The Joint AACC International–ICC Methods Harmonization Project

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Analytical chemistry was the basis of the formation of both AACC International and the International Association for Cereal Science and Technology (ICC). The original concept of an association of cereal chemists originated as long ago as 1915, when a group of mainly flour-mill chemists, of whom the question had been asked “Why can’t you chemists agree on your reports?” met in Kansas City, MO. The American Association of Cereal Chemists was first officially recognized on January 1, 1923 (2). From the humble beginnings of coordinating analytical methods, scientists of AACC Intl. have contributed an imposing volume of elegant and valuable research into many aspects of cereal chemistry. The fundamental research has probed the reasons underlying the complicated behavior of the components of cereals and their derived products. The applied research has addressed mainly the improvement of existing, and the introduction of new, analytical methods, processes, and products. Analytical chemistry remains the basis of *Cereal Chemistry*.

ICC is a global organization with its headquarters in Vienna, Austria, with member countries on all continents. It was established in 1955, with the original concept of the development of internationally approved standard testing procedures for cereals and flour (1). Founded during a disorderly period in European history, ICC provided a platform for cereal scientists to meet, exchange ideas, and cooperate with one another across ideological and political borders.

One of the objectives of ICC is to standardize analytical methods in cereal science and technology, as well as food safety and quality in general. The association has focused on standardization of analytical methods, on exchange of technical information, and extension of knowledge of cereal chemistry and technology among member nations. Both AACC Intl. and ICC have developed independent compendia of analytical methods for application to cereals and their products, and both associations have rigorous procedures for the approval of new methods and of modifications to existing methods. Some uncertainty has existed among laboratories in some ICC membership countries as to whether methods approved by AACC Intl. or ICC would be the most appropriate, and legally acceptable, for use in their laboratories. The same degree of uncertainty has existed in cereal laboratories in North America and elsewhere.

Both organizations hold some kind of liaison to the International Standards Organization (ISO) and the European Committee for Standardization (CEN). To improve global communication, to avoid duplication of efforts, and to avoid confusion of users of both organizations’ methods, the two associations have decided to initialize a harmonization process with global implications. Concentrating efforts and promoting standards with one voice should be achieved by 1) harmonizing existing standard methods issued by AACC Intl. and ICC, and by 2) sharing working groups, and 3) conducting joint validation studies.

About five years ago both associations agreed to develop a set of methods that would be acceptable by any cereal laboratory for the analysis of constituents and parameters that are frequently tested by cereal laboratories all over the world. Constituents are defined as actual components of grains and derived products, such as protein and moisture. Parameters are defined as factors that contribute to the functionality of cereals and their derived products, such as the gluten index and the sedimentation volume.

The development of coordinating the methods involved 1) identifying areas of the individual methods where changes were needed that would make the methods acceptable to all cereal laboratories, and 2) making suggestions for the possible changes.

The process has been documented under the joint AACC Intl.–ICC Methods Harmonization Project. This paper describes the methods that were addressed and the main suggestions made for changes.

The Methods Proposed for Harmonization

Starting in 2003 at meetings held at the AACC Intl. annual conventions, it was decided that nine methods should be standardized. Most of them fell under the responsibility of the Methods for Grains and Flour Testing Technical Committee, which drafted a set of suggestions for harmonization of the methods.

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Table I summarizes the methods as designated by AACC Intl. and ICC in their respective compendia of methods.

The Course of Action

Each method was studied, where applicable, from the aspects of principles, apparatus, reagents, procedure, calculation of results, and reporting as presented by AACC Intl. and by ICC in their respective compendia. Suggestions for changes were made, sent to the chair of the AACC Intl. Approved Methods Committee, and relayed to the ICC representatives in Europe for their evaluation and suggestions. After the suggested changes have been approved by both AACC Intl. and ICC representatives they will be incorporated into single methods for each constituent or parameter, for inclusion in the method compendia of both AACC Intl. and ICC. Meetings were held at each annual conference of AACC Intl., during the period of harmonization.

The Suggested Changes

The main changes that were suggested are summarized by method.

1. Determination of moisture content by the air-oven method. AACC Intl. Approved Method No. 44-15A and ICC Standard No. 110-1. The main changes are shown in Table II. Both the AACC Intl. and ICC methods suggest predrying of grains above 16% moisture (AACC Intl.) and 17% moisture (ICC) because grinding samples at elevated moisture contents for preparation of the samples for testing results in variable and undetected losses in moisture content, with consequent increases in error. The need for predrying of grains at high moisture content was investigated for a series of grains, which were ground by grinders with four different grinding actions. It was found that significant and variable losses in moisture occurred above levels as low as 11.0% moisture (3), so the moisture level at which predrying is recommended has been reduced to 12%.

Table I. Methods involved in the AACC Intl.–ICC Harmonization Project

Constituent/parameter	AACC Intl.	ICC Standard
Ash: incineration	8-01	104-1
Falling number	56-81B	107-1
Gluten index (mechanical)	38-12A	155
Moisture: oven method	44-15A	110-1
Protein: Kjeldahl	46-12	105-2
Protein: Dumas	46-30	167
Protein: NIRS	39-10	Rec. 159[a]
Wet gluten (mechanical)	38-12A	137-1
Zeleny sedimentation	56-61A	116-1

[a] Recommendation number.
NIRS—near-infrared reflectance spectroscopy.

2. Determination of protein content by the Kjeldahl method. AACC Intl. Approved Method No. 46-12 and ICC Standard No. 105-2. The procedures outlined by both AACC Intl. and ICC do not differ significantly, and no suggestions were made as to the actual procedure. The main suggestions addressed reagents, and the changes are summarized in Table III.

It is recommended that AACC Intl. and ICC both remove mercuric oxide or metallic mercury from possible use as catalyst for the Kjeldahl test, due to its extreme toxicity. Selenium dioxide is also highly toxic, and is also not recommended for use in large-scale Kjeldahl testing. Both titanium dioxide and cupric sulfate are regarded as being safe for use as catalysts. The boric acid method is preferred over back titration because the ammonia can be titrated directly against standard sulfuric acid. There is no need to prepare large volumes of standard sodium hydroxide solution, which involves protection from atmospheric carbon dioxide. The standard sulfuric acid used in titration of the ammonia is standardized volumetrically against standard sodium hydoxide solution.

3. Determination of protein content by the Dumas (combustion) method. AACC Intl. Approved Method No. 46-30 and ICC Standard No. 167. Both AACC Intl. and ICC recommend following the operating instructions specified by the instrument manufacturers. The procedure to be prescribed in the list of harmonized methods makes no suggestions for change, but will emphasize the need for use of the most pure gases and reference chemicals.

4. Determination of protein content by the near-infrared reflectance spectroscopy (NIRS) method. AACC Intl. Approved Method Nos. 39.00, 39-10, 39-11, 39-20, 39-21, and 39-25 address application of NIRS to respectively small grains, flour, soybeans, whole soybeans, and whole wheat. Approved Method 39-00 provides guidelines for application of NIRS to any of these (and other) commodities. ICC Standard No. 159 is applicable only to ground wheat and flour. Most of the specific methods of both AACC Intl. and ICC are out of date, with respect to new NIRS instrumentation, software, and general techniques, and the guidelines covered in AACC Intl. Approved Method No. 39.00 are considered to be of the most practical value in a series of harmonized methods. These have recently been updated.

5. The determination of ash content by incineration. AACC Intl. Approved Method No. 8-01 and ICC Standard No. 104-1. The AACC Intl. title does not define the material in which ash is to be determined at all, while the ICC title, which includes "cereals and cereal products for human consumption," is rather broad. "Cereal products" includes cakes, biscuits ("cookies" in North America only), noodles, breads, pasta, and others, and because the preparation of the sample for ashing will certainly differ

Table II. Suggested changes in procedure for testing for moisture content by air-oven

Factor	Changes to AACC Intl. method	Changes to ICC standard
Time of heating	Increase from 60 to 90 min[a]	Decrease from 120 to 90 min[a]
Temperature	130–135°C, no change	130–135°C, no change
Pretreatment (air drying)	Reduce from >16% to >12%[b]	Reduce from >17% to >12%[b]
Moistening of samples of <9%	Not applicable	Remove this step
Grinding apparatus	Wiley Model 4, or Perten KT 3303	Wiley Model 4 or Perten KT 3303
Sample size pretreatment	25 g	25 g (reduced from 100 g)
Sample size single-stage	2–3 g	2–3 g (reduced from 5 g)
Oven temperature regain	15–20 min sufficient[c]	15–20 min (reduced from 45)
Desiccator	Desiccator lid with sleeve suggested	Desiccator lid with sleeve suggested
Desiccant	Drierite[d] suggested	Drierite suggested (in place of P_2O_5)

[a] After oven temperature regain.
[b] See reference 3.
[c] "Temperature regain" means time required for oven to regain initial temperature (130°C) after putting in samples.
[d] $CaSO_4$ impregnated with $CoCl_2$.

among the diverse array of cereal products, perhaps a suitable title for this method would be "Determination of Ash in Flours and Whole-Meals."

For the purpose of harmonization among laboratories that seek to use either AACC Intl. or ICC methods, the method should be restricted to flours and whole-meals. This would mean that the method would be applicable to whole-meals of grains, other than wheat, which would include grains used in the animal feed industry, thereby widening the scope of the method. The main suggestions are summarized in Table IV.

The temperature of 900°C recommended by the ICC standard often results in fusion of the ash. This makes it difficult to clean crucibles after use. Outside of Europe, the temperature of 550–600°C has been found to be adequate for ashing, although in South Africa a temperature of 700°C is preferred. The 2 h specified by the ICC standard has not been found to be sufficient time to ensure complete ashing without use of magnesium acetate, which affects the precision of the test. The temperature of 550–600°C is convenient for some laboratories. Such laboratories can place samples in the furnace overnight and weigh them back in the morning, which saves laboratory time. This method is of less practical value to a flour mill that is producing many tons of flour every hour. Clearly the question of the temperature and time of the ash test for flour and wheat whole-meal requires resolution before a harmonized method can be recommended for adoption by all cereal laboratories.

6 and 7. Determination of wet gluten content and gluten index, mechanical method (Glutomatic). AACC Intl. Approved Method No. 38-12A and ICC Standards Nos. 137-1 and 155. Approved Method No. 38-12A of AACC Intl. includes both the wet gluten and gluten index tests. ICC has two standards for the mechanical determination of wet gluten content: ICC Standard 137-1 is only applicable to wheat flours and ICC Standard 155 is applicable to wheat flours and whole wheat meals, which also gives an objective measure of the gluten quality by the gluten index. The harmonized method will include both. Major changes include that the need to buffer the salt solution wash liquid is no longer recommended, the figures included in ICC Standard No. 137 have been eliminated, and the method is described to include both flour and whole wheat meal. The results of a ring study carried out by ISO in 1994 showed that there was no significant advantage in buffering the wash liquid. The diagrams included in ICC Standard No. 137-1 appeared to serve no useful purpose, and the suggestion is that they will not be attached to the harmonized method.

8. Determination of Falling Number. AACC Intl. Approved Method No. 56-81B and ICC Standard No. 107-1. The falling number is defined as the time in seconds required to stir and to allow a viscometer stirrer to fall a measured distance through a hot aqueous meal, flour, or starch gel undergoing liquefaction due to α-amylase activity. The test indicates α-amylase activity using the starch in the sample as substrate. The method is applicable to the meals and flours of cereal grains. Both AACC Intl. and ICC methods indicate that the method is applicable to malted cereals. This is not strictly correct. The falling number test is not applicable to malted grains. These grains will have large amounts of α-amylase due to the germination, so the results of the test could be misleading.

There are five important sources of error in the falling number test. These are 1) sampling (at least 250 and preferably 300 g of grain should be ground); 2) grinding the sample; 3) cleaning the grinder between samples (unless the grinder is self cleaning); 4) blending of the ground sample, and 5) shaking/mixing the sample in the viscometer tubes after addition of the water. For the most precise results, all of these should be addressed carefully.

The apparatus is automated and is available from Perten Instruments of Huddinge, Sweden (formerly the Falling Number Company). The ICC standard gives specifications for precision tubes and for the stirrer. The AACC Intl. method gives no specifications for the viscometer tubes. The AACC Intl. method includes the Perten Model KT-3100, the Newport Scientific, and the UDY Cyclone grinder as grinders suitable for the test, but also specifies a minimum of 250 g for the test. The receiving jar of the Cyclone grinder holds a maximum of 40 g, so the grinder is not convenient for this test. Both methods recommend that the weight of sample be corrected for moisture content, and that a

Table IV. Suggested changes in procedure for testing for ash content

Factor	Changes to AACC Intl. method	Changes to ICC standard
Temperature	None	550–600°C: change from 900°C
Reagents	None	Suggest take out use of alcohol
Dishes	None	Silica added as option
Desiccator	Sleeve on lid recommended	Sleeve on lid recommended
Time of ashing	6 h or overnight	6 h or overnight: change from 2 h

Table V. Illustrating the sedimentation index

Protein %	Parameter					
10	Sed. vol.(ml)	50	40	30	25	
	Sed. index	5.0	4.0	3.0	2.5	
12	Sed. vol.	60	50	40	30	
	Sed. index	5.0	4.2	3.3	2.5	
14	Sed. vol.	70	60	50	40	30
	Sed. index	5.0	4.3	3.6	2.9	2.1
16	Sed. vol.	70	60	50	40	30
	Sed. index	4.4	3.8	3.1	2.5	1.9

Table III. Suggested changes in procedure for testing for protein content

Factor	Changes to AACC Intl. method	Changes to ICC standard
Concentrated sulfuric acid	SG 1.84: changed from 93–98% pure	No change
Sodium hydroxide solution	40%: changed from 45%	No change
Catalyst	$TiO_2/CuSO_4$: change from HgO[a]	$TiO_2/CuSO_4$: change from $CuSO_4$ or SeO_2
Standard acid	H_2SO_4: no change	H_2SO_4: no standard acid recommended
Standardization chemical[b]	Potassium hydrogen phthalate[c]	Potassium hydrogen phthalate[c]
Standard reference chemical	EDTA: change from $(NH_4)_2C_2O_4$	EDTA: no reference specified
Receiving solution	Boric acid[d]	Boric acid: no change

[a] Recommend deletion of HgO due to extreme toxicity.
[b] For use in standardization of NaOH solution.
[c] No high-purity chemical previously recommended.
[d] Change from back titration of residual H_2SO_4.
EDTA—ethylenediaminetetraacetic acid.

further correction should be made for altitude, which affects the boiling point of water. The formulæ given in AACC Intl. Approved Method No. 56-81B are not clear, because no example is given for their use, but a table is given with approximate corrections and a more detailed table for altitude correction is available from Perten Instruments.

9. The Zeleny Sedimentation Test. AACC Intl. Approved Method No. 56-61A and ICC Standard No. 116-1. The test is applicable to wheat flour. ICC Standard 118 describes the preparation of test flour from wheat for the sedimentation test. The AACC Intl. method is based on the capacity of gluten proteins to swell under the influence of lactic acid. This method measures the relative gluten strength in wheat flour. ICC's test is based on the principle that the degree of sedimentation of a flour suspended in a lactic acid solution during a standard time interval is taken as a measure of baking quality. Swelling of the gluten fraction of flour in lactic acid solution affects the rate of sedimentation of a flour suspension in the lactic acid medium. Higher gluten content and better gluten quality both give rise to slower sedimentation and higher sedimentation test values.

The test relies heavily on the method of sample preparation for reliable results. Flour should be obtained from the wheat by grinding with a grinder with spiral burrs, which have a more gentle action than hammer mills or impeller mills. The main application of the test lies in rough screening for gluten quality in wheat-breeding programs. In the grain intervention system of the European Union (EC 824/2000), there are minimum requirements regarding the Zeleny sedimentation value.

If it is the wish of the AACC Intl. and ICC organizations to retain the method, it should be pointed out that the sedimentation volume according to the Zeleny procedure is affected by both the amount and inherent physicochemical properties of the gluten present in the wheat. The most meaningful approach to interpret the results is to divide the volume by the protein content to realize a "sedimentation index." Table V illustrates the sedimentation index for four protein levels and five sedimentation volumes. Individual laboratories could determine by experiment the sedimentation index appropriate for association with the baking quality for the types of breads predominant in their areas.

The test is also affected by particle size. The meal of soft wheats contains far more fine particles than that of hard wheats. Fine particles have longer settling time, and the results can appear to be higher than they actually should be, so that the suitability for evaluation of gluten characteristics is not given in every case for soft wheats. Accordingly the sedimentation test should be applied to wheats by class. A modification of the test that uses sodium dodecyl sulfate is preferred for use with durum wheat breeding programs (AACC Intl. Approved Method No. 56-70, ICC Standard No. 151).

Conclusion

The end product of the harmonization exercise is that, based on the suggestions made during the project, nine methods will be written up and included in both the *Approved Methods of AACC International* and the *ICC Standard Methods*. The methods will include principles underlying each method, together with full details of apparatus, reagents, steps in procedure, methods of reporting, references, and where applicable, results of ring tests.

Before approval two factors must be decided. First, an agreement has to be reached as to whether the changes suggested to the methods for determination of moisture content using the air-oven, for determination of protein content using the Kjeldahl method, and the determination of ash content require ring tests before approval. Second, a decision must be made on the method of presentation of the harmonized methods by AACC Intl. and ICC. A suggestion is that both organizations include a new section in their respective compendia that include the nine harmonized methods. Provided the harmonized methods receive approval by both AACC Intl. and ICC, all of them would be acceptable for use in any laboratory, for routine use, and for use in official contracts.

For suggestions and feedback on the joint AACC Intl.–ICC Methods Harmonization Project, please contact Phil Williams at philwilliams@pdkgrain.com or Roland Poms at office@icc.or.at.

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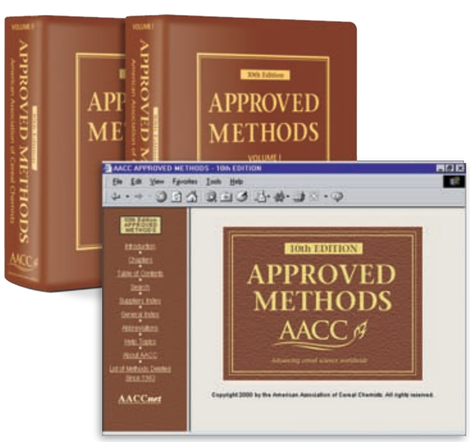

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