

COLLAGEN AND POLYPHENOLS: NEW RELATIONSHIPS AND NEW OUTCOMES. PART 2. PHENOLIC REACTIONS FOR SIMULTANEOUS TANNING AND COLORING*

by

A. D. COVINGTON^{1**}, C. S. EVANS², T. H. LILLEY¹, O. SUPARNO^{1,3}¹British School of Leather Technology

University of Northampton

BOUGHTON GREEN ROAD

NORTHAMPTON NN3 2AL, UK

²Fungal Biotechnology Research Group

School of Biosciences, University of Westminster

115 NEW CAVENDISH STREET

LONDON W1W 6UW, UK

³Department of Agroindustrial Technology

Bogor Agricultural University (IPB)

KAMPUS IPB DARMAGA, PO. BOX 220

BOGOR 16002, INDONESIA

ABSTRACT (IN ENGLISH AND SPANISH)

Polyphenols are the basic building blocks of natural skin and hair pigments: they can be polymerised to create versions of natural black, brown and orange melanins. These reactions can be catalysed by laccase: not only do they create colour, but also, if they are conducted in the presence of collagen, the resulting pigment is bound to the leather in a covalent tanning manner. Therefore, the leather becomes coloured by a lightfast pigment, which is firmly fixed. It has not proved possible to create a pure black, but earth shades from green to brown/black are easily produced.

It appears to be a feature of this type of stabilising mechanism for collagen, the creation of a polyphenol matrix around the triple helix, that it has the added benefit of actually strengthening the leather. Since every traditional process step effectively weakens collagen, especially tanning, this is the first chemical reaction that has been shown to reverse that effect.

Polifenoles son los componentes básicos constituyentes de la piel natural y pigmentos del pelo: estos pueden ser polimerizados para crear versiones naturales de melaninas negras, pardas y anaranjadas. Estas reacciones pueden ser

catalizadas por laccasa: no solo creando color, pero también, si se llevan a cabo en presencia de colágeno, el pigmento resultante queda fijado al cuero de manera covalente como un curtido. Es así como el cuero resulta coloreado por un pigmento firmemente fijo y sólido a la luz. No se ha logrado todavía un negro puro, pero tonos tierra desde verde a pardo negrusco son fácilmente producidos.

Aparentemente un aspecto de este mecanismo de estabilización del colágeno, la creación de una matriz polifenólica alrededor de la triple hélice, trae como beneficio adicional, el reforzamiento del cuero. Ya que todo paso adicional en el proceso tradicional efectivamente debilita el colágeno, especialmente en el curtido, esta es la primera reacción química que sea ha demostrado capaz de revertir este efecto.

INTRODUCTION

Leather is invariably coloured with dyes to enhance its aesthetic/fashion appeal. The science and technology of the use of synthetic dyestuffs are well known in the art of leather making. Typically, color is conferred by applying acid or premetallised dyes, which produce bright colours, even on the blue background of a chrome tanned substrate.

However, common problems associated with these dyes are incomplete lightfastness and colour transfer in rub testing: the former is inherent in the chemistry of the individual dye and the latter may be a consequence of the tanner building the dye concentration on the surface, attempting to achieve deep shade.

An alternative route to achieving colour is to exploit the chemistry of creating the natural skin and hair pigments: orange, brown and black melanins. Certain reaction products of laccase-mediated oxidation and polymerisation of phenolic compounds can give coloured products, of which melanins are examples.^{1,2} Synthesis of melanin is a process occurring in diverse biological systems, in which mono- or polyphenolic substrates are oxidised to their respective quinones, which in turn undergo non enzymatic polymerisation to produce pigmented products.^{3,4} Since the reaction produces quinoid intermediates, it is likely that the products will bind at least partially covalently to the leather substrate:⁵ this would confer a tanning effect and contribute to wash and rub fastness. Furthermore, since the reaction is oxidative, the products should exhibit good light fastness.

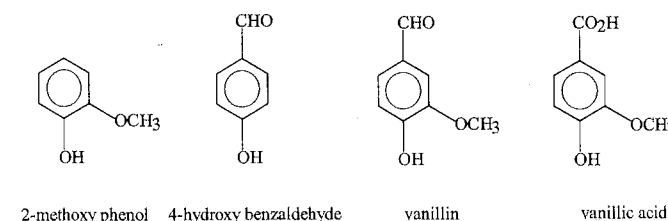


Figure 1. - Products of lignin breakdown.

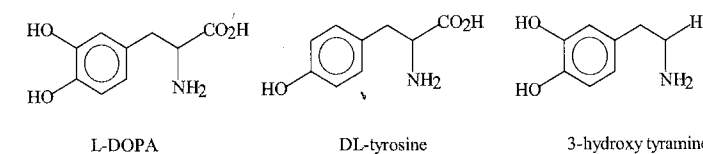


Figure 2. - The structures of aminophenols.

In order to investigate the influence of phenol structure, four groups of phenolic compounds were used in the experiments.

a. Lignin degradation products.

Studies on the biomimetic breakdown of lignin revealed the dominant reaction products are 2-methoxyphenol, 4-hydroxybenzaldehyde, vanillin and vanillic acid,⁶ see Fig. 1. Their use in this context would offer the opportunity to add value to a byproduct of the paper industry.

b. Simple phenols.

This group comprises the di- and trihydroxy phenol mononuclear species: catechol, resorcinol, hydroquinone, pyrogallol and phlorogucinol. They were also used in Part 1 of this series of papers as models for flavonoid tannins, see Part 1 of this series of papers.⁷

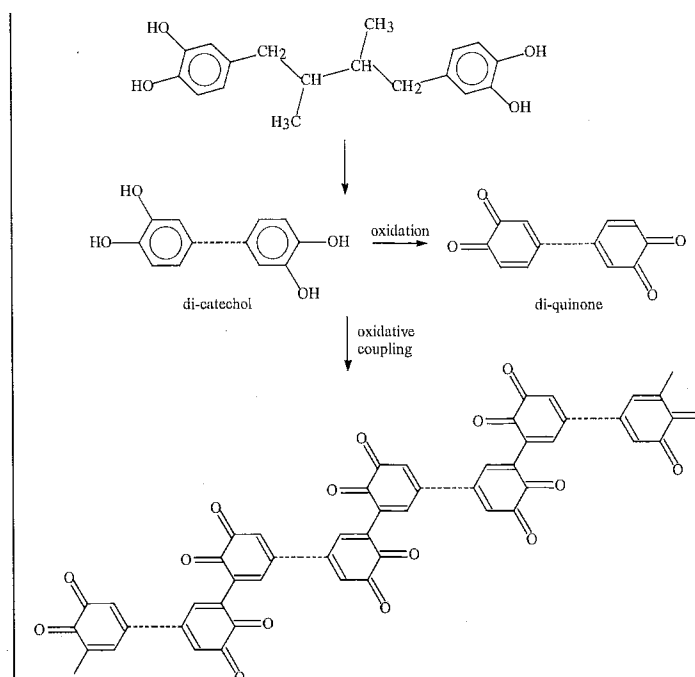


Figure 3. - Model of the polymerisation reaction of NDGA.

c. Flavonoid compounds.

The simplest flavonoid antioxidants are represented by catechin and green tea extract: the latter is composed predominantly of monomeric gallic acid and other catechin species.⁸

d. Amino phenols.

The synthesis of melanin is based on the polymerisation of derivatives of amino phenol, represented here by: DL-tyrosine, dioxy phenylalanine (L-DOPA) and 3-hydroxytyramine, see Fig. 2.

In this study, the phenolic compounds were reacted aerobically with crude laccase, (benzenediol: oxygen oxidoreductase, E.C.1.10.3.2), a copper containing polyphenol oxidase that catalyses the oxidation of electron-rich substrates such as phenols^{9,11} and the polymerisation of phenolic compounds.⁹ It has broad substrate specificity towards aromatic compounds containing hydroxyl and amino groups,¹² and is produced by some basidiomycete fungi, which cause white rot of wood.¹³

It is a commonly experienced observation that processing weakens leather, both in the controlled destructive environments of the beamhouse and by the chemical changes induced in tanning and post tanning. Therefore, any process that has claimed to strengthen leather should more correctly be regarded as less weakening. Recently, it has been suggested that collagenic material in tendon can be strengthened by an oxidative coupling reaction, using nordihydroguaiaretic acid (NDGA), 2,3-bis

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**Corresponding author - email: Tony.Covington@northampton.ac.uk

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(3,4-dihydroxyphenyl methyl) butane.¹⁵ It is proposed that the molecules polymerise via a quinoid type of reaction, indicated in Fig. 3.¹⁶ This is consistent with the proposal that tanning depends on the formation of matrices.¹⁷

EXPERIMENTAL

Lignin degradation products, 2-methoxyphenol, 4-hydroxybenzaldehyde, vanillin and vanillic acid, were purchased from Sigma-Aldrich Company Ltd., Dorset, UK. Flavonoid antioxidant, catechin, was obtained from Sigma-Aldrich and green tea polyphenol was obtained as a gift from Dr. Lijiang Song.⁸ Simple phenols, catechol, resorcinol, pyrogallol, hydroquinone and phloroglucinol, were obtained from BDH, Dorset, UK. Aminophenols, L-tyrosine, L-DOPA and 3-hydroxy tyramine, were received from Prof. Christine S. Evans (University of Westminster, London, UK). Laccase was donated by Klenzyme Ltd., London, UK: crude rather than purified laccase was used to avoid the high cost of the isolated enzyme. Standard hide powder was obtained from BLC The Leather Technology Centre, Northampton, UK.

Dyeing effects of phenols and laccase on hide powder

Hide powder (1g) and 1 mmol of phenol were added to 25ml of distilled water. The phenols that were not readily soluble in water were dissolved first in 3ml of acetone. The mixtures were shaken for 30 minutes at 30°C, to rehydrate the hide powder and dissolve the phenols, and then the mixture was adjusted to pH 6.0 with 0.2M NaOH. Crude laccase (0.1g, containing 10 units of activity) was added for reaction. A control for each phenol omitted the enzyme. The reaction mixtures were shaken at 30°C for 24 hours. After the reaction was finished, the hide powder was filtered and dried at room temperature.

Dyeing effects of phenols and laccase on chrome tanned leather

For each reaction, three 70x75mm pieces of neutralised chrome tanned leather (conventionally manufactured wet blue) were weighed and put in a 500ml glass vessel. 150 ml distilled water was added, the leather was shaken at 35°C for 15 minutes, then the solution was adjusted to pH 4.5-5.5 with 5% sodium bicarbonate. Phenol (0.75 g) was added and the mixture was shaken at the same temperature for 2 hours. Then the solution was adjusted to pH 6.0 with 5% sodium bicarbonate, followed by the addition of 0.3g crude laccase for reaction. The reaction mixtures were shaken at 35°C for 20 hours. On completion, the solution was drained. Fatliquor (8% Truponol BTK), emulsified in water at 50°C, was added and then shaken 90 minutes. The solution was adjusted to pH 3.2-3.4 with 5% formic acid solution to fix

the fatliquor; the mixture was shaken at 35°C for 60 minutes and then the solution was discarded. The dyed leather was toggle dried at room temperature.

Colour measurement

Colour of the dyed hide powder and leather was measured by a Minolta CR210 Chromameter (Minolta, Germany) using the L*a*b* colour space: where L* indicates lightness, 0 is black and 100 is white, +a* is the red direction, -a* is the green direction, +b* is the yellow direction and -b* is the blue direction. Color difference is defined as follows:

$$\Delta E = \{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]\}^{1/2}$$

A difference of 0.5 in ΔE is discernable by the trained eye.

Hydrothermal stability analysis

Hydrothermal stability of the treated hide powder was measured by its denaturation or shrinkage temperature and energy (enthalpy) of shrinking using a STARE System differential scanning calorimeter (DSC) (Mettler Toledo, UK). 10-15mg of wet samples were weighed into a 40l aluminium pan, and then the pans were transferred to the DSC carousel. Samples were heated at a rate of 5°C/minute over the range 35-100°C.

Colour fastness to washing

Colour fastness of the sample to washing was measured using a modified procedure of SLF 423 (IUF 423).¹⁴ In this test, the wash solution used was 0.1M sodium carbonate instead of 5g/l sodium lauryl sulfate, to examine the stripping effect of alkali. The change in colour of the sample and the accompanying textile were assessed on the standard Grey Scale according to SLF 131 (IUF 131)¹⁴ and SLF 132 (IUF 132)¹⁴ respectively. The colour of the sample was also measured by a Minolta CR210 Chromameter.

Rub fastness test

Dry rub fastness of the dyed leather was evaluated

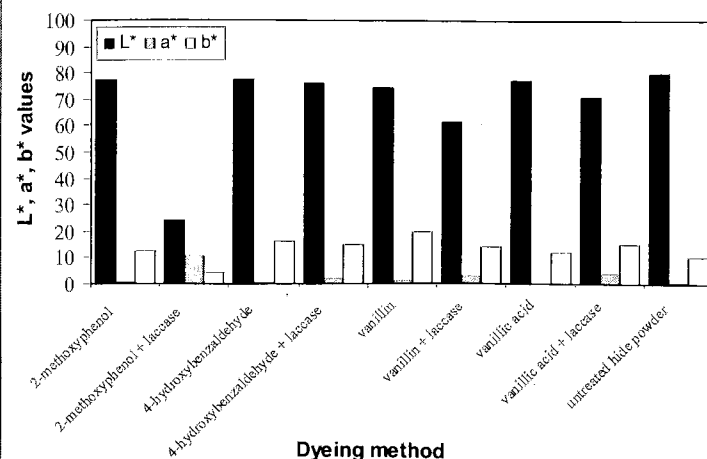


Figure 4a. - The lightness and chromaticity values of hide powder dyed with the lignin degradation products and laccase.

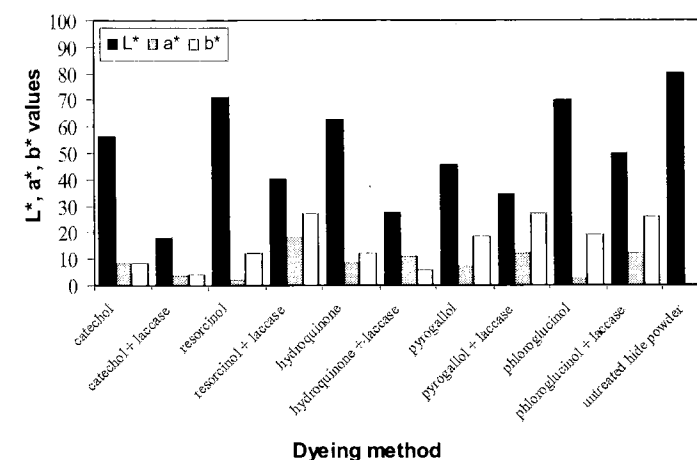


Figure 4b. - The lightness and chromaticity values of hide powder dyed with the simple phenols and laccase.

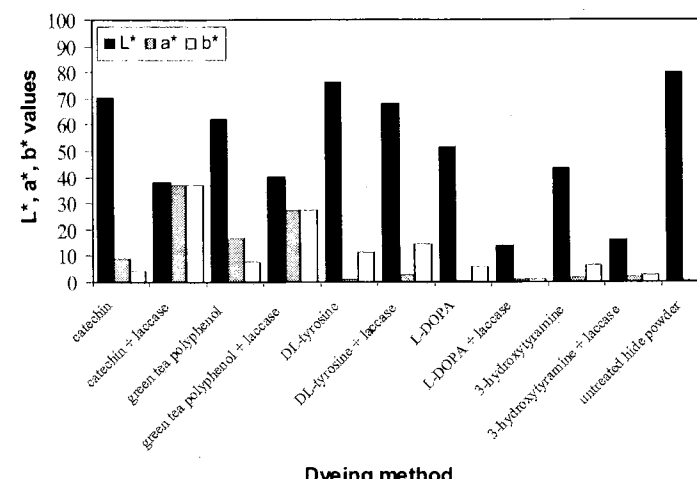


Figure 4c. - The lightness and chromaticity values of hide powder dyed with the flavonoid antioxidants, the aminophenols and laccase.

according to the Official Method SLF 5¹⁴ using a rub fastness tester (STM 461, SAJTRA Footwear Technology

Centre), run for 1024 revs. Colour changes of leather and felt pad were visually assessed according to the Grey Scale.

Measurement of tensile strength and percentage elongation at break

Tensile strength was measured according to the Official Method IUP 6 (SLP 6), using an Instron 1122.¹⁴

Measurement of tear strength

Tear strength (double edge tear) was measured according to the Official Method IUP 8 (SLP 7), using an Instron 1122.¹⁴

RESULTS AND DISCUSSION

Coloring hide powder

All the phenolic compounds were oxidised by laccase and thereby produced various colours. Without the enzyme, low intensity colour was produced, as indicated by the lightness (L*) value of hide powder dyed with each substrate alone. Lightness and chromaticity values of dyed hide powder are shown in Figs. 4a-c and the calculated differences caused by the laccase treatment are given in Table I.

After undergoing reaction with the enzyme for 24 hours, 2-methoxy phenol, catechol, hydroquinone, pyrogallol, L-DOPA and 3-hydroxy tyramine gave dark colours. The darkest colour was produced by oxidation of L-DOPA. Catechol, L-DOPA and 3-hydroxy tyramine produced almost black colours, whereas 4-methoxy phenol, hydroquinone and pyrogallol gave dark purple, purple and brown respectively.

The influence of reaction conditions is illustrated in Fig. 5. Colours produced by reaction of the phenolic compounds

TABLE I
The Effects of Laccase on Lignin Degradation Products to Cause Color Difference.

Dyeing method	ΔE (effect of total treatment, relative to hide powder)	ΔE (effect of laccase alone)
2-methoxy phenol + laccase	57.3	55.0
4-hydroxy benzaldehyde + laccase	6.2	2.0
Vanillin + laccase	19.2	14.3
Vanillic acid + laccase	10.9	7.9
Catechol + laccase	62.8	38.8
Resorcinol + laccase	46.7	34.5
Hydroquinone + laccase	54.2	36.2
Pyrogallol + laccase	50.1	15.0
Phorogucinol + laccase	36.1	23.4
Catechin + laccase	62.1	54.1
Green tea extract + laccase	51.3	31.8
DL-tyrosine + laccase	12.5	8.8
L-DOPA + laccase	66.8	37.7
3-hydroxy tyramine + laccase	64.7	27.6

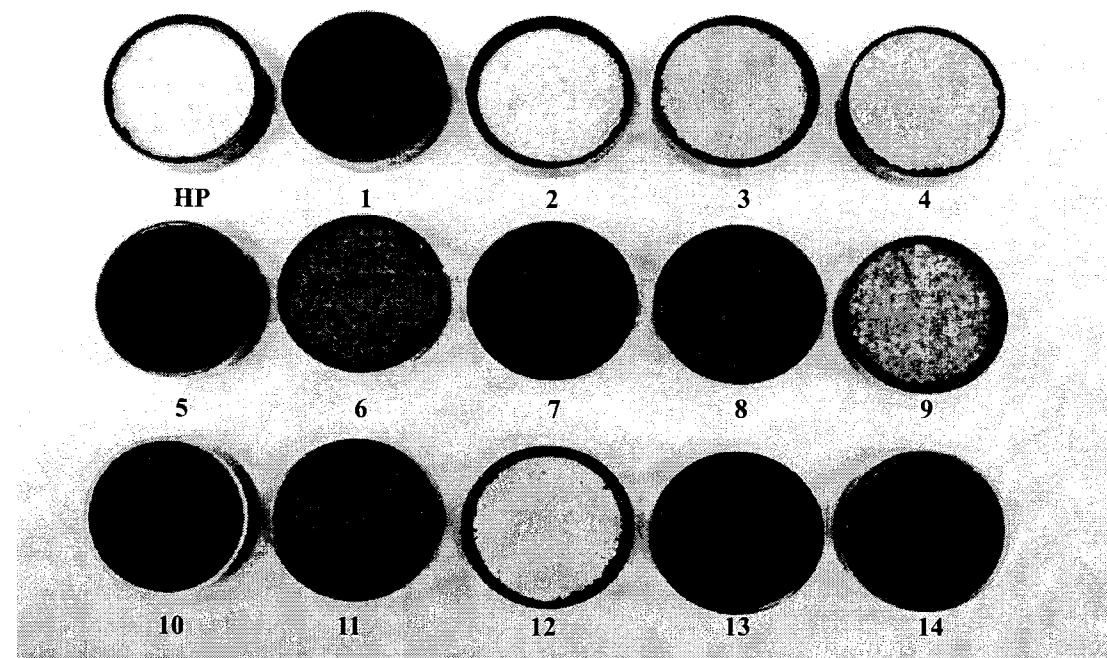


Figure 5. - Hide powder coloured by phenol-laccase treatment

HP = untreated hide powder, 1 = 2-methoxyphenol, 2 = 4-hydroxybenzaldehyde, 3 = vanillin, 4 = vanillic acid, 5 = catechol, 6 = resorcinol, 7 = hydroquinone, 8 = pyrogallol, 9 = phloroglucinol, 10 = catechin, 11 = green tea extract, 12 = DL-tyrosine, 13 = L-DOPA, 14 = 3-hydroxytyramine.

TABLE II
Shrinkage Temperature (T_s), Increase in Shrinkage Temperature (ΔT_s) and Energy of Shrinking (ΔH) of Hide Powder Dyed by Phenol-laccase Reaction. Important Results are Highlighted in Bold.

Dyeing method	With laccase			Without laccase		
	T_s (°C)	ΔT_s (°C)	ΔH (J/g)	T_s (°C)	ΔT_s (°C)	ΔH (J/g)
None	56	0	18	56	0	18
2-methoxy phenol	60	4	31	59	3	35
4-hydroxy benzaldehyde	60	4	40	58	3	38
Vanillin	60	4	32	58	2	39
Vanillic acid	57	1	34	57	1	37
Catechol	71	15	25	61	5	24
Resorcinol	67	11	35	60	4	26
Hydroquinone	74	18	30	61	5	29
Pyrogallol	60	4	26	61	5	27
Phloroglucinol	60	4	25	63	7	25
Catechin	77	21	37	69	13	17
Green tea extract	76	20	41	75	19	34
DL-tyrosine	56	0	26	59	3	20
L-DOPA	59	3	23	58	2	24
3-hydroxy tyramine	57	1	20	56	0	25

with laccase resulted in positive values for a^* chromaticity, therefore, all produced red colours. Phenolic compounds giving a^* values higher than +10 were 2-methoxy phenol, resorcinol, hydroquinone, pyrogallol, phloroglucinol, catechin and green tea polyphenol. The highest a^* value was attained by oxidation of catechin with the enzyme. All colours produced by the reaction of phenolic compounds

and laccase had positive values for b^* chromaticity, therefore, all reactions gave yellow chromaticities. Phenolic compounds giving b^* values higher than +25 were resorcinol, pyrogallol, phloroglucinol, catechin and green tea polyphenol. Therefore, the colouring from phenol oxidation is dominated by shades of brown, so the colouring closest to black might be characterised as brown-black.

TABLE III
The Lightness, Chromaticity a^* and b^* Values of Hide Powder Dyed with Phenolic Compounds and Laccase: before and after Washing.

Dyeing method	L^*		a^*		b^*	
	Before washing	After washing	Before washing	After washing	Before washing	After washing
2-methoxyphenol + laccase	23.3	21.9	10.8	10.3	4.1	3.7
catechol + laccase	21.0	21.9	4.6	3.2	4.4	5.7
L-DOPA + laccase	18.1	15.0	1.7	2.1	1.7	1.5
3-hydroxytyramine + laccase	17.1	15.5	2.6	2.5	2.8	2.3
hydroquinone + laccase	31.3	32.1	12.6	9.5	5.7	12.4
pyrogallol + laccase	38.9	35.4	10.7	6.7	25.4	16.1

TABLE IV
Staining in the Washing Test of Hide Powder Dyed with Phenolic Compounds and Laccase. (Measurements on the grey scale, where higher scale reading means less color contrast after testing).

Dyeing method	Secunder cellulose acetate (dicel)	Bleached unmercerised cotton	Nylon 6.6	Polyester (terylene)	Acrylic	Wool worsted
	2-methoxyphenol + laccase	3/4	3/4	3	3/4	4
catechol + laccase	4	3	3/4	5	5	3
L-DOPA + laccase	5	3	4/5	5	5	3/4
3-hydroxytyramine + laccase	5	4	5	5	5	3/4
hydroquinone + laccase	5	5	4/5	5	5	3
pyrogallol + laccase	3	3/4	3/4	5	5	2/3

Hydrothermal stability

The shrinkage temperatures of the dyed hide powders were higher than those of either untreated hide powder or hide powder treated with phenol alone, as shown in Table II. Moreover, the energies of shrinking indicate that the reactions do not denature the collagen, which would be indicated by a decrease in value. Therefore, the oxidation and polymerisation products were able to cross link with collagen, probably via hydrogen bonding, but also by covalent reaction.

In this study, the phenolic compounds showing the greatest tanning effect after undergoing reaction with laccase were: catechol, hydroquinone, resorcinol, catechin and green tea extract, shown in Table II. It is noteworthy that these results are not totally consistent with the combination reaction with oxazolidine, reported in Part 1 of this series of papers.⁷ However, the response of the reactive sites in the aromatic centres to quinoid formation and to aldehydic reaction with oxazolidine might not necessarily be expected to be the same, even though there may be some similarity in the tanning outcome.

Colour fastness to washing

After washing with Na_2CO_3 (to break hydrogen bonds), the hide powder dyed with L-DOPA and 3-hydroxy tyramine became duller, but gave good fastness resistance, as shown

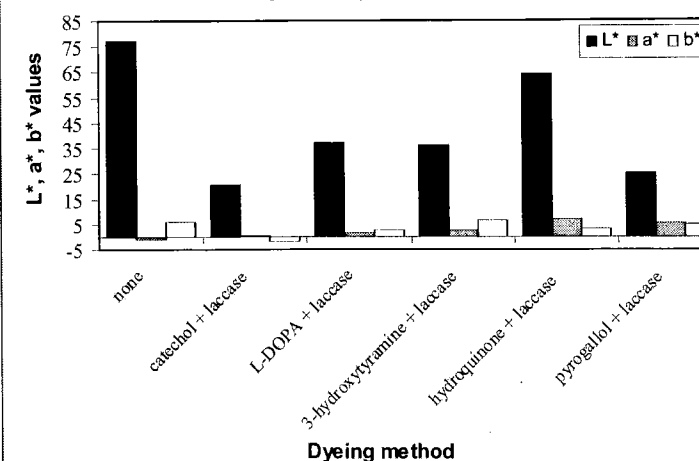


Figure 6. - The lightness and chromaticity values of chrome tanned leather dyed with phenolic compounds and laccase.

by the small decreases in the L^* values of the samples, shown in Table III. After washing, the chromaticity values of hide powder dyed using 2-methoxy phenol remained constant, but hide powder dyed with catechol, hydroquinone and pyrogallol lost some red component of the colour,

TABLE V

Rub Fastness of Chrome Tanned Leather Dyed with the Phenolic Compounds and Laccase.

(Measurements on the grey scale, where higher scale reading means less color contrast after testing).

Dyeing method	Colour transfer to pads		Color change on leather	
	Grain	Flesh	Grain	Flesh
Catechol + laccase	3	2/3	3	3
L-DOPA + laccase	4/5	3	3	3
3-hydroxy tyramine + laccase	4/5	3	4	3
Hydroquinone + laccase	5	5	4	5
Pyrogallol + laccase	4	2/3	3	3

TABLE VI

Strength Properties of Chrome Tanned Leather Treated with Phenolic Compounds and Laccase

Treatment	Tensile strength (MPa)		Tear strength (N/mm)	
	Parallel	Perpendicular	Parallel	Perpendicular
None	9.5	8.8	45	40
Catechol + laccase	15.9	11.9	44	46
L-DOPA + laccase	14.3	13.1	50	49
3-hydroxy tyramine + laccase	16.8	12.6	43	46
Hydroquinone + laccase	13.7	11.6	42	47
Pyrogallol + laccase	16.5	12.7	46	52

TABLE VII

Summary of the Average Effects of Phenol-laccase Treatment on Leather Strength (% Change)

	Tensile strength	Elongation at break	Tear strength
Parallel to backbone	+ 60	+ 15	0
Perpendicular to backbone	+ 40	+ 15	+ 20

whereas hide powder dyed with pyrogallol decreased a little in the yellow component. The hide powder dyed with hydroquinone became slightly more yellow. Of the accompanying textiles in the wash test, hide powder dyed with 2-methoxy phenol showed good fastness resistance to acrylic and wool worsted fabrics; dyeing with catechol, L-DOPA, 3-hydroxy tyramine and hydroquinone gave good fastness resistance to dicel, polyester and acrylic fabrics (Table IV). In general, these results show that dyed hide powder has good colour fastness resistance to washing with

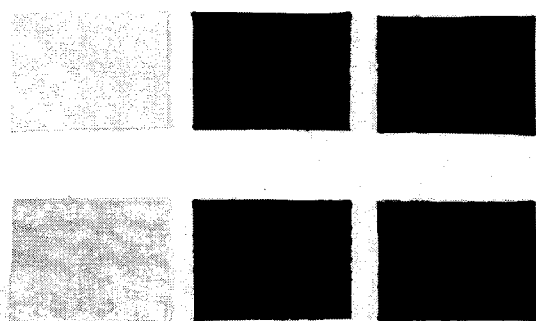


Figure 7. - Colour of chrome tanned leather dyed with phenolic compounds and treated with laccase.

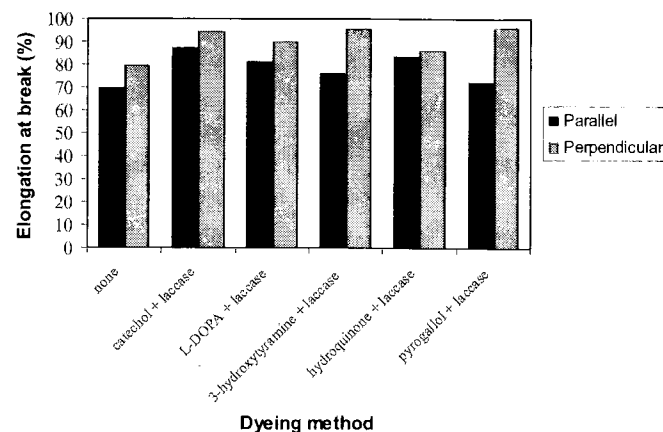


Figure 8. - Percentage of elongation at break of chrome tanned leather dyed with the phenolic compounds and laccase

a hydrogen bond breaker. This indicates that the sample should have good fastness resistance to normal washing conditions.

Dyeing effects on chrome tanned leather

The phenols in conjunction with laccase gave good colouring effects on leather on both grain and flesh sides: catechol

confers the darkest colour, almost black, shown in Figs. 6 and 7. The coloration also penetrates well into the leather; the best penetration was achieved by hydroquinone, but which also produced only pale colour.

The dyed leather performed well in the rub fastness test, as shown in Table V. The rub fastness on the grain side was better than that on flesh side, which is typical. Among the phenols used in this investigation, dyeing using hydroquinone in conjunction with the enzyme gave the best rub fastness on both grain and flesh sides, although this is probably only a reflection of the lower intensity of colour conferred by this reaction.

From Table VII, there is a positive effect on the physical properties of the leathers. The average effect is more pronounced in the tensile properties than the tear strength. In the case of tensile strength, the average effect of the process is to make the material more anisotropic, increasing the difference in the directional property, but the elasticity change (Fig. 8), in terms of stretching at break, is the same in both directions. In the case of tear strength, the anisotropy is reduced.

It is clear that the nature of the matrices created by the phenol-laccase reaction and their binding to collagen do not constitute enough structure to create a high hydrothermal stability product. However, the physical and thermodynamic properties of leathers have never been correlated, because a connection has never been made between the chemical modifications applied for increasing hydrothermal stability and those applied for modifying physical properties. However, these studies suggest that such a relationship might exist.

Therefore, the opportunity arises to define the relationship between the two aspects of stabilising collagen. At the same time, the leather may be coloured. Whilst this contribution to so-called compact processing might be useful, control of the colour is limited. Nevertheless, it may be of industrial interest to initiate colouring prior to dyeing, to provide a base colour and thereby reduce the cost of dyeing with synthetic dyes.

CONCLUSIONS

It is possible to produce coloured and tanned leather without using dyes and conventional tanning agents. This approach might be of use to the industry, as long as it is earth colours that are wanted. Black colour was almost produced, but it is clear that the biochemistry of pure black melanin pigment was not matched under these conditions.

The observed effects of these chemical modifications to collagen indicate that the typical deterioration to leather properties by processing may be avoided and, indeed, the weakening effect can be reversed. The improved characteristics of leathers made by phenolic polymerisation, observed in these studies, may be useful for some applications, such as in manufacturing shoe upper leather.

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