

**STUDY OF THE “INTER PLANT” VARIABILITY IN AGAVE AMERICANA L. FIBER PROPERTIES**

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**ABSTRACT**

This work aims to investigate the inter plant variability in agave fiber physical and mechanical properties. Using Design of experiments techniques, some parameters were defined and considered as controlling factors. These parameters are related to the fiber collecting mode, namely the choice of the plant, the leaf and the position of the fiber in the leaf (fiber maturity).

The main results show that the factor ‘position’ has a highly significant effect on the different studied characteristics except for the initial modulus. This means that fibers located at different positions in the leaf (tip, middle or base) exhibit different properties. On the contrary, the factor ‘leaf’ remains the more negligible for most characteristics. Furthermore, it was noted that the effect of factor ‘plant’ is highly significant for mechanical properties but negligible for fineness and diameter parameters.

**KEYWORDS**

Variability, Tenacity, Extension, Fineness, agave fiber, statistical analyses

**1. INTRODUCTION**

Lignocellulosic fibers, also called “plant” fibers, “natural” fibers or “vegetable” fibers, include bast fibers, leaf (or hard) fibers, seed, fruit, wood, cereal straw, and other grass fibers. These plant fibers have been one of the most attractive fillers for different types of polymers including rubbers as well as for ceramic matrices due to some of their unique characteristics unparalleled with any other reinforcing/filler materials. They include renewability, biodegradability, good availability, low cost and density, limited damage to the processing equipment, reduced health hazard and reasonable strength and stiffness.

Consequently, there is an increased motivation in the use of plant fibres by different industrial sectors, like automotive, to replace glass fibres (Avérous et al., 2006; Bledzki et al., 1999; Klemm et al., 2005; Rao et al., 2007; Saheb et al., 1999).

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The extraction, often called separation, of natural fibers from the plant is the most essential stage of work and the most determinist in terms of fiber properties. Therefore, it must be carefully and wholly treated. Actually to separate fibers, many methods can be used. These methods are classified according to the way of extraction which may be mechanical, chemical or biological (we can also use a combination of these three methods). The biological method may be enzymatic or by retting in seawater. Each method has its own advantages and drawbacks, and the choice of the appropriate method is dictated by the ultimate application of the fiber.

On the other hand, even though plant fibers are extracted with the same method, they can exhibit a great dispersion in results for all studied parameters (Msahli, 2000; Jaouadi et al., 2009; Rezig et al., 2014). Many reasons were introduced to explain this variability. The first reason is related to the nature of the fiber itself since it presents a composite structure with unequal proportions of cellulosic and non cellulosic materials (lignin, pectin, gums...).

Another hypothesis may be raised assuming that the great variability of results is due to the leaves and fibers collection mode. In fact, fibers can be randomly extracted from different plants and positions in the same plant.

In this respect, this work aims to investigate the inter plant variability in the results for vegetable fibers, and particularly for agave fibers. A full experimental design was used to study the variability in fineness parameters and mechanical properties when fibers are taken from different plants and from different parts of a same plant. In this work, many controlling factors were considered namely the plant, the leaf and the fiber maturity (position of fibers within the leaf).

## 2. MATERIALS AND METHODS

### 2.1. Sampling plan

Agave plant grows in the form of a dense rosette composed of long thick leaves. These leaves are disposed, as illustrated in figure 1, in a spiral defining a line drawn onto the plant stalk and holding the different points of intersection of consecutive leaves. Considering this particular arrangement, the factor level is defined as one turn of the spiral.

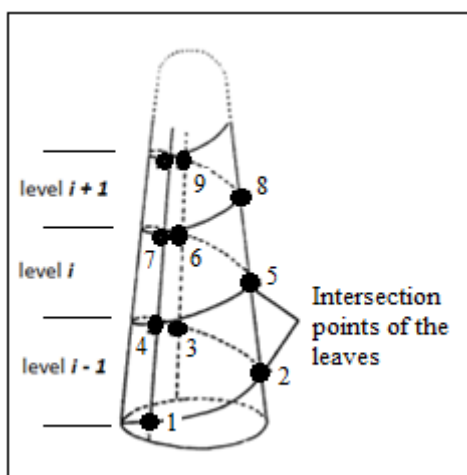


Figure 1: Arrangement of leaves in the plant

The levels indicate about the age of the plant. In this manner, for a plant of 14 levels, those at the bottom of the plant (1, 2 and 3) are composed of falling or dead leaves not useable, and it is the same with level (14) in the top of the plant which include very young leaves, practically without fibers.

Thus, for the factor 'level', we have considered only the levels where the leaves are useable (not falling), namely the levels from 4 to 13, and in the following parts, the level  $N=1$  corresponds to the fourth level of the plant.

In the following part, we describe the mode to choose plants and to collect leaves from each plant.

- Number of plants : 5
- Region : Bembla (Monastir)
- One level of collecting : level 5
- Number of leaves per plant: 3 collected from the same level for all plants
- Total number of leaves: **15**

Extraction of fibers from the leaves was done by degradation of the parenchyma in seawater during a period time of 7 months.

After retrieving from seawater, fibers were rinsed by distilled water, dried at ambient temperature, and then manually individualized. Before testing, fibers were preconditioned as required in the fiber test standard, at a temperature of  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and a relative humidity of  $65\% \pm 2\%$ .

*Fibers sampling plan*

The observation of the extracted fibers revealed a significant variation of the diameter between the tip of the leaf and its root, the reason for which we have introduced another controlling factor in the experimental design. This latter presents the fiber position in the leaf defined by three different levels as illustrated in figure 2.

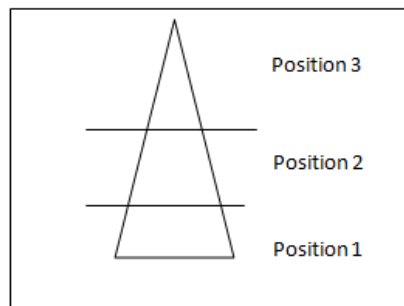


Figure 2: Leaf schematization (position factor)

For this inter plant study, the experimental design is defined by three controlling factors with their different levels. Controlling factors are chosen and defined as follows:

- **PI** = Plant    1        2        3        4        5
- **F** = Leaf     1        2        3
- **P** = Position1    2        3

The factor 'level' is constant **N = 5**.

This leads to a factorial experimental design of type  $5 \times 3 \times 3$ , containing 45 experiments, and each one is repeated 20 times. Thus the total number of rows is 900 (experiments). The samples are coded as follows:

**PI F P**

With            PI = number of the plant (from 1 to 5)

F = number of the leaf (from 1 to 3), the level of the leaf in the plant is fixed at N=5

P = position of the fiber in the leaf (from 1 to 3)

This collecting mode, represented by two different experimental designs, allows us to carry out a vertical study within the same plant taking into account the factor 'level' as principle controlling parameter, and a horizontal study between 5 different plants (figure 3).

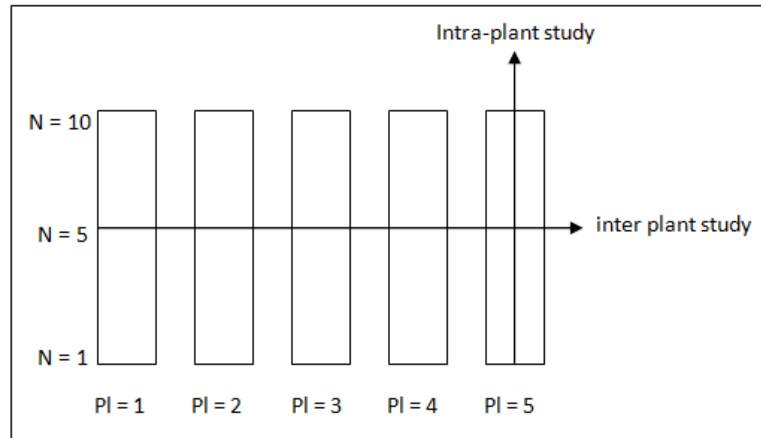


Figure 3: Schematization of the collecting mode of fibers for inter plant studies

The output factors of the process are the fibers characteristics evaluated by:

- fineness : determined by gravimetric method,
- apparent diameter measured with a projection microscope for a cut length  $L=0,75$  mm,
- mechanical parameters: tenacity, elongation at rupture, initial modulus and impact energy.

## 2.2. Fineness assessment

To measure quantitatively the fineness of agave fiber, many techniques may be used:

### - Gravimetric method

As described in the French standard NF G 07-007 (AFNOR, 1983), this method is based on the measurement of fiber weight and length. The linear density, often called 'fineness' is determined (in tex) by the following equation:

$$T \text{ (tex)} = 1000 \frac{m(g)}{L(m)} \quad (1)$$

This technique requires a microbalance with a precision of at least 1%, and another instrument to measure the fiber length as specified in the French standard NF G 07-006.

Before test, fibers have to be conditioned in a standard atmosphere ( $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for temperature and  $65\% \pm 2\%$  for relative humidity).

### - Projection microscope measuring

This method is essentially used to determine the diameter of wool fibers as described in French standard .NF G 07-004 (AFNOR, 1973) : fiber sections with standard length (between 4 and 8 mm) are placed on a specimen slide, and moved with a constant pitch under the objective lense (with magnification x500). For

the case of hard fibers (like agave fibers), the magnification of 500 times is high enough to observe entirely the fiber diameter, for that reason, we have used a magnification of 200 times.

Furthermore, we have defined the 'apparent' diameter characteristic which corresponds to the mean value of the projected diameters, when the fiber takes all the possible orientations with respect to the projection plan (Dreyer et al., 1994), as illustrated in figure 4.

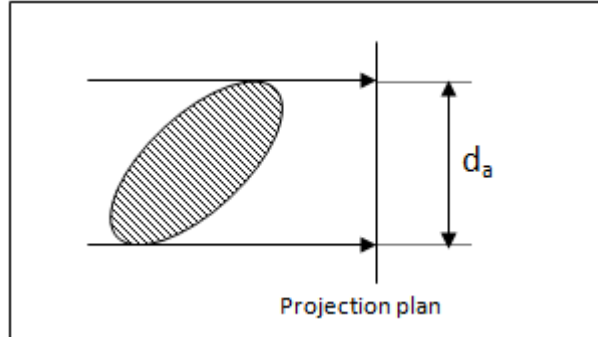


Figure 4: Definition of the 'apparent' diameter for elliptical cross-section fiber

The 'apparent' diameter is calculated by the following formula:

$$D_a = E (d_a / A) \quad (2)$$

With

- $E (..)$  : the mathematical expectations operator
- $E (.. / ..)$  : the mathematical expectations operator conditional
- $A$  = straight section area.

### 2.3. Tensile tests and mechanical parameters

Tensile tests were conducted with a GAC Shirley dynamometer (type Micro 250) with the following characteristics:

- Measuring accuracy (0.5% for load and 0.01 mm for elongation)
- Load measurement cell : 20 N

The breakage time was fixed at  $20 \pm 3$  seconds as recommended by NFG07 002 French standard (AFNOR, 1985).

For all tests, we chose a sample gauge length of 25 mm (1 inch) as specified in ASTM D 3217 -79 American standard for the control of synthetic fibers (ASTM, 1979).

Tests involved a conditioned atmosphere of  $20^\circ\text{C} \pm 2^\circ\text{C}$  and  $65\% \pm 2\%$  RH according to NFG00 003 French standard (AFNOR, 1970). Before testing, fibers were dried at ambient conditions for at least 24 hours and conditioned at  $20^\circ\text{C} \pm 2^\circ\text{C}$  and  $65\% \pm 2\%$  RH for at least 24 hours.

The mechanical parameters we have worked on are deduced from tensile test (stress-strain curve) as follows:

- Ultimate tensile strength: maximum stress supported by fiber in a tensile test and leading to failure. In textile terminology, the ultimate tensile strength is often expressed by "tenacity" with the following formula :

$$\text{Tenacity (cN / tex)} = \frac{\text{Stress (cN)}}{\text{Fineness (tex)}} \quad (3)$$

- Elongation at rupture: increase in fiber length (deformation) expressed in cm or mm. when fiber test length is not considered, we have to use extension expressed in percent (%) of the initial length of fiber.
- Impact energy ( $w$ ): the energy, expressed in Joule, for which the fiber is broken.
- Initial modulus ( $M_0$ ): this parameter is related to the shape of stress- strain curve and informs about the stiffness of the fiber (resistance of fiber subjected to very low stress), it is determined by the expression:

$$M_0 \text{ (N/tex)} = \text{tg} (\alpha) \quad (4)$$

## 2.4. Statistical tools

An extensive statistical study permits us to demonstrate if the variations of results are assigned only to hazardous fluctuation or to the effect of the input controlling factors considered. This study uses statistical tools such as radar chart, response graph, analysis of variance (ANOVA) techniques, developed under Minitab® V14 software.

### - Radar chart

The radar chart is a plot that consists of a sequence of equi-angular spokes, called radii, with each spoke representing one of the variables. The data length of a spoke is proportional to the magnitude of the variable for the data point relative to the maximum magnitude of the variable across all data points. A line is drawn connecting the data values for each spoke. The star plot can be used to locate similar points or dissimilar points. Radar charts are used in our study to better compare the effects of controlling factors for each output parameter.

### - Main effects plot

The main effects plot represents the mean value of the output responses for each level of controlling factors. This plot is basically used to compare the significance of controlling factors effects and to determine the most important factor influencing the process.

### - Multivariate analysis

Multivariate analysis (MVA) is based on the statistical principle of multivariate statistics, which involves observation and analysis of more than one statistical outcome variable at a time. In design and analysis, this graphical technique is used to perform the analysis of variance across multiple dimensions while taking into account the effects of all variables (effect of interactions) on the responses (Aghreshi et al., 1990).

### - Interaction effects

Interaction effects represent the combined effects of factors on the dependent measure. When an interaction effect is present, the impact of one factor depends on the level of the other factor. Part of the power of ANOVA is the ability to estimate and test interaction effects. As Pedhazur and Schmelkin note, the idea that multiple effects should be studied in research rather than the isolated effects of single variables is one of the important contributions of Sir Ronald Fisher. When interaction effects are present, it means that interpretation of the main effects is incomplete or misleading.

In the digram of interaction effects, parallel Interaction results whose lines do not cross (as in the figure at left) are called "ordinal" interactions. If the slope of lines is not parallel in an ordinal interaction, the interaction effect will be significant, given enough statistical power. If the lines are parallel, then there is no interaction effect (Hicks et al., 1982).

### - Homogeneity of variances test

In some statistical tests, for example the analysis of variance, we assume that variances are equal across groups or samples (homogeneity of variances). The Bartlett test can be used to verify whether the data are

normally distributed (Brown et al., 1974). Bartlett's test is sensitive to departures from normality. That is, if samples come from non-normal distributions, then Bartlett's test may simply be testing for non-normality. The Levene test (Levene, 1960) is an alternative to the Bartlett test that is less sensitive to departures from normality.

- *Analysis of variance*

The analysis of variance is a statistical test which allows to see if the controlling factor effects are more or less significant on the response. The method is based on the comparison between the real variances of the model coefficients and the variances of these same coefficients if the controlling factors have no effects (Milliken et al., 1984; Nelson, 1983).

This test consists on calculating a statistic F from the coefficients of the established model and then to compare it with F values taken from statistical tables of *Snedecor* law (Olshen, 1973).

- If  $F_{cal} > F_{1\%}$ , the difference is highly significant with 1% risk of error.
- If  $F_{5\%} < F_{cal} < F_{1\%}$  the difference is significant with 5% risk of error.
- If  $F_{cal} < F_{5\%}$  the difference is not significant with 5% risk of error.

**3. RESULTS AND DISCUSSION**

Figures 5, 6 and 7 show the evolution of fineness and diameter parameters as function of variability factors taken into consideration (plant, leaf, position).

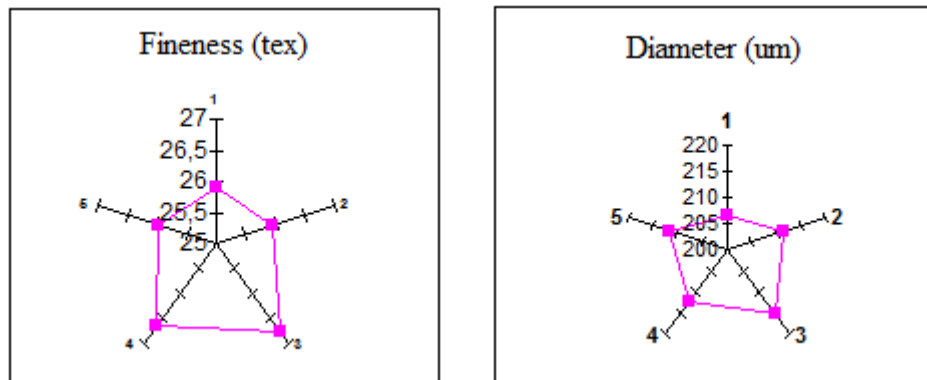


Figure 5: Evolution of fineness and diameter with the factor 'plant'

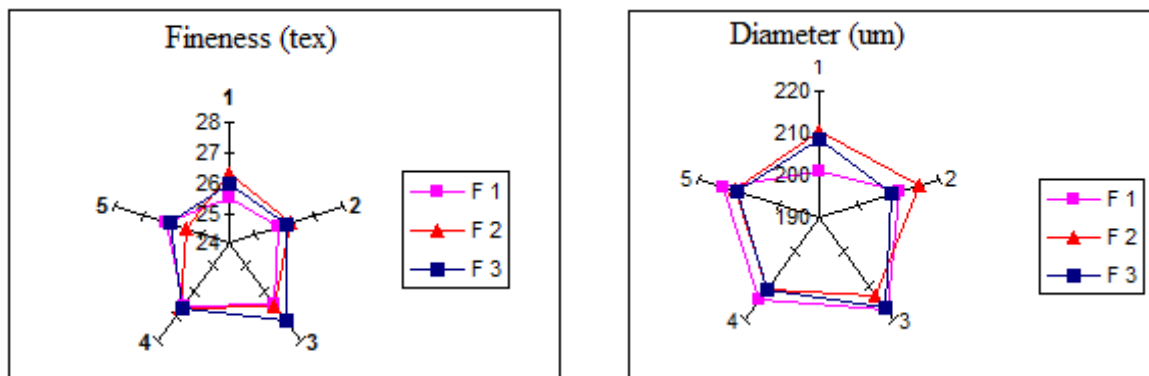


Figure 6: Evolution of fineness and diameter with the factor 'leaf'

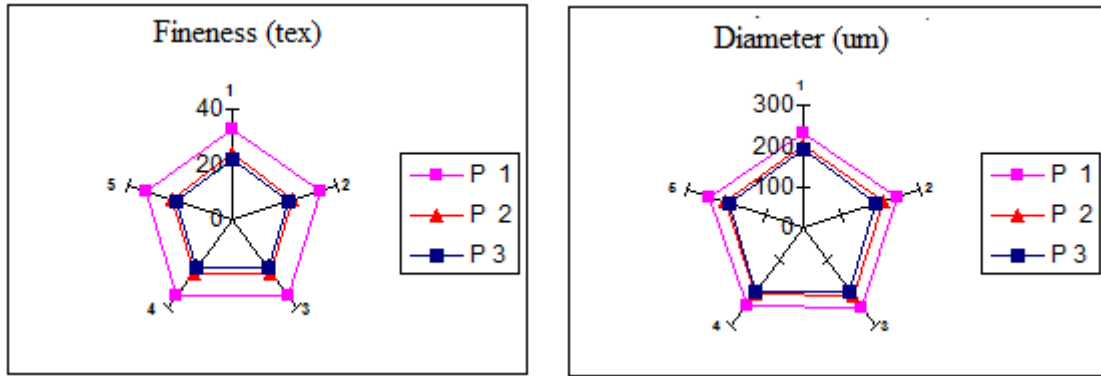


Figure 7: Evolution of fineness and diameter with the factor 'position'

The evolution of the mechanical properties (tenacity, elongation, and impact energy) is presented in figures 8→13.

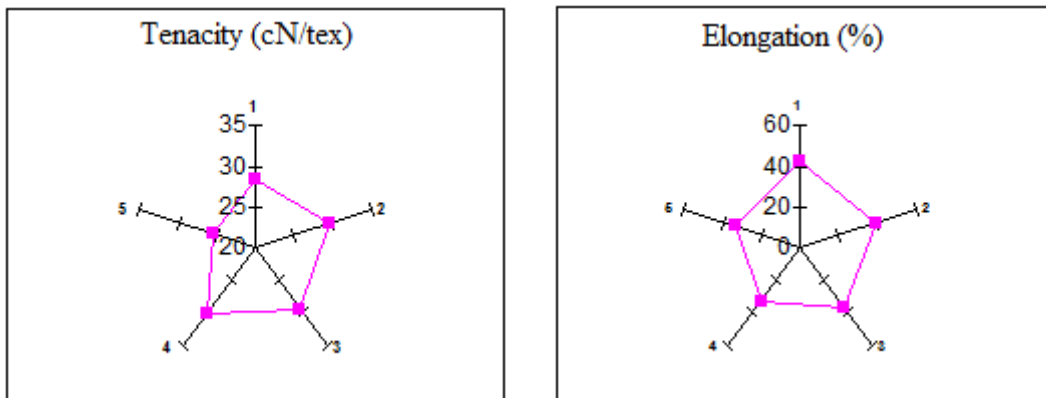


Figure 8: Evolution of tenacity and elongation as function of factor 'plant'

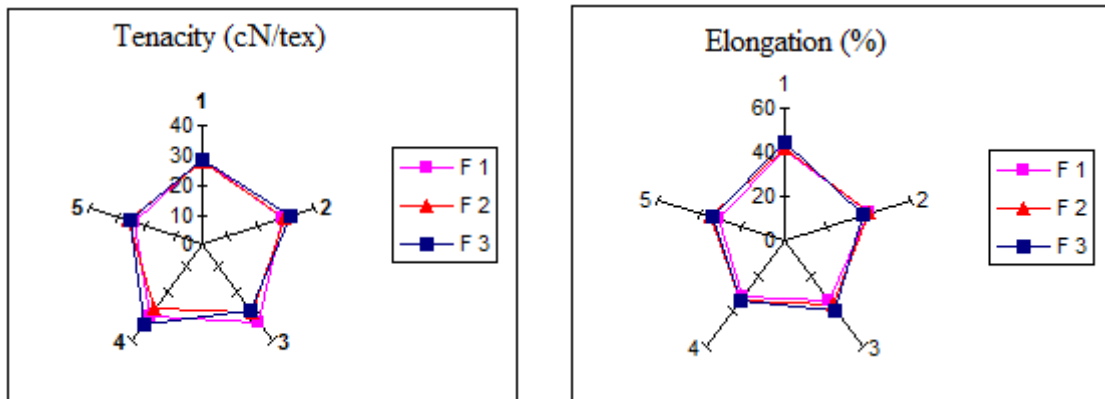


Figure 9: Evolution of tenacity and elongation as function of factor 'leaf'

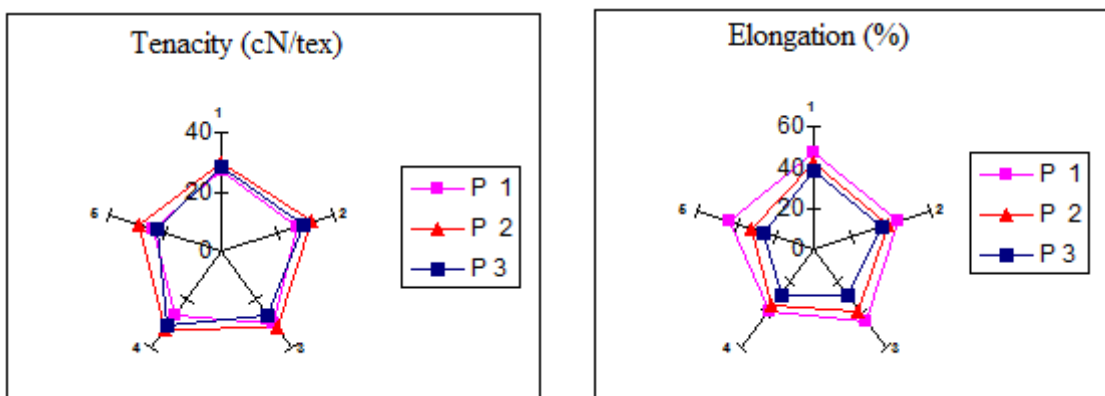


Figure 10: Evolution of tenacity and elongation as function of factor 'position'



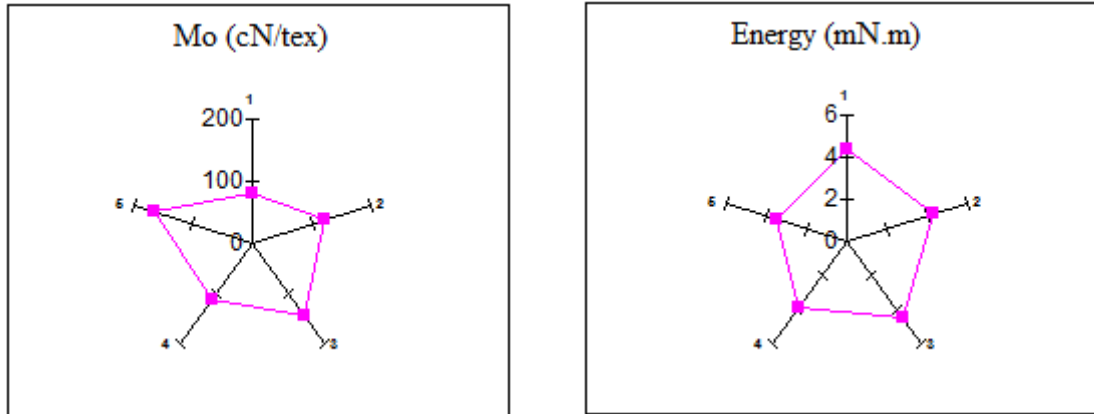


Figure 11: Evolution of initial modulus and energy as function of factor 'plant'

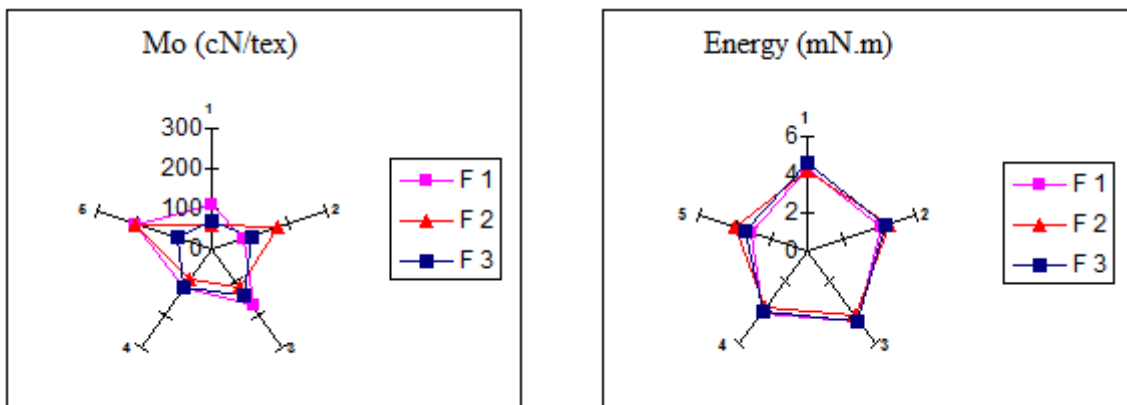


Figure 12: Evolution of initial modulus and energy as function of factor 'leaf'

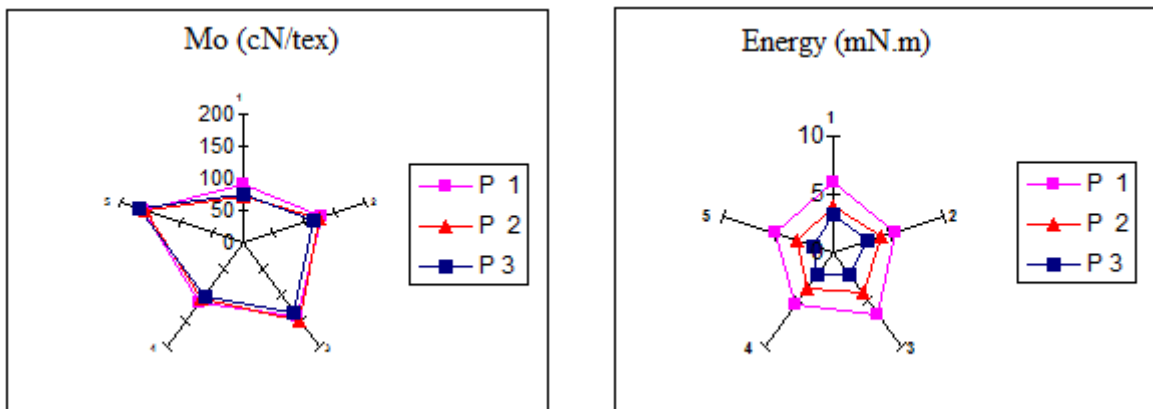


Figure 13: Evolution of initial modulus and energy as function of factor 'position'

From figures 8→13, we can deduce the following points:

- The most relevant effect is the 'position' factor', and this result is in correlation with that found by intra-plant study, in other word, all characteristics decrease towards the tip of the leaf.
- The factor 'plant' is also important and may have statistically significant effects.
- The factor 'leaf' remains the less influent one, and this fits with results previously found for the intra-plant study.
- The interaction between factors 'plant' and leaf' has, a priori, an important effect. This will be verified in the statistical study.



Table 6: Analysis of variance results for tenacity.

	DL	F	F (5%)	F (1%)	P	Conclusion
PI	4	6.49	2.38	3.34	0.000	xx
F	2	1.98	3.00	4.63	0.139	0
P	2	16.78	3.00	4.63	0.000	xx
PI xF	8	2.85	1.95	2.53	0.004	xx
PI xP	8	1.82	1.95	2.53	0.07	0
F xP	4	3.68	2.38	3.34	0.006	xx
PI xF xP	16	2.65	1.65	2.02	0.000	xx

Table 7: Analysis of variance results for elongation.

	DL	F	F (5%)	F (1%)	P	Conclusion
PI	4	56.23	2.38	3.34	0.000	xx
F	2	7.48	3.00	4.63	0.001	xx
P	2	225.1	3.00	4.63	0.000	xx
PI xF	8	3.4	1.95	2.53	0.001	xx
PI xP	8	7.48	1.95	2.53	0.000	xx
F xP	4	4.21	2.38	3.34	0.002	xx
PI xF xP	16	4.39	1.65	2.02	0.000	xx

Table 8: Analysis of variance results for initial modulus.

	DL	F	F (5%)	F (1%)	P	Conclusion
PI	4	41.48	2.38	3.34	0.000	xx
F	2	19.28	3.00	4.63	0.000	xx
P	2	1.41	3.00	4.63	0.245	0
PI xF	8	21.86	1.95	2.53	0.000	xx
PI xP	8	0.6	1.95	2.53	0.779	0
F xP	4	1.97	2.38	3.34	0.097	0
PI xF xP	16	4.29	1.65	2.02	0.000	x

Table 9: Analysis of variance results for impact energy.

	DL	F	F (5%)	F (1%)	P	Conclusion
PI	4	7.4	2.38	3.34	0.000	xx
F	2	0.68	3.00	4.63	0.509	0
P	2	189.37	3.00	4.63	0.000	xx
PI xF	8	1.21	1.95	2.53	0.291	0
PI xP	8	1.42	1.95	2.53	0.183	0
F xP	4	2.28	2.38	3.34	0.059	0
PI xF xP	16	2.09	1.65	2.02	0.007	xx

Table 10 resumes the results found for analysis of variance of all studied parameters.

Table 10: Recapitulation of ANOVA results

	Fineness	Diameter	Tenacity	Extension	M. initial	Energy
PI						
F						
P						
PI xF						
PI xP						
F xP						
PI xF xP						

With :  Highly significant  Significant  Negligible

From table 10, we find that the factor 'position' has a highly significant effect on the different studied characteristics except for the initial modulus. This means that fibers located at different positions in the leaf (tip, middle or base) exhibit different properties. On the contrary, the factor 'leaf' remains the more negligible for most characteristics. Furthermore, it should be noted that the effect of factor 'plant' is highly significant for mechanical properties but negligible for fineness and diameter parameters.

We can summarize the results of statistical analysis by correlation equations established between the output responses and the different controlling factors as well as their interactions. These equations represent the sum of significant effect with 5 % risk of error.

- Fineness = [ P ]
- Diameter = [ P ]
- Tenacity = [ PI ] + [ P ] + [ PI × F ] + [ F × P ] + [ PI × F × P ]
- Elongation = [ PI ] + [ F ] + [ P ] + [ PI × F ] + [ PI × P ] + [ F × P ] + [ PI × F × P ]
- Initial modulus = [ PI ] + [ F ] + [ PI × P ] + [ PI × F ] + [ PI × F × P ]
- Impact energy = [ PI ] + [ P ] + [ PI × F × P ]

Although we have simplified the study by the analysis of variance which allows us to remove negligible controlling factors, the models proposed are still difficult and complicated. As a result, we have proposed to simplify again those models by taking into consideration the weight of factors (importance) and keeping only the most significant factors. These latter are chosen so that the sum of their relative effects represent 80 % of the total effect. The results of this simplification approach are given in table 11.

Table 11: Relative weight (%) of factors held in inter plant study.

Parameter	F (tex)	D ( $\mu\text{m}$ )	$\tau$ (cN/tex)	A (%)	Mo (cN/tex)	w (J)
PI	0	0	18.61	26.03	36.64	6.70
F	0	0	0	1.73	8.51	0
P	100	100	24.06	52.09	0	85.71
PI × F	0	0	16.35	3.15	38.62	0
PI × P	0	0	0	6.92	1.06	0
F × P	0	0	10.54	1.95	0	0
PI × F × P	0	0	30.42	8.12	15.16	7.58

The simplification proposed (80 % of the total effect) induces the following correlation equations:

- Fineness = [ P ]
- Diameter = [ P ]
- Tenacity = [ PI ] + [ P ] + [ PI × F ] + [ PI × F × P ]
- Elongation = [ PI ] + [ P ] + [ PI × F × P ]
- Initial modulus = [ PI ] + [ PI × F ] + [ PI × F × P ]
- Impact energy = [ P ]

In conclusion, the simplified models, established by statistical analysis, direct us to select conveniently the fibers collecting mode by taking into consideration the most significant controlling factors. For instance, in the applications demanding good mechanical properties, the collection mode has to respect the factors 'plant' and 'position'.

#### 4. CONCLUSIONS

The determination of physical and mechanical properties for agave fibers shows a great dispersion in results. The aim of this paper was to investigate this variability which can be assigned to the extraction mode. As a matter of fact, fibers may be extracted from different plants and also, they may be taken from different parts of the same plant and of the same leaf.

The Design of experiment DoE techniques, and other statistical analyses were used by varying the factors of interest in a full factorial design to assess the main effects and the interactions of some factors influencing fiber properties.

The controlling factors were chosen as follows:

- 'Plant'
- 'Leaf' taken from the same level
- 'Position' of fibers in the leaf, that indicates about the maturity of fibers.

The results of the main effects analysis show that the factor 'position' has a highly significant effect on the different studied characteristics except for the initial modulus. This means that fibers located at different positions in the leaf (tip, middle or base) exhibit different properties. On the contrary, the factor 'leaf' remains the more negligible for most characteristics. Furthermore, it was noted that the effect of factor 'plant' is highly significant for mechanical properties but negligible for fineness and diameter parameters.

The results of statistical analysis were summarized by correlation equations established between the output responses and the different controlling factors as well as their interactions. Those models were simplified by taking into consideration the weight of factors and keeping only the most significant one. These latter are chosen so that the sum of their relative effects represent 80 % of the total effect. By this way, the simplified model, direct us to select conveniently the fibers collecting mode by taking into consideration the most significant controlling factors.

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