Major Advances in Testing of Dairy Products: Milk Component and Dairy Product Attribute Testing

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ABSTRACT

Milk component analysis is relatively unusual in the field of quantitative analytical chemistry because an analytical test result determines the allocation of very large amounts of money between buyers and sellers of milk. Therefore, there is high incentive to develop and refine these methods to achieve a level of analytical performance rarely demanded of most methods or laboratory staff working in analytical chemistry. In the last 25 yr, well-defined statistical methods to characterize and validate analytical method performance combined with significant improvements in both the chemical and instrumental methods have allowed achievement of improved analytical performance for payment testing. A shift from marketing commodity dairy products to the development, manufacture, and marketing of value added dairy foods for specific market segments has created a need for instrumental and sensory approaches and quantitative data to support product development and marketing. Bringing together sensory data from quantitative descriptive analysis and analytical data from gas chromatography olfactometry for identification of odor-active compounds in complex natural dairy foods has enabled the sensory scientist and analytical chemist to work together to improve the consistency and quality of dairy food flavors.

Key words: milk component testing, infrared, quantitative descriptive analysis

INTRODUCTION

Milk Component Testing

Lynch and coworkers in 2004 stated that methods to measure milk composition are relatively unusual in quantitative analytical chemistry because the results determine the allocation of very large amounts of money between buyers and sellers of milk. Therefore, there is a large incentive to develop and refine these methods to achieve a level of analytical performance rarely de-

manded of most methods or laboratory staff working in analytical chemistry. In the past 25 yr, improvement of the hardware for mid infrared (**MIR**) milk analysis and improvement in the performance of the chemical methods used to calibrate MIR have enabled the dairy industry to shift from paying for milk based on weight and fat content, to valuing milk based on the weight of each milk component (e.g., fat, true protein, other solids) delivered. In January 2000, the reform of the USDA Federal Milk Market Orders instituted multiple component payment for milk in almost all regions of the United States and changed the basis of milk protein payment from crude to true protein, as described in the Federal Register. Research to improve both chemical and instrumental methods of analysis for milk enabled these advancements. The initiative, leadership, and funding for much of the advancement in performance of milk component testing in the United States in the last 25 yr has come from Test Procedures Committee of the USDA, Dairy Programs, Federal Milk Market Orders.

Dairy Product Composition and Attribute Testing

Consolidation and increased scale of dairy product manufacturing has driven the development of new, faster methods of analysis for composition and quality control. Sensory and instrumental analyses of the attributes of dairy foods are used to judge the success of quality control before the product is sent to the consumer, with the ultimate sensory evaluation done by the consumer. Although detecting, identifying the cause of, and correcting product defects will always be important in dairy food manufacturing, modern instrumental and sensory approaches allow the optimization of the desirable attributes of a product and the development of new products that fit a product attribute profile of a potential market segment. Sensory analysis of dairy foods has evolved from a defect identification-oriented system to an attribute intensity scaling approach that quantitatively describes many dimensions of a product's characteristics. Lawless and Heymann indicated in 1999 that data from sensory methods, such as quantitative descriptive analyses (**QDA**) when combined with objective measures of chemical (e.g., gas chromatogra-

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phy olfactometry, **GCO**) or physical (e.g., rheological measurements such as dynamic mechanical analysis) characteristics of a product can be used to define and optimize dairy food product characteristics for different market segments. The combined sensory and instrumental data can then be analyzed with more advanced statistical protocols such as principal component analysis.

ADVANCEMENTS IN THE LAST 25 YEARS

Milk Component Testing

Milk component testing (i.e., fat, protein, lactose, total solids, solids-not-fat, and other solids) impacts every dairy farmer. Mid infrared milk analysis is a rapid, cost-effective secondary testing method, the results of which are routinely used to determine payment for milk to each dairy farmer. All of the milk composition testing for individual cows used for decision making in feeding and management strategies and genetic selection is done by MIR. Thus, the accuracy and consistency of testing across wide geographic areas is of great economic importance and influences business decisions every day.

Harmonized Method-Validation Procedures. An important development in the field of routine methods of quantitative analysis that has impacted milk component testing was the development in the 1980s and 1990s of internationally harmonized guidelines for interlaboratory studies for validation of analytical method performance. The guidelines, published by the Association of Official Analytical Chemists International (**AOACI**) in 1989 and revised in 1995, were originally adopted in 1987 by a consensus of representatives from methods validation organizations around the world, including the AOACI, the International Organization for Standardization (**ISO**), and the International Dairy Federation. The guidelines apply to validation of method performance across all analytical chemistry, but they have been particularly useful in validation of analytical method performance in the dairy industry. The guidelines provide a framework for interlaboratory study design, minimum requirements for the number of sample materials and laboratories, and standardized statistical procedures for removal of outliers and calculation of statistical indices of method performance. The statistical indices (e.g., *r*-value and *R*-value) that are published as part of each method provide clear validated benchmarks for the analyst for expected within-laboratory agreement on replicates (i.e., repeatability) and expected between-laboratory agreement (i.e., reproducibility) if the method is being carried out correctly.

Traceability and Uncertainty in Analytical Results. Over the past 25 yr, analytical communities worldwide have been actively engaged in developing harmonized standards and procedures for ensuring the quality of analytical results as described by ISO in 1999 and in the Eurachem Guide in 2000, followed by an update in 2003. Although still evolving, these efforts are based on the recognition that confidence in analytical results is not a function of a single activity, but rather the product of multiple activities that include the use of qualified staff, validated methods, internal quality control, proficiency testing, and accreditation. Furthermore, the reliability of analytical results is inextricably linked to the concepts of traceability and uncertainty. Eurachem indicated in 2003 that traceability in analytical chemistry refers to the link between an analytical result and some agreed-upon reference standard (an accuracy benchmark) by means of a series of unbroken comparisons all having a stated uncertainty (an estimate of variability based on the dispersion of the results, including method bias). For milk payment purposes in the United States, the milk components, protein, fat, and total solids, are of indefinite (mixed) composition; thus, the reference standards that define them are the agreed-upon reference methods themselves. Lactose is the exception because it is a single chemical entity and a stable physical standard (lactose monohydrate) can be obtained.

Much of the focus of the USDA Test Procedures Committee has been on implementing these principles within the Federal Milk Marketing Orders. Important first steps were taken in 1989 through 1998, when reference methods for component testing were defined and their method performance documented. An extension of this work was the initiation of a proficiency-testing program for laboratories operated by or under contract to individual Milk Marketing Orders, to assure that method performance specifications could be met on an on-going basis. Results of the USDA Test Procedures Committee progress on the programs were reported by Lynch and coworkers in 1994, 1997, and 2003. The Test Procedures Committee is currently in the process of establishing a program to formally realize traceability of MIR testing by assigning reference values with uncertainty estimates to milk-based materials used for both calibration and validation. Taken as a whole, this program is intended to provide the US dairy industry with a means to produce high quality data that are fit-for-purpose.

Chemical Methods of Analysis: Primary Methods. Although these methods may be viewed by many as old fashioned, the results from validated chemical methods performed on a small number of samples form the basis for calibration of the high-speed testing meth-

ods. Thus the accuracy of these primary, or reference, tests is of paramount importance because all MIR milk testing is dependent on their accuracy. Current versions of these reference methods are available in the 2000 edition of *Official Methods of Analysis* published by AOACI. The chemical method generally accepted as the reference method for fat is AOACI method 995.19, ether extraction. The basis for protein reference testing is Kjeldahl nitrogen analysis, with the United States and some other countries measuring true protein using AOACI method 991.22, whereas other countries measure crude protein using AOACI method 991.20. In 1992, Barbano and Lynch described the improvement in accuracy of MIR measurement of all milk components that could be expected when true protein rather than crude protein is used as a reference because MIR only detects true protein at the classical protein measurement wavelength. Until recently, chemical methods for measurement of lactose have not been as important. However, when an MIR payment test for total solids or other solids is needed, then proper chemical reference methods for lactose and total solids are important. Lactose measurement by polarimetry is the classical approach, but the AOACI method 896.01 is very difficult and generally does not perform at a level comparable to the reference methods for measurement of fat and protein. In the last 25 yr, spectrophotometric-based enzymatic methods such as AOACI method 984.15 have become more common for measurement of lactose. In 2003, Lynch and coworkers reported that the enzymatic lactose method has been improved by converting the method to a weight instead of volume basis and research is continuing to improve method performance. The AOACI forced air oven drying method 990.20 is used as a reference method for total solids measurement.

The following were major improvements in the ether extraction method in the last 25 yr and reported by Barbano and coworkers in 1988: use of a color indicator to help the analyst do a better job removing the ether layer without contamination; addition of water to adjust the level of the interface to facilitate the removal of the ether layer; inclusion of a third extraction; and interlaboratory studies documenting the performance of the method that an analyst should be able to achieve.

One of the major improvements in the Kjeldahl method made in 1990 by Barbano and coworkers was the substitution for mercury catalyst by an optimized level of copper sulfate which is friendly to workers and the environment, and achieves equal or superior recovery of nitrogen. Another advance was the development of a sample preparation procedure by Barbano and coworkers in 1991 to determine true protein directly by Kjeldahl, instead of calculating the difference between

separate total nitrogen and nonprotein measurements. This made it practical to use true protein measurement by Kjeldahl as a reference method for calibration of MIR milk analyzers and was adopted by the USDA as published in 1999 in the Federal Register. The first country to use true protein as a reference for calibration of MIR was France. Today it is becoming more common worldwide for true protein to be used as the reference for milk payment testing and milk production record keeping. Barbano and coworkers conducted interlaboratory studies documenting the performance of the crude protein and true protein (direct and indirect) Kjeldahl methods in 1990 and 1991, respectively. Similar documentation was provided in 1991 by Barbano and coworkers for the Kjeldahl nonprotein nitrogen method, followed by the work of Lynch and her colleagues in 1998 reporting on the performance of direct and indirect Kjeldahl methods for determination of milk casein content. In an effort to help laboratories achieve better performance of the Kjeldahl method for analysis of dairy products, Lynch and Barbano published a troubleshooting guide in 1999.

The major improvements in the total solids method made in the last 25 yr were reported by Clark and coworkers in 1989 and Lynch and coworkers in 1997: the routine use of disposable oil-free aluminum weighing dishes (allows the milk to spread more evenly over the bottom of the pan); standardization of sample size, drying temperature and time; the initiation of drying starting from a hot oven; the generic definition of the temperature recovery rate that the oven must have at the beginning of the test; and interlaboratory studies documenting the performance of the method that an analyst should be able to achieve.

Electronic Methods of Analysis: Secondary Methods. Secondary methods require calibration using a set of samples with established reference values. Electronic milk testing instruments based on light scattering, commonly called Milko Testers, emerged in the 1960s and were the first commercially successful electronic testing methods for fat. In the last 25 yr, the quality and design of the optical bench, electronics, and computer handling of data for MIR analysis has developed rapidly and has almost completely replaced the Milko Tester. For milk component testing, the MIR method measures infrared light absorbance at classical vibration frequencies in the MIR spectra that represent key chemical bonds. The MIR estimation of total solids is done by calculation based on the values determined for fat, protein, and lactose and a regression correlation to reference values for total solids determined by the oven-drying method.

The MIR filter-based instruments use pairs (sample and reference) of optical filters to select a band of wave-

lengths for measurement of fat, protein, and lactose. The first generation of MIR instruments used the carbonyl stretch to measure fat (sometimes called Fat A), whereas the second generation used the carbon-hydrogen (sometimes called Fat B) as well as the carbonyl stretches. Today, instruments with optical filters are still marketed, but more advanced Fourier transform infrared instruments that use interferograms to produce full spectral data are now available and were described by Agnet in 1998. Although Fourier transform infrared is capable of collecting more spectral information and theoretically should enable more accurate testing, it has yet to be demonstrated that this additional information can be successfully used to improve method performance under practical milk payment testing conditions. For testing milk from individual cows, the use of other wavelengths has allowed the measurement of milk urea and this information is useful for evaluation of the efficiency of dietary nitrogen use by dairy cows. When testing dairy product matrices that contain other ingredients (e.g., ice cream mix), the additional spectral information may allow compensation for the interference from nonmilk-based ingredients.

To achieve the performance potential of MIR equipment for payment testing, the accuracy of the reference values and most importantly the design of the calibration sample set (e.g., range and distribution of component concentrations, lack of correlation between individual component concentrations, and the number of samples) will be a more important determinant of the actual MIR method performance in the field. It is impractical for individual laboratories to continually make their own calibration samples and run chemistry on them. Centralized calibration services that can properly formulate calibration samples and spread the cost over the calibration of many instruments will be the course for the future. Moreover, coordinated multilaboratory reference chemical testing of common calibration and validation samples will provide more robust all laboratory mean chemical calibration and validation reference values as well as the associated uncertainty estimates for important analytical testing methods for milk payment testing and for process control.

Dairy Product Composition and Attribute Testing

Composition Analysis. Over the past 25 yr, the dairy foods processing sector has undergone massive consolidation to capture economies of scale in manufacturing, distribution, and marketing. The number of processing factories decreased and the processing capacity of individual factories increased greatly. Thus, in today's large factories, even small mistakes in process control are significant. In large factories the amount of

product being produced per hour is very large and process control has become increasingly important. Although accurate traditional chemical methods are useful for calibration reference testing, high-speed quality control tests are needed for process control decision-making. The electronics and equipment design has allowed the emergence of inline MIR process control hardware for real-time standardization of milk composition for cheese manufacture as described by McKenna in 1999. For composition control of liquid dairy products (e.g., fluid milk, milk for cheese making), inline MIR and near infrared (NIR) milk analyzers have been developed and are commonly used in today's factories. However, the situation is more difficult for solid products like cheese. Near infrared reflectance methods have filled this need. Although NIR equipment still needs calibration and usually a large number of samples from the specific product are required for the calibration, the speed of analysis compared with conventional chemical analyses on a day-to-day basis is critical for process control decisions in which small changes in product composition can have a large impact on product yield, shelf life, or quality.

Sensory Analysis. Historically, dairy product judging and the American Dairy Science Association (ADSA) scorecard system have been used as the basis for sensory evaluation of dairy products but these approaches did not serve research and product developers very well. Although the ADSA scorecard serves an important purpose for the dairy industry, particularly in the identification of defects, Bodyfelt indicated in 1981 that the scorecard is relatively subjective. McBride and Hall in 1979 and Lawless and Claasen in 1993 suggested that the ADSA scorecard approach may not be very useful in quantitatively characterizing the relative intensity of positive attributes of a product or detecting interactions.

Quantitative descriptive analysis was developed in the 1970s. Lawless and Heymann discussed QDA in 1999 as a quantitative method yielding data suitable for statistical analysis and therefore of value for research, development, and basic sensory science but more expensive than the scorecard approach. For a particular type of product, panelists (10 to 12) are given a group of similar products that exhibit the type of variation in attributes likely to be encountered in the population. Panelists, along with a facilitating sensory scientist, develop a lexicon of descriptive terms that allow panelists to describe the difference in attributes among the samples. Where possible, groups of samples showing a range of intensities of each specific attribute are part of the training to allow panelists to practice intensity scaling judgments. The trained panel is then used to collect data in a specific experimental design. Data on

multiple attributes can be analyzed in spider plots to provide a fingerprint of multiple attributes of a product. Principal component analysis can be used to identify the principal components that differentiate populations of samples. Drake and coworkers published in 2001 an example of this approach for sensory analysis of Cheddar cheese.

Instrumental Analysis. The rapid evolution of instrumental methods that interface with olfactory sensory detection has brought a new dimension to the coupling of sensory science and chemical analysis. Acree and coworkers reported in 1984 that the development of GCO approaches to characterization and quantification of the aroma profile of food products has brought a powerful new tool to sensory science. For example, a food may have hundreds of different volatile compounds that can be separated and detected by gas chromatography but only a small number of those compounds may elicit a sensory response in a human. Flow splitters and column effluent conditioners have allowed persons to evaluate each peak by sniffing as it is eluted from the chromatograph, to assign a descriptive term to the retention index, and in some cases, to indicate an intensity or time of persistence of the aroma. In addition, the individual peaks that have a detectable aroma can be isolated and analyzed by mass spectrophotometry to determine their structure and identification, as demonstrated in 2003 by Karagül-Yüceer and coworkers for liquid whey. In 2004, Carpino and coworkers used this approach to identify specific compounds and relate them to the results of QDA analysis. It has been proposed that unique compounds in specific cheese varieties are derived from the consumption of local forages and could be used as biomarkers to authenticate the origin of protected cheese varieties.

SUMMARY

Over the past 25 yr, the development of high-speed instrumental testing methods to measure milk and dairy product composition provided the motivation for improvement of the analytical performance of classical chemical analysis methods that form the basis for instrument calibration and validation. Harmonized statistical procedures and study designs have allowed validation of uniform testing methods that can be used worldwide. Overnight, refrigerated shipping of perishable samples has allowed the development of multiple laboratory proficiency testing programs. This has improved method and laboratory performance on reference and secondary methods for analysis of dairy foods. Although the shipping of proficiency testing and calibration samples between continents is more problematic due to customs restrictions, approaches to maintain

linkages of proficiency testing systems among different areas of the world will become more important in the future. There will be continued development of statistical methods to quantify uncertainty in analytical results and approaches to document and maintain traceability of instrument performance. Uniformity in testing methods, documented analytical performance of laboratories through participation in proficiency testing, and maintenance of traceability to reference standards will become more important as international trade of dairy products increases.

In sensory science, there will be continued development of analytical methods to provide more objective measures of the sensory characteristics of dairy foods. The development of GCO, combined with gas chromatography/mass spectrometry is an example of the development of analytical tools that can be correlated with aroma and odor perception of foods. Future development of rapid analytical methods, such as liquid chromatography coupled with mass spectrometry for protein and peptide sequence analysis, will increase our understanding of the complex processes of production of peptides and flavor development in natural cheeses. Degradation of proteins and amino acids ultimately produce aroma compounds detected by GCO. Methods for peptide analysis may also enable the identification of peptides in dairy food products that have biological activity. The combination of these analytical techniques will allow improved understanding of the biological processes that produce the flavor and nutritional characteristics of aged natural cheeses.

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