Yinghua Tian,
***Xiaolan Liu,**
Xiqun Zheng,
Lu Wang

Antimicrobial Properties of Flax Fibers in the Enzyme Retting Process

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College of Food and biotechnology, Qiqihar University, Qiqihar, China;

***Key Laboratory of Processing Agricultural Products of Heilongjiang Province, Qiqihar University,** 161006 Qiqihar, P. R. China
Corresponding Author: Liu Xiaolan
E-mail: yinghua_tian@163.com, liuxiaolan001@126.com

Abstract
"GB/T 20944.3-2008--Part 3: Shake flask method" was applied to assess the antimicrobial property of flax fibres. The antimicrobial efficiency of flax fibre was represented as the absolute antimicrobial rate and relative antimicrobial rate. Cotton fibre served as a contrast in relation to the antimicrobial rate. The retted flax fibre showed an absolute antimicrobial rate against E.coli and S. aureus, but only presented a relative antimicrobial rate against S. cerevisiae. The contents of pectic substances of flax straw such as cerolipoid, hydrotrope, pectin, hemicellulose and lignin declined during flax retting, especially lignin, whose content declined from 24% to 7.13%, and gaps appeared between the fibre bundles. Consequently the antimicrobial efficiency of flax fibre decreased gradually. This experiment confirmed the existence of antibacterial substances, but exactly what compounds associated with flax exhibiting antimicrobial properties is not discussed.

Key words: *flax fibre, pectic substance content, antibacterial property, retting.*

Introduction

Flax (*Linum usitatissimum*) fibres have excellent qualities for use in textiles, non-woven fabrics, high quality papers and lightweight high-strength composites [1]. Textiles containing flax have been known to possess bacteriostatic or antibacterial characteristics, as well as more resistant to fungal growth than other natural fibres [2, 3]. It was well-known that aromatic compounds such as lignin in plants inhibit microbial degradation of lignocellulosic material [4]. High levels of aromatic components, particularly with the amount and diversity in flax core cells, suggest the possibility of enzyme inhibition during enzyme-retting [5, 6]. Moreover water extracts from late harvest flax have more inhibition for *E. coli* and *Streptococcus sp*., although the identities of such inhibitory substances are unknown [7]. However, the results from the study of Chun, D. T. W., et al. did not indicate that adding flax or increasing the flax content in denim provided any bacteriostatic properties against *S. aureus* or *K. pneumoniae*, and did not support the presumption that flax fabric is bacteriostatic or antibacterial against the bacteria, *S. aureus* or *K. pneumoniae* [8, 9].

Flax fibres are in the phloem tissue of the stem. The individual (i.e., ultimate) fibres are formed in bundles that encircle the core tissue [10]. The initial step for converting flax to linen is called retting. The pectin is partly removed in flax-retting, and the fibre bundles are separated from the core and other nonfibre cells [5, 11, 12]. Retting is one of the most important procedures in flax fibre production. Our previous research showed that bacteriostatic properties against *E. coli*, S*taphylococcus aureus*, *Bacillus subtilis* and *saccharomycetes* declined gradually in the process of flax stem to roving and fabric. This paper will report the relationship between the composition and structure of flax fibre as well as bacteriostatic properties in enzyme flax-retting.

Experimental

Sample

The flax (HeiYa 14) used was of the fibre flax type, grown in Heilongjiang Province. In the retting experiment 150 g of flax stems were soaked in tap-water for 8 h to remove soluble compounds and pigment in the flax stem, dried, then retted with 0.1% pectinase (Sigma, Denmark) in sodium acetate-acetic acid buffer solution, pH 5.5, giving a solution to fibre ratio (v/w) of about 10:1, and finally incubated at 36 °C for 8, 16, 24, 32 or 40 h, respectively. Samples were boiled in a pan of water to inactivate the enzyme and then air-dried. 1 cm of retted straw was tested for general antimicrobial effects.

Analysis of the pectic substance content of flax fibres

Method of quantitative analysis of the Ramie Chemical Components GB5889-86 was used to determine the pectic substance content of flax fibres.

Microorganism

The three microorganism species used in the antimicrobial test were *Escherichia coli* (a gram-negative bacterium), *Saccharomycetes* and S*taphylococcus aureus* (a pathogenic gram-positive bacterium). *Escherichia coli* and *Saccharomycetes,* selected due to their popularity in daily life. S*taphylococcus aureus* was used because it was the major cause of cross-infection in hospitals. The strains were maintained on Nutrient Agar (*Escherichia coli* and S*taphylococcus aureus*) or Potato Dextrose Agar (*Saccharomycetes*) slants, incubated at 37 °C and 28 °C, respectively, and stored at 4 °C.

Culture medium

Escherichia coli and S*taphylococcus aureus* were cultured on Luria Broth (LB) medium, respectively, containing the following: yeast extract 0.5%, peptone 1%, NaCl 1%, agar 2% & pH 7.4, and *Saccharomycetes* was cultured in Yeast Extract Peptone Dextrose Agar (YPD) medium containing as follows: yeast extract 1%, peptone 2%, Dextrose 2% & agar 2% [13] . The above mediums were sterilised at 121 °C for 30 min.

Protocol for assaying the antibacterial property of flax fibres

The protocol used to measure the antimicrobial strength of flax fibre was the modified method described in "Textiles--Evaluation for antimicrobial activity--Part 3: Shake flask method (GB/T 20944.3-2008)". 0.75 g swatches of retted flax fibre or cotton fibre (control sample) was mixed with 70 ml of phosphate buffer solution (PBS) in a 250 ml Erlen-

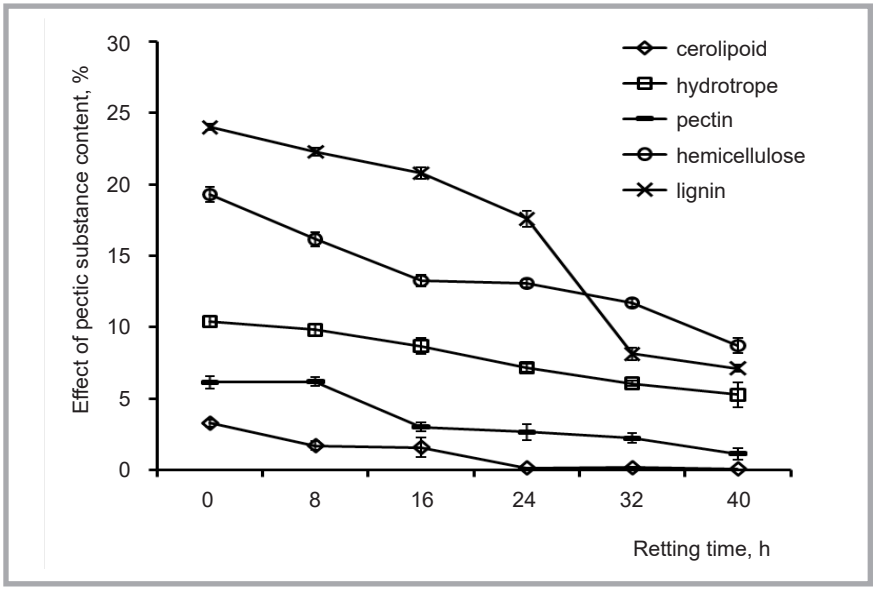

Figure 1. *Effect of enzyme retting on pectic substances content.*

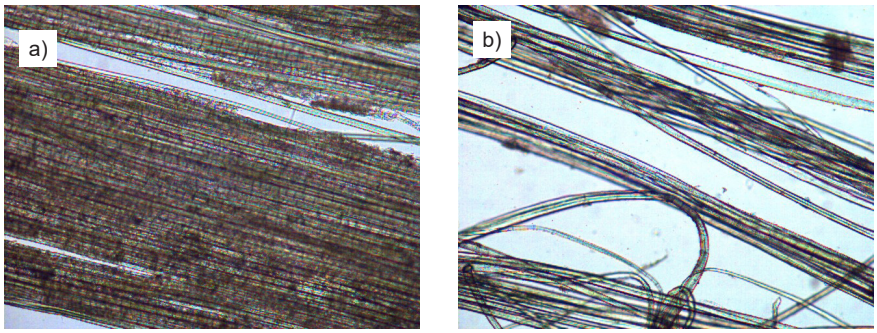

Figure 2. *Effect of enzyme retting on the pectic substance content of flax fibre: a) retted and b) unretted fibres under light microscopy.*

meyer flask, respectively, and then sterilised at 121 °C for 30 min. For each fibre treatment 4 sample replicates were made.

Before each antimicrobial test, *Escherichia coli* and S*taphylococcus aureus* were incubated in Luria Broth (LB) liquid medium, respectively, and *Saccharomycetes* in Yeast Extract Peptone Dextrose liquid medium (YPD) for 10 h. At the end of the incubation the density of cultures was diluted with sterile PBS to 1×10^5 CFU/ml - 5×10^5 CFU/ml by the Turbidimetric method. The turbidity was measured using an SHIMADZU UV 1750 Spectrophotometer (China) at 500 nm. 5 ml of the above-mentioned diluted bacterial suspension and PBS with the swatches of fibres were mixed and then shaken for 5 min to disperse them. 0.5 ml of the mixture was plated in petri dishes after serial dilution and then incubated at 37 °C (*Escherichia coli* and S*taphylococcus aureus*) or 28 °C (*Saccharomycetes*) for 24 h. The rest of the mixture was cultured by shaking for 6 h, then plated and incubated the same as above. The results are expressed as a percent reduction of bacteria (R) by ***Equation 1***, where A and B represent the colony numbers of before shaking the culture for 6 h and after shaking the culture for 6 h, respectively.

$$R = \frac{A - B}{A} \times 100 \text{ in \%} \qquad (1)$$

Table 1. *Antibacterial property of flax fibres during retting period: [a] Absolute antibacterial rate of fibres in %, [b] Relative antibacterial rates in %: D-value of antibacterial rate between flax fibres and cotton.*

retted flax fibre	cotton	0 h	8 h	16 h	24 h	32 h	40 h
E. coli	-72.55	46.62[a]	43.0 [a]	38.18 [a]	31.18 [a]	28.94 [a]	27.54 [a]
		119.17[b]	115.55 [b]	110.73 [b]	103.73 [b]	101.49 [b]	100.09 [b]
S. aureus	-54.80	42.65 [a]	40.23 [a]	38.31 [a]	31.82 [a]	14.95 [a]	14.27 [a]
		97.46 [b]	95.03 [b]	93.11 [b]	88.62 [b]	69.75 [b]	69.07 [b]
S. cerevisiae	-110.24	18.10 [a]	-12.84 [a]	-29.36 [a]	-40.37 [a]	-44.95 [a]	-49.16 [a]
		128.34 [b]	97.40 [b]	80.88 [b]	69.87 [b]	65.29 [b]	61.08 [b]

Results and discussions

The flax bast fibres are located inside the cuticle, where they are glued together to form fibre bundles that, in turn, are glued by middle lamella pectic substances both to the cuticle and woody core [14]. The pectic substances form an amorphous matrix which surrounds the cellulose fibres [15]. Pectic substances were composed of cerolipoid, hydrotrope, pectin, hemicellulose, lignin and so on. In the retting process, the substances above were partly hydrolysed and flax fibres were released from the matrix and other cohesive substances which bind the fibres to the shive via cortex cells and underlying secondary phloem [16].

The change in pectin content of flax fibre during enzyme retting was researched in this experiment. In a 40 h retting period the content of cerolipoid, hydrotrope, pectin, hemicellulose, and lignin of flax fibres was determined every 8 h. The effect of enzyme retting on the pectic substance content of flax fibre is presented in ***Figure 1***. With the proceeding of flax retting, the content of the five kinds of pectic substances above all decreased, but the variation tendency of that was not coincident. Pectin and hemicellulose were hydrolysed rapidly in 16 h retting periods, which led to the sum content of both decreasing from 25.45 to 16.29%, while the lignin content declined by almost 40% in 24 to 32 h retting periods. In the 40 h retting period, the total pectin content (22.39%) was almost one third of the unretted fibre's (63.16%).

The effect of pectinase enzyme retting on pectic substances of the flax fibres was also supported by observing the retted and unretted fibres under light microscopy (***Figure 2***). Unretted fibres (***Figure 2.a***) were observed with a substantial amount of large fragments attached to the shive and epidermal tissue. In contrast, the retted fibres (***Figure 2.b***) presented bundles with separated fibres mostly shive-free.

Three microorganisms (*E. coli*, *S. aureus* and *S. cerevisiae*) were applied in the antimicrobial test. The absolute and relativity antimicrobial rate of the retted flax fibres are given in ***Table 1***. As shown, in the retting process, the absolute antimicrobial activities of retted flax fibres against *E. coli* and *S. aureus* were moderate, and the absolute antimicrobial rates against *E. coli* were higher than *S. aureus;* however, the absolute antimicrobial activities against *S. cerevisiae* were rarely observed, which might be due to *E.coli* being a gram-negative bacterium, whose cell wall is thinner and the content of peptidoglycan is lower than *S. aureus,* which is gram-positive. And *S. cerevisiae* is a fungus whose cell wall is different from prokaryotes on the molecular components and structure [17, 18] .

The absolute antimicrobial rates of cotton fibres against *E. coli*, *S. aureus* and *S. cerevisiae* were all negative, i.e. after 6h incubation the colony numbers of culture with cotton fibres were all increased, with the largest increases of colony numbers for *S. cerevisiae*, resulting in a more than -110% antimicrobial rate of cotton fibre against *S. cerevisiae*. For this reason the highest relative antimicrobial rates of flax fibres against *S. cerevisiae* were observed. Especially the relative antimicrobial rate of unretted flax fibres reached 128.34%. The antimicrobial rate against *S. aureus* during the 16 to 32 h retting period decreased significantly, which was in accordance with the change in lignin content during the same period. A similar result was found for the antimicrobial rates against *S. cerevisiae* and for the pectin and hemicellulose content during the 16h retting period; however, as to which substance has a antimicrobial effect still needs to be studied. On the whole, the antimicrobial efficiency against the three microorganisms all decreased in the retting process, indicating that the antimicrobial substances might be removed or degraded with the flax pectin hydrolysed.

Conclusions

In conclusion, the modified GB/T 20944.3-2008 method used in this experiment is different from the AATCC Test Method performed in antimicrobial tests [19, 20]. The antimicrobial efficiency of flax fibres was characterised by absolute and relative antimicrobial rates in this experiment. The absolute antimicrobial rate represented an exceeding amount of inhibition against the microorganism over that propagation. However, the relative antimicrobial rate might be regarded as the interreaction of the microorganism inhibited and the propagation. Thus, even though the absolute antimicrobial rates were negative, it could not be considered that bacteriostatic properties of flax fibres were not in existence.

The content of lignin [11, 21], which is well known to inhibit microbial degradation of plants, declined from 24% to 7.13% in the retting process, and polysaccharides of flax fibres such as pectin and hemicellulose were hydrolysed to a low molecular carbohydrate weight,which might represent a carbon source for microorganisms. On the other hand, gaps in single fibres or in fibre bundles appeared and extended with the degradation of the pectic substance, which led to an increase in the contact area of bacteria to flax fibres in the retting process. This might be caused by the decrease in antimicrobial effects of retted flax fibres. The identities of the antimicrobial substance were not included in this study, which will be researched in a later experiment.

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