

Soxtec™ SoxCap™ collaborative study



A fully validated solution for total fat analysis

In food, cereals and feed, fat often occurs in the form of phospholipids, and glycolipids or lipoproteins. These bound forms of fat are not recovered by standard solvent extraction procedures, and must first be released by means of acid hydrolysis.

Acid hydrolysis is usually performed by boiling a sample in 3-4 M hydrochloric acid. After filtration and rinsing of the filter cake, the residue is dried and can then be subjected to solvent extraction. The procedure is time-consuming, and involves sample transfer steps that can cause losses as a main source of error. FOSS has simplified the process with the help of its integrated SoxCap™ concept, eliminating the need for sample transfers^[1].

Though comprehensively validated in numerous single lab studies^[2], until recently no validation via an international collaborative study had been made. But now, in conjunction with an inter-lab study for the revision of the ISO 7302 standard^[3], the SoxCap system has been collaboratively studied using the same samples as for the ISO 7302 study and following the

IUPAC/ ISO rules for design and result evaluation^[4].

Validation

Eight participating labs from China, Denmark, Germany, Japan and Sweden received eleven feed, cereal and cereal product samples to be analyzed as blind duplicates:

Sample 1	Parboiled rice
Sample 2	Whole wheat kernels
Sample 3	Rye flour
Sample 4	Sorghum kernels
Sample 5	Couscous (durum wheat)
Sample 6	Flour mix (multi-corn)
Sample 7	Croutons
Sample 8	Cornbread
Sample 9	Cattle feed
Sample 10	Chicken feed
Sample 11	Pig feed

The samples were analyzed for moisture, crude fat (fat A, using direct petroleum ether extraction) and total fat (fat B, after acid hydrolysis using SoxCap).

Results are given in Table 1. Due to the

limited amount of sample material, only five labs could analyze Sample 9. One lab only reported results for the first five samples. A few results had to be removed as Cochran or Grubbs outliers on the basis of statistical analysis.

The performance data in the form of relative error (coefficient of variation of repeatability and reproducibility) is depicted in Figure 1.

As expected, relative error increases at lower fat content. All participants used 1 g sample weights. Doubling this amount for samples with low fat content would obviously improve precision. The values are comparable to those obtained using other methods.

Accuracy

The accuracy of the SoxCap method could be verified by comparison with the revised prEN ISO/DIS 7302 method, as the same samples were used and the analyses were performed at about the same time. Results are shown in Figure 2.

As can be seen, there are no significant

Parameter	Sample										
	1	2	3	4	5	6	7	8	9	10	11
Number of labs participating after elimination of outliers	8	8	7	7	7	7	7	6	4	7	7
Mean total fat content, g/100 g (based on dry matter basis)	1,093	2,207	1,692	3,451	2,014	12,031	15,922	26,15	14,149	6,705	3,814
Repeatability standard deviation (sr), g/100 g total fat	0,047	0,066	0,073	0,076	0,066	0,134	0,281	0,265	0,098	0,109	0,221
Repeatability relative standard deviation %	4,3	3,0	4,3	2,2	3,3	1,6	1,8	1,0	0,7	1,6	5,8
Repeatability limit r [r = 2,8 x sr], g/100 g total fat	0,132	0,185	0,205	0,213	0,185	0,544	0,786	0,742	0,276	0,305	0,618
Reproducibility standard deviation (sR), g/100 g total fat	0,315	0,260	0,149	0,289	0,139	0,743	0,870	0,919	0,447	0,598	0,644
Reproducibility relative standard deviation %	28,8	11,8	8,8	8,4	6,9	6,2	5,5	3,5	3,2	8,9	16,9
Reproducibility limit (R) [R= 2,8 x sR], g/100 g total fat	0,882	0,729	0,417	0,810	0,388	2,079	2,436	2,572	1,252	1,675	1,802

Table 1: Results of validation study

differences in analyses of total fat content between the two methods. All values, with the exception of those for sorghum, overlap within one standard deviation of the reproducibility. A statistical analysis (t-test of means) reveals that there is no difference in the values between prEN ISO/DIS 7302 and SoxCap in 98 out of 100 cases.

Total fat v crude fat content

As can be seen in Figure 3, total fat content is considerably higher than fat content determined by direct extraction. The differences are especially high for ‘natural’ products and less pronounced for processed products, in which fat is added during the production process. There are substantial and significant differences

in total fat and crude fat content for rice (+187%), whole wheat kernels (+33%), rye flour (+25%), couscous (+157%), croutons (+14%), cattle feed (+19%) and pig feed (+23%). Less significant differences are found for sorghum (+2%), flour mix (+6%), corn bread (+6%) and chicken feed (+7%).

Figure 3 illustrates the importance of total fat determination.

Conclusions

For the first time a commercially available, simplified solution for determination of total fat content has been collaboratively studied. Accuracy and precision were comparable to international standard methods presently in use. The results are of importance for labs interested in the

validation and accreditation of a total fat method for routine analysis of cereals, cereal products and animal feeds.

References:

- [1] www.foss.dk
- [2] FOSS Application Notes for the Sox-Cap™ System
- [3] prENISO/DIS7302 Cereals and animal feeds – Determination of fat content (to be published in 2007)
- [4] ISO 5725-2 Accuracy (trueness and precision) of measurements methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method

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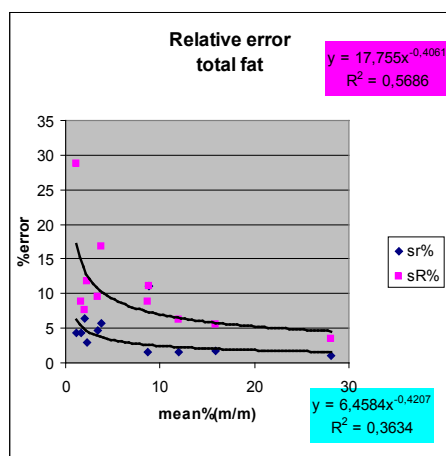


Fig. 1: Relative error in determination of total fat using SoxCap™ as a function of total fat content

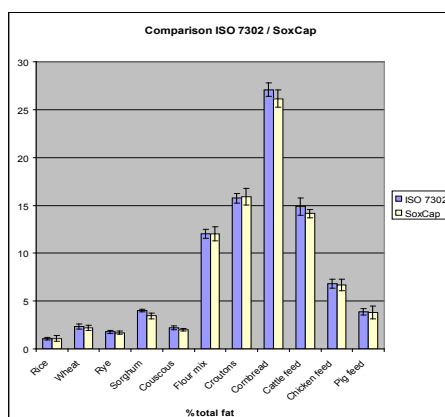


Fig. 2: Mean values for total fat content (% fat) as determined by prEN ISO/DIS 7302 and SoxCap™ (error bars show reproducibility standard deviation)

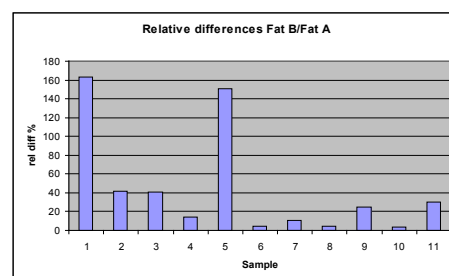


Fig. 3: Relative differences between total fat (Fat B) and crude fat (Fat A) content