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Determination of plant cell wall composition by a physical and chemical Approaches

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Abstract

For many years lignocellulosic plants like flax or hemp have been cultivated. At first, these plants were mainly produced for their fibers. Nowadays, agricultural by-products are changed into bio-based building engineering products like particle boards and mortars. These materials allow good insulation characteristics (acoustics, thermic) and low environmental impact. Thus, the current research is focused on plant valorization into bio-based building products. However, plants are a natural and variable matter that is not submitted to international standards compared to building materials. A better understanding of plant material, which has great characteristics, is required for its valorization. This investigation aims to provide an accurate analysis of the chemical composition of plant materials, especially hemp shiv. A deeper understanding of plant aggregate interactions with building engineering products could be necessary. At first, the Van Soest method (VS) was performed to quantify cell wall compounds. A non-destructive version of the Van Soest method (NVS) allowed us to separate each fraction derived from this method. Thermal Gravimetric Analysis (TGA) has analyzed all these fractions to quantify and identify molecules in crushed samples. The results obtained from these technics are analyzed and compared. Analyses suggested that hemp shives contain 49.0% cellulose, 21.5% hemicellulose, 18.6% soluble compounds (pectins, lipids, oses, ashes, proteins), 8.1% lignin and 2.8% of minerals. TGA results showed different thermal stabilities depending on the considered fraction, which we link to cellulose interactions with lignin and hemicellulose. This physical technic provides another method to quantify cell wall molecules.

Keywords: Hemp; valorization; building; biomaterials

Introduction

For thousands of years, lignocellulosic biomass like hemp has been cultivated. Initially, the culture was based on fibers to produce garments or ropes for the shipbuilding sector. Later, culture was extended to produce new high molecules for the pharmaceutical sector, second-generation biofuels and more recently, bio-based materials for building engineering [1]. This bio-based product is interested in its insulation capacities, low carbon impact, and increasing air quality. Moreover, these products contribute to increasing local employment (agriculture or agronomy) and decrease the impact of carbon linked to importations.

Thus, current research topics are focused on plant valorization into a bio-based material process like particle boards or mortars. However, compared to conventional building materials submitted to rules and international standards, plants are still a variable matter depending on pluviometry, culture condition, or species. Thus, a deeper understanding of plant composition is required to understand the physicochemical

interactions of molecules released by aggregates during the process. Therefore, this could upgrade their quality.

The chemical approach followed by physical characterization of lignocellulosic biomass is investigated. The Van Soest method is a destructive chemical approach used mostly in lignocellulosic biomass to quantify cell wall compounds [2]. The literature suggests that polar molecules like hemicelluloses and some soluble compounds, representing less than 40% of the biomass, are easier to extract by hot or basic pH water than other cell wall compounds. That means they might directly impact particle interactions into binderless particle boards or mortar rigidifications by interacting with other compounds [3,4]. Also, a non-destructive version of the Van Soest method was carried out to produce samples without some cell wall compounds and analyzed by Thermal Gravimetric Analysis (TGA). The results obtained from these techniques will be analyzed and compared to the conventional Van Soest method.

1. Materials and methods

1.1. Plant material preparation

The variety of hemp is FEDORA 17. Hemp shiv is produced in Normandy Region in France by defibrating hemp stems in a local producer transformation unit. The particles of hemp shiv were sieved to remove all residual fibers. Then, aggregates were crushed into powder (1 mm) using a mill with a mill-sieve and stored at 40°C.

1.2. Van Soest method

The Van Soest method [2] is a global cell wall quantification used to quantify by gravimetric methods main cell walls compounds like cellulose, hemicellulose, lignin and soluble compounds. Van Soest method was performed with Fibertec™ 8000 semi-automatic machine with programs. Solvents used during the process are Neutral Detergent Fiber (NDF, VWR Chemicals, 305320.5000) to remove soluble compounds (pectins, oils, minerals, water, sugars...), Acid Detergent Fiber (ADF, VWR Chemicals, 305319.5000) to remove hemicellulose and sulfuric acid (H₂SO₄ 72%, Carlo Erba reagents, 502771) to remove cellulose. After each treatment, samples are dried at 105°C for 16h, weighed, and dried in an oven at 480°C for 6h to remove lignin and obtain minerals or ashes.

1.3. Non-destructive Van Soest method

The non-destructive version of the Van Soest method was applied to hemp shiv powders. All the fractions were obtained after each treatment step (NDF, ADF, and H₂SO₄), followed by drying at 105°C for 16h. Calcination was not carried out. Thus, the method is called "non-destructive".

Theoretically, the first fraction (Fraction A) contains cellulose, hemicellulose, lignin, and ashes; the second (Fraction B) contains cellulose, lignin, and ashes, and the third (Fraction C) contains lignin and minerals. Samples will be stored in plastic pots at room temperature (Figure 1).

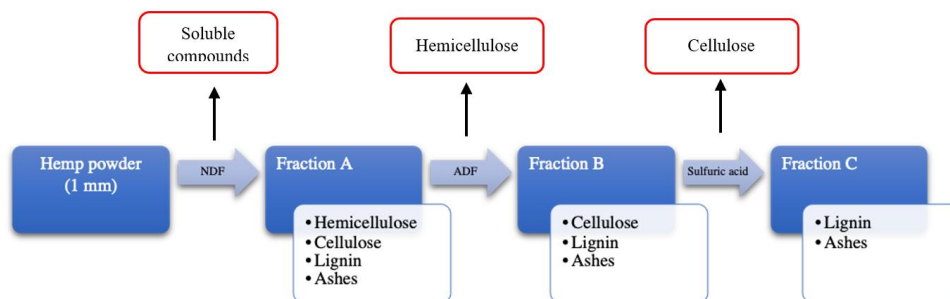


Figure 1. Chemical composition of Van Soest fractions

1.4. Thermal Gravimetric Analysis (TGA)

The experiments were carried out on a Netzsch TG209 F1 machine. The samples were heated from 30°C to 600°C at the rate of 10°C/ min using two atmospheres: argon and oxygen. Both have been employed

independently. The mass variation as a function of temperature was recorded. Mass loss peaks were identified with a determination of onset, peak, and final temperatures of thermal transitions in the first and second derivatives (Table 1, Figure 2) [5,6].

Table 1. Proposed determination protocol of onset (T_o), peak (T_p), and final (T_f) characteristic temperatures of thermal transitions employing TGA data.

Nomenclature	1 st Derivative	2 nd Derivative
T_o	$dm/dT = 0$	Inflexion point of d^2m/dT^2 vs. T
T_p	LM	$d^2m/dT^2 = 0$
T_f	$dm/dT = 0$	Inflexion point of d^2m/dT^2 vs. T

LM: Local

Minimum

2. Results and discussions

Van Soest method is a global cell wall quantification widely used in lignocellulosic biomass like flax or hemp. This experiment quantifies cell wall compounds (cellulose, hemicellulose, lignin, soluble compounds, and ashes) by using solvents following gravimetric quantification [2] (Table 2).

This method shows that hemp shiv contains 49.0% cellulose, 21.5% hemicellulose, 18.6% soluble compounds (pectins, lipids, sugars, minerals, proteins), and 8.1% lignin. All these molecules represent dry matter with 97.2% (w/w) of organic mass, and the rest is the inorganic part (also called ashes or minerals).

These results seem to agree with the bibliography, with approximately the same order of magnitude for cellulose and hemicellulose but different for lignin, soluble compounds, and ashes (Table 2). Hemp shiv contains around 44.0-49.9% of cellulose, 6.4-27.0% of hemicellulose, 8.0-28.0% of lignin, 1.0-29.4% of soluble compounds and 1.2-8.8% of ashes. Some articles explain these differences by growing conditions like pluviometry, weather, temperature, and plant species, which could directly impact chemical composition [4,7,8].

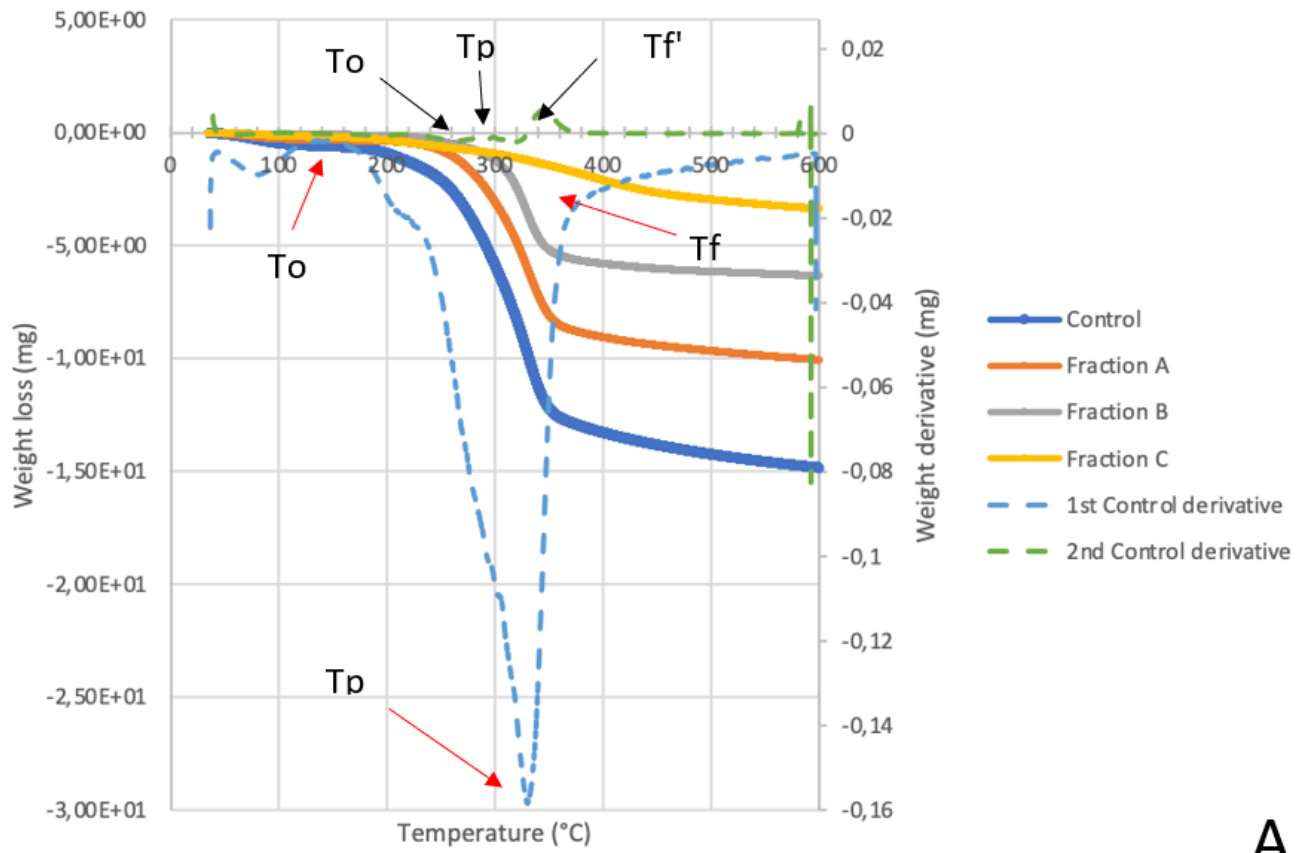
The non-destructive version of the Van Soest method was carried out to generate crushed samples with a specific composition. All generated fractions were used and analyzed to see if cell wall molecules can be quantified in this way. The mass loss curves as a function of temperature for the different fractions (A, B, and C) of hemp shiv and raw hemp shiv (control) are shown in Figure 2 (TGA under argon) and Figure 3 (TGA under oxygen).

Table 2. Hemp shiv chemical composition according to the bibliography and expressed in percentage (% w/w: mass/dry mass)

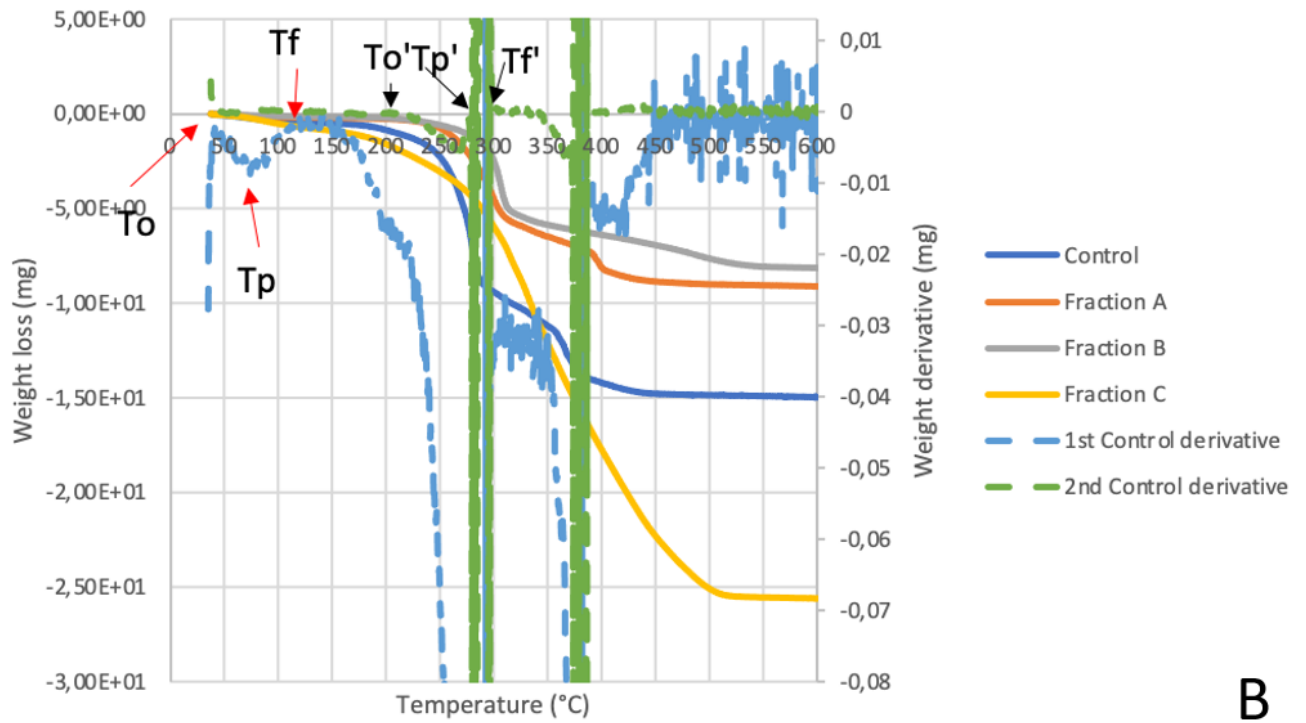
References	Cellulose	Hemicellulose	Lignin	Soluble Compounds	Proteins	Ashes
Present study	49.0 ± 2.8	21.5 ± 1.7	8.1 ± 0.6	18.6 ± 0.8	-	2.8 ± 0.1
Hussain <i>et al.</i> , 2018 [3]	44.0	18.0–27.0	22.0–28.0	1.0–6.0	-	1.0-2.0
Thomsen <i>et al.</i> , 2005 [9]	48.0	21.0–25.0	17.0–19.0	-	-	-
Gandolfi <i>et al.</i> , 2013 [10]	44.0	25.0	23.0	4.0	-	1.2
Cappelletto <i>et al.</i> , 2001 [11]	51.6	21.5	12.9	12.9	-	6.6
Godin <i>et al.</i> , 2010 [12]	47.5	6.4	8.0	29.4	-	8.8
Viel, 2016 [7]	49.9	21.4	9.5	17.7	-	0.6

TGA curves under argon (Figure 2A) suggest that the control sample is the most degraded, followed by Fraction A, B, and C. This means that the more the sample contains cell wall compounds, the more mass losses are. Moreover, Fraction C is less degraded in a large range of temperatures than in other samples. Thus, Fraction C seems more stable under high temperatures than in other samples. This is a link to lignin which is globally stable at high temperatures [6].

On the other hand, TGA curves under oxygen (Figure 2B) show different mass losses. Indeed, Fraction C is the first degraded sample, followed by control, Fraction A and B. In addition, curves of A and B fractions seem overlapped and far away from the control and Fraction C between 350-600°C. However, the curve of the Fraction B is lower than A. Eliminating soluble compounds (Fraction A) with or without hemicellulose (Fraction B) does not change mass loss much. Also, this contributes to increasing the thermal stability of fractions between 300-600°C. This suggests that hemicellulose was partially removed from the sample as CO₂ and char or that xylans were partially depolymerized from the sample [13,14]. Besides, the Fraction C is theoretically constituted by lignin and ashes and is less stable than other fractions. This behavior suggests cellulose decrystallization and elimination make accessible lignin and hemicellulose, which are extremely linked [15]. This event would make accessible, reducing functions and alcohol functions of lignin to produce volatile and oxidative by-products [14].



A



B

Figure 2. Thermal degradation of hemp shiv samples. Figures 2A and 2B correspond to mass losses under Ar and O₂.

The other interest of this technic was to quantify cell wall compounds by mass losses with a decomposition temperature range of cell wall molecules. By this method, we found 2.7% of water and proteins, 3.5% of pectins, 24.7% of hemicellulose linked to cellulose (holocellulose) and 38.8% of lignin linked to cellulose in the control sample. These results represent around 70% of biomass decomposition in inert conditions. (Table 3).

Table 3. Cell wall composition estimation (gx/100g dry mass) under argon and oxygen with TGA¹. Number of repetitions = 3.

	Cell wall compounds	Control	FA	FB	FC
Argon	Water	2.7 ± 0.8 (40–140 °C)	2.0 ± 0.1 (45–130 °C)	1.2 ± 0.2 (40–130 °C)	2.3 ± 1.2 (40–130 °C)
	Pectins	3.5 ± 0.6 (140–220 °C)			4.0 ± 0.3 (130–230 °C)
	Holocellulose [Hemicellulose– Cellulose]	24.7 ± 4.6 (220–300 °C)	23.5 ± 2.1 (190–330 °C)	83.8 ± 1.2 (180–510 °C)	8.6 ± 1.1 (260–345 °C)
	[Lignin– Cellulose]	38.8 ± 6.4 (300–420 °C)	53.7 ± 3.4 (330–500 °C)		17.6 ± 1.0 (345–450 °C)
Oxygen	Water	2.5 ± 0.6 (40–120 °C)	2.0 ± 0.0 (45–150 °C)	1.5 ± 0.1 (40–115 °C)	3.0 ± 0.1 (45–140 °C)
	Pectins	4.4 ± 0.2 (150–220 °C)			5.2 ± 0.3 (145–230 °C)
	[Hemicellulose– Cellulose]	42.6 ± 1.0 (220–285 °C)	74.6 ± 0.2 (160–360 °C)	80.3 ± 1.0 (180–385 °C)	19.7 ± 10.7 (230–300 °C)
	[Cellulose– Hemicellulose]	18.4 ± 1.6 (285–310 °C)			26.4 ± 1.9 (300–355 °C)
	[Lignin– Cellulose]	29.0 ± 0.8 (310–400 °C)	22.7 ± 0.3 (360–430 °C)	16.6 ± 0.9 (410–500 °C)	48.3 ± 3.8 (355–540 °C)
	Lignin	2.0 ± 0.4 (400–430 °C)			

¹ This cell wall composition estimation was based on Table 2.

We found the same percentages for Fraction A, even lignin linked to cellulose with 53.7%. For the Fraction B, we get around 1% of water and 83.8% of cellulose, pectins, and hemicellulose linked. For

the Fraction C, we obtain 2.3% of water, 4% of pectins, 8.6% of hemicellulose-cellulose, and 17.6% of linked lignin.

According to a total percentage under Argon (Table 3), reactions are limited to pyrolysis and limit the degradation yield. Results also suggest that Van Soest treatments do not remove all cell wall molecules. In oxidative conditions, we obtained 2.5% of water and proteins, 4.4% of pectins, 42.6% of hemicellulose linked to cellulose, 18.4% of cellulose linked to hemicellulose, 29.0% of lignin linked to cellulose, and 2% of lignin for the control sample. Total mass decomposition represents near to 100% of the biomass (Table 3).

For Fractions A and B, we obtained the same order of magnitude for water; hemicellulose-linked and lignin-linked percentages. For the Fraction C, we found 3% of water, 5.2% of pectins, 19.7% hemicellulose linked, 26.4% of cellulose linked, and 48.3% of lignin.

Complete combustion could explain these total percentages under the oxygen atmosphere, which allows pyrolysis and combustion reactions [16].

Conclusion

According to these results, chemical interactions of cell wall molecules remain challenging to characterize each molecule. Results seem close to the Van Soest method but not enough to quantify cell wall compounds; that is why TGA/ Mass Spectrometry (MS) on Van Soest fractions could be interesting to identify by-products degradation and, by the same time, cell wall molecules. Also, this experiment shows the limit of the widely used Van Soest method, which represents the greatest tool for a global idea about cell wall composition but is still not enough to characterize plant cell walls.

Acknowledgments

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