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Environmentally friendly wood preservatives formulated with enzymatic-hydrolyzed okara, copper and/or boron salts

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ABSTRACT

Novel biocides, such as copper azole (CuAz) and ammoniacal copper quaternary (ACQ), are extensively used as substitutes for chromate copper arsenate (CCA) in wood preservation. However, the expense of these biocides has necessitated the development of cost-effective and environmentally friendly wood preservatives. This study was conducted to investigate the effectiveness against decaying fungi of the preservatives formulated with enzymatic-hydrolyzed okara (OK), which is an organic waste produced from the manufacture of tofu, $CuCl_2$ (CC) and/or $Na_2B_4O_7{\cdot}10H_2O$ (B). With the addition of NH_4OH as a dissociating agent, the addition of OK facilitated the target retention of most of the OK/CC and OK/CC/B preservative formulations in wood blocks. The OK-based wood preservatives (OK-WPs) were stable against hot-water leaching. When compared with control and CC-treated wood blocks, the leached wood blocks treated with OK/CC and OK/CC/B formulations showed excellent decay resistance against both *Postia placenta* and *Gloeophyllum trabeum*, especially when OK was hydrolyzed by Celluclast at a loading level of 0.1 ml/g. Scanning electron microscopy (SEM) and SEM-energy dispersive X-ray (SEM-EDX) spectrometry analyses demonstrated that preservative complexes, such as OK–CC and OK–CC–B, existed in the wood blocks treated with OK/CC and OK/CC/B formulations. This study results support the potential application of OK-WPs as environmentally friendly wood preservatives capable of replacing CuAz and ACQ.

1. Introduction

The toxicity of chromium and arsenate elements, which are released from chromated copper arsenate (CCA)-treated wood, against human health and the environment has generated a lot of controversy [1–4]. As a result, the use of CCA-treated wood for residential purposes was prohibited by the United States Environmental Protection Agency, but CCA is still extensively used to protect wood for outdoor uses [5]. Additionally, when CCA-treated wood is removed from service, its disposal can cause serious environmental problems because it retains high levels of toxic elements. Humar et al. [6] predicted that the volume of the CCA-treated waste wood would be 16 million m^3 in 2020. Therefore, considerable attention has recently been focused on the development of environmentally friendly, alternative wood preservatives to CCA.

The wood preserving industry has been engaged in several efforts to manufacture effective wood preservatives that are environmentally more acceptable. Consequently, novel biocides, such as copper azole (CuAz) and ammoniacal copper quaternary (ACQ), have become a predominant choice worldwide in today's wood preservation systems from the end of the 1990s [7]. However, due to the high cost of these biocides compared to CCA, several researchers have investigated the development of new effective and economically practicable, preservation systems. For example, natural resources, such as egg albumin, milk casein [8] and soy protein products [9,10], and industrial wastes, such as lignin [11,12] and tannin [13], were used as a raw material in their preservative formulations, because these resources are readily available in large quantities with preservative complexes retained from aqueous solutions, and might have a potential as inexpensive adsorbents. However, in Korea, such resources are unsuitable as ingredients in newly developed preservation systems because of their high cost or rarity.

Okara (OK) is the residue generated as a byproduct during soymilk and tofu production. In Korea, approximately

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310,000 tonnes of OK were obtained from tofu production in 2004, but most of the OK were dumped and burnt as waste [14]. Consequently, OK is readily available in sufficient quantities at very low or zero cost. In addition, the chemical composition of OK, which contains about 27% proteins, 53% fibers and carbohydrates, 12% fats and oils and 8% ashes by oven-dry weight, is promising for its application as an ingredient in a wood preservative system. For instance, protein can be retained by boron and metals such as copper and zinc to form water-insoluble complexes, suggesting that these complexes might provide preservative efficacies against fungal growth. Based on OK's characteristics, Ahn et al. [15] formulated several OK-based wood preservatives (OK-WPs). In the preservative formulations, OK was hydrolyzed chemically to form more complexes with copper and/or boron salts, and the preservatives had good decay resistance against fungal attacks.

The enzymatically hydrolyzed OK might also be used in the formulation of OK-WPs, because only a catalytic amount of enzyme is required, thereby minimizing safety concerns. In addition, the use of enzymes for hydrolyzing OK can significantly reduce the use of heat, pressure, and corrosive chemicals in the OK hydrolysis process. Therefore, the objective of this study was to fully explore the potential of the wood preservatives formulated with enzymatically hydrolyzed OK, copper chloride and/or sodium borate.

2. Materials and methods

2.1. Materials

Okara (OK), obtained from CJ Food Ltd. Co., which is the biggest tofu manufacturer in South Korea, was stored in a freezer at −4 °C before used in the preservative formulations. Three enzymes, Celluclast® 1.5L FG (cellulase produced from *Trichoderma reesei*), Pectinex 5 XL (pectate lyase and arabinase produced from *Aspergillus niger*) and Savinase® 16L Type EX (protease produced from *Bacillus* microorganism), were provided by Enzyme-tech Co. (Yongin, South Korea). Other chemicals, such as copper chloride ($CuCl_2$: CC), sodium borate ($Na_2B_4O_7{\cdot}10H_2O$: B) and ammonium hydroxide (NH_4OH), were purchased from Duksan Chemical Co. (Yongin, South Korea).

Wooden planks of Scots pine sapwood (*Pinus sylvestris*) were obtained from Sanga Timber Co. (Incheon, South Korea), and cut into wood blocks with dimensions of 2.54 cm × 2.54 cm × 2.54 cm.

2.2. Preservative formulations

The OK-WPs formulated for this study are presented in Table 1. To formulate these preservatives, OK (6 g) was suspended in 300 ml of deionized water and stirred for 10 min to obtain a uniform dispersion. The pH/temperature of the dispersion was adjusted to 5.5/60 °C for Celluclast® 1.5L FG, 4.5/50 °C for Pectinex 5XL and 8.3/50 °C for Savinase® 16L, respectively. Then, each enzyme was added into the dispersion, and the mixtures were incubated in a shaker (Lab-Line-Environ-Shaker; Lab-Line Instrument, Inc. Melrose Park, IL) at 37 °C for 12 h. To examine the effect of the enzyme on the treating and leaching properties and decay resistance of the OK-WPs, three enzymes, which were cellulase, pectinase and protease, were used either separately or together. The amount of each enzyme was adjusted to 0, 0.1 and 0.2 ml per 1 g OK. To obtain a suspension of OK-WPs, CC and/or B were added into the OK hydrolyzate solutions at an OK/CC or OK/B weight ratio of 1–1, or 1–0.5, respectively. Before the wood blocks were treated by the suspension, the formulation of the OK-WPs was completed by the addition of 20 ml NH_4OH into the suspension.

Table 1
Type of okara-based wood preservatives formulated in this study and its treatability and leachability.

Salt type	Hydrolysis conditions of okara				Treatability[b] (%)	Leachability[c] (%)
	Enzyme loading[a] (ml/g)			Time (h)		
	Celluclast	Pectinex	Savinase			
$CuCl_2$	0	0	0		62.43 (10.43)	3.18 (0.78)
			0	12	61.31 (13.12)	7.17 (1.18)
		0	0.1	24	57.84 (14.57)	8.10 (1.82)
			0.2	24	60.59 (17.82)	8.26 (1.23)
	0.1		0	24	67.28 (18.24)	8.37 (1.48)
		0.1	0.1	36	98.48 (14.55)	9.19 (0.86)
			0.2	36	100.46 (20.66)	9.32 (1.79)
			0	12	62.48 (5.02)	7.64 (0.66)
		0	0.1	24	71.76 (11.94)	8.35 (1.05)
			0.2	24	79.78 (5.44)	8.44 (1.71)
	0.2		0	24	78.55 (7.67)	8.34 (1.35)
		0.1	0.1	36	96.99 (4.97)	8.20 (0.53)
			0.2	36	116.39 (10.52)	9.43 (1.59)
$CuCl_2 + Na_2B_4O_7{\cdot}10H_2O$	0	0	0		83.51 (10.48)	3.75 (0.49)
			0	12	84.02 (19.43)	7.99 (1.49)
		0	0.1	24	84.26 (11.06)	8.85 (1.94)
			0.2	24	88.32 (24.43)	10.51 (2.09)
	0.1		0	24	98.93 (11.73)	10.38 (2.92)
		0.1	0.1	36	122.45 (50.27)	10.60 (2.68)
			0.2	36	129.91 (12.44)	10.34 (2.99)
			0	12	82.27 (10.88)	8.74 (1.14)
		0	0.1	24	116.04 (17.07)	9.46 (1.75)
			0.2	24	110.23 (9.84)	9.86 (1.31)
	0.2		0	24	96.72 (15.95)	8.17 (2.44)
		0.1	0.1	36	122.24 (8.56)	10.90 (1.41)
			0.2	36	121.78 (10.25)	9.76 (1.29)

Number in parenthesis, which is in the columns of treatability and leachability, is a standard deviation of mean of each formulation.
[a] Enzyme loading means the volume of enzyme (ml/1 g okara) used for the hydrolysis of okara.
[b] Treatability means the percentage of actual retention to the target retention.
[c] Leachability means the percentage preservatives leached from treated specimens.

Fig. 1. Images of the laboratory pressure cylinder used for the treatment of preservative formulations (left) and the extraction apparatus used for the hot-water leaching of the wood blocks treated with preservative formulations (right).

2.3. Treating and leaching procedures

Wood blocks were immersed in each OK-WP solution for 20 min under vacuum (500 mm Hg), followed by 20 min pressure (1 MPa) in a laboratory pressure cylinder (Fig. 1). The weight gain of each wood block by preservative formulations was measured with the procedure reported by Ahn et al. [15]. The difference between the final dry weight after treatment and the original dry weight of each wood block was designated as the treatability of the preservative formulation.

Treated wood blocks were placed in a 3-l extractor and leached in 70 °C water for 72 h (Fig. 1). The hot water was replaced with fresh condensate at a rate of approximately 350 ml per hour. After leaching, the wood blocks were dried, and then weighed. The percentage weight loss of the OK-WP-treated wood blocks due to hot-water leaching was designated as the leachability of each preservative formulation.

2.4. Decay resistance of leached wood blocks

The decay resistance of the OK-WP-treated wood blocks was determined after leaching in compliance with ASTM Standard D 1413-05b [16]. For the decay trial, *Postia placenta* (KCTC No. 6671) and *Gloeophyllum trabeum* (KUC No. 8013), obtained from Korean Forest Research Institute and Korea University, respectively, were used as the test fungi.

For the decay test, fungi cultured on potato dextrose agar were inoculated on the surface of the soil in culture bottles sterilized for 30 min. After the surface was covered with fungal mycelia, sterilized wood blocks were placed onto the surface, three blocks per bottle. After the soil block culture was incubated at 26 ± 1 °C and 75% relative humidity for 12 weeks, the wood blocks were removed from the culture bottles, and their surfaces were completely cleaned of fungal mycelia by brush. The wood blocks were air-dried for 24 h, oven-dried at 80 °C overnight, and then weighed to determine the percentage weight loss attributable to exposure to the decay fungus.

2.5. Analyses of copper and protein

In order to measure the contents of copper and protein leached from the OK-WP-treated wood blocks, the leachates were analyzed by inductively coupled plasma (ICP) emission spectrometer with a Perkin-Elmer Optima 4300 DV. After determining the weight loss of the leached wood blocks, the matchstick-like samples with dimensions of 2.54 cm × 0.2 cm × 0.2 cm (L, R, T) were prepared from the blocks. Then, 2 g of the samples was put in the thimble and extracted with 100 ml distilled hot water at 98 ± 1 °C in the extractor for 2 h. The extracted solutions were subjected to ICP analysis at a detection wavelength of 324.8 nm to detect the copper content.

To examine the protein content in the leachates and extracted solutions, total nitrogen and non-protein nitrogen were determined with an Automatic Kjeldahl protein/nitrogen analyzer (Kjeltec Auto 1035/1038 System, Tecator AB, Sweden) by the modified Kjeldahl method as described by Hambraels et al. [17]. To determine the non-protein nitrogen, the proteins were precipitated by trichloroacetic acid (12%, w/v). Protein nitrogen was calculated indirectly by subtracting non-protein nitrogen from total nitrogen. All analysis results are the averages of three measurements.

2.6. Microscopic observation by SEM

After the decay test of the leached wood blocks was completed, the wood blocks were sliced into thin samples with dimensions of 2.54 cm × 0.2 cm × 0.2 cm (L, R, T) by razor blade. The samples, placed on an aluminum stub, were sputter-coated with a thin layer (approximately 20 nm thick) of platinum. The specimens were observed with a field emission scanning electron microscope (FE-SEM, Supra 55VP; Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 3 kV. Random observations were made of different structures to identify the existence of complexes, such as OK–CC, OK–B, OK–CC–B or CC–B, in the anatomical structure of the samples. In addition, the elemental composition was determined by using an energy dispersive X-ray analyzer (EDX, XFlash 4000; Bruker AXS Microanalysis, Berlin, Germany) combined with SEM. X-rays were collected with a detector fixed at a take-off angle of 35°, and their intensities were recorded in counts per second.

2.7. Statistical analysis

Twelve wood blocks were treated with each formulation, and the treated wood blocks were then leached. For the decay trials, 6 wood blocks were randomly assigned for exposure to either *P. placenta* or *G. trabeum*. The effects of each variable – such as the type

and loading levels of enzymes used for the OK hydrolysis and the addition of B for formulating OK/CC preservative solutions – on the treatability, leachability and decay resistance of the OK-WPs were examined by the General Linear Model procedure with the Statistical Analysis System programming package. A 95% confidence level was used in all statistical tests. If a significant effect was found in a variable at $p < 0.05$, the data of two samples of the variable were compared based on Student's t-test at $\alpha = 0.05$.

3. Results and discussion

3.1. Treatability

When CC or CC/B was added into a suspension of OK hydrolyzates, the viscosity of the mixtures was remarkably increased. Increase of the viscosity is probably due to the existence of various complexes, such as OK–CC, OK–B, OK–CC–B and CC–B, formed by the chelating reaction of OK, CC and/or B, in the mixtures [10,13,15]. However, the addition of ammonium hydroxide into the OK/CC and OK/CC/B formulations as a dissociating agent reduced the molecular weight of the complexes (OK–CC and OK–CC–B) sufficiently to allow them to penetrate freely into the wood blocks, which may have relieved the penetration problem.

The treatabilities of the OK-WPs were considerably affected by the experimental variables. At first, the addition of B into the OK/CC formulations increases the treatability ($p = 0.01$), as shown in Fig. 2. For example, the treatability of OK/CC/B (104.87%) was much higher than that of OK/CC (79.32%), and this was attributed to the difference of molecular weights between the complexes, formed in the OK/CC and OK/CC/B formulations. Thevenon et al. [8] suggested that the molecular weight of the OK–B and CC–B complexes might be smaller than that of the OK–CC complexes, thereby lowering the treatability of OK/CC compared to that of OK/CC/B.

Secondly, the treatability of the OK-WPs was affected by the type of enzymes used for the OK hydrolysis. For example, at the 0.1 ml/g loading level of each enzyme, the treatabilities of the preservatives formulated with CE-OK (cellulase-hydrolyzed OK), PE-OK (pectinase-hydrolyzed OK) and SA-OK (savinase-hydrolyzed OK) were 74.33%, 93.03% and 81.27%, respectively. Additionally, in the OK/CC/B formulations, the treatability of the preservatives formulated with PE-OK (115.34%) was higher than that of CE-OK (101.32%) or SA-OK (111.25%). These results suggested that PE is the most effective enzyme for reducing the OK molecular weight. Gwak et al. [18] identified the chemical composition of the same OK used in our study: carbohydrate (58.9%), protein (18.8%), and fiber (15.8%). Among the carbohydrates, the contents of arabinose (31.80%) and galactose (29.31%) were higher than those of glucose (17.49%), xylose (20.11%) or mannose (1.31%). The PE used for the OK hydrolysis in our study contained pectinase and arabanase with high digestibilities. Therefore, the OK molecular weight might be reduced effectively by PE, and subsequently the treatability of the preservatives formulated with PE-OK might be relatively higher than that of CE-OK and SA-OK.

Thirdly, when PE and SA were used for the OK hydrolysis or the loading level of CE and SA was increased from 0.1 to 0.2 ml/g, the treatabilities of the preservatives were increased (Fig. 2). The results indicated that the OK molecular weight was decreased by the addition of PE or SA and the increase of CE and SA loading level, and therefore suggested that the complexes formed by OK, CC and/or B might be sufficiently small to penetrate easily into the wood structure.

3.2. Leachability

Fig. 3 shows the stability of the OK-WPs against hot-water leaching. Wood blocks treated with OK/CC and OK/CC/B formulations had higher leachabilities than those with CC and CC/B formulations. For instance, the addition of OK, which was used for fixing CC and B salts in wood blocks, adversely affected the stability of OK-WPs against leaching. These results may have been caused by leaching of the degradation products of any OK that were not chelated with

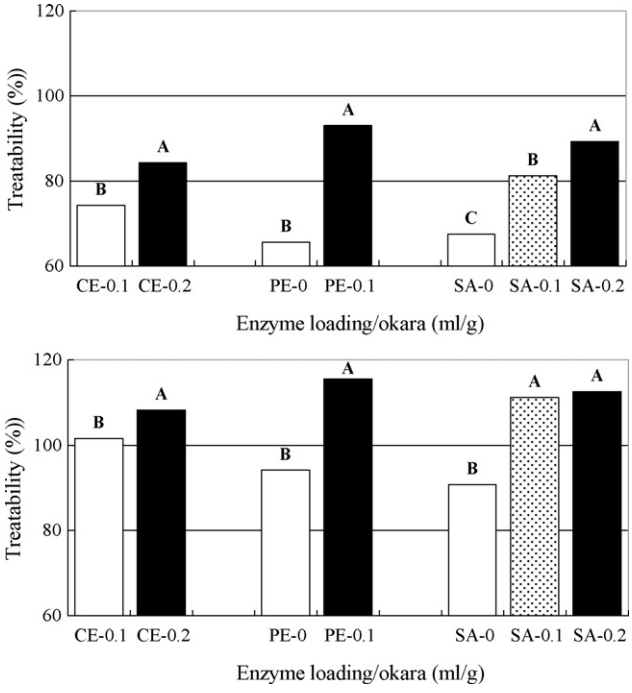

Fig. 2. Effects of type and loading volume of enzymes on the treatabilities of okara-based wood preservatives formulated with OK/CC (top) and OK/CC/B (bottom) formulations. Same capital letters over each column are not significantly different from each other at $p = 0.05$ (least significance difference test).

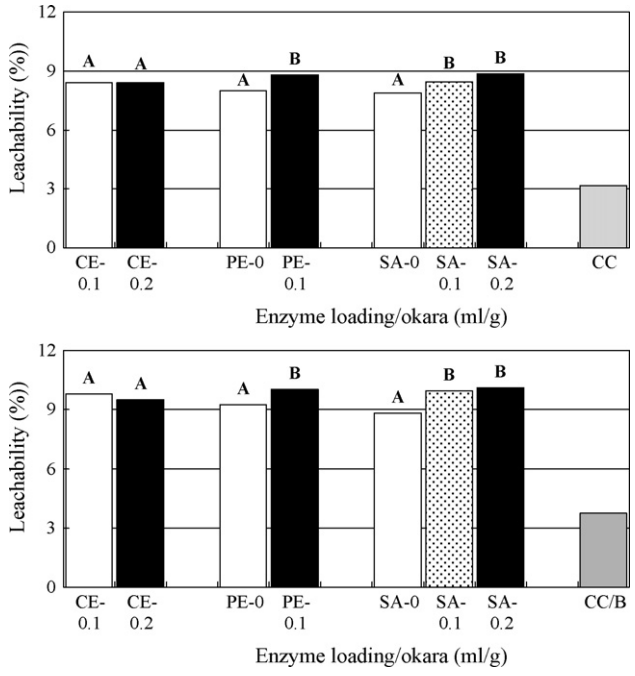

Fig. 3. Effects of type and loading volume of enzymes on the leachabilities of okara-based wood preservatives formulated with OK/CC (top) and OK/CC/B (bottom) formulations. Same capital letters over each column are not significantly different from each other at $p = 0.05$ (least significance difference test).

CC and B, and by the presence of chelated complexes on the surface of the wood blocks. In addition, there was a significant difference between the leachabilities of OK/CC and OK/CC/B ($p = 0.01$). For example, the leachability of OK/CC/B (9.63%) was higher than that of OK/CC (8.40%), which was attributed to the high water-solubility of B.

The enzymes used for the OK hydrolysis also influenced the leachability of the OK-WPs. At an enzyme-loading level of 0.1 ml/g, the leachabilities of CE-OK/CC, PE-OK/CC and SA-OK/CC were 8.40%, 8.81% and 8.46%, respectively. The retention of PE-OK/CC in the wood blocks was lower than that of CE-OK/CC ($p = 0.04$) and SA-OK/CC ($p = 0.04$), but the leachability of CE-OK/CC did not differ from that of SA-OK/CC ($p = 0.39$). These results might indicate that PE, possessing a high digestibility, produces more degraded products than CE and SA. As a result, the degraded products unchelated with CC, which was added into the suspension of OK hydrolyzates at a fixed rate, might be easily leached out from the wood blocks. This suggests that the leachability of PE-OK/CC was higher than that of CE-OK/CC and SA-OK/CC. In the OK/CC/B formulations, the leachabilities of the preservatives formulated with CE-OK, PE-OK and SA-OK were 9.78%, 10.03% and 9.95%, respectively. The leachability of PE-OK/CC/B did not differ from that of CE-OK/CC/B ($p = 0.27$) and SA-OK/CC/B ($p = 0.41$), and nor was any significant difference found between the leachabilities of CE-OK/CC/B and SA-OK/CC/B ($p = 0.31$). These results might have been caused by the increase of the complexes formed by PE-OK, which were not chelated with CC, and B. Therefore, the complexes might have been retained into the wood blocks during leaching, thereby eliminating any differences between the leachabilities of PE-OK/CC/B, CE-OK/CC/B and SA-OK/CC/B. To reliably verify this inference, quantitative analysis of the B salts existing in the leachates is required.

The leachabilities of the preservatives formulated with PE-0.1-OK/CC and SA-0.1-OK/CC were significantly higher than those of PE-0-OK/CC and SA-0-OK/CC, as shown in Fig. 3, which was attributed to the leaching of unchelated OK hydrolyzates. For instance, as the enzymatically hydrolyzed OK contained more degraded molecules than that of non-hydrolyzed OK, the degraded OK hydrolyzates unchelated with CC might have easily been leached out from the wood blocks during hot-water leaching. On the other hand, there were no significant differences between the leachabilities of CE-0.1-OK/CC and CE-0.2-OK/CC ($p = 0.23$), or between those of SA-0.1-OK/CC and SA-0.2-OK/CC ($p = 0.14$). These results indicated that the formation of the preservative complexes retained in the wood blocks was not affected by the increase of the CE and SA loading levels. However, the Cu content of the leachates obtained from the wood blocks treated with CE-0.1-OK/SA-0.1-OK/CC was 43.57 μg/ml, and that of the leachate from the wood blocks treated with CE-0.2-OK/PE-0.1-OK/SA-0.2-OK/CC was reduced to 29.52 μg/ml. In addition, the protein contents were also decreased from 184.49 to 173.87 mg/l when the enzyme-loading levels were increased from CE and SA of 0.1 ml/g to CE and SA of 0.2 ml/g and PE of 0.1 ml/g, respectively. Based on these results, the formation of the OK–CC complexes in CE-0.2-OK/PE-0.1-OK/SA-0.2-OK/CC might have increased compared to that of CE-0.1-OK/SA-0.1-OK/CC. Therefore, we considered that the contents of Cu and protein were decreased.

3.3. Decay resistance

Tables 2 and 3 show the decay resistances of the OK-WP-treated and leached wood blocks against *P. placenta* and *G. trabeum*. The weight losses of the control wood blocks against *P. placenta* and *G. trabeum* were 22.95% and 17.24%, respectively. Leached wood blocks treated with CC alone showed 4.06% weight loss against *P. placenta*, but those with the OK/CC and OK/CC/B formulations, which were prepared with CE-0.1-OK regardless of the use and increasing PE and SA loading level, did not exhibit any decay or only showed very little weight loss (Table 2). In addition, very little or zero decay against copper-intolerant *G. trabeum* was found in the wood blocks treated with OK/CC and OK/CC/B, as well as the CC and CC/B formulations (Table 3). These results indicated that complexes chelated with OK, CC, and/or B might have been retained in the leached wood blocks after leaching, and thus that the OK/CC and OK/CC/B formulations showed better decay resistances against the decaying fungi, especially *P. placenta*, than CC. Ahn et al. [15], Baechler and Roth [19] and Johnson and Gutzmer [20] reported similar results in which a certain amount of Cu and/or B salts should be retained in the leached wood blocks to effectively protect preserved wood against decaying fungi such as *P. placenta* and *G. trabeum*.

When OK/CC and OK/CC/B formulations, including CE-0.2-OK, were used to treat the wood blocks, the decay resistance of the formulations against *P. placenta* was degraded, as shown in Table 2, which was attributed to the existence of the OK unchelated with CC and/or B in wood blocks. For instance, the amount of OK molecules in the OK suspension was increased with increasing enzyme-loading levels, thereby allowing more OKs unchelated with CC and/or B to exist in the wood blocks treated with OK/CC and OK/CC/B formulations. The unchelated OKs, which remained

Table 2
Decay resistance of leached wood blocks treated with okara-based preservatives against brown-rot fungus *Postia placenta*.

Enzyme loading[a] (ml/g)			Preservative formulations[b]				Control
CE	PE	SA	CC	OK/CC	CC/B	OK/CC/B	
0	0	0	4.06	–	0	–	22.95
0.1	0	0	–	0	–	0	–
		0.1	–	0.65	–	0	–
		0.2	–	0	–	0	–
	0.1	0	–	0	–	0	–
		0.1	–	0	–	0	–
		0.2	–	3.13	–	1.02	–
0.2	0	0	–	0.88	–	2.80	–
		0.1	–	2.37	–	4.53	–
		0.2	–	1.46	–	2.96	–
	0.1	0	–	1.12	–	5.20	–
		0.1	–	2.31	–	3.61	–
		0.2	–	7.45	–	5.78	–

Decay resistance is expressed as percent weight loss after exposing specimens to the brown-rot fungus, *Postia placenta*, for 12 weeks. Each value of decay resistances is a mean representing six leached wood blocks treated with different preservative formulations.

[a] Enzyme loading means the volume of enzyme (ml/1 g okara) used for the hydrolysis of okara; CE: Celluclast, PE: Pectinex, SA: Savinase.

[b] CC: copper chloride ($CuCl_2$); B: sodium borate ($Na_2B_4O_7 \cdot 10H_2O$).

Table 3
Decay resistance of leached wood blocks treated with okara-based preservatives against brown-rot fungus *Gloeophyllum trabeum*.

Enzyme loading[a] (ml/g)			Preservative formulations[b]				Control
CE	PE	SA	CC	OK/CC	CC/B	OK/CC/B	
0	0	0	0.33	–	0	–	17.24
0.1	0	0	–	0	–	0	–
		0.1	–	0.43	–	0	–
		0.2	–	0	–	0	–
	0.1	0	–	0	–	0	–
		0.1	–	0	–	0	–
		0.2	–	0	–	0	–
0.2	0	0	–	0	–	0	–
		0.1	–	0.14	–	0	–
		0.2	–	0	–	0	–
	0.1	0	–	0.12	–	0.04	–
		0.1	–	0.25	–	0.67	–
		0.2	–	0.98	–	0	–

Decay resistance is expressed as percent weight loss after exposing specimens to the brown-rot fungus, *Gloeophyllum trabeum*, for 12 weeks. Each value of decay resistances is a mean representing six leached wood blocks treated with different preservative formulations.

[a] Enzyme loading means the volume of enzyme (ml/1 g okara) used for the hydrolysis of okara; CE: Celluclast, PE: Pectinex, SA: Savinase.

[b] CC: copper chloride ($CuCl_2$); B: sodium borate ($Na_2B_4O_7{\cdot}10H_2O$).

in the wood blocks after leaching, might have been used for the growth of *P. placenta*. After the decay resistance of the leached wood blocks treated with OK/CC formulations against *P. placenta* was determined, quantitative analysis of the Cu and protein in the decayed wood blocks was conducted. When the enzyme-loading levels were increased from CE and SA of 0.1 ml/g to CE and SA of 0.2 ml/g and PE of 0.1 ml/g, the Cu content was increased from 2.53 to 3.70 μg/ml, but the protein content was remarkably decreased from 26.29 to 13.84 mg/l. These results appeared to confirm our speculation that the unchelated OKs remaining in the wood blocks supported the growth of the decaying fungi.

3.4. Microscopic observation and SEM-EDX analysis

SEM images offered a clear view of the preservative complexes retained in the OK-WP-treated wood blocks. EDX mapping

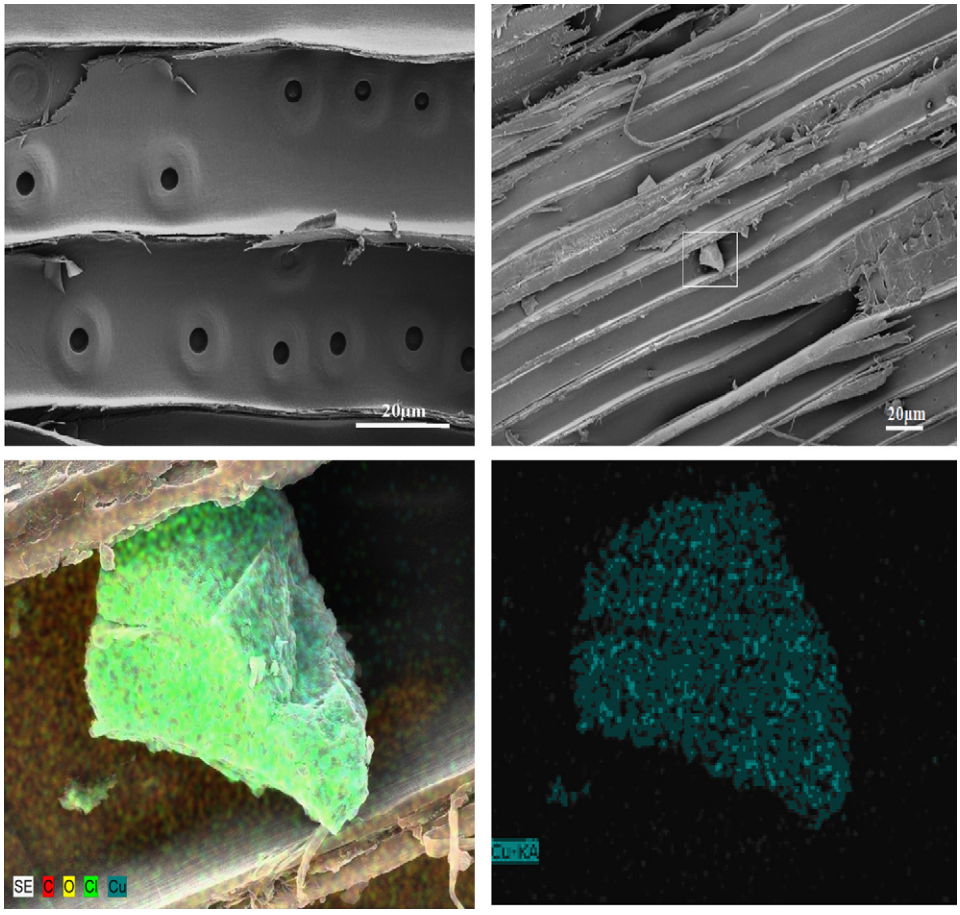

Fig. 4. Scanning electron microscopic (SEM) images of control wood block (top-left). SEM image (top-right) and corresponding SEM-EDX (energy dispersive X-ray spectrometer) maps (bottom-left and right) taken on the same area of wood blocks treated with CC formulations.

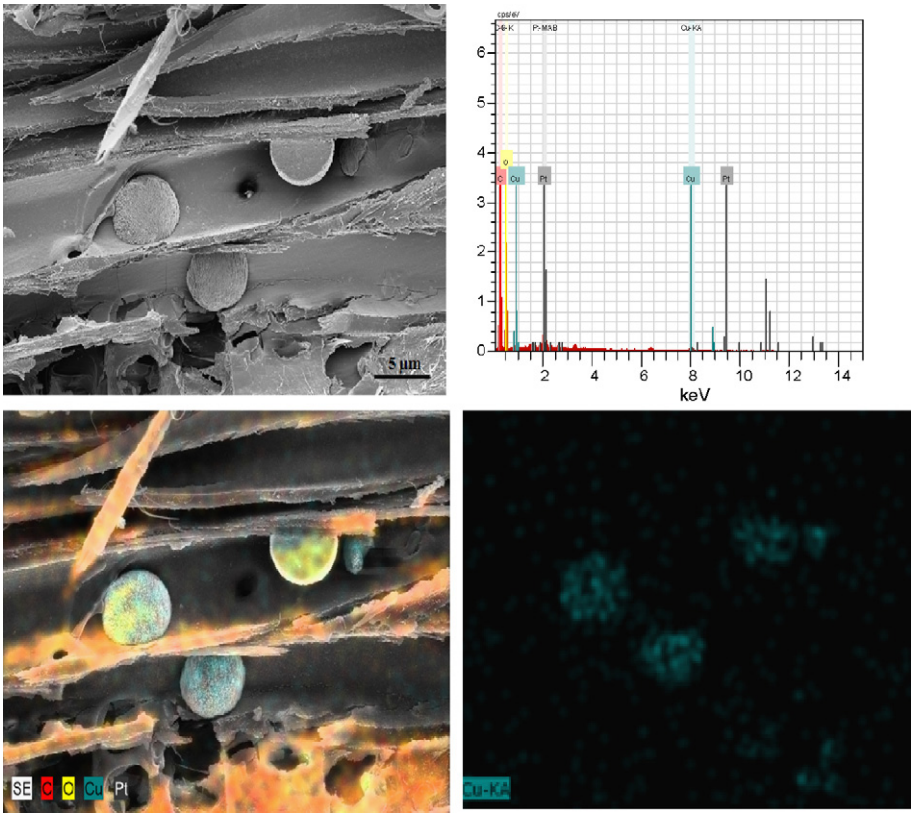

Fig. 5. SEM images (top-left), corresponding spectrum (top-right), and SEM-EDX maps (bottom-left and right) taken on the same area of leached wood blocks treated with OK/CC formulations.

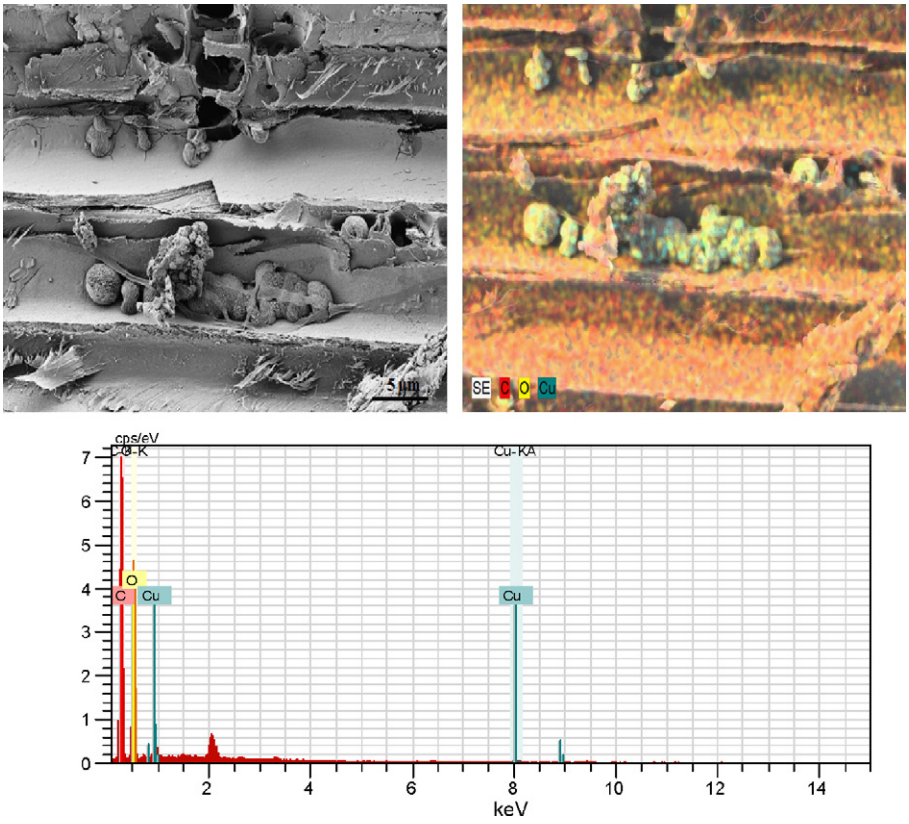

Fig. 6. SEM images (top-left), corresponding SEM-EDX maps (top-right) and spectrum (bottom) taken on the same area of leached wood blocks treated with OK/CC/B formulations.

presented further detail on the location of the preservative complexes inside the wood structure. No preservative complexes were detected in any part of the control wood blocks (Fig. 4top-left). When the leached wood blocks treated with CC were observed, one pyramid-shaped particle was found in the cell lumens (Fig. 4top-right). The SEM-EDX analysis revealed that the particle contained Cu and Cl originating from $CuCl_2$ (Fig. 4bottom).

In the microscopic observation of the leached wood blocks treated with CC/OK, the spherical agglomerates were easily detected in the cell lumens, as shown in Fig. 5. The spot analysis of the agglomerates by SEM-EDX revealed the presence of Cu in the wood blocks. Additionally, the spectrum obtained from the SEM-EDX analysis verified that the agglomerates contained CC (Fig. 5top-right). Fig. 6 clearly shows that the cell lumens inside the leached wood blocks treated with OK/CC/B were filled with the agglomerates containing Cu and probably B, but no B was found by SEM-EDX analysis. This result indicated that the B concentration in the wood blocks may have been too low to be detected, resulting in a non-detectable amount of B in the leached wood blocks treated with OK/CC/B. The SEM and SEM-EDX analysis results suggested that that the presence of the agglomerates containing Cu, probably B, contributed to the good decay resistance of the leached wood blocks treated with OK/CC and OK/CC/B. However, this assumption on the mechanism for the formation of the agglomerates by the chelating reaction of OK–CC and OK–B has to be checked in future experiments.

4. Conclusions

Despite the large molecular weights of OK–CC and OK–CC–B complexes, the uses of OK hydrolyzates and NH_4OH as a dissociating agent might have facilitated the successful target retention of most of the OK/CC and OK/CC/B preservative formulations in the wood blocks. The OK-WPs were stable against hot-water leaching and showed good decay resistance against both *P. placenta* and *G. trabeum*, but the severe OK hydrolysis, especially in the 0.2 ml/g CE formulation, degraded the leachability and decay resistance of the OK-WPs. The SEM and SEM-EDX analysis results suggested that the leached wood blocks treated with OK/CC and OK/CC/B formulations contained preservative complexes, such as OK–CC, probably OK–B, and OK–CC–B, in the cell lumens, and that these complexes might have played important roles in the stability against leaching and decay resistance against decay fungi that was exhibited by the OK-WPs. These conclusions support our proposal for the OK-WPs as new wood preservatives with an environmentally friendly effectiveness equal to that of existing wood preservatives, such as CuAz and ACQ, while significantly reducing the production costs for wood preservation compared to those of CuAz and ACQ. However, further well-defined studies of OK-WPs are needed to gain better insight into the formation of preservative complexes as a function of the wood input, as are long-term field trials to evaluate the applicability of these preservatives.

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