Issues Related to Sampling and Analysis of Milk

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Abstract

Determination of the weight of milk produced, and sampling and testing of milk provides the information to make feeding and cow replacement management decisions in dairy herds. Milking parlor technology has changed from weigh jars to flow sensors and proportional milk sampling devices. With those changes, more management attention needs to be focused on control of the accuracy of the milk flow sensors that estimate weight of milk produced by a cow and representativeness of milk sampling. In addition, central laboratory mid-infrared **(IR)** transmittance milk analysis methods continue to evolve, enabling the measurement of minor milk components, such urea and fatty acids in milk. While, in general, it does not take additional time beyond that need for fat and protein to test milk for these new components using an Fourier Transform **(FT)**-IR, it does take extra time to do additional quality assurance validations of these new parameters, in addition to purchasing and using additional calibration and validation reference materials to control the accuracy of testing these additional milk components. These additional costs for measuring new milk components need to be balanced against the value of the new milk composition and production information for farm management decision making. Equipment is already available to measure the fat and protein of milk as the cow is being milked. For large farms, a cost effective approach for doing this testing at every milking will provide a rich new source of information to make more timely feeding and management decisions.

Introduction

Improvement of milk production efficiency requires information that can be used in support of management decision making. Data on milk production and composition is one of the information inputs needed to conduct the symphony of factors that a farmer can control in the environment and feeding of dairy cattle. Replacing and changing cows and redirection of the genetics of a dairy herd is like buying some new musical instruments to play. Information on milk production and milk composition is used in support of both types of management decisions, and these data also support the off-farm genetic improvement for replacement animals available to dairy farms. It is easy to look at, and accept, large quantities of numeric data without questioning the quality of the data. Multiple factors can influence the quality (i.e., accuracy) of milk composition and milk production data. In the current discussion, I would like to take a moment to systematically review the critical factors that impact the accuracy of milk production data and then discuss some of the new opportunities in milk composition testing that could provide new information to improve the management and productivity of lactating ruminants. I will focus on measurement of milk weight, the process of collecting a representative sample of milk from each cow, the details of testing for fat and protein, current

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testing for other milk components, and new opportunities to measure other characteristics of milk composition that may serve as new markers for management decision making.

Milk Weight and Sampling

In the not too distant past when milking parlors became common, it was typical to see a line of large glass "weigh jars" in the milking parlor. While this technology was not as fast and efficient as current technology, it did have some advantages. The milk quantity measurement calibration by a weigh jar was fixed and did not change with time. It allowed the person milking the cows to see the milk and determine if there was something visually wrong (e.g., blood in the milk) before the milk from each cow was released into the bulk tank. The weigh jar also provided the opportunity to have all of the milk from each cow in one place so it could be mixed and a representative sample of the complete milking of that cow could be collected. Why is this important? The composition of milk from each cow changes through the course of a single milking. The fat content of milk at the end of milking an individual cow is much higher than early in the milking process. The more complete the milk let down and removal, the more extreme the difference in fat content from the beginning to end of milking. This can be demonstrated very clearly by milking a cow and collecting and mixing that milk for fat testing and then administering oxytocin and collecting the residual milk. The fat content of the residual milk can be as high as 6 to 8%.

Today, for the most part, the weigh jars in milking parlors are gone. A variety of milk flow meters and proportionate samplers have replaced the weigh jars. While these devices improve the speed and efficiency of collecting milk weight data and samples, control of both the accuracy of weight measurement and the representativeness of sampling can easily be neglected and diminish the quality of data on milk production and milk composition. Poor maintenance and control of these devices can result in incorrect data used as the basis of feeding management decisions. While most of my effort is spent in the laboratory focused on the accuracy of the milk measurement equipment, I am often shocked by the fact that the process of sampling is not collecting a representative sample of the milk and therefore all the controls of the quality of the milk testing equipment in the laboratory were wasted because the milk sample was not representative of what the cow produced. Just go into a milk parlor and look at the proportional milk sampling containers. Every time you see a milk sampling container that is full and the cow is still being milked, you might just as well dump that sample on the floor and mark it as missing data. The extra penalty of this mistake is that the data on the highest producing individual cows in the herd are likely to be the data that are incorrect if this problem exists in the milking system. The high producing cows are the cows where the sampling device is full before they have been completely milked and also the cows for which you would like the most accurate milk composition. A very low producing cow is easy to identify without milk composition data. Often, no data are better than incorrect data that you assume is correct. These problems can be fixed, but someone needs to see them, place a priority on them, and take action. The milking parlor staff has a certain number of cows to get milked per hour, and they are not going to slow down to fiddle with these things. The hardware needs to be designed and adjusted to work correctly and checked as a part of the information quality control system on the farm.

What is the DHIA and Milk Payment Testing Technology Anyway?

Today, the technology used in Dairy Herd Improvement Association **(DHIA)** and payment testing laboratories for measurement of milk composition is mid-IR transmission spectroscopy. When DHIA labs only measured fat content of milk,

the testing technology was done using the chemical Babcock test (Barbano et al., 1988) and light scattering measurement instruments called call milk-o-testers (Barbano and Lynch, 2006). While the scientific basis for Mid-IR measurements of milk composition has really not changed since the 1970's, the hardware, electronics, and statistics have continued to improve and evolve to increase the accuracy of fat, protein, and lactose measurements while opening up new opportunities to measure other milk composition characteristics (e.g., milk urea nitrogen) with improved versions of the same milk testing technology.

We often don't take the time to reflect on how really awesome is the technology of Mid-IR milk testing. First, the only sample preparation that is needed is to warm the milk to 40°C and mix the sample prior to testing. No chemical reagents required!! How many analytical methods to measure the composition of any type of sample are there which can claim that? The next amazing thing is the number of samples per hour. The first mid-IR milk testing instruments in the early 1960's promoted the small number of minutes that it took to analyze one sample. Today, we have improved the hardware and data handling so that we can test 600 milk samples per hour. As the speed, complexity, and automation of these instruments have increased, the educational and training background required to control the accuracy of these instruments have increased. Manufacturers of the milk testing equipment promote the ease of operation of the technology, but the reality is that a more sophisticated quality assurance and reference sample checking system are required to maintain the accuracy of the equipment and the results. The person running 600 samples per hour on the instrument is often in the same situation as the person milking cows; they have a certain number of samples to test per hour and they don't have time to fiddle with the machine if it has a non-fatal problem. The DHIA and milk testing laboratories need to invest in quality assurance and maintenance to ensure high productivity and high quality results.

How Does the Mid-IR Measurement Work?

The very first mid-IR milk analyzer in the early 1960's was a dual beam spectrophotometer with no control of sample temperature, no in-line homogenizer, no control of moisture in the optical compartment, and a slow moving diffraction grating that produced a full spectra of a milk sample. While it provided the opportunity to make a fat and protein measurement, it was ahead of its time. There was no demand for protein data at that time, and simple light scattering (milk-o-tester) was faster, more accurate, cheaper, and easier to operate. So mid-IR for milk testing went back into the development lab to mature and wait for the dairy industry to ask for both fat and protein content of milk samples.

The first commercially successful mid-IR milk analyzers corrected the limitations mentioned above and focused only on the wavelengths were there was information of value for measuring fat, protein, and lactose by using optical filters that sequentially rotated through the infrared light beam to measure the mid-IR light absorbed at different wavelengths instead of trying to collect a full spectrum. This approach increased the speed of the measurement to make mid-IR milk analyzers competitive with the light scattering technology for fat measurement, with the added benefit of providing data on protein content of milk.

The first measurements of fat concentration in milk using mid-IR were based on the wavelength (5.73 microns) where the carbonyl stretch of the ester bond in triglycerides and phospholipids occurred in the IR spectra (Goulden, 1964). The protein measurement was based on the amide II group in protein (6.46 microns), and the lactose was measured using the absorbance of hydroxyl groups (9.6 microns) in lactose (Kaylegian et al., 2009 a, b). A milk spectra with the wavelengths indicated is shown in Figure 1. After a few years of use of this approach in DHIA laboratories, it was recognized that the fat measurement was not as

accurate as expected because the measurement assumed that the fat in all milk samples had the same fatty acid chain length. The change in fatty acid composition with stage of lactation is relatively large (Sjaunja, 1984a,b; Lynch et al. 1992) and the approach for measurement of fat was improved by adding a measurement of the carbon-hydrogen stretch (3.48 microns) to help compensate for sample-to-sample variation in fatty acid chain length. The measurement of fat using the carbonyl stretch absorbance is commonly called the Fat A measurement and the measurement of fat using the absorbance of the carbon hydrogen stretch is commonly call the Fat B measurement. The Fat B was an improvement, but many laboratories found that a combination of Fat B and Fat A provided improved accuracy of fat testing (Biggs and McKenna, 1989). Intercorrection factors (Lynch et al, 2006) were used in the calculations of concentrations of milk components to correct for background absorbance of one component at the measurement wavelength of another component and for changes in water concentration as the concentrations of the other milk components increased or decreased from one sample to another. The mid-IR approach using optical filters was used very effectively for milk testing until the late 1990's. A filter wheel removed from a mid-IR instrument is shown in Figure 2. Each filter had to rotate into the light beam and move out of the light beam for each sample. This limited the speed of analysis per sample and also produced mid-IR absorbance information only at these discreet wavelengths in the mid-IR spectra.

While simple filter based instruments are still manufactured and marketed, high speed large volume milk testing has, for the most part, changed to FT based mid-IR instruments. While the fundamental spectrophotometric basis of the milk analysis has not changed, the quantity of information, the speed of data collection, and processing of the data have dramatically changed. The key technological changes that supported this were lower-cost computing power, lower-cost laser technology, and development of software to deconvolute a spectral interferrogram to produce a full spectra for a sample at the rate of several full spectra "snapshots" per second. Research using new statistical software to extract information from a full mid-IR spectra has allowed the industry to predict the concentration of other components in milk (e.g., milk urea nitrogen) at high speed and with no chemical reagents required. However, every new component measured requires the development of a statistical prediction model for that milk component and reference samples to calibrate and control the quality of the results of the mid-IR predictions for that component.

Which Calibration Approach: Filter or Spectral?

Instruments with optical filters can only use the traditional fixed filter calibration approach for measuring fat, protein, and lactose contents of milk. Other milk components (e.g., total solids, solids-not-fat, and other solids) can be calculated from the primary measurements of fat, protein, and lactose. Fourier Transform mid-IR milk analyzers (no optical filters) can use either the traditional fixed filter approach by creating "virtual" filters (Kaylegian et al., 2009a,b) in the software or use what have been called spectral (or full spectrum) calibrations. There are advantages and disadvantages of each approach. Most owners of FT instruments do not realize that a combination of both approaches can be used on the same instrument for different purposes.

If only fat, protein, and lactose measurements are needed, then I would recommend that a traditional fixed filter approach be used, but with optimized wavelengths and bandwidths for virtual filter approach, as defined bv Kaylegian et al. (2009a,b). Center wavelengths and bandwidths need to specified. Research has been completed to optimize the wavelengths

(Kaylegian, 2009a), and a summary of those wavelengths and intercorrection factors are given in Table 1. Once the virtual filter wavelengths are set, they do not need to be changed. A further advantage of using a FT instrument in virtual filter mode, compared with a optical filter instrument, is that the filters are consistent from one instrument to another and have no risk for physical movement or mechanical damage to which optical filters are susceptible. The standard precalibration and calibration procedures can be followed (Barbano and Clark, 1989; Lynch et al., 2006). The advantage of this approach is that as characteristics of the instrument change (i.e., output of the light source, cuvette pathlength, and detector), the gain of the primary signal at the defined wavelengths can be adjusted to compensate for these changes. Most instrument owners are not aware that the main milk components can be measured using this approach on an FT instrument, while other new and/or minor components can be measured using full spectra calibrations within the same instrument. No research has been able to demonstrate that the full spectra calibration approach performs better than the traditional approach (Kaylegian et al., 2009b) for measurement of fat, protein, and lactose. The disadvantage of the spectral approach is that if it does not perform well on an instrument, the user cannot modify it because it was developed by the instrument manufacturer and is not modifiable by the user.

The strength of full-spectrum calibrations is that minor milk components have the possibility of being measured by using advanced statistical approaches (e.g., partial least squares regression, principal component analysis, etc.) to develop new calibrations for these milk components. This makes the potential of a FT mid-IR instrument more open ended with respect to new analytical capabilities. Measurement of milk urea nitrogen is an example of a minor milk component that requires a full spectral calibration. While the accuracy and repeatability of measurement of minor components is not as good as the accuracy and repeatability of measurements of fat, protein, and lactose used in milk payment testing, the measurement of minor components has the potential to provide information that is suitable for feeding management decisions and genetic selection that will improve the profitability of dairy farms.

In the end, both fixed filter and spectral calibration approaches require calibration samples for each parameter to be measured. The more components measured, the more calibration samples required. Infrared milk analysis is a secondary method and periodic verification, control, and calibration are required. The extreme case with respect to work and cost of calibration is from components used for payment; however, if the reference testing need to produce calibration samples for a minor component is expensive, then the cost of calibration and control of those components can be a significant cost of testing.

What Are New Central Testing Laboratory Opportunities?

Assuming most DHIA labs are already measuring milk urea nitrogen and they have FT instruments, then the 2 most commonly mentioned new opportunities in automated milk analysis are milk composition indices of ketosis and milk fatty acid composition. One question related to the utility of these milk composition parameters is the frequency of measurement and the return of data to the dairy farm in a timely manner for decision making. The frequency of DHIA sampling on many farms has been decreased to reduce testing costs. This works against the timeliness of data on these new components if they are going to be useful for feeding management decisions.

Also, there is a question of whether this analysis is needed on individual cow samples or if more frequent testing (every pickup) on bulk tank samples would be useful. It seems that the distinction

between a dedicated milk payment testing laboratory and a dedicated DHIA laboratory may be more blurred in the future. Some DHIA labs are starting to do payment testing, and I expect that some milk payment labs may start doing testing for milk components useful in farm management on bulk tank milks and selected individual cows or milking strings. The fact that the milk payment labs use unpreserved milk samples may be important because the preservatives used in some DHIA labs may interfere with measurement of minor milk components when using the full spectral calibration approach.

The measurement of milk fatty acid composition gets complicated pretty quickly because of the large number of fatty acid parameters that could be measured. Each different measurement would require development of separate statistical prediction models and calibration maintenance is required for each prediction. The current work in our research group has developed models for the major individual fatty acids, groups of fatty acids (e.g., *denovo*, mixed origin, and preformed), and we are trying to develop models for C18:1 *trans* 10 and C18:1 *trans* 11 fatty acids. We have prediction models for total *cis* and total *trans* unsaturation. These are all expressed on a grams of fatty acid per 100 grams of milk basis. We also can express the output data on grams per 100 grams of fatty acids. There are several other parameters, such as fatty acid chain length (i.e., mean carbon number) and degree of unsaturation (i.e., double bonds per fatty acid), for which we have been able make prediction models.

For example, one could predict C16:0, C18:0, and C18:1, but how would a dairy farmer or nutrition consultant use this information to make management decisions? Development and maintenance of calibration models for fatty acid predictions is time consuming and costly. However, from a technical perspective, sometimes the analytical quality of some of the fatty acid results is surprisingly good. The challenge in my opinion is to focus on what will be useful for feeding management decision making. What about a milk fat depression index based on fatty acid composition data from IR analysis? If we had milk fat depression index information on a large number of cows, could we identify a relationship between susceptibility to milk fat depression and a particular genetic profile? Could an energy balance index be predicted using IR? It seems these indices would be of more practical value for feeding management than a long list of fatty acids. Developing these approaches will require a collaborative research effort by both milk analysts and feeding management experts.

Are We Ready for a Shift in Milk Testing Paradigm?

The increase in dairy farm size and management approaches used on large dairy farms is changing the North American dairy industry. Will we have large central DHIA milk testing labs in 20 years? Will fat and protein testing on large farms shift to new technology that integrates into the milking system and provides fat and protein tests on every cow plus a milk weight at every milking? This would eliminate the cost and time delay created by collection and transport of samples to a remote laboratory. There are already systems developed and available using near-IR to make real time milk composition measurements during milking. Other new on-farm milk testing technologies are likely to emerge. Will the DHIA system be part of this change or will they be sitting on the sideline wondering what happened? Will the more detailed IR milk testing (e.g. fatty acid analysis, MUN, etc.) be done on bulk tank milk samples and groups of cows at a similar stage of lactation and some feeding management information within a herd provided by payment testing laboratories, while individual cow fat and protein testing will be integrated into the milking system on farm?

Conclusions

More than ever before, management of dairy cows to maximize production efficiency, profitability, and minimize environmental impact is an information intensive system. Control of the accuracy of individual cow milk weight measurements and ensuring that milk samples are representative are both critical in support of feeding and genetic selection decision making. Having more high quality data that are available almost immediately will allow more rapid dairy management decision making for improved profitability. Methods for measurement of milk composition on farm for fat and protein will continue to improve in quality and will become more common in the future.

References

Barbano, D.M., and J.L. Clark. 1989. Infrared milk analysis: Challenges for the future. J. Dairy Sci. 72: 1627-1636.

Barbano, D.M., J.L. Clark, and C.E. Dunham. 1988. Comparison of the Babcock and ether extraction methods for determination of fat content of milk: Collaborative Study. J. Assoc. Off. Anal. Chem. 71:898-914.

Barbano, D.M., and J.M. Lynch. 2006. Major advances in testing of dairy products: Milk component and dairy product attribute testing. J. Dairy Sci. 89:1189-1194.

Biggs, D.A., and D. McKenna. 1989. Alternative methods for infrared analysis of fat in milk: Interlaboratory study. J. AOAC 72:724– 734.

Goulden, J.D.S. 1964. Analysis of milk by infrared absorption. J. Dairy Res. 31:273–284.

Kaylegian, K.E, G.E. Houghton, J.M. Lynch, J.R. Fleming, and D.M. Barbano. 2006a. Calibration of infrared milk analyzers: Modified milk versus producer milk. J. Dairy Sci. 89:2817-2832.

Kaylegian, K.E. , J.M. Lynch, G.E. Houghton, J.R. Fleming, and D.M. Barbano. 2006b. Modified versus producer milk calibration: Mid-infrared analyzer performance validation. J. Dairy Sci. 89:2833-2845.

Kaylegian, K.E., J.M. Lynch, J.R. Fleming, and D.M. Barbano. 2009a. Influence of fatty acid chain length and unsaturation on mid-infrared milk analysis. J. Dairy Sci. 92:2485-2501.

Kaylegian, K.E., D.A. Dwyer, J.M. Lynch, D.E. Bauman, J.R. Fleming, and D.M. Barbano. 2009b. Impact of fatty acid composition on the accuracy of mid-infrared fat analysis of farm milks. J. Dairy Sci. 92:2502-2513.

Lynch, J.M., D.M. Barbano, D.E. Bauman, G.F. Hartnell, and M.A. Nemeth. 1992. Effect of a prolonged release formulation of N-methionyl bovine somatotropin (sometribove) on production and composition of milk fat. J. Dairy Sci. 75:1794-1809.

Lynch, J.M., D.M. Barbano, M. Schweisthal, and J.R. Fleming. 2006. Precalibration evaluation procedures for mid-infrared milk analyzers. J. Dairy Sci. 89:2761-2774.

Sjaunja, L.O. 1984a. Studies on milk analyses of individual cow milk samples. I. Infrared spectrophotometry for analysis of fat, protein and lactose in milk. Acta Agric. Scand. 34:249–259.

Sjaunja, L.O. 1984b. Studies on milk analyses of individual cow milk samples. II. Factors affecting milk analyses by infrared technique under laboratory conditions. Acta Agric. Scand. 34:260–272.

Table 1. Sample and reference center wavelengths and bandwidths and expected intercorrection factors for the optimized virtual filter center wavelengths and bandwidths.[1]

Primary Component	Sample Center	Sample Bandwidth	Reference Center	Reference Bandwidth	Sample Center	Sample Bandwidth	Reference Center	Reference Bandwidth	Intercorrection factor for secondary component: Fat B	Lactose	Protein	Fat A
	—— Wavelength (μm) ——				—— Frequency (cm^{-1}) ——							
Optimized FT MIR center wavelengths and bandwidths (i.e., minimized size of intercorrection factors).												
Fat B	3.508	0.032	3.556	0.030	2851	26	2812	24	1.000	-0.160	-0.065	...
Lactose	9.542	0.182	7.734	0.084	1048	20	1293	14	0.038	1.000	0.015	...
Protein	6.489	0.085	6.707	0.054	1541	20	1491	12	0.065	0.050	1.000	...
Fat A	5.721	0.052	5.583	0.050	1748	16	1791	16	...	0.030	0.025	1.000

[1]FT = Fourier Transform and MIR = mid-infrared.

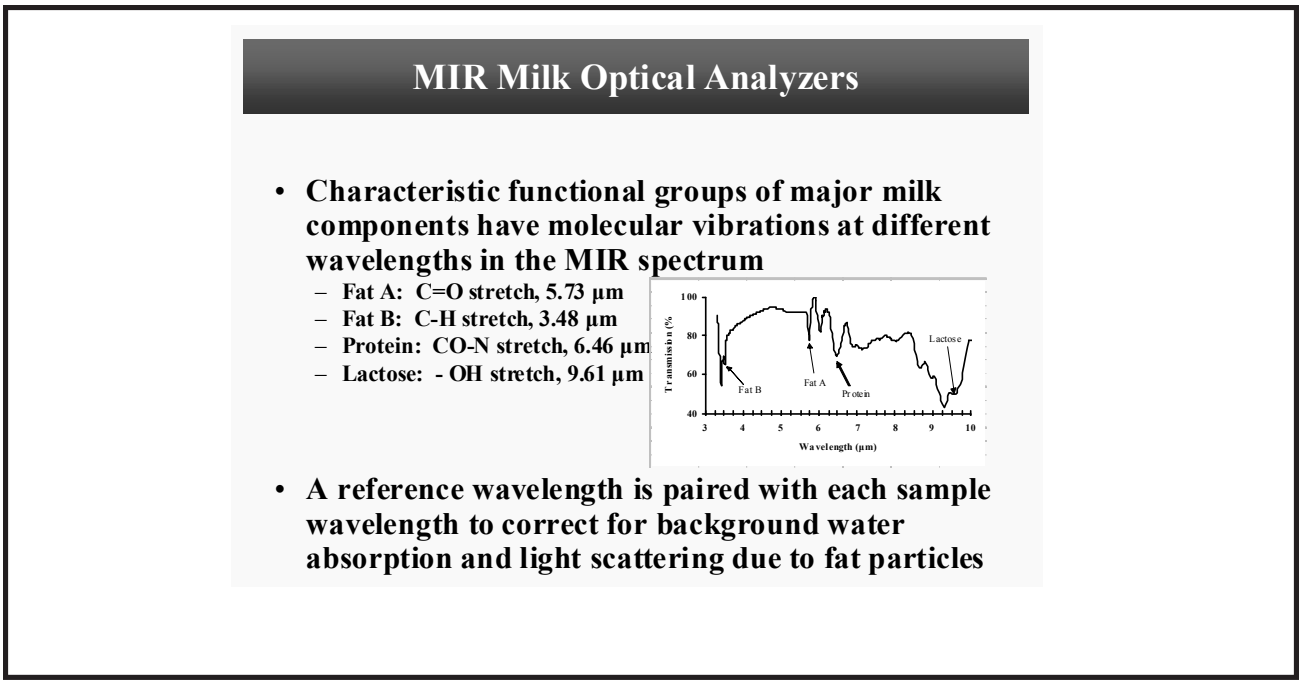

Figure 1. Mid-infrared (MIR) spectra of milk with background absorbance of water subtracted out. Sample wavelengths are indicated.

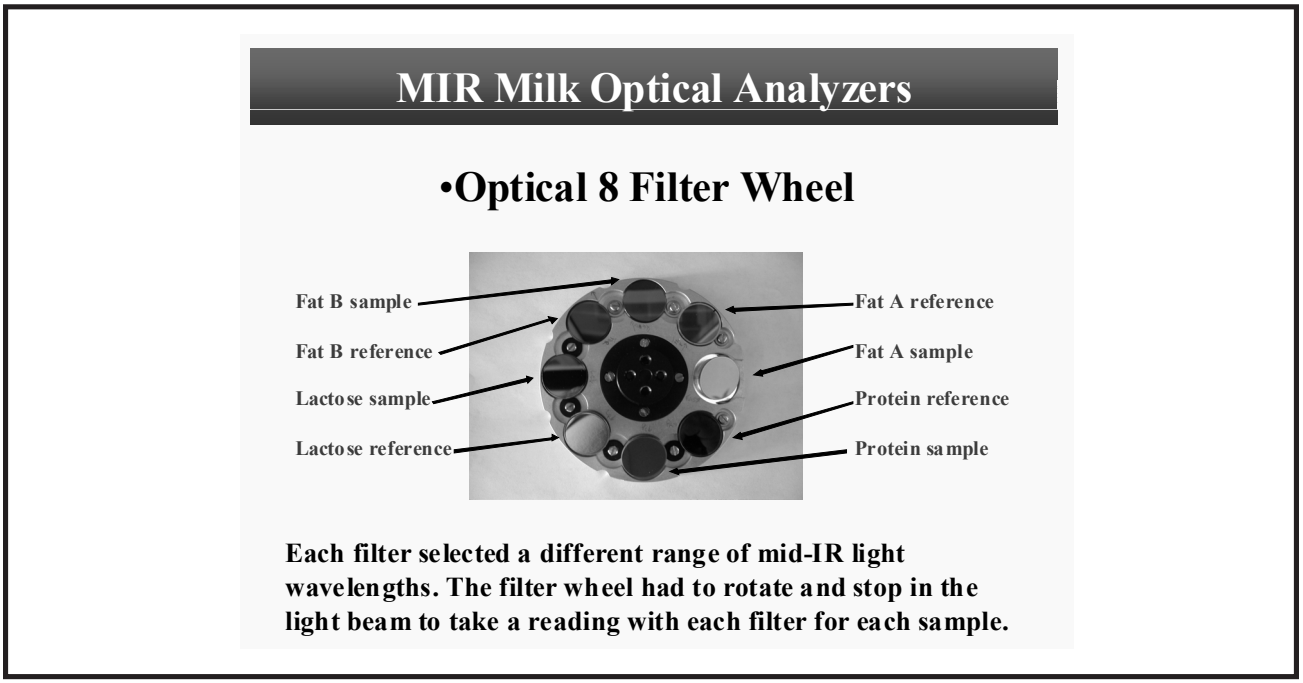

Figure 2. An optical filter wheel removed from a mid-infrared (MIR) milk analyzer. Each sample filter is paired with a reference filter.