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Application of FTIR and Raman Spectroscopy to Qualitative Analysis of Structural Changes in Cellulosic Fibres

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Abstract

Fibres belong to organic materials and are therefore susceptible to external influences, causing structural changes in materials. The consequences of structural changes in fibres are the changes in their properties. Natural cellulosic fibres, which were in the past often used for clothing, decorative and applied arts, are very susceptible to external influences. The fibre structure can be analysed with vibrational spectroscopic methods. Infrared and Raman spectroscopies are complementary methods, allowing us to analyse the fibre supramolecular structure (crystallinity and different polymorph structures of cellulose). In the present work, both methods were applied for the analysis of aged cellulose textiles degraded by fungi. The selected methods turned out to be appropriate for the analysis of supramolecular structural changes in the biodegraded textiles, e.g. depolymerisation of cellulose macromolecules and changes in the arrangement of macromolecules. A prolonged time of active contamination with fungi led to more intensive supramolecular structural changes.

Keywords: cellulose fibres, infrared spectroscopy, Raman spectroscopy, structure of fibres

1 Introduction

Natural cellulosic fibres are due to their composition susceptible to the influences from the environment, including physical and chemical, and biological influences. All these influences cause changes in the structure of materials. Historical materials are especially sensitive to external influences, due to their long exposure to external influences, which already changed their properties. The application of conservation and restoration treatments, and handling with the museum objects are influenced by the changed properties of aged fibres.

Cellulosic fibres are more susceptible to external influences than proteinaceous fibres [1]. Environmental influences, e.g. changing the temperature, relative humidity, ultraviolet irradiation, can cause oxidation, hydrolysis, scission of macromolecular chains and breaking of intermolecular bonds in exposed materials [2, 3]. These processes can often take place simultaneously [4]. The listed structural changes usually cause simultaneous changing of the fibre properties. *Inter alia*, they alleviate deterioration of materials by enzymes excreted by different organisms [5, 6]. Fungi belong to the most intensive decomposers of materials [2].

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The present study was performed on cellulosic textiles inoculated with fungi, a part of them being previously artificially aged. For the analysis, we used cotton and linen fabrics. Cotton (seed fibres) and flax (bast fibres) are present in many dressings and other cultural heritage objects. We analysed the structure of contemporary non-degraded textiles, artificially aged, inoculated and irradiated with gamma rays, as well as naturally aged (museum) textiles, contaminated with fungi and non-contaminated.

Spectroscopic methods were applied for the analysis of the cellulosic fibre structure: Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy, which are complementary methods, both applicable to the analysis of material structures. In Raman spectroscopy, the specimen is irradiated with monochromatic light (laser) of different wavelengths (from 229 nm to 1064 nm). The spectrum is obtained as non-elastic scattering of light, reflected from the specimen [7]. Raman spectroscopy gives the basic information about the skeletal vibrations of molecules, which are intensively Raman active due to their birefringence. On the other hand, side groups are more polarisable and therefore active in the infrared spectra. The properties of Raman spectroscopy allow it to give information about polymer (vibrations of β-1,4-D bonds between glucose molecules) [8, 9].

With Raman spectroscopy, the surface of specimens can be analysed. For the analysis, microscope is attached to the spectrometer. Thus, very small specimens can be analysed, which is very important in conservation – restoration, where often only microscopic grade specimens can be obtained for the analysis, in order to keep the integrity of specimens [10]. Another possibility for smaller objects is to put the whole object under the objective of a microscope.

FTIR is a vibrational spectroscopic method, where the specimen is irradiated with infrared (IR) light. The infrared spectrum is obtained when molecules absorb specific frequencies of the IR light. FTIR spectroscopy can be applied as a non-destructive FTIR ATR (Attenuated total reflectance) method [11] or as a micro destructive method with the application of a diamond anvil cell and microscope attached to the spectrometer. The third possibility in the case of adequately large specimens is the preparation of KBr discs, where powdered specimen is included. Whilst FTIR ATR is a surface analysing method, the other two are transmission methods, allowing us to analyse the whole fibre thickness. Both above presented spectroscopic methods (FTIR and Raman spectroscopy) are sensitive to the structural changes in materials and therefore allow us to analyse the degradation processes in cellulose [12]. By using them, crystalline and amorphous cellulose can be differentiated, i.e. more and less ordered arrangement of macromolecules within (micro)fibrillar structures. Additionally, different polymorph forms of cellulose can be differentiated [13, 14] in a qualitative and quantitative manner [14].

The aim of the present study was to check the applicability of selected spectroscopic methods to the analysis of structural changes in cellulosic fibres as well as to estimate the extension of damage in cellulosic fibres after the inoculation with fungi.

The results of the analysis allowed us to investigate what kind of structural changes occurred in the inoculated and aged cellulosic fibres. For the analysis, laboratory specimens which were artificially aged and the specimens from museum objects were prepared.

2 Materials and methods

2.1 Materials

Laboratory specimens were prepared from a cotton and flax fabric of contemporary production. We chose non-whitened and non-sized cotton, woven in canvas binding (44 threads/cm × 31 threads/cm) as well as non-treated linen fabric in panama binding (20 threads/cm × 14 threads/cm). Each fabric was cut into pieces of 2.5 cm × 5 cm in size.

The sizes of specimens taken from museum objects depended on the object properties and ranged from some fibres to a piece of a fabric of several square centimetres in size. The specimens were taken as near as possible to the fungal spots and we made sure that the appearance of the object was not altered. In Table 1, we present a list of analysed historical objects.

Table 1: List of museum specimens with marks and descriptions of objects

Specimen	Object description	Source
MKS01	Statue of Maria with textile dress	Museum of Christianity in Slovenia, Stična (MKS)
NGS01	Painting on canvas "At the piano" (Rihard Jakopič)	National Gallery (NG)
PMP01	Underskirt	Regional Museum Ptuj Ormož (PMP)
PMP05	Leather belt with textile lining	
RCS01	Painting on canvas "14th station of the Stations", original canvas	Institute for the Protection of Cultural Heritage of Slovenia, Restoration Centre (RCS)
RCS02	Painting on canvas "Jesus heals dropsy man", original canvas	
RCS05	Painting on canvas "St. Francis", original canvas	
RCS07	Painting on canvas "St. Nicholas", original canvas	
RCS10	Painting on canvas "Death of Maria", lining	

2.2 Laboratory specimen preparation

2.2.1 Artificial ageing

A half of contemporary specimens, sewn on Whatman paper, were artificially aged in a Climatic chamber (Vötsch Climatic chamber, Type VC 0020). The specimens were aged at 80 °C and 65% relative humidity in two steps, first for 13 days and then for 12 days at the same conditions.

2.2.2 Inoculation with fungi and incubation

The non-aged and artificially aged specimens were inoculated with six different fungal species (*Aspergillus clavatus* (EXF-5895), *Cladosporium cladosporoides* (EXF-5883), *Fomes fomentarius* (EXF-5903), *Hypoxylon fragiforme* (EXF-5882), *Penicillium chrysogenum* (EXF-5913) and *P. corylophilum* (EXF-5897)). The fungi used for the inoculation were isolated from museum objects in different Slovenian museums (cf. Table 2). The selected fungi grown in test glasses were poured over with 5 ml of physiological solution (9% w/v NaCl9. With the mechanical agitation with an inoculate loop, we removed spores and fragments of hyphae. Distilled water was added to obtain 20 ml of suspension. Two autoclaved specimens of the same type were laid in an open Petri dish and were inoculated with 250 µl of suspension. A smaller Petri dish with the fabric was put into a larger closed Petri dish, in which we put blotting paper soaked with 500 µl of sterile distilled water to obtain a humidifying chamber. The specimens were incubated in the chamber at 25 °C. After 12 days, blotting papers were soaked with an additional 1 ml of sterile distilled water. Half of the specimens were incubated for 8 weeks and the other half for 20 weeks. At the end of the incubation, all specimens were autoclaved in steam at 104 °C for 10 minutes. Prior to the autoclaving, we made a test to make sure that autoclaving did not affect the structure of laboratory specimens.

2.2.3 Irradiation with gamma rays

Autoclaving is not an appropriate method for the sterilisation of museum objects, since humidity and high temperature could damage their structure or change visual appearance. To investigate the possibility of using gamma rays, a part of laboratory specimens was sterilised with irradiation instead of autoclaving. Gamma irradiation has already been used for the sterilisation and disinsection of cultural heritage objects [16]. The sterilisation with gamma rays was performed on non-autoclaved specimens, aged as well as non-aged, which were incubated with fungi for 20 weeks. Gamma irradiation was conducted in Zagreb, Croatia at the Ruđjer Bošković Institute, where the irradiation of cultural heritage objects is a common practice [16]. Irradiation was carried out in a multi-purpose room with the panoramic gamma source ^{60}Co, in which 24

Table 2: List of marks of laboratory specimens and example of specimen designation

Type of textile	
CO	Cotton
LI	Linen
Artificial aging	
n	Non-aged
s	Artificially aged
Inoculation	
5882	*Hypoxylon fragiforme*
5883	*Cladosporium cladosporoides*
5895	*Aspergillus clavatus*
5897	*Penicillium corylophilum*
5903	*Fomes fomentarius*
5913	*Penicillium chrysogenum*
K	Control non-inoculated specimen
N	Reference non-inoculated and non-incubated specimen
Successive numbers	
_1	8 weeks incubated specimen, autoclaved
_2	20 weeks incubated specimen, autoclaved
_22	20 weeks incubated specimen, irradiated with gamma rays of 5 kGy dose
_21	20 weeks incubated specimen, irradiated with gamma rays of 10 kGy dose
Example	
COn5903_22	Cotton, non-aged, inoculated with *F. fomentarius*, irradiated with 5 kGy gamma rays

poles filled with ^{60}Co were arranged in a cylindrical shape. The specimens were irradiated closed in Petri dishes, six at a time. Two different doses were applied. 5 kGy is among the lowest doses effective for disinfection according to the literature [16] and 10 kGy is the upper limit for the doses to be applied in order not to damage fibres too seriously [17, 18]. The dose 5 kGy was achieved in 1360 s (22.67 min) and the dose 10 kGy in 2710 s (45.17 min).

2.3 Methods for analysis of fibre structures

2.3.1 Raman spectroscopy

Raman spectra were scanned on a dispersive Raman spectrometer LabSpec HR 800 (Horiba Jobin-Yvon) with an air cooled CCD detector (Charge Coupled Device). For the analysis, a diode laser in the NIR was used with the wavelength of 785 nm. All specimens were analysed with a microscope at 10× magnification in the range from 100–2000 cm^{-1} at the aperture of 1000 µm and grating 600 g/mm. Two different filters were used for the analysis, i.e. one that transmits half of the energy (D0'3) and another, which transmits a quarter of the laser energy (D0'6). The time of accumulation varied between 50 s and 300 s, and the drench quenching time between a few minutes and several hours. The use of the filter, as well as the time of accumulation and drench quenching time varied according to the properties of each specimen in a way as to obtain the minimum luminescence and the most intensive absorbance bands. Each specimen was placed on an objective glass covered with aluminium foil. The non-inoculated as well as inoculated specimens were analysed. Specimens irradiated with gamma rays produced a luminescence background which

was too large to be investigated with Raman spectroscopy.

2.3.2 Fourier transform infrared spectroscopy (FTIR)

Spectra of laboratory prepared specimens were scanned with the FTIR ATR method on a FTIR Spectrum 100 spectrometer (PerkinElmer) in the range between 4000 cm^{-1} and 675 cm^{-1}. Each spectrum is an average of 32 scans, scanned at the resolution of 4 cm^{-1}. The pressure on a fabric depended on its properties and was always the highest possible. Each piece of fabric (specimen) was scanned in five different spots on both sides, which were usually affected by fungi in different intensities.

The FTIR analysis of museum specimens was performed on a microscope Spectrum spotlight 200 (PerkinElmer), connected to a Spectrum 100 spectrometer (PerkinElmer). Some fibres were pressed in the diamond anvil cell and then investigated in an open cell to increase the energy. In this manner, the range at lower wavenumbers did not diminish and no noise occurred [19]. Each single fibre was analysed with a 20 μm × 20 μm aperture. The spectra were averaged from 64 scans in the range between 4000 cm^{-1} and 600 cm^{-1} at the 4 cm^{-1} resolution.

3 Results and discussion

The non-inoculated, inoculated, as well as inoculated and irradiated laboratory specimens were analysed with both above described spectroscopic methods. In Raman spectra, especially those of flax and partially of more intensively affected cotton specimens, a luminescent background occurred, which made the quality of the spectra worse with the rise of a shapeless background. Only the most intensive bands could be observed in such spectra. The luminescence could occur due to the presence of impurities (improbable), fungi or products of deterioration of the fibres on the fabric surface [20]. The intensity of the luminescent background was reduced with drench quenching, where a specimen was irradiated with a laser beam at diminished power for a longer period (from a few minutes to several hours). During the drench quenching, photodegradation of artefacts is said to occur [21]. With this method, the quality of Raman spectra improved and some of the smaller bands became visible.

The analyses of structural changes were conducted by observing the shape of the Raman and FTIR spectra, peak positions and intensities of bands relatively to the local baseline. The band positions for the Raman spectra are listed in Table 3 and for the FTIR spectra in Table 4.

3.1 Cotton

During artificial ageing, the structure of cotton changed, which could be observed in the Raman spectra as a decrease in the absorption band at 435 cm^{-1}, as well as an increase in the absorption bands

Table 3: Positions of bands and their assignments for cellulose in Raman spectra together with references

Raman shift [cm^{-1}]	Assignment	Reference
1380	CH_2 bending and skeletal vibrations	[8]
1335	CH_2 vibrations	[20]
1120	Symmetric vibrations of glycoside bond	[9]
1096	Asymmetric vibrations of glycoside bond	[22]
1057	CO vibrations of secondary alcohols	[20]
1034	CO vibrations of primary alcohols	[20]
520	CCC of ring and glycoside bond	[10]
458	CCC and CCO vibrations of glucose rings	[10]
435	CCC and CCO vibrations of glucose rings	[10]
380	CCC vibrations of glucose rings	[9]
345	CCC vibrations of glucose rings	[9]

Table 4: Positions of bands and their assignments for cellulose in FTIR spectra together with references

Wavenumber [cm^{-1}]	Assignment	Reference
2900	CH and CH_2 bonds in aliphatic methylene groups	[11]
1710–1745	Carbonyl bands	[12]
1698	CHO vibrations	[23]
1510	Lignin	[12]
1430	CH_2 vibrations; HCH and OCH in-plane bending; intramolecular hydrogen bonds bending	[14]
1372	COH and HCC vibrations of cellulose and hemicelluloses hemiceluloz	[3]
1335	OH and CH_2 vibrations	[11]
1316	COH and HCC vibrations	[3]
1280	CH and OH vibrations	[14]
1160	COC asymmetric vibrations	[14]
1111	Asymmetric vibrations of glucose rings	[14]
900	COC vibrations of glycoside bonds	[14]

at 1096 cm^{-1} and 1335 cm^{-1} (cf. Figure 1). These changes are a consequence of reducing the crystallinity, decomposition of the skeletal structure or increasing the number of OH bonds [9, 10, 22]. They occurred in the processes of hydrolysis and oxidation, and breaking of intramolecular bonds, which were caused by increased the temperature and relative humidity in the chamber for artificial ageing. Similar changes as during artificial ageing were observed in non-aged specimens inoculated with fungi. We assume that fungi caused similar changes in the structure of cellulose as the increased temperature and relative humidity at artificial ageing.

The result analysis shows that more intensive changes occurred in longer (20 weeks) incubated specimens. Although artificial ageing is supposed to alleviate penetration of enzymes into the interior of specimens [5, 6], we did not notice any changes

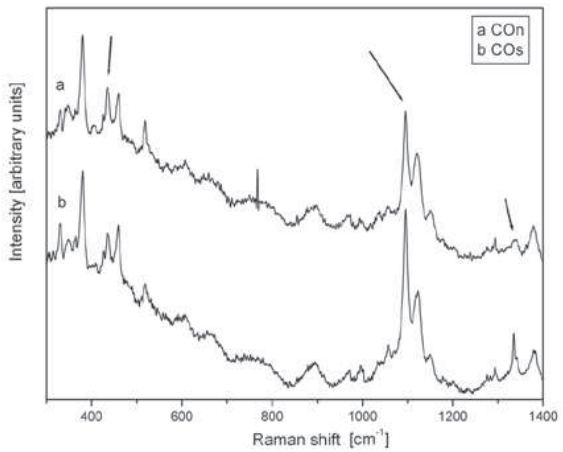

Figure 1: Raman spectra of (a) reference non-aged and (b) artificially aged cotton reference specimens; labelled bands are (from left) at 435 cm^{-1}, 1096 cm^{-1} and 1335 cm^{-1}

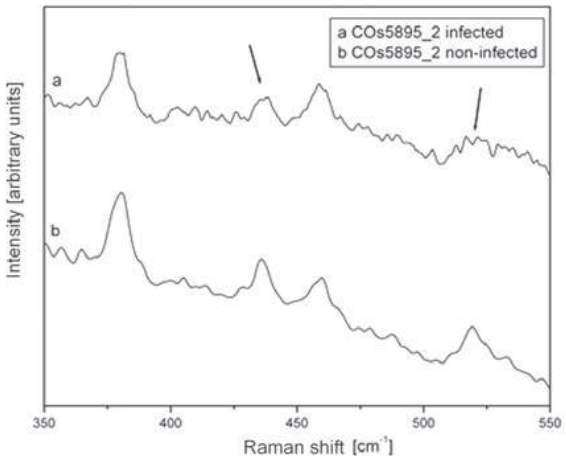

Figure 2: Raman spectra of (a) contaminated and (b) non-contaminated position of artificially aged specimen inoculated with A. clavatus in the range between 350 cm^{-1} and 550 cm^{-1}; marked are bands (from left) 435 cm^{-1} and 520 cm^{-1}, intensities of which decreased during biodeterioration

with FTIR spectroscopy in the vibrations of functional groups of the specimens inoculated with *H. fragiforme*, *P. chrysogenum* and *P. corylophilum* after 8 weeks of incubation. On the other hand, the skeletal vibrations active in the Raman spectra changed, which suggests the changes in intermolecular bonds. The contamination with fungi acted locally, since the structural changes were more intensive in the immediate vicinity of fungal spots as seen from the Raman spectra of artificially aged specimens inoculated with *A. clavatus*, where in a more intensively degraded position, the intensity of the band at 345 cm^{-1} decreased (cf. Figure 2).

The main processes in the inoculated cotton fibres were depolymerisation and decrease of arrangement in the supramolecular structure. The decomposition of the skeletal chain (depolymerisation) can be observed as a decrease in the Raman absorption bands at 520 cm^{-1}, 1120 cm^{-1} and 1380 cm^{-1} (for assignments cf. Table 3). The most intensive decrease in polymerisation according to the Raman spectra occurred in the non-aged and artificially aged specimens inoculated with *C. cladosporoides*, and in the non-aged specimens inoculated with *A. clavatus* (cf. Figure 3).

The proportion of the ordered structure decreased in the specimens inoculated with *Hypoxylon fragiforme* and *Penicillium corylophilum*, which appeared as a decrease in the band at 435 cm^{-1} (similar as in the more degraded specimen inoculated with *A. clavatus* (cf. Figure 2)). Making a comparison of the Raman spectra of artificially aged non-inoculated and non-aged specimens inoculated with *P. corylophilum*, we discovered that some fungi cause after a shorter period of incubation similar changes in the skeletal structure as the ageing process.

The process of depolymerisation is observed in the FTIR spectra of the specimens inoculated with *C. cladosporoides*, where the absorption band for the carbonyl vibrations at the ends of depolymerised chains [12] occurred (cf. Figure 4). Hydrolysis and oxidation of the carbon atoms in the glucopyranose rings lead to the depolymerisation and occurrence of carbonyl groups on the broken ends of macromolecular chains – the first mainly in aldehyde groups and the second in carbonyl groups [4]. The intensity of the carbonyl band in the FTIR spectra increased in most specimens during prolonged incubation times, which is a consequence of a larger number of broken cellulose macromolecules. In the aged specimen inoculated with *C. cladosporoides*, the position of the carbonyl band after 20 weeks of incubation moved to higher wavenumbers as a consequence of an increased number of hydrogen bonds due to the hydrolytic decomposition [4]. The depolymerisation caused the occurrence of aldehyde groups, observed as the occurrence of the

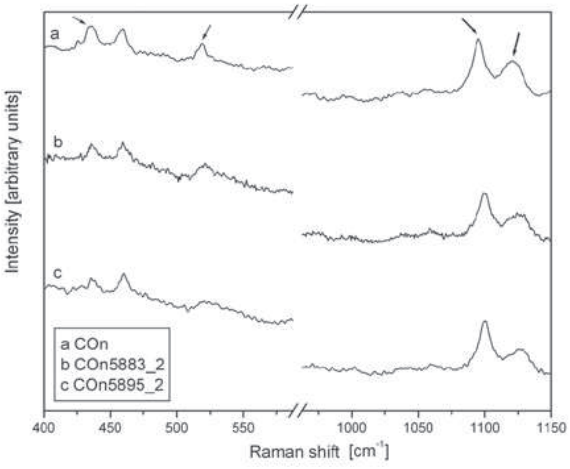

Figure 3: Raman spectra of (a) reference non-aged specimen, (b) non-aged specimen inoculated with C. cladosporoides and (c) non-aged specimen inoculated with A. clavatus; marked are bands (from left) 435 cm^{-1}, 520 cm^{-1}, 1096 cm^{-1} and 1120 cm^{-1}, the intensities of which decreased during biodeterioration

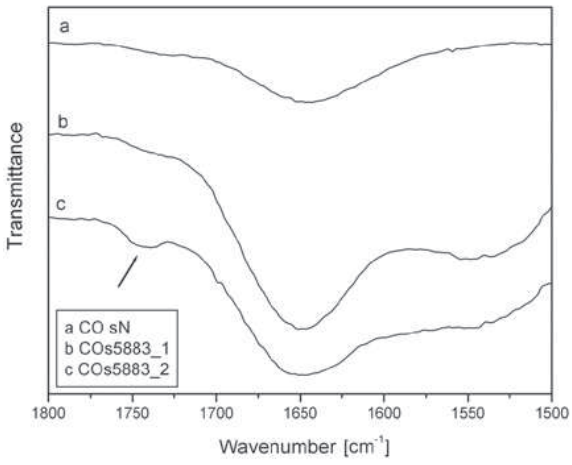

Figure 4: FTIR ATR spectra in range of carbonyl band in (a) reference specimen as well as in artificially aged specimens, inoculated with C. cladosporoides after (b) 8 and (c) 20 weeks of incubation, and after irradiation with gamma rays of doses (d) 5 kGy and (e) 10 kGy

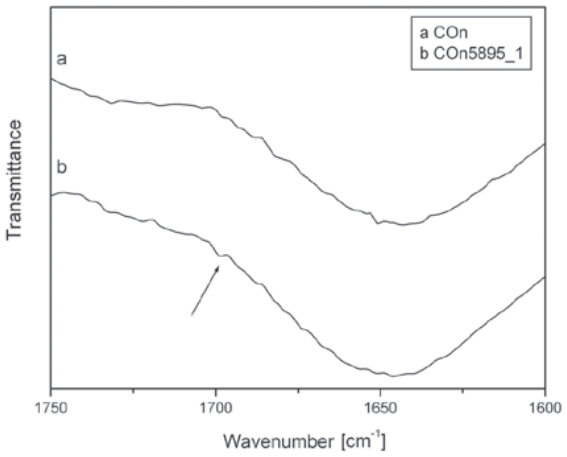

Figure 5: FTIR ATR spectra of (a) non-aged reference specimen and (b) non-aged specimen inoculated with A. clavatus; arrow points at band at 1698 cm^{-1}, assigned to vibrations of aldehyde groups

band at 1698 cm^{-1} in the specimen inoculated with *A. clavatus* (cf. Figure 5) [23] (for assignments of vibrations in FTIR spectra cf. Table 4). This means that the fungus caused predominantly hydrolysis, whereas other fungi caused oxidative reactions.

A decrease in crystallinity was observed as the changes in the FTIR spectra of the non-aged specimens inoculated with *A. clavatus* and *C. cladosporoides*, where the absorption band at 1430 cm^{-1} moved to lower wavenumbers as a consequence of changed vibrations in the C_6 atom region [24].

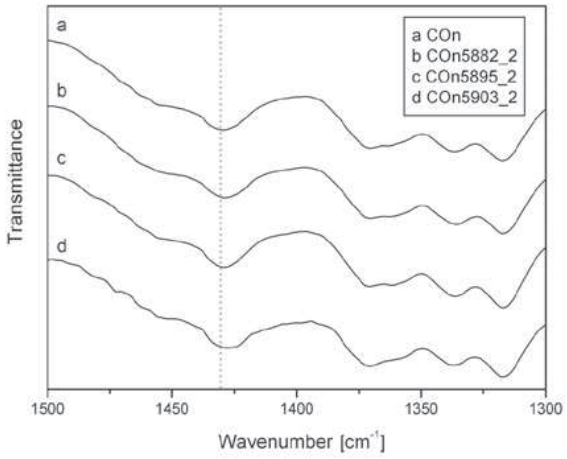

Figure 6: FTIR ATR spectra of (a) reference specimen, (b) non-aged specimen incubated for 20 weeks with H. fragiforme, (c) non-aged specimen incubated for 20 weeks with A. clavatus, and (d) non-aged specimen incubated for 20 weeks with F. fomentarius; band at 1430 cm^{-1} (cf. arrow) moved in infected specimens to lower wavenumbers

Irradiation with gamma rays did not cause significant structural changes, which could be observed with FTIR or Raman spectroscopy.

3.2 Flax

In the FTIR ATR spectra of flax fibres, we observed absorption bands typical of lignin vibrations (cf.

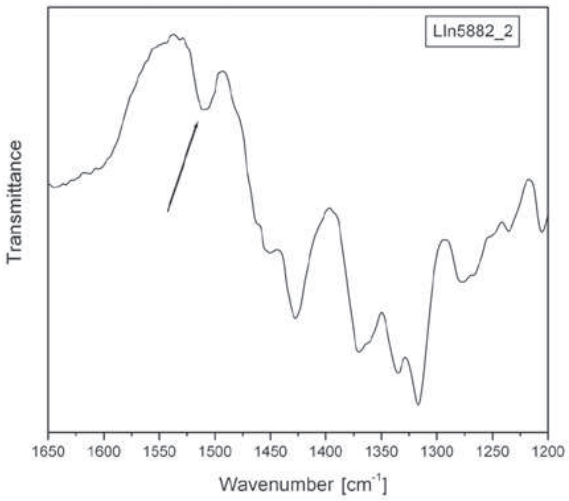

Figure 7: FTIR spectrum of specimen inoculated with H. fragiforme with marked band in region of aromatic vibrations of lignin

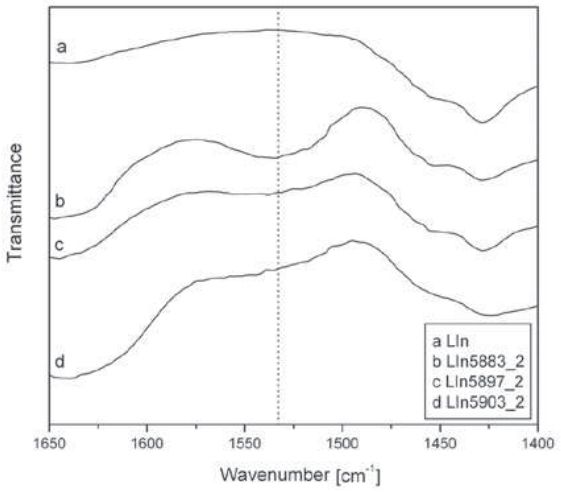

Figure 8: FTIR spectra of (a) reference non-aged flax specimen, (b) non-aged specimen inoculated with C. cladosporioides, (c) non-aged specimen inoculated with P. corylophilum and (d) non-aged specimen inoculated with F. fomentarius in region of amide vibrations, indicating presence of fungi in analysed area

Figure 7). In more intensively infected areas, we observed the amide II absorption band, present due to the presence of fungi, either as a consequence of the present chitin or proteins in the interior of the hyphae [25, 26] (cf. Figure 8).

The structural changes in flax fibres occurred already after eight weeks of incubation with all non-aged specimens, except for those inoculated with *P. corylophilum*, as well as in the aged specimens inoculated with *A. clavatus* and *H. fragiforme* (cf. Figure 9). Due to the inoculation, changes in the supramolecular structure of cellulose occurred. The process was limited to the immediate vicinity of fungal spots. The change in the supramolecular structure can be observed in the Raman spectra as a decrease in the intensities of bands at 345 cm^{-1}, 435 cm^{-1}, 520 cm^{-1}, 1096 cm^{-1} and 1122 cm^{-1}. After 20 weeks of incubation, additional changes were observed in the skeletal vibrations of non-aged specimens inoculated with *H. fragiforme* and aged specimens inoculated with *P. corylophilum*.

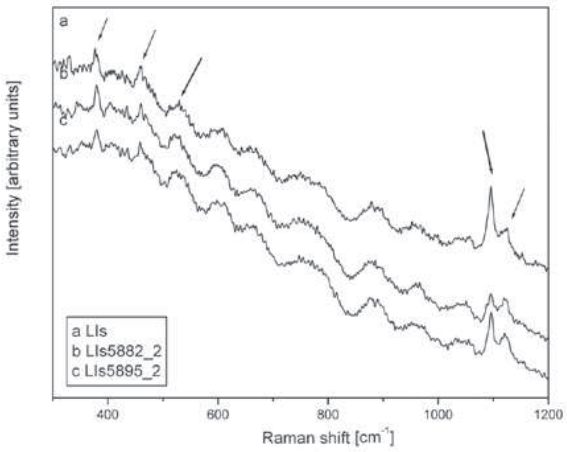

Figure 9: Raman spectra of aged flax specimens: (a) reference specimen, (b) specimen inoculated with H. fragiforme and (c) specimen inoculated with A. clavatus; marked are bands (from left) at 345 cm^{-1}, 435 cm^{-1}, 520 cm^{-1}, 1096 cm^{-1} and 1122 cm^{-1}

Due to the depolymerisation of cellulose macromolecules, carbonyl groups occurred in flax fibres, similarly as in cotton. A consequence of their occurrence is the formation of the carbonyl band in the FTIR ATR spectra (cf. Figure 10, right). The absorption band characteristic of carbonyl group vibrations could be observed already in the artificially aged non-inoculated specimens. We concluded that artificial ageing caused the depolymerisation of cellulose macromolecules in flax fibres. Due to the increased number of hydrogen bonds, the carbonyl band in the aged specimens inoculated with *P. corylophilum* shifted to 1750 cm^{-1} [4]. The intensity of the carbonyl band decreased after the irradiation with gamma rays, with the exception of artificially aged specimens inoculated with *A. clavatus* and *P. chrysogenum*, where its intensity increased, probably due to the continuation of depolymerisation processes. Simultaneously with the occurrence of the carbonyl band, the absorption bands in the region of CH and CH_2 vibrations at 2900 cm^{-1}

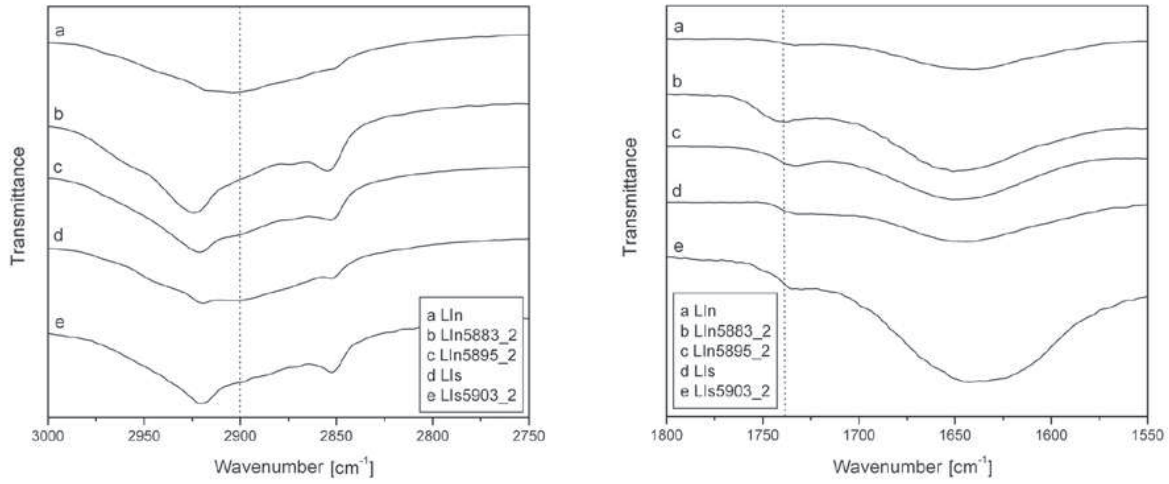

Figure 10: FTIR ATR spectra in region of carbonyl (right) and of CH vibrations (left) of (a) non-aged non-inoculated reference specimen, (b) non-aged specimen inoculated with H. fragiforme, (c) non-aged specimen inoculated with A. clavatus, (d) aged non-inoculated specimen and (e) aged specimen inoculated with F. fomentarius

changed (cf. Figure 10 left). Instead of one band at 2900 cm^{-1} for the CH_2 vibrations, two peaks appeared at 2922 cm^{-1} and 2854 cm^{-1}, typical of the CH vibrations [27]. This change is connected to the carbonyl band occurrence, i.e. to the existence of cellulose deterioration products.

A consequence of reducing the ordered structure in the FTIR spectra is overlapping of the bands at 1335 cm^{-1} and 1318 cm^{-1}, forming a band with a single peak at 1320 cm^{-1}, which is a consequence of the increased number of OH bonds due to hydrolysis [24, 28]. This happened in the aged and non-aged specimens inoculated with *F. fomentarius* (cf. Figure 11).

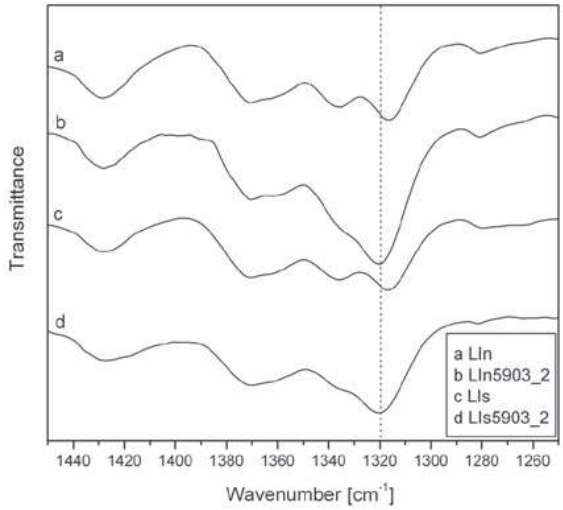

Figure 11: FTIR spectra of (a) non-aged non-inoculated specimen, (b) non-age specimen inoculated with F. fomentarius, (c) aged non-inoculated specimen and (d) artificially aged specimen inoculated with F. fomentarius; in infected specimens, we observed overlapping of two bands, forming single band with peak at 1320 cm^{-1}

Gamma irradiation affected the structure of flax fibres more than the structure of cotton fibres. In the non-aged fibres, the intensity of the carbonyl band in the FTIR spectra decreased. Due to the changed structure of intramolecular bonds after the gamma irradiation, the peak of the carbonyl band in the non-aged specimens inoculated with *P. corylophilum* moved to lower wavenumbers (cf. Figure 12). The process is a consequence of increasing the number of intramolecular bonds. In the non-aged specimens inoculated with *C. cladosporoides*, the peak moved to lower wavenumbers due to the decreased number of intramolecular bonds.

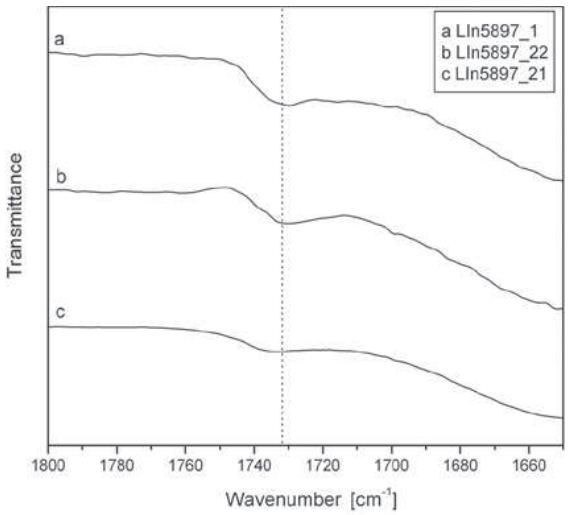

Figure 12: FTIR spectra of non-aged flax specimen inoculated with P. corylophilum: (a) after 20 weeks of incubation, (b) after 20 weeks of incubation and irradiation with gamma rays of 5 kGy dose, and (c) after 20 weeks of incubation and irradiation with gamma rays of 10 kGy dose; spectra are presented in carbonyl vibration region; in spectrum (c), shift of carbonyl band toward higher wavenumbers can be observed

3.3 Specimens from museums

The luminescent background was in the museum specimens more intensive than in the laboratory specimens. We assume that this is a consequence of the degradation of historical fibres as well as impurities on their surface. The spectra of cotton, which

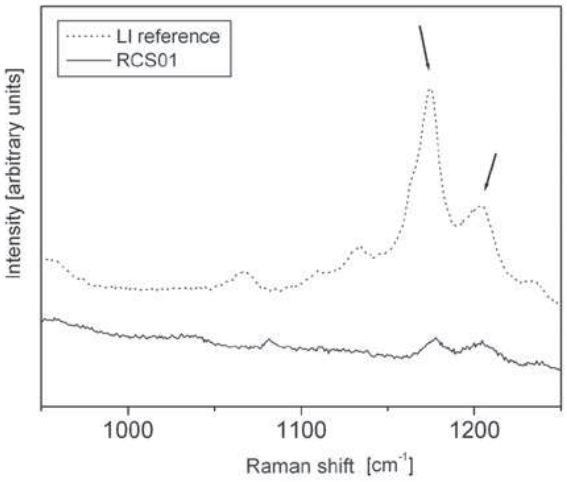

Figure 13: Raman spectra of specimen RCS01 (continuous line) and reference specimen (dashed line) in region of glycoside rings vibrations; marked bands are (from left) 1096 cm^{-1} and 1122 cm^{-1}

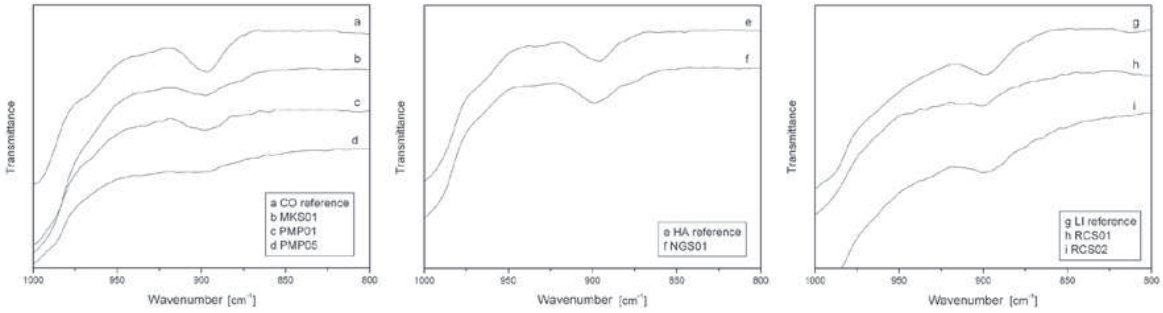

Figure 14: FTIR spectra obtained with microscope in diamond anvil cell in region of band at 900 cm^{-1}; left: (a) reference cotton specimen, (b) MKS01, (c) PMP01 and (d) PMP05; in the middle: (e) reference specimen of hemp and (f) NGS01; right: (g) reference specimen of flax, (h) RCS01 and (i) RCS02

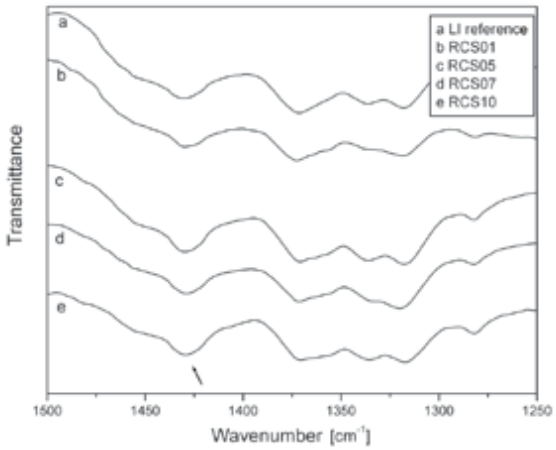

Figure 15: FTIR spectra of (a) reference specimen of flax and specimens of (b) RCS01, (c) RCS05), (d) RCS07 and (e) RCS10, scanned with microscope in diamond anvil cell in region of vibrations significant for crystalline cellulose; band at 1430 cm^{-1} is marked

contains fewer non-cellulosic components, had less intensive luminescent background. With drench quenching, we could lower the background to such an extent that we could analyse the most intensive absorption bands, especially those at 1096 cm^{-1} and 1122 cm^{-1}. In the specimen of the linen RCS01 (cf. Table 1), both bands had approximately equal intensities (cf. Figure 13) [29]. This was a consequence of distinctive contamination with fungi and consecutive changes in the supramolecular structure [11, 22].

The FTIR spectra of inoculated and non-affected historical specimens showed increased crystallinity, contrary to the laboratory specimens. An increase in crystallinity appeared as a decrease in the intensity of the 900 cm^{-1} band (cf. Figure 14). The changes occurred in cotton as well as in flax and hemp fibres. In other specimens, a decrease in the 1430 cm^{-1} band and its shift towards lower wavenumbers (cf. Figure 15) signifies a decrease in the amount of ordered structure [30]. Fungi and other external factors, which influenced the fibres during the ageing processes, have similar impacts on fibres, depending on the conditions objects were exposed to.

4 Conclusions

According to the FTIR and Raman spectroscopy results of the analysis of cotton and flax textiles inoculated with fungi, we can say that both methods are suitable for the analysis of the structural changes in cellulosic fibres. The methods are complementary and supplement each other. Therefore, the use of both methods is reasonable.

Based on the results obtained with selected spectroscopic methods, we analysed the influence of artificial ageing and different species of fungi on the molecular and supramolecular structure of cellulosic fibres. Fungi, which belong to the most intensive decomposers of organic materials, cause structural changes already after a short period of activity, both on the supramolecular level (skeletal vibrations) and on the molecular level (vibrations of functional groups). The structural changes begin earlier in the non-aged specimens, whereas in the artificially aged specimens, the processes begin later on, but are more intensive in the long run. The depolymerisation of cellulosic macromolecules and a decrease in the arrangement of the supramolecular structure occurred due to the impact of fungi. An active inoculation caused more intensive changes

after a prolonged time of incubation. The structural changes on the supramolecular levels are caused by gamma irradiation as well, especially in flax fibres. On the other hand, gamma irradiation caused an interruption of the deterioration process due to the sterilisation.

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