Effects of enzymatic treatment on cellulosic fibres from recycled paper. Analysis using a response curve experimental design.

M. CLAUDIA TALEB[1] AND MIRTHA G. MAXIMINO[2]

SUMMARY

An enzymatic preparation consisting of cellulases and hemicellulases significantly affected the drainability and strength properties of an unbleached softwood Kraft pulp repulped in laboratory.

The independent variables, treatment time and enzyme dose, were analysed using a central composite design for both the whole pulp and its fibrous fractions.

Statistical analysis showed significant improvements in drainability. The effects of enzymatic treatments on strength properties were: increase in tensile index and apparent density and decrease in tear index and light scattering coefficients.

Keywords

Cellulases and hemicellulases, experimental design, recycled fibre, softwood kraft pulp, drainability, stain.

Recycled fibre has become an important element in the pulp and paper industry. Apart from being a low cost source of fibre for paper and board manufacture, it contributes to preserve and economize scarce forest resources, to reduce landfill requirements, and to promote overall water and energy conservation. Worldwide more than one third of the paper is made from recycled fibres (*1*).

It is known that as the cycle number of chemical pulps increases, interfibre bonding decreases and as a consequence, there is a significant loss in the strength properties of paper. This loss is a function of two parameters: fibre flexibility (wet conformability) and changes occurring on the surface of the fibres (*2*).

Mechanical refining is typically used to recover strength properties in recycled fibres, however this generates large amounts of cellulosic fines which decrease the drainage rate of the pulp and therefore its paper production rate. Increased refining also limits the amount of strength that can be recovered in future cycles (*3*,*4*).

The number of potential applications of enzymes in pulp and paper manufacture has grown steadily, and they include enzyme-aided bleaching with xylanases, delignification with oxidative enzymes, energy-saving refining with cellulases, pitch reduction with lipases, drainage improvement with cellulases and hemicellulases, and enzymatic slime control on the paper machine (*5*).

In past years, enzymes have been applied to secondary fibres with the purpose of improving drainability and strength properties. Pommier et al (*6*) and Bhat et al (*4*) reported that celllulases and mixtures of cellulases and hemicellulases, at low concentrations, could be used to increase pulp freeness without affecting physical properties.

Some basic effects of the enzymatic treatment on recycled fibres have been analysed by Jackson et al (*7*), who characterized the effects on fibre surfaces and physical properties. They concluded that modification of secondary fibre with cellulases and hemicellulases could result in a substantial increase in pulp freeness with little or no loss in physical properties. Drainability increased when pulp was treated with cellulases but not with hemicellulases, which implies that hydrolysis of cellulose occurs without affecting fibre length. They also concluded that the fines are preferably attacked due to their high specific surface area, which is related to their flocculation with the enzyme.

Bhardwaj et al (*1*) evaluated the effectiveness of improving the secondary fibre drainability using commercial enzymes such as cellulases, hemicellulases and a mixture of both. For pulps with different initial drainabilities, Pergalase treatment was found to give the highest gain.

Stork et al (*8*) examined the ability of individual enzymes of the cellulase and hemicellulase system to improve recycled pulp properties. They concluded that the presence of endoglucanase activity is a prerequisite to improve the drainage of recycled fibres by enzymatic means. When cellobiohydrolase and xylanase are present, they act synergistically with endoglucanase to enhance its effects.

Eriksson et al (*3*), on the other hand, studied the freeness improvement in recycled fibres using enzymes (Pergalase A40) with refining. Their results confirmed drainage improvement for recycled fibres with minimal strength losses, particularly for board grades.

The effects of the enzymatic treatment with Pergalase A40 (a mixture of cellulases and hemicellulases) on fibres of an unbleached chemical pulp recycled in the laboratory are studied in the present work. The enzymatic treatment of the whole pulps and their fibrous fractions (F>100 in Bauer McNett classifier) were evaluated separately, focusing on the effect of selected factors (enzyme dose and treatment time) and their interactions in the development of the analysed properties.

MATERIALS AND METHODS

Pulp

A virgin unbleached pine Kraft pulp supplied by Papel Misionero S.A. mill (Argentina) in dry laps was used (kappa number 35.2). The pulp was refined in a PFI mill to 445 mL CSF freeness and then dried in a pilot paper machine, according to a previously published method (*9*). Recycled paper was considered as the initial state, and was used for the different enzymatic treatments.

Enzyme

The enzyme used for this study was Pergalase A40, a commercial product by Genencor International, Inc. It is a blend of enzymes, mainly cellulases and hemicellulases, obtained from Trichoderma longibrachiatum [Manufacturer's information].

Enzymatic activity was assayed with

[1]Researcher and corresponding author (email: turtaleb@fiqus.unl.edu.ar), [2]Associate Professor
Instituto de Tecnología Celulósica
Facultad de Ingeniería química
Universidad Nacional del Litoral
CP S3000AOJ Santiago del Estero 2654, Santa Fe, Argentina.

the dinitrosalicylic acid method (DNS), measuring the reducing sugars generated according to the Commission on Biotechnology, IUPAC. Xylanase activity was determined using birchwood xylan as the substrate for 30 min. at 45ºC, adjusted to pH 6 with 0.1 M phosphate buffer. Cellulase activities were assayed using carboxymethylcellulose and Whatman No. 1 filter paper as the substrates for 30 and 60 min, respectively, at 45ºC and pH 6.

One unit of activity, U, was defined as 1 µmol of reducing sugar produced per minute under the assay conditions. The results of enzymatic activities, in units per mL of solution, were:

- CMC: 2,624 U/mL
- Filter paper: 27.4 U/mL
- Xylan: 267.5 U/mL

These results indicate that the enzyme solution used has high cellulase and xylanase activities.

Experimental design and modelling

A central composite rotatable design was applied to analyse the two parameters, enzyme dose and treatment time.

This statistical design provides a systematic and mathematically structured method for investigating several variables simultaneously.

The first step in designing the experiments was to specify the range of factors. Thus,

$$E = (X_1 - 0.2) / 0.1 \quad [1]$$

$$T = (X_2 - 60) / 30 \quad [2]$$

Where:

E and T represents coded levels for enzyme dose and treatment time, respectively.

X_1 represents enzyme dose (% o.d. pulp) and

X_2 represents treatment time (min.)

Factors or variables were coded as shown in Table 1 and the calculations were based on Equations [1] and [2].

The experimental results obtained were subjected to analysis of variance using the Statgraphics plus v. 3-software package.

Experimental data were fitted to the following second–order polynomial equation, which, for two factors, is (see below):

where Y denotes dependent variables or responses and X_1 and X_2 the independent variables, enzyme dose and treatment time, respectively, specified in their original units. Parameters b_0, b_1, b_2, b_3, b_4 and b_5 are the regression coefficients to be estimated from the experimental values or data. The b values are constants and estimated or computed by the least–squares method.

Analysis of variance, regression equation, response surface, contour and residual plots were performed at 95% confidence level.

Treatment conditions

Enzymatic treatments were performed at pH 6; 45ºC and 10% pulp concentration, in a stainless steel batch reactor with indirect heating and mixing.

After the enzymatic treatment, the pulp was filtered on a Büchner funnel using a laboratory vacuum pump. The reaction was stopped by pouring the pulp slurry into a sodium hydroxide solution (pH = 12) and mixing for 15 minutes at room temperature; then the pulp was washed to guarantee complete alkali removal.

Reducing sugars, released during the enzyme treatment and analysed to determine the extent of enzyme hydrolysis, were estimated in the residual liquor using the DNS method.

Pulp fractionation

In order to distinguish the modifications produced on the fibres as a consequence of the different enzyme treatments, initial and treated whole pulps were fractionated in a Bauer McNett classifier according to SCAN-M 6:62 test method. Through microscope examination of the different fractions obtained, the F>100 fraction was chosen as representative of the "fibrous fraction".

$$Y = b_0 + b_1 X_1 + b_2 X_1^2 + b_3 X_2 + b_4 X_2^2 + b_5 X_1 X_2 \quad [3]$$

Table1
Coded and experimental values of treatment factors.

Coded level (E or T)	Enzyme dose (X_1), % o.d.p.	Treatment time (X_2), min.
-1.41	0.05	15
-1	0.1	30
0	0.2	60
1	0.3	90
1.41	0.35	105

Evaluation of fractionated and whole pulp properties

Drainability was evaluated for different treatments, both for the whole pulps and their F>100 fractions, using Canadian Standard Freeness (SCAN-C 21:65).

Hydration changes in the fibre wall were monitored using the water retention method (water retention value: WRV), by centrifuging (30 min at 1,650 x g) 1g of sample previously soaked in water for 24 h. The result is the average of two determinations. Total (WRV_t) and intrafibre (WRV_i) water retention values were determined according to Silvy's methodology (*10*), which applies ether to remove the residual fibre water and then evaporates it.

Fines analysis was performed with Britt's dynamic drainage jar with a 200–mesh screen, according to TAPPI Test Method T 261 cm-00. A portable Hach 2100 P turbidimeter was used to adjust the end point of fractionation so as to achieve consistency in measurements.

Handsheets were prepared with the whole pulp and also its F>100 (fibrous) fraction according to SCAN-C26:76 test method, and then conditioned according to SCAN P2:75 (50 ± 2 % RH, 23 ± 1ºC). The physical properties of the different pulps were determined according to TAPPI standards, except for the tear index and light scattering coefficients, which were determined using SCAN tests.

Optical microscopy was used to observe the surface condition of the initial and the treatment fibres by applying Simons´ differential stain, which is sensitive to variations in accessibility to the internal fibre structure (*11,12*).

RESULTS AND DISCUSSION

Table 2 summarises whole pulp and fibrous fraction properties. The corresponding response surface equations with their R-squared statistic (R^2) are shown in Table 3.

The Equations of the Canadian standard freeness of the whole pulps and their fibrous fractions (Equations 4 and 8, respectively) show that the enzyme dose (X_1) with positive sign and the squared

Table 2
Whole pulp and fibrous fraction properties.

		Whole pulps								Fibrous fraction						
X_1 (%)	X_2 (min)	CSF mL	Fines (%)	WRV_t g_{H_2O}/g_{fib}	WRV_i g_{H_2O}/g_{fib}	Apparent density g/cm³	Tensile index N.m/g	Tear index mN.m²/g	Light scattering coefficient m²/kg	CSF mL	WRV_t g_{H_2O}/g_{fib}	WRV_i g_{H_2O}/g_{fib}	Apparent density g/cm³	Tensile index N.m/g	Tear index mN.m²/g	Light scattering coefficient m²/kg
0	0	495	11.5	1.73	1.38	0.561	42.1	18.4	21.6	745	1.40	1.16	0.549	38.1	17.7	20.9
0.1	30	535	5.9	1.71	1.44	0.603	52.2	13.9	21.0	745	1.51	1.25	0.583	47.1	16.5	19.8
0.3	30	570	6.9	1.75	1.48	0.603	51.2	15.2	19.9	755	1.45	1.29	0.574	42.7	16.4	19.3
0.1	90	525	4.8	1.77	1.48	0.611	56.6	12.8	19.1	735	1.54	1.28	0.588	50.7	14.5	18.9
0.3	90	600	6.5	1.86	1.60	0.595	48.4	12.2	18.6	745	1.50	1.24	0.592	47.0	12.0	18.5
0.05	60	510	4.8	1.76	1.43	0.619	51.9	13.6	20.3	700	1.51	1.31	0.570	47.0	15.1	19.3
0.35	60	580	8.5	1.82	1.49	0.607	53.1	12.3	19.5	710	1.46	1.26	0.588	46.2	13.7	19.4
0.2	15	610	7.1	1.77	1.51	0.590	45.8	13.8	19.7	740	1.44	1.19	0.588	44.2	12.0	19.7
0.2	105	560	4.8	1.89	1.58	0.587	51.2	12.8	19.9	745	1.54	1.29	0.590	47.2	14.2	19.0
0.2	60	565	7.3	1.82	1.43	0.606	48.0	13.0	20.5	755	1.46	1.27	0.582	43.0	15.7	19.8
0.2	60	580	6.2	1.84	1.50	0.583	47.4	14.2	20.1	755	1.48	1.29	0.569	41.8	15.9	19.3
0.2	60	585	6.8	1.87	1.55	0.592	49.5	13.4	19.3	750	1.48	1.26	0.585	43.5	15.4	18.6

Table 3
Response surface equations and R-squared statistic (R^2) for the properties of whole pulp and fibrous fraction after enzyme treatment.

	Regression equations	R^2 (%)	
Whole pulp	CSF (mL) = $462.0 + 908.6X_1 - 1639.1X_1^2$	78	[4]
	Fines (%) = $5.6 + 9.7X_1 - 1.910^{-2}X_2$	78	[5]
	Tensile index (N.m/g) = $36.5 + 2.3X_1 + 0.3X_2 + 182.2X_1^2 - 1.2X_1X_2$	95	[6]
	Tear index (mN.m²/g) = $14.7 - 2.210^{-2}X_2$	47	[7]
Fibrous fraction	CSF (mL) = $682.5 + 707.4X_1 - 1768.4X_1^2$	70	[8]
	WRV_t (g_{H_2O} g^{-1}_{fiber}) = $1.5 - 0.2X_1 + 9.010^{-4}X_2$	82	[9]
	Tensile index (N.m/g) = $60.4 - 133.8X_1 - 0.1X_2 + 284.4X_1^2 + 1.410^{-3}X_2^2$	95	[10]
	Tear index (mN.m²/g) = $14.5 + 32.9X_1 - 6.210^{-3}X_2 - 66.0X_1^2 - 0.2X_1X_2$	95	[11]

X_1: enzyme dose. X_2: treatment time. X_1^2: squared enzyme dose. X_2^2: squared treatment time. X_1X_2: interaction of enzyme dose and treatment time.

enzyme dose (X_1^2) with negative sign had significant effects.

The R^2 statistic indicates that the model, fitted in this way, accounted for 78 and 70 % of the CSF (mL) variability of the whole pulp and its fibrous fraction, respectively.

Figure 1 shows the response surface and contour plot for CSF (mL) for the whole pulp with CSF values between 480 and 600 mL. As can be seen, the greatest values in drainability were obtained for high enzyme doses. In the case of fibrous fractions (Table 2), very little variations in drainability were found.

As shown by regression Equation 5, % fines were influenced by enzyme dose (X_1) with positive sign and treatment time (X_2) with negative sign.

Figure 2 shows the response surface corresponding to fines values from 3.7 to 8.7 %. The R^2 statistic indicates that the model, fitted in this way, accounted for 78 % of the fines (%) variability.

Contour plots in Figure 2 show that fines content increases when enzyme dose

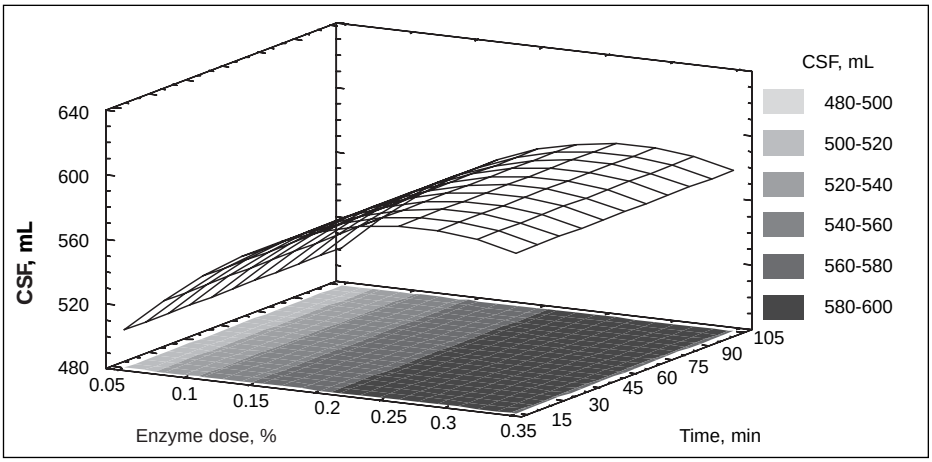

Fig. 1 CSF (mL) for the whole pulp: response surface and contour plot in function of the independent factors.

increases and treatment time decreases.

Regression Equation 6 for tensile index of the whole pulp shows that enzyme dose (X_1), treatment time (X_2), squared enzyme dose (X_1^2) and the interaction between enzyme dose and treatment time (X_1 X_2) had significant effects. All terms except the last one (X_1 X_2) have a positive sign. The coefficient of the squared enzyme dose is higher than that of the other factors.

For fibrous fraction, Equation 10 shows that enzyme dose (X_1) and treatment time (X_2) with negative sign and squared enzyme dose (X_1^2) and squared treatment time (X_2^2) with positive sign had significant effects.

The R^2 statistic indicated that the

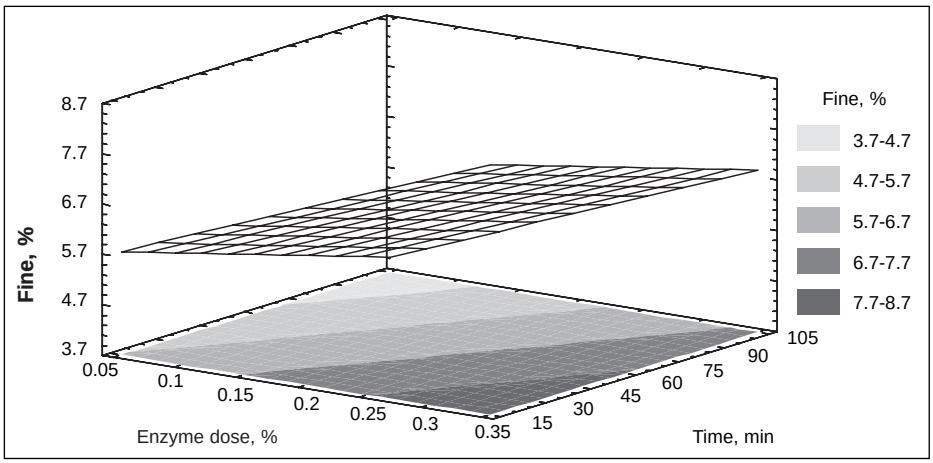

Fig. 2 Fines (%): response surface and contour plot in function of the independent factors.

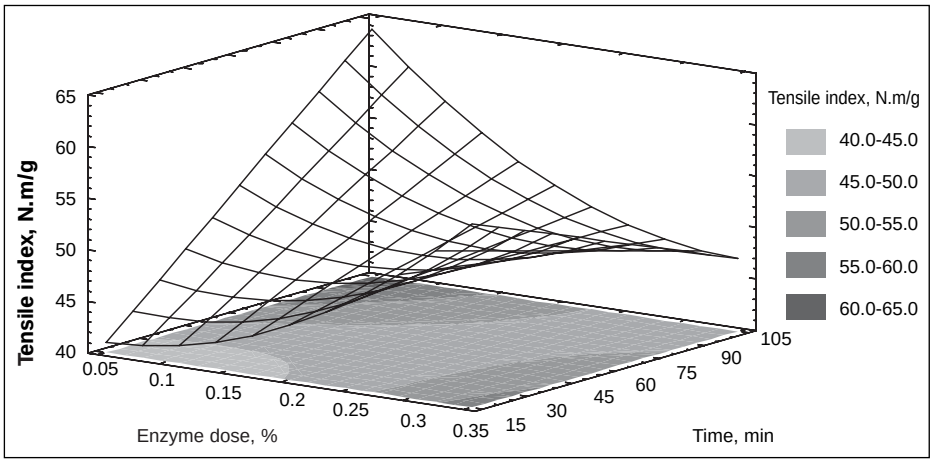

Fig. 3a Tensile index (N.m/g) for the whole pulp: response surface and contour plot in function of enzyme dose (%) and time (min).

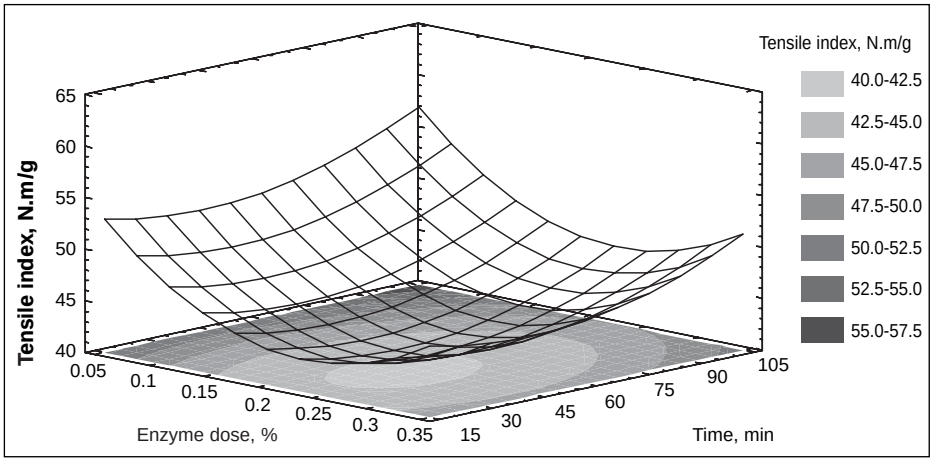

Fig. 3b Tensile index (N.m/g) for the fibrous fraction: response surface and contour plot in function of the independent factors.

model, fitted in this way, accounted for 95 % of the tensile index variability of both whole pulp and its fibrous fraction.

Figures 3a and 3b show the response surface and contour plot for tensile index of the whole pulp and its fibrous fraction, respectively. As can be seen, the greatest values in tensile index were obtained for low enzyme dose and high treatment time in both cases.

Equation 9 indicates the significant effects on total water retention value (g_{H_2O} g^{-1}_{fiber}) in the fibrous fraction. The enzyme dose (X_1) with negative sign and the treatment time (X_2) with positive sign had influence on this property. The R^2 statistic indicates that the model, fitted in this way, accounted for 82 % of the WRV_t variability.

Equations 7 and 11 show the influence of the enzymatic treatment on tear index ($mN.m^2/g$) for the whole pulp and its fibrous fraction, respectively. A low correlation was found for the whole pulp, time being the only significant effect. For the fibrous fraction, treatment time (X_2), squared enzyme dose (X_1^2) and interaction between enzyme dose and treatment time (X_1 X_2) show negative effect on this property, while enzyme dose (X_1) shows a positive effect.

Figure 4 shows the response surface with tear index values from 10 to 18 $mN.m^2/g$. The R^2 statistic indicates that the model, fitted in this way, accounted for 96 % of the tear index variability. A non-linear decrease in tear index was observed as the treatment time increased.

Optical microscopy: Simons´ stain

Simons´ differential stain is a mixture of a blue, low molecular weight dye and an orange, high molecular weight dye. With this mixture, fibres with large micropores (internal delamination, fibrillation or damage) are stained in orange; while the fibres with small micropores (non-refined or dried pulps) are stained in blue. When the fibres are stained in green it means that they have significant amounts of both large and small micropores (*11,12*).

Photographs in Figure 5 show fibres of the initial pulp (a) and a fibre after enzymatic treatment (0.1 % - 90 min) (b).

The untreated fibres showed two well-differentiated zones, a central one indicating large micropores (fibrillation and folds) and an outer one showing a mixture of large and small micropores. The enzymatically treated fibres showed similar characteristics to the central zone of initial fibres and changes in the fibre topography, which indicates the surface effects of the enzymatic action.

CONCLUSIONS

The experimental design chosen, with a limited number of experiments, allowed the analysis of two important factors (enzyme dose and time) of the enzymatic treatment with Pergalase A40, on the paper properties of recycled fibres.

Significant improvements in drainability (measured as CSF) were attained in all enzymatic treatments. The factor most strongly influencing CSF was found to be the enzyme dose. With regard to strength properties, although the enzyme led to a decrease in tear index in all cases, enzymatically treated pulps showed higher tensile values than the initial pulp. A gain

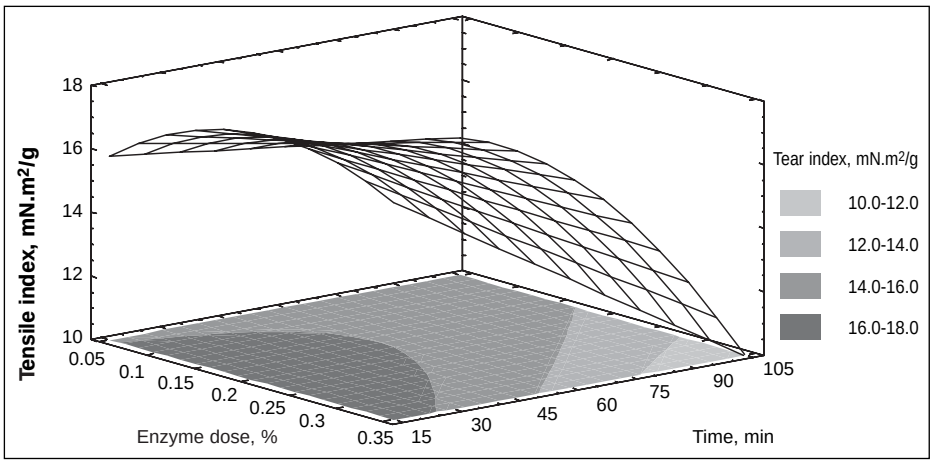

Fig. 4 Tear index (mN.m²/g) for the fibrous fraction: response surface and contour plot in function of the factors.

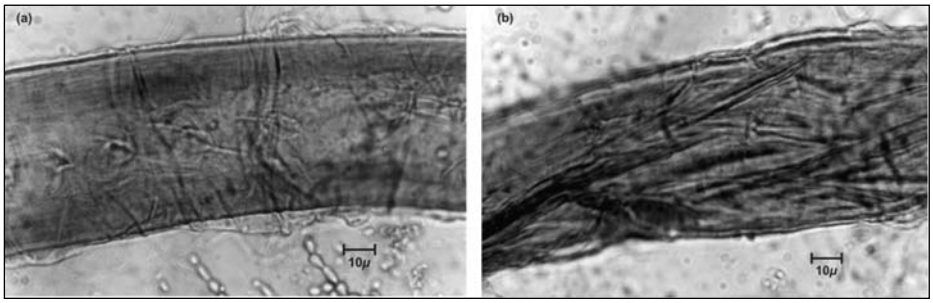

Fig. 5 (a) Microphotograph of a fibre without enzymatic treatment, (b) photograph of a fibre with enzymatic treatment (0.1 % - 90 min). Fibres were stained with Simons' stain.

of 34% in tensile index of the whole pulp was observed with 0.1% enzyme dose and 90 min time.

The statistical analysis did not show any significant effects on properties related to the internal structure of the fibre, such as water retention value, in either the whole pulp or its fibrous fraction. This is in agreement with Siika-aho et al (*5*), who stated that enzymatic treatments of wood and pulp could be considered as specific surface modification of fibre.

The surface effects of the enzyme were clearly shown by the optical microscopy.

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