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Structure and Physical and Chemical Properties of Fibres from the Fifth Larval Instar of Caddis-Flies of the Species *Hydropsyche angustipennis*

Abstract

*Caddis-flies (Trichoptera) belong to insects closely related to butterflies and, like the latter, have a gift for spinning silky floss. It is an especially attractive ability for modern science as it is looking for new natural materials applicable in human life. The aim of our study was to compare the properties of trichopteran silky floss with those of raw silk and fibres from a cobweb. Knowledge of the chemical composition and structure of these fibres, along with their micro- and macroscopic features and morphological properties, will allow to assess the usefulness of this material. It is most likely that the product from the silk glands of caddis-flies could be applied in medicine. The material of our investigations came from one of the Trichoptera species - *Hydropsyche angustipennis*. Fibres were taken from live advanced/matured larvae (in the 5th larval instar). The larvae were raised separately in a plastic box, without food and with a systematic water exchange in order to eliminate the influence of contamination by trichopteran excrement. Fibres of the caddis-flies investigated were elements of their web and the lining of the pupal shelter, while the raw silk came from a *Bombyx mori* cocoon and the spider's silk from a *Tegenaria domestica* cobweb. The investigations included identification of the material and the density of the caddis-fly fibres. Additionally, the structure of these fibres was examined by means of IR absorption spectroscopy, and the FTIR method made it possible to confirm the presence or absence of functional groups in the silky floss of various groups of invertebrates. Observations with the use of scanning electron microscopy (SEM) revealed the spatial structure and cross-sectional shape of the fibres under investigation.*

Key words: natural fibres, silk, silky floss, caddis flies, *Hydropsyche*, density, solubility, FTIR spectroscopy, SEM.

Introduction

Caddis-flies are insects closely related to butterflies. They are merolimnetic organisms with a complete metamorphosis. Their life cycle usually consists of 5 larval instars [1, 2] (**Figure 1**, see page 8).

Larvae, completely dissimilar to imago, have a caterpillar shape and occur in all types of fresh water [4]. Especially the representatives of case-less caddis-flies, including the genus *Hydropsyche*, live in rapid streams, keeping themselves between stones thanks to the strong floss woven by means of secretion from their spinning glands. The larvae of all *Trichoptera* species are capable of making floss owing to their symmetric twin spinning glands, i.e. transformed salivary glands. Prior to discharging through a single spinning orifice near the mouthpart, the filaments from both glands join together to make a double thread [4]. The labium orifice is equipped with a muscle that controls its opening. Thus a so-called "spinning press" is formed [5, 6]. The semi-fluid liquid secreted from spinning glands is solidified in water to form a strong and elastic thread used to make a web. The extent of exploiting the secre-

tion for the formation of floss, web and case parameters are known as ontogenic features, characteristic of the given taxon.

So far the capability of invertebrates to spin fibres has been known only to researchers of these insects. The secretion of spinning glands can have a different character and functions in the world of insects [7].

Recently representatives of various scientific fields have been working together to make use of the potential provided by nature. There are numerous examples of commercial exploitation of new substances extracted from flora and fauna, especially their specific strength, and therapeutic or antiseptic qualities. Thus research forces have been united to examine chitin, which, due to its unique properties, is finding wider and wider applications in medicine [8 - 10]. It is most likely that the fibres produced by caddis-flies could be used, for example, in medicine in bio-bandages (especially the close-woven pupa cocoons of a semi-permeable membrane character) [11]. However, all efforts to use this material in practice must be preceded by studies on its properties.

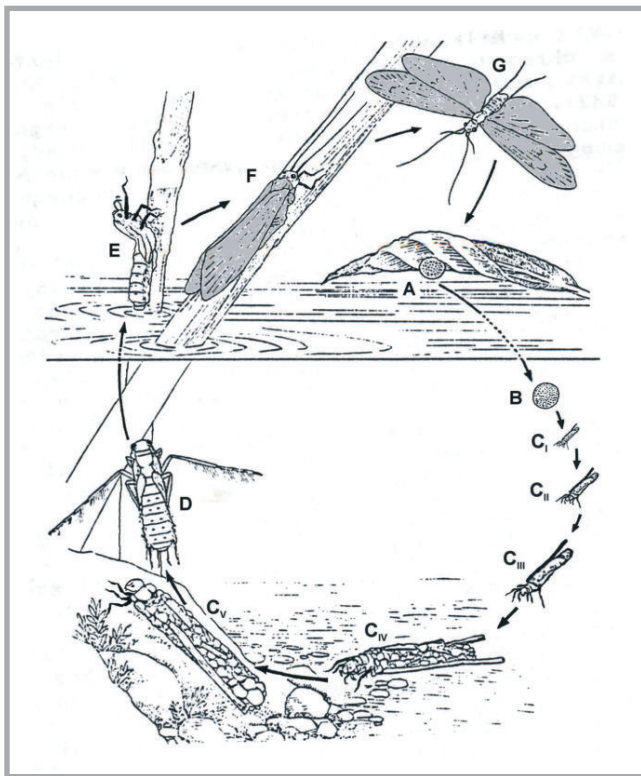


Figure 1. Life cycle of *Trichoptera* (period involving reproduction in one generation), where: A and B - egg deposit, C_{I-V} - larval instars (stages between molts), D and E - pupa, F and G - adults [3].

Table 1. Behaviour of silk and caddis-fly fibres stored in water and ethyl alcohol under the influence of the reagents used in the silk identification tests.

Reagent	T, °C	Silk	Caddis-fly fibres (H ₂ O)	Caddis-fly fibres (C ₂ H ₅ OH)
Cuoxam	20	Fibres dissolve at different rates; they crack before dissolution	Fibres do not dissolve	Fibres do not dissolve
NaOH 18%	20	Fibres neither crack nor dissolve	Fibres do not dissolve	Fibres do not dissolve
NaOH 18%	90	Fibres dissolve	Fibres do not dissolve but swell	Fibres do not dissolve but swell
Formic acid 85%	20	Fibres neither crack nor dissolve	Fibres do not dissolve	Loose fibres are dissolved.

The aim of the present study was to determine the type of material from and the polymeric protein structure of caddis-fly floss and compare them with those of classic silk and spider filament. Knowledge of the composition, chemical structure, and macroscopic and morphologic features of these fibres will allow to assess the application possibilities of the material under investigation.

Material

The material tested consisted of the floss produced by caddis-flies of the species *Hydropsyche angustipennis*. A type of raw silk derived from the pupa cocoon of a silkworm (*Bombyx mori*) and one from the cobweb of a spider (*Tegenaria domestica*) were used for comparison.

Preliminary tests [12 - 14] showed that the floss of caddis-flies is characterised by a high tenacity in some cases, reach-

ing even a value of 45 cN/tex. These values are comparable to the strength parameters of a spider's web, and they considerably exceed the strength of silk fibres.

Caddis-fly fibres were obtained from laboratory-bred larvae in the most advanced developmental stage (the 5th larval instar). The trump card in the studies on the secretion of spinning glands of *Trichoptera* is the ease of larva breeding and the intravital obtainment of floss under laboratory conditions, which is impossible in the case of spiders. In order to eliminate spectra distortion deriving from the pollution of fibres with the products of metabolic transformations, caddis-fly larvae were bred in three successive stages. In the first stage floss was derived from larvae immediately after its obtainment from the environment. In the two subsequent stages larvae were deprived of nourishment, and the water was changed

each time with fresh water and oxygenated to obtain floss without impurities.

Raw silk obtained from the unreeled cocoons of silkworms came from a commercial sericulture that supplies the textile industry with raw materials. As cocoon-formed silk fibres are very thin, they are combined into a single thread of filaments (usually from 4 to 20). During the reeling process, the fibres stick together because they are covered with a viscous polymeric substance - silk gum or sericin. For research purposes the raw silk was degummed, i.e. free from sericin. A cobweb was obtained exclusively from the natural environment.

Test methods

Identification of fibre type

The identification of caddis-fly fibres and the group to which they belong was made on the basis of Polish standard PN-72/P-04604: 'Methods for testing textile raw materials, and fibre identification'.

The standard specifies the following actions to identify the type of fibre:

- Organoleptic evaluation
- Observation of fibre behaviour in a flame
- Recognising the shape of fibres under a microscope in longitudinal and cross-sectional views
- Observation of fibre behaviour in chemical agents
- In doubtful cases, especially in testing synthetic fibres, it is necessary to determine the melting point and density of fibres.

Once it was confirmed that the floss tested was of natural origin and contained a protein substance, specific tests were carried out to identify natural silk fibres using the following chemicals: cuoxam, NaOH, and formic acid. The tests were performed under conditions stipulated for silk fibres.

Determination of the density of caddis-fly fibres

Analysis was carried out by the gradient method. The floss-containing sample tested originated from the second stage of breeding (with no excrement contamination). After deaeration the floss was placed in an immersion medium, in which a specially selected liquid penetrated the sample to fill all the empty internal spaces in it. Tests were performed in a mixture of toluene and carbon tetra-

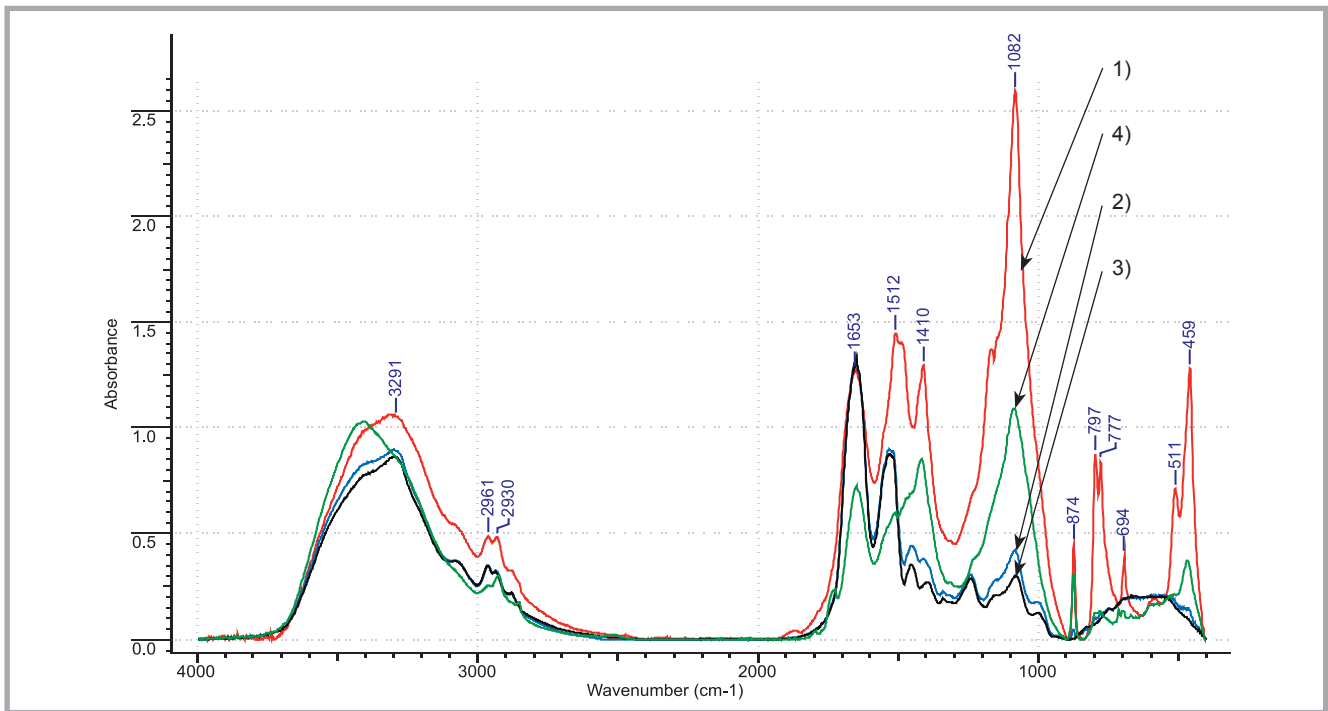


Figure 2. Comparison of FTIR spectra of fibres from the captured webs of a caddis-fly (I, II and III stages of breeding) and the products of the metabolism; 1 - Fibre from the captured web of caddis-fly larva in the 5th larval instar (Stage I), 2 - Fibre from the captured web of caddis-fly larva in the 5th instar (Stage II), 3 - Fibre from the captured web of caddis-fly larva in the 5th instar (Stage III), 4 - Products of the metabolism of larva in the 5th instar (Stage I).

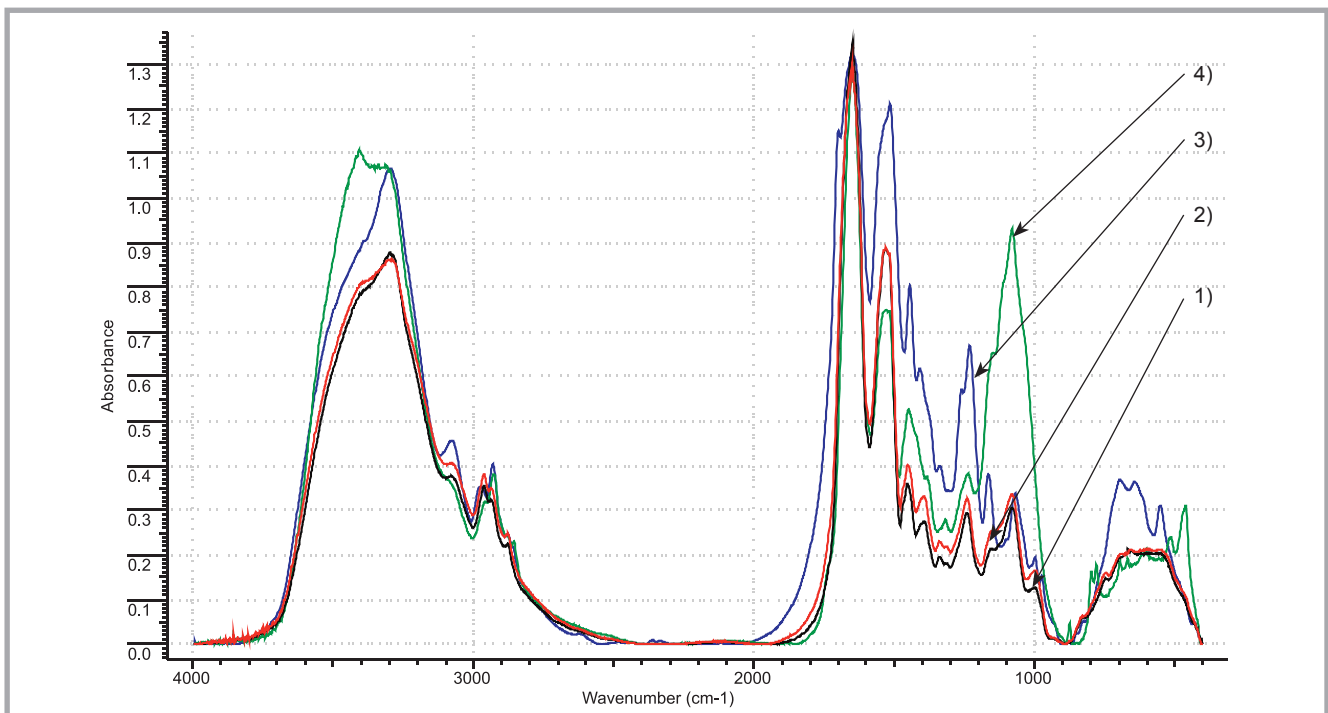


Figure 3. Comparison of the FTIR spectra of fibres from captured webs of a caddis-fly, (breeding, stage III) fibres of raw silk, and fibres from a captured spider's web; 1 - Fibre from a captured web of caddis-fly larva in the 5th instar (Stage III), 2 - Fibre of a caddis-fly from a pupa cocoon, 3 - Fibre of raw silk, 4 - Fibre of a captured spider's web.

chloride. The range of liquid density was from 0.79 kg/dm³ to 1.59 kg/dm³.

Examination of the molecular structure of the fibres by IR spectroscopy

Infrared spectroscopic measurements were performed with the use of a spectro-

photometer from Shimadzu, type FTIR 8101M. Tablet preparations containing 1% wt. of powdered and rubbed caddis-fly fibres were prepared. IR absorption spectra (100 scans) were recorded within a wavelength range of 4000 to 400 cm⁻¹;

in the systems $T = f(1/\lambda)$, and $A = f(1/\lambda)$. The spectrograms obtained were analysed by means of an ACD LABS program to assess changes in the absorption spectra band intensities of the fibres under investigation.

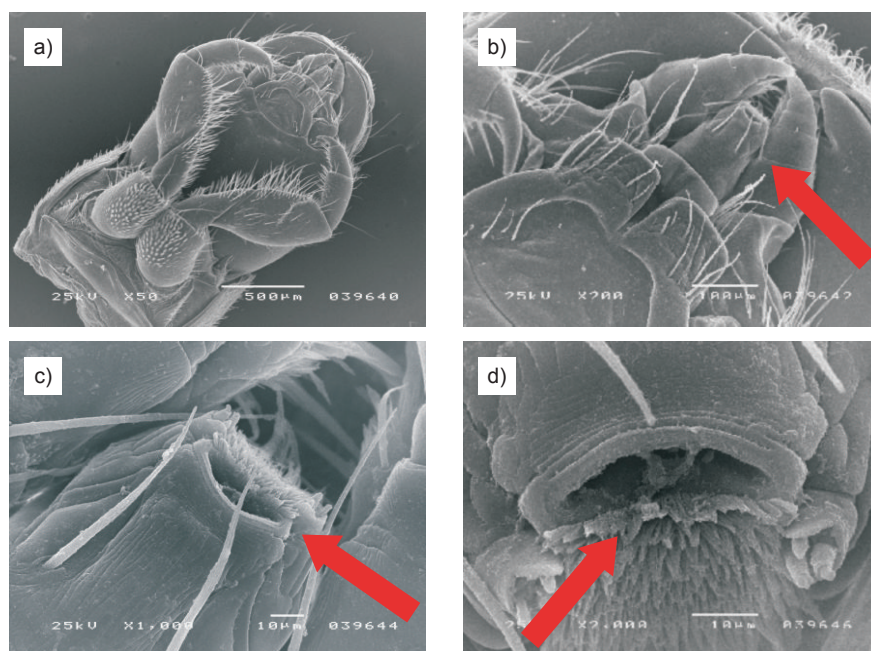


Figure 4. Outlet of silk glands; a) head capsule with one segment of the thorax and first pair of legs (ventral view), b) mouthpart, c - d) spinning organ.

Electron scanning microscopy observations

The morphology of the surface and cross-sections of the fibres produced by caddis-fly larvae was examined by means of a JSM-5200LV electron scanning microscope from JEOL (Japan) using a detector of secondary electrons. Observations were carried out with an accelerating voltage of 25 kV at magnifications of between 1000 \times and 10000 \times . Images were recorded by a digital method using the SEMAFORE system. Scanning macro-photography also served to show the structure of the mouthpart of a caddis-fly larva.

Results and discussion

Test results of fibre type

After identifying the type of fibres tested (see "Identification of fibre type"), especially the behaviour in a flame - the characteristic smell of combusted protein, it was assumed that caddis-fly fibres consist of a protein substance. To confirm their possible similarity to silk fibres, appropriate identification tests were performed. The results obtained are listed in *Table 1* (see page 8), which show the reagent type used and the reactions of silk and caddis-fly fibres stored in separate media. Additionally, the behaviour of fibres under the influence of tetrachloroethane at temperatures of 25 $^{\circ}$ C and 80 $^{\circ}$ C was as-

essed. Tests with cold and hot dimethylformamide (DMF) were also performed.

The effects of tetrachloroethane and dimethylfurane result in no visible changes in the appearance of caddis-fly fibres regardless of the medium in which they were stored. These test results lead to a conclusion that caddis-fly fibres, being protein fibres (like silk and other animal fibres), contain other reinforcing substances (non-organic, for example) that bring about the loss of their solubility in solvents, which is typical for protein fibres.

The tests showed that caddis-fly fibres are characterised by a density of 1.47 kg/dm³, which is higher than that of silk, amounting to 1.37 kg/dm³ (the density of wool fibres is similar to that of silk). Comparison of the density of caddis-fly fibres to that of silk-worm fibres leads to the next conclusion that caddis-fly fibres, besides protein material, contain some additives that increase their density. This confirms the above hypothesis concerning the solubility limitation of caddis-fly fibres compared to typical silkworm fibres.

Results of the examination of the fibre structure by IR absorption spectroscopy

Comparison of the IR spectrum of Trichoptera floss with that of a silk-worm shows a slight increase in the absorption band intensity at wavelength values

$\lambda = 2960 - 2930 \text{ cm}^{-1}$ connected with $-\text{CH}_2-$ and $-\text{CH}_3-$ groups, which indicates a difference between the structure of caddis-fly fibre and that of silk-worm fibre (*Figure 2*, see page 9). This is also confirmed by the increase in the intensity of the band connected with amide group II ($\lambda = 1512 \text{ cm}^{-1}$), with the quantities of bands correlated with amide I ($\sim \lambda = 1650 \text{ cm}^{-1}$) and amide III ($\sim \lambda = 1230 \text{ cm}^{-1}$) being unchanged, and the additional band at $\sim \lambda = 1410 \text{ cm}^{-1}$ correlated with carboxyl groups showing a more than twice higher quantity compared to a similar band of silk fibre. This indicates the increased contribution of α -amine acid radicals of an acidic nature. It can therefore be assumed that their increased content would facilitate the formation of a higher number of intermolecular bonds to increase polymer coherence [10, 12]. The absorption band ($\sim \lambda = 1165 \text{ cm}^{-1}$), which can be associated with tyrosine in the silk spectrum, has a similar intensity. The greatest change in intensity was shown by the band at $\sim \lambda = 1080 \text{ cm}^{-1}$, probably connected with the products of the protein metabolism. What is more, additional bands in the spectrum of the caddis-fly fibres were observed at $\sim \lambda = 775 - 900 \text{ cm}^{-1}$, which may be associated with the presence of low-molecular inorganic compounds, such as carbonates and silicates. Their presence on fibres or inside them is likely to be due to impurities in the form of droppings or undigested food residues, mainly diatomaceous food, which is the diet component of these insects (*Figure 2*, see page 9). These bands are absent in the spectrum of a silk-worm, while they occur to a low extent in the material of a cobweb (*Figure 3*, see page 9). In order to completely avoid interpretation mistakes caused by fibre pollution from protein metabolism products, other spectra of caddis-fly fibres derived from the third stage of breeding with the spectra of larva droppings were listed and compared (*Figure 3*, see page 9). In this spectrum, the presence of amide II groups was not observed ($\lambda = 1512 \text{ cm}^{-1}$), while the intensity of amide bands of a higher order had decreased considerably (*Figure 3*). These bands, corresponding to dropping impurities, disappeared in the IR spectra of captured webs from subsequent stages of breeding, after larva starving and water exchange (the spectrum of caddis-flies II and III); however, they were still observed in the spectrum of metabolism products of the caddis-fly (*Figure 2*, see page 9).

Comparison of the spectra of captured webs of a caddis-fly in the 5th stage (derived from the 2nd stage of breeding) and from the pupa cocoon did not show significant differences in their molecular structure.

Results of fibre examination by means of SEM

The structure of the mouthpart of caddis-fly larva, especially the area of the haustellum (the lower lip), and the lip opening with a “spinning press” are illustrated in *Figures 4.a – 4.d*.

A lengthwise projection of caddis-fly fibres has the shape of twisted ribbons (*Figures 5.a-5.d*). In the central part of the fibres, one can observe a few deep grooves along the fibre axis and local micro-creases situated cross-wise to the fibre axis.

The cross-sections of fibres are characterised by an irregular edge line, a flattened shape and relatively large non-uniformity of thickness (*Figures 6.a-6.d*). The thickness of fibres ranges from about 1 to 2 μm for the minimum size and from about 4 to 7 μm for the maximum size of the flattened shape of a fibre cross-section (*Figures 6.a-6.d*).

In the SEM images it can be noticed that in many places impurities are deposited on the fibres in the form of grains polygonal shape with rounded edges. The size of impurity grains amounts to $\sim 2 \mu\text{m}$ (*Figures 5.b-5.c*). Foreign matter visible on the fibre surface seems to comprise fine mineral systems (characteristic scooping during specimen preparation for IR examinations). However, it is their regular shape and moderately identical dimensions that attract attention. Fibre impurities are most likely to originate from the products of the protein metabolism or excrement. It seems possible that impurities on the fibre surface could slightly affect the value of density, which is higher than that of known natural protein fibres. The presence of impurities was taken into account in the spectroscopic chemical analysis of the fibre material (see “Results of the examination of the fibre structure by IR absorption spectroscopy”). The deposition of impurities on the fibre surface should not influence the behaviour of fibres in the agents used during solubility tests. One may assume that the increased chemical resistance of caddis-fly fibres in

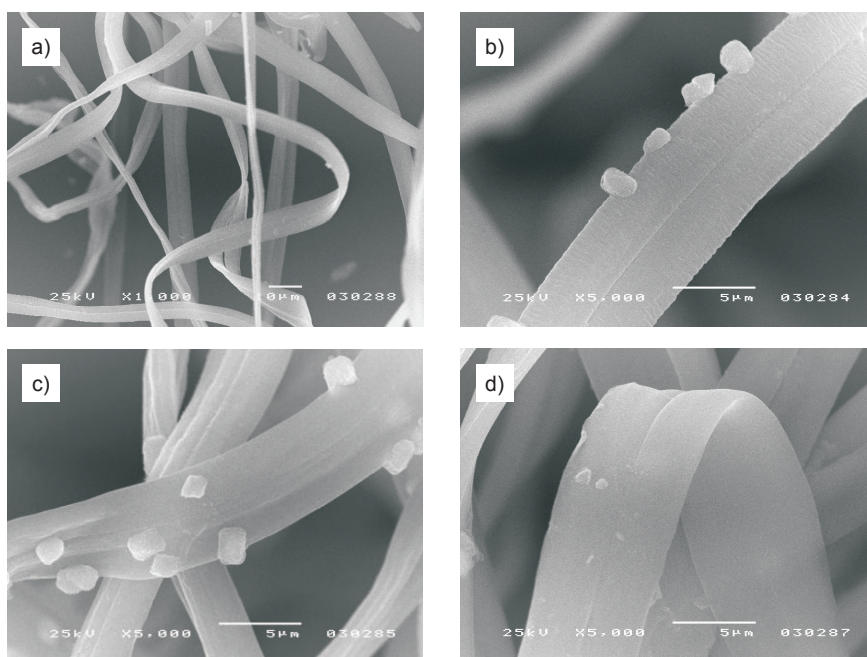


Figure 5. SEM images of lengthwise views of caddis-fly fibres.

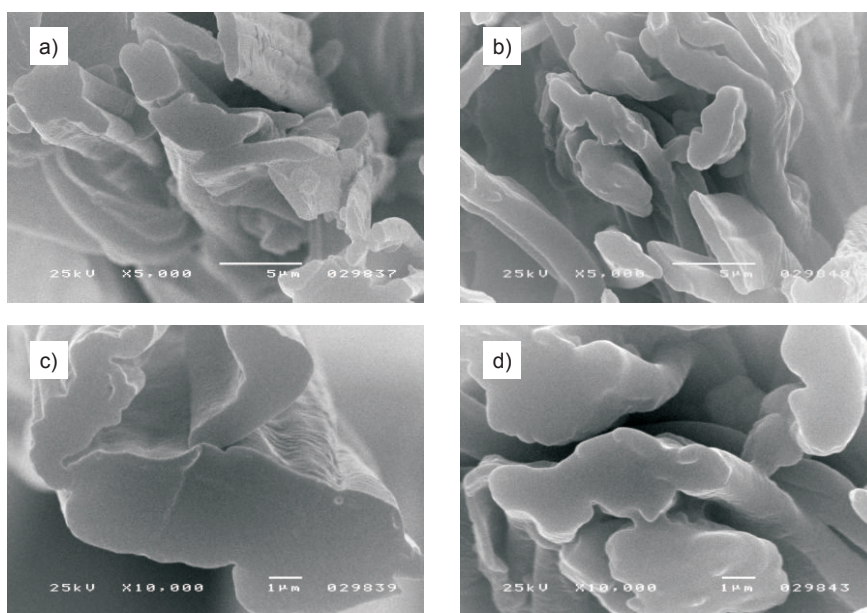


Figure 6. SEM images of caddis-fly fibre cross-sections.

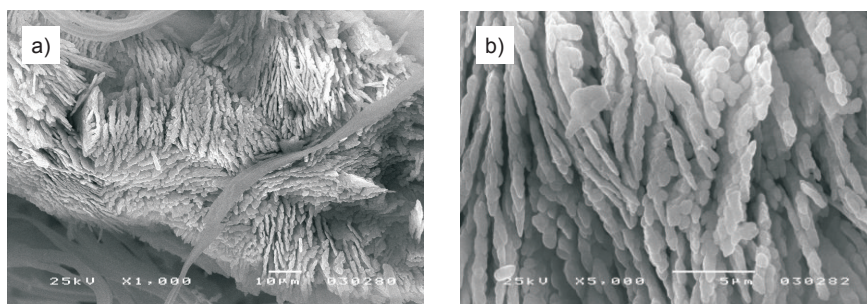


Figure 7. SEM images of impurities in the form of bigger agglomerates.

relation to other protein fibres is due to the high molecular coherence suggested by the results of IR spectroscopic meas-

urements, which would also justify the high tensile strength of fibres found previously [12].

The caddis-fly fibre samples tested also contained greater impurities of an unidentified chemical constitution in the form of agglomerates with dimensions amounting to several hundred micrometers (Figures 7.a-7.b, see page 11). This material was not tested in physical and chemical respects for fear of too large errors resulting from various accidental additions.

Conclusions

1. The fibres produced by caddis-flies have a protein molecular structure like that of silk and cobwebs, which is confirmed by the similarity of IR absorption spectrograms.
2. Slight differences in the IR absorption spectra within the range of low wavelength values indicate the presence of non-protein substances such as carbonates and silicates.
3. The presence of non-protein substances in the form of incrustations of particles on the fibre surface is confirmed by the results of SEM observations.
4. The tests performed indicated that it is possible to control the occurrence of impurities in caddis-fly fibres.
5. In comparison to silk fibres and cobweb filaments, caddis-fly fibres are characterised by a decreased solubility in typical reagents used for dissolving protein fibres and by increased density, which can be attributed to the presence of non-protein substances in the fibre material mentioned above.
6. The fibrous form of the substance produced by caddis-flies, in the form of „stratum” of disordered fibres, can be used as bio-dressings for specified chronic diseases, after confirmation of their beneficial bioactive properties.
7. Considering the fact that the fibres investigated contain carbonate and silicate derivatives, the natural substance produced by caddis-flies can be useful for the preparation of implants for regenerating bone defects.
8. The fibrous protein product obtained after processing the fibre into a continuous filament can be exploited for the preparation of threads for various medical or technical applications.

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References

1. Nielsen A., 1942. Über die Entwicklung und Biologie der Trichopteren. Arch. Hydrobiol. 17: 255-631.
2. Elliott J.M., 1969. Life history and biology of *Sericostoma personatum* SPENCE (Trichoptera). Oikos 20: 110-118.
3. Moretti G.P., 1983. Tricoteri (Trichoptera). Guide per il riconoscimento delle specie animali delle acque interne italiane, 19. CNR, Verona. 1-155.
4. Wichard W., 1978. Die Köcherfliegen (Trichoptera). Die Neue Brehm-Buchererei. Ziemsen Verlag, Wittenberg Lutherstadt, 1-63.
5. Mackay R.J., Wiggins G.B., 1979. Ecological diversity in Trichoptera. Annu. Rev. Entomol. 24: 185-208.
6. Malicky H., 1973. Trichoptera (Köcherfliegen). Handbuch der Zoologie 29: 1-114.
7. Craig L.C., 2003. Spiderwebs and Silk. Tracing Evolution from Molecules to Genes to Phenotypes. Oxford University Press, 1-230.
8. Progress on Chemistry and Application of Chitin and its Derivatives. Edited by Małgorzata M. Jaworska, Monograph, Volume XII Łódź, 2007 ISSN 1896-5644.
9. Austin P. R., Brine C. J., Castle J. E., Zikakis J. P., Chitin: New facts of research, Science 15 May 1981:Vol. 212. no. 4496, 1981.
10. Kumar M.N.V.R., A review of chitin and chitosan applications Reactive & Functional Polymers, 46 (2000).
11. Wichard W., Schmidt H. H., Wagner R., 1992. The Semi-Permeability of the Pupal Cocoon Of *Rhyacophila* (Trichoptera: Spicipalpia). In: Proc. 7th Int. Symp. on Trichoptera, ed. C. Otto, Bachhuys Pub., Umeå, 33-38.
12. Michalak M., Tszedel M., Bilaska J., Krucińska I.. „Products of Caddisfly Larvae (Trichoptera) Silk Glands as a New Natural Textile Fibre” Fibres&Textiles in Eastern Europe, Nr 6, 2005 S. 28-32.
13. Surma B., Tszedel M., Michalak M. and Student Scientific Interest Group of Commodity. Caddis larvae natural filaments, Imtex 2007, October 8-9, Łódź, Poland, pp. 138-140 and CD
14. Brown S. A., Ruxton G. D., Humphries S., 2004. Physical properties of *Hydropsyche siltalai* (Trichoptera) net silk. J. N. Am. Benthol. Soc., 23: 771-779.

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