

Chemical Response of Hardwood Oligosaccharides as a Statistical Function of Isolation Protocol

LAN BAN,[†] XINSHENG CHAI,[†] JIQIANG GUO,[§] WEIPING BAN,[§] AND
 LUCIAN A. LUCIA^{*,#}

Institute of Paper Science and Technology, Georgia Institute of Technology, 500 Tenth Street Atlanta, Georgia 0332-0620; Dalian Polytechnic University, Dalian, China 116034; and Department of Wood and Paper Science, North Carolina State University, Campus Box 8005, Raleigh, North Carolina 26795-8005

Hardwoods contain a substantial amount of hemicellulose-type oligosaccharides, the chemical structures of which are typically categorized in the xylan saccharide class. The efficient and selective isolation of these hemicelluloses is regarded as one of the most critical obstacles to overcome for their eventual biomaterial and bioenergy utilization. Therefore, the objective of the current work was to perform a fundamental exploration of the function of extraction pH, temperature, and time on the final chemical properties of the extracted hemicelluloses. The extraction chemistries employed varied pH and showed that acidic conditions provided a higher extractive yield versus alkaline conditions. An alkaline environment gave higher yields than an acidic environment and also resulted in much higher lignin removal from wood, especially at high temperature. In general, control of the pH chemistry leads to a change in the carboxylic acid groups present in the extracts. When the extraction pH changed from acidic to alkaline, the total carboxylic acid group content in the hemicelluloses removed significantly increased due to more acetic acid group hydrolysis and increased lignin degradation.

KEYWORDS: Hardwoods; hemicelluloses; extraction; pH; temperature

INTRODUCTION

Plant biomass is the most abundant biomaterial available on earth. Due to its renewable and biodegradable character, it has received considerable interest as a resource to replace petroleum-based feedstocks. It is also starting to become important for providing a sustainable source of raw materials for chemical conversions analogous to petroleum-derived chemical conversions. In general, hemicelluloses are oligosaccharides that rank among the three most abundant natural materials. They exist in wood, annual grasses, agricultural residues, and crop byproducts. They occur in plant tissues in a relatively tight and complex chemical network that includes lignin and cellulose (the so-called lignocellulosic matrix); not surprisingly, therefore, it is very difficult to separate the hemicelluloses without significantly modifying their structure (1).

Nevertheless, different ways to separate hemicelluloses from the lignocellulosic matrix have been investigated that include physical methods such as steam treatment (2–4) and microwave irradiation (5, 6), wet chemical methods such as organosolv processes (7, 8), acid or alkali extraction, and enzyme

treatment (9, 10). Heat treatment is often combined with the addition of chemicals such as alkali, acid, or hydrogen peroxide, and the overall success of hemicellulose extraction is dictated not only by factors such as its solubility, but also by competing chemical reactions (11, 12).

Hardwoods are among the most hemicellulose-enriched biomass sources. The main hemicellulose component in hardwoods is O-acetylated 4-O-methylglucuronic acid xylan. Xylan is estimated to account for one-third of all renewable organic carbon available on earth (13). Many research efforts have shown the potential of xylans and their derivatives for various industrial and nonindustrial applications, such as chiral separations (14), blood cholesterol reduction (15), tablet disintegrations (16), HIV inhibition (17), and food additives (18). Although xylans have shown many important applications, the difficulty of their isolation has compromised the scope of their commercial applications. *Nevertheless, the effect of isolation protocols on the chemical properties xylan has surprisingly received little attention.* It is therefore the objective of this effort to provide fundamental data on the effect of extraction pH and temperature on the chemical features of hardwood hemicelluloses.

EXPERIMENTAL PROCEDURES

Materials. The wood chips of a representative hardwood species, birch, were obtained from the industrial supporting members of the

* Author to whom correspondence should be addressed (e-mail lucian.lucia@ncsu.edu).

[†] Georgia Institute of Technology.

[§] Dalian Polytechnic University.

[#] North Carolina State University.

Table 1. Variables and Range

factor	units	variable range
pH		2.0–12.0
extraction temperature	°C	80–160
extraction time	min	30–120

Table 2. Experimental Design

sample order	pH	temperature (°C)	time (min)
4	12	160	75
9	7	80	30
1	2	80	75
12	7	160	120
11	7	80	120
8	12	120	120
14	7	120	75
3	2	160	75
13	7	120	75
10	7	160	30
7	2	120	120
5	2	120	30
6	12	120	30
15	7	120	75
2	12	80	75

Institute of Paper Science and Technology Center at the Georgia Institute of Technology. The chips were screened to retain industrially relevant fractions having a length between 16 and 32 mm and thickness of <10 mm. The solid content of all wood chips was 90.81%, and all bark, knots, and other nonwoody components were removed to maintain as much chip morphological uniformity as possible for the extraction.

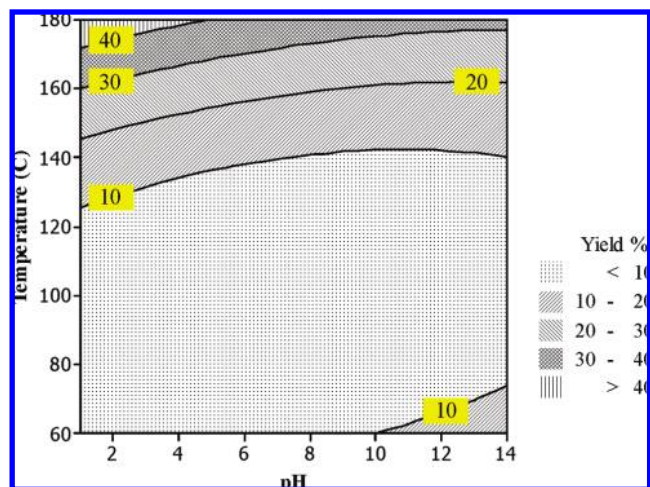
Extraction and Separation Processes. Extraction of wood chips was conducted on a multiunit stainless steel bomb digestive system. Six samples were treated simultaneously. Extraction procedures were controlled by the computer-programmed electric heater; temperature ramps were on the order of 1.5 °C/min. In general, the air-dried wood chips (equivalent to 50 g of oven-dried chips) were placed in each bomb, and the following extraction conditions were applied: ratio of extraction liquor to wood chips = 4:1; extraction temperature and time varied according to the experimental design.

In terms of designed pH conditions, an extraction liquor for each sample was prepared. Chemicals were purchased from Sigma-Aldrich, and reagent grade and deionized water were used for all experimental processes. Sodium hydroxide was used to prepare the alkaline extraction liquor, hydrogen chloride was used to prepare the acidic extraction liquor, and deionized water was used for neutral conditions. pH in all experiments refers to the initial pH in the extraction process.

When the extraction was completed, the extraction liquor was drained and the pH adjusted to neutral. A 4-fold excess (v/v) of ethanol (95% purity) based on the original liquor volume was added to the cooling extraction liquor. The resultant diluted extraction liquor was allowed to stand for 48 h to allow precipitation. All resultant precipitated extracts were filtered by a cheesecloth; the first filtrate liquor was allowed to stand overnight, and it was filtered again to ensure maximum yield. All filtered extracts were thoroughly rinsed using ethanol (75% purity). The extracts were then vacuum-dried and refrigerated.

Analytical Methods. Extract yield was calculated by the weight loss of wood chips after extraction. The extracts were loaded in an autoclave and hydrolyzed with 4% H₂SO₄ at 120 °C for 1 h. Afterward, the monosaccharides were analyzed using high-pressure anion exchange chromatography (HPAEC). Carboxylic acid group content in wood extracts was measured using headspace gas chromatography (HS-GC) as described in the literature (19). The lignin content in the process liquors was analyzed by UV spectrophotometry (20).

D-Optimization Design. A D-optimization design (Box–Behnken) was applied to statistically analyze the current work. Three variables,

**Figure 1.** Interactive effect of temperature and pH on total yield (75 min).**Table 3.** Sugar Composition in Wood and Extracts^a

sample	arabinan (%)	galactan (%)	glucan (%)	xylan (%)	mannan (%)	lignin (%)
birch wood	0.58	0.38	54.79	11.26	4.97	28.02
extracts with pH 2	3.25	5.83	8.43	71.31	4.54	6.64
extracts with pH 12	4.13	2.90	3.32	76.41	1.71	11.53
extracts with pH 7	3.98	10.93	5.02	66.28	3.87	8.36

^a Extraction conditions: 120 °C; 120 min; liquor ratio to wood, 4.

extraction pH, temperature, and time, were selected as the extraction process factors. The variables' range and experimental design are shown in **Tables 1** and **2**. As part of the experimental statistical design, all experimental points were duplicated to ensure that the relative standard deviation was >95%.

RESULTS AND DISCUSSION

Chemical Nature of Extracts. The typical extract composition from the birch wood chips is illustrated in **Table 3**. Overall, the sugar compositions obtained from either alkaline or acidic extraction are similar. Not surprisingly, xylan is the predominant component in the hemicelluloses obtained. The difference between the two hemicellulose extracts is that those from the alkaline process contain more lignin, whereas the extracts from the acidic process contain other sugar components in addition to xylan. This difference will be discussed later.

General Influence of Extraction Process Variables on Hemicelluloses Yield. The fluctuation in the hemicellulose yield over a broad pH range is shown in **Figure 1**. Generally, sufficient energy was critical for hemicellulose recovery. When the extraction temperature was below 120 °C, no substantial extraction was achieved despite the pH. However, higher temperatures always led to a higher extract yield. Moreover, to acquire the same extraction yield, alkaline extraction required a higher temperature than the complementary acidic process, which indicated that acidic pH regimens are more favorable for hemicelluloses isolation.

Figures 2–4 demonstrate the effects of different pH regimens on extract yield. During neutral pH water extraction, temperature is the critical variable for extraction yield. Below 120 °C, no relevant levels of extracts were obtained; only when the temperature reached 130–140 °C were extracts obtained, albeit

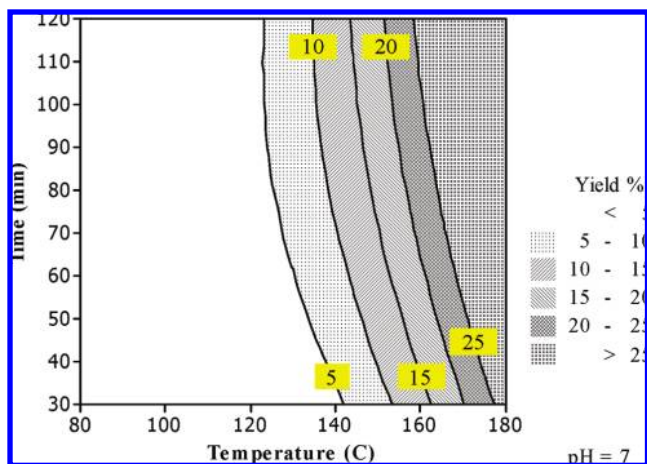


Figure 2. Interactive effect of time and temperature on total yield (pH 7).

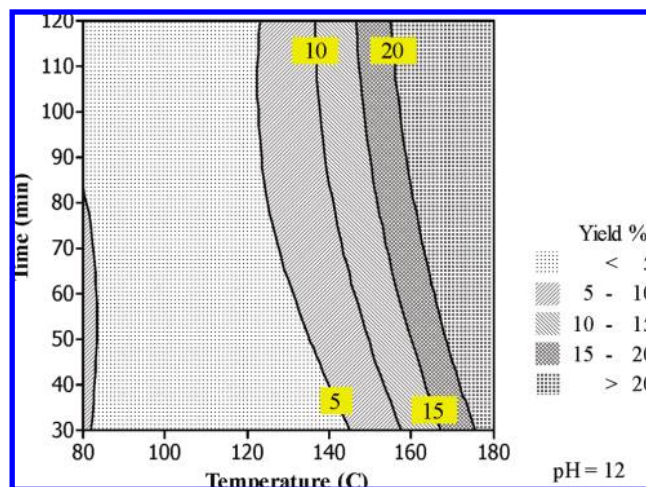


Figure 4. Interactive effect of time and temperature on total yield (pH 12).

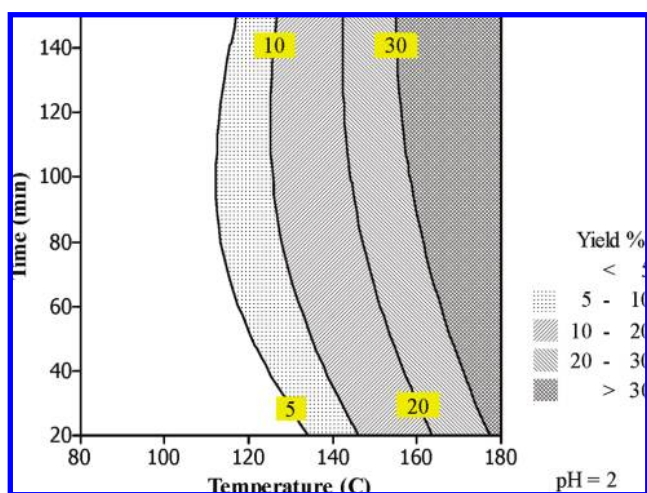


Figure 3. Interactive effect of time and temperature on total yield (pH 2).

in small amounts; above 150 °C, the extraction quantities were more abundant. An approximately 5% increase in the extraction yield was observed for every 10 °C increase in temperature. On the other hand, compared to extraction temperature, extraction time does not exert an important effect on extraction yield. Although extending the extraction period contributes to minimal yield gains at the same temperature, there appears to be no strong interactive effect between temperature and time on yield; in other words, an extension of the extraction time does not compensate for yield loss sustained by lower temperatures, especially at long extraction times.

Likewise to neutral pH values, acidic regimens needed higher extraction temperatures to acquire significant amounts of hemicelluloses, as illustrated in **Figure 3**. However, temperatures lower by approximately 10–20 °C can achieve the same extraction yield in the acidic regimens compared to the neutral case. Overall, acidic regimens provided a more favorable environment for hemicelluloses extraction, that is, 5–10 absolute percentage points higher extraction yield.

Compared to neutral extraction, the predominant effect of alkaline regimens on extract yield is higher hemicellulose extractability. As **Figure 4** illustrates, a small amount of extracts was dissolved at low temperature, believed to be the low molecular weight fraction that is easy to remove in alkaline environments (21). However, to attain substantial extraction, it was necessary to raise the temperature to >100 °C.

To summarize, the common features of the effects of different pH regimens on extract yield are as follows: temperature is the key factor for extraction at all pH ranges; at the current pH ranges, extraction time does not significantly affect extract yield, and there is no strong interaction with temperature; finally, an acidic environment provides the highest yield, whereas an alkaline environment requires higher temperatures to achieve a similar yield.

Solubility of Extracts. The extracts from the birch wood chips contained water-soluble and water-insoluble components. Due to variable extraction conditions, the ratio of the water-soluble fraction to the insoluble fraction in the extracts varied. The soluble and insoluble fractions show several differences in sugar composition and may therefore have different final applications (22).

In the case of the neutral extraction, the water-soluble fraction clearly depended on the extraction temperature and time as shown in **Figure 5**. An increase in either temperature or time led to an increase in the soluble fraction. Overall, over most of the experimental range, the water-soluble fraction in the water extracts is >50% of the total composition. However, to acquire >90% for water-soluble extracts requires temperatures >140 °C or longer extraction time (>2 h).

As discussed above, a higher extract yield may be achieved under acidic extraction conditions. However, the water-soluble fraction in the acidic extracts was less than that of the neutral extraction as illustrated in **Figure 6**. The soluble fraction of the acidic extracts was generally <60%, unless severe extraction conditions were employed (160 °C for 2 h).

As opposed to acidic extraction, alkaline extraction resulted in more soluble components as shown in **Figure 7**. Under alkaline conditions, the availability of the water-soluble fraction mainly depended on the extraction temperature; above 150 °C, most of the extracts were water-soluble. This result indicated that the alkaline regimen provides the best environment for obtaining water-soluble extracts. One of the reasons might be due to the peeling reaction that occurs under alkaline conditions; the other might be because of acetyl group degradation in the hemicellulose polymer chain. Nevertheless, both pathways either increase monosaccharide content or enhance oligosaccharide hydrophilicity, which increase solubility.

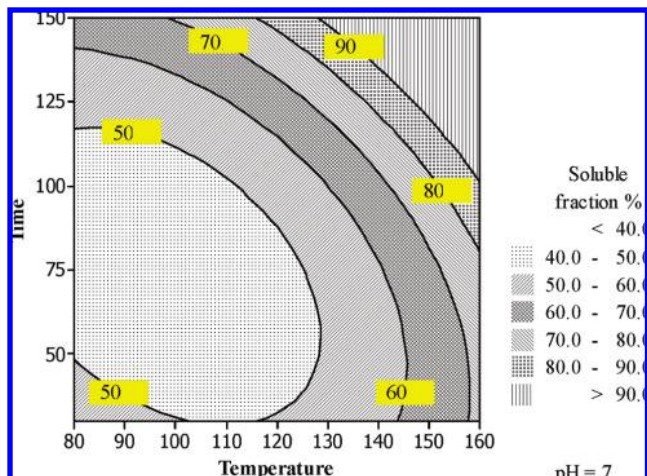


Figure 5. Soluble extract fraction as a function of time and temperature (pH 7).

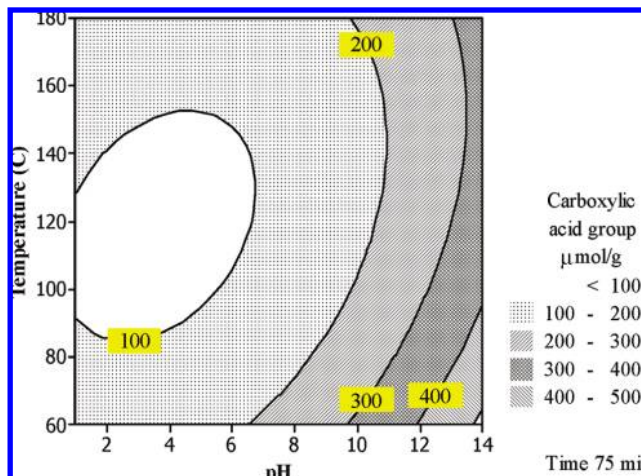


Figure 8. Interactive effect of pH and temperature on carboxylic acid content.

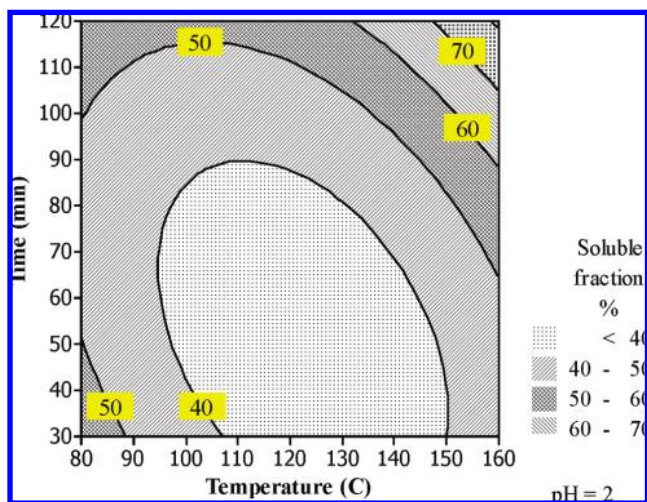


Figure 6. Soluble extract fraction as a function of time and temperature (pH 2).

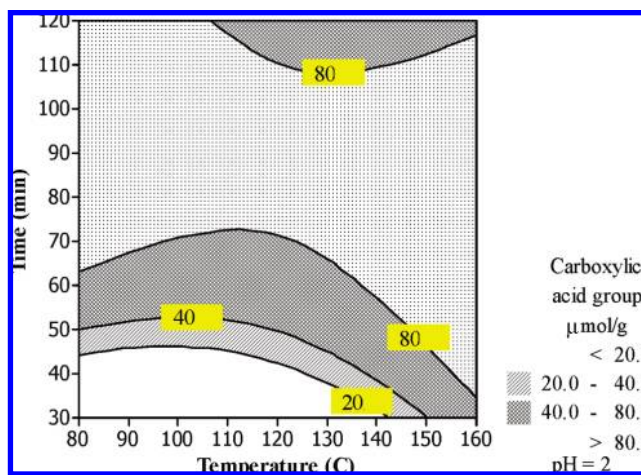


Figure 9. Interactive effect of time and temperature on carboxylic acid group content (pH 2).

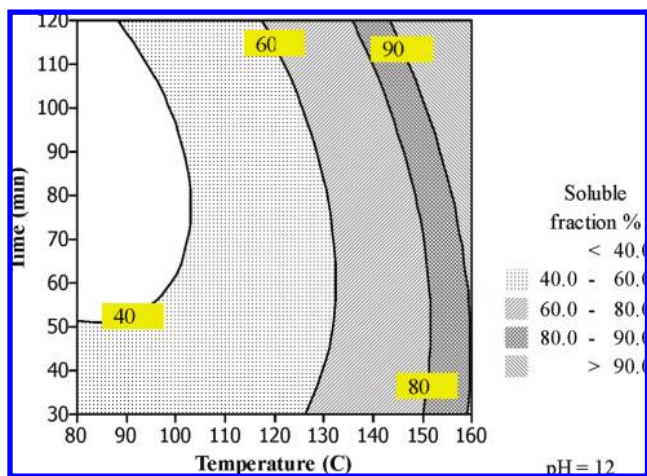


Figure 7. Soluble extract fraction as a function of time and temperature (pH 12).

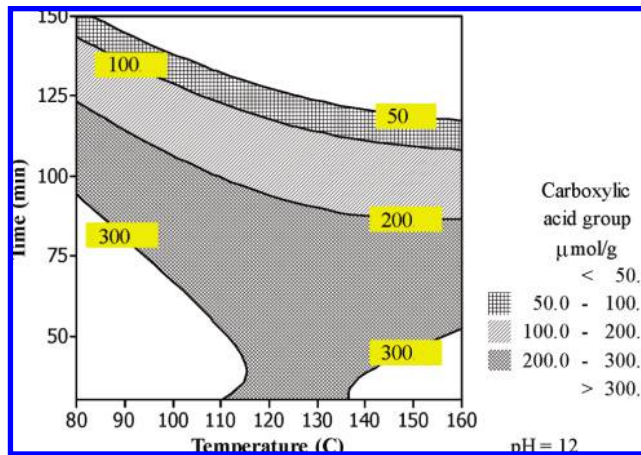


Figure 10. Interactive effect of time and temperature on carboxylic acid group content (pH 12).

In conclusion, when the extraction pH changes from acidic to alkaline, the soluble fraction in the extracts increases. A higher temperature and longer time always lead to higher water-soluble fractions.

Impact of Extraction Environment on Carboxylic Acid Group Content. In general, carboxylic acid groups of the

extracts from hardwood originate from three sources: native carboxylic acid functionalities such as glucuronic acid branched from the xylan backbone; the hydrolysis reaction, such as acetic acid, for example, hexenuronic acid; or nonhemicellulose substances such as lignin. The amount of carboxylic acid groups in the extracts affects the chemical properties and application of the extracted hemicelluloses. Changes in the carboxylic acid

groups in the extracts due to extraction conditions are shown in **Figures 8–10**. Overall, the amount of carboxylic acid group of the extracts increased when the pH changed from acidic to alkaline (as shown in **Figure 8**). The extracts from the acidic extraction contain fewer carboxylic acid groups, usually $< 100 \mu\text{mol/g}$. Alkaline conditions, however, contribute to many more carboxylic acid groups in the extracts, $300 \mu\text{mol/g}$ or more. On the basis of the same wood species, it is reasonable to conclude that the difference in carboxylic group content may be attributed to the different pH regimens.

In native hardwood, the majority of the carboxyl groups in hemicelluloses are methylglucuronic acid groups that are present in the xylan backbone. Xylans, mainly those that are more highly substituted with uronic acid groups, gradually undergo structural changes in alkaline or acidic solutions.

Hexenuronic acid (HexA) in hardwood is substituted xylans, 4-deoxy- β -L-threo-4-enopyranosyluronic acid (23). This uronic acid does not exist in native wood, but is formed during alkaline treatments such as kraft pulping in which 4-*O*-methylglucuronic acid is formed from the β -elimination of a methanol molecule (24). During the alkaline process, 4-*O*-methyl-D-glucuronic acid units, which exist in xylan, are to a great extent converted to unsaturated hexenuronic acid groups (23). However, although the new carboxylic acid groups are generated, it does not affect the total carboxylic acid content because HexA is converted from methylglucuronic acid, which already existed on the hemicellulose backbone.

The new acid groups are mainly liberated from the hydrolysis of ester linkages, which principally generate acetic acid. The hydrolysis of the ester bonds can occur in either alkaline or acidic conditions. However, it proceeds much more rapidly under alkaline conditions than under acidic conditions, especially at high temperatures (25). Hence, it is reasonable to observe more carboxylic acid groups in the extracts from alkaline extraction.

Also, lignin degradation byproduct contain more acidic groups than the native lignin macromolecule (26). Whereas the alkaline regimens demonstrated a higher lignin fraction removal than the acidic extraction process, these lignin fractions provide more acid groups.

Comparison of carboxylic acid contents in acidic and alkaline environments is shown in **Figures 9 and 10**. The change in carboxylic acid content mainly depended on extraction time. The highest carboxylic acid content was achieved within a specific extraction period; a shorter or longer time would result in a decrease in the carboxylic acid content. Extraction temperature shows only a minor effect on carboxylic acid content. These results demonstrate that an alkaline environment results in more acid groups.

Impact of Extraction Environment on Lignin Extraction.

Because hemicelluloses in the plant cell wall are surrounded and connected to lignin, lignin fractions are unavoidably dissolved in the extraction of hemicelluloses. The amount of lignin removal mainly depends on the extraction conditions. **Figures 11–13** demonstrate how extraction conditions influence lignin content.

When the extraction was carried out at neutral pH, there was no significant lignin removed from the wood chips at low temperatures and short times. Raising the temperature or extending the extraction time always led to an increase in lignin dissolution as demonstrated in **Figure 11**. To ensure lower levels of lignin in the extracts, it was necessary to operate at lower

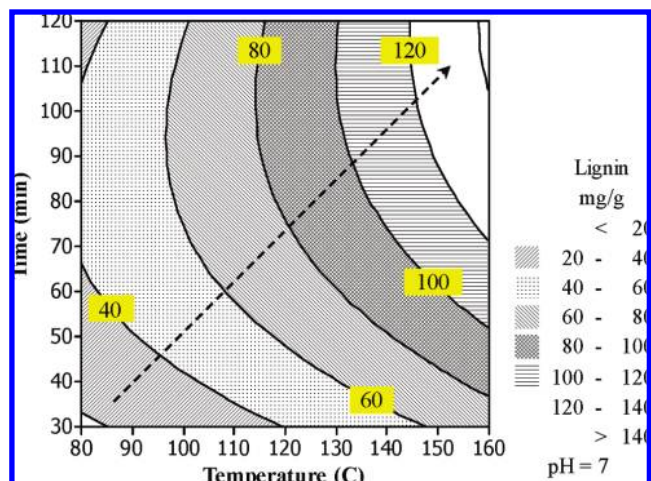


Figure 11. Interactive effect of temperature and time on lignin content in extracts (pH 7).

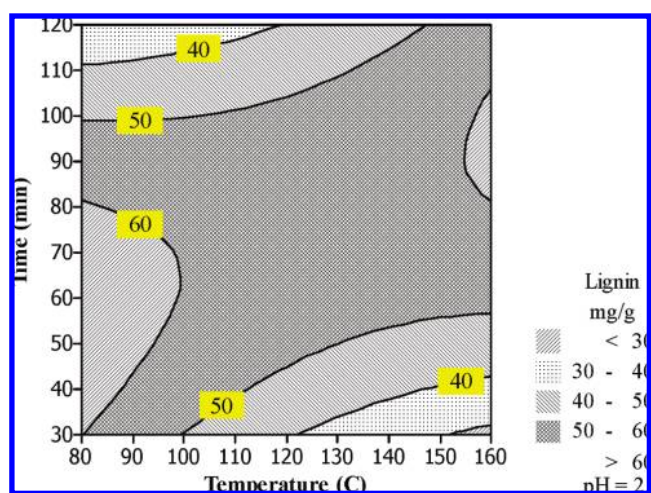


Figure 12. Interactive effect of temperature and time on lignin content in extracts (pH 2).

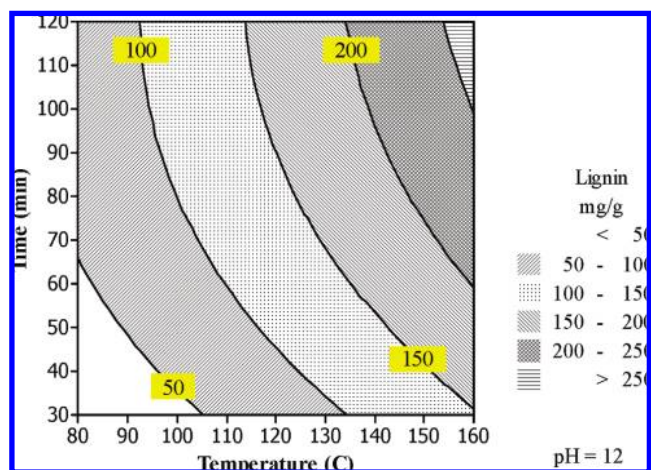


Figure 13. Interactive effect of temperature and time on lignin content in extracts (pH 12).

temperatures. Furthermore, under a neutral pH, the lignin extraction was almost completed in 80 min, but this was a very limited fraction of the total. The result indicates that a higher temperature is the primary driver to dissolve increasing amounts of lignin, yet no significant delignification occurs during the water extraction. On the other hand, high temperatures function

to increase the yield of hemicelluloses extracts, which indicates that there is a minimum temperature for acquiring significant extraction yields. However, due to an interactive effect between temperature and time, extensions in extraction time can compensate for a decrease in temperature. To achieve the same extraction yield, applying lower temperatures and longer times can reduce lignin content in the extracts.

In addition to temperature, extraction pH also showed significant effects on lignin extraction. Between 100 and 120 mg/g of lignin content was observed in the extracts at the neutral pH conditions, which equates to approximately 7–8% of total lignin in the original wood. Compared to neutral water extraction, lignin dissolution under acidic extraction exhibits a unique feature: as **Figure 12** illustrates, at the highest temperatures, the lignin content remained low, 50–60 mg/g, approximately 5–6% of the original lignin content in wood, whereas prolonging the extraction period did not lead to an increase in lignin dissolution. The results indicate two facts: first, an acidic environment leads to less lignin extraction; second, under acidic conditions, lignin dissolution is insensitive to temperature change, that is, higher temperature does not correlate to higher lignin removal.

In contrast, alkaline extraction results in significant lignin dissolution during hemicelluloses extraction, as observed in **Figure 13**. An alkaline environment exerts a strong influence on lignin extraction. Indeed, the lignin fraction in the extracts uniformly increased with increasing extraction temperature. At the same temperature and time, 50–100% more lignin was extracted under alkaline conditions relative to neutral conditions. As much as 15–20% of the original lignin in wood can be extracted under strong alkaline conditions, almost a 4-fold greater lignin level than observed in the acidic conditions, even when performed at higher temperatures.

In summary, the main effects of modifying the extraction environment on lignin extraction are as follows: a change from acidic to alkaline conditions caused the lignin fraction in the extracts to significantly increase, and in the change from acidic to alkaline conditions, the temperature has a pronounced contribution to extraction.

In conclusion, the extracted hemicelluloses from birch wood obtained under various pH conditions resulted in different extractable yields and chemical properties of the extracts.

Generally, an acidic environment provides a higher extract yield compared to alkaline conditions. On the other hand, an alkaline environment provides a greater water-soluble fraction than observed under acidic conditions. Furthermore, alkaline extraction results in much higher lignin removal from wood, compared to acidic environments, especially when higher extraction temperatures are applied.

When the extraction pH environment was varied, differences in the carboxylic acid content of the extracts were observed. When the extraction pH changed from acidic to alkaline, the total carboxylic acid group content in the hemicellulose extracts significantly increased, likely due to increased acetic group hydrolysis and more lignin degradation.

Finally, extraction temperature plays a key role in extract yield. The extract yield always increased with higher temperature, either for total yield or for soluble fraction yield. An increase in extraction temperature also increased lignin dissolution, especially under alkaline conditions. In contrast, the effect of temperature on acid group content in the extracts is not significant.

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