COST Action FP0901
Round Robins of lignin samples
Part 1: Lignin content

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Preface and acknowledgements

The work presented in this report has been carried out within the frame of COST Action FP0901 “Analytical techniques for biorefineries”.

We wish to acknowledge Rickard Drougge (Innventia) for the practical issues regarding the sample distribution, and Anders Reimann (Innventia) for contributions to this report, and Tarja Tamminen (VTT), Richard Gosselink (WUR) and Bodo Saake (vTI) for supplying non-kraft lignin samples.

The laboratories participating in the Round Robin test are gratefully acknowledged for their contribution: Innventia, Latvian State Institute of Wood Chemistry, Lenzing, MoRe Research Örnsköldsvik, Nab Labs, Technische Universität München, The Council for Scientific and Industrial Research, University of Hamburg, University of Liège, Vienna University of Technology, VTT and Wageningen UR Food & Biobased Research.

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Summary

A Round Robin test on the determination of lignin content in lignin samples were performed during 2010–2011 by 12 laboratories within COST Action FP0901. Five different samples were analysed, each sample using two different protocols. The samples were hardwood kraft lignin, softwood kraft lignin, organosolv lignin, soda lignin and enzymatic treated steam explosion lignin. The two protocols employed were based on a conventional acid hydrolysis method, and a simplified acid dissolution protocol, respectively.

It was shown that both protocols were easily executed, and the obtained results were comparable for all participants. The results achieved using the procedure based on standard acid hydrolysis was very reproducible, with a relative standard deviation of only 3–6%.

Furthermore, it was shown that hydrolysis may be omitted when determining the total lignin content in lignin samples. However, some caution should be taken, and the method should be verified for each sample type. Due to the higher throughput when using the procedure without hydrolysis, this method should be especially good for screening purposes.

1 Introduction

The conventional methods for determination of lignin content is based on analysis of the raw material (wood, annual plants), or processed raw materials such as paper pulps. The new interest in valorising the lignin present in the sidestreams of the pulping mill, has high-lightened the need for faster analysis methods for technical lignins and evaluation of suitable procedures to perform quantification of this type of samples.

The purpose of this Round Robin test was to investigate the performance of two different protocols for the determination of lignin content in different lignin samples. One of the protocols was a scaled-down version of conventional methods leading to a slightly shorter analysis time, whereas the other protocol in addition minimize the treatment time for the acid hydrolysis, thereby further decrease the total analysis time.

12 laboratories participated in the test that was performed during 2010–2011.

2 Experimental

2.1 Samples

Five lignin samples were used in this study. The abbreviations, descriptions and origin and supplier are given in Table 1.

Table 1. Overview of samples used in this Round Robin test.

Sample abbreviation	**Description**	**Origin**	**Supplier**
KLHM	Kraft lignin (hardwood)	Birch/aspen	Innventia
KLSM	Kraft lignin (softwood)	Pine/spruce	Innventia
ORGSOLV	Organosolv lignin	Spruce	VTT
SODA	Soda lignin	Wheat straw	WUR
ESEL	Enzymatic treated steam explosion lignin	Poplar	vTI

2.2 Protocols

Two methods were used as described in Appendix 1. In the first method (Procedure 1), the sample is hydrolysed similar to the standard methods for pulp samples [SCAN-CM 71:09, TAPPI T 249 cm-00], but scaled down with a factor 3 since the high lignin content in precipitated lignin samples causes prolonged filtering time. After the hydrolysis, the sample is filtered, the acid-insoluble residue (Klason lignin) is determined gravimetrically [TAPPI T 222 om-02], and the acid-soluble lignin is determined spectrophotometrically [TAPPI UM 250].

The second method (Procedure 2) is similar to the first method, but the “hydrolysis step” is omitted [Aldaeus, *et al.* 2010]. (Note that some hydrolysis of the carbohydrates may occur even during this procedure, but the conditions are drastically less severe.)

3 Results and discussion

3.1 Initial characterization

The lignin samples were initially characterized at Innventia by determining the content of the impurities carbohydrates and ash using standard methods. The results are outlined in Table 2.

Table 2. Contents of impurities in the lignin samples.

Sample	*Total carbohydrates* (mg/g)	*Total ash* (mg/g)
KLHM	15	7
KLSM	12	8
ORGSOLV	30	36
SODA	133	106
ESEL	9	29

The samples KLHM, KLSM and ESEL contained only minor amounts of impurities, whereas ORGSOLV and especially SODA contained several percent carbohydrates and ash.

3.2 Procedure 1

The contents of acid-insoluble and acid-soluble lignin were determined in the samples using Procedure 1 with full hydrolysis, and the results are given in Table 3.

Table 3. Average lignin contents (acid-insoluble, acid-soluble and total lignin) and relative standard deviation *CV* (for the total lignin content) using Procedure 1, and the number of participating laboratories *n*.

Sample	*Acid-insoluble lignin* (mg/g)	*Acid-soluble lignin* (mg/g)	*Total lignin* (mg/g)	*CV* (for total lignin content)	*n*
KLHM	877	82	960	3%	12
KLSM	906	51	957	6%	12
ORGSOLV	741	68	809	6%	12
SODA	692	32	725	5%	12
ESEL	848	26	874	3%	8

None of the samples was considered as outlier. The reproducibilities, expressed as relative standard deviation *CV* were 3–6%.

Detailed results are reported in Appendix 2.

3.3 Procedure 2

The contents of acid-insoluble and acid-soluble lignin were determined in the samples using Procedure 2 without hydrolysis, and the results are given in Table 4.

Table 4. Average lignin contents (acid-insoluble, acid-soluble and total lignin) and relative standard deviation *CV* using Procedure 2, and the number of participating laboratories *n*.

Sample	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**	***CV***	***n***
KLHM	855	81	936	7%	10
KLSM	917	31	948	8%	10
ORGSOLV	711	66	777	14%	10
SODA	730	34	764	10%	10
ESEL	877	19	896	8%	7

None of the samples was considered as outlier. The reproducibilities, expressed as relative standard deviation *CV* were 7–14%.

Detailed results are reported in Appendix 2.

3.4 Comparison

The lignin contents as measured with the two procedures are compared in Figure 1. The differences between the procedures as well as 95% confidence limits for the differences were calculated, and the results are given in Table 5. A student's *t*-test revealed that there were no significant differences in the values of total lignin content obtained by the two procedures. KLSM was the sample that behaved most similar using the two procedures.

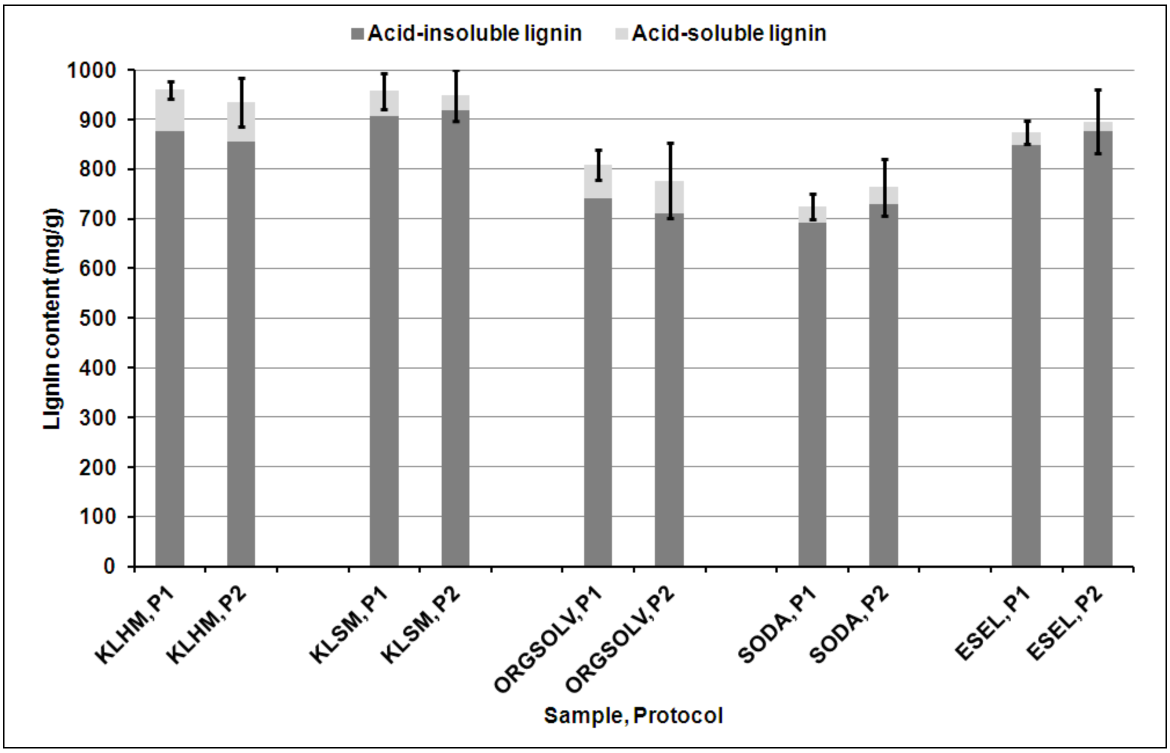

Figure 1. Graphical comparison of the results using the two protocols. The error bars are equal to 95% confidence limits for the total lignin content.

The reproducibilities using Procedure 1 were slightly better (lower) than the reproducibility obtained by Procedure 2. The relative standard deviations were compared using an *F*-test, and the result was that the differences were significant ($F_{calculated}/F_{critical} > 1$) for all samples except KLSM. However, for rapid screening purposes, the difference in reproducibility should not yield any major problems.

Another observation was that the time for hot filtering of the samples generally was longer for the unhydrolysed samples (*i.e.* using Procedure 2).

Table 5. Difference in lignin content (ie. Content using Procedure 2 – content using Procedure 1) with 95 % confidence limits, and the ratio between calculated *F* value and critical F value

Sample	***Difference between the procedures* (mg/g)**	$F_{\mathrm{calculated}}/F_{\mathrm{critical}}$
KLHM	–24 ± 45	2.1
KLSM	–9 ± 57	0.6
ORGSOLV	–32 ± 71	1.7
SODA	39 ± 54	1.5
ESEL	22 ± 57	1.7

4 Conclusions and recommendations

Both the tested methods were easily performed, and the obtained results were similar for all participants.

The results obtained using the procedure based on standard acid hydrolysis was very reproducible, with a relative standard deviation of only 3–6%.

Hydrolysis may be reduced when determining the total lignin content in lignin samples. However, some care should be taken, and the difference should be tested for each sample type. Due to the higher throughput when using the procedure without hydrolysis, this method should be especially suitable for screening purposes.

The filtering procedure is still a problem for samples that has not been hydrolysed during prolonged treatment, in accordance to conventional lignin determinations for *e.g.* pulp samples. The reason should be studied further in order to further simplify the method without hydrolysis.

5 References

SCAN-CM 71:09 (2009)
Pulps – Carbohydrate content
Scandinavian Pulp, Paper and Board testing Committee, Stockholm, Sweden.

TAPPI T 222 om-02 (2002)
Acid-insoluble lignin in wood and pulp
in: 2002-2003 TAPPI Test Methods, Tappi Press, Atlanta, GA, USA.

TAPPI T 249 cm-00 (2002)
Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography
in: 2002-2003 TAPPI Test Methods, Tappi Press, Atlanta, GA, USA.

TAPPI UM 250 (1991)
Acid-soluble lignin in wood and pulp
in: 1991 TAPPI Useful Methods, Tappi, Atlanta, GA, USA.

F Aldaeus, H Schweinebarth, P Törngren, A Jacobs (2011)
Simplified determination of total lignin content in kraft lignin samples and black liquors
Holzforschung 65, 601–604

6 Appendix 1 – Protocol for round robin test of lignin content in lignin samples (COST FP0901)

Version 4 (2011-10-07)
Contact: Fredrik Aldaeus, fredrik.aldaeus@innventia.com

Introduction

This document is a protocol for a first round robin test of lignin content in lignin samples in the COST FP0901 action. The protocol describes two methods for the determination of total lignin content in lignin samples.

In the first method (Procedure 1), the sample is hydrolysed similar to the standard methods for pulp samples [SCAN-CM 71:09, TAPPI T 249 cm-00], but scaled down with a factor 3 due to that the high lignin content in precipitated lignin samples cause prolonged filtering time. After the hydrolysis, the sample is filtered, the acid-insoluble residue (Klason lignin) is determined gravimetrically [TAPPI T 222 om-02], and the acid-soluble lignin is determined spectrophotometrically [TAPPI UM 250].

The second method (Procedure 2) is similar to the first method, but the hydrolysis step is omitted [Aldaeus, *et al.* 2010].

Note that it is assumed that the total lignin is equal to the sum of the acid-soluble lignin and the acid-insoluble residue. No corrections for extractives or ash are included in these procedures.

Reagents

- Water, of high purity (distilled or deionized)
- Sulphuric acid (H_2SO_4), 72 %

Apparatus

Ordinary laboratory equipment and the following:

- Water bath at a temperature of (30 ± 0,5) °C .
- Autoclave at a temperature of (120 ± 5) °C
- Drying oven at a temperature of (105 ± 3) °C for the determination of dry matter content in accordance with ISO 638 and the determination of acid-insoluble residue.
- Laboratory balance
- Glass fibre filters (or equivalent)
- Spectrophotometer capable of measuring the absorption at 205 nm.

Procedures

Procedure 1

1. Determination of dry matter content
Weigh a portion of the sample material and determine the dry matter content by drying at 105 °C until constant weight is achieved [ISO 638].

> *Note 1*
> Samples with high moisture content (*ie.* dry matter content less than approximately 90%) should be air-dried prior to the analysis.

2. Test material preparation
Note: Carry out the preparation and testing in step 2 – 5 in duplicate!
Weigh a test portion of (100 ± 10) mg to the nearest 0,1 mg into a glass beaker with a volume of at least 150 ml. Calculate and record the oven-dry weight of the test portion, in grams.

3. Hydrolysis
To the test material in the beaker, add 1,0 ml of 72 % sulphuric acid with a pipette. Stir the contents of the beaker with a glass rod until the test material begins to dissolve. Place the beaker in a (30 ± 0,5) °C water bath for 1 h. Stir occasionally. Add 28,0 ml of water.
Cover the beaker with aluminium foil and place it in autoclave at (120 ± 5) °C for 1 h. Allow the beaker and its contents to cool to approx. 80 °C.

4 Acid-insoluble residue (AIR)
Filter the content of the beaker while still hot through a single or double pre-weighed glass fibre filter. Transfer the filtrate to a separate beaker (this filtrate is used for the determination of acid-soluble lignin). Wash the retained residue with hot water until acid free (check with pH-indicator paper). Remove the filter with residue from the filter container carefully and allow it to dry overnight at 105°C, cool down in exsiccator and determine weight increase (*i.e.* the acid-insoluble residue).

5 Acid-soluble lignin (ASL)
Determine the content of acid-soluble lignin in the first filtrate (in step 4) by spectrophotometry at 205 nm. Dilute the filtrate until the absorption is in the range 0.2–0.7 AU.

Procedure 2
Same as Procedure 1, but step 3 (Hydrolysis) is replaced by:

3. Acid suspension
To the test material in the beaker, add 1,0 ml of 72 % sulphuric acid (5.3) with a pipette. Stir the contents of the beaker with a glass rod until the test material begins to dissolve. Add 28,0 ml of water. Heat the beaker and its contents to approx. 80 °C, and filter the content of the beaker while still hot.

Calculations

Acid-insoluble residue (AIR)

$$AIR = \frac{m}{M} \cdot 1000 \text{ mg/g}$$

where

m = the weight increase (*ie.* the residue after drying), in g
M = Oven-dry weight of sample (*ie.* as 100% dry matter) before acid hydrolysis/suspension, in g

Acid-soluble lignin (ASL):

$$ASL = \frac{A \cdot D \cdot V}{a \cdot b \cdot M} \cdot 1000 \text{ mg/g}$$

where

A = Absorption at 205 nm
D = Dilution factor
V = Volume of the filtrate, in l (here: 0,029 l)
a = Extinction coefficient of lignin, in g/l cm (here: 110 g/l cm, according to TAPPI UM 250)
b = cuvette path length, in cm (here: 1 cm)
M = Weight of sample (as 100% dry matter) before acid hydrolysis/suspension, in g

Total lignin content:

Total lignin content = AIR + ASL

Reporting

For every sample, please report for each of the two procedures:

- the average *AIR*, in mg/g
- the average *ASL*, in mg/g
- the average *total lignin content*, in mg/g
- which filter that has been used for the determination of acid-insoluble residue
- deviations from the proposed protocol

References

- SCAN-CM 71:09, *Pulps – Carbohydrate content*, 2009, Scandinavian Pulp, Paper and Board testing Committee, Stockholm, Sweden.
- TAPPI T 222 om-02, *Acid-insoluble lignin in wood and pulp*, in: 2002-2003 TAPPI Test Methods, 2002, Tappi Press, Atlanta, GA, USA.
- TAPPI T 249 cm-00, *Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography*, in: 2002-2003 TAPPI Test Methods, 2002, Tappi Press, Atlanta, GA, USA.
- TAPPI UM 250, *Acid-soluble lignin in wood and pulp*, in: 1991 TAPPI Useful Methods, 1991, Tappi, Atlanta, GA, USA.
- F Aldaeus, H Schweinebarth, P Törngren, A Jacobs, *Simplified determination of total lignin content in kraft lignin samples and black liquors*, Holzforschung 65 (2011) 601–604

7 Appendix 2 – Detailed results

Sample KLHM

The results from the participating laboratories for the sample KLHM are given in Table A2.1. Note that the reported values for contents of acid-insoluble lignin and acid-soluble lignin from laboratory are the averages from two measurements (as stated in the protocols).

Table A2.1. Detailed results for the sample KLHM.

Lab no.	**Procedure 1**			**Procedure 2**		
	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**
1	907	86	992	852	105	957
2	843	73	916	879	128	1007
3	827	101	928	851	107	958
4	857	81	939	907	99	1006
5	877	75	952	777	46	823
6	875	95	969	857	86	944
7	976	38	1013	982	27	1009
8	844	127	972	810	25	835
9	846	109	955	761	117	878
10	914	71	985	n.d.	n.d.	n.d.
11	845	104	949	875	65	939
12	920	25	945	n.d.	n.d.	n.d.
Average	877	82	960	855	81	936
Standard deviation	44	29	28	64	37	69
CV	5%	35%	3%	7%	47%	7%

n.d. = did not determine

Sample KLSM

The results from the participating laboratories for the sample KLSM are given in Table A2.2. Note that the reported values for contents of acid-insoluble lignin and acid-soluble lignin from laboratory are the averages from two measurements (as stated in the protocols).

Table A2.2. Detailed results for the sample KLSM.

Lab no.	Procedure 1			Procedure 2		
	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**
1	985	42	1027	917	27	944
2	919	29	948	962	27	989
3	913	41	954	920	40	960
4	905	52	957	946	24	969
5	912	34	946	881	14	895
6	941	57	997	963	34	998
7	983	77	1060	996	65	1061
8	888	73	961	925	22	947
9	825	59	884	758	29	787
10	929	27	956	n.d.	n.d.	n.d.
11	806	40	845	906	29	935
12	865	84	949	n.d.	n.d.	n.d.
Average	906	51	957	917	31	948
Standard deviation	55	19	57	65	14	72
CV	6%	37%	6%	7%	44%	8%

n.d. = did not determine

Sample ORGSOLV

The results from the participating laboratories for the sample ORGSOLV are given in Table A2.3. Note that the reported values for contents of acid-insoluble lignin and acid-soluble lignin from laboratory are the averages from two measurements (as stated in the protocols).

Table A2.3. Detailed results for the sample ORGSOLV.

Lab no.	**Procedure 1**			**Procedure 2**		
	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**
1	766	65	832	647	45	692
2	705	95	800	739	91	830
3	739	71	810	722	114	836
4	718	64	781	702	58	759
5	747	63	810	501	50	551
6	751	71	822	797	15	812
7	858	55	913	910	48	958
8	753	88	842	684	110	794
9	692	82	774	657	79	736
10	774	58	832	n.d.	n.d.	n.d.
11	710	68	778	754	48	802
12	682	33	715	n.d.	n.d.	n.d.
Average	741	68	809	711	66	777
Standard deviation	47	16	48	106	32	106
CV	6%	24%	6%	15%	48%	14%

n.d. = did not determine

Sample SODA

The results from the participating laboratories for the sample SODA are given in Table A2.4. Note that the reported values for contents of acid-insoluble lignin and acid-soluble lignin from laboratory are the averages from two measurements (as stated in the protocols).

Table A2.4. Detailed results for the sample SODA.

Lab no.	**Procedure 1**			**Procedure 2**		
	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**
1	716	24	740	780	28	807
2	707	28	735	741	35	776
3	705	31	736	773	24	797
4	658	26	683	798	27	824
5	699	27	726	571	18	589
6	674	33	707	729	37	766
7	745	28	773	851	21	871
8	662	44	705	637	43	680
9	692	82	774	657	79	736
10	740	22	762	n.d.	n.d.	n.d.
11	611	28	639	759	29	788
12	701	15	716	n.d.	n.d.	n.d.
Average	692	32	725	730	34	764
Standard deviation	37	17	39	84	18	80
CV	5%	53%	5%	12%	52%	10%

n.d. = did not determine

Sample ESEL

The results from the participating laboratories for the sample ESEL are given in Table A2.5. Note that the reported values for contents of acid-insoluble lignin and acid-soluble lignin from laboratory are the averages from two measurements (as stated in the protocols).

Table A2.5. Detailed results for the sample ESEL.

Lab no.	**Procedure 1**			**Procedure 2**		
	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**	***Acid-insoluble lignin* (mg/g)**	***Acid-soluble lignin* (mg/g)**	***Total lignin* (mg/g)**
1	878	21	899	935	17	952
2	802	36	838	921	30	951
3	845	27	872	853	28	881
4	831	24	854	935	17	952
5	872	23	895	766	12	778
6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	865	40	906	820	11	831
9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
11	814	24	838	909	19	928
12	874	14	888	n.d.	n.d.	n.d.
Average	848	26	874	877	19	896
Standard deviation	29	8	27	66	7	69
CV	3%	32%	3%	7%	39%	8%

n.d. = did not determine

8 Uppgifter för Innventia databas

Titel

COST Action FP0901 Round Robins of lignin samples – Part 1: Lignin content

Författare

Fredrik Aldaeus

Referat

A Round Robin test on the determination of lignin content in lignin samples were performed during 2010–2011 by 12 laboratories within COST Action FP0901. Five different samples were analysed, each sample using two different protocols. The samples were hardwood kraft lignin, softwood kraft lignin, organosolv lignin, soda lignin and enzymatic treated steam explosion lignin. The two protocols employed were based on a conventional acid hydrolysis method, and a simplified acid dissolution protocol, respectively.

It was shown that both protocols were easily executed, and the obtained results were comparable for all participants. The results achieved using the procedure based on standard acid hydrolysis was very reproducible, with a relative standard deviation of only 3–6%.

Furthermore, it was shown that hydrolysis may be omitted when determining the total lignin content in lignin samples. However, some caution should be taken, and the method should be verified for each sample type. Due to the higher throughput when using the procedure without hydrolysis, this method should be especially good for screening purposes.

Nyckelord

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