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GRINDING IN ISTA MOISTURE TESTING

A discussion paper (Harry Nijënstein, **harry.nijenstein@dlf.com**)

Annex 1: Grinding in the ISTA Rules moisture chapter

The wording relating to grinding in the Rules will be as follows after the next OM:

9.1.5.4 Grinding

A lower limit for coarse grinding was inserted in order to bring it into line with fine grinding where you have a lower and an upper limit. The objective of coarse grinding is to prevent changes in moisture content that would occur if such seed were ground finely due to increases in temperature.

How to prevent contamination of one sample to another is deleted here, and will be dealt with in the Moisture Handbook.

Large seeds and seeds with seed coats that impede water loss from the seeds must be ground before drying unless their high oil content makes them difficult to grind or (particularly in seed such as *Linum* with oil of a high iodine number) liable to gain in weight through oxidation of the ground material.

If grinding is not possible, then cutting is allowed. See 9.1.5.5 for details.

It is obligatory to grind seed of a particular species if this is indicated in Tables 9.A.1 and 9.A.2.

The grinding mill should be adjusted so that particles of the required dimensions are obtained. For those species requiring fine grinding (Table 9.A.1), at least 50% of the ground material should pass through a wire sieve with meshes of 0.50 mm and not more than 10% should remain on a wire sieve with meshes of 1.00 mm. For those species requiring coarse grinding (Tables 9.A.1 and 9.A.2); at least 50% of the ground material should pass through a sieve with meshes of 4.00 mm, and not more than 55% should pass through a wire sieve with meshes of 2.00 mm.
The total time of the grinding process shall not exceed two minutes.

When using a grinder ensure that there is no contamination from one sample to the other.

9.1.5.5 Cutting

High oil content species may have to be cut instead of ground (Tables 9.A.1 and 9.A.2). Grinding results in a paste that will not pass a sieve, and give other problems.
With large seeds, there can be large variability within a seed lot. It is difficult to obtain representative working samples from the seed lot for moisture tests. To minimise differences between replicates, working samples are recombined and mixed after cutting, before drawing the final working samples.

Large tree seeds (thousand seed weight > 200 grams) and tree seeds with very hard seed coats, such as those of *Fabaceae* (*Leguminosae*), and/or species with high oil contents, should be cut into small pieces of less than 7 mm instead of being ground. The cutting shall be carried out on two sub-samples each of a weight approximately equal to the weight of five intact seeds drawn from the submitted sample.

The sub-samples are quickly cut, recombined and mixed with a spoon prior to dividing into two replicates. The replicates are placed in weighed containers. Exposure to the atmosphere should not exceed 4 minutes

9.1.5.6 Pre-drying

Pre-drying for *Glycine max* shall only be required if the sample is above 12%, not 10%. Many samples are between 10% and 12% moisture. Below 12% no free water is present and seeds are storable, and grinding is no problem at 12% moisture. Deleted information can now be found in Tables 9.A.1 and 9.A.2.

If the species is one for which grinding is necessary and the moisture content is higher than indicated in Table 9.A.1, pre-drying is obligatory. Two sub-samples, each weighing 25 ± 1 g are placed in weighed containers. The two sub-samples, in their containers, are then dried at 130 ºC for 5 to 10 minutes, depending on the moisture content, to reduce the moisture content to below that required in Tables 9.A.1. The partly dried material is then kept exposed in the laboratory for at least two hours.

In the case of very moist seed of *Zea mays* (above 25% moisture content) the seed is spread in a layer not deeper than 20 mm and dried at 65-75°C for 2-5 hours, depending on the initial water content. In the case of other species with a moisture content exceeding 30%, samples should be dried overnight in a warm place.

After pre-drying the sub-samples are then reweighed in their containers to determine the loss in weight. Immediately thereafter the two partly dried sub-samples are separately ground, and the moisture determined as prescribed in 9.1.5.3.

Pre-drying is not obligatory for any seeds that are cut (Table 9.A.2).

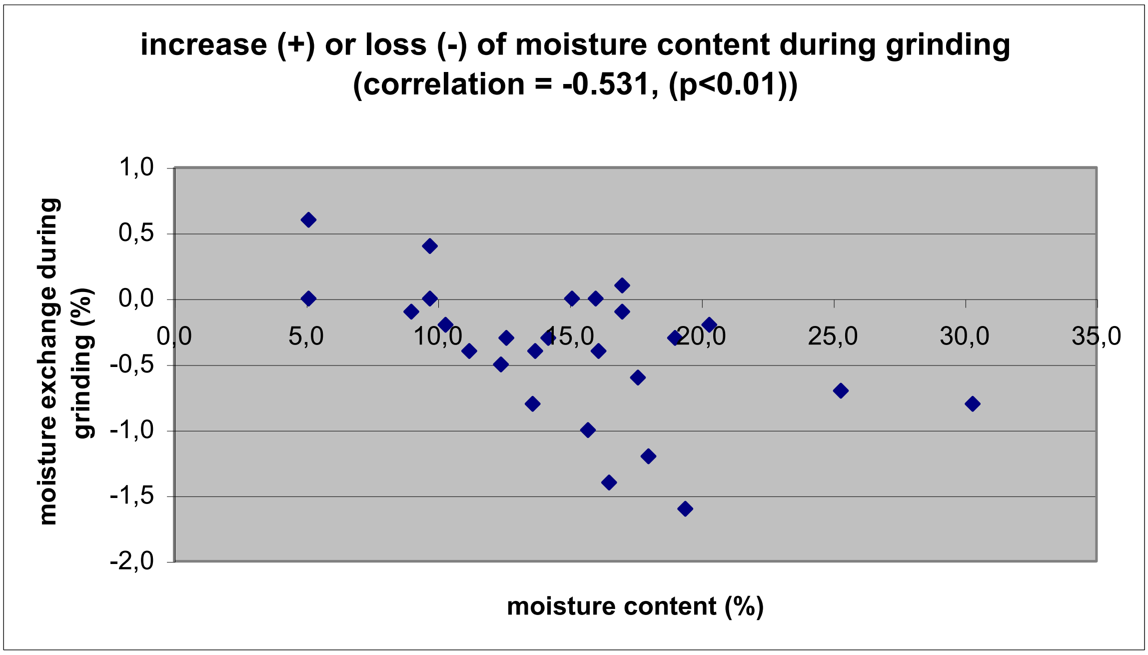
Annex 2.
Effects of grinding on moisture test results in general

Grinding of large seeds shortens the duration of the drying period. Drying periods in official methods are calibrated for a particular particle size, usually an 18 or 20 mesh. Particles larger than prescribed size may not dry completely during the established drying period (Grabe, 1989 a and b).

Seeds may lose or attract water during and after grinding. Exposure of high-moisture samples to a dry atmosphere may cause loss of moisture. Conversely, dry samples exposed to a humid atmosphere may absorb water. In addition, the fineness of milling may also have two effects. If the product is coarse, while its moisture content may not alter much in the mill, it may fail to lose all its moisture in the oven. On the other hand, if it is fine enough for all the water to be removed during drying, it will be in a condition to change its moisture content readily in the mill. In addition the added work done in the mill may generate enough heat to change the moisture content (Warner & Browne, 1963).

Table A2.1.: Relationship between moisture loss or increase during grinding and moisture content during grinding.

moisture content	increase (+) or loss (-)	reference
5.1	+0.6	Warner & Browne, 1963
9.7	+0.4	Warner & Browne, 1963
16-18	+0.1	Hunt & Neustadt, 1966
16	0.0	Hunt & Neustadt, 1966
15.1	0.0	Warner & Browne, 1963
5.1	0.0	Warner & Browne, 1963
9.7	0.0	Warner & Browne, 1963
<17	-0.1	Leendertz, 1948
<9.8	-0.1	Williams & Sigurdson, 1978
10.3	-0.2	Williams & Sigurdson, 1978
20.3	-0.2	Warner & Browne, 1963
12.6	-0.3	Bormuth, 1994 a and b
19	-0.3	Leendertz, 1948
14.2	-0.3	Sair & Fetzer, 1942
11.2	-0.4	Williams & Sigurdson, 1978
13.7	-0.4	Sair & Fetzer, 1942
16.1	-0.4	Sair & Fetzer, 1942
12.4	-0.5	Williams & Sigurdson, 1978
17.6	-0.6	Sair & Fetzer, 1942
25.3	-0.7	Warner & Browne, 1963
13.6	-0.8	Williams & Sigurdson, 1978
30.3	-0.8	Warner & Browne, 1963
15.7	-1.0	Williams & Sigurdson, 1978
16.5	-1.4	Williams & Sigurdson, 1978
18.0	-1.2	Williams & Sigurdson, 1978
19.4	-1.6	Williams & Sigurdson, 1978
>18	erratic	Hunt & Neustadt, 1966



These results seem erratic at first sight. However, the %RH during grinding was not recorded in any of the experiment which may explain some of these differences.

The results may also be confounded by the coarseness of grinding as too short a duration of grinding may result in large particles, leading to incomplete moisture loss during oven drying and an apparent moisture loss because of grinding.

In high-moisture samples or when the oil content of a sample is >25%, grinding may not be satisfactory (Zeleny, 1953). Gumminess of the ground material, excessive loss of moisture during and after grinding, and overheating may occur.

A high moisture content may be overcome by using the two-stage method which involves preliminary drying before grinding.

Apart from seed size, other seed characteristics, such as moisture content, shape, hardness, gumminess, and the volume ratio between seed coat, endosperm and germinative tissues, may influence the grinding product, such that the size and shape of particles resulting from grinding in the same mill are often different (Hart *et al.*, 1959).
Fang & Campbell (2003) indicated that the three parts of the wheat kernel (i.e. bran, germinative tissues and endosperm) differ in relative toughness and friability, resulting in different breakage patterns during roller milling. These differences are influenced by the moisture content of the seed.

Other aspects known to influence the quality of the grinding include
- Sharpness of the blades, as the mc is 0.2% when dull blades are used (Hunt & Neustadt, 1966).
- Rate of feed, which may influence particle size distribution (Hunt & Neustadt, 1966)
- Heating of the mill (Hunt & Neustadt, 1966). Williams & Sigurdson (1978) showed that moisture loss may vary from 0% at seed moisture contents of 9.0% at the start of milling, to 3.5% at 17.8% initial seed moisture content and a grinding interval of 240 minutes.
- Seed type and mc (Fang & Campbell, 2003), although varietal differences did not affect particle size in maize (Paynter & Hurburgh, 1983b).

- Type of mill. Williams & Sigurdson (1978) tested several cereals in four mills. Percentage moisture loss during grinding averaged 1.20% over all mills and all species. Loss over the four mills varied from 0.77 to 1.89%. A mill should (Warner & Brown, 1963; Hunt & Pixton, 1974):
  - provide little or no exposure of the sample to air; thus, for a given power consumption the best mill will be the quickest and also the least dusty since it will be fully sealed in use.
  - be easy to clean between samples
  - allow little or no heating during the grinding
  - produce particle sizes that meet the requirements

Comparative tests with wire screens on a Wiley mill showed no difference in moisture content results between 18- and 20-mesh screens (0.85 and 0.80 mm respectively). However, the moisture content results obtained were lower when the samples were ground with a 16-mesh screen (1.0 mm). An 18-mesh screen was preferred to a 20-mesh screen as the grinding was faster and ‘packing’ difficulties often encountered with oats and barley were reduced. If the grain does pack in the grinding chamber, the packed material and that, which is already ground, must be discarded and the mill be thoroughly cleaned. The easiest way to clean a mill in such cases is to run very dry, hard wheat or other vitreous grain through it before disassembling for brushing (Hunt & Neustadt, 1966).
The Wiley mill is recommended because it produces little heat and allows minimum exposure of ground material. The mill has rotating cutting edges or blades and the grinding chamber and the receptacle for the ground seed is completely enclosed. Seeds are often ground fine enough to pass through a screen with openings of 1.04 mm (Hart & Golumbic, 1963).

Oxley & Pixton (1961b) found correlations (r) of 0.60 and 0.53 between the percentage sample that passed through a 10 wire mesh screen (aperture size 1676 microns) and the moisture content of two wheat varieties. In addition they concluded that much of the variation in moisture content results was ascribable to day to day variations in the performance of the grinder on an unchanged setting.
See table in Warner & Browne (1963) for relation mesh*aperture size).

Samples of the ISTA Comparative Testing of 2000 were used for a methodology study by one lab (Table A2.3, Nijënstein, not published).

Table A2.3.:
Moisture content per lab (ISTA method) and for different methods in one lab;
Further information: the average standard deviation sd(w) between the two replicates within the laboratories, the standard deviation sd(b) between the results of the laboratories and the ratio between the two standard deviations [r = sd(b) / (sd(w) * SQRT(2))]. (Kruse, 2000)

Laboratory	**Oat 1**		**Oat 4**		**Oat 6**		**Wheat 2**		**Wheat 3**		**Wheat 5**	
		ground		**ground**		**ground**		**ground**		**ground**		**ground**
A		15.9		16.0		12.2		14.8		11.6		14.8
B		16.9		16.7		12.2		15.1		11.9		15.0
C		14.8		15.9		11.8		14.8		11.6		14.8
D		16.4		16.5		12.4		15.0		12.0		15.0
E		16.0		16.3		11.9		14.9		11.9		14.9
F		16.2		16.1		12.0		14.8		11.9		14.7
G		16.5		16.5		12.3		15.0		12.0		15.0
H		15.8		16.0		11.8		14.5		11.6		14.6
I		*16.6*		*16.6*		*12.4*		*15.1*		*11.7*		*14.9*
J		16.5		16.4		12.1		14.7		11.7		14.8
Mean		**16.2**		**16.3**		**12.1**		**14.9**		**11.8**		**14.9**
sd(w)		**0.07**		**0.05**		**0.05**		**0.04**		**0.04**		**0.02**
sd(b)		**0.56**		**0.30**		**0.24**		**0.19**		**0.17**		**0.15**
r		**11.3**		**8.5**		**6.9**		**6.2**		**6.9**		**9.7**
min-max		**14.8- 16.9**		**15.9- 16.7**		**11.8- 12.4**		**14.5- 15.1**		**11.6- 12.0**		**14.6- 15.0**

variation min-max		**2.1**		**0.8**		**0.6**		**0.6**		**0.4**		**0.4**

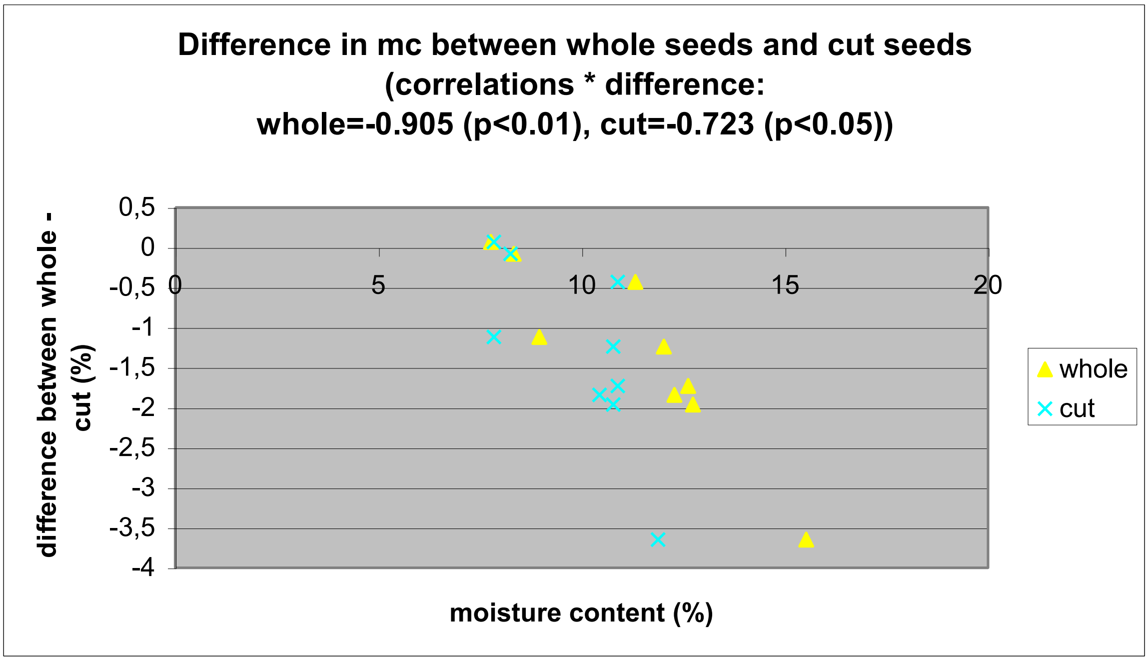
These results showed a very good repeatability of moisture content determination between all the laboratories within the test. On the request by the organiser the test will be repeated according to ISTA Rules 9.6.2 when the difference between the replicates exceeds 0.2. The two replicates (not shown) were dependent replicates because they were tested in the same run; therefore sd(w) was not an estimate for the classical repeatability which assumes independent tests, but an estimate only for dependent replicates. The ratio r as well as the variance analysis showed that differences between each of the laboratories had a significant effect on the results. The variation between the laboratories was much greater than it would have been expected on the basis of variation within the laboratories, on average by factor 8.25 (in the ISTA referee test 97/1 with *Phalaris aquatica* this ratio was only 4.5) (Kruse, 2000).

Table A2.4.:
Moisture contents as influenced by grinding and temperature for several species (Nijënstein, unpublished results).
Botom lines: * = significant effects at p<0.05, ns = no statistically significant effect.
Diff. = difference between whole and ground seed per species.
Underlined in italic: present ISTA method

temp / hrs	**mean**	***Lolium perenne***		***Poa pratensis***		***Triticum aestivum***		***Hordeum vulgare***		***Pisum sativum***		***Zea mays***	
		whole	ground	whole	ground	whole	ground	whole	ground	whole	ground	whole	ground
103°C													
8	**11.44**	11.87	11.87	11.12	11.25	11.25	13.46	10.40	12.46	09.66	12.79	09.14	12.02
16	***12.05***	*12.03*	*11.94*	*11.28*	*11.18*	*12.79*	*13.89*	*11.89*	*12.80*	*11.61*	*12.92*	*10.57*	*11.74*
24	**12.08**	11.83	11.82	11.28	11.27	12.67	13.82	11.75	12.84	11.64	12.94	10.60	12.51
48	**12.42**	11.80	11.86	11.19	11.15	13.53	14.07	12.51	12.99	12.49	12.95	11.75	12.72
72	**12.29**	11.85	11.79	10.95	11.03	13.31	13.78	12.53	12.84	12.20	12.72	11.80	12.69
mean		**11.88**	**11.86**	**11.16**	**11.18**	**12.71**	**13.80**	**11.82**	**12.79**	**11.52**	**12.86**	**10.77**	**12.34**
diff.			**+0.02**		**-0.02**		**-0.09**		**+0.03**		**-1.34**		**-1.57**
130°C													
8	**12.60**	12.54	12.56	11.87	11.75	13.11	14.14	12.39	13.33	12.15	13.25	11.15	13.01
16	**13.08**	12.73	12.61	11.87	11.70	14.26	14.68	13.12	13.60	13.58	13.47	12.27	13.01
24	**13.33**	12.83	12.89	12.00	11.94	14.10	14.54	13.46	14.05	13.81	13.64	12.99	13.74
48	**13.59**	12.82	12.70	12.04	11.89	14.89	14.88	13.98	14.11	14.48	13.92	13.44	13.93
72	**13.62**	12.78	12.76	11.88	12.08	14.65	14.78	14.00	14.13	14.43	14.18	13.78	14.00
mean		**12.74**	**12.70**	**11.93**	**11.87**	**14.20**	**14.60**	**13.39**	**13.84**	**13.69**	**13.69**	**12.73**	**13.54**
diff.			**+0.04**		**+0.06**		**-0.40**		**-0.45**		**0.00**		**-0.81**
LSD (p<0.05)		0.23	0.22	0.17	0.25	0.28	0.21	0.35	0.26	0.35	0.49	0.41	0.18
LSD (p<0.05)		0.20		0.22		0.71		0.65		1.20		0.89	
drying time		ns		ns		*		*		*		*	
grinding		ns		ns		*		*		*		*	
temperature		*		*		*		*		*		*	

Table A2.5.:
Mean moisture contents as determined by three different methods for *Quercus* spp (Bonner, 1974) and for *Fraxinus pennsylvanica* (Bonner, 1972).
Two-stages = ground, then ?? hours at 130°C
Cut = cut into halves, then 15 hrs at 105°C
Intact = whole seeds, 17 hrs at 105°C (ISTA)

Species	**Intact**	**Two-stage**	**Cut**
Quercus alba	47.4	48.0	47.9
Quercus muehlenbergii	36.8	37.1	37.3
Quercus nigra	31.5	32.2	32.4
mean	**38.6**	**39.1**	**39.2**
	Intact	**Cut**	**Difference**
Fraxinus pennsylvanica	7.77	7.84	+0.07
	8.96	7.84	-1.12
	8.33	8.25	-0.08
	12.28	10.44	-1.84
	12.02	10.78	-1.24
	12.74	10.78	-1.96
	15.53	11.88	-3.65
	11.32	10.89	-0.43
	12.62	10.89	-1.73
mean	**11.29**	**9.95**	**-1.33**



The moisture content of intact *Q. nigra* seeds was significantly lower when compared to that of seeds from the same species subjected to the two-stage and cut treatments (Table A2.5.). In contrast, moisture contents of seeds from the two other species did not significantly differ between the different methods used. It should be noted, however, that the pericarps of many of the seeds fromthe latter two species were already split open by emerging radicles (Bonner, 1974).
For *F.pennsylvanica* it was concluded that, even though the cutting was done very quickly with scissors after initial weighing, some moisture was lost during cutting (Bonner, 1972).

Table A2.6.:
Comparison of two methods of moisture determination for some species at two seed testing stations
Ground = 2 hrs at 130-133°C (= ISTA method).
Whole = 192 hrs at 101-105°C.
(Klitgard, 1978)

Species	**Station A**		**Station B**	
	Ground	**Whole**	**Ground**	**Whole**
Avena sativa	13.58	13.56	11.12	11.09
Hordeum vulgare	15.48	15.35	12.15	12.09
Oryza sativa			12.79	12.77
Secale cereale	14.86	14.72	12.04	11.99
Sorghum vulgare			12.27	12.21
Triticum aestivum	14.28	14.07	11.73	11.66
Zea mays	14.51	14.47	11.41	11.47
mean	**14.54**	**14.43**	**10.44**	**10.41**

In addition Klitgard (1978) compared the ISTA method with a drying to constant weight at 103°C for 21 species. The differences between the two methods varied considerably between groups of species. Very small differences varying from 0.1% to 0.3%, were obtained in the cereal species *Avena sativa, Hordeum vulgare, Oryza sativa, Secale cereale, Sorghum vulgare, Triticum aestivum*, and *Zea mays*, and in the pulses *Phaseolus vulgaris, Pisum sativum, Vicia faba* and *Vicia sativa*.
In the small seeded legumes *Lotus corniculatus, Medicago sativa*, and *Trifolium pratense* the moisture percentage measured by the ISTA method was 0.7 to 1.9% lower than drying to constant weight at 103°C.
For the grasses *Dactylis glomerata, Festuca pratense, Festuca rubra, Lolium multiflorum, Lolium perenne, Phleum pratense* and *Poa pratensis*, this difference varied between 0.1 and 1.0%.
The apparent difference between groups of species may have been caused by differences between grinding versus no grinding, since cereals and pulses were tested as ground seed, whereas small-seeded legumes and grasses were tested as whole seeds.

Difference between KF and oven as found by Benjamin & Grabe (1988) for whole seeds were influenced by moisture content: relatively lower results in the oven at lower moisture levels. In Table A2.6 the results are more negative in the oven at low moisture levels for Triticum, but not for the other species that have to be ground.

Table A2.7.:
Effect of moisture level and grinding on hours of drying at 130°C required to obtain moisture percentages in agreement with the Karl Fischer method (Grabe, 1987; Benjamin & Grabe 1988; Grabe, 1990).

	Moisture range of samples					
	low (4-7%)		medium (7-11%)		high (12-16%)	
species	whole	ground	whole	ground	whole	ground
perennial ryegrass	6	2	3	1	2	-
orchardgrass	3	1	2	1	0.7	-
Kentucky bluegrass	3	1	3	1	0.5	-
tall fescue	7	3.5	3	1	2	
red fescue	6	3	3	1.5	2	
Bentgrass	2	1	1	1	0.5	-

Grinding seeds shortens the time required for drying to the true moisture value, and ground and whole seeds gave equivalent values when dried to constant weight.

It is not possible to select one drying period that is entirely accurate at all moisture levels within a species. More time is required to remove all the moisture from low moisture seeds than from high

moisture seeds. From figures in Benjamin & Grabe (1988) it can be estimated that the underestimation at 1 hour at 130°C can be more than 1% at all moisture levels for perennial ryegrass.
It would be impractical to prescribe more than one drying method for each species since to do so would require that the moisture range be known beforehand. The data indicate that selection of one drying period will give results of varying degrees of accuracy, depending on the original moisture content. This is an inherent shortcoming of the oven method and one which must be accepted (Grabe, 1987).
Seed moisture tests on these six temperate-climate grass species should be 3 hours for perennial ryegrass, Kentucky bluegrass, tall fescue and red fescue; 1.5 hours for orchardgrass; and 1 hour for bentgrass. Hart et al (1959) recommended the same drying time for colonial bentgrass, the fescues and perennial ryegrass, but suggested 1 hour for orchardgrass and Kentucky bluegrass.
In contrast, the present ISTA Rules prescribe 1 hour for all six species (Benjamin & Grabe, 1988).
Drying to constant weight at temperatures of 90, 100, 105°C gave moisture percentages up to almost 2% lower than the Karl Fischer value (Benjamin & Grabe, 1988).

Table A2.8.:
Percentage of moisture of total lost over time as influenced my absolute moisture level for Lolium perenne (Nijënstein, unpublished).
Moisture content: level after 6 hours.

	Percentage of total lost over time (minutes)				
moisture content	15	30	60	120	360
12.37	60	81	89	95	100
16.92	76	85	92	97	100
20.70	73	86	95	97	100
23.91	82	89	94	97	100
27.64	84	91	95	98	100
30.50	78	91	96	97	100
32.56	71	91	96	98	100
35.04	69	93	97	99	100
39.34	65	90	97	99	100

Water loss from wet seeds is faster than from dry seeds (Table A2.8). However, in the first fifteen only absolute moisture loss is faster, not relative moisture loss as a percentage of the final moisture loss after six hours. From 30 minutes onwards, both absolute and relative moisture loss are highest for highest moisture contents.

Cabrera & Mourad (1995) tested for moisture in cottonseeds of different moisture levels. It was found that after 30 hours of drying at 103°C, the amount of water extracted from intact seeds and ground seeds was not statistically different. Nevertheless, when the quantity of water removed by the oven at different sampling intervals was compared with moisture titrated by the KF titrator, moisture removed with the oven was almost always significantly lower than the moisture level detected with KF. Thus, it was concluded that 103°C is not a sufficiently high temperature to completely remove all moisture from cottonseed.
At 130°C the oven reaches the level of KF. However the time to this level depends on the moisture content of the seeds: at 8% mc it is more than 120 hours, at 12% it is 120 hours, and at 16% it is 75 hrs.

Table A2.9.:
Comparison of Karl Fischer and oven methods (Tillmann & Cicero, 1996).
Time in hours for the oven methods to reach the level of Karl Fischer.

	Karl Fischer	Oven 24 hrs 105°C whole		Oven 130°C whole		Hours to reach level of KF
Zea mays	12.0	whole				264
Zea mays	13.3	11.2				240
Zea mays	17.4	whole				72
Zea mays	13.3			whole		29
Zea mays	15.0			whole		17
Zea mays	17.1			whole		4
Glycine max	9.6	whole				27
Glycine max	10.6	10.7				15
Glycine max	17.4	whole				9
Glycine max	10.6			whole		2
Glycine max	12.4			whole		1.75
Glycine max	14.6			whole		1

At 103°C it was not possible to reach the KF level for soybean.
For both maize and soybean, wet seed reaches the KF level earlier than dry seeds do.

Table A2.10.:
Comparison methods:

- Hart et al (1959) and Hart et al (1966), methods resulting in approximately equal results to those obtained by the KF method.
- ISTA: ISTA Rules (2003)

	Hart et al (1959)	Hart et al (1966)	ISTA
Cereals	16-20 hrs 130°C (whole)		2 hrs at 130°C (ground)
Grasses	1-3 hrs at 130°C	2.5 hrs at 130°C	1 hr at 130°C
Medicago, Trifolium	2.5 hrs at 130°C		1 hr at 130°C
Allium cepa (Liliaceae)	50 mns at 130°C		17 hrs at 103°C
Linum usitassimum (Linaceae)	4 hrs at 103°C		17 hrs at 103°C
Daucus, Pastinaca, Petroselinum (Umbelliferae)	1-2 hrs at 100°C	2 hrs 100	1 hr at 130°C
Carthamus, Helianthus (Compositae)	1-3 hrs at 130°C		17 hrs at 103°C (only Helianthus)
Brassica (Cruciferae)	4 hrs at 130°C		17 hrs at 103°C
Raphanus sativus (Cruciferae)	1hr10mns at 130°C		17 hrs at 103°C
Lactuca sativa		1.5 hrs 120	1 hr 130
Beta vulgaris		2.5 hrs 110	1 hr 130
Spinacia oleracea		2h35m 130	1 hr 130
Apium graveolens		4 hrs 93	1 hr 130
Cucumis sativus		1h40m 130	1 hr 130

Hibiscus esculentus		3 hrs 130	-
Abies procera+grandis		2 hrs 100	17 hrs 103
Pseudotsuga menziesii		1h12m 100	17 hrs 103
Picea engelmanii		1h45m 130	17 hrs 103
Pinus lambertiana		1h50m 110	17 hrs 103
Pinus ponderosa		2h45m 100	17 hrs 103

Hart et al (1959) compared oven tests of whole seeds to the KF method (Table A2.10).

Table A2.11.:
Comparison of three methods on 521 samples of maize in range of moisture contents between 9-40% (Paynter & Hurburgh, 1983b).

method	Mean	variance
72 hrs 103°C, whole seeds	17.89	0.052
4 hrs 130°C, ground seeds	18.62	0.011
4 hrs 140°C, ground seeds	18.61	0.021

The ground grain oven and KF methods were significantly biased with respect to the whole grain oven. Grain dried by the ground grain oven lost more moisture than when dried by the whole grain oven. This positive bias of approximately 0.5% was most evident at moistures below 20%. Moisture values from KF were less than from the whole grain oven at moistures below 20%. The correlation coefficients were very low, showing that while the methods were biased with respect to each other, much variability existed across samples of similar moisture content.
The within method variance was greatest for KF. This high and erratic variability of the KF method was probably a result of the following experimental errors: the procedure requires numerous weighings, each adding its own measure of imprecision. Further methanol, the solvent used for extraction, is extremely hygroscopic so that exposure to air could easily alter the actual moisture content of an extraction. These factors contribute to a much greater procedural error, and hence higher variability, in the KF method than in the whole or ground grain oven methods. Variance of the whole grain oven is erratic at all moistures. Moisture measurement among ground sub-samples is extremely repeatable. The ground grain oven produced the least within-method variability of all procedures tested, even at high moisture contents (Paynter & Hurburgh, 1983b).

Don Grabe organised a referee between a number of laboratories in the United States to see whether in fact it was possible to get comparable results using the Karl Fischer apparatus just between the various laboratories. These were not entirely successful.

Table A2.12: Comparison of methods (oven at 105°C, average of Wageningen and Farnham). Source: Buszewicz (1962).

species	whole	ground	whole	ground	whole	ground	whole	ground	whole	ground
	toluene	toluene	P_2O_5	P_2O_5	oven	oven	oven	oven	oven	oven
					105°	105°	105°	105°	130°	130°
					6 hrs	6 hrs	24 hrs	24 hrs	1 hr	1 hr
Abies nobilis-1	7.00	7.50	8.43	10.18	8.34	10.11	9.08	10.51	9.05	10.85
Abies nobilis-2	16.97	16.03	18.37	20.32	17.71	19.56	18.29	19.84	18.55	20.35
Abies nobilis-3	14.63	14.47	14.97	18.01	14.75	17.17	15.72	17.33	15.40	17.90
Abies grandis-1	5.16	6.00	6.37	13.29	6.99	12.86	7.74	13.24	7.18	12.98
Abies grandis-2	5.34	5.72	6.09	12.57	6.19	12.56	6.85	12.87	6.36	12.62
Abies alba	18.41	18.44	20.21	25.48	20.83	25.86	22.10	26.32	20.54	25.55
sum	**11.25**	**11.36**	**12.41**	**16.64**	**12.47**	**16.35**	**13.30**	**16.69**	**12.85**	**16.71**
Diff ground-whole		**0.09**		**4.23**		**3.88**		**3.39**		**3.86**
diff 105-130°C									**+0.45**	**-0.02**

The results for ground Abies seeds are always much higher than those for whole seeds (Table A2.12). The discrepancy between the two 105°C methods varies with species, being least with A.nobilis and considerably greater for A.alba and A.grandis. In the latter case the results with ground seed are double as compared with whole seeds. These differences were ascribed by differences in chemical composition of the seed.
The toluene method is generally recognised as an appropriate method for seed containing nonaqueous volatile compounds. In the tests by Buszewicz (1962) the discrepancies between the toluene method and all other methods were explained in terms of loss of nonaqueous volatile materials.
From additional experiments it was concluded that there was a fairly close agreement between the result of the toluene test and those of the oven test after 1 hour at 105°C.

Zeleny (1953) concluded that for finely chopped groundnuts the following procedures resulted in similar moisture contents: 3 hours at 130°C – 16 hours at 105°C – toluene distillation.

Nehring (1952) concluded that for many organic materials 1 hour at 130°C results in similar moisture contents as 12 hours at 105°C does.

Leendertz (1948) found that the P_2O_5 drying method resulted in figures less than 1% different from 105 minutes at 130°C and to 12 hours at 105°C for many species: Lolium perenne, L.multiflorum, Raphanus sativus, Papaver somniferum, Carum carvi, Spinacea oleracea, Lactuca sativa, Cichorium intybus, Daucus carota, Petroselinum sativum, Linum usitatissimum, Allium cepa, Pinus sylvestris, Beta vulgaris.

Table.A2.13.:
Maximum moisture content of whole seeds as affected by temperature (after Hart et al, 1959)

Temperature (°C)	wheat (vacuum oven)	maize (aire oven)	flax (air oven)
94		10.60	
96		10.80	
98.5		10.90	
100		10.97	7.64
103	12.01	11.31	
105	12.05	11.40	7.78

110	12.30		7.90
120			8.12
130			8.18

Table A2.14.:
Moisture content of ground cereals under different drying conditions (average of 7 cereal samples) (Oxley et al, 1960)

method	dry	medium	wet
A = 105°C, 16 hrs, LTE still air oven	12.24	14.64	18.12
B = 113°C, 4 hrs, Gallenkamp ventilated oven	12.36	14.78	18.23
C = 120°C, 4 hrs, LTE still air oven	12.29	14.72	18.33
D = 120°C, 4 hrs, Gallenkamp ventilated oven	12.56	14.96	18.45
E = 130°C, 1.5 hrs, Brabender semi-automatic moist.tester.	12.70	14.97	18.50

The only difference between methods C and D is the type of oven. The ventilated oven results in 0.27-0.24-0.12 higher moisture contents (Table A2.14). Other methods differ in more than one aspect from each other, so effects are confounded.
In addition, there was a significant method and species interaction. Harder, more vitreous materials gave a narrower range of differences between methods. Conversely, soft, non-vitreous materials gave a wider range.

Table A2.15. :
Moisture content of three lots of maize of different moisture content, as determined by using 2 * 5 g of whole seeds (except for TCAO 103) at different drying schedules in two oven types (Christensen et al, 1992).
CAO 130 = circulation air oven at 130°C, CAO 103 = circulation air oven at 130°C , TCAO 103 = two-stage drying in circulation air oven at 103°C, VO = vacuum oven at 95-90°C.

Oven	time (hrs+min)	sample 1	sample 2	sample 3
CAO 130	00 40	04.85	09.91	14.00
CAO 130	01 00	06.01	11.47	15.69
CAO 130	03 00	07.74	13.03	17.27
CAO 130	24 00	10.45	14.45	17.59
CAO 130	48 00	10.54	15.65	19.67
CAO 130	72 00	11.25	15.95	19.86
CAO 103	72 00	09.73	15.15	18.94
TCAO 103	72 00	10.33	14.92	19.32
VO	04 00	06.52	13.22	17.20
VO	12 00	08.37	14.45	18.01
VO	24 00	09.36	15.07	18.13
VO	48 00	10.33	15.58	19.42

Grabe (1989a and 1989b) lists methods for whole and ground seeds of several species (Table A2.16).

Table A2.16.:
Comparison of seed moisture methods for whole and ground seeds (Grabe, 1989a and 1989b). KF = Karl Fischer, Va = Vacuum-P_2O_5, Va-100 = Vacuum – 100°C.

species	whole / ground	sample size (g)	temperature (°C)	drying period (hrs-min)	reference method	reference
Avena sativa	whole	10	130	23-00	KF	Hart et al, 1959
	ground	5	130	02-00	Va	IACC, 1976
Hordeum vulgare	whole	10	130	20-00	KF	Hart et al, 1959
	ground	5	130	02-00	Va	IACC, 1976
Secale cereale	whole	10	130	16-00	KF	Hart et al, 1959
	ground	5	130	02-00	Va	IACC, 1976
Sorghum vulgare	whole	10	130	18-00	KF	Hart et al, 1959
	ground	2-3	130	02-00	Va-100	AACC, 1979
Triticum aestivum	whole	10	130	19-00	KF	Hart et al, 1959
	ground	5	130	02-00	Va	IACC, 1976
Zea mays	whole	15	103	72-00	Va-100	AACC, 1979
	ground	8	130	04-00	Va	IACC, 1976

Samples of the ISTA Comparative Testing of 2000 (see Table A2.3) were used for a methodology study by one lab (Nijënstein, not published). The results can be found in Table A2.17.

Table A2.17.:
Moisture content for different methods in one lab; wh= whole seeds, gr=ground seeds.

lab	oat 1	oat 1	oat 4	oat 4	oat 6	oat 6	wheat2	wheat2	wheat3	wheat3	wheat5	wheat5
	wh	gr	wh	gr	wh	gr	wh	gr	wh	gr	wh	gr
hr/temp												
1-130	14.83	16.44	14.97	16.21	10.20	12.09	11.47	14.83	7.98	11.54	11.18	14.65
2-130	*15.75*	*16.64*	*15.87*	*16.58*	*11.16*	*12.36*	*12.41*	*15.07*	*9.09*	*11.71*	*12.41*	*14.89*
19-130	17.24	17.17	17.35	17.23	13.02	13.12	15.28	15.64	11.95	12.37	15.18	15.57
28-130	17.34	17.29	17.41	17.33	13.10	13.25	15.55	15.76	12.06	12.52	15.44	15.63
72-103	16.77	16.87	16.89	16.85	12.53	12.62	14.81	14.82	11.51	11.71	14.94	15.00
192-103	17.14	17.15	17.25	17.09	12.79	12.89	15.28	15.19	12.05	11.99	15.37	15.23
mean	**16.5**	**16.9**	**16.6**	**16.9**	**12.1**	**12.7**	**14.1**	**15.2**	**10.8**	**12.0**	**14.1**	**15.2**
diff.		**0.4**		**0.3**		**0.6**		**1.1**		**1.2**		**1.1**
variation min-max	**14.8-17.3**	**16.4-17.3**	**15.0-17.4**	**16.2-17.3**	**10.2-13.1**	**12.1-13.3**	**11.5-15.6**	**14.8-15.8**	**8.0-12.1**	**11.5-12.5**	**11.2-15.4**	**14.7-15.6**
variation ground		**0.9**		**1.1**		**1.2**		**1.0**		**1.0**		**0.9**
variation whole	**2.5**		**2.4**		**2.9**		**4.1**		**4.1**		**4.2**	
Difference ISTA-other methods												
hr/temp												
1-130	***0.92***	***0.20***	***0.90***	***0.37***	***0.96***	***0.27***	***0.94***	***0.24***	***1.11***	***0.17***	***1.23***	***0.24***
2-130	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***

19-130	***-1.49***	***-0.53***	***-1.48***	***-0.65***	***-1.86***	***-0.76***	***-2.87***	***-0.57***	***-2.86***	***-0.66***	***-2.77***	***-0.68***
28-130	***-1.59***	***-0.65***	***-1.54***	***-0.75***	***-1.94***	***-0.89***	***-3.14***	***-0.69***	***-2.97***	***-0.81***	***-3.03***	***-0.74***
72-103	***-1.02***	***-0.23***	***-1.02***	***-0.27***	***-1.37***	***-0.26***	***-2.40***	***0.25***	***-2.42***	***0.00***	***-2.53***	***-0.11***
192-103	***-1.39***	***-0.51***	***-1.38***	***-0.51***	***-1.63***	***-0.53***	***-2.87***	***-0.12***	***-2.96***	***-0.28***	***-2.96***	***-0.34***

Average differences ISTA – other methods.

hr/temp	whole	ground
1-130	1.01	0.25
2-130	0.00	0.00
19-130	-2.22	-0.64
28-130	-2.37	-0.76
72-103	-1.79	-0.10
192-103	-2.20	-0.38

The difference between one and two hours at 130°C is between 0.17 and 1.23%. On average, the difference for ground seed is 0.25% and for whole cereal seeds is 1.01%.

Table A2.18.:
Mean moisture content (dry basis) for each oven method (standard deviation in parenthesis), based on 20 replicates per sample.
ISO712 = 2 hrs 130-133°C, ground, 5 g
ASAE S352 = 19/20 (wheat/barley) hrs 129-131°C, whole, 15 g
NIAE = 16 hrs 129-131°C, whole, 10 g.
(Bowden, 1984)

ISO	ASAE	NIAE	ISO	ASAE	NIAE
barley	barley	barley	wheat	wheat	wheat
ground	whole	whole	ground	whole	whole
10.49(0.051)	09.86(0.029)	09.63(0.029)	10.01(0.092)	09.21(0.041)	09.06(0.040)
13.49(0.080)	13.01(0.033)	12.76(0.034)	13.85(0.089)	13.21(0.054)	13.09(0.054)
18.87(0.069)	18.60(0.038)	18.40(0.046)	18.91(0.107)	18.62(0.060)	18.41(0.050)
25.12(0.067)	24.83(0.049)	24.17(0.040)	25.09(0.102)	24.55(0.061)	24.45(0.066)
31.61(0.075)	31.12(0.051)	30.91(0.070)	31.79(0.134)	31.21(0.074)	31.01(0.093)
38.71(0.094)	38.28(0.060)	37.99(0.082)	39.29(0.173)	38.64(0.133)	38.61(0.128)
average					
23.05(0.073)	**22.62(0.043)**	**22.31(0.050)**	**23.16(0.116)**	**22.57(0.071)**	**22.44(0.072)**
difference ISO-ASAE	**difference ISO-NIAE**	**difference ASAE-NIAE**	**difference ISO-ASAE**	**difference ISO-NIAE**	**difference ASAE-NIAE**
0.63	0.86	0.23	0.80	0.95	0.15
0.48	0.73	0.25	0.64	0.76	0.12
0.27	0.47	0.20	0.29	0.50	0.21
0.29	0.95	0.66	0.54	0.64	0.10
0.49	0.70	0.21	0.58	0.78	0.20
0.43	0.72	0.29	0.65	0.68	0.03
average					
0.43	**0.74**	**0.31**	**0.59**	**0.72**	**0.13**

The effect of 3-4 hours extra drying of whole seeds, the difference between the ASAE duration of 19-20 hours and NIAE of 16 hours is 0.13 to 0.31%.
A drying period of 2 hours at 130°C resulted in higher moisture percentages than drying whole seeds at the same temperature for a longer period. This difference between the ISO-method and both other methods is 0.43 to 0.74 %.

Looking at the differences between ISO and the other two methods, it is obvious that there was a distinct difference between the results below and above 20.48% moisture content. Below this value the deviations decrease as the moisture content level increases. This may be due to the milling process of the ISO. As the sample nominal moisture content increases possible more moisture is lost in the mill with a corresponding reduction in indicated moisture content (Bowden, 1984). Alternative possibilities are faster release of moisture from wetter material or chemical reactions resulting in the production of water occurring at higher moisture levels.
The linear regressions showed that for both barley and wheat the standard deviations, at each moisture content level, were greatest for the ISO method followed byh the NIAE then the ASAE method. The latter two had smaller and less consistent difference between them. Some of these differences could be due to differences in sample size and oven drying time. This would indicate that the ASAE method gave more consistent results, over the range of moisture content levels, than the NIAE or ISO methods (Bowden, 1984).

Table A2.19.:
Comparison of two moisture methods for rough rice (Noomhorm & Verma, 1982):
- AOAC = 1 hr 129-131°C, ground, 3 reps of 3 g.
- whole grain = 16 hrs 129-131°C, whole, 3 reps of 3 g.

level	AOAC	whole	difference
1	18.40	19.50	1.10
2	17.63	18.44	0.81
3	16.52	17.36	0.84
4	15.68	16.20	0.52
5	15.01	15.74	0.73
6	13.10	14.82	1.72
7	12.60	12.88	0.28
8	11.74	11.82	0.08
9	10.85	11.24	0.39
avg	**14.61**	**15.33**	**0.72**

Whereas in Table .. for barley and wheat the whole grain method results in lower moisture percentages compared to ground, in Table .. for rough rice the opposite is the case. In the latter experiments the whole grain method showed a significant difference from the AOAC method at a probability level of less than 0.01. This may have been due to moisture loss during the grinding.
In the experiments of Noomhorn et al there is little evidence for an interaction between moisture level and difference between the two methods.

Table A2.20.:
Comparison of two methods for moisture determination barley and wheat:
- 1 hr 130°C, ground
- 16 hrs 130°C, whole

(estimated from graphs of Matthews, 1962).

barley ground	barley whole	barley difference	wheat ground	wheat whole	wheat difference
08.00	07.53	-0.43	08.00	07.77	-0.23
10.00	09.63	-0.37	10.00	09.82	-0.18
12.00	11.69	-0.31	12.00	11.88	-0.12
14.00	13.75	-0.25	14.00	13.93	-0.07

16.00	15.81	-0.19	16.00	15.98	-0.02
18.00	17.86	-0.14	18.00	18.03	+0.03
20.00	19.91	-0.09	20.00	20.08	+0.08
22.00	21.96	-0.04	22.00	22.12	+0.12
24.00	24.00	0.00	24.00	24.16	+0.16
26.00	26.04	+0.04	26.00	26.20	+0.20
28.00	28.08	+0.08	28.00	28.28	+0.24
30.00	30.13	+0.13	30.00	30.27	+0.27
mean					
19.00	**18.87**	**-0.13**	**19.00**	**19.04**	**0.04**

There is a clear interaction between moisture level, differences between methods and species. At approximately 24% for barley and at 17% for wheat, both methods gave similar results. At 8% the 1 hr method gave values of up to 0.4% higher, the error being greatest for barley. At the higher moisture levels the differences were greatest for wheat.

Table A2.21.:
Comparison of moisture oven methods for rough rice (estimated from the equations in Chen, 2003). Quantities tested: 15 g if mc < 25%, 100 g if mc >25%.

	ASAE					
seed	whole	whole	whole	whole	ground	ground
hrs	72	48	20	16	1	5
temp.	105	105	130	130	135	105
	10.0	10.1	10.6	10.5	10.3	10.3
	15.0	15.0	15.6	15.5	15.7	15.8
	20.0	19.8	20.6	20.5	21.1	21.2
	25.0	24.7	25.6	25.5	26.5	26.7
	30.0	29.5	30.6	30.5	31.9	32.2
mean	**20.0**	**19.8**	**20.6**	**20.5**	**21.1**	**21.2**

For the whole seeds there doesn't appear to be an interaction between method and moisture level. For ground seed there is an interaction: at higher moisture levels the difference between whole and ground seeds are bigger.
Drying whole kernels for 72 hours at 105°C had the smallest standard deviation (Chen, 2003).

The size of the test portion is critical in moisture determination. Portions of ground wheat ranging from 3-10 grams were shown to give results ranging from 12.3% for a 3 g sample, to 12.06% for a 10 g sample using dishes 55 mm in diameter and 15 mm high. However, even with larger dishes (83 x 16 mm) samples of material weighing more than 5 g gave low moisture content values (Hunt & Neustadt, 1966).

In determining the moisture content of milled wheat, at a nominal 15% mc, by oven drying at 130°C for 1 hr Warner and Browne (1963) showed that there were variations in indicated moisture content among locations on individual oven shelves of up to 0.2% mc. Among locations on all shelves within an oven differences of 0.4% mc occurred, and between the means of two ovens of 0.2% mc.

The great variation in moisture content among individual acorns seems to be inherent with this genus. Pockets of insect and disease damage, thick spongy pericarps (on some species) and premature sprouting in storage may all contribute to this variation. This variation makes it practically impossible to meet the ISTA requirement of duplicate determinations not differing by more than 0.2% (Bonner, 1974).

Pande (1974) and Henderson (1991) give estimations for a number of sources of error:

- Particle size variation after grinding: 1%.
- Loss of moisture due to heat produced by grinding: 0.2-0.5%.
- Temperature gradients of variations within the oven: 0.2-0.4%.
- Day to day variation in ambient humidity, temperature and barometric pressure variations: 0.2%.
- Buoyancy effect due to the warm air enclosed with the sample: 0.04%.
- Difference in temperature at the time of weighing: 0.02%.
- Temperature fall during weighing (2-3°C): 0.03%.

Henderson concludes however, that the error inherent in sampling is potentially far greater than all errors due to measurement put together: sub-sampling, speed, condensation when removing samples from a refrigerator prior to mc determination.

Table A2.22.:
Comparison of moisture methods for some species of International organisations. Drying period: hrs + temperature at which timing should start after placing samples in the oven.

Species	whole/ ground	sample size (g)	temperature (°C)	drying period (hrs + temp)	estimated diff. to ISTA	organisation
Zea mays	whole	10	130-133	36-40 (130)		ISO 6540
Zea mays	whole	10	130-133	36-40 (none)		ICC 135
Zea mays	whole	15/100	103	72 (102)		USDA
Zea mays	whole	15/100 (****)	103	72 (none)		ASAE S352.2
Zea mays	ground	>8	130-133	4 (130)		ICC 110
Zea mays	ground	8	130-133	4 (130)		ISO 6540
Zea mays	ground	5	130	4 (130)	--	ISTA 2003
Zea mays	ground	15	102-104	72 (none)		AACC, 1999
Zea mays	ground	?	127-133	3		AOAC
cereals	whole	10	130	16-22 (none)		ASAE S352.2
cereals	ground	>5	130-133	2 (130)		ICC 110
feeding stuffs (***)	ground	5	130	2 (130)		EU
cereals	ground	5	127-133	2 (127)		ISO 712
cereals	ground	5	130	2 (130)	--	ISTA 2003
cereals	ground	2.5	130	1 (129)		USDA
cereals	ground	15	129-131	1 (none)		AACC, 1999
cereals	ground	?	127-133	3		AOAC
oil seeds	ground/ whole (*)	5	101-105	3 (**) (103)		ISO 665
oil seeds	ground/ whole	5	101-105	17 (103)	--	ISTA 2003
soybeans	whole	15	103	72 (none)		ASAE S352.2
oil seeds	ground	2.5	130	1 (129)		USDA
soybeans	ground	15	129-131	72 (none)		AACC, 1999
mustard, canola, rape	whole	10	103	1 (102)		USDA
mustard, canola, rape					--	ISTA 2003
mustard, canola, rape	whole	10	130	4 (none)		ASAE S352.2
flax	whole	5-7	103	4 (102/none)		USDA/ASAE
flax					--	ISTA 2003
flax	ground	5-7	102-104	4 (none)		AACC, 1999

safflower	whole	10	130	1 (129)		USDA/ASAE
safflower					--	ISTA 2003
sunflower	whole	10	130	3 (129/none)		USDA/ASAE
sunflower					--	ISTA 2003

(*): Small seeds (linseed, colza, hemp, etc) as well as safflower seed, sunflower seed, soybeans and cottonseed with adherent linters, are analysed without previous grinding.
(**): Return vessels to oven, after 1 hour repeat weighing. Subject the test portion to successive 1 hour periods in the oven, until the difference between two successive weighings is equal to or less than 0.005 g.
(***): For feedingstuffs composed predominantly of oils and fats, dry in the oven for an additional 30 minutes at 130°C. The difference between the two weighings must not exceed 0.1% of moisture.
(****): 100 g if moisture exceeds 25%.

In Brazil the standard method for moisture determination of all species is 24 hours at 105°C, whole seeds (no grinding!) (Tillman, pers.comm.).

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