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FT-Raman Analysis of Cellulose based Museum Textiles: Comparison of Objects Infected and Non-infected by Fungi

FT-Ramanska analiza celuloznih muzejskih tekstilij: primerjava neokuženih in okuženih z glivami

Original Scientific Article/*Izvirni znanstveni članek*
Received/*Prispelo* 03-2018 • Accepted/*Sprejeto* 06-2018

Abstract

It is well-known fact that the supermolecular structure of museum textiles changes during aging and biodeterioration. These structural changes can be observed by different spectroscopic methods such as FT-IR, FT-Raman, and dispersive Raman spectroscopy. The purpose of the presented research is to present the usability of FT-Raman spectroscopy method for the analysis of the cellulose structure of the biodeteriorated historical textile fibers. Although historical textiles have already been analyzed using FT-Raman spectroscopy the method has been rarely used to analyze the changes of supermolecular structure of the biodeteriorated historical textiles attacked by microorganisms. In the research, cellulose textile samples from different museums and religious institutions were analyzed. Contemporary and historical cellulose textiles were scanned by FT-Raman spectra of reference and compared to determine the supermolecular cellulose fiber structure of each material. It has been shown that structural changes such as depolymerization and crystallinity changes can be detected using FT-Raman spectroscopy. The supermolecular changes of the cellulose fiber structure have been detected in biodeteriorated as well as in historical objects not infected by microorganisms. In the spectra of biodeteriorated objects, more intensive changes of spectral features were observed compared to spectra of non-infected samples. The changes were more pronounced at the museum objects made of flax. It can be concluded that biodeterioration causes more intensive structural changes than aging. On the basis of the research work, it has been shown that FT-Raman spectroscopy method can be used for the analysis of supermolecular structure changes of cellulose textiles.
Keywords: historical textiles, cellulose, biodeterioration

Izvleček

Dobro znano dejstvo je, da se nadmolekulska struktura muzejskih tekstilij med staranjem in biorazgradnjo spreminja. Strukturne spremembe opazujemo z različnimi spektroskopskimi metodami, npr. s FT-IR, FT-ramansko in disperzno ramansko spektroskopijo. Namen predstavljene raziskave je prikazati uporabnost FT-ramanske spektroskopske metode za analizo strukture celuloze biorazgrajenih zgodovinskih tekstilnih vlaken. Čeprav so bile zgodovinske tekstilije v preteklosti že analizirane z uporabo FT-ramanske spektroskopije, pa je bila metoda redko uporabljena za analizo sprememb v nadmolekulski strukturi biorazgrajenih zgodovinskih tekstilij, ki so jih napadli mikroorganizmi. V raziskavi smo analizirali celulozne tekstilne vzorce iz različnih muzejev in verskih ustanov. Zgodovinske celulozne tekstilije smo primerjali s sodobnimi referencami, da bi identificirali nadmolekulsko strukturo vsakega materiala. Pokazali smo, da lahko depolimerizacijo in spremembe kristalinosti detektiramo z uporabo FT-ramanske spektroskopije. Spremembe

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Tekstilec, 2018, ***61****(2), 110-123*
DOI: 10.14502/TEKSTILEC2018.61.110-123

nadmolekulske strukture celuloznih vlaken smo opazili tako v biorazgrajenih kot tudi v neokuženih zgodovinskih vlaknih. V spektrih biorazgrajenih predmetov smo opazili intenzivnejše spremembe kot v neokuženih vzorcih. Spremembe so bile izrazitejše na zgodovinskih tekstilijah, izdelanih iz lanu. Zato lahko sklepamo, da biorazgradnja povzroči izrazitejše spremembe kot staranje. Na podlagi raziskovalnega dela smo pokazali, da s FT-ramansko spektroskopijo lahko analiziramo spremembe nadmolekulske strukture celuloznih tekstilij.
Ključne besede: zgodovinske tekstilije, celuloza, biorazgradnja

1 Introduction

The aim of all institutions dealing with cultural heritage is to preserve objects from the past for future generations. Among the most susceptible materials to be preserved are textiles. Materials produced from cellulosic (plant) fibers which consist mainly of cellulose (cotton more than 90%, flax 60-70%, and hemp up to 77%) represent an important part of textile materials in museums. In flax and hemp fibers, the main accompanying materials to cellulose are hemicelluloses (17%) and lignin (2–3%) [1]. Cellulose supermolecular structure in plant fibers is semi-crystalline which means that a certain part of cellulose fibers is ordered, i.e. crystalline, and a certain part is non-ordered, i.e. amorphous. The ratio between the crystalline and the amorphous phase differs between different plant types and depends on growth conditions, processing, use, and storing conditions. Features of the inner structure of cellulose fibers can be detected by spectroscopic methods. External influences, e.g. physical, chemical, or biological agents, cause changes of the textile fiber inner (supermolecular) structure as well as changes of appearance (fading of dyes and yellowing) [2]. The changes of inner structure of textile fibers cause changes of material mechanical properties. Textile fibers and textile materials become more fragile and prone to mechanical damages. Additionally, changes in the inner structure of textile fibers enable easier penetration of enzymes, produced by different types of living organisms into the structure [3, 4]. This is the case especially with fungi, which are among the most severe deteriorating agents of textiles [5] and which attack mainly pre-degraded fibers. Since fungi can cause changes in the supermolecular structure of textile fibers [6–11], an analysis of infected materials is of crucial importance. Such an analysis allows us to see the level of structural changes caused by aging processes or/and fungi attack and to decide what measures need to be taken to prevent further deterioration or even destruction of the historical objects. Biodeterioration of historical textile objects is usually investigated from the point of the infesting microorganisms [12, 13] and only rarely from the viewpoint of structure and properties of the attacked material(s). The latter were mainly analyzed on the buried contemporary samples [6, 14, 15].

Supermolecular structure of materials can be analyzed by different analytical methods, e.g. infrared and Raman spectroscopy, x-ray diffraction, etc. With the results obtained by the above-mentioned methods and their correlation with other visible and mechanical properties, it is possible to understand the changes and the plan of further treatment of historical objects. Spectroscopic techniques present a widely accepted approach for the analysis of historical objects. For the analysis of organic materials, infrared spectroscopy has proved to be a reliable and effective method [16]. Raman spectroscopy, on the other hand, can cause several problems when analyzing degraded organic materials. The research of historical textiles with dispersive Raman spectrometer using the visible laser wavelength 785 nm has already been presented by the authors of this paper [17]. Those problems can, to a certain extent, be solved by drench quenching (photobleaching), a prolonged exposition to a reduced laser power [18]. This method takes a long time to obtain spectra, sometimes more than a single working day. On the other hand, FT-Raman spectroscopy has proved to be a reliable tool when analyzing historical materials of organic origin [19, 20]. The near-infrared laser with 1064 nm wavelength should allow the analyst to obtain spectra with reduced luminescent background and enhanced Raman signal. The method was invented in 1986 as a response to high fluorescence in Raman spectra of certain types of materials [21].

The present study is a part of a broader research on the influence of different fungal species on structure of different natural fibers, being part of historical objects. The aim of the present study is twofold. Firstly,

to analyze historical textile materials infected by fungi using FT-Raman spectroscopy method and to detect whether fungi cause additional structural changes to natural aging, and, secondly, to compare the usefulness of different spectroscopic methods (dispersive Raman spectroscopy and Fourier transform infrared spectroscopy (FT-IR)) in the analysis of the structure of historical cellulose textiles.

2 Materials and methods

2.1 Textile samples

Textile samples for the analysis were obtained from 14 different historical textile objects stored in Slovene museums, different religious institutions or were under conservation at the Restoration Centre of the Institute for the Protection of Cultural Heritage of Slovenia (IPCHS). The historical textile objects originate from different historical periods from the 16th century or later. The analyzed textile objects are listed in Table 1 and presented in Table 2. Figure 1 presents an example of infection on one of the infected historical artefacts. Textile objects were made from different cellulosic fibers, which were identified by optical microscopy as described in our previous work [16]. The objects were selected according to their appearance: objects with visible mycelium growth or uncommon stains appearing like fungal spots were selected. The samples for the analysis were taken from historical objects in form of small pieces of fabrics or single threads according

Table 1: List of the selected historical objects with their description, source institution (and for paintings also storage institution), dating, material, and information about fungal infection in the upper rows non-infected objects are listed and in lower rows the infected

Sample label	Object description	Dating	Institution	Material	Fungal infection
MKS03	cloth, partly embroidered with metal threads	unknown	Slovene Museum of Christianity	cotton	no
PMP02	embroidered tablecloth	½ 20th cent.	Ptuj Regional Museum	flax	no
PMP05	leather belt with textile lining	½ 20th cent.	Ptuj Regional Museum	cotton	no
RCS04	painting on canvas	end of 17 th cent.	IPCHS[a)] (Convent)	flax and hemp	no
RCS06	painting on canvas	1st decade of 18th cent.	IPCHS[a)] (Convent)	flax	no
RCS07	painting on canvas	1582–1584	IPCHS[a)] (Provost church)	flax	no
RCS08	painting on canvas	unknown	IPCHS[a)] (Convent)	hemp and flax	no
MKS02	painting on canvas with a paper patch on the back	unknown	Slovene Museum of Christianity	flax	yes
MKS04	painting on canvas	unknown	Slovene Museum of Christianity	flax	yes
PMP03	underwear	unknown	Ptuj Regional Museum	cotton	yes
RCS01	painting on canvas	½ 19th cent.	IPCHS[a)] (Succursal church)	flax	yes
RCS05	painting on canvas	1729	IPCHS[a)] (Monastery)	flax	yes
RCS09	painting on canvas	17th cent.	IPCHS[a)] (Cathedral)	flax	yes
RCS14	painting on canvas	1821	IPCHS[a)] (Parish church)	flax	yes

[a)]IPCHS = the Institute for the Protection of Cultural Heritage of Slovenia

to the conservators and restaurateurs code [2]. The current storage conditions of all analyzed objects are listed in our previous work [2]. Environmental conditions in the past, which had influence on properties of objects, are not known.

2.2 Isolation and identification of fungi

The historical samples were analyzed for possible fungal contamination [2]. The sampling for the fungi was performed with areas of the material that we supposed could be infected by fungi (e.g. unusual stains, mycelium like spots etc.).

The fungi were sampled from the selected objects by rubbing with a sterile cotton swab that was either soaked in physiological solution (0.9% [w/v] NaCl), or was dry, in the cases of more sensitive objects. The fungi were isolated from these swabs by subsequent inoculation onto malt extract agar medium with the added antibiotic chloramphenicol (50 mg/l), to prevent bacterial growth. The plates were incubated at 25 °C for up to 21 days. Pure cultures of the fungi were obtained from the primary isolation plates by the further culturing of selected colonies with different morphologies. All of the isolated

Table 2: Photographs of all analyzed historical objects and close-up captures of spots which might be infected by fungi (first and second column – infected historical objects; third and fourth column – non-infected historical objects)

fungi are stored in the Microbiological Culture Collection EX of the Department of Biology, Biotechnical Faculty, University of Ljubljana (www.ex-genebank.com).

The fungi in the fungal isolates were identified according to their macromorphological and micromorphological characteristics, and using genus/species-specific molecular markers, according to

taxonomic standards. For the fungal DNA isolation, the strains were grown on malt extract agar medium for 7 days. Their DNA was extracted according to Gerrits van den Ende and de Hoog [22], by mechanical lysis of *ca*. 1 cm^2 of their mycelia.

Figure 1: An example of infection spot on the infected historical artefact (MKS 02)

For the purpose of the research, contemporary cotton and flax fabrics were analyzed using the same testing conditions and procedures as in the case of historic samples (see chapter 2.2). The reference materials were not bleached and were unsized to resemble the historical materials. Reference fabrics with the mixed content of hemp and flax were not available to us to directly compare historical textiles made of hemp/ flax mixture.

2.2 FT-Raman spectroscopy method

For the FT-Raman analysis, Bruker multiRam instrument was used. The instrument is equipped with cryo-cooled Ge detector, Nd-YAG laser with a wavelength of 1064 nm with a line width of ~ 5 cm^{-1} to 10 cm^{-1}, and a resolution of 4 cm^{-1}. Raman spectrometer was calibrated each day before use. The applied laser intensity varied between 30 mW and 150 mW. The number of accumulated scans varied between 100 and 5000. Raw fibers or pieces of textiles were used without additional sample preparation and put under the FT-Raman microscope. The surface area of the analysis was approximately 20 μm in diameter. Several areas, selected randomly, were analyzed on each historical as well as reference sample. By comparison of reference and historical samples' spectra structural changes in historical objects were determined.

All spectra are shown in the range between 150 cm^{-1} and 2000 cm^{-1}. Lower wavenumbers would not be reasonable due to high fluorescence in this region, as observed from obtained the spectra. Spectra were baseline-corrected to give clearer results and better visual comparison.

The ratios I^{1121}/I^{1096} [20] and I^{380}/I^{1096} [23] were calculated for all the samples (where bands were clearly visible). Bands are typical of symmetrical vibrations of glycoside bonds (1120 cm^{-1}), asymmetrical vibrations of glycoside bonds (1096 cm^{-1}) and CCC vibrations of glycoside ring (380 cm^{-1}) [24]. The ratios were selected as the most often occurring and proved as reliable for cellulose crystallinity determination in the available literature. The first ratio has proved to be stable and reliable in several articles (as confirmed by Jähn and co-workers [25]). For the second ratio Agarwal and co-workers [23] have proved that the bands at 380 cm^{-1} and 1096 cm^{-1} are significantly affected by cellulose crystallinity modification and that this ratio generated excellent regression (R2 = 0.992) and showed good sensitivity to crystallinity change.

Heights of the bands were measured using OPUS software. Band heights were measured from a selected baseline in the spectrum as shown in Figure 2.

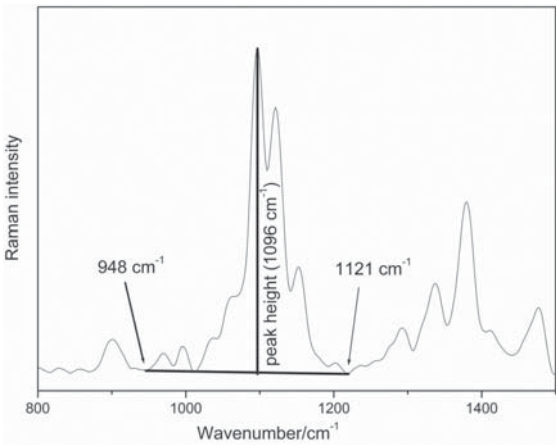

Figure 2: Baseline selection and peak height measurement for quantitative analyses

3 Results and discussion

As it can be seen from the Table 1, three of the selected historical specimens were made of cotton (MKS03, PMP03, and PMP05), nine objects were made of flax, and two were mixed, made of flax in

the warp direction and hemp in the weft (RSC04 and RCS08). The textile materials were identified by optical microscopy as described in our previous work [16].

3.1 FT- Raman analysis of the cotton samples

Two of the analyzed cotton samples turned out not to be infected by fungi. Infection with fungi was confirmed on one sampled underwear (PMP03, Tables 1 and 2) [2]. FT- Raman spectra of different quality were obtained from the cotton specimen.

Two different spectra were obtained from the specimens taken from the infected underwear (PMP03) sample (Figure 3). This fact leads us to the conclusion that the degree of deterioration is not uniform over the whole sample and depends mostly on the fungal contamination and activity. As it can be seen in the Figure 3a, spectrum b resembles contemporary cotton (spectrum a) to a great extent. The band heights are decreased. However, the ratio between them remained similar to those of reference spectra, as confirmed also by quantitative analyses (Table 3). Decreased band heights can be a consequence of luminescent background due to surface dirt or fungi [20]. It can be concluded that no severe deterioration occurred in this area of the sample. In the case of the spectrum c (Figure 3a), structural changes of the cotton fibers could be clearly observed when compared to reference spectra of the cotton (spectrum a). Some bands in the spectrum c decreased relative to other when compared to the reference spectrum. The decrease of the band at 380 cm^{-1}, typical of vibrations of glycoside ring [24], is a sign of decreased crystallinity of the cellulose [23]. The decrease of the band at 437 cm^{-1}, typical of glucose rings vibrations [24, 26], is a sign of cellulose degradation [26]. Additionally, the bands at 1096 cm^{-1} and 1120 cm^{-1}, typical of asymmetric and symmetric β-glycosidic linkages respectively [24], decreased strongly relative to the reference spectrum. This is a sign of a hydrolytic fission of the glycosidic bonds [27]. The decrease of the intensities occurred at the bands between 1250 cm^{-1} and 1450 cm^{-1}, typical mainly of CH_2 vibrations, as well as HCC, HCO, and COH bending, all indicating cellulose deterioration [20, 28]. On the basis of the above-mentioned results, it can be concluded that cotton fabric infected with fungi is subjected to depolymerization and the decrease of crystallinity. The deterioration takes place only in the infected spots.

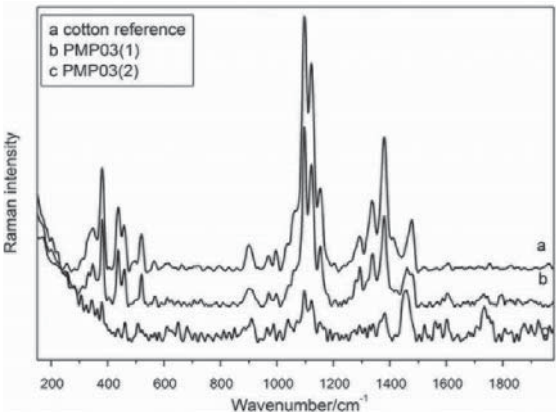

Figure 3a: Comparison of two different spectra form infected cotton object (PMP03; spectra b and c) with cotton reference (spectrum a)

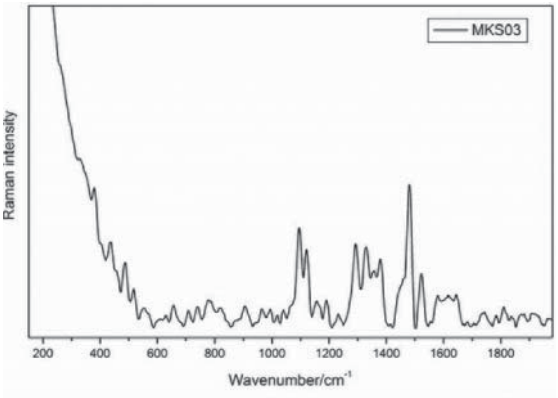

Figure 3b: Spectrum MKS03, showing poorly resolved bands and unidentified bands (around 1500 cm^{-1})

The spectra of belt lining made of cotton (PMP05) did not show any changes in typical cellulose features, only a strong luminescent background because of which a clear analysis of its supermolecular structure was not possible. Luminescence is usually a consequence of severe deterioration or presence of dirt or fungal hyphae on the surface of the analyzed objects [20]. Since the object was not subjected to fungal deterioration, the strong luminescence was probably a consequence of deterioration caused by aging and surface dirt. According to FTIR spectroscopy results obtained previously [16], it has been discovered that the crystallinity of the sample was higher compared to the reference sample, as observed by decreasing of the band at 900 cm^{-1} and the presence of a strong band 1280 cm^{-1}. Higher crystallinity could be a consequence of the deterioration of the amorphous regions caused by aging [29].

The spectra of the third cotton specimen (MKS03) exhibits very low intensity. Since the objects were not infected by fungi and the bands are very low, severe deterioration of the cellulose can be assumed. Additionally, a strong decrease of the bands at 1337 cm^{-1} and 1380 cm^{-1} is a sign of cellulose deterioration. Changes in the structure could have been caused by different environmental factors, e.g. light, as well as fluctuations in humidity and temperature.

The comparison of the state of preservation of the three investigated cotton samples shows that fungi did not cause more severe deterioration than environmental impacts. Processing and environmental impacts of the past decades, which could be of crucial importance for the state of preservation of each object, are unknown to us. However, even in time of sampling, most objects were not kept under the most appropriate conditions (relative humidity 40 to 65% and temperature 16 to 18 °C [30]) as described in our previous article [2].

Intensity ratios of the selected spectral bands were calculated for cotton historical objects and for cotton reference (Table 3). For both analyzed objects, the ratio I^{1121}/I^{1096} decreased, as was expected according to the literature [20, 31]. The ratio in the spectrum of the non-infected object (MKS03) also showed a small decrease relative to the ratio in the reference spectrum of cotton. It can be concluded that non-infected structure of the cotton also changed during aging. The differences in values of different areas (4b and 4c) of the infected object (PMP03) are a consequence with natural processes in fibers and fungal growth which do not affect all the areas equally. Comparing both ratios of cotton samples, it can be seen that in both cases the highest crystallinity ratio exhibits the sample PMP03_4b[a)] which is followed by the sample PMP03_4c[a)]. The lowest crystallinity ratio exhibits the sample MKS03. The degree of crystallinity is a function of many interconnected processes to which a textile material is subjected during its life.

Table 3: Intensity ratios I^{1121}/I^{1096} and I^{380}/I^{1096} for the cotton samples

Sample	I^{1121}/I^{1096}	I^{380}/I^{1096}
cotton reference	0.82	0.33
MKS03	0.77	0.27
PMP03_4b[a)]	0.81	0.43
PMP03_4c[a)]	0.79	0.38

[a)]Infected objects

The ratio I^{380}/I^{1096} was suggested by Agarwal and coworkers [23] to be the most appropriate to determine the cellulose crystallinity. It shows different tendency than the ratio I^{1121}/I^{1096}. According to the ratio I^{380}/I^{1096}, the crystallinity decreased in the sample MKS03 and increased in the sample PMP03 compared to the reference sample. On the basis of the presented results, it can be concluded that aging decreased the crystallinity ratio I^{380}/I^{1096} and fungal infection increased it. This could be explained by faster deterioration of amorphous regions compared to crystalline when subjected to fungal infection and by the fact that the amorphous/crystalline ratio is changing. The ratio I^{1121}/I^{1096} remains relatively similar in all tested samples. This is to be expected, since both the 1121 cm^{-1} and 1096 cm^{-1} bands are assignable to the β-glycosidic COC vibration of cellulose [20].

3.2 FT- Raman analysis of the bast fibers samples

Nine of the analyzed fabrics were made of flax fibers, and two of a mixture of flax and hemp fibers. One sample was taken from an embroidered tablecloth and the rest were taken from painting canvases, most of which are still kept in churches or cloisters, whereas two are kept in a museum in improper conditions with too high relative humidity and fluctuating temperature [2]. Neither of the two samples with mixed composition was infected by fungi. On the other hand, six of nine samples made from pure flax were infected by different fungal species (Tables 1 and 2). Spectra of both samples with mixed fibers had a strong luminescent background, which prevented us to make any conclusions regarding their supermolecular structure. The reason for bad quality is the presence of hemp, as it contains many non-cellulosic substances (e.g. lignin, hemicellulose, pectin, etc.) which, according to Agarwal, cause luminescent background [18].

At FT-Raman spectrum of non-infected flax sample taken from the embroidered tablecloth, (PMP02) a relatively strong band at 457 cm^{-1} (Figure 4, spectrum b), compared to the band at 435 cm^{-1}, appeared which is typical of ring vibrations [20]. This is a sign of relatively high crystallinity [26]. The same is true for the intensive band at 520 cm^{-1}, typical of glycoside links [19, 20]. A relative increase of the band at 1120 cm^{-1} regarding the band at 1096 cm^{-1} is also seen, which is again a sign of more organized structure in cellulose fibers [27]. However, according

to Kovur et al., it could also be a consequence of cellulose degradation and remaining of non-cellulosic compounds (lignin, hemicelluloses) [32]. The latter is, in this case, less probable since the absence of the band at 1276 cm^{-1} signifies the absence of lignin [33]. Therefore, it can be concluded that during the aging process the amorphous regions deteriorated and only the crystalline regions remained. The band at 780 cm^{-1} in Figure 4, spectrum d (RCS07) is most probably due to lignin [34].

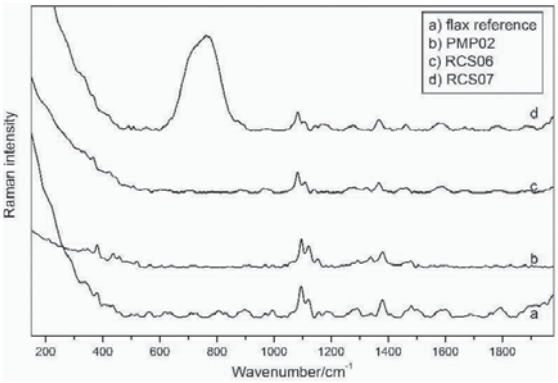

Figure 4: Comparison of non-infected flax objects PMP02 (spectrum b), RCS06 (spectrum c) and RCS07 (spectrum d) with flax reference (spectrum a)

According to the FT-Raman spectra of non-infected specimens of two paintings treated at the Restoration Centre of the IPCHS (RCS06 and RCS07, Figure 4, spectra c and d), it can be seen that not only biodeterioration is the cause of luminescence. The older painting from the 16th century (RCS07) has the more intensive luminescent background and consequently weaker bands, especially in the region between 300 cm^{-1} and 600 cm^{-1}, which makes the evaluation of its structure difficult. On the other hand, the spectra of younger painting from the beginning of the 18th century (RCS06) show relatively well-resolved bands despite moderate luminescent background. The decrease of the band at 1380 cm^{-1} is a sign of cellulose depolymerization [28]. This band decreased at spectra of both objects. Additionally, the ratio between 1120 cm^{-1} and 1096 cm^{-1} bands also decreased, indicating the degradation of cellulose [27]. The cellulose degradation is probably the main reason for low spectra quality.

Six of the investigated linen fabrics were infected by fungi (Tables 1 and 2). All of the infected investigated objects were painting canvases. Two of them were from the Slovene Museum of Christianity and all others were from different religious institutions, treated at the Restoration Centre of the IPCHS.

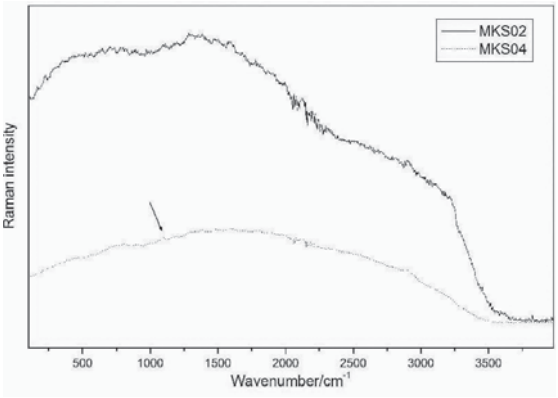

Figure 5: Spectra MKS02 and MKS04 showing an intensive background with only slightly visible main bands around 1100 cm^{-1} (arrow)

The spectrum of one of the two paintings from the Museum of Christianity (MKS02, Figure 5, solid spectrum) had a strong luminescent background and no spectral bands could be observed. Therefore, no estimation of its structure was possible by FT-Raman spectroscopy. At spectra of the other painting from the same museum (MKS04, Figure 5, dotted spectrum), which is approximately one century older, the background is strong. However, weak bands of the cellulose could still have been observed. However, most of the bands are difficult to interpret. Therefore the structure of cellulose could not be estimated properly. This example shows, that the higher age of the sample is not necessary the cause of stronger deterioration. Since the history of neither object is familiar to us, no further conclusions can be drawn about what caused faster deterioration of the later produced textile.

Figure 6 represents a comparison of biodeteriorated flax objects sampled during the conservation processes at IPCHS: RCS01 (spectrum a), RCS05 (spectrum b), RCS08 (spectrum c), RCS09 (spectrum d), RCS14 (spectrum e) with flax reference (spectrum f). The most severely and visibly infected (of all the investigated objects) was the painting from a succursal church, dating from the first half of the 19th century (RCS01). The mycelium growth was visible with a naked eye on the whole back side (Table 2). The FT-Raman spectra of the sample RCS01 (Figure 6, spectrum a) exhibited too strong background

and no spectral bands could be observed. The reason for the strong background is the presence of fungal hyphae as proposed by Edwards and co-authors [20] or severe deterioration of the cellulose, as confirmed by FTIR spectroscopy [1].

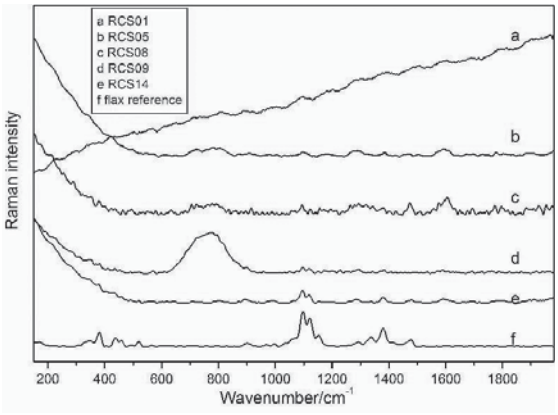

Figure 6: Comparison of biodeteriorated flax objects RCS01 (spectrum a), RCS05 (spectrum b), RCS08 (spectrum c), RCS09 (spectrum d), and RCS14 (spectrum e) with flax reference (spectrum f)

The strong luminescent background can be seen also in some other samples as well (Figure 6, spectra b, c and d). At the spectrum of sample RCS05 from the Franciscan monastery, dating from 1729 (Figure 6, spectrum b), only the strongest bands in the spectrum are visible, therefore the structural determination is difficult. In the region around 1100 cm^{-1}, only a broad band with the peak at 1096 cm^{-1} could be observed (Figure 6, spectrum b), with only a slight shoulder towards 1120 cm^{-1}. According to the FT-Raman spectra of sample RCS05, it can be concluded that the fibers are severely deteriorated and the glycoside vibrations are weak.

In the FT-Raman spectrum (Figure 6, spectrum d) of the painting from the Ljubljana cathedral (RCS09), which was painted in the 17th century, the background was strong as well. However, the most characteristic features of cellulose could still be observed. The disappearance of the band at 995 cm^{-1} and the decrease in intensities of the bands at 1096 cm^{-1}, 1120 cm^{-1}, and 1380 cm^{-1} are a sign of cellulose deterioration [27]. The disappearance of the band at 1480 cm^{-1} is a sign of crystallinity decrease [20]. It can be concluded on the basis of all these findings that the fibers are severely deteriorated and the crystallinity content in the fibers has decreased.

In the FT-Raman spectrum (Figure 6, spectrum e) of the infected sample RCS14, the background is rather intensive. However, the bands could still be distinguished relatively clearly. Several differences could be observed compared to contemporary reference spectra. Decreased intensities were observed at bands at 1096 cm^{-1}, 1120 cm^{-1}, 1380 cm^{-1} and 1480 cm^{-1} indicating cellulose deterioration and the decrease of crystallinity [35, 36].

At the FT-Raman spectrum of the sample RCS08 (Figure 6, spectrum c), all the bands are very weak. Thus, it is difficult to compare visually with reference spectra. All the main bands are visible. Their ratios, however, are difficult to interpret.

The features were less visible and more difficult to interpret in all FT-Raman spectra of flax samples than at cotton samples spectra. The most probable cause for luminescent background masking spectral bands is the presence of lignin and other non-cellulosic features, which are present in bast fibers and absent in cotton. However, when comparing the results of infected and non-infected objects, it seems, that fungi influence the cellulose structure that it becomes more crystalline and the ageing itself to become more amorphous. Due the small number of the samples this cannot be seen as rule, but could be explained that the enzymes can more easily degrade amorphous structures, whereas (mainly) physical influences during ageing influence crystalline structure.

Table 4: Intensity ratios for flax fibers

Sample	I^{1121}/I^{1096}	I^{380}/I^{1096}
Flax reference	0.57	0.16
PMP02	0.75	0.35
RCS05[a)]	0.26	0.05
RCS06	0.53	0.23
RCS07	0.42	0.06
RCS08	0.58	0.18
RCS09[a)]	0.59	0.39
RCS14[a)]	0.61	0.17

[a)]Infected objects

In Table 4, the calculated intensity ratios of flax samples are presented. It can be seen that neither aging nor fungal infection causes the same crystallinity changes in all the cases. An increase or a decrease of the ratios are not directly connected to fungal deterioration. The degree of crystallinity is a

function of many interconnected processes to which a textile material is subjected during its life. The reason for this is unique influences of physical environmental factors (light, relative humidity, temperature) and of different fungal species to a specific investigated object.

3.3 Comparison of FT-Raman, dispersive Raman, and FTIR methods

Selected samples were analyzed using FT-Raman spectroscopy method. The same samples were previously analyzed also with the dispersive Raman and FT-IR spectrometers [11, 12]. Especially with dispersive Raman spectrometer we met with several difficulties, since fluorescence masked spectral bands [17]. Raman spectrometers give information about skeletal structure of the polymers [28], whereas infrared spectra base more on functional groups' vibrations [37]. FT-Raman spectroscopy was invented as an upgrade of dispersive Raman spectroscopy, to reduce the luminescence [21]. The aim of presented work is also to test the applicability of this analytical method in the field of historical materials especially for their analysis, restoration and conservation.

Obtained FT-Raman spectra were analyzed and compared to dispersive Raman spectra and FT-IR spectra. Figure 7a represents a comparison of FT-Raman spectrum of the sample RCS14 (solid line) to dispersive Raman spectrum of the same specimen (dotted line). In FT-Raman spectra, several minor bands can be observed. They are hidden in the case of dispersive Raman spectra by fluorescent background (Figure 7a).

Comparing the FT-Raman and dispersive Raman spectroscopy methods and their results it can be clearly seen that additional information could be gathered from the FT-Raman spectra when compared to dispersive Raman spectra. Additionally, the scanning time for FT-Raman spectra is shorter, since samples need no or only little signal quenching whereas dispersive Raman spectra sometimes needed several hours of signal quenching to obtain informative spectra. Therefore if one has a choice the use of FT-Raman spectroscopy would be advised for the analysis of historical textiles analyses.

Figure 7b shows FTIR spectra of the same specimen (RCS14). In FTIR spectra, all peaks and details can be clearly seen and well resolved. However, the main vibrations which are seen on FTIR spectra are vibrations of functional groups whereas Raman spectroscopy shows more clearly the cellulose backbone structure vibrations [28]. Therefore FTIR and Raman spectroscopy and their results are complementary. In FT-IR spectrum of the sample RCS14 no significant changes were observed, when compared to contemporary reference flax spectra in one spot, but the other one shows some changes in the cellulose vibrations regions (1300 to 1400 cm^{-1} and 1200 do 1000 cm^{-1}), showing cellulose deterioration and crystallinity decrease [38, 39, 40]. FT-Raman spectra as well show decrease of certain bonds (as mentioned above), also indicating cellulose deterioration and the decrease of crystallinity [27, 28]. Since FT-IR spectra are easy and quick to obtain, although not without problem, especially when working in transmission mode [16], it will remain the preferred method for the authors.

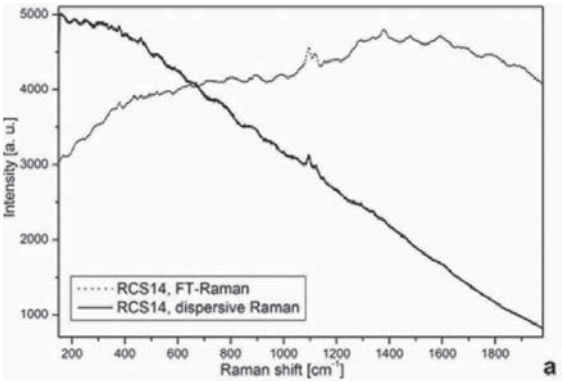

Figure 7a: Comparison of FT-Raman spectrum of the sample RCS14 (dotted line) to dispersive Raman spectrum of the same specimen (solid line)

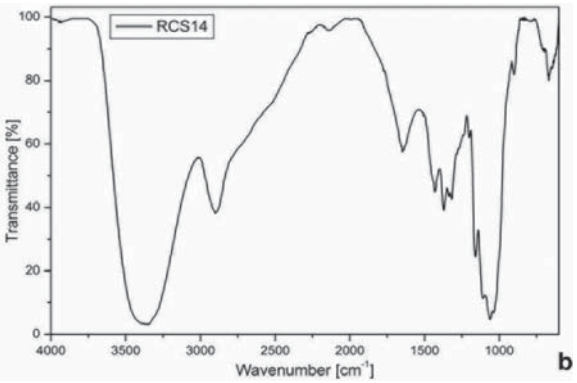

Figure 7b: FTIR spectrum of the sample RCS14

4 Conclusion

FT-Raman spectroscopy has proved to be a useful tool for investigation and analysis of cellulose historical textile objects and fiber supermolecular structure for cotton and in most cases for bast fibers. In the case when cellulose fibers contain many non-cellulosic substances, as it is in the case of hemp fibers, or are subjected to severe deterioration, a strong luminescent background appears which masks spectral bands and prevents the analysis of the cellulose fiber supermolecular structure. Compared to dispersive Raman spectroscopy, FT-Raman spectroscopy enables clearer spectra with more visible spectral bands and with less luminescence which leads to more accurately interpreted supermolecular structure of cellulose fibers. To obtain even more precise information about the supermolecular structure of investigated materials combining FT-Raman method with other analytical methods (especially FT-IR spectroscopy) is highly recommended.

The FT-Raman analysis also clearly confirmed the fact that the physical and biological factors cause severe deterioration of cotton and flax fibers as a consequence of fungi attack or other, especially physical, deteriorating factors (e. g. light and humidity). The results show relatively more intensive changes at infected objects compared to non-infected. This confirms the prediction that the supermolecular structure of cellulose fibers change more at objects subjected to fungal infection than at those which were not infected by fungi and were only only infected by the physical environmental factors. This is confirmed especially for cotton fibers. Other cellulosic fibers (bast fibres) exhibit too high luminescence to be reliably analysed.

Acknowledgements

The authors would like to thank Ingalill Nystrom and the Department for Conservation of the University of Gothenburg, Sweden, for giving us the possibility to use the FT-Raman equipment at their institution. We would also like to thank the Slovene Museum of Christianity, Ptuj, the Regional Museum and the Department for Easel Paintings of the Restoration Centre of the IPCHS for providing the sampling objects.

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