GRINDING IN ISTA MOISTURE TESTING

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1. INTRODUCTION

The revised Moisture chapter in the ISTA Rules, and the new Moisture Handbook will show the present state of the art in moisture testing. The sections about grinding have been updated and clarified where possible. Not all questions and problems have been solved yet, however.

Factors related to seed type that affect the need for grinding and thus the results of an oven seed moisture test are:

- Seed coat:
  - Depending on thickness and material it is made of, it may hamper exchange of moisture and thereby the assessed seed moisture content.
- Seed size:
  - Mainly affects the speed of moisture exchange. Big seeds have to be ground.
- Seed moisture content:
  - If the seed is too wet, it cannot be ground and needs pre-drying. There is discussion about what can and can't be ground. This is probably influenced by species and by type of grinder.
- Seed oil content:
  - High oil contents influence the possibilities and the quality of the grinding.

The effects of these factors are confounded with particle size distribution after grinding and with duration of the test.
In addition, there is the factor of the grinder itself.

A questionnaire was included in the ISTA PT 01-2004. The information was used when revising the Rules Chapter and writing the Handbook. Out of the 53 laboratories that sent in the questionnaire, 11 reported to encounter problems in grinding (table 1.1).
The problems can be grouped in three different subjects:

- Oats. See paragraph 3 of this paper for details
- High oil content and high moisture content. See paragraph 4 of this paper for details.
- Practical problems.

Table 1.1.
Problems related to grinding, as indicate in ISTA questionnaire in 2004.

Subject	Problem
High oil content	• Pulse (large legumes) • Linum, Brassica, Sinapis, Helianthus and ep. Foeniculum needs grinding at low speeds to avoid vaporising of oil, Helianthus wasn't even included in the low constant temp method until recently.(for info only – for reseach there have been problems with peanuts which was overcome by cutting then into quarters using a sharp knife. • Problems with Gossypium spp Sesamun indicum, Helianthus annus, Glycine max, Arachis hipogea. The sample grinding is not sufficient for the test because are viscous for the mill knife. • In principle the mill grinds peas to fine, as nearly 100% passes a sieve with 4.0mm holes. To keep the ground material at the most course level we have set up more narrow limits. The limits are >50% shall pass 4.0mm, but < 50% shall pass a sieve with 2.0mm holes. • Seeds of Phaseolus that have high moisture content cause caking to occur on the grinding wheels. This material is removed with a small wire brush. Citrullus lanatus seeds will also cake the grinding wheels. A small wire brush is used to remove the material. The wheels are then cleaned and dried before using again. • Seeds of any species which is too wet – two stage drying. Pea seed where cotyledons are evenly ground to a constant particle size but test may not be. Thoroughly mix sample before taking working sample. • Bean seeds (some species) by cutting them with a pair of garden scissors
Oats	• Oats, clean grinder between reps • Problems with oats – in case moisture content is high (15% and more). The milling process is performed slowly to overcome this problem. • Avena glumes keep unbroken – no further action. Zea mays: the mill gets warm by grinding several samples – there must be breaks between the samples.
Miscelleneous	• The large variation are seeds between the parallel of seed samples which needs rude grinding. The reason of this will come from the weighing of the grinding seed (because grinding seeds are not homogeneous it will be containing seed powder etc) in drying cup. • Seeds of various crops require different grinding, that is fine grinding for cereal and cotton plant. It means that 50% of the material ground pass through a wire sieve, with a sieve size of mesh of 0.5mm, and no more than 10% should remain at the wire sieve, with a size of mesh of 1mm. Legumes and arboreous crops seeds require course grinding, no less than 50% of the ground material should pass through the sieve with a size of mesh of 4mm.

How to assess the need for grinding will be described in the revised Rules chapter. The requirements for grinding itself (f.e. particle size distribution) have not been changed yet. Comparative testing for this has to start. This paper tries to identify the factors related to grinding that should be taken into account.
The factors seed coat, seed size, seed moisture content and seed oil content are discussed in relation to grinding.

2. SEED COAT

2.1 SEED COAT EFFECTS ON QUALITY OF GRINDING

Seed coat material and seed coat structure influence the exchange of moisture in the oven test, and it also influences the quality of the grinding.

Don (2006) suggests that the present specification for grinding in the ISTA Rules can not be satisfied using plate mills such as the Regent-Maskin that are common in a large number of ISTA laboratories. Although hammer mills can produce ground material of the required specification, they do not give representative samples, with chaffy material being retained within the mill. They can also significantly increase the temperature of the sample, which can lead to a reduction in the moisture content in the ground material.

Kruse (1996) reported results of a comparative test on some cereal species in Germany. The data of oat samples showed the greatest differences between labs. Labs reported to have difficulties in grinding moist oat, because of its tough glumes and because of problems in filling the voluminous ground material into the small glass-bowls.
Kruse also indicated that stations using a centrifugal mill reported on average a 2% lower moisture content compared to stations that used toothed-disk mills or mixers.

2.2. SEED COAT EFFECTS ON EXCHANGE OF MOISTURE IN THE OVEN

In the oven, seed coat material and structure and/or thickness influence the exchange of moisture. If exchange of moisture is hindered by the seed coat, this may be alleviated by

- grinding,
- a higher temperature in the oven,
- a longer duration of the time in the oven.

Bonner *et al*. (1992) tested two leguminous species for the effect of seed coat and thousand kernel weight (tkw) on moisture test results. Honeylocust (*Gleditsia triacanthos* L.) and mimosa (*Albizia julibrissin* Durazzini) of which tkw's were 200 and 42 grams respectively. For both species four samples were tested at two moisture levels each (Table 2.1).

Table 2.1:
Treatment means for both species and moisture levels (Bonner *et al.*, 1992)

Species	**Treatment**	**Seed moisture content**	
		Low	**High**
Honeylocust	intact	9.6 a	15.5 a
	cutting	11.4 b	17.8 b
	Wiley mill	12.0 c	16.7 ab
	coffee mill	11.6 bc	17.6 b
	Karl Fischer	11.6 bc	15.7 a
Mimosa	intact	5.3 a	10.0 a
	cutting	9.4 b	13.4 b
	Wiley mill	9.6 bc	12.9 b
	coffee mill	9.8 c	13.4 b
	Karl Fischer	11.7 d	15.6 c

The results clearly showed that the hard seed coats of honeylocust and mimosa must be ruptured in some fashion to allow all moisture to escape during oven drying. In addition, rupture appeared to be more important for seeds with low moisture contents. It was also apparent that in most cases cutting the seeds in half gave results equal to those obtained with ground seeds; and that fineness of grinding seems to be less important. There was also no difference in moisture contents obtained following grinding with either the Wiley or coffee mills.
In mimosa none of the oven methods used were able to drive out all of the moisture from the seeds (results not shown). This may have been due to stronger chemical bonding of moisture in mimosa seeds (Bonner *et al.*, 1992).

However, even within one species there was no one method that was always in accordance with KF. In addition, significant differences among the different seed lots were found even within one species (results not shown).

Moisture loss on grinding was shown to be dependent on the functioning of the grinder and the species used, with differences in seed size possibly having significant effect (Mullett, 1978, Table 2.2.).

Table 2.2.:
Effect of grinding on seed moisture content determination in samples of *Medicago sativa* and *Lotus penduncculatus*, by using the low constant oven method (Mullett, 1978).
Method: ISTA = 17 hrs 105°C

Species	Station	Sample	Moisture content		Difference
			Ground	Whole	
Medicago sativa	1	HR	7.50	7.08	+0.42
	2	HR	7.25	6.88	+0.37
	1	Wairau A	10.66	10.23	+0.43
	2	Wairau A	9.65	10.15	-0.50
	1	Wairau B	10.50	10.09	+0.41
	2	Wairau B	9.39	9.50	-0.11
	1	Wairau C	10.48	10.14	+0.34
	2	Wairau C	9.39	9.30	+0.09
	mean		**9.35**	**9.17**	**+0.18**
Lotus penduncculatus	1	A	11.71	11.63	+0.08
	2	A	11.98	11.82	+0.16
	1	B	11.74	11.60	+0.14
	2	B	10.05	10.40	-0.35
	mean		**11.37**	**11.36**	**+0.01**

Three years later the above results were confirmed. It was also found now that the between-station variation was much greater when whole seeds compared to ground seeds are tested (Table 2.4). As a consequence, it was suggested that the seed moisture testing methods for these species be revised. But this was never effectuated.

Table 2.3.:
Comparison of several methods for moisture determination for *Trifolium pratense* and *Trifolium repens* with the prescribed ISTA method; i.e. 1hr at 103°C (Klitgard, 1978).

Treatment	*T.pratense* mc	Diff. to ISTA	Diff. to whole	*T.repens* mc	Diff. to ISTA	Diff. to whole
1 hr 130-133°C, whole	8.65			8.43		
2 hrs 130-133°C, whole	9.17	+0.52		9.01	+0.58	
17 hrs 101-105°C, whole	9.16	+0.51		8.96	+0.53	
1 hr 130-133°C, ground	9.67	+1.02	+1.02	9.78	+1.35	+1.35
2 hrs 130-133°C, ground	9.75	+1.10	+0.58	9.90	+1.47	+0.89
17 hrs 101-105°C, ground	9.37	+0.72	+0.21	9.52	+1.08	+0.56

The reported results in Table 2.3 indicate that the present ISTA method for moisture determination of small-seeded legumes gives results that may be too low.

The moisture content as measured by the present ISTA method was 0.6 to 1.0% lower than the moisture content obtained by the other method for whole seeds for all four species. When the seeds were ground, the measured moisture percentage was 0.8 to 1.7% higher than that determined by the

ISTA method. It also appeared that the differences are not the same for all species; it was smaller for Trifolium spp, and bigger for Medicago and Lotus seeds (Table 2.4).
This is supported by Hart *et al*. (1959), who obtained similar results by KF and 2.5 hours at 130°C in the oven. Differences between whole and ground were smallest following oven drying at 17 hours at 103°C.
The mean deviation in seed moisture contents between the stations was considerably higher for all four samples determined by the ISTA method when compared to the other three methods.

Table 2.4.:
Comparative moisture content determinations of *Trifolium repens, T.pratense, Medicago sativa* and *Lotus corniculatus*. Average of results obtained by 11 stations and mean deviations between the stations (Klitgard, 1981).

Treatment	***Trifolium repens***		***Trifolium pratense***		***Medicago sativa***		***Lotus corniculatus***	
		Diff. to ISTA		**Diff. to ISTA**		**Diff. to ISTA**		**Diff. to ISTA**
no grinding, 1 hr 130-133°C (ISTA)	mc = 8.74		mc = 8.64		mc = 7.52		mc = 9.07	
no grinding, 17 hrs 101-105°C	+0.60		+0.62		+1.00		+0.99	
grinding, 1 hr 130-133°C	+1.13	+1.13	+0.99	+0.99	+1.70	+1.70	+1.57	+1.57
grinding, 17 hrs 101-105°C	+1.02	+0.62	+0.78	+0.16	+1.49	+0.49	+1.36	+0.37
	Mean deviations between stations							
no grinding, 1 hr 130-133°C (ISTA)	0.33		0.34		0.51		0.44	
no grinding, 17 hrs 101-105°C	0.16		0.14		0.15		0.15	
grinding, 1 hr 130-133°C	0.21		0.20		0.19		0.16	
grinding, 17 hrs 101-105°C	0.08		0.16		0.14		0.10	

Time of grinding had no effect on the moisture analysis, and very little effect on the particle size distribution observed (latter results not shown here).
Seed moisture content, determined on a whole seed basis, was almost 50% lower than that determined following grinding.

Table 2.5.:
Effect of duration of grinding on moisture content for three varieties of *Vigna radiata*. Method: 1 hour at 130°C (= ISTA method, ground seeds). Each value represents the means of three replicates of 10 g each (Mullett, 1981).

Treatment	**mean**	Green Gram 'Celera'			Black Gram 'Regur'			Green gram 'unknown'		
		lab 1	lab 2	lab 3	lab 1	lab 2	lab 3	lab 1	lab 2	lab 3
whole seeds			8.6	7.1	4.2	6.3	5.6	5.3	5.7	5.7
30 sec grinding	**11.1**	11.6	12.0	12.0	9.0	11.6	11.6		10.4	10.4
60 sec grinding	**11.1**	11.4	12.2	12.1	9.6	11.6	11.6		10.3	10.3
120sec grinding	**11.1**	11.3	11.8	12.1	9.4	11.6	11.8		10.4	10.4

McGill (pers.comm.) tested canary grass. Canary grass was tested by the National Seed Laboratory of New Zealand (McGill, pers.comm., table A2.2.). The canary grass tended to stick in the grinder at the size for cereals.

Table A2.2.: Effects of grinding on the measured moisture contents of canary grass seeds following oven drying.

Treatment	**Moisture contents**	
	Sample 1	**Sample 2**
1 hour (unground)	10.1	10.0
2 hours (unground)	11.1	11.1
2 hours (ground)	11.9	12.0

3. SEED SIZE AND PARTICLE SIZE DISTRIBUTION

The ISTA Rules is not the only place where particle size distribution is described. Many other international standards contain details about particle size distribution as well (Table 3.1).
Although ISTA has to take care of far more different species in comparison to ISO, ICC or EU, the results obtained by these organisations are probably useful to ISTA.

Table 3.1. Summary of minimum requirements by various organisations for the quality of ground seeds

Organisation	**Upper limit**		**Lower limit**
ISTA (general)		<10% is >1mm	>50% is <0.5mm
ISTA (leguminous seeds)	>50% is <4mm		
ISO, ICC (cereals)	100% is <1.7mm	<10% is >1mm	>50% is <0.5mm
EU (feeding stuffs)	100% is <1.7mm	<10% is >1mm	>50% is <0.5mm
USDA (cereals, oil seeds)	100% is <0.85 mm		
ISO 665 (oil seeds)		'coarse'	
Suggested by Don (2006)	100% is <2.0 mm	>20% is <1.0 mm	>20% is <0.5 mm

Some laboratories find it easy to work within these quality limits and may have stricter internal rules. For example the Danish Plant Directorate achieve > 4 mm – max. 50%, < 2.0 mm – min. 55% (Nydam, pers comm.). Other labs have difficulties keeping within the required limits, having approximately only 34% of the particles 0.5 mm or less after grinding. (McGill, pers comm.)

For some species cutting instead of grinding is allowed. Cutting results in samples that will not meet the requirements for particle size distribution after grinding: sizes up to 7 mm are allowed.
In fact the maximum sizes for allowing moisture determinations in whole seeds should be the same or less than the maximum particle sizes that are acceptable after grinding. Preferably less, because most seeds have seed coat structures that may hinder the removal of water during oven drying.

Makower (1950) suggested particle sizes of less than 0.3 - 0.5 mm. In addition, Bonner (1981) suggested that all seeds over 10 mm in diameter or length should be broken.
If the size of the particles are too big, water may be sealed in by "varnish-like" films (Bidwell & Sterling, 1924).

Mani & Tabil (2002) investigated the effects of grinding chickpea seeds on moisture content determination. Whole seeds were tested according to the ASAE-standard S352.2 (i.e. 72 hrs at 105°C; NN ASAE, 2003), while ground seeds were tested according to AOAC-925.10 (i.e. 1 hr at 130°C; NN AOCS, 1997). The mean particle diameter of whole seeds was 5.3 mm, while that of ground material 0.5 mm. The moisture content of whole seeds was 11.4%, whilst that of ground material was 9.6%. The lower mc after grinding was ascribed to the evaporation of water during grinding.

Bormuth (1992 and 1994b) assessed the effects of particle size on moisture content test results in pea (summarised in Table 3.2.).

Table 3.2.:
Effects of particle size and moisture content determination method (i.e. oven drying vs. KF) on the moisture content of pea seeds (Bormuth, 1992 and 1994b). KF = Karl-Fischer Titration. a-c: means and variance respectively with different letters are significantly different.

	Air-oven drying (1 hour at 130°C)						**KF**
	Coarse grinding					**Whole seeds**	**Fine grinding**
Particle size (mm)	0-1	0-2	0-3	0-5	0-7.5	5-7.5	0-1
Mean (%)	13.53 a	13.47 a	13.41 b	13.38 b	12.32 e	12.64 d	13.00 c
Variance	0.013 b	0.012 b	0.008 b	0.007 b	0.089 a	0.028 ab	0.011 b

Coarse grinding 0-1 to 0-5 mm overestimated, whilst coarse grinding 0-7.5 and determination on a whole seed basis underestimated pea seed moisture contents when compared to the results of the KF test. It should be noted, however, that the coarse grinding 0-7.5 mm samples included seeds that were only chipped and could retain their original sizes.
The moisture content of samples coarsely ground to 0-7.5 mm was 0.32% lower than compared to whole, unground seeds. This may, in part be due to moisture loss during grinding. Alternatively, the seeds may have been sufficiently damaged to ensure rapid moisture loss during oven drying.
The ISTA Rules requires that at least 50% of the ground material must pass through a sieve with a mesh of 4.00 mm.
Overestimation of the actual seed moisture content may have been as a consequence of loss of water and or volatiles during grinding. However, this seems unlikely as the samples used for the KF test were also ground.
It is more likely that loss of non-aqueous volatiles and loss of weight because of the decomposition of materials occurred during the air-oven drying.

Some German labs allow particle sizes up to 5 mm in moisture tests (Wiegand, pers.comm.). In Brazil even bigger seeds are kept whole in the oven.

Don (pers.email.comm.) indicates that the ISTA requirements were approved during the Edingburgh congress of 1985. It is not clear why such fine grinding is necessary, nor why we have it for cereals, but not for peas and beans, or tree seeds. No background or evidence for this is available anymore.
Literature (see Annexes 2 and 3) indicates that moisture contents in Karl Fischer are always higher than that of the oven method when fine grinding is required. This suggests moisture loss during fine grinding.
This is supported by anecdotal evidence, indicating that the second replicate of a test invariably has a lower moisture content compared to the first one (Don, 2006).

It is believed that many of the ISTA labs don't conform to the grinding specifications in the Rules, as many labs do not have mills that can deliver ground material of the required specification. And even when mills can grind fine enough it appears that there is a selection process going on and husk and other fibrous material is being retained within these mills. So hammer mills may provide ground material of the required fineness but the material they provide is not representative of the seed that was ground (Don, 2006).

In the past MOI has done some research in this field, although not much.
In the report of MOI over the period 1977-1980 (SST 9 (1981) 229-254) results on particle sizes from USDA research are mentioned, without citation however.

In the 1983-1986 report (SST 15 (1987) 451-462) on page 453 a 1924-paper is mentioned (Buchholz, 1924), indicating that results of the oven test largely depend on the degree of grinding of the sample. The paper itself appears to compare ground and whole seeds only; no details on particle size distribution are given.

In moisture determination of large seeded legumes ISTA prescribes one method and the EU directive 71/393/EEC (for official control of feed stuff) prescribes another. Experimental moisture tests of 20 samples at the Danish Plant Directorate showed, that the two methods gave slightly different results for *Pisum sativum* (table 3.2).

Another problem encountered was the quality of the ground material. A comparison test was made for two mill types, an older Regent mill and a relatively new Cemotec mill. In total 20 samples were analysed on each mill. The differences found are summarised in table 3.3. For the combination of the mill and the method giving the highest moisture content and the mill and method giving the lowest moisture content the difference for the 20 samples were on average 0.4 %. The difference between the two mills was 0,1 or 0,12 depending on the method used.

Table 3.3. Average moisture content in 20 samples of *Pisum sativum,* analysed at the Danish Plant Directorate. Moisture determination made according to ISTA rules and to EU directive 71/393/EEC. Two different mills were used.

	Regent mill	Cemotec mill	Difference between mills
ISTA method	13,1 %	13,3 %	-0,2 %
EU-method	12,9 %	13,0 %	-0,1 %
Difference between methods	0,2 %	0,3 %	

For the long test at 103°C the need may not be present for a certain species, whereas for the same species at 130°C grinding may be necessary.
At the moment we don't allow/specify the possibility for grinding at 130°C for species that don't need grinding at 103°C.

4. SEED MOISTURE CONTENT AND SEED OIL CONTENT

When the seed moisture content is too high, grinders cannot cope with the product. Predrying is necessary in that situation.
The Rules and the Handbook contain guidance for some species. But again, scientific evidence backing up this guidance is lacking. Also the effects of species and moisture level may be confounded.

Experiments by Mannino (Table 4.1.) demonstrate the effect of moisture content.

Table 4.1.
Effect of moisture content on particle size distribution in Soybean. Grinder Ika A10. No. 6, time of grinding: 12 seconds (Mannino, 2001, pers.comm.).

Particle size distribution	9% moisture	14% moisture	19% moisture
>4 mm	17.9%	44.6%	46.5%
<4 mm	82.1%	55.4%	53.5%

As soybean seeds have relatively high oil contents, the effect of the moisture level may be confounded with oil content as well.

Danish unpublished results (Nydam, pers. comm.) on 26 samples of peas resulted in
18-44% of the material >4 mm in a Retch mill (moisture content 16.1%), and
34-46% of the material >4 mm in a Regent mill (moisture content 15.8%).

If the seed oil content is too high, grinders cannot cope with the product. Cutting may then replace grinding. Some examples can be found for tree and shrub seeds in the new Table 9.A.2 in the Rules. In Table 9.A.1 for agricultural and vegetable seeds Arachis and Ricinus can be found as examples for high oil content species that have to be cut.

5. SUMMARY AND CONCLUSIONS

5.1 SEED COAT

The nature of the seed coat affects both, the quality of the grinding and the exchange of moisture in the oven.

Suggestion 1:
Check the method in the ISTA Rules for the species mentioned in section 2.2.
In additions Tables 9.A.1 and 9.A.2 should be checked for more species like this.

5.2. GRINDERS

Differences between methods may be influenced by the performance of grinders. Important aspects are particle size distribution and loss of moisture during grinding.

Suggestion 2:
Organise comparative testing in order to check the temperature increase, and effect on moisture content:

- Temperature increase during grinding (ten samples, to be ground by each lab, after which moisture content will be assessed).
- Particle size distribution:
- Seed moisture content (two levels)
- Different types of grinders (compaction, impact, shear, toothed-disk, …..)

Suggestion 3:
Organise comparative testing on the effect of grinder, seed moisture content and seed oil content on particle size distribution:

- Seed moisture content (two levels).
- Species: oats, wheat, peas or soybean or …?
- Labs grind and return to central lab for assessment of particle size distribution.
- Duration of grinding
- Temperature increase of grinder and sample.

The number of samples, treatments and labs has to be arranged in cooperation with the ISTA Statistics Committee and be in accordance with the ‘ISTA Method Validation for Seed Testing (2007).