PHENOLIC REACTIONS FOR LEATHER TANNING AND DYEING

A thesis submitted to the University of Leicester in partial fulfilment of the requirements for the degree of Doctor of Philosophy

by

ONO SUPARNO
BSc, M Tech, MRSC

British School of Leather Technology
University College Northampton

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Ono Suparno

Abstract

Kraft lignin degradation by a biomimetic system was investigated, using haemin and hydrogen peroxide, which mimics the catalytic mechanism of lignin peroxidase to produce phenolic compounds. The degradation products were identified using spectroscopic methods and gas chromatography-mass spectrometry. 2-methoxyphenol, 4-hydroxybenzaldehyde, vanillin and vanillic acid were produced and their formaldehyde polymerisation products were used for tanning collagen. The denaturation (shrinkage) temperature of collagen was raised to 80°C through hydrogen bonding interactions between the polymers and the protein. For dyeing of hide powder, the lignin degradation products were reacted with laccase (a polyphenol oxidase); 2-methoxyphenol gave the darkest colour. These products have potential to be used as raw materials for tanning and dyeing of animal skins. Therefore, this can add value to the industrial byproduct (Kraft lignin) and reduce its environmental impact.

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 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.

Leather tanning reactions between collagen and dihydroxynaphthalenes (DHNs) and oxazolidine have been investigated, using hide powder and sheepskin pickled pelt. This investigation showed that some DHNs have a tanning effect on collagen. The measurement of combined and cross-linked DHNs on collagen showed that 30-40% of 1,6- and 2,6-DHNs were fixed through covalent bonding. Shrinkage temperature of the leather changed little after the non combined DHNs had been removed from the leather, indicating that the high stability of the combination tanned leather comes from the covalent bonding formed between DHNs and collagen through oxazolidine.
I dedicate this thesis to
my beloved parents, family, and country
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<tr>
<td>°Bé</td>
<td>Degrees Béume, solution density</td>
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<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<td>ΔE*</td>
<td>Total quantitative colour change</td>
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<td>ΔH</td>
<td>Change in enthalpy, energy of shrinking</td>
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<tr>
<td>ΔTₜ</td>
<td>Rise in shrinkage temperature</td>
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<td>a*</td>
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<td>ABTS</td>
<td>2,2’-azinobis(3-ethylbenzthiazoline-6-sulfonate)</td>
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<td>BLC</td>
<td>British Leather Confederation</td>
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<tr>
<td>BSLT</td>
<td>British School of Leather Technology</td>
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<tr>
<td>BSTFA</td>
<td>N,O-bis-trimethylsilyl trifluoroacetamide</td>
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<td>Co-A</td>
<td>Coenzyme-A</td>
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<td>COD</td>
<td>Chemical oxygen demand</td>
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<td>5,6-dihydroxyindole</td>
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<td>Dihydroxynaphthalene-3,6-disulfonic acid, disodium salt</td>
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<td>g</td>
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<td>k</td>
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<td>(M_n)</td>
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<td>Substitution nucleophilic bimolecular</td>
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<td>THN</td>
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LIST OF ORIGINAL PUBLICATIONS AND
CONFERENCE PRESENTATIONS

Publications

1. Suparno, O., Covington, A.D. and Evans, C.S.
   Kraft lignin degradation products for dyeing and tanning of leather.
   Journal of Chemical Technology & Biotechnology. 2004, 80(1), 44-49.

2. Suparno, O., Covington, A.D. and Evans, C.S.
   A new application of biotechnology in leather dyeing.
   PubScie AEIF. 2003, 3(1), 18-24, ISSN 1626-6447.

   Collagen and plant polyphenols: new relationships and new outcomes. Part 2.
   Phenolic reactions.
   Journal of American Leather Chemists Association, accepted for publication.

Conference presentations

1. Suparno, O.
   Biomimetic lignin degradation: implications for leather tanning.
   The Society of Leather Technologists and Chemists (SLTC) Annual

2. Suparno, O.
   Biomimetic lignin degradation.

3. Suparno, O., Covington, A.D. and Evans, C.S.
   Dyeing.
   Proceedings of the 9th Indonesian Students' Scientific Meeting (ISSM 2004),
   0855-8692.

4. Suparno, O., Covington, A.D. and Evans, C.S.
   Tanning.
   Proceedings of the 9th Indonesian Students' Scientific Meeting (ISSM 2004),
   0855-8692.
5. Suparno, O.
A new approach to leather colouring: dyeing without dyes.

6. Suparno, O., Covington, A.D. and Evans, C.S.
A new application of biotechnology in leather dyeing.

7. Suparno, O., Covington, A.D. and Evans, C.S.
Biomimetic degradation of Kraft lignin.
CHAPTER 1

GENERAL INTRODUCTION
1.1 Background

Leather making is one of the oldest crafts performed by humankind. Although there is world-wide trend to exploit alternative materials derived from other sources, leather still finds widespread use\(^1\). In the leather manufacturing, phenolic compounds have an important role, particularly in the tanning process. They are used as materials for the production of synthetic tanning agents (syntans) and in the form of vegetable tannages.

Phenols are used in syntan production, i.e. exchange syntans, which are related to the natural polyphenol tannins. Syntans contain phenolic hydroxyl groups and have the ability to react with collagen to produce leather. The first successful development of a commercially feasible syntan capable of producing leather was by Stiasny\(^2\)\(^-\)\(^3\). The syntan called Novolac was prepared by condensation of phenol or cresol (methylphenol) with formaldehyde by heating at elevated temperatures\(^2\).

Plant polyphenols, also called vegetable tannins, have been used for many thousands of years to convert animal skins and hides to leather\(^4\), because they can interact with collagen and increase the hydrothermal stability of the collagen\(^5\). They are extracted from a wide variety of plants, found in wood, leaves, nuts, twigs, and bark. From the early work of Freudenberg in 1920's and 1930's, they can be classified into two groups based on the chemical nature and structural characteristics, i.e. hydrolysable tannins and condensed tannins\(^6\). Another group of vegetable tannins called complex tannins was introduced in 1980's. The tannins contain both hydrolysable and condensed tannins. They are tannins in which a catechin unit is bound glucosidically to a gallotannin or an ellagitannin unit\(^7\). Their actions towards hydrolytic agents, particularly acids, illustrate the main distinction between the two groups. Hydrolysable tannins are readily hydrolysed by acids or enzymes into a sugar or related polyhydric alcohol and phenol carboxylic acids. Condensed tannins undergo progressive polymerisation under the action of acids to produce tannin reds or the amorphous phlobaphenes\(^4\)\(^,\)\(^8\).
Lignin is one of the most abundant renewable organic materials on earth, found in higher plants. Wood and other vascular tissues generally contain 20-30% lignin\textsuperscript{9,10}, which is a complex polymer, made by the oxidative polymerisation of cinnamyl alcohol precursors\textsuperscript{9}.

Kraft lignin is a polymer that is readily available as a byproduct of the Kraft pulping process. The conversion of wood chips to pulp for manufacturing paper generates huge quantities of byproduct lignins; the best estimates indicate that more than 26 million tonnes of Kraft lignin are generated as byproducts of such pulping operations every year in the United States only\textsuperscript{11}.

The effluents produced during the processing of pulp from lignocellulose have the potential to cause damage to the receiving waters, which may, in turn, be used for domestic, industrial, fishing, and recreational purposes. Lignin and its derivatives impart a dark brown colour to the effluents that is aesthetically objectionable and may reduce the primary productivity of the receiving waters by reducing the transmission of light\textsuperscript{12}. The effluents from pulp industries might be treated to reduce their chemical oxygen demand (COD), solid wastes, and toxicity before discharging into rivers or they could be converted into useful products.

Development of lignin bioconversion processes has considerable potential for the future, as such bioconversions might produce a variety of chemicals. One of the uses of lignin with potential value would be in its conversion to low molecular weight chemicals\textsuperscript{13-16}, for example, vanillin, dimethylsulfide, dimethylsulfoxide, methylmercaptan, and a variety of phenols\textsuperscript{12}. Lignin degradation can yield some phenolic aldehydes, ketones, acids\textsuperscript{17,18}, and phenols\textsuperscript{19,20}. The phenolics might have reactivity towards collagen and therefore be useful to leather processing from renewable organic materials.

It has been shown by Covington and Shi\textsuperscript{21-23} that condensed tannins and oxazolidine combination tannage can confer high hydrothermal stability to leather. This method employed environmentally benign and renewable natural
plant materials, vegetable tannins, and a reactive cross-linking reagent oxazolidine. This makes it suitable for the requirements of the future leather industry and could be a substitute for traditional mineral tannage, chromium(III). The possibility of using other phenolics, instead of condensed tannins for combination tanning, has not been established.

Some phenolic compounds have roles as precursors of melanins, which are pigments, usually dark brown or black, widely distributed in living organisms\textsuperscript{24-26}. Melanins have chemical similarity to lignin; they are macromolecules formed by oxidative polymerisation of phenolic compounds. Phenolics such as catechol\textsuperscript{24,25,27} and 1,8-dihydroxynaphthalene\textsuperscript{25,26,29} are precursors of melanins.

Leather dyeing is colouration of leather, usually with synthetic dyes, to enhance its aesthetic or fashion appeal. An alternative route to achieving colour might be to exploit the chemistry of melanins\textsuperscript{30}. Oxidation and polymerisation by enzymes of simple phenols, including extract of green tea, might produce deep colour shades and bind the pigment to collagen in a tanning reaction. In this way, lightfast colour would be permanently fixed to the leather, using only organic, environmentally sound reactions.

In this work, the main objective is to understand the phenolic reactions which may be applied to leather tanning and dyeing and at the same time to address combination tanning with the phenols and oxazolidine as cross-linking agent.

1.2 Cross-linking of collagen

Collagen cross-linking contributes to the tensile strength of tissues such as tendon and is important in stabilising the collagen fibrils. It can be classified into two types, i.e. natural and artificial cross-linkings.
1.2.1 Natural cross-linking

The action of the enzyme lysyl oxidase on collagen causes formation of covalent cross-links\textsuperscript{33-35}. The cross-links are based on aldehyde formation and condensation involving specific peptidyl lysine and hydroxylysine residues. The process is catalysed by lysyl oxidase, which oxidatively deaminates the amino group of certain lysyl and hydroxylysyl residues in the telopeptide regions of collagen molecules to form reactive allysyl and hydroxylysyl aldehydes, respectively. These aldehyde groups then react with the amino group of lysyl and hydroxylysyl residues and other aldehydes to form a variety of di-, tri-, and tetrafunctional cross-links (Figure 1.1)\textsuperscript{36}.

\textbf{Figure 1.1} Oxidative deamination of lysyl and hydroxylysyl residues by lysyl oxidase\textsuperscript{36}

The monomeric molecules in the supramolecular aggregates of collagen molecules are stabilised by two different pathways. Cross-linking initially takes place through an enzymatic mechanism involving oxidation of specific lysine
hydroxylysine residues, providing divalent cross-linking which subsequently matures to multivalent cross-links. It is followed by a non-enzymic pathway\textsuperscript{31,32}.

Cross-links between the molecules making up the fibrils and between the fibrils making up the fibres determine the strength of the collagen fibres. These cross-links are covalent bonds formed between the individual molecules within the fibre at highly specific points, controlled by the precise alignment of the fibre. They are very important for the optimal functioning of collagen fibres as a framework structure in the body\textsuperscript{31,37}.

1.2.2 Artificial cross-linking

Reconstituted forms of collagen, such as films, fibres or sponges, can lack strength and may disintegrate on handling or collapse under the pressure from surrounding tissue in vivo, due to dissociation of cross-links in the isolation processes. As a consequence, mechanical resilience and collagenase resistance of the collagen is often necessarily conferred by introducing artificial cross-linking into the molecular structure of collagen\textsuperscript{38}. This basic principle is used in leather tanning.

Trivalent chromium salts are used as cross-linking agents to form covalent bonds with collagen\textsuperscript{39}. Aluminium and other polyvalent cations can also be used as cross-linkers\textsuperscript{40}. Covalent cross-links in collagen can be created in a number of ways. Aldehydes are the most commonly utilised reagents, with formaldehyde and glutaraldehyde (GTA) being the most well known examples. As an alternative to aldehyde treatment, strong, and resistant collagen can be obtained using hexamethylenediisocyanate (HDC) as a cross-linker\textsuperscript{41}.

Recently, a matrix theory of collagen-tannin cross-linking mechanism was proposed by Covington and Song\textsuperscript{42}. The theory described that a matrix is composed of the primary tanning agent, covalently bound to collagen, interacting with the supramolecular water and any other secondary tanning or auxiliary. The
matrix may be regarded as cross-linking the fibre structure, it is not necessary to consider cross-linking by individual components of the matrix as a fundamental requirement of the stabilising mechanism; cross-linking may occur depending on the tanning reagent and the availability of reaction sites. The theory indicates that hydrothermal stability derives from a matrix interaction at the triple helix.

1.3 Leather manufacturing

Leather is made by subjecting the protein of animal skin or hide to a process known as tanning, whereby the skin or hide becomes more durable and capable of being used for a wide range of purposes. The leather industry may be regarded as a bridge between production of the hide as a byproduct of the food industry and its manufacture into shoes and wearing apparel, for which it provides basic raw materials. The practice of leather manufacture varies considerably from tannery to tannery and from country to country, so that there is no single universally applied process. The main sequences of operations and their aims are the same, but there are wide differences in detailed techniques.

The production of leather can be regarded as taking place in three steps. The first step is to remove unwanted components, hair, fats, etc., leaving the fibrous network of the hide protein. The second step is to react this network with tanning materials to produce a stabilised fibre structure. The third step is to build in the characteristics of fullness, colour, softness, and lubrication, and to finish the surface, to make a practically useful product.

Cattle, sheep, goat, calf, and pig are the primary sources of hides and skins used for leather. After hides and skins are removed from the animal (flaying), they undergo curing or preservation, in which they receive either long term or short term protection against bacteria. The most common method of preservation of hides and skins is curing with sodium chloride.
The typical tanning process starts in the beamhouse. Classical operations of the beamhouse are the following: (1) soaking and mechanical removal of scud from hides; (2) fleshing, splitting, smoothing of hides; (3) removing the hair, splitting hide fibres and degradation of the epidermis (liming); (4) removal of lime (deliming); (5) enzymatic loosening of hide fibres (bating); and (6) pickling. The purpose of these operations is to increase the amount of water in the hide to the amount close to that of the living hide, remove non-collagenous components and loosen the structure. This loosening makes it easier for the tanning agents, fats, dyestuffs, and other substances to penetrate into the hide. In the beamhouse, the non-collagenous proteins are removed from the hide, so are the epidermis, hair, globular proteins, and melanins, while the collagen fibre skeleton remains practically untouched.

1.3.1 Soaking

Leather manufacture commences with the soaking of cured stock in water to rehydrate and to cleanse it. The soaking of cured hides has as its aim the removal of salt and part of the soluble protein content and imparting an adequate plumpness to the hide. Another purpose of soaking is to remove remaining blood, urine, and dung.

1.3.2 Liming

Opening up of the structure may be considered as an extension of soaking; its purpose is to separate two structural proteins: keratin and collagen. Methods of hair removing can be divided into two groups: (1) methods based on destruction or modification of the epidermis tissue surrounding the hair, so that this may be loosened and mechanically removed, and (2) methods in which hair itself is attacked and its structure destroyed. This more drastic method is in practice connected with use of alkalis (calcium or sodium hydroxide) and of sulfide. The hides and skins are chemically unhaired and the fibre structure is opened up by alkaline swelling in saturated lime at pH 12.5.
1.3.3 Deliming and bating

The removal of combined alkali is achieved by treating the goods with acids or acidic salts, typically ammonium salts\(^43\). Bating is a treatment of the hide with proteolytic enzymes to remove additional non-collagenous proteins from the surface of the hide\(^40\).

1.3.4 Degreasing

Degreasing is the last step in the beamhouse operations. In the majority of technological processes applied there is no need for degreasing, as the fat contained in raw hide becomes saponified and is washed out by the use of surfactants\(^44\).

1.3.5 Pickling

Pickling prepares raw hide for tanning. The purposes of this operation are to stop enzymatic bating and to adjust the pH of the hide for subsequent processes, e.g. storage or chrome or vegetable tannage. To pickle the pelts means to acidify it in such a way as to prevent it from simultaneous swelling under the action of acid: this is usually done by salt (sodium chloride) addition\(^44\).

1.3.6 Tanning

Tanning is the process of introducing a stabilising or tanning agent into the hide or skin. Tanning is usually done with basified trivalent chromium salts. The reaction of chromium salts with carboxylic groups of the hide protein, collagen, leads to high hydrothermal stability and stability against microbial action. After chrome tanning, hides and skins are called wet blue or blue crust\(^40,45\).
1.3.7 Retanning

Most leather requires retanning, using a variety of tanning materials. In general, retannage gives specific properties to the leather such as tightness and fullness. Various tanning agents are used for retanning, including chromium compounds. Retanning with chrome tanning agents makes the leather soft. Vegetable and synthetic tannins also fill leather well in its weaker parts (bellies)\textsuperscript{44}.

1.3.8 Dyeing

Leather dyeing is a transition process between tanning and finishing. It is colouration of leather usually with synthetic dyes typically either acid or direct dyestuffs. The former is used when a penetrating dyeing is required and the latter when surface dyeing is required\textsuperscript{43}. The result of dyeing depends not only on the tanning agent and method used, but also on reaction with fat, surfactant and water\textsuperscript{3}.

1.3.9 Fatliquoring

The process of fatliquoring entails the treatment of leather with a warm dilute emulsion of oil in water. The principle of fatliquoring is the application of an oil-in-water emulsion, which is subsequently induced to break, thus depositing a film of fatty matter over the fibre structure. The functions of the process are lubrication, adjustment of physical properties, waterproofing, and filling\textsuperscript{43}.

1.3.10 Drying

Before drying, leathers are stacked and sammyed (squeezed). Leather is dried usually at relatively low temperature in order to prevent structural and chemical changes. The initial temperature is lower (usually 40-50°C for chrome leather, 35-45°C for vegetable tanned leather), then, as the water content decreases, temperature is increased, however, never higher than 8-10°C below the shrinkage
temperature. It is done so to avoid local overheating and deterioration of components introduced in tanning and finishing.\textsuperscript{44}

1.3.11 Finishing

The final operation in leather manufacturing is finishing. The functions of leather finishing are to produce leather with constant and uniform physical properties and standardised colour and appearance, to protect the leather against adverse chemical and physical influences, and to satisfy fashion and colour fastness requirements.\textsuperscript{43}

1.4 Tanning

Tanning is the most important step in leather production. It is typically carried out in an aqueous environment in rotating drums. During tanning, collagen will fix the tanning agent to its reactive sites, as a result stopping the putrefaction phenomenon.\textsuperscript{40,46} Tanning can be classified into three groups: mineral tanning, vegetable tanning, and other organic tanning (aldehyde, quinone, and synthetic tanning).

1.4.1 Mineral tanning

Four elements play a significant role in the modern leather tanning industry, i.e. chromium(III), aluminium(III), titanium(IV), and zirconium(IV), of which chromium(III) is the most important. Nowadays, more than 90\% of the world’s leather is tanned with chromium, which is a consequence of the easy processing, the broad achievability and the excellent properties of leather. Tanning using Cr(III) sulfate can achieve shrinkage temperatures above 120°C. However, it also has considerable potential for environmental pollution.

The interactions of collagen with chrome have been extensively investigated since the end of the nineteenth century. The fundamental reaction is the formation of
complex bonds with the ionised carboxyl groups of aspartic and glutamic acid residues on collagen fibres\textsuperscript{45,47} (Figure 1.2).

![Figure 1.2 Model of chrome complexation with carboxyl groups of collagen](image)

Other mineral tannages (Al(III), Ti(IV), and Zr(IV)) have similar reaction mechanisms to chromium, although reaction is dominated by electrovalent bonding, thus much lower shrinkage temperature is obtained than with chrome\textsuperscript{45}. The maximum shrinkage temperatures of leather tanned with Al(III), Ti(IV), and Zr(IV) salts are 79, 90, and 97°C respectively\textsuperscript{40}. The development of titanium and zirconium tannage is relatively new\textsuperscript{45}. Empirically, the chemistry of Ti(IV) is dominated by the titanyl ion TiO\textsuperscript{2+} and the species in the tanning agent are chains of (Ti-O)\textsubscript{n}. Zirconium salts are characterised by eight-coordination and high affinity for oxygen, resulting in a tetrameric core structure; the basic unit of structure is four Zr(IV) ions at the corners of a square\textsuperscript{40,45}. The tanning powers of titanium and zirconium are similar and both are better than aluminium.

1.4.2 Vegetable tanning

Vegetable tannins or natural polyphenols are complex higher plant secondary metabolites. They are water soluble, have relative molecular masses in the range 500-3000 and besides giving the usual phenolic reactions, they are able to precipitate some alkaloids, gelatine, and other solution from protein, from solution. They may be extracted from plant material containing polyphenols\textsuperscript{48}. They can be classified into three major groups: hydrolysable (pyrogallol), condensed (catechol), and complex tannins\textsuperscript{7,45,48}.
Hydrolysable tannins are sugar derivatives, based on glucose, but may be larger polysaccharides. Depending on the polyphenolic acids that are obtained as products of hydrolysis, hydrolysable tannins can further be divided into gallotannins (a) and ellagitannins (b) (Figure 1.3)\(^6\). Gallotannins yield gallic acid and glucose on hydrolysis. The ellagitannins produce ellagic acid in addition to gallic acid and glucose on hydrolysis. The hydrolysable tannins give good filling effect and typically raise the shrinkage temperature of collagen to 75-80\(^\circ\)C\(^4\). Commercially used hydrolysable tannins are listed in Table 1.1.

![Figure 1.3 Indicative molecular structures of hydrolysable tannins: (a) gallotannin, and (b) ellagitannin\(^6\)](image)

### Table 1.1 Commercially used hydrolysable tannins\(^6,7\)

<table>
<thead>
<tr>
<th>Tannin</th>
<th>Type of tannin</th>
<th>Plant</th>
<th>Part of the plant</th>
<th>Tannin content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese tannins (tannic acid)</td>
<td>Gallotannin</td>
<td><em>Rhus semialata</em></td>
<td>Galls, leaves</td>
<td>34-71</td>
</tr>
<tr>
<td>Turkish tannin</td>
<td>Gallotannin</td>
<td><em>Quercus infectoria</em> (Aleppo galls)</td>
<td>Galls</td>
<td>25-45</td>
</tr>
<tr>
<td>Sumach</td>
<td>Gallotannin</td>
<td><em>Rhus coriaria</em>, <em>R. typhina</em></td>
<td>Leaves</td>
<td>26-27</td>
</tr>
<tr>
<td>Tara</td>
<td>Gallotannin</td>
<td><em>Caesalpinia spinosa</em></td>
<td>Fruit pods</td>
<td>45-59</td>
</tr>
<tr>
<td>Myrobalans</td>
<td>Ellagitannin</td>
<td><em>Terminalia chebula</em></td>
<td>Fruit</td>
<td>30-50</td>
</tr>
<tr>
<td>Valonea</td>
<td>Ellagitannin</td>
<td><em>Quercus valonea</em></td>
<td>Acorn cups</td>
<td>15-36</td>
</tr>
<tr>
<td>Chestnut</td>
<td>Ellagitannin</td>
<td><em>Castanea sativa</em></td>
<td>Wood</td>
<td>10-12</td>
</tr>
</tbody>
</table>
Condensed tannins are referred to as polyflavanols or proanthocyanidins; they are based on the flavonoid ring system (Figure 1.4)\textsuperscript{6,45}. The A ring usually contains one or two phenolic hydroxyl groups; the B ring, which typically has a catechol structure, has different reactivity\textsuperscript{45}. The fundamental structure of the tannins is the phenolic flavan-3-ol, present in catechin (cyanidin), gallocatechin (delphinidin), fisetinidol, and robinetinidol. The flavan-3-ol units are linked through the C-4 to C-8 positions and C-4 to C-6 positions. General structures of the condensed tannins are shown in Figure 1.5\textsuperscript{8,21,22,45}. Sources of condensed tannins are given in Table 1.2. They typically raise the shrinkage temperature of collagen to 80-85°C\textsuperscript{45}.

**Figure 1.4** The flavonoid ring system of condensed tannins\textsuperscript{8,21,22,45}

**Figure 1.5** General structures for condensed tannins\textsuperscript{8,21,45}
Complex tannins are built up from a gallotannin unit or an ellagitannin unit and a catechin unit. One example of this group is acutissimin A, having a flavogallonyl (nonahydroxytriphenoyl) unit bound glucosidically to C-1, and linked via three further hydrolysable ester bridges to the D-glucose derived polyol.

Vegetable tannins react with collagen primarily via hydrogen bonding, as demonstrated in the model in Figure 1.6. Hydrophobic interactions have also an important role, particularly in hydrolysable tannins. Condensed tannins are more resistant to removal by hydrogen bond breakers because they have an additional mechanism for reaction, which is covalent reaction between the protein and aromatic carbon in tannin molecules via quinoid structures. Quinone itself can tan collagen effectively.
### Table 1.2 Sources of condensed tannins\(^6,7\)

<table>
<thead>
<tr>
<th>Family (Tannin)</th>
<th>Plant</th>
<th>Part of the plant</th>
<th>Tannin content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myrtaceae</td>
<td>Eucalyptus astringens</td>
<td>Bark</td>
<td>40-50</td>
</tr>
<tr>
<td></td>
<td>Eucalyptus wandoo</td>
<td>Bark, heartwood</td>
<td>12-15</td>
</tr>
<tr>
<td>Myrtan/ (Eucalyptus)</td>
<td>Eucalyptus wandoo</td>
<td>Bark, heartwood</td>
<td>12-15</td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Acacia mollisima</td>
<td>Bark</td>
<td>35-40</td>
</tr>
<tr>
<td>(Wattle/mimosa)</td>
<td>Acacia catechu</td>
<td>Heartwood</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Robinia pseudacacia</td>
<td>Bark</td>
<td>7</td>
</tr>
<tr>
<td>Anacardiaceae</td>
<td>Schinopsis balansae</td>
<td>Heartwood</td>
<td>20-25</td>
</tr>
<tr>
<td>(Quebracho)</td>
<td>Schinopsis lorentzii</td>
<td>Heartwood</td>
<td>16-17</td>
</tr>
<tr>
<td>Rhizophoraceae</td>
<td>Rhizophora candelaria</td>
<td>Bark</td>
<td>25-30</td>
</tr>
<tr>
<td>(Mangrove)</td>
<td>Rhizophora mangle</td>
<td>Bark</td>
<td>20-30</td>
</tr>
<tr>
<td>Fagaceae (Oak)</td>
<td>Quercus robur</td>
<td>Bark</td>
<td>12-16</td>
</tr>
<tr>
<td>Pinaceae</td>
<td>Picea abies</td>
<td>Bark</td>
<td>5-20</td>
</tr>
<tr>
<td></td>
<td>Pinus sylvestris</td>
<td>Bark</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Larix decidua</td>
<td>Bark</td>
<td>5-20</td>
</tr>
<tr>
<td>Rubiaceae (Gambier)</td>
<td>Uncaria gambier</td>
<td>Leaves, twigs</td>
<td>-</td>
</tr>
</tbody>
</table>

![Figure 1.6 Model of hydrogen bonding between plant polyphenols and collagen\(^4\)](attachment://image.png)
1.4.3 Aldehyde tanning

Several aldehydes have reactivity towards collagen and prevent putrefaction of the skins and hides. Among the aldehydes, formaldehyde (HCHO) has been known as a tanning agent. The fixation of formaldehyde by proteins is accompanied by changes in the physical properties: thermal stability and resistance to trypsic enzyme digestion are increased. The hydrothermal stability of formaldehyde tanned collagen is increased with increase in concentration, temperature, and time: shrinkage temperature ($T_s$) is increased with pH and fixed amount of formaldehyde up to pH 8.0, but at higher pH values there is no further increase in shrinkage temperature. The shrinkage temperature of formaldehyde tanned leather is 80-85°C, although its use in industry is effectively banned due to its toxicity.

A rise in shrinkage temperature has been reported when collagen was treated by other simple aliphatic aldehydes, such as acetaldehyde, glyoxal, acrolein, and crotonaldehyde. Glyoxal, which is the simplest dialdehyde, is a good tanning agent.

Although the cross-linking reaction of collagen with aldehyde, especially formaldehyde, has been studied for a long time, the mechanism is still not completely clear. Most researchers accept that aldehydic compounds react with the free amino groups of lysine and form cross-links.

$$\text{Collagen-NH}_2 + \text{HCHO} \rightarrow \text{Collagen-NH-CH}_2\text{OH}$$

The $N$-hydroxymethyl group is highly reactive and cross-linking may occur at a second amino group:

$$\text{Collagen-NH-CH}_2\text{OH} + \text{H}_2\text{N-Collagen} \rightarrow \text{Collagen-NH-CH}_2\text{-NH-Collagen}$$

Note, formaldehyde will react as a polymerise species, depending on the conditions.
Glutaraldehyde (OCH-(CH₂)₃-CHO) is a dialdehyde that can be used as tanning agent. It forms semiacetal bonds with the hydroxyls of hydroxyproline, hydroxylysine, and serine. With phenols, it produces insoluble compounds, so it cannot be used with vegetable tannins. Glutaraldehyde gives leather with the characteristics of perspiration resistance, washing resistance, better fullness and density⁴⁴. As with formaldehyde, glutaraldehyde is polymerised in solution (Figure 1.7); the terminal hydroxyl groups of the polymer are active and capable of reacting with amino groups. With regard to health and safety implications, formaldehyde and glutaraldehyde are unlikely to be suitable for the leather industry of the future. Besides that, the hydrothermal stability of the aldehydes tanned leather is not high, i.e. 85°C maximum⁴⁵.

![Chemical Structure](image)

**Figure 1.7 The reactivity of glutaraldehyde⁴⁵**

Oxazolidines, heterocyclic derivatives obtained by the reaction of aminohydroxy compounds with formaldehyde⁵²,⁵³, are an alternative to aldehyde tannage. Under hydrolytic conditions, the rings open to form an N-hydroxymethyl compound, which can react with one or more amino groups to produce effective cross-linking (Figure 1.8)⁵². However, the reaction mechanism of oxazolidine with collagen and its toxicity needs further investigation.
Oxazolidines have been shown to possess high reactivity and good tanning ability. Leather tanned by oxazolidine E (Figure 1.8) has similar shrinkage temperature to glutaraldehyde tanned leather\textsuperscript{32}, but is less full and less hydrophilic, because the molecular weight of monomeric oxazolidine is smaller than that of polymerised glutaraldehyde.

\begin{center}
\includegraphics[width=\textwidth]{figure1_8}
\end{center}

\textbf{Figure 1.8} Ring opening of oxazolidine and cross-linking of collagen with oxazolidine\textsuperscript{53}

\subsection*{1.4.4 Quinone tanning}

Quinone tanning is usually separately discussed as a specific tanning process. The tanning agent involved is a compound of small molecular size, containing a functional group of one kind only. In the time of chromium shortage (e.g. in France during World War II), quinone was used in the pretanning process\textsuperscript{44}. 
Quinones react with the collagen amino groups to form aminohydroquinone or 2,4-aminoquinone, as shown in Figure 1.9. As a result of nucleophilic attack on quinone, ring aromatisation occurs: the redox potential of quinone decreases by about 250 mV, so the compound obtained is easily oxidised by another quinone molecule; hence two hydroquinone molecules may react, so a cross-linking quinone is formed. Products of this type can be separated from the reaction mixture. The reaction kinetics are pH-dependent; in acidic or alkaline (pH 10) medium, the $T_s$ increases rapidly, then it maintains the level reached or drops slightly. In neutral solution, the $T_s$ initially rises quickly, but the reaction remains incomplete for a long time.

![Figure 1.9 Reaction of quinone with collagen](image)

Quinone tanning is conducted in buffer solution, because with pH increase, quinone solutions become dark and so does the leather. The properties of the anion of the buffering compound affect the value of pH at which quinone-collagen binding occurs. For borate buffer, the optimal binding pH is about 5, for
phosphate it is pH 7-8. The tanning process takes 24-48 hours. The shrinkage temperature \(T_s\) of quinone tanned leathers may be as high as 90°C. The amount of quinone bound is high, 20% or more, particularly at optimal pH. The proteolytic resistance of quinone-tanned leather is very high.

1.5 Synthetic tanning materials

Synthetic tanning materials (syntans) are organic compounds capable of converting skins or hides into imputrescible leather. Syntans are produced with the aim of aiding or substituting vegetable tanning, with the incentive of decreasing cost and providing unlimited supply. Syntans can be classified into three types, according to their primary properties, i.e. auxiliary syntans, replacement syntans, and combination or retanning syntans.

Auxiliary syntans are frequently based on naphthalene and are synthesised by the ‘Nerodol’ method, i.e. the base is sulfonated to high degree and then may be polymerised by formaldehyde (Figure 1.10). The presence of the sulfonate groups means that these compounds can interact with the amino sidechains of collagen:
Collagen-NH₃⁺ — 0₃S-Syntan

The auxiliary syntans have low tanning power; they are poor tanning agents, if used alone. They are mainly used to bleach the surface, level dyes, disperse tannins, and fatliquors.

Replacement syntans or exchange syntans are used to replace vegetable tannins, so they can be used as solo tanning agents. They vary in their effects on leather, but they can produce properties similar to vegetable tannins, including raising the shrinkage temperature to 75-80°C.

Combination syntans are usually based on simple phenolic compounds: they are synthesised by the 'Novolac' method, i.e. the base material is polymerised with formaldehyde and then the product is partially sulfonated (Figure 1.11). They are relatively small polymers, with consequently weak tanning power. These syntans are used in mixture with vegetable tanning material to give a better degree of tannage, yield, and fullness. This type can tan on its own, but not to the same degree as replacement syntans, so they are usually used as retanning agents.

![Chemical structure of syntans](image)

*Figure 1.11 The Novolac synthesis of syntans*
Lignosulfonic acid is the main solid component of sulfite liquor, which is produced in great amount by the pulp and paper industries. The structures of the main components of the sulfite liquor are similar to those of condensed vegetable tannins, but they do not have good tanning properties.

1.6 Lignin

Lignin is a major component of vascular tissues in terrestrial plants: it is an amorphous, water-insoluble, three-dimensional aromatic polymer. It is found in higher plants, including ferns, but not in liverworts, mosses, or plants of lower taxonomic ranking. Wood and other vascular tissues generally contain 20-30% lignin, mostly found within the cell wall, where it is intimately interspersed with the hemicelluloses, forming a matrix that surrounds the orderly cellulose microfibrils. In wood, lignin in high concentration is the glue that binds contiguous cells, forming the middle lamella. Reid stated that lignin is the material that confers the qualities of rigidity and durability of wood. In this natural composite material, the cellulose fibrils provide tensile strength, and the hemicellulose and lignin provide cross-linking, binding the structure together.

1.6.1 Biosynthesis of lignin

Lignin is derived from phenylalanine and, in grasses such as bamboo and wheat, from both phenylalanine and tyrosine, which are synthesised from sugars via the shikimic acid pathway. Figure 1.12 shows the pathways in the biosynthesis of lignin from carbon dioxide. L-phenylalanine is converted to trans-cinnamic acid, catalysed by phenylalanine ammonia-lyase (PAL), which is a key enzyme in the syntheses of various phenolic compounds including lignin and is widely distributed in higher plants. Tyrosine ammonia-lyase (TAL), which catalyses the formation of p-coumaric acid from tyrosine, is characteristically found in grasses, the lignin of which contains p-coumaryl alcohol as an additional lignin monomer as well as esterified p-coumaric acid. Deamination, ring hydroxylation,
phenolic methylation, and carboxyl reduction steps lead to the intermediate cinnamyl alcohol precursors of lignin, i.e. \( p \)-coumaryl, coniferyl, and sinapyl alcohols\(^{10,60} \). Figure 1.13 illustrates the chemical structures of lignin precursors.

![Figure 1.12 Metabolic pathway of carbon dioxide to lignin\(^{59} \)](image)

![Figure 1.13 Chemical structures of lignin precursors\(^{60} \)](image)
Lignin is made by the oxidative polymerisation of the cinnamyl alcohol precursors. Single electron oxidation of the phenolic hydroxyls in these precursors within the lignifying cell wall produces radical species, which exist in mesomeric forms. These couple essentially randomly with each other, but primarily with radicals in the growing lignin polymer, which contains phenolic hydroxyls and is itself a substrate for single electron oxidation\(^\text{10,60}\). Figure 1.14 illustrates various structural features in a schematic formula of aspen lignin. Table 1.3 lists the frequencies of the main substructures in gymnosperm and angiosperm lignins as deduced analytically\(^\text{60}\), the substructures are referenced to Figure 1.14.

![Schematic formula for a portion of aspen lignin, qualitatively illustrating important inter unit linkage types\(^\text{10}\)](image)
Table 1.3 Frequencies of the major substructures in representative gymnosperm and angiosperm lignins\textsuperscript{10,60}

<table>
<thead>
<tr>
<th>Substructure</th>
<th>Unit in Figure 1.14</th>
<th>Proportions (% of total C\textsubscript{9}-units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-O-4 ((\beta)-aryl ether)</td>
<td>1-2, 3-4</td>
<td>48 Spruce</td>
</tr>
<tr>
<td>(\beta)-5 (phenylcoumaran)</td>
<td>2-3</td>
<td>9-12 Spruce</td>
</tr>
<tr>
<td>5-5 (biphenyl)</td>
<td>4-5</td>
<td>9.5-11 Spruce</td>
</tr>
<tr>
<td>(\alpha)-O-4 ((\alpha)-aryl ether)</td>
<td>7-8</td>
<td>6-8 Spruce</td>
</tr>
<tr>
<td>(\beta)-1 (1,2-diarylpropane)</td>
<td>7-9</td>
<td>7 Spruce</td>
</tr>
<tr>
<td>4-O-5 (diphenyl ether)</td>
<td>6-7</td>
<td>3.5-4 Spruce</td>
</tr>
<tr>
<td>(\beta)-O-4 (glyceraldehyde-2-aryl ether)</td>
<td>4</td>
<td>2 Spruce</td>
</tr>
<tr>
<td>(\beta)-(\beta) (resinol-type)</td>
<td>5-6</td>
<td>2 Spruce</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>87-94 Spruce</td>
</tr>
</tbody>
</table>

Synthesis of a polymer resembling lignin can be achieved by oxidative polymerisation of coniferyl, sinapyl, and/or \(p\)-coumaryl alcohol using commercially available horseradish peroxidase and hydrogen peroxidase\textsuperscript{61,62}. This synthetic lignin or dehydropolymerisate (DHP) of coniferyl alcohol is a macromolecular material and contains an inter lignol bond identical to those found in native lignin\textsuperscript{62}.

1.6.2 Degradation of lignin

1.6.2.1 Biological degradation of lignin

Lignin is biodegraded by a unique enzymatic ‘combustion’, i.e. non-specific enzyme-catalysed mineralisation. Lignin is degraded by a narrower array of microorganisms than the other major biopolymer, cellulose. Lignin biodegradation is central to the earth’s carbon cycle because lignin is second only to cellulose in abundance and perhaps is more significant, because lignin physically protects most of the world’s cellulose and hemicellulose from enzymatic hydrolysis\textsuperscript{9,63}.
Due to the size, non-hydrolysability, heterogeneity, and molecular complexity of lignin, its initial biodegradation is oxidative and non-specific, and mediated by an extracellular system. It is clear from observation of lignin mineralisation that its conversion to CO$_2$ and H$_2$O is thermodynamically favoured$^{9,63}$.

1.6.2.1.1 Lignin degrading microorganisms

Fungi and bacteria are able to degrade lignin and, while higher life forms such as termites and other xylophagical insects are reputed to digest lignin, this ability is probably conferred through certain elements of their intestinal microflora. Fungi, which degrade wood polymers, including the lignin component, are classified into three specific decay groups: white-rot, brown-rot, and soft-rot fungi$^{10,57}$. Their name is derived from the white, brown, and soft residue in affected wood, respectively$^{64}$. In addition, various soil fungi, e.g. Fusarium, are reported to degrade lignin, although their contribution to polymer transformation in the biosphere remains to be established$^{57}$.

Certain Ascomycetes and Fungi imperfecti attack wood extensively under conditions of high humidity$^{65}$. Buswell and Odier$^{57}$ found that this type of rot is characterised by a softening of wood tissue accompanied by significant weight loss. These fungi penetrate the secondary wall of the wood cell, forming cylindrical cavities in which the hyphae propagate. Soft-rot fungal species are found among several genera, including Chaetomium, Cephalosporium, Allecheria, Graphium, Monodictys, Paecilomyces, Polpulospora, and Thielavia.

Brown-rot fungi mainly decompose the polysaccharides in wood, so attack results in only a limited decrease in the lignin content$^{66-68}$, however, the residual lignin is chemically modified. These basidiomycetous fungi are closely related taxonomically to white-rot fungi, and there are several genera, e.g. Poria, Polyporus, Lentinus. Brown-rot fungi colonise wood in a similar way to white-rot fungi but do not appear able to bore through the fibre$^{69}$, the fungal hyphae
advancing longitudinally through the lumina of the wood cells\textsuperscript{70}. Decomposition is largely confined to the less lignified S\textsubscript{2} and S\textsubscript{1} layers of the secondary wall\textsuperscript{70,71}.

White-rot fungi are able to degrade all the major components of wood and are generally considered to be the main agents of lignin decomposition in nature\textsuperscript{72,73}. While the polysaccharides and lignin are often degraded simultaneously, the relative rates at which the cellulose, hemicellulose, and lignin are attacked may vary depending upon the fungus and conditions\textsuperscript{73}.

There are a lot of species of white-rot fungi, which form a heterogenous group consisting mostly of \textit{Basidiomycetes} belonging to a number of families of \textit{Hymenomycetes} (e.g. \textit{Agaricaceae}, \textit{Corticiaceae}, \textit{Hydnaceae}, \textit{Polyporaceae}, and \textit{Thelephoraceae}) and a few \textit{Ascomycetes} in the order Sphaeriales (e.g. \textit{Ustilina vulgaris}, \textit{Xylaria polymorpha})\textsuperscript{77}. Amongst the white-rot fungi, \textit{Phanerochaete chrysosporium}\textsuperscript{74,75,76}, \textit{Coriolus versicolor}\textsuperscript{77,78}, \textit{Sporotrichum pulverulentum}\textsuperscript{15,16,79}, and \textit{Pycnoporus cinnabarinus}\textsuperscript{80} are the most widely studied organisms.

The association of bacteria with rot in wood is well documented. Many genera of \textit{Actinomycetes} (e.g. \textit{Nocardia}, \textit{Streptomyces}, \textit{Thermomonospora}, \textit{Micromonospora}) and \textit{Eubacteria} (e.g. \textit{Pseudomonas}, \textit{Acinetobacter}, \textit{Xanthomonas}, \textit{Bacillus}, \textit{Aeromonas}) degrade various type of extracted lignin and \textsuperscript{14}C-labeled DHPs. However, reports of bacterial attack on lignin are less numerous and are currently restricted to \textit{Actinomycetes} and unusual wood-degrading bacteria, tentatively identified as \textit{Myxobacteria}\textsuperscript{77}.

\subsection*{1.6.2.1.2 Lignin degrading enzymes}

Three main fungal enzymes that affect lignin structures are lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. All three enzymes can act with low molecular weight mediator molecules to bring about lignin oxidation. Some white-rot fungi do not produce all three, but only two or one of the enzymes.
Peroxidase function requires extracellular hydrogen peroxide, which is supplied by other enzymes involved in lignin degradation system\textsuperscript{9}.

\textit{a. Laccase (E.C. 1.10.3.2)}

The first protein implicated as a lignin degrading enzyme was laccase, a copper-containing polyphenol oxidase produced extracellularly and isolated from the white-rot fungus \textit{Trametes versicolor} (also designated \textit{Coriolus}, \textit{Polyporus} or \textit{Polystictus versicolor}). The primary role of laccase was considered to be polymerisation of phenolics, although there were indications that under specific conditions, such as in the presence of hydrogen peroxide or in organic media, some depolymerisation of lignin occurred\textsuperscript{78}. Kirk and Farell\textsuperscript{9} reported that laccase is produced by most white-rot fungi, with the notable exception of \textit{P. chrysosporium}. This fungus belongs to a minority of white-rot fungi that produce no detectable laccase. This blue copper oxidase catalyses the one-electron oxidation of phenols to phenoxy radicals, eventually transferring four electrons to \textit{O}_2. The effect on the substrate phenols is the same as that of horseradish peroxidase, despite fundamental differences in enzyme mechanism.

Laccase normally oxidises only those lignin model compounds with a free phenolic group, forming phenoxy radicals. However, in the presence of the artificial substrate 2,2'-azinobis(3-ethylbenztbiazoline-6-sulfonate) (ABTS), laccase can also oxidise certain non-phenolic compounds. ABTS functions as mediator for laccase to oxidise non-phenolic compounds. It also enhances the ability of laccase to degrade the residual lignin in Kraft pulps\textsuperscript{81}.

Morohoshi\textsuperscript{82} carried out degradation experiments using some model compounds of lignin. From the degradation of syringylglycerol-β-guaiacyl ether (SOG) by laccase, 3-hydroxy-2-(o-methoxy phenoxy)-propionic acid (I), 2-(o-methoxy phenoxy) ethanol (II), syringaldehyde(III), guaiacol (IV), 2,6-dimethoxy benzoquinone-1,4 (V), and 4-formyl-6-methoxy benzoquinone-1,2 (VI) were detected in the first stage of the reaction (after 1.5 hours). Compounds guaiacol
(IV), 2,6-dimethoxy benzoquinone-1,4 (V), and 4-formyl-6-methoxy benzoquinone-1,2 (VI) were found in the late stage (after 10 hours). Figure 1.15 demonstrates the proposed degradation pathway of SOG by laccase. The main reaction is the cleavage of C-C bonds between the α- and β-carbons in side chains and a side reaction is the cleavage of alkyl phenyl bonds. The subsequent reaction is the cleavage of β-O-4 ether linkages.

![Proposed degradation pathway of syringylglycerol-β-guaiacyl ether (SOG) by laccase](image)

**Figure 1.15** Proposed degradation pathway of syringylglycerol-β-guaiacyl ether (SOG) by laccase

**b. Lignin peroxidase (E.C. 1.11.1.14)**

Lignin peroxidase (LiP) is a haem peroxidase with an unusually high redox potential and low optimum pH. It shows little substrate specificity, reacting with a wide variety of lignin model compounds. Lignin peroxidase from *P. chrysosporium* was initially isolated by various chromatographic procedures and
was shown to contain one mole of protohaem IX per mole of enzyme, and to have molecular mass 41-42 kD. The enzyme is referred to as an H2O2-requiring oxygenase\textsuperscript{83,84}. It is able to oxidise methoxylated aromatic rings without a free phenolic group, generating cation radicals that can attack further by a variety of pathways, including C\textsubscript{\alpha}-C\textsubscript{\beta} cleavage and ring opening\textsuperscript{58}. Hammel et al.\textsuperscript{85} found that LiP can depolymerise DHP in vitro, if the DHP is presented in low concentration in a reaction mixture containing 30% or more of an organic solvent.

Evans\textsuperscript{86} reported that the mechanism of reaction for LiP is characteristic of peroxidases in general, with the first stage in the reaction involving the formation of a radical cation in the substrate molecule. Reactions of this enzyme include C\textsubscript{\alpha}-C\textsubscript{\beta} bond cleavage\textsuperscript{87}, aromatic ring cleavage, aryl-C\textsubscript{\alpha} cleavage, phenolic oxidation, and demethoxylation (Figure 1.16)\textsuperscript{88-91}.

\begin{figure}[h]
\centering
\includegraphics{reactions.png}
\caption{Reactions of lignin peroxidase\textsuperscript{86}}
\end{figure}
Tien and Kirk\textsuperscript{92} reported the isolation of LiP from cultures of \textit{Phanerochaete chrysosporium} which could degrade lignin model dimers and polymeric lignin, both synthetic DHP lignin and milled wood lignin. The enzyme also catalyses the oxidation of veratryl alcohol (3,4-dimethoxybenzyl alcohol) to veratraldehyde in the presence of catalytic amounts of hydrogen peroxide.

\textit{c. Manganese peroxidase (E.C. 1.11.1.13)}

Kuwahara \textit{et al.}\textsuperscript{93} discovered a peroxidase activity different from ligninase in the extracellular growth fluid of ligninolytic cultures of \textit{P. chrysosporium}. The isolated 46 kD enzyme exhibited a requirement for H\textsubscript{2}O\textsubscript{2}, Mn(II), and lactate. Like horseradish peroxidase, it oxidises phenol red, \(\alpha\)-dianisidine, and polymeric dyes.

Manganese peroxidase is another haem peroxidase, but it shows a strong preference for Mn(II) as its reducing substrate\textsuperscript{94}. The enzyme oxidises Mn(II) to Mn(III), which in turn oxidises the organic substrates\textsuperscript{94,95}. Mn(III) forms a complex with organic acids and diffuses away from the enzyme to oxidise other materials, such as lignin. The redox potential of the MnP-Mn system is lower than that of LiP and it does not oxidise non-phenolic lignin models. Phenolic substrates are oxidised to phenoxy radicals, which can react further by demethylation, alkyl-phenyl cleavage, \(C_\alpha\) oxidation or \(C_\alpha-C_\beta\) cleavage\textsuperscript{96}.

1.6.2.2 Biomimetic degradation of lignin

Studies of the biochemical mechanisms of lignin degradation indicate that two types of extracellular haem-containing enzyme play important roles in the decay of lignin by white-rot fungi. These enzymes are peroxidases and include a group of lignin peroxidases\textsuperscript{83,84,99-101} and a group of manganese peroxidases\textsuperscript{95,102-104}. Ligninases appear to initiate lignin degradation by extracting one electron from methoxylated aromatic rings, forming cation radical species, which undergo further non-enzymatic reaction (e.g. reactions with H\textsubscript{2}O or O\textsubscript{2} or both) that lead to
lignin decomposition. Manganese peroxidases oxidise Mn(II) to Mn(III), which may diffuse into wood cells and initiate additional oxidative reactions\textsuperscript{102,104}.

Several researchers have used a biomimetic approach to the study of ligninase mechanisms. Shimada \textit{et al.}\textsuperscript{105} reported that a synthetic tetraphenyl-porphyrin iron(III) chloride [TPP(Fe)Cl] in combination with iodosylbenzene or tert-butyl hydroperoxide (TBH) in organic solvent would cleave C\textsubscript{a}-C\textsubscript{b} bonds in side chains of diarylpropane lignin model compounds in the same manner as LiP does. Habe \textit{et al.}\textsuperscript{106} demonstrated that haemin in the presence of hydrogen peroxide or TBH mimicked LiP by catalysing the same C\textsubscript{a}-C\textsubscript{b} bonds cleavage of the lignin model compound 1,2-bis-(4-methoxyphenyl)propane-1,3-diol, giving \textit{p}-anisaldehyde. The optimal pH for the reaction was 3.0 in 80\% aqueous dimethylsulfoxide, which is similar to that of LiP. Shimada \textit{et al.}\textsuperscript{107} reported that both haemin and TPP(Fe)Cl showed catalytic activity to oxidise various lignin model compounds, but haemin was more effective than TPP(Fe)Cl. Paszczyński \textit{et al.}\textsuperscript{108} used the biomimetic approach to treat wood chips and wood pulps with a variety of natural haems and synthetic porphyrins in the presence of TBH or H\textsubscript{2}O\textsubscript{2} in aqueous solution. They found that the treated wood chips were delignified extensively and were significantly depleted of their hemicelluloses. Huynh\textsuperscript{109} demonstrated that non-haem single-electron oxidants (e.g. copper peroxidisulfate) mimicked LiP in their oxidation of lignin model compounds such as veratryl alcohol and veratryl glycerol-\textit{p}-guaiacyl ether. Hammel \textit{et al.}\textsuperscript{110} used Mn(III) acetate as a biomimetic model for enzymatically generated Mn(III) and reported that this oxidant oxidised non-phenolic lignin models, such as the monomeric lignin model veratryl alcohol and the diarylpropane model 1-\textit{(3,4-dimethoxyphenyl)-2-phenyl propanol. Cui \textit{et al.}\textsuperscript{111} reported that metallophorpyrins are biomimetic catalysts for lignin model compound degradation. The reactions catalysed by the metalloporphyrin include benzylic alcohol oxidation, benzylic methylene hydroxylation, C\textsubscript{a}-C\textsubscript{b} double bond dihydroxylation, aromatic demethoxylation, side chain C-C bond cleavage, side-chain oxidation, and aromatic ring cleavage reactions.
In view of the renewed interest in alternative pulping and bleaching processes, the possibility of using enzymes as mild and environmentally benign agents is being considered by the pulp and paper industry. Biomimetic catalysts such as metalloporphyrins, which can yield highly oxidised metallo-oxo species, have been used as LiP models, and their potentiality for lignin degradation has been subject of several studies. The biomimetic systems with porphyrin catalysts mimicking LiP seem to be more economical and ideal for practical use and industrial application.

Residual Kraft lignin contains significant amounts of 5,5' and diphenylmethane substructures. Crestini et al. reported the oxidation pathway of model compounds of 5,5' and diphenylmethane lignin models in the presence of cationic and anionic water soluble porphyrins, using hydrogen peroxide as an oxidant. The main oxidation products of both model compounds were \( p \)-quinones.

1.7 Melanin

Melanins are pigments of high molecular weight formed by oxidative polymerisation of phenolic compounds and usually are dark brown or black. They are widely distributed in the living world. Melanins occur across the spectrum of living organisms, from higher plants and lower animals to humans. Melanin is the black chemical in human skin, hair, and eyes.

1.7.1 Classification of melanin

Mammalian (animal) melanins can be classified into two major groups: the brown to black eumelanins and the yellow to reddish-brown pheomelanins. Eumelanin is the dark pigment which predominates in black and brunette hair. Pheomelanin is a lighter pigment, which is found in red and blond hair.

In fungi, several different types of melanin have been identified. The two most important types are DHN-melanin (named for one of the pathway intermediates,
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General introduction

1,8-dihydroxynaphthaleine) and DOPA-melanin (named for one of the precursor, L-3,4-dihydroxyphenylalanine)\textsuperscript{118,119}. Most fungal melanins are DHN-melanins\textsuperscript{120}.

1.7.2 Biosynthesis of melanins

1.7.2.1 Mammalian melanins

Mammalian melanin is a polymer derived from the amino acid tyrosine. The synthesis of melanin is catalysed by the enzyme tyrosinase, which is found in only one specialised type of cell, the melanocyte. In this cell, melanin is found in membrane-bound bodies called melanosomes\textsuperscript{116}.

![Biosynthesis of melanins](image)

**Figure 1.17** Raper-Mason scheme for eumelanogenesis, modified on the basis of findings by Pawelek, Prota, and Ito\textsuperscript{24}

Raper\textsuperscript{121} and Mason\textsuperscript{122} proposed the Raper-Mason scheme for eumelanogenesis. The scheme, which is still valid in most aspects, is depicted in Figure 1.17\textsuperscript{24}. The melanocyte-specific enzyme catalyses the first two steps in melanogenesis: the
hydroxylation of tyrosine to DOPA and the oxidation of DOPA to
dopaquinone\textsuperscript{123}. Dopaquinone undergoes intramolecular cyclisation to form
leukodopachrome at very high rate (step 3)\textsuperscript{124}. Leukodopachrome is oxidised by
dopaquinone to form dopachrome and DOPA by a redox-exchange reaction (step
4). It has been assumed that rearrangement of dopachrome (step 5) proceeds
similarly in vivo, producing 5,6-dihydroxyindole (DHI), as a major melanin
intermediate next to dopachrome, and 5,6-dihydroxyindole-2-carboxylic acid
(DHICA). The final stage of eumelanogenesis is the oxidative polymerisation of
DHI and DHICA, leading to the black pigment eumelanin (step 6 to 8)\textsuperscript{24}.

\textbf{Figure 1.18 Prota scheme for pheomelanogenesis}\textsuperscript{24}

Prota, Nicolaus, and collaborators postulated that pheomelanins are derived from
the interaction of cysteine with dopaquinone produced by the oxidation of
tyrosine by tyrosinase\textsuperscript{125,126} (Figure 1.18). The first two steps in pheomelanoge-
nesis are the same as eumelanogenesis. The addition of cysteine to dopaquinone
proceeds extremely fast to produce cysteinyldopas (step 9)\textsuperscript{124}. Oxidation of
cysteinyldopas by dopaquinone proceeds via cysteinyldopaquinones (step 10),
which rapidly undergo intramolecular condensation to give the cyclic quinone-imine intermediates (step 11). They rearrange with or without decarboxylation into the benzothiazine derivatives (step 12)\textsuperscript{127}. The final stage of pheomelanogenesis (step 13) is not well characterised, however, the tendency of 2H-1,4-benzothiazines to undergo oxidative coupling at the 2 positions is well documented\textsuperscript{128}. The coupling between 2 position gives trichochromes, whereas coupling between the 2 position and other positions produces pheomelanins\textsuperscript{24}.

1.7.2.2 Fungal melanins

The best characterised fungal melanin is dihydroxynaphthalene (DHN) melanin, otherwise known as polyketide melanin. The DHN-melanin biosynthesis pathway is based on genetic and biochemical evidence obtained from Verticillium dahiae\textsuperscript{129,130} and Wangiella dermatitidis\textsuperscript{131,132}. A general model for DHN-melanin biosynthesis is depicted in Figure 1.19.

Malonyl-Co-A serves as the starter and extender unit for polyketide synthetase (PKS1) catalysing the first step in the biosynthesis pathway\textsuperscript{133}. The polyketide synthetase (PKS) converts malonyl-Co-A to the first detectable intermediate of the pathway, 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN). Following this, 1,3,6,8-THN is reduced by a specific reductase enzyme to produce scytalone\textsuperscript{134}. Scytalone is dehydrated enzymatically to 1,3,8-trihydroxynaphthalene\textsuperscript{135}, which is in turn reduced, possibly by a second reductase, to vermelone\textsuperscript{136,137}. This reductase can also be inhibited by tricyclazole. A further dehydration step, possibly also catalysed by scytalone dehydratase, leads to the intermediate 1,8-dihydroxynaphthalene (DHN), for which this pathway was named. Subsequent steps are thought to involve a dimerisation of the 1,8-DHN molecules, followed by polymerisation, possibly catalysed by a laccase\textsuperscript{138}.

The final step of the DHN melanin pathway is the conjoining of 1,8-DHN molecules to form the melanin polymer. There is also the possibility that various
dihydroxynaphthalene dimers may act as the immediate precursors to melanin rather than 1,8-DHN\textsuperscript{139}.

Figure 1.19 Schematic representation of fungal dihydroxynaphthalenes (DHN)-melanin biosynthesis pathway\textsuperscript{25,26,140}

1.8 Enzymology

1.8.1 Theory of enzyme action

Enzymes are proteins that catalyse biochemical reactions, so they influence the rate at which equilibrium is obtained, but do not affect the overall equilibrium of
the reaction. The reaction is accelerated by providing a reaction route having a lower free energy of activation for the transition of substrate to products than the uncatalysed process\textsuperscript{141-143}. The rate of a process is determined by the free energy levels of the rate-limiting transition state in the reaction pathway; the higher the free energy barrier the slower the rate\textsuperscript{142}.

1.8.2 Kinetic analysis of enzyme activity

Kinetic studies are used to measure the affinity and the specificity of binding of substrates and of inhibitors to enzymes, to establish maximum rates of catalysis by specific enzymes\textsuperscript{143}.

The first observed rate or initial velocity ($v_o$) of enzyme-catalysed reactions increases with increasing substrate concentration only until a substrate level is reached beyond which further additions of substrate do not increase the initial rate. This phenomenon and the maximum initial velocity ($V_{max}$) are achieved at substrate saturation\textsuperscript{142}.

Plummer\textsuperscript{144} observed that if the activity of an enzyme is determined over a range of substrate concentrations, a rectangular hyperbola curve is often obtained. At low substrate concentrations, $v_o$ varies linearly with $[S]$, which gives first order kinetics:

$$v_o = \frac{d[S]}{dt} = k[S]$$

where $k$ is the rate constant.

At high substrate concentrations $v$ is independent of $[S]$, giving zero order kinetics:

$$v_o = \frac{d[S]}{dt} = \text{constant} = V_{max}$$

At intermediate substrate concentrations there is a mixture of first and zero order kinetics.
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An equation relating $v_o$ and $[S]$ can be obtained for the whole curve and was first derived by Michaelis and Menten in 1913, known as the Michaelis-Menten equation:\(^{141-144}\):

$$v_o = \frac{V_{\text{max}} [S]}{K_M + [S]}$$

where:

$v_o$ = initial velocity of enzyme-catalysed reaction

$[S]$ = substrate concentration

$V_{\text{max}}$ = maximum initial velocity

$K_M$ = Michaelis constant.

The kinetics constants $K_M$ and $V_{\text{max}}$ are most conveniently determined from a linear transformation of the equation that is obtained by taking reciprocals:

$$\frac{1}{v_o} = \frac{1}{V_{\text{max}}} + \frac{K_M}{V_{\text{max}}} \left( \frac{1}{[S]} \right)$$

A plot of $1/v_o$ against $1/[S]$ gives a straight line of slope $K_M/V_{\text{max}}$, as shown in Figure 1.20. The reciprocals of the kinetic constants can then be determined from the intercepts on the axis:

$$1/[S] = 0, \quad 1/v_o = 1/V_{\text{max}},$$

and

$$1/v_o = 0, \quad 1/[S] = -1/K_M.$$  

Plots derived from other formulations of the Michaelis-Menten equation may also be used to obtain the same constants\(^{141-143}\).

Hanes-Woolf plot:

$$\frac{[S]}{v_o} = \frac{1}{V_{\text{max}}} [S] + \frac{K_M}{V_{\text{max}}}$$
Eadie-Hofstee plot:

\[ v = -K_m \frac{V_o}{[S]} + V_{\text{max}} \]

Figure 1.20 Determination of the Michaelis-Menten constant – Lineweaver-Burk plot\textsuperscript{141-144}

1.9 Introduction to this study

In this study, Kraft lignin was degraded to produce simple phenols and then exploited the products for leather tanning and dyeing. Do the degradation products give good leather tanning or dyeing effects towards collagen? In this project, Kraft lignin was degraded and then the products were used in tanning and dyeing studies.

Melanins, pigments found in animals, plants, and microorganisms, have chemical similarity with lignins. They are synthesised by oxidation and polymerisation of phenols by an enzyme called laccase, a polyphenol oxidase. Simple phenols such as catechol are precursors of animal melanin\textsuperscript{25,27}. Can the melanin type reaction be exploited for leather colouring and tanning? Is it possible for other simple phenols or polyphenols to be used for leather colouring? To answer the questions, the dyeing and tanning study using phenols and crude laccase were studied.
1,8-dihydroxynaphthalene, an aromatic diphenol, is a precursor of fungal melanin\textsuperscript{25,26,28,29}. Can other dihydroxynaphthalenes rather than 1,8-DHN be used for melanin synthesis? The possibilities of these as other precursors to melanin, as well as the tanning effects of the DHNs towards collagen were studied.

Oxazolidine will react with the amino groups of collagen to form cross-links to improve the shrinkage temperature of leather\textsuperscript{52,145}. Can combination tanning using phenols including the lignin degradation products and DHNs, and oxazolidine produce leather with high hydrothermal stability? In this work, tanning studies using the phenols and oxazolidine as the cross-linking agent were investigated.

1.10 Objectives and aims of this study

Objectives of the study were to define the potential of use of lignin degradation products and phenols for leather tanning and dyeing and to exploit melanin-type reactions for leather colouring.

The aims of the study were:

1. to degrade Kraft lignin and to identify its degradation products,
2. to undertake tanning and dyeing studies with the lignin degradation products and laccase,
3. to investigate the tanning and dyeing effects of phenolic compounds and laccase,
4. to investigate tanning effects of the phenolic compounds in conjunction with oxazolidine as a cross-linking agent,

by addressing the following aspects:
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1. degradation of Kraft lignin using lignin peroxidase-biomimetic and laccase-mediator systems,
2. tanning and dyeing effects of the phenolic lignin degradation products,
3. dyeing reactions of some simple phenols and polyphenols,
4. reactions of dihydroxynaphthalenes for leather tanning and dyeing,
5. combination tanning studies using the phenolic compounds and oxazolidine.
CHAPTER 2

MATERIALS AND METHODS
2.1 Materials

2.1.1 Chemicals and reagents

Kraft lignin, haemin, and other analytical chemicals and reagents were purchased from Sigma-Aldrich Company Ltd., Dorset, UK, unless otherwise stated. Hydrogen peroxide was obtained from Beecroft & Partners, Rotherham, UK. Catechol, pyrogallol, hydroquinone, and resorcinol were obtained from BDH Ltd, Dorset, UK. L-DOPA and 3-hydroxytyramine were received from Prof. Christine S. Evans, University of Westminster, London, UK.

2.1.2 Enzyme

Crude laccase was produced from culture of Coriolus versicolor, kindly donated by Klenzyme Ltd., London, UK. Activity of the enzyme was 0.1 unit/mg: 1 unit of laccase activity causes a change in absorbance at 440 nm of 1.0 absorbance units per minute for 0.1 M catechol solution.

2.1.3 Hide powder

Hide powder was obtained from BLC The Leather Technology Centre, Northampton, UK. The hide powder was prepared from cattle hide according to the Official Method (SLC 10)\textsuperscript{146}. It had the following specification:

- Moisture content: 14.1%
- Ash content: 2.4%
- pH: 5.2

2.1.4 Sheepskin pickled pelt

New Zealand sheepskin pickled pelt was obtained from the British School of Leather Technology, Northampton, UK. The pickled pelt was degreased prior to tanning studies, see Appendix II.
2.1.5 Wet blue

Chrome tanned leather (wet blue) was conventionally prepared from the sheepskin pickled pelt. Degreased pickled pelt was repickled and tanned with basic chromium(III) sulfate, see Appendix III.

2.1.6 Basic chromium salt

Commercial grade basic chrome salt was supplied by Bayer Ltd, Leverkusen, Germany under the trade name Chromosal B. The chrome powder had the following specification:

- Chrome oxide content: 26%
- Sodium sulphate content: 23-24 %
- Basicity: 33.3%, Schorlemmer definition
- Appearance: dark green

2.1.7 Fatliquor

Fatliquor based on sulfited oxidised fish oil was supplied by Tumpler International Ltd, Worms, Germany under the trade name Truponol BTK. The fatliquor had the following specification:

- Appearance: red brown oil
- Charge: anionic
- Active mater: approx. 63%
- pH 10% emulsion: approx. 6.5
- Acid stability: very good
- Salt stability: very good
- Chrome stability: very good
- Light fastness: good
2.2 Methods

2.2.1 Biomimetic degradation of Kraft lignin

2.2.1.1 Effect of temperature

2.2.1.1.1 Experimental procedure

Haemin stock solution (0.2 ml) was added to 50 ml of Kraft lignin stock solution, followed by the addition of 2 ml of 30% hydrogen peroxide (H₂O₂). The suspension was heated at 90°C under reflux for 1, 3 or 5 hours. Experiments without heating were carried out with vigorous shaking on a reciprocal shaker at room temperature for 1, 3 or 5 hours. Controls were run in the absence of haemin and H₂O₂, with or without heating, for 5 hours. A part of the sample solution was dried to remove water and solvent in an oven under reduced pressure at 50°C and then dried over anhydrous sodium sulfate in a desiccator under reduced pressure.

Lignin stock solution:
1 g of lignin was dissolved in 500 ml of 50% aqueous 1,4-dioxane (1:1 v/v): the lignin was dissolved in dioxane first, before adding distilled water.

Haemin stock solution:
Haemin stock solution was 2 mM, made up from 13 mg of haemin dissolved in 10 ml of 1,4-dioxane and sealed to exclude air.¹⁰⁸

2.2.1.1.2 Sample analysis

Solutions containing degradation products were analysed using paper chromatography, tests for aldehydes and ketones, and ultraviolet (UV) spectroscopy. Dried samples were analysed using infrared (IR) spectroscopy.
a. Paper chromatography

Ascending chromatography, using Whatman chromatography paper grade 1, was used to identify carbonyl compounds and phenols in the degradation products. The procedure described by Smith and Feinberg\textsuperscript{147} was used.

Procedure:
50 ml of solvent was poured into the bottom of the chromatography tank and the lid was replaced. A 20x20-cm sheet of chromatography paper was prepared. One drop of sample was applied to the origin using a capillary pipette. The paper was formed into a cylinder and the chromatogram was run for about 4 hours. The chromatogram was removed and dried in the fume cupboard. The solvent front and spots were marked with a pencil under ultraviolet light. The chromatogram was treated with 2,4-dinitrophenylhydrazine (2,4-DNPH) reagent for carbonyl compounds and FeCl\textsubscript{3}-K\textsubscript{3}Fe(CN)\textsubscript{6} for phenol identification.

Solvents:
Ethanol - petroleum ether (boiling range 80-100°C) 2:1 v/v was used as solvent for carbonyl compound identification and 10\% acetic acid was used as solvent for phenol identification.

Detection reagents:

(1) 2,4-Dinitrophenylhydrazine reagent
The 2,4-DNPH reagent described by Bobbit\textsuperscript{148} was used. The reagent was prepared by dissolving 0.5 g of 2,4-DNPH in 100 ml of 2M HCl. The chromatogram was sprayed with the reagent solution. A yellow or red colour was formed as a reaction by aldehydes or ketones with the acidic 2,4-DNPH.

(2) Ferric chloride-ferricyanide reagent
Ferric chloride-ferricyanide [FeCl\textsubscript{3}-K\textsubscript{3}Fe(CN)\textsubscript{6}] reagent described by Smith\textsuperscript{149} was employed. The reagent was prepared by dissolving 3\% FeCl\textsubscript{3} and 3\% K\textsubscript{3}Fe(CN)\textsubscript{6}
in water. The reagents were prepared and stored in dark bottles. When required, each solution was diluted ten times and equal volumes of both reagents were mixed. The chromatograms were dipped through the resulting clear, bright brown solution, washed with 2M HCl and water and dried at room temperature. The reagent detects less than 1 μg of many phenols and tannins.

b. Test for aldehydes

The test procedure for aldehydes described by Diamond and Denman\textsuperscript{150} was used. Two drops of sample were added to 2 ml of Tollen's reagent in a test tube. The mixture was warmed to about 40°C in a beaker of warm water. Tollen's reagent consists of:

Reagent A: 3 g of silver nitrate in 30 ml of water,
Reagent B: 3 g of sodium nitrate in 30 ml of water.

1 ml of each reagent A and B were mixed, and dilute ammonia solution was added dropwise until the precipitate of silver hydroxide just dissolves. Aldehydes reduce Tollen's reagent and a silver mirror is formed on the wall of the tube; ketones do not reduce the reagent.

c. Test for ketones

The procedure of test for ketones used by Goddard and Brown\textsuperscript{151} was used. Excess (1 ml) 2M NaOH solution was added to 1 ml of sample and 0.1 g of \textit{m}-dinitrobenzene was added. Colour change of the mixture was observed; a violet coloration is produced if the sample contains ketones.

d. Ultraviolet spectroscopy

Samples containing lignin degradation products were diluted with 50% 1,4-dioxane-water, then 3 ml of the sample solution was put into a UV cell. The
absorbance was recorded by a Lambda 2 UV/VIS spectrophotometer (Perkin-Elmer, UK) with a light path of 1 cm. Wavelength range was 240-370 nm.

e. Infrared spectroscopy

Infrared spectroscopy analysis was carried out using KBr discs. The KBr discs were prepared from dried degraded lignin samples: 1.5 mg of the dried sample in 200 mg of KBr. The analysis was carried out using a 781 Infrared Spectrophotometer (Perkin-Elmer, UK). Wavenumber range was 4000-600 cm⁻¹.

2.2.1.2 Effect of reaction time

2.2.1.2.1 Experimental procedure

Kraft lignin (1g) was dissolved in 500 ml of 50% (v/v) aqueous 1,4-dioxane. The pH of the solution was adjusted to 3.0 by adding 2 M HCl. 2 ml of haemin stock solution was added to the solution, followed by the addition of 20 ml of 30% (w/v) H₂O₂. The solution was boiled under reflux for 25 hours. Samples were taken every 5 hours. Concentration of H₂O₂ in the solution was measured by titration, using an iodometric method (Section 2.2.1.2.2). H₂O₂ and haemin were added into the solution, to keep their concentrations constant. A part of the sample solutions was dried to remove water and solvent in a vacuum oven at 50°C and then dried over anhydrous sodium sulfate in a vacuum desiccator.

2.2.1.2.2 Analysis of hydrogen peroxide

The analysis procedure of hydrogen peroxide described by Vogel¹⁵² was employed. 2 ml of sample was diluted in 25 ml of water and then shaken. The sample solution was added gradually and with constant stirring to a solution of 1 g of potassium iodide in 100 ml of 1 M sulphuric acid contained in a stoppered bottle. The mixture was allowed to stand for 15 minutes and the liberated iodine was titrated with standard 0.1 M sodium thiosulfate. 2 ml of 0.1% (w/v) starch
solution was added, when the colour of the iodine was nearly discharged. A control determination was run at the same time.

2.2.1.2.3 Sample analysis

Degradation products were analysed using paper chromatography, tests for aldehydes and ketones, UV spectroscopy, and IR spectroscopy. Analysis procedures were described in Section 2.2.1.1.2.

2.2.1.3 Isolation of the lignin degradation products

Kraft lignin (0.1 g) was dissolved in 250 ml of 50% (v/v) 1,4-dioxane-water. The pH of solution was adjusted to 3.0 with 2M HCl, and then haemin solution containing 100 mg of haemin was added. 1 ml aliquots of H₂O₂ were added every 30 minutes over 2 hour, a total of 5 aliquots. The mixture was boiled at 90°C under refluxing for 5 hours. On completion, the reaction mixture was cooled over crushed ice. Control was prepared omitting haemin and H₂O₂.

Any residual peroxide was reduced with sodium sulfite, then the reaction mixture was centrifuged at 1900 g for 30 minutes. The supernatant, containing the degradation products, was acidified with 6 M HCl to pH 1.0 and then extracted four times with 200 ml of diethyl ether that had been previously treated with a saturated aqueous solution of Fe(NH₄)₂(SO₄)₆·H₂O to remove peroxides. The ether extract was dried over anhydrous sodium sulfate overnight. Solvent was evaporated on a water bath at 35-40°C and then under reduced pressure, using a rotary evaporator at 40°C to remove 1,4-dioxane.

2.2.1.4 Identification of the lignin degradation products

2.2.1.4.1 Ultraviolet spectroscopy

The same method was used as described in Section 2.2.1.1.2.d.
2.2.1.4.2 Fourier transform-infrared (FT-IR) spectroscopy

FT-IR spectroscopy analysis was carried out using KBr discs. The KBr discs were prepared from dried degraded lignin samples: 1.5 mg of the dried sample in 200 mg of KBr. The analysis was undertaken using an FT-IR 1720X spectrometer (Perkin-Elmer, UK) under the following conditions: spectral range 4000-400 cm\(^{-1}\); coated fast response deuterated triglycine sulfate (FR-DTGS) temperature stabilised detector.

2.2.1.4.3 Gas chromatography-mass spectrometry (GC-MS)

The ether extract of the degradation products was dissolved in 0.5 ml of pyridine. The mixture was then silylated by addition of \(N,O\)-bis-trimethylsilyl trifluoroacetamide (BSTFA) and 1% trimethylchlorosilane (TMCS) for GC-MS analysis. The sample solution (2 µl) was injected into a GC-MS system (Varian model CP-3800 GC and Saturn 2000 GC/MS) (Varian, UK) with a SGA BPX5 bonded phase, fused silica column (30m x 0.32 mm inner diameter, 0.1 µm film thickness). The temperature profile for GC operation was isothermal at 80°C for two minutes, followed by a 5°C/minute temperature gradient to 200°C and a 15°C/minute gradient to 280°C and isothermal period at 280°C for 10 minutes\(^{154}\). The MS conditions were electron ionisation mode (70 eV) and scanning rate of 60 scans/minute.

2.2.1.5 Yields of the lignin degradation products

Yields of the lignin degradation products were evaluated using GC-MS. Pure compounds of the degradation products were used as references or standards. The standards were run through GC-MS using the same procedure as used for samples as described in Section 2.2.1.4.3 and then peak area of each product was calculated. Calibrations for the yield measurement are given in Figure 2.1.
2.2.2 Enzymatic degradation of lignin

2.2.2.1 Degradation procedure

Degradation of Kraft lignin was carried out in 0.05M sodium acetate buffer (pH 5). 140 mg of lignin were suspended in 50 ml of the buffer in the presence of 5 mM 1-hydroxybenzotriazole (HBT), N-hydroxyphthalimide (HPI) or 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO). To the suspension was added 50 mg of crude laccase. The mixture was kept at 30°C for 48 hours in an open flask with vigorous shaking in order to ensure constant oxygen saturation of the solutions throughout the experiment.
2.2.2.2 Isolation of the enzymatic lignin degradation products

After the reaction was finished, the reaction mixture was cooled, and then the degradation products were isolated using the same procedure described in the second paragraph of Section 2.2.1.3.

2.2.2.3 Identification of the enzymatic lignin degradation products

2.2.2.3.1 FT-IR spectroscopy

The same method as described in Section 2.2.1.4.2 was employed to identify the enzymatic lignin degradation products.

2.2.2.3.2 GC-MS

The isolated products were identified by GC-MS using the same method as described in Section 2.2.1.4.3.

2.2.3 Tanning and dyeing studies using phenolic lignin degradation products

2.2.3.1 Tanning studies with the lignin degradation products

The Kraft lignin biomimetic degradation products were used in tanning studies on hide powder. The enzymatic degradation products were not used in the tanning studies, because, based on their structures, they are unlikely to have tanning effects on collagen.

2.2.3.1.1 Tanning experiment

1 g hide powder was soaked in 15 ml of water containing 1.5 g of salt (NaCl) at 30°C for 30 minutes. The pH of the hide powder suspension was adjusted to 4.5 with 5% formic acid solution, followed by addition of 0.4 g (40%) of each
degradation product, which was previously dissolved in 3 ml of acetone. Reaction was run at 35°C with shaking for 24 hours; then the mixtures were filtered through a fast filter paper (Whatman no. 541).

2.2.3.1.2 Measurement of tanning effect

Tanning effect of the lignin degradation products on collagen was measured by determining the denaturation or shrinkage temperature ($T_s$) and energy (enthalpy) ($\Delta H$) of shrinking of the treated hide powder. Untreated, wet hide powder was used as a control. The rise in shrinkage temperature ($\Delta T_s$) indicates tanning ability of the products. The $T_s$ and $\Delta H$ were measured using a DSC822e differential scanning calorimeter (DSC) (Mettler Toledo, UK). The DSC offers an objective method to evaluate the hydrothermal stability of collagen matrixes in the solid state. 10-15 mg of wet sample was weighed and placed in a 40 µl standard aluminium pan and then the pan was transferred to the DSC carousel. Samples were heated at a rate of 5°C/minute and measurement range was 35-100°C.

2.2.3.2 Combination tanning studies with the lignin degradation products and oxazolidine

2.2.3.2.1 Tanning experiment

The same procedure as described in Section 2.2.3.1.1 was employed. After discarding the float, 1000% (10 ml) water was added, then the mixture was adjusted to pH 6 with 5% NaHCO₃ solution; 10% oxazolidine (Neosyn TX) was added, then the mixture was shaken at 50°C for 2 hours. The mixtures were adjusted to pH 3.2 - 3.8 by adding 5% formic acid, then shaken at 35°C for 1 hour. On completion, the mixtures were filtered through a fast filter paper.
2.2.3.2.2 Measurement of tanning effect

Tanning effects of the lignin degradation products and oxazolidine were investigated using the same procedure as described in Section 2.2.3.1.2.

2.2.3.3 Dyeing studies with the lignin degradation products

2.2.3.3.1 Dyeing experiment

To produce a dyeing effect with the lignin degradation products, 1 g of hide powder was mixed with 25 ml of distilled water. Each lignin degradation product (1 mmol) was dissolved in 3 ml acetone, and then added to the hide powder suspension. The mixtures were shaken for 30 minutes at 30°C to rehydrate the hide powder and dissolve the lignin product and then the mixture was adjusted to pH 6 with 0.2M NaOH. Crude laccase (0.1g, containing 10 units) was added to catalyse the reaction. A blank for each lignin product omitted the enzyme. The reaction mixtures were shaken at 30°C for 24 hours. After reaction was finished, the hide powder was separated using a fast filter paper and dried at room temperature.

2.2.3.3.2 Colour measurement

Colours of the dyed hide powder were measured by a Minolta CR210 Chromameter (Minolta, Germany) by use of the parameters: L* (0 = black and 100 = white), a* (- = green and + = red), and b* (- = blue and + = yellow). ΔE* is calculated as \[ \Delta E^* = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)} \], and is an expression for the total quantitative colour change. ΔL*, Δa*, and Δb* are the delta values of L*, a*, and b* of samples respectively relative to the reference.
2.2.3.3 Measurement of hydrothermal stability

Hydrothermal stability of the dyed hide powder was evaluated using the same procedure as described in Section 2.2.3.1.2.

2.2.4 The phenolic lignin degradation products for syntans

2.2.4.1 Polymerisation of the phenolic compounds

The lignin degradation products were polymerised using formaldehyde with alkali catalysis\textsuperscript{156}. 0.05 mol of each lignin degradation product was dissolved in 50 ml of distilled water and 50 ml of 2M NaOH. The mixtures were stirred for 15 minutes at constant temperature 40°C and the pH of the solution was measured. 0.05 mol of 37\% (w/v) formaldehyde was added gradually at constant temperature 40°C with vigorous stirring and then the mixtures were stirred for 5 hours. The solution was neutralised with 2M HCl. Due to the difficulty in polymerising 2-methoxyphenol in alkaline conditions, the phenol was sulfonated prior to formaldehyde polymerisation and polymerised by acid catalysis\textsuperscript{2}. Sulfonation was carried out by adding 0.055 mol of concentrated sulphuric acid gradually at 90°C for 30 minutes with vigorous stirring; constant heating and stirring were continued for 2 hours. The sulfonic acid formed was diluted with three times volume of water, and then polymerised using the same procedure as above, except after heating at 40°C for 2 hours, the product was heated at 105°C for 15 minutes to complete the reaction of the formaldehyde.

2.2.4.2 Molecular weight distribution

Molecular weight distribution of the products was measured by gel permeation chromatography (GPC). The GPC analysis was carried out using a data collection unit PL-DCU (Polymer Laboratories, UK), HPLC pump LC 1120 (Polymer Laboratories, UK), UV detector 132 RI (Gibson Ltd., UK) and PL aquagel-OH30 column (polymer 8 µm particle size, 300 mm length, 7.5 mm inner diameter)
(Polymer Laboratories, UK). 10 µl of suitably diluted sample was injected into the column. The samples were eluted by deionised water at pH 7. The HPLC pump was set at a flow rate of 1.00 ml/minute. Polysaccharides (Polymer Laboratories) were used as references. Calibrations for the analysis were given in Figure 2.2.

Figure 2.2 Calibrations for gel permeation chromatography analysis with polysaccharide standards

2.2.4.3 Functional groups analysis

Functional groups of the polymerisation products were analysed using FT-IR spectroscopy. The same method was used as given in Section 2.2.1.4.2.

2.2.4.4 Tanning studies

2.2.4.4.1 Tanning procedure

The polymerisation products were used in tanning studies on hide powder. The same procedure as described in Section 2.2.3.1.1 was employed.
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2.2.4.4.2 Measurement of tanning effect

Tanning effects were investigated using the same procedure as described in Section 2.2.3.1.2.

2.2.4.5 Combination tanning studies using the polymerisation products and oxazolidine

2.2.4.5.1 Tanning procedure

Combination tanning using the polymerisation products and oxazolidine was carried out on hide powder. The same procedure as described in Section 2.2.3.2.1 was employed.

2.2.4.5.2 Measurement of tanning effect

Tanning effects of the polymerisation products and oxazolidine were measured using the same procedure as described in Section 2.2.3.1.2.

2.2.5 Dyeing and tanning studies using phenolic compounds

2.2.5.1 Dyeing and tanning effects on hide powder

Dyeing and tanning experiments using some simple phenols, aminophenols and polyphenols were carried out using the same procedure as described in Section 2.2.3.3.1, except the phenols were not dissolved in acetone prior to addition to the hide powder suspension.

2.2.5.2 Effect of enzyme concentration

The effect of the enzyme concentration on dyeing was studied on catechol, hydroquinone, pyrogallol, L-DOPA, and 3-hydroxytyramine. 1 g of hide powder and 0.001 mol of each substrate in 25 ml of distilled water were shaken for 30
minutes at 30°C, then the mixtures were adjusted to pH 6 with 2M NaOH. Various amounts (2.5, 5, 7.5, 10, 25, 50 mg) of enzyme were added to the reaction mixtures. Blanks of each substrate were prepared omitting the enzyme. The reaction mixtures were shaken at 30°C for 24 hours. On completion, the hide powder was filtered using a fast filter paper, and dried at room temperature. Colours of the dyed hide powder were measured by a Minolta CR210 Chromameter (Section 2.2.3.3.2). Effects of the enzyme concentration on the hydrothermal stability of the treated hide powder were measured using DSC (Section 2.2.3.1.2).

2.2.5.3 Effect of phenol concentration

The effect of phenol (substrate) concentration on dyeing used various concentrations of catechol, hydroquinone, pyrogallol, L-DOPA, and 3-hydroxytyramine. The enzyme concentration of 0.025 g/g hide powder was used.

Hide powder (1 g) and 0.1, 0.25, 0.5, 0.75, 1 or 1.5 mmols of each phenol in 25 ml of distilled water were shaken at 30°C for 30 minutes, then the mixtures were adjusted to pH 6 with 2M NaOH. Crude laccase (0.025g) was added to each reaction mixture. A blank was prepared without the addition of phenol. The reaction mixtures were shaken at 30°C for 24 hours. On completion, the hide powder was filtered using a fast filter paper, and dried at room temperature. Colours of the dyed hide powder were measured by a Minolta CR210 Chromameter (Section 2.2.3.3.2). Effects of the enzyme concentration on the hydrothermal stability of the treated hide powder were measured using DSC (Section 2.2.3.1.2).

2.2.5.4 Colour fastness to washing

Colour fastness to washing of hide powder dyed with catechol, hydroquinone, pyrogallol, L-DOPA, and 3-hydroxytyramine and crude laccase was measured using a modified procedure of SLF 423 (IUF 423)\textsuperscript{146}. In this test, the wash liquor
used was 0.1M sodium carbonate, a hydrogen bond breaker, instead of 5 g/l sodium lauryl sulfate.

Each dyed hide powder (0.9 g) and a sheet of a range of multifibre fabrics were placed in a 100 cm³ glass vessel. The composite sample was washed with 45 ml of 0.1M sodium carbonate and then shaken at 30°C for 30 minutes. The wash float was removed and replaced with 45 ml of distilled water with further shaking at 30°C for 10 minutes. The rinsing procedure was repeated with fresh distilled water. The multifibre fabric was removed and placed between a blotting paper and perspirometer plates. A 5 kg load was added and left for 1 minute. The multifibre fabric was removed and dried at 37°C. The sample of hide powder was filtered with a fast filter paper, then shaken in 45 ml of phosphate buffer (pH 6.0) for 30 minutes, filtered, and dried at room temperature. The change in colour of the sample and the accompanying textile were assessed according to the standard Grey Scale, SLF 131 (IUF 131) and SLF 132 (IUF 132) respectively. The colour of the sample was also measured by a Minolta CR210 Chromameter (Section 2.2.3.3.2); the shrinkage temperatures of the washed samples were measured by DSC (Section 2.2.3.1.2).

2.2.5.5 Kinetics of enzymatic reactions of the phenols with laccase

Kinetics of enzymatic reactions of catechol, hydroquinone, pyrogallol, L-DOPA, and 3-hydroxytyramine and laccase were investigated spectrophotometrically.

2.2.5.5.1 Determination of maximum wavelength

Maximum absorbances in the UV/VIS range for reaction between phenols and laccase were obtained from scans of each reaction mixture, using a Lambda 2 UV/VIS spectrophotometer (Perkin-Elmer, UK) with a light path of 1 cm as presented in Figure 2.3.
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**Figure 2.3** UV/VIS spectra of the phenols and their laccase reaction product:
- phenol, --- reaction product of the phenol and laccase
2.2.5.5.2 Reaction procedure

Each concentration (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.75, and 1 mM) of each phenol was reacted with laccase and the kinetic constants $V_{\text{max}}$ and $K_M$ were determined using the Michaelis-Menten equation by plotting a graph $1/V$ against $1/[S]$ (Lineweaver-Burk plot)\(^{141,142}\).

Enzyme activity was determined by either the appearance of product or the disappearance of the substrate at 30°C for 10 minutes. For catechol and hydroquinone, the enzyme activity was determined by the disappearance of each substrate, measured at 275 and 288.5 nm respectively. For pyrogallol, L-DOPA, and 3-hydroxytyramine, the enzyme activity was measured by the appearance of the product, measured at 435, 475, and 470 nm respectively.

Reaction mixtures consisted of 0.6 ml of 0.1 M potassium phosphate buffer at pH 6.0, 0.2 ml of substrate solution (0.05-1.00 mM), and 0.2 ml of 10 mg/ml of
laccase solution. A blank consisted of the same enzyme assay mixture with boiled enzyme solution (for deactivation), and the substrate was replaced with distilled water. One unit of activity is defined as a change of absorbance of 0.001 per minute.

2.2.5.6 Dyeing and tanning effects of the phenols on chrome tanned leather

2.2.5.6.1 Neutralisation of wet blue

The wet blue was sammed (squeezed) to remove excess water content and shaved in order to get uniform thickness, then it was neutralised. The procedure of neutralisation is given in Appendix IV.

2.2.5.6.2 Dyeing experiment

Three pieces, 70x75 mm, of neutralised wet blue samples were weighed, then put in a 500 ml glass vessel. 150 ml distilled water was added, the mixtures were shaken at 35°C for 15 minutes, then the float was adjusted to pH 4.5-5.5 with 5% sodium bicarbonate. Phenols (2.5%) were added. The mixture was shaken at the same temperature for 2 hours and then the pH of the float was adjusted to 6 with 5% sodium bicarbonate, followed by the addition of 1% crude laccase. The reaction mixtures were shaken at 35°C for 20 hours. On completion, the float was discarded. Fatliquor, sulfited oxidised fish oil, (Truponol BTK) (8%) which was emulsified in water at 50°C was added and then the process was run at 50°C for 90 minutes. The float 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 the float was drained. The dyed leather was toggle dried at room temperature. Offers in this process were based on the neutralised wet blue weight.
2.2.5.6.3 Colour measurement

Colour of the dyed leather was measured using the same procedure as described in Section 2.2.3.3.2.

2.2.5.6.4 Measurement of tensile strength and percentage elongation at break

Tensile strength of the conditioned leather was measured according to the Official Method IUP 6 (SLP 6) using an Instron 1122146.

2.2.5.6.5 Measurement of tear strength

Tear strength (double edge tear) of the conditioned leather was measured according to the Official Method IUP 8 (SLP 7) using an Instron 1122146.

2.2.5.6.6 Rub fastness

Dry rub fastness of the dyed leather was evaluated by the Official Method (SLF 5)146 using a rub fastness tester (STM 461, SATRA Footwear Technology Centre). Colour changes of leather and felt pad were visually assessed according to the Grey Scale.

2.2.5.7 Dyeing and tanning effects of the mixture of catechol and hydroquinone on chrome tanned leather

2.2.5.7.1 Preparation of wet blue

Sheepskin chrome tanned leather was prepared using the same procedure as described in Section 2.2.5.6.1.
2.2.5.7.2 Dyeing experiment

The same method as described in Section 2.2.5.6.2 was employed, except instead of using one phenolic compound, mixtures of catechol and hydroquinone with molar ratios of 1:10, 1:3, 1:1, 3:1, and 10:1 were used.

2.2.5.7.3 Colour measurement

Colour of the dyed leather was measured using the same procedure as described in Section 2.2.3.3.2.

2.2.5.7.4 Tensile strength and percentage elongation at break

The same procedure as described in Section 2.2.5.6.4 was used to measure the strength properties.

2.2.5.7.5 Measurement of tear strength

Tear strength was measured using the same procedure as described in Section 2.2.5.6.5.

2.2.5.7.6 Rub fastness

Rub fastness of the dyed leather was measured using the same method as described in Section 2.2.5.6.6.

2.2.6 Dihydroxynaphthalenes (DHNs) for leather dyeing and tanning

2.2.6.1 Dyeing studies with the DHNs

Dyeing studies using 1,3-; 1,5-; 1,6; 1,7-, 1,8-; 2,3-; 2,6-; and 2,7-DHNs in conjunction with laccase were carried out on hide powder.
2.2.6.1.1 Dyeing procedure

The same procedure as described in Section 2.2.3.3.1 was used.

2.2.6.1.2 Colour measurement

Colour was measured using the same procedure as described in Section 2.2.3.3.2.

2.2.6.1.3 Measurement of hydrothermal stability

Hydrothermal stability of the treated hide powder was evaluated using the same procedure as described in Section 2.2.3.1.2.

2.2.6.2 Tanning studies with DHNs

2.2.6.2.1 Tanning studies with DHNs on hide powder

The same procedure as described in Section 2.2.3.1.1 was employed. Tanning effects were measured using the same method as described in Section 2.2.3.1.2.

2.2.6.2.2 Tanning studies with DHNs on sheepskin pickled pelt

a. Depickling of sheepskin pickled pelt

The degreased pickled pelt was put in a drum; 0.5% sodium formate and 1% sodium carbonate were added, run for 20 minutes. Water (100%) at 20°C was added, then run for 30 minutes. The pH and solution density (°Bé) were checked; the float was discarded.
b. Tanning experiment

All chemical additions were based on depickled pelt weight. Three pieces of the depickled pelt were put into a glass vessel with 150% water and 10% brine solution (NaCl). The pH was adjusted to 4.5 with 5% formic acid solution. 25% of each DHN was added, previously dissolved in 5 ml of acetone. Reaction was run at 35°C with shaking for 20 hours. After the reaction was finished, the float was discarded.

c. Measurement of tanning effect

Tanning effects was investigated using the same procedure as described in Section 2.2.3.1.2.

d. Measurement of tensile strength and percentage elongation at break

The same procedure as described in Section 2.2.5.6.4 was employed to measure the tensile strength and percentage elongation at break of the leather.

e. Measurement of tear strength

Tear strength of the leather was measured using the same procedure as described in Section 2.2.5.6.5.

2.2.6.3 Combination tanning studies using DHNs and oxazolidine

2.2.6.3.1 Combination tanning studies using DHNs and oxazolidine on hide powder

The same procedure as described in Section 2.2.3.2.1 was employed. Tanning effects were measured using the same method as described in Section 2.2.3.1.2, except the measurement range was 35-120°C.
2.2.6.3.2 Determination of tannin combination on leather

The method described by Covington and Song\textsuperscript{23} was used to determine the DHNs combination on leather.

DHN tanned or combination tanned hide powder was soaked in 50 ml of 50% acetone aqueous solution at 20°C for 20 minutes under vigorous shaking and washed with the same solution. The amount of washed out tannin was measured by a gravimetric method. The shrinkage temperature of washed hide powder was analysed by DSC.

2.2.6.3.3 Combination tanning studies using DHNs and oxazolidine on pickled pelt

a. Depickling of sheepskin pickled pelt

The sheepskin pelt was depickled prior to tanning using the same method as described in Section 2.2.6.2.2.a.

b. Tanning procedure

Tanning studies using the DHNs and oxazolidine were carried out on sheepskin pickled pelt. The same procedure as described in Section 2.2.5.2.2.b was employed. After discarding the float, 150% water was added, then the mixture was adjusted to pH 6 with 5% NaHCO\textsubscript{3} solution; 10% oxazolidine (Neosyn TX) was added, then shaken at 50°C for 2 hours. On completion, the float was discarded.

c. Measurement of tanning effect

Tanning effects of the polymerisation products and oxazolidine were measured using the same procedure as described in Section 2.2.3.1.2.
d. **Measurement of tensile strength and percentage elongation at break**

The same procedure as described in Section 2.2.5.6.4 was used.

**e. Measurement of tear strength**

Tear strength was measured using the same procedure as described in Section 2.2.5.6.5.
CHAPTER 3

DEGRADATION OF LIGNIN
3.1 Introduction

In nature, lignin degradation is accomplished by white-rot basidiomycetes fungi. The fungi are able to produce three classes of ligninolytic enzymes: laccases, lignin peroxidases (LiP), and manganese-dependent peroxidases (MnP). Laccases are oxygenases with an active site containing four copper atoms. LiP and MnP are peroxidases with an active site constituted by a haem centre, the protoporphyrin IX\textsuperscript{86,92,157}.

Biomimetic systems with a metalloporphyrin catalyst mimic the catalytic mechanism of lignin peroxidase\textsuperscript{158}. Haemin, a natural porphyrin, has been shown to exhibit catalytic activity in the oxidation of various lignin model compounds\textsuperscript{107}; it is chloroprotoporphyrin IX iron(III) (C\textsubscript{13}H\textsubscript{32}N\textsubscript{4}O\textsubscript{4}FeCl), the reddish-brown crystalline chloride of haem, produced when haemoglobin reacts with glacial acetic acid and sodium chloride. It was used in this biomimetic system, since the natural porphyrin is more closely related to the haemoprotein lignin peroxidase than the system with the synthetic porphyrin and it is more effective than synthetic porphyrin catalysts such as tetraphenylporphyrin iron(III)\textsuperscript{107}. The chemical structure of haemin is given in Figure 3.1.a.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure31.png}
\caption{Chemical structures of haemin (a) and Kraft lignin (b)}
\end{figure}
In this study, we have been concerned with degradation of Kraft lignin (Figure 3.1.b) by biomimetic and enzymatic systems. The biomimetic degradation was carried out using haemin as a catalyst and hydrogen peroxide as an oxidising agent. Effects of temperature and reaction time on the biomimetic lignin degradation were investigated. Laccase and laccase-mediator systems were used in enzymatic degradation of Kraft lignin. The degradation products were isolated, and identified by ultraviolet (UV) spectroscopy, infrared with Fourier transform (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). The biomimetic degradation products were used in the tanning and dyeing studies, which will be discussed in Chapter 4.

3.2 Results and discussion

3.2.1 Biomimetic degradation of Kraft lignin

3.2.1.1 Effect of temperature on the biomimetic degradation of Kraft lignin

3.2.1.1.1 Paper chromatography

After degradation of Kraft lignin using H₂O₂ and haemin at 90°C for 1 hour (A1), 2 hours (A2), and 3 hours (A3), using visualisation with 2,4-DNPH reagent, an orange spot at retardation factor (Rf) 0.63 was obtained from chromatogram of treatment A1, two spots at Rf 0.63 and 0.80 were obtained from samples A2 and A3, and no spot appeared from control (CA) (Figure 3.2.a). The spots indicate the products contain compounds with carbonyl groups: treatment A1 appears to yield one compound with a carbonyl group, treatments A2 and A3 yield two compounds with carbonyl groups, and the control did not produce any compounds with carbonyl groups.

Paper chromatograms of degradation using H₂O₂ and haemin at room temperature (20°C) for 1 hour (B1), 3 hours (B2), and 5 hours (B3) showed one spot at Rf 0.63, which indicates that the samples may contain one compound with carbonyl
groups. No spot appeared from the control (CB), so it did not contain compounds with carbonyl groups. Figure 3.2.b shows the paper chromatograms for carbonyl group identification of treatments at room temperature.

![Paper chromatograms for carbonyl group identification](image)

**Figure 3.2** Paper chromatograms for the carbonyl group identification of Kraft lignin treated by H$_2$O$_2$ and haemin at:

- **a. 90°C** for 1 hour (A1), 3 hours (A2), 5 hours (A3); CA is control
- **b. room temperature** for 1 hour (B1), 3 hours (B2), 5 hours (B3); CB is control

To identify other components of the lignin degradation products, i.e. phenols, the solvent system of 10% aqueous acetic acid was used. After visualisation using ferric chloride-potassium ferricyanide (FeCl$_3$-K$_3$Fe(CN)$_6$) reagent, a blue spot at R$_f$ 0.83 was obtained from treatments A1, A2, and A3. The spot with the same R$_f$ value was also showed by treatments B1, B2, and B3. Figure 3.3 shows paper chromatograms for phenol identification of treatments with heating at 90°C and without heating at room temperature. The spots show that the lignin degradation products may contain a compound with phenolic groups. The spot did not appear from controls CA and CB, which indicates the controls did not contain compounds with phenol.
3.2.1.1.2 Infrared spectroscopy

The infrared spectrum of Kraft lignin indicates the lignin contains hydroxyl groups, indicated by a broad O-H stretching absorption at 2900-3600 cm\(^{-1}\); aromatic, shown by C=C stretching between 1400 and 1600 cm\(^{-1}\) and C-H bending at 1130 cm\(^{-1}\); C\(_\alpha\)-C\(_\beta\) indicated by absorption at 1600 cm\(^{-1}\), and C-S stretching at 620 cm\(^{-1}\) (Figure 3.4). This supports the general structure of Kraft lignin given in Figure 3.1.b.

The degradation products of the treatments A1, A2, and A3 exhibit absorption at 1730 cm\(^{-1}\), due to the stretching of carbonyl C=O groups. A lower intensity of the bands between 1400 and 1600 cm\(^{-1}\), and 1130 cm\(^{-1}\) indicates a decrease in aromatic content, due to ring cleavages. A decrease of the absorption band centred at 1600 cm\(^{-1}\) indicates a decrease of content of C=C group, due to C\(_\alpha\)-C\(_\beta\) cleavage in the lignin structure. The infrared spectra of treatments A1 and control
CA are given in Figures 3.5 and 3.6. The IR spectra of treatments A2 and A3 are given in Appendix V.

Figure 3.4 Infrared spectrum of Kraft lignin

Figure 3.5 Infrared spectrum of Kraft lignin treated by H$_2$O$_2$ and haemin at 90°C for 1 hour (A1)
Figure 3.6 Infrared spectrum of control in the absence of oxidising agent and catalyst for treatment at 90°C (CA)

In the infrared spectra of treatments B1, B2, B3, and control CB, the absorption bands between 1400 and 1600 cm\(^{-1}\), and 1130 cm\(^{-1}\) of the degradation products are slightly decreased. This indicates a decrease in their aromatic content. The spectra show also an increase in the absorption band at 1730 cm\(^{-1}\) and a decrease in the absorption band centred at 1620 cm\(^{-1}\), due to C=O and C=C stretching bands respectively. The infrared spectra of treatment B1 and control CB are shown in Figures 3.7 and 3.8. The IR spectra of treatments B2 and B3 are given in Appendix VI.
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Figure 3.7 Infrared spectrum of Kraft lignin treated by $\text{H}_2\text{O}_2$ and haemin at room temperature for 1 hour (B1)

Figure 3.8 Infrared spectrum of control in the absence of catalyst and oxidising agent for treatment at room temperature (CB)
3.2.1.3 Tests for aldehyde and ketone

Paper chromatograms and infrared spectra of the degraded lignin indicate that the degradation products might contain compounds with carbonyl groups. To investigate whether the carbonyl group is aldehyde, ketone or both, tests for aldehyde and ketone were carried out.

In test for aldehydes, ammoniacal silver nitrate (Tollen's reagent) was used to identify the presence of aldehydic compound in a sample. When the samples from treatments A1, A2, A3, B1, B2, and B3 were tested by the reagent, silver mirrors were formed on the wall of the test tubes, which indicated the samples gave a positive result for the test; the mirror did not appear for the controls CA and CB. This indicates that the treatments A1, A2, A3, B1, B2, and B3 produced a compound with aldehyde, whereas the controls CA and CB did not contain aldehydic compounds.

All of the samples for both degradation methods did not produce a violet colouration, when they were tested using the colour reaction for ketones: this indicates the samples gave negative results for the ketone test. Therefore, the degradation products from the treatments either at 90°C or room temperature did not contain compounds with ketone groups.

3.2.1.2 Effect of reaction time on the biomimetic degradation of Kraft lignin

To understand the effect of reaction time on the Kraft lignin biomimetic degradation, treatments were undertaken at 90°C for 5 to 25 hours. Experiments on the reaction time effect at room temperature were not carried out, because the treatments for 1 to 5 hours, as previously discussed, did not yield a different number of degradation products.
3.2.1.2.1 Paper chromatography

2,4-DNPH and ferric chloride-potassium ferricyanide reagents were used for the identification of carbonyl groups and phenolic groups respectively. Two orange spots, Rf 0.63 and 0.80, were obtained from the treatments for 5, 10, 15, 20 to 25 hour reaction times. The paper chromatogram for carbonyl group identification is given in Figure 3.9; it shows the degradation products of the treatment samples may contain two compounds with carbonyl groups. The degradation products of all reaction times contain one compound with phenol, indicated by one blue spot obtained from the treatments as shown in Figure 3.10. The chromatograms indicate that the mechanism of breakdown may be simple, since the products do not appear to change with reaction time.

![Figure 3.9 Paper chromatogram for the carbonyl group identification of Kraft lignin treated by H₂O₂ and haemin for a variety of reaction times](image)
3.2.1.2.2 Infrared spectroscopy

Infrared spectra of treatments for 5, 10, 15, 20, and 25 hours reaction times were identical (Appendix VII); the spectrum of treatment for 5 hours reaction time is given in Figure 3.11. The IR spectra show an absorption band at 1730 cm$^{-1}$, due to the stretching vibration of carbonyl C=O group. Compared with the initial condition (Figure 3.4), the spectra exhibit a lower intensity of the absorption bands between 1400 and 1600 cm$^{-1}$, indicating a decrease in the aromatic content; and a decrease of the absorption band at 1620 cm$^{-1}$, indicating a decrease in C=C content.

Treatments at reaction times for 5 to 25 hours gave similar degradation products as shown by the similar IR spectra. This is supported by the paper chromatograms (Figure. 3.9 and 3.10), showing the treatments gave the same chromatogram for both carbonyl and phenolic groups. Therefore, the types of reactions for the treatments for 5 to 25 hours are the same.
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3.2.1.2.3 Tests for aldehyde and ketone

The degradation products of 5 to 25 hours reaction times gave positive results for the aldehyde test and negative results for the ketone test. The qualitative tests indicate that the degradation products contain compounds with carbonyl groups and the carbonyl group was aldehyde.

3.2.1.3 Isolation and identification of the degradation products

Kraft lignin degradation products were isolated by solvent extraction, and identified by UV spectroscopy, FT-IR spectroscopy, and GC-MS.

3.2.1.3.1 UV spectroscopy

The UV spectrum of the Kraft lignin degradation products exhibits increased absorption at wavelengths about 260 nm and 310 nm compared with the control in

Figure 3.11 Infrared spectrum of Kraft lignin treated by H$_2$O$_2$ and haemin at room temperature for 5 hours
the absence of the oxidising agent and catalyst (Figure 3.12). The change in spectrum of the degraded lignin at around 310 nm is due to \( \alpha \)-carbonyl groups\textsuperscript{159,160}. This is supported by the FT-IR data shown in Figure 3.13. The UV spectrum also shows a decrease in absorption at 280 nm, which indicates a decrease of aromatic content in the lignin due to benzene ring cleavage.

\[\text{Figure 3.12 UV spectra of Kraft lignin treated by } \text{H}_2\text{O}_2 \text{ and haemin at 90°C for 5 hours and control}\]

### 3.2.1.3.2 FT-IR spectroscopy

The FT-IR spectrum of degraded lignin (Figures 3.13) indicates that the products contain hydroxyl group, indicated by O-H stretching absorption at 2900-3400 cm\(^{-1}\) and O-H bending absorption at 875 cm\(^{-1}\); aldehydic C-H stretching bands at 2690 and 2760 cm\(^{-1}\); carbonyl group, indicated by C=O stretching band at 1729 cm\(^{-1}\); aromatics, showed by C=C stretching band at 1455 cm\(^{-1}\) and C-H bending absorption at 1122 cm\(^{-1}\); C-O stretching band at 1290 cm\(^{-1}\); and C-H stretching band of phenol at 1256 cm\(^{-1}\). Therefore, the products might contain compounds with phenol, aldehyde, and carboxylic acid.
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3.2.1.3.3 Gas chromatography-mass spectrometry

Mass spectra of the positively charged derivatised biomimetic degradation products of Kraft lignin are shown in Figure 3.14.

Figure 3.14 Mass spectra of the positively charged trimethylsilyl derivatised biomimetic degradation products of Kraft lignin: (a) product I, (b) product II, (c) product III, and (d) product IV
The mass spectra indicate that the products are 2-methoxyphenol (guaiacol) (I), 4-hydroxybenzaldehyde (II), 3-methoxy-4-hydroxybenzaldehyde (vanillin) (III), and 3-methoxy-4-hydroxybenzoic acid (vanillic acid) (IV). Chemical structures of the products are given in Figure 3.15.
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\[
\begin{align*}
\text{OH} & \text{OCH}_3 \\
\text{CHO} & \\
\text{OH} & \text{OCH}_3 \\
\text{CHO} & \text{COOH}
\end{align*}
\]  

\begin{align*}
\text{2-methoxyphenol} & & \text{4-hydroxybenzaldehyde} & & \text{vanillin} & & \text{vanillic acid} \\
\text{I} & & \text{II} & & \text{III} & & \text{IV}
\end{align*}

Figure 3.15 Chemical structures of the biomimetic degradation products of Kraft lignin

The mass spectrometric data were supported by the FT-IR spectrum of the reaction products, containing absorption bands of carbonyl group, hydroxyl group, C-H stretching vibration of aromatic and C=C stretching vibration of aromatic rings. The absence of syringyl products indicates that the Kraft lignin used in this work originated from softwood (gymnosperm wood). Degradation products would depend on the type of wood: therefore, syringic aldehyde and syringic acid would be expected from the degradation of hardwood (angiosperm wood) lignin. The small amounts of un-isolated reaction products should not significantly affect the use of the isolated products for the preparation of syntans and the melanin type reaction for dyeing.

3.2.1.4 Yields

Yields of the degradation products are shown in Table 3.1 and could be summarised as follows:

vanillin >> vanillic acid >> 4-hydroxybenzaldehyde > 2-methoxyphenol.

The total yield of the products above was 32%.
Table 3.1 Yields of the biomimetic Kraft lignin degradation products

<table>
<thead>
<tr>
<th>Product</th>
<th>Retention time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methoxyphenol</td>
<td>15.2</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde</td>
<td>19.5</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Vanillin</td>
<td>23.6</td>
<td>19.0 ± 0.8</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>29.1</td>
<td>9.3 ± 0.9</td>
</tr>
</tbody>
</table>

*means ± standard deviation of triplicate experiments

3.2.2 Enzymatic degradation of Kraft lignin

Kraft lignin was degraded using laccase and laccase mediators, i.e. 1-hydroxybenzotriazole (HBT), N-hydroxyphthalimide (HPI), and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO). The degradation products were extracted and identified using FT-IR and GC-MS.

3.2.2.1 Identification of the enzymatic degradation products

3.2.2.1.1 FT-IR spectroscopy

Figure 3.16 demonstrates the FT-IR spectrum of Kraft lignin degraded by laccase-BPI mediator system. It indicates the degradation products contain carboxylic acid and aromatic compounds. Carboxylic acid is indicated by the strong and narrow C=O stretching absorption at 1700 cm⁻¹, strong and narrow C-O stretching at 1280 cm⁻¹, and strong and broad hydrogen bonded O-H stretching absorption at 2500-3300 cm⁻¹. Aromatics are indicated by the stretching absorption band at 1400-1600 cm⁻¹.

The FT-IR spectrum of Kraft lignin degraded by laccase-HBT mediator system (Figure 3.17) indicates the degradation products contain compounds with carboxylic acid and phenol. Carboxylic acid is confirmed by the bonds referred to above. Phenol is indicated by the O-H stretching band at 3100 cm⁻¹, C-H stretching band at 1190 cm⁻¹. Aromatic C=C stretching absorption is at 1450 cm⁻¹ and C-H bend absorption is at 1230 cm⁻¹.
Figure 3.16 FT-IR spectrum of Kraft lignin degraded by laccase and N-hydroxyphthalimide (HPI)

Figure 3.17 FT-IR spectrum of Kraft lignin degraded by laccase and 1-hydroxybenzotriazole (HBT)
Figure 3.18 shows the FT-IR spectrum of Kraft lignin degraded by laccase-TEMPO mediator system. The spectrum indicates the degradation products contain compounds with aromatic and carboxylic acid.

![FT-IR spectrum of Kraft lignin degraded by laccase and TEMPO](image)

**Figure 3.18** FT-IR spectrum of Kraft lignin degraded by laccase and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)

### 3.2.2.1.2 Gas chromatography-mass spectrometry

Mass spectra of the enzymatic degradation products of Kraft lignin are given in Appendices VIII-X. The mass spectra indicate that the products are some phenolic acids, carboxylic acids, and biphenyl as given in Table 3.2, which were supported by FT-IR spectrum of each degradation reaction product (Figures 3.16-3.18). Chemical structures of the products are shown in Figure 3.19.

Table 3.2 shows that a mediator was very important for laccase in lignin degradation. Lignin degradation by laccase in conjunction with TEMPO or HBT as mediators produced more degradation products than the degradation mediated by HPI. This indicates that reaction mediated by TEMPO or HBT took place
more broadly than that mediated by HPI, leading to the production of more degradation products.

Table 3.2 Enzymatic degradation products of Kraft lignin by laccase-mediator systems

<table>
<thead>
<tr>
<th>Degradation method</th>
<th>Degradation product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laccase + HPI</td>
<td>1,2-benzenedicarboxylic acid</td>
</tr>
<tr>
<td>Laccase + HBT</td>
<td>2-hydroxy-3-methylbenzoic acid</td>
</tr>
<tr>
<td></td>
<td>3-hydroxybenzoic acid</td>
</tr>
<tr>
<td></td>
<td>2,3,4-trimethoxymandelic acid</td>
</tr>
<tr>
<td>Laccase + TEMPO</td>
<td>2-phenoxylbenzoic acid</td>
</tr>
<tr>
<td></td>
<td>3,3'-dimethoxy-1,1'-biphenyl</td>
</tr>
<tr>
<td></td>
<td>1,2-benzenedicarboxylic acid</td>
</tr>
<tr>
<td>Laccase alone</td>
<td>no product</td>
</tr>
<tr>
<td>Control</td>
<td>no product</td>
</tr>
</tbody>
</table>

Figure 3.19 Chemical structures of the enzymatic degradation products of Kraft lignin by laccase-mediator systems

1,2-benzenedicarboxylic acid  2-hydroxy-3-methylbenzoic acid  3-hydroxybenzoic acid

2,3,4-trimethoxymandelic acid  2-phenoxylbenzoic acid  3,3'-dimethoxy-1,1'-biphenyl
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Lignin degradations using biomimetic and enzymatic reactions produced different phenolic degradation products. The biomimetic degradation yielded simple phenol, phenolic aldehydes, benzoic (vanillic) acid; the enzymatic reactions produced benzoic and mandelic acid derivatives and biphenyl. This indicates that there is a difference in the catalytic reaction mechanisms between the two systems: the biomimetic degradation mimics the catalytic reactions for LiP, whereas the enzymatic degradation is laccase catalysed reaction.

The structures of the biomimetic degradation products indicated that the lignin was broken down via C-C bond cleavages between the α- and β-carbons and C-O-4 bond cleavages between the β-carbon and O in side chains, which are the reactions for LiPg6. The main reactions of the enzymatic degradation in the presence of mediators are the cleavage of C-C bonds between the α- and β-positions in side chains and the cleavage of alkyl phenyl bonds82.

The degradation products have a potential to be used as raw materials for various applications and manufacturing other compounds. Methoxyphenols are used in manufacturing stabilisers and antioxidants for plastics and rubbers. They are also used in analgesics, local anaesthetic, flavourings, biocides, antiseptics. 2-methoxyphenol is employed in manufacturing vanillin161. Vanillin is a constituent of vanilla, used as a food flavouring agent and in the pharmaceutical industry161,162. Benzaldehyde is used in the synthesis of other organic compounds in pharmaceutical and plastic industries. It is also an important intermediate for the processing of perfumes and flavourings and in the preparation of certain aniline dyes161.

Benzoic acid is employed in the production of glycol benzoates as plasticisers in adhesive formulations. It is used in the manufacture of alkyd resins and drilling mud additive for crude oil recovery application. It is also used in rubber polymerisation activators and retardants. Benzoic acid is converted to its salts and esters for preservative application in foods and drugs, e.g. sodium benzoate. Benzoic acid derivatives, substituted by hydroxyl group or ether containing an
oxygen atom, have active bacteriostatic and fragrant properties. They are typically used in the pharmaceutical and perfumery industries\textsuperscript{161}.

Mandelic acid has an active bacteriostatic property and is used in medicine, mainly for urinary antiseptics. It and its derivatives are also used in antibacterial applications for cosmetics. It is used as an intermediate for the synthesis of amides for industrial and research applications\textsuperscript{161}. Biphenyl is used as an intermediate for the production of a wide range of organic compounds, e.g. emulsifiers, optical brighteners, crop protection products, and plastics as a heat transfer medium in heating fluids, as a dyestuff carrier for textiles and copying paper and as a solvent in pharmaceutical production. Biphenyl derivatives are used as intermediates for the synthesis of organic compounds including pharmaceuticals, antifungal agents, optical brighteners, and dyes\textsuperscript{161}. 

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CHAPTER 4

TANNING AND DYEING STUDIES USING PHENOLIC LIGNIN DEGRADATION PRODUCTS
4.1 Introduction

Phenolic compounds have an important role in leather making, particularly in tanning. They are used in the form of vegetable tannages (plant polyphenols) and as materials for the production of synthetic tanning agents (syntans). Studies on the biomimetic degradation of Kraft lignin revealed the dominant reaction products were simple phenols: 2-methoxyphenol, 4-hydroxybenzaldehyde, vanillin, and vanillic acid\textsuperscript{20}. The phenolic compounds might have potential tanning and dyeing effects towards collagen.

Synthetic tanning materials (syntans) are high molecular weight synthetic organic compounds which capable of rendering skins and hides into imputrescible leather. They are prepared by polymerisation of phenols with or without sulfonation. Syntheses of syntans from phenols, naphthalene derivatives, and anthracene groups have been studied extensively\textsuperscript{2,163-167}. In this work, some syntans were prepared by polymerisation of the phenolic biomimetic degradation products of Kraft lignin.

Leather is invariably coloured to enhance its aesthetic or fashion appeal, usually by applying synthetic dyes. An alternative route to achieving colour is to exploit the chemistry of creating the natural skin and hair pigments, melanins. Certain reaction products of laccase-mediated oxidation and polymerisation of phenolic compounds can give coloured products, of which melanins are examples\textsuperscript{168,169}. The melanin-type reactions might be useful for leather colouring to produce for example black suede. Besides that, since the reaction produces quinoid intermediates, it is likely that the products will bind at least partially covalently to collagen\textsuperscript{45}, which will increase in the hydrothermal stability of collagen.

In this work, the lignin degradation products were used in tanning and dyeing studies of hide powder. The products were polymerised using formaldehyde to obtain high molecular weight polymers that might have tanning ability towards collagen. Molecular weight distributions of the polymers were measured by gel
permeation chromatography (GPC). Combination tanning using the polymerised lignin degradation products and oxazolidine was also investigated. The tanning effects of the phenols were measured by differential scanning calorimetry (DSC). In the dyeing studies, the phenols were reacted aerobically with crude laccase in the presence of collagen.

4.2 Results and discussion

4.2.1 Kraft lignin degradation products for tanning

4.2.1.1 Tanning studies using the lignin degradation products

The phenolic Kraft lignin degradation products were applied to hide powder, and hydrothermal stability of the treated hide powder was measured. Vanillic acid and 4-hydroxybenzaldehyde increased the shrinkage temperature ($T_s$) of hide powder by 10 and 6°C respectively as shown in Table 4.1. Vanillin gave a 'negative' tanning effect as shown by the decrease of $T_s$ of the treated collagen by 4°C. 4-methoxyphenol did not significantly alter the hydrothermal stability of hide powder. This shows that side chains or other functional groups of the phenolic compounds determine the tanning effect of the phenols. The shrinkage temperature of the treated hide powder did not increase significantly indicating that the phenolic product did not possess any tanning property towards collagen.

Table 4.1 Hydrothermal stabilities of hide powder treated with phenolic biomimetic Kraft lignin degradation products

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>$T_s$ (°C)</th>
<th>$\Delta T_s$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>58</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>2-methoxyphenol</td>
<td>57</td>
<td>-1</td>
<td>25</td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde</td>
<td>64</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td>Vanillin</td>
<td>54</td>
<td>-4</td>
<td>30</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>68</td>
<td>10</td>
<td>37</td>
</tr>
</tbody>
</table>

*standard deviation: $T_s=\pm1$, $\Delta H=\pm3$
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Tanning and dyeing studies using phenolic lignin degradation products

The energy or enthalpy of shrinking (ΔH) of collagen treated by 4-hydroxy-benzaldehyde and vanillic acid was slightly higher than that of the control, whereas the T_s of collagen treated by vanillin and 2-methoxyphenol was lower than that of the control (Table 4.1), indicating destabilisation of the collagen. This reflects the shrinkage temperature data of the treated collagen.

4.2.1.2 Polymerisation of the lignin degradation products

It has been shown that the biomimetic-LiP lignin degradation products generally did not give good tanning. By polymerisation, however, the products would contain high hydroxyl and other functional groups (aldehyde or ketone) contents, which might be able to combine by hydrogen or covalent bonds with collagen; therefore, they might confer a tanning effect.

4.2.1.2.1 Molecular weight distribution of the polymerisation products

The weight-average molecular weight (M_w) is defined as the distribution by weight of polymer over the molecular weight states and can be used to understand polymer properties related to viscosity and strength. The number-average molecular weight (M_n) is the distribution by number of molecules over the molecular weight range. Polydispersity (PD) or polydispersity index (PDI) is the ratio of M_w to M_n, essentially a measure of the broadness or relative spread in molecular weight present in a polymer

M_w of polymers of the lignin degradation products were bigger than those of their starting materials as shown by GPC (Table 4.2). The M_w of the polymers were much higher than those of vegetable tannins, but the M_w were only indicators relative to the polysaccharide standards.

Molecular weight distributions of the polymerisation products are demonstrated by GPC chromatograms in Figures 4.1-4.4. The molecular weight distribution parameters show that the lignin degradation products have been polymerised by
formaldehyde. $M_n$ values are lower than $M_w$ values for all samples, which is typical. Polydispersity values of the polymerisation products of 2-methoxyphenol, vanillin, and vanillic acid were around 1.10, indicating that the polymers are uniform, whereas the polydispersity of the polymerisation product of 4-hydroxybenzaldehyde was 1.41, showing that the molecular weights present in the polymer are relatively varied, as shown in Figure 4.2.

Table 4.2 Molecular weight distributions of the formaldehyde polymers of lignin degradation products

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Monomer $M_w$</th>
<th>Polymerisation products$^a$</th>
<th>$M_w$ (k)</th>
<th>$M_n$ (k)</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methoxyphenol</td>
<td>124</td>
<td>216</td>
<td>188</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde</td>
<td>122</td>
<td>122</td>
<td>86</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>Vanillin</td>
<td>152</td>
<td>0.65</td>
<td>0.59</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>168</td>
<td>154</td>
<td>136</td>
<td>1.13</td>
<td></td>
</tr>
</tbody>
</table>

$^a$based on the calibration curve with polysaccharide standards

![Figure 4.1 GPC chromatogram of 2-methoxyphenol polymerisation product](image-url)
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Figure 4.2 GPC chromatogram of 4-hydroxybenzaldehyde polymerisation product

Figure 4.3 GPC chromatogram of vanillin polymerisation product
4.2.1.2.2 Functional groups of the polymerisation products

In order to investigate the functional groups constituting the polymerisation products and to make comparison between the polymerisation products and their starting materials, FT-IR spectroscopy analysis was undertaken.

According to the FT-IR spectra (Figures 4.5 and 4.6), 4-methoxyphenol and its formaldehyde polymerisation product contained phenol, indicated by O-H stretching band at 3500 cm\(^{-1}\) and C-H stretching bands at 1260 and 1225 cm\(^{-1}\). C-H stretching bands at 3010 and 3050 cm\(^{-1}\) and C=C stretching band at 1400-1600 cm\(^{-1}\), both of which may be assigned to the benzene ring (aromatic). This shows that the polymerisation process did not alter the functional groups of its monomer.
4-hydroxybenzaldehyde contained aldehyde, indicated by C=O stretching band at 1670 cm\(^{-1}\), phenol, indicated by O-H stretching absorption at 3170 cm\(^{-1}\) and C-H stretching bands at 1220 and 1270 cm\(^{-1}\), and aromatic indicated by C-H stretching band at 1160 cm\(^{-1}\). The functional groups of the polymerisation products were
the same with those of its starting material (Appendix XI). Therefore, the functional groups of polymerised 4-hydroxybenzaldehyde remained the same as those of its monomer.

The formaldehyde polymerisation product of vanillin had the same functional groups as its starting material. It contained aldehyde, indicated by \( \text{C=O} \) stretching band at 1670 cm\(^{-1}\); phenol, indicated by O-H stretching band at 3180 and C-H stretching absorption at 1270 cm\(^{-1}\); and aromatic, shown by C=C stretching bands at 1400-1600 nm\(^{-1}\). The FT-IR spectra of vanillin and its polymerisation product are given in Appendix MI.

In order to make direct comparison between vanillic acid and its polymerisation product, the polymerisation product was acidified with 2M hydrochloric acid to pH 3 and dried. The infrared spectra of vanillic acid and the acidified polymerisation product are given in Appendix XIII. Vanillic acid and its polymerisation product contained carboxylic acid indicated by \( \text{C=O} \) stretching band at 1685 cm\(^{-1}\) and C-O stretching band at 1300 cm\(^{-1}\) and O-H stretching band at 3485 cm\(^{-1}\) of phenol. Therefore, the functional groups of the polymer vanillic acid were still the same as those of its monomer.

4.2.1.2.3 Mechanism of polymerisation

The phenolic degradation products of lignin are substituted benzenes. Substituents, such as -OH and -OCH\(_3\) which release electrons to the ring, increase the rate of reaction relative to benzene and direct the incoming electrophile preferentially (although not exclusively) to the ortho and/or para positions (activated). Substituents, such as -CHO and -CO\(_2\)H, which withdraw electrons from the ring, decrease the rate of reaction relative to benzene and direct the incoming electrophile preferentially to the meta position (deactivated)\(^{171,172}\). Table 4.3 shows the directing effect of common substituents.
Table 4.3 The directing effect of common substituents

<table>
<thead>
<tr>
<th>Activating groups</th>
<th>Deactivating groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho and para directing</td>
<td>Ortho and para directing</td>
</tr>
<tr>
<td>- Alkyl groups</td>
<td>- F</td>
</tr>
<tr>
<td>- Aryl groups</td>
<td>- Cl</td>
</tr>
<tr>
<td>- NH$_2$</td>
<td>- Br</td>
</tr>
<tr>
<td>- N(CH$_3$)$_2$</td>
<td>- I</td>
</tr>
<tr>
<td>- NHCOCH$_3$</td>
<td>- NH$_3^+$</td>
</tr>
<tr>
<td>- OH</td>
<td>- N(CH$_3$)$_3^+$</td>
</tr>
<tr>
<td>- OCH$_3$</td>
<td>- CH$_3$</td>
</tr>
</tbody>
</table>

According to Table 4.3, the donating and withdrawing electrons of the lignin degradation products are given in Figure 4.7.

![Donating and withdrawing electrons of lignin degradation products](image)

Figure 4.7 The donating and withdrawing electrons of the lignin degradation products

In this study, 4-hydroxybenzaldehyde, vanillin, and vanillic acid were polymerised using formaldehyde by base catalysis. The mechanism of polymerisation can be explained using the general mechanism of phenol polymerisation.$^{156}$

In the presence of alkali, the phenolic degradation products of lignin are present as resonance-stabilised anions, as shown in Figure 4.8. In the case of 4-hydroxybenzaldehyde, the resonance stabilisation leads to possible nucleophilic substitution at C-3 (meta) or ortho from –OH position in the aromatic ring. The first step in the polymerisation involves addition of the anions to formaldehyde to give meta substituted methylolphenolic compounds as shown below (Figure 4.9).
Although reactions involving the \textit{meta} positions only are given below, analogous reactions also occur at C-5 (\textit{ortho} position to -OH).

\begin{align*}
\text{4-hydroxybenzaldehyde} & \\
\text{vanillin} & \\
\text{vanillic acid}
\end{align*}

\textbf{Figure 4.8} Resonance-stabilised anions of 4-hydroxybenzaldehyde, vanillin, and vanillic acid in alkali

Further polymerisation of the methylolphenolic compounds gives methylene bridges linking the benzene rings, resulting from condensation between the methylolphenolic compounds and available \textit{meta} or \textit{ortho} positions. This occurs either by direct S\textsubscript{N}2 displacement of the hydroxyl group (Figure 4.10) or by Michael addition (Figure 4.11) to a quinone methide structure, which is present in equilibrium with the methylol anion (Figure 4.12).
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Figure 4.9 Addition of the resonance-stabilised anion to formaldehyde to give meta substituted methylolphenolic compounds

Figure 4.10 Methylene bridges linking the benzene rings by direct \( S_N^2 \) displacement of the hydroxyl group

Figure 4.11 Methylene bridges linking the benzene rings by a Michael addition to a quinone methide structure

Figure 4.12 Equilibrium of quinone methide with the methylol anion

The proposed overall polymerisation reaction of the phenolic degradation product is given in Figure 4.13. The model shows formaldehyde reacting as a monomer, although it is known it exists in different polymeric forms. The molecular size or
the length of polymerisation product is controlled by the ratio of formaldehyde (condensing agent) to the aromatic base material (phenolic compound).

**Figure 4.13** Proposed overall reaction of formaldehyde polymerisation of 4-hydroxybenzaldehyde

Based on the reaction mechanisms above, the proposed overall reactions involved in the polymerisation of vanillin and vanillic acid are shown in Figure 4.14.

**Figure 4.14** Proposed formaldehyde polymerisation reactions of 2-methoxyphenol, vanillin, and vanillic acid

In this study, 2-methoxyphenol was polymerised using formaldehyde by acid catalysis. The mechanism of the polymerisation is explained using the mechanism of phenol polymerisation. A phenol-formaldehyde polymerisation
mechanism produced by acid catalysis with excess phenol is quite different to that obtained by base catalysis. The mechanism involves protonation of the carbonyl group (Figure 4.15) followed by electrophilic aromatic substitution at *ortho* or *para* positions (Figure 4.16).

![Figure 4.15 Protonation of the carbonyl group in formaldehyde in acidic medium](image)

**Figure 4.15** Protonation of the carbonyl group in formaldehyde in acidic medium

![2-methoxyphenol](image)

**Figure 4.16** Electrophilic aromatic substitution of 2-methoxyphenol at *ortho* or *para* positions

Under acidic conditions further reaction occurs to give methylene bridges. It is formed by electrophilic substitution involving benzylic carbocations and available ring positions, as shown in Figure 4.17. The net result is the formation of complex mixtures of polymer. Based on the reaction mechanisms, the proposed overall reactions involved in the formaldehyde polymerisation of 2-methoxyphenol are depicted in Figure 4.18.

![Methylene bridge formation in acidic medium](image)

**Figure 4.17** Methylene bridge formation in acidic medium
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Figure 4.18 Proposed formaldehyde polymerisation reaction of 2-methoxyphenol

4.2.1.3 Tanning studies with the polymers of lignin degradation products

Shrinkage temperatures ($T_s$) of hide powder increased significantly after the treatments using polymerisation products of 2-methoxyphenol and vanillic acid, showing that the polymerisation products of the phenolic compounds have tanning effects on collagen. $T_s$ of hide powder treated with the polymerisation product of vanillin was lower, therefore, the vanillin polymer gave a 'negative' tanning effect (Table 4.4).

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>$T_s$ ($^\circ$C)</th>
<th>$\Delta T_s$ ($^\circ$C)</th>
<th>$\Delta H$ (J/g)</th>
<th>$\Delta T_{\text{pm}}$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>58</td>
<td>-</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Polymer of 2-methoxyphenol</td>
<td>74</td>
<td>16</td>
<td>29</td>
<td>17</td>
</tr>
<tr>
<td>Polymer of 4-hydroxybenzaldehyde</td>
<td>65</td>
<td>7</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>Polymer of vanillin</td>
<td>54</td>
<td>-4</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>Polymer of vanillic acid</td>
<td>68</td>
<td>10</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

$^4$the effect of polymerisation, $\Delta T_s$ (polymer-monomer)

This result also shows that the polymerisation of 2-methoxyphenol created a tanning agent as shown by its $\Delta T_{\text{pm}}$ in Table 4.4, but tanning effects were not created by the other polymerisation products. It indicates the hydroxyl groups of 2-methoxyphenol polymerisation product can combine with collagen by hydrogen bonding to raise the hydrothermal stability. The other polymerisation products did not react with collagen owing to the following contributions from the component groups:
1. deactivation of phenolic hydroxyl to hydrogen bonding by donated charge,
2. deactivation of aldehyde groups by donated charge,
3. steric hindrance to reaction.

Therefore, the reactivity of a phenolic polymer towards collagen depends on other elements of the structure of the polymer.

4.2.1.4 Combination tanning with polymers of the lignin degradation products and oxazolidine

This section of the work addresses whether or not combination tanning, using polymers of lignin degradation products and oxazolidine, can increase the hydrothermal stability of collagen.

The polymers of lignin degradation products were applied to hide powder and then the treated hide powders were retanned with oxazolidine as a crosslinking agent. The combination tannages increased the shrinkage temperature of hide powder between 10 and 20°C as shown by $\Delta T_s(\text{Oz})$ (Table 4.5), indicating that there is a little synergy from the combination reaction: only vanillin produced an additive tannages, but 2-methoxyphenol, 4-hydroxybenzaldehyde, and vanillic acid all exhibit negative synergy, i.e. antagonistic reactions.

Table 4.5 Hydrothermal stability of collagen tanned with the polymers of lignin degradation products and oxazolidine

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>$T_s$ (°C)</th>
<th>$\Delta T_s$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
<th>$\Delta T_s(\text{Oz})$ (°C)$^a$</th>
<th>$\Delta T_s(\text{synergy})$ (°C)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>58</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxazolidine (Oz)</td>
<td>75</td>
<td>17</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polymer of 2-methoxyphenol + Oz</td>
<td>82</td>
<td>24</td>
<td>33</td>
<td>8</td>
<td>-9</td>
</tr>
<tr>
<td>Polymer of 4-hydroxybenzaldehyde + Oz</td>
<td>76</td>
<td>18</td>
<td>28</td>
<td>11</td>
<td>-6</td>
</tr>
<tr>
<td>Polymer of vanillin + Oz</td>
<td>74</td>
<td>16</td>
<td>23</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Polymer of vanillic acid + Oz</td>
<td>78</td>
<td>20</td>
<td>32</td>
<td>10</td>
<td>-7</td>
</tr>
</tbody>
</table>

$^a$the effect of oxazolidine cross-linking, $\Delta T_s(\text{polymer + Oz})$ - polymer

$^b$ $\Delta T_s(\text{synergy}) = \Delta T_s(\text{polymer + Oz})$ - polymer - Oz
Enthalpies or energies of shrinking of the hide powder treated with the polymers of lignin degradation products and oxazolidine are given in Table 4.5. Shrinking energy of hide powder treated with the polymer of vanillin and oxazolidine indicates a tanning reaction, causing an increase of stability by the interactions with collagen.

From Table 4.5, shrinkage temperatures of hide powder tanned with polymers of 4-hydroxybenzaldehyde, vanillin, and vanillic acid and retanned with oxazolidine were around 75°C, which are the T_s of oxazolidine-tanned hide powder. This shows that the increases in T_s are primarily due to retanning with oxazolidine; therefore, those polymers did not undergo tanning reactions on collagen.

4.2.2 Kraft lignin degradation products for dyeing

4.2.2.1 Dyeing studies using the lignin degradation products

4.2.2.1.1 Colouring hide powder

2-methoxyphenol, vanillin, and vanillic acid are oxidisable by laccase, to create some colour. Reaction of 2-methoxyphenol with laccase conferred a dark purple colour to hide powder, as can be seen in Figure 4.19. The other products, after oxidation by laccase, did not produce high intensity of colour; light green and brown were produced by vanillin and vanillic acid respectively. Reaction of 4-hydroxybenzaldehyde and the enzyme did not change the colour of hide powder. The dyeing reaction using 2-methoxyphenol and laccase may have potential for use in leather colouring, especially in combination with other phenolic species.

The colouring effects of the phenols and laccase towards collagen are shown by the increase of colour intensity of the treated hide powder, as indicated by the decrease of lightness value (L*) of the treated collagen (Figure 4.20).
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Figure 4.19 Colour of hide powder dyed with phenolic lignin degradation products and treated with laccase

Figure 4.20 The lightness ($L^*$) values of hide powder dyed with lignin degradation products and laccase
All of the treatments using the phenols and laccase gave positive chromaticity $a^*$ values (Figure 4.21). This indicates that the colours produced by the reaction of the phenols and the enzyme contained red colour. After undergoing reaction with laccase, the highest $a^*$ value was produced by 2-methoxyphenol followed by vanillic acid and vanillin.

![Figure 4.21 The $a^*$ chromaticity values of hide powder dyed with lignin degradation products and laccase](image)

The treatments resulted in positive values for chromaticity $b^*$, showing that the colours produced contained yellow colour, see Figure 4.22. 2-methoxyphenol in conjunction with laccase gave the lowest the $b^*$ value, and vanillin without the enzyme showed the highest $b^*$ value.
Tanning and dyeing studies using phenolic lignin degradation products

Figure 4.22 The b* chromaticity values of hide powder dyed with lignin degradation products and laccase

Table 4.6 shows the calculated colour differences caused by the phenols and laccase treatments. The greatest effect of the total treatment ($\Delta E^*_{\text{total}}$) was produced by oxidation of 2-methoxyphenol, followed by the oxidation of vanillin. Phenols alone did not give high colour difference on hide powder as shown by their $\Delta E^*_{\text{phenol}}$ values.

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Effect of total treatment ($\Delta E^*_{\text{total}}$)</th>
<th>Effect of phenol alone ($\Delta E^*_{\text{phenol}}$)</th>
<th>Effect of laccase on phenol ($\Delta E^*_{\text{lacc}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methoxyphenol + laccase</td>
<td>57.4</td>
<td>3.6</td>
<td>55.0</td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde + laccase</td>
<td>6.2</td>
<td>6.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Vanillin + laccase</td>
<td>19.0</td>
<td>11.0</td>
<td>14.3</td>
</tr>
<tr>
<td>Vanillic acid + laccase</td>
<td>10.9</td>
<td>3.3</td>
<td>7.9</td>
</tr>
</tbody>
</table>
4.2.2.1.2 Hydrothermal stability of coloured hide powder

The hydrothermal stability of hide powder treated with lignin degradation products and laccase increased slightly (Table 4.7). Although the dyeings did not give good tanning, they did not destabilise the collagen, as indicated by the shrinkage temperature ($T_s$) of the treated collagen and the enthalpy of shrinking ($\Delta H$).

Table 4.7 Hydrothermal stabilities of hide powder dyed with lignin degradation products and laccase

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>$T_s$ (°C)</th>
<th>$\Delta T_s$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>56</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td>2-methoxyphenol</td>
<td>59</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>2-methoxyphenol + laccase</td>
<td>60</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde</td>
<td>58</td>
<td>2</td>
<td>38</td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde + laccase</td>
<td>59</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Vanillin</td>
<td>58</td>
<td>2</td>
<td>39</td>
</tr>
<tr>
<td>Vanillin + laccase</td>
<td>60</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>57</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td>Vanillic acid + laccase</td>
<td>57</td>
<td>1</td>
<td>34</td>
</tr>
</tbody>
</table>

4.2.2.2 Colour fastness to washing

After washing with Na$_2$CO$_3$ (to break hydrogen bonds), the hide powder dyed with 2-methoxyphenol became duller, but gave good fastness resistance, as shown by the small decrease in the $L^*$ value of the samples, shown in Table 4.8. After washing, the chromaticity values of hide powder dyed using 2-methoxyphenol remained constant.
## Table 4.8 Colour fastness to washing of hide powder dyed with 2-methoxyphenol and laccase

<table>
<thead>
<tr>
<th></th>
<th>Before washing</th>
<th>After washing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lightness, L*</td>
<td>23.3</td>
<td>21.9</td>
</tr>
<tr>
<td>Chromaticity a*</td>
<td>10.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Chromaticity b*</td>
<td>4.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Total colour change, $\Delta E_{\text{total}}$</td>
<td>57.4</td>
<td>59.6</td>
</tr>
<tr>
<td>Shrinkage temperature, $T_s$ (°C)</td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>Raise in shrinking temperature, $\Delta T_s$ (°C)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Enthalpy of shrinking, $\Delta H$ (J/g)</td>
<td>31</td>
<td>34</td>
</tr>
</tbody>
</table>

### Table 4.9 Grey scale rating for change in colour and staining of hide powder dyed with 2-methoxyphenol and laccase after washing

<table>
<thead>
<tr>
<th>Colour change</th>
<th>Grey scale rating$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour change 4/5 (duller)</td>
<td></td>
</tr>
<tr>
<td>Secunder cellulose acetate (dicel)</td>
<td>3/4</td>
</tr>
<tr>
<td>Bleached unmercerised cotton</td>
<td>3/4</td>
</tr>
<tr>
<td>Nillon 6.6</td>
<td>3</td>
</tr>
<tr>
<td>Polyester (terylene)</td>
<td>3/4</td>
</tr>
<tr>
<td>Acrylic</td>
<td>4</td>
</tr>
<tr>
<td>Wool worsted</td>
<td>4/5</td>
</tr>
</tbody>
</table>

$^a$higher scale reading means less colour contrast after treatment

From Table 4.9, of the accompanying textiles in the wash test, hide powder dyed with 2-methoxyphenol showed good fastness resistance to acrylic and wool worsted fabrics. This fastness resistance to washing with a hydrogen bond breaker also indicates it should have good fastness resistance to normal washing conditions.
4.3 Summary

The biomimetic degradation product of Kraft lignin, 2-methoxyphenol, in conjunction with laccase gave a good colouring effect and colour fastness to washing. The simple phenols produced by the biomimetic degradation products of Kraft lignin did not give good tanning effects towards collagen; however, after polymerisation using formaldehyde, 2-methoxyphenol can increase the hydrothermal stability of collagen, like a conventional syntan.
CHAPTER 5

TANNING AND DYEING STUDIES USING PHENOLIC COMPOUNDS
5.1 Introduction

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\textsuperscript{119,173}. These types of reactions might be applied for leather colouring and, at the same time, for tanning of the leather due to the covalent binding between the products and the leather\textsuperscript{45}, which would also contribute to wash and rub fastness. Moreover, since the reaction is oxidative, the products should exhibit good light fastness.

In this study, in order to investigate the influence of phenol structure on dyeing effect, three groups of phenolic compounds were used:

a. Simple phenols.
   This group comprises the di- and trihydroxyphenol mononuclear species: catechol, resorcinol, hydroquinone, pyrogallol, and phloroglucinol (Figure 5.1).

b. Flavonoid antioxidants.
   The simplest flavonoid compounds are represented by catechin and green tea extract: the latter is composed predominantly of monomeric galloallocatechin-3-gallate and other catechin species (Figure 5.2)\textsuperscript{174}.

c. Amino phenols.
   The synthesis of melanin is based on the polymerisation of derivatives of amino phenols, represented in this study by: DL-tyrosine, 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA), and 3-hydroxytyramine (Figure 5.3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure51.png}
\caption{The structures of mononuclear polyphenols} \label{fig:5.1}
\end{figure}
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(3,4-dihydroxyphenyl methyl) butane. It was proposed that the molecules polymerise via a quinoid type of reaction, indicated in Figure 5.4. This is consistent with the proposal that tanning depends on the formation of matrices.
In this work, dyeing studies of chrome tanned leather were undertaken using some phenolic compounds in conjunction with laccase. Colour, rub fastness, and strength properties of the dyed leather were investigated.

5.2 Results and discussion

5.2.1 Colouring 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 ($L^*$) values of dyed hide powder are shown in Figures 5.5 – 5.7.
and the calculated colour differences ($\Delta E^*$) caused by the treatments are given in Table 5.1.

**Figure 5.5** The lightness and chromaticity values of hide powder dyed with the mononuclear phenols and laccase

**Figure 5.6** The lightness and chromaticity values of hide powder dyed with the flavonoid antioxidants and laccase
Dyeing method

Figure 5.7  The lightness and chromaticity values of hide powder dyed with the aminophenols and laccase

Table 5.1 The effects of treatments on the phenolic compounds to cause colour differencea

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Effect of total treatment (ΔE*total)</th>
<th>Effect of phenol alone (ΔE*phenol)</th>
<th>Effect of laccase on phenol (ΔE*lacc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol + laccase</td>
<td>62.7</td>
<td>25.4</td>
<td>38.8</td>
</tr>
<tr>
<td>Resorcinol + laccase</td>
<td>46.7</td>
<td>9.5</td>
<td>37.6</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>54.2</td>
<td>19.4</td>
<td>36.2</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>50.0</td>
<td>36.0</td>
<td>14.9</td>
</tr>
<tr>
<td>Phloroglucinol + laccase</td>
<td>36.1</td>
<td>13.7</td>
<td>23.4</td>
</tr>
<tr>
<td>Catechin + laccase</td>
<td>62.1</td>
<td>14.6</td>
<td>54.0</td>
</tr>
<tr>
<td>Green tea polyphenol + laccase</td>
<td>51.3</td>
<td>24.3</td>
<td>31.8</td>
</tr>
<tr>
<td>DL-tyrosine + laccase</td>
<td>12.8</td>
<td>4.1</td>
<td>8.7</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>66.8</td>
<td>29.1</td>
<td>37.7</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>64.6</td>
<td>37.1</td>
<td>27.6</td>
</tr>
</tbody>
</table>

a measured relative to untreated hide powder
Chapter 5
Tanning and dyeing studies using phenolic compounds

The dyed hide powders are shown in Figures 5.8-5.10. After undergoing reaction with the enzyme for 24 hours, 2-methoxyphenol, catechol, hydroquinone, pyrogallol, L-DOPA and 3-hydroxytyramine gave dark colours. The darkest colour was produced by oxidation of L-DOPA. Catechol, L-DOPA and 3-hydroxytyramine produced almost black colours, whereas 4-methoxyphenol, hydroquinone and pyrogallol gave dark purple, purple and brown respectively.

Figure 5.8 Hide powder coloured by simple phenol-laccase treatments
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Tanning and dyeing studies using phenolic compounds

Figure 5.9 Hide powder coloured by flavonoid antioxidant-laccase treatments

Figure 5.10 Hide powder coloured by aminophenol-laccase treatments
Colours produced by reaction of the phenolic compounds with laccase resulted in positive values for $a^*$ chromaticity, therefore, all produced red colours. Phenolic compounds giving $a^*$ values higher than +10 were 2-methoxyphenol, 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 is dominated by shades of brown, derived from the combination of red and yellow, so the colouring closest to black could be characterised as brown-black.

5.2.2 Hydrothermal stability

The denaturation or shrinkage temperatures of the dyed hide powders were higher than those of either untreated hide powder or hide powder dyed with each substrate without enzyme, as shown in Table 5.2. Moreover, the energies of shrinking indicate that the reactions do not denature the collagen, which would be indicated by a decrease in value. 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. This shows that the enzyme oxidised and polymerised the phenolic compounds, so that the molecular weights of the phenols became higher and, in turn, the phenolic polymers reacted with collagen to create a tanning effect, shown by an increase in the $T_s$. Therefore, the oxidation and polymerisation products were able to cross-link with collagen, probably via hydrogen bonding, but also by some covalent reactions.
Table 5.2 Shrinkage temperature ($T_s$), increase in shrinkage temperature ($\Delta T_s$), and energy of shrinking ($\Delta H$) of hide powder dyed with phenolic compounds and laccase

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>With laccase</th>
<th>Without laccase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_s$ (°C)</td>
<td>$\Delta T_s$ (°C)</td>
</tr>
<tr>
<td>None</td>
<td>56</td>
<td>18</td>
</tr>
<tr>
<td>Catechol</td>
<td>71</td>
<td>15</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>67</td>
<td>11</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>74</td>
<td>18</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Catechin</td>
<td>76</td>
<td>21</td>
</tr>
<tr>
<td>Green tea polyphenol</td>
<td>76</td>
<td>20</td>
</tr>
<tr>
<td>DL-tyrosine</td>
<td>56</td>
<td>1</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>59</td>
<td>3</td>
</tr>
<tr>
<td>3-hydroxytyramine</td>
<td>57</td>
<td>1</td>
</tr>
</tbody>
</table>

5.2.3 Effect of enzyme concentration on dyeing effect

Lightness ($L^*$) values of hide powder decreased for laccase concentrations between 0 and 10 mg/g hide powder for all phenolic substrates used, resulting in the dyed hide powder becoming darker. The lightness was not changed significantly for enzyme concentrations between 10 and 50 mg/g hide powder (Figure 5.11).

The $a^*$ chromaticity values of hide powder treated with L-DOPA, 3-hydroxytyramine, pyrogallol, and hydroquinone increased for the enzyme concentration between 0 and 10 mg/g hide powder; whereas the $a^*$ value for that of catechol decreased. Therefore, all phenolic reactions increased red chromaticity, except the reaction of catechol, which decreased the red chromaticity. For enzyme concentration between 10 and 50 mg/g hide powder, there were no large changes in the $a^*$ value for all compounds (Figure 5.12).
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Figure 5.11 The lightness ($L^*$) values of hide powder dyed with phenolic compounds and various laccase concentrations

Figure 5.12 The chromaticity $a^*$ values of hide powder dyed with phenolic compounds and various laccase concentrations

The $b^*$ chromaticity values of the hide powder treated with hydroquinone, catechol, and 3-hydroxytyramine decreased at laccase concentrations lower than
10 mg/g hide powder, indicating the decrease of yellow chromaticities, and then were relatively constant at the enzyme concentrations between 10 and 50 mg/g hide powder. The \(b^*\) values of the hide powder treated with L-DOPA and pyrogallol increased at the enzyme concentrations lower than 10 mg/g hide powder, indicating that the reactions increase yellow chromaticity and were relatively constant at enzyme concentrations between 10 and 50 mg/g hide powder (Figure 5.13).

![Figure 5.13 The chromaticity \(b^*\) values of hide powder dyed with phenolic compounds and various laccase concentrations](image)

The effects of the treatments and laccase on phenol are given in Figures 5.14 and 5.15 respectively. Both figures show that reaction of laccase with pyrogallol did not produce dark colour compared with the other phenols.

These data show that enzyme saturation was achieved at enzyme concentrations higher than 10 mg/g hide powder. Therefore, an enzyme concentration of 10 mg/g hide powder was sufficient for dyeing.
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Figure 5.14 The effects of laccase concentration and phenol to cause colour difference

Figure 5.15 The effects of laccase on phenol treated hide powder to cause colour difference
5.2.4 Effect of phenolic substrate concentration on dyeing

The lightness ($L^*$) values of the hide powder were decreased at phenolic substrate concentrations between 0 and 0.25 mmol/g hide powder for all substrates with the hide powder becoming dark in colour (Figure 5.16).

From Figure 5.17, the $a^*$ chromaticity values of the hide powder treated with the phenolic compounds and laccase were increased at substrate concentrations between 0 and 0.1 mmol/g hide powder. The greatest increase of the chromaticity $a^*$ value was caused by the reaction of hydroquinone.

![Figure 5.16 The lightness ($L^*$) values of hide powder dyed with various concentrations of phenolic compounds and laccase](image)

Treatments of hide powder with the phenols up to 0.25 mmol/g hide powder, in conjunction with laccase, decreased chromaticity $b^*$ values, except the treatment with pyrogallol, as shown in Figure 5.18.
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Figure 5.17 Chromaticity $a^*$ values of hide powder dyed with various concentrations of phenolic compounds and laccase

Figure 5.18 Chromaticity $b^*$ values of hide powder dyed with various concentrations of phenolic compounds and laccase
The effects of the treatments and the phenols alone to cause colour difference of the dyed hide powder are given in Figures 5.19 and 5.20 respectively. These data demonstrate that phenol concentrations higher than 0.25 mmol/g hide powder did not increase dyeing of the hide powder.

The reactions of catechol and hydroquinone with laccase resulted in an increase in hydrothermal stability of the treated hide powder of around 15°C at the phenol offer of 0.25 mmol/g hide powder, as shown in Figure 5.21. No significant increase in the shrinkage temperature of hide powder treated by the other phenols was observed.

Although L-DOPA and 3-hydroxytyramine have catechol in their structures, the laccase-polymerisation products of the phenols did not produce tanning effects. This clearly indicated that the side chains, alanine in L-DOPA, and ethylamine in 3-hydroxytyramine, contribute to this phenomenon owing to deactivation of phenolic group to hydrogen bonding by donated charge and steric hindrance to reaction.

![Figure 5.19](#)  
**Figure 5.19** The effect of phenols concentration and laccase on colour difference
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Figure 5.20 The effect of various phenols concentration alone on colour difference

Figure 5.21 The effects of phenol concentration on shrinkage temperatures of treated hide powder
5.2.5 Colour fastness to washing

To determine the colour fastness of dyed hide powder to washing, changes in colour of the dyed hide powder and the accompanying textiles on washing were investigated. After washing with Na$_2$CO$_3$ (hydrogen bond breaker), the hide powder dyed with L-DOPA and 3-hydroxytyramine became duller, but gave good fastness resistance, as shown by the small decreases in the $L^*$ values of the samples, shown in Figure 5.22 and Tables 5.3 and 5.4.

![Figure 5.22](image-url)  
Figure 5.22 The lightness values of hide powder dyed with phenolic compounds and laccase, before and after washing
Table 5.3 Effect of washing on colour difference of hide powder dyed with phenolic compounds and laccase

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Effect of washing (ΔE* wash)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol + laccase</td>
<td>2.1</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>3.1</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>1.7</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>7.4</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>10.7</td>
</tr>
</tbody>
</table>

![Chromaticity a* values of hide powder dyed with phenolic compounds and laccase: before and after washing](image)

**Figure 5.23** Chromaticity a* values of hide powder dyed with phenolic compounds and laccase: before and after washing

In this test, the chromaticity values of hide powder dyed with the reaction of catechol, hydroquinone, and pyrogallol lost some red component of the colour, whereas hide powder colour dyed by reaction of L-DOPA and 3-hydroxytyramine remained constant (Figure 5.23). Hide powder dyed by the reaction of pyrogallol...
decreased a little in the yellow component, whereas the hide powder dyed with hydroquinone became slightly yellower, shown in Table 5.4 and Figure 5.24.

Table 5.4  Grey scale rating for change in colour of the dyed hide powder after washing

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Grey scale rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol + laccase</td>
<td>4/5</td>
<td>little loss of red</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>4/5</td>
<td>duller</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>4/5</td>
<td>duller</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>4</td>
<td>yellower, loss of red</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>2</td>
<td>loss of yellow and red</td>
</tr>
</tbody>
</table>

Figure 5.24  Chromaticity $b^*$ values of hide powder dyed with phenolic compounds and laccase: before and after washing

Of the accompanying textiles in the wash test, hide powder dyed by the reactions of catechol, L-DOPA, 3-hydroxytyramine, and hydroquinone gave good fastness
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resistance to dicel, polyester, and acrylic fabrics (Table 5.5). In general, these results show that dyed hide powder has good colour fastness resistance to washing with a hydrogen bond breaker. This indicates that the sample should have good fastness resistance to normal washing conditions.

Table 5.5 Grey scale rating for staining of the dyed hide powder after washing

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Secunder cellulose acetate (dicel)</th>
<th>Bleached unmercerised cotton</th>
<th>Nylon 6.6</th>
<th>Polyester (terylene)</th>
<th>Acrylic</th>
<th>Wool worsted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol + laccase</td>
<td>4</td>
<td>3</td>
<td>3/4</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>5</td>
<td>3</td>
<td>3/4</td>
<td>5</td>
<td>5</td>
<td>3/4</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3/4</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>5</td>
<td>5</td>
<td>4/5</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>3</td>
<td>3/4</td>
<td>3/4</td>
<td>5</td>
<td>5</td>
<td>2/3</td>
</tr>
</tbody>
</table>

The effect of washing on the hydrothermal stability of dyed hide powder was measured. No decrease in shrinkage temperature was observed in the washed samples, as shown in Table 5.6.

Table 5.6 Hydrothermal stability of hide powder dyed with phenolic compounds and laccase: before and after washing

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Before washing</th>
<th>After washing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_s$ ($^\circ$C)</td>
<td>$\Delta T_s$ ($^\circ$C)</td>
</tr>
<tr>
<td>Catechol + laccase</td>
<td>67</td>
<td>11</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>57</td>
<td>2</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>57</td>
<td>1</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>71</td>
<td>16</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>None</td>
<td>55</td>
<td>-</td>
</tr>
</tbody>
</table>

160
5.2.6 Kinetics of enzymatic reaction of laccase with phenolic compounds

In order to measure the affinity of laccase with the phenols and to establish the maximum rate of catalysis by laccase, kinetic studies were carried out. Progress curves showing the effect of substrate concentration on enzyme activity for the enzymatic reaction gave a curve similar to a rectangular hyperbola (Figure 5.25). Therefore, the enzymatic reactions obeyed Michaelis-Menten kinetics.

![Figure 5.25](image)

**Figure 5.25** Effect of phenolic substrate concentrations on laccase activities (progress curves)

The kinetic parameters, $K_M$ and $V_{max}$, were determined from the Michaelis-Menten equation using Lineweaver-Burk plots, as shown in Figure 5.26. The $K_M$ and $V_{max}$ values are given in Table 5.7.

The Michaelis constant ($K_M$) gives a measure of the enzyme-substrate affinity: large $K_M$ shows low enzyme-substrate affinity; small $K_M$ shows high enzyme-substrate affinity. In other words, $K_M$ is the dissociation constant of the enzyme-substrate (ES) complex and a measure of the strength of the ES complex: a high $K_M$ indicates weak binding; a low $K_M$ indicates strong binding. Based
on the $K_M$ values (Table 5.7), the order of affinity of laccase with phenolic compounds is (pyrogallol ≈ hydroquinone) > (catechol ≈ L-DOPA) > 3-hydroxytyramine. Therefore, the binding between laccase and pyrogallol was stronger than that between the enzyme and 3-hydroxytyramine. Consequently, if laccase is used to catalyse a reaction of a mixture of pyrogallol and 3-hydroxytyramine, it will prefer to catalyse the reaction of pyrogallol first and then react with 3-hydroxytyramine.

![Lineweaver-Burk plots for the enzymatic reaction of laccase and the phenolic compounds](image)

**Figure 5.26** Lineweaver-Burk plots for the enzymatic reaction of laccase and the phenolic compounds

**Table 5.7** Kinetic parameters for laccase reactions with phenolic substrates (30°C, pH 6.0)

<table>
<thead>
<tr>
<th>Phenolic substrate</th>
<th>$K_M$ (mM)</th>
<th>$V_{max}$ (U.min⁻¹.ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol</td>
<td>0.58 ± 0.08</td>
<td>196 ± 4</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>0.63 ± 0.14</td>
<td>199 ± 2</td>
</tr>
<tr>
<td>3-hydroxytyramine</td>
<td>0.85 ± 0.03</td>
<td>219 ± 34</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.21 ± 0.01</td>
<td>247 ± 4</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.20 ± 0.01</td>
<td>169 ± 9</td>
</tr>
</tbody>
</table>
According to $K_M$ values of laccase reactions with phenolic substrates, as shown in Table 5.7, although laccase has broad substrate specificity towards aromatic compounds containing hydroxyl and amino groups, its enzyme-substrate affinity relating to catalysis rate, is influenced by the structures of the reactants (substrates). The substrates are bound to the enzyme by multiple weak attractions. Hence, the enzyme and substrate should have complementary shapes. The directional character of hydrogen bonds between enzyme and substrate often enforces a high degree of specificity.

Correlations between affinity, colour change ($\Delta E^*$) and rise in shrinkage temperature ($\Delta T_s$), based on Tables 5.1, 5.2, and 5.7, can be summarised as follows:

Affinity (phenol-laccase) order:
- pyrogallol $\approx$ hydroquinone $>$ catechol $\approx$ L-DOPA $>$ 3-hydroxytyramine

$\Delta E$ order:
- L-DOPA $\approx$ 3-hydroxytyramine $>$ catechol $>$ hydroquinone $>$ pyrogallol

$\Delta T_s$ order:
- hydroquinone $>$ catechol $>$ L-DOPA $\approx$ 3-hydroxytyramine $\approx$ pyrogallol.

These correlations show that higher affinity means less colour change, so lower affinity causes darker colour. Roughly, higher affinity means higher $T_s$, so lower affinity means less stabilisation. This shows also that more colour change means less tanning effect. Therefore, the reactions leading to colour development are not the same as the reactions resulting in collagen stabilisation. The reactions also relate to structure differences of the phenolic compounds.

5.2.7 Dyeing effects on chrome tanned leather

5.2.7.1 Colouring of the leather

The phenols in conjunction with laccase gave good colouring effects to wet blue leather on both grain and flesh sides: catechol confers the darkest colour, almost
black, shown in Figures 5.27 and 5.28. Dyeing effects of the reactions are given in Table 5.8. The colouration also penetrates well into the leather; the best penetration was achieved by hydroquinone, but which also produced only pale colour.

Table 5.8 Effect of the dyeing using phenols and laccase to cause colour difference on wet blue leather

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Effect of the dyeing (AE*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol + laccase</td>
<td>57.5</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>40.3</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>41.6</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>15.6</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>52.5</td>
</tr>
</tbody>
</table>

Figure 5.27 The lightness and chromaticity values of chrome tanned leather dyed with phenolic compounds and laccase
5.2.7.2 Rub fastness

Table 5.9 Rub fastness (grey scale\textsuperscript{a}) of chrome tanned leather dyed with the phenolic compounds and laccase

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Colour transfer to pads (staining)</th>
<th>Colour change on leather</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grain</td>
<td>Flesh</td>
</tr>
<tr>
<td>Catechol + laccase</td>
<td>3</td>
<td>2/3</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>4/5</td>
<td>3</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>4/5</td>
<td>3</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>4</td>
<td>2/3</td>
</tr>
</tbody>
</table>

\textsuperscript{a}higher scale reading means less colour contrast after testing

The dyed leather performed well in the rub fastness test, as shown in Table 5.9. 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 may be merely a reflection of the lower intensity of colour conferred by this reaction.

5.2.7.3 Strength properties

Table 5.10 Strength properties of chrome tanned leather treated with phenolic compounds and laccase

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Tensile strength (MPa)</th>
<th>Tensile strength (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>None</td>
<td>9.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Catechol + laccase</td>
<td>15.9</td>
<td>11.9</td>
</tr>
<tr>
<td>L-DOPA + laccase</td>
<td>14.3</td>
<td>13.1</td>
</tr>
<tr>
<td>3-hydroxytyramine + laccase</td>
<td>16.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Hydroquinone + laccase</td>
<td>13.7</td>
<td>11.6</td>
</tr>
<tr>
<td>Pyrogallol + laccase</td>
<td>16.5</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Table 5.11 Summary of the average effects of phenol-laccase treatment on leather strength (% change)

<table>
<thead>
<tr>
<th>Position to the backbone</th>
<th>Tensile strength</th>
<th>Elongation at break</th>
<th>Tear strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel</td>
<td>+ 60</td>
<td>+ 15</td>
<td>0</td>
</tr>
<tr>
<td>Perpendicular</td>
<td>+ 40</td>
<td>+ 15</td>
<td>+ 20</td>
</tr>
</tbody>
</table>

From Table 5.11, 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, in terms of stretching at break, is the same in both directions (Figure 5.29). In the case of tear strength, the anisotropy is reduced.
Chapter 5

Tanning and dyeing studies using phenolic compounds

Figure 5.29 Percentage of elongation at break strength of chrome tanned leather dyed with the phenolic compounds and laccase

5.2.8 Dyeing of chrome tanned leather with mixtures of catechol and hydroquinone

5.2.8.1 Colouring of the leather

As described in the previous Section, in conjunction with laccase, catechol conferred the darkest colour and hydroquinone gave the best penetration into the leather. To combine the advantages of both phenols, dyeing experiments using the mixtures of catechol and hydroquinone were investigated. From Figure 5.30, the L* values decreased, showing the leather became darker, owing to the increase in catechol proportion in the reaction mixture. Penetration of catechol into the leather was also increased by mixing it with hydroquinone.

Figure 5.31 shows that chromaticity a* and b* values of the dyed leather decreased, indicating that the yellow and red components of the colour decreased, due to the increase in molar ratio of catechol to hydroquinone. Catechol
dominated the formation of colour as indicated by the increase of $\Delta E^*$ in accordance with the amount of catechol (Table 5.12).

**Figure 5.30**  The lightness ($L^*$) values of leather dyed with the mixtures of catechol and hydroquinone and laccase.

**Figure 5.31**  The chromaticity $a^*$ and $b^*$ values of leather dyed with the mixtures of catechol and hydroquinone and laccase.
Table 5.12 The effects of mixed phenol dyeing to cause colour difference to chrome tanned leather

<table>
<thead>
<tr>
<th>Molar ratio of catechol to hydroquinone</th>
<th>Effect of dyeing ($\Delta E^*$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>1:10</td>
<td>31.6</td>
</tr>
<tr>
<td>1:3</td>
<td>41.6</td>
</tr>
<tr>
<td>1:1</td>
<td>49.0</td>
</tr>
<tr>
<td>3:1</td>
<td>52.5</td>
</tr>
<tr>
<td>10:1</td>
<td>57.1</td>
</tr>
</tbody>
</table>

5.2.8.2 Rub fastness

The leather dyed by the laccase reaction with mixtures of catechol and hydroquinone performed better in the rub fastness test compared with that dyed with the enzyme reaction with catechol alone (Table 5.13). As with the previous experiments, the rub fastness on the grain side was better than on the flesh side.

Table 5.13 Rub fastness (grey scale$^*$) of leather dyed with the mixtures of catechol and hydroquinone and laccase

<table>
<thead>
<tr>
<th>Molar ratio of catechol and hydroquinone</th>
<th>Colour transfer to pads (staining)</th>
<th>Colour change on leather (damage to the leather)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grain</td>
<td>Flesh</td>
</tr>
<tr>
<td>1:10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1:3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1:1</td>
<td>4/5</td>
<td>2/3</td>
</tr>
<tr>
<td>3:1</td>
<td>4</td>
<td>2/3</td>
</tr>
<tr>
<td>10:1</td>
<td>3/4</td>
<td>2</td>
</tr>
</tbody>
</table>

$^*$higher scale reading means less colour contrast after testing

5.3 Summary

Some phenolic compounds in conjunction with laccase can give a good colouring effect on leather and colour fastness to washing. Oxidation and polymerisation of the phenolic compounds increase the hydrothermal stability of collagen. Phenol
structure and laccase concentration influence the dyeing and tanning effects. The phenols exhibit different affinities towards laccase. Dyeing of wet blue with the phenolic compounds and laccase can improve leather properties, i.e. tensile strength and elongation at break.
CHAPTER 6

DIHYDROXYNAPHTHALENES FOR LEATHER DYEING AND TANNING
6.1 Introduction

Dihydroxynaphthalene (DHN) melanin or polyketide melanin is the best characterised fungal melanin. The final step of the DHN melanin pathway is the conjoining of 1,8-DHN molecules to form the melanin polymer. The biosynthesis of DHN melanin is catalysed by laccase. The melanin has chemical similarity to lignin, since it is a macromolecule and has phenolic groups in its structure. There is also the possibility that various DHNs or naphthols may act as the immediate precursor to melanin, rather than 1,8-DHN. Structures of some DHNs are given in Figure 6.1.

![Structures of dihydroxynaphthalenes used in this study](image)

Figure 6.1 Structures of dihydroxynaphthalenes used in this study

The DHNs contain aromatic and hydroxyl groups. They have similar structures to plant polyphenols, in term of their aromaticity and functional groups. Therefore, they might have tanning properties like plant polyphenols.

In this study, to investigate the dyeing effects of DHN compounds, dyeing studies using some DHNs for synthesising melanin-like polymers were carried out. In these dyeing experiments, laccase was employed as catalyst for the oxidation and polymerisation reactions. In order to investigate tanning capacity and the influence of DHN structure, studies using DHNs and combination tanning
reactions using oxazolidine on hide powder and sheepskin pickled pelt were undertaken.

6.2 Results and Discussion

6.2.1 Dyeing effects of dihydroxynaphthalenes and laccase

6.2.1.1 Colouring hide powder

Figure 6.2 shows the colours of hide powder treated by some dihydroxynaphthalenes and laccase. They resulted in a range of colours: light brown, green, purple, and dark brown.

Figure 6.2 Colour of hide powder dyed with dihydroxynaphthalenes (DHNs) and laccase
The L* values of the treated hide powder are given in Figure 6.3. The treatments increased darkness of hide powder, shown by the decrease of L* values of all of the treatments. The darkest colours were given by oxidation of 1,3-DHN and 1,5-DHN; they produced violet and dark brown colours respectively. This indicates that the DHNs were oxidised and polymerised by molecular oxygen, catalysed by laccase.

![Figure 6.3](image)

**Figure 6.3** Lightness values (L*) of hide powder dyed with dihydroxynaphthalenes and laccase

Figure 6.4 shows the treatments using laccase and most of the DHNs produced positive chromaticity a* values, indicating that the colours produced by the treatments contained a red component; except 2,7-DHN and 1,7-DHN, which gave negative chromaticity a*, showing that the colours produced contained a green component. The greatest chromaticity a* value was obtained by the reaction of 1,8-dihydroxynaphthalene-3,6-disulfonic acid, disodium salt (1,8-DHN-DSA).

The treatments produced positive values for chromaticity b*, indicating that the colours contained a yellow component, as can be seen in Figure 6.5. The
oxidation of 2,3- and 1,7-DHNs catalysed by laccase yielded the highest \( b^* \) values.

The calculated colour differences caused by the DHNs and laccase treatments are given in Table 6.1. The greatest effect of the total treatments (\( \Delta E^*_{\text{total}} \)) resulted from oxidation of 1,3-DHN, followed by the oxidation of 1,5-DHN and 2,7-DHN. Not much colour was produced by the oxidation of 2,6- and 2,3-DHNs. Laccase gave different effects on the DHNs as shown by \( \Delta E^*_{\text{lacc}} \); 1,8-DHN-DSA and 2,7-DHN were highly affected by the enzyme. Without laccase, 1,3- and 1,5-DHNs produced high intensity of colour as shown by \( \Delta E^*_{\text{phenol}} \) values.
Figure 6.5 Chromaticity b* values of hide powder dyed with dihydroxynaphthalenes and laccase

Table 6.1 Effects of the treatments to cause colour difference of hide powder dyed with dihydroxynaphthalenes and laccase

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Effect of phenol alone ($\Delta E^*_{\text{phenol}}$)</th>
<th>Effect of laccase on phenol ($\Delta E^*_{\text{lacc}}$)</th>
<th>Effect of total treatment ($\Delta E^*_{\text{total}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,3-DHN + laccase</td>
<td>55.2</td>
<td>16.8</td>
<td>58.8</td>
</tr>
<tr>
<td>1,6-DHN + laccase</td>
<td>44.0</td>
<td>5.3</td>
<td>47.2</td>
</tr>
<tr>
<td>1,8-DHN-DSA + laccase</td>
<td>11.5</td>
<td>37.0</td>
<td>48.1</td>
</tr>
<tr>
<td>2,3-DHN + laccase</td>
<td>21.5</td>
<td>11.1</td>
<td>27.7</td>
</tr>
<tr>
<td>2,7-DHN + laccase</td>
<td>22.2</td>
<td>28.6</td>
<td>50.1</td>
</tr>
<tr>
<td>1,5-DHN + laccase</td>
<td>48.7</td>
<td>9.3</td>
<td>57.6</td>
</tr>
<tr>
<td>1,7-DHN + laccase</td>
<td>33.3</td>
<td>9.1</td>
<td>33.8</td>
</tr>
<tr>
<td>2,6-DHN + laccase</td>
<td>17.6</td>
<td>2.7</td>
<td>15.4</td>
</tr>
</tbody>
</table>

*measured relative to untreated hide powder

According to these results, dihydroxynaphthalenes can be oxidised and polymerised, catalysed by laccase, to create pigment products. The reactivities of
the DHNs in producing colour and their tanning effects depend upon their structures: 2,7-DHN and 1,8-DHN-DSA were the most reactive to produce colour.

6.2.1.2 Hydrothermal stability

The hydrothermal stability of hide powder treated by 1,8-DHN-DSA decreased (Table 6.2) owing to destabilisation of the collagen. On the other hand, the treatments using the other naphthols increased the stability of the treated hide powder, particularly the treatments using 2,7-DHN without laccase ($\Delta T_s = 13^\circ C$) and 2,6-DHN with laccase ($\Delta T_s = 26^\circ C$). This indicates that some naphthols have tanning affinity for collagen.

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>$T_s$ (°C)</th>
<th>$\Delta T_s$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>59</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td>1,3-DHN</td>
<td>68</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>1,3-DHN + laccase</td>
<td>60</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>1,6-DHN</td>
<td>68</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>1,6-DHN + laccase</td>
<td>68</td>
<td>9</td>
<td>29</td>
</tr>
<tr>
<td>1,8-DHN-DSA</td>
<td>53</td>
<td>-6</td>
<td>28</td>
</tr>
<tr>
<td>1,8-DHN-DSA + laccase</td>
<td>50</td>
<td>-9</td>
<td>19</td>
</tr>
<tr>
<td>2,3-DHN</td>
<td>66</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>2,3-DHN + laccase</td>
<td>63</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>2,7-DHN</td>
<td>72</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>2,7-DHN + laccase</td>
<td>66</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>1,5-DHN</td>
<td>64</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>1,5-DHN + laccase</td>
<td>61</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>1,7-DHN</td>
<td>68</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>1,7-DHN + laccase</td>
<td>64</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>2,6-DHN</td>
<td>63</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>2,6-DHN + laccase</td>
<td>85</td>
<td>26</td>
<td>32</td>
</tr>
</tbody>
</table>

Table 6.2 Hydrothermal stability of hide powder dyed with dihydroxynaphthalenes and laccase (important results are highlighted in bold)
6.2.2 Tanning studies with dihydroxynaphthalenes

6.2.2.1 Hydrothermal stability

The DHNs were used for tanning studies on hide powder. Simple compounds of this type do have some affinity for collagen, exploited for example as dye levelling agents.

Table 6.3 Hydrothermal stability of collagen treated with dihydroxynaphthalenes

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>Ts (°C)</th>
<th>ATs (°C)</th>
<th>ΔH (J/g)</th>
<th>Ts (°C)</th>
<th>ATs (°C)</th>
<th>ΔH (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>57</td>
<td>-</td>
<td>31</td>
<td>55</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>1,3-DHN</td>
<td>61</td>
<td>3</td>
<td>42</td>
<td>-a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,5-DHN</td>
<td>56</td>
<td>-2</td>
<td>25</td>
<td>64</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>1,6-DHN</td>
<td>64</td>
<td>7</td>
<td>38</td>
<td>71</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>1,7-DHN</td>
<td>64</td>
<td>7</td>
<td>35</td>
<td>-a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,8-DHN-DSA</td>
<td>49</td>
<td>-9</td>
<td>31</td>
<td>61</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>2,3-DHN</td>
<td>61</td>
<td>4</td>
<td>40</td>
<td>-a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2,6-DHN</td>
<td>62</td>
<td>4</td>
<td>42</td>
<td>71</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>2,7-DHN</td>
<td>62</td>
<td>5</td>
<td>40</td>
<td>71</td>
<td>16</td>
<td>27</td>
</tr>
</tbody>
</table>

*the pelt denatured when dihydroxynaphthalene was applied*

Table 6.3 shows the 1,6-, 1,7-, 2,6-, and 2,7-DHNs slightly increased the shrinkage temperatures of the treated hide powder, whereas 1,5-DHN and 1,8-DHN-DSA gave a ‘negative’ tanning effect as shown by the decrease of Ts of the treated hide powder by 2 and 9°C respectively. These data indicate that, although DHNs have the same number of -OH groups, they produce different tanning ability towards collagen, due to position difference of the groups in the DHNs. These data show also that the DHNs were not good enough for solo tanning, but if some of the phenols were to be used in combination tanning with a cross linker such as oxazolidine, they might confer a tanning effect.
Tanning studies using the DHNs on sheepskin pickled pelt were investigated. Table 6.3 shows that 1,6-; 2,6-; and 2,7-DHNs increased shrinkage (denaturation) temperatures of sheepskin pickled pelt by 16°C, which were higher than for hide powder. Therefore, the DHNs had limited leather tanning ability.

The hydrothermal stability of the pelt was decreased by the treatment using 1,3-; 1,7-; and 2,3-DHNs, indicated by the shrinkage of the pelt when the DHN solutions applied. Therefore, those DHNs did not have a tanning effect.

Although the treatment of sheepskin pickled pelt using 1,8-DHN-DSA increased the hydrothermal stability of the skin, it did not produce leather; it produced a parchment-like stiff material.

### 6.2.2.2 Strength properties

Table 6.4 shows strength properties of sheepskin pickled pelt treated by four selected naphthol compounds. Strength properties of the pelt treated by 1,8-DHN-DSA were not measured, since the DHN did not produce a leather.

Tensile and tear strengths of the leather in the parallel direction to the backbone were relatively higher than those in the perpendicular direction, which is typical. The DHNs gave relatively the same strength effects on the pelt. In terms of the elongation at break, the elasticities in the perpendicular direction were higher than those in parallel direction.

The average effects of tanning using DHNs on strength properties of the tanned sheepskin are given in Table 6.5. No appreciable change in the strength properties of skin was recorded after tanning using the DHNs. On the other hand, there is an improvement in the elasticity of the leather tanned using the DHNs, as shown by elongation at break.
Table 6.4 Strength properties of sheepskin tanned with DHNs

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>Tensile strength (MPa)</th>
<th>Tear strength (N/mm)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel</td>
<td>Perpendicular</td>
<td>Parallel</td>
</tr>
<tr>
<td>None</td>
<td>8.9</td>
<td>9.8</td>
<td>39.5</td>
</tr>
<tr>
<td>1,5-DHN</td>
<td>11.9</td>
<td>9.5</td>
<td>39.7</td>
</tr>
<tr>
<td>1,6-DHN</td>
<td>11.0</td>
<td>7.1</td>
<td>48.4</td>
</tr>
<tr>
<td>2,6-DHN</td>
<td>11.2</td>
<td>6.9</td>
<td>45.5</td>
</tr>
<tr>
<td>2,7-DHN</td>
<td>13.9</td>
<td>8.1</td>
<td>35.1</td>
</tr>
</tbody>
</table>

Table 6.5 The average effects of tanning using DHNs on leather strength (%)

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>Tensile strength</th>
<th>Tear strength</th>
<th>Elongation at break</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,5-DHN</td>
<td>13</td>
<td>-3</td>
<td>15</td>
</tr>
<tr>
<td>1,6-DHN</td>
<td>-3</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>2,6-DHN</td>
<td>-3</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>2,7-DHN</td>
<td>15</td>
<td>-17</td>
<td>51</td>
</tr>
</tbody>
</table>

6.2.3 Combination tanning studies using dihydroxynaphthalenes and oxazolidine

To investigate the tanning effects of four selected DHNs in conjunction with oxazolidine, combination tanning studies on hide powder and sheepskin were investigated.

6.2.3.1 Hydrothermal stability

Table 6.6 shows that tanning using some DHNs and retanning using oxazolidine increased the hydrothermal stabilities of hide powder and sheepskin between 20 and 55°C as shown by ΔT_s. The highest shrinkage temperatures are achieved using 1,6- and 2,6-DHNs. 1,5-; 1,6-; and 2,6-DHNs produced additive tannages that exhibited positive synergies, as shown by ΔT_s(synergy). Combination tanning using 2,6-DHN and oxazolidine produced a leather with a shrinkage temperature equivalent to conventional chrome tanned leather. Therefore, this type of reaction might be useful as a substitute for chrome tanning leather.
Table 6.6 Hydrothermal stability of collagen treated by dihydroxynaphthalenes and oxazolidine (important results are highlighted in bold)

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>T (°C)</th>
<th>ΔT (°C)</th>
<th>ΔH (J/g)</th>
<th>ΔT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hide powder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>57</td>
<td>-</td>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td>Oxazolidine (Oz)</td>
<td>75</td>
<td>17</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td>1,5-DHN + Oz</td>
<td>85</td>
<td>27</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>1,6-DHN + Oz</td>
<td>90</td>
<td>32</td>
<td>29</td>
<td>8</td>
</tr>
<tr>
<td>2,6-DHN + Oz</td>
<td>110</td>
<td>53</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>2,7-DHN + Oz</td>
<td>79</td>
<td>22</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>Sheepskin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>55</td>
<td>-</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Oxazolidine (Oz)</td>
<td>78</td>
<td>23</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>1,5-DHN + Oz</td>
<td>95</td>
<td>40</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>1,6-DHN + Oz</td>
<td>100</td>
<td>45</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>2,6-DHN + Oz</td>
<td>110</td>
<td>55</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>2,7-DHN + Oz</td>
<td>86</td>
<td>31</td>
<td>27</td>
<td>-8</td>
</tr>
</tbody>
</table>

*ΔT (°C) = ΔT [(DHN + Oz) - DHN - Oz]

6.2.3.2 Strength properties

Table 6.7 Strength properties of sheepskin tanned with four dihydroxynaphthalenes and oxazolidine

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>Tensile strength (MPa)</th>
<th>Tear strength (N/mm)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel</td>
<td>Perpendicular</td>
<td>Parallel</td>
</tr>
<tr>
<td>None</td>
<td>8.9</td>
<td>9.8</td>
<td>39.5</td>
</tr>
<tr>
<td>Oxazolidine (Oz)</td>
<td>6.8</td>
<td>5.8</td>
<td>24.5</td>
</tr>
<tr>
<td>1,5-DHN + Oz</td>
<td>5.2</td>
<td>4.8</td>
<td>24.6</td>
</tr>
<tr>
<td>1,6-DHN + Oz</td>
<td>6.9</td>
<td>5.5</td>
<td>31.8</td>
</tr>
<tr>
<td>2,6-DHN + Oz</td>
<td>7.6</td>
<td>7.4</td>
<td>25.4</td>
</tr>
<tr>
<td>2,7-DHN + Oz</td>
<td>11.1</td>
<td>7.1</td>
<td>26.5</td>
</tr>
</tbody>
</table>

The strength properties of sheepskin tanned with DHNs and retanned with oxazolidine are given in Table 6.7 and the effects of retanning of DHNs tanned leather using oxazolidine are given in Table 6.8. In general, the retanning
decreased strength properties of the DHNs tanned leather, except for the case of
2,6-DHN tanned leather.

Table 6.8 The effects of retanning using oxazolidine on dihydroxynaphthalene
tanned leather strength (% change)

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>Tensile strength</th>
<th>Tear strength</th>
<th>Elongation at break</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel</td>
<td>Perpendicular</td>
<td>Parallel</td>
</tr>
<tr>
<td>Oxazolidine (Oz)</td>
<td>-31</td>
<td>-69</td>
<td>-61</td>
</tr>
<tr>
<td>1,5-DHN + Oz</td>
<td>-57</td>
<td>-49</td>
<td>-38</td>
</tr>
<tr>
<td>1,6-DHN + Oz</td>
<td>-37</td>
<td>-22</td>
<td>-34</td>
</tr>
<tr>
<td>2,6-DHN + Oz</td>
<td>-32</td>
<td>+7</td>
<td>-44</td>
</tr>
<tr>
<td>2,7-DHN + Oz</td>
<td>-20</td>
<td>-12</td>
<td>-24</td>
</tr>
</tbody>
</table>

In general, there is a negative effect on the physical properties of the leathers, as shown in Table 6.9. It is clear that any contribution to weakening, compared to the untanned control, derives from the oxazolidine reaction. In the case of tensile and tear strengths, the average effect of the process is to make the material less anisotropic, decreasing the difference in the directional property. The elasticity, in terms of elongation at break, of the DHN-oxazolidine tanned leather was increased in the parallel direction and was decreased in the perpendicular direction.

Table 6.9 The average effects of retanning using oxazolidine on dihydroxynaphthalene tanned leather (% change)

<table>
<thead>
<tr>
<th>Position to the backbone</th>
<th>Tensile strength</th>
<th>Tear strength</th>
<th>Elongation at break</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel</td>
<td>-35</td>
<td>-35</td>
<td>+15</td>
</tr>
<tr>
<td>Perpendicular</td>
<td>-15</td>
<td>-30</td>
<td>-20</td>
</tr>
</tbody>
</table>

6.2.3.3 Combination of DHNs in the tanning system

6.2.3.3.1 Affinity of DHNs on collagen

Hydrogen bonds in leather can be broken down by lyotropic agents such as 50% acetone, 8M urea or concentrated sodium carbonate solution. The lyotropic
agents can release the tannin molecules from tanned hide powder or pelt. Covalently attached tannins on collagen are more stable and cannot be extracted by lyotropic compounds\textsuperscript{177-179}.

**Table 6.10** The affinity of some dihydroxynaphthalenes on collagen in hide powder

<table>
<thead>
<tr>
<th>Tannins</th>
<th>DHN bound to collagen\textsuperscript{a} (%)</th>
<th>DHN left after acetone washing\textsuperscript{b} (%)</th>
<th>Cross-linked DHN left after acetone washing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,5-DHN</td>
<td>57</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>1,6-DHN</td>
<td>74</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td>2,6-DHN</td>
<td>76</td>
<td>35</td>
<td>49</td>
</tr>
<tr>
<td>2,7-DHN</td>
<td>69</td>
<td>36</td>
<td>35</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Calculated by difference between initial DHN used and DHN left in the after tanning liquor

\textsuperscript{b}Calculated by difference between DHN bound to collagen and DHN in washing liquor after washing with 50% (v/v) aqueous acetone

From Table 6.10, it can be seen that 70-90% DHNs were washed out by 50% (v/v) aqueous acetone from DHN tanned hide powder and less DHN (50-80%) was washed out after the cross-linking using oxazolidine. This indicates that 20-50% of DHNs was irreversibly fixed in combination tanning of collagen. The reason for this phenomenon is that some of the DHN molecules are fixed on collagen chains by covalent bonding, analogous to observations in the combination tanning using condensed plant polyphenols and oxazolidine, reported by Covington and Song\textsuperscript{23}.

**6.2.3.3.2 Tanning fixation studies**

Differential scanning calorimetry analysis (Table 6.11) shows that the highest shrinkage temperatures were achieved by oxazolidine combination tanning using 1,6- and 2,6-DHNs. There was some loss of hydrothermal stability after washing with the hydrogen bond breaker, 50% (v/v) acetone-water, but it remains high. This indicates covalent bonding has an important role in DHN-oxazolidine combination tanning, which is supported by the affinity of the DHNs on collagen.
described in Section 6.2.3.3.1 above. Combination tanning using 2,6- and 2,7-DHNs with oxazolidine gives a synergistic effect to create high hydrothermal stability.

Table 6.11 Shrinkage temperature (°C) of treated collagen (important results are highlighted in bold)

<table>
<thead>
<tr>
<th>Tanning method</th>
<th>Tanned collagen</th>
<th>Acetone washed collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>57</td>
<td>56</td>
</tr>
<tr>
<td>Oxazolidine</td>
<td>75</td>
<td>71</td>
</tr>
<tr>
<td>1,5-DHN</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>1,5-DHN + oxazolidine</td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>1,6-DHN</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td>1,6-DHN + oxazolidine</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td>2,6-DHN</td>
<td>62</td>
<td>57</td>
</tr>
<tr>
<td>2,6-DHN + oxazolidine</td>
<td>110</td>
<td>97</td>
</tr>
<tr>
<td>2,7-DHN</td>
<td>62</td>
<td>58</td>
</tr>
<tr>
<td>2,7-DHN + oxazolidine</td>
<td>79</td>
<td>67</td>
</tr>
</tbody>
</table>

This result shows the reactivities of the DHNs to produce tanning effects towards collagen depend upon their structures, where 1,6- and 2,6-DHNs are more reactive than the other naphthols. It also indicates that 2,6- and 1,6-DHNs undergo cross-linking with oxazolidine and collagen with different reactivities, as shown by the differences in their ability to raise shrinkage temperature.

On the other hand, there was no marked increase in $T_s$ for collagen treated by the other DHNs. This could be attributed to the limited cross-linking between the DHNs, oxazolidine and collagen, perhaps due to positions of phenolic hydroxyl groups in their structures giving rise to unfavourable steric hindrance.

The reactivities of 1,6- and 2,6-DHNs can be explained by the electronic nature of naphthalene, which normally undergoes electrophilic substitution at C-1 ($\alpha$-location) more rapidly than at $\beta$-sites, ortho-coupling is preferred and its substitution reactions tend to proceed in a manner that maintains one intact
benzene ring\(^{180}\). Based on these characteristics, reactive sites of the naphthol compounds, where the substitution reactions most readily occur can be determined. The naphthols tend to maintain one intact benzene ring, i.e. A ring (Figure 6.6), thus polymerisation and substitution reactions will take place on B ring. The reactive sites for 2,6-DHN are at C-1 (\(\alpha\)) and C-3 (\(\beta\)), and for 1,6-DHN are C-2 (\(\beta\)) and C-4 (\(\alpha\)), as shown in Figure 6.6. Both reactive sites for 2,6-DHN are at ortho positions to the phenolic hydroxyl group, so the substitution reaction at both sites are preferred. On the other hand, reactive sites for 1,6-DHN are at ortho (C-2) and para (C-4) positions to the phenolic hydroxyl group; consequently, the coupling reaction at C-4 position will occur after the first coupling at ortho (C-2). Therefore, the 2,6-DHN should be more reactive than 1,6-DHN. As a result, 2,6-DHN can create more cross-linking between the naphthol, oxazolidine, and collagen, leading to higher shrinkage temperature.

![Figure 6.6 Reactive sites of 2,6- and 1,6-dihydroxynaphthalenes](image)

The electronic effect in naphthol is a resonance induced feedback of electrons from the oxygen lone pairs into the ring. This tends to decrease the electron density round oxygen and to decrease the strength of the O-H bond, and therefore encourages proton loss\(^{171}\), which in turn promotes hydrogen bonding to collagen. In other words, the phenolic hydroxyl group of the DHNs is an electron donor to the aromatic ring. Resonance stabilisation occurs by lone pair interaction, as shown in Figures 6.7 and 6.8.
Chapter 6
Dihydroxynaphthalenes for leather dyeing and tanning

Figure 6.7 Resonance-stabilised anions of 2,6- dihydroxynaphthalene at pH > 6

Figure 6.8 Resonance-stabilised anions of 1,6- dihydroxynaphthalene at pH > 6

The resonance forms of the naphthol indicate that electron donation results in negative charges at the ortho and para positions. Therefore, the naphthols will be prone to electrophilic attack at those positions. This accounts for the cross-links between the naphthol with oxazolidine and collagen.

Based on the positions of reactive sites in the compounds, cross-linking reactions of 2,6-DHN and 1,6-DHN with oxazolidine can be predicted, as given in Figure 6.9. Models of the cross-linking for 2,6- and 1,6-DHN-oxazolidine combination tanning are demonstrated in Figures 6.10 and 6.11 respectively.
Chapter 6
Dihydroxynaphthalenes for leather dyeing and tanning

Figure 6.9 Cross-linking reactions of 2,8- and 1,6-dihydroxynaphthalenes with oxazolidine

Figure 6.10 Cross-linking model for 2,6-dihydroxynaphthalene-oxazolidine combination tanning
Chapter 6

Dihydroxynaphthalenes for leather dyeing and tanning

Collagen

--- Covalent bond between tannins
--- Hydrogen bond between collagen and tannins
--- Covalent bond between collagen and tannins

Figure 6.11 Cross-linking model for 1,6-dihydroxynaphthalene-oxazolidine combination tanning

6.3 Summary

Dihydroxynaphthalenes can be oxidised and polymerised, catalysed by laccase to create some coloured products. They have different capacities for colouring hide powder; 1,3-; 1,5-; and 2,7-DHNs give good colouring effects. The reaction of 2,7-DHN does not only produce colour, but also raises the collagen's hydrothermal stability.

Some naphthol compounds possess tanning ability. Combination tanning using 2,6- and 1,6-DHNs with oxazolidine give synergistic effects to produce high hydrothermal stability of leather. This is mainly determined by the covalent bonding formed between the naphthols and collagen through oxazolidine.
CHAPTER 7

GENERAL DISCUSSION
From this study, it is clear that the nature of the matrices created by the laccase reaction with phenolic lignin degradation products, simple phenols, flavonoid antioxidants, and amino phenols 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.

These studies suggest that such a relationship between physical and thermodynamic properties might exist, e.g. the treatments of wet blue using some mononuclear polyphenols and amino phenols in conjunction with laccase can increase strength and elasticity of the leather. From the investigation on the use of DHNs and oxazolidine for combination tanning, some DHNs show that the matrices created by those reactions can create high hydrothermal stability.

Therefore, the opportunity arises to define the relationship between the two aspects of stabilising collagen. At the same time, the leather may be coloured. While this contribution to so-called compact processing as there are two processes, i.e. dyeing and tanning, occur in one process simultaneously might be useful, although control of the colour is limited. Nevertheless, it may be useful to initiate colouring prior to dyeing, to provide a base colour and thereby reduce the cost of dyeing with synthetic dyes. Therefore, this study has shown a new approach and principle in leather manufacturing: colouring of leather without synthetic dyes. An advantage of this approach is that the leather is not only coloured but also tanned, since the pigments are bound to the collagen by covalent bonds, which confer a tanning effect.

Regarding tanning itself, polyphenol reactions are conventionally limited by astringency, i.e. high reactivity, leading to slow penetration into the substrate. This study has addressed the limits to using phenolic reagents for combination tanning. The observation shows that even the lowest molecular weight species
can be used to achieve high hydrothermal stability opens up opportunities to conduct leather making processes entirely novel ways.
CHAPTER 8

CONCLUSIONS
From this study on the phenolic reactions for tanning and dyeing of leather, the following conclusions can be drawn:

1. Biomimetic degradation of lignin yields simple phenolic products. These compounds might have new uses, for example, to exploit the affinity between phenolic compounds and hides or skins; they may form the basis of synthetic organic tanning agents (syntans) and serve to colour leather. Vanillin is also employed in the foodstuff and pharmaceutical industry. Such applications would be beneficial in the developing economies. In this way, value can be added to an industrial byproduct (Kraft lignin), reducing its environmental and economic impact and so environmental pollution from use of chrome tanning may be reduced.

2. It is possible to produce coloured and tanned leather without using dyes and conventional tanning agents, i.e. by using oxidation and polymerisation of some phenolic compounds catalysed by laccase. They can produce earth colours; therefore, this approach might be useful, as long as it is the 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.

3. 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, such as increasing in elasticity, made by phenolic polymerisation, observed in these studies, may be useful for some applications, such as in shoe manufacturing.

4. Tanning using 2,6- or 1,6-DHNs and retanning with oxazolidine can produce a high hydrothermal stability of leather, possibly exploitable as a substitute for chrome tanning, although more likely to constitute a new organic tannage. In this way, use of basic chromium(III) sulfate in leather
manufacturing might be reduced, so environmental pollution from chrome tanning might be decreased. However, the environmental impact of waste naphthol must also be considered.
APPENDICES
APPENDIX I
GLOSSARY OF LEATHER TERMS

Basicity
Relationship between chromium atoms and the hydroxyl groups in a chromium(III) complex. It is ratio of the total hydroxyl groups to the total maximum number of hydroxyls which can associate with the total number of chromium atoms in the complex, expressed as percent. Basicity varies from 0% in chromium(III) sulfate to 100% in chromium(III) hydroxide Cr(OH)₃ (often referred to as Schorlemmer basicity).

Bate (v); bating
To treat unhaired and limed pelt with proteolytic enzyme(s) at pH 8-9.

Degrease (v); degreasing
To remove grease by any method.

Depickle (v); depickling
Neutralise the acid in pickled pelts (raise the pH towards the isoelectric point of collagen) by treating them with basic compounds, in a saline bath.

Fatliquor; fatliquoring
Introduce oil into leather, normally by drumming it with an oil-in-water emulsion, to provide lubrication to the leather.

Float
Refers to the aqueous liquor in which a process such as pickling or tanning is performed.
Neutralisation
Process of raising pH towards neutral from acidic zone, typically of a mineral-tanned leather after tanning by treatment with a solution of alkaline salt or buffer mixture.

Pickle (v); pickling
Treatment of pelts with an acid liquor, such as a solution of sulfuric acid and sodium chloride, to preserve them or to prepare them for tanning, especially chrome tanning.

Pickled weight
Weight of hides or skins after the pickling process and draining to approximately 55 to 60% moisture.

Samm (v); samming
Squeeze the leather to uniformly semi-dry state (approximately 50 to 60% water content) necessary for certain operations, by passing it through the samming machine or by pressing.

Shave (v); shaving
Reduce and/or level the thickness of leather, suitable for its intended end-use, by cutting fine, thin fragments from the flesh side by a machine with a rapidly revolving bladed cylinder.

Shaved weight
Weight of tanned hides or skins after wet shaving.

Shrinkage temperature
Temperature at which leather begins to decrease in length and width (shrinks) when heated under specific conditions, for example, when heated in water at (usually) 2°C per minute.
Tan (v); tanning
Treating prepared hides or skins with suitable chemicals to give a fibrous product, imputrescible when wet, more or less soft and flexible when dry and capable of being wetted and dried without loss of these properties. Note: hides and skins are primarily composed of collagen in a fibrous structure. The aim of tanning is to form irreversible chemical crosslinks to the collagen matrix to prevent degradation by bacterial, chemical or thermal action. Commonly used tanning agents, applied individually or in combination, are chromium(III) salts, giving a typically blue coloured leather, synthetic tanning agents and organic vegetable tannins which give a characteristic pale brown colour. Various other metallic salts such as aluminium(III) and zirconium(IV) are also available as tanning agents.

Toggle (v); toggling
The straining and fixing of leather onto frames with toggles. The purpose is to dry leather, keeping it under tension.

Wet blue
Term for a hide or skin, which has been subjected to the usual beamhouse processes, chrome tanned and left wet; may be stored or exported in this state.
### APPENDIX II
#### DEGREASING OF SHEEPSKIN PICKLED PELT

**Material:** Sheepskin pickled pelt.

Offers in this process were based on pickled pelt weight.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Chemical</th>
<th>%</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degreasing</td>
<td>Baymol AN</td>
<td>3</td>
<td>30</td>
<td>30</td>
<td>Non ionic, solvent free degreasing and wetting agent.</td>
</tr>
<tr>
<td></td>
<td>Sodium chloride</td>
<td>6</td>
<td>35</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drain</td>
<td>Baymol AN</td>
<td>3</td>
<td>35</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Hang to drain (horse)</td>
<td>Sodium chloride</td>
<td>6</td>
<td>35</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>overnight</td>
<td>Water</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slick out</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>To remove grease and fat.</td>
</tr>
<tr>
<td>Washing</td>
<td>Sodium chloride</td>
<td>6</td>
<td>35</td>
<td>10</td>
<td>Repeat.</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>100</td>
<td></td>
<td></td>
<td>To remove the detergent.</td>
</tr>
<tr>
<td></td>
<td>Sodium chloride</td>
<td>6</td>
<td>35</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antimould</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hang to drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Material: Degreased sheepskin pickled pelt. 
Offers in this process were based on pickled weight.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Chemical</th>
<th>%</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanning</td>
<td>Sodium chloride</td>
<td>6</td>
<td>70</td>
<td>20</td>
<td>15 Record density (Bé) and pH</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>70</td>
<td>20</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sulfuric acid</td>
<td>0.2</td>
<td>25</td>
<td>120</td>
<td>Dilute 1:10 with water.</td>
</tr>
<tr>
<td></td>
<td>Formic acid</td>
<td>0.1</td>
<td>25</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25% Cr₂O₃ powder</td>
<td>8</td>
<td>25</td>
<td>60</td>
<td>33% basicity.</td>
</tr>
<tr>
<td>Basification</td>
<td>Sodium bicarbonate</td>
<td>0.25</td>
<td>30</td>
<td>30</td>
<td>Dilute 1:5 with water. Repeat every 30 min until pH 3.2-3.8. Run for a further 120 mins. Measure T₅.</td>
</tr>
<tr>
<td>Drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hang to drain (horse) overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX IV
NEUTRALISATION OF WET BLUE

Material: Shaved wet blue.
Offers in this process were based on shaved weight of wet blue.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Chemical</th>
<th>%</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing</td>
<td>Water</td>
<td>200</td>
<td>35</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Repeat</td>
</tr>
<tr>
<td>Water</td>
<td>Sodium formate</td>
<td>200</td>
<td>35</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Measure pH of the float</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td></td>
<td>1</td>
<td>35</td>
<td>45</td>
<td>pH 5-6</td>
</tr>
<tr>
<td>Measure pH of the float</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hang to drain (horse)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>overnight</td>
</tr>
</tbody>
</table>


APPENDIX V
IR SPECTRA OF KRAFT LIGNIN TREATED BY HYDROGEN PEROXIDE AND HAEMIN AT 90°C FOR 3 AND 5 HOURS

a. 3 hours (A2)

b. 5 hours (A3)
APPENDIX VI
IR SPECTRA OF KRAFT LIGNIN TREATED BY HYDROGEN PEROXIDE AND HAEMIN AT ROOM TEMPERATURE FOR 3 AND 5 HOURS

a. 3 hours (B2)

b. 5 hours (B3)
APPENDIX VII
IR SPECTRA OF KRAFT LIGNIN TREATED BY HYDROGEN PEROXIDE AND HAEMIN AT ROOM TEMPERATURE FOR 10 TO 25 HOURS

a. 10 hours

b. 15 hours
c. 20 hours

d. 25 hours
APPENDIX VIII
MASS SPECTRUM OF THE DEGRADATION PRODUCTS OF KRAFT LIGNIN BY LACCASE-N-HYDROXYPHTHALIMIDE (HPI) SYSTEM

1,2-benzenedicarboxylic acid
(positively charged trimethylsilyl derivatised)
APPENDIX IX

MASS SPECTRA OF THE DEGRADATION PRODUCTS OF KRAFT LIGNIN BY LACCASE-1-HYDROXYBENZOTRIAZOLE (HBT) SYSTEM

a. 2-hydroxy-3-methylbenzoic acid
   (positively charged trimethylsilyl derivatised)

b. 3-hydroxybenzoic acid
   (positively charged trimethylsilyl derivatised)

c. 2,3,4-trimethoxymandelic acid
   (positively charged trimethylsilyl derivatised)
APPENDIX X
MASS SPECTRA OF THE DEGRADATION PRODUCTS OF KRAFT LIGNIN BY LACCASE-2,2,6,6-TETRAMETHYL-1-PIPERIDINYL OXO (TEMPO) SYSTEM

a. 2-methoxy-benzoic acid
(positively charged underivatised)

b. 3,3'-dimethoxy-1,1'-biphenyl
(positively charged underivatised)

c. 1,2-benzenedicarboxylic acid
(positively charged trimethylsilyl derivatised)
APPENDIX XI
FT-IR SPECTRA OF 4-HYDROXYBENZALDEHYDE AND ITS POLYMERISATION PRODUCT

a. 4-hydroxybenzaldehyde

b. Polymerisation product of 4-hydroxybenzaldehyde
APPENDIX XII
FT-IR SPECTRA OF VANILLIN AND ITS POLYMERISATION PRODUCT

a. Vanillin

b. Polymerisation product of vanillin
APPENDIX XIII
FT-IR SPECTRA OF VANILLIC ACID AND ITS FORMALDEHYDE
POLYMERISATION PRODUCT

a. Vanillic acid

b. Polymerisation product of vanillic acid
The publications from Appendix XIV (pp. 213-254) have been removed from the electronic version of this thesis due to copyright restrictions.

Details of the publications can be found on pages 23-24 of this thesis.
REFERENCES
References


References


References


83. Gold, M.H. Kuwahara, M., Chiu, A.A. and Glenn, J.K. Purification and characterization of an extracellular H$_2$O$_2$-requiring diarylpropane oxygenase


105. Shimada, M.T., Habe, T., Umezawa, T., Higuchi, T. and Okamoto, T. The C-C bond cleavage of a lignin model compounds, 1,2-diarylpropane -1,3-diol, with a heme-enzyme model catalyst tetraphenylporphyrinato iron(III)
References


264
References


References


References


