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Hemicellulosic polymer from Vetiver grass and its physicochemical properties

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Abstract

Chemical composition of two ecotypes of the Vetiver grass (*Vetiveria zizanioides nash*) leaves has been investigated using the gravimetric method. The principal polysaccharides found in samples were hemicelluloses (ca. 38%), followed by cellulose (ca. 27%). Protein content determined by bicinchoninic acid assay using bovine serum albumin (BSA) as a standard was approximately 5%. Lignin content determined by acid chlorite method was approximately 10%. Ash content was 3% consisting of mainly silica (ca. 50%). The obtained hemicelluloses with the molecular weight of 30,000 showed the decomposition temperature at 310 °C. In order to determine an optimum condition for extraction of hemicelluloses, effects of process parameters: alkaline type (NaOH, KOH, $Ca(OH)_2$ and $Ba(OH)_2$), alkaline concentration (0.025–4 M), extraction time (2–18 h) and temperature (25–60 °C) were studied. It was found that all parameters influenced on the hemicellulose yield and properties in various degrees. Finally, an optimum condition giving a maximum yield of 35% hemicelluloses was recommended as 4 M NaOH at ambient temperature for 8 h.

Keywords: Chemical composition; Hemicelluloses; Vetiver grass; Process parameters; Physicochemical properties

1. Introduction

Nowadays, earth-environmental pollution or disruption is becoming a serious problem. The polymer industry has numerous problems regarding recycling or disposal of polymeric wastes at the present. Therefore, material developments without an accompanying disruption of the earth-environment are getting much more important even in the polymer industry. Among them, the natural polymers have undergone a re-evaluation regarding their ability to biodegrade. There is a growing interest in exploiting renewable resources for the production of biodegradable polymers. The lignocellulosic waste materials from agriculture and forestry look promising to replace unfriendly environmental hydrocarbons. Due to their abundance, renewability and biodegradation, these raw materials have received considerable interest as a source of chemicals in recent years.

Vetiver grass is one of tropical plants and presumably its origin is in India. The Vetiver grass can grow in wide ranges of area, from highlands to lowlands, and in various soil conditions. In Thailand, there are two species of the Vetiver grass commonly found; *Vetiveria zizanioides nash* and *Vetiveria nemoralis A. Camus*. Principal utilization of the Vetiver grass is for preventing soil erosion and conserving soil moisture according to His Majesty's Initiative. Since His Majesty the King has realized the problems of soil erosion and recognized that the Vetiver grass has a deep thick root system like an underground curtain, it thus enables to form a naturally embankment and to store water or moisture. In addition, roots of the Vetiver grass especially *V. zizanioides* are used to extract volatile oil for making perfume, soap and other fragrant materials. Normally, leaves of the Vetiver grass has been cut every few months. Among these large quantities, only a minor portion of the residues is reserved as animal feed or household fuel. However, huge quantities of the remaining residues are not used as industrial raw materials and are burnt in fields or on the side of road. Thus, investigations

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aiming to improve the value of such a residue are important. In this work, we chose to focus on hemicelluloses although plant materials are composed of several components. In nature hemicelluloses comprise roughly one-fourth to one-third of the most plant materials and some important applications of these polysaccharides have been demonstrated during the past two decades. They showed a potential fermentation feedstock for the production of sugars, furfural and used as an additives in paper-making, pharmaceutical, cosmetic and food areas (Freden et al., 2002; Sun & Tomkinson, 2002). A novel product like hydrogel has been also exploited since hemicelluloses are, in their native form, responsible for the interaction with water (Gabrielii, Gatenholm, Glasser, & Kenne, 2000). In addition, with a chemical modification, a hydrophobic film from maize bran hemicelluloses has been created as a bioplastic (Fenden et al., 2002).

The global aim of the work, being presented here just a part, is to assess the practical various applications of hemicelluloses extracted from the Vetiver grass. A thorough understanding of the chemical composition of the Vetiver grass and influence of the extraction process on the physicochemical characteristics of hemicelluloses is the first step in achieving such an aim. The objectives of this study were to investigate the chemical composition of two ecotypes of the Vetiver grass. Role of the process parameters and the physicochemical properties of the hemicelluloses were also studied and discussed.

2. Experimental

2.1. Materials

Two ecotypes (*Mae hae* and *Songkha* 3) of the Vetiver grass (*V. zizanioides*) were kindly supplied by the Royal Project Foundation (Chiangmai, Thailand) and the Land Development Department (Nakhon Rachasrima, Thailand), respectively. The grasses aged about 1 year were hand-harvested and dried in sunlight. Then, only leaves were ground using a grinder to pass a 1 mm size screen and dried in an oven at 60 °C for 16 h prior to use.

2.2. Chemical analyses

All analyses were performed in five replicates using methods modified from those described by Harper and Lynch (1981). Briefly, the chemical components were determined by sequential extraction and weighing. All weights and calculations were performed on dry basis.

2.2.1. Ash content and inorganic components

About 0.1 g of samples were accurately weighed in porcelain crucibles and then heated in a muffle furnace at 550 °C for 5 h. After that the crucibles and ash were cooled in a desicator and reweighed. The remaining weights of samples were recorded as ash content. Inorganic components in ash were determined using X-ray fluorescence spectrometry (XRF, Phillips PW2400).

2.2.2. Extractives

Extractives from the ground samples (35 g) were extracted by chloroform–methanol (2:1 v/v, 600 ml) for 5 h using a Soxhlet apparatus. After that solvent was removed using a vacuum rotary evaporator and then the extractives were dried and weighed. The extractive free samples were also dried in an oven at 60 °C for 16 h.

2.2.3. Protein

The extractive free samples were added with phosphate buffer (0.2 M, pH 7.5) containing sodium azide (0.003 M). Proteolysis was started by addition of protease type XXIV (EC No 232.642.4, 0.25 mg/g sample). The proteolysis was proceeded with continuous stirring at 40 °C for 6 h. After that the activity of enzyme was terminated by incubation in boiling water for 3 min. Then the samples were filtered, washed with water and dried at 60 °C for 16 h. Protein content in filtrates were determined by bicinchoninic acid protein assay (Stoscheck, 1990) using bovine serum albumin (BSA) as a standard.

2.2.4. Hot water soluble polysaccharides

After proteolysis, the dried grasses were boiled twice in distilled water (100 ml/3 g samples) for 2 h. The sample were filtered and residues were washed twice with hot water and dried and 60 °C for 16 h. The weight loss was defined as the hot water soluble component.

2.2.5. Weakly acidic pectic polysaccharides (WAPP)

The dried residues from previous extraction were added with hydrochloric acid (0.01 M, 800 ml) and ethylenediaminetetraacetic acid (0.02 M, pH 2.7) and heated at 85 °C for 4 h. Then extracts were adjusted to pH 5 with ammonia and filtered. After that filtrates were concentrated, precipitated with ethanol (5 volumes) and left at room temperature for 5 days. After filtration, the resultant white powder labelled as weakly acidic pectic polysaccharides (WAPP) was washed with ethanol and dried at 60 °C for 2 h. The grass residues were washed with water and then dried at 60 °C for 16 h for further extraction.

2.2.6. Ethanol soluble component

The remaining residues after previous extraction were added with 80% ethanol (100 ml/1.5 g samples). The mixture was boiled gently for 3 h. After that the residues were filtered, washed twice with ethanol and water, dried at 60 C for 16 h and weighed. The weight loss was defined as hot 80% ethanol soluble component.

2.2.7. Lignin

The residues from above procedure (3 g) were added with water (150 ml), acetic acid (2 M, 10 ml) and delignified using sodium chlorite (5 g). The mixtures were gently stirred and heated at 75 °C for 1 h. Then acetic acid (2 M, 5 ml) and sodium chlorite (2.5 g) were added again and stirred for another hour. After lignin was oxidized for 2 h, the residues were filtered and washed with water (three times), dried at 60 °C for 16 h and reweighed. The weight loss was defined as sodium chlorite lignin.

2.2.8. Hemicelluloses and cellulose

Hemicelluloses from the remaining residues were extracted with potassium hydroxide (4 M, 100 ml/2.5 g residues) containing sodium borate (0.001 M) at room temperature for 2 h. After filtration, the residues were washed with water (several times) and acetic acid (1 M) and dried at 60 °C for 16 h. The weight loss was defined as hemicelluloses. Weight of residues remained after alkaline extraction and corrected for ash content was taken as cellulose.

2.3. Effect of process parameters on physico-chemical properties of hemicelluloses

The scheme for extraction of hemicelluloses from the Vetiver grass is shown in Fig. 1. Firstly, wax and other extractives were eliminated from the dried and ground samples by extraction with chloroform–methanol (2:1, v/v) for 5 h in a Soxhlet apparatus. The hemicelluloses were isolated from the chlorite holocellulose (2.5 g) with alkaline solution (100 ml). The mixture was stirred at ambient temperature for 18 h. After filtration with a nylon cloth, the hemicelluloses were isolated from the filtrate by precipitation of the acidified filtrate (pH 5) with three volumes of 95% ethanol. The polysaccharide was collected by filtration and dialyzed against deionized water for 18 h. The products were suspended in water and centrifuged. The combined supernatants and pellets were dried using lyophilizer and called as soluble and insoluble hemicelluloses, respectively. Yield of the hemicelluloses was calculated on dry basis and represent as the mean of triplicate.

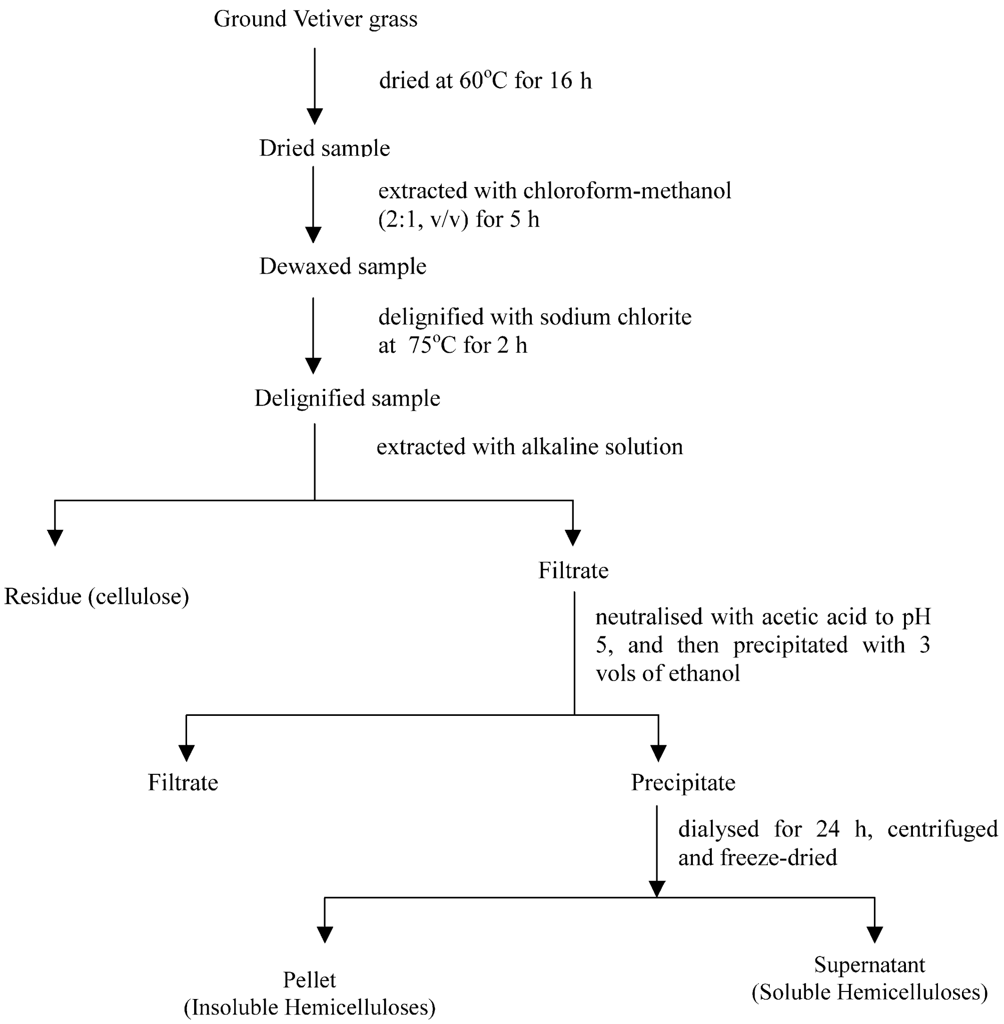

Fig. 1. Scheme for isolation of hemicelluloses from the Vetiver grass.

2.3.1. Effect of extractant

According to Fig. 1, the hemicelluloses were isolated from the Vetiver grass samples using various alkaline solutions (2 M NaOH, 2 M KOH, saturated $Ca(OH)_2$ and saturated $Ba(OH)_2$) at ambient temperature for 18 h.

2.3.2. Effect of alkaline concentration, time and temperature

The hemicelluloses were extracted using various NaOH concentrations (0.025–4 M) and various times (2–18 h). The obtained hemicelluloses were purified and dried in the same manners as mentioned above. For the effect of temperature, the studied conditions were using 2 M NaOH, 2 h at 40 and 60 °C.

2.4. Gel permeation chromatography (GPC)

The chromatographic analysis was carried out using PL-gel permeation chromatography (GPC) 110 (PolymerLab, UK) system equipped with a differential refractomer (RI). An Ultrahydrogel linear column (7.8 ID × 300 mm, Waters, USA) was used for determining the molecular weight of the samples. Calibration curve parameters were calculated using the SEC option of the PL Logical GPC software package (PolymerLab). The universal calibration $\log(M_P)$ versus V_R, where M_P is the peak molecular weight obtained using pullulan standards (PolymerLab) with molecular weight ranging from 738 to 1,660,000. The injection volume was 20 μl (2 mg/ml of sample), the running temperature was 30 °C, and the mobile phase flow rate was set to 0.06 ml/mm. NaOH (0.1 M) solution was used as an eluent.

2.5. Thermal properties

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer TGA7 with continuous nitrogen flushing. Samples (5–10 mg) were heated from room temperature to 900 °C with heating rate of 20 °C/min. The thermograms were recorded as a function of temperature.

2.6. Solubility

Samples (1 mg/ml) were dissolved in various studied solvents: water, acetonitrile, DMSO, chloroform, hexane, and NaOH (0.1–4 M). For a maximum solubility test, samples were added to a constant volume of solvent until saturated. After stirring for overnight, the samples were centrifuged. The remaining residues were dried and weighed.

3. Results and discussion

3.1. Chemical composition of the Vetiver grass

The chemical compositions of two ecotypes (Mae hae and Songkha 3) of the Vetiver grass are shown in Table 1. Like a general plant, three principal components found in the Vetiver grass are cellulose (ca. 30–35%), hemicelluloses (ca. 40%) and lignin (ca. 10%). By comparison the chemical composition between two ecotypes of the Vetiver grass, there were no obvious differences apart from protein and WAPP contents. The protein content found in Mae hae (ca. 1%) was lower than that found in Songkha 3 (ca. 4%) whereas WAPP content in Mae hae (ca. 7%) was considerably higher than that in Songkha 3 (ca. 1%). Ash content of both samples was approximately 3% with significantly lower than that of rice straw from various sources, which ranges from 10 to 17% (Agbagla-Dohnani, Noziere, Clement, & Doreau, 2001; Lam, Bigot, Delmas, & Avignon, 2001; Sun, Tomkinson, Ma, & Liang, 2000). However, there are no differences in the amount of ash between the Vetiver grass samples and rye straw from

Table 1
Chemical compositions of two ecotypes of the Vetiver grass leaves

Composition	Mae hae		Songkha 3	
	Content (%)[a]	Content after normalization (%)	Content (%)[a]	Content after normalization (%)
Ash	4.91 ± 0.30	3.33	4.96 ± 0.34	3.66
Extractives	2.32 ± 0.17	1.57	3.15 ± 0.29	2.33
Protein	1.97 ± 0.20	1.34	5.37 ± 0.50	3.97
Hot water soluble polysaccharides	10.52 ± 0.74	7.13	7.75 ± 1.13	5.72
WAPP[b]	10.00 ± 0.81	6.78	0.90 ± 0.40	0.66
Hot 80% EtOH soluble	2.87 ± 0.20	1.95	2.60 ± 0.57	1.92
Lignin	14.88 ± 1.03	10.09	10.66 ± 1.92	7.87
Hemicelluloses	58.36 ± 1.63	39.57	53.35 ± 1.17	39.40
Cellulose	41.64 ± 1.63	28.24	46.65 ± 1.17	34.46

[a] Mean ± standard deviation, $n = 5$.
[b] WAPP—weakly acidic pectic polysaccharides.

Table 2
Inorganic components in ash of the Vetiver grass

Constituents	Content (%)
SiO_2	50.80
Al_2O_3	1.14
Fe_2O_3	0.61
CaO	6.98
MgO	3.02
Na_2O	0.51
K_2O	19.52
P_2O_5	7.28
MnO	0.43
TiO_2	0.05
Cl	2.51
Other impurities	0.05

UK (Sun, Fang, & Tomkinson, 2000). The predominating elements found in ash of the samples as shown in Table 2 are silica, potassium, phosphorus and calcium whereas these of barley straw are silica, potassium, calcium and chloride (Theander & Åman, 1984). Since the minerals are known to vary widely depending on agronomical factor and with the amount of contaminating soil. Silica (SiO_2) content of the Vetiver grass ash is approximately 50% with slightly lower than that of rice straw (ca. 70%). The role of silica in plant cell wall is even more ambiguous. It has been reported that silica has a positive effect on plant growth, contributes to the plant resistance against insects and fungi and increases mechanical resistance (Sun, 1996). Extractives found in the samples were approximately 2%. Normally, the extractives are contributed as chlorophyll, wax and other polymers such as cutin and seberin. They are produced by plants for photosynthesis and for protecting themselves from the effects of water loss, from the effect of high and low temperatures and from the effects of insect attack. Since there was no obvious difference in hemicellulose content of both ecotypes studied, Songkha 3 was chosen for further studies.

3.2. Effect of alkaline types on hemicellulose yield

To evaluate the importance of alkaline extractant type on the yield of hemicelluloses, aqueous solution of NaOH, KOH, $Ca(OH)_2$ and $Ba(OH)_2$ were used. Two fractions of hemicelluloses, water soluble and insoluble, were obtained from the Vetiver grass. Percentage yields of the hemicelluloses extracted using various type of alkali are shown in Fig. 2. NaOH and KOH represent as a monovalent extractant whereas $Ca(OH)_2$ and $Ba(OH)_2$ represent as a divalent extractant. Efficiency of the extraction tends to be associated with size of a positive charge ($K^+ > Na^+$, $Ba^{++} > Ca^{++}$). The bigger size, the higher yield. Practically, $Ca(OH)_2$ and $Ba(OH)_2$ are not a good alkaline for isolating hemicelluloses from the Vetiver grass since they gave a lower yield compared with NaOH and KOH even though their concentration was increased until saturated. In addition, it seems to be that $Ca(OH)_2$ and $Ba(OH)_2$ cannot release the insoluble fraction from the samples. It was concluded that the variation of the yield depended on the nature of the alkaline. For further analyses, NaOH was used as an extractant due to its easy assesibility.

3.3. Effect of process parameters on hemicelluloses yield

Effects of alkaline concentration, time and temperature on the hemicellulose yield were investigated. For the effect of alkaline concentration, four different concentrations of NaOH (0.025, 1, 2 and 4 M) were used. As would be

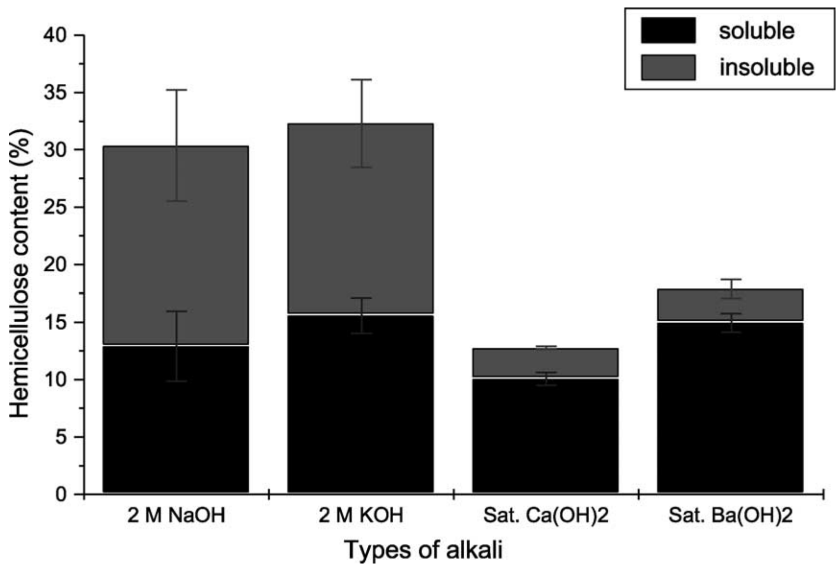

Fig. 2. Hemicellulose contents extracted from Songkha 3 using various alkaline extractants at room temperature for 18 h.

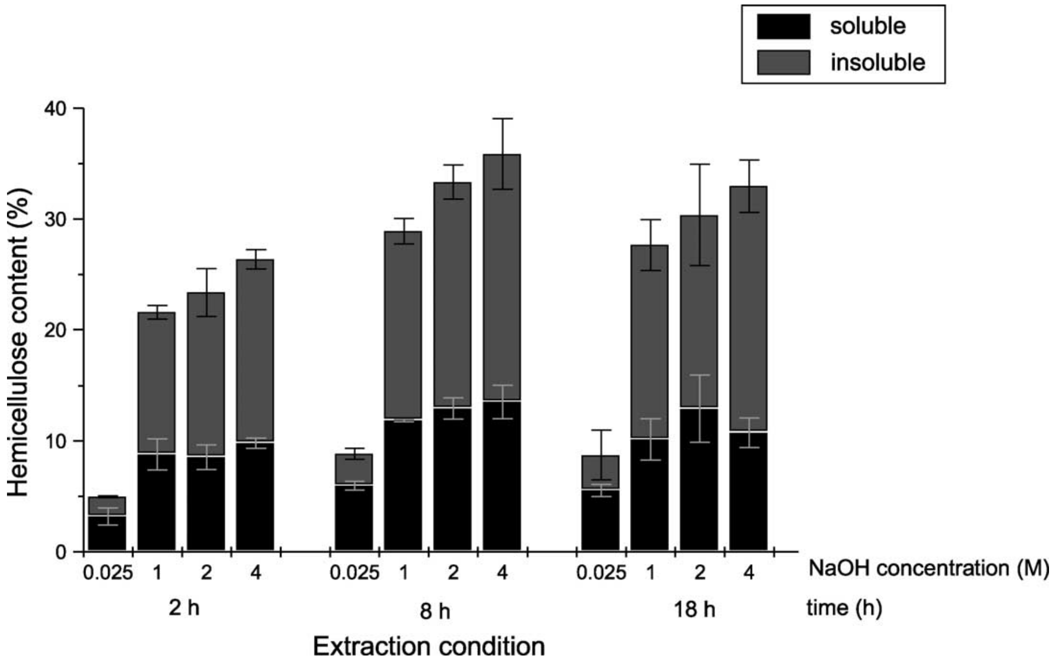

Fig. 3. Effect of alkaline concentration and time on percentage yield of hemicelluloses (at room temperature).

expected, the percentage yield increased when the concentration of alkali increased. The obtained yield dramatically increased when using 1 M NaOH compared with 0.025 M. The lower yield at 0.025 M was probably due to too low concentration of alkali in releasing the hemicelluloses from the carbohydrate–lignin complex structure (Ebringerova & Heinze, 2000). Whereas there were no obvious differences in the amount of hemicelluloses obtained when using NaOH concentration in the range of 1–4 M (Fig. 3). Higher yield of the hemicelluloses could be achieved at longer time (>2 h). However, the yield dropped when the reaction was left too prolonged (18 h). Not only alkaline concentration and time but also temperature contributed to achieve a higher yield of hemicelluloses. Even though temperature attributed the increasing of hemicellulose yield (Fig. 4), a higher temperature than 40 °C did not gave a higher yield. The recommended condition for extracting the hemicelluloses from the Vetiver grass with a maximum yield of 35% is 4 M NaOH at room temperature for 8 h.

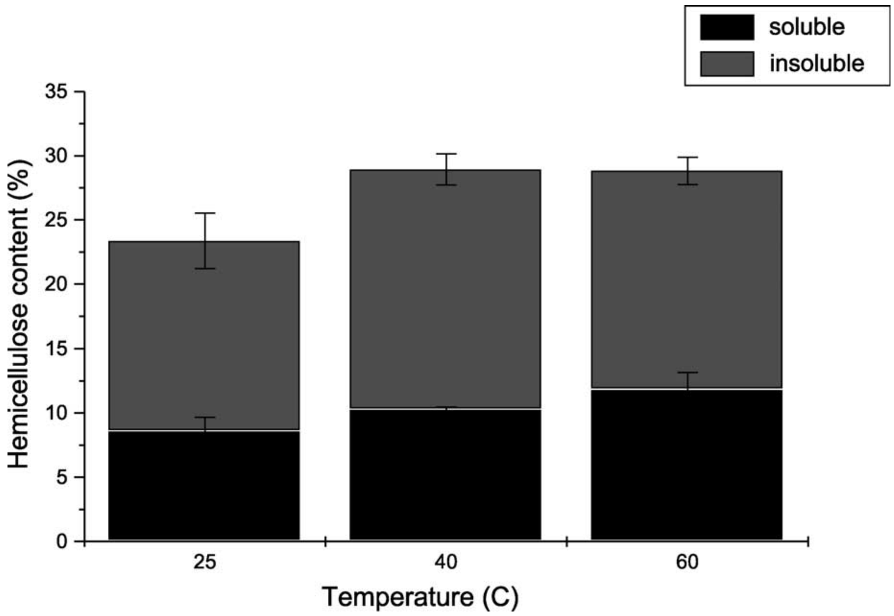

Fig. 4. Effect of temperature on percentage yield of hemicelluloses (2 M NaOH, 2 h).

Table 3
Molecular weights of the hemicelluloses obtained from various extractions

Condition	Molecular weight (kDa) of	
	Soluble fraction	Insoluble fraction
2 M NaOH, 2 h, room temp	33.44, 1.02	25.30, 1.01
2 M NaOH, 8 h, room temp	31.33, 0.94	24.94, 1.03
2 M NaOH, 2 h, 40 °C	29.42, 1.07	24.94, 1.01
2 M NaOH, 2 h, 60 °C	28.54, 0.56	23.19, 0.97
4 M NaOH, 18 h, room temp	33.24, 0.53	25.21, 0.97

3.4. Physicochemical properties of the obtained hemicelluloses

3.4.1. Molecular weight

Effects of process parameters upon the molecular weight of the obtained hemicelluloses were observed. There were two different size components, high (ca. 25,000–30,000) and low molecular weights (ca. 500–1000), in both soluble and insoluble fractions (Table 3). According to the work of Bikova and Treimanis (2002) which combined RI- and UV/VIS detection for monitoring fractions obtained by sequential extraction of *A. incana* xylem, the high- and low-MW components would be expected as hemicelluloses and lignin (aromatic substances). A drastic extraction condition attributes to the degradation of the hemicellulose polymer as would be expected. However, the molecular weights of the samples obtained from various conditions studied were not significantly different.

3.4.2. Thermal properties

Similarly as cellulose, no distinct thermal transitions had been noted. Thermal decomposition of the hemicelluloses extracted from the Vetiver grass began near 220 °C. Maximum rate of weight loss occurred between 290 and 350 °C. This result was well supported by the studies of Ebringerova and Heinze (2000) and of Sun and Tomkinson (2002).

3.4.3. Solubility

The solubility of hemicelluloses, as usual, for polysaccharides and derivatives, is affected by the patterns of intra- and inter-molecular hydrogen bonds. Soluble fraction of the obtained hemicelluloses was very soluble in water and solution up to 500 mg/ml was easily obtained. Insoluble fraction was poorly soluble in water but completely soluble in 0.5 M NaOH solution (up to 350 mg/ml). Both fractions were insoluble in other studied organic media (DMSO, acetonitrile, chlorform, and hexane). Since the rheological behaviour and viscoelasticity of hemicelluloses is important for various practical applications and these properties are a strong structure-dependence, the structural elucidation of these hemicelluloses will be investigated in a further work.

4. Conclusions

Two ecotypes of the Vetiver grass were studied for comparison of the chemical components. They were found to be composed of ash, extractives, protein, hot water soluble, WAPP, 80% ethanol soluble, lignin, hemicelluloses and cellulose. The major polysaccharides in the samples were hemicelluloses (ca. 40%), followed by cellulose (ca. 35–40%). Sodium chlorite lignin was in an account about 10%. There are only obvious differences in protein and WAPP contents. Protein content found in Mae hae was lower than that found in Songkha 3 whereas WAPP of Songkha 3 was higher than that of Mae hae. Besides, the organic components, the leaves of Vetiver grass consisted of inorganic components reported as ash content (ca. 3%). The predominating elements, of ash were mainly silica (SiO_2, ca. 50%) and potassium (K_2O, ca. 20%). Small amounts of other elements such as phosphorous, calcium and chloride also were presented.

Effect of process parameters such as alkaline type, alkaline concentration, time and temperature was studied in order to determine an optimum extraction condition. NaOH is the most practical alkaline extractant compared with KOH, $Ca(OH)_2$ and $Ba(OH)_2$. Alkaline concentration and time are the pronounced effect on the extraction yields. Temperature slightly affected to the obtained yield. The highest yield of 35% hemicelluloses was obtained from a condition using 4 M NaOH at room temperature for 8 h. The reported molecular weight values showed insignificant variations affected by the extraction conditions. Both fractions of the hemicelluloses, water soluble and water insoluble, can be easily dissolved in aqueous NaOH (0.5 M). Their thermal decomposition temperatures started at approximately 220 °C.

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