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Changes of extractives in pruning shoot of Japanese cedar (Cryptomeria japonica) during storing and pelletizing

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Changes of the extractives contents in pruning shoots of Japanese cedar during storing and pelletizing procedure were determined with GC and GC/MS. The extractives are consisted with essential oil and phenols such as terpenoids, flavonoids and lignans. Monoterpenes with high volatile properties were released from the leaves during storing and pelletizing of the pruning shoots. The remaining essential oils in the pellets were mainly consisted of sesquiterpenes, diterpenes, and some monoterpene-alcohols with lower volatility. These remaining oils are well known for having anti-biotic properties. Other extractives were also subjected to qualitative and quantitative analysis. Ethyl acetate and alcohol soluble fractions containing phenols and phenol glycosides, respectively, also remained in the pruning shoots and pellets after storage and pelletizing. These fractions exhibited anti-oxidant activity.

Pellets produced from pruning residues with varied moisture content. No correlation between moisture content and durability was detected. However, the durability did correlate with the pellet length. The durability of the pellets was slightly lower than that of the normal tree trunk pellets. In addition, the higher heating value (HHV) of the pruning residue pellets was higher than that of the normal pellets because of the higher extractive concentrations such as in terpenoids, phenols, and wax.

Key words: Japanese cedar, pellets, essential oil, extractives, antioxidant

1. INTRODUCTION

Recently, a variety of bio-fuels have been produced from biomass. These bio-fuels include fuel pellets from various wood materials, bio-ethanol from corn and molasses, biosolids derived fuel from plant oils, and methane gas from fermentation of livestock manure. These bio-fuels, being carbon neutral, are attractive because they limit greenhouse gas emissions. The more familiar wood biomass fuel pellets represent a promising alternative to fossil fuels for heat and power production; in particular, in the residential sector, where automated heating systems can be implemented. Global pellet markets are growing fast. The global annual production of wood pellets was recently estimated at approximately 6–8 million tons with a net potential of approximately 13 million tons [1]. However, in 2008, only 60,000 tons, approximately, were produced in Japan [2]. The increasing difficulties with low-cost supply of raw materials are perceived as a barrier to its widespread use. Till date, in Japan, the main resources for wood pellets are waste wood from sawmills and wood industry byproducts such as sawdust and cutter shavings. The national price of wood residues has increased to an unsustainable level with respect to pellet production. Recently, a new Japanese government policy recognized thinnings as a new resource for wood pellet production. However, thinnings are priced higher, which is reflected in the increased pellet production cost and the attending reduced marketability as fuels for domestic and industrial use. It is therefore desirable to find new resources for fuel pellet production.

Agricultural by-products and grasses have become a possible feedstock for producing renewable fuels and their characteristics for pellet production have been investigated by many researchers around the world. Kariyan and Morey investigated, using micro-structural analysis, the role of the natural binders in corn stover and switch grass to achieve durable particle–particle bonding in pellets [3]. They concluded that solid bridges between the particles were mainly achieved by natural binders such as lignin and protein, and that activating the natural binders using moisture and temperature in the glass transition is important to create durable particle–particle bonding. Stelte et al. showed that the pellets produced from straw have low strength [4]. Mani et al. investigated the effects of compressive forces, particle size, and moisture content on mechanical properties of pellets from corn stover, wheat straw, barley straw, switch grass, and various other grasses [5]. They concluded that all these properties significantly affected the pellet density except for the particle size of wheat straw.

Forests cover approximately 68% of Japan and 13% of these forests consist of Japanese cedar (*Cryptomeria japonica*). Cedar forest residues from thinning and pruning have been estimated at approximately 20

million m^3. Forest development projects require that these pruning shoots be removed from conifer tree stands, whereas the litters that fall into broad-leaf stands decompose easily and are important sources of fertilizer. However, these residues are usually left in the forests unused, and have been an attractive biomass for the production of chemicals and energy resources. Considering this fact, some transport trials have been conducted in various forest stands to evaluate transportation costs. In general, the forest residues contain many more extractives than tree trunks. It is known that Japanese cedar leaves contain various essential oils such as mono-terpenes and sesquiterpenes and that these compounds have anti-biotic properties [6]. Furthermore, the leaves include polyphenols such as flavonoids and lignans, which exhibit anti-oxidant activity [7]. Therefore, it was expected that these fuel pellets would have additional functions.

On the other hand, it has been suggested that the active sites on the particles may be blocked by hydrophobic extractives that have migrated to the particle surface during drying and hot pressing, thus obstructing wettability and reducing the bond strength [8]. The effect of the extractives on the physical properties of fuel pellets has been extensively studied. Nielsen et al. evaluated the effect of extractives and storage on the pelletizing process of sawdust and the results showed that removal of the extractives significantly increased the pellet strength and the energy requirements in all stages of the pelletizing process compared to the samples with extractive content (Nielsen et al., 2010). They concluded that extractives act as plasticizers and lubricants thereby decreasing the energy requirements for the pelletizing process. In addition, extractives seem to prevent close contact between the bonding sites of the lignocelluloses particles thereby decreasing the pellet strength. Bergstrom et al. obtained the same results with pre-extracted Scots pine sawdust [10]. The results showed that the extracted sawdust yielded pellets of higher density and compression strength than those made from non-extracted sawdust. Stelte et al. investigated the fracture surfaces of straw pellets by infrared spectroscopy [4]. The results indicated that high concentrations of hydrophobic extractives were most likely responsible for the low compression strength of the pellets because of the presence of a chemical weak boundary layer, limiting the adhesion mechanism to Van der Waals forces.

During storage of raw materials, lipophilic extractives are broken down through microbiological and auto-oxidative processes resulting in increased particle surface wettability and improved particle bonding [11, 12]. Arshadi and Gref reported that pellets stored under certain conditions emit high levels of volatile organic compounds, especially volatile aldehydes such as hexanal and pentanal [13].

In this study, changes of extractives in pruning shoots of Japanese cedar during storage and pelletizing procedure were measured. Furthermore, physical properties of pellets produced from stored pruning shoots were evaluated.

2. EXPERIMENT

2.1 Raw materials

Pruning shoots used as raw materials for the production of fuel pellets were prepared from the Japanese cedar (*Cryptmeria japonica*) plantation site in a Niigata prefecture research forest at Murakami city, Niigata. The climate in the area is moderate, with an annual maximum temperature of 26°C and a minimum of 2°C. The mean annual rainfall is 2,230 mm. Pruning shoots were cut from 19-year old cedar trees in November of 2008 and kept on the ground and covered with a sheet for four months. Their shoots (composition rate of branches and leaves was 1:3) were picked up in April of 2009 and broken down into fragments of size less than 20 mm with Oohashi Ogarizer type GS70G. Then, the fragments were dried in a vinyl house with moisture content (MC) ranging from 6.0% to 12.0%.

2.2 Pellet production

Pellets were prepared from pruning shoot particles of Japanese cedar with a Shinko–Kouki TS-55 pelletizer equipped with a flat die. The pellet diameters were 6.5 mm and the temperature of the die during pelletizing was not controlled.

2.3 Preparation and analysis of essential oils

Essential oils from fresh shoots, stored shoots (dried to an MC of 6%), and pellets (prepared from the stored shoots) were prepared with a steam distillation method for 2 hr. The volumes of the essential oils were measured and their contents (V/W, dry base) in the raw materials were calculated by the following formula.

$$Ec = 100 \times Ev/[Sw \times (100 - MC)/100]$$

where Ec = essential oil content (%, V/W)),
Ev = essential oil volume (ml),
Sw = sample weight (g),
MC = moisture content (%).

The essential oils contained in these different tissue types were analyzed using the Shimadzu GC-14A equipped with an FID and capillary column CBP1 (25 m × 0.25 mm i.d.; film thickness, 0.25 um). The injector temperature was maintained at 240°C, and the column oven temperature was programmed from 50°C to 230°C as in the GC-FID analysis. The exact contents of typical oil components were analyzed by calibration curves of peak areas of 14 authentic samples and quantification of other peaks was performed using percentage peak area calculations. The samples were injected into the GC injector with a 1:10 split ratio. The chemical compositions of the essential oils contained in the different tissue types were also analyzed with a Finnigan Trace GC-Polaris Q mass instrument (Finnigan-Spectronex), equipped with a capillary column RTx-5MS (30 m × 0.25 mm i.d.; film thickness, 0.25um). Mass spectra were recorded over the 35–650 amu range at 1 scan/s, with ionization energy of 70 eV and an ion source temperature of 200°C. Helium was the carrier gas at a flow rate of 1 mL/min, and the identification of individual components was done by comparison of their relative retention indices with authentic reference compounds and using the Wiley/NBS Registry of Mass Spectral Database and NIST MS Search.

2.4 Fractionation of alcohol-benzene extractives

Extraction from the fresh shoot particles, stored shoots, and pellets was done with a soxhlet extractor using a mixed solution of ethanol (benzene = 1:2) for 6

hr. After extraction, their mixed solutions were evaporated in a flask, and weighed with the flask before and after evaporation.

The alcohol–benzene extractives were fractionated by solubility of each organic solvent as follows. Dried alcohol–benzene extractives were solved with a small volume of ethanol and the ethanol solution was absorbed into silica gel C100. The ethanol in the silica gel was evaporated and then the dry silica gel was extracted with hexane, diethyl ether, ethyl acetate, ethanol, and water, respectively. Solvents of their solutions were evaporated and weighed.

2.5 Assay of antioxidant activity

The radical scavenging capacity of DPPH was determined according to the method reported on by Kitts et al. [14]. Briefly, the reaction mixtures of test samples and DPPH in an ethanol solution were incubated in test tubes at 37°C for 30 min. Commercial antioxidants including quercetin and ascorbic acid were used as positive controls. The mean value was obtained from triplicates, and the percent inhibition was calculated. The IC_{50} (inhibitory concentration) values denote the concentrations of the samples required to scavenge 50% of the DPPH free radicals.

2.6 Physical properties

Pellet density represents the ratio between the pellet mass and its volume including the pore volume. The volumes of pellets were calculated as volumes of cylinders. Bulk densities were measured according to JIS Z 7302-9. The mechanical durability of the pellets was measured according to the standard method CEN/TS 15210 with a pellet tester type DT-D purchased from the Sanyo trading company. The pellet tester subjected a 500 g pellet sample to controlled shocks by collision of pellets against each other and the wall of a defined rotating chamber. Mechanical durability basically refers to the pellet density and how well it is formed.

3. RESULTS AND DISCUSSION

3.1 Essential oil contents and their composition

The essential oil contents of each sample are shown in Table I. The oil content of fresh shoots was 1.67% in dry base and the content of stored shoots decreased to 0.56% (approximately 34% of the fresh shoot oil content). This change of the oil content is the result of volatilization and breaking down through microbiological and auto-oxidative processes during storage [12, 15]. Furthermore, half of the oil content of the stored shoots (i.e., 0.29% or approximately 17% of the fresh shoot oil content) was lost because of the pelletizing procedure applied to the stored shoots. The GC spectrum of the essential oil from fresh shoots and their chemical structures are shown in Fig.1.

Table I Yields of essential oil from pruning shoots

Sample	Concentration % on dry base
Fresh shoots	1.67
Stored shoots	0.56
Pellets	0.29

*Sample No 2 (see table 5) was used

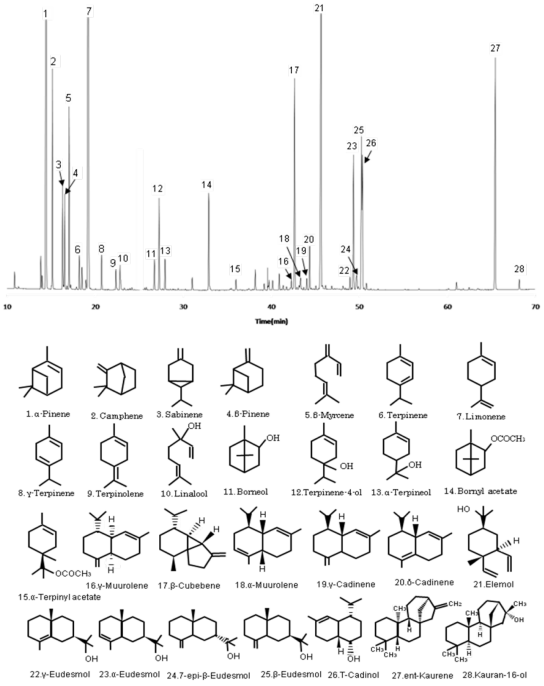

Fig.1 Gas chromatogram of essential oil from fresh shoots and assign of peaks

The identification of peaks was accomplished by comparing the retention times with authentic samples and the fragment patterns with GC/MS. Compounds 1–15 were monoterpene, compounds 16–26 were sesquiterpenes, and compounds 27 and 28 were diterpenes. The presence of these compounds in the Japanese cedar leaves has been reported by Chen *et al* [6]. They studied the chemical composition of the essential oils contained in four different Japanese cedar tissues by GC-MS and suggested that there were large differences in the chemical composition of fresh leaves and bark and that there were nonvolatile compounds present in the bark, which were not detected in the leaves. They also investigated the anti-fungal activity of the essential oils and concluded that the oils in the leaves had the highest resistance to pathogenic fungi. Terpenoids from the Japanese cedar are well known as bioactive compounds such as antifungal [16, 17] antimite [18, 19] and anti-pathogenic [7, 20]. The qualitative results for each compound contained in the essential oils from fresh shoots, stored shoots, and pellets are shown in Table II. A significant decrease of monoterpene contents was observed in the stored shoots and pellets. Approximately 10% of the volatile monoterpenes remained in the fresh shoot pellets and near total of the amount disappeared after storing and pelletizing. The remaining monoterpenes were mainly acetates of monoterpene-alcohols. In the pellets, elemol (compound 21, sesquiterpene), β-eudesmol (compound 25, sesquiterpene), and ent-kaurene (compound 27, diterpenes) were mostly preserved and represented the main components of the pellet oil causing an odor different from that of the fresh shoots. It was suggested

Table II Concentration of terpenoids in samples, mg/kg on dry base

	Fresh shoots	Stored shoots	Pellet
α-Pinene	4041	261	17
Camphene	520	29	ND
β-Pinene	258	274	20
β-Myrcene	225	17	ND
α-Terpinene	459	51	ND
Limoαnene	96	19	ND
γ-Pinene	3063	108	12
Terpinolene	96	85	20
Linalool	50	24	5
Borneol	87	14	9
Terpinene-4-ol	91	8	7
α-Terpineol	251	284	88
Bornil acetate	84	18	7
Terpinyl acetate	277	40	37
α-Terpinyl acetate	26	11	10
16.γ-Muurolene	27	9	7
β-Cubebene	671	28	12
α-Muurolene	48	15	24
γ-Cadinene	30	23	37
δ-Cadinene	134	85	110
Elemol	1622	952	576
γ-Eudesmol	39	15	13
α-Eudesmol	455	203	139
7-epi-β-Eudesmol	71	67	48
β-Eudesmol	542	385	244
T-Cadinol	380	181	161
ent-Kaurene	854	381	312
Kauran-16-ol	30	46	24

that in laboratory bioassays, elemol could act as a repellent for blacklegged ticks (a.k.a. deer ticks) and lone star ticks, which are vectors of Lyme disease and human monocytic ehrlichiosis, respectively [11]. Seo *et al.* determined the pharmacological effect of β-eudesmol as an anti-inflammatory agent. On the other hand, ent-kaurene has been known as one of the biosynthetic intermediates in the production of gibberellins, which are plant hormones [21].

3.2 Extractive fractions and antioxidant activity

Japanese cedar tissues contain various secondary metabolites as well as essential oils. Alcohol–benzene extractives from the shoots and pellets were fractionated by solubility with organic solvents and their contents are shown in Table III. Low polarity fraction contents such as hexane and ether soluble fractions, consisted of volatile compounds, and decreased with aging and pelletizing.

Table III Extractives content in the shoots and pellet (%)

Solvent	Fresh shoots	Stored shoots	pellet
Alcohol-benzene	16.1	7.2	6.3
Hexane	0.8	0.3	0.2
Diethyl ether	4.1	3.2	2,5
Ethyl acetate	2.2	1.9	1.6
Ethyl alcohol	4.3	4.1	4.2
Water	0.9	0.8	1.3

*68g (dry weight) of sample was used for extraction
Yields were calculated based on dry weight samples

On the other hand, the effect of aging and pelletizing on high polarity fraction contents was low because they consist of compounds with high boiling points.

Antioxidant activities of these fractions were measured by the DPPH method and the results are shown in Table IV. The hexane soluble and diethyl ether soluble fractions had little activity and the ethyl acetate, ethyl alcohol, and water soluble fractions had high DPPH scavenging activity because they consisted of phenols. These results suggest that the stored shoot pellets can be used in various fields before they are finally used for combustion.

Table IV DPPH scavenging activity of each fraction

Concentration mg/ml	DPPH scavenging activity, %					
	Alcohol-Benzene	Hexane	Ether	Ethyl-acetate	Ethyl-alcohol	Water
0.323	-	-	-	-	-	-
0.658	12.3	8.7	5.3	10.2	12.9	11.3
1.316	29.1	20.8	17	21.7	26.6	27.1
2.632	41.1	22.3	18.1	36.6	44.8	47.8
6.579	64.5	23.1	19.3	54.5	61.1	64.1
13.158	90.7	28.6	21.6	83.0	96.7	89.8
Ic50	3.98	-	-	5.58	3.77	3.02

* DPPH Concentration =0.13mM

3.3 Pellet characterization

The physical properties of the pellets are shown in Table V. It was found that raw materials with low MC produced pellets with slightly higher bulk density than

Table V Characters of pellets from various moisture contents of raw materials

No	Moisture content (%)		Pellet size (mm)		durability %	Density g/cm^3	Bulk density kg/m^3
	Raw	Pellet	Diameter	Length			
1	6.0	5.3	6.6	9.9	54.2	1.007	570.3
2	7.7	6.9	6.6	11.2	64.5	0.994	556.0
3	8.5	6.9	6.6	12.0	68.8	1.006	577.9
4	9.4	7.7	6.6	8.1	51.6	0.965	515.1
5	10.6	10.3	6.6	14.9	87.3	1.045	536.7
6	12.0	11.3	6.6	10.7	60.1	0.936	483.4

those with high MC. In addition, the correlation of the pellet durability with pellet length was high, whereas the correlation between pellet durability and pellet density was low (Figs.2 and 3). In general, the durability of pellets from pruning shoots was lower than that of normal pellets from tree trunks. Therefore, it is necessary to handle the pellets with care during loading and transporting. The higher heating value (HHV) of pellets from stored shoots of 7.7% MC was 22.6 MJ/kg. This value was higher than for normal pellets because averages of the HHV of normal pellets from tree trunks range from 18 to 20 MJ/kg.

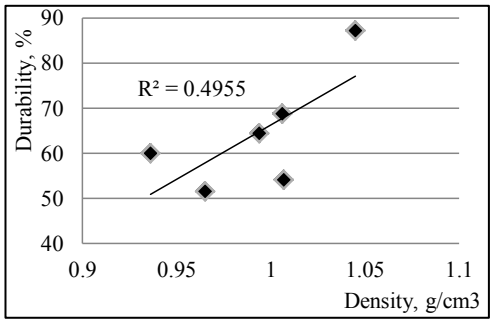

Fig.2 Relationship between durability and density

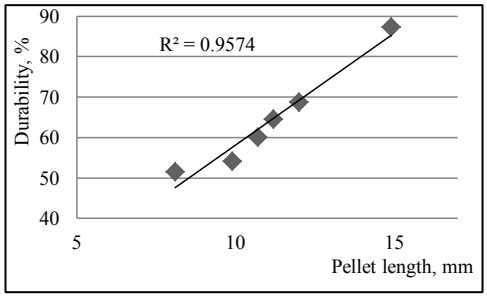

Fig.3 Relationship between durability and pellet length

CONCLUSION

Part of the essential oils remained in the pellets from stored pruning shoots with changing oil compositions. Monoterpenes with high volatile properties were released from the leaves during storing and pelletizing of the pruning shoots. The remaining oils in the pellets consisted mainly of sesquiterpenes, diterpenes, and some of the monoterpene-alcohols with lower volatile properties. These remaining oils also have anti-biotic properties. This constitutes a new use for the pellets, which are expected to be obtained at the stage prior to combustion. Other extractives were subjected to qualitative and quantitative analysis. The ethyl acetate soluble and ethyl alcohol soluble fractions, which were the primary remaining components in the stored shoots and pellets, exhibited high antioxidant activity.

Fuel pellets were produced from stored pruning shoots of the Japanese cedar with varied moisture content. Although the durability of the pellets was slightly lower than that of the normal pellets from tree trunks, the HHV of pellets from pruning shoots was higher than that of the normal pellets because of higher extractive concentrations such as terpenoids, phenols, and wax.

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