Studies on the Use of Enzymes in Tanning Process: Part II. Kinetics of Vegetable Tanning Process**

by

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Abstract

The action of bacterial collagenase as an auxiliary agent in the tanning bath was studied. The use of bacterial collagenase enzyme has resulted in increased tannin absorption and better diffusion of tannins into the leather. Kinetics of tanning involving pretreatment with bacterial collagenase at different concentrations was studied. Tanning isotherms and the fit of different empirical equations (Parabolic, Cegarra-Puente and modified Cegarra-Puente equations) for the process were obtained. The modified Cegarra-Puente equation provides the best fit with the experimental values. The absorption rate constant calculated with this equation shows higher rate constants and faster kinetics for enzyme assisted tanning process. The enzyme treatment at different temperatures produced a significant increase in the exhaustion of tannins and the absorption rate constants, when compared to control. Enzyme treatment of leathers resulted in significant decrease in the apparent activation energy for the tanning process. 60% reduction in activation energy is observed for enzyme assisted tanning process compared to control as calculated from Arrhenius equation for enzyme treated leathers.

Resumen

La acción de colagenasa de origen bacterial como agente auxiliar en el baño del curtido fue estudiada. El uso de la enzima de colagenasa bacterial resultó en incrementada absorción de taninos y mejorada difusión de taninos dentro del cuero. La cinética de la curtición involucrando pretratamiento con colagenasa bacteriana a diferentes concentraciones fue estudiada. Curvas isotérmicas de curtición y el ajuste de diferentes ecuaciones empíricas (Parabólica, Cegarra-Puente, y Cegarra-Puente modificada) al proceso fueron obtenidas. La ecuación modificada de Cegarra-Puente dio el mejor ajuste con los valores experimentales. La constante para la tasa de absorción calculada en base a esta ecuación demuestra incrementadas constantes de velocidad de reacción y cinética más rápida para el proceso de curtición auxiliado enzimáticamente. El tratamiento enzimático a varias temperaturas produjo un significativo incremento en el agotamiento de los taninos y en los valores de las constantes de las tasas de absorción, comparadas al control. Tratamiento enzimático de los cueros resultó en una significante disminución en la energía de activación aparente del proceso de curtición. Reducción del 60% en la energía de activación observada en el proceso de curtición con auxilio enzimático comparada al del proceso de control calculado por medio de la ecuación de Arrhenius.

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INTRODUCTION

The present work describes an attempt to use collagenolytic enzymes in vegetable tanning and ascertain suitable methods for calculating the rate of diffusion of tannins with respect to absorption kinetics. Different types of tannins have different rates of absorption and this can lead to problems in achieving uniform tanning and maximum uptake of tannins. The major reason for the different tanning behavior of tannins in tanning process of leathers is their varying affinity for the leather substrate and the variations among the tannins themselves. The behavior of tannins is primarily determined by the charge characteristics of both the tannin and the leather to be tanned. Several studies on the adsorption kinetics processes of tannins in wastewater are reported in literature as effective methods for the removal of tannins from wastewaters.[1-3] However, as desorption of the tannins from the adsorbent is of no commercial significance to the leather industry, economically viable alternatives for use of such adsorbents are essential. Consequently, high exhaust methodologies with near zero pollution have gained significance. The present investigation reports the use of enzymes in tanning process and the study related to kinetics of absorption, which explains the rate of tannin absorption to the amount of tannin absorbed by the substrate at any time during the tanning process. This work would be of significance especially in developing countries where the effluent standards have become stringent.[4] Theoretical and experimental investigations to determine the nature and efficiency of the absorption process were carried out. A theoretical model which would enable the quantification of the rate of absorption and time required for optimal absorption based on the concentration of tannin in the tanning bath was also evaluated and reported. This work aims at a three-part elucidation consisting of using enzymes as pretreatment for diffusion[5] and absorption of vegetable tannins, followed by evaluation of absorption rate constant using modified Cegarra and Puente equations[6] and determination of the apparent activation energy[7] for the tanning process using Arrhenius equation for enzyme treated leathers. Therefore, in the present study, the tanning kinetics of leather treated at five different enzyme concentrations was carried out. The fit of different empirical equations (Parabolic, Cegarra-Puente and modified Cegarra-Puente equations) to the kinetics was studied.5[-12] The absorption rate constants of the tanning processes were obtained. Tanning isotherms were also obtained at different temperatures in order to determine the apparent activation energy and the absorption rate constants for each tanning temperature. These tanning isotherms were obtained for both control and enzyme treated leathers, at a concentration that produced the most significant kinetic change.

EXPERIMENTAL

Reagents and Chemicals

All the chemicals used up to tanning process were of commercial grade and the chemicals used for the analysis of spent tan liquors are of analytical grade. Post tanning chemicals were procured from M/s BASF India Ltd. The bacterial collagenase enzyme used was obtained from Bioscience Chemicals, Chennai, India, which is of technical grade.

Experimental Trials

Conventionally dehaired and delimed goatskins of area 5 - 6 sq ft from a same lot of similar weight range and grade were selected for the study. Three leathers were taken for each trial – quantity of chemicals calculated on fleshed weight. The samples were processed into tanned leathers as per the process described in Table I. The effect of collagenolytic enzyme pretreatment during vegetable tanning was studied, employing bacterial collagenase in the process mentioned in Table I. Experimental trials were carried out at different concentration, temperature and duration of enzyme treatment; subsequent to the enzyme treatment, tanning process was followed as mentioned in the table.

Control Tanning Process

The control tanning processes were carried out without enzyme treatment as given in Table I. Tanning process was also carried out at different temperatures of 25, 30, 35 and 40°C to study the apparent activation energies for plotting the isotherms. The process liquors from all the experimental and control trials were analyzed for the exhaustion of vegetable tannins. All the leathers after vegetable tanning were washed and piled for 24 h. The leathers were set, hooked to dry and stored at room temperature. Both experimental and control tanned leathers were post tanned using conventional post-tanning process.

Enzyme Assisted Tanning Process

The enzyme treatments were carried out at five different concentrations viz., 0.01, 0.02, 0.03, 0.04 and 0.05% at pH 7.2 with bacterial collagenase as given in Table I. To study the apparent activation energies, pre-treatment of enzyme during tanning were also carried out at different temperatures of 25, 30, 35 and 40°C at enzyme concentration of 0.02%.

Analysis of Exhaustion of Vegetable Tanning Spent Liquors

Spent tan liquors from control, all pickle-less enzyme tanning experiments and matched pair processes spent tan liquors were analyzed for % uptake of vegetable tannins.[13] Wattle was used for preparation of standard graph at various known concentrations. The sample was neutralized to pH of 7 using 0.1N NaOH. From the known concentrations of the sample, 0.5ml was taken and 0.5 ml water was added and made up to 1ml. 5ml of solution A (Solution A: 1ml of (1% $CuSO_4$ and 2% Sodium Potassium Tartrate) in the ratio of 1:1 + 50ml of (1g NaOH in 150ml water and 5g Na_2CO_3 added and made up to 250ml) was added to the samples and allowed to stand for 10 minutes. Then 0.5 ml of Follins reagent (Folin-Ciocalteu reagent - Folin : water (1:1)) was added to the sample and allowed it to stand for 30 minutes and the absorbance was measured at 660 nm using *UV–visible spectrophotometer (Hitachi, Japan)*. The respective absorption value for the particular concentration was plotted. From this plot, the amount of tannins present in the waste liquor (after filtering) was analyzed with the same procedure mentioned above and the exhaustion was calculated as;

$$\% \text{ Exhaustion} = \frac{(\text{Concentration of tannin given} - \text{Concentration of tannin in spent liquor})}{\text{Concentration of tannin given}} \times 100$$

Results And Discussions

Kinetics of tanning involving the enzyme pretreatment using bacterial collagenase for the improvement of the exhaustion of tannins is based on the concept that the enzymes act as biocatalysts in opening up of the fibrous collagen network there by enhancing the diffusion of tannins into the leather matrix on one hand and increasing the availability of more functional groups in the matrix for reaction with the tannins on the other leading to improved uptake of tannins. In the rate of tanning of an anisotropic material like leather, a plot of the relative uptake of tannins against the square root of the tanning time gives a straight line passing through the origin over most of the tanning time. The curve in short time of tanning would reflect the mode of the tannin penetration in the surface of the collagen matrix and the straight-line in the greater part reflect mode of tannin penetration in the bulk phase of the cross-section of the collagen matrix. In the case of vegetable tanning, the tanning curve is initially concave and becomes linear after some time. This leads to the assumption that a 'barrier' with small capacity of tannin exists at the leather surface. Such barrier in an earlier study of diffusion[14] was believed to be responsible for the non-Fickian isotherms.[14] If α/D, (where D is the diffusion coefficient in the cross-section of collagen) is constant, i.e. diffusion coefficient and a barrier to the diffusion of tannin molecules during tanning do not change, the solution for diffusion equation is one formally dealt with equation which is given by Crank and described by Vickerstaff[23]

$$Q_t / Q_\infty = \sqrt[2]{(D.t/\partial)} \qquad (1)$$

Equation (1) sometimes with satisfactory results is used in the early stage of diffusion, i.e. it applies in the initial stage of uptake for small time in connection with diffusion and surface evaporation. A plot of Q_t / Q_∞ against $t^{1/2}$ should therefore be linear with a slope proportional to the square root of the diffusion coefficient. In the presence of diffusion barrier the solution may be expanded in two forms for describing initial and ultimate behavior. The Equation (2) shows that, once the initial lag due to the barrier effect was overcome, a plot of Q / Q_∞ against $t^{1/2}$ approaches a line of slope 2 $(D\ t\ /\pi)^{1/2}$ with ^{t}an intercept on the sorption axis of $-D/\alpha$.

$$Q_t / Q_\infty = \sqrt[2]{(D.t/\partial)} - D/3 + 1/3^2\left(D^3/\partial\right)\sqrt{t} \qquad (2)$$

In the present study of tannin uptake by an anisotropic material like leather, we have assumed the early stage of tanning, which is generally accepted to be responsible for the tanning rate of the collagen matrix and uniformity of tanning in processing of leathers. We expressed the rate of tanning with wattle-vegetable tannin kinetics with standard $t^{1/2}$ plot. In order to compare the results of tanning in the presence and in the absence of enzymes the curves of Q / Q_∞ against $t^{1/2}$ were plotted. The $-D/\alpha$ values comprising the "entry" of the barrier factor were of assistance to understand the origin of surface layer barrier[12] and the role of enzyme in the mechanism of tanning with vegetable tannins. As shown in Figure 1, the asymptote to the curve is extrapolated to intercept the ordinate axis. The slope and the intercept of this line can be used to calculate diffusion coefficient D and the "entry" factor of the barrier α.[12] Q_t/ Q_∞ in Figure 1 represents uptake of tannins at time t as a fraction of the equilibrium uptake. This (Q_t / Q_∞) generally permits to examine the effects of various surface modifications on tanning of collagen fibers, with particular reference to the surface barrier effect. Using this technique and the rate of tanning equation for fibers and fiber bundles that are like cylinders show that modification of collagen matrix with enzyme removes the surface barrier and increases the diffusion coefficient. According to Medley and Andrews,[15] the "entry" of the barrier factor $\alpha = D_bQ_b / \delta Q_\infty$, where D_b is the diffusion coefficient in the barrier, Q_b is the equilibrium concentration of tannin in the barrier of thickness δ. To evaluate the effect on tanning of leathers produced by the enzyme treatment, absorption kinetics corresponding to the tanning isotherm processes was determined. The tannin absorption rate constants were calculated by fitting the experimental values to three empirical kinetic equations, Parabolic, Cegarra-Puente and modified Cegarra-Puente equations.[4-10] The apparent activation energy was also calculated using the Arrhenius equation.

Absorption Studies

Absorption studies were reviewed by several authors with specific reference to various auxiliaries and chemicals used in textile manufacture. Cegarra and Puente[5] had developed an equation that relates the rate of absorption to the amount absorbed by the substrate at any time during any process. Popescu and Segal[6] proposed a non-isothermal system in which the rate of absorption remains constant as temperature is varied. The study of Medley and Holstock[16] confirmed the results. Shibusawa[17] obtained a polynomial model, which agreed well with several auxiliaries. This model relates the ratio of the time related chemical concentration relative to concentration at equilibrium to the activation energy, rate of tannin absorption and time. Other models by Hill, Vickerstaff, Shelton and Patterson were compared by Cegarra and Puente[18] for dyeing polyester with disperse dyestuff. It was found out that the Hill's equation and Cegarra-Puente's model was the nearest to the practical values. Ryes and Sperb[19] had also proposed mathematical models for the absorption behavior of auxiliaries in batch, semi-continuous and continuous production in textile manufacture. In the present investigation, Parabolic equation, Cegarra-Puente equation, Modified Cegarra-Puente equation and Arrhenius equations were used to calculate the rate of tannin absorption to the amount absorbed by the collagen substrate at any time during tanning process.

Parabolic equation

Theoretically, equation (3) is adapted to the experimental results only from the beginning of the tanning process to half the tanning time, although in some cases it can also be used well beyond half time when the exhaustion levels are high. Q_t is tannin concentration at time *t*, '*K*' the absorption rate constant and 't' the tanning time.

Cegarra-Puente equation

$$Q_t = K\sqrt{t} \qquad (3)$$

The equation (4) is suitable for tanning in baths at constant concentration. *Q* is the tannin concentration of the leather at time *t*, Q_∞ is the tannin concentration at equilibrium, *K* is the absorption rate constant and *t* is the tanning time.

$$ln\left[1-\left(Q_t^{2}/Q_\infty^{2}\right)\right] = -Kt \qquad (4)$$

Modified Cegarra-Puente equation

Equation (4) is deduced for tannin baths with constant concentration. However, it can be adapted to tannin baths with varying exhaustion, introducing the appropriate modification, where '*a*' is the coefficient depending on exhaustion. The modified equation (5) is

$$ln\left[-ln\left(1-Q_t^{2}/Q_\infty^{2}\right)\right] = a\,ln\,t + a\,ln\,K \qquad (5)$$

Arrhenius equation

The activation energy may be regarded as the energy, which any molecule must acquire in order to become mobile and may be regarded as a measure of the barrier inhibiting movement of the molecule or substance. The apparent activation energy can be calculated using the Arrhenius equation (6). In common with most processes, a rise in temperature increases the rate at which the substance or tannin is absorbed. The effects of temperature changes are normally expressed in terms of the activation energy of the process. The activation energy is a reflection of the way in which the diffusion coefficient changes with temperature. For this purpose, the changes in diffusion coefficient are described by equation (7). R the gas constant (R = 1.9858 cal/mol °K), T the absolute temperature (°K), D_o is a constant independent of temperature. E is found from the slope of the graph of ln (D) versus 1/T.

$$D = D_o e^{(-E/RT)} \qquad (6)$$

$$ln\,D = -K/RT + lnD_o \qquad (7)$$

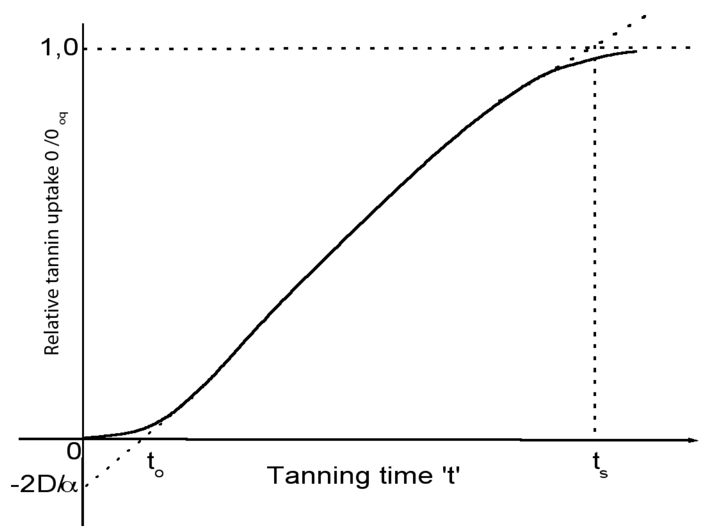

Figure 1. – The surface barrier effect for diffusion of tannins in vegetable tanning of leathers.

Absorption Kinetics of Enzyme Assisted Vegetable Tanning

Enzyme pretreatment was carried out at pH 7.2 because bacterial collagenase used in this study has pH 7.2 as its maximum activity. The absorption kinetics at different enzyme concentrations of control and experimental tanning are given in Figure 2. As seen in the figure, the enzyme pretreatment produces a faster rate of tanning for Wattle. The initial uptake of tannins was predominantly fast and reaches equilibrium after 60 min. The increase in the tannin absorption in the first stages of the tanning process produced by the enzyme treatment is significantly higher than control. Leather treated with 0.02% enzyme presents optimized tanning kinetic behavior faster to that of control leathers. For higher enzyme concentrations, the tanning rate is higher. However, a major significant difference in kinetic behavior for enzyme concentrations above 0.02% was not observed. Hence, tanning kinetics of 0.02% enzyme treated leathers was optimized for studying diffusion kinetics and relative tannin uptake at varying temperatures.

Absorption Rate Constants of Control and Enzyme Assisted Tanned Leathers

The absorption rate constant (*K*) obtained after fitting different kinetic equations and correlation coefficients '*r*' to the experimental results for wattle is given in Table II. These values correspond to the best fit, until values of exhaustion are about 97% of the final exhaustion (C_∞). From Table II, *K* and r^2 values were obtained from the modified Cegarra-Puente tanning isotherm as 0.1033 and 0.9983 for wattle treated at 0.02% enzyme at 35°C. The shape of the isotherm (Figure 3) and the r^2 values support the modified Cegarra-Puente model for absorption of tannins. The highest absorption rate constants correspond to the tanning processes carried out for enzyme treated leathers at 0.02% enzyme concentration. Hence, the results illustrate that the modified Cegarra-Puente equation fits the experimental values best for tannins. Plots of experimental values and the values fitted to the modified Cegarra-Puente equation for control and leathers treated with 0.02% enzyme were made and the same is obtained. As seen in Figure 3, absorption rate constants obtained from this equation or model (modified Cegarra-Puente equation) fits the experimental values the best.

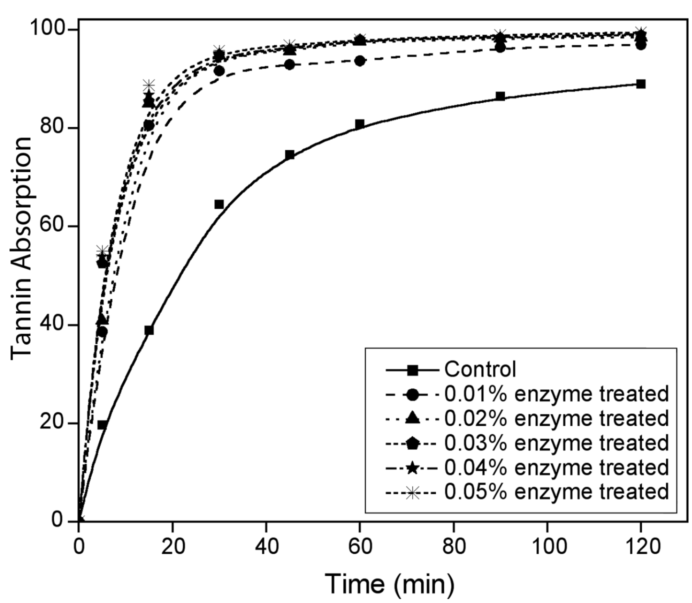

Figure 2. – Absorption kinetics of Wattle at 35 C and pH 7.2 for control and enzyme pretreated leathers.

Considering the values of the absorption rate from equation (5), it can be seen that the tanning carried out without enzyme exhibit the lowest values. From these results, it can be concluded that the enzyme pretreatment helps in the absorption and better diffusion of tannins, hence the kinetics of tanning become faster.

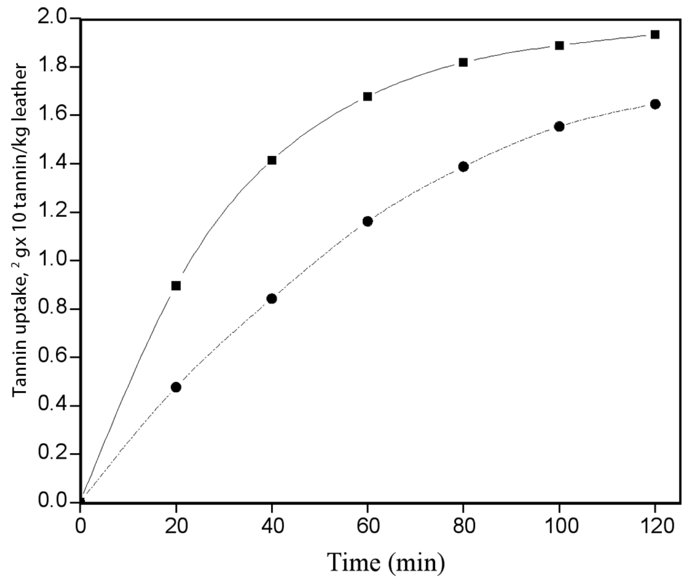

Figure 3. – Modified Cegarra-Puente equation fit to experimental values for control and 0.02% enzyme pretreated leathers with Wattle (■ - Experimental values of 0.02% enzyme treated leather, _____ Equation 3 (fit) of 0.02% enzyme treated leather; ♦ - Control values of leather, --------- Equation 3 (fit) of control leather).

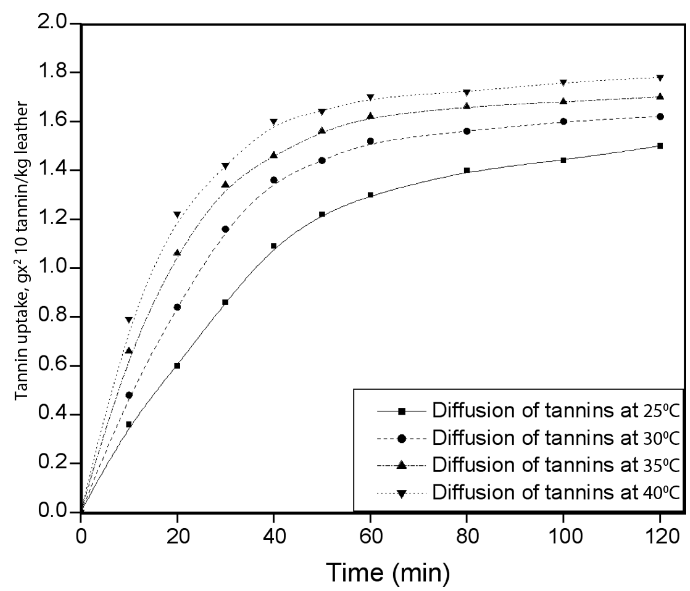

Figure 4. – Kinetics of absorption of Wattle at varied temperatures of enzyme (0.02% at pH 7.2) assisted vegetable tanning.

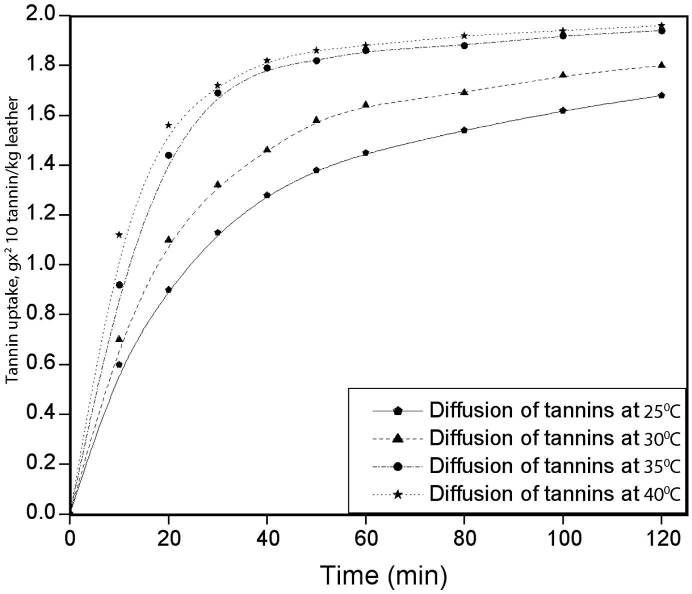

Figure 5. – Kinetics of absorption of Wattle at varied temperatures of control vegetable tanning.

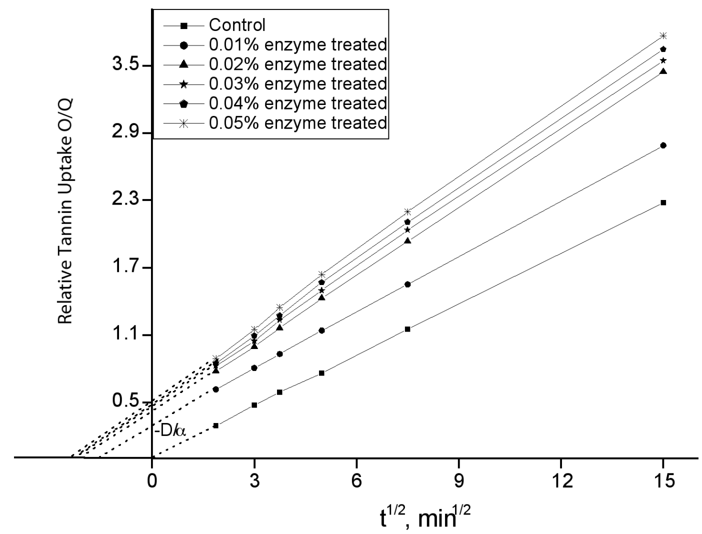

Figure 6. – Relationship of relative tannin (Wattle) uptake versus tanning time ($t^{1/2}$) at 35°C temperature.

Absorption Rate Constant and Correlation Constants at Different Temperatures of Control and Enzyme Assisted Leathers

Absorption rate constants at different temperatures for tanning with wattle at 0.02% enzyme treated leathers and control is given in Table III. The values fit well with the modified Cegarra-Puente equation. An increase in the tanning temperature leads to faster kinetics in the tanning of both control and enzyme treated leathers. The influence of temperature appears to be important for the control leathers, wherein tannin absorption is very low at 20°C. The values of *K* indicate that, for all the temperatures, the enzyme treatment produces a significant increase in the absorption rate constants compared to control leathers. The lower temperature results in greater difference between the absorption rate constants of control and enzyme treated leathers. The effect is more distinguished at lowest tanning temperatures, indicating insufficient diffusion energy when leather is tanned at low temperatures without enzyme treatment. Increase in the tanning rate was exhibited with enzyme treatment. From the present study, it is established that experimental leathers have good bulk properties, which are better in comparison to that of control leather. The increase in the tannin absorption produced by the enzyme treatment can be equivalent to an increase that is produced by ≥ 10°C in the tanning temperature of control leathers. Hence, the enzyme treatment not only improves the bulk properties of leathers but also enables tanning processes at lower temperatures. Tanning at low temperature is always an advantage from the point of view of saving energy.

Apparent Activation Energies of Control and Enzyme Treated Leathers

The apparent activation energies were determined from the absorption rate constants corresponding to the isotherms at different temperatures. From the absorption rate constants given in Tables III, the apparent activation energy (*E*) was calculated using the Arrhenius equation (Equation 6 and 7). The apparent activation energy is defined as the amount of energy required for the tannin molecules to overcome the resistance of the leather matrix to diffuse into them. A lower value of *E* means faster diffusion of the tannin into the cross-sectional matrix and a lower dependence on the temperature. The apparent activation energies and the correlation coefficients of the fit are also obtained as given in Table IV. A significant decrease in the activation energy can be observed when enzyme treatment is carried out prior to the tanning process. The activation energies for the tanning of the leathers treated with 0.02% concentration of enzyme are approximately half of those for the tanning of the control leathers. These results confirm that the enzyme treatment brings about a decrease in the resistance of leather for diffusion of tannin, resulting in enhanced absorption of tannins (Table IV). The tannin isotherms of wattle at varying temperature of enzyme assisted tanning and control are given in Figure 4 and 5. From the figures, it can be observed that diffusion of tannins increased with increase in tanning temperature in enzyme treatment and the control. However, the uptake of wattle in control process increases steadily whereas, the uptake values are significantly higher at 35 and 40°C temperature for enzyme treated leathers. Values for the initial kinetic constant *K* in the absence and the presence of enzymes are given in Table III. The absorption rate constant (*K*) and correlation constant (r^2) is comparatively higher at 35 and 40°C temperature of enzyme treated leathers as given in the Table III. It is clear from these results that the use of enzyme leads to a considerable increase in the apparent uptake, and that addition of enzyme render the whole quantity of tannin to penetrate into the collagen matrix more readily as suggested by kinetic constant *K*. It is well established that the enzymes assist in opening up of the fibrous collagen network at optimized concentration, pH and temperature of its maximum activity, there by enhancing the diffusion of tannins into the leather matrix.

Relative Tannin Uptake of Control and Enzyme Treated Leathers

The rate of tanning in the presence and absence of enzymes by plotting the values of relative tannin uptake Q_t / Q_∞ against tanning time $t^{1/2}$ is given in Figure 6. As shown in Figure 6, the asymptote to the curve is extrapolated to intercept the ordinate axis. The $-D / \alpha$ values comprising the "entry" of the barrier factor was used to interpret surface layer barrier and the function of enzyme in the mechanism of tanning with vegetable tannins. Q_t / Q_∞ in Figure 6 represents relative uptake of tannins at time *t* as a fraction of the equilibrium uptake, which is usually used to calculate the surface modifications, with particular reference to the surface barrier effect and compared to tanning of collagen fibres. From Figure 6, it can be observed that the enzyme treatment at 35 and 40 °C temperature results in higher and increased total tannin absorption and tanning rate when compared to control. Figure 6 also shows that the intercept on tannin absorption axis in the case of enzyme is significantly reduced, while with control it does not bring about any

TABLE I
Control and Experimental Vegetable Tanning Process

Process	%	Chemicals	Duration (min)	Remarks
Material: Delimed Goatskins from a similar lot				
Control Tanning Processes				
Control 1	100	Water		
	5	Sodium Chloride	30	pH 4.5 - 4.7
	0.75	Sulphuric acid	3x15 + 30	
	1	Basyntan P	60	Check Penetration
	20	Wattle	60	
Control trial groups Trial C1 – Treatment at varying temperature (25, 30, 35 and 40°C) Exhaustion at varying running times for control process at varying temperatures (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 and 120 minutes)				
	0.1	Formic acid	45	pH 3.8-4.0
Experimental Tanning Processes				
Experiment	100	Water		pH 7.2
Enzyme Treatment (Experimental trial groups) Trial E1 – Treatment at varying concentration (0.01, 0.02, 0.03, 0.04 and 0.05 %) Trial E2 – Treatment at varying temperature (25°C, 30°C, 35°C and 40°C) Optimized enzyme treatment at 0.020% enzyme, pH 7 for 30min				
Drain liquor				
	100	Water		
	0.1	Oxalic acid	10	
	0.1	Non swelling acid	10	
	1.0	Basytan P	60	
	20.0	Wattle	180	
Exhaustion at varying running times for Trial E1 and Trial E2 (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 and 120 minutes)				
	0.1	Formic acid	2x15 + 15	Check Penetration pH 3.8-4.0
All control and experimental leathers were sammed, split and shaved to uniform thickness (1.0-1.1 mm)				

TABLE II

Absorption Rate Constant (K) and Correlation Constants (r^2) for Wattle – Vegetable Tannin (fit to empirical equations) of Control and Enzyme Pretreated Leathers

Sample	Parabolic		Cegarra-Puente		Modified Cegarra-Puente	
	K (min^{-1})	r^2	K (min^{-1})	r^2	K (min^{-1})	r^2
Control	1.5246	0.9832	0.0514	0.9624	0.0512	0.9896
Enzyme treated at 0.01%	2.1232	0.9358	0.0836	0.9952	0.0798	0.9982
Enzyme treated at 0.02%	2.1246	0.9432	0.0962	0.9943	0. 1033	0.9983
Enzyme treated at 0.03%	2.3198	0.9799	0.1213	0.9951	0. 1048	0.9986
Enzyme treated at 0.04%	2.3139	0.9662	0.1142	0.9957	0.1056	0.9989
Enzyme treated at 0.05%	2.3098	0.9628	0.1129	0.9949	0.1078	0.9992

TABLE III

Absorption Rate Constant (*K*) and Correlation Constant (r^2) for Vegetable Tanning with Wattle at Different Temperatures of Control and Enzyme Pretreated Leathers

Sample Temp (°C)	Control		Enzyme pretreated*	
	K (min^{-1})	r^2	*K* (min^{-1})	r^2
25	0.0088	0.9812	0.0482	0.9869
30	0.0214	0.9878	0.0728	0.9942
35	0.0582	0.9889	0.1484	0.9984
40	0.0718	0.9898	0.1562	0.9986

* - at 0.02% concentration and pH 7.2

reduction. Hence, in the present study, enzyme assisted tanning in comparison with control produced a higher increase in the diffusion of tannins, tannin absorption and tanning rate, particularly in the early stage of tanning. It can also be seen from Figure 6 that at an increase in temperature for the tanning in the absence of enzyme a value – D/α remains significant, i.e. the tannin diffusion barrier at 35 °C as well as for 40 °C was important. As given in Table IV, the tannin absorption values of kinetic constant at lower temperatures for enzyme were significantly higher compared to those obtained for control at 0.02% and above tannin concentration. Using this diffusion concept and studying the rate of tanning equation for fibers and fiber bundles that is like cylinders shows that modifications of the collagen matrix with enzyme removes the surface barrier and increases the diffusion of tannins.

TABLE IV

Apparent Activation Energies and Correlation Constant (r^2) of Vegetable Tanned Control and Enzyme Pretreated Leathers

Sample	Wattle	
	E (Kcal/mol)	r^2
Control	39.948	0.9689
Enzyme pretreated*	16.131	0.9989

* - at 0.02% concentration and pH 7.2

CONCLUSIONS

The use of enzymes for absorption of tannins in leather processing was presented. The enzyme treatment of leather with bacterial collagenase resulted in an absorption efficiency of nearly 98%. All the enzyme-treated leathers showed an increase in tannin absorption when compared to control leathers. The absorption process follows the modified Cegarra-Puente equation and the enzyme treatment was found to produce a tanning process with higher absorption rate constants. Maximum tannin absorption with enzymes was obtained at an enzyme concentration of 0.02% with enhanced bulk properties in leather. The enzyme treatment produced a significant increase in the values of the absorption rate constants, when compared to control. At lower temperatures, higher differences in absorption rate constants were observed in enzyme treated leathers. The apparent activation energy was found to be significantly lowered on enzyme treatment. The decrease in activation energy indicates that the enzyme treatment can bring about enhanced tannin absorption at lower temperatures with saving in terms of energy and time. Since the enzyme treatment has resulted in the richness of shade, there is a possibility in achieving cost saving in leather tanning on optimization of the offer level of tannins.

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