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Technical note: Feasibility of near infrared transmittance spectroscopy to predict cheese ripeness

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ABSTRACT

The aim of the study was to evaluate the feasibility of near infrared (NIR) transmittance spectroscopy to predict cheese ripeness using the ratio of water-soluble nitrogen (WSN) to total nitrogen (TN) as an index of cheese maturity (WSN/TN). Fifty-two Protected Designation of Origin cow milk cheeses of 5 varieties (Asiago, Grana Padano, Montasio, Parmigiano Reggiano, and Piave) and different ripening times were available for laboratory and chemometric analyses. Reference measures of WSN and TN were matched with cheese spectral information obtained from ground samples by a NIR instrument that operated in transmittance mode for wavelengths from 850 to 1,050 nm. Prediction equations for WSN and TN were developed using (1) cross-validation on the whole data set and (2) external validation on a subset of the entire data. The WSN/TN was calculated as ratio of predicted WSN to predicted TN in cross-validation. The coefficients of determination for WSN and TN were >0.85 both in cross- and external validation. The high accuracy of the prediction equations for WSN and TN could facilitate implementation of NIR transmittance spectroscopy in the dairy industry to objectively, rapidly, and accurately monitor the ripeness of cheese through WSN/TN.
Key words: chemometric, cheese quality, ripening time, water-soluble nitrogen

Technical Note

During ripening, cheese undergoes changes in organoleptic and chemical-physical attributes, promoting specific tastes, textures, and flavors. These characteristics are evaluated mainly by cheese factory technicians through sensory analysis, which is a subjective method. Variations during ripening depend on proteolysis, lipolysis, and glycolysis, with proteolysis being the most important process (García-Palmer et al., 1997; McSweeney and Fox, 1997), indicating that chemical analysis is useful to assess cheese ripeness.

Cheese proteolysis involves the degradation of caseins, which are insoluble in many solvents, into large, medium-sized, and small peptides and free AA, which are soluble fractions. Consequently, peptides and free AA increase during the ripening period. These soluble fractions are determined through use of different solvents (McSweeney and Fox, 1997; Moatsou et al., 2002; Panari et al., 2003) and they can be used as objective indicators of cheese ripening. Traditionally, the ratio of water-soluble nitrogen (**WSN**) to total nitrogen (**TN**) has been adopted as an objective index to evaluate cheese ripeness (Innocente, 1997; Mazerolles et al., 2001; Moatsou et al., 2002). Chemical determination of TN and WSN is labor intensive and expensive, and it requires sample alteration. Therefore, the dairy industry and cheese technicians are interested in having a standardized, low-cost, fast, and reliable method to determine cheese ripeness.

Infrared spectroscopy offers a quick, easy to manage, low-cost, nondestructive, and chemical-free analysis, and it can be used to determine several traits concurrently. It is routinely used in the dairy industry to predict gross composition (fat, protein, and moisture content) of the products. Some authors have attempted to predict cheese age (Downey et al., 2005; Fagan et al., 2007), WSN (Karoui et al., 2006a,c; Fagan et al., 2007), TN (Karoui et al., 2006a,b), and WSN/TN (Mazerolles et al., 2001; Karoui et al., 2006a) using near infrared (**NIR**) reflectance (Downey et al., 2005; Karoui et al., 2006c) or mid-infrared reflectance (**MIR**; Mazerolles et al., 2001; Karoui et al., 2006a; Fagan et al., 2007) spectroscopy, but within a restricted ripening time (<12 mo). The aim of the present study was to develop a NIR transmittance spectroscopy prediction model to objectively determine ripeness of several cheeses.

A total of 52 Protected Designation of Origin (**PDO**) cow milk cheeses of 5 varieties—Asiago, Grana Padano, Montasio, Parmigiano Reggiano, and Piave—were chosen according to the most produced, purchased, and ap-

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Table 1. Least squares means ± SE (g/100 g of cheese) of moisture, fat, protein, and salt contents across Protected Designation of Origin (PDO) cheese variety and ripening time

Item	n	Moisture	Fat	Protein	Salt
PDO cheese variety					
Asiago	8	31.17 ± 1.60[a]	35.24 ± 1.04[d]	27.30 ± 0.90[a]	1.52 ± 0.11[a]
Grana Padano	22	35.05 ± 0.96[a]	27.53 ± 0.62[a]	33.18 ± 0.54[b]	1.52 ± 0.06[a]
Montasio	7	30.73 ± 1.71[a]	35.30 ± 1.11[cd]	28.11 ± 0.96[a]	1.60 ± 0.11[a]
Parmigiano Reggiano	7	30.67 ± 1.71[a]	30.75 ± 1.11[abc]	33.77 ± 0.96[b]	1.75 ± 0.11[a]
Piave	8	30.69 ± 1.60[a]	35.05 ± 1.04[bd]	29.52 ± 0.90[a]	1.65 ± 0.11[a]
Ripening time, mo					
1 to <3	13	36.89 ± 1.03[c]	28.72 ± 1.21[a]	29.94 ± 0.90[a]	1.41 ± 0.08[a]
3 to <12	20	33.39 ± 0.83[b]	31.74 ± 0.98[ac]	29.87 ± 0.73[a]	1.59 ± 0.06[ac]
12 to 40	19	28.86 ± 0.85[a]	32.75 ± 1.00[bc]	33.21 ± 0.74[b]	1.70 ± 0.06[bc]

[a–d]Means with different letters within trait and item (PDO cheese variety or ripening time) are significantly different ($P < 0.05$).

preciated Italian PDO products (Gambelli et al., 1999; CLAL, 2015), including a wide ripening time (from 1 to 40 mo; Table 1). Samples were acquired from commercial stores and from the dairy industry, and they were sent at refrigeration temperature to the laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Italy) for analyses. After removal of the rind (1.5 cm from the outer surface), each cheese sample was homogenized by using a knife mill (Retsch Grindmix GM200, Retsch GmbH & Co, Haan, Germany). Ground samples were kept in a sealed plastic bag at 4°C and analyzed within 24 h to avoid variations in cheese composition depending on moisture loss.

Cheese moisture, protein, fat, and salt amounts were determined using FoodScan Dairy Analyzer (Foss Electric A/S, Hillerød, Denmark) precalibrated with Foss Artificial Neutral Networks Dairy Calibration. The WSN fraction was obtained with a dilution of 4 g of each ground sample into 100 mL of distilled water at 40°C (Gazzetta Ufficiale, 1986) and homogenized using an Ultra Turrax PT 2500 homogenizer (Polytron Kinetica, West Yorkshire, UK) at 242 × g for 30 s. After an overnight rest, the sample was filtered and analyzed by using the Kjeldahl method and a Tecator Kjeltek 2300 Analyzer Unit (Foss Electric A/S). The amount of TN was determined using the Kjeldahl method by digesting 1 g of the ground sample (Gazzetta Ufficiale, 1986). The WSN/TN ratio was used as index of cheese ripening. Spectra of ground cheeses were recorded by means of FoodScan Dairy Analyzer (Foss Electric A/S), which operated in transmittance mode from 850 to 1,050 nm every 2 nm. Each spectrum was obtained by averaging 16 sub-spectra recorded at different points during automatic rotation of the Petri cup (10 cm internal diameter) filled with the sample. Spectrum was recorded as log(1/transmittance). An ANOVA followed by Bonferroni's test for multiple comparisons was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC) to test whether moisture, protein, fat, and salt contents differed across cheese varieties and ripening time. Significance was set at $P < 0.05$, unless otherwise indicated.

Prediction equations for TN and WSN were developed for each trait using (1) the whole data set (n = 52) or (2) a subset of the entire data (calibration set) using modified partial least squares (**MPLS**) regression analysis (WinISI III v. 1.60; Foss and Infrasoft International LLC, State College, PA), which is more accurate and stable than the standard partial least squares approach (González-Martín et al., 2011). The algorithm for MPLS considers the standardized residuals after each factor before moving to the next factor (Meagher et al., 2007). The predicted WSN/TN was calculated as ratio of predicted WSN to predicted TN, which was obtained through the model developed for each trait on the whole data set. For an easy implementation of WSN/TN in the dairy industry, a prediction equation for WSN/TN was also developed. The goodness of prediction equations obtained for the whole data set were assessed through cross-validation, where one-fifth of the samples, randomly chosen, were temporarily removed from the initial data set. This procedure was repeated until all subgroups were treated both as calibration and prediction set. The goodness of the prediction equations developed using the calibration set were evaluated by means of external validation. To create the calibration and validation set, the complete data set was split into 2 subsets with comparable mean and standard deviation (**SD**) for each trait. The calibration set (75% of the samples) was used to develop the prediction models, and the validation set (25% of the samples) was used to validate the calibration. Several combinations of scattering correction [no correction; detrend (D); standard normal variate (**SNV**); SNV + D; and multiplicative scatter correction (**MSC**)] and mathematical treatment (0,0,1,1; 1,4,4,1; 1,8,8,1; 2,5,5,1; and 2,10,10,1, with the first digit representing