

Spectroscopic characterization of genetically modified flax fibres enhanced with poly-3-hydroxybutyric acid

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ABSTRACT

Genetically modified flax fibres, derived from transgenic flax with expression of three bacterial genes necessary for synthesis of poly-3-hydroxybutyric acid (PHB), have been analysed. These transgenic flaxes, enhanced with different amount of the PHB, have been studied by FT-IR spectroscopy. The integral intensities of the IR bands have been used for estimation of the chemical content of the normal and transgenic flaxes as well as the differences between the natural and genetically modified flax fibres. The spectroscopic data were compared to those obtained from chemical analysis of flax fibres.

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1. Introduction

The flax fibre properties depend strongly on the growing conditions [1] and the applied fibre processing technique [2]. Among the methods used in the studies of these materials, FT-Raman and FT-IR spectroscopies have been found to be very suitable to detect the major chemical components of the flax stems *in vivo* [3]. These techniques provide information on the molecular changes of the flax fibres caused by ageing [4], mechanical processing [5] and chemical treatment [6].

Many papers have been published on the vibrational spectra of the flax fibres and the components isolated from these materials [6–16]. The application of these methods allowed to recognise several important problems of the flax chemistry. For instance, they gave the information on polarisation behaviour of the IR bands for oriented cellulose fibres [8,9], structural changes of flax fibres in chemical treatment [6,14], the fibre content of flax stems [17], strain induced shifts of the Raman bands of natural cellulose fibres [18,19], effects of the enzymatic retting of flax stems [16] and the role of the hydrogen bonds in the different packing of the celluloses I and II [1].

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These investigations resulted also in the finding of the diagnostic way to differentiate two categories of the native celluloses, i.e. algal and bacterial celluloses I_A, and higher plant celluloses I_B [20].

The biochemical, mechanical and structural properties of flax stems and fibres, derived from field grown genetically modified (GM) flax, were studied in our previous papers [21,22]. An expression of three bacterial genes responsible for synthesis of PHB has been used. This expression led to the accumulation of polymer in the flax stems and fibres. These studies revealed that the content of cellulose, lignin and pectin does not differ substantially for the natural and GM flax. However, the dependence between the PHB content, strength, Young's modulus and energy per unit volume was revealed [21]. The comparison of the IR and Raman spectra of 3-hydroxybutyric acid, bacterial PHB, commercial PHB and PHB extracted from the GM flax allowed to propose the structure of the PHB-flax fibre composite. The nature of the interactions between the fibre and polymer has been analysed [22]. In the present paper the results of FT-IR studies of native and transgenic flax fibres are reported. A number of samples have been prepared using bacterial way of growing, which yields different and controlled amount of the PHB. The chemical content and structure of the fibres are analysed on the basis of the IR spectra.

2. Experimental

About 1500 plants per transgenic line were grown in a field in the vicinity of Wrocław and harvested after 4 months. Those plants

were then retted by the dew method, i.e. the plants were spread out in a field for at least 40 days with the plants being turned every 2 weeks. During this process, bacteria and fungi grew on the plants and caused degrading of the cell-wall polysaccharide and middle lamella. Due to this process fibres were released from the stems. Further details can be found in our previous paper where the cellulose, lignin and pectin contents were also determined [21].

The IR spectra at room temperature were measured in the spectral range 50–4000 cm^{-1} using a FT-IR Biorad 575 $^{\circ}\text{C}$ spectrometer. The samples were prepared in the KBr pellet and Nujol suspension, for the measurements in the mid- and far-IR regions, respectively. The spectral resolution was 2 cm^{-1} . All spectroscopic measurements were performed for the pulverized samples obtained by milling of the dried flax fibres in Retsch mill of the ZM 200 model.

The mathematical processing of the measured spectra was performed using the computer program ORIGIN 5.0. Lorentzian distribution function was used for the data fitting. The fitting parameter χ^2 was order 10^{-6} .

3. Results and discussion

3.1. Chemical content of the studied fibres

The results of the chemical analyses of the studied fibres are presented in Fig. 1. This figure shows that the content of the pectin, lignin, cellulose and PHB is comparable for all samples, i.e. wt (natural flax), M13, M42, M48 and M50 samples. It also shows that concentration of the PHB increases for the studied flax series.

3.2. Vibrational data

The IR spectra of the studied samples are presented in Fig. 2. Table 1 lists the wavenumbers of the observed bands together with the proposed assignment.

The IR spectra consist of several bands grouped in 2000–3750, 800–1800 and 100–800 cm^{-1} spectral regions. The main contours are similar to those reported in the literature for other flax stems

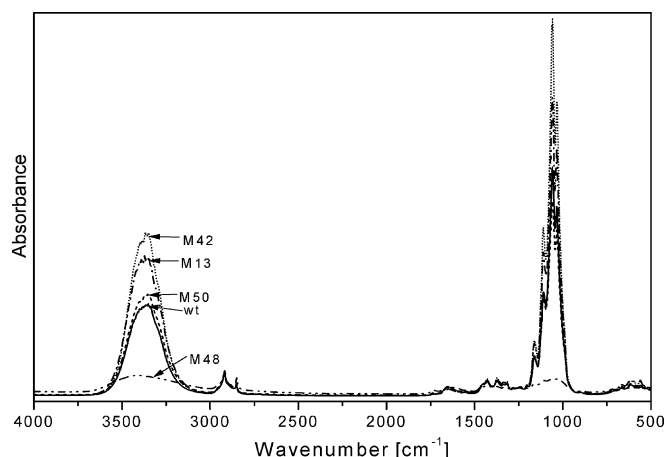


Fig. 2. IR spectra of the studied samples in the 500–4000 cm^{-1} range.

[6–16]. However, the relative intensities of several narrow lines, which appear on the slope of these broad bands, allow to obtain important conclusions on the chemical content of the transgenic flax in respect to the natural plant. The IR spectra are particularly useful for such considerations. Figs. 3–6 show the IR spectra together with the Lorentzian distributions of some contours observed in the three regions mentioned above. The integral intensities of the weak bands corresponding to lignins and pectins have been evaluated by their subtraction from the base line of the cellulose spectrum.

The IR spectra of natural and genetically modified (GM) flax fibres consist of the bands characteristic mainly for cellulose [6,8,9,14–16,19]. The weaker contour, which appears at about 2900 cm^{-1} , corresponds to the $\nu(\text{CH})$ stretching vibrations of the five CH bonds of the glucopyranose ring. ν_s and ν_{as} vibrations of the CH_2 bonds of the $-\text{CH}_2\text{OH}$ terminal group of each ring also contribute to this contour. Therefore, it can be deconvoluted into at least seven Lorentzian components labelled from 1 to 7 (see Fig. 4). Five of them are narrow, with nearly the same bandwidth,

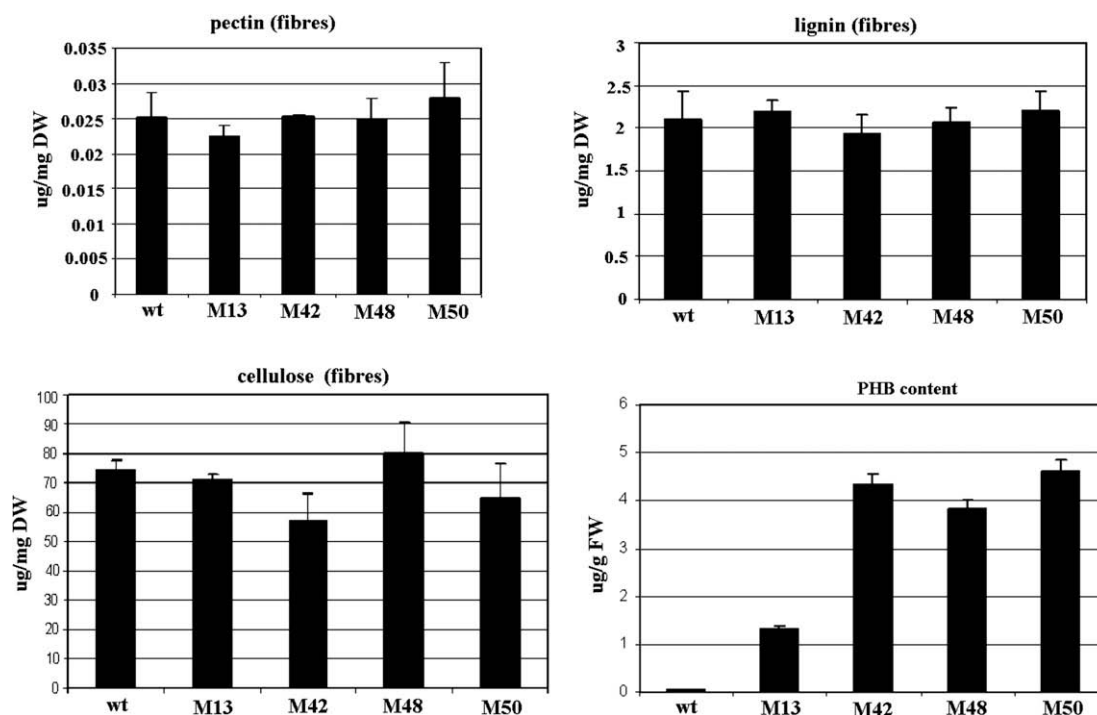


Fig. 1. Content of the pectin, lignin, cellulose and PHB in natural and GM flax fibres [21].

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