Flax Fiber

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FLAX FIBER

1. Introduction

For centuries, natural fibers from a variety of species have been used by humans in a wide range of applications. One of these plants was flax, generally regarded as a dual-purpose plant because of its products, the fiber and seed. Woven and spun, fiber gave yarn which served as a major source to manufacture textiles for tablecloths, bed coverings and clothing, whereas seeds were pressed to extract edible oil. Marginally, shives and straw mainly from linseed flax were also used to seal and thermally insulate homes. However, hardships associated with flax cultivation and processing, as well as some weaknesses of flax fiber, such as poor elasticity, and unpredictable quality, together with the appearance of cheaper and more resilient cotton on the market, caused the devaluation of flax fiber in the world.

Nowadays, due to the great demand for natural fibers, it is a challenge for growers to overcome certain flaws in flax that undermined its commercial viability. With their help, there is a strong chance of a successful comeback of flax fibers. At the moment, many research programs have been launched, which allow a fresh perspective, from which flax will be seen as a multi-purpose plant, or even a no-waste product (1). A multitude of possible applications of flax in the textile industry, nutrition (a valuable source of unsaturated fatty acids and antioxidants), medicine (moisture-absorbing bandages rich in antioxidants, functional food as an important dietary supplement in a variety of diseases), bioremediation, and generation of biocomposites and reinforced composites (used as non-toxic implants in medicine, a biodegradable polymer in engineering) seem to be boosting the chance of renewing flax cultivation. Furthermore, shives, straw and fibers from both fibrous and linseed varieties, might also be used as biomass for special paper and biofuel production or even as a feed (1–9).

Taking into account such a promising and wide spectrum of flax applications, there is a hope that, when scientists and growers join forces, flax will again enter the global scene.

2. Flax Fiber Production in the Past and Present

The use of flax fiber and seeds, as well as flax cultivation has its roots in ancient history. Up to now, it was believed that flax was used as a source of fibers not more than some 10 000 years ago (10). Evidence for this includes fragments of straw, seeds, fibers, yarns, and various types of fabrics found in the remains of Swiss lake dwellings, which date back to about 8000 BC. Quite recently, however, dyed flax fiber samples were found at Dzudzuana cave, in Georgia (11). The discovery dates to the Upper Paleolithic and is over 30 000 years old, indicating that prehistoric hunter-gathers were using woven yarn to manufacture and/or improve everyday objects, such as baskets or tool hafts. These findings indicate that our ancestors in the Upper Paleolithic noted the usefulness of the unique properties of flax fiber, and hence, its use by humans dates back to the beginnings of agriculture and development of advanced tools.

However, the question remains unanswered, where flax species originated. There are speculations on the subject (12,13), whether it was Mesopotamia, Egypt or perhaps regions between the Baltic and Caspian Sea, but probably we will never know. Nevertheless, it is well established, that flax was cultivated for fiber in ancient Egypt (13). Linen textile was highly valued by Egyptians for its unique properties, prized especially in hot climate. Flax textile has high liquid absorptivity, up to 20% of its own weight while still remaining dry. Moreover, thanks to quick evaporation, it leaves the feeling of dryness and coolness. Given these advantages, it is not a surprise that flax was a major textile in ancient Egypt and quickly became a luxurious fabric. Priestly vestments were made of flax textile, as well as shrouds used to wrap mummies (10). The latter were discovered in a perfect state in the tombs of the pharaohs Tutankhamun, who died in 1323 BC, and Ramses II, who died in 1213 BC, which means that linen textile survived around 3 000 years with practically no decomposition.

Settling down in Egypt and surrounding countries, flax made its way from the ancient Middle East, through the Mediterranean countries, central and northern Europe, to the British Isles (10,12). It is considered that Phoenicians introduced flax growing and the making of linen into Ireland more than 2 000 years ago. Through the centuries, apart from wool, flax fiber had become one of the most important sources of textiles in Europe. Growing demand for flax crops meant expansion of the cultivation of flax all over Europe and during the 18th and 19th centuries flax became one of the most important commercial goods. In the 19^{th} century the biggest flax center in Europe was Belfast, which even gained the nickname Linneapolis. During the American Civil War there were disruptions to the supply of cotton reaching Europe, and a shortage of cotton was then replenished by Irish linen. Later in the 19^{th} and early 20^{th} century, before World War II, flax was one of the greatest export items of Russia which met 80% of global demand for flax fiber. At the same time, around the mid-1800s flax was brought to Oregon, and soon the Willamette Valley became a major producer of high quality flax fiber. It was in Oregon, where the mechanization of flax straw and fiber took place and from where it spread all over the world. Earlier, flax was introduced to North America most probably with the first European colonists as the first records about flax come from 1640 (14).

The flax heyday lasted until the middle of the last century, when flax fiber was gradually forced out of the market by synthetic fibers and cotton (10,12,14). Discussing the flax fiber market in the second half of the twentieth century up to now, the world leading producer of flax fiber in the 1970s and 80s was the USSR, up to its cease in 1991. In the 90s flax fiber cultivation decreased dramatically, from over 1,000,000 ha in 1990 to ca 455,000 ha in 2000 (Table 2). There are many reasons for that, first of all, the economic and mechanical advantages of synthetic materials, such as nylon and polyester are huge. Secondly, in the mid-20^{th} century large-scale cotton production developed thanks to the introduction of the cotton gin into industrial cotton manufacturing. Even though cotton fibers do not have as high water absorptivity as flax fibers, they are nothing, but pure cellulose which make them much more elastic and thus easier to process or blend with other fibers. On top of this, cotton cultivation and fiber possessing is much cheaper than flax. The significant cost of linen originates not only from the difficulty of working with the thread, but also because the flax plant itself requires a great deal of attention.

Table 1. **Flax Fiber Production in Last Five Decades (FAO Statistics)**

		Flax fiber Production (tonnes)			
	Country	1970	1990	2000	2011
1	France	40,343	77,000	75,000	52,400
2	Belarus	na*	na*	37,200	46,037
3	Russian Federation	na*	na*	51,000	43,450
	USSR	456,000	245,000	–	–
4	China	76,479	243,260	214,977	39,648
5	United Kingdom	na*	na*	28,000	13,776
6	Belgium	na*	na*	16,200	8,973
7	Egypt	7,280	12,025	14,000	8,300
8	Netherlands	5,287	40,000	30,860	7,954
9	Chile	1,500	2,060	2,100	2,857
10	Argentina	2,271	1,600	1,695	2,323
11	Ukraine	–	–	8,300	800
12	Italy	29	50	438	393
13	Latvia	–	–	1,100	300
14	Poland	52,100	16,268	7,900	249
15	Bulgaria	4,076	1,873	340	98
	Europe	613,517	428,603	293,585	174,603
	World	703,134	687,649	526,364	227,734

*Not Announced

In addition flax yarn is not elastic, and therefore it is difficult to weave without breaking threads.

Nowadays, according to statistics of the Food and Agriculture Organization of the United Nations (FAO) (Table 1) the major flax fiber producer is Europe, where approximately 77% of flax fiber is generated. Next in line are China (17.4%), Egypt (3.6%), Chile (1.25%) and Argentina (1%), which means that one of the biggest flax fiber producers in the past, the USA, at the moment has no shares on the flax fiber market. Eventually, loss of government subsidies for flax growing in Oregon caused the definite eclipse of flax fiber in the US. Meanwhile the European Union and later the Common Agricultural Policy (CAP) boosted flax cultivation in Europe; the CAP ensures large subsidies for agriculture, and that include flax (15,16). Worth stressing is also a dramatic fall in flax fiber production in China. The area harvested in 2011 is ten times lower than it was 10 years ago (Table 2), although the decrease in productivity is smaller, only 5.3 fold. Despite this decline in flax cultivation, China is still one of the leading producers of flax fiber, and the only one outside Europe that count.

Gradually, three leaders of the European flax fiber market emerged: France, and two former Soviet Union countries, Belarus and the Russian Federation (Table 1 and 2). It appears that these countries vary in the yield of flax fiber, because there are some disturbances between the production and area cultivated. Nevertheless in the last twenty years France with a fiber crop reaching 52,400 tonnes in 2011 is the worldwide leader in production of flax fiber. Apparently, France benefits most from CAP subsidies, which explains its position as the market leader. Similarly, Belgium and the UK positions could be explained by CAP subsidies, although it is worth stressing that both the UK and Ireland have a very long tradition in flax cultivation.

Table 2. **The Area of Flax Cultivation for Fiber**

	Area Harvested (Ha) for flax fiber and tow				
	Country	1970	1990	2000	2011
1	Belarus	–	–	82,000	61,177
2	France	39,703	60,927	62,925	60,868
3	Russian Federation	–	–	92,720	48,200
	USSR	1,284,000	772,600	–	–
4	Belgium	na	na	13,561	11,285.68
5	Egypt	8,417	12,910	15,000	10,100
6	United Kingdom	na*	na*	18,000	9,320
7	China	48,125	89,867	97,520	6,524
8	Argentina	3,650	2,500	2,672	2,929
9	Chile	1,700	2,070	2,100	2,607
10	Italy	875	530	3,000	2,399
11	Netherlands	5,188	5,535	4,400	2,156
12	Ukraine	–	–	19,800	1,300
13	Poland	98,300	29,630	4,100	848
14	Latvia	–	–	1,600	141
15	Estonia	–	–	65	78
	Europe	1,535,087	931,867	338,046	197,861
	World	1,598,391	1,039,214	455,338	220,021

*Not Announced

Apart from CAP subsidies, what helped Europe to retain its flax cultivation were promotional programs for flax cultivation and products launched by Northern Ireland and western European countries (12). The best example of the programs promoting flax fiber and other flax products is a program established in 1974 by the FAO and European research institutions within ESCORENA (European System of Cooperative Research Networks in Agriculture) coordinated by the Institute of Natural Fibers (Poznan, Poland). It is a knowledge-sharing platform for people around the world, who aim to popularize flax due to its unique properties, strong and absorptive flax fibers and seeds rich in antioxidants and unsaturated fatty acids (17). Growing demand for flax as a source of new raw material will trigger the renewal of flax cultivation world wide.

Worth mention is also the condition of the linseed flax market, as the straw and fiber derived from such cultivation might also be a source of fiber. Unfortunately, it is mainly treated as a by-product or waste, and as such, it is most commonly burnt or chopped and ploughed back into the soil. According to the FAO, the linseed flax market is also undergoing huge changes (Table 3). Apparently, for years Canada was the top linseed flax producer with a total

Table 3. **The Area Harvested for Oilseed Flax Varieties**

	Linseed Area Harvested (Ha)		
	Areas	2000	2011
1	Russian Federation	87,630	472,700
2	India	593,100	338,810
3	China	497,870	322,100
4	Kazakhstan	1,400	310,000
5	Canada	590,900	273,200

production of over 1,000,000 tonnes in the season 2005/6 (18). However, in the last ten years the area harvested in Canada has decreased heavily, to reach in 2011 only 50% of the area cultivated in 2000. Surprisingly, the Russian Federation has developed linseed flax cultivation in last decades, dedicating nearly half a million hectares to that purpose (Table 3), whereas approximately only 50,000 ha were sown for fibrous flax. Apart from countries which belonged to the former Soviet Union, China, India and Canada are top countries in linseed flax agriculture. If only Canada produces hundreds of thousands of tonnes of seeds, the amount of straw produced while seed collecting is tremendous. Thus, there is a challenge for scientist and engineers to process it and successfully apply it in industry.

3. Fibers Derived From the Plant Stem

Flax (*Linum usitatissimum* L.) is an annual herbaceous plant, widely grown in temperate climate. It is the most popular fibrous plant in our climate zone. Botanically, flax species is classified as an angiosperm dicot. Its height varies from ca. 60 cm up to 120 cm depending on the variety (usually, fibrous flax is measurably higher than linseed flax), weather and sowing density (thick sowing produces taller plants with a singular high stem, while sparse sowing results in plants with many shoots without one main stem) (19).

Focusing on fibrous flax, its silhouette is tall and slender; leaves are green with a silvery shade, lanceolate in shape and 20–40 mm in length and 3 mm in width (20). The developing stem ends with a wisp whose branches blossoms with pale blue color, sometimes with a purple shade or entirely white. The fruit is a round, dry capsule 5–9 mm diameter, containing usually 10–12 glossy seeds brown or golden yellow in color (19).

Fiber might be quite a confusing word. For dieticians and people dealing with nutrition it is a diet component of vegetable origin, consisting of cellulose, hemicelluloses, pectin and lignin. For biologists fiber is a single elongated cylindrical cell with a thick cell wall, and for the industrialists and farmers it is associated with the raw material, bundles of fiber cell filaments that resemble thread and are extracted from straw. Most commonly, the third definition is used when referring to the flax product, whereas the second one is specified as the fiber cell.

According to its botanical origin, flax fiber is a stem fiber, the same as jute, kenaf, industrial hemp, ramie and bamboo. The other kinds of fiber are: leaf fibers (sisal, banana and agave), seed (cotton) and fruit fibers (coconut).

The individual fiber cell is long, typically 27 mm but ranging from 9 to 70 mm in length and has an average diameter of 23 μm (20). Fiber cell bundles are located in the outer parts of the stem, just under the epidermis and cortical parenchyma and are derived from phloem cells. The cross section of a flax stem shows developing fiber cells and their polygonal or circular shape. Their development process can be divided into three phases: specification, elongation, and wall thickening (21). After cell division and specification fiber cells begin to elongate and differentiate from each other. The process of growing is longer than for other cell types in the stem, which allows fiber cells to reach their unusual length (22). During this step the fiber cells gradually lose their protoplast. When growth is over, the last step is intensive cell wall thickening when cells continue to lose the protoplast reaching their mature stage with a thick wall and a small lumen inside. After plant maturation and harvesting, all that remains of the elementary fiber is the cell wall and it is this structure that enters the fabrication of yarns for textiles.

4. Fiber Biochemistry

4.1. Chemical Composition. On the whole, bast flax fibers are a complex assembly of different polymers, polysaccharides such as cellulose, hemicelluloses and pectin, and the phenolic polymer of lignin (23,24). However, what distinguishes flax among other fibers, especially cotton fibers, and gives flax fiber its biological activity, is a wide group of secondary metabolites derived from the phenylpropanoid pathway. The amount of fiber polymers, proportions between them, and monomer composition depend on many factors and vary among species and even within a single cell (25). This also explains the diversity of different fibers properties.

In details, the cell wall of flax fiber is composed of the primary and secondary cell wall. The latter is divided into three layers: S1, S2 and S3. The S2 layer is the one that dominates the cross section of the fiber cell. Cellulose covers about 70% of fiber weight, whereas hemicelluloses are present in approximately 15%. The last two components are pectin and lignin present in the fiber in different amounts, approximately covering together 10% of fiber weight. The remaining 5% is represented by phenolic compounds, proteins residues and ash consisting of waxes and inorganic compounds (26,27).

Cellulose is an unbranched biopolymer of β-1,4-glucose, whose chain of monomers is called micro-fibril and its diameter varies from 0.1 μm to 0.3 μm. In the cell wall, cellulose is present in two conformations, amorphous and crystalline (28). The latter consist of highly oriented cellulose micro-fibrils whose axis is not parallel to the fiber axis but slightly angled by about 10 degrees. This angle plays a crucial role in providing high tensile strength for the fiber (29). The ratio of crystalline areas in the whole structure is called the crystallinity index and also determines the physical and mechanical properties of fiber (28,30). The less the crystalline cellulose, the better the water uptake as spaces between cellulose chains acts as a sponge, absorbing moisture (31,32). The number of free –OH groups gives cellulose a hydrophilic character, which affects fiber blending with the composite matrix (33).

Cellulose fibrils are embedded in concentric lamella composed of pectin and hemicelluloses. In contrast to cellulose, the molecular weight of hemicellulose is much lower. It is a branched, fully amorphous heteropolymer that consist mainly of xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. However, their composition and the level of branching vary between cell types. Hemicelluloses strongly bonds to cellulose *via* numbers of hydrogen bonds. Apart from –OH groups, hemicelluloses also contain a number of acetyl groups making them partially soluble in water and hygroscopic. Cellulose micro-fibrils are cross-linked together via hemicelluloses, which mean that by taking part in ensuring proper cellulose orientation, hemicelluloses contribute to fiber strength.

The cell wall structure is reinforced by pectin. This sugar polymer also has a diverse structure, whose composition depends not only on cell type, but also on cell fraction. Generally, pectin is built mainly from a galacturonic acid backbone polymer, which is branched via α-1,4-glycoside bonds with various sugars. However, in flax fiber pectin could be divided into two groups, those present in the primary cell wall and cell junctions, and those embodied into secondary layers (34). The first include large molecules that ensure cohesion between cells and consist of homogalacturonate (65%) and rhamnogalacturonate-I (25–30%) with short side chains mainly of galactose (34–36). The second kind of pectin, which is bond to cellulose, is mainly represented by rhamnogalacturonan with longer side chains of galactose (34,35).

The main function of pectin in the cell wall is binding together fiber cells to ensure bundle cohesion *via* calcium bridges and other bonds. Pectin also maintains the proper orientation of cellulose micro-fibrils and by extension takes part in ensuring mechanical properties. There are reports suggesting that removing pectin negatively affects fiber strength by reducing the interactions between amorphous and crystalline polymers (37–39). Additionally, the presence of pectin in the primary and secondary walls of fibers increased their cation exchange capacity and water sorption. Pectin is also encrusted with various phenolic acids being one of the carriers of phenolics and a kind of reservoir of them (26). It should be stressed that pectin has many other functions in plants, but they do not relate to fiber.

Owing to the fact that pectin is a linker between cells, it is the main target for microorganisms during extracting fiber from the straw. As retting and its consequences for fiber quality will be discussed in the next paragraph, now it will only be stated that the longer the retting, the worse the fiber quality. This lead to the conclusion that a lower pectin level, to some extent, might improve fiber quality.

Last but not least component of the cell wall is lignin, covalently bound to hemicelluloses (40). Lignin is a highly complex, heterogeneous biopolymer with no defined primary structure. The three main monomers from which lignin is built are: *p*-hydroxyphenyl (H), guaiacyl (G), and syringal (S), which are derived from the phenylpropanoid pathway. Monolignols are linked by various condensed bonds such as ether and carbon-carbon bonds, as well as non-condensed linkages (β-O-4 and α-O-4 bonds) (41). Although lignin structure is already well known, quite recently it was revealed that flax fiber lignin is quite unusual. Flax is an angiosperm; however, its bast lignin strongly resembles gymnosperm lignin whereas the xylem lignin is typical angiosperms. Additionally, in flax bast cells lignification is the lowest among natural fibers, and lignin has the highest ratio of condensed linkages, which practically makes them chemically inactive. In addition, flax bast fibers contain relatively high levels of non-cellulosic glucans (26,42,43).

Generally, such a diverse structure means that lignin is very poorly chemically reactive and quite resistant to undergoing enzymatic digestion; it is also hydrophobic in nature. These properties allows lignin to play the crucial role in water conduction in plants and in ensuring mechanical strength for the cell wall structure and hence, flax fiber (44). Lignin also takes part in plant defense against pathogen infection acting as a mechanical barrier. When it comes to potential application of fiber (or flax straw), lignin is a major stumbling block. In fiber, lignin is responsible for poor elasticity and impaired blending with other cellulosic fibers in the textile industry (45). Lignin also reduces the accessibility of cellulose to degradation both in biofuel synthesis and paper production.

4.2. Spatial Organization. The knowledge on fiber constituents and their quantities is mainly derived from chemical methods. The sequential release of fiber components and measurements of their levels are commonly used. These methods although satisfactory enough are destructive and do not provide any information on these constituents arrangements. To fill this gap, recently several research group have started to use infrared spectroscopy (IR) as noninvasive method. For example IR and Raman spectra were used to characterize the vibrational properties of the major components of flax fiber at the molecular level (Fig. 1).

It was found that the ν(OH) band of the flax fiber is a good diagnostic probe for the study of the hydrogen bond strength between the fibers constituents (46). The band at 3200–3400 cm^{-1} correspond to the $\nu(OH\cdots O)$ vibrations of the intra- and intermolecular hydrogen bonds (HB) of the cellulose (47). The component at 3549 cm^{-1} corresponds to the ν(OH) vibration of the free hydroxyl groups. The IR contours observed in the region 1500–1800 cm^{-1} correspond to four types of carboxyl groups present in pectin and lignin constituents: free COOH groups not bound to cellulose, asymmetric vibrations of the COO— groups, carbonyl groups involved in hydrogen bonds of the C= O···H O type and C—O—C ester bonds. The region at 1000– 1250 cm^{-1} is assigned to the vibrational stretching and bending modes. The stretching modes concern $\nu(CH_3)$, $\nu(CH_2)$, ν(CH), ν(OH) of the hydroxyl group, ν(OH) of the carboxylic group, ν(COO—), ν(C=O) of the hydroxyl group and ν(C—C). The most characteristic bending modes correspond to the vibrations of the CH_3, CH_2, CH, COO— and C—OH groups. Although the wavenumbers of all these vibrations are well defined this region of IR spectra is useless. The reason is that the region is assigned to vibrational spectra of groups common to each constituent of fiber (47).

Flax fibers, beside their strong mechanical properties, have important biological functions. For example, they possess antioxidant activity beneficial for their application as wound dressings for chronic wound healing (4). This is mainly due to phenolic compounds from the phenylpropanoid pathway that

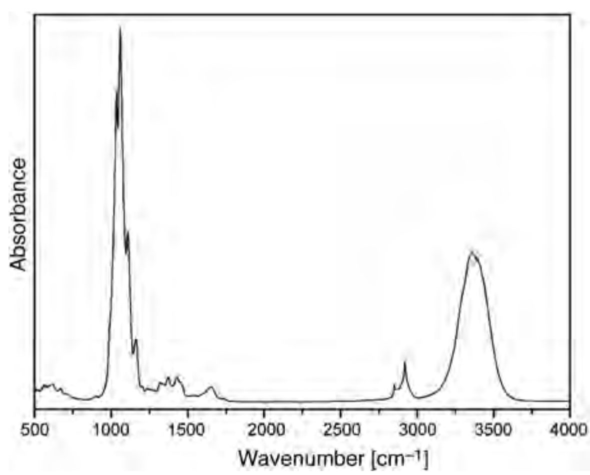

Fig. 1. The example of IR vibrational spectra of flax fiber (43). 335 × 239 mm (96 × 96 DPI).

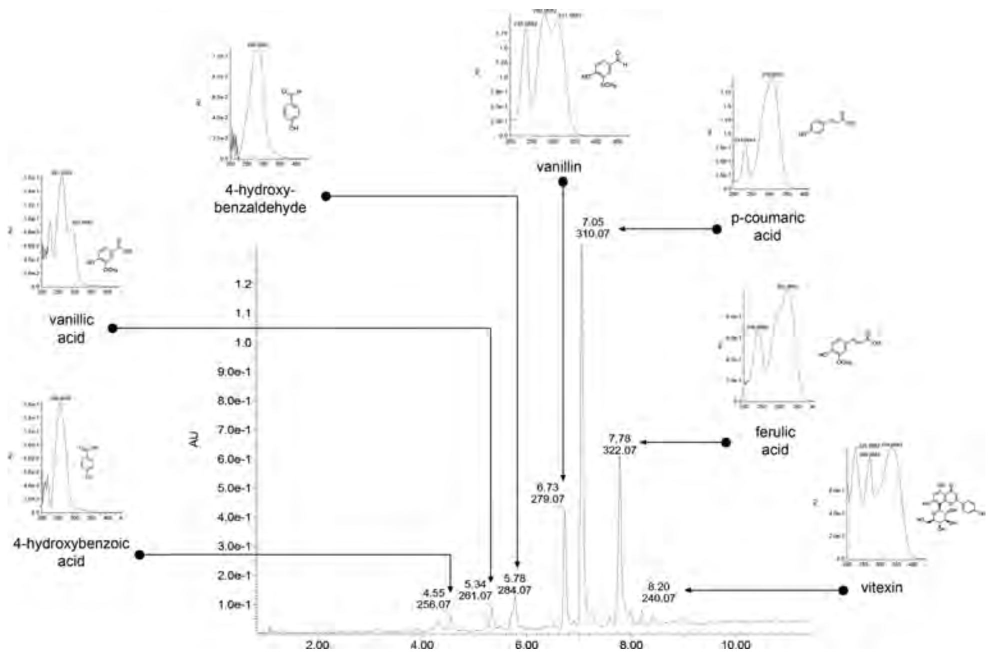

Fig. 2. A chromatogram of phenolic compounds ester bonded to the cell wall components and released from the fiber by alkali hydrolysis. The analysis was performed on Waters UPLC coupled with photodiode detector and Xevo Q- TOF MS. 326 × 213 mm (300 × 300 DPI).

include esterified pectin and lignin constituents of fiber. Examples of compounds identified in flax fiber are presented in Fig. 2 and Table 4.

The presence of vanillin and vanillic acid strongly suggested the fiber's antioxidant and antimicrobial activities (48). This was the case when the growth of several species of human pathogenic bacteria (hospital strains) was inhibited by fiber extract (6,49,50). Both ferulic and p-coumaric acids are hydroxycinnamic acids with antioxidant properties; they are reactive toward free radicals such as reactive oxygen species (ROS). Moreover, recent studies suggested that ferulic acid might have pro-apoptotic effects in cancer treatment (51,52).

Table 4. **A Quantities of Phenolic Compounds Ester-Bond to the Cell Wall and Released by Alkali Hydrolysis**

Ester bound phenolics in flax fiber	μg/g DW
Vanillin	58.57 ± 5.80
p-coumaric acid	20.34 ± 3.76
Ferulic acid	15.74 ± 2.82
Vitexin	18.51 ± 1.91
4-hydroxybenzoic acid	14.48 ± 1.65
Syringaldehyde	13.18 ± 0.27
4-hydroxybenzaldehyde	11.25 ± 1.09

5. Flax Growth and Fiber Extraction

5.1. Flax Planting. The life cycle of fibrous flax starts in early spring and ends in mid-summer, when July ends. Flax should be sown when the temperature of the outer layer of soil is around 7–8°C (20). Sowing density also affects fiber quality, as too sparsely sown it will cause branching of the stem and generally lower height, which means shorter fibers. 1600 – 1800 seeds per square meter are optimal for fibrous flax (20). Ideally, fibrous flax should be grown for approximately 107–110 days, when plants achieve the early-yellow stage of maturity (20). It happens when the bottom quarter part of the stem loses leaves and becomes yellow. The upper part of the stem is still green, but with a yellowish hue, and seed capsules are yellow with fully matured, although not yet colored, flax seeds. It is the best time for flax harvesting with regard to fiber quality, as it affects fiber composition, the longer the plant grows, the more lignified the fiber becomes, increasing fiber stiffness. When growing flax for fiber it is of great significance to keep the field free from weeds. The basic crop, from which fibers are extracted, is straw, and as yet, no mechanical technology has been developed to purify straw and fibers from weeds. The fiber contaminated with weeds residues is of low value and is unsuitable for textile production.

Flax fiber is harvested mechanically with specially designed mowers by plucking out the whole flax plants without breaking the stem and thus maintaining fiber length. The next step in fiber processing is separating seeds from straw to obtain flax stems (threshing).

5.2. Fiber Extraction Technologies. The process of fiber extraction from stems (straw) is called retting. There are a few retting methods, and apart from purely mechanical, the general rule is to remove and/or degrade pectin, and to some extent hemicelluloses, by fungal and bacterial enzymes (26). Enzymes from the polygalacturonase class are key in fiber extraction; the second in line are rhamnogalacturonases (53–55). These enzymes are applied either by treating with their pure form or by employing microorganisms.

For decades, even centuries, the basic method of retting was 'water retting'. This method involves submerging the straw in a water basin, where the fermentation of pectin occurs. It is a quick method, takes only a few days, and gives fiber of fine quality, bright and smooth. However, water retting is not free from disadvantages; it requires the presence of a water basin and produces remarkable amounts of water pollution. Additionally, there is a risk that the straw will start to rot, which cause loses in crop yield. It is also one of the most expensive retting methods (56,57).

Nowadays, the most popular method of retting is dew retting, when the flax straw is spread on the ground and then the microorganisms naturally inhabiting the soil are employed to disengage the fiber bundles from the stem residues (57,58). On average, the process takes ca. 3–4 weeks, but it heavily depends on weather conditions. The straw during retting needs careful attention, otherwise huge losses in fiber quantity and quality occur. For instance, the straw cannot be too wet so it should be turned over during retting at least twice to ensure that the retting is uniform and avoid rotting. Another important thing is to clean the place of retting free of weeds; this applies not only to flax growing, but also to retting. When the retting is over, which could be checked by hand or by measuring the pectin level in a laboratory. The straw should be dried to approximately 17–18% of bundles humidity and then it could be stored for processing (20). Furthermore, to release fiber bundles the straw undergoes mechanical treatment and what remains after the fiber extraction are lignified residues of the stem called shives. The raw fiber is further manufactured to bring the fiber into a certain state of quality for the very finest purposes, such as lace, cambric, damask, and very fine linen. This second part is performed by the refining machine only.

It is the a cheaper method than water retting and produces much less environmental pollutions; however, dew retting also is not free of drawbacks (56). Above all, the fiber obtained with it has decidedly reduced properties compared to that obtained with water retting (mechanical and physical properties are inferior), as well as higher contamination (by epidermis residues and woody fragments of stems). In addition, the type of microorganisms present in a given area has an impact on the color and texture of the fiber. This technique also requires the appropriate geographical location to obtain good results (suitable parameters of temperature and humidity are of concern) (53,59).

Discussing this method, the question arises, whether enzymes degrading pectin may affect fiber structure, and further, if any microorganisms' enzymes, such as cellulases, hemicellulases, or pectinases might affect fiber composition during retting. The experiments carried out so far have shown that in fiber treatments with pectinase the xylan and xyloglucan polymers in the fibers were not accessible to the enzymes. Moreover, the polymers entrapped by the cellulose could be released by the hydrolysis of this cellulose. This means, that unless the straw is exposed to a highly humid environment or water stream, the pectin in the fiber will be untouched (60).

In addition to climate specifications, significant impact on the effectiveness of dew retting also has the degree of cell wall lignification. An optimal retting process is when stems' lignification is low and there is relatively high temperature and humidity. The prolongation of dew retting results in deterioration of fiber quality. Worse texture and fiber darkening (even to brownish grey) are accompanied by an increase in the number of micro-damages which is related to cellulose degradation in the fiber structure. All these reduce the market value of the fiber and its suitability for textile production (56,57).

Accordingly, ways of improving the retting process are researched, which would result in better quality of flax fiber, while reducing costs and environmental pollution. Experiments conducted on enzymatic retting (61), showed that it is an expensive method and suitable only for preparation of fibers for a particular purpose (56,57).

Pectinases in combination with calcium chelators appeared to be the most specific in enzymatic retting (61). Akin and co-workers showed that a pectinase-rich commercial enzyme product in combination with ethylenediaminetetraacetic acid (EDTA) tested on straw gave satisfactory results to develop commercial-grade, short staple flax fibers for use in textiles (62). There is recent evidence, that retting might by carried out by some commercially available enzymes, such as Texazym SCW and DLG that might highly accelerate separation of fiber from stem residues (63).

Nevertheless enzymatic treatment is often just a straw pretreatment step when extracting fibers with other retting methods.

Mechanical fiber possessing, which is called decortication, and further chemical purification of fiber gives mid-quality fiber, with absolutely no biologically active compounds such as phenylpropanoids that have antioxidant properties. High temperature (boiling or steaming the straw under pressure) or extracting fiber by boiling it in alkaline lye or acidic solutions gives fiber of low quality, with no biological activity. What is more, all these methods are time- and energy-consuming and generate huge amounts of pollution, especially chemical pollution (57).

Recently, there has been some studies on novel ways of retting. Sampio and co-workers tested stand-retting and decortication with promising results. Stand retting is a new method where the crop is not cut after achieving maturity but is left standing. This retting method takes longer than dew retting but it has the potential of producing larger quantities of higher quality fiber. The advantages include reduced contamination from soil and stones, reduced straw losses and easier baling. Moreover, decortication produced fibers fine enough for blending and spinning with wool, suitable for processing with cotton machinery and subsequently susceptible to blending and spinning with cotton (64).

Mechanical decortication is the most popular in the Canadian flax industry where they cultivate linseed flax, and straw is a by-product. Thus, this short fiber is not suitable for textile production but it is used for various other applications. Hence, fiber of high quality is not needed. So far, Canadians have developed two main mechanical approaches to decortication: roller breaker and hammer mill systems. Most likely, further development and combining decortication with other treatments, enzymatic or (bio)chemical, will allow these technologies to be applied to fibrous flax. More recently, a novel biotechnological method for fiber extraction was proposed (65). It combines decortication with chemical and enzymatic treatment to achieve consistently high-quality fibers that can be used to produce fine linen yarns. The approach completely eliminates the weather-associated risks of traditional fiber retting.

Straw and fiber might undergo many treatments to improve retting efficiency and/or later fiber quality. Lignin elongates retting time as it blocks the enzymes access to pectin and hemicellulose whereas pectin couples together fiber bundles and the bundles to other stem cell. Hence removing lignin and/or pectin would shorten the retting time and thereby improve fiber quality.

Pectin in the epidermal regions of the flax stems is rich in non-methoxylated units and calcium bridges, conversely to the pectin in fiber. Non-methoxylated carboxyl groups on galacturonic acids are often cross-linked by Ca^{2+} to form stable bridges across pectin molecules. High amount of calcium in the cuticule and epidermis reinforce the lignin barrier for retting enzymes. Hence, using calcium chelators or other divalent cations would improve retting (66). One of the most common chelators is CDTA, which extracts pectin from the surface of fibers, namely the middle lamella and partly from the primary cell-wall (35). Using the $Na_2CO_3 + NBH_4$ solution is not advisable, as it extracts the pectic matrix of the secondary cell wall and thus disturbs fiber composition (35).

To sum up, retting efficiency and thus fiber quantity and quality heavily depend on the method and parameters of the flax fiber extraction from the straw, which is determined by the amount of pectin and lignin in the straw (32). So far studies on retting refer to the amount and composition of pectin in flax and their relation to the retting (43,67) as well as the search for enzyme that support the retting process (53,62,63). Nowadays, the vast accumulation of genomic data on different plants, including flax, gives the opportunity for innovative breeding to improve the retting process by reducing pectin and/or lignin levels in plants. Surely, such modifications might also affect fiber composition, properties and quality, which should be the subject of further careful examination.

6. Fiber Properties and Quality Assessment

6.1. Fiber Properties. To begin with, flax fiber is extremely strong; it is one of the strongest among vegetable fibers. However, the amount of lignin is quite low; it makes the flax fiber relatively stiff and vulnerable to breaking. Another important feature of flax fiber is its high liquid absorptivity. Moreover, flax fiber is non-static, non-allergenic, naturally insect-repellent and gives UV protection. Many of these properties have their roots in the fiber composition and cell wall structure. What distinguishes flax fiber from other natural fibers is that it is biologically active, thanks to the phenylpropanoid compounds.

When it comes to flax fabric, it gives the feeling of coolness in hot temperatures thanks to fiber absorptivity; it very quickly absorbs perspiration, then swells and releases the moisture to the outside air. Therefore, it is a kind of a self-drying textile. It is worth stressing that linen can absorb up to 20% of its own weight in moisture while still feeling dry to the touch.

With such a complex structure, it comes as no surprise that there are many factors that have an influence on the fiber properties. These factors could be divided into three main groups. The first one is related to the anatomic structure of the fiber cell and includes such parameters as: cell length, diameter, and cell wall thickness. The second group of factors affecting fiber quality refers to the fiber's chemical structure, the proportion between the polymers, their spatial arrangement and the number and kind of chemical bonds with which they are connected.

Any changes in one of these factors have an impact on fiber quality and quantity, influencing its tensile strength, stiffness, absorptivity, biological activity, susceptibility to further treatment and chemical reactivity.

The third group of factors is connected with flax cultivation and straw processing. What distinguishes flax fiber from other crops is the fact that its quality and yield depend on many factors, including the careful treatment of plants not only during their growth but also when dealing with straw after harvesting. Mature flax straw contain up to 30% of fiber (20), whose extraction from the stem is conducted by retting. There are many types of retting, employing only natural microorganisms or carried out mechanically, chemically, enzymatically, with osmosis or various combinations of them. But the most important is the fact that the chosen retting method and carefulness with which it is done strongly affect fiber value, quality and properties. To be more precise, fiber cleanliness (the amount of shives contaminating fiber), color (it depends on microorganisms degrading the straw's bark), hue, texture, the number of micro-defects, and to some extent even composition depends on the retting method. Thus, the retting degree is one of the most important factors when determining the market value of flax fiber.

On top of this, flax is a delicate plant, whose growth and development are also dependent on weather conditions. In particular fiber yield and composition are factors strongly sensitive to weather.

6.2. Improving Fiber Properties by Chemical Treatment. One of the flax fiber features is its hydrophilic character due to the presence of –OH groups from cellulose and lignin, which cause difficulties when blending with hydrophobic polymers. Chemical treatment with various compounds decreases the number of –OH groups and thus leads to the improvement of their physical properties, such as melting point (68–72). Moreover, such treatments not only improve the adhesion but also reduce the liquid absorptivity, which is rather a negative feature for composites. Silane and peroxide are commonly used agents. Silane increases the degree of crosslinking in the interface region while free radicals from decomposition of peroxides react with the hydrogen group of the matrix and cellulose fibers (73). Acetylation leads to the esterification of –OH groups from the amorphous fraction of cell wall polymers (lignin, hemicelluloses and to some extent cellulose). Two research teams showed that up to 18% of acetylation in flax fiber (30%)-polypropylene (70%) composites increased composite strength, but a higher level of acetylation led to deterioration of the mechanical properties (33,74). Treatment with alkali (mercerization) has a great impact on fiber, leaving it clean by removing contaminations and polysaccharide residues. Moreover mercerization affects fiber chemical composition by removing pectin, hemicelluloses, and to some extent lignin. Finally, it has an effect on the degree of polymerization and molecular orientation of the cellulose crystallites; it also converted the crystalline form of cellulose I into cellulose II (75). Polystyrene composites reinforced with alkali treated flax fiber showed improved mechanical properties (76).

6.3. Fiber Quality Assessment. While discussing fiber applications it is worth highlighting which factors contribute to the fiber market value. Traditionally linen assessment includes: fineness, length and shape of fibers, strength, density, luster, color, handle, parallelism, cleanliness, and freedom from naps and knots (56). Sadly, there are no objective standards developed for these parameters. Akin in his works presents results of ASTM International Committee D13 on Textiles, which established some standards for flax fiber (56,77). First of all, they standardized flax terminology; secondly, they defined a test method for color measurement. This is only the beginning to fully standardize flax fiber characteristic.

7. Fiber Industrial Applications

No longer is flax seen only as a source of textiles for clothes, bedclothes and edible oil. With the development of civilization and advanced technology, an increase in the demand for new sources of biodegradable raw materials, various goods and generally accepted eco-friendly policies can be observed. To meet these challenges a vast number of studies are carried out and it seems that flax straw and fiber are again in the spotlight.

7.1. Fiber Derived From Fibrous Plant. Apart from the traditional perception of flax, flax fiber is successfully used in reinforced composites replacing glass fibers. A number of studies characterize fibers from different plant sources and their usefulness for composites (78). Apparently, bast fibers, which include flax one, are of the most appropriate quality for use as the reinforcement of polymer matrix-composites. They have weight-specific properties and they are considered as less problematic in the context of environmental issues (78). Generally, in terms of density, weight, costs, availability, degradability and biodiversity, flax fibers have great advantages over glass fibers, whereas their mechanical properties are very similar (79,80). Especially biodegradability and weight are of great importance; they are main reasons for which flax fibers are now successfully used as reinforcement in composites for the automobile industry and some other industries, where small parts of resistant plastic are used. Tests of mechanical properties of some already generated composites, such as flax fabric reinforced with epoxy matrix or polypropylene matrix, showed in all cases improved tensile strength of composites (2,33,81,82). More recently, due to the legislation requirements, the automobile industry was forced to employ innovative recycling and eco-friendly components in cars. As a result, one of the cars and aircraft producing companies launched a program to produce elements of flax-polymer composites (polylactic acid, PLA) (83). Apparently, not only small elements will be produced from flax composites, also large ones such as body shell fragments, seats, trunk load floor or foot mats.

Another pressing problem is the search for new sources of energy; this is both because of shrinking supplies of fossil fuels, and for environmental reasons, to reduce greenhouse gas emissions. In the case of flax, the raw materials for biofuel production are coarse fibers and shives left after fibrous flax straw processing and linseed fiber and straw. The biggest problem is the presence of lignin in these materials, which blocks saccharification enzymes' access to cellulose. Although possible and commonly applied, delignification is an expensive and environment-polluting process (84–86). Despite these drawbacks, there is evidence that flax might be useful as a source of biomass for bioethanol production. In one study different feedstocks were used to verify how the mixture of bioethanol and traditional gasoline works in cars. The results were promising. In comparison to alfalfa, poplar and hemp, flax yield of ethanol production from dry mass was in the top three. The lowest yield was obtained for alfalfa – with 22%, hemp and poplar have very similar results, 31.4 and 31.2% respectively, whereas flax's yield reached 29.6%. It shows that flax could be as good a source of biomass for bioethanol production as poplar and hemp. Additionally, the emission of greenhouse gases was also reduced, although acidification, eutrophication and photochemical smog increased. Nevertheless flax had relatively low emissions of acidic contaminations.

7.2. Fiber From Linseed Plants. It is worth stressing that, for many industrial purposes other than textiles, fiber and straw used as a source of biomass and reinforcement are derived from linseed varieties. This fiber has shorter cells and slightly worse composition, which make it useless for clothes production. The straw from flax cultivated for seeds is quite often treated as a waste, burnt or chopped and ploughed back into the soil. The vast majority of flax linseed straw is treated in this way. Hence, finding new applications for this straw and derived fiber not only make flax an almost no-waste product, but also gives scientists ready, biodegradable, new raw material.

Currently, flax fiber is used in many branches of industry (87). Long, fine fibers are mainly used in the textile industry, but not only. Both short fiber and linseed fiber are used to produce blotting paper and banknotes. The short flax fibers (tow) from linseed straw are also used to generate cottonized flax. Since cotton took over the spinning market, 90% of the world's spinning and weaving equipment has been designed to use fibers with the approximate length and diameter of cotton fibers. Hence, a mechanical process was developed to break down flax fiber bundles into ultimate fibers to produce a flax-based fiber that could be spun on the cotton equipment. Such flax is generally referred to as cottonized flax and it is used in clothing production.

Flax fiber is also used as a geotextile to produce mesh that protects soil from erosion or to reduce the level of dust and erosion along roads, railroads and building sites. Another usage is in paper recycling, which requires re-pulping. The process results in the loss of paper strength in the next generation. To overcome this flaw a portion of virgin fibers is added to the pulp. Such reinforcement is called the pulp sweetener. Insulation is another branch of industry where flax fiber finds its usage. Coarse fibers and shives generated as a wastes when fine long fibers are produced, as well as linseed flax and straw, are used to produce insulation batts that are of the same quality as glass fiber batts. Furthermore, such material is used to build horse and pet lairs, boxes and travel containers.

Bioremediation, especially in the context of contamination of water with metal cations, is also a pressing problem. Marshall showed that flax shives, whose carbon was activated by means of pyrolysis, might serve as a binder for selected divalent cations (cadmium, calcium, copper, magnesium, nickel, zinc). This makes flax a prospective component of industrial water filtration systems (88).

8. Flax Fiber Engineering

The rapidly developing field of genomics also concerns flax, whose genome is now published and available. Consequently, the enormous accumulation of data on flax genomics (89) gives the opportunity to find the exact mechanisms of fiber formation, and hence such breeding that will significantly improve the plant productivity and quality of fiber. It is expected that better understanding of the genes involved in fiber productivity, quality and novel properties will provide targets for fiber improvements by genetic engineering and the novel, broadly accepted epigenetic method, leading to more diverse products based on flax fibers. It is quite possible that increased knowledge of cell wall development in relation to fiber properties will help to develop novel plant fibers (longer, thinner, soft, resilient, etc.) for textile applications, composite fibers (containing polyhydroxyalkaonate) for biodegradable composite materials and improved fibers for adding functionality to composite materials. Development of stronger fibers for specific applications by modifying the plant cell wall, flax with modified cell walls with easy extractable fibers and simplified procedures for extracting plant fibers from the natural source are also expected when the respective knowledge increases.

Nowadays, novel applications of fiber are mainly based on a new flax types generated by gene engineering technology. These include modified flax fiber for chronic wound healing, biocomposites for biodegradable packaging material and scaffold for tissue engineering, micronized fiber for drug carrying, etc. (90).

Since the beginning of agriculture, breeders have selected plants with the best possible properties, talking into consideration their suitability for human needs (91). A fiber of good quality was highly important due to its usefulness for clothes and bed coverings. In recent decades, studies on plant genetics have developed rapidly, giving scientists and breeders tools for the development of more efficient plant breeding programs in order to improve plant/fiber quality (89,92,93). Recent research approaches the task of obtaining the highest quality fiber from two different perspectives. The quantitative approach includes boosting such features as plant growth, productivity and crop yield, whereas qualitative engineering relies on the manipulation of fiber composition in such a way that it will have a highly positive effect on its quality and further properties.

8.1. Engineering Fiber Productivity. Quantitative improvements include manipulating parameters referring to the efficiency of nutrient assimilation, the plant resistance to biotic stresses manifested mainly in pathogen infections, and abiotic stress, from which of greatest importance at the moment is drought resistance (94). To improve the growth rate, we can manipulate the biosynthesis of key hormones involved in the signal transduction in plants (95,96). Nowadays, due to the changing climate and global warming, the most pressing problem is plant resistance to the lack of water (97). There are many recent studies on the subject, both on commercially important and fibrous plants, but only a few concern flax (98,99). In one study, introducing a gene encoding β-1,3-glucanase, a pathogenesis-related (PR) protein, resulted in resistance improvement against the main flax pathogen, *Fusarium oxysporum* (100). In another study on flax, genes involved in pectin and formate metabolism were correlated with their metabolite levels after *Fusarium* infections (101). The research gives valuable contribution to pectin and formate metabolism in the early stage of pathogen infection and might serve as an important starting point to improve flax resistance to biotic stress. A different approach for flax protection against pathogen infection was applied by a Chinese group; introduction of the rabbit defensin gene, NP-1, increased the plant resistance to *Fusarium* wilt and thus fibers productivity (102). A very recent study on flax gave an important insight into plant growth. It appeared that the LuWD40-1 gene encoding WD Repeat Protein regulates proper flax growth (103). It turned out that overproduction of the WD-Repeat Protein led to ca. 80% non-viable pollen, reduced branching, and delayed flowering and maturity when compared to the male fertile genotypes.

8.2. Engineering Fiber Quality. Qualitative improvements particularly concern the plant cell, its diameter, length, cell wall thickness and composition. These are factors that heavily affect fiber quality and thus are the aim of the various modifications. Another aspect of fiber quality is the process of extracting the fiber from the stem. It might be carried out mechanically, with chemical and/or enzymatic treatment, or with the help of genetic method. This stage of fiber processing affects fiber hue, texture and the number of micro-defects in it.

The recent progress in the understanding of cell wall formation and biochemistry, polymer synthesis and arrangements, clearly shows that targeted genetic modifications are capable of modifying cell wall structure. Activating/ reducing genes controlling the biosynthesis of the main structural polymers that are cellulose, lignin, pectin and hemicelluloses or secondary metabolite biosynthesis, clearly improves fiber quality and broadens its application (104–107).

Both in model plant species, such as *Arabidopisis*, and commercially important fibrous plant species, such as cotton or flax, genes that are responsible for fiber development are tracked (73,81,108–110). These studies are the first step to qualitative fiber improvements and the first attempts aiming to modify fiber composition are just underway. Flax plants overexpressing β-1,3-glucanase, a PR protein, appeared to have fiber enriched in pectin and phenolics which strengthen fiber mechanical properties and antioxidant capacity (111).

Lignin Polymer Engineering. The biosynthesis of lignin metabolism is currently under careful investigation for many reasons; lignin is a stumbling block for plant products and biomass application in the various branches of industry (112). This is why functions and effects of down-regulation of particular genes from lignin biosynthesis are being thoroughly examined (113). Earlier research indicated that modification of the lignin biosynthesis pathway may cause an increase in cellulose level in transgenic aspen (114); in various species *cinnamyl alcohol dehydrogenase* (*CAD*) down-regulation led to altered lignin structure, and increased its susceptibility to enzymatic digestion and/or content (115–121). In flax, plants engineered to under-express the *CAD* gene showed reduction in the stem lignin content between 16% and 40% (depending on the line analyzed), and a soluble phenolic content increase (122). The reduction in lignin content was accompanied by improved mechanical properties of the whole stem. The investigation of fibers from these plants confirmed lower lignin level and improved tensile strength which was followed by more uniform retting (123). The result seems promising since lowering the lignin level does not cause alteration of plant growth.

Similar research on flax was presented by Day and co-workers in 2009 (124). Another enzyme, Caffeoyl CoenzymeA O-Methyl Transferase (CCoAOMT) engineered to down-regulate its activity in plants, also showed reductions (8–18%) in stem lignin content associated with decreased cell wall thickness and reduced mechanical strength as indicated by the irregular outline of certain xylem cells.

Lately, thirteen partial cDNAs of the critical genes involved in flax lignin biosynthesis were amplified from flax bark mRNA. The sequence analysis revealed eight new genes, including two *CCoAOMT*, three 4-coumarate-CoA ligase (4CL), and three ferulate-5-hydroxylase (F5H) genes, which implies that lignin biosynthesis is regulated by multi-gene families and of these, three full length cDNAs (COMT, 4CL, CCoAOMT) were obtained (125,126). The research gives a valuable contribution to the lignin biosynthesis pathway and might serve as an important starting point to improve flax fiber quality.

Very recently a new approach has been tested for lignin reduction in a model plant (*Arabidopsis*). The promoter of the key lignin gene C4 H was replaced by vessel-specific promoter of VND6 transcription factor. Concomitantly the promoter of the cell wall glycosyltransferase gene (IRX8) was used for expression of the NST1 gene, whose product (transcription factor) induces the IRX8 promoter. In this way a positive feedback loop is generated which results not only in lignin reduction exclusively in the vessel tissues, but also in polysaccharide accumulation in stems (127). This technology, although tested only in a model plant appears to be a prospective tool for modifications of other plant species including flax.

Pectin Polymer Engineering. More and more concern is given to the pectin component in the cell wall structure. Recently, it was shown that pectin methylesterases (PME) are of great importance to the secondary cell wall development in cotton fibers (128). PME catalyzes de-methylesterification of homogalacturonans, resulting in gelation of the pectin and cell wall stiffening. Targeted alteration of *PME* gene expression and PME enzyme activity could therefore be a key to improving cotton fiber quality as PME influence final fiber diameter and length, two key quality attributes of cotton fibers.

One of the latest studies showed that rhamnogalacturonanII (RGII) plays a crucial role in cell wall integrity. In a model plant *Arabidopisis* mutants with a defect in two (out of four) genes encoding the enzyme UDP-glucose dehydrogenase (UGD) had shortened RGII side chains by 60% without changing their structure. Mutants showed swollen plant cell walls and severe developmental defects associated with changes in pectic polysaccharides. Another enzyme that degrades pectin and is strongly suspected of playing an important role in cell wall integrity is endopolygalacturonase (PG). PGs by cleaving linkages between α-1,4 D-galacturonic acid residues in non-methylated homogalacturonan release short cell wall fragments (OGs) from the pectin matrix (129). It appeared that cell wall associated kinases (WAK) bind OGs that activate invertase, which can modulate soluble sugar levels in plants, in turn affecting turgor pressure (130–134). PGs are also present in fungi and they are secreted by pathogens during infection. This indicates that putatively, overstimulation of PG in plants might imitate pathogen infection and thus cause changes in the plant cell wall composition which further improve plant resistance to pathogen infection. However this hypothesis is not yet verified (129).

The generation and analysis of GM flax with reduced pectin synthesis support this view. For example, introducing cDNA of genes encoding fungal enzymes degrading pectin, polygalacturonase I from *Aspergillus aculeatus* (PGI) and rhamnogalacturonan lyase from *A. aculeatus* (RHA) resulted in 50% decreases of pectin content and improved retting of the straw demonstrated in significantly earlier appearance of retted fiber as compared to the control (135).

An alternative to PGI and RHA plants might be the generation of plants with modification of genes encoding the key enzymes of pectin biosynthesis pathway. In the last decade several publications have been dedicated to these issues.

For instance, the overexpression of the grapevine polygalacturonase-inhibiting protein 1 in tobacco results in the cell wall remodelling and reorganization of the cellulose-xyloglucan network (136), which could be a consequence of changes in the expression of genes involved in cell wall metabolism (137). The overexpression of bean polygalacturonase-inhibiting protein 1 in wheat (138) improves its resistance to the fungal pathogen *Bipolaris sorokiniana*. The overexpression of a *Petunia inflata* pectin methylesterase in *Solanum tuberosum* improves stem elongation and modifies cation distribution, but has no effects on the chemical structure of tuber cell wall's pectin (139). Wheat transgenic lines overexpressing the PMEI from *Actinidia chinensis* were characterized by changes in the pectin structure caused by the increase of pectin methyl esterification (140). Antisense transgenesis of tobacco with a flax pectin methylesterase affects the demethylation of pectin in the pollen cell wall-specific structure (141).

These examples show the different possibilities of the generation of GM flax in order to analyze the structure and composition of the main cell wall component. Thus, the great progress made in recent years with respect to the understanding of fiber polymer synthesis and arrangement is a way to determine key genes regulating fiber productivity and quality. These genes will be the basis to generate gene constructions for the creation of stable flax transformants with improved fiber fineness.

Engineering of Other Cell wall Constituents. Flax cell walls are a complex assembly of different polymers (polysaccharides such as cellulose, hemicelluloses and pectin, proteins, and the phenolic polymer lignin) and the amount of particular polymers differs in fiber depending on the plant species, tissue and even cell region. The variability of cell wall composition might be responsible for the different properties of different fibers. The correctness of this suggestion is supported by the experiment (cited above) concerning generation and analysis of transgenic plants with reduction of lignin synthesis.

Cell wall associated genes other than lignin or pectin were also the targets for fiber engineering. It was revealed (142) that β-galactosidase is essential for normal secondary wall development in flax fibers. The developing stems of flax plants with repressed β-galactosidase were characterized by remarkably thinner galactan-enriched matrix, and a unnaturally thick cellulosic gelatinous-layer. Further analysis showed that cellulose crystallinity was reduced which was responsible for the fiber's weaker mechanical properties.

In addition to modifying fiber quality by targeting native cell wall polymers, researchers have also engineered fiber plants to accumulate secondary metabolites and to produce novel polymers that are not normally produced by plants. An example is a flax type (called W92) overproducing phenolics whose fibers were successfully used for preparing wound dressing for chronic wound healing (5). In one study, genes encoding PHB (poly-β-hydroxybutyrate) synthesis were introduced into cotton plants (143). The amount of PHB in the cotton fibers was very low (0.34% of fiber weight), but even so improved the thermal properties of fibers. The reason is that poly-β-hydroxybutyrate is a hydrophobic and thermoplastic agent. A similar strategy has also been used to increase the elastic properties of flax fibers (144,145). In these studies, three bacterial genes (*phb A*, *phb B, phb C*) encoding different enzymes of the PHB biosynthetic pathway were introduced under the control of a vascular bundle specific promoter into fiber flax (*L. usitatissimum* L. cv Nike) by *Agrobacterium*. Analyses of modified plants showed that different mechanical properties (elasticity, flexibility, tensile strength) were improved as compared to control plants. Further experiments evidenced that PHB binds to cellulose polymer by chemical bonds. Data from IR spectra analysis suggested that during plant growth natural a complex of PHB and cellulose matrix is formed as a result of ester C—O—C and C= O···H O hydrogen bonds (for the predicted scheme of these structure see Fig. 3). However the exact molecular mechanism for this remains unknown (46).

In other studies (3,146,147), fibers from PHB-engineered flax plants and polypropylene (PP) and polylactide (PLA) were used to produce composite materials. Scanning electron microscopy (SEM) showed that modified fibers showed enhanced adherence to the PP and PLA matrix as compared to control fibers. The corresponding composite material also showed improved mechanical properties as

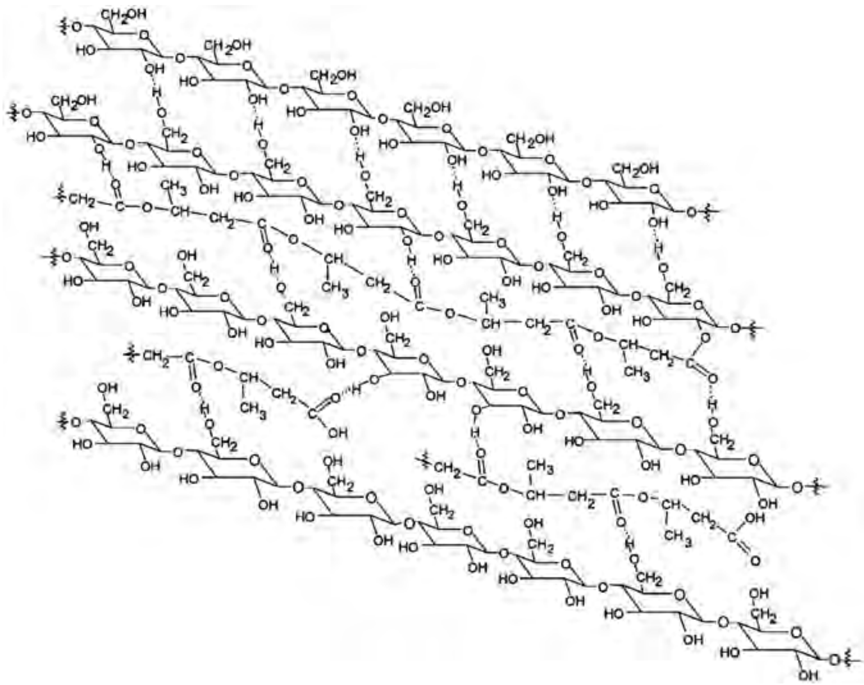

Fig. 3. Predicted ester and hydrogen bonds between the flax fibres and PHB. 279 × 222 mm (96 × 96 DPI).

compared to composites prepared with control fibers. In addition, bio-compatibility tests indicated that modified fibers provoked little platelet aggregation, and do not show any inflammation response after subcutaneous insertion thereby opening up the possibility of using such materials for the development of biomedical devices that are in regular blood contact (146,147).

8.3. Synchronization of Fiber and Seed Maturation of Flax. It appeared that stem and by extension fiber maturation, as well as seed maturation, do not occur simultaneously. Fibrous flax is harvested when stems are almost entirely defoliated and the development of the secondary phloem fiber cells is complete. At this time flax combines good fiber yield and quality. However, harvesting the plant at this stage means risking loss of sowing seed quality. On the other hand, later harvesting causes increasing lignification and results in poor fiber quality. Thus a more synchronized maturation of stem fibers and seeds will reduce harvest risk. To minimize this risks during the production of both high quality fiber and flax sowing seed, a breeding program is being carried out to synchronize the maturation of fiber and grains of flax (148).

Nowadays, growing interest in flax means that more attention is being paid to linseed straw and fiber as they are by-products of linseed cultivation in North America. The relationships of stem fiber content with quantitative (plant height, number of days from emergence to end of flowering, petal width, seed weight, seed, oil content and proportion of linolenic acid out of all fatty acids) and qualitative (stem branching, petal color, petal overlap, petal margin folding and seed color) plant characters were investigated (149). The results showed that the wide variation in the fiber content of flax germplasm will be a highly useful tool for determining the germplasm relevance for breeding dual-purpose flax.

For the same reason, to make one flax cultivar with a dual-purpose crop, genomic regions controlling both stem fiber and seed quality traits were investigated (108). The authors concluded that core collection of flax (*Linum usitatissimum* L.) is suitable for advanced studies targeting multiple agronomic and quality traits to achieve the goal. Candidate regions were indicated as affected by divergent selection in flax which after further investigation gives the opportunity to detect loci influencing complex traits.

In another study (150), using hormone treatment with gibberellic acid (GA3), the relationships between GA3, stem architecture, and bast fiber properties in linseed were investigated. The results were promising, even though the impact of GA3 on phloem tissues was less apparent. However, GA3 application in combination with IAA led to an increase in the thickness of bast fiber secondary walls. The research provides useful information for the manipulation of fiber properties for breeders and field treatments.

9. Flax Genetic Engineering and Genome Modulation Methods

Application of genetic engineering in basic and applied plant breeding research was possible because gene technologies and transformation techniques have been developed. It is strongly believed that this method will allow further progress in flax breeding. The most exploited method in modern plant biotechnology is generation of transgenic organisms for both scientific and commercial purposes. While scientific usage of transgenic crop plants is broadly accepted, their introduction to the market and commercialization of their products is doubtful. This is mostly due to the European countries. In 2010, the EU Commission delivered a publication which summarized ten years of research on the potential environmental impact of genetically modified organisms (GMOs), food safety, the coexistence of GM and non-GM crops and risk assessment strategies. The main conclusion drawn is that biotechnology and in particular GMOs are not more risky than conventional plant breeding technology. Thus despite public concerns about the safety of engineered plant crops, there is no scientific evidence to indicate that they are more dangerous than any other plant varieties produced through conventional breeding techniques.

The modification of fiber structure can be obtained by targeting genes associated with fiber development and polymers biosynthesis. In both cases, the aim is to modify the gene/genes expression by either up-regulation or down-regulation. This might be accomplished by introducing a piece of DNA (in sense or antisense orientation) into the targeted plant with a number of different mechanisms that already exist in the plant kingdom.

The most commonly used method to introduce DNA fragments into the plant genome is the *Agrobacterium* method. In this method, the DNA fragment, which might be the whole gene or its part, is firstly introduced into the binary vector (expressed in both bacteria and plant cells) carrying the sequence that facilitates the DNA transfer to the plant cell and its insertion into the plant genome. Then the binary vector carrying the desired DNA fragment is introduced into *A.*

tumefaciens cells, which, in turn, are transferred into plant cells. The *Agrobacterium* method is also widely used to improve fibrous plants productivity and quality (111,122,124,142,145,151,152).

Another method to generate transgenic plants is the biolistic method in which metal particles coated with multiple copies of the target gene are introduced into plant tissues mechanically by firing them from a special apparatus called a 'gene gun' (153). The particle bombardment method is an alternative way to modify plants which are resistant to the *Agrobacterium* method.

Despite the fact that transgenesis is commonly used as a method for gene function study and the plant improvement which lead to the upgrade of their commercial value, the method is laborious, time-consuming and controversial in public perception. Thus, recent research has concentrated on the development and optimization of epigenetic modulation. This method based on the introduction of small pieces (12–21nt) of DNA or RNA (called OLIGO) into the cell, has been successfully used for several years to modify mammalian cells. To date, three potential mechanisms of small DNA/RNA (OLIGO) action are recognized (Fig. 4).

The first is based on the recruitment of protein complexes (Dicer, RISC) by OLIGO and then the complex binds to the targeted mRNA, inhibiting its activity; this mechanism is collectively called RNA interference. The introduction of small OLIGO into the cell might also results in mRNA activation; the mechanism has so far only been detected in mammalian cells and occurs perhaps due to histone modification. The third mechanism includes small RNA-directed targeted

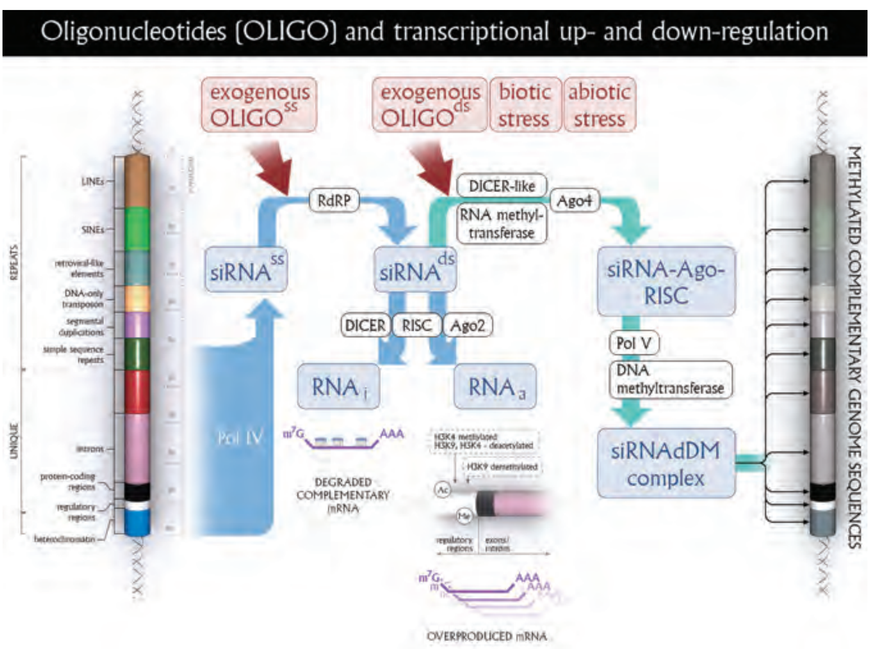

Fig. 4. The scheme of endogenous/exogenous oligonucleotides impact on homologous mRNA metabolism and modification of homologous DNA. 390 × 292 mm (100 × 100 DPI).

gene methylation. This requires the recruitment of complexes containing DNA methyltransferase and Argonaute 4. However, this thesis should be studied in more details.

Plant OLIGO treatment (called ODN technology) was first successfully used for silencing of the SUSIBA2 gene in barley (154). Our own preliminary experiments showed that OLIGO might induce either the gene's repression or activation in flax plants (155). Introducing 18-nt long OLIGOs derived from flax lycopene epsilon-cyclase or from β-glucanase reduced or induced targeted gene expression, respectively. β-glucanase is capable of hydrolyzing β-1,3-glucans, the major component of the cell wall in many groups of fungi. Its expression in transgenic flax was effective in preventing the development of disease. Demethylation of the β-glucanase gene in its coding region was detected upon plant exposure to OLIGO and this modification is stable along three generations. The plant called EMOβ-glu of the third generation (F3) showed the same methylation profile as that of F0 and was at least twice as resistant against *Fusarium* infection as the control.

10. Future Prospects for Flax Fiber Improvement

Plants as photosynthetic organisms are the primary source of energy, nutritional ingredients and tissue-building substances for most non-plant organisms. Photosynthesis results in the biomass which is needed by all living organisms.

Over the centuries, the people have strived through classical breeding to develop crops better suited to their needs. The demand for healthy, safe and sufficient food and feed has been increasing worldwide for decades. Similar trends are also evident for plant-based products – chemicals and energy. As a matter of fact, both healthy, safe and sufficient food and feed and plant based products are among the five challenges specified by the European Technology Platform as a research strategy for the next decade (up to 2025).

The worldwide demand for feed will increase dramatically as a result of the growing demand for high-value animal protein. Since the effectiveness of animal protein production is relatively low, the new markets are looking for higher value-added food products from low-cost plant proteins.

It is now quite clear that feed and food are increasingly competing for acreage with non-food products like bio-energy and industrial products, such as bioplastics for packaging. For instance, some bulk materials are already obtained from plant and wood, including carbohydrates, protein, oil and fibers. It was calculated that these make up ca. 5% of industrial raw material demand. Thus, one of the main goals for the EU is to increase the percentage of renewable resources used for synthesis of chemicals and materials. The recent progress in plant research proved that plant-based resources can certainly provide far more facilities for society and industry than at present.

New plant raw materials may include medicines, specialty chemicals and enzymes, industrial feedstock, polymers and fibers produced from genetically/epigenetically modified plants.

A certainly achievable goal is to develop plants as "green factories" for the production of new compounds and to explore the uses of new raw plant materials with better performing features. The benefits may come from cheaper, safer or more environmentally friendly production methods to the development of new and improved products for the consumer.

The progress in genetic engineering resulted in generation of several new flax types. An example is a flax type (called W86) over-accumulating tannins, producing oil with “ideal characteristics” which include a suitable ratio of $\omega 6/\omega 3$ fatty acids recommended by the FDA, the presence of tocopherols and water soluble antioxidants which guarantee oil stability (156). Another example is flax type (called W92) overproducing phenolics fibers of which were successfully used for preparing wound dressings for chronic wounds healing (5).

Even so flax acreage has recently declined. There are perhaps two reasons for this: the reduction in financial support for flax cultivation, and public concerns about genetic engineering methods that weaken market interest in flax. Recently, however, renewed interest in flax products might be detected. There are several reasons: firstly, the accumulated data have demonstrated that modifications in gene expression can change fiber cell wall structure and properties. Nowadays gene targets are chosen mainly on the basis of results obtained from cell wall analysis in model species. The recent progress in understanding of cell wall formation, polymer synthesis and arrangements clearly shows that targeted genetic modifications are capable of modifying cell wall structure. For example, a number of studies have underlined the role of transcription factors in regulating (activating/reducing) the whole secondary cell wall developmental program by activating genes controlling the biosynthesis of the main structural polymers that are cellulose, lignin, pectin and hemicelluloses (104–107).

Secondly, the capabilities of plants as green factories for the production of significant groups of green products such as recombinant pharmaceuticals, recombinant proteins, industrial enzymes and chemicals are well known. For most of the firstly anticipated products, the important genes and metabolic pathways need to be identified. Currently, about two-thirds of all antimicrobials and half of all anticancer drugs are directly or indirectly derived from natural resources. The dependence of human life on natural plant-derived products is clear, and the concept of plants as green factories provides the basis for ground-breaking new possibilities in medicine. The example of antibacterial compounds isolated from flax has been already reported (1).

Thirdly, after flax genome sequencing, the next challenge is to find out the role of genes in the formation and development of fibers. To accomplish this, both forward- and reverse-genetics can be used to determine the role of different genes. Complete understanding of a given gene’s role in fiber wall formation and development requires the development of an approach involving the integration of transcriptomics, proteomics and metabolomics (157,158). These are currently under extensive development. For example, transcriptomes of *Arabidopsis*, rice and maize have been used for such research discoveries including developmental gene activity networking, identification of key genes regulating secondary metabolite pathways, plant response to biotic and abiotic stresses at transcriptome and metabolom levels, etc. (e.g. (107)).

Finally, another extremely important aspect is a method of plant modification which might be thoroughly accepted and this should be other than GMO. A method that might be satisfactory is based on epigenetic modulation of DNA and is under development (155). The method does not include breaking the integrity of the genomic DNA, no heterologous DNA is incorporated into the genome, and it does not need to be mediated by bacteria infection. Therefore, it can be classified as a non-GMO method. The efficacy of this method in transient gene modification is already well established. For example, silencing of the SUSIBA2 gene in barley was successfully accomplished (154).

It is expected that better understanding of the genes involved in flax productivity, quality and novel properties will provide targets for fiber improvements by the novel, broadly accepted epigenetic method leading to more diverse products based on flax fibers. It is quite possible that increased knowledge of cell wall development in relation to fiber properties will help to develop novel plant fibers (longer, thinner, soft, resilient, etc.) for textile applications, composite fibers (containing polyhydroxyalkaonate) for biodegradable composite materials and improved fibers for adding functionality to composite materials. The development of stronger fibers for specific applications by modifying the plant cell wall, as well as generating flax with modified cell walls with easily extractable fibers and simplified procedures for extracting plant fibers from the natural source, are also expected when the respective knowledge increases.

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