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REVIEWS

Analysis of Dairy Products by Near-Infrared Spectroscopy: A Review

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Near-infrared spectroscopy analysis of foodstuffs is a relatively recent technique. Its principal advantage is speed of analysis, and it does not require sample pretreatment either. In this paper we review its application to powder dairy products, liquid milk, cheese, butter, and fermented milk products, mainly from the analysis of the major components point of view and also for the detection of adulterations and other determinations as well.

Keywords: *Near-infrared spectroscopy; dairy products*

INTRODUCTION

The technological processes used in the dairy industry have made very important progress in recent years. This fact combined with the increase in interest by quality on the part of consumers, the availability of laboratories of control in factories, and the regulatory arrangements to which the marketed products have to be adjusted have contributed to the sensibilization toward the control of raw materials, processes, and finished products. In relation to elaborated products, one must consider in a special way the composition characteristics, which have so much interest from different points of view: nutritional aspects, processing control, and yields.

The traditional analysis methods of major food components like fat, protein, and moisture are slow and expensive and need highly qualified staff. At present, with the great development of the food industry, a great number of analyses from both the raw material and the elaborated products is required. The chemical methods are not effective enough to cover the growing demand and low costs that industry needs. To comply with this request, a great number of instrumental analytical techniques has been developed.

Within the field of dairy science and concretely milk analysis, methods based on the turbidity and dye binding for the fat and protein determination, respectively (Casado and Blanco, 1978; van Reusel and Oger, 1987; van Reusel and Klijn, 1987; Grappin et al., 1991), have been used, but the technique that indeed revolutionized the dairy laboratories was middle-infrared spectroscopy (Andersen et al., 1986; Biggs et al., 1987; Grapin et al., 1991), which shows, as great advantages, the simultaneous determination of fat, protein, and lactose in a unique sample without previous treatment, the rapidity (up to 400 samples/h), the absence of chemical reactives, and the total automation of the process. However this technique, which displaced all

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those previously used for the determination of the major components of liquid milk and which is currently a method approved by the IDF (Standard 141A, 1990), APHA (1992), and AOAC (1995), does not solve the problem for milk powder and other dairy products.

Near-infrared spectroscopy upon presenting inferior absorption bands in, at least, 1 order of magnitude for each successive overtone allows the use of moderately greater concentrated samples and greater optical paths than those used in middle infrared spectroscopy. This means that the transmission spectrum of some materials can be obtained without previous treatment of the sample. However, its greater advantage for food analysis lies in the possibility of obtaining reflectance spectra for opaque and intact materials. In this way various food constituents can be determined in a time not longer than 1 min and without previous preparation of the sample. The near-infrared spectrophotometers can measure the light transmitted through a sample or reflected by its surface (Giangiacomo and Nzabonimpa, 1994).

The near-infrared region was traditionally avoided by the spectroscopists due to the difficult interpretation of band overlapping since acute peaks do not appear in this region of the spectrum as they do in middle infrared, due to its much lower sensibility than that of middle infrared (in the order of 10−100 times smaller), not to mention how difficult assigning the bands to the different functional groups proved to be due to the numerous overtones and combination bands (Burns and Margoshes, 1992; Giangiacomo and Nzabonimpa, 1994).

For the last decades near-infrared spectroscopy has become more popular due to a series of factors like the great advance in data processing technology and computer programming which made possible the treatment of the spectral information obtained by the instruments, the development experimented by the multivariate analysis techniques (Martens and Naes, 1993), and the advance in the construction of the spectrophotometers (better sources of radiation, detectors, and optical parts in general). The conjunction of all these factors made possible the application of near-infrared spectroscopy for the determination of major components in food for human consumption as well as in feeds and forage for animal nourishment (Osborne et al., 1993). Recent developments make near-infrared spectroscopy coupled with optical fiber probes a suitable technique for on-line control in the food industry. The development of near-infrared spectroscopy in the last years takes form in the number of published articles (Burns and Margoshes, 1992):

years	articles
1930−1940	3
1940−1950	4
1950−1960	23
1960−1970	23
1970−1980	152
1980−1990	>1000

APPLICATION TO DAIRY PRODUCT ANALYSIS

The near-infrared spectrum of casein, fat, lactose, and powdered milk was obtained for the first time by Goulden (1957) who used several wavelengths to determine the composition of the sample. Thereafter Ben Gera and Norris (1968) studied the influence of the fat content of milk in its near-infrared spectrum and applied, for the first time in this technique, multiple linear regression (MLR) to determine each component.

Casado et al. (1978) analyzed fat, protein, and moisture in milk powder, with standard error of calibration values (SEC) of 0.125, 0.193, and 0.095 for fat, protein, and moisture, respectively. But it was Verbiese (1981) who determined for the first time the moisture of milk powder in the production line on a conveyor belt using optical filter equipment and taking the measure at 1.94 μm which corresponds to the principal water absorption band in the near-infrared region and 1.80 μm as reference, for which the absorption of water is of little importance; from the results, he only indicates that the precision for both whole and skim milk was 0.1%, not indicating the number of samples, the residuals, nor the correlation coefficient. In 1983, de Vilder and Bossuyt, continuing in this working line, determined moisture, fat, and protein in milk powder with a Technicon InfraAlyzer 400 instrument supplied with 19 filters, of which six alone were used for the calibrations of the instrument by MLR. With the same Technicon equipment, Baer et al. (1983a, 1983b) determined the moisture, fat, protein, and lactose in skim milk powder and whey powder by reflectance spectroscopy, using three wavelengths for fat and lactose, four for water, and eight for protein; the calibration was also made by MLR.

In addition to fat, protein, lactose, and moisture, Frankhuizen and van der Veen (1985) analyzed by reflectance spectroscopy the lactate and ash contents from different types of milk powder of several European countries, using for this a 19-optical filter equipment and making the calibrations by MLR.

Near-infrared spectroscopy has also been successfully used for the classification of skim milk powders according to the thermal treatment received (Downey et al., 1990).

Weaver (1984) studied the application of near-infrared spectroscopy to the analysis of liquid and skim milk, milk powder, cream, butter, and cottage cheese, using for this equipment provided with optical filters capable of measuring 20 different wavelengths and calibrated by MLR. In liquid milk, he determined the fat and total solids. For this determination he used as a reference method the value calculated from density and fat instead of the oven-drying method, the one he did use when he calibrated the equipment for the determination of moisture in skim milk powder, butter, and cottage cheese; while in the case of the cream, the calibration of the instrument was made with the Gerber method. Thereafter, Robert et al. (1987) applied principal components analysis (PCA) to the study of near-infrared spectra for liquid milk samples, relating the wavelengths of 1724, 1752, 2308, and 2344 nm to the fat content, 2050 and 2180 nm to protein, and 2094 nm to lactose. Kamishikiryo-Yamashita (1994) modified a method for protein determination in emulsions of fat/water to determine protein in liquid milk, using the absorption measurement at 2170 nm and calibrating by linear regression of the second derivative. The statistic values are SEC = 0.124, correlation coefficient $r = 0.887$, and standard error of prediction SEP = 0.177.

Near-infrared spectroscopy has been recently used for the analysis of goat and ewe milk. Díaz-Carrillo et al. (1993) quantified the fat, protein, lactose, total casein, and different casein fractions (α_s-casein, β-casein, and κ-casein) of goat milk using the calibration obtained by partial least squares (PLS), while Pascual and Molina (1994) determined, by reflectance spectroscopy, the total protein, real protein, and casein contents in liquid

samples of ewe milk with an InfraAlyzer instrument supplied with 19 optical filters, making the calibration by MLR.

Near-infrared spectroscopy has also been used for adulteration detection; Sato et al. (1990) studied the presence of strange fats in milk samples: they used butter and margarine mixtures from which they obtained the spectrum between 1100 and 2500 nm and concluded that it is a good screening method, even though subsequent confirmation of the suspected samples is necessary via chromatographic method. In that same investigation line Giangiacomo et al. (1991) used satisfactorily an Infra-Alyzer 500 instrument in a 1100−2500 nm interval to detect milk powder adulterations with whey powder, calibrating the instrument by MLR. While the results obtained by Pedretti et al. (1993) upon applying near-infrared spectroscopy to detect soluble substances that can be added to the milk like water, sodium chloride, and skim milk powder are satisfactory to detect the sodium chloride and skim milk powder additions, it is not appropiate for the detection of added water.

The application of near-infrared spectroscopy to cheese analysis is subsequent to its first applications to powdered dairy products; Frank and Birth (1982) determined the fat, protein, and moisture contents in a total of 30 samples of different cheese varieties (Cheddar, Colby, Parmesano, Romano, and Gruyere). For the determination they previously grated the sample, freeze-dried a subsample of the grated cheese, and made measurements of the grated and freeze-dried subsamples. For calibration the second derivative and multiple linear regression with three wavelengths for each component were used. Better results were obtained with the freeze-dried subsample, though in this case only fat and protein were determined and not water. Thereafter Frankhuizen and van der Veen (1985) applied near-infrared spectroscopy to the study of different Dutch cheeses (Edam, Gouda, and processed cheese). For that, once the rind was eliminated, they grated the sample through a Hobart mill and made the measurement with a Technicon optical filters Infra-Alyzer 400 instrument, at 19 different wavelengths; when calibrating by MLR they used six wavelengths for moisture determination, the most significant one corresponding to 1940 nm (characteristic band of water absorption); for protein, between six and ten wavelengths were used, including the characteristic protein bands of 2139 and 1734 nm; for fat, seven wavelengths between 2310 and 1759 nm were included. Upon using a group of different samples for prediction, they concluded that better results are obtained when the instrument is calibrated in a specific way for each variety of cheese. The Cheddar cheese moisture content, with a ripeness interval between 3 and 6 months, was determined by reflectance spectroscopy by Wehling and Pierce (1988), using three wavelengths: 1818, 1734, and 1445 nm, the measurements at 1818 and 1445 nm being proportional to the moisture content and the measurement at 1734 nm being directly related to the fat and protein contents and inversely related to moisture. The sample was introduced in the instrument in a plastic bag, after having been crumbled in cylinders of 1 mm in diameter and 20 mm in length, and the calibration was made by MLR; in spite of the influence of the ripeness time on the near-infrared spectrum, satisfactory results were obtained.

After the calibration by PLS, Molt and Kohn (1993) determined by transmission, in the region between 850 and 1050 nm, the major components of processed cheese, obtaining acceptable results for the fat, protein, and dry matter contents; to obtain the spectrum, the sample was melted before filling the measurement cell.

Klenke and Schneemann (1993) used near-infrared spectroscopy to control on-line the dry matter during a quark production process. In this same product, after testing the calibration by different mathematical algorithms (MLR, PCR, and PLS) for the determination of the major components (fat, protein, and total solids), Wüst (1994) obtained the best results by using PLS.

Pierce and Wehling (1994) studied various mathematical treatments to determine moisture and fat in cheese samples by near-infrared spectroscopy; they collected the spectra by transmittance and reflectance of previously grated samples: the smaller SEP were obtained upon using the PLS of the first derivative in the case of moisture and the PLS of the direct spectra in the case of fat. In the same way, the use of optical fiber on intact samples was tested, thus obtaining worse results than with grated samples. Rodríguez-Otero et al. (1995) determined by reflectance, without any previous handling of the sample, the content in fat, protein, and total solids of different varieties of cheese manufactured in Galicia, using sections of the same ones and calibrating the instrument by PLS after correcting the radiation dispersion with two different mathematical treatments.

Frankhuizen (1992) applied near-infrared spectroscopy to the study of ripeness age of different varieties of Dutch cheese (Edam and Gouda), with the purpose of being able to classify them in four categories: young (minimum 28 days), young-matured (minimum 2 months), matured (minimum 4 months), and extra matured (minimum 7 months), since the price of the product is related to the ripeness. As the ripeness conditions tend to vary little for each type of cheese, a relation between the degree of ripeness and the age of the cheese may exist. After calibrating the apparatus by MLR, he obtained a correlation coefficient of 0.92 and a SEC of 28 days for samples with a ripeness interval between 25 and 412 days, while the statistic values are more advantageous, $r = 0.96$ and SEC = 11 days, when testing specific calibrations for subgroups of more reduced samples with ripeness intervals of 160 days.

Rodríguez-Otero and Hermida (1996) used with success near-infrared reflectance spectroscopy to determine fat, protein, and total solids of fermented milks avoiding any pretreatment of the sample. However better results were obtained by the same authors when they analyzed the samples by near infrared transflectance spectroscopy (Rodríguez-Otero et al., 1997). In spite of the great expansion that these kind of products have shown in recent years, in the consulted literature no additional applications of near-infrared spectroscopy to fermented milks have been found.

CONCLUSIONS

Near infrared spectroscopy is an adequate technique for the analysis of major components (fat, protein, lactose, and moisture) in dairy products without sample pretreatment. It is also useful to classify milk powders according to heat treatment and as a screening method to detect adulterations of dairy products.

Better calibrations are obtained with full spectra methods like PLS than with local methods like MLR.

In our opinion the main limitation to this technique lies in its least accurate measurement of the mineral content which does not absorb in that region of the spectrum, as it is being determined indirectly.

The use of fiber optic probes to conect near infrared spectroscopy instruments with the production lines, providing real time information of the process, thus saving raw materials and improving the finish product quality, has a promising future in the dairy industry.

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