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Understanding Wood Polysaccharide Depolymerization and Denaturation Under Different Toasting Conditions Through Analysis of Sugars Extracted from French Oak Chips

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Abstract: By analyzing the sugars extracted from oak chips toasted at various temperatures (180 to 280 °C) for various durations (10 to 30 min) in a model wine, we examined how wood polysaccharides are affected by toasting. The responses induced by toasting significantly differed among the major sugars constituting the wood. The main components of wood polysaccharides—glucose, arabinose, galactose, and xylose—were analyzed, and the results showed that galactose had the highest extraction amounts at around 220 °C of toasting, xylose at around 240 °C, and glucose at around 280 °C. On the other hand, the extraction amounts decreased with longer toasting durations. These results suggest that wood polysaccharides undergo temperature-dependent depolymerization while simultaneously undergoing denaturation. In addition, these depolymerization reactions tended to shift towards lower temperatures with longer toasting durations. The results of this study elucidate the chemical changes that occur within the wood during the toasting of oak chips and highlight the importance of the relationship between toasting temperature and duration. Additionally, this study demonstrated that by using the sugars extracted from oak chips as indicators, it is possible to partially visualize the reactions that occur within oak chips during toasting.

Keywords: oak chips; *Quercus petraea*; polysaccharides; toasting; wine

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1. Introduction

Barrels used for wine have various functions, including acting as containers, imparting flavors, and aging wine through oxygen permeability [1]. However, in the present day, with the development of varietal containers, such as stainless-steel ones, the primary role of barrels in winemaking is largely the addition of flavor. Specifically, aromatic compounds and phenolic compounds such as tannins present in barrel wood dissolve into wine, enhancing its flavor complexity and depth [2]. On the other hand, as the importance of the barrel's role as a container has diminished, alternatives such as oak chips and staves are increasingly used to impart flavor to wine [3,4].

Barrel woods used for wine undergo a process known as "toasting" during their production. Toasting induces various reactions within wood, significantly impacting the compounds derived from barrel woods. The barrel-derived compounds that influence the flavor of wine can be broadly classified into volatile and non-volatile compounds. Volatile compounds derived from barrel wood have a significant impact on a wine's aroma. For instance, key aromatic compounds, including vanillin (vanilla), furfural (caramel), and oak lactone (coconuts), are produced through pyrolysis reactions within wood, which are

induced by the toasting of barrel wood [5,6]. On the other hand, the major non-volatile compounds extracted from barrel wood are phenolic compounds. It has been reported that ellagitannins, the primary components of phenolic compounds derived from barrel wood, significantly influence the bitterness, astringency, and color stability of wine [7,8]. Ellagitannin contents are significantly affected by toasting, with higher toasting levels reported to reduce the amount of ellagitannins extracted [2,9,10].

Recently, we reported that certain amounts of sugars were extracted when French oak chips were added to a wine-like solution (model wine) [11]. The concentration of the extracted sugars was relatively low, making it unlikely that they would directly influence the sweetness of the wine. On the other hand, it was considered that these sugars could reach considerable concentrations when the surfaces of barrels or chips are moistened during washing processes at wineries and may lead to microbial contamination. In our previous experiments, the toasting duration of oak chips was fixed at 10 min, while the toasting temperature was varied between 180 and 280 °C [11]. As a result of the study, both monosaccharides and polysaccharides were extracted from the oak chips, and their composition varied significantly depending on the toasting temperature. This indicates that not only aromatic compounds and phenolic compounds, but also sugars extracted from the barrel are greatly influenced by toasting temperatures.

The primary components of wood are polysaccharides, such as cellulose and hemicellulose. Cellulose is composed of glucose units linked by β-1,4-glycosidic bonds, forming a crystalline structure. On the other hand, hemicellulose has an amorphous structure and is a heterogeneous polymer composed of pentoses (xylose (Xyl) and arabinose (Ara)) and hexoses (glucose (Glc) and galactose (Gal)) [12]. Research in the field of wood science has reported that these wood polysaccharides begin to be affected by heat treatment at around 180 °C, with significant changes occurring around 250 °C, which aligns with the temperature range typically used for toasting wine barrels and chips [2,13]. In our previous report, monosaccharides such as Glc, Ara, Xyl, and Gal, which are components of wood polysaccharides, were detected either as free sugars or as part of polysaccharides. The monosaccharides significantly decreased with the increase in toasting temperature, suggesting that these sugars underwent thermal denaturation and were transformed into other compounds [14]. Since some of the degraded sugars are thought to convert into aromatic compounds, this reaction is considered highly significant in the toasting of barrel wood. On the other hand, the concentration of polysaccharides fluctuated under different toasting temperatures, leading us to hypothesize that two reactions occur simultaneously: one is a reaction in which the polysaccharides in wood are depolymerized by heat, making them more soluble, and the other is a reaction in which sugars are denatured by heat to become different compounds.

Cooperage companies manage toasting levels using terms such as “light toast”, “medium toast”, and “heavy toast”, but no standardized criteria have been established across the industry [2]. Generally, barrel toasting is conducted based on the empirical knowledge of each cooperage, which can lead to variations between manufacturers [15]. Furthermore, toasting is performed manually by cooperages, relying on indicators such as changes in the color of the wood surface and the temperature of the barrel surface, making it unsurprising that variations can occur even within the same cooperage [16].

The toasting of wine barrel wood is controlled by two parameters: temperature and duration, and it is well known that variations in these parameters significantly affect the compounds extracted from the wood [2,17,18]. Therefore, it is crucial to understand the internal reactions that occur within the wood as a result of differences in toasting temperature and duration. The aim of this study was to analyze the sugars extracted from oak chips in a model wine solution and thereby visualize the chemical changes that occur within the oak wood by varying the parameters of toasting temperature and duration.

2. Materials and Methods

2.1. Reagents and Chemicals

D-(+)-galactose (Gal), L-(+)-arabinose (Ara), D-glucose (Glc), acetonitrile (HPLC grade), and trifluoroacetic acid (TFA) were obtained from Fujifilm Wako Pure Chemical Industries (Osaka, Japan). D-(+)-Xylose (Xyl) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). An ABEE labeling kit for sugar derivatization was purchased from MGC Woodchem Corp. (Tokyo, Japan). Other reagents used were of analytical grade.

2.2. Preparation of Oak Chips

The staves were derived from untoasted French oak heartwood (*Quercus petraea*) sourced from France. Approximately 30 kg of oak staves was chipped using a woodchipper (KCM181D; Kioritz Corp., Tokyo, Japan) and mixed well. In this experiment, oak chips with dimensions of approximately 1.5 × 1.0 × 0.1 cm (width × length × thickness) were used. Oak chips were toasted under various conditions using a muffle furnace (Yamato FP313; Tokyo, Japan). The muffle furnace was preheated to the specified temperature before toasting the oak chips. After the designated heating time, the chips were immediately removed from the furnace and cooled at room temperature. The toasting temperatures were adjusted to 180, 200, 220, 240, 260, and 280 °C, and the toasting durations were set at 10, 20, and 30 min. The toasting of the oak chips was conducted under atmospheric pressure and in the presence of air.

2.3. Measurement of Oak Chip Surface Color

A spectrophotometer (CM-5; Konica Minolta, Inc., Osaka, Japan) was used to measure changes in the color of the French oak chip samples caused by toasting. The color of the chip surfaces was measured based on the CIE Lab* color system, yielding values for L* (luminosity), a* (red/green coordinate), and b* (yellow/blue coordinate). Measurements were taken under the conditions of a D65 light source, a 3 mm sensor aperture diameter, and a 10° observation angle. The measurement values for each sample were obtained from the average of ten randomly selected oak chips.

2.4. Measurement of Weight Loss and Moisture Contents of Oak Chips

For measurement of weight loss, about 30 g of chips was weighed before the toasting treatment. After the toasting treatment, the toasted oak chips were allowed to stand in a room (25 °C) and weighed several times until the weight remained constant. Weight loss from the initial weight was calculated.

The moisture content of the oak chips was determined according to the Japanese Industrial Standard (JIS Z2101: 2009) [19]. Briefly, the initial weight of the oak chips was measured before drying. The samples were then dried in a muffle furnace at 103 °C until a constant mass was achieved. After drying, the oak chips were cooled at room temperature in a desiccator, and the final mass was measured to calculate the moisture content of the oak chips. All experiments were conducted in five replications.

2.5. Extraction Experiments on Oak Wood Sugars from Model Wine Solutions

The model wine solutions used in this study contained 5 g/L potassium tartrate and 12% (*v*/*v*) ethanol, with the pH adjusted to 3.6 using hydrochloric acid. Oak chips (6 g) were added to 1 L of model wine solution, which was placed in glass bottles with the headspace purged using N_2 gas [20]. The extraction experiments were conducted at 25 °C in the dark, with the bottles shaken at 80 rpm using a shaker (BW400; Yamato Scientific Corporation, Tokyo, Japan). After 21 days, the oak chips were removed, and the remaining liquid was used for sugar measurements. All extraction experiments were performed in triplicate.

2.6. Determination of Neutral Sugars

Neutral sugars were quantified using the phenol–sulfuric acid method as described by Dubois et al. [21]. The amounts of neutral sugars were expressed as xylose equivalents. All measurements were performed in triplicate.

2.7. HPLC Analysis of Sugars

The composition of sugars was determined using HPLC as described in a previous report [11]. Total sugar composition was analyzed by hydrolyzing samples with 8 M TFA and using an ABEE labeling kit (MGG Woodchem Corporation, Tokyo, Japan). The monosaccharide composition was analyzed by the same method, excluding the acid hydrolysis step. The amount of polymeric sugars was determined by calculating the difference between the monosaccharide content and the total sugar content, expressed as polysaccharides hereafter, though it may have contained small polymers known as oligomers. The HPLC system was equipped with an autosampler (AS-2055 Plus), a pump (PU-2089 Plus), a column oven (CO-2065 Plus), and a UV detector (UV-2075 Plus), which were all obtained from JASCO Corp. (Tokyo, Japan). The sample was filtered through a 0.45 μm PTFE filter, and a 10 μL aliquot was injected into a column (Honenpak C18, 75 × 4.6 mm i.d.; MGC Woodchem Corporation, Tokyo, Japan). The separation was carried out using the method provided by the manufacturer of the ABEE labeling kit. Briefly, the column oven temperature was set to 30 °C, and detection was performed at 305 nm. The flow rate was 1.0 mL/min. The eluent used for the separation consisted of boric acid buffer–acetonitrile (93/7, *v*/*v*, A eluent) and 0.02% (*v*/*v*) trifluoroacetic acid–acetonitrile (50/50, *v*/*v*, B eluent). The total run time was 65 min. The gradient system was as follows: 0–45 min, 0% B; 45–50 min, 100% B; 50–65 min, 0% B. The sugars in the samples were quantified using calibration curves prepared with standard solutions of four neutral sugars (Gal, Glc, Ara, and Xyl). All measurements were performed in triplicate.

2.8. Statistical Analysis

Tukey's honestly significant difference test was used to compare samples across groups. These statistical analyses were performed by JMP® Pro ver. 17.2.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Change in Oak Chip Color by Toasting

Figure 1 shows the color changes of oak chips under each toasting condition, as well as those of the untoasted chips (Control). Table 1 shows the measurement results of the color differences (L*, a*, and b*) on the surfaces of the toasted chips.

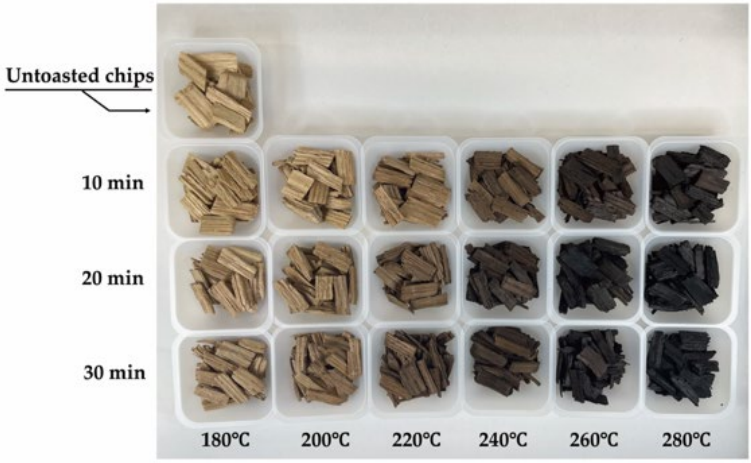

Figure 1. Changes in French oak chip surfaces caused by toasting.

Table 1. Color parameters of French oak chips toasted with different conditions.

Temperature	Duration	L*	a*	b*
Untoasted		66.2 ± 1.46	7.40 ± 0.73	21.9 ± 1.18
180 °C	10 min	62.2 ± 1.74 aA	8.53 ± 0.57 aA	23.2 ± 0.61 aA
200 °C		60.7 ± 1.97 aA	8.30 ± 0.48 aA	23.0 ± 0.50 aA
220 °C		54.1 ± 2.89 bA	8.98 ± 0.57 aA	20.9 ± 1.10 bA
240 °C		38.5 ± 2.40 cA	6.89 ± 0.73 bA	12.9 ± 1.66 cA
260 °C		28.6 ± 1.61 dA	4.65 ± 0.86 cA	7.5 ± 1.68 dA
280 °C		25.9 ± 1.31 eA	2.26 ± 0.63 dA	3.2 ± 1.05 fA
180 °C	20 min	61.2 ± 3.10 aA	8.00 ± 1.06 aA	21.7 ± 0.64 aB
200 °C		55.1 ± 1.94 bB	8.52 ± 0.46 aA	21.2 ± 1.01 aB
220 °C		42.6 ± 2.96 cB	7.93 ± 0.65 aB	15.1 ± 1.68 bB
240 °C		31.8 ± 2.07 dB	4.84 ± 0.61 bB	8.0 ± 1.36 cB
260 °C		26.5 ± 1.39 eB	2.52 ± 1.11 cB	3.6 ± 1.61 dC
280 °C		25.6 ± 1.25 eA	0.80 ± 0.21 dB	0.8 ± 0.26 eB
180 °C	30 min	60.0 ± 2.34 aA	8.10 ± 0.90 aA	21.3 ± 0.72 aB
200 °C		51.5 ± 2.02 bC	8.66 ± 0.33 abA	19.4 ± 1.01 bC
220 °C		38.5 ± 1.96 cC	7.48 ± 0.64 bB	13.6 ± 1.61 cB
240 °C		32.1 ± 1.54 dB	4.74 ± 0.57 cB	7.4 ± 1.72 dB
260 °C		28.1 ± 1.40 eA	4.09 ± 0.67 dA	5.8 ± 1.28 eB
280 °C		24.8 ± 1.15 fA	0.89 ± 0.20 eB	1.2 ± 0.16 fB

Different lowercase letters indicate the differences between the toasting temperatures for the same duration of toasting ($p < 0.05$). Different capital letters indicate the differences between the different durations of toasting at the same temperature ($p < 0.05$).

It was confirmed that as the toasting temperature of the oak chips increased, the surface color of the oak chips became significantly darker compared to the untreated chips (Figure 1). In addition, it was confirmed that as the toasting duration increased, the color of the chips darkened. The measurement of the oak chip color parameters revealed that as the toasting temperature increased, the values of the color differences (L*, a*, and b*) decreased. Additionally, when the toasting temperature was the same and the toasting duration was extended, a trend of decreasing color parameter values was observed. However, in the cases of 20 and 30 min, there were instances where the color difference values were smaller at 20 min.

3.2. Weight Loss of Oak Chips Due to Toasting

Weight loss due to the toasting treatment was calculated from the weights of oak chips before and after toasting, as shown in Figure 2.

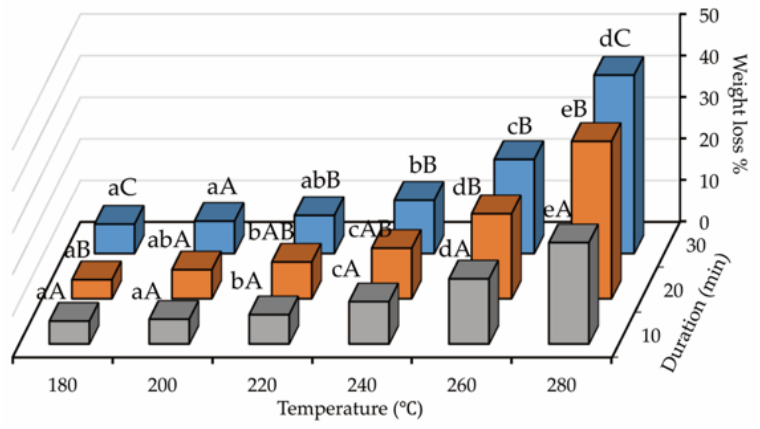

Figure 2. Weight loss of oak chips after toasting. Different lowercase letters indicate the differences between the toasting temperatures for the same duration of toasting ($p < 0.05$). Different capital letters indicate the differences between the different durations of toasting at the same temperature ($p < 0.05$).

The higher the toasting temperature applied to the oak chips, the greater the weight loss observed. Additionally, the longer the toasting duration, the more significant the weight loss. Notably, when the toasting temperature exceeded 260 °C and the toasting duration exceeded 20 min, substantial weight loss was observed, with a 43% weight loss observed at 280 °C after 30 min of toasting.

3.3. Determination of Neutral Sugar Contents

Neutral sugars extracted from the oak chips in the model wine solutions sampled on day 21 were determined (Figure 3). The results of the following experiment were calculated by taking into account the weight loss and converting the sugar amounts to the amount per weight of the chips before toasting.

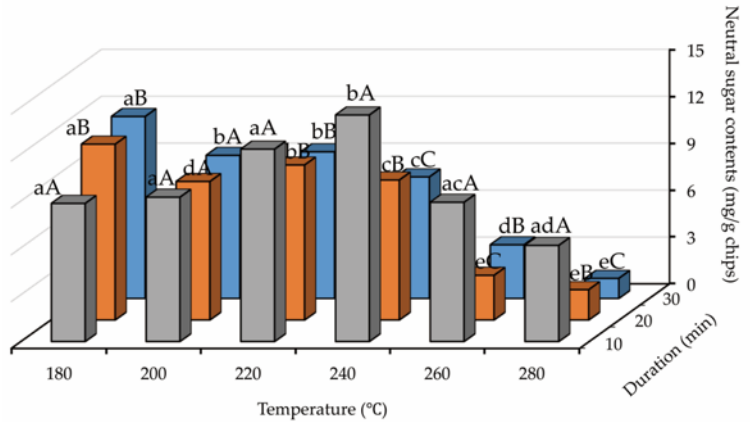

Figure 3. Amounts of neutral sugars extracted from oak chips on day 21. Different lowercase letters indicate the differences between the toasting temperatures for the same duration of toasting ($p < 0.05$). Different capital letters indicate the differences between the different durations of toasting at the same temperature ($p < 0.05$).

For toasting at 180 °C, the amount of neutral sugars extracted from the oak chips increased with longer toasting durations. At 200 °C, the amount of neutral sugars remained almost the same after 10 min of toasting, but as the toasting duration increased, the amount of neutral sugars decreased compared to toasting at 180 °C. When the toasting temperature exceeded 200 °C, the amount of neutral sugars increased for all duration conditions, peaking between 220 and 240 °C. Moreover, the maximum extraction amount (14.5 mg/g chips) was obtained by toasting at 240 °C for 10 min. Subsequently, as the toasting temperature increased, the extraction amount of neutral sugars decreased sharply. Compared to 10 min of toasting, the amount of neutral sugars decreased with longer durations of toasting.

3.4. Analysis of Monosaccharides in the Wood

Figure 4 shows the relationship between toasting temperature and duration for the monosaccharides predominantly extracted from the model wine solution, which are components of the major polysaccharides (cellulose and hemicellulose) in wood.

Glc was detected under relatively low toasting temperatures, such as 180 and 200 °C, with the maximum extraction amount (1.66 mg/g chips) observed at 180 °C after 10 min (Figure 4A). At these toasting temperatures, there was a tendency for the extraction amount to decrease as the toasting duration increased. On the other hand, Glc was also detected at temperatures above 260 °C, but the extraction amount was low at 10 min, increasing with toasting durations of 20 min or more. Ara showed a higher extraction amount at lower temperatures and shorter toasting durations, with the maximum extraction (1.39 mg/g chips) occurring at 180 °C after 10 min (Figure 4B). As the toasting temperature and duration increased, the extraction amount of Ara decreased. Gal was not detected at temperatures above 260 °C (Figure 4C). At 180 °C, the extraction amount was about 0.1 mg/g chips, regardless of the toasting duration. However, during toasting at 200

to 240 °C, the extraction behavior of Gal varied with toasting duration. At 10 min, the extraction amount was relatively high at 240 °C, while after 20 and 30 min, the highest extraction was observed at 220 °C. Additionally, there was a tendency for the extraction amount to increase with longer toasting durations. Some Xyl was extracted at 180 °C, but the amounts decreased as the toasting temperature increased (Figure 4D). However, as the temperature continued to increase, the extraction increased again before finally decreasing. The maximum extraction occurred at around 240 °C (or at 260 °C with 10 min of toasting). There was also a tendency for the extraction amount to increase with longer toasting durations.

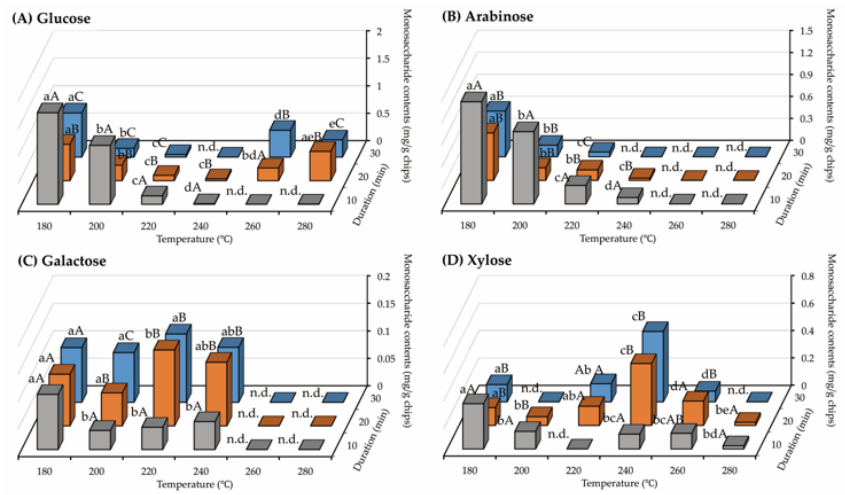

Figure 4. Amounts of monosaccharides extracted from oak chips: (**A**) glucose; (**B**) arabinose; (**C**) galactose; (**D**) xylose. n.d.: not detected. Different lowercase letters indicate the differences between the toasting temperatures for the same duration of toasting ($p < 0.05$). Different capital letters indicate differences between different durations of toasting at the same temperature ($p < 0.05$).

3.5. Analysis of Polysaccharide Components in the Wood

Figure 5 shows the relationship between toasting temperature, toasting duration, and the extraction amounts of polysaccharide components.

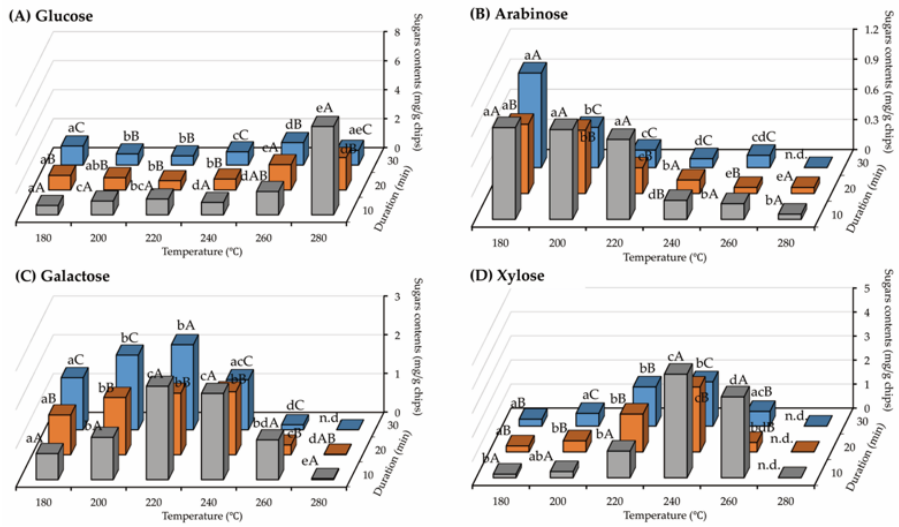

Figure 5. Amounts of polysaccharide components extracted from oak chips: (**A**), glucose; (**B**), arabinose; (**C**), galactose; (**D**), xylose. n.d.; not detected. Different lowercase letters indicate the differences between the toasting temperatures for the same duration of toasting ($p < 0.05$). Different capital letters indicate the differences between the different durations of toasting at the same temperature ($p < 0.05$).

Glc had low extraction amounts at lower toasting temperatures, but the amount increased significantly between 260 and 280 °C, especially when the toasting duration was shorter (Figure 5A). The highest amount of Glc was observed at 280 °C after durations of 10 and 20 min, but for 30 min, the peak extraction temperature was 260 °C, with a slight reduction in the amount. Ara showed higher extraction amounts at relatively low toasting temperatures, and its extraction decreased at temperatures above 240 °C (Figure 5B). At 180 to 220 °C, the extraction amount generally decreased with longer toasting durations, with the exception of 180 °C for 30 min. For Gal, the extraction amount increased as the toasting duration lengthened between 180 and 200 °C (Figure 5C). Additionally, under all toasting conditions, the extraction amount tended to increase with toasting temperature. The highest extraction occurred at 220 °C for durations of 10 and 30 min of toasting and at 240 °C for 20 min of toasting. After that, at higher toasting temperatures, the extracted amount significantly decreased, with almost no detection at 280 °C. Xyl had very low extraction amounts at lower temperatures, but as the toasting temperature increased, the extraction amount increased, peaking at 240 °C (Figure 5D). Between 180 and 220 °C, longer toasting durations led to increased extraction amounts. However, at 240 °C, the extraction amount decreased with longer toasting durations. At 260 °C, the highest extraction was observed with 10 min of toasting, followed by a decrease after 20 min and then a slight increase after 30 min. Xyl was not detected at 280 °C.

4. Discussion

The color of the toasted chips became darker as the toasting temperature increased or as the toasting duration lengthened. The values of L*, a*, and b* showed a relatively large difference between the 10 min toasting and the 20 and 30 min toasting durations, suggesting that significant chemical changes may occur between the 10 min and 20 min toasting durations. On the other hand, the differences in the values of L*, a*, and b* between the 20 min and 30 min toasting durations were minimal, suggesting that it would be difficult to detect differences in toasting conditions based on visual color assessment.

As shown in Figure 2, when the toasting temperature was below 240 °C, the weight loss was less pronounced compared to samples toasted at higher temperatures. On the other hand, severe weight loss was observed when chips were treated at 260 and 280 °C. In general, the addition of chips to wine is based on weight. Therefore, when the same weight of toasted chips is added to wine, oak chips subjected to higher toasting temperatures and longer toasting durations will introduce a greater amount of wood into the wine. Regarding weight loss, three factors were considered to be important, i.e., the evaporation of water in wood (free and bound water), pyrolysis, and carbonization [22,23]. The moisture content of the staves used in this experiment was around 15%, as determined by drying them in a 103 °C oven according to the JIS Z2101:2009 method. Based on these results, the oak chips used in the samples were considered to be sufficiently dried under the ambient conditions in Japan. The weight loss during this process is likely attributable to the evaporation of both free and bound water from the wood components [22]. On the other hand, when toasted to higher temperatures, some wood components are likely to undergo pyrolysis. Furthermore, as pyrolysis progresses, the carbonization of wood components like cellulose and hemicellulose occurs, potentially producing smoke and other emissions. Indeed, in this experiment, toasting at temperatures above 260 °C resulted in noticeable smoke, suggesting that volatile components other than water were evaporating from the wood. High-temperature toasting conditions require careful attention, as they can lead to the formation of hazardous and toxic substances, such as benzo(a)pyrene and other polycyclic aromatic hydrocarbons (PAHs) [23].

As shown in Figure 3, at a toasting temperature of 180 °C, the extraction amount of neutral sugars increased with longer toasting durations. This suggests that, at 180 °C, some of the polysaccharides in the wood were depolymerized, making them more soluble. At temperatures exceeding 200 °C, the concentration of neutral sugars increased, particularly around 220 to 240 °C. In our previous our study and in the one presented here, the 10 min

data were obtained under identical experimental conditions. While reproducibility was shown for the behavior of sugar extraction relative to temperature, the concentration of sugars extracted from the model wine was slightly higher. This discrepancy was attributed to differences in the raw stave material [11] On the other hand, there was a tendency for longer toasting durations to result in lower extraction amounts of neutral sugars. Additionally, under high toasting temperatures and longer toasting durations, the extraction of neutral sugars significantly decreased. Therefore, within these temperature ranges, both depolymerization of wood polysaccharide components leading to solubilization and thermal denaturing due to elevated temperatures occur simultaneously, suggesting that the effects of toasting on wood are complex [11,24]. Based on this, we considered that analyzing the major compositional sugars of wood polysaccharides separately could enhance our understanding of the reactions that occur within wood due to toasting. Moreover, to comprehend these reactions thoroughly, it was essential to analyze the sugars extracted from wood polysaccharides in the model wine solution, categorizing them into monosaccharide and polysaccharide fractions.

The results from Figure 4 confirm that the major sugars in wood polysaccharides, namely, Glc, Ara, Gal, and Xyl, were extracted in their monosaccharide forms. Smith et al. reported the presence of free Glc and Ara in the interior of wood [25]. Our data also indicated that Glc (Figure 4A) and Ara (Figure 4B) exhibited higher extraction amounts at lower toasting temperatures, particularly with shorter toasting durations at around 180 °C. This suggests that these monosaccharides were already present in the raw oak chips. In fact, these monosaccharides were detected at amounts comparable to those observed at 180 °C in untoasted chips [11]. Gal, detected as a monosaccharide (Figure 4C), exhibited a consistent extraction amount at 180 °C, irrespective of toasting duration; however, upon increasing the toasting temperature to 200 °C, the extraction amount decreased. This suggests that the Gal present as a monosaccharide may have undergone thermal denaturation. Similarly, Xyl, also detected as a monosaccharide (Figure 4D), showed a reduction in extraction amounts as the toasting duration increased between 180 and 200 °C, indicating that the monosaccharide likely undergoes thermal denaturation as well. These results suggest that at least the four monosaccharides analyzed in this study (Glc, Ara, Gal, and Xyl) undergo thermal denaturation at approximately 180 °C, with the reaction being facilitated by both higher toasting temperatures and longer toasting durations. It is speculated that these monosaccharides are converted to volatile compounds during toasting [14]. Madelaine et al. reported that heat treatment of hardwood caused acid hydrolysis of wood polysaccharides to produce monosaccharides, which were further converted to furfural and hydroxymethylfurfural (HMF) by dehydration of these sugars [24]. And it has been reported that furfural is generated through the dehydration of hexoses such as Glc, while HMF is produced from the dehydration of pentoses like Xyl [26]. Therefore, the denatured monosaccharides constitute important fractions of aromatic compounds in toasted oak chips. On the other hand, it has been reported that the thermal decomposition of wood polysaccharides leads to the formation of anhydrosugars and fragmentation compounds [27,28]. Therefore, the denaturation of sugars induced by chip toasting is speculated to occur by competitive reactions leading to the formation of these compounds.

The amount of Glc contained in both monosaccharide and polysaccharide fractions increased at toasting temperatures above 260 °C. These sugars were thought to be extracted by depolymerizing wood polysaccharides, which made them easier to solubilize. On the other hand, after shorter toasting durations, the polysaccharide fraction contained a greater amount of Glc; however, as the toasting duration increased, the amount of the polysaccharide fraction decreased, leading to an increase in the amount of Glc detected as a monosaccharide. Based on the above results, it is speculated that depolymerized wood polysaccharides undergo further depolymerization with longer toasting durations, with some being broken down into monosaccharides. However, under the conditions of this study, Glc as a monosaccharide is believed to undergo denaturation, suggesting that both depolymerization and denaturation of the wood polysaccharides occur simultane-

ously during treatment at temperatures between 260 and 280 °C. With the exception of the polysaccharide fraction at 180 °C for 30 min, the amount of Ara in both monosaccharide and polysaccharide fractions decreased as the temperature increased and the toasting duration lengthened (Figures 4B and 5B). Therefore, it is suggested that polysaccharides containing Ara are present within the wood but do not generate further due to depolymerization. And high-temperature, long-duration toasting likely promotes their denaturation. As an exception, large amounts of monosaccharides and polysaccharides containing Ara were detected after toasting at 180 °C for 30 min. This suggests that polysaccharides containing Ara may be depolymerized when treated for a long period at 180 °C, but denaturing may become dominant at temperatures higher than 180 °C. On the other hand, Xyl showed an increase in both monosaccharide and polysaccharide fractions when toasted at temperatures around 240 °C. Moreover, this reaction is similar to that which occurs with Glc at temperatures between 260 and 280 °C, where shorter toasting durations result in a higher proportion of polysaccharide components, while longer toasting durations lead to an increase in Xyl as a monosaccharide. Therefore, it is suggested that at toasting temperatures around 240 °C, the polysaccharides within the chips undergo depolymerization, which progresses further to yield monosaccharides with extended toasting durations. Simultaneously, it is likely that degradation of the monosaccharide Xyl also occurs. Gal shows higher amounts in both the monosaccharide and polysaccharide components around 220 °C, suggesting that depolymerization occurs in this temperature range. The amount of the monosaccharide Xyl was relatively low at 10 min but increased significantly with toasting durations exceeding 20 min. When comparing the polysaccharide fractions of Gal at the same temperature, a tendency for higher amounts was observed after both 10 and 30 min, suggesting that depolymerization and degradation reactions occur more competitively for Gal compared to other sugars.

The sugars analyzed in this study—Ara, Xyl, Gal, and Glc—are components of the hemicellulose found within wood. On the other hand, cellulose is composed solely of Glc. Additionally, it is known that hemicellulose is amorphous, while cellulose is crystalline, exhibiting strong bonding characteristics [15]. Given this experiment, it is speculated that Glc undergoes depolymerization at temperatures above 260 °C due to the robust bonding characteristics of cellulose. On the other hand, Ara does not depolymerize or depolymerizes at relatively low temperatures around 180 °C, while Gal depolymerizes near 220 °C and Xyl depolymerizes near 240 °C, both of which are lower temperatures than that of Glc. Moreover, these temperatures tend to shift to the lower side with longer toasting durations. From these results, it is suggested that the reactions that occur within oak chips during toasting are complex, with the initial decomposition of hemicellulose followed by the depolymerization of cellulose occurring at temperatures exceeding 260 °C [13,29]. Additionally, while these reactions exhibit relatively high temperature dependency, it was observed that Ara and Glc denatured when toasted for about 10 min at temperatures around 180 to 200 °C. In contrast, Xyl and Gal were maintained in certain amounts even after approximately 30 min of toasting, suggesting that they possess a certain resistance to thermal denaturation with respect to toasting duration. The thermal decomposition of cellulose and hemicellulose is known to involve highly complex reaction mechanisms, with reports indicating that depolymerization of wood polymers leads to the formation of oligosaccharides [30]. Therefore, the polysaccharide fractions identified in this study are suggested to be sugars extracted as a result of these reactions. Moreover, it has been reported that further thermal degradation of these oligomers leads to various reactions, such as dehydration, ring fragmentation, cleavage, rearrangement, and char formation [28]. However, additional research is required to elucidate the relationship between chip toasting and the formation of these compounds.

This study demonstrated that varying toasting temperatures and durations elicited highly complex reactions within oak chips. Furthermore, the responses induced by toasting significantly differed among the major sugars constituting the wood. The results of this study elucidate the chemical changes that occur within the wood during the toasting of oak

chips and highlight the importance of the relationship between toasting temperature and duration. In particular, the use of electric furnaces for precise toasting during the production of oak chips allows for the careful consideration of the reactions that occur within the wood. Therefore, determining the optimal toasting conditions for the resulting wine becomes critically important. In the case of toasting wine barrels, considering factors such as the thickness of the wood, it is expected that the reactions observed at different toasting temperatures and durations occur simultaneously [31]. Future research should focus on staves and other barrel woods to gain a deeper understanding of the effects of wood-derived sugars and other compounds on wine. This approach is expected to contribute not only to the understanding of the reactions that occur within wine barrel woods but also to the scientific knowledge regarding the structure of wood and the subsequent formation of aromas.

5. Conclusions

The results of this study revealed that the composition of sugars extracted from oak chips varies significantly depending on toasting temperature and duration. It is believed that both depolymerization and denaturation of wood polysaccharides occur simultaneously within the wood. The high extraction amounts of Gal at around 220 °C, Xyl at around 240 °C, and Glc at around 280 °C suggest that the depolymerization reactions are both site-specific and temperature-dependent. Moreover, these depolymerization reactions tended to shift towards lower temperatures with longer toasting durations. On the other hand, the denaturation reactions were not only temperature-dependent but also significantly influenced by the length of the toasting duration. In addition, it was found that in the case of Gal, the depolymerization and denaturation reactions induced by toasting occur competitively, making the analysis difficult. While the reactions triggered by toasting within oak woods are highly complex, it is speculated that these reactions initially occur from the thermal depolymerization of hemicellulose, with thermal depolymerization of cellulose potentially occurring during high-temperature toasting above 260 °C. While this study focused on oak chips, it is not surprising that similar reactions occur in wine barrels during the toasting process. However, due to the differences in wood thickness in wine barrels, it is likely that the reactions observed in this study occur simultaneously. This study demonstrates that by using sugars as indicators, it is possible to partially visualize the reactions that take place within oak chips during toasting. Given that the structure of wood polysaccharides likely varies depending on the species and origin of the wood, the sugar analysis conducted in this study is expected to be valuable in determining precise toasting conditions for specific wood types and regions in the future. Further research is necessary to deepen our understanding of the reactions that occur within the wood of wine barrels and other barrel woods.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages10040118/s1, Table S1: The weight loss of toasted oak chips; Table S2: The amount of neutral sugars extracted from oak chips; Table S3: The amounts of four types of monosaccharides extracted from oak chips; Table S4: The amounts of four types of polysaccharides extracted from oak chips.

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