuated total reflectance (ATR) sampling technique for the infrared spectroscopy measurements.

2. Materials and methods

2.1 Experimental site

This study was conducted at AFTER, located in the Greater Sydney region, New South Wales (NSW), Australia. A description of the facility and the environmental conditions are documented in Knobel et al.[8]. A 74 year old deceased male, weighing approximately 60-65 kg, was received through the University of Technology Sydney (UTS) Body Donation Program, with consent provided in accordance with the NSW Anatomy Act (1977). Ethics approval was provided by the UTS Human Research Ethics Committee (HREC ETH15-0029). The cause of death for the donor was pulmonary and heart failure. The remains were kept in cold storage at 4°C for 5 days prior to the commencement of the study. The donor was clothed in a white cotton t-shirt, grey cotton underwear and black polyester pants (all purchased from Kmart, Australia), and placed in a supine position on the soil surface. A wire mesh cage was placed over the donor to avoid vertebrate scavenging activity. A control site containing identically sourced clothing was also created at an adjacent location. Weather data were collected using a HOBO® U30 weather station within the

field site. The study took place from October 2018 to January 2019, with an average daily temperature during the period of investigation was 24°C with a total rainfall of 357 mm.

2.2 Sample collection

Two 3 cm x 3 cm sections of the textiles were collected using sterilized scissors from the anterior and posterior (in direct contact with the soil below) aspect of the donor and the control site on a series of days: 0, 1, 2, 4, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, 32, 39, 46, 59, 75 and 101 post-placement. The scissors were cleaned with acetone between each section. The samples were photographed, and any visible changes were documented. Each textile sample was individually packaged and labelled in a paper envelope and stored in a cooler for transportation to the laboratory. Adhering soil and hair was removed from the samples in the laboratory, which were then dried at ambient temperature to impede bacterial and fungal growth. The textile samples were then repackaged into individually labelled envelopes and stored for a maximum of 3 months at -18°C until required for analysis.

2.3 Infrared spectroscopy

Infrared spectra were obtained using a Nicolet Magna-IR 6700 spectrometer (Thermo Scientific, USA) with an ATR accessory consisting of a diamond crystal with a 45° angle of incidence. Spectra were recorded over a range of 4000-400 cm^{-1}, with a spectral resolution of 4 cm^{-1} and averaged over 128 scans. Three replicates were collected for each sample and acetone was used to clean the ATR crystal between each sample.

OMNIC software (version 8.2, Thermo Scientific, USA) was used to collect and auto-baseline correct the spectra. The spectra were processed using Microsoft Excel 2016, with the

replicate spectra converted into CSV files and averaged to produce representative data for each sampling day. Spectra were pre-processed taking the second derivative using the Savitzky-Golay algorithm. Multiplicative and extended multiplicative scatter correction (MSC/EMSC) was applied to normalize the data prior to principal component analysis using UnscramblerX (version 10.3, CAMO, Norway) multivariate statistical software.

3. Results and discussion

3.1 Physical observations

Changes to the human remains were categorized as early, middle and late stages of decomposition using recognised post-mortem observations[9]. The early stage accounts for the fresh and onset of bloat stages and included sampling days 0-7. The middle stage is representative of the transition between the bloat and active decay stages and included sampling days 8-21. The late stage marks the transition of the remains from active decay to the advanced decay stages. This stage was sub-divided to allow for increased discrimination within the late stage (days 22-101), being divided into late 1 (days 22-42), late 2 (days 43-53) and late 3 (days 54-101) stages.

Visual changes to the cotton materials used in this study have been previously described by Collins et al.[7], where it was noted that darker discolouration was observed to the posterior regions as a result of decomposition process. Minimal visual differences were noted for the polyester pants until late stage 1, where the posterior region of the polyester showed areas of yellow discolouration and adipocere formation. No significant visual changes were noted for the control textile specimens throughout the study.

3.2 Comparison of infrared spectra

A number of infrared bands were identified by Collins et al.[7] that prove useful for monitoring human decomposition products collected in cotton textiles. The presence of lipids can be determined by the observation of distinctive sharp bands in the 3000-2800 cm^{-1} region of the spectrum. Strong bands at 2925 and 2850 cm^{-1} are due to the asymmetrical and symmetrical C-H stretching of the methylene chain, respectively, while bands at 2955 and 2870 are attributed to the asymmetrical and symmetrical methyl group stretching, respectively. A small band observed in the C-H stretching region at 3010 cm^{-1} due to cis -C=CH stretching can be used to provide an indication of the degree of saturation in the fatty acids. This band is recognized as a means of monitoring the presence of C=C bonds retained in the fatty acid chains of lipids[10]. The carbonyl region 1800-1500 cm^{-1} also provides information regarding the presence of lipids and the associated by-products. Triacylglycerols produce C=O stretching bands at a wavenumber of 1745 cm^{-1}, while free fatty acids produce a band near 1710 cm^{-1} [11]. Triacylglycerols hydrolyse to free fatty acids as part of the decomposition process, so these bands may be used to monitor decomposition processes. Carbonyl compounds such as ketones and aldehydes, established as late decomposition products, are known to produce a C=O stretching band near 1720 cm^{-1} [2,14]. The carboxylate bands of fatty acid salts appear at 1575 and 1540 cm^{-1} and provide evidence of adipocere, a decomposition product of lipids[11,13]. The presence of proteins may also be determined in the infrared spectra using two bands centred at 1650 and 1545 cm^{-1} that are characteristic of the amide I and II bands of proteins, respectively[14,15]. A decrease in the intensity of the amide I band and a corresponding increase in the intensity of the amide II band is established as a measure of the breakdown of the protein molecules and has been used as a means of monitoring proteolysis[16].

Figure 1 illustrates examples of ATR spectra collected for cotton material before and after exposure to decomposition fluids, indicating recognizable lipid and protein infrared bands that

appear in addition to the cotton spectrum. The spectra for both the cotton t-shirt and underwear samples for each sampling event were inspected to identify the presence of representative lipid and protein infrared bands. Table 1 summarizes the findings where specific marker bands were observed for the anterior and posterior cotton samples (including observations for either t-shirt or underwear) at the various decomposition stages.

For samples collected from the posterior region, sharp lipid methylene bands first appear at an early stage of decomposition and are observed in the spectra collected throughout all stages of the study. There is evidence of unsaturated lipids, known to appear as early decomposition products, in the early and middle stages for the posterior samples, but these lipids are not apparent in the late stages due to conversion to saturated lipids. Triacylglycerols are observed in the early to middle decomposition stages for the posterior cotton, but not in the late stages due to their breakdown to free fatty acids. Free fatty acids in the posterior samples are observed throughout the study as predicted, due to their appearance early in the decomposition process[2]. Recognised late decomposition products, such as aldehydes and ketones, are observed only in the late stages for the posterior samples. Fatty acid salts are formed from the middle stage and are often associated with the formation of adipocere[2]. Proteins are apparent in the posterior spectra from an early stage.

The first appearance of the different compound classes used to monitor decomposition differed for the anterior cotton samples. The methylene lipid bands were first observed for anterior samples during the middle stage of decomposition. Likewise, unsaturated lipids and triacylglycerols were initially observed during the middle stage and were still observed for the late sampling events. No aldehydes and ketones were evident throughout the study for the anterior samples. Fatty acid salts only appear during the last late stage investigated. Proteins are observed

from the middle stage. Overall, there is a delayed appearance of decomposition products in the anterior samples compared to the observations made for the posterior samples.

The migration of decomposition fluids is gravity driven and so leads to pooling of fluids beneath the remains, explaining the earlier appearance of the decomposition products in the posterior region. The appearance of decomposition products in the anterior region of the cotton clothing during the middle decomposition stage correlates with the bloat stage of the remains. The distended remains during the bloat stage enables close contact with the clothing and an increased likelihood of absorption of decomposition fluids by the clothing material.

The monitoring of the appearance of lipid and protein infrared bands in the polyester spectra is less clear than for the cotton spectra due to a very strong polyester contribution in the 1800-1500 cm^{-1} region (Figure 2). However, the appearance of strong lipid bands in the 3000-2800 cm^{-1} region, as well as distinct sharp fatty acid bands, are still able to be identified. The presence of the characteristic protein amide bands is also able to be confirmed. The decomposition product marker bands observed for the anterior and posterior polyester samples at the various decomposition stages are shown in Table 2.

Lipids are observed in the polyester from the middle decomposition stage onwards in samples collected from both the posterior and anterior regions. Fatty acid salts, associated with an advanced stage of adipocere formation[11], are observed in both anterior and posterior polyester samples collected in the late stage 3. Protein amide bands are present in the spectra for the posterior samples collected from the middle stage onwards, but are not observed for the anterior samples throughout the course of the study. Polyester is known to retain less moisture than cotton so the contact between the remains and the polyester pants may not have been sufficient to allow absorption of proteins in significant enough quantities to be observed in the spectra.

3.3. Principal component analysis

Given the complex mixture of decomposition products identified in the clothing samples, PCA was carried out to clarify the observed post-mortem changes. The PCA results for the cotton samples are illustrated by the score plot shown in Figure 3. The first principal component PC1 accounts for 69% of the variability in the data, while the second PC2 component accounts for 23% of the variance, so these components contribute to 92% of the total variance in the data. The plot shows a clear difference in the behaviour of decomposition products contained in the cotton from different anatomical locations. The posterior samples are positively separated, with late stage samples most separated, with the major variation associated with PC1. By comparison, the anterior samples are clustered and overlap in the plot region where early to middle stage posterior samples are observed.

The loadings plots for the cotton samples shown in Figure 4 demonstrate that lipid bands in the 3000-2800 cm^{-1} region has a significant influence on the separation in the score plot. A positive association due to the 1600-1400 cm^{-1} region (connected to lipid C-H bending bands and protein bands) is also noted in the PC1 loadings plot. There are also contributions to the variation in data observed in the PC2 loadings plot that are associated with bands in the 1300-1000 cm^{-1} region, a complex region with overlapping C-O and C-C stretching and C-H bending contributions.

Although the t-shirt and underwear materials sampled were placed on the torso and groin regions of the remains, respectively, the location of the cotton on the body does not appear to impact the chemistry of the decomposition products for the duration of the sampling. In Figure 3, there is no clear separation or clustering of t-shirt or underwear data. Indeed, the fact that polyester pants were placed over the cotton underwear appears to have no significant impact on the observed trends.

Figure 5 illustrates the PCA score plot for the polyester samples. PC1 accounts for 93% of the variability in the data, while the PC2 component accounts for 5% of the variance, so these components contribute to 98% of the total variance in the data. The plot demonstrates a similar pattern of separation for the anterior and posterior polyester composition to that determined for the cotton samples. That is, positively separated posterior samples with later stage samples most separated. The anterior samples are also more clustered with some overlap with the earlier posterior sample behaviour. The separation is not as distinct as that observed for the cotton samples and this is likely due to overlap in more spectral regions of decomposition products and the polyester material.

Figure 6 illustrates the loadings plots for the polyester samples. The PC1 loadings plot shows contributions at 730 cm^{-1} and 1720 cm^{-1} being responsible for the variation in the data. These bands occur at wavenumber values associated with lipid CH_2 bending and C=O stretching modes. It is notable that these regions overlap with polyester bands, but that changes due to the presence of lipids are being detected using this approach. The PC2 loadings plot shows that lipid bands at 3000-2800 cm^{-1} also contribute to variation.

Conclusions

The uptake of human decomposition fluids by two textile types, cotton and polyester, at different anatomical locations has been successfully investigated using infrared spectroscopy. A clear difference in behaviour is observed for clothing covering the anterior and posterior regions of a cadaver in the first months following death. An accumulation of decomposition fluids beneath the body sees the appearance of decomposition products from an early stage in the material located in the posterior region of the body. There is a delay in the detection of the decomposition compounds in the anterior region of the material, with recognized compounds appearing after the

bloat stage has occurred and has enabled sufficient contact between the cadaver and the clothing. It has been demonstrated that fewer individual decomposition products may be identified by inspection of the spectra for polyester samples exposed to human remains due overlap of the respective chemical components.

PCA has provided a means of differentiating the behaviour of the decomposition products contained in the anterior and posterior clothing samples, with lipids providing a stronger marker for decomposition behaviour. The behaviour of decomposition products in both cotton and polyester clothing was able to be monitored at the different post-mortem stages.

These findings are of particular value to those investigating human remains surface deposited in an outdoor environment. The nature of the lipid and protein products identified in textiles provide an indicator of contact with early to late stage human decomposition products, thus adding to the understanding of the time of deposition. It is also important to note that although there are differences in the spectral identification of products in the different textile types, cotton and polyester, based on their own chemical differences, the analysis described is still able to demonstrate clear differences in behaviour for the posterior and anterior regions examined. By understanding that decomposition products migrate into the textiles and concentrate toward the posterior aspect of the body, it is possible to understand the initial physical positioning of a body and also determine if a repositioning event has occurred post-mortem.

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Disclosure statement

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Table 1. Identification of decomposition products in cotton (t-shirt and/or underwear) post-mortem stages using infrared spectra.

infrared band present	**location**	**early stage**	**middle stage**	**late stage 1**	**late stage 2**	**late stage 3**
lipid methylene (3000-2800cm^{-1})	anterior					
	posterior					
unsaturated lipids (3010 cm^{-1})	anterior					
	posterior					
triacylglycerols (1745 cm^{-1})	anterior					
	posterior					
free fatty acids (1710 cm^{-1})	anterior					
	posterior					
aldehydes, ketones (1720 cm^{-1})	anterior					
	posterior					
fatty acid salts (1575, 1540 cm^{-1})	anterior					
	posterior					
protein amide I / II (1650, 1545 cm^{-1})	anterior					
	posterior					

Table 2. Identification of decomposition products in polyester pants during post-mortem stages using infrared spectra.

infrared band present	location	early stage	middle stage	late stage 1	late stage 2	late stage 3
lipid methylene (3000-2800cm^{-1})	anterior					
	posterior					
fatty acid salts (1575, 1540 cm^{-1})	anterior					
	posterior					
protein amide I / II (1650, 1545 cm^{-1})	anterior					
	posterior					

Figure 1. Example infrared spectra for cotton t-shirt before and after exposure to decomposition fluid, illustrating common lipid and protein bands.

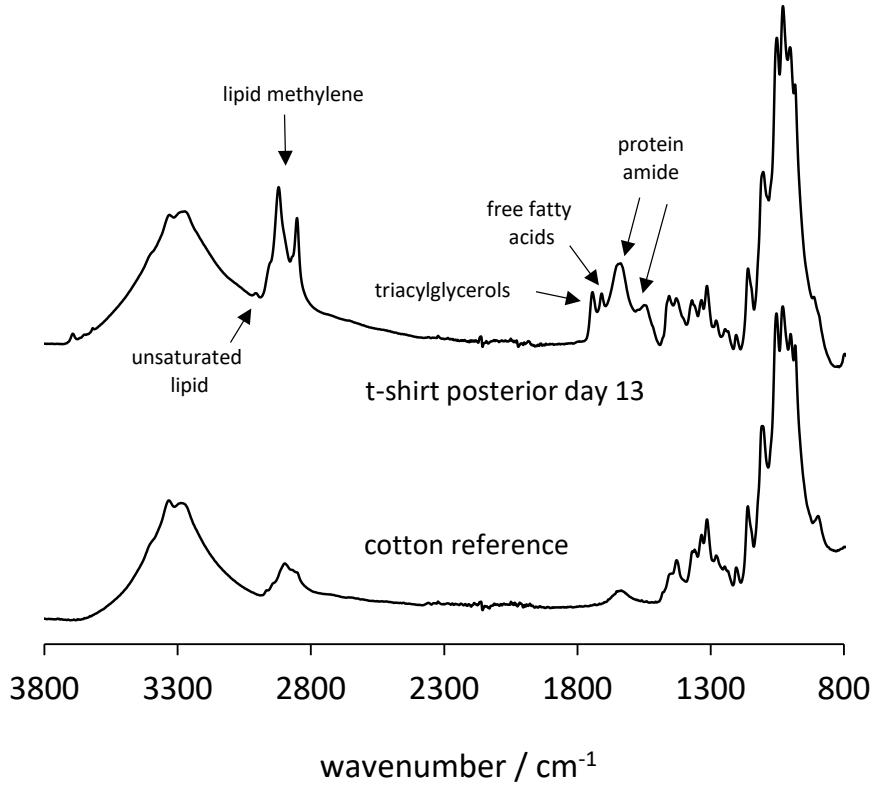


Figure 2. Example infrared spectra for polyester pants before and after exposure to decomposition fluid, illustrating common lipid and protein bands.

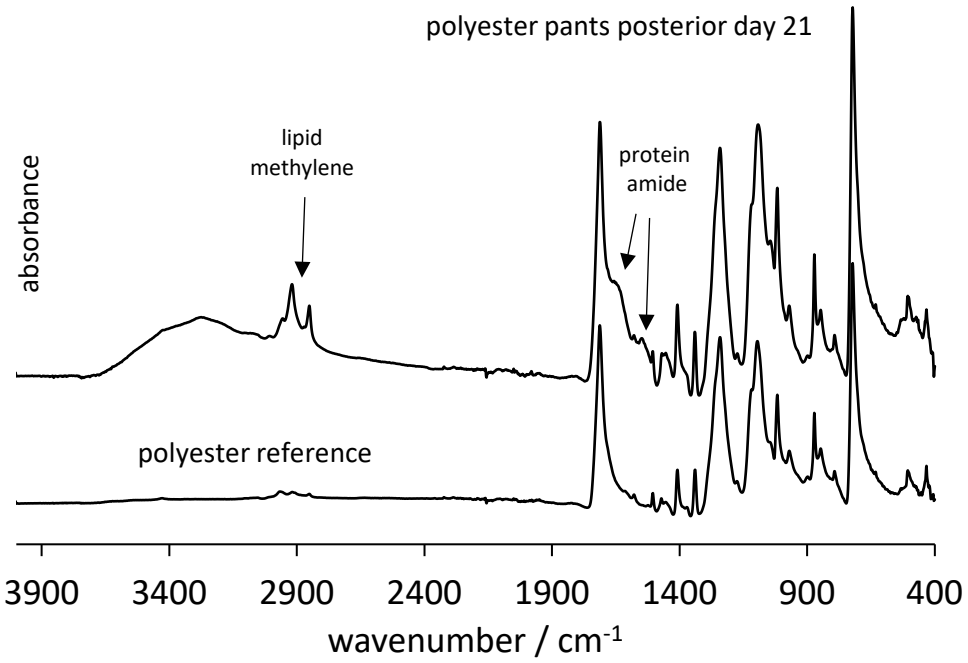
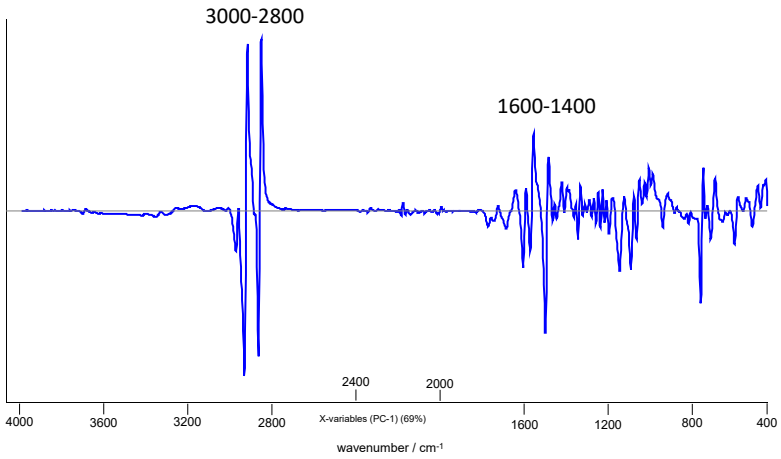


Figure 3. PCA score plot for cotton samples. Squares represent anterior samples and circles represent posterior samples. Anterior t-shirt (TA), posterior t-shirt (TP), anterior underwear (UA), posterior underwear (UP). Numbers correspond to the post-placement sampling day, with replicate samples per day averaged, n = 3.

Figure 4. Loadings plot for (a) PC1 and (b) PC2 for all cotton samples.

(a)



(b)

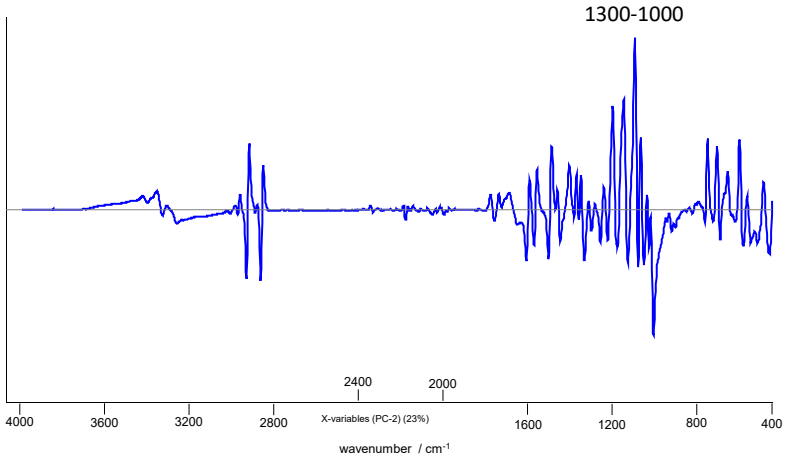


Figure 5. PCA score plot for polyester samples. Squares represent anterior samples and circles represent posterior samples. Numbers correspond to the post-placement sampling day, with replicate samples per day averaged, n = 3.

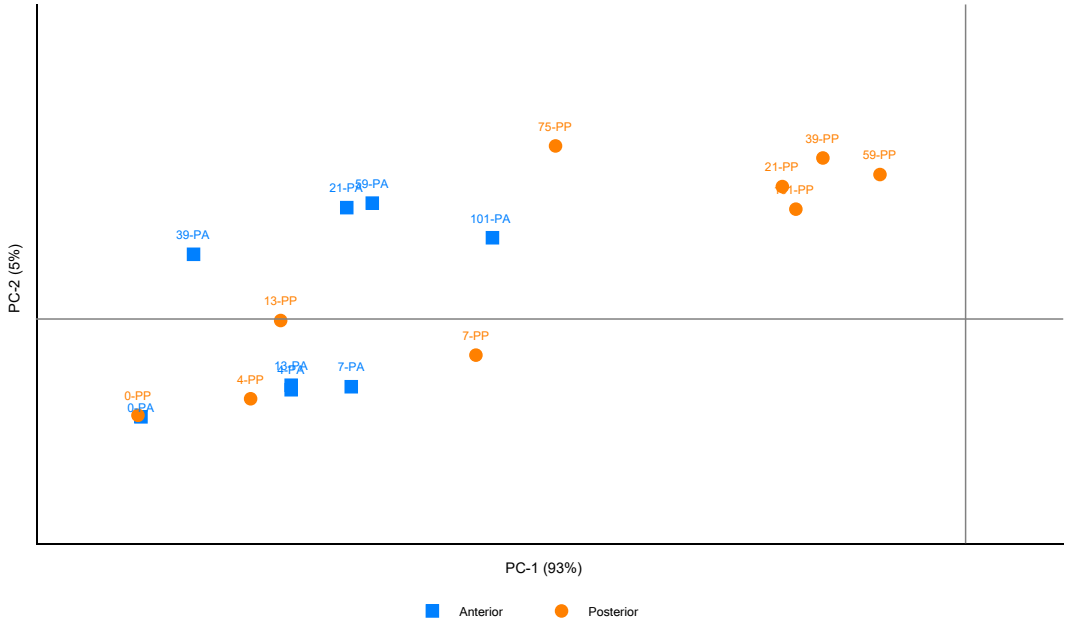
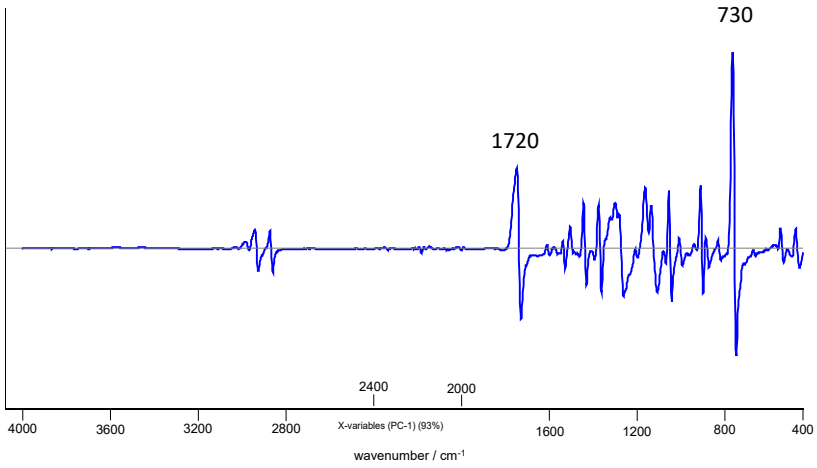


Figure 6. Loadings plot for (a) PC1 and (b) PC2 for polyester samples.

(a)



(b)