

# Starch Gelatinization Measured By Pulsed Nuclear Magnetic Resonance

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## ABSTRACT

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The extent of starch gelatinization was quantified by pulsed-proton nuclear magnetic resonance (NMR) analysis. To quantify the gelatinization of starch, a relative liquefying index (RLI), calculated from the ratio of liquid to solid phases determined by pulsed NMR, was defined. A correlation coefficient  $>0.94$ , at 5% significance level, was found between the RLI and the degree of gelatinization determined by a method of enzymatic susceptibility. Studies on the kinetics of gelatinization of

wheat starch in starch-water systems by the NMR method showed a first-order reaction until the degree of gelatinization reached a fixed and constant value, which increased with the temperature and with the amount of water in the system. The minimum activation energy occurred at 45.0% water. The similarity between the activation energies found at 55.0 and at 65.0% water indicated that 55.0% water is the minimum water content for the complete gelatinization of wheat starch.

Gelatinization is the term used to characterize the transformations occurring when a starch-water dispersion is heated. Above a certain temperature, the starch granules swell and their structure is altered (Leach 1965, Collison 1968). A gradual loss of birefringence occurs, and the low molecular weight components are leached out into the water medium (Greenwood 1979). During this process, the secondary bonds that maintain the granule structure are broken and the micellar network is pulled apart.

Several methods can be used to follow the gelatinization process: loss of birefringence, increase in viscosity, increase in enzyme susceptibility, decrease in enthalpy, and loss of X-ray diffraction pattern. Of these, only the measurements of enzyme susceptibility and variation of enthalpy are used to quantify the extent of gelatinization. These methods however, are either time-consuming (enzyme susceptibility) or do not show good reproducibility (calorimetry).

Lelievre and Mitchell (1975) showed that when a starch-water dispersion is heated above 60°C, an increase in the relaxation time as measured by pulsed-proton nuclear magnetic resonance (NMR) occurs. This behavior was attributed to an increase in the mobility and hydration of the starch polymers, suggesting an increase in the number of protons linked to the liquid phase. Although the relaxation time due to the protons in the solid phase was not measured, it would be lower than those of the liquid phase. Thus, in a starch sample with different degrees of gelatinization, there would be different relations between the protons in the liquid phase and those in the solid phase. According to this hypothesis, it should be possible to measure the degree of gelatinization using the ratio between the protons in the liquid and those in the solid phase as determined by pulsed-proton NMR.

The objective of this work was to study the possibility of using pulsed-proton NMR to measure the degree of gelatinization. Simultaneously, the kinetics of the gelatinization of wheat starch-water systems was investigated.

## MATERIAL AND METHODS

### Raw Material

The starch used in this study was isolated from a Brazilian wheat variety using the method of Wolf (1964).

### Chemical Analysis

Moisture, protein, ash, and fat were determined by approved methods 44-15, 46-11, 08-03, and 30-20, respectively (AACC 1995).

### Sample Preparation and Liquid-Solid Determination

Starch samples containing 35.0, 45.0, 55.0, and 65.0% water were poured into 5.0- × 40.0-mm Duran tubes. To avoid moisture loss, the tubes were glued closed with special plastic lids and placed in special 10.0- × 170.0-mm NMR tubes.

Gelatinization was performed for different time periods in an adapted oven. The temperature inside the oven was checked constantly with a thermometer and periodically with a thermocouple. After heating, the samples were chilled to 0°C to stop the gelatinization process. After chilling for a period no longer than 2 hr, the liquid-to-solid ratio was determined in a pulsed-proton NMR spectrophotometer, according to the method of Waddington (1977).

The liquid-solid ratio was obtained from the free induction decay (FID) signal. The signal that immediately registered after a "dead time" of 2-5 μsec ( $T$ ) was considered to be proportional to the signals of the protons in the liquid and solid phases. The signal of the protons in the liquid phase ( $L$ ) was determined at a time longer than the time corresponding to the FID of the protons in the solid phase (~20 μsec).

Assuming that the content of the solid phase ( $S$ ) is proportional to the difference  $T - L$ , then  $S = fs \times (T - L)$ , where  $fs$  is a factor that depends on the nature of the solid phase and on the time at which the NMR signal ( $T$ ) is taken in the FID. In this work, it will be assumed that the  $fs$  factor does not depend on the thermal treatment of the samples.

TABLE I  
Chemical Composition of the Isolated Starch

Component	Percent
Moisture	12.61
Ash	0.25
Protein	0.17
Lipids	0.37
Carbohydrates <sup>a</sup>	86.59

<sup>a</sup> Calculated by difference.

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The percentage of liquid and solid may be calculated as:

$$\%L = L/[L + fs(T - L)] \times 100$$

$$\%S = \{fs(T - L)/[L + fs(T - L)]\} \times 100$$

The *fs* factor was calculated for various nongelatinized starch samples with a known percentage of solids (87.39%), determined by AACC Method 44-15. From these operational conditions, the factor *fs* was calculated from an average of 10 measurements. Each system showed a different *fs* factor. Thus, %*L/S* can be written as: %*L/S* = *Lfs*(*T* - *L*) × 100.

To better determine the degree of gelatinization by the Waddington(1977) method, a relative liquefying index (RLI) was defined. This index corrects for the initial percentage of liquid phase for each sample before the gelatinization process and compares the degree of gelatinization of all treated samples with that for a completely gelatinized standard sample.

$$RLI = [(\%L/S)_t - (\%L/S)_o]/[(\%L/S)_f - (\%L/S)_o]$$

where (%*L/S*)<sub>t</sub> = percentage of liquid to solid in a partially gelatinized sample; (%*L/S*)<sub>o</sub> = percentage of liquid to solid in a nongelatinized sample with a known percentage of solids (this sample was used to calculate the *fs* factor); and (%*L/S*)<sub>f</sub> = percentage of liquid to solid in a completely gelatinized sample. This last sample was a dispersion of starch in water (65.0%) treated at 130°C for 5.0 min. The %*L/S* was 96.36.

### Experimental Conditions

The FID values were measured at 40°C in a low-resolution NMR spectrophotometer of (20 Mhz, model Minispec 20, Brücker, Bonn, Germany), which was linked to an analog computer programmed to calculate liquid-solid ratios from *T* and *L* values. The registered times were taken at 11 and 59 μsec, with pulses repeated every 1.0 sec. These lines were selected by trial and error, after preliminary measurements of many samples with different degrees of gelatinization. The values for the liquid-solid ratio were taken as an average of nine measurements. The signal amplification was *x* = 25.

### Degree of Gelatinization

The degree of gelatinization was determined by the method of Chiang and Johnson (1977). The values obtained by this method were compared with the RLI.

TABLE II  
Effect of Heating Time (sec) on Liquid-Solid Ratio (*L/S*)  
for a Starch-Water (65%) System Treated at 80°C

Heating Time	% <i>L/S</i>
120	65.02 ± 2.54
150	65.78 ± 2.57
180	65.77 ± 2.50
210	67.69 ± 2.64
240	74.38 ± 2.90
270	81.04 ± 3.16
300	89.54 ± 3.49
330	91.12 ± 3.55

TABLE III  
Effect of Water Content (%) of a Starch-Water System  
on Value of the *fs* Factor for Wheat Starch

Water Content	<i>fs</i> Factor
35	2.33 ± 0.10
45	2.19 ± 0.10
55	2.00 ± 0.12
65	1.63 ± 0.06

### Kinetics of Starch Gelatinization

To determine the rate constant, it was assumed that the gelatinization kinetics followed a first-order reaction such that:  $dx/dt = k(1 - x)$ , where  $dx/dt$  = rate of gelatinization ( $\text{min}^{-1}$ ),  $k$  = rate constant ( $\text{min}^{-1}$ ),  $x = (x_t - x_0)/(x_f - x_0)$  = RLI, and  $x_t = \%L/S$  at time *t*,  $x_f = \%L/S$  of totally gelatinized sample, and  $x_0 = \%L/S$  of a nongelatinized sample.

Thus,  $k = -\ln(1 - x)/t$ . The linear regression obtained in an Arrhenius plot ( $\log k$  vs.  $1/T$ ) was used to calculate the activation energy ( $E_a$ ) for each starch-water system by means of the relationship: slope =  $-E_a/2.303 \times R$ , where  $E_a$  is expressed in kcal/mole,  $R$  is 1,987 cal/mole × °K, and temperatures (*T*) are expressed in °K.

## RESULTS AND DISCUSSION

### Measurement of Starch Gelatinization by RLI

The results shown in Table I indicate that the isolated wheat starch was sufficiently pure to be used in this work. Table II shows the values for %*L/S* obtained after treatment of a starch-water dispersion containing 65.0% water at 80°C for different periods. The increase in %*L/S* with gelatinization time indicated a possible relationship between the degree of gelatinization and the parameter defined to quantify the gelatinization phenomena.

The *fs* factor values for the different starch-water systems are shown in Table III. The *fs* factors are in the same range as those for multiphase fat mixtures to which the FID signal was applied (Waddington 1973). These results indicated that the measured signals were big enough to be used as a parameter to follow the gelatinization process.

Figure 1 shows the effect of the time after gelatinization on the liquid-solid ratio for starch samples treated at 130°C for 240 sec. The %*L/S* determined after 1 hr was practically constant at each starch concentration. Variance analyses of these systems showed that the *L/S* values after 4 hr of gelatinization were not significantly different at the 5% level from those determined at earlier times. These results indicated that during the time that elapsed between gelatinization and the determination of %*L/S*, no transformation occurred that would alter the percentage of solids in the gelatinized sample, such as the retrogradation phenomena. For practical purposes, the elapsed time was always <2 hr.

The results in Table IV show the RLI and the degree of gelatinization of starch samples with 35 and 65% water treated at 130°C for different times. To obtain more conclusive data about a possible correlation between RLI and the degree of gelatinization, systems containing starch-water and gluten, sucrose, stearic acid, and salts were analyzed.

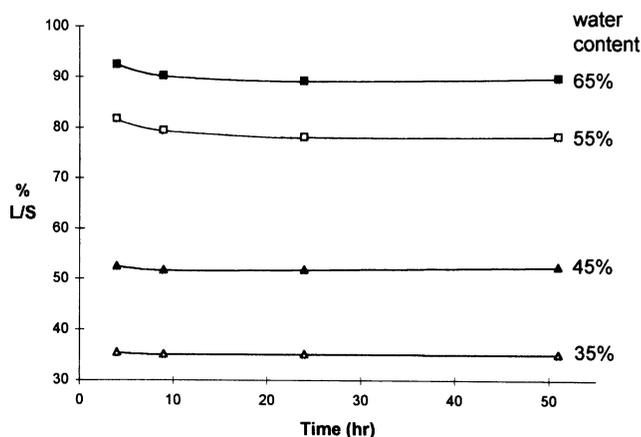


Fig. 1. Effect of the time after gelatinization on the percent liquid to solid ratio (%*L/S*) of starch-water systems treated at 130°C for 240 sec.

The correlation between RLI and degree of gelatinization for all systems (except that containing 35% water) was >0.94, at a 5% significance level. These results showed that the RLI may be used as a measurement of the percent of gelatinization. The limiting amount of water for the gelatinization process in the system with 35% moisture may have caused the low correlation obtained.

### Kinetics of Starch Gelatinization

Figure 2 illustrates the variation of RLI as a function of time for starch-water systems treated at 80, 90, 110, 120 and 130°C. All treatments showed an increase in RLI up to a certain time,

becoming constant thereafter. The RLI steady-state values increased with increasing temperatures and with the amounts of water in the system. Birch and Priestley (1973), Kubota et al (1979), and Wirakartakusumah (1981) also observed a maximum and constant gelatinization value as a function of the heating temperature. Thus, once the temperature reaches a determined level, the RLI would remain constant, and only the velocity of gelatinization would increase. It is possible that, with the occurrence of the gelatinization process, the water is distributed between crystalline and noncrystalline zones in a temperature-controlled process.

Table V shows values for the rate constant and activation

TABLE IV  
Comparison Between Relative Liquefying Index (RLI) and Degree of Gelatinization

System <sup>a</sup>	Moisture	fs Factor	Heating Time (sec)	RLI	Degree of Gelatinization (%)	Correlation Coefficient <sup>b</sup> of System
Starch-water	35.0	2.33 ± 0.10	120	10.21 ± 0.36	10.6 ± 0.3	0.5319
			135	11.41 ± 0.44	12.2 ± 1.4	
			150	10.33 ± 0.54	13.5 ± 1.3	
			165	18.00 ± 0.79	14.0 ± 1.7	
			180	12.00 ± 0.50	14.1 ± 2.4	
Starch-water	65.0	1.65 ± 0.06	105	50.47 ± 0.76	51.7 ± 3.4	0.9997
			120	93.81 ± 2.11	96.4 ± 0.4	
			135	89.11 ± 2.67	92.4 ± 1.6	
			150	96.63 ± 2.31	100.0 ± 0.3	
			180	94.52 ± 1.16	98.5 ± 1.3	
Starch-gluten(7%)	45.0	2.32 ± 0.08	75	1.57 ± 0.02	9.3 ± 0.4	0.9873
			105	22.66 ± 0.78	40.5 ± 1.3	
			120	36.19 ± 1.24	81.6 ± 1.1	
			165	39.37 ± 1.18	85.4 ± 1.3	
			180	38.37 ± 0.92	85.2 ± 0.8	
Starch-gluten(28%)	45.0	2.47 ± 0.09	90	0.58 ± 0.01	3.4 ± 0.5	0.9658
			105	1.67 ± 0.14	25.4 ± 0.6	
			120	12.97 ± 0.45	58.1 ± 0.8	
			135	21.00 ± 0.29	77.0 ± 1.0	
			180	27.99 ± 1.01	82.1 ± 1.4	
Starch-sucrose(10%)	55.0	2.01 ± 0.07	105	52.41 ± 1.90	61.9 ± 1.3	0.9893
			135	66.63 ± 2.40	72.4 ± 1.2	
			165	80.39 ± 1.31	88.1 ± 1.2	
			180	74.82 ± 2.72	80.3 ± 2.0	
			210	80.07 ± 1.89	89.6 ± 2.0	
Starch-sucrose(40%)	55.0	2.60 ± 0.10	120	0.00 ± 0.00	5.0 ± 0.3	0.9990
			135	0.00 ± 0.00	6.5 ± 0.6	
			150	2.59 ± 0.10	7.1 ± 0.5	
			165	53.51 ± 1.28	56.0 ± 3.3	
			180	60.76 ± 1.41	66.4 ± 0.9	
Starch-stearic acid (5%)	55.0	1.35 ± 0.01	105	35.17 ± 0.11	38.4 ± 1.2	0.9616
			120	42.72 ± 0.20	45.3 ± 1.6	
			150	43.00 ± 0.43	48.9 ± 1.1	
			165	46.98 ± 0.71	50.7 ± 1.8	
			195	50.10 ± 0.15	52.1 ± 1.8	
Starch-stearic acid (20%)	55.0	1.00 ± 0.01	105	2.10 ± 0.02	4.1 ± 0.5	0.9825
			120	32.77 ± 0.30	33.7 ± 1.7	
			150	32.55 ± 0.35	38.9 ± 2.6	
			195	33.39 ± 1.03	39.4 ± 1.8	
			210	32.13 ± 0.43	40.6 ± 3.0	
Starch-NaCl (0.43N)	55.0	1.41 ± 0.02	135	32.05 ± 0.32	38.7 ± 1.3	0.9473
			150	50.26 ± 0.56	57.6 ± 2.2	
			165	51.26 ± 0.65	59.8 ± 1.9	
			180	52.40 ± 0.68	60.2 ± 2.3	
			210	53.50 ± 1.29	70.9 ± 2.4	
Starch-NaF (0.43N)	55.0	1.49 ± 0.02	105	0.00 ± 0.00	3.0 ± 0.2	0.9975
			120	14.25 ± 0.22	16.3 ± 0.7	
			135	32.43 ± 0.82	40.9 ± 2.2	
			150	36.74 ± 0.56	49.1 ± 0.9	
			180	51.56 ± 0.78	54.0 ± 0.8	

<sup>a</sup> Percentage of gluten, sucrose, and stearic acid expressed on a starch basis.

<sup>b</sup> Confidence interval, 95.0%.

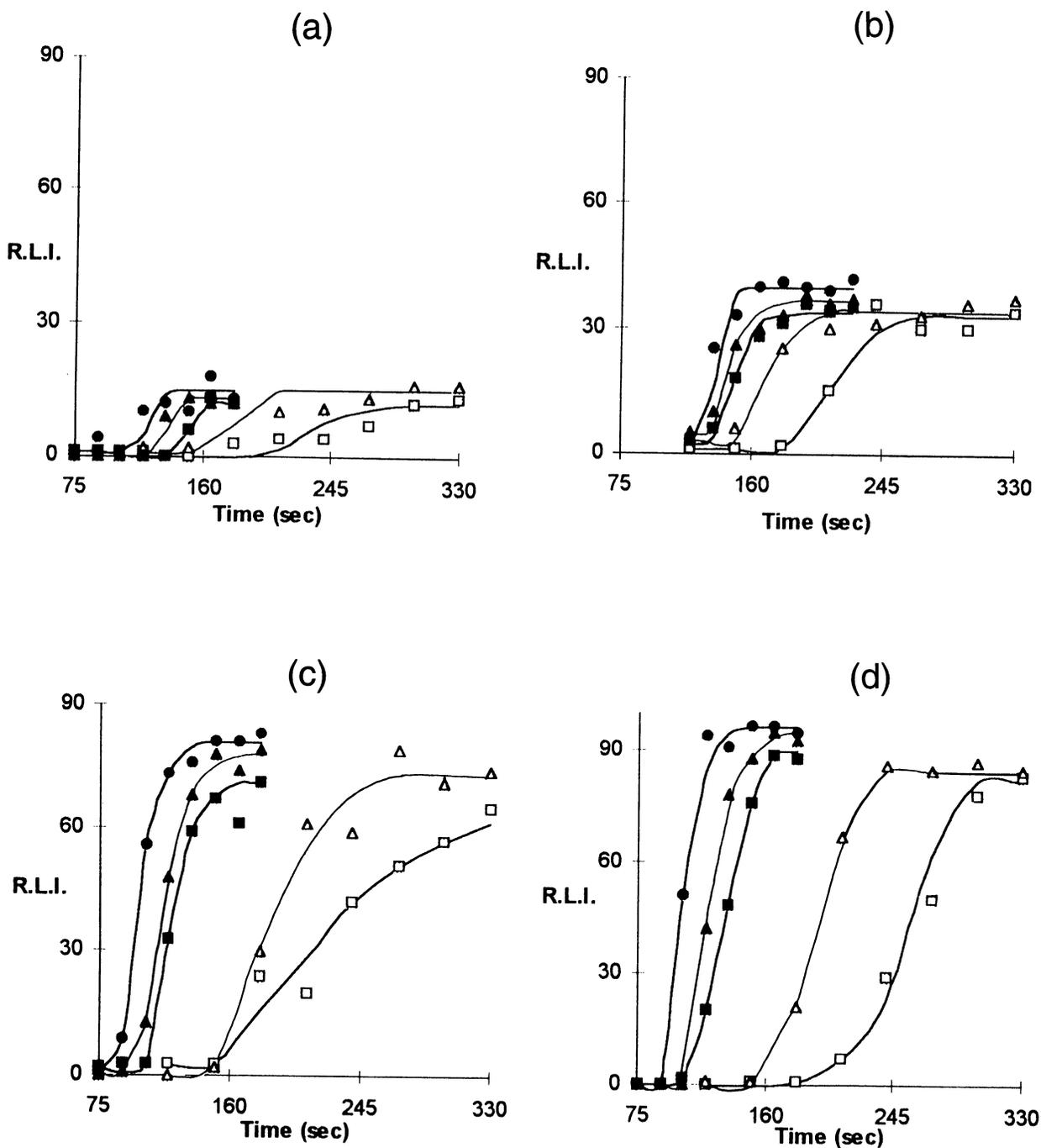


Fig. 2. Variation of the relative liquefying index (RLI) as a function of time for the starch-water systems treated at 80 (□), 90 (Δ), 110 (■), 120 (▲), and 130°C (●). Water content (%): 35 (a), 45 (b), 55 (c), and 65% (d).

energy of the different starch-water systems. Figure 3 illustrates the variations of activation energy as a function of water content. Values for the activation energy at 35, 45, 55, and 65% water were 12.05, 6.79, 8.59, and 8.97 kcal/mol, respectively.

The minimum activation energy occurred at 45% water, indicating that the process would be affected by other kinds of energy besides that caused by the increase of temperature. It is possible that the hydration of the noncrystalline zones would result in an increasing granule volume, causing a tearing effect in the crystalline zones and therefore a decrease in the activation energy. At 45% water, the hydration sites in the noncrystalline zones would be saturated, and the resulting increase in volume would contribute to the breaking of the secondary bonds that maintain the crystal-

line zones. On the other hand, at 35% water, the thermal energy required to initiate the gelatinization process would be higher than at 45% water, since the mechanical stress, resulting from the increase in volume, would not be sufficient to break the hydrogen bonds. Above 45.0% water, additional energy would be necessary to hydrate sites that would be liberated by the breaking of the hydrogen bonds, causing an increase in the activation energy.

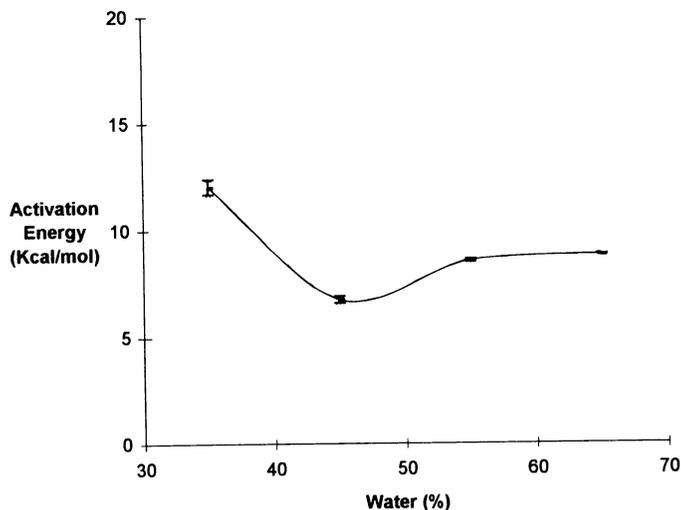
The similarity between the activation energies found at 55 and 65% water indicated that 55% water is the minimum water content for complete gelatinization of the wheat starch granule. Collinson and Chilton (1974) and D. Reid and S. Charoenrein (*unpublished*) found 58 and 60% as the minimum water content for complete gelatinization of potato and corn starches, respectively.

**TABLE V**  
Rate Constant and Activation Energy for the Starch-Water Systems

Water Content (%)	Temperature (°C)	Rate Constant <i>k</i> (min)	Activation Energy at Given Water Content (kcal/mol)
35.0	80.0	0.0041	12.05
	90.0	0.0074	
	110.0	0.0200	
	120.0	0.0220	
	130.0	0.0260	
45.0	80.0	0.0169	6.79
	90.0	0.0283	
	110.0	0.0393	
	120.0	0.0464	
	130.0	0.0637	
55.0	80.0	0.0067	8.59
	90.0	0.0075	
	110.0	0.0160	
	120.0	0.0206	
	130.0	0.0304	
65.0	80.0	0.0085	8.97
	90.0	0.0149	
	110.0	0.0266	
	120.0	0.0340	
	130.0	0.0436	

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**Fig. 3.** Variations of activation energy as a function of the water content.

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