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AN ECOLOGICAL PROTECTION FOR WOOD MATERIAL BY HYDROLYZED FEATHER KERATIN

Within the scope of this study, the hydrolyzed keratin which is an ecological and harmless material, was applied to the wood material surfaces by dipping and spraying method in different concentration rate 1%, 3% and 5%. Within the scope of the research, as wood materials Scots pine (Pinus sylvestris L.*) and beech (Fagus orientalis* L.*) were used. Wood samples were exposed to white and brown rot fungi for 16 weeks and water absorption rate tests for 48-hour period to in order to determine protection performances. According to the results of the water absorption tests, it was observed that the keratin concentrations reduced the water absorption of wood material at least 7 times than control samples. Keratin concentrations were determined to reduce mass loss by at least 50% compared to control samples against rot fungus. As a result; it has been determined that keratin has positive effects on the protection of wood material in tests and that keratin substance can be applied as a natural preservative on wooden surfaces.*

Keywords: hydrolyzed keratin, ecological preservation, white rot, brown rot, water absorption

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Introduction

Wood material has a wide range of usage area in building and furniture industry. This renewable unique natural material is always biggest winner candidate of competition between metals, plastics etc. because of its physical, chemical and esthetical properties. On the other hand, wood can destroyed by fire, water and humidity, UV effect and biological attacks this also requires protection against mentioned factors. Coating with various protective and decorative finishes, impregnation by some chemicals are among the main protection processes applied today. Chemicals used for protection such as arsenic, copper, chromium etc. have an undeniable role in wood preservation industry, but otherwise, their toxic effects to living creatures and environment limits their use. Many countries have restricted or banned the use of these chemicals. This situation has led researchers to discover more ecological and nontoxic preservatives for wood materials.

Keratins are insoluble fibrillary proteins found in the skin of mammals, reptiles, and birds. Owing to such structural characteristics as a high content of disulfide and hydrogen bonds, keratins are resistant to both chemical and biological hydrolyzing agents [Arai et al. 1996]. The protein keratin, formed by all vertebrates, is the chief structural component of hair, scales, horn, wool, nails, and feathers [Lehninger 1984]. Keratins has been used as an active ingredient in many fields such as hair care materials shampoo and conditioner [Rouse and Dyke 2010], tanning in leather industry [Karthikeyan et al. 2007] and filtering waste water.

Robbins [2002] studied keratin as a new method based on peptide formulation and is an alternative to traditional chemical treatments for green hair care cosmetics industry. Basma et al. [2020] have developed bio-plastic films by different ratio of keratin and cellulose. Rabe et al. [2019] studied Mexican tannery waste-derived natural keratin fiber coconut processing waste as a backfill material in thermoplastic starch-polyester combination, which are biologically fissionable. These can also be provided commercially to maintain sustainable bio-composites. Pyrolysis, flammability and flame burning behaviors as well as morphological, rheological and mechanical properties of those bio-composites. Hierarchical structure of horse nail wall as a natural energy absorbing polymer composite and energy absorbing mechanism examined. In the recent research [Wang et al. 2019] combined polyurethane, keratin, and silver nanoparticles to generate a novel nano-fiber mat for wound dressing.

Villanueva et al. [2020], studied on a smart antibacterial biomaterial, based on keratin hydrogel that has an optimum pH behaviour and zinc oxide nano plats as biocidal agent. Endo et al. [2010]; developed of a new method to protect archaeological woods by using goose feather-derived keratin. When keratin treatment added into the wood, water, an increase from 10% to 40% in hydrolysed

feather solution concentration is observed. In the case that polyethylene glycol to be used as protection method treatment, an increase up to 90% is observed.

According to the literature, there have been few research on the use of keratins as a preservative on wood products. The aim of the present work is to develop an ecological protection way for wood material by utilization of hydrolyzed keratin.

Materials and methods

Materials

Wood specimens prepared from pine (*Pinus sylvestris* L.) and oriental beech (*Fagus orientalis* L.), decay resistance samples according to standard EN 113 (1996) with a sample size of 50 × 25 × 15 mm, according to TS 4083 (1998) for water absorption rate 20 × 20 × 20 mm. All specimens conditioned at 20 ± 2 °C and 65 ± 3 % RH for 3 weeks before the feather keratin treatments. Hydrolyzed keratin in 95% purity purchased from TCI America Company (K0043). Brown (*Rhodonia placenta*) and white (*Trametes versicolor*) obtained from culture collection of Mugla Sitki Kocman University, Mushroom research center.

Methods

Hydrolized keratin was diluted with water in the ratios 1%, 3% and 5%. The concentrations achieved applied to ten wood specimens for each group by dipping and spraying methods. Dipping method executed in a glass container such that the wood specimens remained completely in the liquid at 22 °C for 1 hour. In the spraying method application applied to wood species in two layers by a non-pressure spray gun.

Keratin load on wood material was calculated by following equation as mass percent gain (WPG) (%, w/w):

$$WPG\ (\%w/w) = \frac{(Wof - Woı)}{W_{oı}} \times 100 \qquad (1)$$

In this equation;
W_{of} is the oven-dry mass (g) of a wood specimen after impregnation,
$W_{oı}$ is the oven-dry mass (g) of a wood specimen before impregnation.
The treated wood blocks were stored for four weeks according to standart [TS 5563 EN 113] in a conditioning room at 20 ± 2 °C and 65 ± 3% relative humidity until they reached a stable mass before the decay resistance tests.

Fungal Decay Resistant Tests

Fungal decay resistant tests operated in Mugla Sitki Kocman University, Mushroom Research Center Laboratory according to TS 5563 EN 113. Wood specimens treated with keratin and untreated control group were exposed to a brown rot *Rhodonia placenta* (Fr.) M.J. Larsen & Lombard (Mad-698-R) and a white rot, *Trametes versicolor* (L: Fr.) Pilat. (FFPRI 1030). Ten replicates were used for each group. Wooden test samples were placed in glass jars and sterilized in an autoclave of Hiramaya brand at 121 °C for 15 minutes. Sterilized control group and treated samples were placed in un-inoculated petri dishes. Specimens were placed on the growing mycelium in each petri dish, and then were incubated at 20 °C and 70% relative humidity for 16 weeks. Percent mass losses were calculated by equation according to TS 5563 EN 113 in the 103 ± 2 °C oven-dried masss of each specimen before and after the decay test.

$$Mass\ loss\ (\%) = [100 \times \frac{T_1 - T_2}{T_1}] \quad (2)$$

In this equation:
T_1 is the mass of the wood specimen plus remaining preservative after conditioning and before exposure to the test fungus,
T_2 is the mass of the wood specimen after test and after final conditioning.

Water absorption rate

For determination water absorption rate (WA) of treated and untreated wood specimens, 190 samples (10 replicates per group) were prepared. Deionized water at room temperature. The wet mass measured by sensitive scales after 2, 8, 12, 24 and 48, immersion periods. The WA calculated according to:

$$WA = [(M_a - M_b) \div M_b] \times 100 \quad (3)$$

M_a: wet masss after immersion period (g)

M_b: bone dry mass before immersion period (g)

Statistical Analysis

The data obtained within the framework of the study were analyzed using the SPSS (Statistical Package for the Social Sciences) statistical package program and based on the 95% confidence level, and the statistical difference between them was revealed by analysis of variance. Duncan test was applied to determine the factors among which the obtained differences were.

Results and discussion

Water absorption rate

Amount of water absorption rate of pine wood samples treated with keratin concentrations in various proportions are given in Table 1. Water absorption rate in pine samples, after 48 hours were measured between 5.44% and 67.02% on average.

According to Table 1 it was seen that the samples absorbed most of the total amount of water in the first 8-24 hours. In the first 2 hours period, it was observed that the keratin applied samples did not absorbed water, while the control samples absorbed 42.84%. In fact, the rate of water absorption for up to 4 hours was measured as 0% in samples treated with 1% keratin by dipping method. Generally, it observed that the dipping method better performance than the spraying method.

Water absorption rate of oriental beech wood samples treated with keratin concentrations in various proportions are given in Table 2.

It was seen in the Table 2 that the oriental beech wood samples absorbed most of the total amount of water at the first 8-24 hours. In general, it was found that in the first 2 hours period, almost no water was absorbed on keratin applied samples. Minimum water absorption rate, measured on 3% keratin applied group as 5.12 % by dipping method and maximum rate measured on control group 70.18 %.

Because of immersing the samples in water for 48 hours, it was observed that the water uptake rate in the keratin applied samples remained below 10%. On the other hand, controls showed a progressive increase in water absorption over time. It can be said that keratin application on wood materials by the dipping method better protection than the spraying method, the reason for this may be that the amount of keratin retention to the wood material in the dipping method is higher. However, it has been observed that as the rate of keratin concentration applied increases, the water uptake rate of the wood material increases. Meanwhile, it has been reported that water logged wood treated with feather keratin, have good dimensional stability of its high crystallinity and anti-alkali, structure [Endo et al. 2008].

Table 1. Water Absorption Rate of Pine (%)

Rate	Method	2 hours		4 hours		8 hours		24 hours		48 hours	
		Mean	*Std. Dev.*	*Mean*	*Std. Dev.*	*Mean*	*Std. Dev.*	*Mean*	*Std. Dev.*	*Mean*	*Std. Dev.*
Control	-	42.84	8.74	46.37	6.15	52.57	6.05	59.87	7.58	67.02	7.22
1 %	*Dipping*	0.00	0.00	0.00	0.00	2.50	0.23	3.99	1.25	5.44	1.34
	Spray	0.00	0.00	1.13	0.56	3.54	1.22	6.42	2.63	8.73	2.42
3 %	*Dipping*	0.00	0.00	2.10	0.94	4.77	1.51	7.10	2.17	8.41	2.70
	Spray	0.00	0.00	2.27	1.33	5.64	1.14	7.43	2.00	9.37	1.18
5 %	*Dipping*	0.13	0.02	1.64	0.50	3.35	1.28	4.81	1.21	7.49	2.20
	Spray	0.24	0.01	2.29	0.92	5.55	1.98	6.49	1.09	9.43	2.68

Table 2. Water Absorption Rate of Oriental Beech (%)

Keratin Ratio	Method	2 hours		4 hours		8 hours		24 hours		48 hours	
		Mean	*Std. Dev.*	*Mean*	*Std. Dev.*	*Mean*	*Std. Dev.*	*Mean*	*Std. Dev.*	*Mean*	*Std. Dev.*
Control		30.96	3.98	49.38	5.67	54.51	7.64	64.28	1.79	70.18	1.84
1 %	*Dipping*	0.05	0.00	1.00	0.27	1.85	0.31	3.11	1.01	5.57	1.09
	Spray	0.00	0.00	0.49	0.09	1.03	0.21	4.86	1.92	5.69	1.49
3 %	*Dipping*	0.00	0.00	0.38	0.84	1.61	0.09	4.27	1.22	5.12	1.02
	Spray	0.00	0.00	0.48	0.06	2.26	0.96	4.76	1.40	5.61	1.55
5 %	*Dipping*	0.00	0.00	0.81	0.11	1.48	0.86	4.47	1.54	6.98	1.83
	Spray	0.00	0.00	0.48	0.06	2.36	0.52	5.70	1.34	7.80	1.39

Mass Losses

Table 3 shows the average percentage of retention rate, pH value of concetration and mass loss for the untreated samples (control) and for the samples treated with various concentrations of keratin after to brown rot (*Rhodonia placenta*) and white rot (*Trametes versicolor*) 16 weeks.

According to Table 3 minimum retention rate measured on 1% keratin application by spray method on pine and beech wood samples 5.33% and 4.68%. In the pine samples, the highest retention amount was measured in samples where 3% keratin was applied by dipping method, while on 5% keratin application by dipping method 8.89%. It has been observed that the application method increases the amount of retention and generally, the highest retention rate was obtained by the dipping method. There was no significant difference on pH values keratin concentration.

The highest mass loss average due to brown rot fungus on and beech wood samples was measured respectively as 23.41% and 21.96 % in the control group. The lowest mass loss value for booth wood species where 1% keratin was applied by spraying method pine wood 8.45% and for beech wood 7.63%. Mass loss after white rot fungus. The lowest average value of mass loss for pine wood samples was measured as 13.42% in the group where 5% keratin was applied with the dipping method. Beech wood samples 23.03% in the group where 5% keratin was sprayed.

The mass loss arithmetic mean of the experimental samples coated with hydrolyzed keratin concentrations exposed to brown rot fungus were found to be different, and the results of the multiple variance analysis performed to determine the factors causing differentiation are given in Table 4.

According to the Table 4 of multiple variance analysis, it was determined that the wood type was statistically significant ($P < 0.05$) in the mass loss due to brown rot fungus in the samples, while the other factors were insignificant ($P > 0.05$). The results of the paired comparison Duncan test on the mass loss values of the hydrolyzed keratin due to the brown rot fungus at the wood species level are given in Table 5.

Table 3. Mass Losses of Wood Species Exposure to Brown rot and White rot

			Scots pine			*Beech*		
Keratin Ratio	*Method*	*pH value of concetration*	*Retention Rate % (Std. Dev.)*	*Brown rot Mass Loss % (Std. Dev.)*	*White rot Mass Loss % (Std. Dev.)*	*Retention Rate % (Std. Dev.)*	*Brown rot Mass Loss % (Std. Dev.)*	*White rot Mass Loss % (Std. Dev.)*
Control	*Non Treated*		---	23.41(2.53)	26.91(2.49)	---	21.96(1.60)	40.70(2.30)
1 %	*Dipping*	8.01	6.14(1.00)	9.65(1.35)	20.96(2.09)	5.37(1.42)	9.16(1.14)	29,87(1.59)
	Spray		5.33(0.10)	8.45(1.34)	23.18(2.73)	4.68(1.18)	7.63(1.69)	24,12(2.16)
3 %	*Dipping*	7.84	11.41(0.86)	10.25(1.89)	17.12(1.76)	5.81(1.51)	10.61(2.14)	24,66(2.27)
	Spray		9.37(1.30)	12.65(2.18)	15.31(1.51)	5.61(1.55)	9.17(1.41)	23,69(1.94)
5 %	*Dipping*	7.78	8.08(0.49)	12.23(2.15)	13.42(1.12)	8.89(1.57)	9.96(1.03)	23,18(2.15)
	Spray		6.16(0.66)	11.90(2.16)	13.50(1.69)	7.80(1.39)	12.35(2.17)	23,03(1.97)

Table 4. Multiple Variance Analysis for Mass Losses Brown rot

Factors	*Sum of Squares*	*Degrees of Freedom*	*Mean Square*	*F-value*	*P-value**
A: Wood species	187.43	2	93.71	7.91	.00*
B: Keratin ratio	67.66	2	33.83	2.86	.06 NS
C: Method	1.01	1	1.01	0.09	.77 NS
*Interaction A*B*	36.53	4	9.13	0.77	.55 NS
*Interaction A*C*	2.04	2	1.02	0.09	.92 NS
*Interaction B*C*	0,55	2	0,28	0.02	.98 NS
*Interaction A*B*C*	63.17	4	15.79	1.33	.26 NS
Error	995.26	84	11.85		
Total	16778.21	105			

NS: not significant

Table 5. Mass Loss Values at Wood Species Level Due to Brown Rot Fungus

Keratin Ratio	Mean	Group of homogeneity
Beech	11.54	AB*
Scots Pine	12.64	B

Table 5 shows the Duncan test results regarding the mass loss values due to brown rot fungus the wood species, the highest mass loss value was found to be 12.64% in beech wood and the lowest mass loss value was found as 11.54% in pine wood.

The mass loss arithmetic mean of the experimental samples coated with hydrolyzed keratin concentrations exposed to white rot fungus were found to be different, and the results of the multiple variance analysis performed to determine the factors causing differentiation are given in Table 6.

Table 6. Multiple Variance Analysis for Mass Losses White rot

Factors	*Sum of Squares*	*Degrees of Freedom*	*Mean Square*	*F-value*	*P-value**
A: Wood species	1760.12	2	880.06	57.16	.00*
B: Keratin ratio	777.31	2	388.66	25.24	.00*
C: Method	9.70	1	9.70	0.63	.43 NS
*Interaction A*B*	76.36	4	19.09	1.24	.30 NS
*Interaction A*C*	30.05	2	15.02	0.98	.38 NS
*Interaction B*C*	9.35	2	4.68	0.30	.74 NS
*Interaction A*B*C*	77.10	4	19.27	1.25	.30 NS
Error	1293.32	84	15.40		
Total	54040.26	105			

NS: not significant

Table 6 shows the multiple variance analysis, it was determined that the wood species and the keratin ratio were statistically significant ($P < 0.05$), while the other factors were not ($P > 0.05$).

The results of the paired comparison Duncan test regarding the mass loss values of the hydrolyzed keratin due to the white rot fungus at the wood species level and keratin concentration ratio are given in Table 7.

Table 7. Mass Loss Values at Wood Species and Keratin Ratio Levels Due to White Rot Fungus

Homogeneity Group of Wood Species		
Wood Species	**Mean**	**Group of homogeneity**
Scots Pine	18.62	A*
Beech	27.03	B
Homogeneity Group of Keratin Concentration Ratio		
Keratin Ratio	**Mean**	**Group of homogeneity**
5%	16.67	A*
3%	18.86	A
1%	23.71	B
Control	31.50	C

According to the results of the paired comparison Duncan test results regarding the mass loss values due to white rot fungus at the tree species level, the

lowest mass loss value was found to be 18.62% in pine wood and highest 27.03% in beech wood samples.

Results of the paired comparison of the mass loss values due to white rot fungus at the mixing ratio level, the lowest mass loss value was found as 16.67% in the 5% keratin applied group, and the highest mass loss was found as 31.50% in the control group without keratin. Wood preservatives usually used to protect the wood materials against rot fungi. In the present study, the three different keratin concentration rate had a significant antifungal activity against wood decay fungi. With respect to standard TS EN 350, species with mass loss less than 5% is classified as very durable, 5 to 10% as durable, 10 to 20% as moderately durable, 20 to 30% as slightly durable and above 30% as not durable. In this study, from the above classifications it has been found that all applied keratin concentration rates can be categorized in very durable or durable class against brown rot and durable or moderately durable class against white rot.

In both wood species, it was observed that keratin ratio increased the protection rate against the white rot fungi increased, while the protection against brown rot fungi decreased. Keratin contains; a large amount of the amino acid cysteine compared to other proteins depending on the keratin source [Barone et al. 2005; Vicent 1990 and Fraser 1972]. Cysteine (C) is a sulfur-containing amino acid and can form sulfur–sulfur (S–S) cystine bonds with other intra- or intermolecular cysteine molecules [Barone et al. 2005]. The crosslinks plus other protein structural features, such as crystallinity and hydrogen-bonding, gives keratin high strength and stiffness [Barone 2005 et al.; Fraser 1980]. We deduced that, at the low concentration cysteine and other substances in keratin can easily be dissolved in concentrations the fungus. However, the amount of these substances increases at high concentrations. Degradation mechanism of fungal attack to wood can be oxidative attack and breakdown of lignin by hydroxyl radicals. Hence, these compounds may act as a sink to react with hydroxyl radicals, preventing attack against the wood structure [Temiz et al. 2013a,b; Yilgor and Kartal 2010; Temiz et al. 2010; Mohan et al. 2008; Mourant et al. 2005]. The increase in mass loss at the high concentrations on brown rot affects may be explained as keratin is predicted to dissolve nutrients in the wood material that can be easily destroyed by brown rot fungi and mass loss increases. However, it can be said that keratin provides adequate protection and can be an alternative wood preservative to chemicals, known toxic and limited compounds.

It can be concluded that the protection mechanism against brown and white rot fungi of keratin concentrations applied to wood material can be attributed to the presence of antifungal structures in its chemical composition. Hydroxyl radicals can explain degradation mechanism of fungal attack to wood because of oxidative attack and breakdown of lignin. Hence, these compounds may act as a sink to react with hydroxyl radicals, preventing attack against the wood structure [Mourant et al. 2005; Mohan et al. 2008; Yilgor and Kartal 2010; Temiz et al. 2010; Temiz et al. 2013

a,b]. Keratin can be an alternative wood preservative to chemicals and known toxic and limited compounds.

Conclusion

As conclusion, some of experiments were performed to determine the properties of feather keratin applied to wood samples such as white and brown rot fungus and water absorption rate tests. Results were showed that the mass losses of both wood species was reduced by at least 50% compared to the control group with keratin application. In addition, keratin provided an extraordinary protection on the test samples against water absorption. Because of the tests, keratin concentrations were observed to reduce the water absorption rates of wood material by at least 7 times. The great performance of keratin in wood material highlighted the possibility of using keratin obtained from feathers, an ecological waste resource, in wood preservation, according to these findings.

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TS 4083:1983 Determination of Shrinkage in Radial and Tangential Direction in Wood, Turkish Standards Institution, Ankara, Turkey

TS EN 350:2016 Durability of wood and wood-based products - Testing and classification of the durability to biological agents of wood and wood-based materials. Turkish Standards Institution, Ankara, Turkey

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