J. Dairy Sci. 89:1926–1937

Utilization of Fourier Transform Infrared Spectroscopy for Measurement of Organic Phosphorus and Bound Calcium in Cheddar Cheese

P. Upreti and L. E. Metzger[1]
MN-SD Dairy Foods Research Center, Department of Food Science and Nutrition, University of Minnesota, St. Paul 55108

ABSTRACT

The methods available for measuring organic P and bound Ca in cheese are either cumbersome or involve dilution of the cheese. Dilution of the cheese can lead to erroneous results, particularly in the case of bound Ca. Hence, the objective of this study was to evaluate the feasibility of Fourier transform infrared (FTIR) spectroscopy for direct measurement of organic P and bound Ca in Cheddar cheese. Two hundred sixteen samples of cheese were analyzed for protein-bound organic P, bound Ca using a water-extraction based method, and buffering curves. Additionally, the infrared spectra of the cheeses were collected between 4,000 and 650 cm^{-1}, at a resolution of 4 cm^{-1}, and 256 scans per sample. The spectral shifts in the infrared region from 1,050 to 900 cm^{-1}, in addition to the measured concentrations of organic P, bound Ca, and buffering peak area at pH 5.1, were used to develop calibration models using partial least squares (PLS) regression analysis. The spectral region of 956 to 946 cm^{-1} correlated with the measured concentrations of organic P and the overall PLS model had a correlation (R^2) of 0.76 between the predicted and measured concentrations. The spectral region at ~980 cm^{-1} was correlated with the measured concentrations of bound Ca, and the overall PLS model had a correlation (R^2) of 0.70 between the predicted and measured concentrations. A similar spectral region at ~980 cm^{-1} was also correlated with the measured buffering peak areas and the overall PLS model had a correlation (R^2) of 0.64 between the predicted and measured peak areas. A linear regression analysis between the bound Ca and buffering peak area demonstrated that bound Ca was correlated ($R^2 = 0.73$) with buffering peak area. This study demonstrates that FTIR can be used to measure organic P in cheeses. It also has the potential to be used for measuring bound Ca in undiluted cheeses, and for prediction of the buffering capacity of cheese.

Key words: calcium, phosphorus, Fourier transform infrared spectroscopy, buffering

INTRODUCTION

Calcium and phosphorus exist in different forms in Cheddar cheese. The portion of the Ca and P that is associated with the serum phase of cheese is referred to as soluble Ca and P, whereas the portion that is associated with casein is called bound Ca and P. Bound P in cheese can be further divided into 2 categories. The first category includes P that is covalently linked to the protein as phosphoserine residues, and is referred to as organic P. The second category of bound P (referred to as bound-inorganic P), in addition to bound Ca, constitute the inorganic constituents trapped in the structural network of casein (Schmidt, 1980). These inorganic constituents in caseins interact with phosphoserine residues of casein (organic P) and act as a cross-linking agent within casein micelles (Aoki et al., 1987).

The importance of total Ca and P content in determining the texture and physicochemical characteristics (proteolysis, pH) of cheese has been well recognized (Geurts et al., 1972; Lawrence et al., 1984; Kimura et al., 1992; Lucey and Fox, 1993; Metzger et al., 2001; Pastorino et al., 2003). However, as mentioned earlier, only a fraction of the total Ca and P (i.e., bound Ca and P) plays a role in cross-linking protein. Hence, studies based on this hypothesis have successfully demonstrated that distribution of Ca in cheese influences its textural characteristics (Metzger et al., 2001; Hassan et al., 2004). However, the importance of bound P, particularly organic P, in defining cheese characteristics has been studied to a lesser extent. Studies conducted on caseins have indicated that organic P is important for several functional interactions in food systems in which caseins are involved (Yamauchi and Yoneda, 1978; Van Hekken and Strange, 1993; Van Hekken et al., 1996). It appears that a hindrance in investigating the influence of organic P or bound Ca on cheese characteristics is the cumbersome analytical methods used for measuring these constituents in cheese.

In the absence of a method to measure organic P in cheese, a method was recently developed in our laboratory (see Materials and Methods) based on a previously

Received October 17, 2005.
Accepted January 10, 2006.
[1]Corresponding author: lmetzger@umn.edu

suggested fractionation technique for measuring organic P in milk (Jenness and Patton, 1959). However, the method developed for cheese involves multiple extraction and ashing steps, and takes at least 2 d to analyze a sample. Therefore, a rapid and less cumbersome method would be valuable. In contrast, numerous techniques for measuring bound Ca in Cheddar cheese have been described in the past, but each method has its own limitations. Morris et al. (1988) suggested a method for determining bound Ca by extraction of the aqueous phase of cheese by pressing. In this method, the grated cheese is mixed with sand, packed in a perforated hoop, and pressed (32 MPa) at room temperature for 1 h. The Ca content in the liquid that is extracted from the cheese is the soluble Ca content of cheese. Later, a water-extraction method, based on previous water-extraction methods (Sinha et al., 1979; Kimura et al., 1992), was developed and used to evaluate the bound and soluble Ca in Mozzarella cheese (Metzger et al., 2001). A more recently developed method uses acid-base buffering curves to measure the bound Ca in cheeses (Hassan et al., 2004). This method is based on the principle that bound Ca in cheese is related to its pH buffering peak at pH 5.0. The titration method proposed by Hassan et al. (2004) for bound Ca measurement also requires measurement of the pH buffering curve of the milk used for cheese manufacture. However, it may be possible to use only the buffering peak of the cheese and still obtain an accurate estimate of bound Ca. A common limitation with the analytical methods based on water extraction and acid-base buffer curves is that they are time consuming, and more importantly, involve dilution of the cheese. Dilution of the cheese can alter the distribution of Ca, which would lead to erroneous results for bound Ca. Therefore, none of the methods developed to date has been universally accepted. The ability of the latter 2 methods to explain observed physicochemical phenomena in cheese indicates that they are related, although a study comparing the 2 methods for bound Ca has never been conducted. Nonetheless, a rapid method of analysis that does not involve dilution or numerous steps would be useful.

Graves and Luo (1994) used Fourier transform infrared (**FTIR**) spectroscopy to identify phosphates in proteins, and suggested that FTIR spectroscopy could be used to obtain information about the ionization state of phosphate esters and their binding with metal ions. Van Hekken and Dudley (1997) also used FTIR to study the covalently bound phosphate (i.e., organic P) in caseins. In a more recent study, Fernandez et al. (2003) used FTIR spectroscopy to unravel the changes in the interactions of phosphate ester bonds in a model α_s-casein system on precipitation by chitosan. They demonstrated that the dianionic symmetric stretching band of the covalently bound phosphate (organic P) in α_s-casein at 976 cm^{-1} is sensitive to changes in the ionization state of phosphate and electrostatic interactions with Ca ions. This study prompted us to investigate the possibility of utilizing the IR region of 1,050 to 900 cm^{-1}, as identified by Fernandez et al. (2003), for measurement of organic P and bound Ca in cheese.

Infrared spectroscopy has been used for several decades to quantify fat, protein, and lactose in milk (Adda et al., 1968; Biggs, 1972). With the developments in spectroscopic instrumentation and computational ability to mathematically manipulate the spectra, FTIR spectroscopy has gained popularity for food analysis (van de Voort, 1992). Although FTIR spectroscopy offers a high signal-to-noise ratio and a significantly reduced scan time (Ingle and Crouch, 1988), it poses sample handling challenges (Chen and Irudayaraj, 1998), and complicated spectral analysis (van de Voort, 1992). Because of the texture and opacity of cheese, it is difficult to analyze cheese spectroscopically using a traditional transmission sample handling technique. Hence, the use of an attenuated total reflectance (**ATR**) sample handling technique is more appropriate for cheese samples. Use of ATR-FTIR requires minimal sample preparation; and variations in sample thickness do not affect the intensity of the bands (Ingle and Crouch, 1988). Fourier transform infrared spectra collected from a complex sample matrix like Cheddar cheese are difficult to interpret using simple linear regression models because the spectra may consist of broad overlapping bands of various other substances that are present. Additionally, the number of data points in the spectra necessitates use of multivariate statistical tools, such as partial least squares (**PLS**) regression, to extract relevant information from the spectral data (van de Voort, 1992). A large number of samples that cover a wide range in variability of the desired attribute will contribute to the robustness of the calibration model.

The objective of the present study was to determine if ATR-FTIR spectroscopy and available multivariate statistical tools could be used to measure organic P and bound Ca in cheese. Also, the relationship between bound Ca measured by a water-extraction method and the area of the pH buffering peak at pH ~5.1 of cheese was assessed.

MATERIALS AND METHODS

Experimental Design

Cheese samples used in the study were obtained from 3 replicates of Cheddar cheeses that were manufactured with 2 levels (high and low) of Ca and P, residual lactose, and salt-to-moisture ratio (**S/M**). The 8 different treatments included high Ca and P-high lactose-high