



Bryant University

HONORS THESIS

Assessment of Silk Degradation Using Pyrolysis

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Submitted in partial fulfillment of the requirements for graduation
with honors in the Bryant University Honors Program

APRIL 2015

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ABSTRACT

The purpose of this research is to investigate the molecular constitution of silk and the specific causes of post-production degradation in silk material. This capstone project focused specifically on the conditions and degradation of ancient Chinese silk products provided by the Chinese National Silk Museum. Modern silk products were artificially degraded and compared to the ancient silk using Py-GC-MS and comparative analytic techniques. This investigation revealed that the ancient silk products most likely underwent degradation in elevated temperatures while in the presence of moisture and the absence of sunlight or UV radiation during long term burial.

INTRODUCTION

Background

Ancient silk products contain crucial information for understanding the culture and history of China where silk textiles were first produced and later monopolized. Silk products are also the critical material link between East Asia and Europe through both the ancient terrestrial and maritime “Silk Roads”. These trading routes began during the Han dynasty (220 - 280 AD). A boom in silk production in China occurred during the Tang dynasty (618 - 907 AD), which inspired the further expansion of silk across the continent and into Europe. This is an important archeological link. As we discover more silk products throughout the ancient world, we can begin to characterize trading patterns and better understand the lives these peoples lived. Within the regions producing silk, specific cities monopolized the production methods of silk materials. These cities and regions are noted on the map of China in Appendix A. Molecular and isotopic studies of ancient silk excavated from archaeological sites can provide insights into silk production, trade routes, and other cultural information significant to history. Using isotope analysis will allow us to discover the exact city site that much of the discovered silk came from, learning much more about these ancient people. However, ancient silk products, of hundreds or thousands of years old, are more or less degraded at the time of discovery.

In order to understand what conditions these ancient silk products experienced during their lifetime, we must first understand how and what degrades the specific material. In order to better understand the silk burial and degradation process, a degradation study was conducted on modern silk samples which were decayed artificially under different extreme burial conditions by the China National Silk Museum (Hangzhou, China). The museum is currently

examining these modern degradation samples via high performance liquid chromatography (Wouters and Verhecken, 1991), while Bryant University examines them using pyrolysis-gas chromatography-mass spectroscopy (Py-GC-MS).

Although Py-GC-MS analysis is a widely used molecular-level tool to analyze the level of decay and preservation conditions of ancient material, it has never been used to examine silk degradation. Previous silk research that used Py-GC-MS only focused on specific aspects of silk, such as dyes, neglecting the actual silk products. As compared with previous studies on silk degradation, the method we will use, Py-GC-MS, offers the examination of individual compounds, provides excellent peak separation, and has relatively quick results without time-consuming sample preparation or large sample quantities. The lowered analytic requirement is particularly important, as we do not have very much of the ancient silk samples for analysis. These advantages will also allow us to better understand the degraded compound within silk and create a master list of common compounds found in the analyte. Given an understanding of the degradation process, not only will the ancient Silk Road have a comprehensive and complete history, but can also help us understand how to handle and preserve the ancient silk products unburied from archeological sites.

Questions

This research will answer several important and related questions in line with silk degradation and later preservation: 1) what compounds of silk degrade under given burial conditions, 2) what are the main components of silk that can be used in future studies and in comparative analysis, and 3) what conditions have the ancient silk samples been subjected to since their creation. In order to answer these questions, the goals and objectives are to use Py-GC-MS

technology (see methods) for the comparative analyses between ancient and modern degraded silk.

This research represents the first use of Py-GC-MS to examine degradation in silk samples. The findings from this research will hopefully provide insights into the conditions that the ancient silk samples have undergone. The master list will also help to serve further exploration in silk using Py-GC-MS. With this master list, any other researchers wishing to pursue silk studies using Py-GC-MS will have a list of common compounds already determined, so that they might proceed with comparative analysis or their specific study faster, and without much of the preliminary research. Based on the archeological site's given burial conditions, they can provide insights into the biological, histological, and cultural features of ancient silk as well as providing methods to properly handle and preserve the treasured ancient silk found at archeological sites.

Silk

The silk fiber is a natural dual protein fiber, containing two independently secreted proteins, and produced by the silk worm (*Bombyx mori*). *B.mori*, the worm which produces the best silk for human use in textiles was domesticated in China during the Longshan period (3000-2000 BC) from the wild silk worm (*Bombyx mandarina*) through years of selective breeding. (Goldsmith, 2005; Yu, 2011) in comparison to *B. mandarina*, *B.mori* grows and matures at a much more rapid rate leading to enhanced cocoon size and efficiency. *B.mori* is entirely human created though thousands of years of selective breeding for the most efficient use of time and resources. This unique species does not exist in the wild except where they were introduced, they lack basic survival skills including fear of predators, making them entirely dependent on humans for their continued existence. The cultivation of silk, and selective

breeding of the silk worm is known as sericulture. During the early stages of larval development, the silk worm is fed the leaves of white mulberry (*Morus alba*). It is this specific consumption that allows for isotopic analysis and geographic placement of different silk fibers. When degraded, worms living off *M.alba* leaves from different regions will show different isotopic signatures, and allow for reverse construction of environmental factors and the eventual geographic placement of the silk production site.

The silk worm produces the two protein components of silk during its metamorphosis stage between larva and adulthood in the final stage of larval development. During this process, twin exocrine glands combined with modified salivary glands within the worm produce the proteins sericin and fibroin which are stored in the silk gland secreted via the anterior duct and spinneret of the worm near its head upon cocoon spinning (Shimizu, 2000). In its unaltered state, silk consists of a double filament thread, made up of the structural protein fibroin, and held together by the gummy sericin, a macromolecular protein. ($C_{30}H_{40}N_{10}O_{16}$) (Mondal, 2007) (Appendix B). Sericin is a soluble protein, and was historically mostly removed from the fibroin during the common process of boiling the silk cocoon. Fibroin is the main component of the silk fiber, and consists of sheets of repeating amino acids, especially glycine. This structure contributes to the strength and durability of the fibers. Each of these materials is also valued by different industries based on their unique properties. For the textile industry, silk is coveted for its lightweight, luster, and affinity for dyes. In engineering and biomedical industries, silk is useful because of its resistance to oxidation, anti-bacterial qualities, as well as its ability to absorb and release water easily. Both these uses benefit from the fact that silk also does not require harsh chemicals for its production

(Mondal, 2007).The cocoon produced is between three and nine hundred meters long and each fiber is ten micrometers in diameter.

The production of silk from the cocoon of *B.mori* continues with the death of the pupa. This process is often completed by boiling the cocoon whole, thus killing the pupa and removing some of the sticky sericin protein. This must be done for all worms that will not become breeding moths, as the adult moth secretes an enzyme which degrades the silk fiber thus destroying the continuity of the silk thread. The silk is then unwound from the cocoon and woven in to textile materials. Since its inception, the process of producing silk has remained largely unaltered, availing slightly from improvements in technology. The process today is much more efficient and large scale, as sericulture has become an extremely profitable industry (Goldsmith, 2005)

METHODS & INSTRUMENTATION

Degradation

Two major sample types will be used for this study: modern laboratory-decayed silk samples which have undergone extreme degradation conditions and ancient silk samples that have been buried for several thousands of years under natural but unknown conditions. All samples used in the study were graciously provided by the China National Silk Museum.

Modern silk samples were all taken from the same piece of silk fabric (No. 11160 silk fabric made by the Hangzhou Wensli Silk Sciences & Technology Co. Ltd) and then exposed to different degradation methods both physical and chemical conditions, a process known as “aging” in textile studies. The different test conditions were based on conditions that may affect the preservation condition of ancient silk unearthed from archeological sites, including:

dry environments and intense heat degradation (at 125 °C and at 150°C), wet environments and intense heat degradation (at 125°C in water and at 150°C in water), hydrolyzing degradation (in a solution of pH 12 at 70°C), and light degradation (under a xenon lamp of 340 nm wave length with 70% relative humidity at 62°C), along with one control sample (Appendix C). Samples were taken from each degradation condition on the second, sixth, tenth, sixteenth, and twentieth days in order to better understand the process of degradation at different stages. All samples after artificial degradation were air-dried and kept in room temperature for analyses and to not degrade the samples further.

Four ancient silk samples were unearthed from the M1 Chu Tomb at Wufu, Anji County, Zhejiang Province, China (Warring States period, 475-221 BC), the Shi SongZhi's Tomb at Hemudu, Yuyao County, Zhejiang Province, China (Southern Song Dynasty, 1127-1279), and the Munchak-tepa Burial in Ferghana Valley, Uzbekistan (4th-7th Centuries). All questions of comparative analysis will use these samples to compare to modern samples.

PY-GC/MS

The experiment instrumentation is pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Py-GC/MS is common in scientific and industrial uses and is useful for examining trace amount of samples, or samples which are very rare, as the mechanism requires only small amount of samples. Furthermore, solid analytes can be used; analytes do not need to be altered or prepared because pyrolysis thermally decomposes analytes by instant heat, vaporizing the solid into a gaseous state. For this reason, Py-GC/MS is frequently used on fossils and ancient materials as they both are rare to find and in limited abundance.

From pyrolysis, the vaporized sample is forced into the Gas Chromatography (GC). As its name suggests, GC is a type of chromatography that use analytes with gaseous compounds. First, the analyte is injected into a narrow tube known as a column using a microsyringe. GC differs from the most basic forms of column chromatography in that analytes are pushed through a hollow column with silica gel lining, as opposed to a packed column as seen in basic column chromatography. Furthermore, instead of using a liquid mobile phase, a carrier gas stream is used as the mobile phase to push the analyte through the column. Although hydrogen is the most efficient carrier gas for separation, helium is the most commonly used gas because it is inert, non-flammable, unlike highly combustible hydrogen, and has comparable efficiency to hydrogen for most experiments. As compounds move through the column, some exhibit adsorption to the column walls. The strength of adsorption, due to the partition coefficient and other physical properties of the molecules, affects how long the compounds are retained in the column. Therefore, the time that it takes a compound to exit the column is referred to as the retention time. This retention time is generally greater for heavier compounds than for lighter more volatile compounds, however, this is not the case for all compounds. This is the reason the first few compounds out of the GC are often compounds such as toluene.

This retention time is displayed on the chromatogram or graphic representation of the relative abundance of each compound within the sample material. This chromatogram is produced by the last step in the mechanical process, the mass spectrometry, or MS. This process involves bombarding the sample with electrons which ionizes the sample. The ionized compounds are then accelerated and subjected to an electromagnetic field. Ions are then separated and sorted based on their mass-to-charge (m/z) ratio in the mass analyzer module; ions of the same m/z

ratio are equally deflected, which helps demonstrate the abundance of particular compounds in a given sample. Once separated, ions are measured on a detector and graphically represented on an adjacent computer. This process can be compared to a highly sensitive and specific scale measuring mass and the charge of compounds. This graphic representation produced by the MS is known as a chromatogram. These chromatograms depict the compound within the sample by relative abundance. The concept of relative abundance is important for further analysis, as each peak and amount depicted in the chromatogram is a percentage of a whole, and not an absolute value. This method allows for easy comparison between samples with comparative analysis. The relative abundance of each chromatogram is denoted by the height of the peaks on the graph (as seen in Appendix D). Essentially, the higher the peak, the more of that specific compound within the analyte relative to the other compounds.

Py-GC/MS was chosen for this experiment for its significant advantages in sample preparation and comparative analysis. For this study we will only be using one milligram of sample for each test. This method also allows for solid state samples to be used. This unique circumstance is a significant advantage when using delicate partially degraded material. In addition, the results produced are easily compared through the use of computer software programs, and will provide clear results when properly analyzed.

New Method

For the purpose of this specific study, a new method was developed within the Py-GC/MS machinery which allows for much better peak separation. In performing these experiments, it is noticeable that all compounds being studied appear within the first twenty periods of the retention scale, and all compounds after this point were essentially unresolved complex

compounds, or unresolved peaks (Appendix E). The elimination of these peaks would allow for much better peak clarification and separation, so to this end, the Py-GC/MS was initially ramped to 610 degrees °C for thermal decomposition, then allowed to run at a normal 300 degrees °C temperature until the retention time reaches twenty, at which time the temperature is again ramped up, and all unresolved compounds remaining in the GC are burned off. This allows us the compounds within the first section to have a higher relative abundance leading to better peak separation for identification and comparative analysis.

The advantages of this new method are the optimization of the amount of sample used, the minimization of unresolved peaks, and a shorter run time that allows for consistency with the ancient samples. This new method shortens the run time for each sample by twenty minutes, from an hour to forty minutes for each sample, without losing essential key peaks. This method also minimizes the amount of heavy and complex material remaining in the GC column post-run, which often allows for the elimination of blank runs in between sample runs, which further shortens the time for each sample. In addition, the ancient silk samples displayed much fewer unresolved peaks than the modern degraded samples. This new method allows for a more accurate comparison between the two sample sets, as it eliminates the unresolved peaks from the modern samples.

Comparative Analysis

The first step in comparative analysis is to establish the baseline compounds most commonly found within the analyte. These compounds should appear in most if not all of the chromatograms revealing their mother compound's existence within the analyte, in this case silk. Many of the compounds revealing themselves on the chromatograms are pieces, or degraded parts of a whole. To compare the abundances of these degraded remaining

compounds is equivalent to the study of the proportion of the larger compounds within the sample, mainly the complex fibroin protein.

The chromatograms produced from the Py-GC/MS process can be easily analyzed using the Py-GC/MS software on the connected computer. One of the most useful tools for comparative analysis with this specific software is the overlay tool which allows chromatograms to be directly imposed on top of one another. This tool quickly shows trends and correlations between different samples.

Once we have established the baseline elements in the sample, we will combine these into a master list of the degraded compounds found in silk fiber. This master list, featured in Appendix F, will be very useful in future research on silk fiber, as researchers will not need to perform any of the preliminary analysis performed in this study if they already have a list of common compounds found across the board in silk fiber. The only remaining work would be to perform analysis on the specific compounds of their research.

This master list was used to compare trends within the different silk degradation methods, and establish tentative causality in the methods. These trends are denoted by a comparative increase or decrease in the relative abundance of the chromatogram peaks. A comparative increase belies a specific resilience of a compound to the particular stressor being tested. A comparative decrease on the other hand belies a comparative susceptibility to that stressor or degradation method.

RESULTS

Each silk sample was degraded and analyzed both in comparison with samples in other series and control samples. The control sample used was cut from the same cloth as the degraded

samples, but went under no degradation process. This analysis developed patterns for different conditions. These patterns were strengthened through cross examination of similar degradation. For example, if phenol is found at a lower quantity in both the 125 and 150 wet series, but not in the 125 and 150 dry series, then we can reasonable assume that phenol's precursor is degraded more in wet conditions than in dry conditions compared to other compounds in silk. This can also be noted as a comparative susceptibility to moisture. These patterns are then compared to the ancient silk in an attempt to show what condition the ancient silk have undergone during their degradation. For example if both the moisture series and the ancient sample show a comparatively low amount of phenol, which is not found in other test samples, it is reasonable to assume that the ancient sample was degraded in the presence of moisture.

In analyzing the data, the results showed:

When the temperature is increased to 150 degrees Celsius, we found benzyl nitrile (compound 10), to be unusually resilient, appearing in higher abundances in all samples increased to this temperature, but not to the same degree in the 125 degrees Celsius sample series.

When the samples were exposed to water during their degradation, concentrations of propanenitrile (compound 1), were increased steadily. Prolonged exposure resulted in higher relative concentrations.

When samples were kept dry, the relative abundance of indole (compound 16) was increased, suggesting that this compound degrades at a greater rate when exposed to moisture.

When samples were exposed to high levels of pH (pH 12 basic solution), there was little to no alteration in their composition. This suggests that determining the existence of high or low pH during the degradation process will be very difficult, as there will be no way of measuring the degradation, and very low levels of pH will destroy the sample.

When samples were exposed to intense ultraviolet light conditions, the relative levels of phenol, 4-methyl (compound 7) dramatically increased.

The ancient samples showed abnormally high levels of phenol, 2, 4-dimethyl (compound 12), benzyl nitrile (compound 10), propanenitrile (compound 1). Ancient samples also showed a general decrease in levels of phenol, 4-methyl (compound 7) and Phenol, 4- ethyl (compound 13).

The correlations expressed above are not an exhaustive list, however, they are the most consistent and most significant results in determining the situational exposure of the ancient silk compounds.

CONCLUSIONS

In comparing the ancient sample's relative abundance to that of the degraded samples, we can reasonably assume based on the similarity and abundance in the ancient samples and trends in the degradation process of the modern silk, that the ancient samples were exposed to elevated temperatures, moisture, and also lacked exposure to ultraviolet radiation from the sun. Unfortunately, the pH exposure of the samples could not be determined, as the portions of compounds in this series altered very little from the control sample. This means there is little ability to determine if the ancient samples were degraded by pH as comparative analysis was

utilized for the bulk of this testing. The data indicates that high temperatures were present in the degradation process, however, this does not necessarily mean that the sample experiences temperatures of this intensity. In addition, the common trends within the sample exposed to intense light were not only absent in the ancient samples, but in many cases exposed in reverse. This suggests that the ancient samples were exposed to very little light during their degradation process, as those compounds most susceptible to degradation by light were found in higher abundances in the ancient samples, conditions that likely occurred during long term burial.

With this data we have been able to successfully answer all of the questions posed for this project. Using a comparative analysis this study has successfully shown which compounds degrade more or less under specific burial conditions. The Master list of compounds in Appendix F will provide future silk researchers with a starting point for Py-GC/MS studies. Lastly this study was able to infer the burial conditions and degradation process of the Ancient Chinese silk material previously stated.

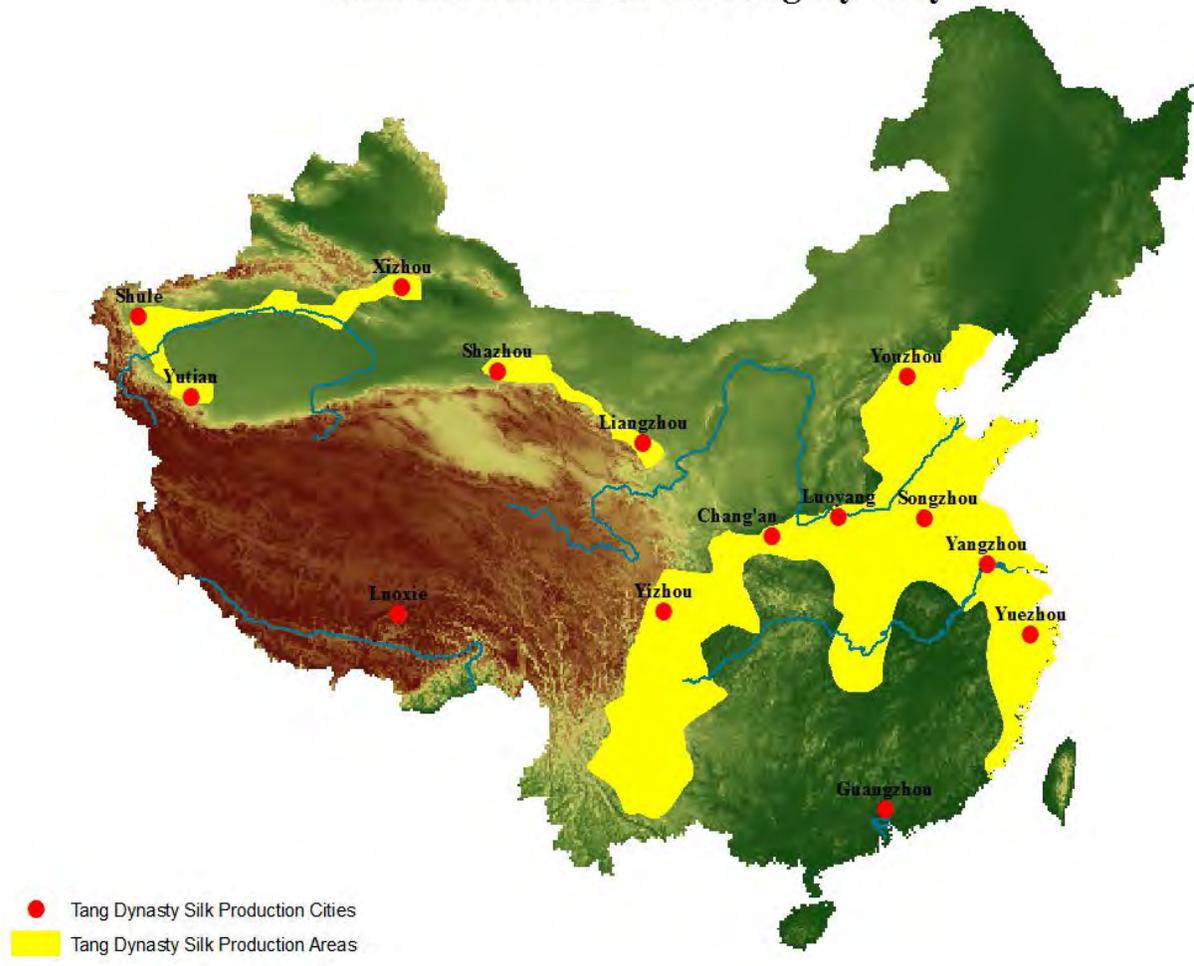
ACKNOWLEDGEMENTS:

1. China National Silk Museum
2. Bryant University Lab for Terrestrial Environments
 - o Hong Yang
 - o Qin Leng
 - o Cait Witkowski
 - o Fellow researchers
3. NASA RISG
4. NASA-RID

APPENDICES

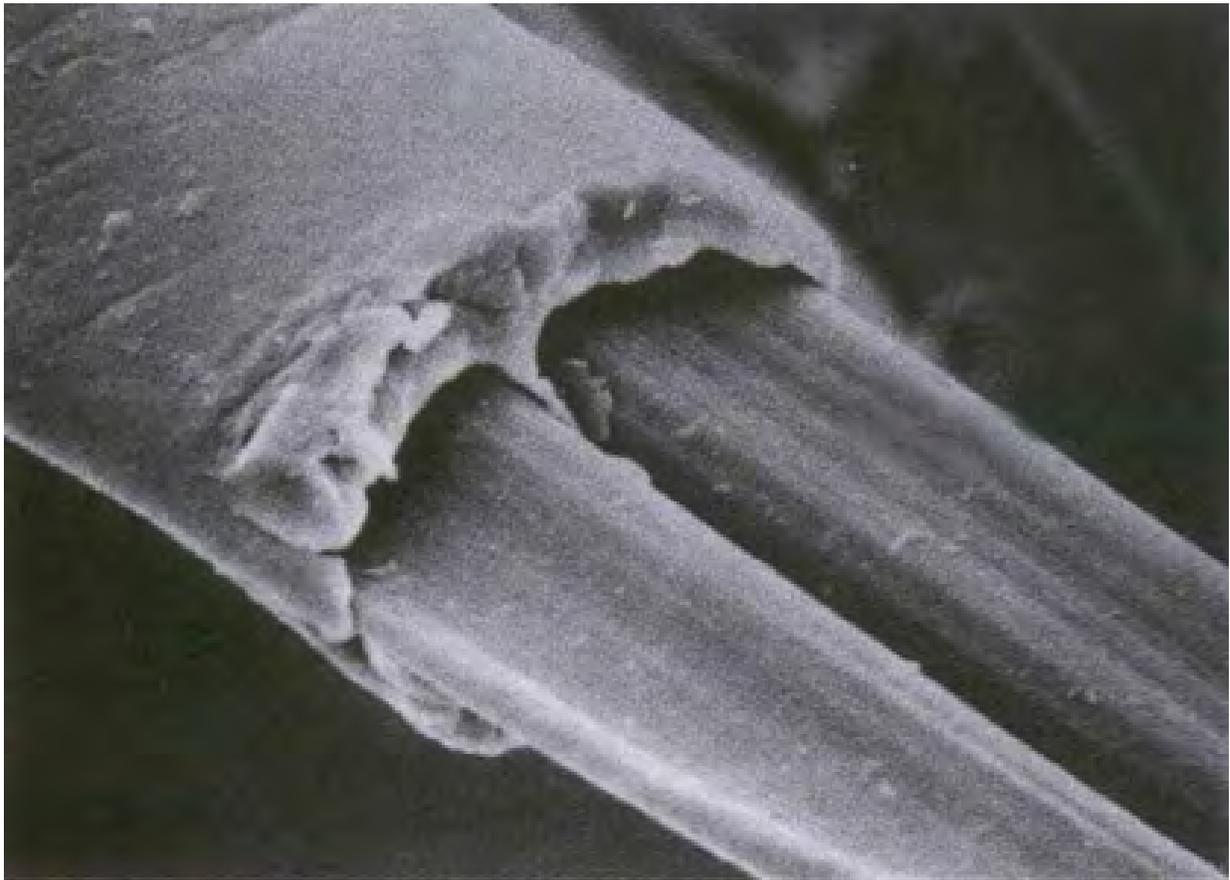
Appendix A – (Map of China's Silk Production)

Silk Production in the Tang Dynasty



Patalano, Robert

Appendix B – (SEM Image of Silk Protein Structure)

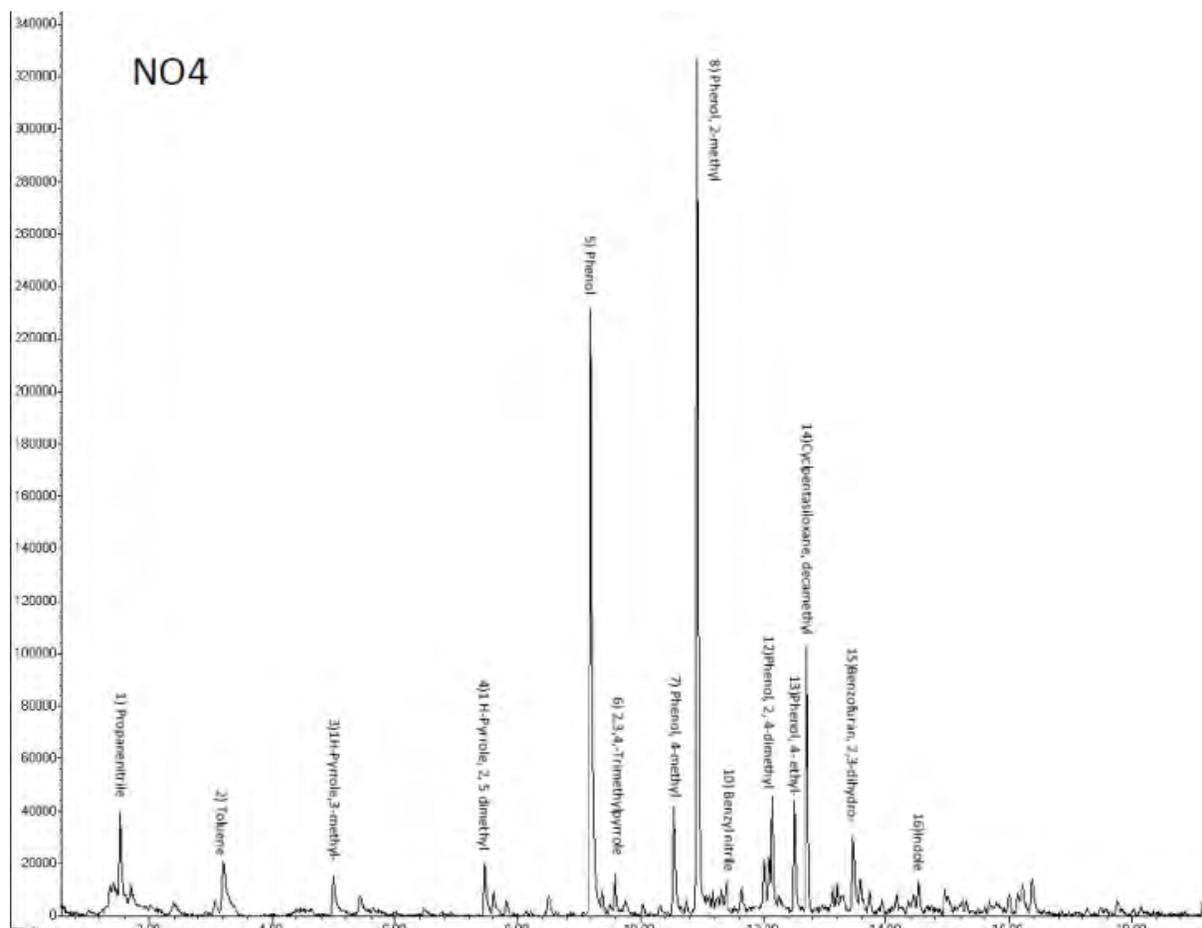


DermaSilk

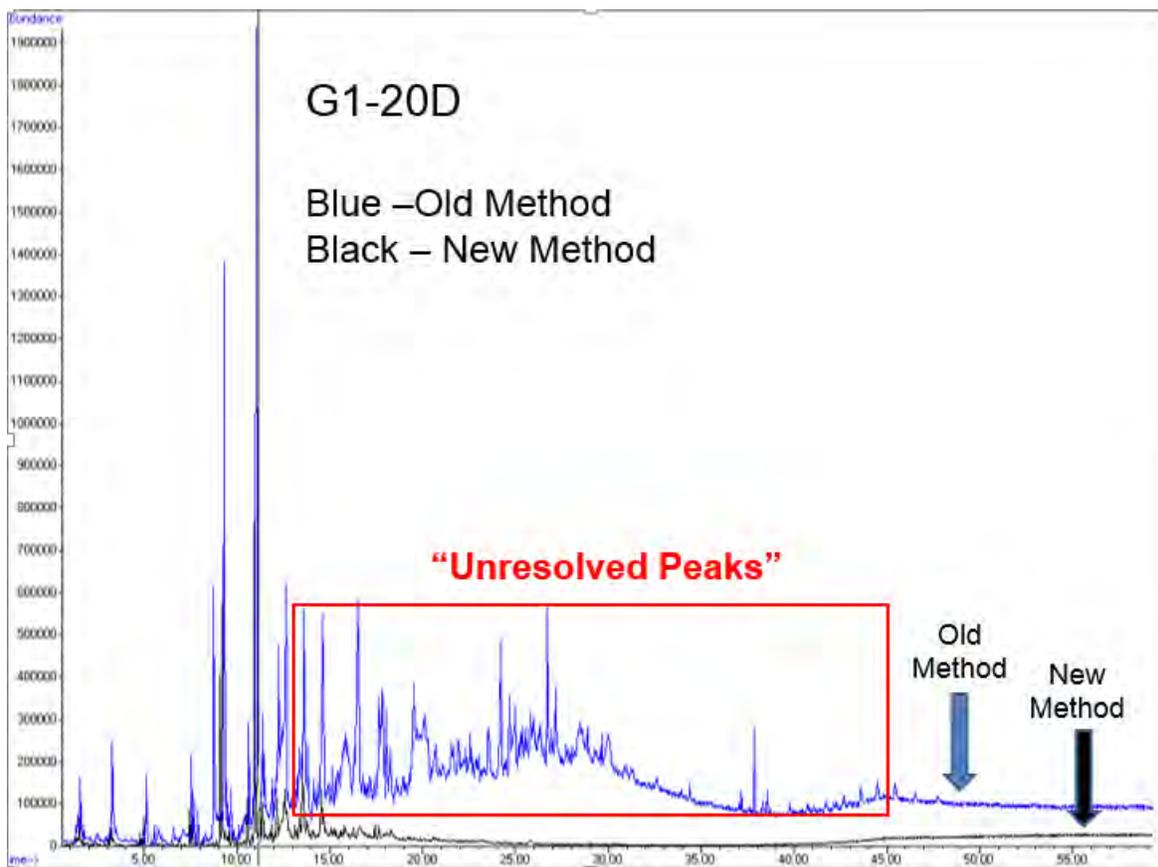
Appendix C – (Variations in Modern Silk Degradation)

Sample Identification	Series Name	Sample Identification
13011110	R2	Silk Fabric without water at 125°C
13011115	R3	Silk Fabric without water at 150°C
13011120	RH2	Silk Fabric with water at 125°C
13011125	RH3	Silk Fabric with water at 150°C
13011130	S4	Silk Fabric pH 12 solution 70°C
13011135	G1	Silk Fabric RH 70% light 62°C
13090601	No4	Ancient Silk Samples
13090602	No5	Ancient Silk Samples
13090605	No62	Ancient Silk Samples
13090606	No147	Ancient Silk Samples
13090613	Box	Ancient Silk Samples

Appendix D – (Example Chromatogram)



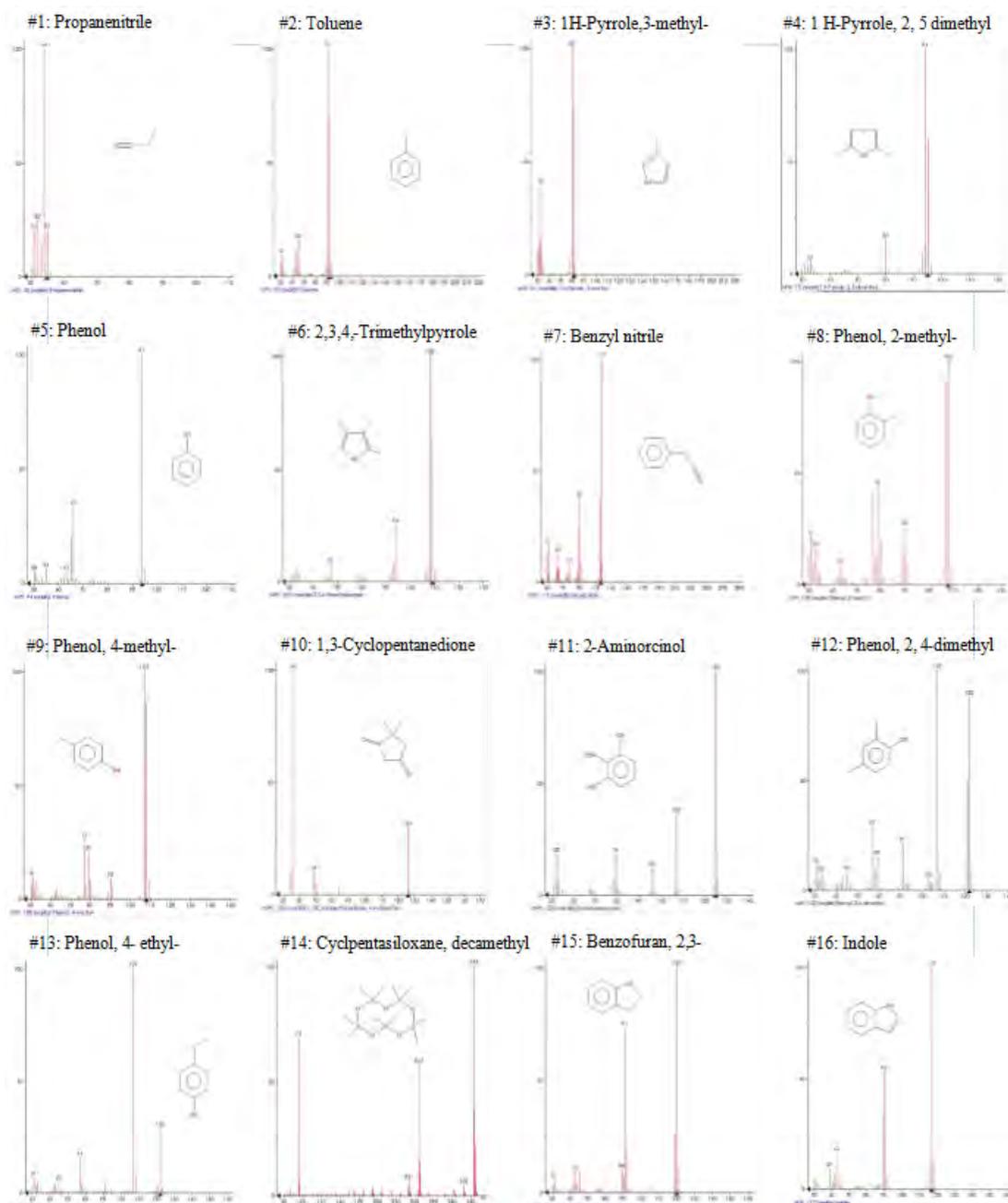
Appendix E – Unresolved Peaks



Appendix F – (Master List and Compound Images)

- 1) Propanenitrile
- 2) Toluene
- 3) 1H-Pyrrole,3-methyl-
- 4) 1 H-Pyrrole, 2, 5 dimethyl
- 5) Phenol
- 6) 2,3,4,-Trimethylpyrrole
- 7) Phenol, 4-methyl
- 8) Phenol, 2-methyl-
- 9) 1,3-cyclopentanedione, 4, 4-dimethyl
- 10) Benzyl nitrile
- 11) 2-Aminorcinol
- 12) Phenol, 2, 4-dimethyl
- 13) Phenol, 4- ethyl-
- 14) Cyclopentasiloxane, decamethyl
- 15) Benzofuran, 2,3-dihydro
- 16) Indole

Appendix F continued; Master list compound images



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 - This source details the method of comparative analysis of specific compounds and asserting their significance in the overall composition of the sample. This source also makes assumptions on the meaning of these compounds on the surrounding environment.
2. Erwin R. Kaal, Mitsuhiro Kurano, Margit Geißler, Hans-Gerd Janssen (2008). "Hyphenation of Aqueous Liquid Chromatography to Pyrolysis-Gas Chromatography and Mass Spectrometry for the Comprehensive Characterization of Water-Soluble Polymers". *Journal of Chromatography A* 1186.1 (2008): 222-227.
 - Details methods used to break down and characterize water-soluble compounds using PY-GC/MS. This outline helped to plan out our studies, and also gives valuable background into the advantages of using PY-GC/MS.
3. Goldsmith, Marian R., Toru Shimada, and Hiroaki Abe. "The genetics and genomics of the silkworm, *bombyx mori**." *Annu. Rev. Entomol.* 50 (2005): 71-100.
 - Goldsmith's research is based on the domestication and morphological differences between the wild silk worm and the domesticated. This journal also explores modern day methods of sericulture including genetic analysis of *B.mori*.

4. Hakimi, Osnat, et al. "Spider and mulberry silkworm silks as compatible biomaterials." *Composites Part B: Engineering* 38.3 (2007): 324-337.
 - Provides an overview of the fibroin protein produced by the silk worm, and other species such as spiders. This report was created to assess silk's biomedical purposes and possible uses.
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 - This source broke down the metallic compounds of silk into the individual elements found in the fibers, and helped to determine what metallic elements and compounds would be common during our own analysis.
7. Liu, Hongtao, et al. "Silk-Inspired Polyurethane Containing GlyAlaGlyAla Tetrapeptide. I. Synthesis and Primary Structure." *Journal of Applied Polymer Science* 117.1 (2010)

-
- This source gives an in depth structural analysis of the main protein of silk, fibroin. The source examines the protein's use in the medical field, it is the initial analysis which is most useful in this case.
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- Mondal presents an easy to understand and comprehensive look at the protein structure of silk's two major proteins (sericin and fibroin) as well as the domesticated silk worm *B.mori*.
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- This source uses the PY-GC/MS process on several textiles, and discusses the various methods and advantages of the process. This source is one which focuses mainly on the dyes of textiles rather than the textiles themselves.
12. Poulin, Jennifer. "Identification of Indigo and its Degradation Products on a Silk Textile Fragment Using Gas Chromatography-Mass Spectrometry." *Journal of the Canadian Association for Conservation= Journal de l'Association canadienne pour la conservation et la restauration* 32 (2007)
- This source uses the process of GC-MS to identify compounds in the dyes of silk, but does not use a thermal degradation process. The process described here is meant to be an improvement on the traditional liquid chromatography approach, and is further improved by using pyrolysis, which requires less sample preparation. This procedure also focused on the dyestuff, specifically indigo.
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- Details the process of quantitative and comparative analysis as far as its relevance to historical and cultural information. The process described uses various analytical methods including PY-GC/MS to examine a variety of compounds which would carry significant historical and cultural information, the most critical of which is their study of proteins.
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intensive and requires a significant amount of sample preparation before the use of the HPLC.

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- This source provides a solid understanding of the use of comparative analysis and use of Py-GC-MS

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- Evidence of silk worm variation during domestication and distinction between independent species is shown

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- Thoroughly explains the proteins in silk (fibroin and sericin), where the two originate, as well as their specific structure.

Images

21. DermaSilk (image) http://www.dermasilk.at/index.php?dermasilk_was-ist-dermasilk

22. Patalano, Robert (silk production image)