



Characterization of proteinaceous and other organic materials in historical stringed musical instruments by off-line analytical pyrolysis with silylation

Alessandro G. Rombolà^{a,*}, Daniele Fabbri^{a,b}, Ryota Shibuya^a, Marco Malagodi^{c,d}, Tommaso Rovetta^c, Giacomo Fiocco^{c,d}

^a Department of Chemistry "Giacomo Ciamician", University of Bologna, via Dario Campana 71, Rimini, Italy

^b C.I.R.I. FRAME, University of Bologna, Tecnopolo di Rimini, via Dario Campana 71, Rimini, Italy

^c Arvedi Laboratory of Non-Invasive Diagnostics, CISRIC, University of Pavia, Via Bell'Aspa 3, 26100 Cremona, Italy

^d Department of Musicology and Cultural Heritage, University of Pavia, Corso Garibaldi 178, 26100 Cremona, Italy

ARTICLE INFO

Keywords:

Musical instruments
Proteins
Pyrolysis
Collagen
GC-MS

ABSTRACT

Historical stringed musical instruments such as violins, violas, and violoncellos are a unique class of cultural heritage objects. The production of these precious musical instruments reached its peak of prosperity in the 16–18th century in a northern Italy city, Cremona, represented by the great Masters of violin-making art. The multi-layered coating system and the pre-treatments applied to the surfaces of wood substrates are assumed to be related to the special sounds and esthetics of these antique instruments. However, due to the absence of written historical records, their construction and finishing methods are still a mystery. For this purpose, chemical analyses are useful to recover historical recipes by providing information about the material composition, methods of manufacture, and past restoration and maintenance procedures. Pyrolysis gas chromatography-mass spectrometry provided useful information on material composition by using specific markers, but protein determination remains an open analytical problem. A comprehensive study on various proteinaceous materials was undertaken to evaluate the performance of off-line pyrolysis combined with solid-phase microextraction with and without on-fiber silylation. The molecular markers were selected for each method. The methods were applied to historic instrument samples from Jacob Steiner, Luigi Baioni, Andrea Guarneri, Francesco Ruggeri, Lorenzo Storioni instruments and a violin from Pietà of Venice. For the first time, the presence of proteinaceous materials was found by analytical pyrolysis in Stainer, Guarneri, Ruggeri, Storioni and probably Pietà of Venice. Different combinations of the markers were observed in each specimen, with pyrocoll, diketopiperazines or silylated 3-hydroxypyridine the most common. Besides collagen, markers indicative of wood components (methoxy phenols, anhydrosugars), gums (anhydrosugars), and resins (dehydroabietic acid, larixyl acetate) provided a complete picture of the organic materials in stringed instruments obtainable by a single analysis of a micro-sample.

1. Introduction

Modern violins and other instruments of the bowed string quartet family originated in Italy, as valuable products of the long-lasting tradition of musical instrument making that flourished in Brescia and Cremona since the 16th century [1,2]. The most famous and precious violins, violas and cellos were built in the renowned workshops of Nicola Amati (1596–1684), Antonio Stradivari (1644–1737), and Giuseppe Guarneri "del Gesù" (1698–1744) placed in Cremona, during the 17th and 18th centuries. It has been shown that each workshop has its own

"know-how" in musical instrument making: the wood choice, the shape, the thicknesses and finally the finishing treatments, are all peculiar aesthetical and functional parts strongly influencing the acoustic features of each instrument [3–5]. The traditional manufacturing techniques, and the recipes employed in the finishing treatments were passed down from Masters to apprentices, gradually changing through the decades. The decline of the art of violin making in Cremona, which occurred from the beginning of the 19th century, caused the loss of these traditions. Therefore, due to the scarcity of written documentation on the Cremonese violin-making traditions, the construction of these

* Corresponding author.

E-mail address: alessandro.rombola@unibo.it (A.G. Rombolà).

<https://doi.org/10.1016/j.jaap.2023.105957>

Received 3 October 2022; Received in revised form 3 March 2023; Accepted 16 March 2023

Available online 20 March 2023

0165-2370/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

masterpieces of inimitable beauty has largely been a mystery for centuries.

Scientific studies of the finishing treatments have been undertaken since the mid-20th century to provide information about the materials used by the Cremonese luthiers and their construction procedures [4,6,7]. However, historic stringed instruments are complex objects that often consist of complex finishing layers applied on the wood substrate, in which pigments, fillers and other inorganic matter are mixed with organic materials like resins, dyes, and glues. Their top plates were crafted from spruce and other structural parts from maple wood, which was often chemically pre-treated causing oxidation and hydrolysis, the consequent change in vibro-mechanical properties of the wood and acoustic characteristics of the instruments [8]. Previous studies confirmed the use of proteinaceous materials in the ground coat layer [9,10]. However, the identification of proteinaceous materials in samples from historical stringed musical instruments is still a difficult task [11,12] due to the presence of a multi-layered coating system which contains a variety of different materials (e.g. pigments, siccatives, fillers). Five potential types of proteinaceous materials in terms of origins can be found in the historic stringed musical instruments [13,14]: (1) animal glue from bones, skins, muscles, and intestines of animals (fish, goats, sheep, goats, cattle, horses, etc.) whose main component responsible for the adhesive properties of glue is collagen, the primary structural protein of animals, (2) glues made from milk or casein, (3) egg yolk containing hydrophobic proteins (e.g., phosvitin) which is one of the most representative phosphoproteins, (4) egg white containing water-soluble proteins, more than half of which is ovalbumin [15] and (5) plant gums that contain glycosylated proteins whose amount is very low and considered difficult to detect [16].

Many analytical methods for the identification and characterization of proteins in Cultural Heritage sample, including artistic, archeological and paleontological objects, have been proposed [17,18], based on staining, immunology, spectroscopic, chromatographic, and proteomics techniques. In the field of musical instruments, due to their high value, sampling is seldom granted by conservators and owners. Nevertheless, non-invasive approaches do not always allow researchers to provide adequate and accurate identification of materials. Chromatographic techniques using high-performance liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC-MS) permit the identification of the proteinaceous material based on the determination of the amino acid composition evaluated after hydrolysis [17,19–22]. The thermal decomposition of the sample by pyrolysis coupled with GC-MS (Py-GC-MS) generates pyrolytic profiles which are useful for the identification of the proteinaceous materials [18,23]. Despite the potential of this technique, relatively few examples in the literature discuss the identification of proteins in artistic, archeological and paleontological objects [24,25]. While the Py-GC-MS has not been applied to study proteinaceous materials in historical bowed string musical instruments. In particular, analytical pyrolysis combined with trimethylsilylation is a powerful approach in GC-MS analysis capable of producing information on a variety of organic components of interest in the field of historical stringed instruments [26]. Off-line and on-line to GC-MS (Py-GC-MS) have been used for the identification of the organic materials, which include components of wood, such as cellulose and lignin, as well as organic components of the resin and oils in varnishes [4,22,26]. However, in on-line pyrolysis silylation the complete derivatization is a major challenge, probably due to the short contact time, partially silylated pyrolysis products are often observed along with persilylated derivatives increasing the complexity of the pyrograms and increasing the difficulty of accurate quantitative approaches [27]. In order to facilitate derivatization, an alternative approach consists of the use of off-line pyrolysis, in which pyrolysis products are firstly trapped and thereafter silylated. Trapping the pyrolysis products can be achieved by solid-phase microextraction (SPME) and the gas-phase silylation can be accomplished directly on the SPME fiber placed in the headspace of a silylating solution. The off-line pyrolysis SPME GC-MS procedure with

on-fiber headspace silylation (Py SPME Syl GC-MS) has been more recently adopted in the field of historical stringed instruments to identify organic components [26,28]. However, the performance of the Py SPME Syl GC-MS method was not evaluated in detail when applied to identify proteinaceous materials in historical bowed string musical instruments.

The present paper is mainly focused on the identification and characterization of proteinaceous materials in historical bowed string instruments. The pyrolytic behavior of protein standards albumin and collagen, and furthermore of sturgeon glue, rabbit glue, and casein glue was investigated by Py SPME with and without on-fiber silylation. The usefulness of pyrolytic markers for the identification of unknown materials was tested by applying Py SPME Syl GC-MS to stringed instrument samples. Specifically, samples from seven historic stringed instruments made by Stainer, Baioni, Guarneri, Ruggieri, and Storioni (Table 1) were investigated by Py SPME Syl GC-MS.

2. Materials and methods

2.1. Samples

2.1.1. Reference materials

The protein standards bovine serum albumin, collagen from bovine Achilles tendon were purchased from Aldrich (St. Louis, MO, United States). The collagen sample contained a non-negligible amount of fatty acids, therefore it was cleansed of these impurities by acetone on a stirrer for two hours and dried after filtration. Another set of protein samples was purchased from Kremer Pigmente: rabbit skin glue (No. 63025), purified sturgeon glue (No. 63114), casein glue (No. 63210).

2.1.2. Historical samples

Seven samples from seven historic stringed instruments were provided by the Arvedi Laboratory of Non-Invasive Diagnostics of the University of Pavia at the Museo del Violino of Cremona. Five of the samples under investigation by analytical pyrolysis are from the Sgarabotto collection, which consists of parts of historic bowed string instruments detached during past restorations by Gaetano Sgarabotto (1878–1959) and his son Pietro (1903–1990) [10,29]: in particular, the five original cellos were made by Jacob Stainer (F01 and F02), Luigi Baioni (F04), Andrea Guarneri (F11), Francesco Ruggeri (F23). Other two samples were detached from the back plate of the Bracco small violin made by Lorenzo Storioni in 1793 (SL793) and from a violin from Pietà of Venice (VEN11) (Table 1). The Bracco small violin, made by Lorenzo Storioni in 1793 (SL793), is from the Civic Violin Collection of the Municipality of Cremona, currently preserved and exhibited at the Museo del Violino of Cremona. The sample VEN11 was taken from the back plate of a violin from the collection of the Istituto di Santa Maria

Table 1
Historic stringed instrument samples selected for the project [10,26,29].

ID	Violin maker	Provenance	Name / Instrument	Part	Collection
F01	Jacob Stainer	Absam, 1621–1683	Cello	Back plate	Sgarabotto
F02	Jacob Stainer	Absam, 1621–1683	Cello	Rib	Sgarabotto
F04	Luigi Baioni	Milan, 1838–1878	Cello	Back plate	Sgarabotto
F11	Andrea Guarneri	Cremona, 1626–1698	Cello	Top plate	Sgarabotto
F23	Francesco Ruggeri	Cremona, 1645–1700	Cello	Rib	Sgarabotto
SL793	Lorenzo Storioni	Cremona, 1744–1816	Bracco / Small violin		Museo del Violino
VEN11	Pietà of Venice	-	Violin		Istituto di Santa Maria della Pietà of Venice

della Pietà of Venice. All the samples were obtained using a scalpel by removing a small area of the surface and reducing it to powder (Table 1).

F01 and F02 samples are fragments composed of maple wood with varnish taken from the back plate with the right lower corner and from the rib near the neck, respectively, of a restored Stainer cello. Sample F04 is from the maple wood backplate of a Luigi Baioni cello and it is composed of a mixture of wood and varnish. Sample F11 is a varnish and wood fragment taken from a violoncello made by Andrea Guarneri, which is mainly composed of spruce wood. Fragment F23 is a varnish and wood fragment taken from the rib near the neck of a cello made by Francesco Ruggeri. The powder sample SL793 is from Bracco small violin. The Bracco small violin made by Lorenzo Storioni has an important value thanks to its rare shape and conservation conditions. It measures around 40 mm less than a full-size instrument usually used by violinists, classified as a half-size violin in modern criteria to be used by a 10-year-old child. The fragment VEN11 is from the back plate of the violin of the collection of Istituto di Santa Maria della Pietà of Venice.

2.2. Materials

Pyridine and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) were purchased from Aldrich (St. Louis, MO, United States). SPME Carboxen/Polydimethylsiloxane (CAR/PDMS) fibers of 75 μm , Polydimethylsiloxane (PDMS) fibers of 7 μm and the fiber holder were purchased from Supelco (Bellefonte, PA, United States).

2.3. Analytical pyrolysis

During method development experiments were performed on protein standards with and without silylation and with two types of fused silica fibers: 75 μm CAR/PDMS and 7 μm PDMS. The results obtained during method evaluation highlighted that several protein markers were produced by off-line pyrolysis without silylation, diketopiperazines among the most specific, suggesting that direct pyrolysis would be better suited for the identification of proteins rather than pyrolysis silylation. However, the relevant pyrolysis markers were important peaks also in the pyrograms obtained after silylation. By considering that several silylated pyrolysis products are employed for the identification of a variety of organic materials, it was decided to apply the Py SPME GC-MS with silylation as an elective technique to investigate the chemical composition of historic stringed instrument finishes. Regarding the fiber selection, for comparison of the results obtained during method evaluation by off-line pyrolysis with CAR/PDMS and PDMS fiber, commercial CAR/PDMS was selected. In fact, the results indicated that the Carboxen/Polydimethylsiloxane composite coated fiber had better extraction capacity of the pyrolysis products from nitrogen flow. The off-line pyrolysis solid-phase microextraction gas chromatography-mass spectrometry procedure with CAR/PDMS on-fiber headspace silylation (Py SPME Syl GC-MS) was selected as final analytical procedural.

The analytical procedure with headspace silylation step before GC-MS analysis was described in a previous work [26]. Briefly, a quartz sample tube containing the sample (< 0.5 mg) was inserted into the platinum coil of the probe (CDS Analytical, Inc. Pyroprobe 1000). The probe was introduced into the pyrolysis chamber and the apparatus was flushed with a nitrogen stream at 10 $\text{cm}^3 \text{min}^{-1}$ for the duration of the analysis. Before pyrolysis, the exposed fiber was placed at the exit of the quartz tube (about 1–2 mm from the probe) and the sample was pyrolyzed at 500 °C for 100 s at the maximum heating rate. At the end of pyrolysis, for the silylation procedure, the SPME fiber was placed in the headspace of a 2 mL glass vial, closed with SIL/PTFE perforable septum, containing 50 μL of silylation agent, BSTFA with 1% TMCS + 10 % pyridine (Aldrich, St. Louis, MO, United States) for 10 min. The Py SPME GC-MS analyses with and without silylation were carried out in duplicates for each standard material. A sample of collagen from bovine Achilles tendon was analysed in triplicates by Py SPME Syl GC-MS in

order to estimate the reproducibility that can be expected when the same, homogenous, sample is analysed with the same technique. Extracted ion chromatograms were obtained for selected *m/z*: *m/z* 167-hydroxypyridine (TMS), *m/z* 166-phenol (TMS), *m/z* 180-methylphenol (TMS), *m/z* 186-pyrococoll, and *m/z* 194-cyclo(Pro-Pro). Areas were integrated, and normalized for their sum. The relative standard deviations (RSD) of the normalized areas and retention times were calculated, and resulted below 24 % and 1 %, respectively. Procedural blanks (pyrolysis/silylation) were performed between each analysis to avoid cross-contamination.

2.4. GC-MS

After the exposure time, SPME desorption was performed at 280 °C in splitless mode in the injection port with SPME liner of a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. Analytes were separated by a HP-5 fused-silica capillary column (stationary phase poly[5% diphenyl/95% dimethyl]siloxane, 30 m, 0.25 mm i.d., 0.25 μm film thickness) using helium at a flow rate of 1 mL min^{-1} as the carrier gas. The following thermal program was used: 50 °C for 5 min, 10 °C min^{-1} until 325 °C, and 10 min at 325 °C. Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the 30–650 *m/z* range. Pyrograms were interpreted with the Agilent MSD ChemStation software using NIST reference library for mass spectral identification or comparison to published literature data.

3. Results and discussion

3.1. Reference materials

3.1.1. Proteins

Py SPME GC-MS with and without silylation has been performed of collagen, albumin, sturgeon, rabbit and casein glue to determine the distinctive molecular markers upon pyrolysis.

3.1.1.1. Collagen. The presence of protein signature peaks in infrared spectra in previous work [7,10,11] confirmed that in historical stringed musical instruments proteinaceous materials were used for violin assembly and repairs. Collagen refers to a group of structurally distinctive triple-helical proteins that contains a high proportion (>30 %) of glycine and (~20 %) proline amino acids. It also contains the unusual amino acids hydroxyproline and hydroxylysine and is considered to be the most abundant protein in mammals. The results of collagen from bovine Achilles tendon analysis clearly show in the pyrogram the presence of pyrococoll and DKP which are related to proline (Tables SM1 and SM2). Moreover, proline formed DKPs with other species of amino acids like hydroxyproline and glycine while cyclo(Pro-Gly) presumably be produced from other amino acids besides glycine when their side chain are lost. Pyrococoll, cyclo(Pro-Pro) and cyclo(Pro-Gly) were previously detected by Py-GC-MS as major compounds from pyrolysis of leather collagen [30], and bones [24,31]. Some nitriles with phenyl groups were also observed. Especially benzenethanamine suggest the presence of phenylalanine together with bibenzyl.

3.1.1.2. Albumin. Albumin is possibly found in various materials of animal origin and the bovine serum albumin, in particular, is rich in albumin where more than half of its protein accounted for albumin. Specifically, protein sealer, as animal and albumin glue, was used as a primer on the wood treatments of historic objects [15]. Py SPME GC-MS analysis with and without silylation was conducted for bovine serum albumin characterization. The results clearly show some fragments related to protein including benzenethanamine, benzylnitrile without silylation and 2-pyrrolidinone, pyroglutamic acid, pyrococoll, cyclo(Pro-Pro) with silylation (Tables SM3 and SM4). Pyroglutamic acid is

a relevant pyrolysis product of albumin, produced by pyrolysis of glutamic acid, which is one of the most abundant amino acids of albumin. The pyrolysis of amino acids lateral chains leads to the formation of aromatic compounds (benzenepropanenitrile, phenol and indole) and cyclic compounds, among them DKPs, obtained by pyrolytic scission and depolymerization of the polypeptides chain, followed by condensation reactions [23]. Fabbri et al. [32] reported toluene, phenol, 4-methylphenol; benzylnitrile, benzenepropanenitrile; indole, pyrrole, pyrrolidine and cyclo(Pro-Leu) as major compounds obtained from Py-GC-MS of bovine serum albumin. Cyclo(Pro-Pro) and pyrocoll were identified in the pyrograms of our study. However, these pyrolysis products are not useful in distinguishing albumin from other proteins. Phenyl amines or nitriles are quite many in the number of species while a relatively low proportion amounts to phenylalanine and tyrosine.

3.1.1.3. Sturgeon, rabbit and casein glue. The results obtained from the analysis of purified glue from sturgeon are provided in Table SM5. As it was observed in the former analyses for collagen, proline, one of the most abundant components of collagen, was converted in pyrocoll and DKP (cyclo(Pro-Pro)), upon pyrolysis. These two compounds are often found in the pyrolysates of other proteins, so they are useful markers for the presence of generic proteinaceous material, but not useful for identifying specific types of proteins. Another DKP, cyclo(Pro-Ala) is only found from collagen, and appeared also in the analyses of collagen from bovine achilles tendon while these were without silylation, therefore it may be a strong candidate for collagen to distinguish it from other proteinaceous materials. There are many other peaks with ion fragment m/z 70, attributed to the pyrrolidinium cation in the mass spectra of DKP containing proline [32], which may serve as preliminary screening for the presence of protein markers in the pyrolysate.

In the same way as the sturgeon glue, rabbit glue was analyzed, and the results are provided in Table SM6. The pyrolysate of this sample contained fewer peaks than that of sturgeon glue. In addition, the presence of peaks of fatty acids and other unidentified pyrolysis products could indicate the presence of contaminants in rabbit skin glue or contamination during sample analyses. Several markers which were found in the sturgeon glue were detected, among which 2-ethyl-4-methylpyrrole, pyrocoll and cyclo(Pro-Pro). In the pyrogram of rabbit glue nitrogen-containing aromatic compounds as benzylnitrile is detected. Benzylnitrile is an important marker for protein but it is not informative about the type of protein occurring in the sample. 3-hydroxypyridine and 3-hydroxy-6-methylpyridine are silylated compounds still observable in this result. 2-pyrrolidinone and pyrrole-2-carboxylic acid are most remarkable ones as the markers, keeping a clear connection to the original amino acids and being subject to silylation.

Gel sample of casein glue was also studied by pyrolysis method and the results are provided in Table SM7. Pyrolysis products containing indole and bibenzyl indicated that the casein glue sample is somewhat abundant in tryptophan and phenylalanine [33]. Indole and bibenzyl, pyrolysis products from some characteristic side chains, are present in the results. This implicating the casein glue sample is somewhat abundant in tryptophan and phenylalanine. No silylation, unfortunately, took place on any of the fragments which are clearly generated from the protein. The most remarkable finding in this analysis is the variety of DKPs. It did not end up with pyrocoll and cyclo(Pro-Pro) as in collagen, it contained some more DKPs, cyclo(Pro-Leu) and cyclo(Pro-Phe).

Py SPME Sy GC-MS data indicate that sturgeon, rabbit and casein showed pyrolytic profiles in agreement with literature observations [25, 33–35]. Pyrrole, alkyl pyrroles, benzyl nitrile and 3-phenylpropionitrile are the most abundant compound in animal glue. Toluene and methyl-phenol are the main pyrolysis products of casein glue, followed by indole, benzenepropanenitrile, phenol, benzeneacetoneitrile, methyl-indole and diphenyl-ethylene. Regarding the presence of the more diagnostic DKPs, they are associated to the amino acid sequences in the protein: cyclo(Pro-Leu) and cyclo(Pro-Phe) dominate the DKPs in

casein glue, cyclo(Pro-Gly), cyclo(Pro-Hyp), cyclo(Pro-Pro) and diketodipyrrole prevail in the DKPs of animal glue [25,34]. Different silylated pyrolysis products were observed upon silylation, among which some pyridine derivatives, 3-hydroxypyridine, 3-hydroxy-6-methylpyridine and 2-pyrrolidinone. The trimethyl silyl ester of pyrrole-2-carboxylic acid was also tentatively identified. The silylated DKP were previously reported [32]. In order to identify the origin of pyridines, some aminoacids have been analysed under the same conditions of proteins, namely tryptophan, phenylalanine, histidine, proline, and glutamic acid. The results (data not shown) indicated that the TMS ether of 3-hydroxypyridine was formed from the analysis of tryptophan.

In summary, the possible molecular markers for each proteinaceous material are present in Table 2. The most significant among them was pyrocoll which always appeared when proline was abundant. The DKP of proline also appeared in some analyses, however its absence in some pyrograms may imply this would not be a marker as strong as pyrocoll. In addition to them, in the pyrogram of standard samples aromatic nitriles compounds (i.e., benzylnitrile, benzenepropanenitrile) are detected as well as bibenzyl compounds. However, aromatic nitriles and bibenzyl are markers for protein but do not give further information about what kind of protein the sample contains since pyrolysis products of a variety of proteinaceous materials are abundant in these compounds. In all of the analyses, maleimide, succinimide, glutarimide and 2-pyrrolidinone, lactams related to glutamic acid were never observed probably because of cross-link or some other reactions of the carboxyl side chain. Among silylated products, the derivatives of 3-hydroxypyridine, 2-pyrrolidinone are proposed as general markers of proteins along with the highly specific marker cyclo(Pro-Hyp).

3.2. Historic violin samples

The results of Storioni's Bracco and the violin from the collection of the Istituto di Santa Maria della Pietà of Venice, as well as the 5 samples from the Sgarabotto collection (Table 1) are reported and discussed in the following paragraphs within the frame of the morphological and chemical characterization reported in previous papers [10,26,28,36].

The pyrograms of historic samples and tentative peak identification of all six instruments are reported in Table 3 and Figs. 1 and 2. Interpretation of results in terms of probable material composition is summarized in Table 4, which provides a schematic comparison of materials identification of historical instruments. Pyrolysis products revealed in this study which were especially relevant for the identification of specific proteinaceous materials are listed in Table 2.

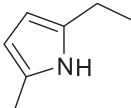
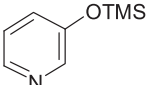
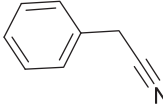
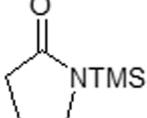
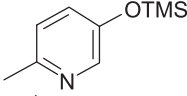
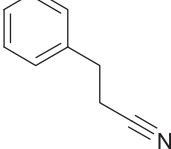
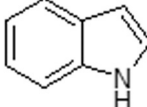
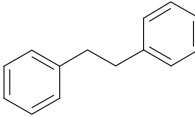
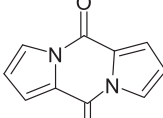
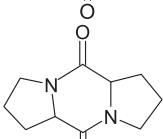
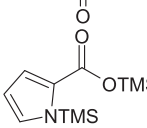
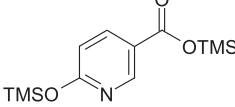
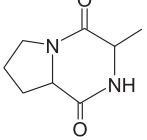
3.2.1. Historic samples containing protein materials

The results of historic samples show the presence of protein molecular markers signature peaks in the pyrogram of F01, F02, F11, F23 and SL793 samples (Figs. 1 and 2). The presence of protein molecular markers signature peaks in the pyrogram of F01 sample confirms the results obtained in a previous FTIR analysis [11]. The pyrogram of Stainer F01 showed the peak of pyrocoll (#62) characterized by the intense ion at m/z 186 at the retention time almost the same as the pyrolyses of proteinaceous standard materials while cyclo(Pro-Pro), its hydrogenated form, was not confirmed here. An unidentified peak (#33, Fig. 1, Table 3) might be a marker for collagen since it was found only in the pyrogram of collagen. A possibility that a bibenzyl product resulted typical of phenylalanine since similar bibenzyl compounds have been observed from the analyses discussed above.

A small peak attributed to pyrocoll (#62), that was identified as a probable marker of collagen, was present in the pyrogram of F02 sample by Jacob Stainer. The presence of unknown peak (#33, Fig. 1, Table 3), determined in the pyrolysis product of collagen, would confirm the occurrence of collagen. Moreover, pyrolysis markers of general proteins as 2-hydroxypyridine (#7) and 3-hydroxypyridine (#18) were identified in the pyrogram of F02 sample. The presence of proteinaceous material in the F02 sample confirmed the results obtained in previous research by

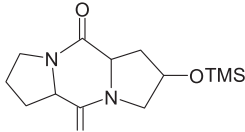
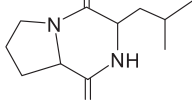
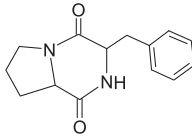
Table 2

Principal molecular markers of proteinaceous markers by off-line pyrolysis with and without silylation.

Material	Silylation	R _t (min)	Compound	Structure	MW	m/z
Most of Proteins		10.0	2-ethyl-4-methylpyrrole		109	80, 94, 109
	X	12.3	3-hydroxypyridine (TMS)		167	152, 166
		12.4	Benzyl nitrile		117	63, 90, 117
	X	12.5	2-pyrrolidinone (TMS)		157	73, 100, 142, 157
	X	13.3	3-hydroxy-6-methylpyridine (TMS)		181	75, 113, 136, 165, 180
		14.0	Benzenepropanenitrile		131	65, 91, 131
		14.8	Indole		117	63, 90, 117
		17.8	Bibenzyl		182	91, 182
		20.1	Pyrocoll		186	65, 93, 130, 186
		22.5	Cyclo(Pro-Pro)		194	70, 96, 110, 138, 166, 194
Collagen	X	16.0	Pyrrole-2-carboxylic acid (bisTMS)		255	73, 152, 193, 211, 240, 255
	X	17.5	6-hydroxy-3-pyridinecarboxylic acid (bisTMS)		283	73, 263, 283
		20.7	Cyclo(Pro-Ala)		168	70, 97, 125, 168

(continued on next page)

Table 2 (continued)

Material	Silylation	R _t (min)	Compound	Structure	MW	m/z
	X	25.1	Cyclo(Pro-Hyp) (TMS)		282	70, 124, 156, 239, 267, 282
Casein		21.2	Cyclo(Pro-Leu)		210	70, 125, 154
		26.1	Cyclo(Pro-Phe)		244	70, 91, 125, 153, 244

SEM-EDX and μ FT-IR [10].

The results of Py SPME Syl GC-MS analysis of F11 sample suggest that it might contain proteins. This hypothesis is supported by the presence of hydroxypyridine, biphenyl compound and unknown peak found in standard collagen. However, the evidence of protein is still insufficient without conclusive markers like pyrocoll (#62) or DKPs. Previous studies have provided information on the chemical characteristics of this sample [11,26]. However, it was not clear if there was proteinaceous material in Guarneri F11 sample.

The pyrogram and detailed interpretation of F23 Ruggeri sample analysis are presented in Fig. 1 and Table 3. A small peak attributed to pyrocoll (#62), characteristic markers for collagen, was observed in pyrolysates of F23 Ruggeri sample. The presence of cyclo(Pro-Pro) was not confirmed here. The unidentified peak #33 (Fig. 1, Table 3), observed in the pyrogram of collagen, was also identified in the results of F23 sample analysis. Hydroxypyridine (#7, #18) is perhaps related to protein for the absence of other N-containing material in this sample. Remarkably 2-pyrrolidinone (#19) was identified in the pyrogram, which was observed in the pyrograms of glutamic acid but not in those of proteins. The chromatograms of the Storioni Bracco showed the probable presence of collagen (Fig. 2). There is a piece of decisive evidence only found in collagen, cyclo(Pro-Hyp) (*29) since hydroxyproline is exclusively contained in collagen among proteins commonly applied as glue. Pyroglutamic acid (*16), pyrrole-2-carboxylic acid (*13) as well as smaller fragments 2-ethyl-4-methylpyrrole (*3), 3-hydroxypyridine (*8), 3-hydroxy-6-methylpyridine (*11) and 2-pyrrolidinone (*7) also were markers for proteins. However, the presence of these products (except 2-ethyl-4-methylpyrrole) is not enough to support the assumption that collagen is present in this sample. Pyrocoll (*24), the most significant marker, is not so easy to recognize in this result because the peak overlapped by neighboring stronger peaks of silylated 1,6-anhydromannofuranose (*22) and 1,4-anhydromannopyranose (*23).

The materials of the finishing layers on the Storioni Bracco were characterized in previous studies [7,11,12,37]. In Fiocco et al. [11] a varnish layer, composed by natural resins and possibly siccativ oil, was clearly identified by non-invasive FTIR in reflection geometry, as well as the presence of proteinaceous glue was determined by μ ATR-FTIR analysis by Albano et al. [7]. In this study, it was assumed that the presence of proteinaceous glue can be justified by following restoration actions undertaken to prevent the mechanical damage likely produced by the strain exerted by the strings. Moreover, proteinaceous material in the upper varnish layer was evidenced by Fiocco et al. [12] by using synchrotron radiation FT-IR micro-spectroscopy. The results obtained through Py SPME Syl GC-MS analysis are consistent with those reported in these previous studies [7,11,12,37].

3.2.2. Historic samples without protein markers

The results of historic violin analysis show the absence of protein molecular markers signature peaks in the pyrogram of F04 and VEN11 samples. The absence of protein material established in a previous study [29] for Baioni cello was confirmed in this study. In fact, Py SPME Syl GC-MS here did not give any protein marker either. Based on the fact that almost no peak contained nitrogen, it may be concluded that F04 does not contain proteinaceous material. The powder sample F04 from the maple wood backplate of a Luigi Baioni's cello was composed of a mixture of wood and varnish as reported in a previous study [26].

The fragment VEN11 from the backplate of the violin of the Pietà of Venice did not show almost anything for resins and proteinaceous materials. Only 3-hydroxypyridine (#18) may be attributed to protein while even pyrocoll (#62), a fragment present in most of the protein analysis is not found here to lower the probability of proteinaceous materials' presence.

3.2.3. General characteristic of organic materials in historic musical instruments

The interpretation of the probable composition of the historic violins is summarized in Table 4. The chromatograms were dominated by pyrolysis products of holocellulose and lignin, due to the wood substrate being the main material. The chromatograms of all the historic violins showed many peaks attributed to cellulose such as 1,2-ethanediol (#3), 2-hydroxymethylfuran (#6), 2-hydroxy-1-methyl-1-cyclopenten-3-one (#24) and levoglucosan (#63). The typical pyrolysis products of lignin of type G (softwood) and S (hardwood) were common to all the examined samples except in the chromatogram of the Guarneri sample. Guaiacol (#27) and syringol (#42), 4-methylguaiacol (#34), 4-ethylsyringol (#53), 4-vinylsyringol (#55) and several other lignin pyrolysis products were identified. The fact that the substrates of Stainer, Baioni, Ruggeri, Storioni and Pietà of Venice were made of maple wood can be confirmed by the presence of the molecular markers for both G Lignin and S Lignin, e.g. guaiacol and its derivatives for G Lignin and syringol for S Lignin. The wood substrate of Guarneri cello is probably spruce wood, only G lignin and no S lignin derivatives were observed. The G lignin derivatives include guaiacol (#27), 4-vinylguaiacol (#46), and isoeugenols (#52, #54).

Typical markers of drying (siccativ) oils such as glycerol (#30), palmitic acid (#75) and stearic acid (#82), as well as the characteristic oxidation products of drying oils (e.g., azelaic acid, #66), were detected in all the varnish samples. Azelaic acid was shown to be a predominant compound in the pyrolysis/silylation of pure linseed oil, however, matrix components such as certain pigments may drastically reduce its detection [37]. The results show the probable presence of pinaceae resin in Stainer, Baioni, and Ruggeri instrument varnishes by the presence of

Table 3

Pyrolysis products identified in pyrograms obtained by Py SPME Syl GC-MS of Stainer F01 and F02, Baioni F04, Guarneri F11, Ruggeri F23 and Pietà of Venice VEN11 samples.

#Peak ^a	R.T. ^b	Compound	<i>m/z</i> ^c	Origin	Sample Id.					
					F01	F02	F04	F11	F23	VEN11
1	9.2	4-pentenoic acid (TMS)	39, 75, 96, 157	Linseed oil		X	X	X	X	
2	9.4	Pentanoic acid (TMS)	75, 105, 117, 159	Linseed oil				X	X	
3	9.60	1,2-ethanediol (bisTMS)	73, 103, 147 , 191	Cellulose	X	X	X	X	X	X
4	9.61	Hexanol (TMS)	41, 75, 159	Linseed oil		X	X			
5	9.70	Pentanoic acid (TMS)	73, 105 , 117	Linseed oil		X			X	
6	9.77	2-hydroxymethylfuran (TMS)	73, 81, 125, 155 , 170	Cellulose		X	X	X		X
7	10.5	2-hydroxypyridine (TMS)	67, 110, 152 , 167	Protein	X	X		X	X	
8	10.7	Indene	39, 63, 74, 89, 116			X		X		
9	10.9	Phenol (TMS)	95, 151 , 166		X	X	X	X	X	X
10	11.1	Hydroxypropanoic acid (TMS)	73 , 147, 191, 219				X			
11	11.20	Hydroxyacetic acid (bisTMS)	73, 117 147 , 178	Cellulose			X			
12	11.23	Hexanoic acid (TMS)	41, 75, 117, 131, 173	Linseed oil	X	X		X		
13	11.31	Octanol (TMS)	39, 75 , 103, 143, 171	Linseed oil	X	X		X	X	
14	11.35	Unknown	82, 96, 119, 147, 207			X	X		X	
15	11.38	Benzylnitrite	63, 90, 117	Protein					X	
16	11.4	5-undecanol (TMS)	41, 75, 97, 119, 173	Linseed oil		X		X	X	
17	12.2	<i>o</i> -cresol (2-methylphenol) (TMS)	73, 91, 135, 149, 165 , 180	Lignin	X	X	X	X	X	X
18	12.3	3-hydroxypyridine (TMS)	73, 125, 152 , 167	Protein		X	X	X	X	X
19	12.40	2-pyrrolidinone (TMS)	73, 100, 142 , 157	Protein					X	
20	12.41	<i>m</i> -cresol (3-methylphenol) (TMS)	91, 135, 165 , 180	Lignin			X	X	X	X
21	12.6	<i>p</i> -cresol (4-methylphenol) (TMS)	91, 115, 165 , 180	Lignin	X	X	X	X	X	X
22	12.7	Heptenoic acid (TMS)	75 , 117, 132, 185, 200	Linseed oil				X		
23	12.8	Heptanoic acid (TMS)	75 , 117, 187, 202	Linseed oil	X	X	X	X	X	
24	13.3	2-hydroxy-1-methyl-1-cyclopenten-3-one (TMS)	75 , 128, 169 , 184	Cellulose	X	X	X		X	X
25	13.4	4-octanol (TMS)	73, 75, 119, 143, 171	Linseed oil		X				
26	13.5	3,5-xyleneol (3,5-dimethylphenol) (TMS)	75, 105, 149, 179 , 194	Lignin		X		X		
27	13.8	Guaiaacol (TMS)	73, 136, 151, 166 , 181, 196	G Lignin	X	X	X	X	X	X
28	14.0	Benzoic acid (TMS)	73, 77, 105, 135, 179 , 194		X	X	X	X		
29	14.2	Octanoic acid (TMS)	73, 75, 117, 129, 201, 216	Linseed oil	X	X	X	X	X	
30	14.5	Glycerol (trisTMS)	73, 117, 147 , 205, 218, 299	Linseed oil	X	X	X	X	X	X
31	14.60	Hexanoic acid (TMS)	45, 75, 101, 131, 173	Linseed oil				X		
32	14.62	3-methoxyphenol (TMS)	73, 181 , 196	Dragon's blood		X				
33	14.9	Unknown	73, 91, 119, 147, 167, 180, 193 , 210, 239, 256	Protein?	X	X			X	
34	15.0	4-methylguaiaacol (TMS)	50, 76, 104, 142, 165, 180 , 195, 210	G Lignin		X				
35	15.11	3,4-diethyl-1,1'-biphenyl	73, 91, 149, 165, 180 , 195, 210	Protein?	X			X		
36	15.13	Unknown	73 , 115, 142, 166, 207, 254, 341, 429	Shellac?					X	
37	15.14	Catechol (1,2-benzenediol)	45, 73 , 115, 136, 151, 166, 180, 207, 239, 254	Lignin	X			X		
38	15.5	Methyl benzoic acid (TMS)	75, 91, 119, 149, 193 , 208	Shellac				X	X	
39	15.6	Nonanoic acid (TMS)	41, 73, 75, 117, 129, 215	Linseed oil	X	X	X	X	X	
40	15.7	Methylbenzoic acid (TMS)	45, 65, 91, 119, 149, 159, 193 , 208		X	X	X	X	X	
41	16.23	Resorcinol (1,3-benzenediol) (bisTMS)	73, 196, 239 , 254	Lignin		X		X		
42	16.24	Syringol (TMS)	73, 153, 181, 196, 226	S Lignin			X			
43	16.25	1,4-dihydroxybenzene (bisTMS)	73 , 239, 254	Lignin		X				
44	16.3	D-Xylofuranose (tetrakisTMS)	45, 73, 117, 147, 217 , 230	Cellulose	X					X
45	16.6	Unknown	73, 91, 115, 128, 141, 156 , 161, 207	Linseed oil?			X			
46	16.7	4-vinylguaiaacol (TMS)	73, 192 , 207, 222	G Lignin	X	X	X	X	X	
47	17.2	Eugenol (TMS)	73, 206 , 221, 236	Lignin	X		X	X	X	X
48	17.3	4-methylsyringol (TMS)	73, 167, 210 , 225, 240	Lignin						X
49	17.4	3-methoxy-1,2-benzenediol (bisTMS)	73 , 115, 163, 254, 269, 284	Lignin	X	X				X
50	17.8	Hexanedioic acid (bisTMS)	73, 75, 147 , 185, 275	Linseed oil		X				
51	18.0	Vanillin (TMS)	73, 194 , 209, 224	Lignin			X	X		X
52	18.35	Z-4-isoegenol (TMS)	73, 206 , 221, 236	Lignin	X					X
53	18.39	4-ethylsyringol (TMS)	73, 209, 224 , 239, 254	S Lignin		X				
54	18.4	E-4-isoegenol (TMS)	73, 206 , 221, 236	Lignin		X		X		
55	18.7	4-vinylsyringol (TMS)	73, 147, 179, 222 , 237, 252	S Lignin	X	X	X			X
56	19.0	1,1-bis(3,4-dimethylphenyl)ethane	193, 208, 223 , 238							X
57	19.2	1,4a,6-trimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalene-1-carboxylic acid (TMS)	73 , 251, 267, 283, 293	Sandarac, Manila Copal			X			
58	19.3	Unknown	73, 127, 170 , 207, 257	Linseed oil?	X					
59	19.4	Unknown	73, 147, 179, 207 , 281, 355 , 385, 401	Shellac?	X					
60	19.7	1,4a,6-trimethyl-1,2,3,4,4a,5,8,8a-octahydronaphthalene-1-carboxylic acid (TMS)	73, 161 , 176, 279, 294	Sandarac, Manila Copal	X				X	
61	19.8	Unknown	73, 207, 225 , 241, 256	Protein (Collagen)?	X					
62	20.0	Pyrocoll	73, 93, 129, 147, 186	Protein	X	X			X	
63	20.12	Levoglucosan (triTMS)	73, 147, 204 , 217, 333	Cellulose		X	X	X		

(continued on next page)

Table 3 (continued)

#Peak ^a	R.T. ^b	Compound	<i>m/z</i> ^c	Origin	Sample Id.					
					F01	F02	F04	F11	F23	VEN11
64	20.13	Glycerol monolinoleate (bisTMS)	73, 147, 207, 221 , 265, 279, 295, 310, 327, 341, 355, 369, 385, 399, 415, 429	Linseed oil				X		
65	20.2	<i>E</i> -4-isopropenylsyringol (TMS)	73, 205, 236 , 251, 266	S Lignin			X			X
66	20.3	Nonanedioic acid (Azelaic acid) (bisTMS)	73 , 75, 129, 201, 217, 317, 332	Linseed oil	X	X	X	X	X	X
67	20.9	Glycerol monooleate (bisTMS)	73, 103, 207, 221, 251, 295	Linseed oil				X		
68	21.3	Tetradecanoic acid (TMS)	73,117, 207, 285, 300	Linseed oil		X		X		
69	21.40	Dihydroxynaphthalene (bisTMS)	73, 207, 304	Madder	X			X		
70	21.48	1,4a,5,6-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalene-1-carboxylic acid (TMS)	73, 175 , 190, 293, 308	Sandarac, Manila Copal				X		
71	21.51	1,4a,5,6-tetramethyl-1,2,3,4,4a,8a-hexahydronaphthalene-1-carboxylic acid (TMS)	73 , 91, 119, 133, 173, 188, 291, 306	Sandarac, Manila Copal			X			
72	22.0	Unknown	73, 91, 131, 145 , 189, 207, 269	Shellac?			X			
73	22.1	Methyldihydroxynaphthalene (bisTMS)	73, 207 , 318	Madder	X					
74	22.4	1-methoxy-2,3-dihydroxy-5-allylphenol (bisTMS ether)	294 , 309, 324	Lignin						
75	23.3	Hexadecanoic acid (Palmitic acid) (TMS)	73, 117, 313 , 328	Linseed oil	X	X	X			X
76	23.8	Oleic acid (octadecenoic acid) (TMS)	73 , 117, 129, 339, 354	Linseed oil						X
77	24.0	Unknown	73, 105, 133, 143 , 185, 207, 255, 281	Sandarac, Manila Copal?	X					
78	24.1	Unknown	73, 143 , 185, 255	Linseed oil?			X			
79	24.3	Unknown	73, 143 , 244, 257	Linseed oil?			X			
80	24.7	9,12,15-Octadecatrienoic acid, glycerol ester (bisTMS)	73, 143 , 221, 255, 295, 369	Linseed oil			X			
81	25.0	Dehydroabietic acid (TMS)	73, 207, 357 , 372	<i>Pinaceae</i> resin			X			
82	25.1	Octadecanoic acid (Stearic acid) (TMS)	73, 117, 129, 341 , 356	Linseed oil	X	X	X			X
83	26.1	Pimaric acid (TMS)	73 , 207, 343, 359, 374	<i>Pinaceae</i> resin			X			
84	26.3	Dehydrodehydroabietic acid (TMS)	73, 153, 195, 237 , 370	<i>Pinaceae</i> resin			X	X		
85	26.5	Dehydroabietic acid (TMS)	73, 173, 239 , 357, 372	<i>Pinaceae</i> resin			X	X		
86	26.9	Larixyl acetate (TMS)	73, 143 , 255, 270, 327, 355	Venice Turpentine			X			

NOTES:

^a Peak referred to GC peak labels in Fig. 1.

^b R.T. retention time in minutes.

^c *m/z* of characteristic ions (italics molecular ion or molecular mass, bold base peak).

some characteristic markers as well as pimaric acid (#83), dehydro-dehydroabietic acid (#84) and dehydroabietic acid (#85). The *Pinaceae* resin was identified to be Venice turpentine through the presence of larixyl acetate (#86) [38,39]. The evidence of *pinaceae* resins is not clear in Guarneri sample. The results showed some peaks such as 1,4a,5,6-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalene-1-carboxylic acid that suggest the presence of *pinaceae* materials, e.g., although its intensity is low.

Shellac was identified in chromatograms of all the historic violins, except in the Pietà of Venice sample, by the peak of methylbenzoic acid (#40). Diterpenic resin such as sandarac or manila copal are probably contained in the Stainer, Baioni, Guarneri and Ruggeri samples. It was identified by 1,4a,6-trimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalene-1-carboxylic acid (#57), 1,4a,5,6-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalene-1-carboxylic acid (#70) and 1,4a,5,6-tetramethyl-1,2,3,4,4a,8a-hexahydronaphthalene-1-carboxylic acid (#71) through comparison with the standards. Previous analysis of the F11 sample with μ -FTIR revealed the presence of natural resins, mainly diterpenic resins such as sandarac and copal, and the possible presence of shellac and wax [10] confirmed in a previous study by Py SPME GC-MS [26].

Dragon's blood resin was identified as the possible red colorant in Stainer and Baioni samples. The presence of Dragon's blood was supported by 3-methoxyphenol (#32) identified as the most abundant pyrolysis product in the dragon's blood standard [26]. However, the presence of silylated 3-methoxyphenol (#32) was found in Stainer and Baioni samples at a very low amount, probably not enough to firmly confirm its presence. Moreover, no dragon's blood flavonoids were observed.

The Storioni small violin sample stands out among the other samples studied for the presence of several anhydrosugars was found which

could be originated from plant gum besides that cellulose and hemicellulose. They are characterized by a mass fragment *m/z* 217 which corresponds to a [TMSO-CH=CH-CH-OTMS]⁺ [27]. In particular, the following peaks were identified: 1,6-anhydrogalactopyranose (*20), 1,6-anhydroglucopyranose (*21), 1,6-anhydromannofuranose (*22), 1,4-anhydromannopyranose (23). There are probably some more to be categorized in the same group including peak *18 which has ion fragment *m/z* 217. These compounds could not only be derived from cellulose and hemicellulose but also from plant gum as Torri et al. [40] found the same patterns in the study of natural gums. Moreover, this material is seemingly used as glue since it is listed as one of five types of glue [16,41]. However, it is not possible to distinguish anhydrosugars formed from gums and those from wood.

A summary of analytical results of the historic stringed instrument varnish samples is provided in Table 4. Three samples F02, F04 and F11 have been already studied by Kasprzak et al. [26] using the same technique. Most of the important markers correspond in these two studies as shown in Table 3. Protein was not found by Kasprzak et al. [26] before making research on standard protein. On the other hand, fragments of some resins were absent in this study probably because the amount of samples was fewer in this study to lower their peak intensities.

Protein is identified by the presence of several compounds in F01, F02, F23, SL793 and perhaps F11. Different combinations of the markers are observed in any of these, but some compounds like pyrocoll, diketopiperazines or 3-hydroxypyridine were present in most cases. Especially pyrocoll and diketopiperazines would be the strongest marker to find proteins in the mixture sample. Pyrocoll is an important marker for proline and protein, but does not give further information about what kind of protein the sample contains since a variety of proteinaceous

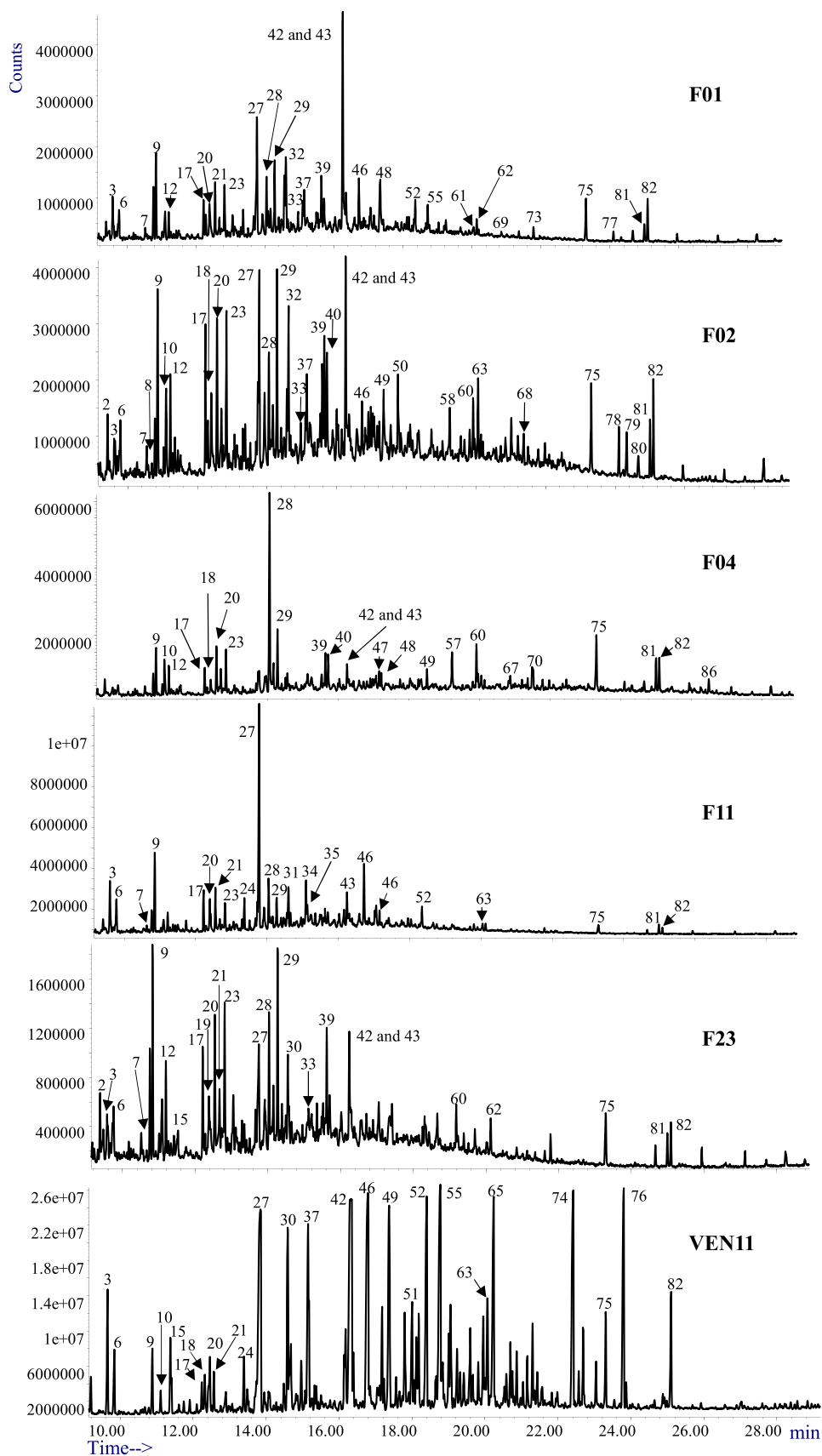


Fig. 1. Chromatograms obtained from the Py SPME Syl GC–MS of Stainer F01 and F02, Baioni F04, Guarneri F11, Ruggeri F23 and Pietà of Venice VEN11 samples. Compounds were observed as persilylated derivatives and peak numbers refers to compounds listed in Table 3.

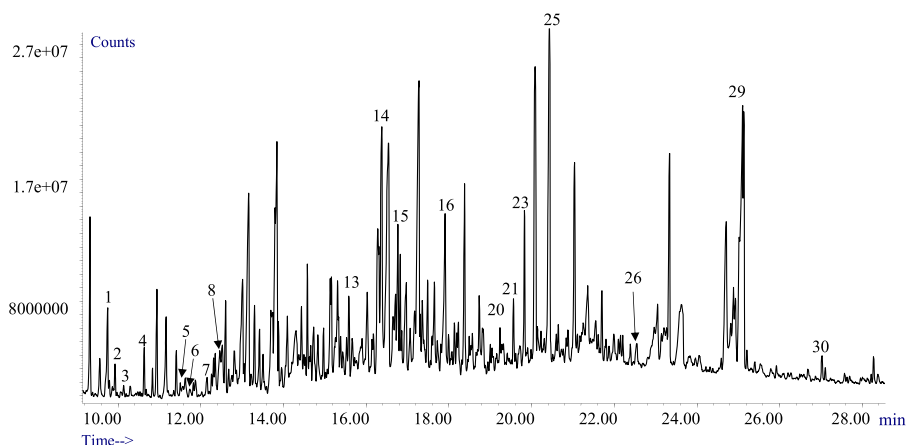


Fig. 2. Total ion chromatogram obtained from analytical pyrolysis with SPME and on-fiber silylation of the Storioni small violin sample. Structural assignment (as persilylated derivatives) peak *1: 1,2-ethanediol; *2: 2-hydroxymethylfuran; *3: 2-ethyl-4-methylpyrrole; *4: 2-hydroxypyridine; *5: 3-hydroxy-6-methylpyridine; *6: imidazole; *7: 2-pyrrolidinone; *8: 3-hydroxypyridine; *9: piperidinecarboxylic acid (bisTMS); *10: 4-hydroxypyridine; *11: 3-hydroxy-6-methylpyridine; *12: guaiacol; *13: pyrrole-2-carboxylic acid; *14: cyclo(Gly-Gly); *15: cyclo(Pro-Leu); *16: pyroglutamic acid; *17: pyrogallol; *18: 4-vinylsyringol; *19: galactopyranoside; *20: 1,6-anhydrogalactopyranose; *21: 1,6-anhydroglucopyranose; *22: 1,6-anhydromannofuranose; *23: 1,4-anhydromannopyranose; *24: pyrocoll; *25: 1,4a,6,trimethyl-1,2,3,4,4a,5,8,8a-octahydronaphthalene-1-carboxylic acid; *26: cyclo(Pro-Pro); *27: hexadecanoic acid; *28: octadecanoic acid; *29: cyclo(Pro-Hyp); *30: cyclo(Pro-Glu). More details on peak assignments are reported in Table SM8 of the supplementary data.

Table 4

Summary of results of Py SPME Syl GC-MS of the samples from Stainer, Baioni, Guarneri, Ruggeri, Storioni and Pietà of Venice historic stringed instruments. Identification of the materials was achieved through observation of molecular markers (Table 2) and analysis of reference standards. "X" indicates present, and "x" indicates trace. If followed by a "?" identification is uncertain.

	F01	F02	F04	F11	F23	SL793	VEN11
Instrument maker	Jacob Stainer	Jacob Stainer	Luigi Baioni	Andrea Guarneri	Francesco Ruggeri	Lorenzo Storioni	Pietà of Venice
Wood	Maple	Maple	Maple	Spruce	Maple	Maple	Maple
Protein (not collagen)	X	X		X?	X		x?
Protein (collagen)						X	
Plant gum						X	
Cellulose	X	X	X	X	X	X	X
G Lignin	X	X	X	X	X	X	X
S Lignin	X	X	X		x?	X	X
Drying oil	X	X	X	X	X	X	X
Pinaceae resin (Venice Turpentine)	X	X	X	X?	X		
Shellac	X?	X	X	X	X	X	
Diterpenic resin (sandarc, manila copal)	X?	X	X	X	X		x?
Dragon's		X?	X?			X	
Blood	X			X			
Madder	X	X		X?	X		x?

materials is abundant in proline. The diketopiperazine of proline and hydroxyproline was also confirmed in SL793 and, because only collagen is abundant in hydroxyproline, this implies that collagen was applied as glue on the Bracco small violin made by Lorenzo Storioni. Other peaks, hetero rings containing N atoms such as pyrrole, pyrrolidine and pyridine as well as 2-pyrrolidinone and pyroglutamic acid, imply there is a material rich in nitrogen and in case no other material known for such fragments, these compounds will be supplemental evidence of protein. The unknown peak with ion fragment m/z 256 is possibly related to collagen.

4. Conclusions

On-line analytical pyrolysis with silylation is known to be a powerful technique for the analysis of complex organic samples in cultural heritage, but often scarcely effective in derivatizing some pyrolysis products, such as anhydrosugars with hindered OH groups and N-bearing pyrolysis products. Off-line pyrolysis with on-fiber silylation was developed to improve derivatization and was evaluated in this study with a special focus on the identification of proteinaceous materials in historic stringed instrument finishes. The results from the analysis of reference proteinaceous materials highlighted the difficulties to reach a quantitative formation of N-trimethylsilyl derivatives. Silylated pyrolysis markers of general proteins that were identified for their use in real sample analysis included 2-pyrrolidinone and 3-hydroxypyridine, while silylated cyclo

(Pro-Hyp) served as a distinctive marker of collagen. Several protein markers were produced by off-line pyrolysis without silylation, diketopiperazines among the most specific, suggesting that direct pyrolysis would be better suited for the identification of proteins rather than pyrolysis silylation. Nevertheless, the relevant pyrolysis markers pyrocoll and cyclo(Pro-Pro) were important peaks also in the pyrograms obtained after silylation. By considering that several silylated pyrolysis products are employed for the identification of a variety of organic materials, pyrolysis with silylation was confirmed to be the elective technique to investigate the chemical composition of historical instruments.

The presence of silylated and non-silylated markers of proteinaceous materials was identified upon off-line pyrolysis with on-fiber silylation of cellos by Stainer, Guarneri, Ruggeri, Storioni and probably the violin by Pietà of Venice. Collagen was positively identified by the presence of cyclo(Pro-Hyp). Besides proteins, the same analysis enabled the identification of other constituents of the varnishes. Notably, a series of fully silylated anhydrosugars featuring the pyrogram of Storioni's small violin suggested the use of gum. The outcome of these investigations is very important in the field of chemistry applied to the study of Cultural Heritage. In fact, it allows to add new information on the way of working of some important luthiers of the past and to define new methodologies for the recognition of materials in lutherie.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

We would like to thank the International Violin Making School "Antonio Stradivari" the Museum of Violin and CR Forma institution of Cremona, and Provincial Institute for Childhood "Santa Maria della Pietà" of Venice for providing insight and expertise that greatly assisted the research. Lastly, we would also like to thank the Bracco Foundation and the Arvedi-Buschini Foundation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jaap.2023.105957](https://doi.org/10.1016/j.jaap.2023.105957).

References

- A. Eddy, W. Kolneder, R.G. Pauly, *The Amadeus Book of the Violin: Construction, History, and Music*, Music Library Association, Notes Second Series 56, No. 2, 1999, pp. 379–381.
- C. Chiesa, Antonio Stradivari and his "rivals" in the late seventeenth-century Cremona, in: A. Zanrè (Ed.), *The 1690 "Tuscan" Stradivari violin in the Accademia di Santa Cecilia*, Scrollavezza & Zanrè, Parma, 2017, pp. 37–40.
- H. Meinel, Regarding the sound quality of violins and a scientific basis for violin construction, *J. Acoust. Soc. Am.* 29 (1957) 817–822, <https://doi.org/10.1121/1.1909064>.
- B.H. Tai, Stradivari's varnish: a review of scientific findings – Part I, *J. Violin Soc. Am.: VSA Pap.* (2007) 119–144.
- E. Skrodzka, B. Linde, A. Krupa, Modal parameters of two violins with different varnish layers and subjective evaluation of their sound quality, *Arch. Acoust.* 38 (2013) 75–81, <https://doi.org/10.2478/aoa-2013-0009>.
- J.-P. Echard, B. Lavédrine, Review on the characterisation of ancient stringed musical instruments varnishes and implementation of an analytical strategy, *J. Cult. Herit.* 9 (2008) 420–429, <https://doi.org/10.1016/j.culher.2008.03.005>.
- M. Albano, M. Ghirardello, G. Fiocco, C. Manzoni, M. Malagodi, D. Comelli, Complementary mapping techniques to characterize the wood finish of musical instruments, *Eur. Phys. J.* 136 (2021) 1054, <https://doi.org/10.1140/epjp/s13360-021-02033-3>.
- R. Malvermi, M. Albano, S. Gonzalez, G. Fiocco, F. Antonacci, M. Malagodi, A. Sarti, The impact of alkaline treatments on elasticity in spruce tonewood, *Sci. Rep.* 12 (2021) 13335, <https://doi.org/10.1038/s41598-022-17596-z>.
- C. Stani, C. Invernizzi, G. Birarda, P. Davit, L. Vaccari, M. Malagodi, M. Gulmini, G. Fiocco, A nanofocused light on Stradivari violins: infrared s-SNOM reveals new clues behind craftsmanship mastery, *Anal. Chem.* 94 (2022) 14815–14819, <https://doi.org/10.1021/acs.analchem.2c02965>.
- G. Fiocco, T. Rovetta, M. Gulmini, A. Piccirillo, M. Licchelli, M. Malagodi, Spectroscopic analysis to characterize finishing treatments of ancient bowed string instruments, *Appl. Spectrosc.* 71 (2017) 2477–2487, <https://doi.org/10.1177/0003702817715622>.
- G. Fiocco, C. Invernizzi, S. Grassi, P. Davit, M. Albano, T. Rovetta, C. Stani, L. Vaccari, M. Malagodi, M. Licchelli, M. Gulmini, Reflection FTIR spectroscopy for the study of historical bowed string instruments: invasive and non-invasive approaches, *Spectrochim. Acta-Part A Mol. Biomol. Spectrosc.* 245 (2021), 118926, <https://doi.org/10.1016/j.saa.2020.118926>.
- G. Fiocco, S. Grassi, C. Invernizzi, T. Rovetta, M. Albano, P. Davit, M. Gulmini, C. Stani, L. Vaccari, M. Licchelli, M. Malagodi, Chemometric tools to investigate complex Synchrotron radiation FTIR micro-spectra: focus on historical bowed musical instruments, *Acta IMEKO* 10 (2021) 201–208, https://doi.org/10.21014/acta_imeko.v10i1.836.
- J.S. Mills, R. White, *The Organic Chemistry of Museum Objects*, second ed., Butterworth, Oxford, 1994.
- C.D. Cennini, in: D.V. Thompson (Ed.), *The Craftsman's Handbook*, 1933 ed., Dover, New York, NY, 1960 (Engl. Transl.).
- J.A. Huntington, P.E. Stein, Structure and properties of ovalbumin, *J. Chromatogr. B* 756 (2001) 189–198, [https://doi.org/10.1016/S0378-4347\(01\)00108-6](https://doi.org/10.1016/S0378-4347(01)00108-6).
- B.H. Tai, Stradivari's varnish: a review of scientific findings – Part II, *J. Violin Soc. Am.: VSA Pap.* (2009) 1–31.
- S. Dallongeville, N. Garnier, C. Rolando, C. Tokarski, Proteins in art, archaeology, and paleontology: from detection to identification, *Chem. Rev.* 116 (2016) 2–79, <https://doi.org/10.1021/acs.chemrev.5b00037>.
- I. Bonaduce, E. Ribechini, F. Modugno, M.P. Colombini, Analytical approaches based on gas chromatography mass spectrometry (GC/MS) to study organic materials in artworks and archaeological objects, *Top. Curr. Chem.* 374 (2016) 6, <https://doi.org/10.1007/s41061-015-0007-x>.
- M.P. Colombini, F. Modugno, E. Menicagli, R. Fuoco, A. Giacomelli, GC-MS characterization of proteinaceous and lipid binders in UV aged polychrome artifacts, *Microchem. J.* 67 (1–3) (2000) 291–300, [https://doi.org/10.1016/S0026-265X\(00\)00075-8](https://doi.org/10.1016/S0026-265X(00)00075-8).
- P. Prikryl, L. Havlíčková, V. Pacáková, J. Hradilová, K. Štulík, P. Hofta, An evaluation of GC-MS and HPLC-FD methods for analysis of protein binders in paintings, *J. Sep. Sci.* 29 (2006) 2653–2663, <https://doi.org/10.1002/jssc.200600171>.
- A. Lluveras-Tenorio, S. Orsini, S. Pizzimenti, S. Del Seppia, M.P. Colombini, C. Duce, I. Bonaduce, Development of a GC-MS strategy for the determination of cross-linked proteins in 20th century paint tubes, *Microchem. J.* 170 (2021), 106633, <https://doi.org/10.1016/j.microc.2021.106633>.
- M. Albano, D. Comelli, G. Fiocco, M. Mattonai, J.J. Lucejko, L. Zoia, M. P. Colombini, M. Malagodi, Chemical modification of wood induced by the traditional making procedures of bowed string musical instruments: the effect of alkaline treatments, *Herit. Sci.* 10 (76) (2022), <https://doi.org/10.1186/s40494-022-00718-1> (2022).
- S. Orsini, C. Duce, I. Bonaduce, Analytical pyrolysis of ovalbumin, *J. Anal. Appl. Pyrolysis* 130 (2018) 249–255, <https://doi.org/10.1016/j.jaap.2018.01.026>.
- A. Adamiano, D. Fabbri, G. Falini, M. Giovanna Belcastro, A complementary approach using analytical pyrolysis to evaluate collagen degradation and mineral fossilisation in archaeological bones: the case study of Vicenne-Campochiaro necropolis (Italy), *J. Anal. Appl. Pyrolysis* 100 (2013) 173–180, <https://doi.org/10.1016/j.jaap.2012.12.014>.
- S. Orsini, F. Parlanti, I. Bonaduce, Analytical pyrolysis of proteins in samples from artistic and archaeological objects, *J. Anal. Appl. Pyrolysis* 124 (2017) 643–657, <https://doi.org/10.1016/j.jaap.2016.12.017>.
- L.M. Kasprzak, D. Fabbri, A.G. Rombolà, T. Rovetta, M. Malagodi, Identification of organic materials in historical stringed instruments by off-line analytical pyrolysis solid-phase microextraction with on-fiber silylation and gas chromatography-mass spectrometry, *J. Anal. Appl. Pyrolysis* 145 (2020), 104727, <https://doi.org/10.1016/j.jaap.2019.104727>.
- D. Fabbri, G. Chiavari, S. Prati, I. Vassura, M. Vangelista, Gas chromatography/mass spectrometric characterisation of pyrolysis/silylation products of glucose and cellulose, *Rapid Commun. Mass Spectrom.* 16 (2002) 2349–2355, <https://doi.org/10.1002/rcm.856>.
- T. Rovetta, C. Invernizzi, G. Fiocco, M. Albano, M. Licchelli, M. Gulmini, G. Alf, D. Fabbri, A.G. Rombolà, M. Malagodi, The case of Antonio Stradivari 1718 ex-San Lorenzo violin: history, restorations and conservation perspectives, *J. Archaeol. Sci.* 23 (2019) 443–450, <https://doi.org/10.1016/j.jasrep.2018.11.010>.
- G.V. Fichera, T. Rovetta, G. Fiocco, G. Alberti, C. Invernizzi, M. Licchelli, M. Malagodi, Elemental analysis as statistical preliminary study of musical instruments, *Microchem. J.* 137 (2018) 309–317, <https://doi.org/10.1016/j.microc.2017.11.004>.
- A. Marcilla, A.N. García, M. León, P. Martínez, E. Bañón, Study of the influence of NaOH treatment on the pyrolysis of different leather tanned using thermogravimetric analysis and Py/GC-MS system, *J. Anal. Appl. Pyrolysis* 92 (2011) 194–201, <https://doi.org/10.1016/j.jaap.2011.05.014>.
- T. Deviese, E. Ribechini, D. Querci, T. Higham, Assessing the efficiency of supercritical fluid extraction for the decontamination of archaeological bones prior to radiocarbon dating, *Analyst* 144 (2019) 6128–6135, <https://doi.org/10.1039/C9AN00859D>.
- D. Fabbri, A. Adamiano, G. Fallini, R. de Marco, I. Mancini, Analytical pyrolysis of dipeptides containing proline and amino acids with polar side chains. Novel 2,5-diketopiperazine markers in the pyrolysates of proteins, *J. Anal. Appl. Pyrolysis* 95 (2012) 145–155, <https://doi.org/10.1016/j.jaap.2012.02.001>.
- G. Chiavari, N. Gandini, P. Russo, D. Fabbri, Characterisation of standard tempera painting layers containing proteinaceous binders by pyrolysis/(methylation)-gas chromatography-mass spectrometry, *Chromatographia* 47 (1998) 420–426, <https://doi.org/10.1007/BF02466473>.
- G. Chiavari, G. Lanterna, C. Luca, M. Matteini, S. Prati, I.C.A. Sandu, Analysis of proteinaceous binders by in-situ pyrolysis and silylation, *Chromatographia* 57 (2003) 645–648, <https://doi.org/10.1007/BF02491743>.
- I. Bonaduce, M.P. Colombini, Gas chromatography/mass spectrometry for the characterization of organic materials in frescoes of the monumental cemetery of Pisa (Italy), *Rapid Commun. Mass Spectrom.* 17 (2003) 2523–2527, <https://doi.org/10.1002/rcm.1222>.
- G. Fiocco, T. Rovetta, C. Invernizzi, M. Albano, M. Malagodi, M. Licchelli, A. Re, A. Lo Giudice, G.N. Lanzafame, F. Zanini, M. Iwanicka, P. Targowski, M. Gulmini, A micro-tomographic insight into the coating systems of historical bowed string instruments, *Coatings* 9 (2) (2019) 81, <https://doi.org/10.3390/coatings9020081>.
- C. Invernizzi, G. Fiocco, M. Iwanicka, P. Targowski, B. Blümich, C. Rehorn, V. Gabrielli, D. Bersani, M. Licchelli, M. Malagodi, Non-invasive mobile technology to study the stratigraphy of ancient Cremonese violins: OCT NMR-MOUSE, XRF and reflection FT-IR spectroscopy, *Microchem. J.* 155 (2020), 104754, <https://doi.org/10.1016/j.microc.2020.104754>.
- J. Van den Berg, J.J. Boon, I. Pastorova, L.F.M. Spetter, Mass spectrometric methodology for the analysis of highly oxidized diterpenoid acids in old master

- paintings, *J. Mass Spectrom.* 35 (2000) 512–533, [https://doi.org/10.1002/\(SICI\)1096-9888\(200004\)35:4<512::AID-JMS963>3.0.CO;2-3](https://doi.org/10.1002/(SICI)1096-9888(200004)35:4<512::AID-JMS963>3.0.CO;2-3).
- [39] J.P. Echard, C. Benoit, J. Peris-Vicente, V. Malecki, J.V. Gimeno-Adelantado, S. Vaiedelich, Gas chromatography/mass spectrometry characterization of historical varnishes of ancient Italian lutes and violin, *Anal. Chim. Acta* 584 (2007) 172–180, <https://doi.org/10.1016/j.aca.2006.10.048>.
- [40] C. Torri, E. Soragni, S. Prati, D. Fabbri, Py-SPME-GC-MS with on-fiber derivatization as a new solvent-less technique for the study of polar macromolecules: application to natural gums, *Microchem. J.* 110 (2013) 719–725, <https://doi.org/10.1016/j.microc.2013.08.006>.
- [41] F.N. Howes, *Vegetable Gums and Resins*, Chronica Botanica, Waltham, MA, USA, 1949.