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DETERMINATION OF WATER IN PLANT SAMPLES: A COMPARATIVE THERMOGRAVIMETRIC AND NMR STUDY ON DIFFERENT SPECIES OF SEEDS

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ABSTRACT

Detailed comparative measurements by TG and NMR in order to evaluate these techniques in the determination of the water content of some vegetable seeds is described. Dried seeds, of different types, were analyzed. TG also yields data on ash content, and NMR can distinguish between free and bound water.

INTRODUCTION

Several studies are concerned with the role of water in different biological processes. This role is particularly important in the case of plant systems, such as seeds when germination needs optimum hydration levels [1,2]. Nevertheless, it is still not clear what the influence of residual water present in previously-dried seeds has on the metabolic processes and their kinetics in storage conditions, which depend not only on water content, but also on its physical state. Control of the water content in the seeds is therefore a problem of biological and commercial interest. Obviously this control can be performed approximately or rigorously, in the first case by determining the weight loss, on vacuum drying of the seeds at a fixed temperature. This method has the disadvantage of requiring a consistent sample and is not suitable for the poor quantity of seeds in some commercial products; also drying is time-consuming (taking some hours) and requires accurate temperature control. The application of Karl Fischer's method is not easy, as the complete release of water contained in the seeds, can be difficult and may

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take a long time. On considering previous work [3,4], in which we determined, with satisfactory precision and accuracy, the water content of some commercial solid matrices by employing thermogravimetric analysis, we felt that this problem too could be solved, rapidly and easily, by thermogravimetric analysis of small samples of dried seeds of some of the vegetable species readily available commercially, stored in different containers. The data obtained were compared with results from physicochemical techniques, such as NMR, which have assumed, during the last years, an increasing importance in the problem of determining water content and physical state in biological and food samples [5,6] and in other solid substrates of commercial interest, using NMR parameters such as relaxation time T_1 (spin–lattice) and T_2 (spin–spin). We have applied this method to the samples analyzed by thermoanalysis, for an experimental comparison.

EXPERIMENTAL

For all the seeds examined (basil, cabbage, parsley, carrot, tomato, onion, lettuce, kindly provided by Asgrow Seed Co., 1982 harvest), samples were stored in tin sealed paper at +4°C.

The TG and DTG curves of the compounds examined were obtained with a Mettler TG50 thermobalance, coupled with a Mettler TC10-TA Processor system and a print Swiss Matrix. The heating rate used was 10°C min^{-1}; the atmosphere was an air stream at a flow rate of 100 ml min^{-1}, or static air.

NMR measurements were performed by a pulsed low-resolution spectrometer (Minispec P20) produced by Bruker Co., Karlsruhe, Germany, operating at 20 Mhz for protons and equipped with an analog computer B-AC5 from the same Company. The NMR experiments were performed at 20°C and then at 10°C, in order to avoid mistakes in the calculation of water content, due to the presence of lipids in the same liquid phase.

The longitudinal magnetization decay was detected by a $180°/t/90°$ pulse sequence. The curve was obtained by plotting the functions $\ln(M_0 - M_t)$ vs. time (t), where M_0 is the equilibrium nuclear magnetization and M_t is the magnetization detected at time t. The slope of this curve gave the longitudinal relaxation time, T_1. The transverse magnetization decay was detected by a $90°/t/180°$ pulse sequence (spin–echo sequence). The curve was obtained by plotting the echo amplitude vs. time $2t$. The slope of this curve gave the transverse relaxation time, T_2.

The 180° and 90° pulses were empirically adjusted by varying their respective widths ($\sim$ 18 and 9 μs on Minispec P20). The time delay between two consecutive measurements varied in the range 4–20 s to allow the nuclear magnetization to return to its equilibrium value.

Calculation of the quantitative determination of water content was performed as previously reported [7].

RESULTS

A series of thermogravimetric analyses was performed on samples of seeds (~ 10 mg) from eight different vegetable species. The analyses were carried out between 20 and 180°C in an air stream (100 ml min^{-1}) with a heating rate of 10°C min^{-1}. These conditions were used since in this temperature range the total loss of water contained in the seeds, can be observed, while at higher temperatures the beginning of other decomposition processes is already evident. The results of the tests in an air stream are more reproducible in this temperature range, than those in static air (as observed in preliminary studies), probably because of the more reproducible and faster removal of water vapour from the crucible containing the sample.

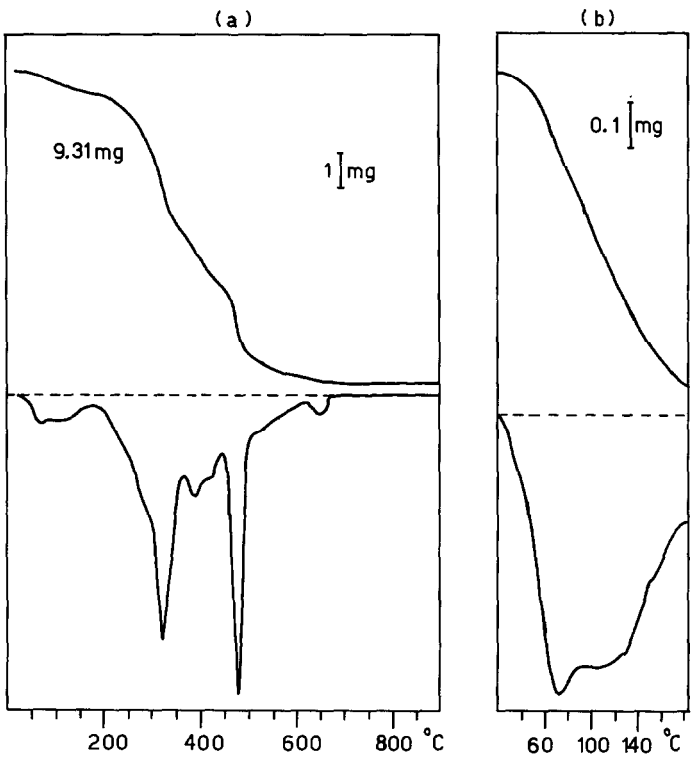

Fig. 1. TG and DTG curves for analysis of basil seeds. (a) Full TG and DTG curves in the temperature range 20–900°C; (b) TG and DTG curves relative to water loss process only. In an air stream (100 ml min^{-1}) heating rate 10°C min^{-1}.

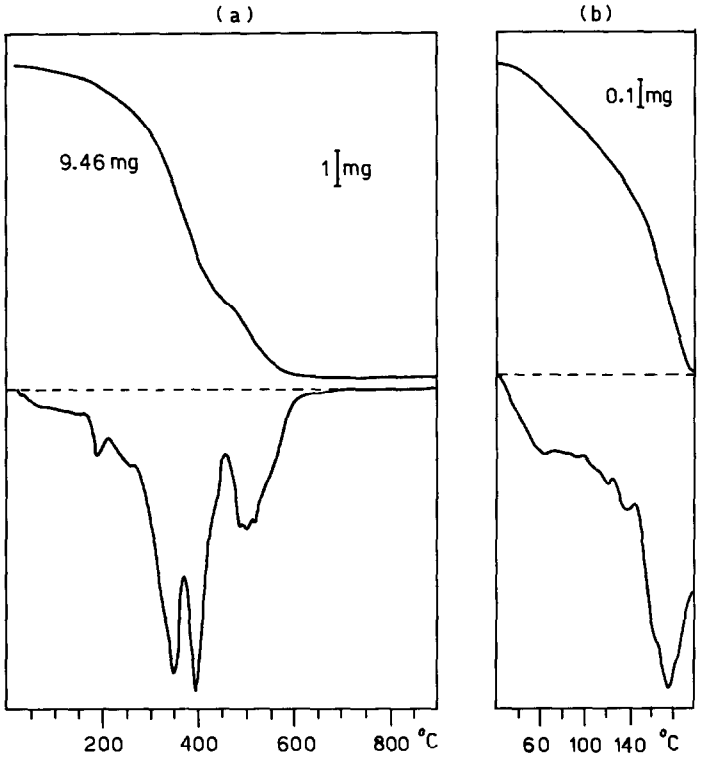

Fig. 2. TG and DTG curves for analysis of cabbage seeds. (a) Full TG and DTG curves in the temperature range 20–900 °C; (b) TG and DTG curves relative to water loss process only. In an air stream (100 ml min^{-1}), heating rate 10 °C min^{-1}.

The heating rate adopted is a compromise between the requirement for reproducible and homogeneous warming and dehydration of the sample in a short analysis time as this itself is one of the main advantages of the method we propose. With the heating rate adopted the time required to perform the analysis does not exceed about 20 min. If heating rates between 30 and 5 °C

TABLE 1

Precision of the analysis of water in basil seeds. Water contents (% w/w) obtained by TG and NMR

Thermogravimetric method			NMR method		
Found	Mean	% Relative SD	Found	Mean	% Relative SD
7.01			5.75		
6.89			6.05		
6.66	6.81	2.1	5.85	5.97	2.5
6.63			6.15		
6.85			6.07		

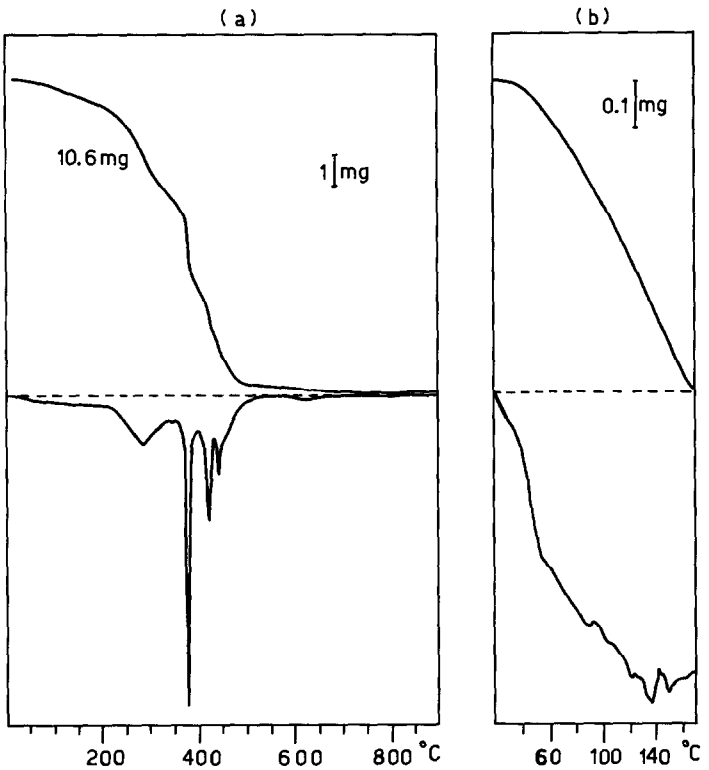

Fig. 3. TG and DTG curves for analysis of parsley seeds. (a) Full TG and DTG curves in the temperature range 20–900 °C; (b) TG and DTG curves relative to water loss process only. In an air stream (100 ml min^{-1}), heating rate 10 °C min^{-1}.

min^{-1} are used, the variations of the results fall in the precision range of the method and if necessary, analysis time can be reduced to one-half or one-third of the indicated value. In Figs. 1(b)–8(b) TG and DTG curves are

TABLE 2

Percentage by weight of water in the seeds examined: comparison between the results by TG and NMR. Reported values are the mean of at least three determinations

Nature of the seed	TG (a)	NMR (b)	$\left(\frac{a-b}{a}\right)$ (%)
Basil	6.81	5.97	12.3
Cabbage	6.80	6.15	9.6
Parsley	6.19	5.35	13.6
Carrot	5.54	5.05	8.8
Tomato	6.37	6.05	5.0
Onion	5.97	5.85	2.0
Lettuce	6.08	5.59	2.1

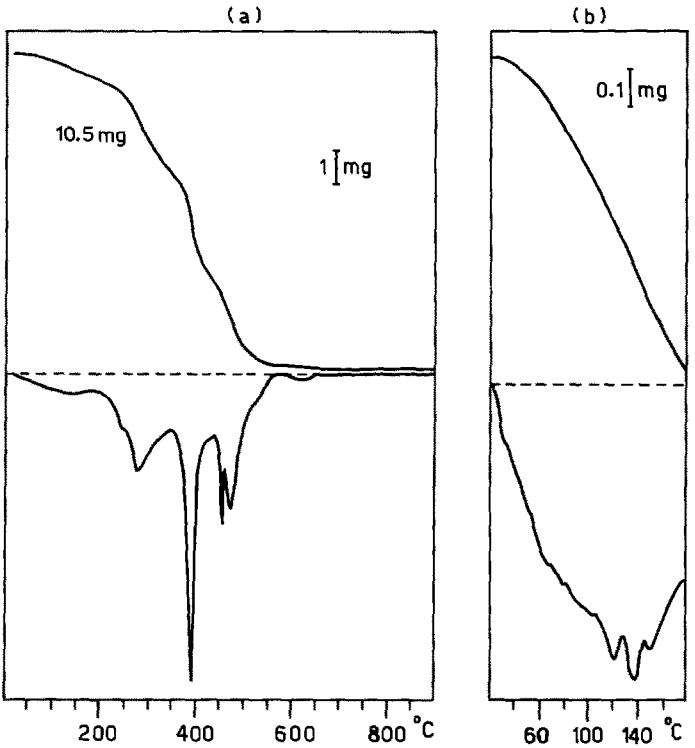

Fig. 4. TG and DTG curves for analysis of carrot seeds. (a) Full TG and DTG curves in the temperature range 20–900°C; (b) TG and DTG curves relative to water loss process only. In an air stream (100 ml min^{-1}), heating rate 10°C min^{-1}.

presented in an expanded scale and show the transition due to water loss for each of the samples analyzed using the experimental conditions described.

In Table 1 repeatability data are given for five tests on the same sample (parsley seed). In the same Table the SD% values of the thermogravimetric and NMR methods are compared for the same sample. In Table 2 results obtained for different samples by both these methods are presented, while in Fig. 9 the correlation between the results is shown.

In order to show the other advantages of each of the two methods, related to the considered matrix, the complete TG and DTG curves in the temperature range 20–900°C are given in Figs. 1(a)–8(a). In this range, beyond the dehydration step, the full oxidative decomposition process of the sample occurs, generally in three or four steps (not always well resolved) beginning at 170–200°C and ending at 650–710°C. These thermograms were also recorded in an air stream; in the case of lettuce seeds, however, static air was employed, as these seeds easily burn in an air stream.

The principal thermogravimetric data from the dehydration and decomposition processes are summarized in Table 3. In the last two columns of

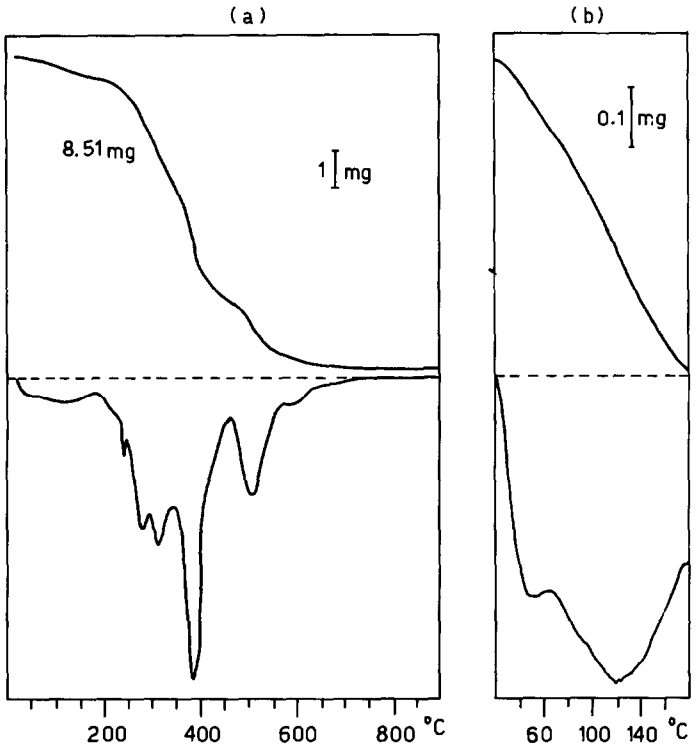

Fig. 5. TG and DTG curves for analysis of tomato seeds. (a) Full TG and DTG curves in the temperature range 20–900 °C; (b) TG and DTG curves relative to water loss process only. In an air stream (100 ml min^{-1}), heating rate 10 °C min^{-1}.

this Table the weight% of the final residues (ashes), obtained both at about 700 °C and at 900 °C, are reported. We report the data at both temperatures as, at the first temperature, the oxidative decomposition process of the organic content ends. Also it is nearer to the value reported in the literature from methods which ash large samples of seeds in the oven. On the other hand, at 900 °C, the ashes are completely free of carbon residues, even if some salts such as carbonate in the ashes, can be decomposed partially at this temperature. Nevertheless it may be observed that the values for the residual percentages, at both temperatures, always differ by less than 5%.

In Table 4, data from analysis by NMR using thermostatted samples at 10 °C, are presented. In particular the amounts (generally ~ 0.5 g) of the different samples analysed and the experimental relaxation time values T_1, from which the solid/liquid ratio and thence the water content (Table 2) were calculated, are reported; they were obtained by the method reported in ref. 8 and at 10 °C, in order to avoid the protons of the lipids which constitute about 30% of the total, contributing to the signal of the liquid phase. Three typical FIDS (free induction decay), obtained for the seeds of

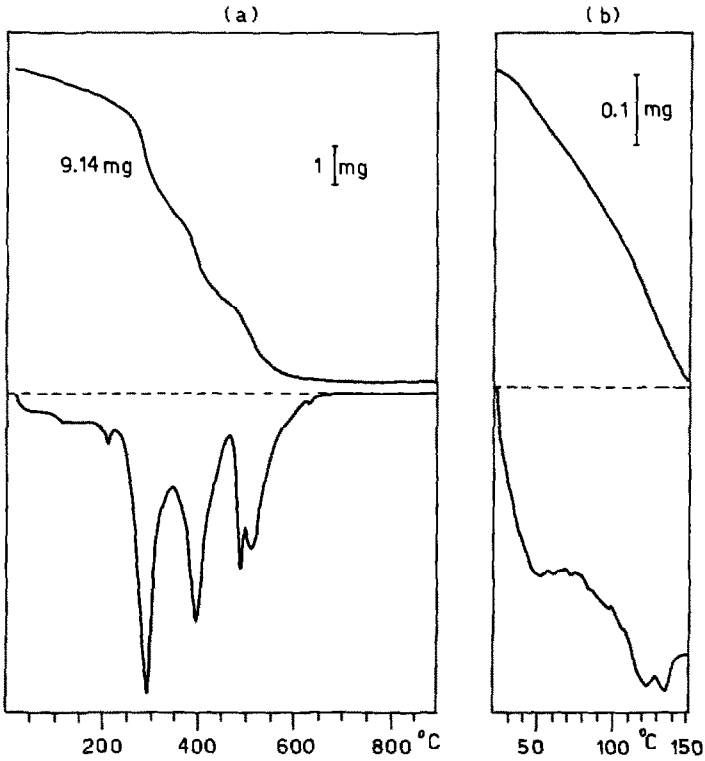

Fig. 6. TG and DTG curves for analysis of onion seeds. (a) Full TG and DTG curves in the temperature range 20–900°C; (b) TG and DTG curves relative to water loss process only. In an air stream (100 ml min^{-1}), heating rate 10°C min^{-1}.

basil, tomato and lettuce, are shown in Fig. 9. From measurement of the reported relaxation times T_2, it was possible to show that, in seeds not submitted to hydration, such as those analyzed by us, it is possible to distinguish at least two types of water [8] even at the low total content; of these two types, 20–24% is bound water (to proteins and polysaccharides) and the remainder free water.

DISCUSSION

TG analysis in an air stream is found to be a useful method for rapid and advantageous control of the water content in the seeds in commercial products and is also useful for obtaining the ash content, although for this thermoanalysis in static air is preferred in some cases.

Precision is good (SD% = 2.1) and is of the same order and sometimes higher than for data by NMR (SD% = 2.5). Correlation between the two

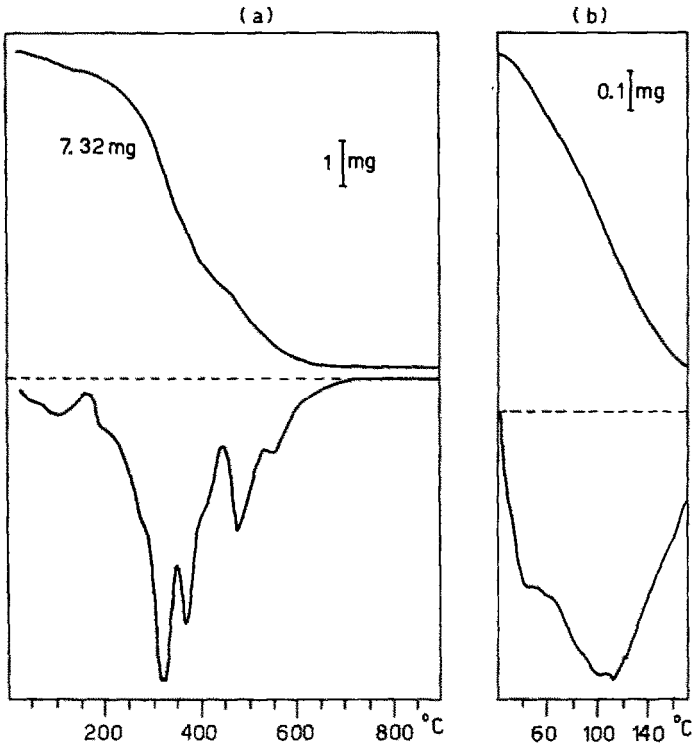

Fig. 7. TG and DTG curves of lettuce seeds. (a) Full TG and DTG curves in the temperature range 20–900 °C, in static air; (b) TG and DTG curves relative to water loss process only, in an air stream (100 ml min^{-1}), heating rate 10 °C min^{-1}.

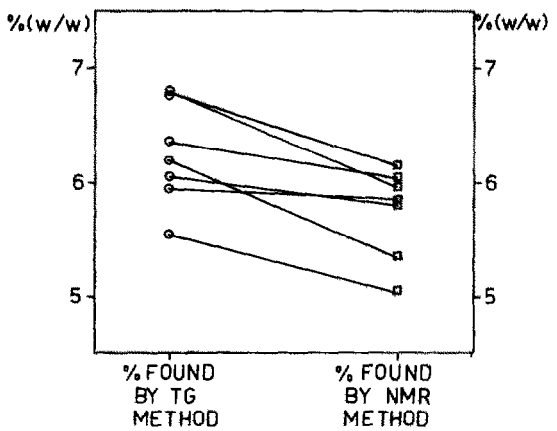

Fig. 8. Correlation of results obtained by TG and NMR methods, in the analysis of water content of seeds.

TABLE 3

Thermal analysis of the vegetable seeds in an air stream (100 ml min^{-1}) and residue ash at 900°C

Nature of the seeds	H_2O loss		Decomposition process			Ash residue at 900°C (as weight%) (mean of three determinations) SD% = ±1.0
	Found %	Pdt [a]	% Found loss	Pdt [a]	Ash residue (as weight%) at the end of the process	
Basil	6.8	25	87.9	190	5.2	5.19
		73		478		
		180		650		
Cabbage	7.6	25	88.1	205	4.3	4.27
		177		393		
		199		700		
Parsley	6.2	24	88.0	180	5.8	5.57
		137		379		
		170		700		
Carrot	5.5	25	88.2	180	6.3	6.17
		137		390		
		156		700		
Tomato	6.4	24	89.7	190	3.9	3.99
		119		382		
		180		720		
Onion	6.0	24	89.9	180	4.1	3.93
		136		291		
		152		700		
Lettuce [b]	6.1	25	88.9	170	5.0	4.77
		114		316		
		160		720		

[a] Procedural decomposition temperatures. [b] TG in static air.

TABLE 4

Pulsed low-resolution NMR data, at 10°C, for the vegetable seeds (values are the mean of three determinations)

Nature of the seed	Weight (mg of the analyzed sample)	Found relaxation time T_1 (ms) ± SD	Found relaxation time T_2 (ms) ± SD
Basil	711.1	89.8 ± 10.5	17.4 ± 5.6
Cabbage	777.4	90.5 ± 12.1	31.9 ± 6.1
Parsley	659.2	84.8 ± 11.2	24.4 ± 6.5
Carrot	518.8	65.5 ± 12.8	28.8 ± 5.3
Tomato	476.2	97.5 ± 9.6	15.3 ± 6.7
Onion	667.6	64.1 ± 11.8	26.5 ± 6.1
Lettuce	494.8	90.7 ± 8.7	17.7 ± 4.2

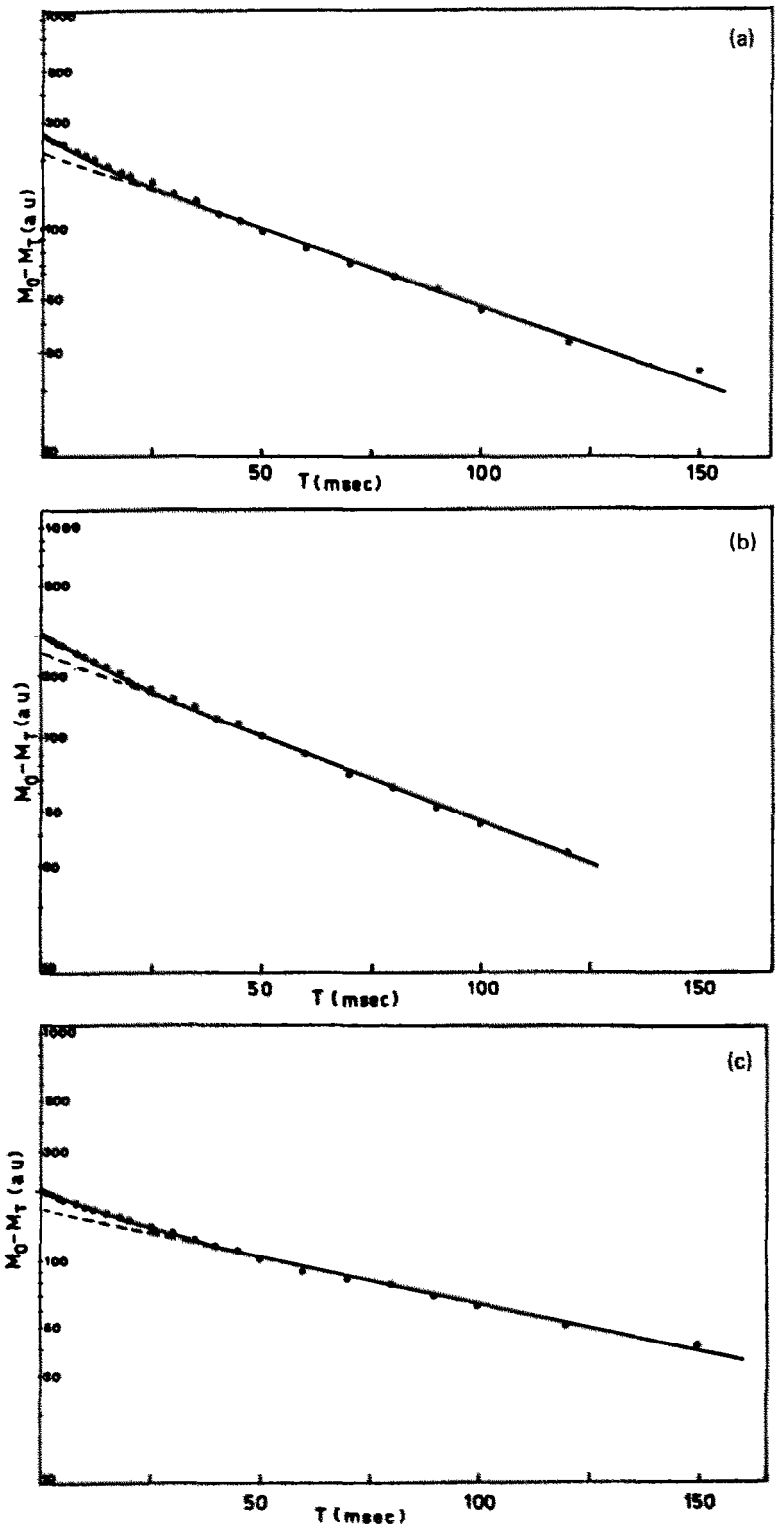

Fig. 9. Semilogarithmic plot of the longitudinal magnetic decay in the seeds examined: (a) basil; (b) tomato; (c) lettuce.

methods is satisfactory, but the values from TG are generally higher than NMR values. This is probably due to the different physical phenomena on which the two methods are based e.g. small amounts of other volatile substances can contribute to the weight loss recorded by TG analysis, or the ratio liquid/solid, obtained by NMR, can be systematically affected by small errors from the determination of empirical coefficients accounting for the magnetization decay during the dead time of the receiver, or of the experimental constant K, used to transform the ratios of the hydrogen abundances between the two phases into weight% [6,7]. A better agreement could be obtained by improving the software of the elaboration programs of both methods.

CONCLUSIONS

The possibility of determining the water content in a vegetable substrate, by two different experimental methods, results from which agree well is presented. The advantage of NMR is that qualitative information on the different types of water can be obtained, but the disadvantage is the long experimental time (~ 1 h). Thermogravimetry is cheaper, faster and easier; it yields more information if the temperature range is extended beyond the narrow limits of the analysis so that ash content can be determined. This is of great commercial and analytical interest, and also may be used for botanic problems.

ACKNOWLEDGEMENT

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