CFW AACC INTERNATIONAL REPORT

TECHNICAL COMMITTEE

Collaborative Study Concerned with Measuring Damaged Starch Using an Amperometric Method

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INTRODUCTION

Starch represents 67–68% of wheat grain and 78–82% of the resulting flour. Composed of amylose (26–28%) and amylopectin (72–74%), starch is the main reserve polysaccharide of superior vegetables (3). Its long glucose chains overlap to form spherical granules the size of which varies between 20–25 μm (starch A) and 2–10 μm (starch B).

Starch is essential for plant development and also for numerous second-stage processing products. Indeed, starch is one of the most important functional polymers of foodstuffs due to its capacities with regard to gelling, viscosity, and fixing water.

The semi-crystalline structure gives the starch granules solid properties that can be damaged by mechanical operations. According to Viot (7), irrespective of the type of mill used, 5–12% of starch granules are harmed during milling. The importance of damaged starch in bread making is considerable. Firstly, damaged starch absorbs between two and four times its weight in water while native starch only absorbs 0.4 times its weight.[2] Secondly, the damaged granules are given "preferential status" by enzymes (including β-amylases) in terms of their attacking ploys, which can only have an effect when a damaged granule is involved.[3]

Damaged starch initially has a positive impact (increased water absorption capacity of the flour), but can later become a negative (dough sticking, slackening, intense coloring of the baking products). An optimum solution can be defined on the basis of the protein content of the flour, the α-amylase content, and the type of bread-making process. The miller can modify the damaged starch content of his flour. The choice of wheat, its preparation, and the mill settings condition the quantity of damaged starch in the flour. During the milling process, some of the starch granules invariably suffer mechanical damage. According to Dubois (2), the biggest grains suffer the most damage. He also showed that the granule has certain elastic properties that can result in several types of damage. The damage may then take the form of cracks or granule "breaking." Claude Willm (8) provided more details with regard to milling factors favoring the production of damaged starch. The two authors both underlined the notable impact of crushing resistance. Furthermore, all things being equal, the greater the wheat's crushing resistance (hardness) during milling, the more damaged starch it will produce. This hardness may be partially modified while the wheat is being prepared. Special attention must be paid to the water conditioning of the wheat and the resting time.

It can be seen that the information provided by starch damage can optimize mill performance. The existing methods (enzymatic) have an advantage when determining a large number of samples (batches), but are often too long and sometimes too complicated for the instantaneous measurement of a sample taken from the mill. The amperometric method, thanks to its simplicity and speed, is a solution tailored to industry's needs. This method can be used by personnel following basic training, which represents a definite advantage (4).

The amperometric method was described by Medcalf and Gilles in 1965 (5). His principle used the amperometric determination of the kinetic absorption of iodine by means of a diluted flour suspension (1).

A new starch damage measuring device is commercially available. AACC International's Physical Testing Methods Committee decided to submit this method to a group study in order to measure the precision-related values. The results will be used as a basis for drafting a new AACC International Standard describing a fully fledged amperometric method for the determination of starch damage.

METHOD

The Principle

The amperometric method is based on the affinity of starch for iodine and on the increase in the resistivity of a solution containing I^{3-} ions in a solution. In other terms, this concerns creating I^{3-} ions in a solution. These ions generate an electrical current (measured in μA) directly related to their concentration in the solution. The iodine is adsorbed (then absorbed) by the starch and is done so to a greater extent when the starch is damaged. The method consists of forming a known quantity of I^{3-} ions and leaving them in contact with the flour for a constant period of time (in general 10 minutes). The μA current is measured at the end of the tests. The weaker the current, the greater the extent to which the I^{3-} ions have been "caught" and the more significant the starch damage of the sample tested.

Numerous authors have compared amperometric and enzymatic methods. Rogers et al. (6) showed that the electrical value measured can indeed be used as a valid indicator to measure starch damage. The author then improved the correlations by separating the high values obtained with the hardest wheat samples from those obtained with the softest ones.

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[2] When the granule is intact following the milling process, this is referred to as native starch.

[3] The α-amylases can attack intact grain (this is the case with sprouted wheat) and the damaged grain (amylase and amylopectin chains cut in an anarchic fashion).

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The Device

The study focused on the SDmatic (Chopin Technologies, Villeneuve la Garenne, France www.chopin-sa.com.) (Fig. 1), the only device available to conduct this test. The device consists of a reaction vessel, a stirrer, a heating resistor, a special probe (Fig. 2) enabling users to 1) generate iodine electro-chemically by applying voltage to the terminals of a pair of platinum electrodes, 2) measure the electrical current (μA) created by the appearance of free iodine (I^{3-} ions) in the solution, and 3) measure the solution temperature; a vibrating motor enabling the flour sample to be introduced automatically; a user interface; and software that manages the test and returns the analysis result as a percent of absorbed iodine (IA%) and converts it into Chopin Dubois Units (UCDs).

Procedure

The operator prepares a solution containing 120 ml of distilled water with 3 g (±1 g) of boric acid and 3 g (±1 g) of potassium iodide. The solution is placed in the device and the measuring head is put in position. A heating resistor heats the solution to 35°C and a thermometer checks the temperature in real time. One g of flour (±0.1 g) is put in the device on a vibrating system. As soon as the temperature is reached, a pair of electrodes generates an electrical current in the solution that will create the free iodine for a period of time depending on the sample (Fig. 3). A second pair of electrodes provides an exact measurement of the electrical current generated, I_M (therefore the quantity of iodine). The flour is automatically fed into the reaction bowl. The test continues for 360 s after which the device measures the residual current, I_R. It is then possible to measure the iodine absorbtion ($IA\% = 1 - (I_R/I_M)$), which is proportional to the quantity of damaged starch (Fig. 3).

Parameters Obtained

At the end of the analytical process, the device measures the IA%, which represents the percent of iodine fixed by the sample. This data is converted into UCDs on an arbitrary scale of 0–36 units, indicating the relative damage of a wheat flour sample.

Organizing a Collaborative Study

The study focused on eight wheat flour samples obtained industrially in various countries and representing a wide starch damage range (from 2 to 25 UCDs) representative of the majority of internally produced flours.

Sixteen laboratories took part in the study including laboratories from the United States, Canada, the United Kingdom, France, Italy, and Turkey (Table 1).

Each laboratory had to conduct duplicate tests on each sample. Furthermore, two samples were repeated as blind duplicates (samples 3 and 8 and samples 7 and 4). The tests took place in April 2004.

The flours were prepared, mixed, and divided by Chopin Technologies and forwarded to the laboratories to be analyzed. Each laboratory then reported the IA% and UCD values obtained.

Results

The results were statistically analyzed by AACC International statisticians in accordance with the ISO 5725 recommendations.

Using the gross values obtained during the collaborative study (Tables 2, 3, 4, and 5), we can compare the standard deviation values with the average value. In this case, it can be seen that the higher the average value of the damaged starch constituent, the lower the measurement standard deviation. This is all the more the case when the reproducibility standard deviation is under consideration. In such a case it would be illogical to attribute a constant value to the repeatability and reproducibility limits. Use would be made of the formulas enabling standard deviations to be linked up with the value measured as described below.

Repeatability Limits (r)

Repeatability is defined here as the value below which we find, with 95% probability, the absolute value of the difference between two results obtained under repeatability conditions. The repeatability

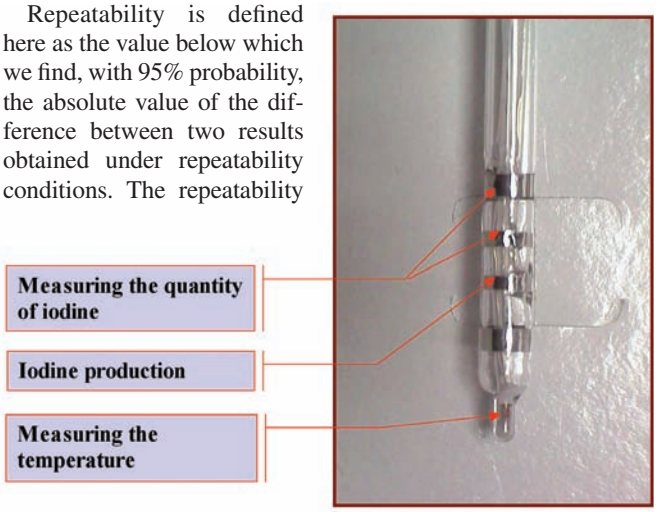

Fig. 2. Chopin SDmatic measuring probe.

Fig.1. Chopin SDmatic.

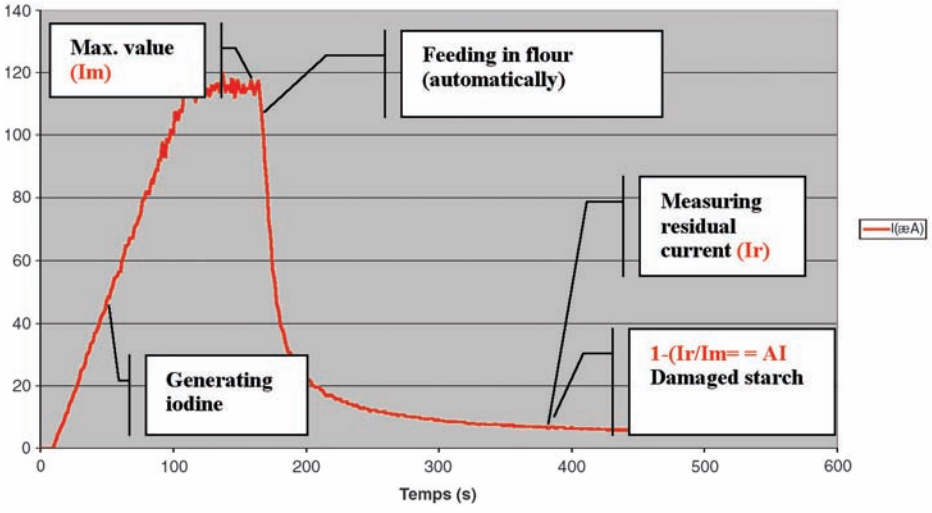

Fig. 3. How the Chopin SDmatic works.

limits (r) are obtained by the equations shown in Figures 4 and 5.

For IA%: r = (–0.0061 IA% + 0.6941) × 2.8
For UCD: r = (–0.007 UCD + 0.4433) × 2.8

In order to make it easier to use this method, an overview table is presented in Appendix 1, which can be accessed online at http://dx.doi.org/10.1094/CFW-52-6-0319S.

Table I. List of laboratories that participated in the intercomparison series

John Ross	ADM Milling Co.
Fatih R. Alaybeyi	Alaybeyi Un
Janette Gelroth	American Institute of Baking
David Cliffe	Calibre Control
Elaine J. Sopiwnyk	Canadian Intl. Grains Institute
Sonia Geoffroy	Chopin Technologies
Ron Heddleson	General Mills Inc.
Ann Lester	General Mills Inc.
Venkat Reddy	General Mills Inc.
Richard Keeping	Heygates Ltd.
M. Provot	LABORAGRO
Jan Levenhagen	Mennel Milling Co.
John McCammon	Siemer Milling Co.
M. Monti	SIMA
Bon Lee	Wheat Marketing Center

Reproducibility Limits (R)

Reproducibility is defined here as the value below which we find, with 95% probability, the absolute value of the difference between two test results obtained under reproducibility conditions. The reproducibility limits (R) are obtained by the equations shown in Figures 4 and 5.

For IA%: R = (–0.0294 IA% + 2.993) × 2.8
For UCD: R = (–0.0332 UCD + 1.3191) × 2.8

In order to make it easier to use this method, an overview table is presented in Appendix 2, which can be accessed online at http://dx.doi.org/10.1094/CFW-52-6-0319S.

Critical Difference

Critical difference (CD) is defined here as the deviation between two averaged values obtained through two test results under repeatability conditions.

Comparison of Two Measurement Groups in the Same Laboratory

Critical difference, the deviation between two averaged values obtained using two test results under repeatability conditions, is equal to:

$$CD = 2.8S_r\sqrt{\frac{1}{2n_1}+\frac{1}{2n_2}} = 2.8 \times S_r\sqrt{\frac{1}{2}} = 1.98 \times S_r,$$

where Sr is the repeatability standard deviation and n_1 and n_2 are the number of test results corresponding to each of the averaged

Table II. Gross results obtained by the laboratories for the IA% parameter

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
Lab No.	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test
1	89.03	89.32	91.84	91.65	95.38	95.39	90.12	90.43	87.01	87.03	93.48	93.76	90.08	90.21	94.96	95.33
2	89.61	89.51	91.89	91.75	95.1	95.33	90.76	90.84	87.87	87.94	93.57	93.56	90.53	91.26	95.06	95.21
3	89.57	89.44	91.89	92.11	95.31	95.34	90.68	90.7	87.69	87.46	93.69	93.65	90.52	90.61	95.15	95.3
4	88.73	88.89	90.8	90.92	94.83	94.74	90.13	89.88	87.21	86.98	93.43	93.31	89.92	90.28	94.82	94.9
5	89.34	89.31	91.63	91.74	95.22	95.13	90.21	90.38	87.36	87.34	93.49	93.73	90.26	90.23	95.27	95.11
6	89.6	89.66	91.51	92	95.08	95.34	90.61	90.54	87.21	88.06	93.67	93.94	90.79	90.67	95.07	95.24
7	89.18	89.67	91.7	91.72	94.98	94.79	90.66	90.93	87.91	88.16	93.73	93.64	90.55	90.57	94.93	94.76
8	89.24	89.06	91.16	91.09	95.16	95	90.13	90	86.9	86.98	93.86	92.82	90.22	90.14	95.27	95.24
9	88.85	89.3	92.02	91.91	95.23	95.26	90.67	90.56	87.54	87.36	93.79	93.48	90.75	90.59	95.12	95.25
10	89.25	89.31	91.3	91.67	94.94	95.07	90.04	90.54	87.41	87.22	93.5	93.46	90.28	90.31	94.97	94.98
11	89.31	89.11	91.76	91.85	94.97	95.06	90.44	90.48	87.81	87.27	93.01	93.66	91.49	90.56	95.13	95.12
12	88.81	89.05	91.51	91.61	94.85	94.95	90.04	89.66	87.03	87.07	93.25	93.5	89.84	89.64	94.91	94.8
13	89.49	89.73	92.17	92.21	95.3	95.34	90.8	90.79	87.75	87.33	93.78	93.69	90.49	90.62	95.31	95.25
14	89.2	89.05	91.47	91.3	94.84	95.06	89.89	89.8	86.47	86.43	93.61	93.43	89.9	89.73	95.01	94.76
15	88.99	89.28	91.71	92.01	94.93	95.15	90.23	90.26	87.1	86.97	93.5	93.58	90.43	90.68	94.94	95.14

Table III. Gross results obtained by the laboratories for the UCD parameter

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
Lab No.	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test	1st test	2nd test
1	7.6	8.4	15.1	14.6	24.6	24.6	10.5	11.4	2.3	2.3	19.5	20.3	10.4	10.8	23.5	24.4
2	9.2	8.9	15.3	14.9	23.8	24.4	12.3	12.5	4.5	4.7	19.8	19.7	11.6	13.6	23.8	24.1
3	9.1	8.7	15.3	15.9	24.4	24.5	12	12.1	4.1	3.5	20.1	20	11.6	11.9	24	24.4
4	7	7.1	13.8	13.9	23.1	23.2	10.5	10.7	1.4	1.1	19.4	19.6	9.3	9.6	23.5	23.8
5	6.8	7.3	12.4	12.7	23.1	22.9	10.6	9.9	2.8	2.2	19.4	19.1	10	11	23.1	23.3
6	8.5	8.4	14.6	14.9	24.2	23.9	10.8	11.2	3.2	3.1	19.5	20.2	10.9	10.8	24.3	23.9
7	9.2	9.3	14.3	15.6	23.8	24.5	11.9	11.7	2.8	5.1	20	20.7	12.3	12	23.8	24.2
8	8	9.3	14.8	14.8	23.5	23	12	12.7	4.7	5.3	20.2	19.9	11.7	11.7	23.4	22.9
9	8.2	7.7	13.3	13.1	24	23.6	10.6	10.2	2	2.2	20.5	17.8	10.8	10.6	24.3	24.2
10	7.2	8.4	15.6	15.3	24.2	24.3	12	11.7	3.7	3.2	20.3	19.5	12.2	11.8	23.9	24.2
11	8.2	8.4	13.7	14.7	23.4	23.8	10.3	11.7	3.3	2.8	19.6	19.5	11	11.1	23.5	23.5
12	8.4	7.9	14.9	15.2	23.5	23.7	11.4	11.5	4.4	2.9	18.3	20	14.7	11.7	23.9	23.9
13	7.1	7.7	14.3	14.5	23.2	23.4	10.3	9.3	2.3	2.4	18.9	19.6	9.8	9.3	23.3	23
14	8.9	9.5	16	16.1	24.4	24.5	12.4	12.3	4.2	3.1	20.3	20.1	11.5	11.9	24.4	24.2
15	8.1	7.7	14.2	13.7	23.1	23.7	9.9	9.7	0.8	0.7	19.9	19.4	10	9.5	23.6	22.9

values. In order to make it easier to use this method, an overview table is presented in Appendix 3, which can be accessed online at http://dx.doi.org/10.1094/CFW-52-6-0319S.

Comparison of Two Measurement Groups in Two Laboratories

Critical difference, the deviation between two averaged values obtained in two different laboratories, using two test results under repeatability results under repeatability conditions, is equal to:

$$CD = 2.8\sqrt{S^2_R - S^2_r\left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)} = 2.8\sqrt{S^2_R - 0.5S^2_r},$$

where Sr is the repeatability standard deviation, SR is the reproducibility standard deviation, and n_1 and n_2 are the number of test results corresponding to each of the averaged values. In order to make it easier to use this method, an overview table is presented in Appendix 4, which can be accessed online at http://dx.doi.org/10.1094/CFW-52-6-0319S.

Uncertainty

Uncertainty (Ue) is a parameter characterizing the scattering of values that could reasonably be attributed to the result. This uncertainty is established using the statistical distribution of the results of the interlaboratory test and characterized by the experimental standard deviation. For each parameter, Ue is equal to more or less twice the reproducibility standard deviation appearing in this standard.

For IA%: Ue = (–0.0294 IA% + 2.993) × 2
For UCD: Ue = (–0.0332 UCD + 1.3191) × 2

Blind Duplicates Test Study

Two samples were blind duplicate tested, i.e., the laboratories were unaware that these samples, named differently, corresponded to the same flour. This made it possible to better appreciate the performance of a method in normal use.

Table 5 shows that the average values obtained for the IA% parameter by the laboratories are 95.1 and 95.1, respectively, for samples 3 and 8 and 90.4 and 90.4 for samples 7 and 4. With regard to the repeatability standard deviations, we obtained 0.09 and 0.10, respectively, for samples 7 and 4. As far as reproducibility standard deviations are concerned, we obtained 0.20 and 0.18 for samples 3 and 8 and 0.35 and 0.45 for samples 7 and 4.

The similarity of the results shows the robust nature of the method and proves that the same sample tested several times will provide similar values provided the method is correctly applied.

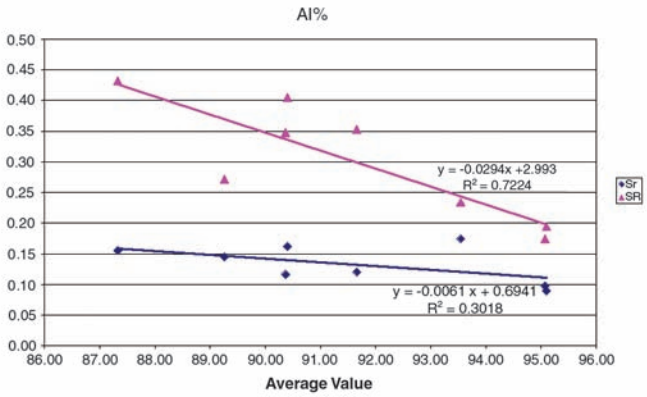

Fig. 4. Relationship between the precision standard deviation and the average IA% values.

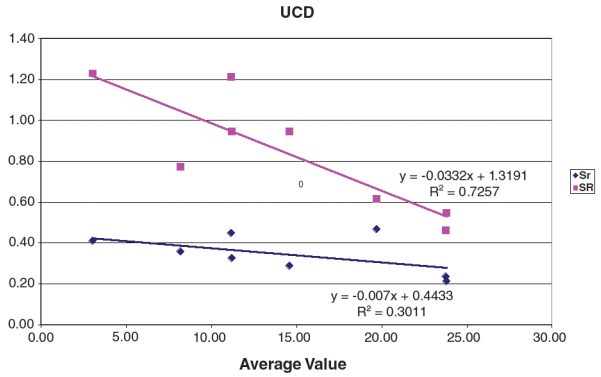

Fig. 5. Relationship between the precision standard deviation and the average UCD values.

Table IV. Statistical analysis of the intercomparison test

Variable	Statistics	S1	S2	S3	S4	S5	S6	S7	S8
Lab No.		15	15	15	15	15	15	15	15
	r	0.14	0.12	0.09	0.12	0.16	0.17	0.16	0.10
	R	0.27	0.33	0.25	0.35	0.43	0.23	0.40	0.17
	CV_r	0.16	0.13	0.09	0.13	0.18	0.17	0.18	0.10
IA%	CV_R	0.3	0.38	0.20	0.35	0.49	0.23	0.45	0.18
	μ	89.3	91.7	95.1	90.4	87.3	93.6	90.4	95.1
	2.8 × r	0.39	0.34	0.25	0.34	0.45	0.48	0.45	0.28
	2.8 × R	0.76	0.92	0.70	0.98	1.20	0.64	1.12	0.48
	r	0.36	0.29	0.21	0.33	0.41	0.47	0.45	0.24
	R	0.77	0.94	0.59	0.95	1.23	0.61	1.21	0.65
	CV_r	4.4	2.0	0.09	2.9	13.5	2.4	4.0	1.0
UCD	CV_R	9.4	6.5	2.3	8.4	40.5	3.1	10.8	1.9
	μ	8.2	14.6	23.8	11.2	3.0	19.7	11.2	23.8
	2.8 × r	1.01	0.81	0.59	0.92	1.15	1.32	1.26	0.67
	2.8 × R	2.16	2.63	1.65	2.66	3.44	1.71	3.39	1.82

R—reproducibility standard deviation; r—repeatability standard deviation; CV_r—repeatability variation coefficient; CV_R—reproducibility variation coefficient; μ—sample average; 2.8 × r—repeatability limit; and 2.8 × R—reproducibility limit.

Table V. Statistical results obtained from the double blind tests

Variable	Statistics	S3	S8	S4	S7
Lab No.		15	15	15	15
	r	0.09	0.10	0.12	0.16
	R	0.25	0.17	0.35	0.40
	CV_r	0.09	0.10	0.13	0.18
IA%	CV_R	0.20	0.18	0.35	0.45
	μ	95.1	95.1	90.4	90.4
	2.8 × r	0.25	0.28	0.34	0.45
	2.8 × R	0.70	0.48	0.98	1.12
	r	0.21	0.24	0.33	0.45
	R	0.59	0.65	0.95	1.21
	CV_r	0.09	1.0	2.9	4.0
UCD	CV_R	2.3	1.9	8.4	10.8
	μ	23.8	23.8	11.2	11.2
	2.8 × r	0.59	0.67	0.92	1.26
	2.8 × R	1.65	1.82	2.66	3.39

Conclusions

The method used to measure starch damage by using the amperometric method was tested during a collaborative study. Fifteen laboratories, representing six countries, tested in duplicate eight samples including two double-blind tests. The statistical analysis of the results shows that the standard deviation value depends on the average starch damage value. The repeatability and reproducibility limits, like the critical difference and uncertainty (essential values for laboratories above all in the event of comparing results), must therefore be factored into this phenomenon. Tables are provided to make it easier to use the method. The blind duplicate test study shows the excellent robustness of the method making it possible to find exactly the same average values and very similar standard deviation values on two test pairs.

This primary method has been validated by the AACC International Physical Testing Methods Committee and is proposed under No. 76–33. It has already been published as a French standard under No. V03–731 and soon ICC Standard under No. 172.

Acknowledgments

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