

# Fat Determination in Feed Samples by Soxhlet Extraction according to European Directive 2009/152/EC

*HydroEx H-506, FatExtractor E-500 Soxhlet: Fat determination in feed samples by Soxhlet extraction*

A simple and reliable procedure for fat determination in feed products according to European Directive EC No. 152/2009 is introduced. Commission Regulation No. 152/2009 includes both direct extraction (procedure A) and extraction after acid hydrolysis (procedure B). Which of the two procedures is used depends on the nature and composition of the sample. In all cases where a higher result is obtained by using Procedure B than by Procedure A, the result obtained by Procedure B shall be accepted as the true value [1].

## 1. Introduction

Fat determination is one of the key analysis performed in the feed industry. For procedure A the sample is extracted with the FatExtractor E-500 Soxhlet. For procedure B the sample is hydrolyzed with the HydroEx H-506 prior the extraction with the FatExtractor E-500 Soxhlet. After the extract has been dried to a constant weight, the total fat content is measured gravimetrically.

## 2. Experimental

Equipment: HydroEx H-506, FatExtractor E-500 Soxhlet

Sample: Feed for domestic turkey (certified reference material, fat content: 4.76 %, limit of tolerance 4.35 - 5.19 %).

Procedure A: 5 g of homogenous sample and 10 g of sodium sulfate were weight into a cellulose thimble. The extraction was performed using the E-500 SOX applying the parameters specified in Table 1.



Figure 1: FatExtractor E-500

Procedure B: 20 g of quartz sand and 2 g Celite® were added to a glass sample tube. The sample was weighed into a hydrolysis vessel containing 2 g of Celite®. After adding 2 x 50 mL hydrochloric acid (4 M) into each vessel the sample was hydrolyzed for 30 min using the HydroEx H-506. The hydrolyzate was transferred into the glass sample tubes and the vessels washed with warm (50 °C) deionized water, until a neutral pH was obtained. The glass sample tubes were dried. After cooling down in a desiccator another layer of quartz sand (20 g) was added to the glass sample tube.

The extraction was performed using the FatExtractor E-500 Soxhlet applying the parameters specified in Table 1.

Table 1: Parameters for the Soxhlet extraction with the FatExtractor E-500

Parameter	Value	Heating level [-]
Solvent	Petroleum ether	
Extraction	120 min resp. 360 min	6
Rinse	5 min	6
SmartDrying	on	-
Solvent volume [mL]	100	

## 3. Results

The determined fat content of the feed sample is lower than the certified value for total fat, independently of the extraction time (2 h or 6 h) by using Procedure A. The results are shown in Table 2. These findings were independent of the extraction time.

Table 2: Free fat content (Procedure A) of feed for domestic turkey, determined with FatExtractor E-500; expected fat content: 4.76 % (limit of tolerance: 4.35-5.19 %).

	Extraction time: 120 min	Extraction time: 360 min
Mean value [%]	4.08	4.15
rsd [%]	0.29	0.76

The determined total fat content using Procedure B of the feed sample is in good correlation to the certified reference value. The directive EC No. 152/2009 requires an extraction time of 6 h. The results of the extraction with 2 h and 6 h show that the extraction with 2 h is sufficient and in accordance to the expected value, although the recovery of fat was increased with longer extraction times. The results are shown in Table 3.

Table 3: Total fat content (Procedure B) of feed for domestic turkey, determined with FatExtractor E-500; expected fat content: 4.76 % (limit of tolerance: 4.35-5.19 %).

	Extraction time: 120 min	Extraction time: 360 min
Mean value [%]	4.79	5.13
rsd [%]	2.57	0.53

## 4. Conclusion

The determination of the total fat content using the HydroEx H-506 and the FatExtractor E-500 SOX provides reliable and reproducible results. The determined total fat content corresponds well to the declared value of the feed sample.

The determined crude fat content using Procedure A of the feed sample is lower than the certified value for total fat, independently of the extraction time (2 h or 6 h). According to the directive, Procedure B including acid hydrolysis has to be used for this sample. It was shown that an extraction time of 2 h is sufficient to receive reliable and reproducible results for total fat determination using Procedure B.

## 5. References

[1] European Directive EC No. 152/2009 downloaded for free at [www.eur-lex.europa.eu](http://www.eur-lex.europa.eu)

For more detailed information and safety considerations please refer to the Application Note No. 366/2019.