Microbial quality of linseed and fibre hemp plants during growing and harvest seasons

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Linseed (Linum usitatissimum L.) and fibre hemp (Cannabis sativa L.) can be used as raw materials in various applications. In this study, microbiological quality and meteorological measurements were made during the growing seasons and harvesting periods of 2001 and 2002. The microbiological analyses were carried out with Hygicult® TPC and Y&F dipslides, and with a surface spreading method using Plate Count and Potato Dextrose agars. During the growing season of 2001 the conditions were mostly humid, whereas the growing season of 2002 was rather dry and warmer than that of 2001. The lack of water during the growing season of 2002 affected the growth of the plants. In the case of both hemp and flax, the mould and bacterial contents (cfu g⁻¹) increased markedly at the end of the growing season of 2001. During the growing season of 2002 the increase in mould and bacterial contents was noticeable but more constant throughout the whole growing season. At the end of the growing seasons, the mould and bacteria contents were higher in 2001 than in 2002. The genera of moulds identified included Cladosporium, Fusarium, Penicillium, Mucor and Alternaria. The microbiological safety should be controlled during the whole production chain, beginning with the cultivation and harvesting periods.

Key words: Linum usitatissimum L., Cannabis sativa L., microbial quality, growing season

© Agricultural and Food Science
Manuscript received March 2004
Introduction

The applicability of the raw material from fibre plants to new non-textile applications has been studied with increasing interest. Fibres and shives can be used for several products, such as insulation materials, composites, packaging materials and paper products. The environmental conditions partly regulate the growth of microbes in the raw material. In favourable conditions of nutrient level, moisture and temperature, microbes can proliferate and spores germinate, leading to damage of organic materials. The fungi can cause losses of dry matter (by utilising the carbohydrate reserves), and they can also deteriorate quality by destroying the structure of the fibres. In order to ensure the hygienic quality of the products, the microbiological safety must be measured and controlled throughout the whole production chain from the field to products. In this study the focus was on the microbiological quality of the plants that will be used as raw materials for thermal insulation materials. The measurements were made in order to be able to understand the effects of the meteorological growing conditions on the plant material, to be able to compare the amount of microbes in the plant material in different times of the year and to obtain basic data concerning the level of microbes in the straws.

*Linum usitatissimum* (flax and linseed) plants are annual bast fibre plants, of which the oil-seed types are bushy in habit and often bear basal branches arising just above the surface of the soil. The bast fibre bundles are located in the pericycle, each bundle containing about 10 to 40 individual fibres. Flax consists of cellulose, hemicelluloses, pectins, lignin, fat and wax, and some water soluble matter. Considerable quantities of nutrients are returned to the soil before maturity, mainly via the fallen leaves yet also by being washed out (Berger 1969). The bast fibre bundles are freed from the surrounding tissue by the combined action of moisture and bacteria which are found on the flax straw. In dew-retting fungi degrade the epidermis and thin-walled and un lignified parenchyma cell walls. Highly cellulosic fibres are less easily degraded by dew-retting microorganisms than the surrounding pectin- and hemicellulose-rich tissues (Akin et al. 1996).

Hemp (*Cannabis sativa* L.) is an annual bast fibre plant ranging in height from 1.2 to 5 m and with a diameter from 4 to 20 mm. The plant is naturally dioecious and the male plants grow more quickly, which leads to uneven maturity at harvest (Berger 1969). The volume of nutrients assimilated reaches its peak at the beginning of maturity.

Retting is a partial degradation of the tissues of the stem of a bast fibre, allowing the fibre bundles to be easily separated from the stem. During retting the components that bind the fibre bundles to the other plant tissues are broken down by the action of enzymes. Enzymatic action may be derived from the activity of microorganisms (Easson and Molloy 1996). Traditional dew-retting is based on naturally occurring fungi, and water retting is based on the presence of bacteria (Easson and Molloy 1996). The population of fungal colonisers, i.e. *Cladosporium*, *Fusarium* and *Epicoccum*, present on the straw after retting can also act as spoilage organisms in humid environments. Hemicellulose and pectins support a profuse growth of fungi (Sharma 1992). The duration of wet and dry periods is critical for the development of fungal growth under fluctuating conditions, because the oscillation between favourable and unoptimal periods slows the fungal growth compared to continuous optimal conditions. In addition, fungal spores possibly survive the fluctuating temperature and moisture conditions (Pasanen et al. 2000). On the other hand, alternating periods of wet and dry conditions can be highly effective in spreading some diseases, as the wet periods encourage the formation of spores while the dry ones encourage their distribution (Mercer 1992). The occurrence of microorganisms on aerial plant parts is well known (Dazzo 1980).

Frost-retting (Pasila 2004) has previously been used as a low-cost way to produce porous fibre material e.g. for insulations, filters and packages. In the dry-line method seeds are harvested in autumn and the rest of the plant is left in the field to be stand-retted and dried during the winter. Frost-retting results in overretted fibres that are
very absorptive in the case of hemp, and relatively resistant to moulding due to the decrease of nutrients during retting (Kymäläinen and Pasila 2000, Kymäläinen et al. 2001). On the other hand, the microbial content has been suggested to be higher in frost-retted fibres than in stalks harvested in the autumn (Kymäläinen et al. 2002).

The aim of the present study was to investigate the microbiological quality of linseed and fibre hemp plants during the growing season until harvesting in autumn and in spring. The ultimate aim was to gather more information concerning the quality of the raw materials for technological purposes, especially for insulation materials.

Material and methods

Cultivation of the plants

Fibre hemp (*C. sativa* L., variety Uso 31) and linseed (*L. usitatissimum* L., variety Helmi) examined in the study were cultivated in Siuntio, southern Finland. The cultivation was carried out with the procedure commonly used in Finland (Sankari 1998, Hongisto et al. 2000). Linseed was sown on both years (2001 and 2002) at a seed density of 68 kg ha⁻¹ and hemp at ca. 30 kg ha⁻¹. The soil type was silty clay with pH 6.1 and a phosphorus balance of 8.0 mg l⁻¹. The linseed was fertilized with 70 kg N ha⁻¹ and 10.5 kg P ha⁻¹ (2001) or with 60 kg N ha⁻¹ and 9 kg P ha⁻¹ (2002). The hemp was fertilized with 140 kg N ha⁻¹ and 21 kg P ha⁻¹ (2001) or with 92 kg N ha⁻¹ and 12 kg P ha⁻¹ (2002). The climate in Finland can vary significantly in different years and the date of sowing depends on the weather during spring. In the year 2001 both hemp and linseed were sown on 17 May. In 2002 hemp was sown on 25 April, and linseed on 26 April. Linseed was not harvested at all in 2001 because of a large amount of weeds in the field. The hemp crop of 2001 was harvested on 14 May 2002. In 2002 about 90% of the linseed crop was harvested on 12 September; a small area was left for frost retting over the winter. The growing season of 2001 started on 22 April and ended on 19 October. The length of the growing season 2001 was 181 days (of normally 175 days in this cultivation area). The sum of effective temperature during the growing season 2001 was 1575ºC (normally 1400–1500ºC). The growing season of 2002 started on 20 April and ended on 19 September. The length of the growing season 2002 was rather short, 153 days. The sum of effective temperature during the growing season 2002 was 1595ºC. The hemp crop of 2002 was harvested on 6 May 2003. Linseed was not harvested in spring, because the linseed plants were very short and not optimal for frost-retting. During the summer of 2002 the use of fungicide on hemp and linseed was pretested. There were five untreated test squares and five test squares treated three times with the fungicide Tilt® 250 EC (Kemira Agro Oy) at the hemp field, and five untreated squares, five squares treated once and five squares treated twice with the fungicide Tilt at the linseed field. The size of each test square was 5 m × 1.5 m.

Microbiological quality of the air and meteorological data

The amount of microbes in field air was measured with an MAS-100 sampler (Merck Eurolab), the growth media used were plate count agar and malt agar. The daily standard meteorological data (air humidity and temperature) were recorded at the Metsähovi Research Station of the Finnish Geodetic Institute, Kirkkonummi, Finland. The data was recorded with Vaisala HMP 35D PT100 and Vaisala HMP 35D HUMICAP. The meteorological conditions in the middle of the hemp field were also checked regularly to provide knowledge of the microclimate in the field among stalks. During the summer of 2001 the meteorological data in the field was recorded with a thermohygrograph (Lambrecht, Germany) and during the summer of 2002 with a Data Logger (Tinytag Ultra, Gemini, England). The field measurements were carried out at a height of 20 cm above the ground.
Microbiological sampling of the plants

The microbiological samples were taken 2–4 times a month during the growing seasons. During the summer of 2001 the samples were taken from a diagonal line through the field by taking one hemp stem or one bunch of flax stems (approximately 15–20 plants in each flax bunch) from five points of the field. During the growing season of 2002 the samples were taken from randomly selected test squares on each sampling occasion. The head and the lower part of the plants were handled separately.

Microbiological analysis of the plants

All heads of the plants were combined and cut into smaller pieces, some of which were randomly taken for further treatments. The same procedure was carried out with the lower parts of the plants. The dry weight of the plants was measured by drying a separate, weighed sample in a kiln (24 hours, 104°C).

The moist samples (m = 5–10 g) consisting of 2–5 cm straw pieces cut by scissors, were extracted with 100 ml sterile salt solution (9 mg NaCl ml⁻¹). The samples were extracted for 30 minutes and then homogenized (5 minutes, 230 rpm) with a Stomacher 400 Circulator (Merck Eurolab). Samples were analyzed by dilution plating. The microbial analyses were carried out using Hygicult® plates (Orion Diagnostica). The media used was Hygicult® Y&F for analysing yeasts and fungi and Hygicult® TPC for analysing total bacterial counts. Serial dilution was used to make the amount of colonies per plate countable. An amount of 50 μl of the diluted solution was pipetted to each Hygicult® plate. Plates were incubated for 5 days at room temperature, after which the colonies were counted and the microbial content (cfu per gram of dry mass) was calculated.

More profound microbiological analyses were carried out in order to determine the genera of the moulds. Samples taken during harvesting on 14 May 2001, before frost retting (linseed on 12 September 2002 and hemp on 19 September 2002) and during frost retting on 5 February 2003 were analyzed by the following method: The total numbers of aerobic bacteria and filamentous fungi were analysed by stomaching 10 grams of the plant material for 5 minutes. The aerobic bacteria were cultivated from three subsequent dilutions of the homogenate by surface spreading on Plate Count Agar (Difco 0479) supplemented with 0.05% cycloheximide. The incubation was carried out for 5 days at 30°C. Potato Dextrose Agar (Difco 0013) with 0.01% chloramphenicol and chlorotetraacycline and incubation for 7 days at 25°C was used for the enumeration of moulds. The moulds were identified to genus level by light microscopy.

Bivariate correlation analysis (Pearson’s correlation coefficients, two-tailed test of significance) of the SPSS statistical tool was used to examine the possible correlation of the microbial contents between the growing seasons 2001 and 2002.

Results

During the growing season of 2001 the conditions were mostly humid, warm during the daytime and rather cold during the night. The growing season of 2002 was rather dry but warmer than the growing season of 2001. The meteorological data are presented in Figures 1a and 1b. The air humidity in the middle of the hemp field was on average 10 percent higher than that measured outside the field. In 2001 the hemp stems reached a maximum height of over 3.5 m and a maximum diameter of 4 cm, but in 2002 the highest individual hemp plants were only about 2 m high and the diameter was less than 2 cm. In 2001 the stalks of the linseed grew to a height of 50 cm, but in 2002 the stalks were only approximately 30 cm high. At the harvesting time in autumn linseed stems were still unripened (seed mature). Hemp stems were also unripened. At the time of spring harvesting the hemp stems were overretted. Evaluation of retting degree was performed visually. In overretted stems the fibre strips were totally or almost totally detached from the stems.
The amount of microbes in field air during the growing season of 2001 varied from $1.6 \times 10^2$ to $1.6 \times 10^3$ cfu m$^{-3}$, which corresponds to the normal summertime outdoor air quality in Finland. During the growing season of 2002 the amount of microbes in field air was noticeably higher than during 2001. Fungal counts in the air during the growing season of 2002 were generally higher than $2.5 \times 10^5$ cfu m$^{-3}$.

The dry weight content of the hemp varied between 20% and 30% at the beginning of the growing season of 2001 and between 15% and 30% at the beginning of the growing season of 2002. At the end of the growing season of 2001 the dry weight content of the head of the mature hemp increased to 60%, but in the lower part of the mature hemp it was still only 30%. In the growing season of 2002 the dry weight content of both head and lower parts of the hemp plants increased to over 70%. In linseed the dry weight content was 20–30% at the beginning of the growing seasons of 2001 and 2002. At the end of the growing season of 2001 it was approximately 70% and at the end of the growing season of 2002 it was 70–80% at the head but only 40–50% at the lower part of the plant. In linseed the difference between the head and lower parts of the plants was not noticeable during the growing season of 2001.

As can be seen in Figures 2 and 3, the total bacterial count and the amount of moulds (cfu g$^{-1}$ dw) in
hemp varied between different parts of the plant. At the beginning of the growing seasons of 2001 and 2002 the amounts of both moulds and total bacteria (cfu g\textsubscript{dw}^{-1}) were higher on the lower part of the hemp than on the head of hemp. At the end of the growing seasons of 2001 and 2002 the mould and bacteria contents (cfu g\textsubscript{dw}^{-1}) were higher on the head than on the lower part of the hemp. In 2002 the difference between the head and lower parts was smaller than in 2001. As can be seen in Figures 4 and 5, in the case of linseed the difference between the top and lower parts of the plant was smaller than in hemp. In the case of both hemp and flax the mould and bacterial contents (cfu g\textsubscript{dw}^{-1}) increased at the end of the growing season of 2001 when the plants started to mature. During the growing season of 2002 the increase in mould and bacterial contents was also evident, but occurred more evenly throughout the whole growing season.

During the growing season of 2001 the total bacterial count in hemp varied from $3 \times 10^2$ to $3 \times 10^9$ cfu g\textsubscript{dw}^{-1} and during the growing season of 2002 from $1 \times 10^4$ to $3 \times 10^7$ cfu g\textsubscript{dw}^{-1} (Fig. 2). During the growing season of 2001 the total bacterial count in linseed varied from $9 \times 10^4$ to $2 \times 10^{10}$ cfu g\textsubscript{dw}^{-1} and during the growing season of 2002 from $3 \times 10^5$ to $6 \times 10^9$ cfu g\textsubscript{dw}^{-1} (Fig. 4).
The mould content in hemp during the growing season of 2001 varied from less than $1 \times 10^2$ to $5 \times 10^7$ cfu g\text{dw}^{-1} and during the growing season of 2002 from $6 \times 10^3$ to $5 \times 10^5$ cfu g\text{dw}^{-1} (Fig. 3). The mould content in linseed during the growing season of 2001 varied from $3 \times 10^3$ to $2 \times 10^7$ cfu g\text{dw}^{-1} and during the growing season of 2002 from $1 \times 10^4$ to $1 \times 10^6$ cfu g\text{dw}^{-1} (Fig. 5). According to the statistical analysis, there was no significant correlation of the microbial contents of hemp between the growing seasons of 2001 and 2002. Similarly, no correlation was observed in the case of linseed. The genera of moulds analyzed from the samples included Cladosporium, Fusarium, Penicillium, Mucor and Alternaria. The effect of fungicide on moulds appeared to be negligible both in hemp and in linseed.

The microbiological quality of spring-harvested plants from the growing season of 2001 (harvested in the spring of 2002) and from the growing season of 2002 (harvested in the spring of 2003) was measured. In addition to the results from the spring harvesting time, Tables 1 and 2 also contain results from the samples taken in autumn and during the frost-retting. There were some differences between the years examined. It is evident that the amount of spores and fungal filaments varied because of the weather conditions during the growing seasons.
### Table 1. The amount of bacteria in the field samples from Siuntio, Finland during the harvesting periods and frost-retting.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total aerobic bacteria (cfu g⁻¹)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spring 2001¹</td>
<td>spring 2002¹</td>
<td>spring 2002²</td>
<td>autumn 2002¹</td>
<td>autumn 2002²</td>
<td>winter 2002-2003¹</td>
<td>winter 2002-2003²</td>
</tr>
<tr>
<td>Hemp</td>
<td>3.7 × 10⁶</td>
<td>2.6 × 10⁸</td>
<td>3.7 × 10⁸</td>
<td>3.6 × 10⁶</td>
<td>1.9 × 10⁷</td>
<td>5.4 × 10⁷</td>
<td>7.2 × 10⁷</td>
</tr>
<tr>
<td>Linseed</td>
<td>4.4 × 10⁶</td>
<td>2.9 × 10⁹</td>
<td>3.1 × 10⁹</td>
<td>1.1 × 10⁶</td>
<td>3.4 × 10⁹</td>
<td>2.8 × 10⁹</td>
<td>7.2 × 10⁸</td>
</tr>
</tbody>
</table>

¹ Plate Count Agar (Difco 0479) + 0.05% cycloheximide. Incubated at 30°C for 3 days
² Hygicult TPC. Incubated at 22°C for 5 days

cfu = colony forming unit
– = not measured

### Table 2. The amount of moulds in the field samples from Siuntio, Finland during the harvesting periods and frost-retting.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mould content (cfu g⁻¹)</th>
<th>Mould genera recognized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spring 2001¹</td>
<td>spring 2002¹</td>
</tr>
<tr>
<td>Hemp</td>
<td>1.2 × 10⁶</td>
<td>4.4 × 10³</td>
</tr>
<tr>
<td>Linseed</td>
<td>6.7 × 10⁵</td>
<td>2.2 × 10⁶</td>
</tr>
</tbody>
</table>

¹ Potato Dextrose Agar (Difco 0013) + 0.01% chloramphenicol and chlorotetracycline + 0.02% Triton X-100. Incubated at 25°C for 7 days
² Hygicult Y&F. Incubated at 22°C for 5 days

cfu = colony forming unit
– = not measured
examined. The results from the samples analyzed by the Hygicult® methods TPC and Y&F, and the surface spreading method using Plate Count Agar and Potato Dextrose Agar were at approximately the same level, although the Hygicult® method appeared to give slightly higher results. The samples analyzed with different methods were separate but were collected from the same test square and processed at the same time.

Discussion

By combining the microbiological data with the meteorological data, the microbiological results can be better understood. Only a few reports are available combining both cultivation of hemp or linseed and the observation of microbiological and meteorological data. The lack of water during the growing season of 2002 affected the growth of the plants. Especially the dry beginning of the growing season of 2002 had an effect on the growth. In the case of linseed the effect of dry conditions during the growing season of 2002 can be seen in the smaller height of the plants. By comparing the dry weights of hemp in the years 2001 and 2002 it can be seen that the dry conditions during the growing season of 2002 also affected the dry weight content of the matured stalk. High moisture content will affect the quality properties due to increased microbial activity. Costs of drying and transportation have also been mentioned as a disadvantage of plants with a high moisture content (Amaducci et al. 2000). In that study the moisture content of hemp (36%) at the end of the cropping season was considered to be low compared with other plant species investigated. On the other hand, moisture contents in excess of 16% will lead to continued retting of fibres (Sultana 1992). Therefore all harvested bast fibre plant material should be dried if this moisture content is exceeded.

When evaluating our microbiological results it can be considered that the Hygicult® TPC and E dipslides were validated against swabbing and control plate methods and the results were at the same level (Salo et al. 2000).

When comparing the microbiological results of both hemp and linseed in the years 2001 and 2002 it can be seen that the air humidity affected the amounts of both moulds and other microbes in plants. During the growing season of 2001 the conditions were more preferable for microbes than during 2002. The air humidity was high at the time when the plants were no longer growing and were mature. According to Mercer (1992) the fungal growth tends to be poorer under wetter conditions, especially when these occur towards the end of the growing season.

The genera of the moulds analyzed in the samples included genera common in outdoor air and in soil. Some species in those genera can be toxic, and for example many species in the genus *Fusarium* can cause plant diseases (Horst 1990, Tuomi et al. 2000). The frost-retted raw material harvested in the spring of 2003 will be used for investigations of the preparation of thermal insulators. Because the plants analyzed will be used in experiments to test raw materials for insulators, it is important to know whether there are any toxic species in the raw material.

According to the result of the pretest the effect of the fungicide used appeared to be negligible. According to Mercer (1992) the use of fungicidal seed-treatment can help to reduce disease incidence. More data is needed to determine whether the amount of moulds in fibre plants can be controlled with any other fungicide.

Frost for any long period has been found to destroy young hemp plants (Berger 1969). Early frost has also been found to be a problem in the case of kenaf because it killed the crop before harvest and the plants developed fungus or mildew. Excessive fungus or mildew was found on the base of the stem, which could retard bacterial activity. More fibre was extracted from the head than from the base of the stem, which may be related to the presence of fungus (Ramaswamy et al. 1999). Hemp and kenaf have shown comparable results in crop yield and quality parameters (Amaducci et al. 2000).

According to the recommendations of the Ministry of Social Affairs and Health in Finland, the amount of fungal spores should not exceed $10^4$
cfu g⁻¹, and the amount of bacteria should be below 10⁵ cfu g⁻¹ in building materials (STM 2003). However, these limits are not used e.g. for thermal insulations. During the growing season of 2001 the mould content in fibre plants analyzed was higher than this limit value, but during the manufacturing of the insulators the raw materials will be heated and finished insulators contain various anti-mould agents, which reduce their tendency to mouldiness (Koivula et al. 2005). Further research is needed to provide more information on the distribution of the microbes in different parts of the plant and in the separated fractions. Only the fibre fraction is used for insulation materials, but most of the shives are separated before manufacturing of the insulators. In a study of Kymäläinen et al. (unpublished results), mechanically separated fibre contained less microbes than the dried stalk. The microbiological quality of hemp and linseed insulation materials is published in a separate investigation (Koivula et al. 2005). The present study provides a basic understanding of the microbial quality of stems during the growing and harvest seasons. The microbiological safety should be controlled throughout the whole production chain, beginning with the cultivation and harvesting periods.

Acknowledgements. This study is a part of the project “Emissions from thermal insulations” in the research program SUNARE (Sustainable Use of Natural Resources) funded by the Academy of Finland, which we thank for financial support. We also thank Irma Redsven, Leena Laitala and Hanna Kinnunen for assistance in this project.

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