Collaborative Evaluation of a Simplified Assay for Total Starch in Cereal Products (AACC Method 76-13)

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ABSTRACT

A procedure for the quantitative analysis of total starch in plant materials has been developed and subjected to a comprehensive interlaboratory study involving 32 laboratories, in accordance with the protocol for collaborative studies recommended by American Association of Cereal Chemists and AOAC International. The method involves treatment of a sample at approximately 95°C with thermostable α-amylase to obtain starch depolymerization and solubilization. The slurry is then treated with purified amyloglucosidase to give quantitative hydrolysis of the starch fragments to glucose, which is measured with glucose oxidase/peroxidase reagent. Test samples used in the interlaboratory study included modified and native starches, cereal flours and brans, processed cereal products, animal feeds, and plant material. Results were statistically analyzed according to AOAC International guidelines (1). The procedure was shown to be highly repeatable (relative standard deviation 2.1–3.9%) and reproducible (relative standard deviation 2.9–5.0%), and on the basis of these results has gained first approval status with AACC (AACC Method 76-13) and approval as AOAC Method 986.11. The method is more robust than a method previously reported (AACC Method 76-12), and 20 samples can be analyzed within 2 hr.

Many currently accepted procedures for the analysis of total starch are not quantitative for high-amylose starches and many processed cereal products (2,3). This problem was addressed by the development of an enzymatic procedure, in which starch is dispersed in dimethyl sulfoxide (DMSO) and then quantitatively hydrolyzed to glucose by sequential treatment with thermostable α-amylase, pullulanase/β-amylase, and amyloglucosidase (glucoamylase) (2). The resultant glucose is measured colorimetrically with a glucose oxidase/peroxidase (GOPOD) reagent. This procedure is quantitative for a wide range of modified starches and cereal products, and an interlaboratory evaluation of precision demonstrated its high repeatability and reproducibility (3). Consequently, the method received first approval status by AACC (AACC Method 76-12). However, feedback from a number of users of this procedure indicated that several steps in the method were tedious and needed to be simplified.

Several studies have reported starch assay procedures in which starch was hydrolyzed by sequential treatment with thermostable α-amylase and amyloglucosidase (4–9). Widespread acceptance of this approach has been limited by the cost of high-purity amyloglucosidase, which is free of contaminating activities of cellulase and catalase. Cellulase contamination contributes to false high-starch values in many cereal products because of cellulose hydrolysis, and catalase reduces the stability of the chromogen formed in glucose assay methods based on the use of GOPOD reagent. Amyloglucosidase of the necessary quality is now affordable and available commercially, and it was used in an assay procedure for total starch based on the use of thermostable α-amylase and amyloglucosidase.

This article details an interlaboratory evaluation of the new simplified format and evaluates the precision of the procedure for starches and cereal products performed in accordance with protocols for collaborative studies recommended by AACC and AOAC International.

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MATERIALS AND METHODS

Assay and Purity of Amyloglucosidase

Amyloglucosidase activity was routinely assayed using a reagent mixture containing *p*-nitrophenyl β-maltoside and excess β-glucosidase (10), and units were expressed in terms of micromoles of *p*-nitrophenol released per minute. Activity was also assayed using soluble starch (10 mg/ml) as substrate, with measurement of released glucose. One unit of activity of *p*-nitrophenyl β-maltoside is equal to 16.3 units of soluble starch at the same temperature and pH. In this communication, activity is expressed as units of *p*-nitrophenyl β-maltoside unless otherwise stated.

The amyloglucosidase used in this study was purified from an industrial *Aspergillus niger* preparation by ion exchange and gel permeation procedures (Megazyme, Bray, Ireland). The degree of contamination by cellulase was determined viscometrically. In this procedure, amyloglucosidase (0.3 ml, 60 U) was incubated with barley β-glucan (10 ml, 10 mg/ml) in sodium acetate buffer (50 m*M*, pH 4.5) at 40°C in a type C, U-tube viscometer. At time intervals up to 90 min, viscosity measurements were made and the specific viscosity calculated as $(t - t_o)/t_o$, where t_o is the time of flow of the solvent and t is the time of flow of the digest.

Assay of Thermostable α-Amylase

α-Amylase was routinely assayed using Ceralpha reagent at pH 6.0 and 40°C. Ceralpha reagent contains end-blocked *p*-nitrophenyl maltoheptaoside in the presence of excess quantities of α-glucosidase and amyloglucosidase (11). One unit of enzyme is defined as the amount of enzyme required to release one micromole of *p*-nitrophenol per minute under the defined assay conditions.

Total Starch Assay Procedure

Finely milled (to pass a 0.5 mm screen) sample material (100 ± 5 mg) in a glass test tube (16 × 120 mm) was wet with

aqueous ethanol (0.2 ml, 80%, v/v). Diluted thermostable α-amylase (3 ml, 300 U) was added with stirring and the tubes were heated in a boiling water bath for 5 min (the tube contents were stirred vigorously on a vortex mixer after 2 and 4 min). The tubes were equilibrated at 50°C and 4 ml of sodium acetate buffer (200 m*M*, pH 4.5) was added, followed by amyloglucosidase (0.1 ml, 20 U). After stirring, the tubes were incubated for 30 min at 50°C. The tube contents were then quantitatively transferred to a 100-ml volumetric flask and the volume was adjusted with distilled water. After thorough mixing, aliquots (0.1 ml) were treated with 3 ml of GOPOD reagent and incubated for 20 min at 50°C. The absorbance at 510 nm was then measured against a reagent blank. This format is referred to as the standard or non-DMSO format.

Four samples were also analyzed after an initial dispersal/dissolution of the starch in DMSO (DMSO format). In this format, DMSO (2 ml) was added to the ethanol-wetted sample with vigorous mixing and the tube heated for 5 min in a boiling water bath to disperse the starch prior to the addition of thermostable α-amylase.

Collaborative Evaluation of Precision

Full instructions and the following reagents were provided in kit form to each collaborator.

1) Thermostable α-amylase (EC 3.2.1.1) (10 ml, 3,000 U/ml in 50% glycerol) purified from Termamyl 120 L (Novo Nordisk, Bioindustrial Group, Novo Alle 2880 Bagsvaerd, Denmark) by ion exchange and hydrophobic chromatography. This was diluted 30-fold with 50 m*M* MOPS buffer, pH 7.0 before use.

2) Amyloglucosidase (EC 3.2.1.3), (10 ml, 200 U/ml in 50% ammonium sulfate) purified to electrophoretic homogeneity by ion exchange and gel permeation chromatography from an industrial *A. niger* preparation. This enzyme was used without dilution.

3) GOPOD reagent buffer concentrate and glucose standard solution (100 μg/0.1 ml), supplied as described previously (3).

4) Regular maize starch for use as a reference sample (starch content ~98% d.w.).

Test samples were the same materials as used previously (3) except that covalently cross-linked starch was omitted. The samples represented materials ranging from pure starches to processed cereal products and included samples with resistant starch. Calculations for total starch on an "as is" basis were as described by McCleary and coworkers (3).

Design of the Collaborative Study

Sixteen homogenous test samples were provided as eight blind duplicates to 32 collaborators, who were asked to become familiar with both the DMSO and non-DMSO formats of the method by repeated analysis of the reference sample supplied. All samples were assayed by the non-DMSO format and samples of high-amylose maize starch and wheat starch were assayed by the DMSO format. Collaborators assayed each test sample once only and reported the analyses on an air dry basis. All results were adjusted for moisture content prior to statistical analysis.

The results were analyzed as in McCleary and coworkers (3) according to AOAC International guidelines (1) using two outlier tests (both at $P < 0.01$). The Cochrans test identifies results with extreme differences between replicates and the Grubbs test identifies extreme average results. Any outliers were omitted from further calculations. Within (s_r) and between (s_R) laboratory standard deviations were determined and repeatability (r) and reproducibility (R) values were 2.8 s_r and 2.8 s_R, respectively. Relative standard deviations (RSD_r and RSD_R) were calculated from s_r and s_R as percentages of the mean values. Horwitz ratios (HORRAT) were calculated as:

$$RSD_R \text{ (determined)}/RSD_R \text{ (predicted)}$$

where $RSD_R(\text{predicted}) = 2^{(1-0.5 \log C)}$ and C is the starch concentration as a decimal fraction (1% = 0.01) (12). HORRAT values normalize the RSD_R values with respect to concentration, allowing comparison of precision characteristics across concentrations, analytes, and methods (13).

RESULTS AND DISCUSSION

Development of the Modified Method

The method described for total starch measurement simplifies the format described previously (2,3). This simplification was made possible with the availability of a highly purified amyloglucosidase, which allowed the removal of several of the manipulative steps in the original procedure (2,3). The sample is treated with a high concentration of thermostable α-amylase followed by treatment of the whole digest with amyloglucosidase. The use of thermostable α-amylase ensures that starch-lipid complexes are hydrolyzed and minimizes the formation of resistant starch (4,8).

Cellulase is a common contaminant in industrial amyloglucosidase preparations (15) and it can result in overestimation of starch in plant materials (particularly if β-glucosidase is also present) or underestimation of β-glucan in dietary fiber determinations. The enzyme used was shown to be essentially free of cellulase activity using dyed-substrate based assays and viscometric assays. In Figure 1, the level of β-glucanase (cellulase) in two amyloglucosidase preparations (commonly used in dietary fiber determinations) was measured using a viscometric assay using barley b-glucan as substrate. The amyloglucosidase used in the current study is essentially devoid of cellulase (very slight viscosity drop), whereas the other preparation is highly contaminated with this activity.

The absence of catalase in the currently employed amyloglucosidase preparation was demonstrated by the stability of the quinoneimine dye complex formed in the GOPOD reaction. Catalase causes a fading of the color complex after color formation. With even high concentrations of amyloglucosidase in the starch assay, the color complex was very stable, with no fading over 2 hr at 50°C (Fig. 2).

For the analysis of high-amylose-containing starches and samples containing high levels of resistant starch, it is essential that the starch is first dissolved with DMSO before α-amylase treatment (2). Consequently, some high amylose starches requiring pre-treatment with DMSO were included in the collaborative study.

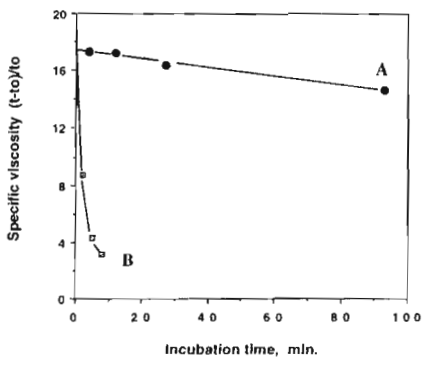

Fig. 1. Assay for cellulase contamination in amyloglucosidase preparations using a viscometric assay with barley β-glucan (10 ml, 10 mg/ml) at pH 4.5 and 40°C, as substrate. A = the amyloglucosidase used in the current starch assay procedure (0.3 ml, 60 units); B = an alternative amyloglucosidase preparation commonly used in dietary fiber determinations (0.3 ml, 60 units).

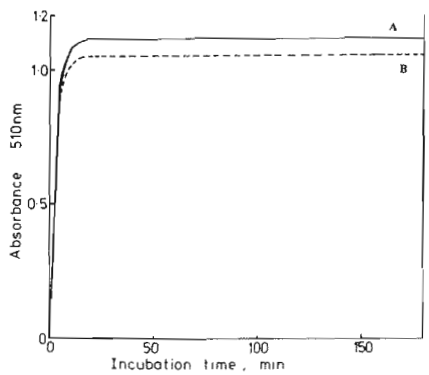

Fig. 2. Stability of the quinoneimine color complex formed in the glucose oxidase/peroxidase reaction for glucose determination at 50°C. A = glucose standard solution (0.1 ml, 100 mg); B = an aliquot (0.1 ml) of solution from a typical starch hydrolysate using the current assay procedure and reagents.

Collaborative Evaluation

Tables I and II show the total starch values determined by each collaborator using the new α-amylase/amyloglucosidase total starch method, with both the non-DMSO and DMSO formats. Results are presented as a percentage, on a dry-weight basis. Means, repeatability values (r), repeatability relative standard deviations (RSD_r), reproducibility values (R), reproducibility relative standard deviations (RSD_R), HORRAT values and ranges for test results on each sample are also given, as are results requested but not supplied by the collaborator. The Cochrans test identified as outliers the results for the following samples: high-amylose maize starch (collaborator 12 [non-DMSO format] and collaborator 19 [DMSO format]), white wheat flour (collaborator 32), oat bran (collaborator 5), spaghetti (collaborator 19), and wheat starch (collaborator 5 [DMSO format]). The Grubbs test identified as outliers the results of collaborator 18 for green peas.

RSD_r values ranged between 2.1 and 3.9%, and RSD_R values ranged between 2.9 and 5.0%. The RSD_R value was 4.8% for high-amylose maize starch analyzed by the non-DMSO format, however, the value was reduced to 2.9% when the DMSO format (recommended for these types of samples) was used. HORRAT values ranged from 1.4 to 2.8, with the lowest and highest values derived from the analyses of high-amylose maize starch by the DMSO and non-DMSO formats, respectively. The differences in the performance of the two assay formats for the analysis of high-amylose maize starch emphasizes the need for DMSO pre-treatment for these sample types. It also highlights some of the problems experienced in the analysis of samples containing high levels of resistant starch.

Although the use of thermostable α-amylase followed by amyloglucosidase in the measurement of starch has been reported in literature, the results of this current collaborative study show that this method (both the non-DMSO and the

Table I. Collaborative Results for Total Starch Determination in Processed Cereal Products and Plant Materials (part 1)

	Chicken Feed Pellets		White Bread		Green Pea		High-Amylose Maize Starch		White Wheat Flour	
Laboratory No.	A	L	B	F	C	J	D	E	G	O
1	49.75	49.86	66.59	66.03	42.69	43.26	81.02	81.25	76.95	76.72
2	54.94	53.81	70.51	70.73	46.00	46.00	76.98	80.55	81.31	79.47
3	50.54	47.60	69.28	65.36	42.35	42.58	–[a]	–	76.83	80.16
4	52.00	50.65	71.52	71.52	44.98	44.75	–	–	82.91	78.10
5	53.69	49.29	63.46	62.67	40.41	40.75	–	–	77.52	72.36
6	46.25	45.35	62.67	63.01	36.64	42.12	78.94	79.86	69.50	73.74
7	48.39	50.31	67.15	64.02	44.86	43.84	90.36	87.94	76.26	75.92
8	50.54	49.63	68.38	68.72	44.29	44.52	–	–	77.75	77.41
9	51.66	52.23	68.49	69.05	45.09	43.72	88.98	88.40	79.24	81.77
10	48.05	47.60	67.49	67.15	42.58	43.95	80.78	83.21	76.61	76.49
11	51.89	49.07	64.91	67.15	44.18	43.38	–	–	78.78	75.23
12	45.23	45.46	60.44	63.46	40.87	44.29	$87^{C}25$	$72^{C}48$	74.43	69.84
13	56.51	51.10	77.11	69.28	42.24	44.06	86.90	90.83	83.37	88.19
14	49.97	51.89	70.84	70.06	45.21	46.00	90.83	88.86	79.70	80.50
15	54.26	50.31	78.79	70.96	43.49	40.87	89.90	86.56	80.85	75.34
16	49.07	49.63	64.35	62.90	44.18	41.89	83.67	86.67	72.82	74.54
17	49.75	48.84	65.70	67.82	43.04	43.72	86.79	85.05	75.92	77.87
18	49.86	49.97	69.17	69.39	$26^{G}48$	$23^{G}52$	88.52	90.48	79.47	79.47
19	55.39	55.27	71.29	69.50	46.92	47.95	–	–	80.96	75.46
20	49.75	50.65	67.60	67.71	44.63	43.38	85.29	87.71	77.64	76.49
21	51.10	49.75	67.71	66.03	43.38	42.47	83.44	81.13	75.92	73.39
22	51.21	48.73	72.64	69.17	45.55	46.58	96.83	86.67	87.16	78.21
23	49.97	52.23	68.38	68.38	48.63	45.55	86.90	88.75	78.67	79.24
24	52.57	50.54	70.96	70.51	44.29	44.86	89.32	89.21	80.16	78.78
25	49.63	49.63	66.37	66.59	43.95	42.58	83.21	83.90	76.83	77.18
26	51.78	54.03	69.95	72.86	46.23	45.78	90.71	91.06	81.88	79.13
27	50.54	50.87	67.49	67.82	44.29	44.41	88.52	88.98	78.33	77.52
28	51.21	51.10	67.15	67.38	43.95	43.15	85.98	86.56	78.21	78.33
29	53.69	50.08	62.56	64.47	49.89	43.84	75.59	85.29	79.36	78.67
30	49.52	51.10	70.51	67.26	43.49	39.61	88.29	84.25	76.61	76.72
31	52.23	51.78	68.94	67.82	43.26	44.41	86.21	89.32	79.82	79.01
32	54.37	49.75	73.87	71.85	44.75	47.03	88.63	90.36	$83^{C}37$	$69^{C}15$
Moisture %	11.35		10.65		12.4		13.35		12.8	
Number of labs	32		32		31		25		31	
Outliers[b]	0		0		$1(^{G})$		$1(^{C})$		$1(^{C})$	
Average %	50.7		68.1		44.0		86.9		78.0	
s_r[c]	1.6		1.8		1.5		2.5		2.2	
RSD_r[d]	3.1		2.7		3.4		2.9		2.9	
r[e]	4.4		5.2		4.2		6.9		6.3	
s_R[f]	2.4		3.4		2.1		4.9		3.3	
RSD_R[g]	4.6		5.0		4.8		5.7		4.2	
R[h]	6.6		9.5		6.0		13.8		9.2	
HORRAT[i]	2.1		2.4		2.1		2.8		2.0	
Range	45.4–55.3		62.0–74.9		39.4–47.4		78.7–96.8		71.6–85.8	

[a] Results requested but not supplied.
[b] Number of outlier laboratories, not included in calculations (^{C} = Cochran, ^{G} = Grubbs outlier).
[c] Repeatability standard deviation.
[d] Repeatability relative standard deviation.
[e] Repeatability value ($2.8 \times s_r$).
[f] Reproducibility standard deviation.
[g] Reproducibility relative standard deviation.
[h] Reproducibility value ($2.8 \times s_R$).
[i] Horwitz ratio, an indication of the precision of the method.

DMSO formats) demonstrates a greater precision (RSD_r range 2.1–3.9%, RSD_R range 2.9–5.0%) than that determined for AACC Method 76-12 (3), where RSD_r and RSD_R values ranged from 1.5 to 7.3% and 4.1 to 11.3%, respectively (Table III). This is particularly noticeable for the spaghetti, oat bran, and chicken feed samples, where RSD_R values decreased from a range of 9.4–11.3% in the older format to a range of 4.6–5.0% in the current assay format. It should be noted that although the interlaboratory evaluations of AACC Method 76-12 and the new method (AACC 76-13) were performed about 2 years apart, essentially all of the samples used in the two studies were identical, and many of the collaborators were the same.

HORRAT values obtained with the new assay format were lower than those reported for AACC Method 76-12 (3) and were close (mean HORRAT value = 2.1) to the suggested general maximum value of 2 for methods with "acceptable" precision (12,13). The lower precision parameters for the modified format reported here probably reflect the simplification of the procedure, to the benefit of the analyst with less familiarity with the assay. These values are lower than those reported by an "Analytical Working Party of the Starch Experts Group" (16) for a precision study on both a polarimetric and an alkaline dispersion/enzymatic procedure on purified starches. The low s_r and RSD_r values reported elsewhere for analytical procedures to measure total starch in food and cereal products (4,5,7,8) were derived from analyses by a single laboratory rather than a collaborative study involving several laboratories.

CONCLUSIONS

The introduction of a cost-effective amyloglucosidase, free of cellulase and catalase activities, and simplification of a previously described (2) enzymatic test

Table II. Collaborative Results for Total Starch Determination in Processed Cereal Products and Plant Materials (part 2)

	Wheat Starch		Oat Bran		Spaghetti		High-Amylose Maize Starch[a]		Wheat Starch[a]	
Laboratory No.	H	M	I	P	K	N	D	E	H	M
1	95.84	95.84	40.79	41.23	76.87	75.62	94.86	93.59	94.47	94.25
2	101.99	100.74	43.09	43.97	81.41	78.57	98.33	99.48	99.03	97.55
3	–[b]	–	37.17	40.24	77.78	75.28	101.67	99.60	97.44	96.18
4	–	–	44.63	44.74	80.73	80.61	101.67	102.14	89.23	100.40
5	–	–	44[C]85	23[C]68	78.57	67.46	96.94	89.44	2[C]96	78[C]52
6	93.45	93.11	45.72	47.92	71.88	74.38	100.06	101.44	104.84	103.25
7	96.52	99.03	42.11	40.02	79.48	76.64	96.02	100.29	97.55	93.79
8	–	–	44.19	41.78	78.46	78.23	97.40	97.63	96.18	96.98
9	95.50	100.06	42.11	42.65	81.63	81.41	99.37	97.63	98.46	92.42
10	95.38	95.95	42.65	40.90	76.30	77.10	96.13	95.21	94.36	95.61
11	–	–	40.68	41.45	69.61	79.93	89.32	93.94	86.72	85.36
12	89.23	102.45	39.91	38.49	74.38	72.45	94.40	102.37	89.12	95.84
13	99.49	110.54	42.76	42.21	76.76	63.49	98.67	100.08	97.66	98.12
14	101.99	100.51	43.31	42.43	79.02	75.62	97.17	97.29	98.80	99.03
15	98.80	91.51	42.87	37.50	72.79	73.02	100.98	99.02	84.33	94.36
16	87.98	95.61	39.91	39.69	71.20	73.70	93.25	95.44	94.70	94.93
17	97.21	94.25	40.90	41.67	76.30	77.89	98.56	99.37	100.17	103.25
18	96.07	95.84	44.63	43.20	73.24	73.36	97.17	97.86	98.12	98.12
19	–	–	45.07	42.32	83[C]45	17[C]69	10[C]39	103[C]29	101.42	105.98
20	96.87	96.30	41.56	42.76	77.44	77.44	100.75	98.56	100.97	99.26
21	98.80	95.38	46.38	40.46	76.08	73.81	94.06	95.44	94.59	96.41
22	92.99	99.60	42.11	45.18	77.78	85.71	100.52	94.40	103.13	95.61
23	99.15	96.41	43.20	41.67	77.10	78.46	97.17	97.52	96.30	95.04
24	97.89	98.12	43.97	42.54	78.80	78.12	96.36	98.79	97.44	98.69
25	96.18	95.95	41.34	40.46	76.98	77.10	95.79	96.48	92.99	92.42
26	99.49	99.94	46.60	43.75	80.39	79.82	97.29	97.52	99.60	99.03
27	97.44	96.75	44.74	40.90	77.21	78.12	94.75	94.86	96.07	95.61
28	96.52	96.98	43.09	41.78	77.21	77.55	94.40	94.86	94.59	94.70
29	95.84	96.75	41.23	42.11	77.21	76.42	95.44	94.86	99.49	97.55
30	92.31	91.85	41.01	39.36	70.86	71.32	97.63	97.40	94.25	91.85
31	101.54	100.97	43.75	40.90	80.84	77.55	94.75	96.71	98.12	88.66
32	102.79	97.66	39.36	40.57	80.39	76.87	99.02	95.33	105.07	99.37
Moisture %	12.25		8.8		11.8		13.35		12.25	
Number of labs	26		31		31		31		31	
Outliers[c]	0		1[C]		1[C]		1[C]		1[C]	
Average %	97.2		42.2		76.6		97.2		96.5	
s_r[d]	3.2		1.6		3.0		2.0		3.0	
RSD_r[e]	3.3		3.8		3.9		2.1		3.1	
r[f]	9.0		4.5		8.4		5.7		8.4	
s_R[g]	3.7		2.1		3.7		2.8		4.4	
RSD_R[h]	3.8		5.0		4.8		2.9		4.6	
R[i]	10.4		6.0		10.3		7.8		12.4	
HORRAT[j]	1.9		2.2		2.3		1.4		2.3	
Range	91.8–105.0		38.7–46.8		70.1–81.8		91.6–101.9		86.0–104.0	

[a] Dimethyl sulfoxide method.
[b] Results requested but not supplied.
[c] Number of outlier laboratories, not included in calculations ([C] = Cochran, [G] = Grubbs outlier).
[d] Repeatability standard deviation.
[e] Repeatability relative standard deviation.
[f] Repeatability value ($2.8 \times s_r$).
[g] Reproducibility standard deviation.
[h] Reproducibility relative standard deviation.
[i] Reproducibility value ($2.8 \times s_R$).
[j] Horwitz ratio, an indication of the precision of the method.

Table III. Comparison of Results Obtained from Interlaboratory Evaluation of Analytical Procedures for the Measurement of Total Starch Contents of a Range of Samples

Sample	Method[a]	Starch Contents (dry wt)	Outliers	RSD_r	RSD_R
White wheat flour	A	80.0	1	2.6	5.4
	B	78.0	1	2.9	4.2
White bread	A	69.8	2	1.5	4.1
	B	68.1	0	2.7	5.0
Chicken feed	A	47.5	0	5.7	10.9
	B	50.7	0	3.1	4.6
Oat bran	A	45.6	0	7.3	9.4
	B	42.2	1	3.8	5.0
Green peas	A	44.2	0	3.6	5.7
	B	44.0	1	3.4	4.8
Spaghetti	A	75.1	1	6.2	11.3
	B	76.6	1	3.9	4.8
High-amylose maize starch	A	98.2	1	1.5	4.9
	B	86.3	1	2.9	5.7
	C	97.2	1	2.1	2.9

[a] A = AACC Method 76-12 employing DMSO, α-amylase, pullulanase/β-amylase and amyloglucosidase (3); B = AACC Method 76-13 employing α-amylase and amyloglucosidase (no DMSO); and C = AACC Method 76-13 except that pre-treatment with DMSO was included.

procedure for starch, have markedly improved the convenience and precision of quantitative starch analysis in cereal products.

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