CEREAL FOODS WORLD®

A PUBLICATION OF AACC INTERNATIONAL

JANUARY-FEBRUARY 2018

In This Issue

aaccnet.org

The Association. AACC International® is a professional organization of individual and corporate members who specialize in grain research and the use of grains in foods. For more than 100 years, AACC International has been proactively supporting its members' professional needs by 1) promoting scientific and technical research on cereal grains, oilseeds, pulses, and related materials, including their processing, utilization, and products; 2) supporting the development and standardization of analytical methods related to grains; 3) promoting cooperation and professional growth among those working with grains and grain-based products through its meetings, Technical Committees, Divisions, Sections, and other activities; and 4) encouraging recognition of the value of professionals in the grains and grain-based products industries.

Membership. Membership in AACC International is open to individuals anywhere in the world. Corporate memberships are available for those companies wishing to lend industry support to the scientific work of the Association. An application form is available online at aaccnet.org.

Production. Editorial content and circulation for *Cereal Foods World*® (*CFW*®) are handled at AACC International headquarters, 3340 Pilot Knob Road, St. Paul, MN 55121, U.S.A.; Tel: +1.651.454.7250.

Mailing. *Cereal Foods World* (ISSN 0146-6283; PUB 099080) is published bimonthly by AACC International, 3340 Pilot Knob Road, St. Paul, MN 55121, U.S.A.; Tel: +1.651.454.7250. Periodicals postage paid at St. Paul, MN, and additional mailing offices. **Postmaster: Send address changes to *Cereal Foods World*, 3340 Pilot Knob Road, St. Paul, MN 55121, U.S.A.**

Responsibility. All articles published in *Cereal Foods World*—including editorials and columns—reflect the individual views of the authors and not the official position of AACC International or the institutions with which the authors are affiliated.

Reprints. Single reprints are not available from *Cereal Foods World*. Address requests for single reprints to authors. Copies of articles appearing in *Cereal Foods World* can be ordered, for a fee, from AACC International headquarters.

Subscriptions (2018 Rates). Nonmember subscription rate: $525/year within the United States, $621/year elsewhere. Prices of single and back issues (any year prior to current) are available on request. **Canadian customers:** Goods & Services Tax and/or Harmonized Sales Tax may apply for print subscriptions. *Cereal Foods World* is available in print or online by subscription to companies and institutions (www.aaccnet.org/publications/_layouts/aaccforms/grainsciencequote.aspx). The subscription rate is per location and online access is delivered by I.P. access. Multiple locations require separate subscriptions for each.

Claims for missing issues must be received within 60 days from the date of mailing plus time normally required for postal delivery of journal and claim. Claims are not accepted when the subscriber has failed to notify the Circulation Department of a change of address or only because a copy is missing from the subscriber's files. Send claims to claims@scisoc.org.

Change of Address. Notify the Circulation Department when changing addresses. Include a recent mailing label and new address with postal code. Allow two to four weeks for change to become effective.

Classified Ads. Classified ads appear in the News section of *Cereal Foods World* and are available in two formats: newspaper style and display ad style. Contact: Jordana Anker at janker@scisoc.org for more information. Classified ads should be e-mailed, mailed, or faxed to Jordana Anker, 3340 Pilot Knob Road, St. Paul, MN 55121, U.S.A.; Fax: +1.651.454.0766; E-mail: janker@scisoc.org. Ads must be received six weeks before the month of the issue in which they should appear.

Advertising Sales. Contact: Brianna Plank at +1.651.994.3819 or bplank@scisoc.org. Advertising orders and materials should be mailed to Brianna Plank at 3340 Pilot Knob Road, St. Paul, MN 55121, U.S.A.

Electronic Access. AACC International provides online access to *Cereal Foods World* at aaccnet.org for all members.

AACC International, 3340 Pilot Knob Road, St. Paul, MN 55121, U.S.A.; Tel: +1.651.454.7250; Fax: +1.651.454.0766.

ISSN (Print) 0146-6283
ISSN (Online) 2576-1056

aaccnet.org

Cereal Foods World® • Volume 63, Number 1 • Analytical

NEXT ISSUE — Baked Products

AACC International, Inc. ■ An International Society Since 1915

Touch Screen Simplicity

Viscosity Measurement and Yield Stress Determination at Your Fingertips

DV3T™ Rheometer

For results in a whole new light!

Full color display for easy viewing

Viscosity data capture and analysis on screen

Perfect for QC and R&D

Controlled user access and secure data integrity complies with 21 CFR Part 11

BROOKFIELD AMETEK®

TEL 800-628-8139 or 508-946-6200 www.brookfieldengineering.com

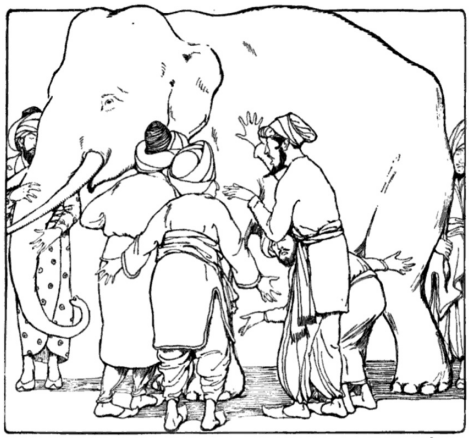
Padmanaban Krishnan

Wayne Moore

What Do "Blind Men and the Elephant" Have to Do with Laboratory Analytics?

Padmanaban Krishnan and Wayne Moore
Guest Editors

A group of blind men heard that a strange animal, called an elephant, had been brought to their town. Out of curiosity, they found the elephant and began to touch it to determine what sort of creature it was. The first man found the trunk and said, "This being is like a thick snake." The man who touched its leg claimed it was a tree-trunk. Feeling its side, one knew it as a wall. "No, it's a rope," shouted another. Grabbing the tusk, the last declared "You fools, it's hard and smooth, like a spear."



This image is in the public domain. Source: Holton, M. A., and Curry, C. M., Holton-Curry Readers, *Rand McNally & Co., Chicago, p. 108, 1914.*

In this parable from India, the blind men describe the elephant based on their partial experience, leading them to distrust and disagree about what an elephant is. The parable reveals how humans have a tendency to project their partial experiences as the whole truth and discount other people's experiences.

Like the blind men in this parable, analytical scientists sometimes become mired in the minutia of individual data points or complex data sets, yet lose sight of what the data actually means. In both academia and industry, laboratories collect data and generate reports, but they miss other relevant factors and are lulled into a false sense of accuracy. For example, a lab that acquired a new instrument began generating data to multiple decimal points, believing that the decimal points signaled precision. But, the instrument was not calibrated properly: precision does not equal truth.

This issue of *Cereal Foods World* presents a collection of articles that explore the theme of "seeking truth in data" from a few different angles. As a director of quality, these articles will help you design and manage quality control systems that are informed—not derailed—by analytical data. Cereal scientists who only peripherally touch on analytical issues will benefit from how these articles inspire different approaches when interpreting data related to their own work.

Hare explores how the data reported by an analytical lab is only as good as the capabilities of the lab. If a lab does not have adequate controls, then their results may be questioned. Some useful techniques are offered to help labs improve their performance using appropriate statistical methods for evaluating performance. He also urges plotting of data to enhance interpretation and communication of data.

Bettge shares a case study in which the price of wheat was affected by flawed interpretation of laboratory data. Grain prices to growers were discounted because of low falling numbers, despite the fact that the particular measurement is not a precise indicator of enzyme activity. Too much reliance on a method applied beyond its intended scope can have real-world consequences. Analytical scientists might focus on educating uninitiated readers and nontechnical business partners on how to extract meaning from their lab data.

Near-infrared spectroscopy (NIRS) has been used to measure moisture and protein of grains for many years, and more recently, calibrations for other constituents have come into practice. Krishnan has expanded the use of NIRS measurements to overlay spectra data on top of other analytics, which unveils a more holistic picture that includes β-glucan, amino acids, and fatty acids—you can touch more parts of the elephant.

From Szpylka's article, we learn how new technologies with increased sensitivities can measure contaminants at ever-lower levels. However, we must be cautious when interpreting results of analyses that show very low levels and understand the significance of these results.

This issue also includes three application articles that offer practical guidance. As a practicing statistician, Nelsen advises on how to design an experimentation plan rather than jumping into data collection and analysis. Without a clear objective and plan, you will be left at the back end asking, "What do I do with these data and what does it mean?" DeVault provides guidance on environmental testing for microbial and allergen food safety testing and addresses laboratory/plant food safety requirements. Guilfoyle presents a practical approach with "how to" steps for data analysis, including use of software.

Because many companies use external labs for specialized, or even routine, analyses, Stier explores the perennial topic of how to select an outside laboratory.

As you peruse this issue of *Cereal Foods World*, keep Ockham's razor in mind: among competing hypotheses, the one with the fewest assumptions should be selected.

https://doi.org/10.1094/ CFW-63-1-0003

President's Message

Envision the Future—Change Is Underway!

Laura Hansen
AACC International President

It's 2018, and we have a year of exciting new changes for AACC International ahead of us! First, I would like to thank all of you for the opportunity to be the president of this wonderful organization. It's a true privilege, and my goal is to exceed your expectations.

The start of a new year is always a good time to reflect on the accomplishments of the past year and build a vision for the coming year. To that end, I have picked a few areas from 2017 to highlight:

- Members of the technical committees advanced many new methods and completed four new methods for publication.
- We continued to collaborate with the International Association for Cereal Science and Technology (ICC), and we will be holding a joint symposium on ancient grains in Cape Town, South Africa, in April 2018 during Sorghum in the 21st Century.
- We transitioned our *Cereal Chemistry* journal to Wiley Publishing, which should increase our visibility and extend our global reach.
- We completed an exciting revamp of *Cereal Foods World* (*CFW*). We have transitioned the editorial process and updated our issue notifications. Each issue now has two volunteer guest editors working with Jon Faubion, newly appointed editor-in-chief, to develop content and enlist authors. In 2018, *CFW* will migrate to a new technology platform that will further enhance readers' experience with the addition of mobile-friendly reading options.

Strengthened by our many recent accomplishments, we must also resolve to think about our organization differently as we head into 2018. We find ourselves in the middle of a transformation across the food sector. Although we have all experienced the challenges of the food industry's changing dynamics, many of us have found that we must approach problems from different angles and try new ideas without fear of failure or setback. Embracing changes can be difficult, but opportunities to evolve and grow can also be invigorating. I am excited by the bright future that lies ahead for AACC International.

Our growing global membership presents both challenges and expanded opportunities. Most of you are aware that over the past year we have been evaluating whether the name of our organization continues to reflect our membership. Although our name has served us well for more than 100 years, our membership is much broader than an association of American cereal chemists. Over the last year we have been working on a rebranding effort to more effectively reach our community. We have received feedback from members concerning the value of the organization and what it means to them. I am energized when I hear members speak with passion about AACC International. Several key themes have risen to the top from member input: connections, networking, science, grains, nutrition, and innovation. Our challenge will be to build on these themes and weave them into a new name and future-focused brand for membership consideration. A name change will be put forth for membership approval this year. Stay tuned and look for communications on the rebranding effort over the coming year!

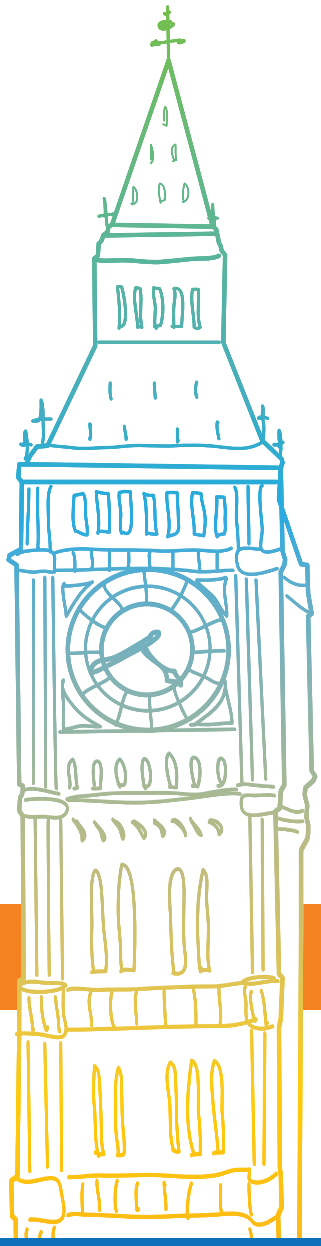
As an international organization, we have finally taken the monumental step to hold an annual meeting outside North America. October 21–23, 2018, we will be in London, England, for Cereals & Grains 18! We envision a meeting that will be new and different from past annual meetings, but I can assure you that the scientific and technical content of the meeting will still be top-notch. Check the AACCI website frequently for travel tips, links to travel sites for more information about traveling within and around England, hotel information, and registration details. We pledge to be transparent about the cost of the meeting so that members understand all that is included. We are looking forward to expanded participation from our members on the European and Asian continents, and we look forward to our members in North America crossing the ocean for the event!

Finally, AACC International is an organization for and of its members, and we cannot survive without member participation and engagement. I encourage all of you to stay involved and share with your colleagues the benefits of being a member. Help us envision the future and change the organization to reflect that vison.

Never hesitate to reach out with questions or to share your concerns. I look forward to meeting many of you in London! Until then, may you all have a happy, healthy, and successful 2018!

https://doi.org/10.1094/ CFW-63-1-0004

CEREALS & GRAINS 18



The 2018 AACCI Annual Meeting is crossing the pond!

Join us in London to discuss the latest research and trends in cereal grain science.

SAVE THE DATE

October 21–23, 2018
Hilton London Metropole
London, United Kingdom

Get ALL the Latest Updates. Follow AACCI!

aaccnet.org/meet
#CerealsGrains18 #AACCI2018

AACC INTERNATIONAL

Using Data for Laboratory Performance Improvement

Lynne B. Hare[1]
Statistical Strategies, LLC, Plymouth, MA, U.S.A.

ABSTRACT

The quality of a laboratory's most important product, information, can be assured through the proper use of statistical tools that support the quest for method accuracy and precision together with routine programs designed to assure their consistency over time. Methods for applying these tools are presented, along with examples and recommendations for routine applications. An additional topic, useful in assuring analytical accuracy, is the determination of sample sizes needed to detect crucial product defect and contamination levels.

"Healthy" analytical laboratories contribute to organizational success by delivering accurate and precise information to both internal and external clients of their services. A high-quality contribution does not happen by accident. It requires hard, continuous work and great attention to detail. Managers of analytical laboratories may entreat their staff members to pay close attention to protocol, engage in discussions of analytical procedures with colleagues, and compare occasional matched samples. These efforts, however, usually wane and lack sufficient impact to assure the long-term, consistent accuracy and precision that results in the client credibility necessary to maintain a truly productive laboratory that provides high-quality contributions.

For laboratory managers to lead their organizations to a healthier status, they must remember that all analytical values are estimates of the true state of nature and that the state of nature is inevitably contaminated by "error" associated with experimental variation. Error in this sense, is the difference between result and actuality. The estimate of the natural state is often referred to as a "signal," and the associated error (or variation) is referred to as "noise." Much focus should be directed to the signal-to-noise ratio. It has been said that there is never a signal without noise. More information on this subject can be found in Box (1) and Box et al. (2).

The immediate focus of a laboratory must be on attaining analytical accuracy and precision. An overall technology for obtaining the necessary data to measure both is measurement systems analysis (MSA). This is a divide-and-conquer process for first measuring accuracy in terms of linearity and bias against known values and then measuring precision by its component parts of reproducibility and repeatability. Reproducibility refers to consistency across equipment and analysts, whereas repeatability addresses uniformity among replicate readings by one analyst using the same equipment.

Accuracy can be assessed through the application of regression models as aids to calibration and detection of bias. More information on regression analysis can be found in Montgomery et al. (11).

Precision is measured by specially designed experiments tailored to the laboratory equipment and staff configuration. As a result, there are many varieties of these studies, commonly called Gage (or Gauge) repeatability and reproducibility (R&R) studies. Much of the literature on Six Sigma contains sections on MSA, including Gage R&R. Leadership aspects of Six Sigma, including MSA, are presented by Snee and Hoerl (12), whereas technical, statistical details are offered by Breyfogle (3) and Hare (5).

A laboratory's credibility is increased when its staff reports response estimates accompanied by intervals stating the uncertainty of the estimates. This can take the form of confidence intervals, standard deviation or standard error estimates, or any of a number of other expressions of uncertainty. More information on statistical intervals can be found in Meeker et al. (8).

As important as assessment of methodological accuracy and precision are, laboratory health does not end there; as with good human health, good laboratory health must be maintained. One maintenance device is the control chart. It provides a graphical representation of laboratory health by displaying the means and standard deviations among blind replicate samples over time. As such, it aids in the detection of unusual results and trends away from stability. Montgomery (10) provides details on many types of control charts and examples of their use.

Another maintenance device is the interlaboratory check sample program. As the name suggests, such a program is designed to compare the output of multiple laboratories in an effort to assure uniformity among laboratories within an association of laboratories commonly assessing the same analytes. As with Gage R&R studies, design-of-experiments technology is used to plan the allocation of samples to potential sources of variation. With interlaboratory check samples, however, the sources of variation include laboratory differences and may also include differences among technicians within laboratories, equipment differences, and even protocol differences. Designs can be quite complex, but they should include blindly submitted random samples and sample replication. Details of the statistical design of experiments are described in Box et al. (2) and Montgomery (9).

Laboratory managers and staff have opportunities to extend their reach by offering advice on the determination of the sample sizes necessary to detect differences that may be important to business success. Although this might typically be considered the domain of the statistician, questions of sample size might be better answered through multidisciplinary collaboration. Background information can be found in Hogg et al. (6), as well as other references on statistical inference.

In the following sections each of these topics is discussed and examples are presented.

Accuracy

The assessment of method accuracy usually is made against a known standard, and linear regression models are used to learn about the relationship between the unknown and the known. Some hypothetical data are shown in Table I. Vacuum-oven moisture data from 15 production samples are listed together with the corresponding readings from two competitive moisture

[1] Tel: +1.908.627.2309; E-mail: lynne.hare@comcast.net

https://doi.org/10.1094/ CFW-63-1-0006

meters. When known standards are not available, chemists must resort to other special methods, such as the use of known ingredient additions. These methods are not discussed here.

My first rule of data analysis is, "Always, always, always, without exception, plot the data—and look at the plot." One would think that the "look at the plot" part would not be necessary, but experience suggests otherwise. Moisture readings for each of the two meters plotted against their corresponding vacuum-oven moisture values are shown in Figure 1.

A close look shows that a linear fit for meter 1 might be reasonable, whereas the same fit for meter 2 might not. The graphical results of the two linear fits with confidence intervals shown in Figure 2 bear this out—notice the downward bias near the center of the vacuum-oven data for meter 2.

The linear, or first-order, model is

$$\eta = \beta_0 + \beta_1 x + \varepsilon \qquad (1)$$

and the second-order, or quadratic, model is

$$\eta = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon \qquad (2)$$

Table I. Hypothetical vacuum-oven moisture determinations with corresponding readings from two competitive moisture meters (data are sorted by vacuum-oven moisture)

Sample	Vacuum-Oven Moisture (%)	Moisture Meter 1 Reading (%)	Moisture Meter 2 Reading (%)
1	10.1	10.5	11.0
2	10.5	10.2	10.8
3	11.1	11.0	10.7
4	11.6	11.7	11.0
5	11.7	11.6	10.9
6	12.7	12.3	11.8
7	13.0	13.2	11.7
8	13.2	13.0	12.0
9	13.2	12.8	11.9
10	13.6	13.4	12.6
11	14.4	14.2	14.4
12	14.7	15.0	15.0
13	15.3	15.2	15.7
14	15.5	15.5	16.2
15	15.7	15.7	16.4

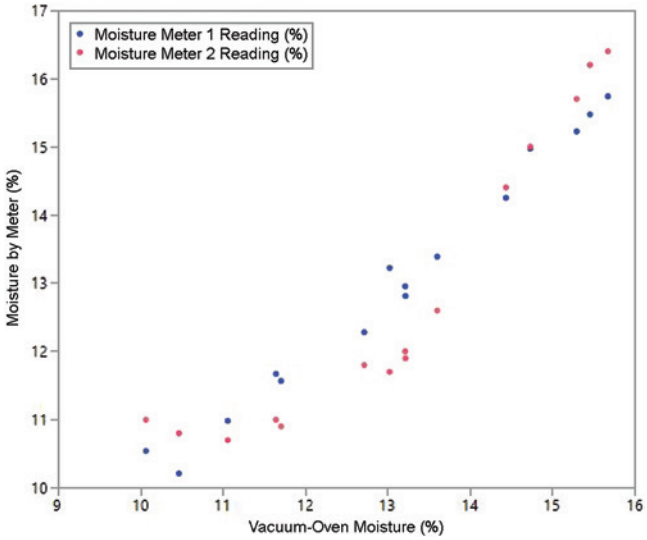


Fig. 1. A scatter plot of readings from two competitive moisture meters against their corresponding vacuum-oven moisture values.

In these models, η is the expected vacuum-oven moisture; the βs are the coefficients to be estimated from the data; x is the moisture resulting from the meter; and ε is the error or difference between actual and predicted values.

The variation of each of the two data sets partitioned into the total, that due to the model and that due to the errors, is shown in Table II. Note that for the linear fit, model 1, the error or root mean square error for meter 2 is much larger than that for meter 1. However, if the second-order model 2 is fit to the meter 2 data, the error matches that for meter 1.

When the second-order model is fit to the data representing meter 2, the coefficient of the squared term shows statistical significance (Table III), meaning that its high value relative to its standard error did not happen by chance alone. There is a specific cause.

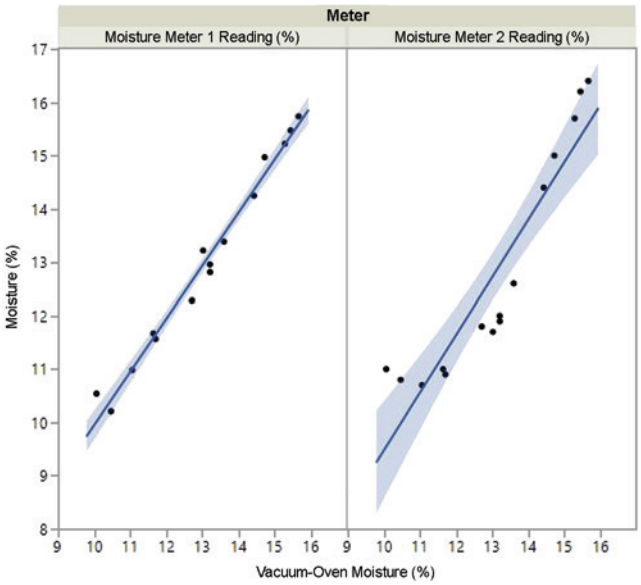


Fig. 2. Graphical summaries of linear regression model fits for two moisture meter readings against the corresponding vacuum-oven moisture readings. The line is the regression fit, and the shaded region surrounding the regression line represents the 95% confidence interval for the mean.

Table II. Regression analysis tables corresponding to first (linear) and second (quadratic) order models by meter[a]

Meter	Source	DF	Sum of Squares	Mean Square	Root Mean Square
Linear model					
1	Model	1	45.62	45.62	
	Error	13	0.85	0.07	0.26
	Total	14	46.47		
2	Model	1	53.69	53.69	
	Error	13	8.84	0.68	0.82
	Total	14	62.53		
Second-order model					
2	Model	2	61.91	30.96	
	Error	12	0.62	0.05	0.23
	Total	14	62.53		

[a] The root mean square error corresponding to the first-order model for meter 2 is much larger than that for meter 1. When the second-order model is fit to the data for meter 2, the root mean square error reduces to that for meter 1.

None of the above discussion is intended to rule out meter 2, so much as it is intended to show that the relationship between meter and truth can be approximated by regression analysis for calibration purposes. It may be that a linear relationship is more persuasive of veracity, but if a curved relationship, such as that of meter 2, can be confirmed, the corresponding equipment may be preferred, especially if it is associated with higher reliability or lower cost.

Precision

There is a hierarchy of variation inherent in all processes (Fig. 3). Total process and product variation consists of both the product variation and the variation associated with our ability to measure product attributes. Measurement variation, in turn, comprises both accuracy, as described above, and precision, which is comprised of repeatability and reproducibility.

Specially tailored studies, called Gage R&R studies, are designed to measure these latter two elements of measurement variation. Factors to be taken into account are analytical equipment differences, operator differences, and product sampling variation. In larger studies, differences among laboratories can also be taken into account. Part of the basic thinking focuses on equipment differences. If there are differences among devices, they can be resolved through adjustment or, if necessary, replacement. Another part of the thinking focuses on operator differences. They might be resolved through training to include benchmarking and reexamination of protocols.

The ultimate objective of a Gage R&R study is to quantify the variation due to reproducibility and repeatability. As described earlier, reproducibility is the variation experienced by multiple operators examining the same sample, perhaps with different instruments, whereas repeatability is the variation due to repeated measurement of the same sample.

Table III. Coefficient estimates of model 1 for meter 1 and model 2 for meter 2, with standard errors, *t* ratios, and probabilities[a]

Term	Estimate	SE	*t* Ratio	$P > \|t\|$
Linear model for meter 1				
Intercept	0.01	0.50	0.0	0.99
Linear term	0.99	0.04	26.4	<0.0001
Second-order model for meter 2				
Intercept	–2.94	0.46	–6.4	<0.0001
Linear term	1.14	0.03	33.9	<0.0001
Quadratic term	0.26	0.02	12.7	<0.0001

[a] The low probabilities confirm the significance of the model fits to the two data sets. The slope of the equation for meter 1, 0.99, is easily within the expected value of 1.0 given that its error estimate is 0.04. This suggests a likely one-to-one relationship between meter 1 and the vacuum oven.

Water activity data generated in a simple Gage R&R study are listed in Table IV. In this study, 10 production samples were homogenized and then divided among 3 operators whose duplicate samples were submitted blindly to them. For operators, it was business as usual. They knew neither the sample number nor the fact that they were analyzing replicates of the same sample for each of 10 samples as part of a designed study.

Typically, analysis of variance (ANOVA), together with its calculated variance components, is used for the analysis of Gage R&R studies. Although it is tempting to plunge into this analysis, it is very important to remember the first law of data analysis (described earlier) and plot the data. Plotting the data can help us avoid the wasted time involved in do-overs due to late discoveries of typos and outliers. We may see things in graphs, e.g., someone writes down 0.271 instead of 0.721, which we are not as likely to see in a table of numbers.

Replicate observations plotted for each of the three operators for each of the 10 samples are shown in Figure 4. Close inspection gives rise to the suspicion that operator 2 may have obtained lower water activities than the other two operators. To see this, it is necessary to spend some time carefully looking at the graph. After examining the graph, it may be concluded that, beyond the operator difference, there are no other obvious mes-

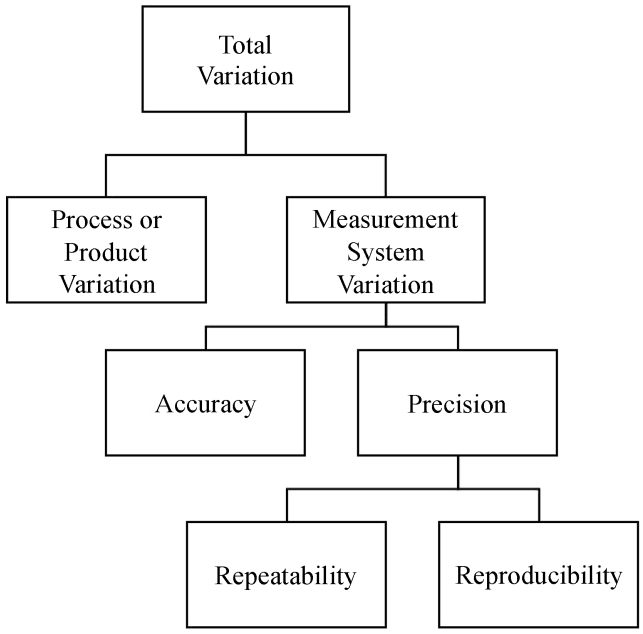


Fig. 3. The hierarchy of variation.

Table IV. Water activity data generated by a simple Gage repeatability and reproducibility (R&R) study

	Operator 1		Operator 2		Operator 3	
Sample	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	0.721	0.720	0.718	0.726	0.720	0.723
2	0.715	0.711	0.694	0.699	0.716	0.710
3	0.730	0.720	0.719	0.718	0.730	0.733
4	0.717	0.729	0.710	0.721	0.729	0.723
5	0.724	0.711	0.703	0.706	0.718	0.706
6	0.711	0.711	0.709	0.705	0.719	0.725
7	0.717	0.722	0.705	0.710	0.724	0.727
8	0.712	0.719	0.715	0.716	0.728	0.737
9	0.733	0.722	0.729	0.725	0.729	0.737
10	0.717	0.725	0.723	0.716	0.735	0.731

sages, and no typos or other quirks are evident. It is safe to proceed with the ANOVA.

The ANOVA partitions the total variation into separate, assignable sources of variation. It lists the amount of variation due to sample differences, operator differences, differences in operator results depending on operators (this is called an interaction), and the replicate variation that we take as random variation or random "error." Excellent statistical software packages are available to perform these calculations.

The source of variation, the corresponding degrees of freedom, sums of squares and mean squares, by line, are shown in Table V. Mean squares are sums of squares divided by degrees of freedom. The mean squares are divided by the error or "Reps(S×O)" (read replicates within the sample-by-operator interaction) mean square to create corresponding *F* ratios. *F* ratios have a known probability distribution that depends on numerator and denominator degrees of freedom, so it is possible to determine when an *F* ratio is larger than chance alone would allow. This study had no provision for repeated measures, but if it did, we would be able to determine from the ANOVA how much variation was due to repetitions as well.

The *F* ratios for operator and sample differences are so large that their probabilities are nearly zero. Differences among samples should come as no surprise. Samples taken from manufacturing processes over the long run will, in fact, show differences unless analytical method differences are insensitive. However, an opportunity for improvement is revealed by the low probability of the *F* ratio corresponding to operators. As illustrated in Figure 5, operators differ significantly from each other, and because they do, they contribute to the uncertainty of laboratory results. Laboratory health can be improved if differences among operators are removed.

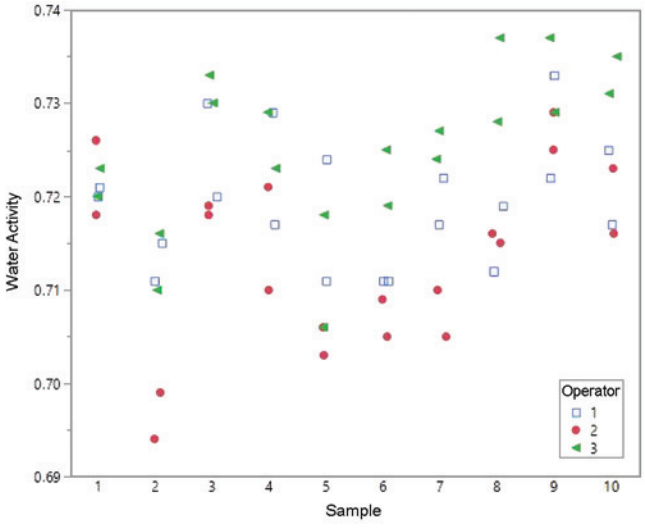


Fig. 4. Gage repeatability and reproducibility (R&R) data: water activity versus sample.

Table V. Analysis of variance (ANOVA) table for Gage repeatability and reproducibility (R&R) data

Source	DF	Sum of Squares	Mean Squares	*F* Ratio	*P* > *F*
Total	59	0.005241			
Operator	2	0.001358	0.000679	27.97	<0.0001
Sample	9	0.002465	0.000274	11.29	<0.0001
Sample × operator	18	0.000690	0.000038	1.58	0.1304
Reps(S×O)	30	0.000728	0.000024		

Maintaining Laboratory Health

Once established, accuracy and precision estimates will go adrift unless maintained. Routine monitoring and maintenance are essential. Two devices are useful for this purpose—one for within-laboratory control and the second for multilaboratory comparisons.

Within-Laboratory Control. The presence of a laboratory information management system (LIMS) and suitable statistical software greatly facilitate internal checks. A relatively simple device is a standard Shewhart chart for detecting errors and drift. Using it, the laboratory supervisor inserts blind replicate samples into the stream of routine samples to the analyst. It should be noted that the purpose in doing this is not so much to catch the analyst out as it is to aid in the detection of assignable causes of wayward results so they can be discovered and eliminated. Usually these samples are duplicates, but multiple blind replicates can be used if a procedure with greater power of detection is desired. Data from a stream of water activity data showing only those production samples that have blind duplicates are listed in Table VI.

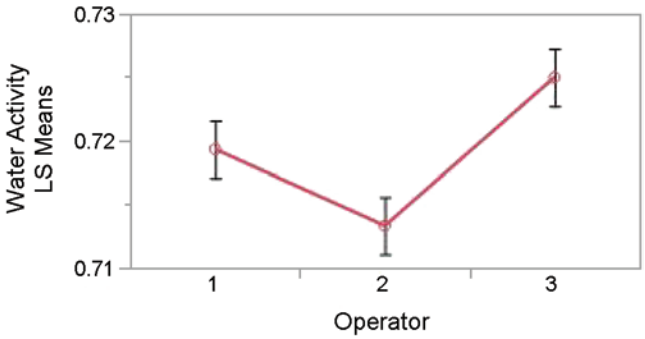


Fig. 5. Gage repeatability and reproducibility (R&R) example—operator differences are illustrated by software-drawn "least significant (LS) intervals," which are calculated such that if they fail to overlap differences among means may be declared significant. There is no overlap. All operators are different from each other.

Table VI. Blind replicate water activity determinations

Sample	Replicate 1	Replicate 2
1	0.731	0.741
2	0.714	0.706
3	0.727	0.737
4	0.716	0.718
5	0.719	0.716
6	0.704	0.72
7	0.713	0.723
8	0.723	0.711
9	0.721	0.716
10	0.714	0.726
11	0.706	0.711
12	0.727	0.725
13	0.718	0.731
14	0.711	0.703
15	0.723	0.721
16	0.712	0.705
17	0.727	0.728
18	0.724	0.782
19	0.728	0.744
20	0.718	0.703

The corresponding Shewhart control charts for the mean and range of the samples are shown in Figure 6. For purposes of analytical reproducibility, the mean chart might seem to be of little value. It simply shows the manufacturing variation, or so one might believe. Notice, however, that the range chart shows greater variation between duplicates at observation 18 than expected. At that same time point, the mean is out of control on the high side. Although the cause of the variation could be either analytical or production, there is greater wisdom in first checking the analysis before alerting the manufacturing department.

Multiple Laboratory Comparisons. Corporate and association laboratories should be in alignment with regard to reported analytical values. Often, interlaboratory test samples are circulated in round robin tests: several samples are created, subdivided, shuffled, and distributed to participating laboratories for analysis. Of course, it is best if blind duplicates are included. When all the data are gathered, they are subject to ANOVA modeling, partitioning sample, laboratory, interaction, and replicate variation so that laboratory differences may be identified. The data gathering and analysis process is similar to that described earlier for Gage R&R studies. The thinking is that once the outlying laboratories are identified, corrective action can be taken to bring their results into alignment with the other laboratories.

A useful graphical device for comparing laboratories when there are two replicates for each sample is the Youden plot (4). (Hare [4] describes the device in the context of a good statistical bedside manner; hence, the unusual title.) Jack Youden, a chemist and statistician at the National Institute for Standards and Technology (NIST), developed this plot in an effort to communicate the results of interlaboratory testing without the obfuscation many associate with statistical analyses.

The data in Table VII are used as an example. For each sample that appears in an interlaboratory test, Youden would plot the first replicate on the horizontal axis of a square graph and the corresponding second replicate on the vertical axis. The plotting symbol for each point is accompanied by a letter code designating the laboratory. A quick glance at Figure 7 shows that the two replicates for laboratory M are not in agreement. They are excluded from the calculation of the replication variation, which is then used to form a circle centered on the mean (or median) with a radius of 2.45 times the replication standard deviation. (Note, this is only an approximate radius. For information on how to obtain an exact radius visit the NIST web pages [www.nist.gov] on Youden plots.) The circle is intended to contain approximately 95% of laboratory means if laboratories do not differ.

Points outside the circle identify laboratories whose means differ from the mean (or median) of all results. Points that stray substantially from the diagonal line identify laboratories whose replicates do not agree with each other. An advantage of using a Youden plot is that the full diagnosis is communicated in one simple graph.

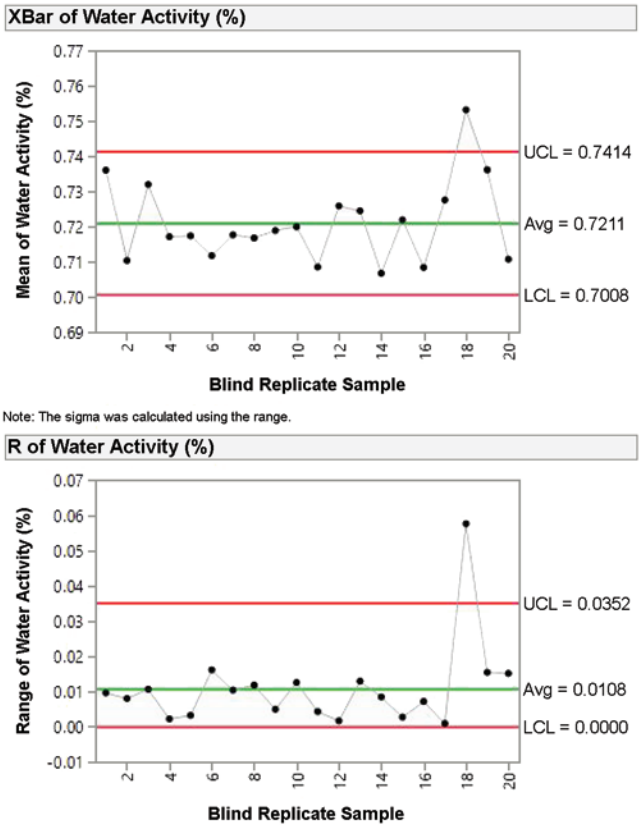


Fig. 6. Mean and range Shewhart control charts for blind analytical sampling assurance.

Table VII. Interlaboratory study data

Laboratory	Replicate 1	Replicate 2
A	57.9	70.9
B	57.3	71.1
C	67.9	77.9
D	84.0	78.9
E	84.0	55.3
F	58.1	57.6
G	61.9	55.3
H	39.2	33.9
I	56.4	58.8
J	45.3	51.6
K	63.0	62.7
L	60.9	75.6
M	35.4	112.7
N	81.7	77.6
O	58.9	60.5
P	51.9	55.8

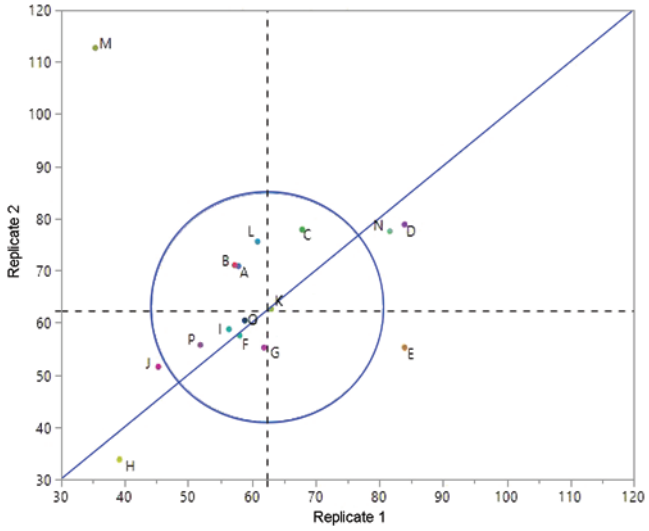


Fig. 7. Youden plot of vitamin A data: replicate 1 versus replicate 2.

Sample Size Determination

For most analytical measures, the determination of sample size depends on the error variation, the difference that might be considered important, and the risk of getting it wrong in both directions, i.e., the risk of declaring a difference when none exists and the risk of failing to detect a difference of a particular size. Most statistical software packages are able to calculate sample sizes given this input. First-time users are usually overly ambitious in the selection of tiny risks that drive sample sizes skyward, discrediting even remote use of statistical sampling. The result can be weakened opportunities for sound organizational decision making.

Many other means of sample size determination require special statistical attention. Two examples are provided.

Defect and Defective Item Detection. Seemingly inevitably, organizations will encounter the problem of suspect defects or defective items in otherwise normal production. The issue may arise as a result of customer complaints or chance encounters. For the laboratory, a first task may be to confirm their existence.

This might be considered a "needles in haystacks" problem, but for confirmation, a hypothetical proportion, p, must be assumed. The probability distribution of defects or defective items is assumed to be binomial and is given by

$$f(x) = \binom{n}{x} p^x (1-p)^{n-x}$$

where x is the number of defects or defective items in an individual sample; n is the number of units in the sample; and p is the hypothetical proportion of defects or defective items.

The expression $\binom{n}{x}$ is called the binomial coefficient and is equivalent to $\frac{n!}{x!(n-x)!}$. The "!" symbol is used to designate the product of the integers up to and including the letter preceding it. For example, $n! = 1 \cdot 2 \cdot 3 \cdots n$.

The probability of not finding a defect or defective unit in a sample of n units is

$$f(0) = \binom{n}{0} p^0 (1-p)^{n-0} = (1-p)^n$$

This means that the probability of detecting one or more defects or defective items in a sample of size n is $P = 1 - (1 - p)^n$.

If P is fixed at some specific value, such as 0.95 or 0.99, corresponding to 95% or 99% chance of detecting a defect or defective unit, then when the true proportion is p, the sample size, n, required is given by

$$n = \frac{\log(1-P)}{\log(1-p)}$$

For example, if it is speculated that the true defect or defective level is 1 in 1,000 or 0.001 and a 95% chance of detecting that level is desired, it will take

$$n = \frac{\log(1-0.95)}{\log(1-0.001)} = 2{,}994.2 \text{ or } 2{,}995 \text{ samples}$$

Accidental Inclusion. If, instead, the issue is one of accidental inclusion of an undesired substance, the probability distribution is often Poisson. The Poisson probability distribution function is

$$f(x) = \frac{e^{-\lambda}\lambda^x}{x!}, x = 0,1,2, \ldots$$

As an example of its application, consider International Commission on Microbiological Specifications for Foods sampling recommendations (7). For *Salmonella* sampling they recommend taking multiple 25 g samples to determine lot disposition. A standard is defined as 0 colony-forming units (CFU) in 100 g of product. In this case, the equation above reduces to

$$f(0) = e^{-\lambda}$$

The mean, λ, is expressed as the sample size, n, times the proportion (or probability) of a single CFU. If we seek detection of a single CFU, we might take 10 25 g samples for a total of 250 g, remembering that the standard is 0 CFU in 100 g of product. The probability function shows

$$f(0) = e^{-\frac{250(1)}{100}} = e^{-2.5} = 0.082$$

indicating an 8.2% chance of detection.

Concluding Comments

On first reading this article, and perhaps even beyond, the topics presented may seem daunting. Beginners and even those with some statistical experience should seek the assistance of a statistician who can collaborate with the laboratory team to tailor methods aimed at improving laboratory health. It is only by engaging in these practices that a laboratory can become a team fully engaged in continuous improvement and integrated with the vision and mission of the overall organization.

Author's Note

Graphs were produced using JMP 13 software (SAS Institute, Cary, NC).

References

1. Box, G. E. P. Signal-to-noise ratios, performance criteria and transformations. Technometrics 30:1, 1988.
2. Box, G. E. P., Hunter, J. S., and Hunter, W. G. *Statistics for Experimenters*. John Wiley and Sons, Hoboken, NJ, 2005.
3. Breyfogle, F. W. *Implementing Six Sigma*. John Wiley and Sons, Hoboken, NJ, 2003.
4. Hare, L. B. It's not always what you say, but how you say it. Qual. Prog., August, 2007.
5. Hare, L. B. Gage R&R reminders. Qual. Prog., January, 2012.
6. Hogg, R. V., Tanis, E. A., and Zimmerman, D. L. *Probability and Statistical Inference*, 9th ed. Pearson, Upper Saddle River, NJ, 2015.
7. International Commission on Microbiological Specifications for Foods. *Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance*. K. M. J. Swanson, ed. Springer, NY, 2011.
8. Meeker, W. Q., Hahn, G. J., and Escobar, L. A. *Statistical Intervals*, 2nd ed. John Wiley and Sons, Hoboken, NJ, 2017.
9. Montgomery, D. C. *Design and Analysis of Experiments*. John Wiley and Sons, Hoboken, NJ, 2012.
10. Montgomery, D. C. *Statistical Quality Control*. John Wiley and Sons, Hoboken, NJ, 2013.
11. Montgomery, D. C., Peck, E. A., and Vining, C. G. *Introduction to Linear Regression Analysis*, 5th ed. John Wiley and Sons, Hoboken, NJ, 2012.
12. Snee, R. D., and Hoerl, R. W. *Leading Six Sigma*. John Wiley and Sons, Hoboken, NJ, 2003.

Low Falling Numbers in the Pacific Northwest Wheat Growing Region: Preharvest Sprouting, Late Maturity Amylase, Falling Number Instrument, or Low Protein?

A. D. Bettge[1]
ADB Wheat Consulting, Moscow, ID, U.S.A.

A kernel of wheat is an edible product as a result of years of selection pressure by humans, which has led to kernels that when milled produce flour with the properties required for proper mixing and baking. A kernel, left to its own devices, is predisposed only to produce another wheat plant. As such, kernels are storehouses of calories and nutrients that not only enable germination, but also have the secondary ability to be used to make tasty products people enjoy eating.

The energy and nutrients within a kernel are stored primarily as large polymers of glucose in the form of starch (endosperm), amino acids in the form of proteins (endosperm), and lipids in the form of triacylglycerols and other fats (scutellum and embryo, which collectively make up the germ). Minerals and other compounds necessary to propagate the plant also exist within the kernel. However, none of the nutrient and caloric components of the kernel can be accessed by a sprouting, growing plant without the presence of enzymes that are synthesized as part of the germination process.

As nutrient-containing compounds are converted from polymers to their monomeric constituents by enzymes, these polymers become less useful for baking and other food processing needs. Chief among the detrimental impacts of enzymatic activity, from a human point of view, is the hydrolysis of starch into glucose by α-amylase, an endo-acting enzyme that randomly hydrolyzes starch polymers and with amyloglucosidase produces glucose monomers used by the growing plant for energy.

From the perspective of cereal grain utilization as a food product, starch is necessary to allow for proper baking profiles, in terms of water and heat, to make a functional end-use product. Starch granules swell, imbibing water that is present in the baking system at specific temperatures and controlling the amount of water available for other reactions within the system. Removal of water during this gelatinization process initially creates viscosity and then, through the process of starch recrystallization or retrogradation, "sets" the baked product as the system cools postbaking.

Without large, intact starch polymers, neither gelatinization nor retrogradation can occur sufficiently well to produce an acceptable product—sticky doughs and collapsed and gooey end products are the result (8). The presence of α-amylase, an endo-acting enzyme that specifically acts on α1-4 glycosidic bonds, deleteriously affects starch gelatinization and retrogradation processes through starch polymer hydrolysis. The result is serious economic losses if the presence of amylase is not discovered prior to utilization of the grain.

The need to characterize grain lots for the presence of amylase was recognized many years ago (9,14). The most direct test method was to measure the ability of flour, or ground grain, to form a gel sufficiently robust to produce acceptable products. If amylase had hydrolyzed starch beyond a certain arbitrary point, the grain was judged to be of inferior quality. Hagberg (9) and Perten (14) developed the falling number test to quantify the soundness of wheat grain lots relative to the presence of α-amylase (i.e., low enzyme activity) (AACCI Method 56-81.03 [1]). The falling number test involves suspending a measured amount of ground grain or flour in a long cylindrical tube with water and then mixing the suspension in a boiling water bath for 60 sec, past the point of gelatinization and denaturation of α-amylase. Intact starch polymers produce a more robust gel than granules affected by enzymic hydrolysis, and longer times are required for a standardized, weighted plunger positioned on top of the gelatinized starch to fall through the gel. The number of seconds it takes for the plunger to fall through the gel is recorded from the start of the test. Because starch that has been degraded by α-amylase produces a less firm gel, the plunger falls more quickly, resulting in a lower falling number.

The falling number test actually measures the impact of α-amylase on starch pasting properties, as well as other factors, and not the amount of enzyme itself. This falling number test has been adopted by the grain trade industry as a rapid and useful test to determine starch quality and, therefore, the suitability of a grain lot for baking. Milling and baking companies also utilize falling number in their specifications. Economic value is assigned to wheat as it is traded and utilized as a result of this test.

To properly place falling number results into a useful end-use context for estimation of performance and establishment of functional value placed on a grain lot, it is necessary to understand the falling number test. The instrument itself, how it operates, the cereal chemistry components and factors that confound results, and sources of amylase all need to be discussed to understand the falling number results that were obtained in the U.S. Pacific Northwest wheat growing region in 2016.

The Falling Number Test

The falling number test indirectly measures α-amylase by evaluating its impact on starch pasting properties. The test is a widely accepted standard for detection of degradation of starch due to amylolytic action. It is important to remember, however, that the falling number method does not test for amylase itself, but rather tests the results of enzymatic hydrolysis of starch and other starch properties linked to starch pasting.

The test itself involves placing a sample of ground grain in a long cylindrical tube with distilled water, suspending the sample, and then placing the tube and its contents in a boiling water bath where it is mixed for 60 sec. At the end of mixing, a standardized,

[1] Tel: +1.208.301.5161; E-mail: adbwheat@gmail.com

https://doi.org/10.1094/CFW-63-1-0012

weighted plunger is positioned on top of the gelatinized starch, and the number of seconds (in addition to the initial 60 sec) it takes for the plunger to fall through the gel is recorded. Therefore, 60 sec is the lowest falling number that can be recorded.

Different grading systems have different, somewhat arbitrary, cut-off points established for the characterization of grain as sound or as negatively affected by amylase. In the U.S. system, as established by the U.S. Department of Agriculture Grain Inspection, Packers and Stockyards Administration and Federal Grain Inspection Service (GIPSA/FGIS), samples with falling number values greater than 300 sec are considered sound (6,16). In France, the standard is 250 sec. The characterization of wheat as sound or sprouted would not be as problematic if economic value were not rigidly applied based on falling number test results. For example, discounts are applied in the U.S. Pacific Northwest at a rate of $0.01/sec between 300 and 275 sec, and greater discounts exist for wheat with falling numbers values below 275 sec. This discount schedule can result in enormous losses for growers who are already in financial peril due to preexisting low grain prices.

Broadly, as amylase concentrations increase in flour, issues associated with starch functionality increase dramatically. As starch from soft white wheat, the predominant class of wheat produced in the Pacific Northwest, is hydrolyzed into shorter chain lengths and eventually monosaccharides, water absorption increases, gelatinization temperature decreases, and retrogradation of starch after baking, frying, or boiling is hindered, resulting in collapsed, wet, structurally inadequate end products. In dry products such as crackers or cookies and noodles, postproduction structure is compromised due to starch chain lengths that are insufficient for adequate retrogradation: crackers and cookies tend to fall apart too easily due to the poor structure, and noodles snap apart during drying after production.

Further, a falling number lower than 250 sec for ground wheat creates difficulties, because as falling number decreases issues with product quality, especially in batter-based products, increase. Cakes, doughnut batters, and other similar products are likely to have deficient quality characteristics as a result of amylase activity. Arabic-style flat breads and some baguettes are the most resilient products when it comes to issues caused by amylase and can withstand lower falling number values than can other products.

Clearly, millers and bakers want to purchase only sound grain for their respective processes. Although enzymes are added during bread production to reduce falling number and enhance bread quality, bakers prefer to utilize enzymes in a controlled fashion, starting with a sound flour that is unaffected by amylase. Commercial purchasers want a falling number value that provides assurance that the grain is unaffected by amylase activity, including a "buffer" for within-test variation produced by the analysis itself. Therefore, purchase specifications often include falling number values greater than 300 sec and, in some cases, 350 sec.

Falling number for ground wheat does not always correlate well with actual amylase concentrations measured by AACCI Methods 22-02.01 and 22-05.01 (1) because falling number measures the impact of the enzyme on starch (Fig. 1). There is great variation in actual amylase in the 300–250 sec falling number range. Above 300 sec, and especially 350 sec, amylase and falling number value are in good agreement. Similarly, below 250 sec the two tests are in considerable agreement.

In addition, falling number does not always correlate well with product quality (Fig. 2). Even with falling number values of 200 sec, acceptable cake volume can be achieved, depending on the wheat cultivar. Falling number values above 300 sec do not guarantee good cake performance either. Clearly, other factors, in addition to the presence of amylase, are involved in grain quality. Although falling number provides information on the extent of amylase-induced degradation of starch, it does not provide as concrete an estimate of end-use functionality as is sometimes imagined.

End users, such as bread bakers, prefer falling number values to be 350 sec or higher to assure the grain is sound. Subsequently, bakers frequently treat wheat flour with malt (a source of amylase) to reduce the falling number value to around 250 sec to provide glucose residues for yeast consumption in bread baking. Additionally, malt (and preharvest sprouted wheat) contains an array of other enzymes that modify other grain storage polymers, such as proteins, lipids, and nonstarch carbohydrates (e.g., arabinoxylan). Enhanced control over the amount and performance of dough-modifying enzymes present in a formulation is the primary reason for the seemingly contradictory practice of adding malt or amylase back into sound flour. The assurance provided to mills and bakeries by high falling number results also takes into consideration any implicit variation within the test itself.

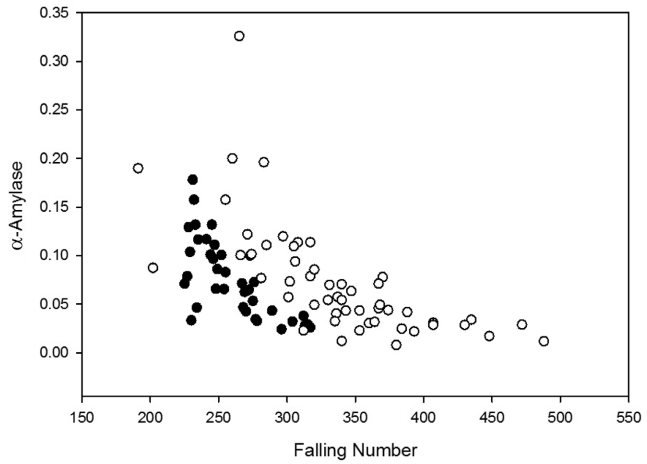


Fig. 1. Falling number (sec) versus α-amylase content (U/g). Black dots (●) and open dots (○) represent soft white wheat samples from two different sources—both from the 2016 Pacific Northwest harvest. (Graph courtesy of A. Kiszonas, USDA-ARS-WWQL)

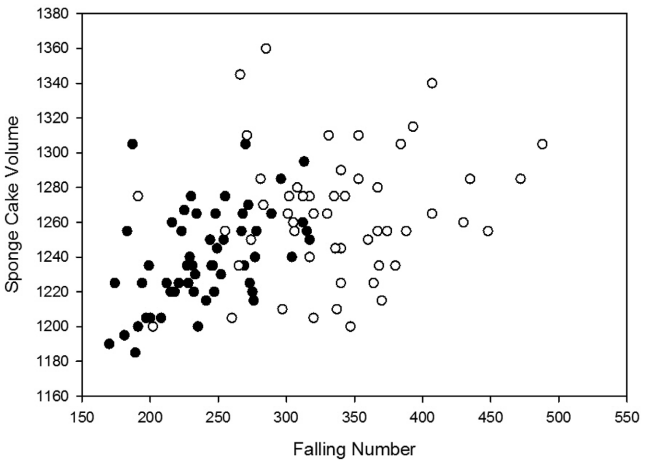


Fig. 2. Falling number (sec) versus Japanese sponge cake volume (cm^3). Black dots (●) and open dots (○) represent soft white wheat samples from two different sources—both from the 2016 Pacific Northwest harvest. (Graph courtesy of A. Kiszonas, USDA-ARS-WWQL)

Falling Number Test Variation

The falling number test itself can contribute to variation in the results that are used to place an economic value on a grain lot based on a fixed falling number value (300 sec). Any test will produce variation in measurements. An examination of variation in falling number results was performed by the AACCI Food Safety, Regulatory, and Quality Task Force Specifications Working Group (2). In the case of falling number, for soft white wheat (the predominant wheat class grown in the Pacific Northwest), variation was measured (with outliers removed) as average standard deviation. A critical value was calculated from the results of collaborative testing in the study (2). When results were pooled and outliers removed, the critical value at $P = 0.05$ was 51.1 sec for falling number. In other words, when comparing two falling number measures at that probability level, if two measures are less than 51.1 sec apart, they can be considered to be the same. Only when two values are greater than 51.1 sec apart can they be considered different at $P = 0.05$.

To base an economic value on a hard-and-fast falling number cutoff value of 300 sec when variation of this magnitude exists may lead to incorrect devaluation of a functional grain lot, resulting in loss of income to farmers. On the other hand, a falling number value of 300 to 350 sec provides insurance that a sample is in fact unaffected by enzyme activity. This provides confidence to the buyer and end user of the grain lot that the wheat will function properly. However, if one is a farmer, the economic impact of a sample that is graded as having a falling number of 275 sec is great, even though it is not significantly different than a sample graded as having a falling number of 325 sec.

Much of the variation in the falling number test can be attributed to a combination of factors, including operator error, poorly calibrated equipment, worn or dirty equipment, and other strictly mechanical factors. However, other factors also contribute to variation.

Table I. A partial list of partial waxy wheat varieties grown in the United States

Variety	Class[a]	Waxy State[b]
Snow Crest	HWS	2
WB7328	HWS	1
BR7030	HWS	1
Dayn	HWS	1
Klasic	HWS	1
Lolo	HWS	1
Macon	HWS	1
Otis	HWS	1
Star	HWS	1
Alturas	SWS	1
Whitebird	SWS	1
Treasure	SWS	1
Jubilee	SWS	1
Cataldo	SWS	1
Centennial	SWS	1
Penewawa	SWS	1
Edwall	SWS	1
Nick	SWS	1
Whit	SWS	1
WaxyPen	SWS	3
Parshal	HRS	1
Mattern	HRW	3
Mattern (sibling)	HWW	3
Ike	HRW	2

[a] HWS: hard white spring wheat; SWS: soft white spring wheat; HRS: hard red spring wheat; HRW: hard red winter wheat.

[b] Waxy state indicates the number of granule-bound starch synthase (GBSS) deletions—increasing waxy state implies greater amylopectin percentage in total starch.

Other Contributors to Falling Number Variation

Falling number, because it measures starch pasting properties in a heated, aqueous environment, is subject to physical and biochemical factors that influence the result and need to be considered. Starch pasting properties are modified, as discussed earlier, by α-amylase. The source of amylase can differ, however, and low falling number results can surprise farmers and grain elevator operators. Both preharvest sprouting (PHS) and late maturity amylase (LMA) play prominent roles in reduction of falling number results. These sources of amylase are discussed in more detail below.

The altitude at which the falling number test is performed also has an effect on results. In an unsealed container, such as is the case with falling number testing, water boils at a lower temperature as altitude increases (6). Within the falling number protocol is an equation used to remove the influence of altitude on falling number results. This equation is to be used only for testing at elevations above 2,000 ft (600 m). There exists, however, a significant difference in water boiling point between sea level and 2,000 ft (100°C versus 98°C) that can affect results through differences in rate of heating of starch and inactivation of amylolytic enzymes (6).

The ratio of the two polymeric components of starch, the amylose/amylopectin ratio, also plays a role in falling number results. Amylopectin, which typically makes up about 78% of starch composition, with the remaining 22% made up of amylose, swells to a greater extent than does amylose. The ratio of amylose to amylopectin is controlled by three granule-bound starch synthase (GBSS) enzymes from each of the three wheat genomes. With sequential deletion of each controlling *waxy* gene, less GBSS is synthesized, leading to a decrease in amylose content. One deletion leads to amylose content of about 20%, two deletions lead to an amylose content of 18%, and deletion of all three GBSS genes results in fully waxy wheat with almost 0% amylose content (18).

Because amylopectin swells to a greater extent than does amylose, a fixed weight of ground grain used in falling number testing results in a larger volume of gel when partial waxy wheat is used. The same weight of solids dispersed over a larger swollen pasted volume means the gel is less dense and, therefore, more easily penetrated by the falling number test probe, leading to a reduced falling number value. This is construed as indicating starch has been hydrolyzed by amylase when, in fact, no amylase is present, resulting in a lower falling number value and discounted prices offered to growers for otherwise sound grain.

The differential swelling phenomenon is the basis of AACCI Method 56-21.01 for measuring flour swelling volume (1). The test measures the relative degree of swelling and reflects the amylose/amylopectin ratio present in the grain lot. Although there are not many varieties commercially grown that exhibit these traits, they are grown in the United States (Table I) and may be inaccurately assessed as having issues with amylase when none exist. Most of these varieties are grown in the Pacific Northwest and, therefore, may be a contributor to the overall lower falling number values found in this region.

A more direct measurement of α-amylase through the use of specific substrates can be made using AACCI Methods 22-02.01 and 22-05.01 (1). The results of these methods provide a more robust analysis of the enzyme without other compounds, physical influences, or protocols introducing variation.

When grain is graded, falling number results for waxy wheat can lead to a reduction in wheat price if the phenomenon is not well-understood and documented. Because *waxy* is a genetic trait, identification of partial waxy cultivars (one or two *waxy* genes) should be possible if the cultivars are reported at the point of sale.

The rapid visco analyzer (RVA) provides a different, although equally indicative, assessment of PHS or LMA than does the falling number test. The RVA method is not influenced by altitude relative to temperature profile and provides a positive relationship between pasting viscosity and *waxy* gene state. The greater the amylopectin content, the greater the registered viscosity, in contrast to the decrease seen in falling number testing. If amylase is present in the sample, low viscosity is recorded, just as with falling number testing. The RVA, therefore, presents a potentially better assessment of PHS and LMA than does falling number and is widely used for this purpose in wheat producing areas such as Australia.

To further assist in determination of whether amylase is present using RVA testing, comparing pasting characteristics between samples run with water and samples run with 1 mM silver nitrate ($AgNO_3$), an amylase inhibitor, can help separate which grains are affected by amylase and which grains with low falling number values may be affected by other processes (5).

Additionally, it has been shown that soft white wheats with very low protein contents (≲7% protein) may also have low falling number values (16). This observation would seem to contradict prevailing theories on the impact of starch on falling number results, in that greater functional (not affected by amylase) starch content in grain should imply greater falling number values. In a study conducted by Ross et al. (16), grain with no amylase but a low falling number was associated with low grain protein content. No theory or explanation for this phenomenon is known. Low protein content occurs with some regularity in the Pacific Northwest, so further study of this phenomenon would be useful, since low-protein wheat can lead to a reduction in crop price due solely to low protein content without the presence of amylase.

In another study, high grain protein content in bread wheat was associated with low falling number results (4). In the case of high protein content, the reduction in starch content due to high protein content might reduce falling number values.

Sources of Amylase

Sources of variation in accuracy and precision of the falling number test notwithstanding, results do successfully reflect the presence of amylase in grain through the impact of the amylase on starch. The most common source of amylase in grain is PHS. PHS occurs when mature grain, postripening and postdormancy, is wetted. Under damp conditions, kernels begin to sprout.

PHS induces production of a large array of enzymes, not just amylase, during germination. Lipase, protease, xylanase, phytase, etc. are all produced in large quantities (10,13). PHS enzymes begin migrating from the germ end of the kernel and work their way up and around the kernel, through the aleurone layer toward the distal end of the kernel, diffusing into the endosperm from the outside to the inside. If grain dries out after being wetted, the chance of hydrolytic damage to the starch by amylase is somewhat deferred. Due to the need for moisture to allow amylase to migrate through the endosperm, amylase can be present but not have a deleterious effect on starch until the grain is dampened a second time, during tempering or dough or batter formation during processing, at which time the amylase will begin to hydrolyze starch immediately.

It has been noted that small amounts of PHS may increase flour functionality (15) in terms of product quality, especially loaf and cake volume, due to gluten structure modifications promoted by proteases and xylanases produced during sprouting that enable increased volume. It is a fine line, however—too much sprouting or amylase will result in to unacceptable end-product quality. Commercial bakers prefer to perform their own malting to having uncontrolled amylase and other enzymes present.

Once grain kernels have broken their dormancy and the presence of amylase may be suspected due to untimely rain at harvest, falling number can provide useful information about both the potential disposition of the grain and the economic value of the grain. As mentioned earlier, however, placing a hard-and-fast falling number value on sound versus sprouted wheat may do a disservice to wheat growers economically.

A more insidious form of amylase that occasionally presents itself is LMA. LMA production requires a genetic predisposition and a narrow set of environmental circumstances to be manifested. Recessive genes are purported to be present on chromosomes 3BL and 7BL (11), although loci on chromosomes 6A, 6B, and 6D, as well as others, are also implicated (17). Other genes and interactions may also be factors in genetic predisposition to LMA, including recombination with dwarfing genes that confer gibberellic acid insensitivity (*Rht1*, *Rht2*, and *Rht3*) (11). Some of the LMA genes appear to be derived from synthetic hexaploid germplasm used to introgress additional D-genome genes into normal hexaploid wheat. The LMA predisposition is on the B genome of the tetraploid contributor to the synthetic hexaploids that were generated for use in breeding (12). LMA genes may have been inadvertently incorporated in wheat germplasm generated through breeding approaches using this genetic source.

LMA is very difficult to screen for in breeding programs and poses a series of very difficult problems in commercial production. Wheat with a genetic predisposition to LMA only produces amylase under certain environmental conditions. Rapid temperature swings, either up or down, at 25–30 days postanthesis during grain development is needed to activate LMA in susceptible varieties (4,7). The environment that leads to production of LMA does not occur naturally very often. Therefore, LMA may go undetected in breeding programs, or even in grain production channels, for years, until the exact, required circumstances arise. LMA then manifests itself when these circumstances are present.

In addition, environmental conditions leading to production of LMA may be quite localized. For example, the temperatures at the top of a hill may be relatively stable, but at the bottom of the same hill, cold air may accumulate on a calm night, leading to LMA synthesis. Temperature swings of ±5 degrees Celsius are sufficient to initiate amylase production in susceptible varieties (4). The amount of LMA produced depends on how many recessive genes are inherited—one or two (12).

Unlike PHS, LMA produces only amylase, not the rest of the suite of enzymes produced during PHS. LMA also appears to be synthesized with a higher pI than that of PHS amylase. In all other respects, the grain appears to be normal. There is no physical impact on the kernel; test weight and visual grading characteristics all appear normal. If there is no rain or wetting of the grain postmaturity, there is no suspicion that the grain may have issues with amylase content. If the LMA-affected wheat is subjected to a falling number test during the grading process, however, a low falling number will result, providing a shock to the grower who would have no idea that LMA had occurred, and will greatly reduce the value of the wheat in the marketplace.

The effects of LMA are as detrimental to end-use quality as the effects of PHS when measured by falling number. However, although there is an impact on starch from LMA, there may be less impact on functional quality compared with PHS (3).

It is exactly this scenario that played out in the Pacific Northwest during the 2016 harvest. Rapid temperature fluctuations of up to 7 degrees Celsius occurred several times during the critical 25–30 day postanthesis window. Because the Pacific Northwest has such a diversity of environments and planting dates, LMA manifested only spottily throughout the region. However, millions of dollars were lost due to the LMA phenomenon that did appear in a limited number of cultivars. Growers were taken by surprise and unable to adjust their marketing and sales strategies to mitigate the fiscal impact, and bankruptcies occurred as a result. The susceptible cultivars that were identified are shown in Table II.

Conclusions

Falling number is a rapid test that measures the impact of amylase on starch. Falling number results are used to place an economic value on wheat in the U.S. marketplace, which uses a cutoff of 300 sec. There are multiple factors beyond amylase that influence falling number values that are not accounted for in this grading system, however, and the test itself has inherent variation. Other tests may be more precise and robust but are not widely used in grain grading or the marketplace. The falling number test serves both the buyer and end user well in providing assurance that a grain lot is truly sound if falling number results are above 300 sec, or even 350 sec.

When amylase results from the presence of LMA, the condition is concealed and goes unnoticed until the grain is harvested and graded. At that time, low falling number test results occur. Clearly, the low falling number results in 2016 in the U.S. Pacific Northwest were due to LMA—significant damage to the crop had occurred early in grain development and was not known until an amylase test was performed.

In other crop years, low falling number values for very low protein wheat or partial waxy wheat may lead to downgraded grain lots even though amylase is absent. The unanticipated loss of revenue for farmers is great and has catastrophic impacts on their business.

Reexamining grain grading standards relative to their use in establishing grain prices, with an emphasis on the degree of variation implicit in falling number testing, should be considered. Alternative tests may assist in better interpreting falling number results, including direct amylase testing or alternative starch pasting tests.

With regard to the presence of LMA as an unpleasant surprise at harvest due to sporadic required weather conditions at the critical moment of grain development, removal of LMA genes from breeding germplasm and released cultivars is the only sure way to rectify the situation.

Table 2. Wheat cultivars suspected of predisposition to late maturity amylase in the Pacific Northwest

Hard White Spring	Soft White Winter	Club
Blanca Grande	SY Ovation	Bruehl
Klasic	Mela CL+	
Express	Curiosity CL+	
	Xerpha	

References

1. AACC International. Method 22-02.01, Measurement of α-Amylase in Plant and Microbial Materials Using the Ceralpha Method; Method 22-05.01, Measurement of α-Amylase in Cereal Grains and Flours—Amylazyme Method; Method 56-81.03, Determination of Falling Number. *Approved Methods of Analysis*, 11th ed. Published online at http://methods.aaccnet.org. AACC International, St. Paul, MN.
2. AACCI Food Safety, Regulatory, and Quality Task Force Specifications Working Group. Specification limitations for hard and soft wheat flour. Cereal Foods World 61:24, 2016.
3. Allen, H. M., Pumpa, J. L., and Stapper, M. LMAA and wheat quality. Page 58 in: *55th Australian Cereal Chemistry Conference and AACCI Pacific Rim Symposium*. C. L. Blanchard, H. Truong, H. M. Allen, A. B. Blakeney, and L. O'Brien, eds. Royal Australian Chemical Institute, North Melbourne, Australia, 2004.
4. Craven, M., Barnard, A., and Labuschagne, M. R. The impact of cold temperatures daring grain maturation on selected quality parameters of wheat. J. Sci. Food Agric. 87:1783, 2007.
5. Crosbie, G. B., Chiu, P. C., and Ross, A. S. Shortened temperature program for application with a rapid visco analyser in prediction of noodle quality in wheat. Cereal Chem. 79:596, 2002.
6. Delwiche, S. R., Vinyard, B. T., and Bettge, A. D. Repeatability precision of the falling number procedure under standard and modified methodologies. Cereal Chem. 92:177, 2015.
7. Farell, A. S., and Kettlewell, P. S. The effect of temperature shock and grain morphology on α-amylase in developing wheat grain. Ann. Bot. 102:287, 2008.
8. Finnie, S., and Atwell, W. A. Wheat and flour testing. In: *Wheat Flour*, 2nd ed. AACC International, St. Paul, MN, 2016.
9. Hagberg, S. A rapid method for determining α-amylase activity. Cereal Chem. 78:485, 1960.
10. Kruger, J. E., and Reed, G. Enzymes and color. Page 441 in: *Wheat Chemistry and Technology*, 3rd ed. Vol. 1. Y. Pomeranz, ed. AACC International, St. Paul, MN, 1988.
11. Mares, D., and Mrva, K. Late-maturity α-amylase: Low falling number in wheat in the absence of preharvest sprouting. J. Cereal Sci. 47:6, 2008.
12. Mares, D., and Mrva, K. Genetic variation for quality traits in synthetic wheat germplasm. Aust. J. Agric. Res. 59:406, 2008.
13. Ohm, J.-B., Lee, C. W., and Cho, K. Germinated wheat: Phytochemical composition and mixing characteristics. Cereal Chem. 93:612, 2016.
14. Perten, H. Application of the falling number method for evaluating α-amylase activity. Cereal Chem. 41:127, 1964.
15. Richter, K., Christiansen, K., and Guo, G. Wheat sprouting enhances bread baking performance. Cereal Foods World 59:231, 2014.
16. Ross, A. S., Flowers, M. D., Zemetra, R. S., and Kongraksawech, T. Effect of grain protein concentration on falling number of ungerminated soft white winter wheat. Cereal Chem. 89:307, 2012.
17. Wrigley, C. Late-maturity α-amylase—Apparent sprout damage without sprouting. Cereal Foods World 51:124, 2006.
18. Zeng, M., Morris, C. F., Batey, I. L., and Wrigley, C. W. Sources of variation for starch gelatinization, pasting and gelation properties in wheat. Cereal Chem. 74:63, 1997.

Before retiring in 2011, **Arthur D. Bettge** spent 32 years at the USDA-ARS Western Wheat Quality Lab in Pullman, WA. He was the research lab manager and collaborated on cultivar development and research into the biochemical and genetic underpinnings of wheat functionality. After retiring, he became a consultant with ADB Wheat Consulting, Moscow, ID, and has worked on projects for the Washington Grains Commission and the U.S. Wheat Associates, as well as numerous private companies. Art is an AACCI member and can be reached at adbwheat@gmail.com.

A Single Analytical Platform for the Rapid and Simultaneous Measurement of Protein, Oil, and β-Glucan Contents of Oats Using Near-Infrared Reflectance Spectroscopy

Devendra Paudel, Melanie Caffe-Treml, and Padmanaban Krishnan[1]
South Dakota State University, Brookings, SD, U.S.A.

ABSTRACT

Effective near-infrared reflectance spectroscopy (NIRS) predictive calibrations were developed for simultaneous multiple component measurement of constituents (protein, oil, and β-glucan contents) in whole and ground oat groats. The use of whole oat groats as a starting material represents an advancement in the science as it precludes the need for sample grinding. Samples were collected from the 2015 and 2016 crop years from various locations in the United States (South Dakota, North Dakota, Washington, Iowa, and Wisconsin), representing a large geographical region and diverse genetic range ($N = 500$). Predictive calibration equations were developed based on the modified partial least squares (MPLS) regression technique. Reference analyses were done using standard methods approved by AACC International and AOCS (AACCI Method 32-23.01 for β-glucan content, AACCI Method 46-30.01 for crude protein content, AOCS Standard Procedure Am 5-04 for oil content, and AACCI Method 44-15.02 for moisture content). The use of validation sample sets for each constituent, which were independent of samples used in NIRS calibration development, served as additional evidence of accuracy and precision. High coefficient of determination (R^2) and one minus variance ratio (1-VR) and low standard error of calibration (SEC) and standard error of cross-validation (SECV) values provided evidence supporting the accuracy and precision of the calibration models developed for estimation of oat β-glucan, protein, and oil contents. The NIRS calibration for estimation of β-glucan content of ground oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.94, 0.16, 0.22, and 0.87, respectively. Protein calibration for ground oat groats yielded R^2, 1-VR, SEC, and SECV values of 0.94, 0.93, 0.61, and 0.64, respectively. Calibration employing ground oat groats for oil content estimation yielded high R^2 and 1-VR values of 0.93 and 0.92, respectively, and low SEC and SECV values of 0.23 and 0.26, respectively. Whole oat groat NIRS calibrations proved to be as effective as ground groat calibrations. Whole oat groat β-glucan calibrations yielded excellent R^2, SEC, SECV, and 1-VR values of 0.93, 0.18, 0.23, and 0.89, respectively. For protein calibrations of whole oat groats, R^2, SEC, SECV, and 1-VR values were 0.92, 0.70, 0.80, and 0.89, respectively. Oil content calibration developed with whole oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.90, 0.27, 0.30, and 0.88, respectively. This study showed that NIRS is an accurate and effective technology for oat quality measurement in plant breeding programs and food processing.

Knowledge about the composition of agricultural materials used in foods is essential for sound decision-making by geneticists, plant breeders, millers, and bakers. Food processors rely on accurate information on the quality of crops that arrive from the field. Reference methods based on chemical and enzymatic analyses provide the basis for determination of accuracy and precision. However, these methods are hard to implement and require highly trained analysts. Such methods also take time to implement and may be cost-prohibitive, especially if large sample throughput is required. Plant breeding programs, in particular, need cost-effective and fast screening techniques. Near-infrared reflectance spectroscopy (NIRS) provides an analytical platform for rapid and robust measurement of multiple constituents once reference methods have been perfected. NIRS, thus, is a correlative tool that relies on the existence of methods that accurately quantify the target compound, be it protein, carbohydrates, oils, or dietary fiber.

Measurement of individual constituents provides little information about the causes of variability of a constituent. Interrelationships between multiple grain constituents can be gauged through a more comprehensive understanding of seed composition and makeup. Plant breeders, for example, need to interpret variability of nutrients and their relationships to other agronomic traits, such as yield and kernel size, in the context of sources of variations such as genetics, environment, and interactions (20). Cereal chemists may have a better understanding of grain functionality based on a more comprehensive knowledge of variations in nutrient composition. This article reports on the development of calibrations for estimation of protein, oils, and β-glucan in oats (both in whole and ground oat groats).

NIR spectra of samples also serve as a repository of information that could be used to devise analytical methodologies for grain constituents whose importance have not yet been fully realized. Information contained within the spectra may be valuable for future development of correlative methods once sound analytical methods can be developed for those constituents. Routine noninvasive measurement of multiple constituents such as amino acids, fatty acids, dietary fiber moieties, and bioactive ingredients may be possible using a single analytical NIRS platform if they are supported by the existence of sound reference methods.

Oat (*Avena sativa*) is a unique cereal crop with significant benefits for human health and nutrition. This cereal grain has been shown to have multiple health benefits for humans, including mitigating the risks for heart disease (2,13,21,27). β-Glucan, a soluble dietary fiber found in the range of 2 to 6% in oats, is associated with the reduction of postprandial serum glucose levels in humans and animals (6). This effect of β-glucans on blood glucose is significant for the management of diabetes. Oats are also endowed with other beneficial compounds, such as antioxidants, an excellent amino acids profile in their protein, and polyunsaturated fatty acids in their oils. The oat protein content also contributes to their health benefits. Oat protein content is unique among grains because it contains significant levels of

[1] Corresponding author. E-mail: Padmanaban.Krishnan@SDSTATE.EDU

https://doi.org/10.1094/CFW-63-1-0017

legumin-like globulins (23) and has a high biological value as a food source (11). Although oats are used primarily in breakfast cereals and breakfast bars, expanding the range of products made with oats would greatly increase the number of consumers who could benefit from its health-promoting attributes. To expand the range of products made with oats, we need to better understand the chemical and nutritional properties of oat constituents and how they affect the quality of final products.

The United States is the world's largest importer of oats. The U.S. oat milling industry currently relies on Canada as the source of most of its oat grain imports (3.1 million tons in 2016). The United States is also the world's fourth largest oat producer, and it imports more than 75% of the world's shipments. The United States generally uses about one-third of its total yearly oat supply for food or industrial uses and about two-thirds for feed. There is interest among U.S. millers in expanding the domestic supply of oat grain. U.S. oat production in 2016 was 939,121 tons (26). As the leading oat-producing state, South Dakota produced 193,050 tons in 2016. An increased emphasis on oat production has prompted the need for effective and efficient tools to monitor the baseline data on quality traits for this cereal grain. Quality evaluation of oats needs to encompass both food and feed quality to ensure a sustainable crop that has diversified functionality and end-use applications. The existence of analytical methods for rapid analysis, therefore, is important for the long-term tracking of oat quality parameters.

In addition to its health benefits, oat crops present advantages for soil health and agricultural sustainability. Since 2013, farmers have shown increased interest in including oats in their crop rotations and, thereby, increasing the diversity of the crops grown in the region. Oat production can be used to break weed and pest cycles in corn–soybean rotations, and it is a low input crop. The focus of U.S. oat breeding programs is to develop new adapted oat varieties with improved agronomic characteristics and improved milling and nutritional qualities. Further improvements for milling and nutritional qualities are necessary to ensure that new varieties meet the needs of the developing food market and provide as many health benefits for consumers as possible.

BACKGROUND

NIRS employs wavelengths in the near-infrared region (850–2,500 nm) of the energy spectrum to scan samples. Chemical constituents within samples, such as proteins and lipids, absorb some of this energy while reflecting the rest. The reflected energy is detected and correlated to the amount of the compound present in the sample. Reliable and accurate reference chemical measurements, thus, are indispensable to good calibration development. NIRS methods have been used in agriculture as screening tools for fast-throughput breeding program selection. Advancements in spectroscopy and refinements in reference analytical methods have improved NIRS analysis significantly over the years to advance the use of the technology as an analytical tool and not merely as a screening protocol. NIRS can rapidly (within seconds) and simultaneously measure the protein, oil, and moisture contents in oats with accuracy and precision (4,16). Krishnan et al. (15) used NIRS to measure oat grain properties. NIRS fatty acid calibrations were shown to be effective as a screening tool in ground oat groat samples from the South Dakota oat breeding program (16). NIRS was used to determine the β-glucan content in barley (7,10,14,24), naked barley (22), and naked oats (4). Schmidt et al. (22) used a Fourier-transformation NIRS instrument to obtain a correlation coefficient of 0.96 to 0.98 between their prediction equation and enzymatically determined barley grain β-glucan content. Osborne (18,19) has provided several reviews of NIRS use.

NIR technologies offer the potential for nondestructive, high-throughput sampling with a low cost per sample once calibrations are in place. These are correlative techniques, however, that rely on the skillful performance of wet chemistry analyses for calibration and validation to generate accurate and precise output.

Although previous studies have reported on NIRS estimation of single constituents, the ability to measure multiple components simultaneously and nondestructively in a single run has enormous advantages for data interpretation. Oat groats represent the edible portion of the oat seed after the hull is removed. NIR calibrations reported in the literature were developed using ground oat groats. NIR calibrations for whole oat groats, however, would remove the time-consuming step of sample grinding. In addition, whole oat groat calibrations do not harm the seed, which remains intact and viable and can be planted to obtain a new generation. Samples can be cleaned, dehulled, and read directly in the NIR spectrometer. This represents a significant technological advancement in oat quality measurement. NIRS, thus, may potentially be used to obtain comprehensive compositional information that can be used effectively for product innovation.

The purpose of this study was to develop predictive calibration models for estimating β-glucan, protein, and oil contents of U.S. oat cultivars using NIRS and validated AACC International (AACCI) reference methods and an AOCS standard procedure (1,3). A rapid, nondestructive (whole oat groat) NIRS method was also developed to estimate β-glucan, protein, and oil contents based on the standard reference analyses procedures approved by AACCI (1). This represents a single analytical platform for reliable and nondestructive multiple-component analysis that provides decision-making tools for producers, plant breeders, and food processors. The influence of genetics and growing environments on the variability of multiple oat constituents was also a research objective.

METHODS AND MATERIALS

General Approach

A schematic of the experiment design used in NIRS calibration development and the validation of those methods is provided in Figure 1. More than 500 oat samples grown in diverse growing regions in the United States and representing two growing years (2015 and 2016) were collected, cleaned, and dehulled to yield oat groats. Sampling included cultivars grown in Washington, Iowa, Wisconsin, Minnesota, and North Dakota, as well as samples from the South Dakota oat breeding program. Dehulling was accomplished using a huller (Codema LLC). A subset of the samples was ground using a mill (Ultra Centrifugal mill, Retsch) equipped with a 0.5 mm screen to yield ground oat groats. Both whole and ground oat groat samples were scanned in an NIRS feed analyzer (DS2500, FOSS NA) to yield spectra from 250 to 2,500 nm. All samples were subjected to reference chemical and enzymatic analyses using officially accepted methods for moisture, protein, oil, and β-glucan contents. Predictive calibration equations were developed for whole and ground oat groat samples using a modified partial least squares (MPLS) regression technique. Calibration and validation sample sets were

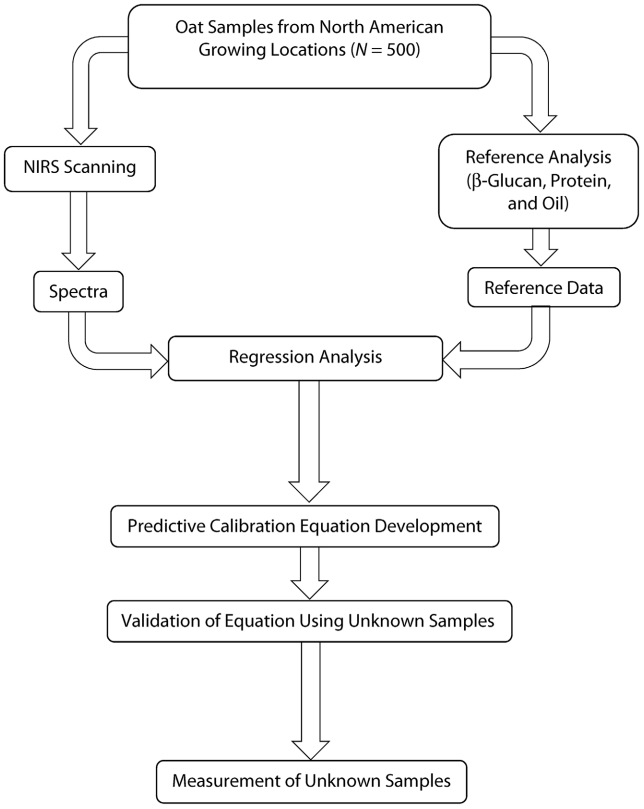


Fig. 1. Flow diagram summarizing experimental methodology for near-infrared reflectance spectroscopy (NIRS) calibration development and validation.

identified for each of the constituents using NIRS software. The validation sample sets, which were not part of the calibration development, were used as independent checks on the accuracy and precision of NIRS predictive equations for the target constituents. A fixed sample set containing varieties (16 cultivars) common to the two growing years and grown in the same locations (4 locations) each year was evaluated by both NIRS and reference analyses to determine the influence of genetics and environmental effects on the variability of protein, oil, and β-glucan contents. A total of 128 samples, thus, were used in the variety by environment study. The effectiveness of NIRS was evaluated both as a screening and analytical tool relative to chemical and enzymatic analytical methods. Ranking of varieties was done to determine if NIRS and reference analyses provided essentially the same information on oat quality.

Reference Analyses

Analytical Methods. Moisture content analysis was performed using the standard air-oven method approved by AACCI (AACCI Method 44-15.02) (1). Crude protein content was measured using the Dumas combustion method (AACCI Method 46-30.01) (1) and employing a protein analyzer (FlashEA 1112, Thermo Fisher Scientific). Nitrogen content measured by this procedure was expressed as percent protein using a conversion factor of 5.83 (% N × 5.83). Crude oil content was measured using AOCS Standard Procedure Am 5-04 (3). Enzymatic analysis of β-glucan content was performed using AACCI Method 32-23.01 (1) with a mixed β-glucan linkage kit (Megazyme International Ireland Limited). Standard barley flour and oat flour samples with known levels of β-glucan provided with commercial kits were used as controls and checks on analytical accuracy. All data were expressed on a moisture-free basis for ease of data interpretation.

NIRS Calibration Development. Oat samples from different North American growing locations and breeding programs were scanned on the NIR spectrometer (N = 500). Based on spectral analyses, and depending on the target constituent, calibration sample sets were identified for each of the constituents: N = 308 for β-glucan content; N = 394 for protein content; and N = 385 for oil content. Separate sample sets, designated as the validation set (N = 104 for β-glucan content; N = 134 for protein content; and N = 133 for oil content), that were independent of those used in the calibration set were employed to test the accuracy and precision of NIRS measurements. The effectiveness of the calibrations was evaluated by statistical comparison of NIRS estimations against true values measured using official reference procedures. High correlation coefficients and low standard error terms provided a good estimate of accuracy and precision, respectively, of NIRS methods. NIRS spectral data are multivariate in nature because they contain large numbers of data points, i.e., one data point in each wavelength for each sample. In addition, differences between samples are discerned only through small spectral variations. For this reason, for multivariate data analysis it is important to obtain accurate information from the spectra. Multivariate data analysis consists of two processes: spectral preprocessing and calibration model development (17). Different calibration models, such as principal component regression (PCR) (25), partial least squares (PLS) (9,12), artificial neural network (ANN) (8), and support vector regression (SVR) (5), are used in quantitative calibration analysis. In a population of 200–300 samples, a small subset of samples may be identified for reference analysis and calibration development. Such samples are selected by the software based purely on spectral information. This sample set is designated as the calibration sample set.

RESULTS

Reference analyses of oat and barley flour standards employing the β-glucan assay kit yielded values of 4.1 and 7.98%, respectively, and a coefficient of variation values of 1.82 and 1.64%, respectively, based on repeated analyses (N = 49). The reported values for the control oat and barley flours were 4.1 and 8.0%, respectively. Our results showed good accuracy and precision in laboratory implementation of the AACCI procedure for β-glucan content. The inclusion of barley and oat flour standards in the day-to- day analyses provided an excellent tool for quality assurance and served as a measure of confidence in minimized laboratory error.

NIRS Calibration Development Using Ground and Whole Oat Groat Samples

The graphs in Figures 2–4 illustrate the accuracy of the NIRS calibrations for estimation of β-glucan, protein, and oil contents in ground oat groats. Reference values for these constituents were plotted against their corresponding NIRS estimates. Both calibration sample sets (Figs. 2A, 3A, and 4A) and validation sample sets (Figs. 2B, 3B, and 4B) for each of the three oat constituents showed strong correlations between reference and predicted values. Validation sample set plots effectively showed how NIRS estimates were close to the true values, even in unknown samples. The graphs in Figures 5–7 illustrate the accuracy of the NIRS calibrations for estimation of β-glucan, protein, and oil contents in

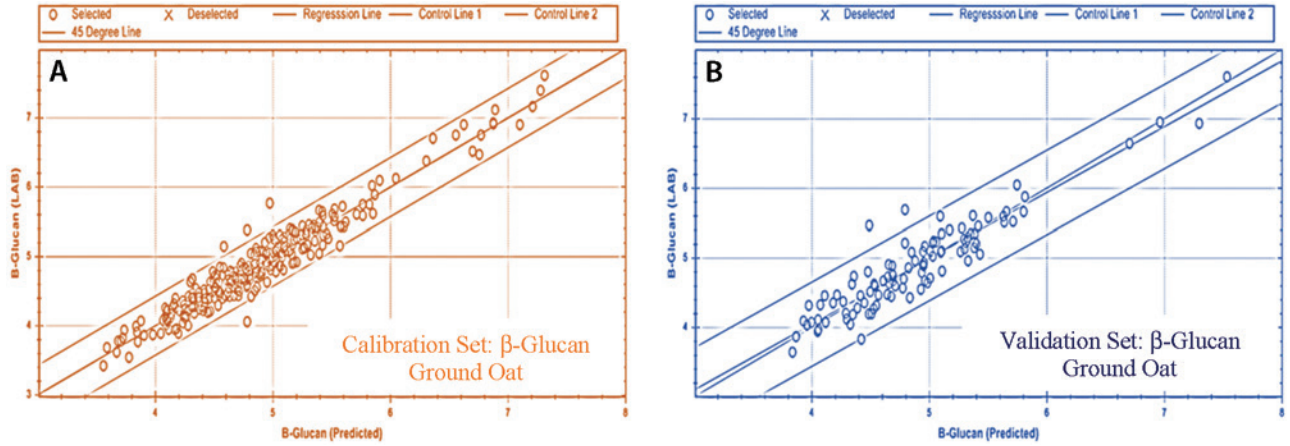


Fig. 2. Ground oat groat β-glucan calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of β-glucan content.

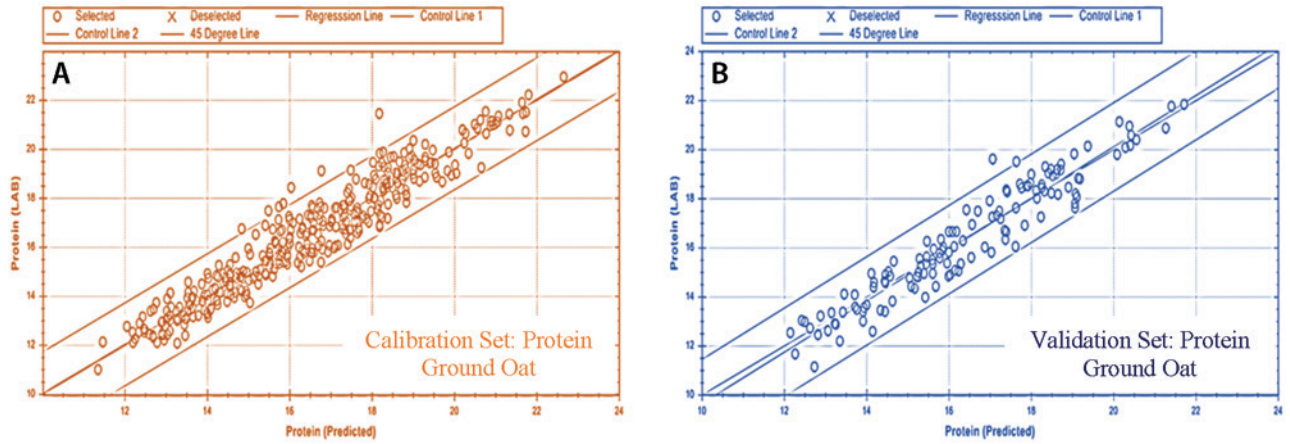


Fig. 3. Ground oat groat protein calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of protein content.

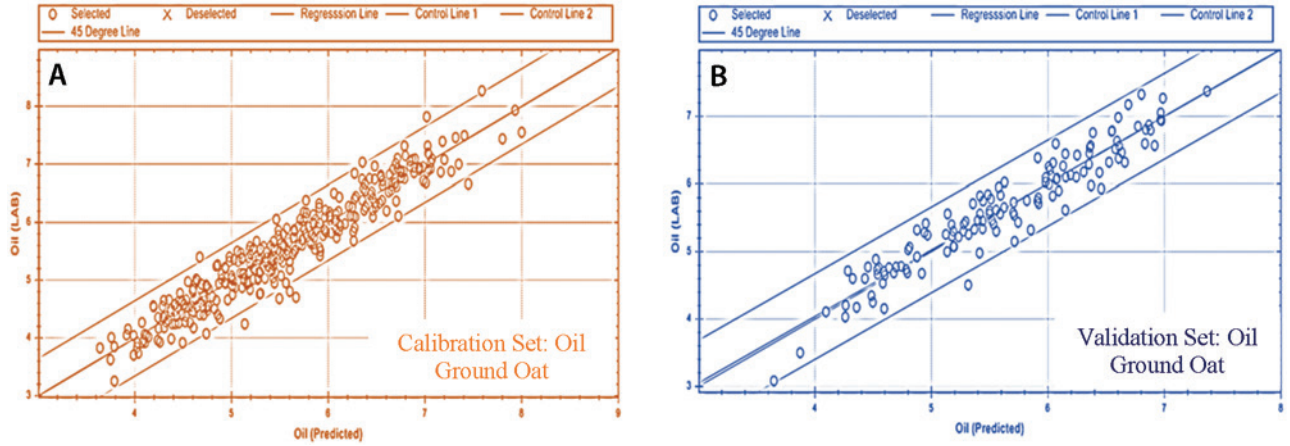


Fig. 4. Ground oat groat oil calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of oil content.

whole oat groat samples. Reference values for these constituents were plotted against their corresponding NIRS estimates for both the calibration (Figs. 5A, 6A, and 7A) and validation (Figs. 5B, 6B, and 7B) sample sets. The graphs in Figures 2–7 also provide an idea of the range of occurrence of the three analytes. Strong predictive NIRS calibration equations require the existence of a large range of target nutrient concentrations that encompass diverse growing environments and broad genetic variability. Robust and effective NIRS calibrations, thus, may require longitudinal evaluation of crop composition and large sample numbers, which in turn, require painstaking reference analysis. All samples in our study, whether they were used in calibration development or in validation of the methods, were analyzed chemically or enzymatically as well as by NIRS methods.

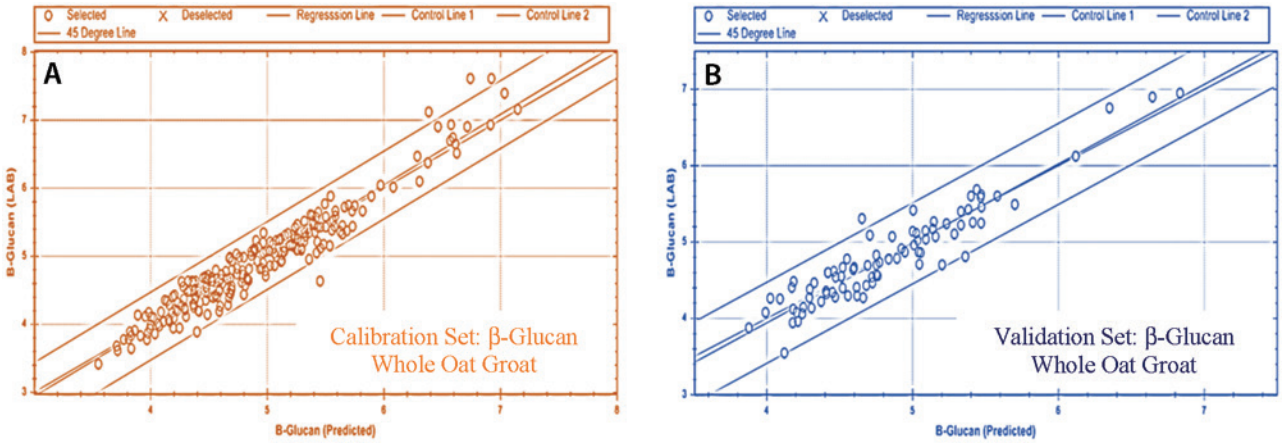


Fig. 5. Whole oat groat β-glucan calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of β-glucan content.

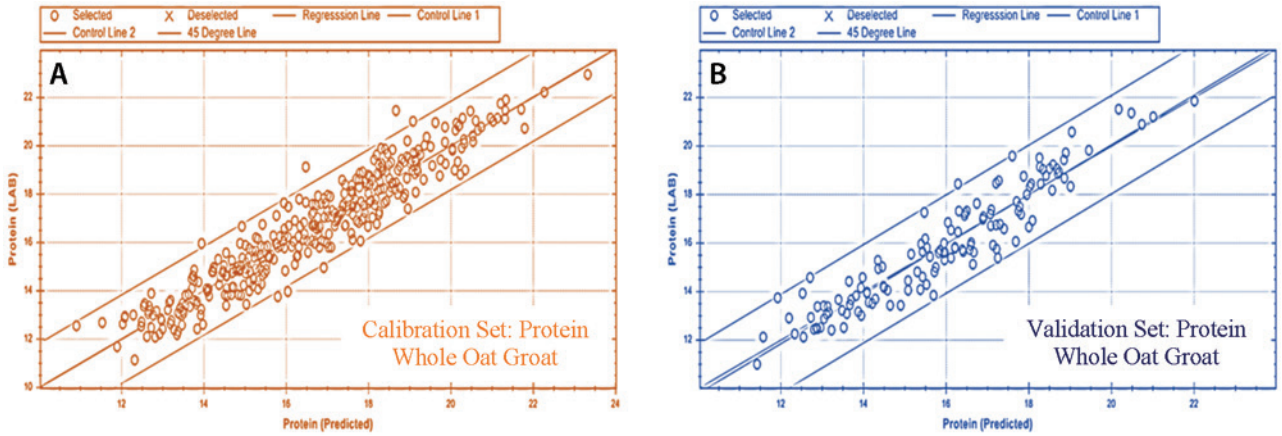


Fig. 6. Whole oat groat protein calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of protein content.

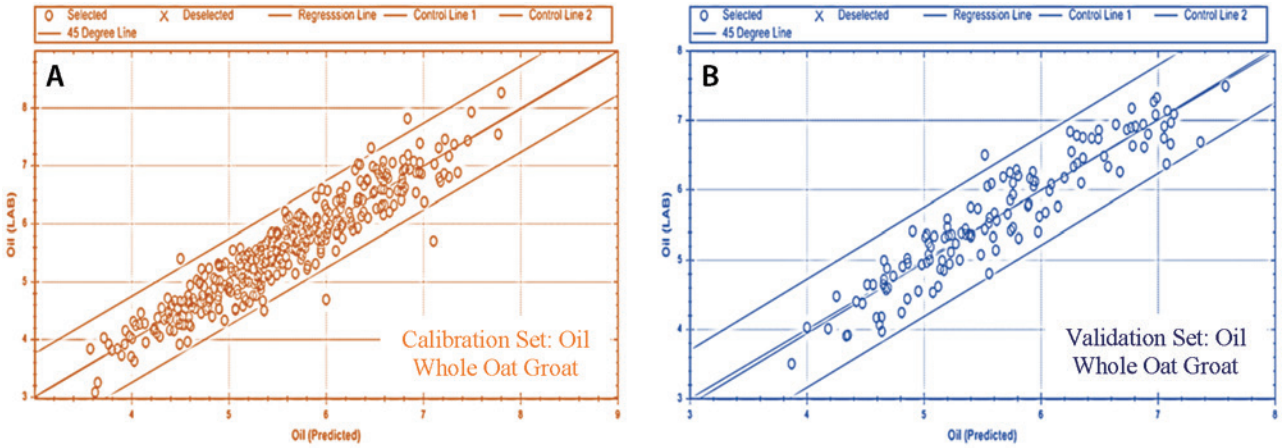


Fig. 7. Whole oat groat oil calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of oil content.

NIRS calibration and validation statistics for β-glucan, protein, and oil content estimation were computed using PLS analysis (Tables I–III). The NIRS terms define the effectiveness of the calibration as well as the validation of the calibration equations using sample sets that were independent of calibration development. Good predictive equations were developed for the three constituents in both ground and whole oat groat matrices.

The accuracy of NIRS predictive equations was based on coefficients of determination (R^2), standard errors of calibration (SEC), standard errors of cross-validation (SECV), standard error of prediction (SEP), and one minus variance ratio (1-VR, where VR is explained by variance divided by total variance). Ideal R^2 and 1-VR values approaching 1.0 and low standard errors are indicative of good calibrations. An ideal R^2 of 1.0 in-

Table I. Near-infrared reflectance spectroscopy (NIRS) calibration and validation statistics on β-glucan content in oats[a]

	Calibration Set						Validation Set				
Constituent	***N***	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	***N***	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Ground oat	308	4.84	0.94	0.16	0.22	0.88	104	4.89	0.87	0.001	0.24
Whole oat	247	4.87	0.93	0.18	0.23	0.89	85	4.82	0.89	–0.025	0.21

[a] RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross-validation; 1-VR: one minus variance ratio; SEP: standard error of prediction; RSQ_{val}: coefficient of correlation.

Table II. Near-infrared reflectance spectroscopy (NIRS) calibration and validation statistics on protein content in oats[a]

	Calibration Set						Validation Set				
Constituent	***N***	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	***N***	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Ground oat	394	16.35	0.94	0.61	0.64	0.93	134	16.40	0.91	–0.052	0.72
Whole oat	394	16.59	0.92	0.70	0.80	0.89	133	15.88	0.89	–0.045	0.81

[a] RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross-validation; 1-VR: one minus variance ratio; SEP: standard error of prediction; RSQ_{val}: coefficient of correlation.

Table III. Near-infrared reflectance spectroscopy (NIRS) calibration and validation statistics on oil content in oats[a]

	Calibration Set						Validation Set				
Constituent	***N***	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	***N***	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Ground oat	385	5.52	0.93	0.23	0.26	0.92	133	5.64	0.91	0.018	0.25
Whole oat	394	5.52	0.90	0.27	0.30	0.88	133	5.62	0.88	–0.016	0.31

[a] RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross-validation; 1-VR: one minus variance ratio; SEP: standard error of prediction; RSQ_{val}: coefficient of correlation.

Table IV. Comparison of AACCI method and near-infrared reflectance spectroscopy (NIRS) prediction for β-glucan content in calibration and validation samples of ground and whole oats

		AACCI Method (%)		NIRS Prediction (%)	
Constituent	***N***	**Mean**	**SD**	**Mean**	**SD**
Calibration set					
Ground oat	313	4.84	0.66	4.84	0.64
Whole oat	251	4.90	0.69	4.90	0.74
Validation set					
Ground oat	104	4.90	0.67	4.90	0.66
Whole oat	85	4.82	0.63	4.84	0.57

Table V. Comparison of AACCI method and near-infrared reflectance spectroscopy (NIRS) prediction for protein content in calibration and validation samples of ground and whole oat groats

		AACCI Method (%)		NIRS Prediction (%)	
Constituent	***N***	**Mean**	**SD**	**Mean**	**SD**
Calibration set					
Ground oat	401	16.40	2.42	16.36	2.32
Whole oat	402	16.60	2.41	16.59	2.29
Validation set					
Ground oat	134	16.40	2.44	16.46	2.23
Whole oat	133	15.89	2.40	15.92	2.20

Table VI. Comparison of AOCS procedure and near-infrared reflectance spectroscopy (NIRS) prediction of oil content in calibration and validation samples of ground and whole oat groats

		AACCI Procedure (%)		NIRS Prediction (%)	
Constituents	***N***	**Mean**	**SD**	**Mean**	**SD**
Calibration Set					
Ground oat	402	5.53	0.91	5.53	0.88
Whole oat	402	5.53	0.89	5.53	0.84
Validation Set					
Ground oat	133	5.64	0.84	5.63	0.81
Whole oat	133	5.62	0.90	5.6	0.83

dicates highly correlated methods. The SEC is computed as the standard error of the difference between the true values of the constituents and their corresponding estimated values. Low SEC values are indicative of low errors in NIRS estimation. Validation sample sets are designed to test how accurately the models predict unknown values. Standard errors computed between true values and NIRS estimates in the validation sample set were designated as the SEP. SEC and SEP, thus, are parallel statistical terms corresponding to standard errors in the calibration sets and validation sets, respectively. High R^2 and coefficients of cross-validation along with low SEC or SEP are hallmarks of good NIRS predictive models.

Calibration for the estimation of β-glucan content in ground oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.94, 0.16, 0.22, and 0.88, respectively. Calibrations for estimated β-glucan content in whole oat groats yielded excellent R^2, SEC, SECV, and 1-VR values of 0.93, 0.18, 0.23, and 0.89, respectively. Calibration for the estimation of protein content in ground oat groats yielded R^2, 1-VR, SEC, and SECV values of 0.93, 0.93, 0.61, and 0.64, respectively. Calibration for the estimation of protein content in whole oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.92, 0.70, 0.80, and 0.89, respectively. Calibration for the estimation of oil content in ground oat groats yielded higher R^2 and 1-VR values of 0.93 and 0.92, respectively, and lower SEC and SECV values of 0.23 and 0.26, respectively. Calibration for the estimation of oil content in whole oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.90, 0.27, 0.30, and 0.88, respectively.

Table VII. Analyses of variance in β-glucan, protein, and oil contents of oats grown in South Dakota in 2015 and 2016

		Official Method[a]			NIRS Method (Ground Oat Groat)[b]			NIRS Method (Whole Oat Groat)[c]		
Constituent	df	Mean Square	*F* Ratio	Significance Level[d]	Mean Square	*F* Ratio	Significance Level[d]	Mean Square	*F* Ratio	Significance Level[d]
β-Glucan										
Cultivar	15	0.188745	104.458	***	5.6659	160.095	***	4.7967	199.306	***
Location	3	0.054769	30.311	***	1.2325	34.826	***	0.913	37.9369	***
Year	1	0.039736	21.9911	***	0.7165	20.2459	***	0.3503	14.5535	***
Cultivar × location	45	0.003021	1.6718	*	0.0632	1.7868	*	0.0656	2.7246	***
Location × year	3	0.020693	11.4521	***	0.4456	12.59	***	0.4076	16.9376	***
Cultivar × year	15	0.003602	1.9937	*	0.0554	1.5656		0.0575	2.3904	**
Cultivar × location × year	43	0.001341	0.742		0.0372	1.0506		0.0365	1.5151	
Protein										
Cultivar	15	0.000117	10.699	***	6.22	8.0097	***	5.53	17.4145	***
Location	3	0.00027	24.7656	***	19.49	2.51E+01	***	25.13	79.0605	***
Year	1	0.006655	611.067	***	506.52	6.53E+02	***	451.43	1,420.47	***
Cultivar × location	45	1.91E-05	1.7496	*	0.87	1.1177		0.72	2.2603	**
Location × year	3	0.000725	66.6074	***	28.01	3.61E+01	***	36.53	114.933	***
Cultivar × year	15	1.56E-05	1.4308		0.63	0.8102		0.58	1.8297	
Cultivar × location × year	43	1.26E-05	1.1577		0.56	0.72		0.64	2.0073	**
Oil										
Cultivar	15	9.3077	155.1	***	8.738	335.7736	***	7.2278	99.1164	***
Location	3	8.9094	148.461	***	6.6824	256.7857	***	7.8262	107.323	***
Year	1	0.2936	4.8916	*	0.3722	14.3011	***	0.6265	8.5914	**
Cultivar × location	45	0.1368	2.2789	**	0.1216	4.6743	***	0.135	1.8515	*
Location × year	3	6.8996	114.971	***	6	230.561	***	4.4507	61.0341	***
Cultivar × year	15	0.2544	4.2385	***	0.0819	3.1464	***	0.0721	0.9888	
Cultivar × location × year	43	0.111	1.8497	*	0.0721	2.7721	***	0.0907	1.2441	

[a] AACCI Approved Methods 32-23.01 for β-glucan analysis and 46-30.01 for protein analysis (1); AOCS Standard Procedure Am 5-04 for oil analysis (3).
[b] Near-infrared reflectance spectroscopy method for ground oat groats.
[c] Near-infrared reflectance spectroscopy method for whole oat groats.
[d] ***, **, and * indicate significant at $P = 0.001$, 0.01, and 0.05, respectively.

High R^2 and 1-VR and low SEC and SECV values, thus, provide evidence supporting the accuracy and precision of calibration models developed for estimation of β-glucan, protein, and oil contents in oats. The study shows that NIRS is an efficient technology for oat quality measurement for high-throughput breeding programs and in oat processing.

Side-by-side comparisons of overall means for β-glucan, protein, and oil contents in all oat samples used in the study are provided in Tables IV–VI. They show how well NIRS estimations of β-glucan, protein, and oil contents matched AACCI and AOCS methods of analysis (or true values) for the same samples. These results show that NIRS-derived β-glucan, protein, and oil content estimations were not statistically different from their corresponding chemically determined values for the same samples. In our study, almost all of the samples were used in both NIRS method development and in reference analysis.

Analysis of variance (ANOVA) statistics, employing chemical reference data and showing significant effects of cultivar (genetics), growing location, and growing year on the variability of β-glucan, protein, and oil contents of oats grown in the South Dakota oat breeding program during the 2015 and 2016 crop years, are provided in Tables VII. All three factors showed a statistically significant influence on data variability. Similar ANOVA tables generated with NIRS methods showed the same results for the influence of cultivar, growing location, and growing years (not included in this article).

Oat cultivar rankings based on β-glucan, protein, and oil contents, as measured by AACCI and NIRS methods (whole oat groat and ground oat groat) are provided in Tables VIII–X. The rankings visibly demonstrate the effectiveness of the NIRS ground whole oat groat predictive calibrations in discriminating between named cultivars grown in South Dakota in two years on the basis of β-glucan, protein, and oil contents.

CONCLUSIONS

- **Calibrations** – High R^2 and 1-VR and low SEC and SECV values indicate high accuracy and precision of calibration models developed for estimation of oat β-glucan, protein, and oil contents.
- **Validation** – Similarly, high R^2 and low SEP values in the validation data set show the calibration models were effective in estimating the β-glucan, protein, and oil contents of oat samples that were not part of the calibration set.
- **Cost-Effectiveness** – NIRS is cost-effective for evaluating large numbers of samples in view of the reduced need for labor-intensive reference analyses. At a cost of $330 per enzyme assay kit, and with 50 samples/kit for duplicate determinations, more than 500 determinations were made in this study for β-glucan content alone. This translates to $3,300 in enzyme assay costs for β-glucan analysis, not including analyst costs and investments in time and effort.
- **Robustness** – The inclusion of a large number of samples from varied growing regions and with a varied range of analyte concentrations strengthened the predictive power of NIRS for oat quality determination.
- **Single Analytical Platform** – This study demonstrates that NIRS can serve as a single platform for rapid multicomponent analysis of oat quality determinants.

Table VIII. Ranking of oat cultivars based on β-glucan content in 2015 and 2016 South Dakota samples[a]

AACCI Reference Analysis		NIRS[b] Analysis (Ground Oat Groat Calibration)		NIRS[b] Analysis (Whole Oat Groat Calibration)	
GMI423	6.93 a	GMI423	6.86 a	GMI423	6.60 a
Newburg	5.35 b	Newburg	5.37 b	Newburg	5.35 b
Jury	5.20 bc	Horsepower	5.24 bc	Jury	5.30 b
Horsepower	5.14 c	Jury	5.14 cd	Horsepower	5.14 c
Rockford	5.13 c	Rockford	4.98 de	Rockford	5.06 c
Goliath	4.92 d	Goliath	4.93 e	Souris	4.87 d
Souris	4.89 de	Souris	4.88 e	Hayden	4.87 d
Hayden	4.75 ef	Hayden	4.86 e	Goliath	4.77 de
Deon	4.65 fg	Deon	4.62 f	Deon	4.68 e
Shelby427	4.53 gh	Shelby427	4.49 fg	Streaker	4.46 f
Streaker	4.38 hi	Streaker	4.36 gh	Shelby427	4.42 f
Stallion	4.32 ij	Stallion	4.31 hi	Stallion	4.35 fg
Jerry	4.18 j	Jerry	4.24 hi	Jerry	4.25 gh
SD110466	4.17 jk	SD110466	4.16 ij	SD110466	4.17 h
Colt	4.00 kl	Colt	4.02 jk	Colt	4.13 h
Natty	3.90 l	Natty	3.99 k	Natty	3.97 i

[a] Means followed by the same letter within each column are not significantly different from each other ($P < 0.05$).
[b] NIRS: near-infrared reflectance spectroscopy.

Table IX. Ranking of oat cultivars based on protein content in 2015 and 2016 South Dakota samples[a]

AACCI Reference Analysis		NIRS[b] Analysis (Ground Oat Groat Calibration)		NIRS[b] Analysis (Whole Oat Groat Calibration)	
SD110466	17.24 a	SD110466	17.47 a	SD110466	17.53 a
Stallion	16.88 ab	Stallion	17.23 ab	Jerry	16.90 b
Jerry	16.79 ab	Jerry	17.20 ab	Streaker	16.83 b
Streaker	16.79 ab	Streaker	16.59 bc	GMI423	16.78 b
GMI423	16.49 b	GMI423	16.58 bc	Stallion	16.71 b
Goliath	15.91 c	Shelby427	16.20 cd	Goliath	16.06 c
Deon	15.87 c	Deon	16.08 cde	Deon	16.04 c
Shelby427	15.83 c	Goliath	15.82 cdef	Jury	15.90 cd
Horsepower	15.68 cd	Horsepower	15.80 cdef	Shelby427	15.86 cd
Natty	15.42 cd	Jury	15.79 cdef	Rockford	15.82 cde
Rockford	15.41 cd	Rockford	15.67 def	Natty	15.61 cdef
Jury	15.40 cde	Newburg	15.48 def	Hayden	15.43 def
Colt	15.37 cde	Souris	15.48 def	Colt	15.42 def
Hayden	15.15 de	Natty	15.42 def	Horsepower	15.42 def
Newburg	15.15 de	Hayden	15.33 ef	Souris	15.34 ef
Souris	14.77 e	Colt	15.24 f	Newburg	15.31 f

[a] Means followed by the same letter within each column are not significantly different from each other ($P < 0.05$).
[b] NIRS: near-infrared reflectance spectroscopy.

Table X. Ranking of oat cultivars based on oil content in 2015 and 2016 South Dakota samples[a]

AOCS Reference Analysis		NIRS[b] Analysis (Ground Oat Groat Calibration)		NIRS[b] Analysis (Whole Oat Groat Calibration)	
Rockford	6.91 a	Rockford	6.89 a	Rockford	6.77 A
Stallion	6.49 b	Stallion	6.54 b	Stallion	6.45 b
GMI423	6.46 b	Streaker	6.29 c	GMI423	6.37 bc
Streaker	6.36 bc	GMI423	6.27 c	Hayden	6.29 bcd
Hayden	6.23 cd	Hayden	6.26 cd	Streaker	6.12 cde
Jury	6.20 cd	Jury	6.22 cd	Jury	6.06 def
Newburg	6.10 d	Newburg	6.11 de	Newburg	6.03 efg
Shelby427	6.07 d	Horsepower	6.00 e	Shelby427	5.85 fg
Horsepower	5.78 e	Shelby427	5.98 e	Horsepower	5.82 g
Deon	5.40 f	Deon	5.55 f	Deon	5.42 h
Goliath	5.26 fg	Goliath	5.29 g	Goliath	5.31 hi
Souris	5.07 g	Souris	5.07 h	Souris	5.08 ij
Jerry	4.66 h	Jerry	4.74 i	Jerry	4.83 jk
Colt	4.54 h	Colt	4.40 j	Colt	4.82 k
SD110466	4.25 i	SD110466	4.35 j	SD110466	4.39 l
Natty	3.99 j	Natty	4.10 k	Natty	4.10 m

[a] Means followed by the same letter within each column are not significantly different from each other ($P < 0.05$).
[b] NIRS: near-infrared reflectance spectroscopy.

- **Efficiency** – Effective NIRS calibrations for whole oat groats represent a significant advancement in view of the elimination of the need to grind samples.

Acknowledgments

We thank General Mills Inc., Grain Millers, Inc., and the South Dakota Agricultural Experiment Station for their support of this project.

References

1. AACC International. Method 32-23.01, β-Glucan Content of Barley and Oats—Rapid Enzymatic Procedure; Method 44-15.02, Moisture—Air-Oven Methods; Method 46-30.01, Crude Protein—Combustion Method. *Approved Methods of Analysis*, 11th ed. Published online at http://methods.aaccnet.org. AACC International, St. Paul, MN.
2. Anderson, J. W., Spencer, D. B., Hamilton, C. C., Smith, S. F., Tietyen, J., Bryant, C. A., and Oeltgen, P. Oat-bran cereal lowers serum total and LDL cholesterol in hypercholesterolemic men. Am. J. Clin. Nutr. 52:495, 1990.
3. AOCS. AOCS Official Procedure, Approved Procedure Am 5-04, Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. American Oil Chemists Society, Urbana, IL, 2005.
4. Bellato, S., Del Frate, V., Redaelli, R., Sgrulletta, D., Bucci, R., Magrì, A. D., and Marini, F. Use of near infrared reflectance and transmittance coupled to robust calibration for the evaluation of nutritional value in naked oats. J. Agric. Food Chem. 59:4349, 2011.
5. Belousov, A. I., Verzakov, S. A., and von Frese, J. Applicational aspects of support vector machines. J. Chemom. 16:482, 2002.
6. Bhatty, R. S. The potential of hull-less barley. Cereal Chem. 76:589, 1999.
7. Blakeney, A. B., and Flinn, P. C. Determination of non-starch polysaccharides in cereal grains with near-infrared reflectance spectroscopy. Mol. Nutr. Food Res. 49:546, 2005.
8. Borggard, C., and Thodberg, H. H. Optimal minimal neural interpretation of spectra. Anal. Chem. 64:545, 1992.
9. Chen, D., Hu, B., Shao, X., and Su, Q. A new hybrid strategy for constructing a robust calibration model for near-infrared spectral analysis. Anal. Bioanal. Chem. 381:795, 2005.
10. De Sá, R. M., and Palmer, G. H. Analysis of β-glucan in single grains of barley and malt using NIR-spectroscopy. J. Inst. Brew. 112:9, 2006.
11. Frey, K. J. Improvement of quantity and quality of cereal grain protein. Page 9 in: *Alternative Sources of Protein for Animal Production: Proceedings of a Symposium*. B. R. Baumgarten, T. J. Cunha, J. Milton Bell, J. E. Halver, W. H. Hale, N. L. Jacobson, R. R. Oltjen, M. L. Sunde, and D. E. Ullrey, eds. National Academy of Sciences, Washington, DC, 1973.
12. Geladi, P., and Kowalski, B. R. Partial least-squares regression: A tutorial. Anal. Chim. Acta 185:1, 1986.
13. Herrera, M. P., Gao, J., Vasanthan, T., Temelli, F., Henderson, K., and Navabi, A. β-Glucan content, viscosity, and solubility of Canadian grown oat as influenced by cultivar and growing location. Can. J. Plant Sci. 96:183, 2016.
14. Jacobsen, S., Søndergaard, I., Møller, B., Desler, T., and Munck, L. A chemometric evaluation of the underlying physical and chemical patterns that support near infrared spectroscopy of barley seeds as a tool for explorative classification of endosperm genes and gene combinations. J. Cereal Sci. 42:281, 2005.
15. Krishnan, P. G., Park, W. J., Kephart, K. D., Reeves, D. L., and Yarrow, G. L. Measurement of protein and oil content of oat cultivars using near-infrared reflectance spectroscopy. Cereal Foods World 39:105, 1994.
16. Krishnan, P. G., Reeves, D. L., Kephart, K. D., Thiex, N., and Calimente, M. Robustness of near infrared reflectance spectroscopy measurement of fatty acids and oil concentrations in oats. Cereal Foods World 45:513, 2000.
17. Manley, M. Near-infrared spectroscopy and hyperspectral imaging: Non-destructive analysis of biological materials. Chem. Soc. Rev. 43:8200, 2014.
18. Osborne, B. Review: Applications of near infrared spectroscopy in quality screening of early-generation material in cereal breeding programmes. J. Near Infrared Spectrosc. 14:93, 2006.
19. Osborne, B. G. Near-infrared spectroscopy in food analysis. In: *Encyclopedia of Analytical Chemistry* (Online). DOI: 10.1002/9780470027318.a1018. John Wiley & Sons, Ltd., Hoboken, NJ, 2006.
20. Peterson, D. M., Wesenberg, D. M., Burrup, D. E., and Erickson, C. A. Relationships among agronomic traits and grain composition in oat genotypes grown in different environments. Crop Sci. 45: 1249, 2005.
21. Queenan, K. M., Stewart, M. L., Smith, K. N., Thomas, W., Fulcher, R. G., and Slavin, J. L. Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. Nutr. J. 6:6, 2007.
22. Schmidt, J., Gergely, S., Schönlechner, R., Grausgruber, H., Tömösközi, S., Salgó, A., and Berghofer, E. Comparison of different types of NIR instruments in ability to measure β-glucan content in naked barley. Cereal Chem. 86:398, 2009.
23. Shewry, P. R. Seed storage proteins: Structures and biosynthesis. Plant Cell Online 7:945, 1995.
24. Sohn, M., Himmelsbach, D. S., Barton, F. E., Griffey, C. A., Brooks, W., and Hicks, K. B. Near-infrared analysis of whole kernel barley: Comparison of three spectrometers. Appl. Spectrosc. 62:427, 2008.
25. Thomas, E. V., and Haaland, D. M. Comparison of multivariate calibration method for quantitative spectral analysis. Anal. Chem. 62:1091, 1990.
26. U.S. Department of Agriculture National Agricultural Statistics Service. Statistics by subject: National statistics for oats. Published online at www.nass.usda.gov/Statistics_by_Subject/result.php?D71E4540-3A48-302A-834B-537275051111&sector=CROPS&group=FIELD%20CROPS&comm=OATS. USDA-NASS, Washington, DC, 2016.
27. Wolever, T. M., Tosh, S. M., Gibbs, A. L., Brand-Miller, J., Duncan, A. M., Hart, V., Lamarche, B., Thomson, B. A., Duss, R., and Wood. P. J. Physicochemical properties of oat-glucan influence its ability to reduce serum LDL cholesterol in humans: A randomized clinical trial. Am. J. Clin. Nutr. 92:723, 2010.

Significance of Low Analytical Limits of Component Detection

John Szpylka[1]
Mérieux NutriSciences, Crete, IL, U.S.A.

When you hear the term, "food safety," an immediate thought concerns reducing the risk of microbiological contamination; a logical thought since microbial contamination can cause acute illness and has the potential to quickly cause fatalities. Another critical component of food safety concerns chemical contamination, as exemplified by allergens, which are now the number one cause of food recalls (4). Although this is another example of an immediate-impact contaminant, many chemical contaminants can also impact health over a longer period of exposure, and they must, therefore, also be assessed and monitored.

The U.S. Food and Drug Administration (FDA) recently published the Food Safety Modernization Act (FSMA) guidance document, "Draft Guidance for Industry: Hazard Analysis and Risk-Based Preventative Controls for Human Food" (8), which lists potential chemical hazards to consider when designing a risk-based safety program. The list contains both immediate and long-term chemical risks:

- Undeclared allergens
- Drug residues
- Heavy metals
- Industrial chemicals
- Mycotoxins/natural toxins
- Pesticides
- Unapproved colors and additives
- Radiological elements

As analytical instruments become more sensitive, the corresponding test methods are able to detect lower and lower levels of these contaminants. In fact, there seems to be a race to see who can create the most sensitive instrument or measure "the analyte du jour" at the lowest possible level. To properly use this additional information we must factor in scientifically sound reasoning on the impact of contaminants on short- and long-term health.

Measuring Lower Levels of Targeted Compounds

When we measure an analyte at a low concentration, how much are we actually seeing? To put this in analogous terms, standard analytical units of measurement are expressed in more common units in Table I.

Analytical instrumentation has evolved over time and can now measure compounds at the lower levels (ppb and ppt) shown in the farthest right columns of Table I. Early measurements were originally performed using gravimetric analyses, titrations, and some basic chromatographic techniques. Measurement levels were steadily decreased by using more stable instruments to quantify compounds using more precise weighing and more sensitive absorption, emission, fluorescence, and mass measuring instruments. Hybrid techniques such as ICP-MS (inductively coupled plasma–mass spectrometry), HPLC-MS (high-performance liquid chromatography–mass spectrometry), HPLC-MS/MS (high-performance liquid chromatography–tandem mass spectrometry), GC-MS (gas chromatography–mass spectrometry), GC-MS/MS (gas chromatography–tandem mass spectrometry), and 2-D chromatography have further lowered the limits of detection. There has also been much work conducted to significantly concentrate analytes before introduction into the instruments listed above. These technologies are summarized in Table II. As can be seen, the capability to measure extremely low levels of contaminants has steadily improved.

Measuring Levels of Contaminants

When a chemical found in a food is designated as a contaminant, the allowable level is ideally at least one or two orders of magnitude lower than the amount found to be harmful in the short-term (contaminants causing acute toxicity) or long-term (contaminants that accumulate over time). Many times these safety levels are unknown, especially for a newly identified contaminant. In this case, the default allowable level is typically set at "zero," or in general terms, "not detectable by the most sensitive method." This approach mirrors the FDA "*Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed*" (6), which lists allowable levels for specific contaminants as, "action levels and tolerances [that] represent limits at or above which FDA will take legal action to remove products from the market. Where no established action level or tolerance exists, FDA may take legal action against the product at the minimal detectable level of the contaminant."

Bearing in mind the ongoing lowering of limits of detection, let's consider the following scenario. A chemical is recognized as a contaminant on January 1, and on that day, the contaminant can be detected down to a level of 100 ppm. Because no established tolerance level exists, 100 ppm would be the actionable level. Manufacturers in the food supply chain use this analytical method as their tool to minimize the risk of the contaminant being present in their products. On June 1, a group develops and releases a fully validated method that can accurately measure the contaminant down to 1 ppm. According to the wording in the FDA guidance document (6), the actionable level has now dropped by two orders of magnitude, which from a safety standpoint is appropriate and defensible because no proven health-affecting level has been established. Because the potential effect on monitoring of the food supply chain can be dramatic, this typically forces decisions to be made concerning the definition of a safe or allowable level. The good news is this series of events does accelerate the pace for proper, "safe" levels to be scrutinized and defined.

[1] E-mail: john.szpylka@mxns.com

https://doi.org/10.1094/CFW-63-1-0026

Table I. Measurement units expressed in common terms

	Unit				
	% (parts per hundred)	ppth (parts per thousand)	ppm (parts per million)	ppb (parts per billion)	ppt (parts per trillion)
Distance	1 yd in 1 football field	1 yd in 10 football fields	1 yd between Orlando, FL, and Gulfport, MS	1 yd in the diameter of the orbit of Earth's moon	1 yd between the sun and Jupiter
Money	1¢ in $1	1¢ in $1,000	1¢ in $10,000	1¢ in $10 million	1¢ in $10 billion
Volume	1 drop of vermouth in 99 drops of gin	1 drop of vermouth in 2 oz of gin	1 drop of vermouth in 64 qt of gin	1 drop of vermouth in 500 bbl of gin	1 drop of vermouth in a football field 43 ft deep in gin
Mass	1 pinch of salt on 16 potato chips	1 pinch of salt in 8 single-serve bags of potato chips	1 pinch of salt in 553 lb of potato chips	1 pinch of salt in 276 tons of potato chips	1 pinch of salt in potato chips equal in weight to 5 QE2 ocean liners

Table II. Effects of the evolution of technology on detection limits

Technologies[a]	Concentrations	Costs	Comments
Wet chemistry and original LC	1–100%	Low	Instruments can be portable
GC, HPLC, AA, and ICP	0.01–1%	Medium	Instruments became more complex
GC, AA, and ICP	1–100 ppm	Medium/high	Instrument improvements
UHPLC-MS/MS, GC-MS, and ICP-MS	ppb to ppm	High	More sensitive detectors
UHPLC-MS/MS, GC-MS, and ICP-MS	ppt	Higher	Instrument improvements
Preanalysis concentration of analytes	<ppt	Higher+	Add-ons to already sensitive instruments
What's next?	Even lower?	???	???

[a] LC: liquid chromatography; GC: gas chromatography; HPLC: high-performance liquid chromatography; AA: atomic absorption spectroscopy; ICP: inductively coupled plasma spectroscopy; UHPLC-MS/MS: ultra high-performance liquid chromatography tandem mass spectrometry; GC-MS: gas chromatography–mass spectrometry; ICP-MS: inductively coupled plasma–mass spectrometry.

Currently, this process is occurring in the field of allergen testing. In 2004, the Food Allergen Labeling and Consumer Protection Act (FALCPA) was enacted, and eight foods and food groups were identified as major food allergens: milk, eggs, fish (e.g., bass, flounder, cod), crustacean shellfish (e.g., crab, lobster, shrimp), tree nuts (e.g., almonds, walnuts, pecans), peanuts, wheat, and soybean (7). FALCPA requires a food to be labeled if it includes an ingredient containing protein from one or more of the foods listed above (7). Because no allowable limits were included in the act, the existing ELISA methods used to monitor foods and ingredients for allergens set the action limit based on their detection limits, which range from 10 to 20 ppm. Recently, LC-MS/MS (liquid chromatography–tandem mass spectrometry) analysis for allergens has been shown to provide greater sensitivity in allergen detection (3). The improvement in testing sensitivity is facilitating discussions on reevaluation of acceptable levels for foods to be declared allergen-free.

Using Data from Improved Detection Limit Technology

It is important that the information obtained using instruments with increased sensitivity be placed in the proper context. The additional data we collect must be carefully interpreted, as was demonstrated to me while I was waiting for a flight at an airport. A gentleman seated next to me was upset over an article reporting lead being found in a food he eats—the level was 1 ppt. The main point he focused on was lead being detected in the food, not the fact that the measured 1 ppt level is 1,000 times lower than the action level. This is a clear example of a quote from science fiction writer Douglas Adams, "Nothing travels faster than the speed of light, with the possible exception of bad news, which obeys its own special laws" (1).

As summarized in English in the *Beverage Daily* (2) in 2002, *Oeko-Test*, an ecology-friendly publication, reported acrylamide was present in several brands of roasted coffee. The article described the health issues as, "German researchers said they found traces of the potentially cancer-causing chemical acrylamide, although not in as high concentrations as in fatty foods such as potato crisps, french fries, or bread" (2). What was not noted was that the measured levels in the coffee were below legal action levels. This is a clear example of the need to place the information gained from the ability to detect lower concentrations of contaminants in the proper and scientifically sound context.

More recently, in 2009 *CNN News* reported "90 percent of U.S. bills carry traces of cocaine" (5). The average amount of 28 μg/bill was measureable due to instrument improvements. What was great to see in this article were the following sentences that placed this information in the proper context: "Research presented this weekend reinforced previous findings that 90 percent of paper money circulating in U.S. cities contains traces of cocaine. Scientists say the amount of cocaine found on bills is not enough to cause health risks." And later, "When the ATM machine gets contaminated, it transfers the cocaine to the other bank notes. These bills have fewer remnants of cocaine. Some of the dollars in his experiment had 0.006 micrograms, which is several thousands of times smaller than a single grain of sand."

Lower Detection Limits Also Mean Obtaining Useful Information

Let's now look at lower limits of detection from a different angle. As the ability to measure lower levels of compounds evolves and increases, we are able to gain a deeper understanding of the structure and identity of a material because the amount of information we gain about the material rises at a dramatic rate. Using a simplified example for illustration, let's test for the components in a sample of brown sugar.

Let's assume the lowest level we can measure of any component in the brown sugar is 1%. Sample testing would only be

Table III. Analysis of brown sugar sample with increasingly lower limits of detection

Limit of Detection	Measureable Components in Brown Sugar Sample
1.0%	Sucrose, water(?)
0.1%	Sucrose, water, protein, something unexpected(?)
1 ppm	Sucrose, water, protein, Ca, something unexpected, something else(?)
1 ppb	Sucrose, water, protein, Ca, Fe, K, Na, something unexpected, something else, or worse(?)
1 ppt	Sucrose, water, protein, Ca, Fe, K, Na, who knows what else(?)

able to measure sucrose and perhaps water, with any additional compounds being "not detected" because of the 1% detection limit. As the technology improves and enables lower levels of components to be detected and measured, additional information concerning the brown sugar sample is obtained (Table III).

As lower limits of detection are attained, more compounds will be detected in materials, and this additional knowledge can be used to create a detailed "fingerprint" of each material. This approach is generally described as "nontargeted testing" and is being explored by a number of organizations, instrument suppliers, software developers, and academia. The development of a well-defined fingerprint or "identity standard" allows comparisons with newly obtained or purchased materials (such as food ingredients) to be made. If the data profile for the material being examined is within normal variation when compared with an identity standard, the risk of contamination (or adulteration) is reduced. If the test material differs significantly from the fingerprint, a deeper examination can be performed. Detecting lower levels of components generates an increased amount of information, and new approaches to using this information can further improve the safety and integrity of the food supply chain.

Summary

Analytical instrumentation and corresponding methods of analysis are constantly improving, allowing lower concentrations of contaminants to be detected in foods. With some compounds now being detected at the parts per trillion level, the impact of finding lower levels of contaminants must be properly interpreted so that sound regulations and the correct food safety protocols can be constructed. This will involve effective communication between scientists, regulators, instrument suppliers, food producers, consumers, and media participants.

John Szpylka, scientific affairs director, chemistry N.A., for Mérieux NutriSciences, manages nutritional analytical method development for Mérieux NutriSciences and is a technical leader for chemistry testing in North America. John is a representative to key scientific organizations and regulatory agencies, including active involvement with AOAC International, AACC International, AOCS, AAFCO, ASTA, and GMA. John is a Fellow of AOAC International and past chair of the AOAC Official Methods Board. He currently serves on numerous AOAC stakeholder panels and expert review panels concerning analyses of nutrients and contaminants. Before joining Mérieux NutriSciences, John was a principal scientist with General Mills and Medallion Laboratories. John received his doctorate in analytical chemistry from The Ohio State University after receiving a B.S. degree in chemistry from Rensselaer Polytechnic Institute. John is an AACCI member and can be reached at john.szpylka@mxns.com.

References

1. Adams, D. *A Hitchhiker's Guide to the Galaxy*. Ballantine Books, New York, 1979.
2. BeverageDaily.com. Acrylamide found in coffee. Published online at www.beveragedaily.com/Article/2002/08/28/Acrylamide-found-in-coffee. William Reed Business Media Ltd., Crawley, England, 2008.
3. Christofakis, M., and Xila, A. LC–MS/MS techniques for food allergen testing. Page 49 in: *Food Allergen Testing: Molecular, Immunochemical and Chromatographic Techniques*. G. Siragakis and D. Kizis, eds. John Wiley & Sons, Ltd., Hoboken, NJ, 2014.
4. Maberry, T. A look back at 2016 food recalls. Published online at www.foodsafetymagazine.com/enewsletter/a-look-back-at-2016-food-recalls. FSM: eDigest, FoodSafetyMagazine.com, 2017.
5. Park, M. 90 percent of U.S. bills carry traces of cocaine. Published online at www.cnn.com/2009/HEALTH/08/14/cocaine.traces.money. CNN U.S. Edition. Cable News Network, Atlanta, 2009.
6. U.S. Food and Drug Administration. *Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed*. Published online at www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/chemicalcontaminantsmetalsnaturaltoxinspesticides/ucm077969.htm. FDA, Silver Spring, MD, 2000.
7. U.S. Food and Drug Administration. Food Allergen Labeling and Consumer Protection Act of 2004 questions and answers. Published online at www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106890.htm. FDA, Silver Spring, MD, 2006.
8. U.S. Food and Drug Administration. Draft guidance for industry: Hazard analysis and risk-based preventive controls for human food. Published online at www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm517412.htm. FDA, Silver Spring, MD, 2017.

Keeping It Simple

Terry C. Nelsen[1]
T. Nelsen Consulting, Port Byron, IL, U.S.A.

Just as the modern electronic age has changed the way we study chemistry, it has also changed the way we perform statistical analysis. In the old "wet chemistry" days, we worked for hours to produce a few measurements. Today we use electronic instruments that spew out data. Statistical analysis was once limited by what we called calculation intensity, because it took hours or even days to perform all the calculations required. Computers are especially good at rapid calculations and can do the job in seconds. We have also been blessed with software packages that combine analytics with graphics and are generally user-friendly.

I probably sound like a grumpy old timer, but I believe some of the old tried-and-true statistical methods are still useful, especially when combined with modern rapid analysis and good graphics. I am not saying we should ignore newer methods. They are useful and necessary, especially when a chemist shows up with a huge data set and asks a statistician to conduct a "salvage job." However, problems can arise with interpretation of results. For example, do you know what the first principal component and a latent variable are? Do you know what assumptions you are making about your data? Are you concerned about multicollinearity or heteroscedasticity? Many of these modern multivariate methods are used for "dimension reduction," which is a statistician's way of saying simplification. If your multivariate analysis is successful in reducing the dimensions of your data set, then consider further experiments or trials on only those variables you have identified as important.

The Basics

Many problems can be avoided with careful planning. After 35+ years, I am convinced the two biggest problems we have in research and quality assurance are 1) sampling and 2) lack of a clear objective.

I am pleased that others in this issue of *Cereal Foods World* are addressing sampling. I will simply remind you that your results will pertain to the materials on which you performed your tests. In methods evaluation, we ask method developers to test their methods using the materials that are expected to be used in the method. Will a method developed to estimate a constituent in wheat flour provide good results for other types of flours? If you grab a sample of grain for a test and then carefully clean and sort the kernels before the tests are performed, can you assume the mill will produce the same results for grain that has not been cleaned and sorted as carefully? Sample preparation is a part of the sampling method. When considering numbers of samples, remember that analyzing five separate samples of a variety grown in five different locations is not the same as bulking the five samples into one bag and then grabbing five samples for analysis.

The lack of a clear objective is not always obvious. When I read an objective such as "determine which proteins affect quality," my first question is, "How exactly do you plan to measure quality?" My next question is, "Does this quality pertain to the grower, the shipper, the miller, the baker, the retailer, or the final consumer?" I have found communications are not always clear between management and the technical people doing the work or between two or more laboratories working on the same project. When you are told to test or compare varieties or optimize processes, be sure everyone agrees on exactly what is being measured before you start.

Initial Considerations

Sometimes you need to describe an entity. What is the range of its occurrence in specific materials? Does it occur alone or always in the company of another entity? Does its level of occurrence depend on the presence, absence, or level of the other entity? Statistics can be used to clarify descriptions. For example, technical people know that a mean and standard deviation tell us more about a measurement than a simple range of the data. A regression formula can be more useful than a simple correlation.

Using a graphics package to look at the data is helpful. If you plan to test hypotheses, then you will need to know how your data are distributed. If you use normal statistical tests, you will usually assume normal distributions. If you run the usual tests on non-normal data, however, it will be more difficult to find real differences. Many measurements are not normally distributed. For example, time intervals or microbial growth can be skewed. Censored distributions are common when measuring at lower concentrations, and some of the data are reported as less than LOD (limit of detection), BDL (below detectable limit), or ND (not detected). Multimodal distributions are common for multiple overlapping peaks in chromatography. Particle size distributions can be confusing if chemical or physical factors cause different types of particles.

Hypothesis testing includes a probability statement. Instead of saying "the difference was statistically significant," try saying "the difference was consistent." Decide how large a difference interests you—a difference can be statistically significant but practically trivial. Usually, the finer the difference you want to detect, the more data you will need to collect. Decide on your acceptable level of significance. Do you need to be 99% sure, or will you accept 95% or even 90%?

How much variation is acceptable? Will this treatment or additive change the final product and by how much? We usually compare means (averages) in hypothesis testing. Be sure to consider comparing variances as well. When comparing the qualities of plant varieties over several environments, they can have similar properties on average, but one of the varieties may be quite stable in several environments, whereas another may be more responsive, being either above or below average as environments differ. Box plots of percent protein content in four varieties of wheat (A, B, C, and D), each grown in several locations, are shown in Figure 1. Looking at this plot, I would describe varieties A and B as similar in average protein content, but variety A appears to be more susceptible to environmental

[1] E-mail: nelsent@gmail.com

https://doi.org/10.1094/CFW-63-1-0029

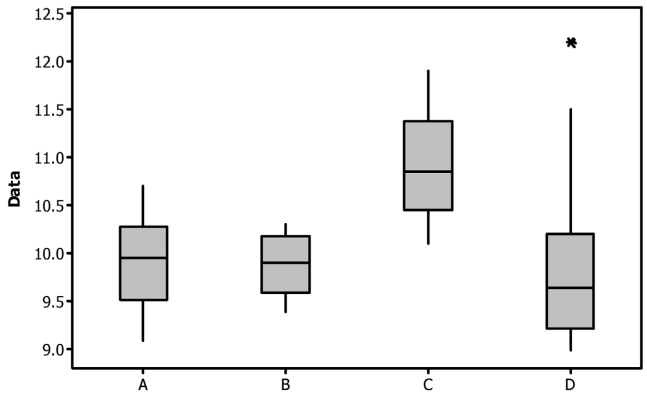


Fig. 1. Box plots.

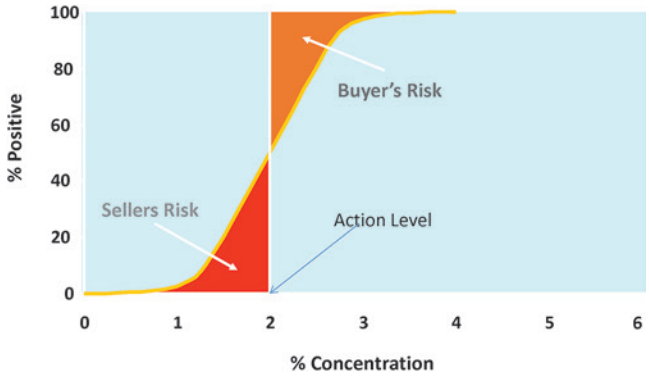


Fig. 2. Operating characteristic curve.

differences. Variety C has a higher average protein content and is similar in variance to variety A. Variety D has an overall lower protein content but appears to be very responsive to some environments. Your statistical software has box plot capability, and I highly recommend using it.

Identifying an Area of Interest

Determine your "area of interest." The area of interest can be those factors (e.g., proteins, additives, or treatments) that consistently affect your measurement. Or, it can be an adjustable range of a continuous variable, such as temperature, concentration, time, pressure, speed, and so on. In what range do you see the greatest effect? If you already know a lot about your subject matter, then concentrate on your expected area of interest. You can collect some data outside of your area of interest just to be sure you have not missed something important, but you can usually do these "checks" with fewer samples. If you are unsure about what you will find, then consider doing your investigation in stages.

A screening design can be used to efficiently determine which factors are important, and you can then concentrate on only those factors and avoid needlessly spending resources by collecting data on settings or combinations of factors that do not affect your measurements.

If you are looking for the effects of a continuous variable, then gather your initial data across a range of that variable. Your objective is to bracket the area of interest. Instead of low versus high or low/medium/high, plan to measure at points along a line. If you expect a straight-line response, then gather data at several points along that line. If you expect a curved response, such as the effects of temperature on some enzyme activity, then gather some data at the extremes but more data at the temperatures at which the peak or curve occurs. If you do not know what shape of curve to expect, then plan your project in stages: first gather some data across a wide range and then go back and gather more data where changes appear (i.e., the area of interest). If you already have a lot of data, then consider asking a statistician to use a dimension-reduction technique to find your area of interest.

An operating characteristic curve for a test kit detecting a particular toxin is shown in Figure 2. How could I have produced this curve most efficiently from the beginning if my objective was to evaluate the test kit at concentrations below 5%? My first trials could involve preparing concentrations at 1% intervals from 0 to 6% (bracketing the area of interest). Depending on the price of the kits, I could test three each at each percentage point. If we suppose the results show all three negative at 0 and 1%, all three positive at 4, 5, and 6%, and a mixture of positive and negative at 2 and 3%, that would narrow my area of interest to between 1 and 4%. Rather than continue to collect data below 1% or above 4%, I would concentrate my resources on gathering data in the area of interest. If the test kits were relatively inexpensive, I would double-check the results with a couple of tests outside the area, just for peace of mind. My advice is not to try to produce the curve all in one trial, because you could waste resources in gathering data outside the area of interest.

Also, consider what would happen if you conducted stage 1 of the trial and then got conflicting results in stage 2. Statisticians often recommend conducting a balanced, complete trial all at one time so that unknown factors do not pop up and bias the results. This is usually good advice for basic research, but your objective is to look for applied results. If an unknown factor causes your test kit or analytical method to produce different results at different times, then it is important for you to recognize this. If you can discover the unknown factor, you can make adjustments. If you cannot determine why you are obtaining different results under different circumstances, then maybe you should not trust the kit or method.

Refining the Design

If you are looking for effects of the presence or absence of several additives plus possible effects of time, temperature, or other variables all at once, then talk with a statistician about using a screening design such as a fractional factorial. Technical people complain that statisticians always want more data, but this is one area in which more information can be gathered with less data using efficient designs. Again, you can gather your data in stages: first a broad-based strategy to identify the area of interest and then a finer design to better describe responses. An efficient early-stage design can tell you which variables are important at which levels and in which combinations.

Chemists seem to love correlations, and statisticians love to tell them correlation does not imply causation. Think of correlations as hunting licenses. When you see a correlation between two measurements, then you get to hunt down and describe the specific relationship between those two measurements. Conversely, when you do not find a correlation where you expect to find one, then find out why not.

An example of an unexpected result (hidden variable) is shown in Figure 3. Data were gathered over three years to evaluate the effect of protein on loaf volume. In Figure 3 the graph on the left shows no relationship between protein and loaf volume—an unexpected result. The graph on the right is the same data looked at by year. It is obvious there is a relationship, but we also have to consider the effect of years. Another common cause of an unexpected nonrelationship is the result of restricted range. If I

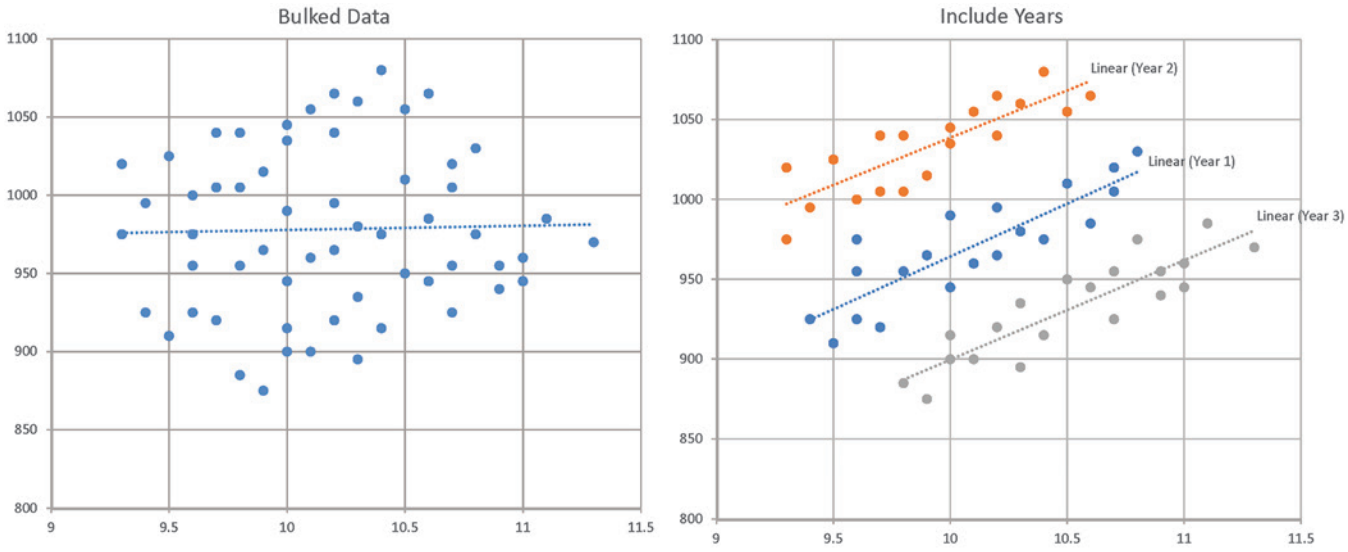


Fig. 3. Example of a hidden variable.

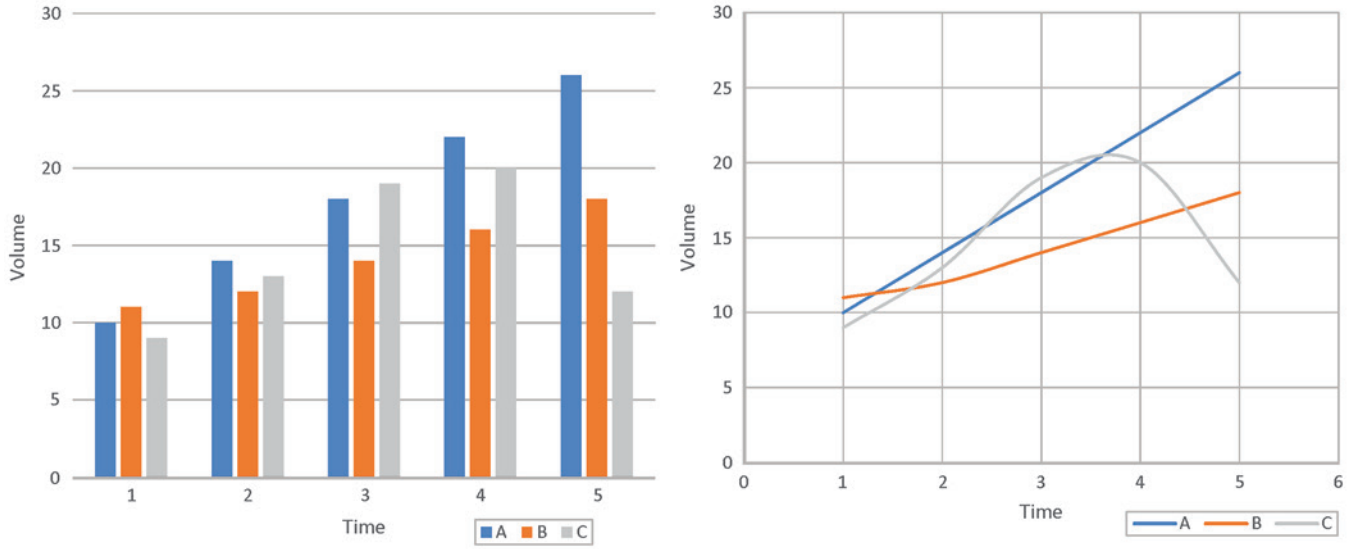


Fig. 4. Histogram versus line plot.

had looked at loaf volumes for a narrow range of proteins, I might not see the relationship.

Presenting Your Results

The best advice I can give someone who is planning to run an experiment or trial is first to consider how you want to present your results. I do not mean for you to anticipate your exact results but rather to consider if you want a table full of means with little letters attached to them, a series of curves, or something else. A statistician can then help you design your experiment to produce results in your preferred format.

The same results are presented in two different ways in Figure 4. The histogram is commonly used and easy to create (Fig. 4, left). You can even put little error bars on the top of the columns. A line plot of the same data is more informative, however (Fig. 4, right). Varieties A and B respond in a linear fashion to time. If their lines are not parallel, then you can assume there is an interaction between varieties A and B and time (i.e., they both respond positively to time but variety A more so than B). The response of variety C to time is more complicated, but it is different than the responses of the other two varieties. I always recommend using line plots and regression analysis when appropriate. Try to keep the values on the x-axis evenly spaced. The results will be easier to analyze and will look better on a graph. Use of a line graph would be inappropriate, for example, if the x-axis is not a continuous variable (e.g., five different locations or varieties). I sometimes cheat a bit in preliminary analysis and use a line graph just so I can spot interactions. Do not try to present your final results this way, however. If the values on the x-axis can be presented in any order, then a line graph is probably not appropriate.

Final Thoughts

In conclusion, I recommend you think through your experiment or trial before you start. Where, when, and how will you gather your samples? What will you do with them to prepare for analysis? What, exactly, do you plan to measure, and how would you like to present your results? Do not be afraid to run your trial in stages. Do not, however, use the term "pilot study." For some reason, management seldom likes to fund pilot studies.

Environmental Monitoring in the Milling and Baking Industry

James D. DeVault[1]
Great Plains Analytical Laboratory, Kansas City, MO, U.S.A.

Over the course of the next 12 months, an estimated one in six Americans will succumb to a foodborne bacterial disease (1), with 130,000 people requiring hospitalization and as many as 3,000 dying (2). Although the exact numbers are open to debate, more than 15 million Americans have food allergies (5), and it is estimated that an additional 3,000 people will seek medical treatment for exposure to food allergens (8). Food manufacturers, including those in all segments of the milling industry, try to provide safe products to their customers. Toward this end, the quality manager or director at any food manufacturing facility would state that they employ stringent safety and quality processes for every aspect of their production facility. Despite these efforts and commitments to food safety, 2016 saw 764 food recalls in the United States (7). In a recent survey conducted by the Grocery Manufacturers Association, it was reported that of the companies subjected to a major food recall (class I), roughly one-quarter of the respondents estimated their direct financial costs at more than $30 million (4), whereas the majority of food recalls were estimated to cost between $10 million and $30 million. This same report showed that 81% of respondents believed the financial risk from a major recall could be significant—if not catastrophic—for the company, thus encouraging them to take a proactive approach to food safety.

The big question, then, is what can be done to prevent the production of contaminated or unsafe foods? When picturing the entire process involved in a milling facility, the potential sources of contamination are many. Some are out of the production facility's control. Therefore, the focus should be on what can be controlled—the product (including raw ingredients) once it enters the facility. The goal is to provide a manufacturing environment that is as free of any type of contaminant as possible.

Good hygiene throughout the mill is vital, and cleaning schedules should be set up, if they are not already in place, to cover all areas of the facility. The key is to remember that milling is food manufacturing and to take the same approach as other food manufacturers. Two of the biggest risks are the introduction of microbes and allergens. Therefore, a key component of any sanitation or HACCP (hazard analysis critical control point) program is the development of an environmental monitoring plan to look for the presence and occurrence of contaminants.

Why Monitor the Production Environment?

The purpose of environmental testing and monitoring programs is 1) to determine the efficacy of general cleaning and sanitation for the removal of transient contaminants; 2) to monitor for the presence of specific pathogens that may be present as transient or resident microorganisms; and 3) to reveal potential sources of contamination. Potential contaminants are ever-present in food-handling environments. They can be introduced into the food manufacturing environment through raw materials, water, the physical environment (HVAC or heating, ventilation, and air conditioning systems), pests, other food products, and employees. Therefore, food processors need to employ environmental sampling programs to monitor production areas for general levels of hygiene and the presence of contaminants, even areas or materials that are not immediately obvious potential sources of contaminants.

For example, because flour is a low-moisture product some might think that there is no reason to be concerned about the presence of pathogens. The basic premise is that there is a key water activity level required for bacteria to grow. It is true that most bacteria cannot grow in low-moisture foods; however, they are able to survive in low-moisture environments. Species such as *Salmonella* bacteria are capable of slowing their metabolism to the point that they become resistant to common antimicrobial treatments. When looking at the mean values for more than 100 flour samples (unpublished data), Great Plains Analytical Laboratory obtained a water activity value of ≤0.60, a level that will not support active microbial growth. However, if bacteria are present, growth can occur when the flour is processed further into a food product that has a higher moisture content.

The next argument against microbial contamination is that flour most often undergoes a kill step, either through baking or cooking, at some point during production and is generally not considered a ready-to-eat product. Despite this perception, in 2008 *Salmonella* spp. in flour was indicated in food-poisoning outbreaks in New Zealand (2) and the United States (9). In 2009 and as recently as 2016, hemorrhagic *Escherichia coli* was also associated with flour recalls (11). The general thought was that the raw flour would go through a kill step before consumption, and producers relied on the public to facilitate the final process required to guarantee a safe product. The U.S. Food and Drug Administration (FDA) has issued a number of warnings and statements directed to the public about the potential dangers of eating raw flour in products such as cookie dough (11). Consumers, however, do not always heed these warnings, which places the responsibility for ensuring a product is safe to consume back on the producer.

There are many steps along the way at which pathogens can enter the food manufacturing process, from the field to the end product. Obviously, millers have control only once the grain enters their custody. While the grain is under their control, it is the miller's responsibility to prevent the introduction of pathogens into the product. This is best achieved through a vigorous and effective HACCP system. The standard for the grain industry is outlined in the Home Grown Cereals Authority guide, which describes the steps recommended by Codex to establish an effective HACCP program (6). Of particular interest is the declared need to monitor manufacturing processes, including sanitation procedures. The entire facility is part of the process

[1] E-mail: jdevault@gpalab.com

https://doi.org/10.1094/CFW-63-1-0032

that contributes to the safety of the products millers are manufacturing. This is where an environmental monitoring program comes into play.

How to Monitor the Production Environment

There are basically three types of organisms that millers can test for: pathogens, indicators, and spoilage organisms. In flour processing, the spoilage organisms of most interest are yeasts and molds, the most common being *Aspergillus*, *Fusarium*, *Alternaria*, and *Penicillium*. Testing for the presence of molds requires both air and surface testing. To perform air monitoring, either a passive system or an active system can be used. The most common passive method involves placing bacterial growth plates in key sampling areas. Upon placement, the plate is opened up to the environment, a timer is set, and contaminants are allowed to settle for a predetermined amount of time (3). Following exposure, the plates are incubated and tested accordingly. A useful rule of thumb is that one colony is acceptable for each minute of plate exposure. Active sampling is performed by passing a known volume of air across an agar strip (or plate) and incubating as with the passive method. The results are then equated back to the number of colonies per the tested volume of air.

Testing for indicator organisms is conducted to monitor for transient organisms that are constantly assaulting a facility's hygiene program. From the microbial side, this could include coliform bacteria, aerobic bacteria, Enterobacteriaceae, as well as others. Bacteria are generally thought to be associated with poor hygiene practices. Testing for these organisms allows for the monitoring or trending of data and can signal a potential issue before it arises. On the surface, trending of data is quite simple. Facilities collect an appropriate number of samples to establish an acceptable baseline. Trending the data using any number of readily available graphing programs, a quality manager can immediately observe how the data are trending compared with the baseline. A trending increase suggests that the sanitation system is not as effective as it could be, whereas a decrease clearly shows that the process is improving. Spikes in trending data may signal a lapse in the sanitation program at a given point in time.

Environmental testing to detect specific pathogens serves two important roles. First, it identifies the presence of important food pathogens that may have been introduced into a food-handling environment that have not been eliminated by routine sanitation practices and, therefore, could be passed on to the food materials being processed. Second, it assists in determining the sources of contamination with important pathogens. There is no hard-and-fast rule for determining which pathogen-monitoring program is required. Toward that end, it is necessary to determine what the manufacturer is trying to control. *Salmonella* spp. is the target organism for monitoring of product-contact and non–product-contact surfaces in a low-moisture manufacturing facility. It is the responsibility of the manufacturer to determine where the hazards are (they exist; one just has to find them). After finding the sources, it is the manufacturer's responsibility to limit their access to the product. It is counterintuitive that the goal of a pathogen-monitoring system is to find pathogens. The key here is to find them before they contaminate the product. Environmental monitoring for pathogens allows the manufacturer to assess the effectiveness of cleaning, sanitation, and employee hygiene practices.

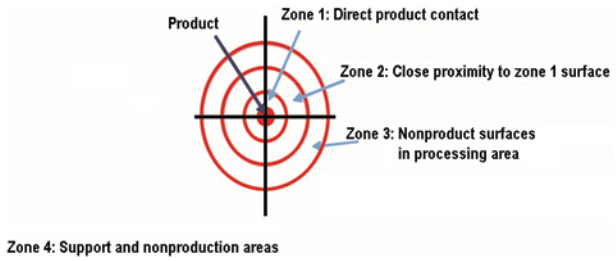


Fig. 1. Target approach used to define zones in a production facility.

When developing an environmental monitoring program, the environmental monitoring zone concept is the easiest to establish and maintain. Understanding the basis of the zone program can be confusing, however. Perhaps the easiest way to understand the zone idea is to picture the product itself as the bull's-eye on a target. In general, the classification of the zones is based on the proximity of the zone to the product and the risk of contamination (Fig. 1). Essentially, the entire facility is broken down into zones, and each zone radiates out from the product as it relates to the product. Zone 1 refers to all direct product-contact surfaces. This includes conveyors, mixers, utensils, racks, etc.—essentially, anything and everything that can come in contact with the product. Zone 2 encompasses those areas directly adjacent to zone 1. This includes non–food-contact surfaces in the processing area (e.g., carts, equipment housing and exterior, etc.). Zone 2 also refers to any pathways that lead to the area where a product is located. Therefore, think along the lines of cart, forklift, and foot traffic. Zone 3 is the area in immediate contacting zone 2. Zone 3, if contaminated, could lead to contamination of zone 2 by means of accidental human traffic or machines and includes hallways and doorways leading into food-production areas, drains, wheels on carts, etc. Zone 4 is the area immediately surrounding zone 3. Zone 4 encompasses the remaining areas that support production, including offices, dry goods storage warehouse, finished product warehouse, cafeterias, hallways, and loading dock area.

When using the zone approach, the surfaces in direct contact with the final product are not tested for pathogens but are tested for allergens (Table I). Allergen testing is only suggested for these areas if product changeover involves cleaning the existing equipment prior to changing the product produced. If there is a dedicated line for the allergen-free product, testing for allergens within zone 1 is not necessary. The unique environment of each facility results in the need for a unique monitoring plan. The plan should clearly demonstrate the effectiveness of the established program and allow for the detection and monitoring of allergens and pathogens before they enter the food product.

From the viewpoint of monitoring for allergens and their presence, it is the responsibility of the individual facility to determine which allergens pose a risk to their products and subsequently to their customers. This is particularly true if the facility is switching back and forth between production of products that contain the specific allergen and products they claim are allergen free. For example, suppose a manufacturer produces snack cakes. During the first part of the week, they produce cakes with almonds. During the last two days of the week, they produce cakes they claim are free of tree nuts. Whether they are performing a full breakdown of the production line or are producing the second batch on a dedicated line, they should demonstrate that no transient tree nut allergens have come in contact with production equipment.

Allergens can be introduced into a product or a process from the unlikeliest of sources. Recently, there was a major food re-

Table I. An example of zone labeling (targets and frequencies are presented only for demonstration and should be determined by each separate facility)

Area	Description	Examples	Possible Targets	Testing Frequency
Zone 1	Product contact	Conveyors, mixers, utensils	Indicators Allergens	Weekly New production
Zone 2	Close proximity to zone 1 surfaces	Conveyor frames, equipment handles, shields and guards, controller buttons, maintenance tools	*Salmonella* spp. *Listeria* spp. Indicators Allergens	Weekly
Zone 3	Interacts with zone 2	Drains, walls, undersides of tables and equipment, lifts, pallets	*Salmonella* spp.	Weekly
Zone 4	Support areas not in processing area	Break rooms, rest-rooms, locker rooms, offices	*Salmonella* spp.	Monthly

call resulting from the undeclared presence of peanut allergens in flour (10). This was believed to result from cross-contamination of a rail car that was used to transport product to a flour-processing facility. With regard to allergens, the best approach is to view them as if they are pathogens and monitor for their presence similar to monitoring for pathogens. The FDA labels milk, eggs, fish, shell fish, tree nuts, peanuts, wheat, and soy as the most common allergens affecting the U.S. population. Reactions to allergens can range from minor irritation all the way to death (12). Therefore, do not discount allergens from the development of an environmental testing plan.

Data Collection and Analysis

Analysis and interpretation must follow data collection. For nonpathogens, start trending the data. Trending the data can be as complicated or as simple as desired. The basics begin with establishing a baseline, which involves an investment of time on the front end. The goal here is to generate a statistically significant amount of data to determine what the actual baseline is. The next step involves setting the control limits. Again, each production facility must define these locally. A good rule of thumb for those just seeking some guidance is to set an alert at 2 standard deviations from the mean that has previously been determined. This, by definition, includes 95% of the samples tested. If something falls out of that range, address it according to internal standard operating procedures. Each production plant, production line, and product is unique and should be treated as such.

The next step when monitoring data is to look for developing trends. Graphing the results will indicate if a trend is occurring. Of course, in an ideal world, the data will trend toward zero, indicating that the processes and plans are working to their successful maximum. If, on the other hand, there is an increase in the trending line, this could be a predictor of problems down the road. An increasing trend line may indicate that additional training is required or that procedures need to be reviewed and amended. The method by which a company monitors and trends its data is an individual decision.

Summary

Monitoring the production environment for bacteria and other pathogens constitutes a proactive approach to guaranteeing the production of a safe product. In the past, these programs were not thought to be required in the baking and milling industry. However, as more recalls and adverse events occur, it is becoming apparent that the industry must reevaluate its practices. Establishing and maintaining an environmental testing plan requires not only the analysis and monitoring of sites within and around the facility. It also requires that management believe in the process. In fact, it requires total buy-in from management at all levels. When management champions a process, programs begin to take on a life of their own, and they become a part of the corporate culture.

References

1. Centers for Disease Control. Foodborne disease outbreak surveillance. Published online at www.cdc.gov. Centers for Disease Control, Atlanta, GA, 2011.
2. Eglezos, S. Microbiological quality of wheat grain and flour from two mills in Queensland. Aus. J. Food Protect. 73:1533, 2010.
3. Evancho, G. M., Sveum, W. H., Moberg, L. J., and Frank, J. F. Microbiological monitoring of the food processing environment. Page 25 in: *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed. F. P. Downes and K. Ito, eds. American Public Health Association, Washington, DC, 2001.
4. Grocery Manufacturers Association. Capturing recall costs: Measuring and recovering the losses. Published online at www.gmaonline.org/file-manager/images/gmapublications/Capturing_Recall_Costs_GMA_Whitepaper_FINAL.pdf. Grocery Manufacturers Association, Washington, DC, 2011.
5. Gupta, R. S., Springston, M. R., Warrier, B. S., Rajesh, K., Pongracic, J., and Holl, J. L. The prevalence, severity, and distribution of childhood food allergy in the United States. Pediatrics 128(1):e9, 2011.
6. Home Grown Cereals Authority. HACCP explained: A supplement to the HGCA Grain storage guide. Published online at https://cereals.ahdb.org.uk/media/178273/g52-haccp-guide.pdf. Home Grown Cereals Authority, London, 2011.
7. Mayberry, T. A look back at 2016 food recalls. Food Safety Mag. February 7, 2017.
8. National Academies of Sciences, Engineering, and Medicine. *Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management, and Public Policy*. National Academies Press, Washington, DC, 2017.
9. Neil, K. P., Biggerstaff, G., MacDonald, J. K., Trees, E., Medus, C., Musser, K. A., Stroika, S. G., Zink, D., and Sotir, M. J. A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: Multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough—United States, 2009. Clin. Infect. Dis. 54:511, 2012.
10. Sosland, J. Recalls prompt milling industry to tighten wheat sourcing efforts. Food Bus. News, June 21, 2016.
11. U.S. Food and Drug Administration. Raw dough's a raw deal and could make you sick. Published online at www.fda.gov/ForConsumers/ConsumerUpdates/ucm508450.htm. FDA, Silver Spring, MD, 2016.
12. U.S. Food and Drug Administration. 2017 Food allergies: What you need to know. Published online at www.fda.gov/Food/ResourcesForYou/Consumers/ucm079311.htm. FDA, Silver Spring, MD, 2017.

The Value of SPC and PC Analytics in Food Processing

Peter Guilfoyle[1]
Northwest Analytics, Inc., Portland, OR, U.S.A.

Analytics is seemingly everywhere these days, especially as it relates to Industry 4.0, IoT (the internet of things), and the other technical buzzwords currently in vogue. Analytics is the core of how manufacturing data is transformed into information that drives decisions that impact productivity and food quality.

Of the types of analytics and analytics approaches that are available, statistical process control (SPC) and process capability (PC) analytics seem to receive the least amount of attention in the industry press and at industry events. SPC and PC have been around for several decades and do not enjoy the same cachet these days as the analytics approaches associated with big data. However, they also do not present the same barriers to success as other analytics approaches—namely, high costs, limited usability, and required data scientist–level analytics expertise. SPC and PC may not be flashy, but they provide a foundation for other analytics approaches and have earned their reputation as the workhorse of manufacturing analytics by delivering easily understood and cost-effective analytics for every level of food manufacturing.

SPC and PC are a necessary part of modern food processing. The software selected to satisfy basic food processing needs will determine whether SPC and PC are awkward and intrusive or smoothly operate as part of the overall process.

Software Selection Considerations

The successful implementation of SPC and PC begins with the selection of the tools and methods best suited to a company's quality goals. SPC and PC must not only connect to the data captured and held in a laboratory information management system (LIMS) to produce control charts, but also provide additional capabilities and analysis to production data systems (e.g., manufacturing execution systems [MES], quality systems, and process historians).

Numerous SPC/PC software packages are readily available. Most, however, were created for discrete manufacturing processes such as auto parts machining and, consequently, are limited in their applicability for other manufacturing processes. Food processors evaluating SPC/PC software need to be aware of several key components when selecting SPC/PC software:

- **Usability.** Can the software handle both process and laboratory data? Is the software able to present critical process data in a way that is effective for each user's role in the organization? Will one package meet the needs of all users? Can routine charting tasks be automated to reduce training time? Is unattended operation possible?
- **Data Types.** Can descriptive, measurement, and defect data be viewed in and analyzed from the same data file?
- **Flexibility.** Can charts be configured to precisely meet internal quality control needs and still meet customer and regulatory reporting requirements?
- **Data Access.** Can the software easily analyze data from all sources through industry-standard connectivity technologies? Can it accept instrument data? Can it share or exchange data with corporate or plantwide information systems? Can it easily deliver analytical results and reports to users throughout the organization?
- **In-house Analytics Expertise.** Are the analytics results easily understood by all users, from operators to executives, or is a data scientist required to analyze, interpret, and present meaningful results?

Driving Quality Control and Process Analysis

SPC and PC is used by food companies of all types and sizes, from small independent to major multinational companies. Applications range from internal quality control and process improvement to vendor certification and regulatory compliance.

As an example, in the case of one large food processing company, SPC and PC are used to monitor quality in the dill pickle packing line. Finished jars of pickles are pulled from the production line for routine data collection and charting. Samples are then drained, weighed, and inspected for defects. The description variables entered into a data set are used to label routine SPC charts and provide easy reference points for later process improvement studies:

Description Variables

- ***Date:*** Sampling date
- ***Stock:*** Pickle size being packed
- ***Lot:*** Lot code

Measurement Variables

- ***Weight:*** Drained weight of pickles

Defects and Counts

- ***Count:*** Number of pickles per jar
- ***Nub:*** Nub, crook, misshapen
- ***Broken:*** Broken, mechanical damage
- ***Rot:*** Rot, shriveled
- ***Dirty:*** Dirty, scarred
- ***Size:*** Incorrect sizing
- ***Hollow:*** Hollow

Note, all of this information is collected at the same time and entered into a single data set (Fig. 1), enabling charting to be launched directly from the data entry screen.

The full value of SPC and PC became apparent when the company was considering alternative solutions to a potential supply shortage. The company's standard operating procedure (SOP) for its 46 oz (filled weight) jar required a 3A pickle size ($1^1/_8$ to $1^1/_4$ in.). When supplies ran low, the limitation forced the company to choose between buying more expensive 3A pickles on the open market or changing the SOP to allow use of another stock pickle size, 3B ($1^1/_4$ to $1^3/_8$ in.). If the weight specification could be maintained, the alternate size would be acceptable. To find out, the company conducted a trial run using 3B

[1] E-mail: pguilfoyle@nwasoft.com

https://doi.org/10.1094/CFW-63-1-0035

pickles. All of the data needed to analyze 3A and 3B stock could be entered into a single data set.

The apparent success or failure of using 3B stock could be indicated in a PC histogram—a chart showing the distribution of pickle weights and their relationship to specifications. However, the weights first needed to be analyzed using a control chart to verify the packing process was within statistical control. As illustrated in Figure 2, SPC software allows users to display histograms and control charts simultaneously. The x-bar and range chart shows the packing process was within statistical control for both stocks, thus validating the PC study.

Labeling regulations allow up to 20% variation from the target. The Cpk index, a commonly used numeric representation of the capability of a process, showed both stock sizes met production requirements. However, PC does not always reveal the whole story. Another view of the data suggested further analysis was in order.

Because the most commonly used statistical charting techniques can all be launched from a tool bar above the data entry screen, users are able to examine their processes from a variety of perspectives. A routine review of defects using Pareto analysis revealed that the defect "broken" increased during the test run (Fig. 3).

Row	DATE 1----(D)	STOCK 2----(A)	LOT 3----(A)	WEIGHT 4----(I)	COUNT 5----(Z)	NUBS 6----(P)	BROKEN 7----(P)	ROT 8----(P)	DIRTY 9----(P)	SIZE 10----(P)	HOLLOW 11----(P)	TOTAL 12----(P)
1	10/19/95	3A	FD292RA	31	57	0	1	0	0	1	0	1
2	10/19/95	3A	FD292RA	29.5	51	0	2	0	0	1	0	2
3	10/19/95	3A	FD292RA	29	48	0	0	1	0	0	0	1
4	10/19/95	3A	FD292RA	29	48	1	1	0	0	0	0	2
5	10/19/95	3A	FD292RA	30	51	0	1	0	0	1	1	2
6	10/20/95	3B	FD334RB	29	38	1	3	0	0	4	0	7
7	10/20/95	3B	FD334RB	27	36	0	2	0	0	0	0	1
8	10/20/95	3B	FD334RB	29	39	1	2	0	0	0	1	2
9	10/20/95	3B	FD334RB	29.5	42	0	2	0	0	0	0	1
10	10/20/95	3B	FD334RB	28.5	36	1	1	0	0	1	0	2
11	10/20/95	3B	FD334RB	29.5	37	0	5	0	0	1	1	1
12	10/20/95	3B	FD334RB	28	36	1	3	0	0	0	1	2
13	10/20/95	3B	FD334RB	30	40	0	4	0	0	2	0	2
14	10/20/95	3B	FD334RB	28	33	1	2	0	0	0	1	1
15	10/20/95	3B	FD334RB	28	39	0	2	0	0	1	0	1
16	10/20/95	3B	FD334RB	29.5	35	1	1	0	0	3	1	2
17	10/20/95	3B	FD334RB	26	33	0	2	0	0	0	0	2
18	10/20/95	3B	FD334RB	30	37	1	3	1	0	0	2	1
19	10/20/95	3B	FD334RB	27	38	0	3	0	0	2	1	0

Fig. 1. Statistical process control (SPC) and process capability (PC) analytics data collection and charting for monitoring quality. Information is collected at one time and entered into a single data set.

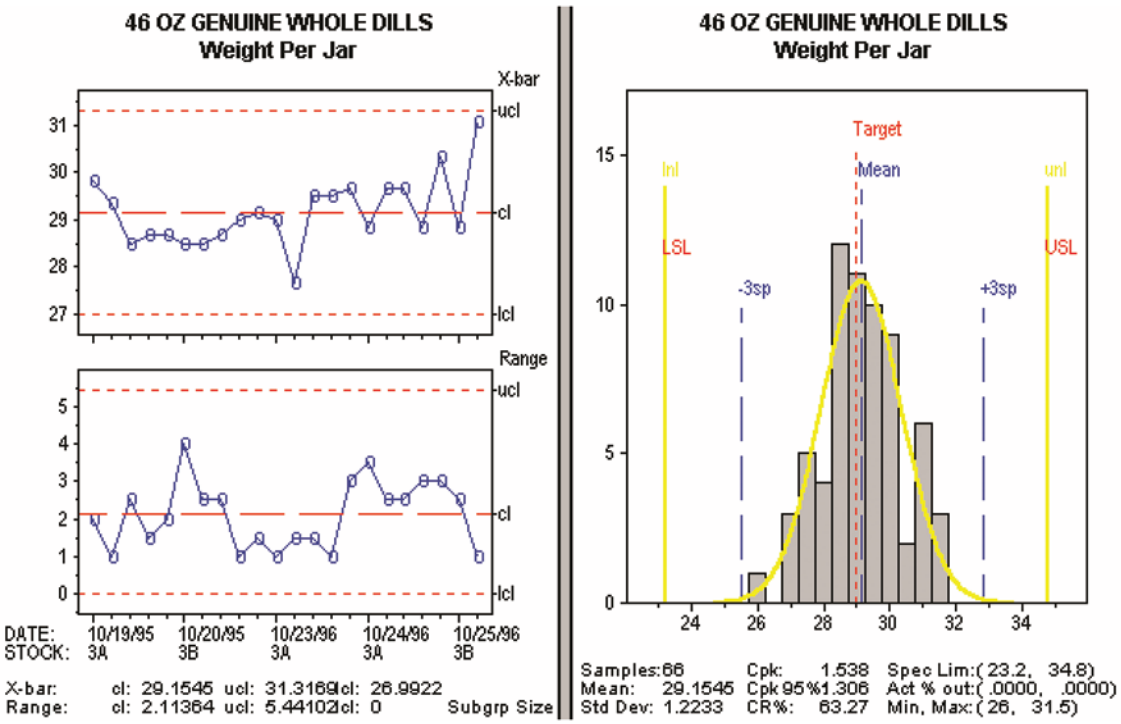


Fig. 2. Left: process capability (PC) histogram chart showing distribution of pickle weights and their relationship to specifications. Right: control chart used to analyze weights and verify packing process is within statistical control.

For further analysis, the lab produced a p-chart (percent defective SPC chart) (Fig. 4) and found two points above the upper control limit. Pattern rule violations, shown on the chart by asterisks, provided further warning. The operator then clicked on each suspect data point to "drill down" for more information. The resulting dialogue boxes pointed to the 3B stock. Separate p-charts for each stock type quickly confirmed 3B stock as the source of the unacceptable levels of breakage (Fig. 5).

Further study revealed that 3B pickles frequently had to be forced into the jar, causing breakage. However, the p-chart showed the process itself to be within statistical control, because breakage was a natural part of the process. The processors concluded that although the 3B stock could be used to remain in label weight compliance, breakage might be excessive.

From Data to Information

Using the information provided by the analyzed data, the processor drew several conclusions:

- They could maintain statistical control and process capability while using either or both pickle stocks.
- Excessive broken pickles resulted when using larger 3B stock.
- SPC analysis of broken 3B stock pickles showed the process was within perfect statistical control; this meant the higher breakage rate was characteristic of the process and was not attributable to any specific cause.

With a clear understanding of their packing process, the company recognized they had three distinct choices:

- Live with the breakage and risk customer displeasure.
- Continue to study the process to determine whether the process could be modified to reduce 3B breakage in a cost-effective manner.
- Meet shortages by continuing to purchase 3A stock on the open market.

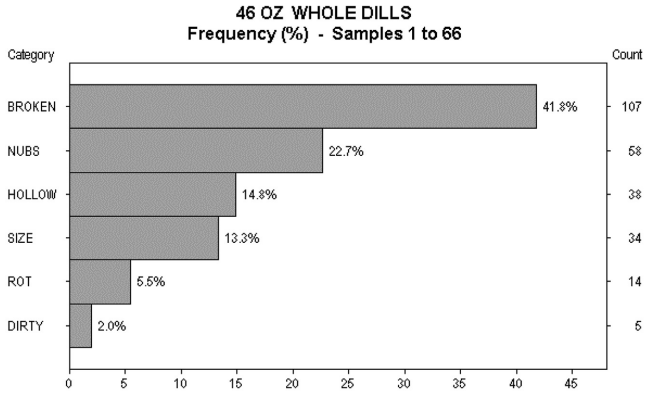


Fig. 3. Pareto analysis used to review defects shows the defect "broken" increased during the test run.

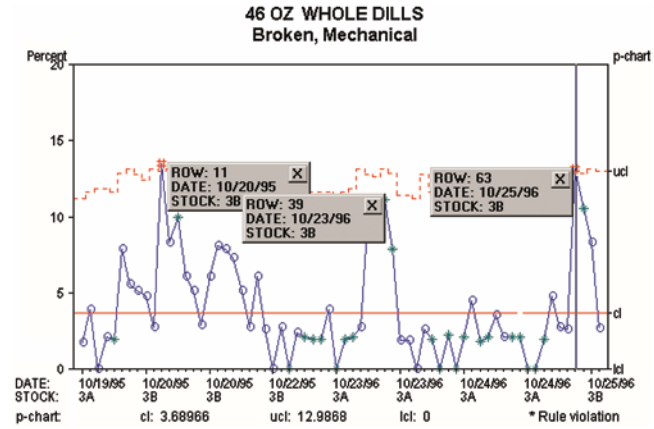


Fig. 4. Analysis using a p-chart (percent defective statistical process control [SPC] chart) shows two points above the upper control limit.

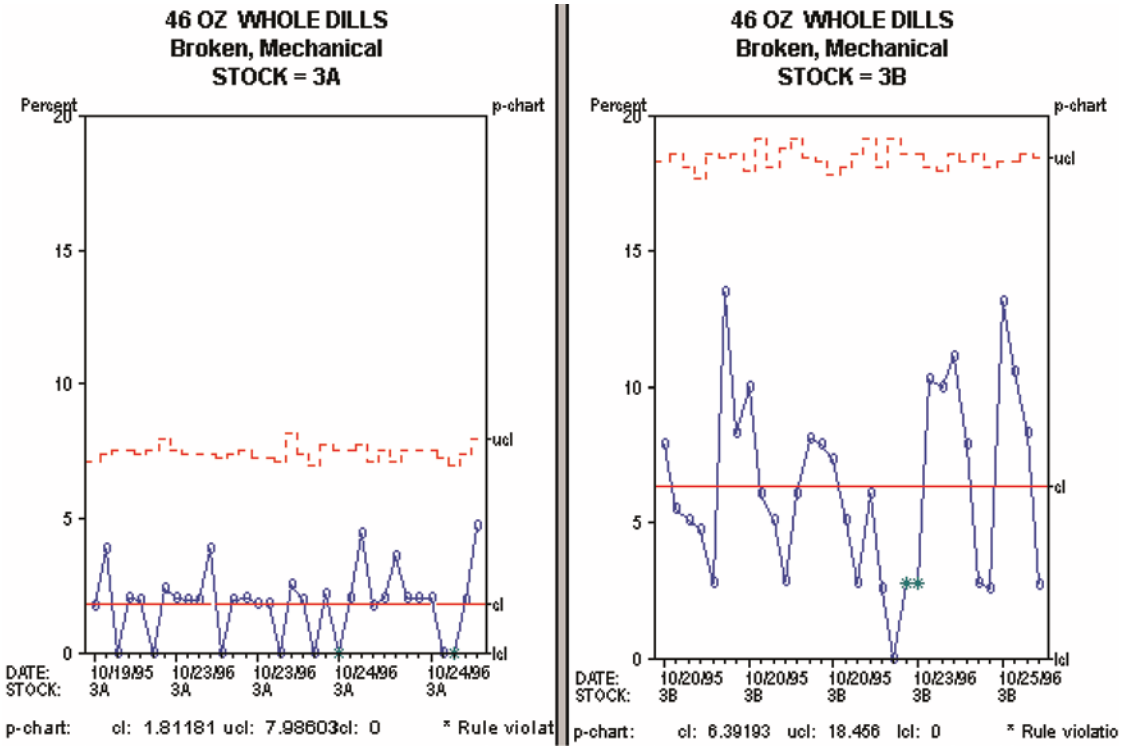


Fig. 5. Separate p-charts for each stock type confirm 3B stock as the source of unacceptable levels of breakage.

SPC and PC delivered valuable information to all levels of the organization, helping both managers and production staff to understand their processes and enabling them to make confident decisions that impacted both product quality and costs.

Application-Specific Charts

Two special-purpose charts that apply to the food processing industry are cumulative sum (CUSUM) and median/individual measurements (M/I) control charts.

CUSUM and Regulatory Requirements. CUSUM produces a control chart based on the accumulated deviations from a target (Fig. 6). It is particularly well suited to examining processes that may "drift," and it can be tuned to different levels of sensitivity. It is especially useful in regulated industries such as meat and poultry processing. For example, as the U.S. Department of Agriculture (USDA) adds CUSUM methods to inspection procedures, such as the protein fat-free (PFF) regulations, CUSUM charting routines can be adjusted to match new requirements.

M/I and Fill Weight Control. The M/I chart solves the SPC challenges presented by "family" processes such as multihead filling machines in which multiple individual processes (separate fill heads) are combined within a larger process (Fig. 7).

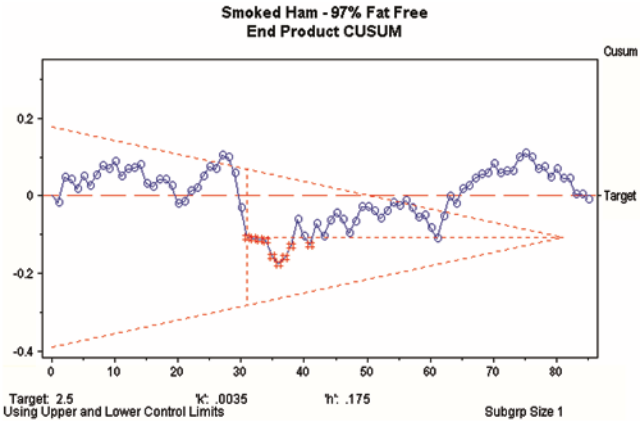


Fig. 6. CUSUM (cumulative sum) produces a control chart based on accumulated deviations from a target.

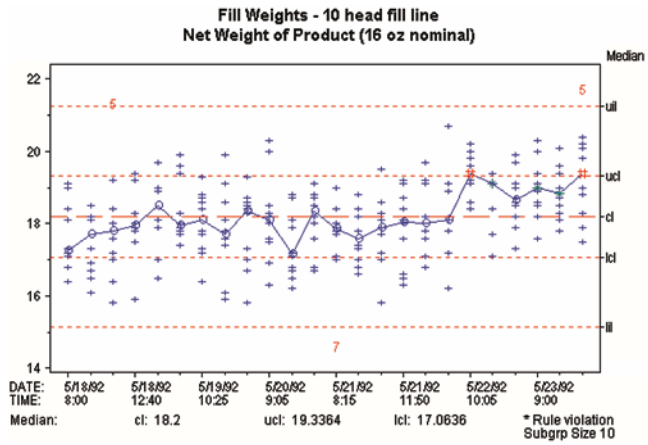


Fig. 7. Median/individual measurements (M/I) charts used for "family" processes such as multihead filling machines in which multiple individual processes (separate fill heads) are combined within a larger process, allowing users to simultaneously monitor the overall process and behavior of individual fill heads.

With conventional control charts, it is virtually impossible to separate the behavior of the individual heads from the global process. To completely monitor a 36 head filling machine, 37 charts would be needed—1 per head and 1 for the overall process.

M/I charts allow users to simultaneously monitor the overall process and the behavior of the individual fill heads. The resulting chart is easy to read and interpret and is far quicker to create than the alternative. (An in-depth examination of M/I charting capabilities and applications can be found in *Median/Individual Measurements Control Charting*, by Perry Holst and John Vanderveen, developers of the M/I technique.)

SPC and PC Provide a Foundation for Analytics Success

SPC and PC may not be the latest-and-greatest analytics approaches in food manufacturing, but they deliver what other analytics approaches cannot: a defined path to quickly improve quality, reduce variation, monitor production or process changes, and ultimately increase productivity. The successful selection and implementation of SPC/PC software in a food processing environment does not require the level of commitment that MES or enterprise resource planning does (in terms of time, personnel, expertise, and financial resources) to deliver actionable results and quick returns on investment. In addition, once a solid analytics foundation is built with SPC and PC the chances of attaining the additional benefits promised by more esoteric big-data analytics approaches increase as well.

Guidance for Selecting an Outside Laboratory

Richard F. Stier[1]
Consulting Food Scientists, Sonoma, CA, U.S.A.

Most food, beverage, and ingredient processors, even multinational operations, will utilize an outside or third-party laboratory on occasion. There are some operations that utilize third-party laboratories for almost all quality, safety, and verification activities because they either do not have the resources to do such work in-house or they have decided they do not want to do so. Work that may done by outside laboratories includes microbiology, chemistry, microanalysis, product and process development, and much more. Cereal grain processors and handlers may require special types of testing, so finding and developing a working relationship with a laboratory may present additional challenges.

One of the elements that make up the U.S. Food Safety Modernization Act (FSMA) of 2010 is supplier verification. In fact, one of the regulations enacted to ensure proper enforcement of the act is the Foreign Supplier Verification Program. In addition, the Preventive Controls Regulation (21 CFR, Part 117, Subpart G) specifically addresses supply chain programs (3). The expectation is that food, beverage, and ingredient processors should develop, document, implement, and maintain programs to identify, select, and maintain working relationships with food, ingredient, and packaging material suppliers. One of the primary elements of this program is conducting risk assessments on all purchased materials, which includes assessing risks associated with the country of origin and the supplier itself—the assessment should be much more than an assessment of the purchased material. As an example, the melamine issues associated with dairy ingredients originating from China a few years ago made purchasers leery about buying these ingredients from China. The risk is now deemed to be higher than if one sources such products from, for example, New Zealand or the United States.

In fact, consideration should be given to applying the same rigid program required for establishing a supply chain program to the selection process for a third-party laboratory. Like a food, raw materials ingredient, or packaging supplier, contract laboratories are partners for progress and for ensuring the safety, quality, and wholesomeness of what a company manufactures. Therefore, selecting a contract laboratory from the Internet, phone book, or out of a hat is not a good idea. A program should developed that documents precisely how a laboratory should be selected and how that operation will be evaluated on a regular basis. It is surprising how few companies have such programs, and even those that have established selection criteria often don't include an evaluation process in the equation.

Evaluating and Approving Laboratories

So, what might a laboratory approval program look like? As noted earlier, such a program should be properly documented, which includes assigning responsibility for managing the program. A generic version of what such a program might look like is provided in Figure 1.

[1] E-mail: Rickstier4@aol.com

https://doi.org/10.1094/CFW-63-1-0039

Although generic, the form does provide the general structure for what a company might do when selecting and evaluating a potential laboratory partner. One extremely important element is noted under the Procedures: "Visit the contract laboratories and prepare a summary report." Get out and visit prospective laboratories. Doing an audit of a potential laboratory partner is absolutely essential for any processor, and it should not be a huge burden to the company. Processors may utilize hundreds or even thousands of vendors when it comes to ingredients, raw materials, and packaging. With these operations, relying on third-party audits is part of doing business. How many laboratories will a processor utilize—one, two, three, maybe more? There is no excuse for not getting out to see and learn about specific laboratory operations. If your company is small and lacks the necessary technical expertise, a consultant or technical advisor can be engaged to accompany you on laboratory visits. The laboratory will want to add you as a client, and a technical advisor can help you to better understand what a laboratory is offering and whether it would be a good fit.

Criteria for Selecting a Laboratory

There is one thing a processor must determine before they visit a potential contract laboratory—what criteria are important to the company when it comes to laboratory support. The following is a list of potential criteria a processor might use to select and evaluate a laboratory partner (2). There may be more or fewer criteria depending on the processor and what is needed.

1) The laboratory has the ability to do the work needed using official and recognized methods.
2) The laboratory is ISO/IEC 17025 certified (1).
3) The work can be started as soon as the samples arrive, and results will be available as the testing protocol dictates.
4) The laboratory can provide references with whom the company can speak.
5) The laboratory will pick up samples.
6) The laboratory allows audits.
7) The company has trained employees who participate in check sample programs.
8) The laboratory has experience with the company's product matrix or matrices that are very similar.
9) The laboratory will not only provide results but will interpret results if needed.
10) The laboratory demonstrates a sense of urgency with testing the company's samples.
11) The laboratory has the ability to suggest tests that are possibly more appropriate, in addition to or in place of the testing the company is requesting.
12) Cost of services.

What Are the Laboratory's Capabilities? The processor must determine what kind of testing they want done by the contract laboratory before they begin their search for a partner. Do they want to do water testing only? If so, the goal will be to find a

certified water testing facility. Are they going to ask the laboratory to develop certificates of analysis that can be shared with their clients? If so, the goal is to not only find a facility that has some familiarity with their products or ones that are similar, but a facility that can develop the necessary documentation. Is the processor manufacturing a ready-to-eat (RTE) pasta product and needs to develop and implement an environmental monitoring program per the requirements of the Preventive Controls Regulation (21 CFR Part 117) (3)? If this is the case, they should look for a microbiological laboratory that can help build the program, which may include training staff to collect and ship samples for testing. This would be a major undertaking and is topic that requires an article all by itself.

Another FSMA requirement is that processors must validate their processes to ensure they are adequate to eliminate food pathogens. If this is a concern in the operation, the goal would be to find a facility that understands how to properly conduct process validation trials that will not only demonstrate that the products are safe but will stand up to scrutiny by regulators and customers. As an example, a supplier of specialty flours or seeds may want to offer their customers the option of purchasing heat-treated products. As part of installing such a system, it would be imperative that validation studies be conducted on the processing equipment.

The needs of a processor may be much less complex, however. They may only require only a few simple tests to be done. Before any search for a laboratory partner is initiated, it is imperative that the operator define what is needed.

Verify ISO/IEC 17025 Certification. ISO/IEC 17025 certification (1) is becoming more and more prevalent throughout the food industry. One of the driving forces is the expectation in the Global Food Safety Initiative (GFSI) food safety audit schemes that if a food processor is using any kind of laboratory support services, they will select an ISO-certified laboratory. What does this mean? The laboratory must have established, documented procedures for managing all aspects of laboratory operations,

SUBJECT: Selection and approval of contract laboratories

OBJECTIVE: To ensure that any contract laboratory needed by the company meets criteria established by the company for service, quality, and potential performance

PROCEDURE:

- Establish what the company needs from outside laboratories. Support services may include, but need not be limited to,
  a) Microbiology
  b) Chemistry
  c) Product development
  d) Nutritional analyses
  e) Environmental monitoring
- Conduct a search to select potential contract laboratories.
- Submit a contract laboratory approval form to the laboratories that have been selected for evaluation. Information on the form may include, but need not be limited to,
  a) Contact information
  b) Capabilities
  c) Certifications – ISO 17025, water testing, etc.
  d) Response times
  e) References
  f) Pick-up program
  g) Audits/visits by customers
  h) Check sample program
  i) Data interpretation
  j) Experience with the company's products or type of products
  k) Ability to offer other options
  l) Pricing
- Review the contract laboratory approval forms.
- Visit the contract laboratories and prepare a summary report.
- Determine which laboratory or laboratories with which to work.

RECORDS (Append All Forms to Procedure):

- Contract laboratory approval form
- Results of laboratory visit or audit
- Contracts with laboratory

RESPONSIBILITY(IES): Purchasing manager

VERIFICATION ACTIVITIES:

- All laboratories providing analytical services must participate in a check sample program and share results with the company.
- Once a year, laboratory performance will be reviewed with the laboratory and company management.

Fig. 1. Generic laboratory approval program.

from receipt of samples to testing to reporting. For many, if a laboratory has achieved an ISO/IEC 17025 certification that is more than enough to approve the operation. However, is that really adequate to meet the needs of a food or ingredient processor? This is one reason why it is imperative to develop guidelines for selecting and approving a contract laboratory, especially for smaller processors. Smaller processors often rely on contract laboratories for technical expertise, so the requirement that the laboratory understand and have experience with their products, or similar ones, should be a prime selection criteria. This topic will be addressed later.

According to Jay McBurney, a consultant who works with both processors and laboratory operations and conducts audits for both the GFSI food safety audit schemes and ISO/IEC 17025 certification (1), there are several areas that present challenges when it comes to obtaining and maintaining certification. These areas are proficiency testing, traceability, and proper documentation of these and other areas. When selecting a laboratory, treat the laboratory as one would an ingredient supplier—ask for the audit to see if there were any issues noted. Remember, ISO certification does not mean that all the tests being conducted in the laboratory have been certified. Have the laboratory define the scope of the testing procedures that have been ISO certified. At a minimum, pathogen analysis should be included in the ISO/IEC 17025 (1) scope for the laboratory's certification. Routine tests may or may not be included in the scope of certification. Laboratories may exclude routine tests, such as total counts, yeast, and mold, from the scope of testing due to the requirement and cost for including those tests in the required third-party check sample program.

Work Is Started Promptly. When selecting a laboratory, find out how long it will take them to complete the different analyses that the company will need them to conduct. As part of this question, ask about samples collected at the end of a week. Will they be cultured immediately or will they sit in the refrigerator over the weekend? Allowing samples to sit may be okay for some analyses, but it would certainly be a mistake, for example, to allow an edible oil sample submitted for peroxide analysis to sit. Ideally the list of tests performed by the laboratory would also provide information on turnover times. Along these lines, require that the laboratory provide information on any tests that are not done in-house and are contracted out to another laboratory, because this will affect turnover time. In addition, if samples are contracted out, there is a high probability that the laboratory does not have the knowledge to interpret results.

Finally, if the laboratory does conduct rapid tests, verify that they are using tests that have been validated against an official method. One client with whom I have worked sent seed samples to a new laboratory. They had never been out-of-specification for aerobic plate count until they began working with the new operation. In an effort to figure out what happened, they sent duplicate samples to the new laboratory plus three other testing laboratories. The sample submitted to the new facility was again out-of-specification, while those submitted to the other laboratories were all within established specifications. It turned out that the new operation was using a rapid test that should not have been used for seeds.

Laboratory Will Provide References. Before selecting a laboratory, make sure the potential contractor will provide references, preferably from operations that produce similar products and are similar in size to your operation. References should include not only names of companies, but potential contacts and contact information as well. This may be even more important if the company is small. What difference does this make? Every client should be treated the same, whether their operation is small or large. Ask references whether smaller companies feel they may have been ignored in favor of larger companies.

Laboratory Will Pick Up Samples. Many laboratories provide pickup services, i.e., they will make arrangements to pick up samples at client facilities. The contract laboratory will coordinate with their customers to arrange schedules for regular pickup of samples. As part of this service, the laboratory will provide information on how to collect, label, and package samples. Of course, sample collection, labeling, and packaging should be included in the documented quality procedures for every processor. This is a service and a convenience, however, not a requirement.

Pickup services are something that processors should consider carefully. If samples are not picked up, it is up to the processor to pack and ship samples. Shipping entails ensuring that the sample remains in good condition (e.g., using blue ice, insulated shipping containers, etc.). Shipping adds costs and takes work to do properly. Weigh the pros and cons as part of the selection process.

Laboratory Will Allow Audits. The importance of visiting the contract laboratory as part of the selection process was alluded to earlier. As a food or ingredient processor, treat the laboratory as you would an ingredient or packaging supplier. Although food processors, especially large operations, may have hundreds of vendors that provide ingredients or packaging, most only utilize a handful of laboratories. So, visit each potential contract laboratory. Meet the account managers, the technical personnel, and make an effort to understand the operation. The laboratory should become your partner in assuring the safety and quality of your products, so make sure you are not only comfortable with the operation but feel that they can become a viable partner. This is also a great time to determine whether the laboratory has experience with your products and the type of processes that you employ.

Laboratory Has Trained Employees and Participates in a Check Sample Program. One of the basic elements for ensuring competence for most laboratories is a check sample program. As part of the evaluation of a facility, ask whether they participate in such a program. These are designed to evaluate the technicians who do the work using samples prepared by a third party. Third parties may include associations such as AACC International, allied industry groups, or other laboratories. Participation in a third-party check sample program is also a requirement for being ISO certified. In addition, many global food processors run their own check sample programs, both with the laboratories with which they work and also with their suppliers.

Laboratory Knows Your Products and Processes and Will Interpret Results. These two points go hand-in-hand. Interpretation of results is something that was alluded to earlier. Large companies often have the expertise in-house to look at results from a contract laboratory and make sense of a report. This is not always the case for small- to medium-sized processors. In fact, many small processors rely on their contract laboratory to let them know if results indicate there is a problem or issue.

Data interpretation has been an issue in recent years. Some laboratories do not provide interpretation because they deem it to be consulting, whereas others feel that if they provide an interpretation of results they are at risk if there are problems. In

other words, they would be liable if there was a legal issue. There are also some who believe that under the ISO/IEC 17025 standard, they cannot interpret data. This is not entirely true, however, according to McBurney. Under ISO/IEC 17025, clause 5.10, laboratories can offer opinion and interpretation if they are qualified to do so (1). Referring back to capabilities, be sure that your laboratory selection criteria includes an understanding of your products and processes. If the laboratory knows your products, then they may be qualified and comfortable enough to evaluate and interpret data.

Laboratory Has a Sense of Urgency. Be sure that the contract laboratory has a sense of urgency concerning testing your samples. It has already been mentioned that it is not a good idea to pick up samples on a Friday and leave them sitting over the weekend. Good laboratories will assign staff, especially people in their microbiology group, to work weekends and keep samples moving through the laboratory. There may be an extra charge for weekend work, especially if the laboratory is working on a special project.

Laboratory Has Capability to Suggest Other Tests. A processor's relationship with its contract laboratory should be a partnership that benefits both parties. A good laboratory will work with its customers to not only perform the requested work, but will offer advice when it comes to testing. In addition, the contract laboratory should provide guidance on other tests that could be performed. This kind of support is especially valuable for small companies or those without in-house technical expertise.

One of the hottest issues today, thanks to FSMA, is environmental monitoring. Food processors, especially those manufacturing RTE products, need to evaluate what they are doing and set up such a program. Many laboratories will assist with the development of an environmental monitoring program, which would include determining sampling sites and collection and analysis of samples. An environmental monitoring program should focus on what is needed to meet both the letter and the spirit of the regulation.

Cost of Services. There is an old adage, "You get what you pay for." This applies across many industries, especially the food industry. Selection of a contract laboratory should never be based solely on price. When evaluating laboratories, keep track of how each operator prices goods and services, but make your selection based on your established criteria.

Are There Things to Worry About?

When looking at contract laboratories, there are several issues that could potentially create problems. Some have been alluded to earlier. The laboratory that you ultimately select should ideally do all the work in-house. Verify this up-front, because testing that is subcontracted may still be reported on the company's letterhead. If your main contractor cannot do the testing, ask for recommendations as to who can do the testing and contact the source directly.

The use of the wrong test method has also been mentioned. The test methods a contract laboratory utilizes should be official methods approved by groups such as the AOAC International, AACC International, the American Oil Chemists Society (AOCS), the International Union of Pure and Applied Chemistry (IUPAC), or others. Microbiological testing methods should be drawn from the *Bacteriological Analytical Manual* (BAM). If the laboratory does use rapid methods, ask them whether these methods have been validated against official methods.

This is not to say that there is anything wrong with a rapid method or quick test, but you do need to be sure they are accurate. The best example that I have heard of was a situation in the Southern Hemisphere a few years ago. A pet food manufacturer was involved in a situation where their products were making pets ill. Tests of the products revealed that they had high levels of aflatoxin. The company used a rapid test to screen every lot of corn they received prior to use. Unfortunately, the rapid test did not work with corn. The test produced false negatives, and it turned out that the test itself had never been validated against the official method.

Summary

The bottom line is that selecting a contract laboratory should be an organized process. Any company that utilizes, or is considering utilizing, a third-party laboratory should develop, document, and implement procedures to do this (Fig. 1). Third-party audits are a fact of life in modern food processing. The expectation is that food processors, both large and small, will be able to pass a third-party audit; for most, this would be a GFSI, Safe Quality Foods (SQF), British Retail Consortium (BRC), Food Safety Systems Certification 22000 (FSSC 22000), or International Featured Standard (IFS) food safety audit scheme. Selection of vendors, including laboratories, is included in these audit schemes. Very small companies might maintain, "The Food Safety Modernization Act of 2010 (FSMA) excludes my company." However, if your customers want food safety programs that are FSMA equivalent or a GFSI audit scheme, you will need to develop and maintain such a program or risk losing customers. Laboratory selection is part of this equation.

Acknowledgments

I thank Jay McBurney for his insights on the ISO/IEC 17025 standard and for reviewing the article.

References

1. International Organization for Standardization. ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories. ISO, Geneva, 1999.
2. Stier, R. F. Food testing: Selecting a contract laboratory. Food Safety Mag. 22:3, 2016.
3. U.S. Food and Drug Administration and Department of Health and Human Services. Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food. 21 CFR, Part 117. *Code of Federal Regulations*. Published online at www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=117&showFR=1. Government Printing Office, Washington, DC, 2015.

Spotlight on Jon Faubion

AACC International members each have their own story, and we want to highlight all of their amazing accomplishments. "Spotlights" is a series of individual and institutional member interviews capturing the unique stories of our many volunteers and their journeys with AACCI.

Jon Faubion
Kansas State University
Member for 34 years

Q: What is your current position and what type of work do you do?

A: I am a professor of cereal science in the Department of Grain Science & Industry at Kansas State University. I'm JYB (just your basic) academic—I teach, direct graduate students, and write.

Q: When and how did you first decide you wanted to work in cereal grain science?

A: It's a long story, so I'll apologize for it up front. I was about to finish a Ph.D. degree in developmental biology when I had a change of heart and went on a search for a degree that would allow me to do interesting science, hopefully to teach, and that had employment possibilities. As luck would have it, I had gone to high school with Dr. Bill Hoover's son, Mike, so I knew Dr. Hoover. I made an appointment to talk with him, and my interest was kindled. He introduced me to Carl Hoseney and Paul Seib, and I was completely hooked. Carl kindly took me on as a doctoral student, and I haven't regretted my decision once in approximately 38 years. (I told you it was a long story.)

Q: When did you join AACCI and why?

A: I joined AACCI as a graduate student, at Carl's strong suggestion. He stressed the opportunities for networking, authorship, technical education, and volunteering (giving back).

Q: Why did you decide to volunteer with AACCI? How did you get involved, and what has been most rewarding about this experience?

A: My first job after graduate school was as a member of the Texas A&M faculty, working with Lloyd Rooney. Lloyd was all about the value of volunteering and was active with AACCI. I followed his example, made my interest known to Ray Tarleton, a truly amazing person, and he introduced me to committee chairs, etc., etc. Over the years, I have been fortunate to be able to do a fair bit of volunteer service for AACCI—this gig as CFW editor-in-chief is an example. Volunteering with AACCI has given me the opportunity to use my modest talents for an exceedingly good cause and to work with a tremendous group of people. What's not rewarding about that?

Q: This issue of *CFW* explores current opportunities and challenges in analytical practices. Have you had any experiences with analytical practices, and how are they affecting your work?

A: During the time I was on the AACCI headquarters staff, my responsibilities included oversight of the Check Sample Program and the Technical Committee, including the Approved Methods Committee. A significant part of those responsibilities involved analytical methods, their use, validation, etc.

Q: What's next for you?

A: To be honest, what's next is retirement. That will happen at the end of this year. It is time to give some other young scientist the opportunity to have the wonderful career I have enjoyed. That said, I plan on staying active in AACCI as long as AACCI is interested in having me.

A Tribute to Raymond J. Tarleton, AACCI and APS Executive Vice President Emeritus

Steven C. Nelson
Ray's Mentee

Raymond J. Tarleton was the right person, at the right time, with the right skills to guide AACC International (AACCI) and The American Phytopathological Society (APS) through a period of amazing advancements. What started with happenstance, culminated in a long and storied career. When volunteer officers of the two small scientific organizations realized they needed a minimal workforce to accomplish the work of their organizations, they found a graduate student who was completing studies in both science and journalism. Over the next 40 years Ray demonstrated organizational and leadership skills that made the partnership between AACCI and APS a success and provided their members with a vast array of services.

Raymond J. Tarleton passed away in late October 2017 in his favorite locale, Tucson, AZ, after 26 years of retirement from AACCI and APS. Ray's career was devoted to these organizations, and while much of their history has been well documented, most are unaware of Ray's tremendous influence on their success.

First Full-Time Employee

As AACCI Annual Meetings grew bigger and more complex and its publications increased in size, the motivation to hire a staff grew. The AACCI journal *Cereal Chemistry* and a cardboard box of records were located at the University of Minnesota when the volunteer journal editor started paying Ray for part-time technical editing. Ray was hired as the first full-time employee of AACCI in 1950. He continued with his classes and finished his master's degree in 1952. In 1967 APS volunteers had reached a point where they also needed help, and they heard about Ray's work for AACCI. Following much discussion, an agreement was reached to create a joint headquarters operation.

The plan was unique—to form a nonprofit cooperative owned jointly by AACCI and APS. While most small scientific organizations were managed by for-profit companies and their journals were published by commercial publishers, AACCI and APS, through their nonprofit cooperative, could manage themselves. Determining how to serve two organizations with very different mixes of members was a challenge: the vast majority of AACCI members were employed in industry, and most APS members were employed in academia. Both groups needed very similar services, however, and Ray's ability to help them leverage their unique characteristics made the partnership successful.

Innovating with Technology

Starting with membership databases and production of their journals, Ray set out to use new technologies to improve the quality of member services and create efficiencies that would save money that could be used to initiate new services. As part of his journalism class, Ray designed a new publication for AACCI, *Cereal Science Today* (now *Cereal Foods World*). Part journal and part magazine, it became a very popular member publication.

Publishing and printing went through many changes over the course of Ray's career. When the AACCI and APS partnership was just getting started, printing was primarily done via hot-metal type and letterpress printing. The process was labor-intensive and commonly performed by printing companies. Thus, most of the journals produced by scientific organizations were published by large for-profit publishing companies. The ability to self-publish afforded AACCI and APS the opportunity to sell subscriptions at lower prices and pass on any surplus to their members.

In 1969 Ray brought "strike-on" typesetting to headquarters, allowing AACCI and APS to manage production in-house. The new technologies kept coming, and Ray took advantage as the return on investment allowed. Ray was also an early adopter of computers. The first computer filled an entire office and provided the ability to organize the membership database in new ways and helped with financial recordkeeping. This eventually allowed the office to keep pace with the sale of thousands of books and other products. Technological advancements in printing and publishing continue to this day, with production of digital publications.

Strength of Partnership

The success of the AACCI and APS partnership has brought benefits to all. The organizations were able to remain independent and financially sound, and members were provided a vast array of services that they would not have had access to otherwise.

Ray made great efforts to remain close to the members, understanding that any new service or product had to have a high value to justify its adoption. He recognized early on that if this nonprofit structure was going to be successful, it was essential that the relationship between the members and staff be collaborative. It would need volunteer members who cared about their organization and would provide leadership and scientific expertise. It also required staff with the ability to support and work with members. Only when this relationship works well is there the innovation and spark needed to make it happen.

APS Past President R. James Cook stated it well, "Combine the energy, innovative ideas, and time commitments of the members with the hard work and dedication of the highly qualified paid professional staff and we have an explanation for past accomplishments and a recipe for continued success."

Ray was adept at welcoming new board members, providing training and financial background, and focusing on the strategic direction of each organization. He ensured that volunteers concentrated on the big issues rather than the details of daily operations. He always took great care to help them be successful in their roles.

In a letter to Ray on his birthday, AACCI Past President William Atwell said, "Thank you for welcoming me to the AACCI Board of Directors years ago when I was fairly junior in the industry and certainly not familiar with the processes, activities, or responsibilities of any board of directors. I suspect I was a little unrefined with respect to your other board members, and I also suspect it may have taken an extra dose of your gentle mentoring to get me where I needed to be. I look back at my time on the

board as a major growth experience for me. I remember you telling me that it was your job to make me look good. That must have been quite a task!"

Ray also recognized and valued diverse thinking. APS Past President Anne Vidaver wrote, "Ray was the man in charge at APS headquarters when I became part of APS's administration, from secretary and board member ultimately to president, becoming the second woman president and the first married one. Ray was extremely helpful and highly professional in all his dealings with me and set the tone for other men at the table—some of whom were highly skeptical of me. Ray was there at one of the most crucial points of my personal life as well, for it was the year before my presidency that my husband was diagnosed with pancreatic cancer. Ray offered help and sent flowers at the time of George's death. At the following APS national meeting, he was by my side at the time of the presidential address, ready for anything."

In the late 1960s the volunteer leadership and Ray decided APS and AACCI should build their own office building to house the growing operations. Many members and companies contributed to the cause, and a headquarters building was constructed in a suburb of St. Paul. A substantial addition was later built. The building has served the organizations well, but as technology brings greater efficiencies and changes in the work environment, a different type of building will soon house APS and AACCI headquarters.

Growth of Services

Over Ray's tenure, the list of member services and products he impacted was substantial. The business model utilized by AACCI and APS was different from that of many organizations. Dues played a minor role in their financial health, and instead a robust list of products and services was the lifeblood of the organizations.

Ed Kendrick recalled that in working with Ray he gained an appreciation for and better understanding of the business operations of a scientific association: "When I became treasurer of APS that was the beginning of my close association and a wonderful friendship with Ray Tarleton. Working with Ray while I was treasurer, I gained an appreciation and better understanding of the business operations of a scientific society. When Ray retired, I believe I had a strong influence on convincing him and Andy that Tucson, AZ, was a great place to retire. Under Ray's executive leadership, the course was set for the strong position that APS enjoys today among scientific societies."

Some of the services established or expanded during during Ray's tenure include AACCI Local Sections and APS Divisions; AACCI and APS Foundations, awards programs, student scholarships, websites, short courses, specific topic meetings, books, government relations, and annual meetings; and the AACCI Check Sample Program.

Jon Faubion, who has the unique perspective of having worked with Ray as an AACCI member and then serving as AACCI's technical staff expert, remembers Ray warmly: "He was a true force of nature and a role model. I owe him a great deal, for his friendship was an accolade and a passport. May he be long remembered."

Ray's impact on AACCI and APS was broad and deep. His ability to work with volunteer leaders, to listen to members, and to hire, train, and challenge staff to make it all happen is a testament to his talents. Nice job, Raymond J. Tarleton!

Thank You AACC International Corporate Members

Happy Anniversary! The following corporate members celebrated major anniversaries with AACCI. Their dedication and support to the association and cereal grain science is greatly appreciated.

10 Years

Brabender GmbH & Co. KG
Cereal Ingredients
J. Rettenmaier USA LP

15 Years

Cereal Science and Technology South Africa

20 Years

Monsanto Co.
Tastepoint By IFF

25 Years

California Natural Products
CPM Wolverine Proctor
Great Plains Analytical Laboratory
Healthy Food Ingredients
MGP Ingredients, Inc.
Richardson Milling

30 or More Years

ADM Milling Co.
Baker Perkins
Bay State Milling Company
Budenheim USA Inc.
Buhler Inc.
C.W. Brabender Instruments, Inc.
Canadian Grain Commission
CHOPIN Technologies
General Mills
Grain Craft
Grain Processing Corporation
ICL Food Specialties
Innophos, Inc.
Kellogg Co.
Malt Products Corp.
MANE, Inc.
McCormick & Co., Inc.
Morton Salt, Inc.
National Manufacturing Co.
Neogen Corp.
Nisshin Seifun Group Inc.
PGP International Inc.
REPCO
Riviana Foods Inc.
Sensient Colors LLC
Symrise Inc.
The Hershey Co.
The Mennel Milling Company
Wenger Manufacturing Inc.

Thank you to all our corporate members, who contribute their knowledge, expertise, and professional involvement to ensure the continued strength of the association and to promote excellence in cereal grain science worldwide. We appreciate their support and encourage you to contact them directly for detailed information on their products. Visit the AACCI Corporate Member web page for comprehensive company and contact information.

ADM/Matsutani LLC
ADM Milling Co.
Agrifood Technology
Agri-Neo Inc.
Agtron
AmSpec LLC
ANKOM Technology
Ardent Mills
ARVALIS Institut du Vegetal
Atyab Foodtech Trading & Services LLC
Baker Perkins
Bastak Food Machine Medical
Bayer CropScience Division
Bay State Milling Company
Bell Flavors & Fragrances
BENEO
BENEO Belgium
Bepex International LLC
Brabender GmbH & Co. KG
Briess Malt & Ingredients Co.
Budenheim USA Inc.
Buhler Inc.
California Natural Products
Campden BRI
Canadian Grain Commission
Canadian International Grains Institute
Cargill
C-Cell C/o Calibre Control
Cereal Ingredients
Cereal Science and Technology South Africa
CHOPIN Technologies
CPM Wolverine Proctor
C.W. Brabender Instruments, Inc.
Dakota Specialty Milling, Inc.
Deibel Laboratories
Delavau
DSM Food Specialties USA, Inc.
DSM Nutritional Products
Emirates Grain Products Co. LLC
EnviroLogix Inc.
Enzyme Development Corp.
Foss Analytical
FOSS North America Inc.
FSFI Centre for Grain Quality Assurance
General Mills
George Weston Foods
GlycoSpot
Gold Coast Ingredients, Inc.
Grain Craft
Grain Millers, Inc.
Grain Processing Corporation
Grains and Legumes Nutrition Council
Granotec Grupo
Great Plains Analytical Laboratory
Healthy Food Ingredients
Hunter, Walton & Co., Inc.
ICL Food Specialties
ILC Micro-Chem
InfraReady Products (1998) Ltd.
Innophos, Inc.
J. Rettenmaier USA LP
Kellogg Co.
Kudos Blends Ltd.
Lallemand Baking Solutions
Malt Products Corp.
MANE, Inc.
McCormick & Co., Inc.
Merieux NutriSciences
MGP Ingredients, Inc.
Miller Milling Company
Minot Milling
Monsanto Co.
Morton Salt, Inc.
National Manufacturing Co.
Neogen Corp.
Nexira
Nisshin Seifun Group Inc.
Northern Crops Institute
NP Analytical Laboratories
Oatly AB
OMIC USA Inc.
P&H Milling Group Limited
Palsgaard Incorporated
Perten Instruments
Perten Instruments AB
PGP International Inc.
Polypro Intl. Inc.
Productos Ramo S.A.
QualySense AG
Radio Frequency Co., Inc.
R-Biopharm Inc.
Red Star Yeast Co. LLC
REPCO
Richardson Milling
Riviana Foods Inc.
Romer Labs Inc.
Seaboard Overseas Trading Group
SEMO Milling, LLC
Sensient Colors LLC
Sensus America, Inc.
Shakhe Zeytoon Lian
Shur-Gain Nutreco Canada
Stable Micro Systems Ltd.
Suzanne's Specialties
Symrise Inc.
Takenouchi Barley Processing Inc.
Tastepoint By IFF
Texture Technologies Corp.
The Hershey Co.
The Mennel Milling Company
The Southern African Grain Laboratory NPC
The XIM Group, LLC
Tree Top Inc.
United Flour Mill Co. Ltd.
US Energy Partners LLC
Wenger Manufacturing Inc.

News

2018 Student Research Leadership Program—Application Process Now Open

To encourage enhanced student participation within AACCI, the Student Research Leadership Program provides funds to facilitate research in key areas of interest that will have an immediate impact on cereal science. Awards are available up to US$4,000, including funds for scholarships ($500), laboratory supplies ($1,500), and travel to an AACCI meeting ($2,000). Projects are typically expected to run 12 months, but some may be longer or shorter; presentation of work would be expected in 2019. For more information, including research areas and upcoming deadlines, visit aaccnet.org/membership/StudentAssociation/Pages/StudentLeadershipProgram.aspx.

Call for Best Student Research Paper Competition—Show Off Your Hard Work!

The AACCI Professional Development Panel (PDP) is hosting the 10th AACCI Best Student Research Paper Competition, which will take place at Cereals & Grains 18 in London, U.K., October 21–23, 2018. We encourage all academic departments to nominate a student to participate in this year's competition. The top chosen submissions will compete at the meeting.

This competition is an excellent way for students to hone their presentation skills and showcase their exciting research. Winners will be announced during the meeting, and awardees can receive up to $1,250! All finalists will receive a travel grant to attend the meeting. Visit aaccnet.org/membership/StudentAssociation/BSRPC for information on rules and important dates.

New Members

Anderson, K. L., project manager, Iowa State University, Ames, IA, U.S.A.
Arbaugh, J., chemist II, West Virginia Department of Agriculture, Charleston, WV, U.S.A.
De Beleyr, A., project manager, Lotus Bakeries NV, Lembeke, Belgium
Gulkirpik, E., University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.
Nakatsuka, Y., president, Henriette, Tsukuba, Ibaraki, Japan
Rao, M. H., process and product development lead, Cargill India Pvt. Ltd., Gurgaon, India
Russo, G., Farmer Ground Flour, Trumansburg, NY, U.S.A.
Schaefer, J., Intellifarms, Archie, MO, U.S.A.
Sudhersanana Unni, S., Coimbatore, India

People

DSM has appointed Frédéric Boned as EMEA vice president of its Human Nutrition & Health business. Boned joined DSM in 2012, holding a variety of senior roles in DSM Personal Care & Aroma Ingredients , including EMEA director of personal care, senior director of global marketing and innovation, and vice president of global sales, personal care, and brings a wealth of knowledge and expertise from a diverse working background. As well as bringing fresh insights and his own vision for the Human Nutrition & Health business, Boned will be responsible for providing strategic direction to help further accelerate DSM's development. Royal DSM is a global science-based company active in health, nutrition, and materials.

Companies

Frutarom Industries Ltd. has announced the acquisition of AB-Fortis activities, including a patent-protected microencapsulation technology that enables delivery of iron with increased biological absorption. The advanced AB-Fortis system provides highly stable encapsulation, ensuring no release of free iron in the food matrix. The unique microencapsulation technology stabilizes and protects the iron, rendering it flavorless and allowing for adequate dosages without negative side effects, while maintaining high bioavailability. Frutarom Industries Ltd. is a leading global company operating in the flavors and natural fine ingredients markets.

2018 Themes

January-February—Analytical
Ad Close: January 8, 2018

March-April—Baked Products
Ad Close: March 13, 2018

May-June—Health & Nutrition
Ad Close: April 30, 2018

July-August—Processing/Pre-annual Meeting
Ad Close: June 6, 2018

September-October—Grains & Pulses
Ad Close: August 10, 2018

November-December—Product Development & Innovation
Ad Close: October 30, 2018

Important AACCI Dates

February 2018
28. AACCI Best Student Research Paper Competition applications deadline

March 2018
13–15. AACCI Rheology Division: Rheology & Texture of Cereal Foods course, West Lafayette, IN, U.S.A.

17. 17th European Young Cereal Scientists and Technologists Workshop abstract submission deadline

30. AACCI Student Research Leadership Program nomination deadline

April 2018
4–6. AACCI Milling & Baking Division Spring Technical Conference, Nashville, TN, U.S.A.

12. AACCI-ICC Organized Symposium: Bringing Ancient Grains to the World's Dinner Tables (at Sorghum in the 21st Century), Cape Town, South Africa

18–20. 17th European Young Cereal Scientists and Technologists Workshop, Warsaw, Poland

May 2018
15. *Cereal Chemistry* Asian Products Focus Issue submission deadline

24. AACCI Rice Division and University of Arkansas Rice Processing Program: Rice Quality & Evaluation Short Course: An Outreach Effort to Rice Processors, Fayetteville, AR, U.S.A.

October 2018
21–23. Cereals & Grains 18 – AACCI Annual Meeting, London, U.K.

For more information visit
aaccnet.org

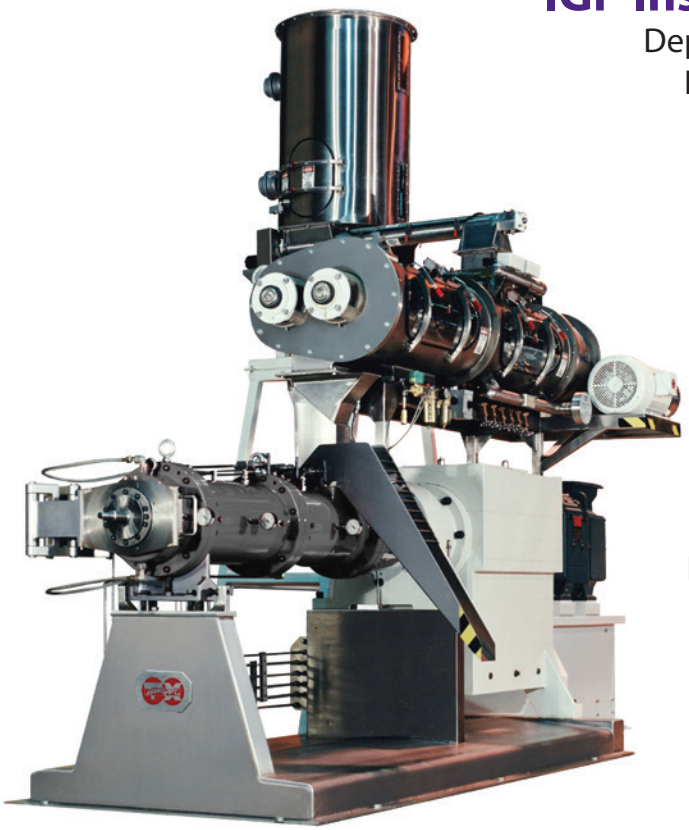
Advertisers' Index

Volume 63, Number 1

IGP Institute

Extrusion Processing:
Technology and Commercialization

An IGP Institute Short Course • August 7–10, 2018



IGP Institute Conference Center

Department of Grain Science and Industry
Kansas State University • Manhattan, KS

Learn fundamentals of extrusion technology, equipment, product development, and economics. The short course includes hands-on lab sessions and an industry field trip.

Optional Supplement:

Pet Food Workshop
August 6, 2018

For details and registration information please visit the IGP Institute website:
www.grains.k-state.edu/igp

Or contact:

IGP Institute
Email: *igp@ksu.edu*
Phone: 785-532-4070

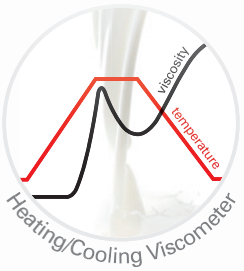
KANSAS STATE UNIVERSITY | IGP Institute
Department of Grain Science and Industry

1980 Kimball Avenue, 102 IGP Institute Conference Center | Manhattan, KS 66506–7000
Tel. 785-532-4070 | Fax 785-532-6080 | *www.grains.k-state.edu/igp* | *igp@ksu.edu*

RVA – The Performance Analyzer


Powerfully Simple

Unique Tool – The RVA measures performance characteristics of foods and ingredients by simulating typical cooking processes. It tests samples by measuring changes in viscosity following standard methods or user-definable heat, shear, and time conditions.



Heating/Cooling Viscometer

Relevant Information – The RVA tests foods and ingredients where the behavior of starches, hydrocolloids or proteins is critical. The curve it produces is characteristic of the sample's attributes such as pasting peak, pasting time, gelling and others. A typical test takes only 10-15 minutes.


Ingredients

Versatile – Researchers use the RVA to measure fundamental characteristics of materials. Product developers use the RVA to test new formulations and effects of new/less expensive ingredients. Production and QC use the RVA to screen ingredients to avoid production issues and reduce waste, and to verify finished product quality.

www.perten.com/rva

Perten INSTRUMENTS
a PerkinElmer company

CEREAL FOODS WORLD®, JANUARY–FEBRUARY 2018, Vol. 63, No. 1, pp. 1–48