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Introduction

Most plant seeds can be dried to conditions of low moisture content and relative humidity. They can also be stored at dry - and preferably cool - conditions for several years. When they are dry they can withstand considerable stresses; some hard coated seeds can be boiled for several hours and most dry seeds store well near 0 K (Priestley 1986). Seeds are thus well adapted to preserve the genetic code of plants from one season to the next or over longer time periods.

However, seeds cannot be stored forever. They slowly deteriorate and loose their viability (a possible exception to this is storage near 0 K). After 5-10 years at low relative humidity and 20°C most vegetable seeds have lost at least half their initial viability. There are thus deterioration processes in dry seeds which one will have to study to be able to optimize storage conditions.

In this paper relative humidity (RH) is used as a measure of the moisture state. For the present purposes RH can be assumed to have the same numerical value as the water activity.

Microcalorimetry is the measurement of low thermal powers (μW) on small samples (g). It is a very general measurement technique as nearly all processes (biological, chemical and physical) will produce heat. The aim of this study was to test microcalorimetry as a method to investigate deteriorating processes in seeds.

Materials and method

Two types of seeds were used: *Trifolium resupinatum* (persian clover) "Accadia" (Lindbloms Frö, Kivik, Sweden) and *Chenopodium quinoa* ("Inca grain", Anpqui, Bolivia). The seeds were sterilized by 12 minutes immersion in the filtrate From an 8% suspension of calcium hypochlorite. After this they were washed in deionized water and dried at 40°C. The seeds had still a high viability after this sterilization as nearly all seeds of both types germinated within 24 h on a wet filter paper at 25°C.

Small lots of both types of seeds were then placed above saturated aqueous salt solutions in closed glass jars. Three salts were used: LiCl, $Mg(NO_3)_2$ and NaCl, corresponding to 11, 53 and 75% relative humidity at 25°C (Greenspan 1977).

Two grams of each seed at each humidity were then used for the microcalorimetric experiments which were performed in 3 ml glass ampoules in a Thermometric TAM microcalorimeter (Suurkuusk and Wadsö 1982). Three experiments were made at different

times and conditions. Table 1 gives an overview of the measurements. The moisture content of the seeds were measured by drying at 60 or 100°C. After the experiments the viability was once more tested by germinating the seeds on wet filter paper at 25°C.

Table 1. An overview of the experiments

#	days in salt jars
1	26
2	30
3	69

Results

Table 2 gives the result of the moisture content measurements. The samples were dried under different conditions, so the moisture contents are not directly comparable. Note also that the moisture contents are given on a dry mass basis (in seed science moisture content on a "wet" mass basis is the most common).

Table 2. Measured moisture contents (MC is moisture content, RH is relative humidity and #1 etc. refers to the measurements number given in Table 1). MC is given on a dry mass basis (mass of water divided by mass of dry seeds).

seed	RH	MC #1 (a)	MC #2 (b)	MC #3 (c)
Trifolium	0,11	0,0164	0,008	0,038
	0,53	0,0955	0,079	0,114
	0,75	0,1284	0,141	0,182
Chenopodium	0,11	0,0236	0,006	0,029
	0,53	0,0848	0,090	0,137
	0,75	0,1435	0,124	0,180

(a) After drying for 3 days at 60°C.
(b) After drying for 2 days at 60°C.
(c) After drying at 100°C.

Table 3 and Fig. 1 give the results of the calorimetric measurements. All measurements were made at 25°C. They showed a relatively quick stabilization at the constant thermal powers given.

Table 3. Measured thermal powers (in μW/g)

seed	RH	P #1	P #2	P #3
Trifolium	0,11	2,4	1,3	1,5
	0,53	0,3	0,3	0,15
	0,75	4,2	2,6	1,9
Chenopodium	0,11	3,0	1,6	0,8
	0,53	0,5	0,1	0,45
	0,75	0,9	1,8	0,9

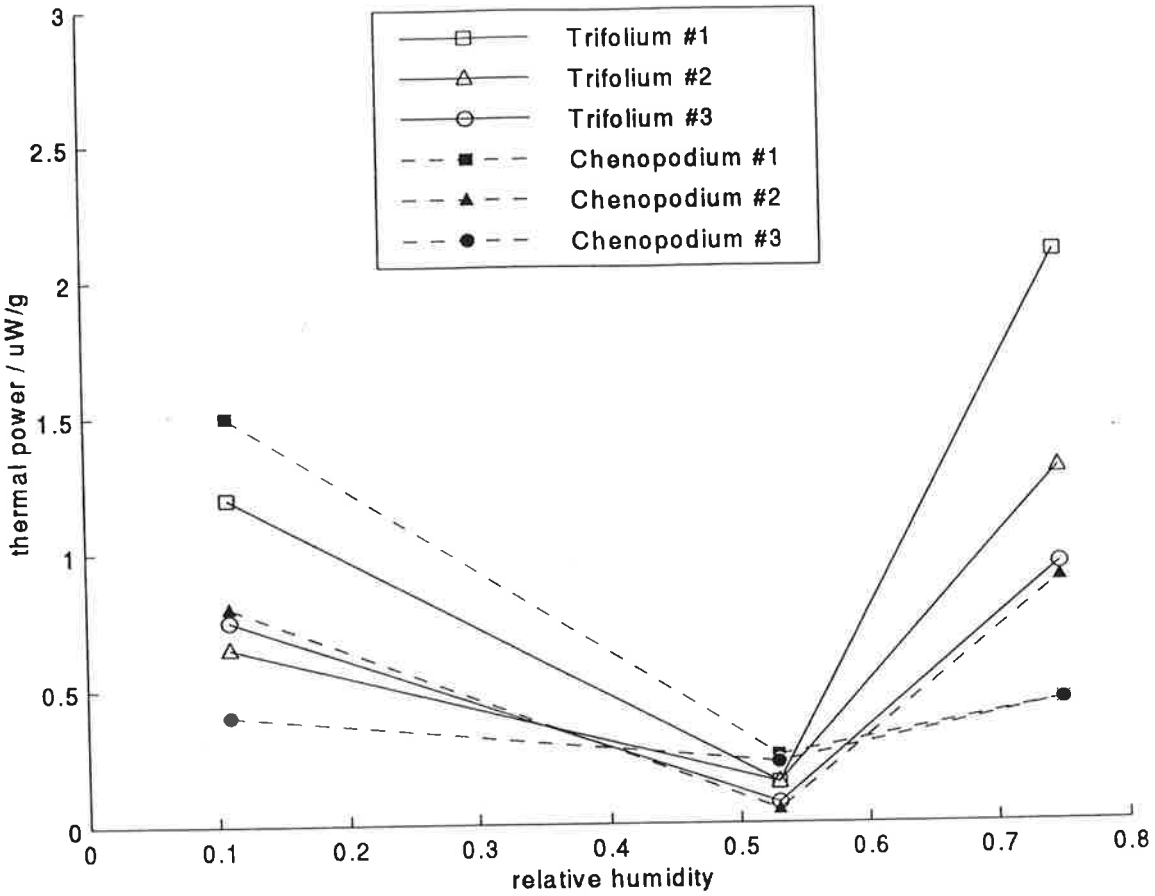

Figure 1. The measured thermal powers as function of the relative humidity.

Seed viability was tested twice: after the sterilization process and after the last measurement. The result is given in Table 4.

Table 4. Result of viability tests. The seeds were germinated on wet filter paper at 25°C under dark conditions. For the first test a large number of seeds were used. For the second test 25 seeds of each species and RH were used. The figures given are the fraction of the seeds that germinated in 48 h (rounded to one decimal).

seed	RH	after sterilization	after measurement
Trifolium	0,11	1,0	1,0
	0,53	1,0	1,0
	0,75	1,0	0,8
Chenopodium	0,11	1,0	0,8
	0,53	1,0	0,0
	0,75	1,0	0,7

Discussion

As is clearly shown in Table 3 and Fig. 1, the thermal powers measured at a relative humidity of 0,53 are lower than those measured at 0,11 and 0,75. This indicates that the thermal output

from more than one process is being measured, and this is of course possible for such a complicated system as a seed.

What processes are being monitored? At a relative humidity of 0,75 the activity may be high for a number of reasons: general deterioration, repair processes and microbiological activity. With "general deterioration" I mean that the rate of most deterioration processes increase as the humidity level is increased.

At a relative humidity of 0,53 almost no thermal power is measured, so the process active at 0,11 must be a process that is inhibited by higher relative humidities. A possible candidate for such a process is lipid oxidation that is known to proceed at higher rates at low relative humidities than at high relative humidities (van den Berg and Bruin 1981, Wilson and McDonald 1986).

A number of other observations may also be made:

- The moisture contents were measures by drying in an oven at 60 or 100oC. The differences seen between similar measurements (e.g. #1 and #2 at 0,11) may be caused by variations in the ambient vapor pressure. Drying should in the future be performed at elevated temperatures in the presence of a drying agent or in the absence of air.
- The scatter in the result is quite large at both 0,11 and 0,75. This may indicate that one or many factors were not the same for the different measurement..
- The smallest difference between the thermal powers at the three relative humidities studied is seen for *Chenopodium* #3. At the same time *Trifolium* #3 was more like the rest of the measurements.

A problem with all monitoring calorimetric measurements is that it is impossible to calculate rates of processes from an unknown process (with an unknown process enthalpy, ΔH). It would, however, be interesting to test the hypothesis that lipid oxidation is responsible for the thermal power at low relative humidities, and also to investigate if this could be linked to the viability of a seed stored at low temperatures.

The germination tests before the measurements (after sterilization) gave nearly 100% germination. After the measurements the *Trifolium* seeds still retained a high viability, while the *Chenopodium* seeds showed a very low viability. The reason for this is not known. It may be that the *Chenopodium* seeds absorbed the sterilization agent, and that this damaged the seeds over the period of the experiments. The *Trifolium* seeds are hard coated.

Conclusions and future measurements

Microcalorimetry is a promising technique for the study of seed deterioration. Future measurements could include the following:

1. Use of a larger number of relative humidities to get a more detailed picture of the heat production as a function of the relative humidity
2. Measurements with and without oxygen to test whether oxidation processes are important.
3. Measurements with seeds with different lipid contents to test the idea of lipid oxidation giving the heat production measured at low relative humidities.
4. Measurements at different temperatures to assess the temperature dependence of the measured processes.

A better control of the sterilization technique is also important.

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