

# Chemical, Chromatographic, and Thermal Analysis of Rosemary (*Rosmarinus officinalis*)

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**ABSTRACT:** Rosemary (*Rosmarinus officinalis*) is mainly known for its cooking qualities, but it possesses an essential oil with pharmacological properties. Moreover, this plant was the start of forest fires in Provence, France. The presence of biopolymers can contribute to electrical energy production by biomass combustion. The aim of this work was to study a stalk of rosemary during its biological cycle (November 1998 to June 1999). The chemical analysis (water, mineral ashes, cellulose, lignin, holocellulose, and extractives) showed few variations during this period. Leaves and branches differentiated themselves according to their chemical composition. Analyzed by chromatography gas–liquid chromatography/mass spectroscopy (GC-MS) and gas–liquid chromatography/flam ionization detector (GC-FID), the essential oil of this rosemary had a high level of camphor (30–45%), which could be used in new therapeutics (cardiac and respiratory analeptic). Finally, the simultaneous thermal analysis (DTA-TG) was run on the fresh plants and the dried powdered samples. Several kinetic constants of the biopolymers had been computed: activation energies of cellulose and holocellulose. During heating, the rosemary's lignin decomposed more than others plants, thereby increasing the fire risks. The chemical, chromatographic, and thermal analysis run on the rosemary could be applied on other species in the Mediterranean basin. © 2002 John Wiley & Sons, Inc. *J Appl Polym Sci* 83: 747–756, 2002

**Key words:** biopolymers; cellulose; essential oil; GC/MS; holocellulose; lignine; *Rosmarinus officinalis*; thermal decomposition; TG-DTA

## INTRODUCTION

The use of biomass is very important for the Mediterranean countries, first, in agricultural resources, but also, more and more often, in the production of electricity by the flash pyrolysis process. On the other hand, forest fires of the Mediterranean plants and their spreading fit well with the presence of the essential oils and evolved gases during the thermal decomposition of less

volatile products, such as the biopolymers and mainly the cellulose. The knowledge of their thermal behavior is very important.

Besides its applications in perfumes and cooking, the essential oil of rosemary has good pharmacological properties. Popular medicine uses it for antibacterial and antifungal activities as oils can be absorbed by the skin (via bath, massage, etc.).

For these reasons, the results of the chemical, chromatographic, and thermal analysis run on the same stalk of rosemary (*Rosmarinus officinalis*, with camphor chemotype) are presented. The tests were done during the biological cycle. The identification of the plant was made by P. Regli

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### SAMPLING, PROCEDURE, APPARATUS

The studied samples had been gathered on the same plant during the period between November 22, 1998 and June 26, 1999 in the village of Entrevennes near Manosque (Alpes de Haute Provence). The gathering dates are characterized by six digits: day, day, month, month, year, year. Some hours after picking, leaves, branches, and flowers were separated. The flowers had been always present, but sometimes in very small quantities.

The level of water was determined (weight loss desiccation at 110°C) at the beginning, when freshly picked.

The essential oil was obtained according to the European Pharmacopae<sup>1</sup> on a second part of the sample; about 50 g (weighed accurately) was put in a 1-L flask with 0.5 L water. The time of distillation was 5 h. This time was sufficient to recover all the oil.

A third part of the plant was dried at 110°C for 15 h. More than 99% of the essential oil was volatilized during the heating at 110°C. Then, this part was pounded and sieved. On this powder, accepted by the 1-mm side sieve, the following were determined: the mineral ashes obtained after 1 h at 650°C; the cellulose by gravimetry, after the action of a 1 : 4 volume mixture of concentrated nitric acid and ethyl alcohol;<sup>2</sup> and the extractives by the 2 : 1 volume mixture of benzene and ethyl alcohol with the continuous extraction Soxhlet apparatus.<sup>3</sup>

The lignin and the holocellulose were calculated on the residue that remained after the Soxhlet method. The lignin was analyzed by gravimetry after 24*N* sulfuric acid attack.<sup>3</sup> The holocellulose (i.e., cellulose and hemicellulose) was also determined by gravimetry after the reaction of the powder with sodium chlorite in acetate buffer at pH 4.9.<sup>2</sup> The hemicellulose is the difference between holocellulose and cellulose. All

**Table I** Repeatability of the Different Techniques Used

Analysis	Average (%/DM)	Standard Deviation (%/DM)	Relative Standard Deviation (%)
Mineral ashes	6.33	0.125	1.97
Cellulose	35.4	0.72	2.03
Lignine	33.9	0.74	2.18
Holocellulose	55.0	0.98	1.78
Extractives	20.0	0.96	4.80
Essential oil <sup>a</sup>	1.95	0.108	5.54

Note. Ten tests for each one. DM, dry matter.

<sup>a</sup> In mL/100 g DM.

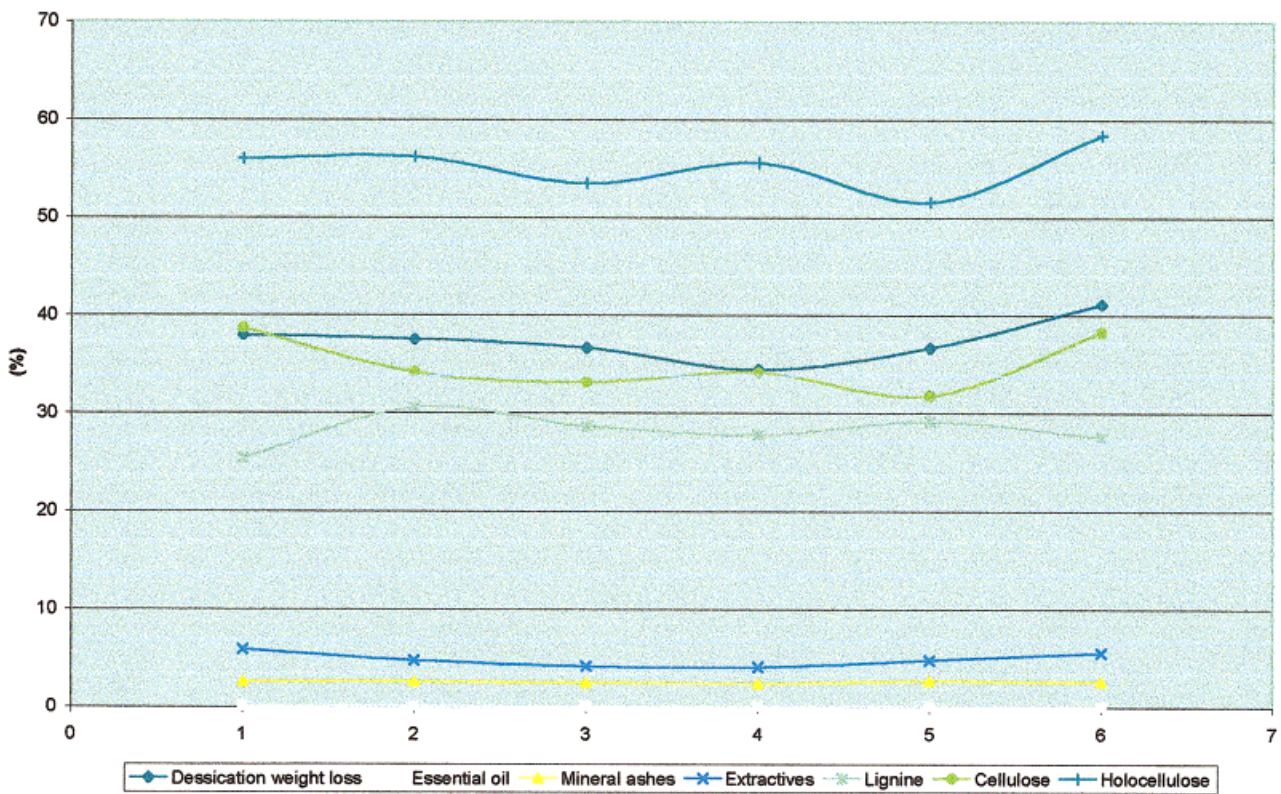
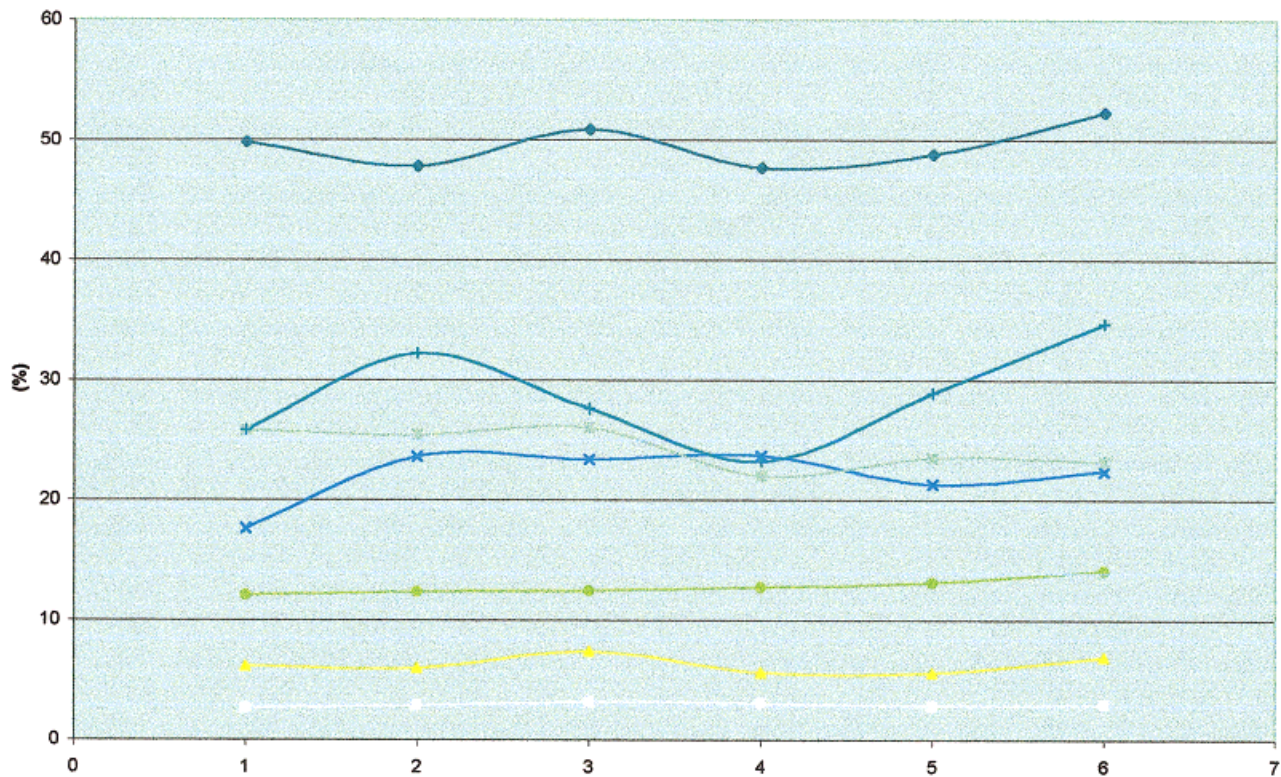
the results are expressed according to the dry matter (%/DM).

Two gas-liquid chromatography (GC) techniques had been used. Gas-liquid chromatography/mass spectroscopy (GC/MS) was carried out by using total ion monitoring on a Varian 3400 apparatus with a detector MS ion trap. The column HP5MS (30 m × 0.32 mm, 1-μm film thickness), in solid support silica, was used. The heating rate was 3°C/min from 60 to 200°C. Injector temperature was set at 250°C. Helium was used as carrier gas with a flow rate of 1.0 mL/min. Gas-liquid chromatography/flame ionization detector (GC/FID) Hewlett-Packard 5700 apparatus was also used. The column (2 m × 0.32 cm, solid support glass, Chromosorb 80/100 Mesh, OV225) was held at 50°C for 4 min and programmed at 8°C/min to 200°C. Injector temperature and detector FID temperatures were 250°C. The flow rates were 30 mL/min for hydrogen, 240 mL/min for air, and 8.7 mL/min for nitrogen carrier gas (Aldrich-Chemie, L'Isle d'Abeau, Saint Quentin, France).

All the standards were from Aldrich-Chemie Co. The three significant components of the oils (camphor, 1,8-cineol, α-pinene) were determined by GC; *n*-dodecane was used as internal standard at 0.75 mg/mL. For the calibration curves of each component, six standard solutions, from 0 to 1.5 mg/mL in hexane, were used as data points.

**Figure 1** Chemical analysis results of the studied rosemary (1998–1999): top, leaves; bottom, branches. (Batch 1, 22/11/98; batch 2, 10/12/98; batch 3, 09/01/99; batch 4, 06/02/99; batch 5, 06/03/99; batch 6, 04/04/99. All the results are in g/100 g DM, except the essential oil in mL/100 g DM, and the desiccation weight loss is in percentages).





Legend: Densitation weight loss (blue diamond), Essential oil (green circle), Mineral ashes (yellow triangle), Extractives (blue cross), Lignine (grey asterisk), Cellulose (green diamond), Holocellulose (blue plus)

**Table II Pluviometry in Manosque (1998–1999)**

Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Water (mm)	72.5	26.3	19.2	96.7	15.3	53.0	80.4	53.4	13.1

Thermal analysis was run on DTA-TG simultaneous apparatus (Setaram 92) with a heating rate of 60–3000°C/h from the ambient temperature to 800°C under 0.5 L/h air sweeping and platinum crucibles.

The sample mass was about 20 mg, and the kaolin was used as a inert thermal reference. Tests were done on freshly cut samples and on 110°C dried powders.

The standards used to calibrate DTA, in temperature, were as follows: indium, tin, lead, and aluminum, and calcium oxalate monohydrate in weight loss for TG.

### Chemical Analysis

The chemical analysis contributed to the determination of the water, the mineral ashes, the cellulose, the lignin, the holocellulose, the Soxhlet extractives, and the essential oils. All the presented results are in g/100 g DM, except the essential oil in mL/100 g DM, and the water was measured in the percentage of weight loss desiccation.

The repeatability of the different techniques of chemical analysis is about 2% for the gravimetry and 5% for the extraction and the distillation (Table I).

The chemical analysis, executed on the leaves and the branches of the rosemary, showed a higher level in water (weight loss desiccation), essential oil, mineral ashes, and Soxhlet extractives for the leaves than the branches (Fig. 1). Oppositely, the reverse was observed for the lignin, the cellulose, and the holocellulose. In the considered period, the variations were low, except for the holocellulose in the leaves, 23.2%/DM for batch 4 (06/02/99). The highest level of humidity in the leaves, for batch 3 (09/01/99) and batch 6 (04/04/99), respectively, 50.8 and 52.3%, can be due to the pluviometry (Table II).

### Chromatographic Analysis

The two maximums of essential oils in the leaves were observed for batch 3 of January 9 (3.15 mL/100 g DM) (Fig. 1) and for batch 7 of June 26 (3.59 mL/100 g DM) (not presented in Fig. 1), corre-

sponding to a dryness in the previous days. The flowers presented a level of 2.47–1.85 mL of essential oil/100 g DM from November 1998 to January 1999.

The rosemary's essential oil composition is mentioned in the French Pharmacopea.<sup>4</sup> As an example, a chromatographic curve of the essential oil of the rosemary type Spain is shown in Figure 2. Another example, issued from the studied rosemary (batch 3), is shown in Figure 3.

The different samples of leaves showed the following levels:  $\alpha$ -pinene, 4.12–8.53%; 1,8-cineol, 13.4–21.3%; camphor, 31.1–44.5%. So, the studied rosemary is near the type Hungary (majority of camphor in the oil, freezing resistance, altitude of 600 m).

If we consider a middle density of 0.90<sup>4</sup> and with the knowledge of the humidity present in the samples, we can determine the values of  $\alpha$ -pinene, 1,8-cineol, and camphor, according to the dry matter (in g/100 g DM), (Fig. 4). If we take into account the concentration of these components, a maximum is observed in January and June.

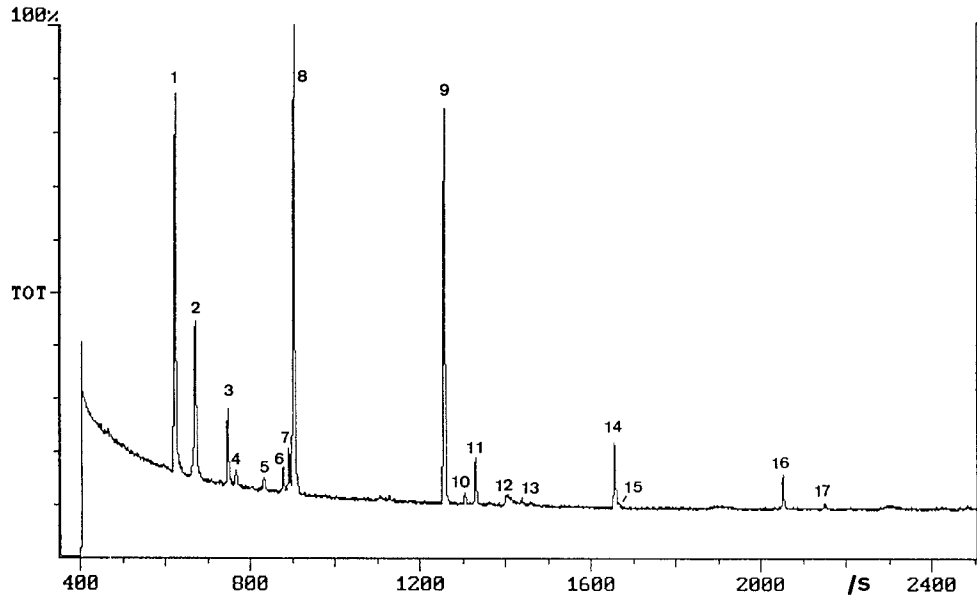
To use the best pharmacological actions, the gathering of the rosemary must be done in January (high level of oil and camphor). The actions of the camphor in the essential oil (30–45%) would have new applications in therapeutic uses.<sup>5</sup>

### Thermal Analysis

In the Mediterranean basin, the start of the forest fires often coincided with a high period of dryness and fierce wind, and also, to the nature of the plant. In the case of electric energy production, by biomass combustion, the kinetic decomposition of this process must be perfectly known. Even if the cellulose represents 20–50% of the biomass, the knowledge of the decomposition kinetic of the other biopolymers seems essential.

The thermal analysis run on the leaves (Fig. 5), or on the branches, under air sweeping is characterized by three stages with weight loss, as follows: until about 135°C, water volatilization (endothermic reaction); between 135 and 360°C, decomposition of the biopolymers, with char formation (exothermic reaction); between 360 and 500°C, char combustion (exothermic reaction).

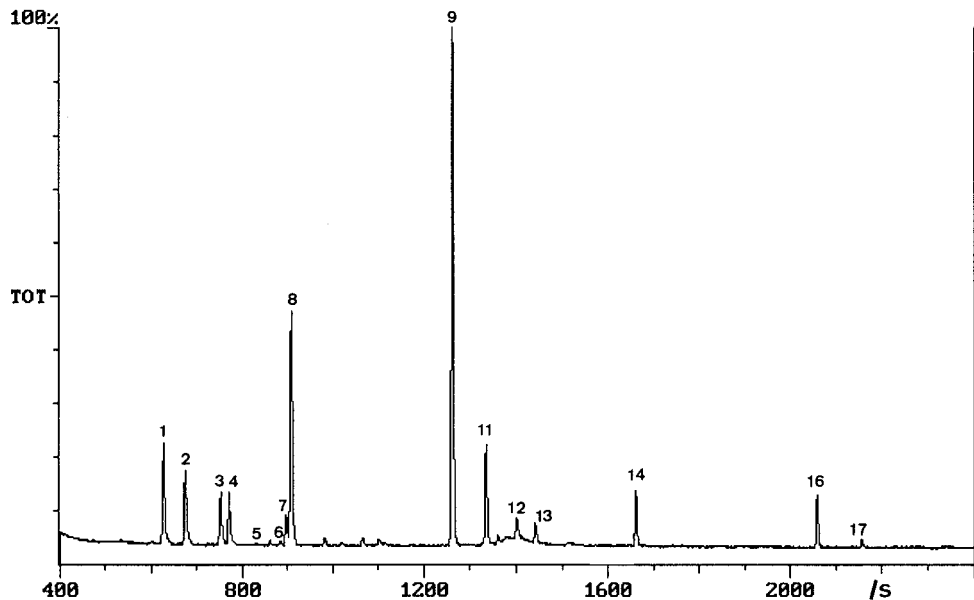




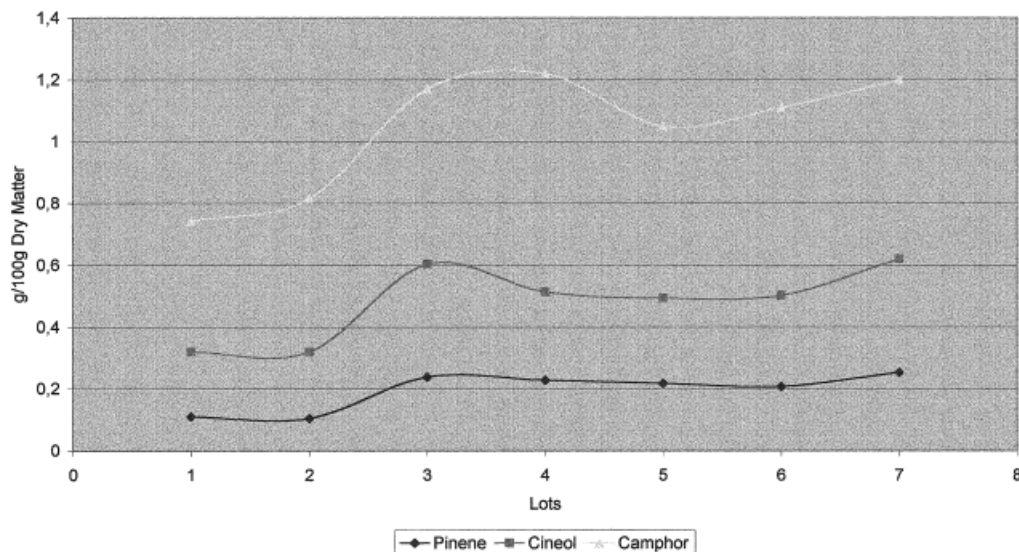
**Figure 2** GC/SM chromatogram of the essential oil of the rosemary type Spain. [1,  $\alpha$ -pinene; 2, camphene; 3,  $\beta$ -pinene; 4, myrcene; 5,  $\Delta$ -3 carene; 6, cymene; 7, limonene; 8, 1,8-cineol; 9, camphor; 10, isoborneol; 11, borneol; 12,  $\alpha$ -terpineol; 13, verbenone; 14, bornyl acetate; 15, isobornyl acetate; 16, trans (or  $\beta$ -caryophyllene); 17,  $\alpha$ -humulene.]

The second stage is of high interest in the evaluation of the fire risks. It corresponds, mainly, to the decomposition of the wooden biopolymers.

Among all the thermal analysis results, we have chosen to present only those affecting the temperatures of the derivative curves of thermo-



**Figure 3** GC/SM chromatogram of the essential oil of the studied rosemary (batch 3; 09/01/99). [1,  $\alpha$ -pinene; 2, camphene; 3,  $\beta$ -pinene; 4, myrcene; 5,  $\Delta$ -3 carene; 6, cymene; 7, limonene; 8, 1,8-cineol; 9, camphor; 10, isoborneol; 11, borneol; 12,  $\alpha$ -terpineol; 13, verbenone; 14, bornyl acetate; 15, isobornyl acetate; 16, trans (or  $\beta$ -caryophyllene); 17,  $\alpha$ -humulene.]



**Figure 4** Titration of the three components of the essential oil of the studied rosemary's leaves. (Batch 1, 22/11/98; batch 2, 10/12/98; batch 3, 09/01/99; batch 4, 06/02/99; batch 5, 06/03/99; batch 6, 04/04/99; batch 7, 26/06/99.)

gravimetry (DTG) peaks, and the weight losses at 135°C (humidity) and 650°C (total volatilized mass). The volatilization loss (EXO/DM), which corresponds to the centesimal ratio between the weight loss, in the interval of the DTG peak at 300°C, and the total dry matter volatilized, is also presented in Table III. The repeatability of the DTG method is applied, first, on the fresh plant, and later, on the same dried and powdered sample.

The desiccation started, and whatever the stocking conditions were after gathering, it would be difficult to determine the true level of humidity.

The temperature of the DTG peaks decreased slightly from the fresh sample to the dried and powdered sample. This variation is higher for the third peak. Moreover, an increase of the volatilization, characteristic of the dry matter decomposition, was observed in our drying conditions (110°C for 20 h). Finally, it is easier to stock the sample in powder form, because its granulometry seems constant. By thermal analysis, the different batches present similar values.

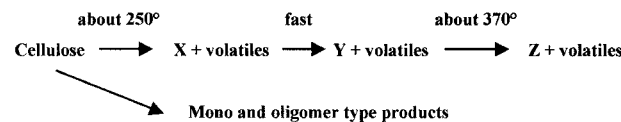
### Thermal Decomposition of the Wooden Biopolymers

Cellulose and hemicellulose components of the whole biomass decompose independently of one another. Lignin decomposition is formally de-

scribed by a very broad DTG peak at about 300°C.<sup>6</sup>

The rich variety of reaction products that evolves when cellulose is heated implies a very complex pyrolysis chemistry. The products of cellulose pyrolysis include many oxygenated products.<sup>7</sup>

The smoldering and flaming combustion of cellulose was studied by Broido and colleagues,<sup>8-9</sup> Bradbury et al.<sup>10</sup>, and Alves and Figueiredo.<sup>11-12</sup> Varhegyi et al.<sup>13</sup> presented the following reaction scheme:



(X, Y, Z are solid intermediate products)

Between 250 and 370°C, a model of first-order kinetics would be adequate, of the form:

$$d\alpha/dt = A \exp(-E/RT)(1 - \alpha)$$

where  $\alpha$  is the fraction of volatiles formed at time  $t$ ,  $E$  is the apparent activation energy, and  $A$  is the preexponential constant.

Using this model, Cooley and Antal<sup>14</sup> obtained a good fit to the experimental weight loss curves with an  $E$  value of about 193 kJ/mol.

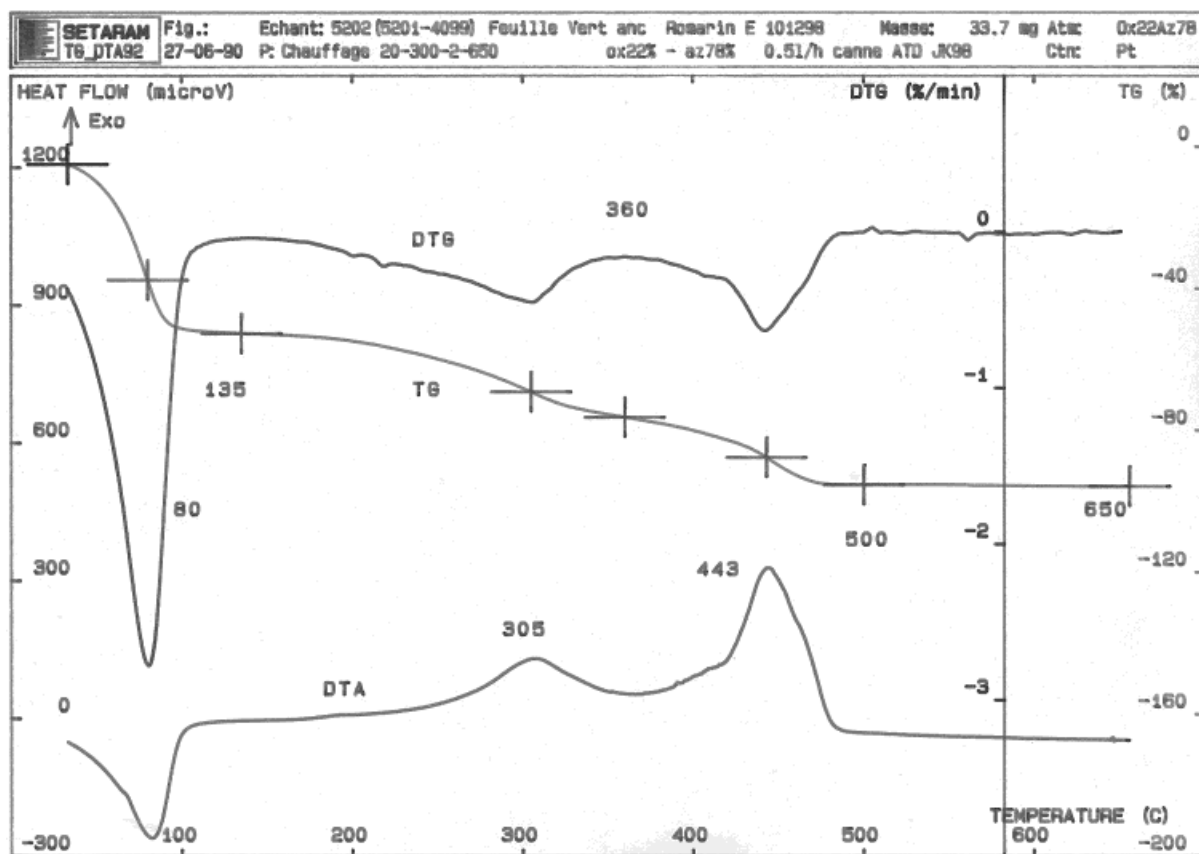


Figure 5 Thermal analysis curves of the studied rosemary's leaves.

In thermogravimetry, using the selected values of conversion, the temperature,  $T$  (in K), at the conversion level is measured for each thermal curve obtained for different heating rates. A plot of the logarithm of heating rate ( $\beta$ ) versus the

corresponding reciprocal temperature at constant conversion is prepared. The plotted data should produce a straight line. The determination of activation energy ( $E$ ) from the slopes uses the method of Flynn and Wall:<sup>15,16</sup>

Table III Repeatability of the Thermal Analysis of the Branches (fresh and powdered) of the Studied Rosemary

	First DTG Peak (°C)	Weight Loss at 135°C (%)	Second DTG Peak (°C)	EXO/DM (%)	Third DTG Peak (°C)	Weight Loss at 650°C (%)
Fresh branch sample						
M	80.2	61.5	296.6	54.6	438.5	96.4
SD	4.20	6.15	1.67	0.91	3.65	1.17
RSD (%)			0.56	1.66	0.83	1.22
Dried and powdered branch sample						
M	64.9	3.6	290.2	70.1	392.3	98.6
SD	3.48	0.55	2.87	1.31	3.24	0.67
RSD (%)			0.99	1.87	0.83	0.68

Note. Ten tests. M, average; SD, standard deviation; RSD, relative standard deviation.

**Table IV** Activation Energy of the Thermal Decomposition of the Cellulose and Holocellulose of the Studied Rosemary (300–350°C)

Samples	E (kJ/mol)
Branches—Cellulose	187.1
Branches—Holocellulose (with 62% cellulose)	190.1
Leaves—Holocellulose (with 51% cellulose)	149.3

$$E = -R \frac{d \log \beta}{d(1/T)}$$

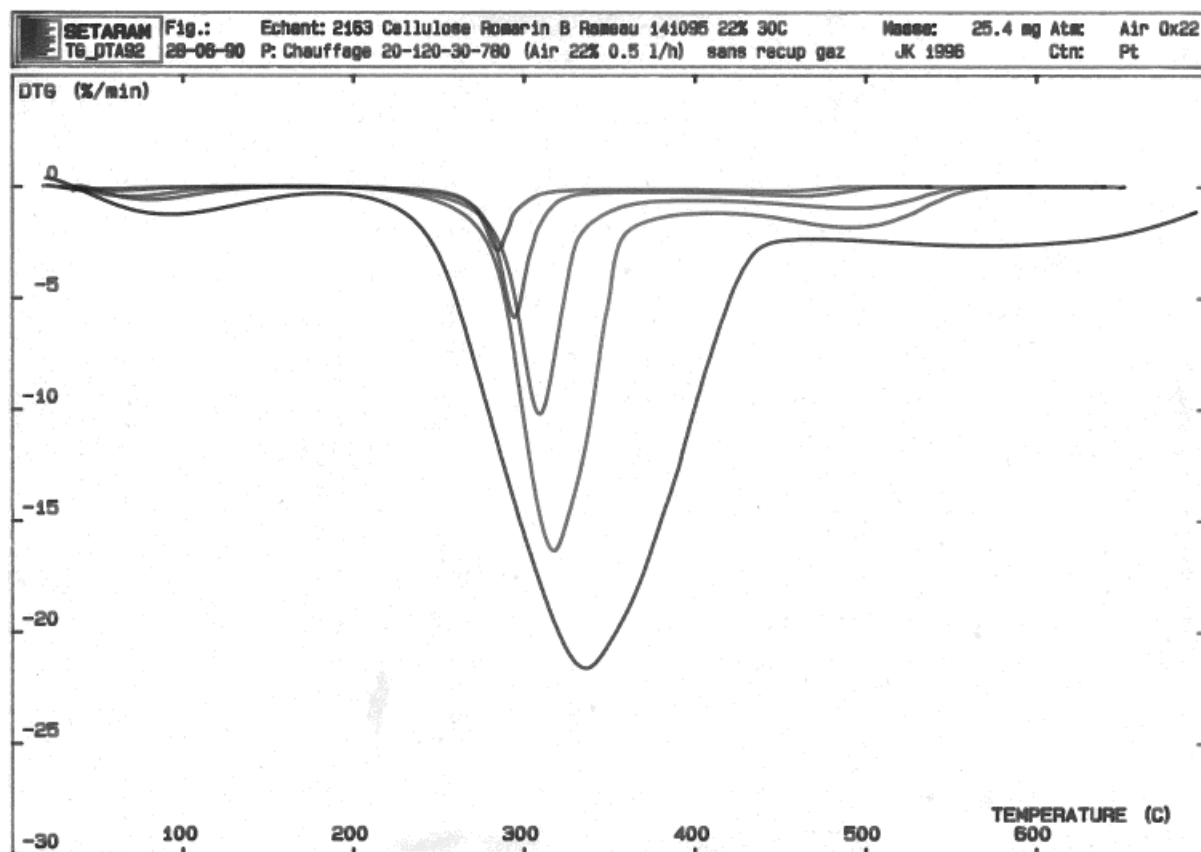
Kissinger<sup>17</sup> supposed that the maximum of the reaction rate corresponds practically with the DSC maximum peak ( $T_{\max}$ ). Ozawa<sup>18–19</sup> proposed an approximative method for  $E$  determination from  $T_{\max}$ . In the Ozawa method,  $\log \beta$  is plotted against  $1/T_{\max}$  and the slope of this plot is again

$-E/R$ .<sup>20</sup> We observed in an earlier study<sup>21</sup> practically the same values of  $E$  computed, on one hand, by the Flynn method,<sup>22</sup> and on the other hand, by using the temperature of the minimum DTG peak.

The DTG data presented clearly support the finding that the weight loss and thus the thermal degradation rate depend strongly on the heating rate, which could be evidenced by evaluating the activation energies of the weight loss process according to Freeman and Carroll.<sup>23</sup>

By using the first-order kinetic model, we evaluated the apparent activation energy ( $E$ ) during the pyrolysis of cellulose and holocellulose, in the case of the rosemary, at about 300–350°C (Table IV).

First, we determined the temperature at the minimum of the DTG curve, which corresponds to the maximum decomposition rate (Fig. 6). Then, we represented  $\log \beta$  versus the reciprocal of the minimum DTG peak temperature  $T$  ( $\beta$  being the heating rate).



**Figure 6** DTG curves of the cellulose issued from the studied rosemary's branches (from top to bottom 1, 2, 5, 10, and 30°C/min).



The obtained values of the cellulose, issued from the branches of rosemary, correspond to those cited in references 24–26.

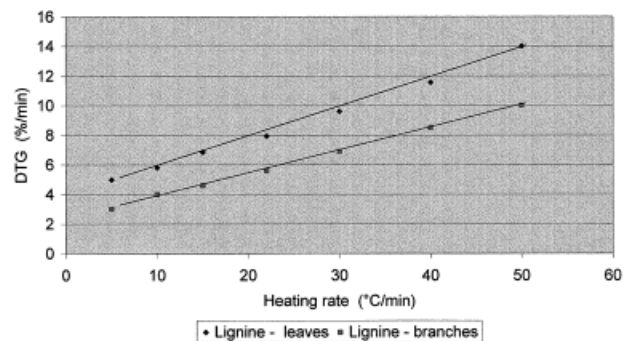
The activation energy of the holocellulose is lower for the leaves than for the branches. This variation will involve an upper fire risk for the rosemary's leaves.

Earlier, it was shown that the decomposition kinetics model of lignin occurs differently from the first-order kinetic observed for cellulose and holocellulose.<sup>21,25,26</sup> For the lignin, a correlation between the maximum weight loss rate and the heating rate was observed. This dependence presents a particular form: a straight line from 5 to 50°C/min.

For the lignin, a more important decomposition occurs for the leaves than for the branches, when we take in consideration the variation of the maximum decomposition rate (DTG in %/min) versus the rate of heating (°C/min) between 5 and 50°C/min (Fig. 7 and Table V).

## CONCLUSION

To value the forest fire risks, it is necessary to know the level of cellulose and/or holocellulose (responsible of flammable gas emission at about 300°C) in the different aerial parts of the rosemary. A high level of the lignin and, mainly, a quickest decomposition kinetic, can increase these risks. The determination of the essential oil and the knowledge of its composition are also important. A high level of this oil and a majority of low boiling components can also increase these risks. On the other hand, the analytical evalua-



**Figure 7** Thermal decomposition of the lignin issued from the studied rosemary. Correlation between the maximum DTG peak (%/min) and the heating rate (°C/min).

**Table V** Thermal Decomposition of the Lignine in the Studied Rosemary [ $Y = a + bX$ , where  $Y$  is maximum decomposition (DTG in %/min),  $X$  is heating rate of the TG (°/min), and  $r$  is correlation coefficient]

	$a$	$b$	$r$
Leaves (Nov 98)	3.558	0.217	0.999
Leaves (March 99)	3.683	0.202	0.998
Branches (Nov 98)	2.302	0.154	0.999

tion of the oil can be used in pharmacological applications.

Finally, the interest of the presented study was to monitor these different parameters all along the biological cycle of the rosemary.

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