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Influence of Age, Height and Growing Site on the Chemical Composition of *Yushania alpina* Grown in Central and Northwest Ethiopia

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Abstract: The aim of this study was to determine the age, height and growth site effect on the selected chemical composition of *Yushania alpine* (Highland bamboo). The sample culms were collected from three, sites, age groups and culm positions. All chemical analyses were conducted according to the American Society for Testing and Materials (ASTM) standards except for cellulose content which was determined by Kurschner and Hoffer method. The results were analyzed by SAS Statistical Software. Except for ash content, the study showed that the chemical properties of *Y. alpina* were significantly ($p<0.001$) affected by growing site, age and culm position. Mean values of ash content significantly ($p<0.001$) varied by culm position and growing site. The top culm position of age three years had the maximum cellulose content (64%) while the minimum lignin content (23.04%) was observed for age four from the *Injibara* growing site. Extractive yield highly significantly occurred in the bottom culm position of age three (12.28%) from *Shenen* growing site. The least ash content was recorded for the middle culm position (1.48%), which yielded 38.84% lower than the maximum (2.48%).

Key words: Cellulose · Extractive Yield · Lignin · Yushinia Alpina

INTRODUCTION

Bamboo is a familiar name for a member of a particular taxonomic group of perennial grass with a large woody stem or culm belonging to the family of grasses, Gramineae (Poaceae), subfamily Bambusoideae [1, 2] with distinctive qualities [3]. It is a self-sustaining, self-regenerating, fast growing species and renewable non- timber natural resource [4] and it has gained a considerable attention as a sustainable and renewable fast growing energy resource with short growth cycles of 4-7 years [5]. However, its utilization is fundamentally undeveloped due to lack of awareness about its multiple uses and lack of scientific knowledge about their production and main properties.

In recent years, bamboo has attracted more attention with the greatest potential as an alternative to timber due to its easy propagation, fast growth and high yield [6, 7]. It provides important raw material for pulp and paper production industries, construction as well as for other versatile uses and services. Bamboo properties (chemical composition) differ with age, location, species and external factors [8, 9].

The main constituents of bamboo culms are cellulose, hemi-cellulose and lignin, which amount to over 90% of the total mass. The minor constituents of bamboo are resins, tannins, waxes and inorganic salts. Compared with wood, however, bamboo has higher alkaline extractives, ash and silica contents [10, 11]. Bamboo contains other organic composition in addition to cellulose and lignin. It contains about 2-6% starch, 2% deoxidized saccharide, 2-4% fat and 0.8-6% protein [12] Investigated the chemistry of the immature culm of a mosobamboo (*Phyllostachys pubescens Mazel*). The results indicated that the contents of cellulose, hemicellulose and lignin in immature bamboo increased while proceeding downward of the culm. The increase of cellulose in the lower position was also accompanied by an increase in crystallinity [13] studied the chemical composition of one, two and three year old bamboo (*Gigantochloa scortechinii*). The results indicated that the holocellulose content did not vary much among different ages of bamboo. Alpha-cellulose, lignin, extractives, pentosan, ash and silica content increased with increasing age of bamboo.

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Many studies indicate that chemical composition of bamboo varies with age, height, layer and growing site. Such variation can lead to obvious physical and mechanical properties changes during the growth and maturation of bamboo. Nevertheless, due to lack of knowledge about the chemical properties of *Y. alpina,* its potential for industrial utilization in the country is not yet well-understood. Moreover, age, height and growing site-dependent changes in chemical composition are less well characterized. Hence, this study is conducted to determine the influence of age, height and growing site on the chemical compositions of *Y. alpina* culms that will finally be used to improve its utilization.

MATERIALS AND METHODS

Study Area and Sample Collection: Bamboo 3, 4 and 5 years of age were collected from Injibara (northwest Ethiopia), Shenen and Tikur Inchini (central Ethiopia) areas. The age of culms was determined based on visual inspection (i.e. internode cover and color, sheaths in culms and surface lichen growth) by local experienced field personnel familiar with the history of the clump. Then; ten representative *Y. alpina* sample culms were harvested, coded and transported to forest products innovation research and training center for further processing. Each of the culms were divided into three equal parts then labelled as top, middle and bottom of the culms. Then, the coded culms were chipped and dried in an oven at 40°C for three days. The dried samples were ground into powder with Willey mill in order to pass through a No. 40 mesh (425 ìm) sieve but retained on a No. 60 mesh (250 ìm) sieve. The resulting material was placed in a polyethylene bag and labeled accordingly for further chemical analysis. A total of 27 treatment combinations were used for the study. The design for the experiment was a completely randomized design with three replications [14]. Then the major chemical constituents such as extractive, ash, cellulose and lignin contents were determined.

Chemical Characterization of *Y. alpina*: Except for cellulose content, all laboratory tests were conducted following the standards of the American Society for Testing and Materials (ASTM). There was a minor modification for extractive content test; in this case, instead of benzene solution, toluene solution was used. The method followed for each chemical property analysis is presented below.

Cellulose Determination [15]: One-gram milled culm samples from each age group and culm position were placed into a 250 ml round bottom flask. It was refluxed with three successive portions of a mixture of concentrated nitric acid (20 percent volume by volume) in ethyl alcohol. The reflux process was conducted on average for one hour each. At the end of the extraction process, the flask containing the extractive nitric acid solution was transferred into a known weight of gauche crucible to proceed to the filtration process by using vacuum suction. The extractive materials were washed several times by distilled water. Then the acid mixture extractives were dried in an oven at 105°C for an average of 1 hour, cooled in desiccators and weighed until a constant weight was obtained.

$$Cellulose(\%) = \frac{W2}{W1} x100$$

where:
W1 = the amount of extract free samples taken for analysis
W2 = the residual mass of cellulose

Klason Lignin of Bamboo Culms [16]: One gram oven-dried extractive-free culm powders from each age group and culm position were placed in 100 ml beakers. 15 ml of cold sulfuric acid (72%) was added slowly to each beaker while stirring and mixing well. The reaction proceeded for two hours with frequent stirring. Thereafter, the specimens were transferred into 1, 000 ml flasks by washing them with 560 ml of distilled water and diluting the concentration of the sulfuric acid to three percent. The flasks were placed on hot plates for four hours. The flasks were then removed from the hot plates and the insoluble materials were allowed to settle. The contents of the flasks were filtered by vacuum suction into G-3 glass crucibles of known weight. The residues were washed with distilled water and then oven-dried at 105 ± 2°C. Crucibles were then cooled in desiccators and weighed until a constant weight was obtained. The following formula was used to obtain the Klason lignin content of culm samples:

$$Lignin\% = \frac{(W2 - W1)x100}{O.D.\ weight\ of\ sample}$$

where:
W2 = weight of crucible + sample.
W1 = weigh of empty crucible.
O.D = oven-dry test specimen

Alcohol-Benzene Extractive Solubility [17]: Two grams oven-dry culm samples from each age group and culm posation were placed into filter paper extraction thimbles. The thimbles were placed in a soxhlet extraction tubes. The boiling flasks contained a 2:1 solution of benzene and distilled alcohol respectively were placed on heating mantles. The extraction was conducted for eight hours at a rate of approximately six siphoning per hour. After the extraction process is completed, the thimbles were removed from soxhlet tubes and dried at 105 ± 2°C for overnight. The materials were removed from the thimbles and weighed. The following formula was used to obtain the alcohol-benzene solubility content of bamboo:

Extractive (%) = ((W2 – W1)/(W1)100

where:
W2 = oven-dry weight of the sample before extraction.
W1 = oven-dry weight of the sample after extraction.

Ash Content [18]: The ash content is an approximate measure of the mineral salts and other inorganic matter in plant fiber. The ash in bamboo culms is the inorganic residue after combustion at a temperature of 575±25°c. An empty crucible was ignited and covered in the muffle at 600 °C for 3 hours, cooled in desiccators containing silica gel up to room temperature and weighed to the nearest 0.1 mg. About 2 gm of the samples from each age group and culm position were taken with crucible and kept in a muffle furnace at 600 ±500C for 4hours. Removal of all the carbon particles was ascertained by blocking air from the formation of black char particles. The crucible was cooled in desiccators with silica gel to room temperature and weighed. The process of heating and cooling was repeated until the difference in two successive weightings is less than 1 mg. The lowest weight was recorded as follows:

Ash content (%) = (W1/W2) x 100

where,
W_1 = weight of ash, g
W_2 = weight of test specimen, g moisture-free

Data Analysis: Effects of height (position), age and growing site on chemical composition were assessed by the analysis of variance using SAS software version 9.0 and SAS Studio. The classical general linear model with two-way ANOVA was used to fit the data very well as shown on the results. Mean separation was carried out using LSD at ($p < 0.001$).

RESULTS AND DISCUSSION

Variation in Chemical Properties of *Y. alpina culm*: Cellulose content, lignin content and extractive yield of *Y. alpina* culms were significantly ($p < 0.001$) affected by growing site, culm age and culm position (Table 1). While the ash content of culms taken at three positions and the different growing sites also showed a highly significant difference at p = 0.001 (Table 2). The interaction effect between growing sites, age and culm position showed highly significant variation cellulose and lignin contents and extractive yield. Ash content was highly significantly ($p < 0.001$) different for bamboo culm position versus growing site. The chemical composition of the culms of *Y. alpina* found in this study was in agreement with that reported by Xiaobo [19] which showed significant variations among bamboo ages of 1, 3 and 5 years old and top, middle and bottom culm positions.

Interaction Effect of Culm Age, Position and Growing Site on Chemical Composition of *Y. alpina*

Cellulose Content: As presented in Table 1, the interaction effect between the comparison factors (age, culm position and growing site) on cellulose content of *Y. alpina* varied significantly. [6] Which is stated that there is variation in the chemical composition bamboo depending on their age. In this study, maximum cellulose content was found on culm age of three years (64%) in top position followed by culms of age five (58.5%) in the middle culm position at the *Injibara* site. The next higher value of cellulose content occurred in middle and bottom culm position on bamboo culms of age 4 years at the *Shenen* site with the values of 52.5% and 54%, respectively. The smallest cellulose content was obtained on bamboo culms of age 3 years (43.69%) in the top Bamboo culm position which was grown at the *Tikur Inchini* site and followed statistically similar values was observed middle (43.95%) and bottom (43.64%) bamboo culm position at *Shenen* and *Injibara* growing sites, respectively. Different authors have confirmed that the previously studied cellulose content varied for different bamboo species; they include bamboo *Kumamoto* (Japan) and Moso bamboo (*Phyllostachys pubescens* Mazel) with the value of 47% and 45%, respectively[20, 21].

Klason Lignin Content: The interaction effect between growing site, Bamboo age and Bamboo culm position on Klason lignin content of *Yushinia alpina* has shown highly significant and statistically similar lignin content were recorded in bottom and middle with the value of

Table 1: Analysis of variance of chemical composition at different age, position and location for *Y. alpina* grown in Central and Northwest Ethiopia

Source of Variation	DF	Cellulose	Klason Lignin	Extractive Solubility
Location	2	534***	64***	27.95***
Bamboo Age	2	63***	67***	10.15***
Bamboo culm Position	2	36***	11***	8.77***
Loc.*Age*Pos.	8	61***	5.53***	4.73***
Error	54	54	53	54
R^2		0.94	0.89	0.83
CV		3.21	3.56	11.89

***=Significant at p <*0.001*; **= Significant at p<*0.01*; *= Significant at p< 0.05; ns= Non-significant at P< 0.05,

EOC_0 = Essential oil content at harvest, EOC_w = Essential oil content at wilting day

Table 2: Analysis of variance of ash content on different growing site and culm position of *Y. alpina* grown in Central and Northwest Ethiopia

Source of Variation	DF	Mean Square Ash
Location	2	0.35***
Position	2	2.05***
Location*Position	4	3.35***
CV	4.75	
R^2	0.99	

***= Significant at p <0.001; **= Significant at p <0.01; *= Significant at p <0.05; ns= Non-significant at p <0.05,

31.56% and 31.55%, respectively on Bamboo culms of age 3 years at *Shenen* growing site. The next higher value of lignin content was determined in the top bamboo culm position at the *Shenen* growing site and followed statistically uniform in bottom culm position at *Injibara* growing site together on Bamboo culms of age 3 with the value of 30.59% and 30.03%, respectively. The least lignin content was a witness on Bamboo age 4 (23.04%) in the top and followed on Bamboo age 5 (23.16 %) in middle culm position together at *Injibara* for *Yushinia alpina* (Table 3). When computing Klason lignin separately in each growing site on bamboo age of 3 years across the bottom to top culm position observed statistically similar and higher yield recorded than on ages 4 and 5 years at *Shenen*. Klason lignin for bamboo ages 4 and 5 culm appeared to show relatively small difference across the study sites, which is almost similar observation mad by Li *et al.* [22] which showed that no significant difference was observed for Klason lignin between bamboo ages (1, 3 and 5) and bamboo culm position.

Extraction Yield: The interaction effect (Bamboo culms age, Bamboo culm position versus growing site) on extractive yield of *Yushinia alpine* is highly significantly occurred in bottom bamboo culm position on Bamboo culms age of 3 (12.28%) at the *Shenen* growing site and followed *Injibara* and *Shenen* growing sites with the respective value of 11.37% on Bamboo culms of age 4. The least extractive yield was found in the top (5.79%) bamboo culm positions at grown *Tikur Inchini*and followed statistical uniform values have been observed in the same culm position on Bamboo culms of age 3 (6.48%), which grew at *Shenen* (Table 3). These results comparing with the previous ones reported by Tolessa *et al.* [23], which is much higher than in *Oxytenanthera abyssinica* (5.6%). Li *et al.* [22] in their studies reported that alcohol-toluene extractive content increased from the base to the top of the stem in the three- and five-year-old bamboos and showed a continuous increase with age. Nevertheless, the present studies revealed that the extractive yield decreases from the bottom to the top culm position in the three, four and five-year-old bamboo at each growing site. However, average extractive yield increased significantly with age both growing site and five years of age bamboo having the highest average content (9.54%) at *Injibara.* These variations might be permissible because of the nature of wax material attached to inner, middle and outer layers in the cellular structure of the plant depending on culm position and bamboo age maturity and also the particle size of the extracted material influences on the accessibility of the required components (tannins, gums, sugars, starches and coloring matter) present in bamboo and indicated easy access and penetration of chemicals to the cell wall materials.

Ash Content: As shown in Table 4, the ash content was highly significantly affected by the bamboo culm position versus the growing site in the studies. Maximum and minimum ash content was found on top (3.77%) and bottom (0.94 %) Bamboo culm position both collected at *Shenen.* In this study, the overall mean value of ash content revealed a significantly higher value for top Bamboo culm position (2.42%), which is in conformity with other findings mad by Xiaobo [19]. The ash content in the top portion of the culm was found maximum across the age (1, 3 and 5) with the value of 1.95%, 1.41% and 1.35%, respectively. The least ash content was recorded for the middle Bamboo culm position (1.48%), which yielded 38.84% lower than the maximum (2.42%) (Table 4).

Table 3: Interaction effect between culm age, position and growing site on cellulose, lignin and extraction content of Y. alpina

		Tikur Inchini			Shenen			Injibara		
Bamboo age	Bamboo culm position	Cellulose	Lignin	Extractive	Cellulose	Lignin	Extractive	Cellulose	Lignin	Extractive
3	Bottom	45.34^{hijk}	28.03^{fghij}	9.95^{bcd}	45.99^{ghijk}	31.56^{a}	12.28^{a}	51.83^{cd}	30.03^{abcd}	7g^{hij}
	Middle	47.14^{efgh}	27.7^{ghijk}	6.64^{ij}	48.59^{ef}	31.55^{ab}	9.21^{cde}	54.33^{c}	24.89^{no}	8.56^{defg}
	Top	43.69^{k}	29.56^{cdef}	4.71^{k}	45.42^{hijk}	30.59^{abc}	6.48^{ij}	64^{a}	29.92^{bcde}	7.83^{efghi}
4	Bottom	45.12^{hijk}	28.31^{fghij}	8.08^{efghi}	44.47^{ijk}	27.34^{hijk}	11.37^{ab}	54^{c}	26.49^{iklmn}	11.37^{ab}
	Middle	46.63^{fghi}	26.30^{klmn}	7.45^{fghij}	46.4^{fghij}	26.55^{jklm}	8.89^{ed}	52.5^{c}	25.45^{mno}	9.75^{bcd}
	Top	49.53d^{e}	27.3^{hijkl}	6.88^{hij}	45.51^{hijk}	27.11^{ijkl}	7.05^{fghij}	58.33^{b}	23.04^{p}	9.17^{ed}
5	Bottom	45.34^{hijk}	28.03^{fghij}	9.95^{bcd}	47.51^{efgh}	28.93^{efgh}	8.65^{def}	43.64^{k}	24.5^{op}	10.83^{abc}
	Middle	44.38^{ijk}	28.31^{efghi}	9.22^{cde}	43.95^{jk}	29.26^{cdefg}	8.43^{defgh}	58.5^{b}	23.16^{p}	9.11^{ed}
	Top	46.27^{fghij}	28.58^{efghi}	5.79^{jk}	45.83^{ghijk}	27.21^{ijkl}	7.63^{efghi}	47.17^{efgh}	25.92^{imno}	8.67^{def}

Table 4: Interaction effect of culm position and location on ash content of *Y. alpina*

	Ash content (%)			
Bamboo culm position	*Tikurinchini*	*Shenen*	*Injibara*	Mean
Bottom	2.72^{b}	1.21^{h}	2.41^{c}	2.11^{b}
Middle	1.84^{e}	0.94^{i}	1.67^{f}	1.48c
Top	2.09^{d}	3.77^{a}	1.40g	2.42^{a}
Mean	2.22^{a}	1.97^{b}	1.83^{bc}	

CONCLUSIONS

In this study, we have observed that cellulose, lignin and extractive contents of *Y. alpina* culms significantly varied by growing site, culm age and culm position. Ash content also demonstrated similar trend on bamboo age and culm position. Bamboo culms grown at Injibara site where the top of the culm position reached age of three to five had better cellulose content. Cellulose content of *Y. alpina* as found to be 44 – 64%, indicating suitability of the species as raw material for paper and pulp industry. In addition, if technologies are available to process, it can be used for the production of bio-ethanol and other similar gas products. Extractive yield for *Y. alpina* was found to be maximum at the bottom culm position, indicating suitability of the species for different industrial applications such as pharmaceuticals, creams and beverages products. Bamboo culm of age three grown at Shenen and Injibara sites possessed relatively better lignin content at the bottom culm position (i.e. for Shenen 31.56% and Injibara 30.03%). *Y. alpina* ash content was found low (i.e. minimum mean value 1.48% and maximum mean value 2.42%) indicating suitability of the species for charcoaling and using it in the form of pellet and briquette. Besides, bamboo ash can be used to polish jewels and manufacture electrical batteries. Our studies provide a good description of the chemical composition profile and support the idea that *Yushania alpina* bamboo species have potential use for chemical and biochemical industrial applications in the country.

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