

Microwave Irradiation of Lignocellulosic Materials

IV. Enhancement of Enzymatic Susceptibility of Microwave-irradiated Softwoods

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Abstract—Effect of microwave irradiation on the enzymatic susceptibility of various softwoods was investigated. The pH values of the reaction liquor dropped with increasing temperature to 2.9–3.3 at 230°C, consistent with increase in acidity (0.5–0.85 meq at 230–239°C). Above approximately 180°C, hemicellulose underwent acid-mediated autohydrolysis and became water-soluble yielding a mixture of oligosaccharides and monosaccharides. The composition of water-soluble portion was similar for all wood species tested.

The maximum extents of saccharification below 240°C ranged between 36–62% for softwoods, while those for hardwoods were between 88–93%. The present investigation confirmed that microwave pretreatment enhanced the enzymatic susceptibility of various softwoods. However, further attempt should be needed to give higher values equal to those for hardwoods.

1. Introduction

Polysaccharides in softwoods have been known to be more resistant against enzymatic attack than those in hardwoods and herbaceous plants^{1~6)}. This might be ascribed to the differences in the chemical structures between softwood and hardwood lignins¹⁾. Previously, a new pretreatment using microwave irradiation in the presence of water^{6~8)} was attempted to transform wood components into more enzyme-susceptible forms for saccharification. The pretreatment was found to improve the enzymatic susceptibility of a softwood, AKAMATSU⁶⁾. This prompted us to further investigate the applicability of the pretreatment to wider range of softwood species. In this report, the effects of microwave pretreatment on the extent of enzymatic saccharification are described for other nine softwood species.

2. Materials and Methods

2.1 Materials and general methods

The sapwoods of the five domestic and four exotic softwood species were used: EZOMATSU (*Picea jezoensis* CARR.), HINOKI (*Chamaecyparis obtusa* ENDL.),

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KARAMATSU (*Larix leptolepis* GORD.), SUGI (*Cryptomeria japonica* D. DON), TODOMATSU (*Abies sachalinensis* FR. SCHM.), LOBLOLLY PINE (*Pinus taeda* LINN.), METASEQUOIA (*Metasequoia glyptostroboides* HU et CHENG), BALD CYPRESS (*Taxodium distichum* RICH.), and SLASH PINE (*Pinus elliottii* ENGELM.). The former five species are domestic and the latter four exotic. The hardwoods used for comparison were SHIRAKANBA (*Betula platyphylla* SUKAT. var. JAPONICA HARA) and a mixture sample of *Eucalyptus* spp. (EUCALYPT): *E. regnans*, *E. delegatensis*, *E. oblique*, *E. diversicolor*, *E. calophylla*, *E. marginata*, etc.⁹⁾ All samples were extracted with methanol-benzene (1 : 1, v/v) and ground to 60-80 mesh. METASEQUOIA and BALD CYPRESS were supplied from Dr. Norimoto (Section of Wood Physics, Wood Research Institute). A mixture of *Eucalyptus* spp. was a gift from Sanyo-Kokusaku Pulp Co., Ltd. The other woods were of laboratory collection. A cellulase-hemicellulase mixture (Meicelase CEPB-5042, 8000 u/mg) was supplied from Meiji Seika Industry Co., Ltd. Unless otherwise specified, other materials and general methods were the same as reported earlier⁶⁾.

2.2 Microwave irradiation

Two grams of each wood sample was soaked in water contained in a glass vessel of 50-ml capacity (Type A-3-L₂, Taiatsu Glass Industry, Co., Ltd.) and degassed by aspiration. The amounts of water were 20 ml for SUGI, 23 ml for METASEQUOIA and 14 ml for the other species to get a complete soaking of each wood meal. Microwave irradiation was performed at $2,450 \pm 50$ MHz and 2.4 kW as reported earlier^{6,7)}. The temperature of the sample solution was estimated with an infra-red thermometer (San-ei model 6T16 thermospot, San-ei Sokki Industry, Co., Ltd.).

2.3 Evaluation of microwave irradiation

Each microwave-irradiated sample was filtered through a glass crucible (porosity 4), and the residue was thoroughly washed with distilled water. Acidity, pH, reducing sugar content, furfural content, and neutral sugar composition of the filtrate were determined as previously described⁶⁾. Molecular weight distribution of the depolymerized neutral sugars was analyzed by gel-permeation chromatography on Toyoparl HW40S at 40°C using water as an eluent. The elution was monitored with changes in refractive indices (Toyosoda RI-8). The residue remained in the crucible was dried at 105°C and weighed to evaluate the amount of the water-soluble components (weight loss).

2.4 Enzymatic saccharification

The microwave-irradiated samples were saccharified with Meicelase at 40°C for 48 h in 0.05 M sodium acetate buffer, pH 4.8, with a few drops of toluene as an antiseptic. The substrate and enzyme concentrations were 2.0% and 0.2%, res-

pectively. After enzymatic treatment the sample was filtered through glass crucible and the residue was thoroughly washed with distilled water to determine the extent of saccharification as described above.

3. Results and Discussion

3.1 Production of acid and furfural

The time for heating to 230°C was below 7 min for SUGI and METASEQUOIA. This was 2–4 min shorter than for the other species, indicating that these two softwoods are more susceptible to microwave energy than the other species. The pH of the irradiated liquor dropped down to 2.9–3.3 at 230°C due to the production of acid (Figs. 1–11, Table 1). The major acid was identified as acetic acid originated from acetylated-hemicelluloses. The productions of acid and furfural were not prominent by 200°C, but their amounts were low in the ranges of 0.52–0.85 meq, and 0.2–0.9% at 230–239°C. This is in agreement with the previous results with AKAMATSU (*Pinus desiflora* SIEB. et ZUCC.), BUNA (*Fagus crenata* BLUME), and POPLAR (*Populus euramericana* (DODE) GUIMIER)^{6,8)}. These results indicate that the degradation of pentoses by microwave irradiation should be considered above 210°C.

3.2 Autohydrolysis of hemicellulose by microwave irradiation

Increase in reducing sugar production was incipient at about 180°C and then rapid with increasing temperature up to 220–230°C (Figs. 1–11). Thereafter it

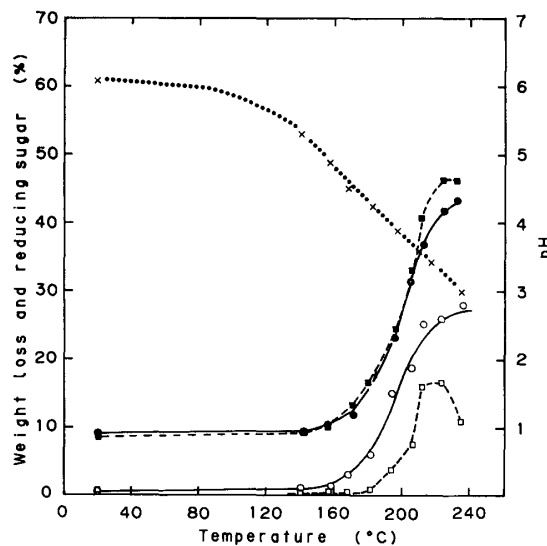


Fig. 1. Microwave irradiation and enzymatic saccharification of EZOMATSU (*Picea jezoensis* CARR.). Symbols: ○, ●, weight loss; □, ■, reducing sugar production; ×, pH. Open symbols are for microwave pretreatment alone and closed, for enzymatic saccharification after microwave pretreatment.

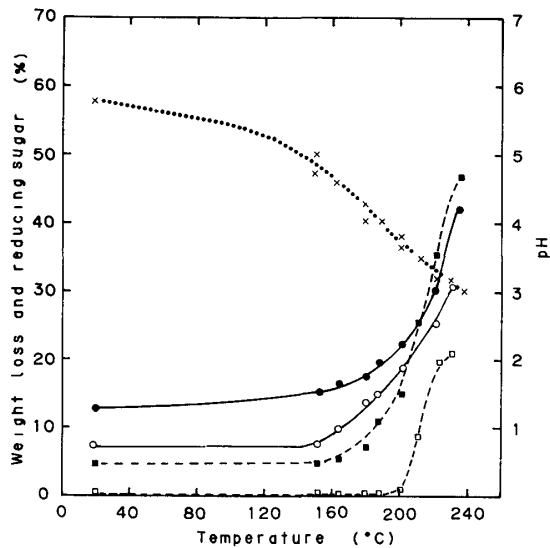


Fig. 2. Microwave irradiation and enzymatic saccharification of HINOKI (*Chamaecyparis obtusa* ENDL.). Symbols: see Fig. 1.

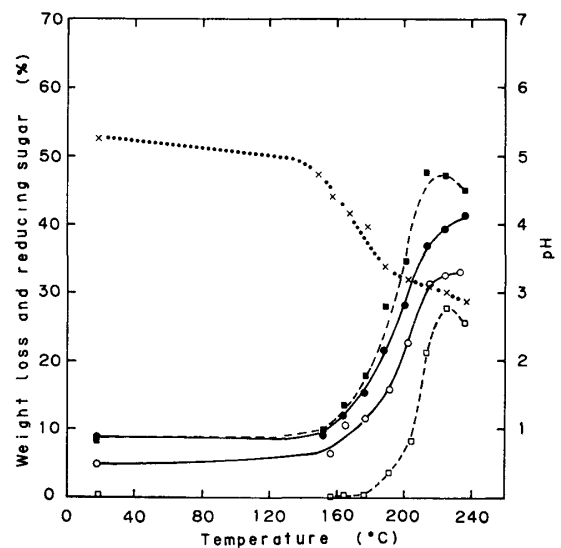


Fig. 3. Microwave irradiation and enzymatic saccharification of KARAMATSU (*Larix leptolepis* GORD.). Symbols: see Fig. 1.

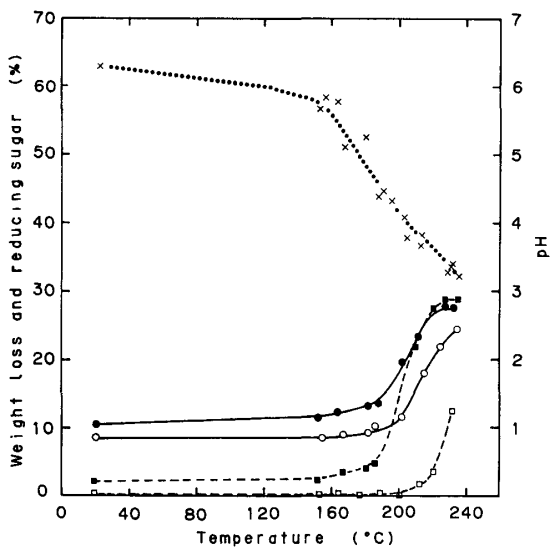


Fig. 4. Microwave irradiation and enzymatic saccharification of SUGI (*Cryptomeria japonica* D. DON). Symbols: see Fig. 1.

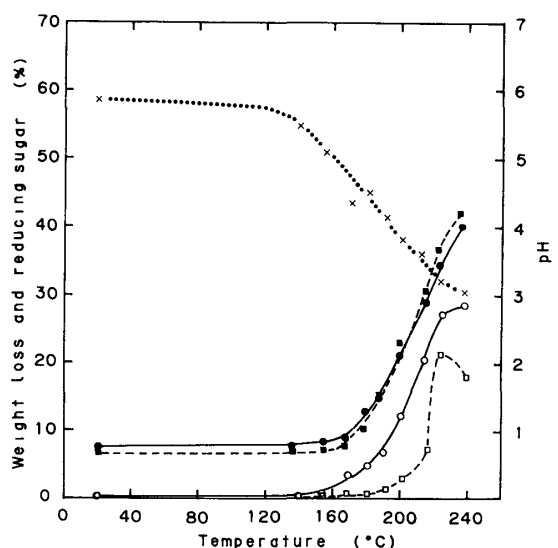


Fig. 5. Microwave irradiation and enzymatic saccharification of TODOMATSU (*Abies sachalinensis* FR. SCHM.). Symbols: see Fig. 1.

declined in EZOMATSU, KARAMATSU, SLASH PINE, TODOMATSU and EUCALYPT in accordance with the degradation of pentoses and subsequent formation of furfural (Figs. 1–11, Table 1). The reducing sugar production of the other woods did not reach maximum under the present condition, although the rate of its increase was smaller above 230°C. The amounts of reducing sugars at 225–230°C were in the range 12–28% similar to the values with AKAMATSU, BUNA and agricultural

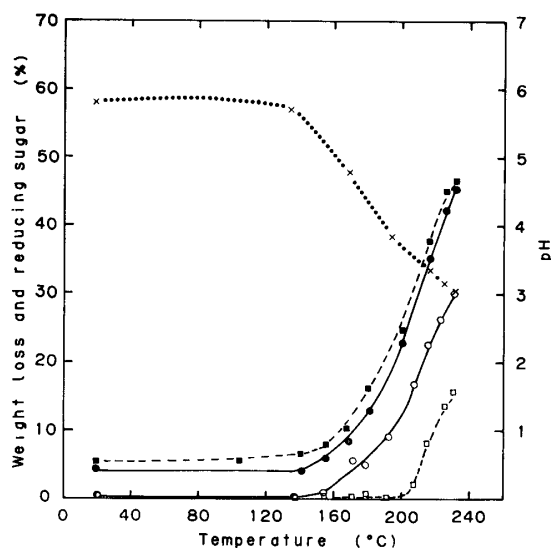


Fig. 6. Microwave irradiation and enzymatic saccharification of LOBLOLLY PINE (*Pinus taeda* LINN.). Symbols: see Fig. 1.

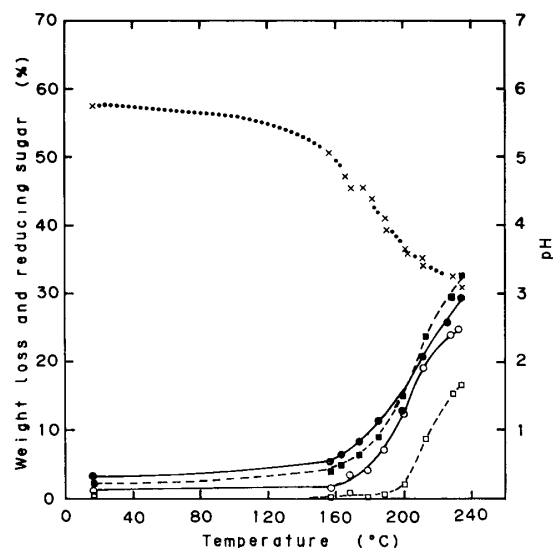


Fig. 7. Microwave irradiation and enzymatic saccharification of METASEQUOIA (*Metasequoia glyptostroboides* Hu et CHENG). Symbols: see Fig. 1.

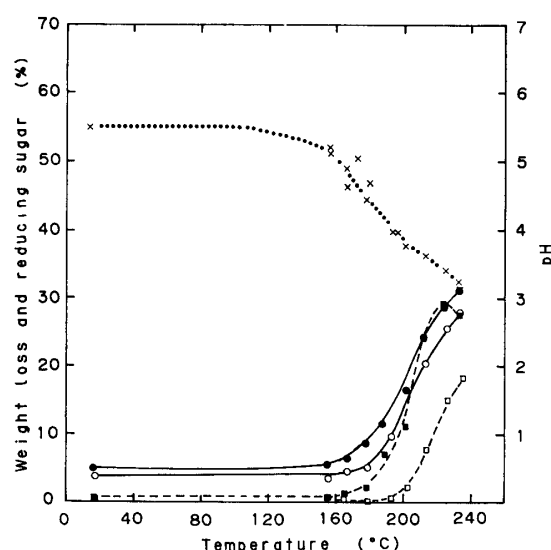


Fig. 8. Microwave irradiation and enzymatic saccharification of BALD CYPRESS (*Taxodium distichum* RICH.). Symbols: see Fig. 1.

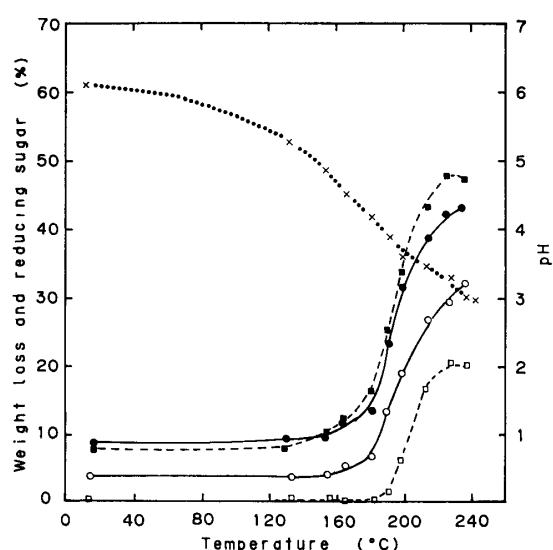


Fig. 9. Microwave irradiation and enzymatic saccharification of SLASH PINE (*Pinus elliottii* ENGELM.). Symbols: see Fig. 1.

lignocellulosic wastes^{6,7)}. In contrast to the reducing sugar production, the amount of the weight loss started to increase at about 20°C lower temperature than that with the reducing sugar production, and increased rapidly with temperature up to 230–240°C (Figs.1–11). The weight loss at 230°C ranged between 25–37%, which was 1.2–2.8 fold higher than that of the reducing sugar production. This deviation may be due to the formation of oligosaccharides and water-soluble lignin. Oligosaccharides

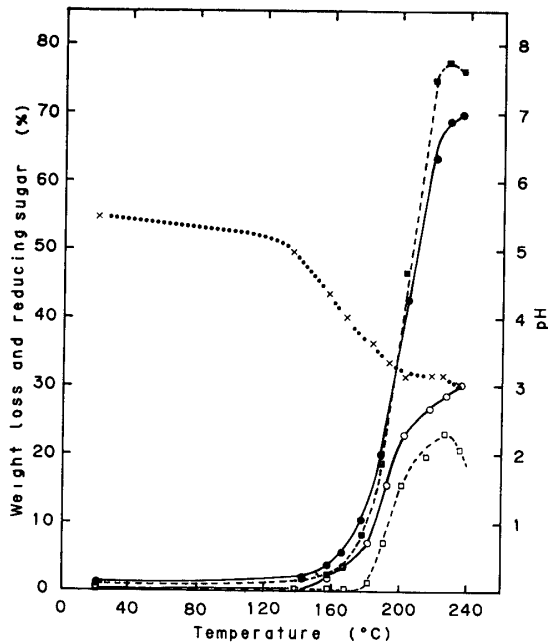


Fig. 10. Microwave irradiation and enzymatic saccharification of EUCALYPT (*Eu-calyptus* spp.). Symbols: see Fig. 1.

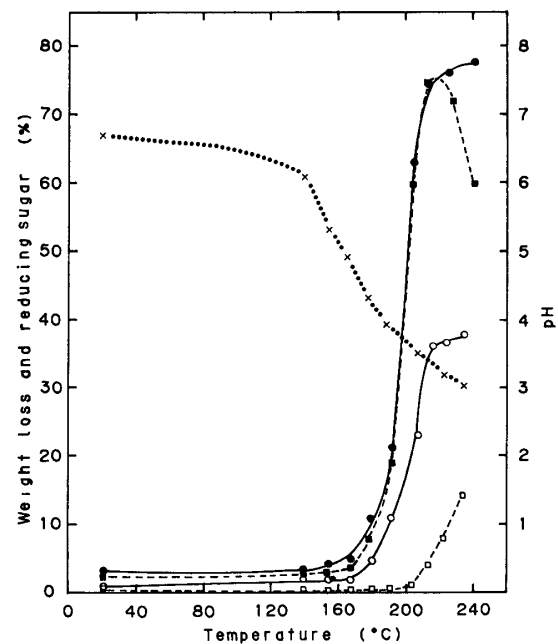


Fig. 11. Microwave irradiation and enzymatic saccharification of SHIRAKANBA (*Betula platyphylla* SUKAT. var. JAPONICA HARA). Symbols: see Fig. 1.

Table 1. Acid and furfural production caused by microwave irradiation

Wood species	Temperature (°C)	Acidity ^a (meq)	Furfural ^b (%)
EZOMATSU	207	0.16	0.04
	215	0.27	0.19
	223	0.33	0.32
	235	0.70	0.33
HINOKI	201	0.20	0.02
	213	0.28	0.13
	224	0.48	0.51
	232	0.72	0.87
KARAMATSU	203	0.20	0.03
	214	0.32	0.48
	225	0.40	0.63
	233	0.85	0.68
SUGI	200	0.16	0.03
	213	0.20	0.07
	224	0.40	0.14
	236	0.56	0.58

Table 1. (Continued)

Wood species	Temperature (°C)	Acidity ^a (meq)	Furfural ^b (%)
TODOMATSU	201	0.15	0.02
	215	0.32	0.08
	223	0.46	0.12
	239	0.73	0.23
LOBLOLLY PINE	206	0.12	0.01
	215	0.24	0.07
	223	0.50	0.30
	230	0.80	0.82
METASEQUOIA	201	0.20	0.05
	213	0.32	0.25
	228	0.56	0.71
	233	0.64	0.87
BALD CYPRESS	203	0.20	0.07
	214	0.24	0.16
	225	0.44	0.49
	234	0.52	0.84
SLASH PINE	200	0.06	0.01
	215	0.20	0.01
	226	0.40	0.14
	237	0.78	0.62
EUCALYPT	201	0.36	0.08
	216	0.40	0.36
	226	0.65	0.62
	236	0.73	0.62
SHIRAKANBA	204	0.27	0.01
	213	0.38	0.15
	223	0.56	0.35
	233	0.62	0.35

a: Values of the filtrated liquor after microwave irradiation.

b: Based on the dry matter of the original samples.

were detected by gel-permeation chromatographic analysis. Since all softwoods and hardwoods gave similar elution profiles, typical elution profiles with TODOMATSU and EUCALYPT are shown in Fig. 12. The water-soluble carbohydrate portions contained mainly a series of xylo-oligosaccharides with the degree of polymerization of 2–6. They appeared together with a small amount of hexose oligomers as shoulders and monosaccharides. The amount of monosaccharides increased with temperature (Table 2). As shown in the table, the distinct difference in the

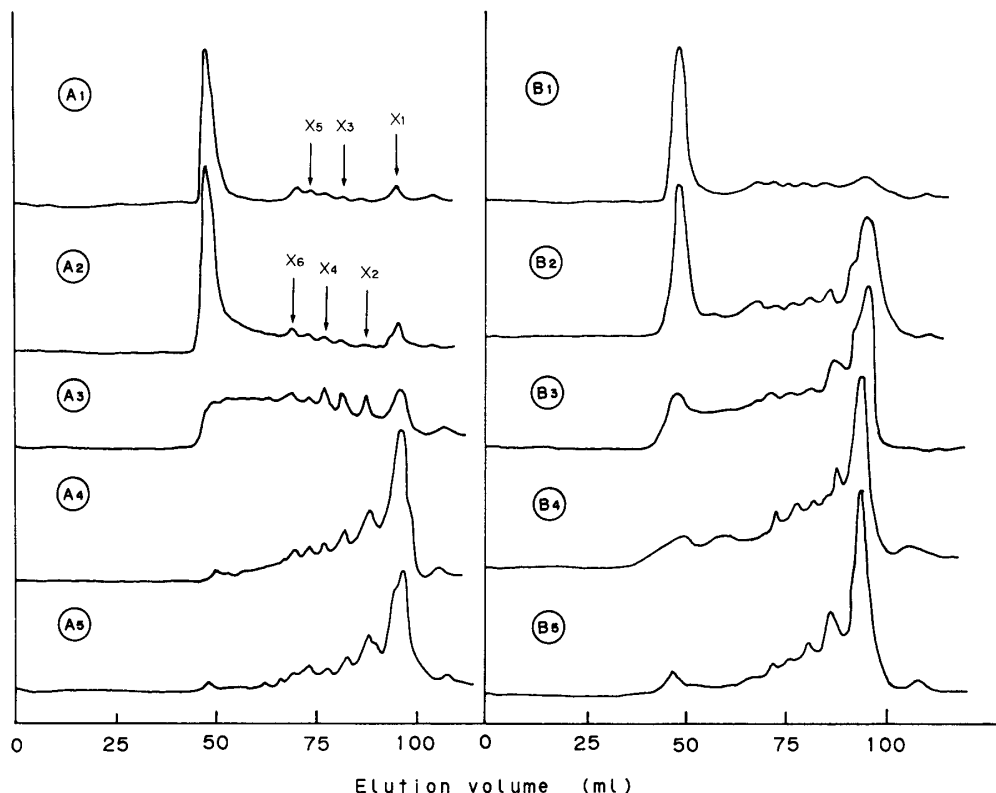


Fig. 12. Gel-permeation chromatography of the water-soluble components of TODOMATSU (*Abies sachalinensis* FR. SCHM.) and EUCALYPT (*Eucalyptus* spp.). Symbols: A₁₋₅, TODOMATSU (A₁, 180°C; A₂, 201°C; A₃, 215°C; A₄, 223°C; A₅, 239°C); B₁₋₅, EUCALYPT (B₁, 180°C; B₂, 201°C; B₃, 215°C; B₄, 223°C; B₅, 239°C); X₁₋₆, Positions of elution of xylose and xylo-oligosaccharides having degree of polymerization of 2-6.

neutral monosaccharide compositions is remarkably clear between softwoods and hardwoods. In softwoods, arabinose and xylose residues are predominating below 210°C above which appreciable amounts of mannose, galactose and glucose residues appeared, while only xylose is the dominant monosaccharide released by microwave irradiation from hardwoods. The increase in monomeric glucose below 230°C has been shown without any change of cellulose crystallinity^{6,10}, indicating the degradation of amorphous region of cellulose and hemicelluloses with glucose residues. No differences in water-soluble components were detected for all wood species during microwave treatment. Therefore, softwood hemicelluloses are considered to be readily acid-hydrolyzed as well as hardwood hemicelluloses¹⁻⁶.

3.3 Enzymatic saccharification of the microwave-irradiated samples

Because the yields of the monosaccharides were low and substantial amount of oligosaccharides were formed by microwave-irradiation alone, the whole microwave-irradiated samples were used for enzymatic saccharification. The extent of sac-

Table 2. Neutral monosaccharide composition of the water-soluble fractions

Wood species	Temperature (°C)	Ara	Xyl	Man	Gal	Glc	Yield ^a (%)
EZOMATSU	182	73.0	15.2	6.7	3.9	1.2	1.18
	195	70.6	20.3	6.5	2.0	0.6	3.99
	207	35.2	39.0	9.8	10.9	5.1	5.86
	215	22.2	39.4	20.2	12.4	5.8	7.94
	223	12.3	38.0	28.5	12.3	8.8	11.07
	235	7.2	18.2	44.5	9.0	21.1	9.14
HINOKI	179	56.6	20.4	5.5	6.1	1.4	0.15
	188	52.7	14.6	14.8	11.5	6.4	0.66
	201	65.9	13.3	7.6	11.1	2.1	0.52
	213	32.4	30.3	16.6	15.5	5.3	1.36
	224	9.1	30.6	35.3	13.0	12.0	3.50
	232	10.9	35.2	6.7	18.7	28.5	3.18
KARAMATSU	176	81.9	4.3	4.9	4.4	4.6	0.71
	191	61.7	11.5	7.1	13.1	6.6	1.28
	203	53.7	18.5	7.9	15.7	4.2	1.62
	214	14.8	32.6	24.0	19.2	9.4	5.67
	225	13.9	32.5	24.4	19.5	9.7	6.98
	233	9.5	24.1	32.0	19.7	14.7	7.75
SUGI	177	57.7	21.1	6.1	9.6	5.4	0.22
	189	62.7	22.3	4.3	6.3	4.4	0.67
	203	63.0	17.0	8.4	8.3	3.3	0.89
	213	56.5	19.6	8.2	12.5	3.3	0.88
	224	37.6	33.2	10.8	14.4	4.0	1.01
	234	9.2	38.9	27.8	11.8	12.3	2.81
TODOMATSU	180	70.0	9.8	9.6	5.8	4.8	0.29
	191	74.7	16.4	0.3	5.3	3.3	0.41
	201	69.5	11.7	4.9	7.5	6.4	0.73
	215	26.9	33.4	20.8	10.7	8.2	1.64
	223	4.2	9.5	60.4	4.8	21.0	7.33
	239	5.2	7.9	48.4	3.8	34.7	5.77
LOBLLOLY PINE	177	56.2	24.4	6.5	11.2	1.7	0.96
	190	64.5	20.9	5.0	6.6	3.0	0.84
	206	62.3	20.4	7.6	7.0	2.7	1.54
	215	28.1	27.0	29.9	9.5	5.5	3.08
	223	20.7	38.0	22.6	11.7	7.1	4.40
	230	11.4	27.8	35.4	12.3	13.1	5.74
METASEQUOIA	179	71.7	8.4	6.8	4.1	9.0	0.26
	190	77.7	9.2	4.4	6.5	2.2	0.40
	201	67.3	15.1	2.9	12.6	2.1	0.69
	213	30.3	38.4	12.7	13.9	4.7	1.90

Table 2. (Continued)

Wood species	Temperature (°C)	Ara	Xyl	Man	Gal	Glc	Yield ^a (%)
	228	13.3	46.2	21.4	11.3	7.8	3.44
	233	9.8	44.1	25.6	10.0	10.3	3.79
BALD CYPRESS	177	82.6	9.8	2.1	3.5	2.0	0.24
	192	81.0	7.0	2.1	7.4	2.5	0.47
	203	70.9	14.6	3.3	8.7	2.5	1.04
	214	48.5	29.7	7.6	10.7	3.6	1.51
	225	13.8	46.2	18.0	14.0	7.9	3.38
	234	13.2	44.2	22.9	12.0	7.7	4.00
SLASH PINE	183	53.5	24.7	7.0	12.7	2.2	1.03
	192	45.7	25.2	10.9	13.4	4.8	2.80
	200	38.1	37.7	4.1	15.3	4.8	2.45
	215	32.0	34.3	12.9	16.9	3.9	3.13
	227	18.3	37.1	21.0	17.1	6.5	5.28
	237	7.6	13.0	39.6	13.6	26.3	8.05
EUCALYPT	181	10.2	68.4	4.0	13.0	4.5	1.15
	192	10.1	71.8	3.3	11.0	3.9	1.79
	201	5.1	80.5	2.6	8.6	3.3	5.59
	216	4.4	76.1	4.3	9.5	5.8	7.66
	226	4.9	72.2	5.7	9.3	7.9	9.09
	236	3.7	66.3	7.7	7.7	14.7	7.00
SHIRAKANBA	178	15.7	84.3	—	—	—	0.10
	190	27.7	59.8	2.0	6.4	4.1	0.30
	204	27.0	60.4	2.5	6.4	3.6	0.49
	213	17.0	69.9	3.0	6.5	3.6	1.14
	223	9.1	77.9	4.9	4.4	4.3	6.08
	232	6.2	71.7	3.3	2.0	4.6	5.48

a: Based on the dry matter of each original sample.

charification was evaluated by reducing sugar production and weight loss (Figs. 1–11). The reducing sugar production and the weight loss became remarkable at about 160°C and rapidly increased within the range 180–215°C. Reducing sugar production of softwoods reached a maximum at 219–228°C in the five softwood species: EZOMATSU, KARAMATSU, SUGI, BALD CYPRESS, and SLASH PINE. No maximum was shown in the other four softwood species: HINOKI, LOBLOLLY PINE, METASEQUOIA, and TODOMATSU. The hardwoods used had a maximum production of reducing sugars at 219–226°C. The optimum temperature range for enzymatic saccharification was similar for all wood species in agreement with the results reported earlier^{6~8)}. Prolonged microwave irradiation above 235°C should be ex-

amined to obtain the optimum temperature for enzymatic saccharification of HINOKI, LOBLOLLY PINE, METASEQUOIA and TODOMATSU. The reducing sugar weights were converted to anhydrosugar weights by multiplying with a factor of 0.9. The maximum extents of saccharification obtained were 59.7% (EZOMATSU), 60.8% (HINOKI), 60.8% (KARAMATSU), 36.4% (SUGI), 54.2% (TODOMATSU), 59.0% (LOBLOLLY PINE), 45.8% (METASEQUOIA), 48.6% (BALD CYPRESS), 61.7% (SLASH PINE), 93.1% (EUCALYPT), and 89.9% (SHIRAKANBA), on the basis of the hydrolyzable polysaccharides present in the original samples. Present and previous results indicate that four hardwoods (BUNA, EUCALYPT, POPLAR, and SHIRAKANBA) had a similar higher enzymatic susceptibility (88–93%) than that of all softwoods used. SLASH PINE was the most susceptible softwood to enzymatic attack while SUGI was the most resistant. Shimizu *et al.*³⁾ reported the different enzymatic susceptibilities for four softwood species, although the effects of autohydrolysis were quite low: AKAMATSU > HINOKI > SUGI > KARAMATSU. Present results, however, were not similar for KARAMATSU. Several factors including intraspecific variance¹¹⁾ should be inspected to clarify this discrepancy. Since the crystallinity of cellulose is stable^{6,10)}, increase in available surface area of cellulose might be a very important factor in enzymatic saccharification of woody plants as already pointed out^{6,7)}.

In conclusion, microwave irradiation pretreatment has been proved to improve the enzymatic susceptibility of various softwood polysaccharides but the effects of microwave energy varied with species and smaller than those for hardwoods. Therefore, further attempts are awaited to enhance the enzymatic susceptibility of softwoods to the same level as with hardwoods and agricultural lignocellulosic wastes.

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References

- 1) F. BENDER, D.P. HEANEY and A. BOWDEN: *Forest Prod. J.*, **20**, 36–41 (1970).
- 2) M.A. MILLETT, A.J. BAKER and L.D. SATTER: *Biotechnol. Bioeng. Symp.*, **6**, 125–153 (1979).
- 3) K. SHIMIZU, K. SUDO, S. NAGASAWA and M. ISHIHARA: *Mokuzai Gakkaishi*, **29**, 428–437 (1983).
- 4) H. MORIKAWA: *Kagaku to Seibutsu* (Japanese), **19**, 286–291 (1981).
- 5) M. TANAHASHI: *Mokuzai Kenkyu-Shiryō* (Japanese), **18**, 34–65 (1983).
- 6) J. AZUMA, F. TANAKA and T. KOSHIIJIMA: *Mokuzai Gakkaishi*, **30**, 501–509 (1984).

- 7) J. AZUMA, F. TANAKA and T. KOSHIIJIMA: J. Ferment. Technol., **62**, 377-384 (1984).
- 8) J. AZUMA and T. KOSHIIJIMA: Mokuzai Kenkyu-Shiryō (Japanese), **20**, 22-30 (1985).
- 9) N. SAKURAI: Kamipagikyoshi (Japanese), **31**, 414-422 (1977).
- 10) E. YAGI and K. OKAMURA: Private communication.
- 11) M. NORIMOTO, T. OHGAMA, T. ONO and F. TANAKA: J. Soc. Reo. Jpn., **9**, 169-175 (1981).