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Effect of Isothermal Heat Treatment on Oregonin Content in Black Alder Bark Extract

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Abstract

Black alder (*Alnus glutinosa* (L.) Gaertn.) wood is characterized by rapid and frequently non-uniform color changes after felling and during hydrothermal processing. These changes are largely associated with extractive compounds, among which oregonin, a phenolic diarylheptanoid glycoside, has been identified as a precursor of the characteristic orange-red coloration. However, information on the thermal stability of oregonin remains limited. This study investigated the effect of temperature and exposure time on oregonin content in black alder bark extract. Aliquots of the extract were exposed to isothermal conditions of 30, 40, 50, and 60 °C for up to 24 h. In a separate trial, a treatment at 98 °C was conducted to simulate the temperature conditions associated with wood saturated water steaming at atmospheric pressure. Oregonin concentration was determined using reversed-phase high-performance liquid chromatography with diode-array detection. Oregonin content remained comparatively stable during 24 h exposure at 30, 40, 50 and 60 °C, with reductions of 6.00%, 3.00%, 2.78%, and 1.64%, respectively, without clear increase in oregonin loss with increasing temperature. Exposure to 98 °C resulted in the most pronounced decrease in oregonin content, with a reduction of 43.93%. The results indicate comparatively high stability of oregonin during 24 h exposure at 30–60 °C, whereas a pronounced decrease was observed in the separate aqueous treatment at 98 °C. These findings are relevant for understanding chemical changes in alder extractives during wood drying and steaming and demonstrate the importance of minimizing unnecessary heat exposure during sample preparation, extraction, and storage.

Keywords: *Alnus glutinosa*; bark; black alder; discoloration; heat treatment; HPLC-DAD; hydrothermal processing; oregonin; thermal stability; wood

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1. Introduction

Black alder (*Alnus glutinosa* (L.) Gaertn.) is a diffuse porous hardwood species native to most of Europe, typically occurring on moist or waterlogged soils along rivers, streams, and lakes, and in wetland forests [1,2]. Although its wood is relatively easy to process and has attractive esthetic properties, it remains underutilized in higher-value wood products [3]. A notable technological limitation is its pronounced and often non-uniform color change after tree felling, sawing, drying, or steaming. Freshly exposed black alder wood can rapidly develop an orange-red coloration [4] (Figure 1), which may reduce visual uniformity and complicate its use in furniture, veneer, and other appearance-oriented products.

(**a**) (**b**)

Figure 1. (**a**) Color change in black alder wood 24 h after felling (photo: Klarić M.). (**b**) Color change in black alder wood 48 h after felling (photo: Klarić M.).

Wood color is strongly influenced by low-molecular-weight extractives. These compounds are predominantly nonstructural secondary metabolites, and include, among others, simple phenols, flavonoids, tannins, lignans, stilbenes, and diarylheptanoids. Although extractives generally constitute only a small fraction of wood in temperate zone species, they occur in both wood and bark and can substantially influence its color, odor, natural durability, and technological properties [5]. The composition and concentration of extractives may vary among species, individual trees, anatomical positions within the tree, and harvesting seasons [6,7].

During hydrothermal processing, wood extractives and other wood components may undergo oxidation, hydrolysis, condensation, and related thermal degradation reactions, which can lead to either desirable or undesirable color changes [8]. Color changes in alder wood may result from both enzymatic and non-enzymatic reactions. In fresh wood, oxidative enzymes can react with phenolic compounds and contribute to discoloration [9], while at higher temperatures thermal transformations of extractives and structural wood components may become more important [10,11]. Conventional kiln drying and steaming with saturated water steam are the principal hydrothermal processes used in wood processing. Conventional kiln drying is generally conducted at moderate temperatures over prolonged periods, whereas steaming exposes wood to higher temperatures and moisture. Both treatment temperature and exposure time may affect the stability of wood extractives. Consequently, understanding the thermal stability of individual extractives is important not only for explaining wood discoloration but also for improving processing conditions and minimizing losses in product quality.

Oregonin, chemically identified as (5*S*)-1,7-bis(3,4-dihydroxyphenyl)-5-(*β*-D-xylopyranosyloxy)-heptan-3-one, is the first discovered and reported naturally occurring phenolic diarylheptanoid glycoside characteristic of several species of the genus *Alnus* [12]. Oregonin was first isolated from the bark of red alder (*Alnus rubra* Bong.) and was named after the US state of Oregon [13–15]. Oregonin belongs to the group of linear 1,7-diarylheptanoids, whose structures contain two aromatic rings connected by a seven-carbon chain. It has subsequently been identified in the bark, leaves, seeds, and reproductive tissues of several alder species, including black alder [4,12]. Oregonin has been associated with the development of orange-red coloration in alder extracts. Earlier studies indicated that it may act as a precursor of colored compounds formed through hydrolytic and oxidative reactions, including reactions involving peroxidases and hydrogen peroxide [13,14]. This

relationship suggests that changes in oregonin content or chemical structure may contribute to the discoloration observed in alder wood and bark during handling and hydrothermal processing. However, published information on the behavior of oregonin under controlled thermal conditions remains limited.

The thermal sensitivity of oregonin is also relevant to analytical procedures. If oregonin is susceptible to degradation at moderate temperatures, uncontrolled heating during sampling, grinding, drying, extraction, concentration, or sample storage could alter its concentration. Analytical protocols should therefore minimize thermal exposure unless the compound has been demonstrated to remain stable under the applied conditions. Determining its stability in an extract can provide an initial basis for assessing both technological and analytical risks. Previous research on black alder has mainly addressed wood properties, discoloration, extractive composition, and the occurrence of oregonin in different plant tissues [3,4,7]. However, the effects of temperature and exposure time on oregonin content have not been sufficiently characterized, particularly within temperature ranges relevant to conventional kiln drying and wood steaming with saturated water steam. Lea et al. [16] reported less than 10% thermal degradation of oregonin during the extraction and spray drying of extract at temperatures between 25 and 50 °C, and 20.8% at 65 °C, under their experimental conditions. However, their study focused primarily on the extraction and purification of oregonin from red alder bark and leaves, whereas systematic information on changes in oregonin content during prolonged, controlled isothermal exposure is still limited. The present study therefore investigated changes in detectable oregonin content during controlled exposure for up to 24 h at 30–60 °C, together with a separate high-temperature treatment at 98 °C. Moreover, most existing studies have focused on the isolation [12,16–18] or biological activity [19–22] of oregonin rather than its thermal stability during wood processing conditions.

Therefore, the aim of this study was to determine the effect of controlled isothermal heat treatment on the oregonin content of black alder bark extract. The results were used to evaluate the thermal stability of oregonin and its potential sensitivity during hydrothermal wood processing and laboratory sample preparation.

2. Materials and Methods

2.1. Material

A black alder (*Alnus glutinosa* (L.) Gaertn.) tree was sampled in early September in the *Đurđevačke nizinske šume* management unit, *Preložnički berek* forest area, compartment 40d, managed by *Hrvatske šume* Ltd., Forest administration *Koprivnica*, Forest Office *Đurđevac*, Croatia.

The sampled 52-year-old tree had a diameter at breast height of approximately 40 cm and was healthy, without visible defects, mechanical damage, central decay, or abnormal discoloration. After felling, a 20 cm long stem section (i.e., cross section segment—disk) was cut immediately above a height of 1.5 m. A central radial strip approximately 10 cm wide was then removed from the cross-section for chemical analysis.

Immediately after collection, the sample was tightly wrapped in vapor-impermeable stretch film, placed in a dark, insulated container containing dry ice at approximately −78.5 °C, and transported to the laboratory to minimize exposure to oxygen and heat. The sample was maintained under these conditions to minimize biochemical and chemical changes before further processing and analysis. The bark was removed from the stem section and maintained frozen on dry ice till grinding and homogenization. The scope of this study was limited to oregonin stability in a representative extract, rather than assessing natural variation among individual trees.

2.2. Methods

2.2.1. Grinding and Homogenization

The frozen bark samples were initially reduced in size by sawing and subsequently ground and homogenized using a cutting mill (RETSCH SM 300, Retsch GmbH, Haan, Germany) equipped with a six-disk cutting rotor designed to prevent heavy metal contamination and a 1 mm trapezoidal-hole sieve.

Dry-ice pellets were added continuously during grinding to minimize sample heating. Sublimation of the dry ice also maintained a carbon dioxide-rich atmosphere within the grinding chamber. The mill was operated at 1500 rpm.

After grinding, the homogenized samples were immediately returned to dry ice and stored frozen until further analysis.

2.2.2. Lyophilization

The ground bark samples were transferred into amber glass vials fitted with vapor-permeable cellulose membranes to prevent sample loss or mixing under vacuum. Before lyophilization, the samples were pre-frozen on dry ice at approximately −78.5 °C for at least 24 h.

Lyophilization was performed using a CHRIST Alpha 1–2 LD freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) to remove water prior to further analysis. After drying, the moisture content was checked using a halogen moisture analyzer to ensure that a constant mass had been reached and that the water had been removed. The lyophilized samples were then vacuum-sealed and stored in the dark at approximately −30 °C, until further analysis.

2.2.3. Extraction

Black alder bark was used as the source material because its oregonin content is substantially higher than that of wood, providing a higher initial analyte concentration and thereby facilitating the detection of induced changes. The use of homogenized bark extract allowed for the thermal response of oregonin to be studied under controlled conditions, while minimizing effects associated with the wood matrix. However, this approach does not fully reproduce the complex interactions occurring in solid wood during drying or steaming. The extract used to evaluate the effect of heat on oregonin was prepared from the bark. Extraction was performed by maceration for 24 h in a 1 L Erlenmeyer flask using a temperature-controlled magnetic stirrer (IKA C-MAG HS 7, IKA-Werke GmbH & Co. KG, Staufen, Germany) (Figure 2).

Figure 2. Extraction using a heated magnetic stirrer with temperature control (photo: Klarić, M.).

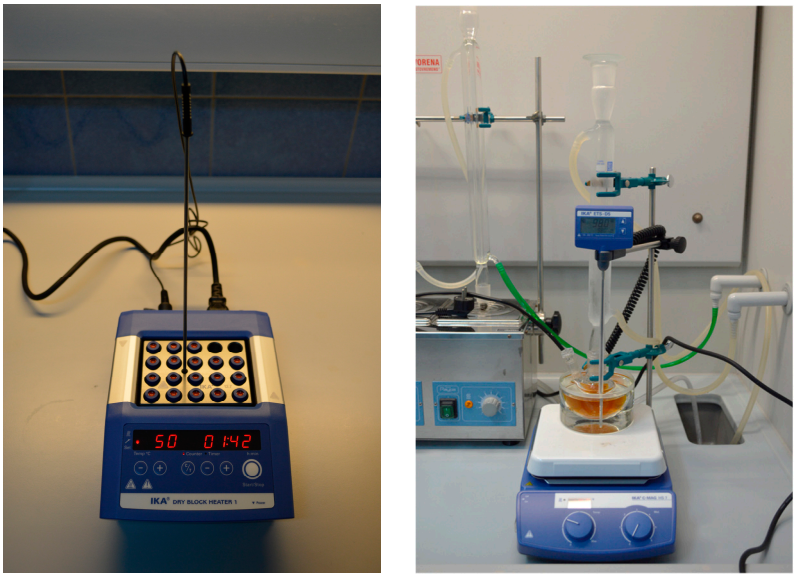
A total of 10 g of ground, homogenized, and lyophilized bark was extracted with 1 L of 90% aqueous methanol (*v*/*v*) at 20 °C. HPLC-grade methanol (MeOH) was obtained from J. T. Baker (Phillipsburg, NY, USA), while ASTM Type II deionized water (dH_2O) was produced using a TKA/Thermo Scientific MicroMed Pure water purification system (Thermo Fisher Scientific, MA, USA).

The temperature was monitored using a glass temperature probe, and mixing was performed at stirrer setting 3 using a 6 × 35 mm PTFE-coated magnetic stir bar.

After extraction, the mixture was filtered through filter paper (Munktell, black ribbon, grade 388). The filtrate was transferred into amber glass bottles and stored under refrigerated conditions until further analysis.

2.2.4. Thermal Treatment of the Extract

Before thermal treatment, 1.5 mL aliquots of the black alder bark extract were filtered through 0.22 µm nylon membrane filters of 25 mm diameter and transferred into sealed amber glass vials. The samples were exposed to four isothermal temperature conditions (30, 40, 50 and 60 °C) Table 1 in a dry block heater (IKA Dry Block Heater 1 with DB 5.1 single block, IKA-Werke GmbH&Co. KG, Staufen, Germany) for 24 h (Figure 3a). At 2 h intervals, three replicate vials were removed from each temperature treatment and stored under refrigerated, dark conditions until analysis by reversed-phase high-performance liquid chromatography system coupled with a diode-array detector (HPLC-DAD) was performed.



(**a**) (**b**)

Figure 3. (**a**) Thermal treatment of the vials in a dry block heater (photo: Klarić, M.). (**b**) Thermal treatment of the extract at 98 °C in a glycerol bath under reflux (photo: Klarić, M.).

A separate experiment was conducted by exposing the extract to 98 °C, corresponding approximately to the steam temperature used during industrial wood steaming. Prior to the 98 °C treatment, methanol was removed under vacuum at 20 °C, because heating the methanolic extract above its boiling point in sealed septum-capped vials would generate elevated internal pressure and pose a risk of leakage or vial failure. The residue was redissolved in an equivalent volume of deionized water and heat-treated under reflux. As methanol had to be removed before reflux treatment, the experiment at 98 °C was conducted in an aqueous medium and should therefore be regarded as a separate high-temperature experiment rather than as a directly comparable extension of the 30–60 °C treatments. The 98 °C treatment was performed for 24 h using a glass apparatus consisting

of a 100 mL two-neck round bottom flask connected to a Liebig condenser and placed on a temperature-controlled magnetic stirrer (IKA C-MAG HS 7) (Figure 3b). A glycerol bath was used to maintain a constant temperature of 98 °C. Every 2 h, a 5 mL aliquot was withdrawn, transferred into amber glass bottles, and stored under refrigerated conditions until further analysis.

Oregonin content was determined in triplicate in both the untreated extract (which served as the reference) and the heat-treated extracts using high-performance liquid chromatography according to the analytical method developed and described in this study.

Table 1. Heat treatment process parameters.

Process	Solvent	t [°C]	τ [Hours]
1	90% (v/v) aqueous MeOH	30	24
2	90% (v/v) aqueous MeOH	40	24
3	90% (v/v) aqueous MeOH	50	24
4	90% (v/v) aqueous MeOH	60	24
5	dH_2O	98	24

2.2.5. HPLC-DAD Analysis

Black alder bark extracts were analyzed by HPLC-DAD (Walnut Creek, CA, USA) to determine oregonin content. HPLC-DAD analyses were performed with a Varian ProStar 500 chromatographic system equipped with a ProStar 230 ternary pump, a ProStar 410 autosampler, a ProStar 500 thermostatted column compartment, and a ProStar 330 diode-array detector.

Chromatographic separation was performed on a NUCLEOSIL C18 analytical column (150 × 4.6 mm, 5 µm particle size, 120 Å pore size; Supelco Analytical). The mobile phase consisted of eluent A (0.1% formic acid in methanol) and eluent B (0.1% formic acid in Milli-Q water), applied in a gradient elution mode (Table 2). The methanol was of Ultra Gradient HPLC grade (J.T. Baker), while the formic acid was of analytical grade (Orka Lab, Zagreb, Croatia). Milli-Q water was produced using a Millipore Simplicity UV System (Millipore Corporation, Billerica, MA, USA). The flow rate was set at 0.5 mL/min, and the injection volume was 10 µL. Each sample was analyzed in triplicate. Identification and quantification of oregonin were performed at a detection wavelength of 280 nm, and retention time was 16.50 min. An oregonin analytical standard with a purity of ≥95% was obtained from Sigma-Aldrich (Darmstadt, Germany).

Table 2. Gradient elution used for HPLC separation.

t [min]	A [%]	B [%]
0	10	90
25	100	0
30	10	90

Chromatographic data were collected and processed using Star Chromatography Workstation software, version 5.5.

Stock standard solution of oregonin was prepared by dissolving an accurate quantity of the standard in MeOH and storing in the dark at 4 °C. The working standard solutions of different concentrations were prepared by appropriate dilution of the stock solution. The calibration curve for oregonin was prepared using six working standard solutions.

The method showed linearity over the concentration range of 5–100 mg/L, with coefficients of determination greater than 0.9993.

To assess method precision, repeatability and intermediate precision were deter-mined and expressed as relative standard deviation (RSD, %). Repeatability was evaluated from three consecutive injections of a 40 mg/L oregonin standard solution, whereas intermediate precision was assessed by injecting the same solution on three consecutive days. The RSD was 0.4% for repeatability (n = 3) and 1.4% for intermediate precision (n = 3).

2.2.6. Statistical Analysis

At each sampling time, HPLC measurements were performed using three replicate injections and the results were expressed as mean ± standard deviation (SD). Spearman's rank correlation analysis was used to assess the relationship between treatment time and relative oregonin content (A/A_0) at each investigated temperature. The initial time point (0 h) was excluded from the correlation analysis because A/A_0 equals 1 by definition at t = 0. Differences in relative oregonin content among the comparable temperature treatments of 30, 40, 50, and 60 °C were additionally evaluated using Friedman's test, with sampling time as the blocking factor. The 98 °C treatment was not included in the Friedman analysis because it was performed in an aqueous extract under reflux, whereas the 30–60 °C treatments were conducted in 90% methanolic extract in sealed vials. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using XLSTAT, ver. 2026.11.

3. Results and Discussion

The effect of heat on oregonin content was evaluated to assess its stability under temperature conditions relevant to wood conventional kiln drying, wood steaming with saturated steam and laboratory sample handling. Changes in oregonin content were expressed relative to the initial peak area value (A_0) measured in the untreated extract, (A/A_0). The results for temperature conditions 30, 40, 50 and 60 °C are shown in Figure 4, while the results of Spearman's rank correlation analysis are summarized in Table 3.

Table 3. Spearman's rank correlation between treatment time and relative oregonin content (A/A_0) at different temperatures.

Temperature [°C]	n	Mean ± SD	Spearman's ρ	*p*-Value
30	12	0.974 ± 0.019	0.035	0.920
40	12	0.987 ± 0.010	−0.408	0.190
50	12	0.972 ± 0.013	−0.239	0.455
60	12	0.975 ± 0.022	−0.337	0.285

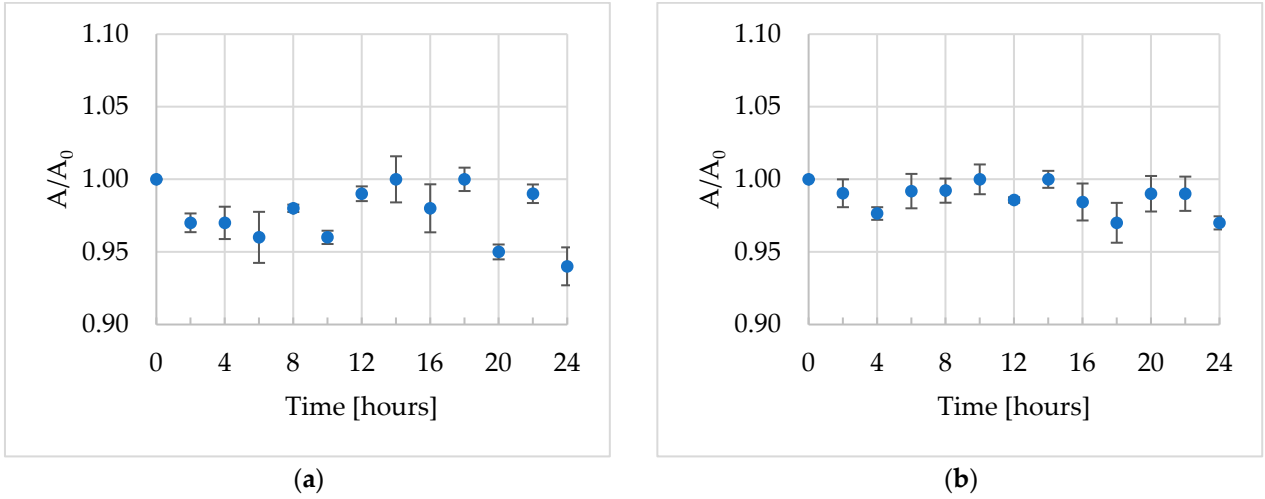


Figure 4. *Cont.*

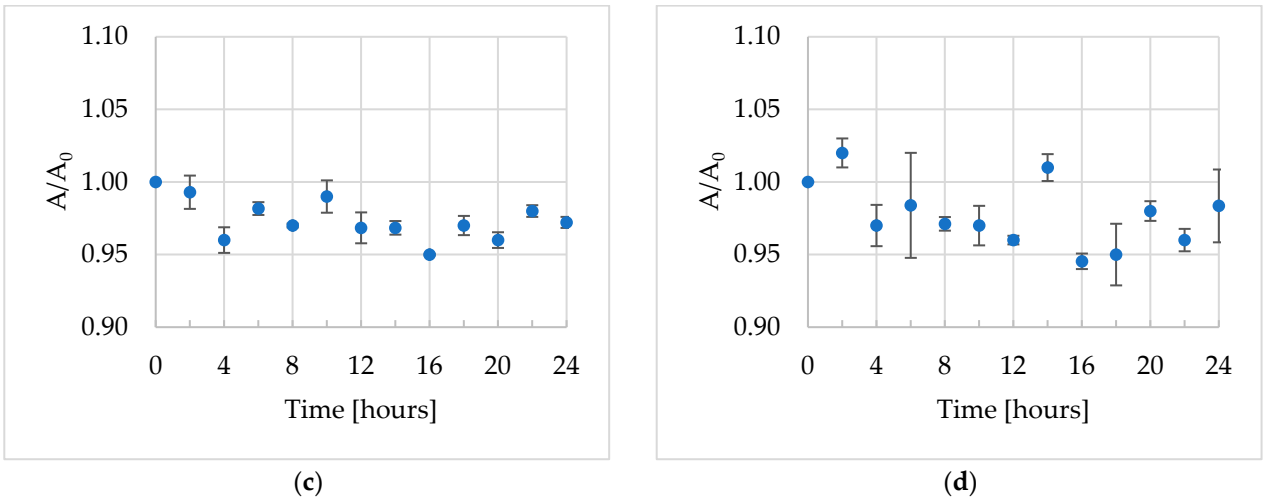


Figure 4. Change in oregonin concentration during 24 h of exposure to heat. Values are presented as mean ± SD of three replicate HPLC injections ((**a**) 30 °C; (**b**) 40 °C; (**c**) 50 °C; (**d**) 60 °C).

At 30 °C, no statistically significant monotonic relationship was observed between exposure time and relative oregonin content (Spearman's $\rho = 0.035$; $p = 0.920$). Similarly, no significant relationship was observed at 40 °C ($\rho = -0.408$; $p = 0.190$), 50 °C ($\rho = -0.239$; $p = 0.455$) or 60 °C ($\rho = -0.337$; $p = 0.285$). Overall, no statistically significant relationship between treatment time and relative oregonin content was observed at any of the investigated temperatures ($p > 0.05$). Furthermore, the Friedman test showed no statistically significant overall difference in relative oregonin content among the investigated temperature treatments ($Q(3) = 6.672$, $p = 0.083$). After 24 h, A/A_0 values were 0.940, 0.970, 0.972, and 0.984 at 30, 40, 50, and 60 °C, respectively, corresponding to decreases of approximately 6.0%, 3.0%, 2.8%, and 1.6% relative to the initial value. Thus, no consistent temperature-dependent decrease in detectable oregonin content was observed within the investigated temperature range. The relatively limited decrease in oregonin content observed at 30–60 °C is consistent with Lea et al. [16], who reported less than 10% thermal degradation of oregonin during extraction at temperatures between 25 and 50 °C in their research conditions, whereas 20.8% thermal degradation was observed only at 65 °C during spray drying of the extract.

These results suggest that oregonin is relatively stable during short-term exposure to lower temperatures within 24 h. Although the decrease in oregonin content was limited during 24 h of exposure at the applied temperatures of 30, 40, 50, and 60 °C, longer treatment periods, such as those encountered during conventional wood drying, may result in a greater content reduction.

The results obtained at 98 °C are shown in Figure 5. Changes in oregonin content are expressed relative to the initial peak area value (A_0) measured in the untreated extract, (A/A_0).

At 98 °C (Figure 5), the oregonin content began to decrease sharply after 6 h of exposure, as indicated by a strong negative linear trend ($m = -0.0202$; $R^2 = 0.9669$). After 24 h of exposure, the oregonin content had decreased by 43.93% relative to its initial value. This decrease may be influenced not only by elevated temperature, but also by oxidation and by the aqueous environment, which may promote glycosidic hydrolysis, although the resulting products were not identified. The treatment at 98 °C is particularly relevant because a similar temperature is applied when wood is being steamed with saturated water steam at atmospheric pressure, during industrial processes. The substantially greater decrease observed at 98 °C should be interpreted in the context of the different treatment conditions, because this experiment was conducted in an aqueous medium under reflux, whereas

the lower-temperature experiments were performed in methanolic extract contained in sealed vials.

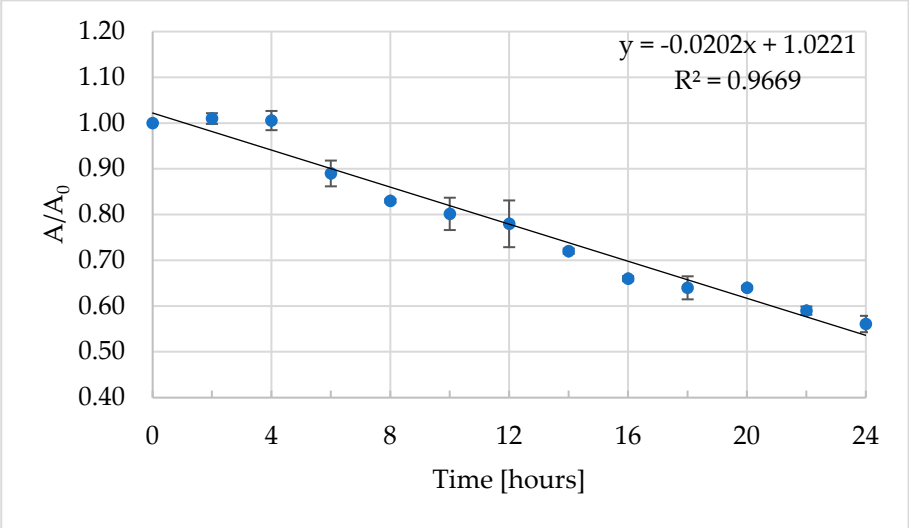


Figure 5. Change in oregonin concentration in aqueous bark extract treated under reflux at 98 °C. Values are presented as mean ± SD of three replicate HPLC injections.

In contrast to the treatments at 30–60 °C, a strong and statistically significant decrease in relative oregonin content was observed during the 98 °C reflux treatment (Spearman's $\rho = -0.998$, $p < 0.001$). The mean A/A_0 value over the 24 h period was 0.761 ± 0.153. However, this treatment was performed in an aqueous extract under reflux and therefore cannot be directly attributed to temperature alone.

Given that oregonin has been described as a precursor of the characteristic orange-red coloration, the decrease in its measurable content at elevated temperature may be related to Kozlik's observation [23,24] that high-temperature presteaming reduces mottling and improves color uniformity in red alder wood. Although a direct causal relationship cannot be established from the results, both findings support the hypothesis that thermal transformation of oregonin contributes to changes in the chromophoric system responsible for uneven orange-red discoloration. However, this proposed relationship should be interpreted with caution, because the present study was conducted on bark extract, whereas Kozlik investigated color changes in solid wood; the studies also examined different alder species and different response variables.

Thompson et al. [25] suggested that the orange-red chromophoric compounds formed in red alder wood are thermolabile and may be degraded by prolonged exposure to heat or by treatment at higher temperatures. This interpretation is partly supported by the pronounced decrease in detectable oregonin content observed in the separate 98 °C experiment, whereas no consistent temperature-dependent decrease was observed within the 30–60 °C range. Dudiak and Dzurenda [26] reported that steaming black alder wood for 12 h with a saturated steam–air mixture at 95 ± 2.5 °C decreased its lightness (L^*) in mean values from 78.3 to 68.6, indicating substantial darkening. Over the same period, the red color coordinate a^* increased moderately from 9.2 to 12.5, whereas the yellow color coordinate b^* decreased slightly from 22.6 to 20.5. Thus, treatment at this temperature did not prevent an increase in redness, but the overall color change was dominated by wood darkening rather than by a pronounced development of red or yellow coloration. The comparatively moderate increase in a^*, despite the substantial decrease in L^*, may indicate that steaming altered the balance between the formation and degradation of red-orange chromophores [26]. The decrease in detectable oregonin content observed in

the study at a similar temperature may have contributed to limiting the development of the characteristic orange-red coloration, although this relationship cannot be confirmed without simultaneous measurements of oregonin content and wood color.

Enzyme activity was not measured in the present study; therefore, its contribution to the discoloration of black alder wood cannot be directly assessed from these results. However, heat also influences enzyme activity [9], as well as oregonin stability. Enzyme-catalyzed reaction rates generally increase with temperature until an apparent optimum is reached, after which thermal inactivation and conformational changes progressively reduce catalytic activity [27]. The extent of inactivation depends on both temperature and exposure time, as well as on the particular enzyme and its chemical environment. Plant oxidative enzymes involved in discoloration, such as polyphenol oxidases and peroxidases, are generally substantially inactivated by sufficiently intense heat treatment, although their thermal resistance varies among enzymes and plant tissues [28]. Therefore, the beneficial effect of presteaming on alder wood color may result from the combined inactivation of discoloration-related enzymes and heat-induced transformations of oregonin and other extractive compounds. It has been reported that to overcome the mottled orange color after felling, sawn alder lumber is steamed at temperatures of 66 to 100 °C for varying periods of time [9,23,24,29,30]. Further studies combining oregonin analysis with measurements of enzyme activity and wood color changes would be useful to clarify this relationship.

The observed temperature-dependent behavior suggests that prolonged or unnecessary exposure to elevated temperatures should be minimized during sample preparation, extraction, and storage to reduce the risk of oregonin loss. However, the apparent stability observed at lower temperatures was evaluated only over a 24 h period and should not be extrapolated to longer exposure times.

Oregonin has been reported in several species of the genus *Alnus* [12,31], suggesting that thermally induced changes in this compound may contribute to similar color development mechanisms across alder species. Nevertheless, species-specific differences in extractive composition and wood chemistry should be considered when extrapolating results between black alder and other alder species.

Black alder is considered a promising short rotation species with considerable potential for the production of furniture, veneers, plywood, interior elements, sauna components, and other value-added wood products [3,32]. Its good workability, attractive appearance, and suitability for surface finishing support its wider industrial use. However, the pronounced color changes that occur during processing remain an important challenge for its industrial utilization, particularly when a uniform wood color is required. The results of the study indicate that the measurable oregonin content tends to decrease with increasing temperature and prolonged exposure, with the most pronounced reduction observed under conditions approaching those used for wood steaming. A better understanding of its thermal stability may therefore contribute to the optimization of drying and steaming schedules aimed at achieving more uniform and predictable color in black alder products. At the same time, the observed decrease in detectable oregonin content should be regarded as only one part of a broader system of heat-induced reactions involving other extractives, hemicelluloses, lignin, and chromophoric compounds.

4. Conclusions

During 24 h of isothermal treatment in methanolic extract 30–60 °C, only limited and variable changes in detectable oregonin content were observed. No statistically significant relationship between exposure time and relative oregonin content was found at any of these temperatures, and no significant overall difference was observed among the temperature

treatments. Thus, no consistent temperature-dependent decrease in detectable oregonin content within the investigated range was demonstrated.

In the separate high-temperature experiment conducted in an aqueous medium under reflux, detectable oregonin content decreased markedly at 98 °C, particularly after 6 h, and was 43.93% lower than the initial value after 24 h. These findings indicate a substantial loss of detectable oregonin under conditions approaching wood steaming temperatures at atmospheric pressure. However, direct quantitative comparison with the 30–60 °C treatments is limited because the 98 °C experiment differed in solvent composition and experimental configuration.

These findings support minimizing unnecessary exposure to elevated temperatures during sample preparation, extraction, concentration, and storage when oregonin is to be quantified. They also support the hypothesis that thermal transformation of oregonin may contribute to changes in the chromophoric system of alder during hydrothermal processing; however, this relationship cannot be confirmed without simultaneous measurements of oregonin content and wood color.

Further studies should simultaneously determine oregonin content, degradation products, enzyme activity, and CIE $L^*a^*b^*$ color changes in solid black alder wood under controlled drying and steaming conditions. Such an integrated approach could help clarify the mechanisms underlying black alder wood discoloration and help improve color uniformity during processing, enhance product quality, and facilitate wider industrial utilization of black alder.

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