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ORIGINAL ARTICLE

Cereals, cereals-based products and animal feeding stuffs – determination of crude fat and total fat content by the Randall extraction method: a collaborative study

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Keywords
crude fat; total fat; Randall/Soxtec submersion method; cereals; feed; collaborative study.

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Abstract

A method for determining crude fat (fat A) and total fat (fat B) in cereals, cereal products and animal feeding stuff using 11 cereal and feed samples has been collaboratively studied, involving 15 participants from nine countries. The samples analyzed covered a range from about 0.5% to 26% crude fat (fat A) and from 1% to 27% total fat (fat B). Relative repeatability standard deviations ranged from 0.6% to 8.5% for crude fat and from 0.6% to 4.4% for total fat. The relative reproducibility standard deviations ranged from 1.0% to 22.6% for crude fat and from 2.6% to 13.4% for total fat. Analyzed samples comprised parboiled rice, whole wheat kernels, rye flour, sorghum kernels, couscous (durum wheat), a multicorn flourmix, croutons, cornbread, cattle feed, chickenfeed and pig feed. On basis of the obtained results the studied method has been published as international standard.

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Introduction

The submersion method according to Randall (1974) for the determination of fat has recently been collaboratively studied for the determination of crude fat in animal feed (Thiex *et al*., 2003a,b) and AOAC Official methods of analysis for the determination of crude fat in animal feed using diethyl ether (AOAC, 2003a) and hexane (AOAC, 2003b) have been published.

In combination with an acid hydrolysis step, the method is also widely spread for the analysis of total fat in foodstuff including cereals and cereal products.

In connection with the revision of the standard method ISO 7302:1982 Cereals and cereal products – Determination of total fat content – it showed that the method was no longer in use and that some countries opted for withdrawal of the standard. The matter was discussed within the technical committee (TC) 34 (Food Products)/Subcommittee (SC) 4 (Cereals and Pulses) of the International Organization for Standardization (ISO) and it was decided to develop a new standard on basis of the Randall submersion method including procedures with and without acid hydrolysis and specifying petroleum ether as a solvent. It was further decided to withdraw the ISO 7302 standard on publication of the new standard.

After ISO TC 34/SC 10 (Animal feeding stuff) joining the project, the scope was extended to include also animal feeding stuff.

A publication of the collaborative study report is of interest as the new standard has been issued (ISO 11085:2008).

Materials and methods

The studied method specifies a procedure for the determination of the fat content of cereals and cereal products as

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well as animal feeding stuff. The method is not applicable to oilseeds and oleaginous fruits.

The method describes two procedures.

Procedure A – directly extractable crude fats

Fat is extracted using light petroleum as a solvent and the Randall modification of the Soxhlet method. The test portion is submerged in boiling solvent before rinsing in cold solvent, reducing the time needed for extraction. The solvent dissolves fats, oils, pigments and other soluble substances. After extraction the solvent is evaporated and recovered by condensation. The resulting fat residue is determined gravimetrically after drying.

This method is applicable to all materials, except those included within the scope of procedure B.

The crude fat content is being defined as the mass fraction of substances extracted from the sample by the procedure A.

Procedure B – total fats

For total fat determination the sample is treated under heating with hydrochloric acid. Hydrolysis makes chemically or mechanically bound fats accessible to solvent extraction. The mixture is cooled and filtered. The residue is washed and dried and submitted to the above extraction procedure. For samples with a relative high fat content (at least 100 g kg^{-1}) a preliminary extraction has to be made.

This method is applicable to all materials from which the oils and fats cannot be completely extracted without prior hydrolysis.

The total fat content is being defined as the mass fraction of substances extracted from the sample by the procedure B.

In both cases the fat content is expressed in grams per kilogram or as a mass fraction in percent.

The use of the described procedures depends on the nature and composition of the material analyzed and the reason for carrying out the analysis.

Method details are described in (ISO 11085:2008).

Design of the study

The study was designed in accordance with IUPAC/AOAC/ISO/CEN protocol (ISO 5725-2:1994). Participating laboratories had to be nominated by their respective national standardization bodies and had to return an application form, confirming their participation and qualification for this test.

In total 15 laboratories from nine countries participated in this study.

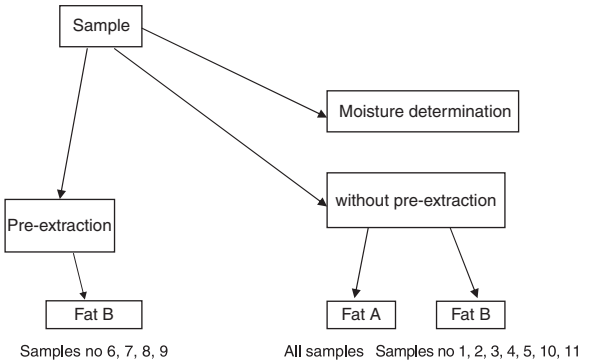

Figure 1 Analysis scheme.

All laboratories received a copy of the method to be validated (ISO 11085:2008), an analysis scheme (see Figure 1), 11 test samples as duplicates and a reporting scheme.

The cereal and cereal products samples chosen were (1) parboiled rice, (2) whole kernel wheat, (3) rye flour, (4) sorghum kernels, (5) couscous – durum semolina, (6) multicorn flour mix, (7) croutons and (8) cornbread. They covered a range of about 0.5–29 % fat. In addition three feed samples, (9) cattle feed mixture, (10) chicken feed mixture and (11) pig feed mixture, were selected. About 1 kg of each sample was ground using a Cyclotec cyclone mill with 1 mm sieve or a Retsch ZM1 with a 1 mm sieve. The homogenous flour was subdivided and packed into plastic bags, sealed airtight in aluminium foil. The moisture content of the samples was between 5% and 13%.

No specific homogeneity tests were performed.

The stability was tested by determining the fat content before and after the validation period. No significant changes in the fat content were found.

All samples had to be analyzed for moisture content according to ISO 712 (ISO 712:2009), as well for fat A (crude fat). If the moisture content was higher than 10% the thimbles with the samples had be pre-dried for 2 h at 103 °C.

For fat B content (total fat) samples no 6, 7, 8 and 9 had to be pre-extracted, as they had a fat content of about 10% or higher. The samples no 1, 2, 3, 4, 5, 10 and 11 could be analyzed without pre-extraction for fat B content (total fat).

Each participating laboratory received about 50 g per sample to be analyzed according to the scheme in Figure 1.

Results

Reported results are compiled in Tables 1 and 2.

Fifteen participants submitted results for crude fat (procedure A). Two laboratories used the traditional Soxhlet

method instead of the described method according to Randell and were therefore excluded from the statistical evaluation. Additional results were excluded after outlier tests according to Cochran and Grubbs (ISO 5725-2:1994).

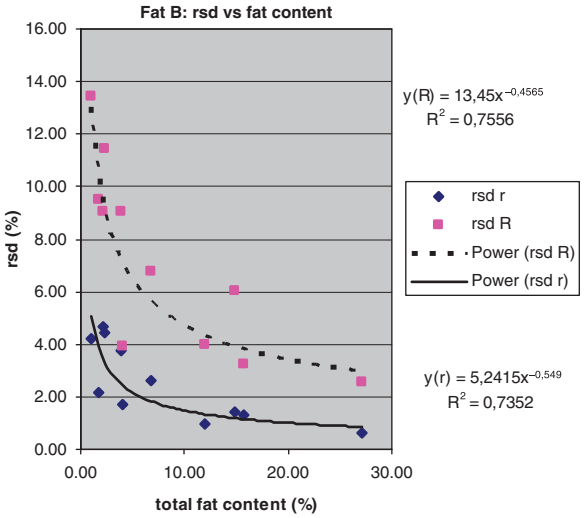

Figure 2 Relative errors (%) as a function of the total fat content (procedure B).

Fourteen laboratories submitted results for the total fat content. Two laboratories were excluded from the statistical evaluation due to non-compliance with the drafted method. The data of the remaining 12 labs were submitted to outlier tests according to Cochran and Grubbs (ISO 5725-2:1994).

The statistical evaluation was made in accordance with ISO 5725-2 (ISO 5725-2:1994) using the Excel spreadsheet CLSTD.XLT version 4.0 from Ken Mathieson, CSL, York, UK. The results are summarized in Tables 3 and 4.

Discussion and conclusions

Figure 2 shows the relative standard deviations for the repeatability and reproducibility as a function of the fat concentration for the determination of total fat (procedure B). As expected the method performance is decreasing with decreasing fat content.

The performance of the studied method (ISO 11085:2008) is comparable to the method ISO 7302:1982.

Table 1 Compilation of submitted results (on dry matter basis) – procedure A (crude fat)

		Sample										
Lab	Value	1	2	3	4	5	6	7	8	9	10	11
1	1	0.48	1.75	1.41	3.52	0.91	11.38	13.99	25.72	10.98	6.26	2.95
	2	0.45	1.72	1.40	3.49	0.78	11.35	13.97	25.82	10.97	6.32	2.94
2	1	0.59	1.79	1.53	3.51	0.96	11.39	13.91	25.84	12.29	6.45	3.18
	2	0.58	1.78	1.49	3.39	0.97	11.35	14.00	25.65	12.20	6.42	3.25
3	1	0.77	2.26	1.87	3.81	1.03	11.62	14.28	26.09	12.57	6.41	3.09
	2	0.68	2.04	1.67	3.82	1.05	11.43	14.09	25.69	12.21	6.56	3.06
5	1	0.27	1.51	1.27	3.39	0.67	11.32	14.05	26.02	13.60	5.93	2.74
	2	0.36	1.53	1.29	3.30	0.78	11.34	14.09	25.79	13.31	5.95	2.84
6	1	0.49	1.72	1.48	3.55	0.80	11.33	14.05	25.75	12.15	6.31	2.98
	2	0.45	1.73	1.46	3.51	0.79	11.31	13.97	25.85	12.11	6.28	2.92
7	1	0.45	1.49	1.23	3.30	0.67	11.18	13.95	25.98	11.65	5.81	2.76
	2	0.45	1.49	1.01	3.19	0.67	11.07	14.48	26.09	11.65	5.58	2.64
8	1	0.45	1.60	1.05	3.19	0.83	11.38	13.98	25.72	11.66	6.29	2.97
	2	0.48	1.62	1.19	3.32	0.81	11.32	13.78	24.99	11.86	6.29	2.80
9	1	0.46	1.70	1.46	3.54	0.84	11.56	14.14	26.01	11.90	6.42	3.01
	2	0.48	1.72	1.44	3.68	0.85	11.66	14.24	25.94	11.92	6.40	3.04
10	1	0.45	1.82	1.57	3.63	0.89	11.37	14.25	25.80	12.12	6.33	2.98
	2	0.57	1.82	1.57	3.63	0.89	11.37	14.25	25.80	12.12	6.33	2.98
12	1	0.12	1.24	0.78	3.38	0.89	11.29	13.90	23.68	11.60	6.06	2.71
	2	0.01	1.13	1.10	3.40	1.05	11.07	13.73	25.12	11.40	6.11	2.98
13	1	0.11	1.26	0.90	3.19	0.78	11.41	13.62	25.53	11.60	6.58	3.10
	2	0.11	1.14	0.90	3.19	0.78	11.52	13.73	25.53	11.60	6.69	3.10
14	1	0.50	1.74	1.39	3.58	0.89	11.53	14.22	26.07	12.06	6.36	3.01
	2	0.45	1.71	1.38	3.46	0.88	11.49	14.33	25.98	11.86	6.30	3.01
15	1	0.42	1.68	1.37	3.31	0.81	11.17	13.75	25.41	12.32	6.04	2.93
	2	0.41	1.59	1.30	3.30	0.77	11.21	13.90	25.49	11.87	5.98	2.93

Table 2 Compilation of submitted results (on dry matter content) – procedure B (total fat)

		Sample										
Lab	Value	1	2	3	4	5	6	7	8	9	10	11
1	1	1.10	2.48	1.81	4.07	2.19	12.31	15.95	27.23	15.32	7.19	3.85
	2	1.02	2.37	1.74	3.86	2.02	11.99	15.96	27.22	15.11	6.94	3.88
2	1	0.97	2.23	1.70	3.99	2.50	12.27	16.20	27.47	16.03	6.95	4.18
	2	0.95	2.26	1.68	3.87	2.48	12.22	16.17	27.50	15.65	7.02	4.18
3	1	1.14	2.25	1.63	3.85	2.13	12.77	15.91	27.08	14.77	6.79	3.88
	2	1.15	2.04	1.59	3.89	2.18	12.69	16.16	27.60	15.23	6.99	3.60
5	1	1.34	3.00	2.16	4.07	2.12	12.17	16.68	27.54	15.83	6.95	4.41
	2	1.27	2.63	2.05	4.24	2.40	12.12	16.62	28.12	15.91	7.14	4.13
6	1	1.10	2.43	1.86	3.90	2.15	12.14	15.99	27.43	15.42	6.92	3.72
	2	1.11	2.50	1.85	3.91	2.12	12.09	15.90	27.32	15.27	6.83	3.69
7	1	0.79	2.06	1.57	3.87	1.88	11.29	14.81	26.20	12.75	6.15	4.14
	2	0.79	1.94	1.57	3.99	2.00	11.18	15.45	26.31	13.08	6.49	3.91
9	1	0.97	2.39	1.68	3.90	2.14	12.04	15.92	27.34	15.07	6.64	3.84
	2	0.99	2.40	1.68	3.82	2.06	12.05	15.87	27.34	14.96	6.67	3.77
10	1	1.13	2.50	1.90	3.98	2.22	11.92	15.94	27.41	15.22	6.90	3.79
	2	1.13	2.50	1.90	3.98	2.22	12.03	15.94	27.52	15.12	7.01	3.90
12	1	0.89	1.98	1.62	3.20	2.48	12.41	15.68	28.72	13.31	6.54	3.70
	2	1.16	2.36	1.85	3.40	2.44	12.46	15.99	29.39	13.38	6.52	3.58
13	1	1.14	2.74	1.91	4.32	2.11	12.28	15.42	27.04	15.14	7.83	4.48
	2	1.14	2.74	1.91	4.32	2.22	12.18	15.52	27.04	15.14	7.71	4.48
14	1	1.23	2.57	1.93	4.22	2.46	12.10	18.68	27.34	15.37	7.14	7.27
	2	1.16	2.55	1.89	4.05	2.51	12.16	16.20	27.39	15.35	7.34	4.17
15	1	1.13	2.34	1.87	3.95	2.24	11.97	15.29	26.92	15.14	6.74	3.89
	2	1.13	2.29	1.87	3.94	2.01	11.72	15.79	26.93	14.79	6.22	3.78

Table 3 Results of statistical analysis for crude fat – procedure A

	1	2	3	4	5	6	7	8	9	10	11
Sample	Rice, parboiled	Wheat kernels	Rye flour	Sorghum kernels	Durum (Couscous)	Flour mix	Crou-tons	Corn-bread	Cattle feed	Chicken feed	Pig feed
Number of laboratories	13	13	13	13	13	13	13	13	13	13	13
Number of laboratories retained after elimination of outliers	11	13	13	13	13	12	12	11	11	13	13
Mean of fat content, procedure A (crude fat in g 100 g^{-1})	0.481	1.621	1.316	3.412	0.842	11.362	13.969	25.773	11.943	6.19	2.928
Repeatability standard deviation (s_r) (g 100 g^{-1})	0.041	0.058	0.092	0.058	0.046	0.070	0.137	0.193	0.145	0.063	0.073
Repeatability relative standard deviation (s_r) %	8.5	3.5	6.9	1.7	5.5	0.6	1.0	0.8	1.2	1.0	2.5
Repeatability limit r ($r = 2.8 \times s_r$) (g 100 g^{-1})	0.114	0.161	0.257	0.164	0.129	0.204	0.384	0.542	0.407	0.177	0.203
Horrat value Ho$_r$	2.9	1.4	1.3	0.78	2.0	0.35	0.55	0.46	0.67	0.51	1.1
Reproducibility standard deviation (s_R) (g 100 g^{-1})	0.109	0.258	0.259	0.210	0.111	0.157	0.137	0.270	0.303	0.324	0.182
Reproducibility relative standard deviation (s_R) %	22.6	15.8	19.5	6.2	13.1	1.4	2.3	1.0	2.5	5.2	6.2
Reproducibility limit R (g 100 g^{-1})	0.304	0.723	0.725	0.588	0.310	0.440	0.891	0.757	0.849	0.906	0.509
Horrat value Ho$_R$	5.1	4.3	2.7	3.4	3.2	0.5	0.85	0.43	0.92	1.72	1.8

Table 4 Results of statistical analysis for total fat – procedure B

	1	2	3	4	5	6	7	8	9	10	11
Samples	Rice, parboiled	Wheat kernels	Rye flour	Sorghum kernels	Durum (Couscous)	Flour mix	Crou-tons	Corn-bread	Cattle feed	Chicken feed	Pig feed
Number of participating laboratories	12	12	12	12	12	12	12	12	12	12	12
Number of laboratories retained after elimination of outliers	12	12	12	10	12	12	9	11	12	12	11
Mean of total fat content (procedure B) in g 100 g^{-1}	1.066	2.366	1.780	4.003	2.193	12.035	15.751	27.080	14.872	6.813	3.883
Repeatability standard deviation (s_r) (g 100 g^{-1})	0.045	0.105	0.039	0.069	0.103	0.118	0.203	0.170	0.215	0.178	0.146
Repeatability relative standard deviation (s_r) %	4.2	4.4	2.2	1.7	4.7	1.0	1.3	0.6	1.4	2.6	3.8
Repeatability limit r ($r = 2.8 \times s_r$) (g 100 g^{-1})	0.125	0.293	0.109	0.193	0.288	0.330	0.567	0.476	0.601	0.498	0.302
Horrat value Ho$_r$	1.6	1.9	0.9	0.8	2.0	0.5	0.7	0.4	0.8	1.3	1.8
Reproducibility standard deviation (s_R) (g 100 g^{-1})	0.143	0.271	0.169	0.158	0.199	0.480	0.511	0.698	0.896	0.463	0.351
Reproducibility relative standard deviation (s_R) %	13.4	11.5	9.5	3.9	9.1	4.0	3.2	2.6	6.0	6.8	9.0
Reproducibility limit R ($R = 2.8 \times s_R$) (g 100 g^{-1})	0.401	0.759	0.475	0.442	0.557	1.343	1.432	1.954	2.509	1.295	0.982
Horrat value Ho$_R$	3.4	3.3	2.6	1.2	2.6	1.4	1.2	1.1	2.3	2.3	2.8

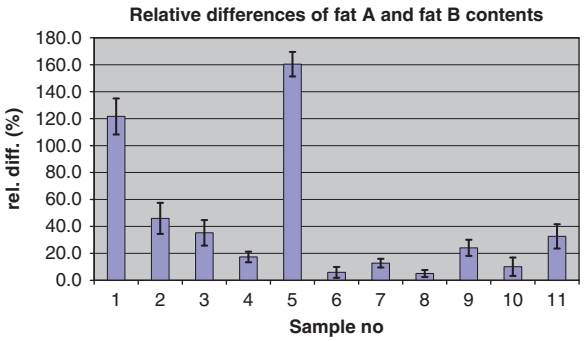

Figure 3 Comparison of relative differences between total and crude fat (error bars indicate ± 1 relative standard deviation of reproducibility.

The Horrat values given in Tables 3 and 4 may lead to the conclusion that the studied method is not fit for purpose. As discussed by Horwitz (1990) the inherent variability of gravimetric methods, in which the analyte is defined empirically, may limit the application of Horrat values. A comparison with the AOAC method no 2003.06 (Thiex *et al.*, 2003b) shows for comparable samples no significant differences of obtained Horrat values. The AOAC method 2003.06 does also utilize the Randall principle for fat extraction, i.e. a hot extraction step in boiling solvent with a subsequent rinsing step with refluxing solvent. The difference is that the AOAC method does not apply a hydrolysis step and thus only determines the crude fat.

A comparison of crude and total fat contents shows that for all samples the total fat content was higher than the crude fat content. The differences were highest for cereals and some feeds and lowest for processed cereal products (Figure 3).

On basis of the obtained results the studied method has been published as international (ISO 11085:2008) and European standard (EN ISO 11085:2010).

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Institute, SVA, Uppsala, Sweden; Hope Kamusiime, Uganda National Bureau of Standards (UNBS), Kampala, Uganda.

References

AOAC. (2003a) Official Method of Analysis, crude fat, diethyl ether extraction, Randall method. Available at http://www.eoma.aoac.org [Last accessed May 2003].

AOAC. (2003b) Official Method of Analysis, crude fat, hexane extraction, Randall method. Available at http://www.eoma.aoac.org [Last accessed June 2003].

EN ISO 11085:2010. Cereals, cereals-based products and animal feeding stuffs – Determination of crude fat and total fat content by the Randall extraction method.

Thiex N.J., Anderson S., Gildemeister B. (2003a) Crude fat, diethyl ether extraction, in feed, cereal grain, and forage (Randall/Soxtec/Submersion Method): collaborative study. *Journal of AOAC International*, **86**, 888–898.

Thiex N.J., Anderson S., Gildemeister B. (2003b) Crude fat, hexanes extraction, in feed, cereal grain, and forage (Randall/Soxtec/Submersion Method): collaborative study. *Journal of AOAC International*, **86**, 899–908.

Horwitz W., Albert R., Deutsch M.J., Thompson J.N. (1990) Precision parameters of methods of analysis required for nutrition labeling. Part I. Major nutrients. *Journal of AOAC International.*, **73**, 661–680.

ISO 7302:1982. Cereals and cereal products – Determination of total fat content (standard withdrawn 2008).

ISO 11085:2008. Cereals, cereals-based products and animal feeding stuffs – Determination of crude fat and total fat content by the Randall extraction method.

ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

ISO 712:2009. Cereals and cereal products – Determination of moisture content (Routine reference method).

Randall E.L. (1974) Improved method for fat and oil analysis by a new process of extraction. *Journal of AOAC International*, **57**, 1165–1168.