# Determination of plant cell wall composition by physical and chemical approach

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## Abstract

Since many years lignocellulosics 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 have a low environmental impact. Thus, the current research is focused on plant valorisation into bio-based building products.
However, plants are a natural and variable matter which is not submitted to international standards in compared to building materials. A better understanding of plant material is required to better value these products with great characteristics.
The aim of this investigation is to provide an accurate analysis of chemical composition of plant materials especially hemp shiv. A deeper understanding could be necessary to understand plant aggregate interactions with building engineering products.
At first, Van Soest method (VS) was performed to quantify cell wall compounds. Non-destructive version of Van Soest method (NVS) allowed us to separate each fraction derived from this method. All these fractions have been analysed by Thermal Gravimetric Analysis (TGA) to quantify and identify molecules in crushed samples. The results obtained from these technics are analysed and compared.
Analyses suggested that hemp shiv contain 49% of cellulose, 21% of hemicellulose, 21% of soluble compounds (pectins, lipids, oses, ashes, proteins…) and 9% of lignin. TGA results showed different thermal stabilities depends on considered fraction, which be link to cellulose interactions with lignin and hemicellulose. By this physical technic we provide another method to quantify cell wall molecules.

**Keywords:** Hemp; valorization; building; biomaterials

## Introduction

Since thousands of years lignocellulosic biomass like hemp have been cultivated. At the beginning, the culture was based on fibers to produce garments or ropes for shipbuilding sector. Later on, culture was extended to produce new high molecules for pharmaceutic sector, second-generation biofuels and more recently bio-based materials for building engineering (**FAO, 2000**). This kind of bio-based products have interests according to their insulation capacities, low carbon impact or air quality increasing. Moreover, these products contribute to increase local employment (agriculture or agronomy) and by the way, decrease impact carbon link to importations.
Thus, current research topics are focus on plant valorisation into bio-based material process like particle boards or mortars. However, plants still are a variable matter depending on pluviometry, culture condition or species, in compared to conventional building materials submitted to rules and international standards.
Thus, a deeper understanding of plant composition is required to understand the physico-chemical interactions of molecules released by aggregates during process. Therefore, this could upgrade their quality.
Chemical approach following by physical characterization of lignocellulosic biomass is investigated. Van Soest method is a destructive chemical approach, mostly used in lignocellulosic biomass, in order to quantify cell wall compounds (**Van Soest P.J. *et al.*, 1985**). The literature suggests that polar molecules like hemicelluloses and some soluble compounds which represent less than 40% of biomass, are easier to extract by hot or basic pH water, than other cell wall compounds. That mean they might directly have an impact in particles interactions into binderless particle boards or mortars rigidifications by interacting with other compounds (**Hussain A. *et al.*, 2018; Vignon M.R. *et al.*, 1995**). Also, a non-destructive version of Van Soest method was carried out to produce samples without some cell wall compounds and analysed by Thermal Gravimetric Analysis (TGA). The results obtained from these technics will be analysed and compared to conventional Van Soest method.

## Materials and methods

- **Plant material preparation**

The variety of hemp is FEDORA 17. Hemp shiv are produced in Normandy Region in France by defibration of 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 using a mill with mill-sieve of 1 mm and storage at 40°C.

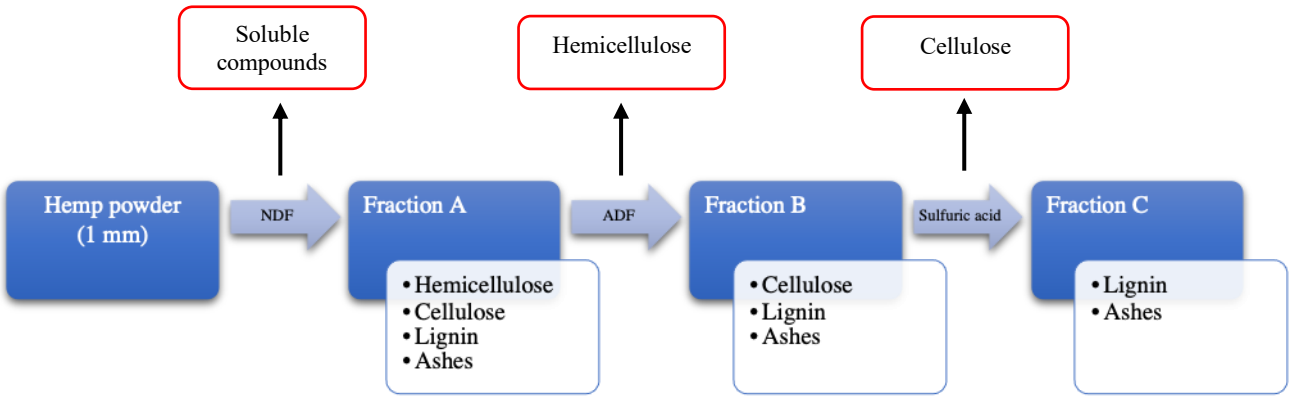
- **Van Soest method**

Van Soest method (**Van Soest P.J. *et al.*, 1985**) is a global cell wall quantification used to quantify by gravimetric method all cell wall compounds like cellulose, hemicellulose, lignin, soluble compounds and ashes. 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 ($H_2SO_4$ 72%, Carlo Erba reagents, 502771) to remove cellulose.
After each treatment, samples are dried at 105°C during 16h, weighed and dried in oven at 480°C during 6 h to remove lignin and obtain ashes.

- **Non-destructive Van Soest method**

The non-destructive version of Van Soest method was applied on hemp shiv powders. All the fractions were obtained after each step treatment (NDF, ADF and $H_2SO_4$) following by drying at 105°C during 16h. Calcination was not carried out. This is why 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. (**Fig.1**)

**Figure 1.** Chemical composition of Van Soest fractions




- **Thermal Gravimetric Analysis (TGA)**

The experiments were carried out on a Netzsch TG209 F1 machine. The samples were heated 30°C to 600°C at the rate of 10°C/ min. Two atmospheres were used: argon and oxygen, both have been employed independently. The mass variation as a function of temperature was recorded. Mass losses peaks were identified with a determination of onset, peak and final temperatures of thermal transitions in first and second derivative (**Table 1, Figure 2**). (**Arufe S. *et al.*, 2019; Brebu and Vasile, 2010**)

**Table 1.** Proposed determination protocol of onset (To), peak (Tp) and final (Tf) characteristic temperatures of thermal transitions employing TGA data

| Temperature (°C) | | First derivative | Second derivative |
|---|---|---|---|
| ~40-120 | To | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| | Tp | LM | $dDM^2/d^2T=0$ |
| | Tf | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| ~142-200 | To | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| | Tp | LM | $dDM^2/d^2T=0$ |
| | Tf | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| ~200-300 | To | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| | Tp | LM | $dDM^2/d^2T=0$ |
| | Tf | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| ~300-400 | To | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| | Tp | LM | $dDM^2/d^2T=0$ |
| | Tf | dDM/dT=0 | Inflexion point $dDM^2/d^2T=0$ |
| ~350-400 | To | Undeterminable: | Inflexion point $dDM^2/d^2T=0$ |
| | Tp | stackable peak | $dDM^2/d^2T=0$ |
| | Tf | | Inflexion point $dDM^2/d^2T=0$ |

LM: Local Minimum

## 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 of solvents following by gravimetric quantification (**Van Soest P.J *et al.*, 1985**) (**Table 2**).

This method shows that hemp shiv contains 49% of cellulose, 21% of hemicellulose, 21% of soluble compounds (pectins, lipids, sugars, minerals, proteins, …) and 9% of lignin. All these molecules represent dry matter with 97% w/w of organic mass and the rest is the inorganic part (also called ashes or minerals).

These results seem be agree with bibliography, with approximately the same order of magnitude for cellulose and hemicellulose but different results for lignin, soluble compounds and ashes (**Table 2**). Hemp shiv contains about 45% of cellulose, 23% of hemicellulose and lignin, 6% of soluble compounds and 1,5% of ashes Some articles explain these differences by growing conditions like pluviometry, weather, temperature and also plant species, which could have a direct impact on chemical composition. (**Vignon M.R. *et al.*,1995; Viel M., 2016; Kymäläinen and Sjöberg, 2008**).

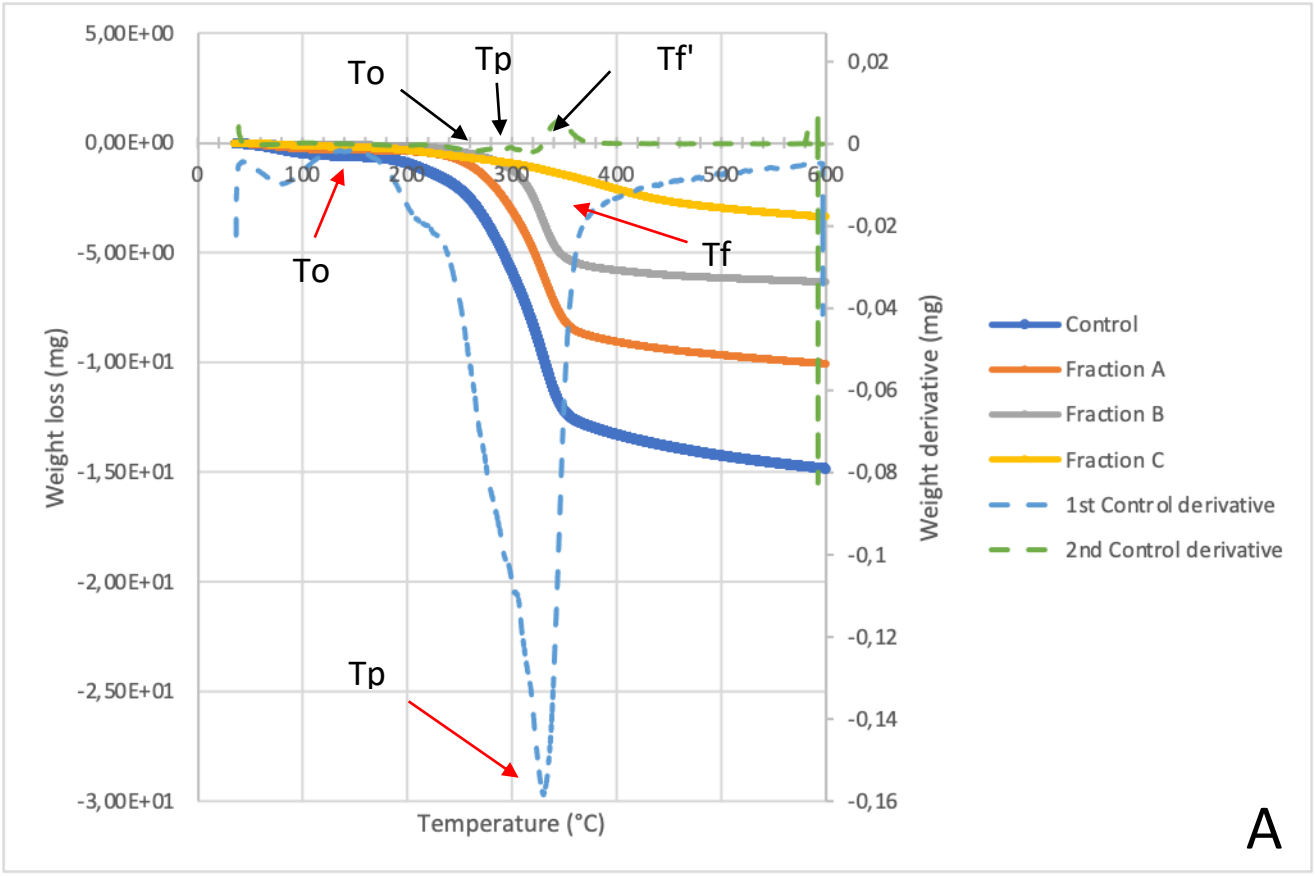
**Table 2.** Hemp shiv chemical composition according to the bibliography and expressed in percentage (%w/w),

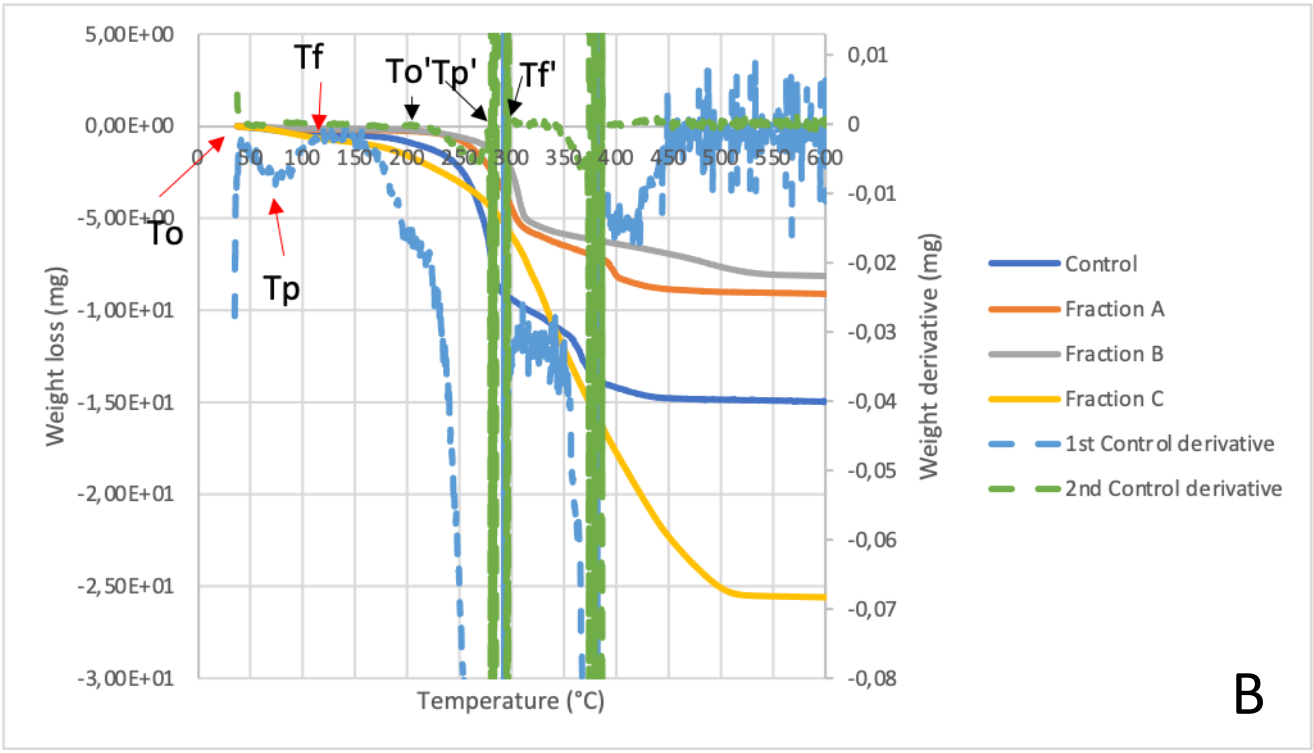
| | Present study | Hussain .A *et al.*, 2018 | Vignon .M.R *et al.*, 1995 | Thomsen .A.B *et al.*, 2005 | Gandolfi .S *et al.*, 2013 | Garcia-Jaldon C., thesis, 1995 | Yuuhong .J *et al.*, 2019 |
|---|---|---|---|---|---|---|---|
| Cellulose | 48,79+/-0,3 | 44 | 44 | 48 | 44 | 48 | 34-44 |
| Hemicellulose | 20,6 +/-0,41 | 18-27 | 18 | 21-25 | 25 | 12 | 31-37 |
| Lignin | 8,99 +/-0,11 | 22-28 | 28 | 19-28 | 23 | 28 | 22-30 |
| Others : Pectins/Oils/ Fatty acids/sugars/waxes/ minerals | 21,61 +/- 0,21 | 1-6 | Pectins : 4 Waxes + fats : 1 | - | 4 | Pectins : 6 Fats and waxes : 1 | Pectins : 4-5 Waxes : 0,5-1,4 |
| Proteins | | - | 3 | - | | 3 | - |
| Ashes | - | - | - | - | 1.2 | 2 | - |

w/w: mass/dry mass

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

**Figure 2.** Thermal degradation of hemps shiv samples. Figure 2A and 2B respectively correspond to mass losses under Ar and $O_2$




TGA curves under argon (**Fig.2A**) suggest that control sample is the most degraded, following by A, B and C Fraction. Which mean that more the sample contains cell wall compounds, the more mass losses are. Moreover, Fraction C is less degraded in large range of temperature than other samples. In this way, Fraction C seems to be the more stable under high temperature than other samples. This is link to lignin which is globally stable at high temperature. (**Brebu and Vasile, 2010**).

On the other hand, TGA curves under oxygen (**Fig.2B**) show different mass losses. Indeed, fraction C is the first degraded sample following by control, A and B fraction. In addition, curves of A and B fractions seem overlapped and far away from control and C fraction between 350 – 600°C. However, curve of B fraction is lower than A. The elimination of soluble compounds (fraction A) with or without hemicellulose (fraction B) does not change mass loss much. Also, this contributes to increase thermal stability of fractions between 300-600°C. Which suggests that hemicellulose was partially removed from sample as $CO_2$ and char or that xylans were partially depolymerized from sample. (**Zhou X. *et al.*,2018; Shen D. *et al.*,2015**). Besides, C fraction which is theoretically constitute by lignin and ashes is less stable than other fractions. This behaviour suggests cellulose decrystallization and elimination make accessible lignin and hemicellulose which are extremely linked (**Kang X. *et al.*,2019**). This event would make accessible reducing functions and alcohol functions of lignin to produce volatile and oxidative by-products **(Shen D. *et al.*,2015)**

**Table 3. Cell wall composition estimation under Ar with TGA method**

| | Control | | Fraction A | | Fraction B | | Fraction C | |
|---|---|---|---|---|---|---|---|---|
| | A | SD | A | SD | A | SD | A | SD |
| Water, proteins | 2.7 | +/- 0.9 | 2.1 | +/- 0.2 | 1.1 | +/- 0.1 | 2.6 | +/- 1.4 |
| Pectins | 3.5 | +/- 0.6 | - | - | 79.4 | +/- 1.4 | 3.8 | +/- 0.5 |
| Hemicellulose, cellulose | 24.7 | +/- 4.6 | 23.6 | +/- 1,4 | | | 7.0 | +/- 1.9 |
| Lignin, cellulose | 38.8 | +/- 6.3 | 48.5 | +/- 0,9 | | | 6.9 | +/- 0.6 |
| **Total** | **69.7** | **+/- 0.6** | **74.3** | **1.2** | **80.5** | **+/- 1.4** | **20.3** | **+/- 1.6** |

**Table 4. Cell wall composition estimation under $O_2$ with TGA method**

| | Control | | Fraction A | | Fraction B | | Fraction C | |
|---|---|---|---|---|---|---|---|---|
| | **A** | **SD** | **A** | **SD** | **A** | **SD** | **A** | **SD** |
| Water, proteins | 2.6 | +/- 0.4 | 2.0 | +/- 0.2 | 1.5 | +/- 0.1 | 3.0 | +/- 0.1 |
| Pectins | 4.3 | - | 73.0 | +/- 0.4 | 80.5 | +/- 1.0 | 5.2 | +/- 0.3 |
| Hemicellulose, cellulose | 42.8 | +/- 1.3 | | | | | 19.7 | +/- 10.7 |
| Cellulose, hemicellulose | 17.5 | +/- 1.0 | | | | | 26.4 | +/- 1.9 |
| Lignin, cellulose | 19.2 | +/- 0.1 | 22.9 | +/- 4.0 | 13.6 | +/- 1.5 | - | - |
| Lignin | 4.1 | +/- 1.0 | | | | | 4.0 | - |
| **Total** | **90.5** | **+/- 1.1** | **97.7** | **+/- 3.3** | **95.5** | **+/- 0.3** | **67.2** | **+/- 25.6** |

SD: Standard Deviation; A: Average

The other interest of this technic was to quantify cell wall compounds by mass losses with 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 link to cellulose and lignin and 38.8% of lignin link to cellulose into control sample. These results represent 70% of biomass decomposition in inert condition. (**Table 3**)

For A fraction we found same percentages even pectins with 2,1% and lignin linked to cellulose with 48,5%. For B fraction we get 1% of water and 79,4% of cellulose, pectins and hemicellulose linked. For C fraction we obtain 2,6% of water, 3,8% of pectins, 7% of hemicellulose-cellulose, 7% of lignin.

According to total percentage (**Table 3**) under Argon, reactions are limited to pyrolysis and by the way limit the yield of degradation. Results also suggest that all cell wall molecules are not removed by Van Soest treatments.

In oxidative condition, we obtained 2.6% of water and proteins, 4.3% of pectins, 42.8% of hemicellulose linked to cellulose, 17.5% of cellulose linked hemicellulose, 19.2% of lignin linked to cellulose and 4% of lignin for control sample. Total mass decomposition represents 91% of the biomass. (**Table 4**)

For A and B fractions we obtained the same order of magnitude for water; hemicellulose linked and lignin linked percentage. For C fraction we found 3% of water, 5,2% of pectins, 19,7% of hemicellulos linked, 26.4% of cellulose linked and 4% of lignin.

These total percentage could be explained by the complete combustion under oxygen atmosphere which allow pyrolysis and combustion reactions.

## Conclusion

According to these results, chemical interactions of cell wall molecules still remain a challenge to characterize each molecule. Results seem close to Van Soest method but not enough to quantify cell wall compound, that's 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 is represent a greatest tool to have a global idea about cell wall composition but still not enough to characterize plant cell wall.

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