Effect of Cryogenic Process on *Trametes versicolor* and *Fomitopsis palustris* Fungal Destruction in ThermoWood® Products of Some Wood Species

Ayhan Aytin,[a,*] Cihat Tascioğlu,[b] İlyas Uygur,[c] and Wakako Ohmura[d]

Aspen, ash, spruce, and fir trees naturally grown in Turkey were obtained directly from the forest and processed using ThermoWood®(TM) processing at two different temperatures (190 °C: TW1 and at 212 °C: TW2). Then, cryogenic treatment (Cr) was applied to the samples, and they were placed in a jar containing *Trametes versicolor* (COV) and *Fomitopsis palustris* (TYP) mushrooms. Their weight loss (WL) was measured after 12 weeks. Although WL results varied depending on the tree type and the applied processes, it has been understood that Cr application can be effective in reducing WL. According to the study, the lowest WL was determined to be in COV fungus with 1.2% in cryogenically treated fir tree TW2 samples. The highest WL was in TYP mushrooms with 56.1% in spruce TW1 samples that received heat treatment (HT). The results obtained from the study show that cryogenic treatment may be an alternative method for reducing WL in heat treated wood species.

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*Contact information: a: Duzce University, Duzce Vocation School, Design Department, Düzce; b: Duzce University, Faculty of Forestry, Forest Industry Engineering Department, Düzce; c: Duzce University, Faculty of Engineering, Department of Mechanical Engineering, Düzce; d: Kyoto University, Research Institute of Sustainable Humanosphere, Kyoyo, Japan; *Corresponding author: ayhanaytin@duzce.edu.tr*

INTRODUCTION

Trees, which have a wide range of uses as a natural and renewable raw material (Ramage *et al*. 2017), and the products (derivatives) obtained from them have been used by societies for thousands of years (Reinprecht 2016). Trees and their derivatives are under the influence of many external factors, mainly due to the climatic characteristics of the areas of use. Many of these factors cause some damage to the tree and its derivatives and shorten the lifespan of the materials. Dimensional changes occurring due to contraction and expansion in water exchange below the fiber saturation point, biological degradation resulting from fungal damage, and color change due to the distorting effects of light are considered as the most important disadvantages that arise in the use of wood material.

Biological degradation caused by fungi causes a significant decrease in the service life of wood materials. Wood materials, which are used extensively in all areas of life, such as construction, household products, and outdoor furniture products, are generally susceptible to fungal decay under suitable living conditions for fungi (such as 27% to 60% moisture content and 3 to 38 °C temperature). This not only reduces the service life of wooden materials but also causes huge economic losses (Bi *et al*. 2024). These decompositions caused by fungi in wooden materials are generally expressed as rot. The main fungal rots that cause significant value losses in wood materials are white (corrosion rot), brown (destruction rot), dry rot, and soft rot.

Although each type of rot has its own characteristics, the common feature of all of them is that they are more or less affected by the cell wall components cellulose, hemicellulose, and lignin. In white rot, all three main components of the cell wall are destroyed, but primarily because the lignin is destroyed, the tree acquires a whitish color, the appearance of the coloration is scattered, and the structure of the stained tree becomes spongy. Dark colored border lines are seen as the most striking characteristic feature of white rot. Brown rot fungi destroy cellulose and hemicellulose, which are cell wall components of the tree, and because lignin is not destroyed, the tree turns red or brown, which is the color of lignin. In this rot, there is a decrease in the volume of the tree, and in advanced stages, cracks in the transverse and longitudinal directions and loss of strength are observed, and the tree is divided into small cubes and prisms. In the soft rot type, the fungus shows its effect on the middle lamella of the wood cell walls (Bozkurt *et al*. 1993).

Fungi that damage cell wall components result in weight loss (WL) in varying amounts during the degradation of wood and its derivatives. In addition to the fungal rots examined in areas of use, studies conducted with various fungi in open field and laboratory environments show that many factors, such as the type of fungus, tree species, and climatic conditions, affect the WL. For example, Mendonça *et al*. (2002) in their studies on *Ceriporiopsis subvermispora* mushroom and *Pinus teada* chips, reported that mushroom treatment caused weight loss from 2.3% to 13.8%. In the same study, it was understood that there was a loss of lignin from 9.6% to 22%, glucan from 0.9% to 2%, and extractive loss from 28% to 65%. Ferraz *et al*. (2000) reported 12% weight loss in *Eucalyptus globulus* chips treated with the same fungus for 90 days; Blanchette *et al*. (1988) stated that weight loss was 38% and lignin loss was 73% in *Betula papyrifera* chips treated with the white-rot fungus *Phanerochaete chrysosporium* BKM-F-1767 for 12 weeks.

Among the well known and applied methods, the most effective process to prevent fungal rots and therefore WL in wood and wood products is to impregnate the wood material with various chemicals and methods. The aim of impregnation is to penetrate various chemicals into the wood material in sufficient quantities and depth using different methods. Thus, the wood material, whose durability is increased by impregnation, is similarly protected from both insect damage and flammable effects (Bozkurt *et al*. 1993). With the impregnation process, the resistance of wood material against fungal rot can be increased as much as desired. However, substances, such as copper, creosote, and arsenic, in the composition of the chemicals used negatively affect living organisms and the environment. In addition, the impregnation processes pose a danger to non-target organisms, dimensional stability cannot be achieved, or dimensional changes may occur (Furniture decoration 2013). Because of their additional negative effects on the environment and human health, the use of impregnation classes with harmful properties has been limited worldwide since the recent past and today. With this potential, 117 harmful preservatives have been banned in Sweden since the 1990s to prevent harmful systems (Johansson 2005). At the same time, the development and use of more environmentally friendly production techniques, methods, and improvements that can be alternatives to harmful impregnation materials and methods in wood technology are encouraged (Korkut and Kocaefe 2009).

As an environmentally friendly production technology, heat treatment (HT) is one of the methods that improve wood material against biological degradation and to increase its resistance against biological threats such as fungi and insects (ThermoWood®

Handbook 2003). Johansson (2005) defines HT as a physical process that results in permanent changes in the chemical composition of cell wall compounds. According to the general definition, the application temperature in HT ranges between 160 and 240 °C (TS CEN/TS 15679 2010). Yildiz *et al*. (2010) report that HT gives new properties to wood material, creates lower equilibrium moisture content, and improvement in dimensional stability, and reduces the amount of moisture exchange and equilibrium moisture. As a result, the amount of work required by HT is minimized and the performance of surface treatments increases by increasing biological resistance and permeability against destructive organisms. Belt *et al*. (2024) report that HT increases the resistance of wood material to decay, and the main reason for the increase in the decay resistance of wood is related to decreasing hygroscopicity. Biological durability tests performed using *Coniophora puteana* and *Poria placenta* fungi on thermally modified pine samples indicate that the resistance of HT wood materials to decay increases.

Heat treatment of wood materials is a production technique that dates back to the 10th century, and similar studies were carried out and reported mostly in Germany and America from the 1930s to the 1970s. It is an important point that, together with the studies carried out in Finland, Germany, France, and the Netherlands since the 1990s, its potential in terms of both installed capacities and product diversity is quite valuable (ThermoWood® Handbook 2003). As a result of these studies, many HT methods, such as ThremoWood®, Platowood, oil heat treatment, rectification, and Les Bois Perdure have been developed. Among these methods, TM is more common, has a higher production volume, and is also widely commercialized compared to the others. Today, TM is the name given both as a HT method and to the modified wood material obtained after HT (Aytin 2013).

The heat treatment and similar applications in wood materials provide improvements in many properties such as fungus resistance. However, as in HT, some properties, mostly mechanical properties, are negatively affected, especially by high temperature applications. It is important to eliminate these drawbacks, which are vital for use, with different but environmentally and human-friendly production techniques. In his study on cryogenic treatment, which he considers as a new modification method for wood materials, Pamukçu (2019) states that an application such as HT can initially improve the physical properties of wood materials, while the mechanical properties of wood materials treated with high temperatures are mostly negatively affected. Pamukçu also states that for this reason, efforts are being made to overcome the problem by combining the applied modification methods with different modification methods to eliminate the negative aspects, as in HT. In addition, he states that the search for new modification methods that do not include these negativities is among today’s current issues. A limited number of studies on cryogenic (Cr) processing reveal that the decreases seen in mechanical strength values in HT wood materials can be tolerated with Cr treatment (Aytin *et al*. 2016; Aytin 2019). Opening new areas of use for wood materials and improving the performance of wood materials in existing areas of use make it possible to consider the expectations regarding the increase in fungal resistance with Cr process, in addition to the recoveries obtained with Cr process. Considering the structural features, the changes in the chemical properties of the wood material with Cr treatment (Aytin *et al*. 2022) strengthen and support the idea that such an expectation can be realized. As the number of trees treated with HT increases and its use becomes more widespread, many studies have been conducted and continue to be conducted on the determination of resistance to fungal damage in terms of suitability for the intended use of HT-treated products. Such a study has not yet been found in the literature on both natural and HT wood derivatives that have been cryogenically treated.

Cryogenic treatment is a secondary specialized process that involves rapid cooling materials to extremely low temperatures, typically below -80 °C to -195 °C, to enhance their mechanical properties and performance. This treatment has gained significant attention in various fields, particularly in materials science and mechanical engineering, due to its ability to improve wear resistance, hardness, and overall durability of metals and alloys. One of the primary benefits of cryogenic treatment is the transformation of retained austenite into martensite, which significantly enhances the wear resistance of materials. For instance, Uygur *et al*. (2015) demonstrated that Cr treatment leads to less retained austenite and more uniform distribution of carbides and the precipitation of fine carbides, which collectively contribute to improved wear resistance in AISI D3 tool steel punches. Similarly, Kara *et al*. (2021) reported that deep Cr treatment enhances the hardness and homogeniziting microstructure of Sleipner cold work tool steels by facilitating carbide precipitation and reducing residual stresses and reducing retained austenite. Moreover, the optimization of Cr treatment parameters, such as soaking time and temperature, plays a vital role in achieving desired material properties. The effectiveness of Cr treatment can vary significantly based on these parameters, which must be tailored to the specific material (Çicek *et al*. 2012; Kara *et al*. 2021, 2023). This adaptability allows for targeted improvements in performance, making Cr treatment a valuable tool in material engineering. Furthermore, Savas *et al*. (2023) indicated that Cr treatment can cause 30% energy saving the machining of GGG-42 cast iron material by uniform microstructure and increased hardness. The application of Cr treatment is not limited to ferrous materials; it has also been effectively utilized in nonferrous alloys. For instance, Ates *et al*. (2017) highlighted that deep cryogenic treatment serves as a supplementary process to conventional heat treatment, enhancing the mechanical properties of AA5xxx series Al-alloys, which were joined by MIG welding technique. This versatility underscores the importance of Cr treatment across different material types and applications. The ongoing research and development in this field continue to reveal new benefits and applications, solidifying the importance of cryogenic treatment in advancing material performance.

Cryogenic applications of wood materials are almost non-existent. In this respect, it is still unclear what kind of changes the Cr process causes in the microstructural, physical, mechanical, and chemical properties of wood materials. In the literature, only limited studies can be seen. Aytin *et al.* (2016) subjected TM *Populus tremula* samples to deep Cr treatment at -145 °C for 24 h and then measured the compressive strength parallel to the fibers, and determined that the resistance values of the Cr-treated TM samples increased 24% to 54% compared to the control samples. Aytin (2019) in another study shallow Cr was applied to TM *Sorbus torminalis* samples at -80 °C for three different periods (6, 18, and 54 hours) and an increase of up to 43% in compressive strength was observed compared to the control samples. Moreover, the findings revealed that the improvement in shrinkage and swelling continued with the heat treatment (dimensional stability increased), with an average increase of 18% and 14.5% in the pressures parallel to the fibers compared to the samples observed in the control and heat treatment, respectively. The FT-IR analysis showed that the wood compound structure was mostly cellulosic. The difference between the carbon-oxygen ratio in the cryogenically-treated wood decreased compared to the percentage change in the three basic elements, and the amount of hydrogen increased proportionally (Aytin *et al.* 2022).

Therefore, because there is no research on fungal damage testing on Cr-applied wood materials in the literature, this study is novel in terms of testing natural state, HT, and Cr-treated samples for fungus damage in wood materials. Within the scope of the study, aspen (*Populus tremula* L.), narrow-leaved ash (*Fraxinus angustifolia* Vahl), spruce (*Picea orientalis* (L.) Link.), and fir (*Abies bornmuelleriana* Mattf.) that grow naturally in Turkey and have commercial potential were used. Tree research is of particular importance as it has the potential to contribute to the most efficient use of wood raw material resources.

EXPERIMENTAL

Materials

Within the scope of the study, aspen (*Populus tremula* L.), ash (*Fraxinus angustifolia* Vahl), spruce (*Picea orientalis* (L.) Link.), and fir (*Abies bornmuelleriana* Mattf.), trees that grow naturally in Turkey, were evaluated. Trees were taken from the stand according to TS 4176 (1984) standard and then sawn into 60-mm-thick planks with the sharp cutting method according to TS 2470 (1976). Then, after drying to an average of 12% moisture, the planks were made ready for HT with dimensions of 20 × 100 × 500 (mm × mm × mm) and subjected to heat treatment with the TM method.

Heat Treatment

Wood samples prepared from working trees were subjected to HT together with air-dried wood materials in the factory of Nova Forest Products Inc. in Gerede / Bolu and TM products were produced.

Shallow Cryogenic Treatment

Afterwards, samples with nominal dimensions of 20 × 20 × 300 (mm × mm × mm) were prepared from TM templates and Cr was applied together with the control sample (CON). Shallow cryogenics was applied to the samples in a specially manufactured deep freezer.

A Core DF 490 type deep freezer with 611 L capacity and 1 °C temperature sensitivity without icing was used in the shallow cryogenic process. An N-SmArt™ control system that can store temperature data numerically and graphically for ten years with 1-h recording intervals, was used in the Core DF 490 type deep freezer and the device could be cooled down to -86 °C. After all the samples were placed in separate compartments, a 12 h pre-waiting was performed to allow the ambient temperature to reach -80 °C, and then Cr was started. At this stage, the samples were kept at -80 °C for 72 h.

Fungus Test and the Development of the Working Pattern

Test samples of 10 mm size were subjected to 12 weeks of decay test. The decay test was performed according to Japanese Industrial Standards (JIS) K 1571 (2010). Sugi (*Cryptomeria japonica* (L.f.) D. Don) sapwood specimens were also prepared in the same size and utilized as controls. All specifications were oven-dried at 60 °C before their pre-decay test weights were recorded. The sterilization was carried out under vacuum conditions with ethyhlene oxide gas for 18 h. Five species were exposed to a monoculture of either the white-rot fungus *Trametes versicolor* (L:Fr.) Pilat (Source or Fungi : Forestry and Forest Products Research Institute of Japan, FFPRI 1030) the brown-rot fungus *Fomitopsis palustris* (Berk et Curt.) Gilb & Ryv. (Source of fungi FFRPI 0507).

The nutritional media was prepared from glucose (2%), peptone (0.15%), malt extract (0.75%), and distilled water (97.1%) and immersed into white quartz sand at the bottom of glass decay containers. Three decay containers were used for 9 replicates from each wood species and treatment conditions and for each test fungus. The total number of specimens were 288, representing all four wood species and all CON, HT, and Cr groups. Furthermore, 18 sugi specimens were also tested as controls for each fungus type (Table 1).

Table 1. Classification of Working Samples and Abbreviations

Control and HT Variations				Control, HT, and Shallow Cryogenic (Cr) Variations			
Name of Group	Abbr.	Fungus		Name of Group	Abbr.	Fungus	
		COV	TYP			COV	TYP
Control (*)	CON	CONCOV	CONTYP	Control + Cr	CONCr	CONCrCOV	CONCrTYP
190 °C,1 h	TW1	TW1COV	TW1TYP	190 °C,1 h + Cr	TW1Cr	TW1CrCOV	TW1CrTYP
212 °C,1 h	TW2	TW2COV	TW2TYP	212 °C,1 h + Cr	TW2Cr	TW2CrCOV	TW2CrTYP
Sugi	SUG	SUGCOV	SUGTYP	(*) Number of samples: 24 pcs for one group			

One group of control and HT variants were used for control purposes without undergoing shallow Cr treatment and were expressed as control samples (CON) within the study. Samples subjected to shallow Cr were named as working samples and expressed with the abbreviation Cr.

Determining Weight Loss

At the end of the decay tests, immediately after removal of the surface mycelium, the specimens were oven-dried at 60 ± 2 °C for 48 h and weighed to determine their weight loss in terms of percent (%):

$$WL = \frac{M_i - M_e}{M_i} \times 100 \tag{1}$$

In the formula, WL is Weight loss, M_i is Initial weight, and M_e is End weight.

Statistical Evaluation

The SPSS package program (IBM, SPSS 15.0 for Windows, Armonk, NY, USA) was used for the statistical evaluation of the data. Analysis of variance was used to determine whether the factors influenced the results obtained, and Duncan's test was performed to determine the size of the difference on the factors that were found to be significant.

RESULTS AND DISCUSSION

The results of multiple variance analysis (MVA) regarding the WL values found after the weight loss test of *Trametes versicolor* and *Fomitopsis palustris* mushrooms in TM products are given in Table 2.

Table 2. Multiple Analysis of Variance Results of WL Values Found after the Weight Loss Test of *T. versicolor* and *F. palustris* Fungi in TM Products

Source	Type III Sum of Squares (SUM)	df	Mean Square (MS)	F	Sig.	Partial Eta Squared (PEA)
Corrected Model	58917.43	47	1253.56	12.25	0.000	0.706
Wood types (WT)	6599.48	3	2199.83	21.49	0.000	0.212
Variation (VAR)	26852.60	5	5370.52	52.46	0.000	0.522
Fungus (FUN)	1904.55	1	1904.55	18.60	0.000	0.072
WT & VAR	5881.56	15	392.10	3.83	0.000	0.193
WT & FUN	11810.21	3	3936.74	38.46	0.000	0.325
VAR * FUN	254.71	5	50.94	0.50	0.778	0.010
WT * VAR *FUN	5614.32	15	374.29	3.66	0.000	0.186
Error	24568.72	240	102.37			
Corrected Total	83486.15	287				

Based on the PEA values in Table 2, it was understood that each of the WT, VAR, and FUN factors was effective on WL. Starting from the control samples (CON), the PEA value of the groups treated with HT and Cr and classified as subgroups was determined to be 0.522 at the highest VAR factor. To see these differences more clearly, the arithmetic mean values and Duncan test results, where all sub-variations for each tree are analyzed as a whole, are given in Table 3.

Table 3. Average Values of WT in the Weight Loss Study and Results of the Duncan Test

WT	Number of Samples (N)	Duncan		
		A	B	C
Fir	72	21.55		
Ash	72		27.93	
Spruce	72		31.00	
Poplar	72			34.58

The degradation of wood material by fungi occurs at different rates in broad-leaved and coniferous trees due to the different structural properties of the trees (Bozkurt *et al.* 1993). As shown in Table 3, the WL due to *Trametes versicolor* and *Fomitopsis palustris* mushrooms varied according to tree species. Some analysis results of both mushroom species and all tree species indicate that WL occurred the least in fir and the most in poplar. However, to better understand the effect of both Cr and HT on WL, it would be useful to see the average WL values and Duncan test results of the sub-main groups for both mushrooms. For this purpose, WL values and Duncan test results of the sub-main groups are given in Table 4.

Table 4. Arithmetic Mean Values and Duncan Test Results after Weight Loss Test in Subgroups

VAR	N	DUNCAN			
		A	B	C	D
TW$_2$Cr	48	14.92			
TW$_2$	48	17.67			
TW$_1$Cr	48		27.41		
TW$_1$	48			34.70	
CON	48			38.54	38.54
CONCr	48				39.36

According to Table 4, the lowest weight loss was in TW$_2$Cr with 14.9, and the highest WL is in the CON and CONCr subgroups. According to these results, as the HT temperature increased, the decrease in WL also increased. The results also indicate that Cr further reduced WL in HT-treated samples.

To date, no fungal testing of Cr-treated wood samples appears in the known literature. In this regard, the results obtained from Cr applied samples are of particular importance in this regard. However, the fungal resistance acquired by HT against both rot fungi of the same tree species is of great importance in terms of both HT and general comparison. For this reason, the rot test results were analyzed separately for each tree with all its variations, and the results were discussed in detail for both HT and Cr. In Table 5, simple variance analysis results (BVA), averages (WL), standard deviation values of the averages (SD), and homogeneity test (HG) results for the ash tree values are given together.

Table 5. BVA, WL, SD, and HG Values of Ash Tree

BVA Results for Ash Tree		**SUM**	**df**	**MS**	**F**	**Sig.**
	Between	15120.814	11	1374.619	15.439	0.000
	Within	5342.274	60	89.038		
	Total	20463.088	71			
Average WL, Standard Deviation (SD) Values, and Homogeneity Test (HG) Results of Ash Tree	**Variation**	**WL ***	**SD**	**Variation**	**WL**	**SD**
	TW$_2$CrTYP**	5.56 (A)	1.52	TW$_1$TYP	35.52 (BCD)	7.17
	TW$_2$CrCOV	9.18 (A)	9.86	TW$_1$COV	36.90 (BCDE)	10.33
	TW$_2$TYP	9.77 (A)	6.45	CONCr TYP	39.33 (CDE)	7.64
	TW$_1$CrCOV	11.72 (A)	12.17	CONTYP	40.43 (CDE)	3.48
	TW$_2$COV	26.10 (B)	9.19	CONCr COV	42.70 (DE)	19.07
	TW$_1$CrTYP	29.39 (BC)	1.96	**CONCOV**	**48.61 (E)**	6.06
(*) Numbers in parentheses indicate standard deviation (SD).				(**) Mushroom type is given next to the subgroup abbreviations.		

According to the BVA results given for the ash samples in Table 5, it is understood that there are differences between the WL values. The Duncan test for differences found the highest and lowest WL, respectively, to be 48.6% and 5.6% in CONCOV and TW$_2$CrTYP variations. In the rot test performed on ash tree samples with the white-rot fungus *Trametes versicolor*, Cr treatment reduced the WL compared to both CON and HT, and the difference between the WL of Cr-treated samples and HT-treated samples was 68% in TW_1 samples and 64.8% in TW_2 samples. According to the results of the brown-rot fungus *Fomitopsis palustris*, WL, TW_1 samples it was 17.2% and 43% less in TW_2 samples. In the case of ash wood, WL results indicate that fungal resistance increases with Cr treatment in both fungal species. In contrast, it was determined that WL decreased with HT, and in both fungal species, the WL of TW_1 samples had lower values than the WL of CON samples.

Cross-sectional images of ash samples (UF) that were not subjected to the fungus test and ash samples after the fungus test are given in Fig. 2. In the images, mycelia belonging to fungi can be seen on the samples subjected to fungal resistance testing.

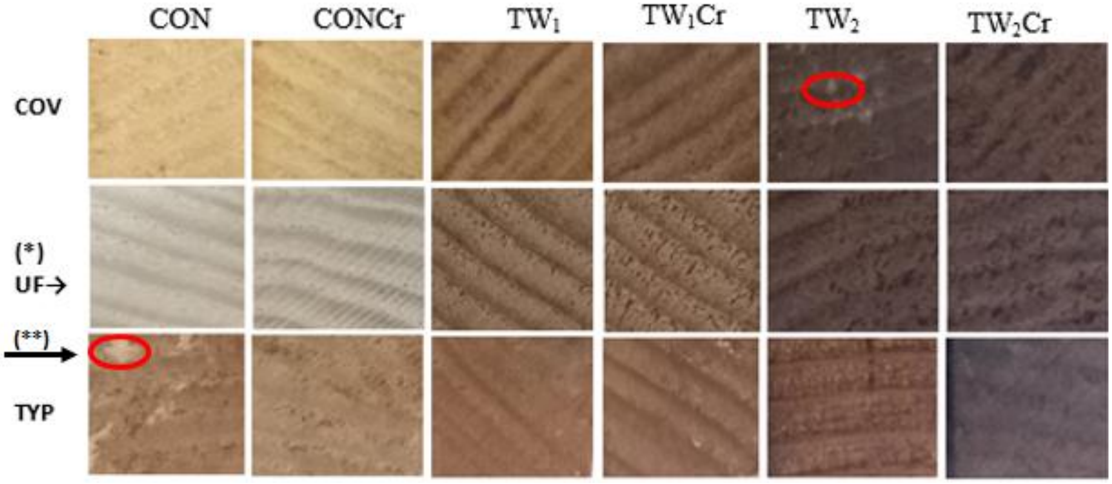

Fig. 2. Cross-sectional view of ash tree samples after the weight loss experiment (*) UF: Comparison samples that did not undergo the fungus test, (**): Fungal mycelia

According to the cross-sectional views given in Fig. 2, fungal degradation on the surface was very clearly apparent in all CON and other TW_1 variations except TW_1CrCOV. The results were compatible with the visuals of the variations and the WL ratios given in Table 5.

In Table 6, simple variance analysis, averages, standard deviation values of the averages and homogeneity test results for aspen tree values are given together.

According to the BVA results given for Aspen samples in Table 6, it is understood that there were differences between the WL values. According to the results of the Duncan test for differences, the highest and lowest WL in % were found to be 55.14 and 7.26 in CONCrCOV and TW_2CrTYP variations, respectively. In the rot test performed with *Trametes versicolor* on Aspen wood, WL occurred in 47.4% and 45.4% in TW_1 samples and TW_1Cr samples, and 24.6% and 29.6% in TW_2 and TW_2Cr samples, respectively. According to these results, there was a 4% decrease in TW_1 samples and a 20.3% increase in WL in TW_2 samples. In Aspen, WL occurred at percentages of 35.2% and 38.3% in *Fomitopsis palustris* TW_1 samples and TW_1Cr samples, respectively, and 7.3% and 11.2% in TW_2 and TW_2Cr samples, respectively. Tests performed with the *Fomitopsis palustris* mushroom show that there was an 8.7% increase in WL in TW_1 samples and a 35.1% decrease in TW_2 samples. Figure 3 shows cross-sectional images of Aspen samples that were not subjected to the fungus test and aspen samples after the fungus.

Table 6. BVA, WL, SD, and HG Values of Aspen Tree

		SUM	df	MS	F	Sig.
BVA Results for Aspen Trees	**Between Groups**	13884.702	11	1262.246	9.492	0.000
	Within	7978.675	60	132.978		
	Total	21863.377	71			
WL, SD, and HG Results of the Aspen Tree	**Variation**	**WL (*)**	**SD**	**Variation**	**WL**	**SD**
	TW$_2$CrTYP	**7.26 (a)**	5.27	TW$_1$TYP	35.20 (BCD)	18.95
	TW$_2$CrCOV	29.65 (BC)	8.90	TW$_1$COV	47.37 (DE)	8.76
	TW$_2$TYP	11.18 (A)	11.53	CONCrTYP	33.94 (BCD)	20.30
	TW$_2$COV	24.65 (B)	11.33	**CONCrCOV**	**55.14 (E)**	5.45
	TW$_1$CrTYP	38.27 (BCD)	9.82	CONTYP	46.47 (DE)	6.89
	TW$_1$CrCOV	45.38 (DE)	4.63	CONCOV	40.23 (CD)	13.55

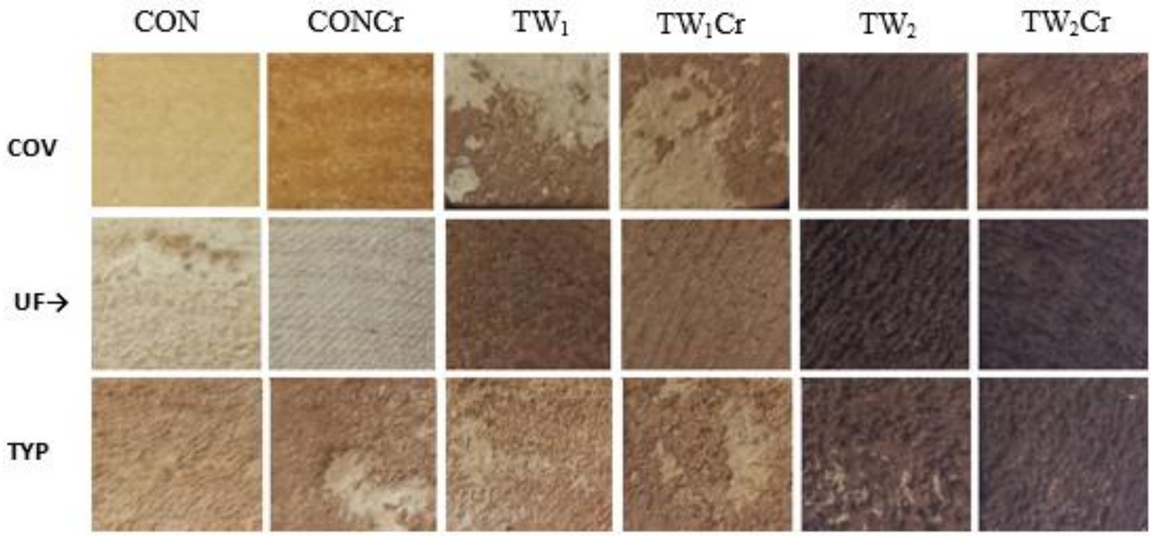

Fig. 3. Cross-sectional view of Aspen samples after the weight loss experiment

When Fig. 3 and Table 6 are examined, it is seen that both data were compatible with each other.

In Table 7, the simple variance analysis results, averages, standard deviation values of the averages and homogeneity test results regarding the fir tree values are given together.

According to the BVA results given for the fir samples in Table 7, it is understood that there were differences between the WL values. According to the results of the Duncan test for differences, it was understood that the highest and lowest WL were 38.6 and 1.20 in CONTYP and TW$_2$CrCOV variations, respectively. In the rot test performed on fir tree samples with the white-rot fungus *Trametes versicolor*, it was determined that the WL of the samples treated with Cr was lower, and there was a difference of 16.4% in the TW$_1$ samples and 66% in the TW$_2$ samples between the WL of the Cr-applied HT samples and the heat-treated samples only. According to the results of the brown-rot fungus *Fomitopsis palustris*, it was understood that WL did not change in TW$_1$ samples, but increased 28.6%

in TW$_2$ samples. In the case of fir tree, WL results show that fungal resistance produces different results between the two fungi. Cross-sectional images of fir samples that were not subjected to the fungus test and fir samples after the fungus test are given in Fig. 4.

Table 7. BVA, WL, SD, and HG Values of Fir Tree

BVA Results for Fir Trees		**SUM**	**df**	**MS**	**F**	**Sig.**
	Between	9560.349	11	869.123	7.739	0.000
	Within Groups	6738.493	60	112.308		
	Total	16298.842	71			
WL, SD, and HG Results of the Fir Tree	**Varyasyon**	**ML**	**SD**	**Varyasyon**	**ML**	**SD**
	TW$_2$CrTYP	25.24 (BCD)	10.22	TW$_1$TYP	26.27(CDE)	5.57
	TW$_2$CrCOV	**1.20 (A)**	0.64	TW$_1$COV	13.56 (ABC)	26.36
	TW$_2$TYP	19.62 (BCD)	12.27	CONCrTYP	36.80 (E)	6.13
	TW$_2$COV	3.54 (A)	1.43	CONCrCOV	25.19 (CDE)	9.74
	TW$_1$CrTYP	26.27 (CDE)	9.90	**CONTYP**	**38.58 E**	**6.16**
	TW$_1$CrCOV	11.33 (AB)	6.35	CONCOV	30.97 (DE)	7.38

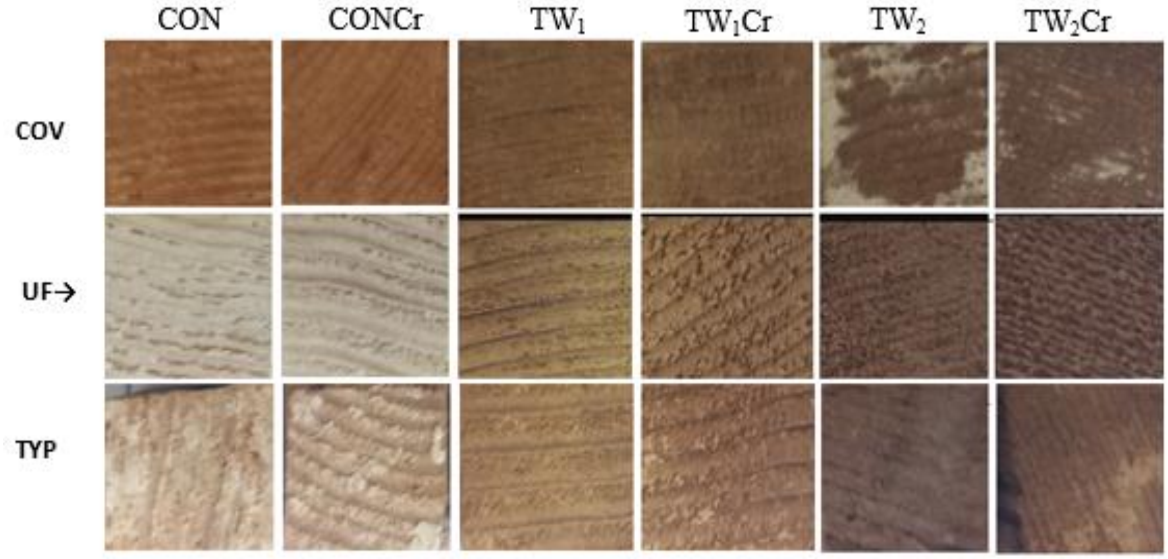

Fig. 4. Cross-sectional view of fir samples after weight loss experiment

When Fig. 4 and Table 7 were examined, it was seen that both data were compatible with each other.

In Table 8, the simple variance analysis results, averages, standard deviation values of the averages, and homogeneity test results for the spruce tree values are given together. In Table 8, it is understood that there were differences between the WL values according to the BVA given for the spruce samples. According to the Duncan test for the differences made, the highest and lowest WL values were determined as 56.2% and 7.0%, respectively, TW$_1$TYP and TW$_2$COV. In the rot test performed on spruce tree samples with *Trametes versicolor*, WL is 6.4% lower in TW$_1$ rates and 48.9% higher in TW$_2$ variations in Cr applied samples compared to HT samples alone. It is seen that the WL values in all samples

(TW_2) where Cr was applied at high HT in the same mushroom are lower than CON samples. According to the *Fomitopsis palustris* test, WL was determined to be 42.9% less in Cr-treated TW_1 samples and 30.19% less in TW_2 samples. Cross-sectional images of spruce samples that were not subjected to fungus testing and spruce samples after fungus testing are given in Fig. 5.

Table 8. BVA, WL, SD, and HG Values of the Spruce Tree

		SUM	df	MS	F	Sig.
BVA Results for Spruce Tree	**Between Groups**	13752.087	11	1250.190	16.635	0.000
	Within	4509.277	60	75.155		
	Total	18261.364	71			
WL, SD, and HG Results of the Spruce Tree	**Variation**	**WL**	**SD**	**Variation**	**WL**	**SD**
	TW_2CrTYP	27.58 (BC)	6.04	**TW_1TYP**	**56.15 (E)**	6.91
	TW_2CrCOV	13.73 (A)	2.11	TW_1COV	26.59 (BC)	1.77
	TW_2TYP	39.51 (D)	9.81	CONCrTYP	54.94 (E)	14.42
	TW_2COV	**7.01 (A)**	7.95	CONCrCOV	26.85 (BC)	5.64
	TW_1CrTYP	32.04 (BCD)	15.11	CONTYP	36.53 (CD)	11.61
	TW_1CrCOV	24.88 (B)	6.47	CONCOV	26.24 (BC)	2.24

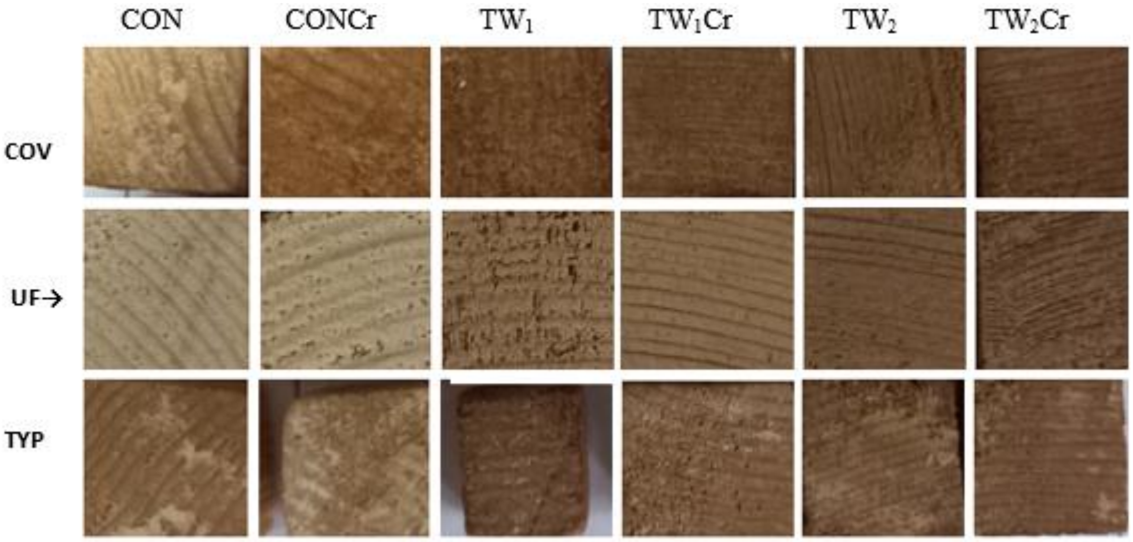

Fig. 5. Cross-sectional view of spruce samples after weight loss experiment

When Fig. 5 and Table 8 were examined, it is seen that both data were compatible with each other.

Research and review studies in the literature on the effect of HT on fungal degradation show that HT increases the resistance of wood material against fungal decay. In one of these repairs (Suri *et al*. 2023) HT-treated *Paulownia tomentosa* wood was exposed to the brown-rot fungus (*Fomitopsis palustris*) in diseases where they were exposed to the brown-rot fungus (*Fomitopsis palustris*) with cycles of WL and CON at 180 and 220 °C were determined as 13.4%, 14.7%, and 1.2%. Accordingly, although the low temperature has increased slightly in amount, the WL value, which remained almost the same, decreased quite sharply in parallel with the increase in temperature and supports 91.0%. The same researchers found WL in *Pinus koraiensis* wood as 21.8%, 32.4%, and 8.8% in terms of its sequence, and reported that WL, which increases in low HT, decreases with increasing temperature (72.8%). Belt *et al*. (2024) reported that heat treatment increases the decay resistance of wood by reducing its hygroscopicity. Candelier *et al*. (2016) in their review study on the control of wood heat treatment and its effects on decay resistance, showed that HT makes wood species with low natural durability resistant to decay. They also report that HT temperature has more effect than time on decay resistance. However, the same studies indicate that fungi also increase the hygroscopicity of wood material over time. They state that this situation will gradually deteriorate the quality of use due to fungal degradation as the period of use increases.

Determining the changes in the chemical structure of the study samples through decay tests is planned as the next step in the study to better understand the differences between the results and to serve as a source for future research studies on the subject.

Classifications are made for the tested trees according to the results obtained after rot tests carried out in various national and international standards. The WL values obtained as a result of this study were classified according to the Standard Test Method of Accelerated Laboratory Test of Natural Decay Resistance of Woods (ASTM D2017-5 2017), and are given in Table 9.

Table 9. Classification of Weight Loss Values according to ASTM D2017-5 (2017)

	COV		TYP		COV		TYP		COV		TYP	
WT*	**CON**	**CON Cr**	**CON**	**CON Cr**	**TW$_1$**	**TW$_1$Cr**	**TW$_1$**	**TW$_1$Cr**	**TW$_2$**	**TW$_2$Cr**	**TW$_2$**	**TW$_2$Cr**
Ash	-+	+	+	+	+	++	+	+	+	+++	++	+++
Aspen	+	-+	-+	+	-+	+	-+	+	++	+	++	+++
Fir	+	+	+	+	++	++	+	+	++	+++	++	+
Spruce	+	+	+	-+	+	++	-+	+	++	++	+	+
Sugi	+		+									

(*) (-+): Slightly resistant or nonresistant; (+): Moderately resistant; (++): Resistant; (+++): Highly resistant

Although HT generally increases fungal resistance, it has been determined that some variations also have opposite results. In all tree species, HT causes an increase in resistance, especially against the white-rot fungus *Trametes versicolor*, except for the TW1COV variation, while in the brown-rot fungus *Fomitopsis palustris*, resistance increases with HT in ash and Aspen, and variable resistance in fir and spruce samples. A fungal resistance is noteworthy. In contrast, because no fungus test has been performed on Cr-treated wood samples in the literature yet adds a special meaning to the results. In Fig. 6, all sub-variations and WL values of the Sugi tree are presented graphically.

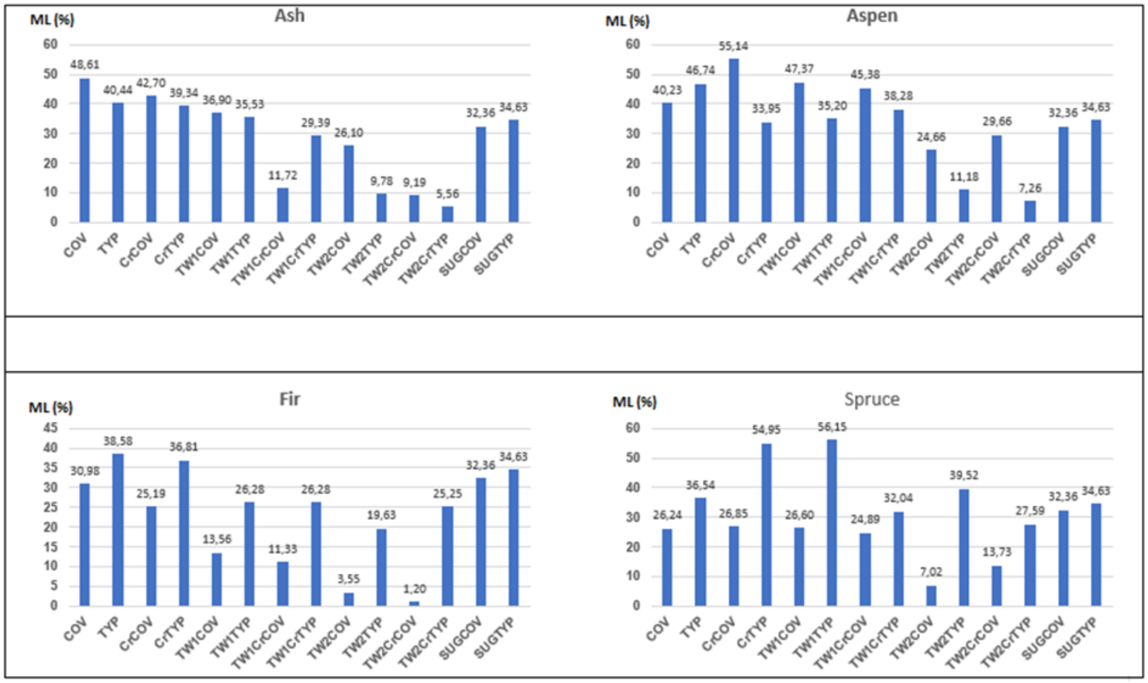

Fig. 6. WL values of working test samples and Sugi control tree

According to Fig. 6, the WL of the TW_2 test samples in both HT only and HT+Cr, except for the test with spruce TW_2 *Fomitopsis palustris*, was less than the weight losses in Sugi.

Since this study is the first in a series, the next step will be to conduct more detailed studies to better understand the decrease in weight loss in samples treated with HT through cryogenic treatment. In this context, it is planned to examine the changes in the chemical structure.

CONCLUSIONS

1. According to the general trend of the study results of the effect of cryogenic process on fungal destruction of *Trametes versicolor* and *Fomitopsis palustris* in Thermowood® products of some wood species, it has been understood that cryogenic process reduces weight loss (WL) in fungal damage. However, there are some differences between the variations, and these differences naturally arise from the tree type, mushroom type, and the modification processes applied.
2. In this study, the fungal degradation was determined in samples that were only heat-treated along with cryogenic treatment, and showed that heat treatment temperature contributed significantly to fungal resistance and that resistance increased as the temperature increased. Thus, the effect of heat treatment (HT) on the reduction of weight loss (WL) in fungal destruction is important, and as the temperature increases, weight loss also decreases. Accordingly, considering the suitability of the values in other wood properties required for the usage areas, it is recommended to apply the highest possible HT temperature.

3. According to tree species, the highest resistance in the effect of Cr treatment on fungal resistance was obtained in ash, aspen, and fir at 212 °C treatment, and in terms of heat treatment (HT) only, the same variations were obtained in ash, fir, and spruce trees. It appears that the nonheat-treated samples, which are the main control samples of the trial trees, had little or no resistance to decay.
4. All wood species that had undergone both cryogenic and heat treatment exhibited higher resistance values in almost all variations compared to the control samples. This makes it clear that it is important to highlight the fungal resistance of both processes as environmentally friendly production technologies and to consider their further improvement.

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