Forensic identification of bast fibres

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Abstract: By definition, bast fibres are the structural components of the stems of plants. They are of interest in the context of composite materials as they have lower density, but may possess similar specific modulus and specific strength to glass fibres. They are also generally perceived as more "sustainable" than synthetic fibres. Bast fibres are ligno-cellulosic systems which have many similarities imposed by their common role in the plant. In the context of industrial supply chain quality, forensic techniques could be employed to confirm that each batch of fibres is from the specific plant species required for the composite application before the materials/structures go into production. In forensic fibre examinations, there is a requirement for analysis to be robust and reliable, such that it can be presented in a court of law, whilst also being cost effective. This chapter reviews the technologies that have been utilised in both the textile industry and in forensic analysis, which would enable the discrimination of the respective fibres. It is assumed that reinforcement fibres are used in their native form or after treatments which change the fibre surface. The review does not consider regenerated cellulose or apparel fabrics where there is a requirement for dyes, pigments, delustrants, and other inclusions. In addition to their use for the confirmation of unadulterated materials supplied to the composites industry, the forensic techniques do find applications in archaeological or criminal investigations (especially for apparel and furniture textiles).

Keywords: A: Fibres; D: Optical microscopy; D: Physical methods of testing; X: Natural fibres.

Introduction:

The use of natural fibres as the reinforcement in composites is the subject of a number of books [1-8]. The use of bast (stem) fibres in composites is the subject of a number of reviews by the author [9-13] and others [e.g. 14-17]. Bast fibres are grown in the temperate zone (e.g. flax, hemp, white ramie/China grass) or the tropical zone (e.g. kenaf, green ramie/rhea, roselle).

There is increasing interest in the use of natural fibres as the reinforcement for polymer matrix composites. The most common synthetic reinforcement fibres can be clearly distinguished, within a manufacturing facility or the component, by colour (carbon, aramid or glass are seen as black, gold or transparent respectively) and they are normally supplied as continuous fibre tows. However, plant fibres are generally coloured from white through to brown (except where dyed for clothing/furniture uses) dependent on the species and fibre treatments and are inevitably fibres of finite length. Bast fibres generally have the best mechanical performance amongst the plant fibres, at relatively high cost, and are also inherently discontinuous (albeit often long) fibres. This opens up the opportunity for unscrupulous activity within the supply chain, especially where the high value fibre may be adulterated by partial replacement with lower value fibres. A rigorous sampling method would be required within quality assurance procedures to detect the presence of cheaper substitutes within the raw materials.

Bast fibres find, or are being proposed for, use in numerous composites applications including marine vessels (e.g. *Araldite* which has raced across the Atlantic Ocean), aircraft interiors (including luggage lockers) and automotive applications. The inadvertent use of compromised raw materials could lead to catastrophic failure of the composite below the design stresses. Any consequent litigation could require forensic investigation.

There is an extensive literature on the forensic discrimination of textile fibres, e.g. ASTM D276-12 [18] and Houck [19]. Textile fibres in forensic science are a form of trace evidence which is part of the broad category called physical evidence. Physical evidence can be any material but, in a legal context it refers to materials which may be bought into court and are formally entered as exhibits. The significance of fibres evidence, and other physical evidence, is based upon Locard's Exchange Principle. The Exchange Principle can be summarised as 'whenever two objects come into contact, a transfer of material will occur' [20]. The type of contact which occurs and the material transferred are dependent upon the circumstances of the crime. For example, fibres may be transferred from a suspect's jumper to a car seat whilst driving. Any fibres evidence found at a major crime scene is initially treated as relevant, as it may have come from the offender and therefore link that person with the scene. Further analysis and comparison of fibres evidence to suspect samples can help to provide information about events leading up to the crime, at the crime scene and events

after the crime scene. This is known as reconstruction of the crime scene. Trace evidence has also proven in the past to be very useful for two other reasons. These are their use as an investigative aid and their use as associative evidence. Fibres, among other trace evidence types, can provide very good investigative leads as their characterisation may provide information about the source of the fibre and the object that it was shed from. As associative evidence, fibres can provide links or relationships between people or between people and objects after suspects have been identified. For example, fibres from a balaclava can be found in the head hair of the wearer [21].

To provide this information in a criminal case, information about the fibre evidence is required, such as its microscopical and chemical characteristics. It is also common for forensic analysts to identify the fibre type, which helps inform the analysis methods to be used, the possible source of the fibre and its potential evidential value. Forensic determination of fibre type may also be necessary to detect counterfeiting or in policing fair trade programmes, trade embargos, protection of biodiversity by detection of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and/or for other reasons.

Although there is a large amount of literature surrounding the identification of synthetic fibre types, for example, [22-28], there is very little literature directly devoted to the ability to distinguish between the different bast fibres which may find application as reinforcement fibres within the composites sector. The principal sources include Menzi and Bigler [29], Marshall [30], Greaves and Saville [31] and Robertson and Grieve [32], all published in the previous century. The latter authors present data for typical dimensions and chemical composition of the key bast fibres (Table 1) but the data does not indicate growth stage and plant maturity.

Table 1: Characteristics of dry natural unprocessed bast fibres (after Catling and Grayson*,1982 via Robertson and Grieve$^{\Delta}$, 1999 [32] or Jonoobi et al°, 2009 [33])

Fibre	Ultimates (dia. x length)	Cellulose	Hemi-cellulose	Pectin	Lignin	Wax *etc*
Flax$^{\Delta}$	15-20 µm x 3 mm	75%	15%	2.5%	2%	1-1.5%
Flax*	length: 1.6-24 mm	~	~	~	~	~
Hemp$^{\Delta}$	15-50 µm x 0.5-5.0 mm	75%	17%	1%	3.6%	2.8%
Hemp*	length: 1.0-34 mm					
Jute$^{\Delta}$	15-25 µm x 1-6 mm	71%	13%	0.2%	13%	2.8%
Jute*	length: 0.6-5.3 mm	~	~	~	~	~
Kenaf° (raw)	~	63.5±0.5%	17.6±1.4%	~	12.7±1.5%	6.2±1.8%
Kenaf° (bleached)	~	92.0±1.4%	5.2±0.6%	~	0.5±0.4%	0.5±0.3%
Ramie$^{\Delta}$	40-75 µm x 2.5-3 mm	75%	16%	2%	1%	6%
Ramie*	13.0-82.7 mm	~	~	~	~	~

Gordon [34] states that cotton fibres, after scouring and bleaching, contain nearly 99% cellulose, whereas the bast fibres (specifically those in Table 1) are typically three-quarters cellulose, wood fibres have 40-55% cellulose and other plant species and parts have even lower cellulose contents. As the main chemical entity in bast (and other vegetable) fibres is cellulose; techniques that would be used to distinguish between synthetic fibre types are not useful for these natural types [31]. In forensic examinations of these fibres, the main forms of analysis method are microscopy based; these are discussed later in this chapter.

Natural fibres from a single plant species can be considerably more variable than synthetic fibre types, especially given the range of growth stages, processing and consequent features (*e.g.* dimensions, thermal and mechanical properties). In forensic analysis, this variation, along with limited characteristics to identify, means that natural fibres are generally less evidentially useful than synthetic fibres. This is due to the possibility of a target fibre having differing characteristics to a control fibre, even if it has come from the same source. To reach a conclusion, great knowledge of the breadth of this variation is required; something which is generally unknown in natural fibre sources and as such leads to weak evidence in court. Due to the variation in the morphological and mechanical properties of natural fibres, the focus in forensic analysis is regularly placed upon the presence and quantification of any colourants in the natural fibre [35], something which may not be a priority when testing fibres in composite manufacturing.

Analysis Methods

The Forensic Approach to Analysing Fibres

Analysis of textile fibres has been carried out by manufacturers for many years to allow the desired qualities of fibres to be identified and monitored. Characteristics such as fibre extensibility, softness, density, shrinkage and affinity for dyes are tested [36]. Though these properties are important in the textile industry, they are not often analysed when fibres are characterised for forensic purposes.

Currently, forensic examination of fibres is mainly a comparative analysis between control fibres (known fibres) and target fibres (unknown fibres, a.k.a. questioned fibres), where controls may be taken from a victim or suspect of a crime and target fibres are retrieved from a scene(s) of crime. This form of analysis requires layers of information (from simple observations to detailed composition) to be obtained about both the target and control fibres so as to make a conclusion about whether these fibres have originated from the same source. Alternatively, when "gathering intelligence information", a fibre analyst may be requested to identify the source or possible end-use of the fibre which has been collected as evidence [32]. For example, in the Atlanta child murders, it was helpful to establish which manufacturer produced a green trilobal carpet fibre found on many of the bodies [37, 38]. In fibre comparisons and in intelligence gathering, fibre *type* is commonly determined and can form the foundation upon which screening for target fibres is carried out, for example, cotton fibres are easily identifiable from their morphology and can be searched for or screened out from bulk samples quickly. In addition to fibre type, other observations are crucial for forensic comparisons, including; colour quantification (commonly using microspectrophotometry), morphological features (e.g. thickness (μm), cross-sectional shape, presence or absence of inclusions), optical properties (e.g. birefringence, sign of elongation and dichroism), any fluorescent properties and chemical composition (using Fourier Transform Infrared Spectroscopy, Raman Spectroscopy and/or Pyrolysis-Gas Chromatography). The analysis methods used in forensic casework is dependent upon the fibre type (as synthetic fibre types generally lend themselves to greater characterisation than natural fibres) and whether intelligence information is sought about the fibres, as generally greater information is required to identify the manufacturer of a sample. The gathering of intelligence information from fibres is generally employed in major crimes when there is no control sample for comparison and as much information as possible is sought about the fibres in order to try and identify a potential source and therefore suspect. Cost effectiveness of the analysis methods is a key consideration in forensic science as it is in the composite manufacturing industry. The budget to be spent on a case depends on many factors, including whether the crime is a volume crime (e.g. theft, or in composites manufacturing supply of adulterated material) or a major crime (e.g. murder). Due to the reduction in Police budgets over the years, greater focus has been placed upon creating cost effective forensic strategies and reducing the cost of existing techniques and methods. This is particularly true for fibres investigations, where the costs of analysis is high and in the perceived value low compared to other evidence types. Work is ongoing in forensic fibres work to decrease cost by speeding up sampling and screening processes [39] and to increase the evidential value of fibres evidence by creating better discriminating techniques and generating large reference data sets for sample comparisons.

Commonly, the forensic identification of natural fibres relies upon microscopy techniques and the use of reference collections which contain authentic samples which can be compared to unknowns. Preliminary observations may be conducted upon the sample under a low-powered microscope to determine the fibres colour and texture, this is often conducted on the fibre bundle (i.e. a collection of ultimates along with other cells/components that were part of the stem, sometimes called the technical fibre) [32]. A series of tests may be applied to the technical fibre such as scraping the fibre (to observe the epidermal tissue and the calcium oxalate crystals), ashing (an alternative to scraping), preparing cross-sections and testing for lignification using phloroglucinol. In addition to these tests, observations of the individual fibre ultimates under a high powered microscope may be carried out, including the average length and width of the ultimates, the presence of transverse lines (a.k.a. cross marks) and parallel striations, the presence, shape, number and average distance between any nodes/dislocations, the Herzog test and the morphology and thickness of the lumen compared to the overall thickness of the ultimate [31, 32].

The process for the analysis of natural fibres in forensic investigations currently differs to the approaches used by the composite industry in the following ways:

1. Focus is placed upon the quantification of any colourants in forensic analysis, not the fibres affinity to dyes, as this characteristic has the potential to better discriminate between natural textile fibres of the same type.

2. Characteristics that are fundamentally stable to the environment are chosen as any properties that could readily change between the time of deposition at a crime scene and the apprehension of a suspect would not enable any links to be made.
3. Tensile strength and related characteristics are not analysed in forensic science as these may not be consistent to a particular source, unless a fabric was being analysed for textile damage which may have occurred during an incident. In this latter situation, the tensile strength of the fabric may be investigated within the context of a case, e.g. the force required to tear a fabric during an assault.
4. Initial techniques in forensic analysis should be quick and effective in screening for relevant target samples so as to reduce the overall cost of analysis. Subsequent more detailed analysis techniques may be more expensive in terms of time and equipment but would be employed on fewer samples.

Sampling of Textiles

Sampling for fibres in forensic investigations includes two areas; obtaining target fibres which have been transferred to surfaces of interest, e.g. the outer surface of a victim's clothes and gathering control samples from textile items for comparison. The latter of these is the most in line with testing of samples for the composite industry with the following ideas being an important consideration in both forensic science and industrial testing:

1. Obtaining a representative sample so as to account for any variation seen in the textile. For the biocomposite industry this is to identify the uniformity of the sample in terms of its mechanical properties and surface characteristics,
2. Using a retrieval method that does not damage or contaminate the fibres,
3. Using a robust labelling system so as to prevent sample mix-up,
4. Sampling and storing samples in an appropriate environment; although fibres are relatively stable, fibrous materials can be sensitive to temperature and humidity so testing should therefore normally be undertaken under appropriate internationally-agreed standard conditions (e.g. 20±2°C and 65±2% relative humidity);
5. Preparing samples appropriate for the technique being used; as much of forensic testing of bast fibres involves microscopy techniques, this involves creating microscope slides using an appropriate mounting medium.

Booth [40] and Saville [41] present a deeper discussion of issues relating to sampling of textile materials.

Optical Techniques

Microscopy within forensic science has almost limitless applications. This is due to the microscope's ability to detect, resolve and image very small items of evidence. This ability is very important when analysing trace evidence such as textile fibres [42]. It has been noted on numerous occasions that microscopy is the essential core for fibre identification and comparisons and is normally the first choice for fibre identification [32, 42-47]. Microscopy is dominant in this field primarily because it:

1. is non-destructive,
2. allows visualisation of fibres due to fibres small size,
3. is a relatively quick means of sample analysis (important for timeliness of analysis, case throughput and if repeat measurements are needed) [45],
4. is inexpensive after the initial outlay (very few consumables),
5. has the ability to identify microscopic characteristics and polymer type (for synthetic fibres),
6. allows point-to-point, side-by-side microscopic comparison which is the most discriminating method of determining if two or more fibres are consistent with originating from the same source,
7. has the ability to distinguish between fibres of the same type but from different sources, for example, different manufacturers.

The popularity of microscopy techniques in forensic fibre analysis was highlighted in Wiggins review of fibre examination [43]. As previously mentioned, brightfield and polarized light microscopy are used by the majority of US and European laboratories but also fluorescence and comparison microscopy are routinely employed. Microscopy is the only area where ASTM D276-12 [18] offers comments specific to natural fibres: §4.3 "For plant (native) cellulose and animal hair fibers microscopical examination of longitudinal and cross-sections is used to distinguish species" and §9.1 "Examine and observe fibre characteristics as directed in the AATCC Test method 20".

There is an arsenal of microscopes available for fibres analysts. Table 2 outlines the different types available and their main uses.

Table 2: Microscope types and the uses for fibre examinations.

Microscope Type	Uses	Comments
Stereomicroscope	Primarily used to search, recover and manipulate individual fibres from tapings. Also used for the examination of textile damage and textile construction. It is not suitable for accurate identification of fibre type.	Usually low power, for example, a typical range of magnification is between x 0.5 and x 10. Stereomicroscopes should be equipped for observation with both transmitted and reflected light [46]
Polarising Light Microscope	Arguably, the most useful and versatile of all the microscope types available to fibre analysts. This microscope allows the same observations as a compound microscope but also permits observations and measurements using plane polarized light and between crossed polars (the arrangement of two polars in sequence so that the vibration directions are at 90° to each other). The polarised light microscope allows qualitative information such as sign of elongation to be gained and also quantitative information such as birefringence.	Although this information can lead to preliminary identification of the generic type of man-made fibres, it must be noted that polarising microscopy cannot be used to identify exact chemical composition.
Comparison Microscope	Provides a side-by-side microscopic comparison between multiple fibres in a single field of view.	A discriminating method for determining if two or more fibres are consistent with originating from the same source.
Fluorescence Microscope	Used to search for and observe fluorescence in fibres originating from some dyes, optical brighteners or contaminants.	The microscope is set up in incident light with a selection of filters that cover the excitation range of ultraviolet through violet, blue and green [46]
Hot-Stage Microscope	Melting point determination of fibres [46].	Technically an accessory which fits upon the stage of a light microscope. The hot stage should reach to above 300°C and should allow the user to increase the temperature by 4°C min^{-1} or less [32].
Scanning Electron Microscope (SEM)	As an imaging tool, it provides high resolution, three dimensional images at very high magnifications, particularly useful for visualising fibre surface characteristics. SEM can also yield additional physical and analytical information.	SEM utilises high-energy electrons to scan the surface of the fibre. Images of surface topography are either derived from backscattered electrons or secondary electrons.
Interference Microscopy	To determine the refractive indices of fibres and combined with a Standort diagram can determine sign of elongation.	This utilises an interferometer to split polarised light. An interferogram is produced of the recombined light beams to enable the refractive indices of the fibre to be determined. By plotting the refractive indices on a Standort diagram the sign of elongation can be determined [24].

Microscopical Observations of Bast Fibres

Natural fibres can be distinguished from each other by observing the fibres' microscopical morphological characteristics, e.g., cross-sectional shape, length of ultimates, presence of nodes and thickness of lumen [32]. In addition to these morphological features, the optical properties of natural fibres may be useful for identifying fibre type.

By definition, fibres have a high aspect ratio and it is appropriate to examine the fibre both longitudinally (side on) and transversely (cross-section). For longitudinal examination, the fibre may be mounted between a glass slide and a cover slip with a few drops of mounting liquid to enhance optical contrast. Cook and Norton [48] have evaluated a wide range of media against ideal properties but analysts still must be aware that interaction between the fibre and the mounting liquid may result in dimensional changes. Appendices 1 and 2 summarise key features which might be observed under the microscope. A comprehensive selection of bast fibre plan view and sections is presented by Kicińksa-Jakubowska et al [49], including eight different bast fibres (flax, hemp, kenak, jute, ramie, isora, nettle and Spanish broom).

Figures 1-3 show jute, flax and hemp fibres, respectively, in DPX mounting medium (RI = 1.52) under plane polarised light taken using a Nikon Optiphot2 polarizing light microscope at x 400 magnification. At this magnification it is possible to see the internal features of the fibres and key characteristics such as cross marks and striations. Length of ultimates can be particularly useful for differentiating between certain bast fibre types, for example, flax and ramie ultimates can be very long (can reach 25 mm in length) [32].

Figure 1: Jute fibre at showing cross marks and longitudinal striations (image width ~ 144 µm)

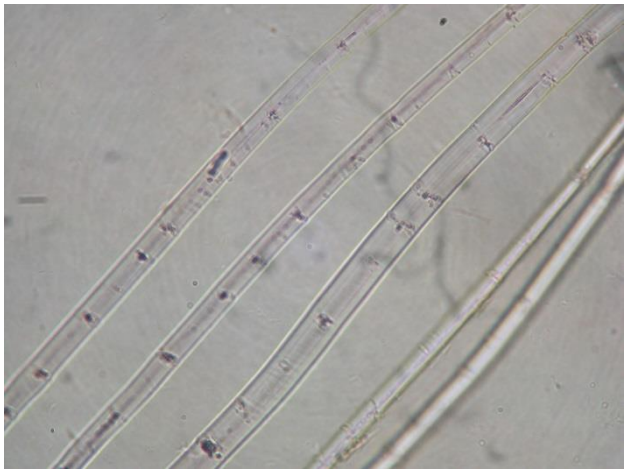

Figure 2: Flax fibre ultimates (image width ~ 144 µm)

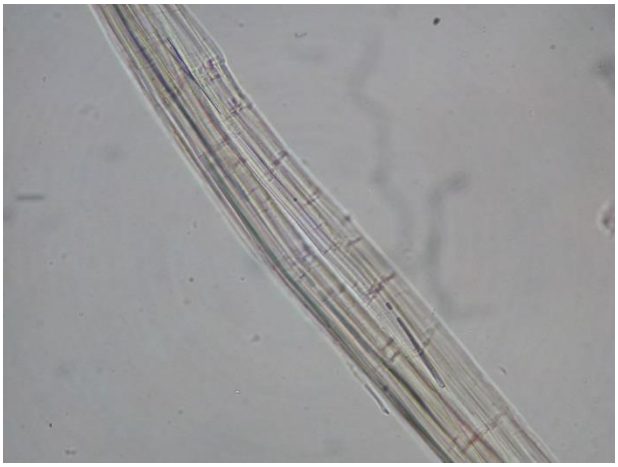

Figure 3: Hemp technical fibre bundle (image width ~ 144 µm)

The cross-sectional shape can be a simple but effective characteristic to observe to distinguish bast fibres from leaf fibre substitutes as bast fibres have a polygonal shape whilst leaf fibres are more round in appearance [32]. Goodway [50] has suggested that "optical sectioning" can be a quicker and much less tedious procedure than cutting cross-sections for *initial* screening. This is achieved by focussing on the fibre at "fairly high powers" (*i.e.* high magnification) while moving the focal plane slowly through the fibre. Although the approximate cross-section can be identified by viewing the fibre longitudinally, it is more accurate to make a transverse cross-sectional cut and view the actual cross-section mounted in a section under a microscope [32, 51-52]. There are a variety of different methods for preparing a fibre for cross-sectional determination depending upon the number of fibres and the experience of the analyst. The classic method for creating sections is utilisation of a microtome. Two other common methods utilised by fibre analysts to create fibre sections are the use of polyethylene film 'sandwich' technique developed by Palenik and Fitzsimons [52] or the Joliff Plate method developed by E.C. Joliff [52, 53].

The anisotropic nature of fibres with oriented molecules results in birefringence (the longitudinal $\eta_{||}$ and transverse $\eta_{\perp}$ refractive indices differ) which is defined as $\Delta\eta = \eta_{||} - \eta_{\perp}$. When the fibre is viewed at 45° to the cross-hairs, under a polarising light microscope, the intensity of the image will change as the analyser filter is rotated, exhibiting colours within the fibres. The specific colours (called interference colours) will differ between fibre types, and represent the retardation (also called Optical Path Difference (OPD)) of the fibre. The OPD along with the thickness of the fibres can be used to determine the birefringence. Figure 4 shows flax fibre ultimates under cross-polar conditions exhibiting interference colours.

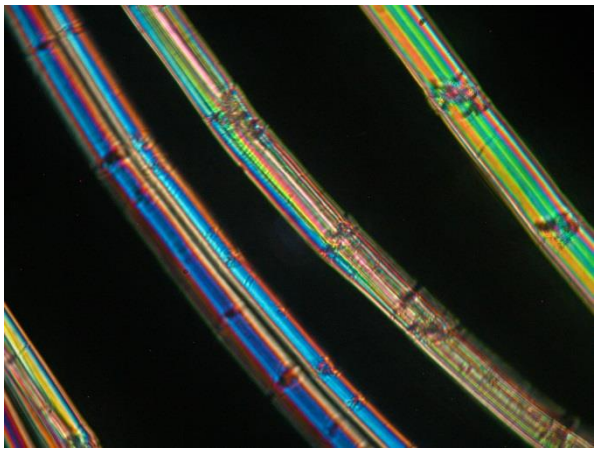

Figure 4: Flax fibre ultimates under cross-polars (image width ~ 144 µm)

A common forensic science approach for measuring the OPD (and therefore birefringence) is the use of compensators, *e.g.* quartz wedge or tilting compensator. Compensators consist of thin sections of birefringent minerals such as quartz, gypsum, mica and calcite. Compensators have controlled thicknesses and optical orientation so as to provide known values of retardation and direction of high and low refractive indices [54]. Each compensator type may be utilised for specific fibre types depending on its range of retardation. The Berek compensator, which is a tilting compensator, is utilised regularly for fibres with retardation values between 0-20λ, where λ is wavelength [54, 55].

In addition to compensators, the immersion method can be utilised to identify the longitudinal $\eta_{||}$ and transverse $\eta_{\perp}$ refractive indices separately. If there is a perfect match between the refractive indices of the fibre and the mounting liquid then the sample will become invisible, while the definition and contrast will increase with the difference in these refractive indices (Table 3). Use of a range of liquids will permit the identification of the refractive index of the fibre (the closest match may be taken as that of the fibre). For better precision, interpolation can be used or the Becke test in which a bright line appears as the objective lens is moved away from the subject causing the bright Becke line in the out-of-focus image to move towards the medium with the higher refractive index. Accuracy can be improved using monochromatic light and an interference microscope. However, immersion refractometry is rarely used in forensic science to prevent cross-contamination and evidence loss. This method is also very time-consuming which increases the cost of analysis; therefore compensators are the preferred method and could be employed within the biocomposite industry more readily.

Table 3: Refractive indices and birefringence of bast fibres

Fibre	η longitudinal	η transverse	Birefringence	Source
Flax	1.58-1.60	1.52-1.53	< 0.06	[56]
Hemp	1.58-1.59	1.53-1.54	<0.05	[57]
Ramie	1.59-1.60	1.53-1.54	<0.07	[58]

Herzog [59] and Luniak [60] noted that flax and hemp fibres could be differentiated by their opposite behaviour in polarised light under crossed polars when a selenite Red I plate was inserted in the 45° position. Flax fibres show addition colours when parallel to the plane of the polariser and subtraction colours when normal to the plane of the polariser, while the hemp fibres have the opposite response and the differences may be less pronounced. The use of known comparison samples is recommended with emphasis on dimensions, shape, colour and texture. Every fibres analyst must have access to a comprehensive reference collection in order to make more confident identifications. The use of a comparison microscope that allows side-by-side analysis of reference samples and unknowns is an effective method. A current reference collection, available to analysts from Microtrace LLC, is the Arbidar collection of natural fibres, fur and hair which contains 145 different samples.

The helical orientation of the cellulose molecules in plant cell walls is specific to the species. In flax, nettle and ramie, the fibrillar orientation corresponds to S-twist, while in hemp and jute the orientation corresponds to Z-twist as illustrated in Figure 5 [61]. Bergfjord and Holst [61] have presented a simple procedure based on measurement of the fibrillar orientation of textile bast fibres using polarised light microscopy and on detection of the presence of calcium oxalate CaC_2O_4 crystals (COX). Star-like cluster COX with many facets protruding from a small central volume (named druses by botanists) occur in nettle, ramie, hemp and jute with single COX in hemp and jute but no COX in flax. The retting process will significantly reduce the presence of oxalate crystals as they are found in the associated tissue around the fibre. Single crystals have anisotropic optical properties and can be identified under polarising light while cluster crystals will be visible independent of orientation. The crystal test is complemented by focussing the polarised light into the upper fibre surface to determine the fibrillar orientation (Table 4).

Table 4: Identifying features for bast fibres (after Bergfjord and Holst[#] [61] or Houck Ch.2[*] [62])

Fibre	Fibrillar orientation	Oxalate crystals[#]	Features[*]
Flax	S-twist[#] (clockwise[*])	absent	Polygonal ultimates, thick walls, small lumina, dark dislocations normal to fibre axis
Hemp	Z-twist[#] (counter-clockwise[*])	Cluster (and rarely single)	Wider lumen and fewer nodes than flax, cross-section rounder and more flattened than jute.
Jute	Z-twist[#] (counter-clockwise[*])	Single (and rarely cluster)	Polygonal ultimates, medium-sized lumina, angular X- or Y-shaped dislocations
Nettle	S-twist[#]	Cluster	
Ramie	S-twist[#]	Cluster	Very long and very wide ultimates, thick walls, flattened cross-section, frequent short dislocations and longer transverse striations, radial cracks in cross-section
NB: absence of crystals does not imply that the fibre is flax[#].			

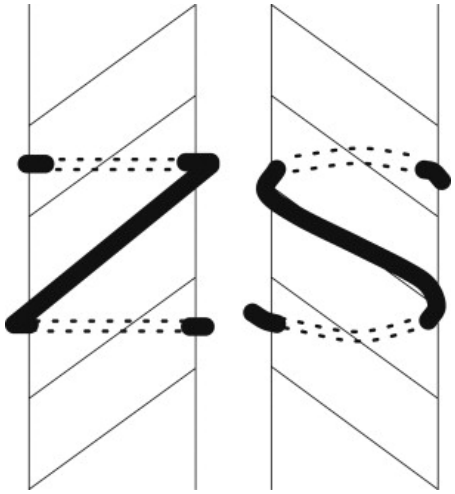

Figure 5: A diagram of Z-twist (left) and S-twist (right) (after Bergfjord and Holst, 2010 [61]).

Dai and Fan [63] treated hemp fibres with copper nitrate or cobalt chloride solutions in an ultrasonic bath at 80°C. Optical photomicrographs and image analysis were used to measure the microfibril angle (MFA). It was found that the S1 layer had an S-helical orientation with an average MFA of 80.35° and the S2 layer had a Z-helical orientation with average MFA of 23-30° (outer part) and 2.65° (inner layer).

Müller et al [64, 65] used x-ray micro-diffraction to distinguish between archaeological samples and claimed to unambiguously identify cotton, flax, ramie and wool. However, the technique uses a synchrotron and hence may not be cost-effective for numerous samples.

Scanning electron microscopy (SEM) is used in forensic fibre investigations when the surface of the fibre needs to be viewed. The biggest advantage of SEM is its ability to produce high resolution images enabling analysts to identify surface features that otherwise would not be seen under normal microscopy conditions. The excellent depth of field has allowed textile scientists to identify fibre fatigue, abrasion and deterioration caused by mechanical damage for many years; this is also particularly useful for forensic scientists examining textile damage [66]. Samples are bombarded with a fine beam of electrons and an image created from the resultant radiation; these may be secondary electron emission or backscattered electrons [31]. Fibres are generally non-conducting which can cause a build-up of charge and create a distorted image. Samples are often coated with a conductive coating to prevent charging but this coating is permanent thus meaning that SEM should be completed after light microscopy. Figures 6-8 show SEM micrographs of the same fibres seen in Figures 1-3, namely jute, flax and hemp at between x 500 and x 750 magnification.

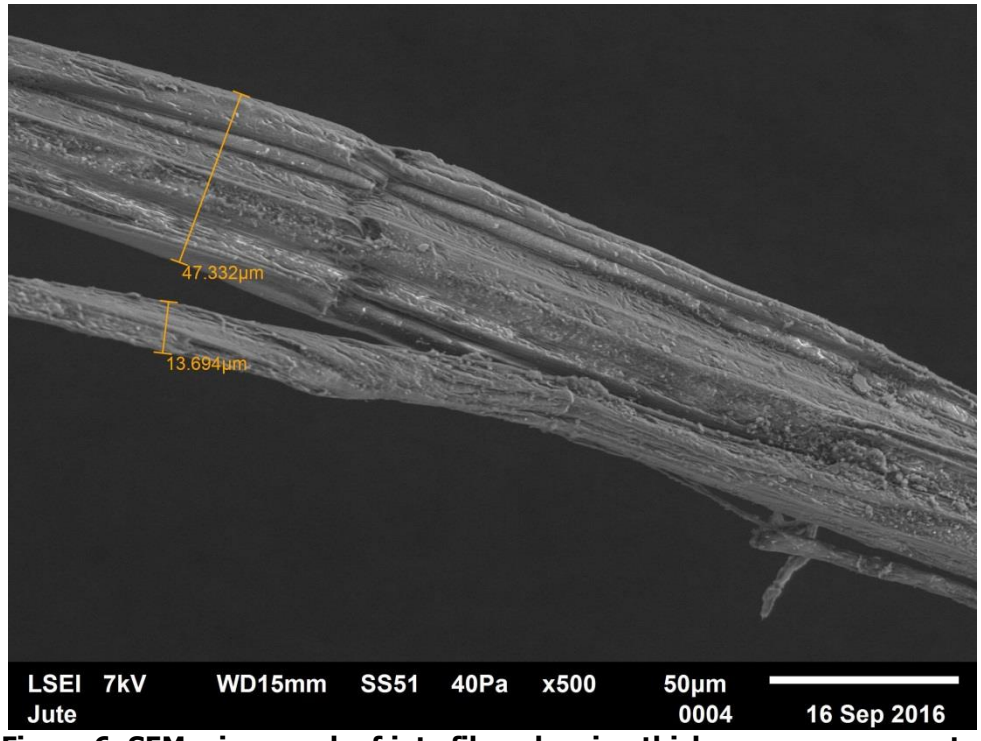

Figure 6: SEM micrograph of jute fibre showing thickness measurements.

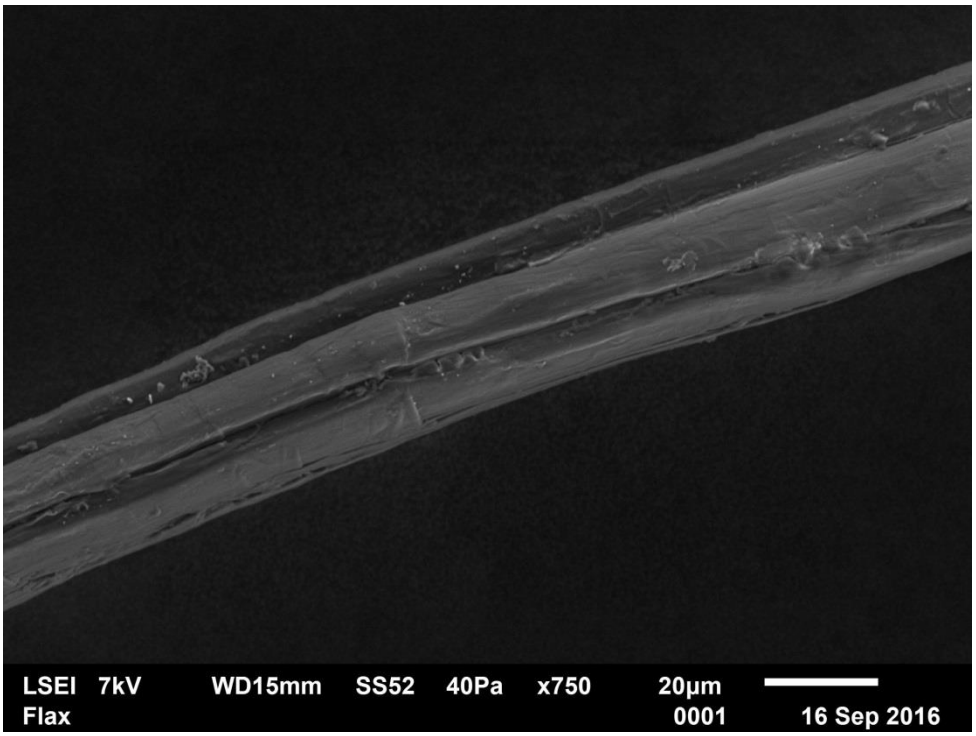

Figure 7: SEM micrograph of flax fibre

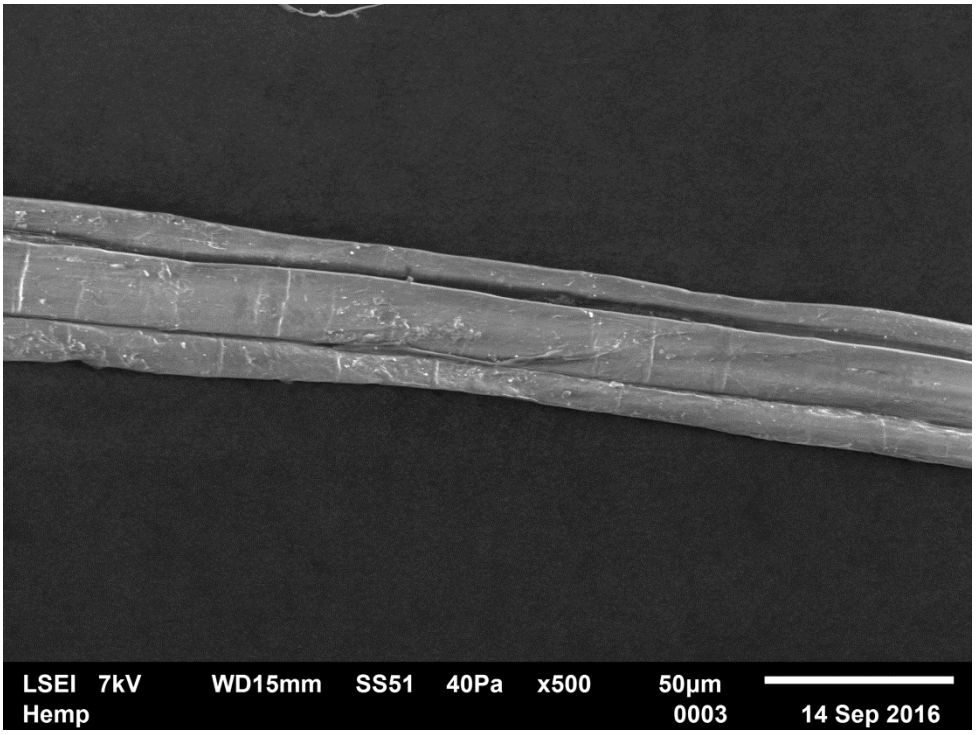

Figure 8: SEM micrograph of hemp fibre

Using microscopy techniques is a fundamental part of the forensic analysis of fibres and by utilising a range of microscopical techniques and characteristics it is possible to identify a large range of bast fibres. Practically, light microscopy is the easiest method to introduce at industrial levels due to the possibility for fast screening and relatively low cost equipment. SEM would be a very useful technique in composite manufacturing but access to, and the expense of, the equipment combined with time consuming sample preparation mean that this is likely not to be used on an industrial scale.

Chemical Tests

Solubility tests will not distinguish chemically similar materials such as cellulosic plant fibres [67], however, a wide range of chemical tests do exist for the identification of undamaged plant fibres [67]:

- cuprammonium hydroxide (Schweitzer's reagent) causes swelling and dissolution of cellulose: the pattern and rate of this process can characterise different species.
- phloroglucinol: selectively stains lignin
- zinc chloroiodide (Herzberg reagent): highlights structures within cell walls and differentially stains cellulose, lignified cellulose and lignin.

and for damaged fibres [67]:

- Congo red: dyes the inner fibre more strongly than the exterior indicating swollen or slip cell walls.
- Fehling's solution: precipitates red copper I oxide in regions of acid damage
- Turnbull blue: dark blue stains in regions of acidic or oxidative damage

The most commonly used chemical test in forensic examinations is the phloroglucinol test which can be added onto cross-section cuts and observed for a colour change which ranges from pink to deep red. No colour change indicates fibres that are practically pure cellulose, e.g. hemp and flax, a bright red colour for jute fibres and pink for semi-lignified fibres, such as manila [32]. Marshall [30] proposed a test protocol for the identification of flax, hemp, jute or ramie fibres using multiple chemical tests, which is summarised in Appendix 3.

Chemical tests are quick and easy to carry out and are therefore beneficial for screening samples on a large scale. These tests are not able to identify bast fibres solely by themselves and therefore should be used in conjunction with other tests, such as microscopy techniques. The nature of the tests subsequently changes the colour of the fibres and/or causes damage to the fibres; due to this these tests should be used after initial microscopical characterisation and thought should be given as to the effect of these tests upon other analytical techniques. In addition to this, some of the chemicals, e.g. the phloroglucinol reagent requires an equal quantity of concentrated hydrochloric acid, which needs specialist storage and handling and therefore may not be suitable for some industrial environments.

Physical/mechanical tests

If individual fibre samples are wetted with distilled water, then a clamped sample will show a specific rotational twist on drying [67-69]. The sample is normally oriented with the fibre principal axis in the horizontal plane and viewed from above. However as with viewing a transparent clockface from the front (obverse) or back (reverse), the motion may be described as clockwise or counter-clockwise respectively. There is general (but not universal) agreement in the literature that flax and ramie turn in one direction while hemp and jute have the opposite rotation. Throughout this chapter, all reported responses have been referenced to the counter-clockwise rotation reported by e.g. Hock [68] and Marshall [30] for flax and ramie, rather than the rarer clockwise response reported on occasions. Wiener et al [70] used an 80ºC hot-plate with the sample behind an air convection current screen and a specimen protrusion length of ~20 mm (10 mm minimum and 30 mm maximum).

Thermal Techniques

The interaction of heat with fibres will result in changes due to glass transition, desorption of water, crystallisation, fusion (melting), chemical reactions and irreversible decomposition. Differential Thermal Analysis (DTA) monitors changes in temperature of a sample and an inert reference material to determine the temperatures at which changes occur but can only provide limited quantitative information. Differential Scanning Calorimetry (DSC) uses a similar configuration to monitor the relative heat flows and can provide the magnitude of enthalpy and entropy changes at each transition.

Thermogravimetric analysis (TGA) monitors the sample mass on a thermal balance during a linear temperature ramp. Changes in the recorded mass may indicate drying, loss of volatiles, chemical reactions or chemical degradation. For control hemp fibres, Özmen [71] reported three regions of thermal decomposition: I (25-250°C, related to the release of moisture), II (150-420°C, related to the degradation of cellulose, hemicelluloses and lignin) and III (425-670°C, related to degradation of the char) with complete decomposition of the fibre at 670°C. There was a clear shift in the active temperature of decomposition from 376°C for control samples to 392°C after acetylation. Acetylation was also found to reduce char formation from 33% of total weight for the control fibres to 14-22% dependent on the modifying agent. Kaith et al [72] found that grafting flax fibres with MMA reduced the thermal stability of the fibre (Table 5).

Table 5: Thermal behaviour of ungrafted (UG) or methyl methacrylate grafted (MMAG) mercerised flax fibres (FF) [72]

	Thermogravimetric data					DTA peaks (°C)	
Material	**Temp (°C)**	**Weight loss (%)**	**Residue (%)**	**IDT (°C)**	**FDT (°C)**	**Exo**	**Endo**
UG-FF	307.2-345.4	31.5	29.17	307.2	559.3	309.5	360
-ditto-	345.4-559.3	39.33				465.6	
MMAG-FF	277-331.6	59.5	3.83	277	446.6	346.8	370
-ditto-	331.6-446.6	36.67				415.1	

Singha and colleagues [73-78] have reported combined TGA/differential thermal analysis (DTA) and derivative thermogravimetry (DTG) measurements on buel (Grewia optiva: GO) and roselle (Hibiscus sabdariffa: HS) fibres (Table 6). The differences between untreated GO and HS fibres are minimal, but changes between untreated and silane-treated fibres were larger.

Table 6: Combined TGA/DTA and DTG measurements on buel (GO: Grewia optiva) and roselle (HS: Hibiscus sabdariffa) fibres.

Parameter	**Technique**	**Units**	**GO [73-76] untreated**	**GO [76] silane**	**HS [77, 78] untreated**	**HS [78] silane**
Initial decomposition temperature (IDT)	TGA/DTA	ºC	200	199	199	197
… weight loss at IDT	TGA/DTA	%	7.25	9.1	7.65	8.3
Final decomposition temperature (FDT)	TGA/DTA	ºC	501	510	500	512
… weight loss at FDT	TGA/DTA	%	87.44	60.57	85.71	55.50
… residue wt. at FDT	TGA/DTA	%	12.66 (sic)	39.43	14.29	44.50
Exothermic peak 1	TGA/DTA	µV	-2.0 at 61ºC	~	-0.9 at 63ºC	-0.7 at 51 ºC
Exothermic peak 1	DTG	µg/min	98 at 61ºC	~	98 at 56ºC	62.3 at 49 ºC
Exothermic peak 2	DTG	µg/min	237 at 293ºC	~	237 at 293ºC	342.8 at 304 ºC
Exothermic peak 3	DTG	µg/min	1126 at 355ºC	~	1126 at 355ºC	
Exothermic peak 4	TGA/DTA	µV	-3.3 at 361ºC	~	-1.5 at 361ºC	

A bomb-calorimeter measures the heat created by a sample burned in an oxygen atmosphere in a closed vessel under controlled conditions. A similar technique is pyrolysis gas chromatography (Pyr-GC) where flash pyrolysis of a fibre in an inert atmosphere produces volatile components that can be analysed by gas chromatography. Smith [79] has reported preliminary calorific values for natural fibres measured in a Parr 1356 Bomb calorimeter (Table 7).

Table 7: Calorific values for various natural fibres obtained using a bomb calorimeter

	Flax (Top-F)	**Hemp (Strick H)**	**Jute (Wingham)**	**Jute (Virk)**
Calorific value (GJ/tonne)	16.36±0.05	17.20±0.24	17.46±0.14	17.75±0.17

Thermal analysis of fibres is not a common approach used in forensic investigations due to the destructive nature of the test and the overlap in thermal characteristics, e.g. melting points of fibres. Pyrolysis Gas Chromatography holds additional benefits for synthetic fibres as it has the ability to effectively classify fibres to sub-types, but this is not true for bast fibres. In order for thermal analysis to be more reliably used in bast fibre identification, a comprehensive collection of thermal data of true bast fibres and other fibres, commonly used as inexpensive alternatives, is required. This would allow the full extent of variation in thermal characteristics between bast fibres to be known.

Chemical Spectroscopy

Infrared (IR) spectroscopy interrogates the stretching and bending vibrations in adjacent atoms of a polar molecule. The transition from near- (NIR) to mid- (MIR) IR occurs at 2000 nm (4000 cm^{-1} wave number: wavelength (nm) = 10^7/wavenumber (cm^{-1})). Infrared microscopes may operate in either transmission or reflectance modes: IR normally penetrates between 6-15 µm dependent upon the refractive index of the material [80]. Fourier Transform IR (FTIR) spectroscopy simultaneously collects a wide spectral range of data with high resolution, whereas the older dispersive IR instruments scan through a range of wavelengths over a longer time period.

Special techniques in IR spectroscopy include Attenuated Total Reflection (ATR), Diffuse Reflectance IR FT-Spectroscopy (DRIFT) and Photo-Acoustic Spectroscopy (PAS) [80]. In ATR, the infrared beam enters an

optically dense crystal with a high refractive index. At a certain angle, the internal reflectance creates an evanescent wave that extends into the sample close to the surface of the crystal. ATR requires pressure to be applied to achieve close contact between the crystal and the sample. The evanescent wave interrogates only a few micrometres (0.5-5.0 μm) into the sample. ATR can be an excellent identification technique for many fibres revealing useful differences between the bulk and the surface material.

In DRIFT, the infrared radiation reflected from roughened surfaces is collected and analysed. The incident radiation is divided between regular reflection (specular reflection), diffuse scattering and penetration into the sample. The Kubelka–Munk function, developed for radiation transport in scattering media, is used to correct for peak shifts so that absorbances remain true.

The photoacoustic technique (PAS) can yield excellent quality FTIR spectra from highly-absorbent samples, *e.g.* dark coloured fibres [80]. The PAS spectrum has different physical characteristics to an optical technique with a tendency to give higher intensity at the lower frequencies (1600-400 cm^{-1}). PAS does require an in-house library of authenticated samples for on-line spectral searching and comparison.

Raman spectroscopy is a complementary technique that considers the change in bond polarisability during bond vibration and the effect on inelastic scattering of visible light. Fan et al [81] and Kavkler and Demšar [82] have reviewed the use of spectroscopy for the determination of the structure, morphology and chemical composition of natural fibres.

Appendix 4 present pairs (different positions from the same batch of fibres) of typical IR spectra for hemp, ramie and two separate batches of jute. Differences between these samples include:

- split peaks at 2917/2849 (hemp) which are less pronounced in the other fibres,
- no peak at 1731 in ramie (present in the other three fibres),
- a single peak at 1636/1638 in ramie/hemp but a double peak at 1638/1594 in both jute fibres,
- four clear peaks at 1423/1364/1316/1237 in jute but not in hemp or ramie.

The fibres had minimal information on pre-delivery processing (otherwise considered as raw fibres), but were conditioned together for an extended period. A compilation of the assignments of FTIR spectral bands for natural fibres can be found in Appendix 5. More comprehensive sampling than this limited set of eight indicative tests would be necessary to confirm these features as appropriate for the confirmation of fibre identity or stage of processing. The compilation of an Atlas of Spectra mapping changes according to the stage of processing and surface treatment of the fibre would be a useful addition to resources.

Garside and Wyeth [83] used ATR-FTIR to distinguish between flax, hemp, jute, ramie (bast), sisal (leaf) and cotton (seed) fibres. Two ratios were calculated from the respective spectral intensities: $R_1 = I_{1595}/I_{1105}$ and $R_2 = I_{1595}/I_{2900}$, where the numerical subscript corresponds to the specific absorbance wave number (band). Each fibre occupied a unique region in a plot of R_1 against R_2.

Garside and Wyeth [83] defined a crystallinity index, X, as the ratio of the two band intensities, I_{1160}/I_{1060} for ATR FTIR spectra of hemp and flax fibres progressively rotated from 0º to 180º in 7.5º steps. The minimum X-value for flax occurred at a negative angle (roughly corresponding to the 6.5º S-twist) while the X-value for hemp occurred at positive angle (corresponding to the 7.5º Z-twist).

Garside and Wyeth [84] have used surface-sampling polarised ATR-IR spectroscopy (Pol-ATR) and transmission polarised infrared microspectroscopy (Pol-μIR) to study ramie (bast), sisal (leaf) and two viscose (regenerated) fibres. Pol-ATR was able to derive the principal alignment of the cellulose molecule with respect to the fibre axis and an indication of the degree of crystallinity. The S-direction twist ramie fibres were identified as having a 7.5° winding angle.

Sohn et al [85] used near infrared (NIR) spectra to distinguish between flax fibres subjected to successive cycles of cleaning in a Shirley Analyser. Systematic changes were observed in the absorbance bands at 1730, 1766, 2312 and 2350 nm. An index was calculated from the second derivative of the absorption at the four wavelengths and was found to decrease with increasingly pure fibre. The absorbances were ascribed to the epidermal layer which is progressively removed with each cleaning cycle. Further to this, the band absorption around 1724 nm was present in the spectra for dew-retted Natashja flax, but absent from the spectra of enzyme-retted Jordan flax.

Edwards et al [86] used FT-Raman spectroscopy with laser excitation at 1064 nm. They provided tabulated wave numbers and vibrational assignments for seven natural plant fibres: flax, jute and ramie (bast), sisal (leaf) and coir, cotton and kapok (seed). To discriminate between the fibres they define two ratios of band intensities: $R = I_{1096}/I_{2900}$ (i.e. glycosidic C-O-C link ring stretch/unresolved C-H band) and $R' = I_{1121}/I_{1096}$ (i.e. doublet of glycosodic C-O-C link stretching modes). R' shows remarkable consistency (except for coir and kapok fibres) while R may provide a suitable method for non-destructive characterisation of the fibres (Table 8).

Table 8: Relative FT-Raman band intensities for natural fibres (after Edwards et al, 1997: typical deviation is ±0.03) [86]

Sample	R' (I_{1121}/I_{1096})	R (I_{1096}/I_{2900})
Bast: flax	0.72	0.85
Bast: ancient linen	0.75	1.08
Bast: modern linen	0.76	1.12
Bast: jute	0.77	0.92
Bast: ramie	0.70	1.63
Leaf: sisal	0.73	0.91
Seed: coir	0.96	0.32
Seed: cotton	0.79	0.75
Seed: kapok	0.89	0.35

Ho and Bismarck [87] used electro kinetic analysis (EKA) to determine the zeta (ζ) potential at surfaces in natural fibres. The ζ-potential correlates well with maximum moisture uptake of natural fibres with typical values for 100% RH given in Table 9. Adhesion in fibre reinforced polymers can be correlated to ζ-potential measurements.

Table 9: Zeta (ζ) potential measurements determined at 100% RH $(\zeta_0-\zeta_\infty)/\zeta_0$ and dissociations of functional groups ($\zeta_{plateau}$) properties for some common natural fibres (after Ho and Bismarck, 2011* [87] and Stamboulis et al, 2001$ [88])

Fibre	$(\zeta_0-\zeta_\infty)/\zeta_0$	$\zeta_{plateau}$ (mV)
Abaca bold* (Philippines/leaf fibre)	0.82	0
Abaca fine* (Philippines/leaf fibre)	0.76	-0.6
Coir* (India/fruit fibre)	0.22	-3.8
Flax* (Belgium)	0.95	-1.1
Green flax fibre$	0.88	ζ_∞ = -1.7
Dew-retted flax fibre$	0.85	ζ_∞ = -2.7
Duralin flax fibre$	0.55	ζ_∞ = -5.3
Hemp* (Central Asia)	0.91	-0.1
Henequen* (Mexico/leaf fibre)	0.76	-0.6
Lechuguilla* (Mexico/leaf fibres)	0.62	-2.5
Lyocell* (regenerated wood cellulose)	0.55	-3.2
Jute* (India)	0.18	-2.6
Sisal* (India/leaf fibre)	0.76	-1.7
Sisal* (Mexico/leaf fibre)	0.88	-0.4

Spectroscopic techniques are fundamental techniques in forensic fibre analysis and are commonly employed after microscopy methods, particularly IR spectroscopy in the form of Fourier Transform Infrared Spectroscopy (FTIR). Like many techniques, these are used primarily for synthetic fibres, but research from the textile industry could be employed for natural fibres in forensic examinations. An important aspect of fibre identification is the generation of data collections of spectra to allow for fast and reliable interpretation.

Molecular probes

Blake *et al* [89] used a series of monoclonal antibodies (protein responses to antigens or foreign/toxic substances) and carbohydrate-binding modules (CBM) as molecular probes in conjunction with optical microscopy to assess the occurrence of cell wall epitope (the portion of a molecule to which an antibody binds) in hemp and flax stems. The LM5 galactan epitope was found to be abundant in flax fibre secondary cell walls but absent from hemp fibre cells whereas the LM11 xylan epitope was present only at the cell periphery and not in the cell walls (as seen in hemp). The LM6 arabinan epitope was present in flax fibre secondary cell walls but only weakly present in early development fibres and at the inner region of cell walls of mature secondary fibres in hemp. The LM10 xylan epitope was not detected in flax fibre cell walls.

DNA analysis

Enzymes are proteins which catalyse specific biochemical reactions. They are named using the target species followed by –ase (*e.g.* cellulase and pectinase break down cellulose and pectin respectively).

Techniques exist to extract and purify DNA (deoxyribonucleic acid) from animal fibres in sufficient quantity for DNA fingerprinting. The quantities can then be increased by in vitro DNA amplification using the polymerase chain reaction (PCR). The extraction of identifiable DNA from plant materials is possible but relies on having undamaged associated tissue [90], *i.e.* raw (no wet processing) fibres [34]. Rogers and Bendlich [91] used Cetyl Trimethyl Ammonium Bromide (CTAB) with subsequent DNA precipitation of cotton fibres with seed material present.

In 2008 [92], Applied DNA Sciences Incorporated announced fiberTyping™ textile genotyping using DNA isolation from cotton fibre cell nuclear and chloroplast genes [92, 93]. The assay can discriminate between Pima (*G.Barbadense*) and Upland (*G.Hirsutum)* cotton. Gordon states that:

> *It remains to be seen whether the genetic variation between varieties of the same species is large enough to be a measurable point of differentiation. Determining the point of origin or place of production will remain unlikely without the application of a DNA-based, electro- or nano- based labelling procedure.*

Dunbar and Murphy [90] used DNA analysis to distinguish between different fibres used to manufacture rope. The crude procedures used to extract fibres from plant materials generally produce dead fibre cells (with no nucleus or other cytoplasmic organelles containing DNA) with associated parenchymal, collenchymal and epidermal cells (with nuclei or other cytoplasmic organelles) attached. The ribulose biphosphate carboxylase/oxygenase large protein sub-unit (rbcL) was chosen for analysis as it is present in all plant species, is plant specific (hence animal or fungal contamination will not distort results) and present in multiple copies. Different enzymes were used to cut the 771 bp amplicon (the base pair small replicating fragment of DNA synthesis using amplification techniques) into two or three fragments. For pure samples of each fibre type, clear differences were found in the results (Table 10).

Table 10: DNA fragment sizes from restriction analysis of rope fibres
NB: the sum for each cell is either zero or 771 (after Dunbar and Murphy [90])

		Bast (stem) fibres			Leaf fibres	
Enzyme	**Sequence**	**Flax**	**Hemp**	**Jute**	**Abaca**	**Sisal**
***Acc* I**	GT'(A/C)(G/T)AC	578+193	518+60+193	230+288+253	230+541	230+541
***Bam* HI**	G'GATCC	uncut	115+656	115+656	uncut	uncut
***Dra* I**	TTT'AAA	35+736	uncut	332+439	uncut	uncut
***Pst* I**	CTGCA'G	uncut	386+385	uncut	uncut	386+385
***Sac* II**	CCGC'GG	385+386	uncut	734+37	uncut	uncut

The whole genome sequencing of the chromosome sets for flax [94, 95] and hemp [96] should facilitate DNA analysis for the bast fibres. CoGePedia [95] states that flax has a small total genome size (estimated to be ~350 megabases). The current assembly v1.0 was produced entirely by Illumina sequencing and consists of a huge number of scaffolds (>88,000). However 290 megabases of the flax genome are present in only 664 scaffolds. The National Center for Biotechnology Information (NCBI) [97] provides access to a comprehensive database of biomedical and genomic information (Appendix 6).

Both molecular probes and DNA analysis are not conducted on natural fibres in forensic investigations as the cost of the analysis cannot be justified for the limited additional information gathered about the sample. DNA analysis is a powerful but expensive tool, is utilised for identification of individuals not plant materials in forensic analysis, as the additional cost of using this technique would have to allow for greater discrimination so as to increase the evidential value of the fibres. With this in mind, it is unlikely ever to be a commonly employed technique for the analysis of fibres.

Discussion

This chapter has identified a range of techniques that have potential for the forensic identification of, or discrimination between, natural fibres. Some of these techniques are already prolifically used in forensic casework and others have only been addressed within textile research. However, there is considerable scope for research to extend knowledge in the effectiveness of some of these techniques, including their ability to

differentiate between bast fibres and fibres commonly used to replace these fibre types. There is also a need for extensive databases of known natural fibres to help address these gaps in knowledge. Development, standardisation and validation of the various procedures currently not being used in a forensic context would assist in their acceptance within the legal system although cost of the techniques would ultimately be the deciding factor as to whether these were used. Many of the forensic techniques to fibres analysis may find a place within the analysis of fibres for the composite industry as both industries have similar drivers, including the need for fast, reliable and cost effective techniques. A compilation of readily accessible fibre micrographs at various magnifications, and representing each stage of fibre treatment, would be indispensable for fibres analysts, particularly those working in the composite industry.

A more problematic issue is that composite failure analysis could require the removal of fibres from the composite before examination with the chosen analytical technique. The interaction between the matrix and the fibre during manufacture and/or the extraction of fibres from the cured matrix could change the nature of the fibre and hence compromise analysis. The development of a procedure for separation of the undamaged natural fibres from the matrix would find wider application given the potential for the determination of fibre weight (and hence volume) fractions within the composite.

It is essential that any analysis undertaken is accompanied by adequate sampling of the source materials and ideally using materials where the statistical ranges of a parameter do not significantly overlap.

Conclusions

A variety of techniques exist to discriminate between the bast reinforcement fibres, including optical and electron microscopy, chemical reactions, mechanical twist, thermal properties, chemical spectroscopy and genetic probes. Some of these techniques are already well established in forensic analysis but others have limitations for forensic science. The nature of the questions asked in a forensic investigation compared to those asked in the composite industry differ to the degree that many techniques employed in textile science are not fit-for-purpose in forensic analysis. In addition to this, the generally low evidential value of natural fibres and variation means that the emphasis is placed more heavily on discrimination of natural fibres samples by colour. Having stated this, some analysis methods overlap, notably those which allow for the quick identification of fibre type. There are a number of discernible differences between species and/or fibre treatment. However, in many cases the analytical data set is limited and there is scope for the development of more comprehensive databases. No single technique in isolation can give definitive characterisation for a specific fibre but combinations of techniques can be used to identify the respective species. There is considerable scope for research to refine the above techniques leading to the development of internationally accepted standard procedures for the identification of bast fibres.

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Appendix 1: Identification of bast fibres (extracted from Robertson and Grieve [32])

Fibre	Colour	Lumen	Cross marks	Miscellaneous	Pits on maceration	Crystals on ashing
Flax	Usually white	Narrow regular	Few, Faint	-	Very fine, difficult to see	None
Hemp	Brown	Variable in width	Frequent	Hairs	Parallel to long axis (slit-like)	Clusters in short chains and singularly, Occasionally rhombic/cubic crystals
Jute	Brown	Constricted	Few, Faint	Few spirals	Bordered	Mainly rhombic and cubic in chains Single cluster crystals
Kenaf/Roselle	Brown	Constricted	Few, Faint	-	Bordered	Cluster crystals in chains and singularly Very occasionally cubic/rhombic crystals

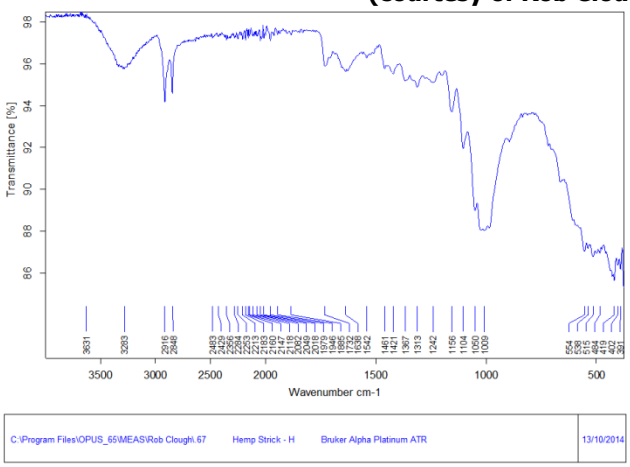
Appendix 2: Features of common bast fibres (data from Catling and Grayson via Robertson and Grieve [32])

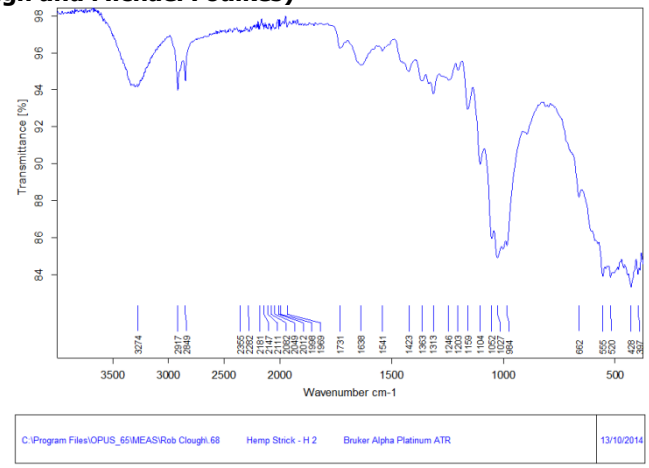
Species	Extraneous material	Cross-markings	Lumen and cell wall	Pits	Crystals / silica ash
Flax	Epidermis with paracytic stomata. Parenchyma. Xylem elements.	Rare. When seen, often very regular along whole length of fibre cell.	Cell wall occasionally striated. Cell wall thick. Lumen narrow, regular.	Very fine. Not obvious. Can be seen in polarised light.	No crystals reported or seen.
Hemp	Laticiferous elements in unmacerated fibre. Parenchyma of various types. Cluster crystals free or in cells. Hairs. Epidermis. More rarely, blocks of tissue and xylem elements.	Variable. Some cells with fine regularly spaced marks in every specimen. Marks from chambered cells. Several series on one fibre cell. More frequent than in flax. Occasional remains of cells attached to marks.	Cell wall striated. Lumen most commonly 3-5 x width of cell wall.	Slit-like, parallel to long axis of cell, sometimes coalescing.	Cluster crystal in short chains, often 3-4 together. Single cluster crystals. *Very* occasional cubic or rhombic crystal in some specimens.
Jute	Few. Mostly parenchyma, sometimes with cubic or cluster crystals. Very occasional vessels.	Few, faint. Occasional marks from chambered cells. Scalloped edges to fibre cells.	Lumen of varying width, often varying regularly along the whole length of cell.	Bordered, funnel-shaped inside view.	Cubic crystals in chains sometimes mixed with occasional cluster crystals. Single cluster crystals.
Ramie	Parenchyma. Cluster crystals free or in parenchyma. Cluster crystals in chambered cells. Few hairs and vessel elements.	Common, fine, nearly always with attached remains of parenchyma cells. Several series on one fibre cell. Occasional marks of chambered cells.	Lumen difficult to see because (a) it varies, (b) cell wall is striated, (c) fibres tangle. Lumen commonly 2-3 x width of cell wall.	Elongated, slit-like parallel to long axis of cell, sometimes coalescing.	Cluster crystals in chains. Single cluster crystal.

Appendix 3: Test protocol for the identification of flax, hemp, jute or ramie fibres (after Joan Marshall, 1992 [30]).

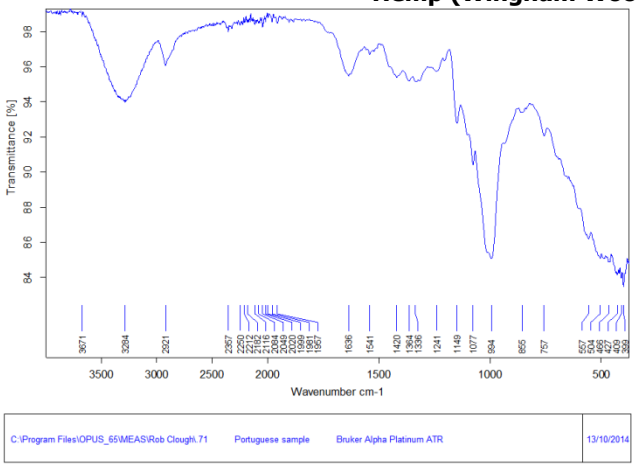
TEST	Flax	Ramie	Hemp	Jute
Optical microscopy and electron microscopy	Single fibres. Bamboo-like appearance caused by frequent swollen nodes.	Single fibres. Flat twisting ribbon-like cells.	Bundles. Fewer nodes but appearing across several fibre cells.	Bundles. Fewest and unswollen nodes.
Twist test (looking down on the fibre after Hock, 1942)	Counter-clockwise.	Counter-clockwise.	Clockwise.	Clockwise.
Phloroglucinol and HCl	Normally no magenta colour change when processed.	Normally no magenta colour change when processed.	Pink or magenta.	Deeper magenta than hemp.
Herzberg's reagent: Zinc chloroiodide	Turns violet. Highlights cellular structures. Lumina more easily visible. Yellow staining of extraneous tissue.	Turns violet. Highlights cellular structures.	Turns violet. Highlights cellular structures. Cross-markings.	Turns greenish brown. Highlights cellular structures. Dark streaked lumen, nodes and cell ends.
Schweitzer's reagent: Cuprammonium hydroxide	Swells. Dissolves quickly. Leaves threads of protoplasm.	Swells. Dissolves quicker than flax. Islands of cells, then no residue.	Swells. Dissolves slower than flax. Ruffling and pleating of middle lamella.	Swells. Dissolves very slowly. Cell ends break away from the bundle and twist. Pale blue transparent residue.
Fibre diameter and range	14.7±5.3 μm 5.1-25 μm	21.6±9.5 μm 10-40 μm	58.7±69.7 μm 1-100 μm	54.7±27.7 μm 20-100 μm

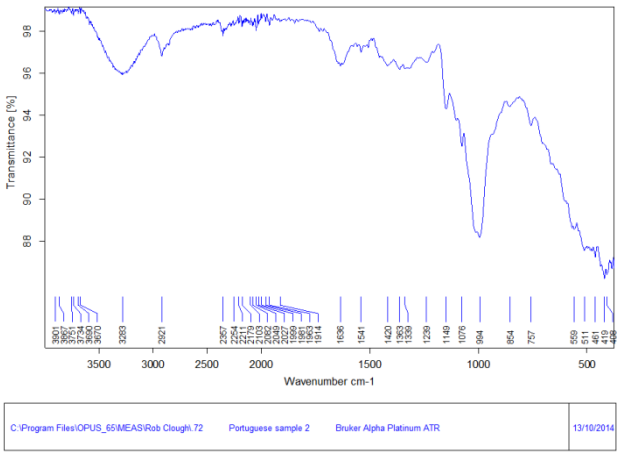
Appendix 4: FTIR spectra from the Bruker Alpha Platinum ATR FTIR (courtesy of Rob Clough and Michael Foulkes)



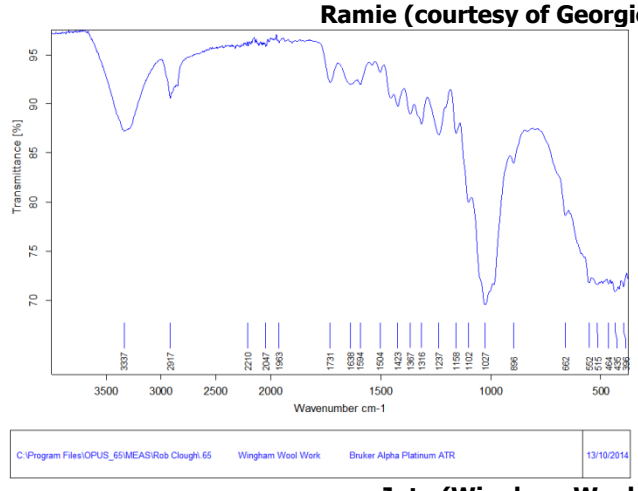


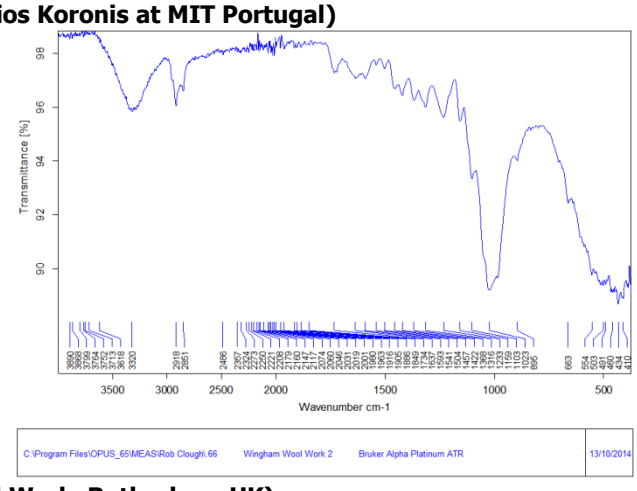
Hemp (Wingham Wool Work, Rotherham UK)



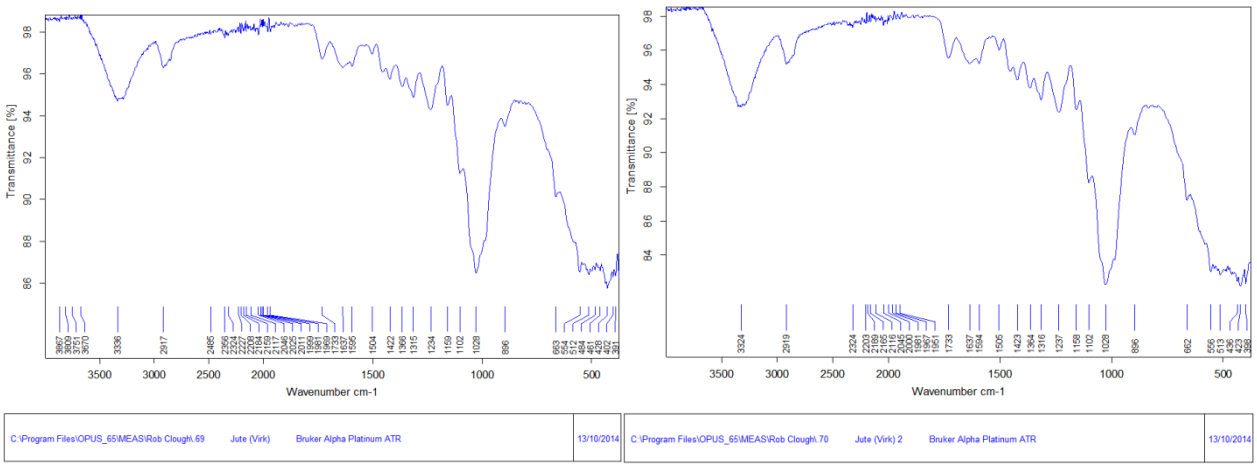


Ramie (courtesy of Georgios Koronis at MIT Portugal)





Jute (Wingham Wool Work, Rotherham UK)



Jute (IJIRA/IJSG - India via [AS Virk doctoral studies])

Appendix 5: FTIR spectral lines of natural fibres

* for dichroic bands, the notation is ° (non-dichroic), ∥ (parallel) or ⊥ (perpendicular)
* for fibres without/with dislocations, the wavenumbers are outside/within brackets respectively
* for the various motions of atoms within the normal modes, the notation is δ bending; ν stretching; ρ rocking; τ torsion; ω wagging.

Wavenumber (cm^{-1})	Vibration	Sources
3350	OH stretching (H bonded)	jute [98]
3336	OH stretching	cellulose, hemicellulose [63]
~3335 {3300}	ν (OH) free ∥	[83, 102]
3327 (3332)	OH stretching	hemp [81]
3300	OH linked shearing	hemp polysaccharides [101]
3200-3600	OH stretching	jute [99]
2922	C-H vibration (doublet with 2854) replacing single band at 2900 on depolymerisation	flax [82]
2900	C-H stretching in methyl and methylene	jute [98, 99]
~2900	ν (C-H) ⊥	[83, 102]
2887	C-H symmetrical stretching	cellulose, hemicellulose [63, 99]
2885	C-H symmetrical stretching	hemp polysaccharides [101]
2883 (2882)	C-H symmetrical stretching	hemp [81]
2854	C-H vibration (doublet with 2922) replacing single band at 2900 on depolymerisation	flax [82]
2850	CH_2 symmetrical stretching	hemp wax [101]
~2850	ν(CH_2) symmetrical stretching (non-dichroic °)	[83, 102]
1750	shifted carbonyl band	flax aged with *Penicillium corylophilum* [82]
1740	C=O stretching vibration (disappears after treatment)	jute hemicelluloses [99]
1735	C–O stretching in carbonyl and unconjugated β-ketone	jute [98]
~1735	ν(C=O) ester	[102]
1732	C=O unconjugated	hemp xylan hemicellulose [101]
1730-1733	present in untreated fibre, not in NaOH treated fibres, reappears with MPP treatment	jute [100]
1729	C=O stretching vibration	pectin, waxes [63]
1724 (1724)	C=O stretching vibration	hemp [81]
~1635	adsorbed water	[102]
1630-1650	OH in water	water [101]
1623	OH bending of absorbed water	water [63]
1623 (1624)	OH bending of absorbed water	hemp [81]

~1595	v(C=C) aromatic in-plane	[102]
1506	C=C aromatic symmetrical stretching	lignin [63]
1506 (disappear)	C=C aromatic symmetrical stretching	hemp [81]
1505	C=C aromatic symetrical stretching	hemp lignin [101]
~1505	v(C=C) aromatic in-plane	[102]
~1475	δ(CH_2) scissoring	[102]
1455	C–H deformation and CH_2 bending	jute [98]
~1455	δ(C-H); δ(C-OH) primary and secondary alcohol	[102]
1432	CH_3 asymmetric deformation	jute lignin [99]
1425	CH_2 symetrical bending C=C stretching in aromatic groups	hemp (pectin. lignin, hemicelluloses, calcium pectates) [101]
1423	HCH and OCH in-plane bending vibration	cellulose [63]
1423 (1423)	HCH and OCH in-plane bending vibration	hemp [81]
~1420 {1425}	δ(C-H) ‖	[102]
1376	C-H symmetric deformation	jute lignin [99]
1370	C–H deforming (asymmetric)	jute [98]
1370	In-the-plane CH bending	hemp polysaccharides [101]
1368	In-the-plane CH bending	cellulose, hemicellulose [63]
1368 (1367)	In-the-plane CH bending	hemp [81]
1363 (1363)	In-the-plane CH bending	hemp [81]
~1365 {1370}	δ(C-H) ‖	[83, 102]
1362	In-the-plane CH bending	cellulose, hemicellulose [63]
1335	C-O aromatic ring	hemp cellulose [101]
~1335 {1355}	δ(CH_2) wagging ‖	[83, 102]
1325 (1325)	S ring stretching	hemp [81]
1320	overlapping of 1335 and 1315 bands due to hydrolysis	flax inoculated with *Fomes fomentarius* [82]
1317	CH_2 rocking vibration	cellulose [63]
~1315	δ(C-H)	[102]
1314 (1313)	CH_2 rocking vibration at C6	hemp [81]
~1280	δ(CH2) twisting ‖	[83, 102]
1259 (1261)	G ring stretching	hemp [81]
1250	C-O stretching in acetyl groups (disappears after treatment)	jute hemicelluloses [99]

1246	C=O and G ring stretching	lignin [63]
1245 (1244)	C-C plus C-O plus C=O stretch; G condensed > G etherified	hemp [81]
1240-1241	present in untreated fibre, not in NaOH treated fibres, reappears with MPP treatment	jute [100]
1240	C-O aryl group	hemp lignin [101]
~1235	δ(C-OH) out-of-plane	[102]
1232 (1231)	C-O-H bending at C6	hemp [81]
1230-1240	C–O stretching in acetyl group	jute [98]
1204 (1199)	C-O-C symmetric stretching, OH plane deformation	hemp [81]
1202	C-O-C symmetric stretching	cellulose, hemicellulose [63]
~1200	δ(C-OH); δ(C-CH)	[102]
1162	C-O-C asymmetrical stretching	hemp cellulose and hemicellulose [101]
1155	C-O-C asymmetrical stretching	cellulose, hemicellulose [63]
~1155 {1160}	v(C-C) ring breathing, asymmetric ‖	[83, 102]
1152 (1156)	C-O-C asymmetrical stretching	hemp [81]
~1105	v(C-O-C) glycosidic	[102]
~1050 {1060}	v(C-OH) secondary alcohol (non-dichroic °)	[83, 102]
1048	C-C, C-OH, C-H ring and side group vibrations	cellulose, hemicellulose [63]
1046 (1043)	C-C, C-OH, C-H ring and side group vibrations	hemp [81]
1030	Aromatic C-H in plane deformation	jute [98]
~1025	v(C-OH) primary alcohol	[102]
1020 (1018)	C-C, C-OH, C-H ring and side group vibrations	hemp [81]
1019	C-C, C-OH, C-H ring and side group vibrations	cellulose and hemicellulose [63]
~1005	ρ(-CH-)	[102]
995	C-C, C-OH, C-H ring and side group vibrations	cellulose and hemicellulose [63]
994 (996)	C-C, C-OH, C-H ring and side group vibrations	hemp [81]
~985	ρ(-CH-)	[102]
900	decreased intensity with increased crystallinity	cotton, flax, hemp [82]
896	C-O-C,C-C-O and C-C-H deformation and stretching	cellulose [63]
895 (894)	C-O-C, C-C-O, C-C-H deformation and stretching	hemp [81]
895	glycosidic bonds	hemp polysaccharides [101]
~895	v(C-O-C) in plane, symmetric	[102]

830	Aromatic C–H out-of-plane vibration	jute [98]
670	C-OH out-of-plane bending	bast [101]
662 (663)	C-OH out-of-plane bending	hemp [81]
662	C-OH out-of-plane bending	cellulose [63]

<Do not publish the following, retained simply for reference checking when reference list renumbered >
Master list at https://www.fose1.plymouth.ac.uk/sme/mats347/FTIR_of_natural_fibres.htm for reference checking!
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Appendix 6: NCBI genome nucleotide dataset samples for flax and dataset numbers for bast fibres (surveyed at 13 March 2014)

	Accession	Genome/Gene	GI					
		Flax (Linum usitatissimum)						
1	AFSQ00000000.1	Whole genome shotgun sequence	344029616	48,397 rc linear DNA	GenBank			
2	HM991839.1	SP2047 fatty acid desaturase 3C (FAD3C)	319999847	3,739 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
3	HM991837.1	M5791 fatty acid desaturase 3C (FAD3C)	319999843	3,874 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
4	HM991838.1	UGG5-5 fatty acid desaturase 3C (FAD3C)	319999845	3,737 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
5	HM991836.1	fatty acid desaturase 3C (FAD3C)	319999841	3,732 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
6	HM991835.1	SP2047 fatty acid desaturase 3B (FAD3B)	319999839	4,573 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
7	HM991834.1	UGG5-5 fatty acid desaturase 3B (FAD3B)	319999837	4,569 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
8	HM991833.1	M5791 fatty acid desaturase 3B (FAD3B)	319999835	4,626 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
9	HM991832.1	fatty acid desaturase 3B (FAD3B)	319999833	4,570 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
10	HM991831.1	SP2047 truncated fatty acid desaturase 3A (FAD3A)	319999831	5,332 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
11	HM991830.1	UGG5-5 fatty acid desaturase 3A (FAD3A)	319999829	5,385 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
12	HM991829.1	M5791 fatty acid desaturase 3A (FAD3A)	319999827	5,500 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
13	HM991828.1	fatty acid desaturase 3A (FAD3A)	319999825	5,383 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
14	L24120.1	peroxidase precursor (FLXPER2) mRNA, 3'end	1854580	1,153 bp linear mRNA	GenBank	FASTA	Graphics	Related Sequences
15	JX174449.1	clone LuBAC346C18, complete sequence	395146555	130,251 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
16	JX174448.1	clone LuBAC395P20, complete sequence	395146541	184,896 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
17	JX174447.1	clone LuBAC375N24, complete sequence	395146526	180,098 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
18	JX174446.1	clone LuBAC375M9, complete sequence	395146501	214,610 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
19	JX174445.1	clone LuBAC364K11, complete sequence	395146479	179,112 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
20	JX174444.1	clone LuBAC317I7, complete sequence	395146470	172,458 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
21	JN133301.1	cultivar CDC Bethune clone FLA-fosmid sequence	355430106	26,231 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
22	JN133300.1	cultivar CDC Bethune clone LTP-fosmid sequence	355430066	31,478 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences
23	JN133299.1	cultivar CDC Bethune clone SAH-fosmid sequence	355429955	34,640 bp linear DNA	GenBank	FASTA	Graphics	Related Sequences

Flax (Linum usitatissimum)	10890 nucleotide records	5 transcriptome or gene expressions	reference genome
Hemp (Cannabis sativa)	93653 nucleotide records	3 transcriptome or gene expressions	reference genome
White Jute (Corchorus capsularis)	83 nucleotide records	<none>	<none>
Dark Jute (Corchorus olitorius)	2036 nucleotide records	<none>	<none>
Kenaf (Hibiscus cannabinus)	194 nucleotide records	2 transcriptome or gene expressions	<none>
Nettle (Urtica dioica)	268 nucleotide records	<none>	<none>
Ramie (Boehmeria nivea)	159 nucleotide records	6 transcriptome or gene expressions	<none>