

Deterioration of ancient Korean paper (*Hanji*), treated with beeswax: A mechanistic study



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ABSTRACT

In the early 15th century, beeswax coating was applied to some of the cellulosic documents in a futile attempt to better conserve the paper. However, this treatment caused much more severe degradation compared to untreated *Hanji*. In the current study, the degradation pathway of this beeswax-treated *Hanji* has been clarified for the first time. The degradation of cellulose was investigated by labeling of oxidized groups combined with gel permeation chromatography, providing profiles of carbonyl and carboxyl groups relative to the molar mass distribution. The beeswax caused purely hydrolytic damage, leading to a decrease in molar mass to about one fifth of the original value. Oxidative degradation, by contrast, did not occur to any significant extent. Hydrolysis was not caused by acids but by microorganism feeding on the beeswax and excreting cellulolytic enzymes, which cause similar cellulose damage patterns. The hydrolytic enzymes were identified by typical metabolites present in the *Hanji*.

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1. Introduction

Korean traditional handmade paper, *Hanji*, is considered to be one of the most stable and durable papers in the world. It is traditionally made of bast fibers from one-year-old paper mulberry (*Broussonetia kazinoki* Siebold), and its cellulose has a very high average degree of polymerization (DP) of 7000–9000. Only celluloses produced by bacteria or tunicates reach comparably high molar mass values.

The Annals of the Joseon Dynasty (*Joseon Wangjo Sillok*) recorded between AD 1392 and AD 1863, are the most representative paper heritage made of *Hanji*. The Annals are the longest continuous authentic records of a single royal dynasty in the world, and they have been listed on the Memory of the World Register by UNESCO (KOCIS, 2009). They consist of 1187 volumes in total. The pages are printed on with Chinese ink, a mixture of soot, glue, and a little perfume (Jeong, Cheon, & Park, 1991), using movable metal letters.

This principle had been used since the 14th century in Korea when the *Jikji* was printed, one of the first books ever printed according to this principle (78 years earlier than the more prominent, similar invention in Europe by Johannes Gutenberg in Germany around 1450).

The partly surprisingly good state of preservation of the Annals of the Joseon Dynasty today can mainly be attributed to the high quality of *Hanji* used. However, 475 volumes of these historically very important records had been treated with beeswax at the time of their writing in an intention to provide even better long-term stability (Song, Shin, Park, & Lee, 2005). While the volumes without beeswax still exhibit an amazingly fine paper quality considering their high age, the beeswax-treated volumes show signs of serious damage, such as discoloration, fissures, mechanical worsening, and formation of fine powder. The occurrence of such deterioration is directly related to the beeswax treatment (Jeong, Lee, Chung, Hong, & Eom, 2004; Jo, 2006; Song et al., 2005). However, the underlying reasons and the degradation mechanisms are not yet understood. It is not clear, for example, whether the deterioration is the result of radical-induced aging processes triggered by the beeswax components, induced autoxidation, or if acidic degradation products from the wax are responsible. Fig. 1 shows examples of beeswax-treated paper and untreated paper from the Annals of King Sejong.

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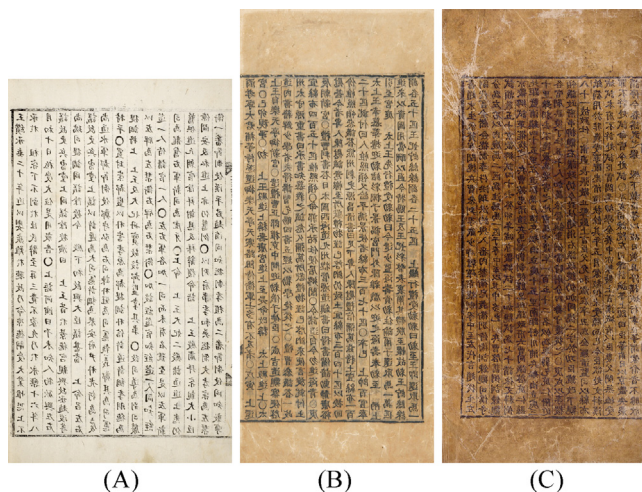


Fig. 1. The Annals of the Joseon Dynasty. (A) Beeswax-free page (1665 AD), (B) beeswax-treated page (1473 AD) and (C) beeswax-treated page with brown staining (1473 AD).

Although several methods exist to analyze and describe the strength and the state of deterioration of common paper (mechanical testing, analysis of DP and functional groups), most of them cannot be readily applied to the characterization of historic papers, which in most cases require non-destructive or at least micro-destructive approaches. In the present case, only micro-destructive approaches were applied on very small pieces of material (3–5 mg). The question of substance analysis was two-fold. First, the degradation status of the paper had to be determined. From the optical and haptical appearance, the beeswax-treated paper seemed to be significantly more damaged compared to the untreated material, as it was more brittle, discolored, and speckled with deposits. Hence, the second task was to find out how the cellulose in the paper was degraded, i.e., whether it suffered from hydrolytic or oxidative damage or a combination of both (Henniges, Bürger, Banik, Rosenau, & Potthast, 2006; Henniges, Prohaska, Banik, & Potthast, 2006; Potthast et al., 2003; Röhring, Potthast, Rosenau, Lange, & Borgards, et al., 2002; Röhring, Potthast, Rosenau, Lange, & Ebner, et al., 2002). This question is crucial with regard to the final conservation measures to be taken. The hydrolytic cleavage of cellulosic β -1,4-glycosidic linkages is caused by water, catalyzed by acids or enzymes, eventually decreasing the overall mechanical qualities of the paper. In this process, cellulose chains are cleaved and new reducing end groups are generated. Upon oxidation, carbonyl and carboxyl groups are introduced along the cellulose chain, which trigger further degradation (chain cleavage), especially under alkaline conditions according to β -elimination mechanisms. Oxidative cellulose degradation is commonly accompanied by rather severe chromophore formation (Röhring, Potthast, Rosenau, Lange, & Borgards, et al., 2002; Röhring, Potthast, Rosenau, Lange, & Ebner, et al., 2002).

2. Materials and methods

2.1. Materials

Small paper fragments from the Annals of Joseon Dynasty were used. Nine samples from the Annals of King Sejong and six samples from the Annals of King Seongjong were selected. The average beeswax content (wt%) of the Annals of King Sejong and Seongjong are about 39% (brown stains: 50%) and 21% respectively. More detailed information of test samples is given in Table 1. The chemicals used were of the highest grade available from commercial suppliers, solvents were of HPLC grade and reagents of p.a. grade.

Table 1

Samples of the Annals of King Sejong and King Seongjong. The assessment of the state of conservation is based on visual inspection and follows the common grading for historic papers.

Sample code	Treatment	State of conservation	Printed year	Dynasty
154-24	Beeswax-free	Best	1665	King Sejong
154-89	Beeswax-free	Best	1665	King Sejong
154-140	Beeswax-free	Best	1665	King Sejong
150-46	Beeswax-free	Best	1606	King Seongjong
154-114	Beeswax	Good	1473	King Sejong
154-123	Beeswax	Good	1473	King Sejong
150-4	Beeswax	Good	1499	King Seongjong
150-8	Beeswax	Good	1499	King Seongjong
150-30	Beeswax	Good	1499	King Seongjong
150-42	Beeswax	Good	1499	King Seongjong
150-93	Beeswax	Good	1499	King Seongjong
154-88	Beeswax	Brown stains	1473	King Sejong
154-97	Beeswax	Brown stains/very poor	1473	King Sejong
154-121	Beeswax	Brown stains/very poor	1473	King Sejong
154-122	Beeswax	Brown stains	1473	King Sejong

Carbazole-9-carboxylic acid [2-(2-aminooxyethoxy)ethoxy] amide (CCOA) and 9H-fluoren-2-yl-diazomethane (FDAM) were synthesized according to Röhring et al. (2001) and Bohrn, Potthast, Rosenau, Sixta, & Kosma (2005).

2.2. Fluorescence labeling of functional groups and dissolution of the cellulosic paper matrix

The CCOA and FDAM labeling were performed as described by previous reports (Bohrn et al., 2005; Bohrn et al., 2006; Röhring, Potthast, Rosenau, Lange, & Borgards, et al., 2002; Röhring, Potthast, Rosenau, Lange, & Ebner, et al., 2002). The labeling procedure was scaled down to 3–5 mg of paper. The beeswax layer and hydrophobic degradation products thereof were removed prior to labeling by extraction with chloroform (3 h) by shaking a piece of paper (few mg) in 5 mL of chloroform at room temperature. The CCOA- and FDAM-labeled samples were dissolved in DMAc/LiCl (9%, w/v) at room temperature for one day as described earlier (Röhring, Potthast, Rosenau, Lange, & Borgards, et al., 2002; Röhring, Potthast, Rosenau, Lange, & Ebner, et al., 2002). The resulting solution was diluted 1:3 with pure DMAc, filtered through a 0.45- μ m filter, and injected into the chromatographic system.

2.3. Gel permeation chromatography (GPC)

The molar mass distribution (MMD) and the carbonyl/carboxyl profiles (the carbonyl and carboxyl distribution relative to the MMD) were analyzed directly by GPC. The GPC system was modified with regard to previous applications (Bohrn et al., 2006; Röhring, Potthast, Rosenau, Lange, & Borgards, et al., 2002; Röhring, Potthast, Rosenau, Lange, & Ebner, et al., 2002) as follows: DMAc/LiCl (0.9%, w/v) was used as the eluent after filtering through 0.02- μ m filters. The sample (100 μ L) was injected automatically and chromatographed on four serial PL Mixed-A LS 20- μ m columns (300 cm $\times$ 7 cm, Polymer Laboratories, UK). The detection system consisted of multi-angle laser light scattering (MALLS), fluorescence, and RI detection. A refractive index increment of 0.136 mL/g for cellulose in DMAc/LiCl (0.9%, w/v) at 25 $^{\circ}$ C and 488 nm was used. All polymer-relevant parameters and the MMD were calculated by ASTRA 4.73 and Grams software programs.

The number of total carbonyl groups (keto and aldehyde functionalities) is the total carbonyl content as obtained by the CCOA

method. REGs were calculated according to Eq. (1), carbonyl groups other than REGs (“along-chain carbonyls”) were calculated according to Eq. (2).

$$\text{Reducing end – groups(REGs)} = \frac{1}{M_n} \times 10^6 \quad (1)$$

where, M_n = number-average molar mass in g mol^{-1} .

$$\begin{aligned} \text{Along-chain carbonyl content (mmol/g)} \\ = \text{Total carbonyl content – REGs} \end{aligned} \quad (2)$$

2.4. Methylation and GC–MS analysis

GC–MS analysis was applied after esterification of carboxylic acids with diazomethane (DAM).¹ DAM was prepared from Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) using the Diazald Kit (Sigma–Aldrich) prior to the derivatization according to a procedure described by Black (Black & Muir, 2003). For the methylation of samples, the excess DAM solution in diethyl ether was added to 3–7 mg beeswax-treated paper samples extracted in chloroform and left to react for 24 h with magnetic stirring. Excess DAM can be readily recognized by the yellow color of the reaction mixture and can be destroyed by addition of acetic acid, which generates methyl acetate as a rather volatile by-product. The solution was dried over sodium sulfate, filtered through a 0.45 μm syringe filter, and analyzed by GC–MS.

GC–MS was performed on a GC 6890N/MSD 5973B instrument with a fused silica HP-5 ms (30 m, 0.25 mm, 25 μm) column and helium as the carrier gas. Total flow was 27.5 mL/min at 46.9 kPa carrier gas pressure, and the resulting column flow was 0.9 mL/min. The following temperature program was used: 50 °C (5 min), 10 °C/min to 280 °C (20 min). At 230 °C inlet temperature in a splitless mode, 0.2 μL aliquots of the dissolved samples were injected. Ionization was performed in EI mode at 70 eV. Data was acquired and processed with ChemStation software MSD Chemstation E.2.01.1177 (Agilent Technologies, USA).

3. Results and discussion

3.1. Annals of the Joseon Dynasty without beeswax treatment

The MMD and carbonyl plots of cellulose from pages without beeswax treatment show that the samples still have a high molar mass and there was no significant oxidation as shown in Fig. 2 and Table 2. Weight-average molar masses (M_w) of the Annals were between 770 and 970 kg mol^{-1} , which corresponded to about 50–60% of the molar mass of non-aged, fresh material. Considering the age of the historic paper, on average about 400 years, it is startling that these historic samples are still much higher in molar mass than European rag paper.²

It is important to consider the factors influencing the molar mass of the sample. The molar mass is always a function of the starting material. In the present case, the average length of the one-year-old bast fibers of paper mulberry (*Broussonetia kazinoki* Sieb.) species in Korea is between 5.78 and 7.66 mm, which depends on the location of cultivation, even within Korea (Choi, Cho, Lee, & Oh, 2007). Before any degradation by aging influences can set in, the papermaking

Table 2

GPC–CCOA data of historic Hanji paper without beeswax treatment and fresh Hanji paper (Hanji H).

Parameter	Sample				
	154-24	154-89	154-140	150-46	Hanji H
M_n (kg mol^{-1})	319.3	218.6	278.0	362.4	1139
M_w (kg mol^{-1})	971.1	949.6	769.7	869.9	1566
M_z (kg mol^{-1})	1675	1628	1309	1461	1836
PolyDispersity index (PDI)	3.0	4.3	2.8	2.4	1.4
REG ($\mu\text{mol g}^{-1}$)	3.1	4.6	3.6	2.8	0.6
Total carbonyl groups ($\mu\text{mol g}^{-1}$)	14.7	15.2	12.1	13.1	24.3
Keto/aldehyde other than REG ($\mu\text{mol g}^{-1}$)	11.6	10.6	8.5	10.3	23.7
Uronic acids ($\mu\text{mol g}^{-1}$)	–	–	83.8	–	67.2

procedure and, in particular, the nature of the base used in pulping are two factors that are crucial with regard to the integrity of the cellulose. The application of potassium carbonate is considered rather mild as compared, for instance, the use of sodium hydroxide. This was shown by several simulations of historic Hanji-making protocols.

The total carbonyl content proved to be a very sensitive indicator of cellulose aging. The carbonyl contents of the historic samples ranged between 12 and 15 $\mu\text{mol g}^{-1}$, which were even slightly lower than that of non-aged Hanji (cf. Table 2). Keto and aldehyde groups without reducing ends were between 8 and 12 $\mu\text{mol g}^{-1}$ – again, a similar value to the non-aged, fresh Hanji. The low values for carbonyls were mainly due to the high molar mass, which means a small number of reducing ends.

In general, a very low degree of oxidative damage was observed for these aged papers. The distribution of carbonyls (aldehyde and keto groups apart from reducing ends) in the lower molar mass areas is characteristic for well-preserved historic papers. Some of the carbonyls in the low-molar mass region may also result from accompanying polysaccharides, e.g., hemicelluloses or mucopolysaccharides. Only very little oxidized functionalities are present in the high-molar mass region, an important prerequisite to long-term durability.

The uronic acid content of sample 154–140 was 83.8 $\mu\text{mol g}^{-1}$, which seems to be a rather high value in comparison to other annual plant fibers (cotton linters: 18.9 $\mu\text{mol g}^{-1}$, flax cellulose: 23 $\mu\text{mol g}^{-1}$, wash: 48 $\mu\text{mol g}^{-1}$), or wood pulp fibers (eucalyptus prehydrolysis kraft: 9 $\mu\text{mol g}^{-1}$) (Bohrn et al., 2006). The content corresponds to the non-aged Hanji H. It resulted mainly from mucopolysaccharides.³

From the data presented in Table 2, it was evident that the historic Hanji samples without beeswax were in excellent condition even after 400 years of natural aging. They suffered hydrolysis and oxidation only to a very minor extent.

3.2. Beeswax-treated pages of the Annals of the Joseon Dynasty

Seven sample fragments from different volumes of the beeswax-treated Annals have been investigated (cf. Table 2): two types of the Annals of King Sejong (1473 AD) and five types from the period of King Sejongjong (1499 AD). Fig. 3 shows representative MMD curves with the carbonyl profiles, and Table 3 summarizes the GPC data. The weight-average molar masses (M_w) of the King Sejong samples

¹ Caution: DAM is toxic by inhalation or by contact with the skin or eyes. It may explode in contact with sharp edges, such as ground-glass joints or even scratches in glassware. All reactions have to be performed behind a glass shield and in the hood.

² For comparison, well-preserved European rag paper has a weight-average molar mass of about 300–400 kg mol^{-1} .

³ Typically, Hanji papermakers added mucopolysaccharides from *Hibiscus manihot* Linne to prevent the fast settlement of paper fibers during papermaking.

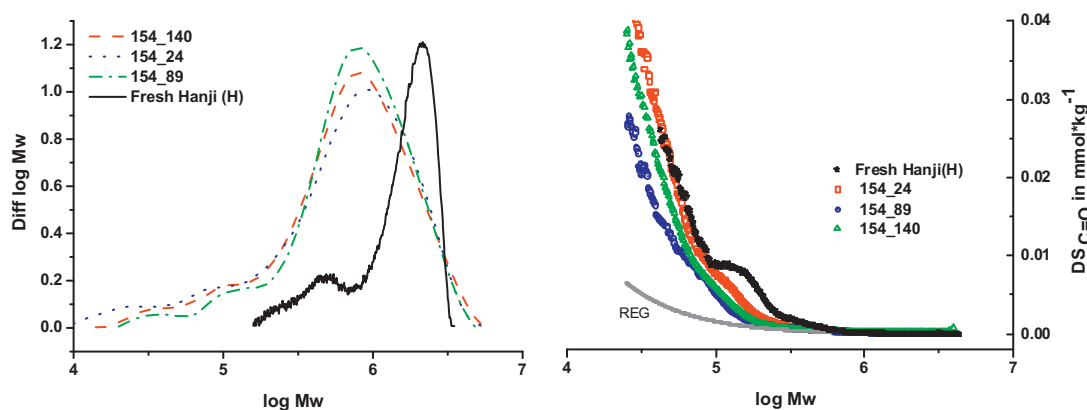


Fig. 2. Molar mass distribution (left) and distribution of carbonyl groups (right) of historic Hanji without beeswax compared to fresh Hanji (H) that was prepared according to historic recipes.

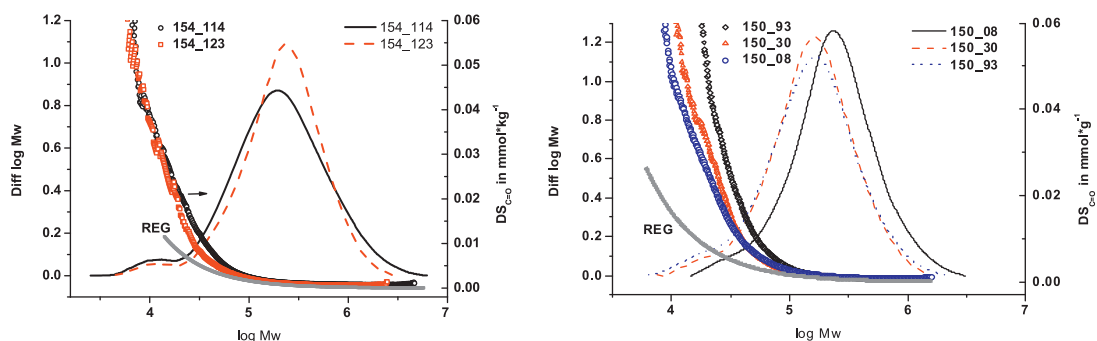


Fig. 3. Distribution of carbonyl groups and mass distribution of the beeswax-treated papers of the Annals of King Sejong (left) and of King Sejongjong (right).

Table 3
Molecular mass and carbonyl/carboxyl data of the beeswax-treated Hanji paper samples.

Parameter	Sample						
	154-114	154-123	150-4	150-8	150-30	150-42	150-93
M_n (kg mol ⁻¹)	92.8	111.8	111.9	92.5	103.7	96.9	160.3
M_w (kg mol ⁻¹)	352.0	306.9	227.7	214.2	207.1	230.7	321.8
M_z (kg mol ⁻¹)	970.9	590.0	383.8	412.9	359.0	469.2	595.4
PDI	3.79	2.74	2.03	2.32	2.00	2.38	2.01
REGs (μmol g ⁻¹)	10.8	8.9	8.9	10.8	9.6	10.3	6.2
Total carbonyl group content (μmol g ⁻¹)	22.5	17.7	24.0	28.2	23.1	25.1	19.8
Keto/aldehyde other than REG (μmol g ⁻¹)	11.8	8.7	15.1	17.4	13.5	14.8	13.5
Carboxyl group content (μmol g ⁻¹)	–	–	–	40.1	49.6	–	63.7

were 306.9 and 352.0 kg mol⁻¹, respectively, while those of King Sejongjong were even lower for four samples (between 207.1 and 230.7 kg mol⁻¹), and similar for sample 150–93 (321.8 kg mol⁻¹).

In comparison to the Annals samples without beeswax, the M_w of the beeswax-treated papers was radically decreased. The values correspond to only 20–35% of those from samples containing no beeswax (cf. Fig. 4 and Table 3). The reason for this drastic difference was evidently not the minor age difference. The important question was what the reason for this rather severe degradation was. Although a direct relation to the applied beeswax seemed to be obvious, there were no indications as to the actual mechanisms. The beeswax treatment would protect the paper from water (or moisture and to some extent oxygen) penetration, the paper being in a more enclosed state. The optical appearance of the paper showed a strong discoloration, which usually indicates oxidative processes in paper. Those are often accompanied by hydrolytic reactions. Hence, the prime question was whether oxidative and/or hydrolytic processes were involved in the evident cellulose degradation.

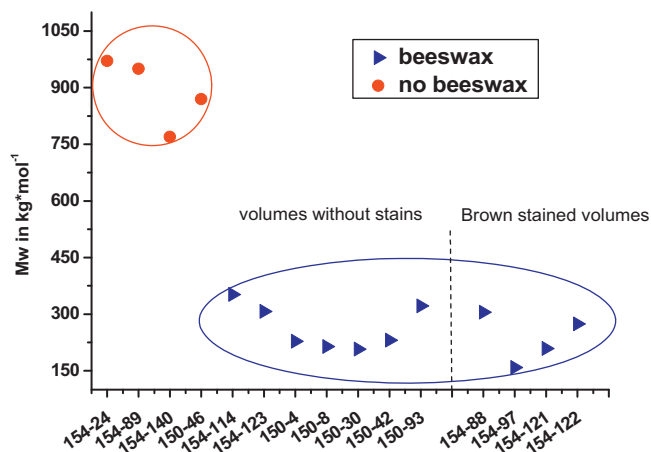


Fig. 4. Clustering comparison of weight-average molar mass data (M_w , y-axis) for beeswax-treated and untreated Hanji paper samples.

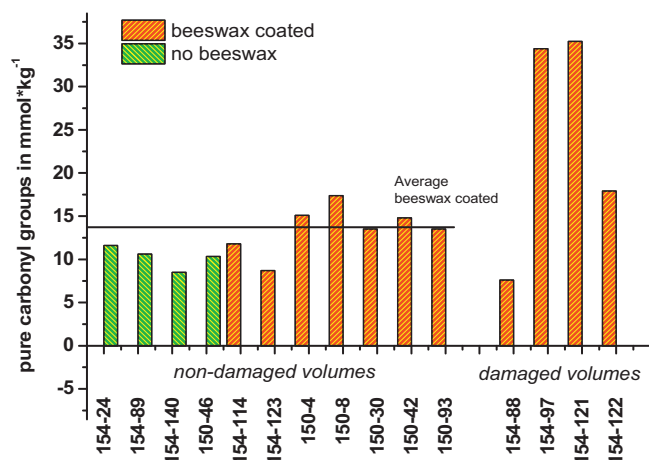


Fig. 5. Along-chain carbonyl values in beeswax-treated and untreated samples as indicators of cellulose oxidation. The horizontal line marks the average content of that type of carbonyls.

The CCOA fluorescence labeling approach, which allows differentiating between the origins of carbonyls (cf. data in Table 2) in cellulose, was now applied to analyze and compare the degradation pathways for historic Hanji with and without beeswax. The total number of carbonyl groups in the beeswax-treated samples increased significantly compared to the untreated ones. However, the number of along-chain carbonyls (=difference between total carbonyls and REGs) remained unchanged, and thus no significant oxidation compared to the untreated papers occurred: the increase in total carbonyls was due to the new reducing ends generated by chain cleavage. Fig. 5 gives a comparison of the values of carbonyls other than REGs (i.e., along-chain carbonyls) in beeswax-treated and untreated papers. The average value was $13 \mu\text{mol g}^{-1}$ for beeswax-treated and untreated Hanji. No significant oxidation was observed for either type of paper. The extent of oxidation for the beeswax-treated cellulose was similar to that of the papers without beeswax. Hence, the beeswax did not trigger oxidation reactions of the cellulosic paper matrix, although this oxidation had appeared as a plausible scenario: it would not be unreasonable to assume that autoxidation reactions of the lipidic moieties of the wax would cause concomitant oxidation of the cellulose matrix. With regard to the visual appearance, this seemed to be a quite surprising result. Only the volumes with additional damage patterns, such as those with brown or red stains or formation of fine powders, exhibited different, sometimes higher, carbonyl values, all the others offered carbonyl values that were merely attributable to newly generated reducing end groups caused by chain cleavage.

In summary, the molecular-level damage pattern in the beeswax-coated papers was characteristic of hydrolytic cellulose degradation without serious oxidation. Since the hydrolytic damage had been quite severe, we were hopeful to be able to address the question as to the agent triggering that hydrolysis.

3.3. Origin of the cellulose hydrolysis

Based on the GPC/labeling results, oxidative damage induced by beeswax components, beeswax degradation products or derived radical species was ruled out. This was in agreement with the observation that no significant concentrations of metal ions, which would be potent promoters of free-radical reactions, were found (Jo et al., 2007). The degradation was reflected by a loss in molar mass by cleavage of glycosidic bonds with simultaneous generation of novel REGs, which, in turn, caused the observed increase in the total carbonyl group content. The most prominent reason for hydrolytic degradation would be acidic constituents that

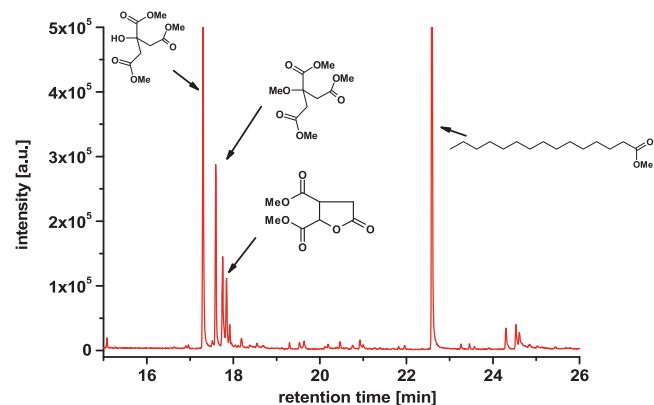


Fig. 6. Gas chromatogram of extracts of methylated beeswax-treated Hanji paper samples. The low-molar mass organic acids are metabolites from microorganisms.

trigger cleavage of glycosidic bonds. The beeswax used for coating contains some acidic compounds (Lattuati-Derieux, Egasse, Regert, Chung, & Lavédrine, 2009; Song et al., 2005), mainly long-chain acids, typically 10–14% in fresh beeswax. These components may induce an acidic environment in the beeswax-treated paper without being ventilated out during natural aging. However, the acidity of fatty acids is so low (pKa about 5) that it cannot account for such severe cellulose hydrolysis: other and more potent triggers would be needed.

Also the uronic acid contents of the polymeric materials, which were within the range of non-aged Hanji, could not be the cause of the hydrolysis. Carboxyls originate mainly from the acidic sugars in mucopolysaccharides-like additives (see footnote 3).

The aging of wax components might have generated acidic degradation products, such as short chain acids, hydroxy acids and keto acids typical of lipid peroxidation. Although their acidity is higher than that of fatty acids, they are rather volatile and their possibility to have a long-term impact on the cellulose matrix is rather limited. Moreover, it would be hard to explain why lipid peroxidation processes that produce sufficient acids to cause cellulose hydrolysis would not have affected the cellulose oxidatively. The extensive hydrolytic damage pattern observed would require the presence of rather strong acids or other similarly active hydrolysis catalysts.

Our initial working hypothesis suggested that degradation of beeswax components caused the formation of considerable amounts of acids capable of hydrolyzing cellulose. The characterization of extracts from the papers by high temperature-gas chromatography–mass spectrometry (HT-GC–MS) did not support this theory: a number of aldehydes and acids were found (Lattuati-Derieux et al., 2009), but their long-chain character and low concentration could not account for the observed strong hydrolysis of the paper.

In order to analyze non-extractable low- M_w acids, being tightly bound to the cellulose matrix by strong H-bond forces, we methylated a small paper fragment with diazomethane. The reaction proceeded neatly without leaving traces of the reagent. The corresponding methyl esters, now readily extractable, were then analyzed by GC–MS, see Fig. 6 for a representative chromatogram from such an analysis. The main peaks correspond to citric acid and the corresponding lactone from isocitric acid. Citric acid is not a compound that can result from degradation of cellulose, nor can it be derived from the beeswax in natural aging processes. Rather than that, it is a typical metabolite of microorganisms. Molds and other microorganisms well accept wax as their nutritional basis – commonly known, for instance, from waxed shoes that start to grow mold if not properly stored. The citric acid, which is stronger (pKa = 3.13) than long-chain fatty acids (pKa = 4.8) might well have

contributed to the hydrolytic damage of the paper. Noticeable acidic hydrolysis of cellulose starts around pH 3–4, but also long-term exposure to slightly higher pH will have a degrading effect. Cho, Kim, Jeong, Jo, and Lee (2009) identified microorganisms, such as *Biscogniauxia atropunctata*, *Aspergillus versicolor*, *Penicillium polonicum*, *Ceriporia lacerata*, *Irpex lacteus*, directly from beeswax treated papers of the annals. The observed pronounced degradation is highly likely to be caused by cellulolytic enzymes secreted by these different microorganisms that had grown on the wax as nutrient source. The damage pattern of cellulose exposed to cellulolytic enzymes is identical to that suffering acidic hydrolysis. It might be reasonably assumed that the hydrolytic action of the enzymes had been complemented or even potentiated by the acidic hydrolysis of the acidic metabolites, such as citric acid.

4. Conclusion

The present study provides a comprehensive analysis of celluloses from ancient Korean Hanji. The stability of Hanji celluloses compared to other papers of comparable age was superior. After almost 400 years of natural aging, the average molar mass was still in the range of about 900,000 g mol⁻¹, corresponding to a DP of 5500. This high molar mass was the major reason for durability and mechanical stability over time.

For the first time, the degradation mechanism of beeswax-treated ancient celluloses was unambiguously identified. Even though appearance and analogy considerations suggested oxidative damage, pure hydrolysis was determined as the underlying mechanism. The cause of this hydrolysis were microorganisms secreting cellulolytic enzymes – previously detected on similar samples – along with acidic metabolites of low volatility excreted from these microorganisms. Citric acid as the main metabolite was identified as a prominent constituent of organic extracts from methylated paper samples. No strong acids directly from the beeswax or from its oxidative degradation were present. The beeswax thus caused the degradation of the cellulose matrix by providing a source of carbon for cellulolytic microorganisms rather than by direct hydrolysis of the cellulose or by generating acidic species through autoxidation processes.

The absence of oxidative damage, i.e. along-chain carbonyl groups, will reduce the risk of damage during conservational treatment. Alkaline conditions, which are prohibitive for oxidatively damaged celluloses due to highly degradative beta-elimination reactions, would be well tolerated by the Hanji papers, which widens the scope of possible conservational treatment options. If the beeswax samples are kept under conditions not supportive of microorganisms, the risk of further degradation is minimized and the beeswax does not any longer pose a danger for the cellulosic paper matrix.

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References

- Black, R. M., & Muir, B. (2003). Derivatisation reactions in the chromatographic analysis of chemical warfare agents and their degradation products. *Journal of Chromatography A*, 1000(1/2), 253–281.
- Bohrn, R., Potthast, A., Rosenau, T., Sixta, H., & Kosma, P. (2005). Synthesis and testing of a novel fluorescence label for carboxyls in carbohydrates and celluloses. *Synlett*, 20, 3087–3090.
- Bohrn, R., Potthast, A., Schiehser, S., Rosenau, T., Sixta, H., & Kosma, P. (2006). The FDAM method: Determination of carboxyl profiles in cellulosic materials by combining group selective fluorescence labeling with GPC. *Biomacromolecules*, 7, 1743–1750.
- Cho, S. E., Kim, Y. T., Jeong, S. Y., Jo, B. M., & Lee, J. K. (2009). Cultural characteristics of fungal species associated with deterioration or foxing of paper and chemical removal. In *Proceedings of the spring conference of the Korea Technical Association of the Pulp and Paper Industry* (pp. 295–303). Taegu: Korea TAPPI. ISBN 1229-4012.
- Choi, T. H., Cho, N. S., Lee, S. H., & Oh, S. K. (2007). In morphological characteristics of paper mulberry bast from different areas in Korea. In *Proceedings of the spring conference of the Korea Technical Association of the Pulp and Paper Industry* (pp. 298–303). Chuncheon: Korea TAPPI. ISBN 1229-4012.
- Henniges, U., Bürger, U., Banik, G., Rosenau, T., & Potthast, A. (2006). Copper corrosion: Comparison between naturally aged papers and artificially aged model papers. *Macromolecular Symposia*, 244, 194–203.
- Henniges, U., Prohaska, T., Banik, G., & Potthast, A. (2006). A fluorescence labeling approach to assess the deterioration state of aged papers. *Cellulose*, 13, 421–428.
- Jeong, S. W., Cheon, J. G., & Park, C. W. (1991). *Munbangsawoo: Ink stick Research Report*. Seoul: National Folk Museum of Korea.
- Jeong, S. Y., Lee, H. Y., Chung, Y. J., Hong, J. K., & Eom, D. S. (2004). Investigation of conservation state on the waxed volumes of Annals of the Joseon Dynasty. *Korean Conservation Science Research*, 25, 119–132. ISBN 1976-3077.
- Jo, B. M. (2006). *Research report – The study of restoration technique of wax-treated volume for the Annals of the Joseon Dynasty (06C001J 6C001Y)*. Daejeon: National Research Institute of Cultural Heritage.
- Jo, B. M., Eum, T. J., Choi, T. H., Kim, H. J., Park, J. S., Shin, B. J., & Ahn, C. Y. (2007). *Research report – Study on the standardization of Indigenous handicraft manufacturing techniques*. Daejeon: National Research Institute of Cultural Heritage.
- Korean Culture and Information Service (KOCIS). (2009). *Korean Documents on UNESCO's Memory of the World Register*. Seoul: Ministry of Culture, Sports and Tourism of the Republic of Korea.
- Lattuat-Derieux, A., Egasse, C., Regert, M., Chung, Y.-J., & Lavédrine, B. (2009). Characterization and degradation pathways of ancient Korean waxed papers. *Journal of Cultural Heritage*, 10, 422–427.
- Potthast, A., Röhring, J., Rosenau, T., Borgards, A., Sixta, H., & Kosma, P. (2003). A novel method for the determination of carbonyl groups in celluloses by fluorescence labeling. 3. Monitoring oxidative processes. *Biomacromolecules*, 4, 743–749.
- Röhring, J., Potthast, A., Rosenau, T., Lange, T., Borgards, A., Sixta, H., & Kosma, P. (2001). Synthesis and testing of a novel fluorescence label for carbonyls in carbohydrates and celluloses. *Synlett*, 5, 682–684.
- Röhring, J., Potthast, A., Rosenau, T., Lange, T., Borgards, A., Sixta, H., & Kosma, P. (2002). A novel method for the determination of carbonyl groups in celluloses by fluorescence labeling. 2. Validation and applications. *Biomacromolecules*, 3, 969–975.
- Röhring, J., Potthast, A., Rosenau, T., Lange, T., Ebner, G., Sixta, H., & Kosma, P. (2002). A novel method for the determination of carbonyl groups in celluloses by fluorescence labeling. 1. Method development. *Biomacromolecules*, 3, 959–968.
- Song, K. J., Shin, B. J., Park, J. S., & Lee, I. S. (2005). *Investigation of conservation state on the Annals of the Joseon Dynasty (vol. I)*. Seoul National University Press: Seoul.