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Assessment of Flax Fibers Quality after Retting and Degumming Using Highly Efficiency Pectinase and Laccase

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ENZYMATIC retting and degumming are considered a promising replacement for traditional methods in terms of time-saving and ecology friendliness. The aiming of this study was the use of pectinase enzyme to accelerate the flax retting and reduce the use of harsh chemical in fiber bleaching with improving of fiber quality. The determination of weight loss, tensile strength, elongation, whiteness and yellowness of the fibers after treatments were evaluated. Pectinase activity in flax tissue was determined after application by immersion, soaking and spraying. The immersion of flax straw in pectinase enzyme solution after ultrasonication pretreatment was the best in enzyme activity in the tissues. *Aspergillus flavus* was potent in pectinase and laccase production. The pectinase and laccase enzymes were extracted from *Aspergillus pulverulentus*, and *Fusarium udum*. Mixture of pectinase and laccase resulted in reducing the retting completion time by 75%. The effect of four concentrations of mixed pectinase and laccase following the extra bleaching was studied in flax retting and degumming. The results showed that, the enzyme treatments reduced the retting completion time by 83.4%. The effect of the use of different chemical and physical pretreatments followed by laccase bleaching were showed that the best pretreatment of sodium hydroxide. Four concentrations of additional laccase bleaching treatment of lenin fabrics were assessed after pretreatment with NaOH 0.25%. The results showed that the whiteness and yellowness indices improved in the treatment receiving the highest laccase concentration (800 U/kg of fabrics).

Key words: Flax fibers, lenin fabrics, enzymatic retting, enzymatic bleaching, pectinase and laccase.

1. Introduction

Retting is the applied process of separation of fibers and fiber bundles from non-fiber tissues in the stems of flax plants. Degumming and bleaching are applied process for removal of waxes, gums and lignins from flax fibers. Bast fibers are processed by various means that may include retting, breaking, scutching, hackling, and combing (Kozłowski, et al., 2020). The quality and amount of extracted fibers depends on the retting and degumming processes and the mechanical decortication made during the breaking and scutching. In general, traditional retting and degumming include various methods for bast fiber extraction such as; physical, chemical, mechanical, biological and enzymatic methods. Classical water retting of flax fibers is an ancient process dating to the beginning of civilization. Traditional retting is based on an indigenous microbial population for release cellulosic fibers from fiber bundles. A modification of water retting is the enzymatic treatment, also called bioscouring, where degrading enzymes are directly added to tank water or in a bioreactor (Summerscales, 2021**)**. In recent years, a few fundamental studies have been initiated on the enzymatic retting process. Enzymatic retting is faster than traditional retting, readily controlled, and produces fewer odors, but further development is required to make it competitive with traditional

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methods. Pectinases were found in of flax retting to improve the mechanical properties of the fibers, increasing flexibility with good retention of tensile strength. Commercial enzymes as Viscozyme, containing cellulase as a component, can weaken the bast fibers, since the nodes of the fibers are particularly sensitive to the attack by this enzyme (Vahora, 2023). Pectinases are believed to play a leading role in the retting of flax fibers, in order to separate the fibers from stems, since 40% of the dry weight of plant cambium cells is comprised of pectin (Angulu & Gusovius, 2024). Pectinases effectively assist in degumming and retting of flax bast fibers by degrading the pectin located in the middle lamella and primary cell wall (Hossain et al., 2022). Pectinolytic enzymes are a heterogeneous group of related enzymes that hydrolyze the pectic substances, mostly present in plants. These enzymes are widely distributed in higher plants and microorganisms (Ayele, 2022). They also help maintain ecological balance by causing decomposition and recycling of waste plant materials (Koul et al., 2022). Pectinolytic enzymes, which are classified according to the main mechanism catalyzing the respective reactions, gather pectin esterase, polygalacturonase and pectin lyase. The pectate lyase also has been shown to be potentially important for retting bast plants (Datta et al., 2020). Polygalacturonase and pectate lyase are both depolymerizing enzymes for pectin. Also, pectate lyase carries out a non-hydrolitic breakdown of pectates and pectinases by a transillumination split of the pectic polymer (Bahtiyari et al., 2021). In order to bleach the flax and to keep the fiber tenacity high enough it is necessary to remove the lignin and partially to preserve the pectin. The problem of the classical hydrolyzing treatment with alkalis and oxidizers is due to the effect of these chemicals not only on the pectin and the lignin but also the cellulose itself resulting on the drastically decrease the material strength (Kostić, 2021). The studies on lignin biodegradation have been carried out mostly using white-rot fungi, *A. niger* which produce extracellular lignin modifying enzymes such as laccases and peroxidases particularly lignin peroxidases and manganese peroxidases (Wafaa, 2006; Abd El-Rahim et al., 2019; Wafaa et al., 2016). The white-rot fungi and their oxidative enzymes are environmentally friendly than harmful chemicals. Laccases catalyze the one-electron oxidation of phenolic compound and cleavage of alkyl-phenyl, Cα-Cβ bonds and phenolic lignin dimers (Houston, 2020). The relationship between lignin molecule degradation and decolonization of linen is important study area. For each enzyme, specific conditions are identified for employment in retting, since the activity can change dramatically with pH, temperature, and enzyme concentration. The aiming of this study was the use of pectinase enzyme to accelerate the flax retting and reduce the use of harsh chemical in fiber bleaching with improving of fiber quality.

2. Materials and Methods.

2.1. Intensity of pectinase activity in flax straw tissues treated with pectinase enzyme

Fungal pectinase enzyme obtained from strain *Aspergillus pulverulentus* described by Wafaa et al, (2020) was used in the concentration of (98.97U/mg) in this experiment. The main treatments applied in this experiment were as follows;

1. Control treatment of raw flax straw treated with pectinase enzyme solution.
2. Ultrasonic pretreatment of flax straw in 70% ethanol for one hour prior to enzyme application.
3. Heating pretreatment (50°C) of flax straw in water bath for 1 hour.

All samples were then rinsed five times in distilled water after all pre-treatments. Each of the above-mentioned treatments was supplemented with pectinase crude enzyme in the ratio of 25U/10g of straw. The pectinase activity of treated flax straw was measured each 2 hours throughout 30 hours by DNS method (Miller, 1959). The pectinase treatment was conduction by several approaches as follows:

A. Immersion of flax straw in pectinase enzyme dissolved in100 ml citrate buffer, then incubate in sealed plastic bag for 30 hours.
B. Soaking in pectinase enzyme dissolved in 100 ml citrate buffer for 2 minutes, then incubate in sealed plastic bag for 30 hours.
C. Spray of flax straw with pectinase enzyme dissolved in 10 ml citrate buffer, then incubate in sealed plastic bag for 30 hours.

All treated flax straw by the previous three methods w ere kept at 40^{o}C in incubator till the end of the experim ent. Assessment of retting flax completion was done by testing fibre separation periodically throughout 30 hours of retting.

2.2. Specially designed experimental bioreactor for retting of flax straw

An experimental cylindrical bioreactor (PVC pipes 6 inches diameter and 120cm length) was designed for flax retting using consortium of microbes. The bioreactor allows the testing of units separates 5 treatments each as 3 replicates. The volume of each treatment 3 replicate has the capacity of 30L. The flax straw retting was performed by immersion retting

method that uses consortium of microbes and enzymes. The bioreactor consists of 5 units each one 3 replicate three tires by covered covers 6inches diameter. Holes were opened in the covers for fixing funnels for adding mixtures of consortium microbes and enzymes. Vent holes were done for either aeration by compressor or pumping of the liquor solution. Valves with taps were fixed in each pipe for sampling. These units were painted in black colour to collect sun energy to accelerate retting and degumming of flax straw. The liquor to straw ratio (LR) in each unit of bioreactor was adjusted to30:1 (v/w).

2.3. Testing the efficiency of fungal pectinase and laccase enzyme in retting and degumming process of flax straw

In this study three fungal strains *Aspergillus flavus, Fusarium udum* and *Aspergillus pulverulentus* were used according previous studies (Moawad et al., 2019, Wafaa et al, 2020). The first strain was identified as effective producer of both enzymes, whereas, the second and third strain was distinguished by production of higher amount of laccase and pectinase respectively. Enzymatic treatment of fibres was pretreated followed the straw with ultrasonic pretreatment in 70% ethanol (LR 30:1 v/w) at room temperature for 1 hour. The free and combined enzyme treatments were as follows;

1. Control retting using ground water (collected from commercial retting factory).
2. Retting using fungal inoculate of strain *Aspergillus flavus,* F30.
3. Crude laccase enzyme (100 U/Kg straw) obtained from strain *Fusarium udum,* F25in acetate buffer.
4. Crude pectinase enzyme (2500U/Kg straw) obtained from strain *Aspergillus pulverulentus* in citrate buffer.
5. Mixture of free crude laccase (100 U/Kg straw) and pectinase (2500U/Kg straw) enzymes obtained from fermentation fungal strains.

The completion of flax retting was evaluated as previously described. Extra bleaching of flax fibres from the five above mentioned treatment was conducted using H_2O_2 (0.25%) at 95°C for 30 minutes.

2.4. Enzymatic retting and degumming of flax as compared with classical industrial retting

This experiment focused on the comparison between traditional industrial retting that include consortium of native microbes and enzymatic retting of fibres. Laccase and pectinase enzymes were used either in free state or by using fungal strains known to produce these two enzymes in large quintets. Fungal pectinase was obtained from supernatant of strain *Aspergillus pulverulentus and laccase from strain Fusarium udum*. Pretreatment of fibbers by heating at 60°C for one hour preceded the enzymatic treatment. This step is important for scouring the flax stems and facilitating enzyme attack to the propose sits of the fibers. All samples were then rinsed five times in distilled water. The treatments were as follows:

1. Conventional industrial retting.
2. Mixture of laccase (100 U/Kg straw) and pectinase (2500U/Kg straw) in retting liquor.
3. Mixture of laccase (200 U/Kg straw) and pectinase (3000U/Kg straw) in retting liquor.
4. Mixture of laccase (300U/Kg straw) and pectinase (3500U/Kg straw) in retting liquor.
5. Mixture of laccase (400 U/Kg straw) and pectinase (4000U/Kg straw) in retting liquor.

Enzymatic treatments were done in LR 30:1 v/w in citrate buffer (pH 5 and 0.1 M) at 40°C. All above mentioned treatments received extra bleaching of flax fibres using H_2O_2 0.25% in LR 30:1v/w at 95°C for 30 minutes. The completion of flax fibres retting was evaluated as previously described**.**

2.5. Enzymatic bleaching of flax fibres

Flax retted fibres were bundled in 50g sample and were exposed to several pretreatments in a trial to enhance bleaching of the fibres. The chemical pretreatments were used as follows; sulfuric acid 0.25%, sodium hydroxide 0.25%, sodium hypochlorite 0.25%, sodium carbonate 0.25% and hydrogen peroxide 0.25%. All pretreatments were performed in liquor ratio 40:1(v/w) at 95°C for 30 minutes. The physical pretreatments of bundles were soaked in70% ethanol and sonicated (35 kHz) at room temperature for 30 minutes. Other bundles treatment was the soak of the fibres in hot water at 50^{o}C for 30 minutes to facilitate removal of coating material on the cellulosic flax fibres. Bundles were then rinsed five times in distilled water after all pre-treatments. Enzymatic bleaching of the pretreated flax fibres was by fungal laccase isolated from *Fusarium udum* described by Wafaa et al, (2020) in concentration 400 U/Kg fibres. Then, incubated at 35°C for 2 hours in a liquor ratio of 30:1 v/w in 100 mM Na-acetate/acetic acid buffer pH 5. Laccase enzymatic bleaching of flax fibres was compared by traditional industrial bleaching. Flax bundles were impregnated in a solution containing 1% $NaClO_3$, 0.5% hexamethylenetetramine (HMTA) activator, 0.5% non-ionic wetting agent using a liquor ratio LR 40:1 v/wat 95°C and pH 10 for 2 hours followed by extra bleaching using hydrogen peroxide (0.5%) at 95°C for 30 min in the same ratio according to Elbana Tex Company, El-Gharbia, Egypt. After bleaching, the samples were thoroughly washed with hot and cold water and finally dried at ambient conditions. The bleached fibres quality was evaluated via measuring percent loss of fibre weight, tensile strength, elongation, whiteness index and yellowness index.

2.6. Enzymatic bleaching of linen fabrics using laccase

Ten grams of Lenin fabrics were submerged in laccase enzyme solution. The enzyme was extracted from fungal strain *Fusarium udum,* F25 producing large amounts of the enzyme (specific activity 0.93 U/mg). Pretreatment of the lenin fabrics was done using NaoH 0.25% at 95°C for 30 minutes. All samples after the previous pretreatment were rinsed five times with distilled water. Laccase enzymes were applied in100 ml 0.1 M acetate buffer pH 5 and kept at 35°C for 2 hours. Treatments are as follows:

1. Laccase enzyme (500 U/Kg fabrics).
2. Laccase enzyme (600U/Kg fabrics).
3. Laccase enzyme (700U/Kg fabrics).
4. Laccase enzyme (800U/Kg fabrics).
5. Control (regular chemical bleaching).

Extra washing of lenin fabric after the previous treatments was performed using diluted H_2O_2 0.25% for 30 minutes at 95°C.

2.7. Evaluation of bleaching and dying properties of lenin fibres and fabrics

- The microbial load in retting liquor was determined by measuring the optical density at λ_{600} nm by JENWAY-6305 UV-VIS spectrophotometer.

- The retting liquor colon was measured at λ_{300} nm. After colorization scanning by JENWAY-6305 UV-VIS spectrophotometer.

- Determination of fibres and fabrics weight loss was done by oven-drying method of 5g straw at105°C before and after retting until constant weight. The loss (%) in weight was calculated from a mean of three replicates (Ruan et al., 2015).

- The tensile strength (kg/den) and elongation (%) of fibers and fabrics were determined by the ASTM machine using test D5035 method. The strength of a fiber is determined by its ability to resist strain or rupture induced by tension. Tensile strength is expressed as the breaking load per unit cross-sectional area of the test specimen. It is an important physical property for fibers or fiber bundles in textile applications because the properties of textile structures such as ropes or geotextiles depend on a complex interrelation between fiber arrangement and fiber properties. The strength of a fiber is related to its internal molecular arrangement (Ruan et al., 2015& Ijaz et al., 2023).

- The whiteness index (WI) and yellowness index (YI) of fibers and fabrics were determined for treated and untreated fibers samples on Ultra Scan Pro. Hunter lab (Ruan et al., 2015& Raslan et al., 2024).
- The morphology of flax straw surface after retting of treated and untreated straw and fibers were examined by Scanning Electron Microscope S-530 (SEM).

3-Results & Discussion:

3.1. Intensity of pectinase activity in flax straw tissues treated with pectinase enzyme

The results in Table (1) show that the pectinase activity in the flax straw tissue was significantly higher in the straw pretreated with ultrasonication or heating as compared with the control treatment. The highest pectinase activity was recorded in the flax straw tissue pretreated with ultrasonication, followed by the heating pretreatment. The different methods of pectinase application to the pretreated flax straw showed that the immersion of the ultrasonically pretreated flax straw gave the highest pectinase activity as compared with the soaking and/or spray pectinase application. This may be due to the micropores created by ultrasonication which allow the penetration of pectinase to the flax tissue, which might accelerate retting of the flax straw. Similar results were obtained by (Desai, 2020; Babu and Chandrasekhara, 2022; Ahmed, 2024). Ultrasound is an ecofriendly technology being used in the textile industry, enzymatic treatment used in the bioscouring of cotton fibres (Grujić et al., 2023 & dos Santos et al., 2024). It would be worthwhile studying the impact of this ecofriendly technology for degradation of pectin from flax fibres (Shadhin, et al., 2023). The enzyme activity has been reduced dramatically in the three method of application (immersion, soaking and spray) after 8 hours of incubation. The extra reduction in pectinase activity was recorded after 18 hours of incubation. Similar treats in pectinase activity were observed in the heat pretreated of flax straw and the control samples. However, the reduction in pectinase activity in the heat pretreated of flax straw was reached after 10 hours whereas, in the control was reached after 12 hours. The results show that the ultrasonication of the flax straw helps in enzymatic retting of the flax fibres. Practically, when this treatment is combined with immersion the straw in pectinase enzyme solution. This may be due to the depletion of pectin in the flax tissue and the accumulation of D-Galacturonic acid in fermentation fluid which interferes with the enzyme activity. These results are in harmony with those obtained by another scientist (Chandel et al., 2022 & Wan Chik et al., 2024).

Table 1. Pectinase activity (U/ml) in flax straw tissue treated with different methods.

Sampling hours	Ultrasonic pretreatment			Heating pretreatment			Control treatment		
	Immersion	Soaking	Spraying	Immersion	Soaking	Spraying	Immersion	Soaking	Spraying
0	0.118 a	0.102 a	0.089 a	0.108 a	0.097 a	0.087 a	0.099 a	0.087 a	0.076 a
2	0.118 a	0.102 a	0.089 a	0.108 a	0.097 a	0.087 a	0.099 a	0.087 a	0.076 a
4	0.118 a	0.102 a	0.089 a	0.108 a	0.097 a	0.087 a	0.099 a	0.087 a	0.076 a
6	0.118 a	0.102 a	0.089 a	0.108 a	0.097 a	0.087 a	0.099 a	0.087 a	0.076 a
8	0.118 a	0.102 a	0.089 a	0.108 a	0.097 a	0.087 a	0.099 a	0.087 a	0.076 a
10	0.103 ab	0.090 ab	0.081 ab	0.108 a	0.097 a	0.087 a	0.099 a	0.087 a	0.076 a
12	0.093 bc	0.081 b	0.073 ab	0.093 ab	0.084 ab	0.075 ab	0.099 a	0.087 a	0.076 a
14	0.088 bc	0.077 b	0.069 b	0.083 bc	0.077 ab	0.069 abc	0.087 ab	0.075 ab	0.066 ab
16	0.086 bc	0.075 bc	0.068 b	0.079 bc	0.073 ab	0.065 bc	0.078 abc	0.070 ab	0.061 abc
18	0.084 c	0.074 bc	0.066 b	0.076 bc	0.070 ab	0.062 bc	0.073 abc	0.064 b	0.056 bc
20	0.065 d	0.057 cd	0.051 c	0.070 c	0.061 bc	0.054 c	0.067 bc	0.058 bc	0.048 cd
22	0.046 e	0.040 de	0.036 d	0.045 d	0.039 cd	0.034 d	0.054 c	0.043 c	0.033 e
24	0.027 f	0.024 ef	0.021 e	0.025 de	0.022 de	0.020 de	0.021 d	0.017 d	0.014 e
26	0.011 fg	0.010 fg	0.009 ef	0.010 ef	0.009 e	0.008 ef	0.006 d	0.004 d	0.002 e
28	0.003 g	0.003 g	0.002 f	0.003 f	0.002 e	0.001 f	0.001 d	0.001 d	0.001 e
30	0.000 g	0.000 g	0.000 f	0.000 f	0.000 e	0.000 f	0.000 d	0.000 d	0.000 e

Duncan's test: values followed by different letters are significantly different

3.2. Specially designed experimental bioreactor for retting of flax straw

A bioreactor was designed for performing the retting of lax straw. The pervious section showed that the pectinase activity in flax straw was the highest in the treatment of straw immersion in pectinase enzyme solution. The retting was performed in 30 L retting fluid, where 1 kg of flax straw was submerged. Each treatment was replicated 3 times. Fig. (1) shows the experimental cylindrical bioreactor. The bioreactor used in this experiment is made of 6inches PVC cylinders fixed in vertical stands. The bioreactor consists of 5 separate units each one replicated three tires. Each column is covered with plastic cover opened in the covers for fixing funnels used for adding mixtures of microbial consortium and any required chemical. Another venation holes for aeration of the fermentation liquor were also opened in the cover. Valves 1 inch diameter with installed taps were fixed in each pipe for sampling. These units were painted in black colour to collect sun energy to accelerate retting and degumming of flax straw. The liquor to straw ratio at the beginning of the experiment was 30:1 (v/w). Experimental cylindrical bioreactor was submitted as patent No. 31248 of flax retting and degumming using microbial consortium and mixture enzymes.

Fig. 1. Flax retting experimental cylindrical bioreactor.

3.3. Testing the efficiency of fungal pectinase and laccase enzyme in retting and degumming process of flax straw

The retting and degumming of flax straw was done in the small-scale cylindrical bioreactor using *Aspergillus flavus* strain F30 identified as potent of pectinase & laccase. The laccase and pectinase enzymes were extracted from culture filtrates of fungal strains *Fusarium udum* efficient in laccase production and *Aspergillus pulverulentus* active producer of pectinase described by Wafaa et al, 2020. The flax straws were submerged in cylindrical tubes of the bioreactor. The completion of retting process was different in the treatments presented in Table (2). The inoculation with fungal strain *Aspergillus flavus* and pectinase treatments accelerated the retting process as compared with control. The pectinase enzyme treatment highly accelerated the retting process. The laccase alone did not show any acceleration of retting process after 24 hours. Whereas, the pectinase alone or in mixture with laccase completed the retting process in 24 hours (reduced the retting completion time by 75%). The inoculation with fungal strain *Aspergillus flavus*, F30 active in production of pectinase and laccase enzymes completed the process in 84 hours. Laccase enzyme did not affect retting process completion. The fungal and enzymatic treatments effect on retting indices (Table 2) show that the pectinase treatment followed by the mixture of pectinase and laccase gave the best retting indices as compared with fungal and laccase treatments. The weight loss of flax straw, the strength and elongation of fibres and whiteness & yellowness were superior using pectinase enzyme as compared with other treatments (Table 2). The best whiteness indicator was found with laccase treatment. These results are in harmony with those obtained by (Mojtabavi et al., 2022; Ren et al., 2023; Pandey and Gupta, 2024). In contrary other reports recorded that pectinase used in acidic conditions weaken the treated flax fibres (Zhao et al., 2022; Eyupoglu et al., 2024; Wojtasik et al., 2024). The use of pectatlyase in alkaline conditions, however, was shown to maintain fibre strengths (Rehman et al., 2012; Zhao et al., 2022; Suhendar et al., 2024).

The pectinase enzyme is the enzyme responsible for the pectin degradation and separation of fibres from flax straw. Whereas, the degumming of flax straw is the responsibility of laccase. The electron micrographs of longitudinal sections of flax straw retted by conventional, fungal strain *Aspergillus flavus* and enzymatic treatments are illustrated in Figure (3) shows that all fungal and/or pectinase enzymatic treatments resulted in the degradation of pectin connecting the fibers with the straw. However, the pectin degradation was more pronounced in the treatment receiving pectinase enzyme compared to the fungal treatment or classical industry treatment. The use of laccase enzyme treatment alone did not act on pectin material in the flax straw. These results are in line with those obtained by other scientist (Różańska et al., 2023; Parameswaranpillai et al.,2023; Darie-Nita et al., 2022), who stated that, this result confirms the positive effect of enzymatic retting on the inter-fiber decohesion within bundles and thus on the fiber individualization of the flax retted. The use of hydrogen peroxide for extra bleaching of flax fibers after the retting process using different microbial and enzymatic treatments was studied. The best treatment for improving fibers quality (weight loss, strength and elongation) was achieved with retting using pectinase enzyme. However, the best treatment for whiteness and yellowness of fibers was the application of laccase and pectinase. The application of H_2O_2 treatment in the concentration of 0.25% for 30 minutes at 95^oC improved the whiteness of fibers. This promising treatment uses much less concentration of H_2O_2 as compared with commercial bleaching of fibers in industry plants which use 0.5% H_2O_2.The use of low dose of H_2O_2 after the enzymatic treatment for retting of the fibers proved to be more suitable for obtaining better fiber quality. The bleaching of flax fibers after retting is important particularly for improving whiteness index. The results (Table 3, Fig. 3) show that pectinase retting followed by bleaching gave the best values of weight loss (1.17%), fiber strength (0.597 g/den) and elongation (1.152%) as compared with other treatments. The whiteness and yellowness indices were the best in laccase treatment either alone or in combination with pectinase.

Table 2. Indices of flax straw retting and fibre quality after retting in cylindrical bioreactor using fungal and enzymatic treatments.

Indices	Treatments				
	Control	***A. flavus***	**Laccase**	**Pectinase**	**Laccase + Pectinase**
Retting completion	96	84	96	24	24
(h, %)	(0%) a	(12.5%) b	(0%) a	(75%) c	(75%) c
Weight loss (%)	9.12 cd	9.87 bc	11.35 a	8.74 d	10.87 ab
Strength (g/den)	0.738 a	0.713 a	0.598 a	0.794 a	0.682 a
Elongation (%)	1.567 a	1.598 a	1.681 a	1.534 a	1.624 a
Whiteness Index	-106.49 b	-101.76 c	-87.05 e	-111.9 a	-93.57 d

Duncan's test: values followed by different letters are significantly different.

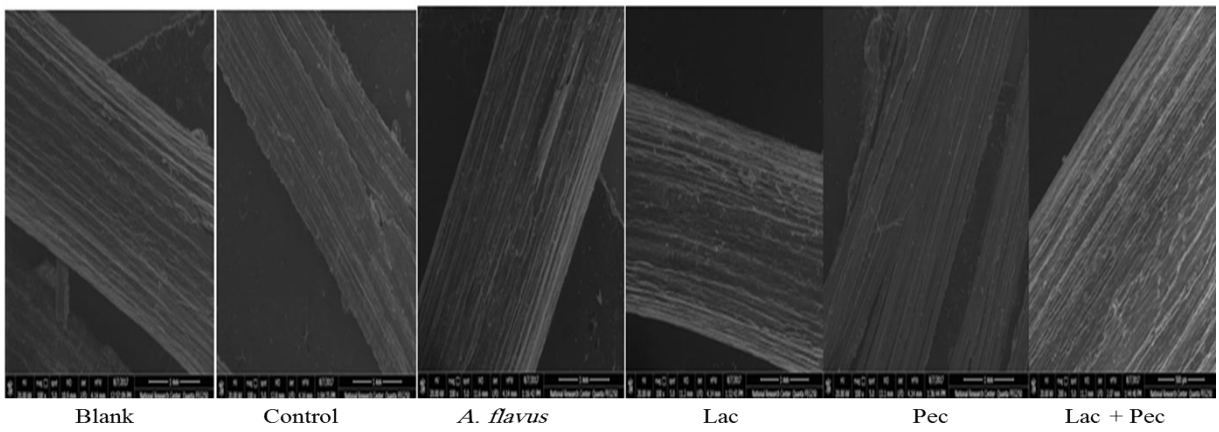

Fig. 2. Scanning electron micrographs of flax straw resulting from different retting treatments.

Table 3. Effect of fungal and enzymatic treatments on the bleaching of flax fibres using H_2O_2.

Indices	Treatments				
	Control	***A. flavus***	**Laccase**	**Pectinase**	**Laccase + Pectinase**
Weight loss (%)	1.23 a	1.35 a	1.54 a	1.17 a	1.47 a
Strength (g/den)	0.556 a	0.535 a	0.448 a	0.597 a	0.513 a
Elongation (%)	1.176 a	1.198 a	1.263 a	1.152 a	1.217 a
Whiteness Index	-63.89 ab	-61.056 b	-52.23 c	-67.14 a	-53.16 c
Yellowness Index	34.77 ab	33.51 ab	28.64 c	35.55 a	30.84 bc

Duncan's test: values followed by different letters are significantly different.

Fig. 3. Whiteness of fibers after improvement bleaching using 0.25% H_2O_2.

3.4. Enzymatic retting and degumming of flax as compared with classical industrial retting

In previous experiment using mixture of laccase and pectinase resulted in the acceleration of flax retting and fibre quality. In this experiment the retting and degumming of flax straw was done in the cylindrical bioreactor using two fungal enzymes. The fungal laccase and pectinase enzymes were extracted from culture filtrates of the two fungal strains; *Fusarium udum and Aspergillus pulverulentus* respectively. Laccase enzyme is known for degumming (removing of natural colour of lignin) of plant straw and fibres; however, it cannot complete the whole retting process which requires pectinase enzyme is for degradation of pectin layer responsible of adhesion of fibres with stem flax. In this experiment, mixtures of both

enzymes in different ratios were assessed in relation to retting and degumming of the flax straw. Four concentrations of mixed laccase and pectinase enzymes were used. The amount of pectinase in the enzyme mixture increased from 2500 to 4000 U/Kg straw. Since the role of pectinase is significant in the retting process, whereas, the concentration of laccase was increased in smaller increment between 100 to 400 U/Kg straw. The completion of retting using different concentrations of laccase and pectinase enzyme is presented in Table (4). All enzyme treatments significantly accelerated the retting completion with highest two enzyme concentrations in the mixture being the best in early completion of the retting process (20 hours). These results are very promising since it reduced the retting completion time from 120 hours in the industrial plant to 20 hours in the bioreactor with the mixture of the two enzymes. The best treatment for retting completion (20 hours) was correlated with an increased in straw weight loss (11.93%), strength of 0.654 g/den, modest elongation (2.292%), more whiteness index (-91.86) and lowest yellowness index (43.26) Table (4) and Fig. (4). These results suggest the advantage of using enzymatic retting to reduce the cost of retting technology in addition to improving the fibre quality. Similar results were obtained by (Lee et al., 2020, Ray et al., 2022, Huang et al., 2024). Laccase enzyme did not affect retting completion time. This likely may be due to the fact that laccase enzyme is known only for degumming (removing of natural colour of lignin) of plant straw and fibres. These results are in harmony with those obtained by (Al-Mamun 2020; Parameswaranpillai et al., 2023; Huang et al., 2024), who stated that, laccases are play important role in the degradation of lignin in natural fibres and catalyzes ring cleavage of aromatic compounds, therefore, such can be classed as lignin-modifying enzymes. The bleaching of flax fibres after retting is important to Additional improvement of the fibre quality. The use of hydrogen peroxide for extra bleaching of flax fibres after the retting process using different enzymatic concentration treatments was studied. The application of H_2O_2 treatment in the concentration of 0.25% improved the whiteness of fibres (Table 7 and Figure 7). This extra bleaching uses much less concentration of H_2O_2 as compared with the bleaching commercial used in industry. In this experiment the fibres previously exposed to enzymatic treatments were heated at 95^{o}C for 30 minutes in the bleaching solution (0.25% H_2O_2). The results in (Table 5, Fig. 5) show that H_2O_2 treatment on previously retted flax straw using mixture of laccase and pectinase resulted in better fibre quality as compared with the untreated fibres which were not soaked in H_2O_2 solution.

3.5. Enzymatic bleaching of flax fibres

In the previous section a mixture of laccase and pectinase enzymes was used of retting of flax straw. In this section the laccase enzyme either alone or with variety of chemicals was used for extra bleaching of flax fibres obtained by enzymatic retting in the previous section. The pretreatments of flax fibres after enzymatic retting were performed by soaking of the fibres in different chemicals (Table 6) for 30 minutes at 95^{o}C. The laccase enzyme was used in the concentration of 400 U/kg fibres. The fibres after pretreatments were soaked in laccase solution at 35^{o}C for 2 hours. The laccase enzyme was used in the concentration of 400 U/kg fibres. The pretreatment with chemicals were performed to help in bleaching of fibres particularly after extra bleaching with laccase enzyme known for removal /degradation of lignin. Two controls were used in this experiment. One is the flax fibres obtained from commercial bleaching factory and other is the fibres not treated with any pretreatments but only treated with laccase. Table (6) and Fig. (6) show indices of flax fibres quality after enzymatic treatment with laccase. The statistical analysis of the obtained date shows that the pretreatment with sodium hydroxide prior to laccase application induced significant effect in fibre tensile strength (0.61 g/den), elongation (1.229%), whiteness (-13.69) and yellowness indices (28.52) compared with other chemical and physical pretreatments. These results may be due to the important role of laccase enzyme in degrading the lignin (Immerzeel and Fiskari 2023; Sukyai 2023; Gupta et al., 2023; Alifia et al., 2024).

Table 4. Enzymatic retting and degumming of flax straw using laccase and pectinase.

Indices	Treatments				
	Control	**100 U Lac + 2500 U Pec**	**200 U Lac + 3000 U Pec**	**300 U Lac + 3500 U Pec**	**400 U Lac + 4000 U Pec**
Retting completion (h, %)	120 (0%) a	24 (80%) b	24 (80%) b	22 (81.7%) c	20 (83.3%) d
Weight loss (%)	9.65 a	10.15 a	10.79 a	11.37 a	11.93 a
Strength (g/den)	0.711 a	0.694 a	0.679 a	0.665 a	0.654 a
Elongation (%)	1.431 d	1.652 cd	1.871 bc	2.083 ab	2.292 a
Whiteness Index	-64.67 c	-98.42 b	-95.97 ab	-94.02 a	-91.86 a

*Lac, laccase, Pec, pectinase. Duncan's test: values followed by different letters are significantly different.

Fig .4. Whiteness degree of fibres after enzymatic retting.

Table 5. Effect of extra bleaching using 0.25% H_2O_2 on flax fibre properties previous enzymatic treatments.

Indices	Treatments				
	Control	100 U Lac + 2500 U Pec	200 U Lac + 3000 U Pec	300 U Lac + 3500 U Pec	400 U Lac + 4000 U Pec
Weight loss (%)	0.96 c	1.21 bc	1.38 abc	1.53 ab	1.67 a
Strength (g/den)	0.548 a	0.526 a	0.516 a	0.504 a	0.496 a
Elongation (%)	1.047 b	1.165 ab	1.299 ab	1.453 ab	1.587 a
Whiteness Index	-64.67 c	-52.59 b	-50.21 ab	-48.12 ab	-46.01 a

*Lac, laccase, Pec, pectinase. Duncan's test: values followed by different letters are significantly different.

Fig. 5. Whiteness of fibres after extra bleaching using H_2O_2.

Table 6. Quality of enzymatic bleached fibres after some additional chemical and physical.

Pretreatments	Indices				
	Weight loss (%)	Strength (g/den)	Elongation (%)	Whiteness Index	Yellowness Index
Traditional bleaching	2.86 a	0.302 c	2.965 a	-11.98 a	24.18 i
None	1.46 def	0.451 abc	1.64 e	-54.34 e	33.96 d
H_2O_2	2.45 ab	0.338 c	2.334 b	-12.21 a	26.78 h
Na OH	2.16 bc	0.601 a	1.299 g	-13.69 b	28.52 g
$NaClO_3$	1.89 cd	0.367 c	2.054 c	-39.41 c	31.32 f
Na_2CO_3	1.71 cde	0.409 bc	1.892 d	-48.63 d	32.69 e
Ultrasonic	1.09 fg	0.594 a	1.412 fg	-61.33 g	37.41 b
Heating	1.28 efg	0.539 ab	1.542 ef	-58.24 f	35.57 c
H_2SO_4	0.89 g	Nd d	Nd h	-67.55 h	39.33 a

*Duncan's test: values followed by different letters are significantly different.

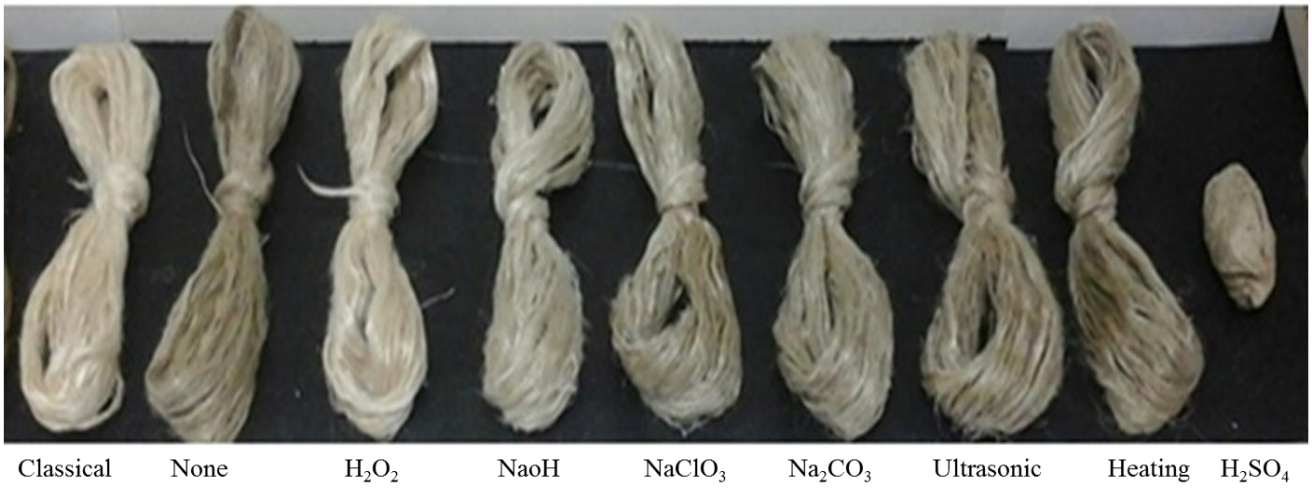

Fig. 6. Fibers whiteness degree after pretreatment with some chemical and physical methods followed by laccase enzymatic treatment.

In trail to ensure high bleaching and better fibre quality, the hydrogen peroxide was used as extra bleaching of flax fibres after using different chemical pretreatments followed by enzymatic bleaching. The application of H_2O_2 in the concentration 0.25% for 30 minutes at 95^{o}C extra improved the whiteness of fibres. The best treatment for improving fibres quality (strength, whiteness and yellowness) was achieved by using NaOH pretreatment as primary bleaching followed by enzymatic bleaching then extra bleaching of flax fibres using 0.25% H_2O_2. The fibres quality using the low dose of $H_2O_2$0.25%after the enzymatic bleaching of fibres proved to obtain better fibre quality. In commercial plants the H_2O_2 issued in concentration of 0.50%. The results (Table 7, Fig. 7) show that pretreatments using NaOH followed by enzymatic bleaching gave the best result of fibre strength (0.468 g/den), whiteness index (-5.66) and yellowness index (29.55) which were significantly different as compared with other pretreatments. The whiteness and yellowness of fibres obtained from NaOH followed by laccase bleaching by superior than any other pretreatments.

Table 7. Effect of extra bleaching by H_2O_2 0.25% after enzymatic bleaching on quality of flax fibres.

Pretreatments	Indices				
	Weight loss (%)	Strength (g/den)	Elongation (%)	Whiteness Index	Yellowness Index
Traditional bleaching	2.34 a	0.134 cd	2.235 a	-0.97 a	24.31 i
None	0.97 e	0.274 bc	1.501 de	-23.01 e	36.17 d
H_2O_2	1.97 b	0.184 c	1.896 b	-1.09 a	28.95 h
NaoH	1.63 c	0.468 a	1.687 c	-5.66 b	29.55 g
$NaClO_3$	1.38 d	0.213 c	1.632 cd	-11.05 c	33.22 f
Na_2CO_3	1.24 d	0.241 c	1.547 cd	-21.78 d	34.57 e
Ultrasonic	0.65 f	0.427 ab	1.084 f	-43.16 g	38.51 b
Heating	0.78 f	0.308 abc	1.349 e	-28.01 f	37.07 c
H_2SO_4	0.44 g	Nd d	Nd g	-44.75 h	39.83 a

*Duncan's test: values followed by different letters are significantly different.

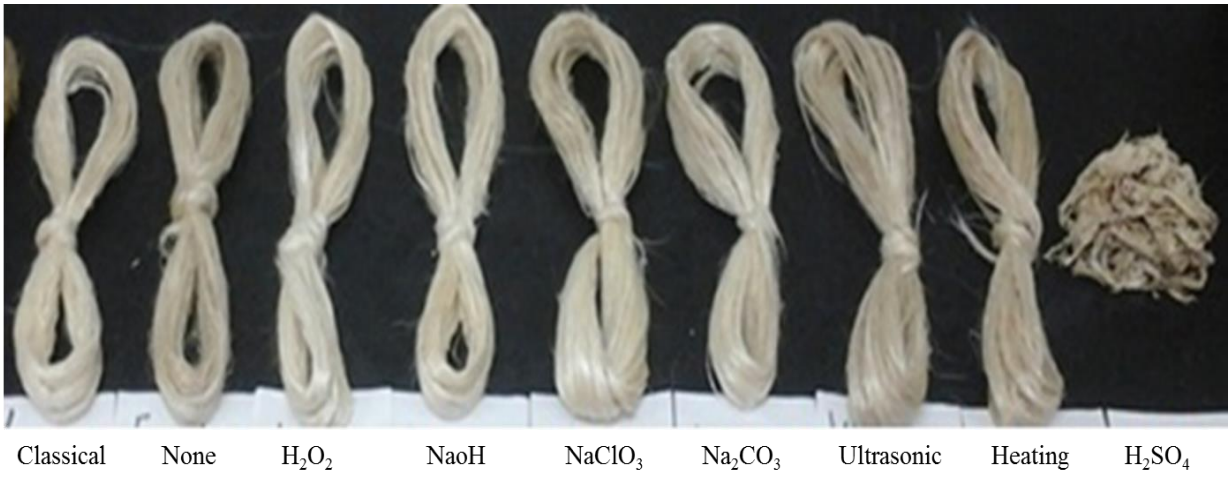

Fig. 7. Whiteness of fibres after extraH_2O_2 bleaching of fibres obtained from enzymatic retting followed by bleaching with laccase and chemicals.

3.6. Enzymatic bleaching of linen fabrics using laccase

In previous experiment the pretreatment of flax fibres with NaOH 0.25% prior to laccase bleaching treatment was found to enhance the bleaching process (Table 7). In the experiment four concentration of additional laccase bleaching treatment of lenin fabrics were assessed after pretreatment with NaOH 0.25%. The results in Table (8) the Lenin fabrics were exposed to different concentrations of laccase treatments after pretreatment by sodium hydroxide for 30minutes at temperature 95^{o}C. The duration of enzymatic bleaching of lenin fabrics was two hours. In Table (8) and Fig. (8), illustrate the results of laccase bleaching using of different concentration of the enzyme. Two controls were used in this experiment. One is the flax fibres obtained from commercial bleaching factory and other is the fibres without any treatment as raw fabric. The laccase treatment improved fibre quality as indicated by weight loss (1.52%), tensile strength (0.517g/den) and the elongation (1.542%) as compared with other treatments. Figures (42-44) show that better whiteness (-2.39) and yellowness indices (26.35) was observed in the treatment receiving the highest laccase concentration (800 U/kg of fabrics). These results are in line with those obtained by (Olczyk et al., 2021; Tülek et al., 2021; Niyonzima et al., 2023; Kujović et al., 2024). These authors reported the positive effect of laccase in delignification of fibres. On the contrary alkaline products such as treatment with NaOH used for bleaching resulted in unsatisfactory effect on fibre quality (Steffen, et al., 2024), in addition to the non-cost effective and not environmentally friendly approach (Lawal et al., 2024). To maximize economic yield and productivity, Egypt's cash crops, such as beans and flax, require new cultivation management and special care of procedures for post-harvest and processing (Moawad et al., 2005; Shamseldin, et al., 2014; Moawad et al., 2019; Wafaa et al., 2020).

Table 8. Effect of different concentrations of enzymatic bleaching by laccase on linen fabrics.

Indices	Treatments					
	Raw fabric	**Traditional bleaching**	**Laccase 500U/kg fabric**	**Laccase 600U/kg fabric**	**Laccase 700U/kg fabric**	**Laccase 800U/kg fabric**
Weight loss (%)	0 e	2.36 a	1.52 d	1.74 cd	1.91 bc	2.09 b
Strength (g/den)	0.679 a	0.403 e	0.517 b	0.478 c	0.457 cd	0.432 de
Elongation (%)	1.287 d	1.923 a	1.542 c	1.632 bc	1.749 abc	1.846 ab
Whiteness Index	-99.28 d	-1.64 a	-5.69 c	-4.76 c	-3.18 b	-2.39 ab
Yellowness Index	53.79 a	25.21 e	31.11 b	29.23 c	27.96 cd	26.35 de

*Duncan's test: values followed by different letters are significantly different.

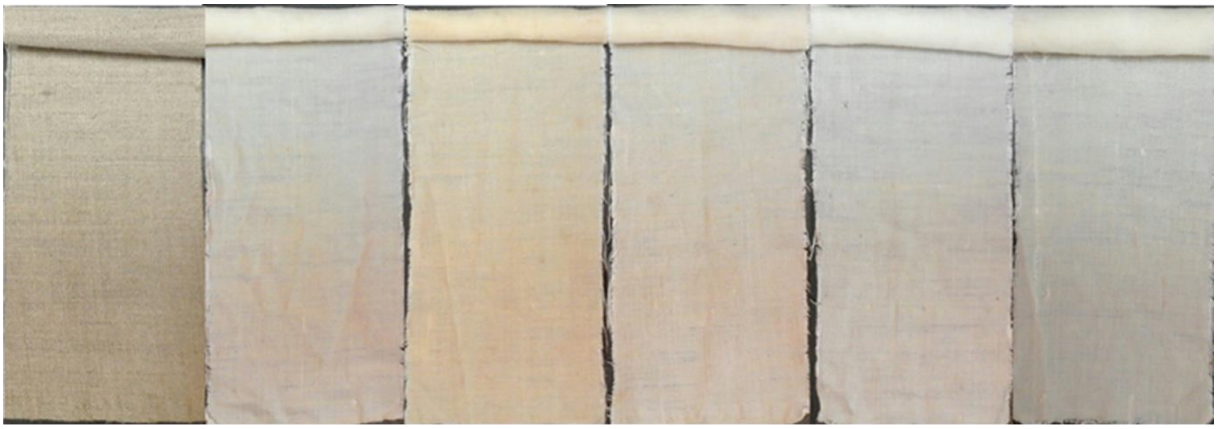

Fig. 8. Whiteness index of fabrics after bleaching using laccase.

4. Conclusions

The retting and degumming of flax straw is an important process through which the fibres are released from the cellular tissues. The results showed that the best activity of the pectinase enzyme and its effect on the flax tissue were in the pretreatment by ultrasonication followed by immersion in the pectinase enzyme solution. Thus, an experimental cylindrical bioreactor had been designed to retting the flax straw by immersion. The study resulted that the immersion of flax straw in the mixture of pectinase and laccase together led to a shortening of the time of retting completion by 75%. Also, the increase of concentration of the enzyme mixture from 100 to 400 units of laccase and 2,500 to 4,000 units of pectinase accelerated the process of flax retting at a rate of 83%, with retaining the quality of fibres in terms of strength and whiteness. The enzymatic bleaching of fibres and fabrics by laccase which was the cloth previously treated with 0.25% NaOH produced the most significant results in terms of whiteness and strength when compared with the use of environmentally harmful bleaching chemicals. This promising study is based on the development of modern biotechnological methods for the retting, degumming and bleaching of fibres, with a safe and ecofriendly way to reduce the harmful effects of chemical pollutants on health and the environment and on biodiversity. These treatments improved the fibre industrial and export qualities.

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