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Prediction of Dry Matter, Fat, pH, Vitamins, Minerals, Carotenoids, Total Antioxidant Capacity, and Color in Fresh and Freeze-Dried Cheeses by Visible-Near-Infrared Reflectance Spectroscopy

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Visible-near-infrared reflectance spectroscopy was used to predict dry matter, fat, pH, retinol, α-tocopherol, β-carotene, xanthophylls, sodium chloride, calcium, potassium, magnesium, zinc, total antioxidant capacity, brightness, redness, and yellowness in both fresh and freeze-dried cheeses. A total of 445 cheeses of four cheese varieties were investigated. Composition of samples was analyzed by reference methods. Samples were scanned (400−2500 nm) and predictive equations were developed using modified partial least-squares with both cross-validation and external validation. Coefficient of determination (R^2) and residual predictive deviation (RPD) in external validation were satisfactory for dry matter (0.97; 5.99), fat (0.90; 3.22), β-carotene (0.92; 3.43), sodium chloride (0.89; 2.94), calcium (0.95; 4.62), Zinc (0.93; 3.75), brightness (0.96; 4.88), redness (0.96; 5.23), and yellowness (0.93; 3.73) in fresh cheeses. Poor predictions were obtained for pH, retinol, α-tocopherol, xanthophylls, potassium, magnesium, and total antioxidant capacity ($R^2 < 0.81$; RPD < 3).

KEYWORDS: Near infrared spectroscopy; cheese; vitamins; carotenoids; minerals; color

INTRODUCTION

Cheese represents a large part of the total consumption of dairy products in countries such as France, Greece, or Italy (*1*) and in higher socioeconomic classes (*2*). The consumption of cheese is of great nutritional interest due in particular to its composition of micronutrients. Cheese is an important source of minerals (calcium, phosphorus, and zinc) and vitamin A. It also contains nonessential constituents such as carotenoids, which could favorably affect human health (*3*). Beyond their nutritional interest, carotenoids also influence the color of dairy products, which is important to the acceptance of these products by consumers (*4*).

The nutritional composition of cheese strongly varies both between the different cheese varieties and within each cheese variety due to the influence of the conditions of milk production and the cheese-making process (*5, 6*). Accurate and reliable information of consumers about the nutritional characteristics of cheese requires therefore frequent measurements of its composition. However, the methods for measuring the micronutrients are generally time-consuming and expensive. Many of them involve chemical steps such as saponification, esterification, or enzyme treatments and the extraction of the nutrients by solvents.

Visible and near-infrared reflectance spectroscopy (VIS/NIRS) can be an alternative technique to the current methods used for the quantification of nutrients. It is nondestructive, rapid, cheap, and multiparametric. It has been successfully used to quantify the gross composition in moisture, proteins, fat, and total solids of cheese (*7–9*), pH (*10*), sensory attributes (*11*), free aminoacids (*12*), and color parameters (*13*). The global information contained in the near-infrared (NIR) spectra of foods is related not only to the macronutrients but also to the interactions that they can develop with other constituents. Some studies have thus shown the ability of this method to quantify the composition of vitamins, carotenoids, and minerals of foods (*14, 15*). According to Jiménez (*15*), the most representative absorbance bands of carotenoids in olive oil are situated in the VIS region, in regions related with the absorption of C−H bond from the aromatic groups (1625−1875 nm and 1125−1250 nm), and also in regions related with the combination bands of the C−H bond of unsaturated groups beyond 2100 nm. Brenna and Berardo (*16*) also found high correlation coefficients in maize between the region of 1150−1250 nm and the content of lutein.

The presence of high percentage of water in foods could sometimes make VIS/NIRS analysis difficult (*17*). Consequently, removing water from cheese before VIS/NIRS analysis could

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increase the accuracy of quantification. However, some reference methods included a freeze-drying process, and we are not sure about possible losses of compounds during this process that could influence the precision of results obtained in fresh cheeses. The main objective of this research was to investigate the ability of VIS/NIRS to predict dry matter, fat, pH, retinol, α-tocopherol, β-carotene, xanthophyll, calcium, magnesium, potassium, zinc, sodium chloride, total antioxidant capacity, and color of cheeses. A second objective was to investigate the influence of the freeze-drying of cheese on the accuracy of prediction.

MATERIALS AND METHODS

Samples. A total of 445 cheese samples of 4 cheese varieties were supplied by 74 farmhouse producers: Abondance cheese (pressed semicooked curd, cow's milk, $n = 92$), Tomme de Savoie cheese (pressed uncooked curd, cow's milk, $n = 107$) Cantal-type cheese (pressed uncooked curd, cow's milk, $n = 113$) and Rocamadour cheese (lactic coagulation curd, goats' milk, $n = 133$). The main characteristics of these cheeses have been previously described (*6*). Cheese samples were selected in order to promote a wide compositional variability. Cheese aliquots submitted for dry matter, fat, and sodium chloride analyses were stored at +4 °C, while those submitted for vitamin, carotenoid, mineral, and visible-near-infrared spectroscopy (VIS/NIRS) analyses were protected from light with aluminum foil and stored at −20 °C until analysis. A proportion of each cheese sample was freeze-dried as previously described by Lucas et al. (*6*).

Reference Analyses. Dry matter and fat contents were determined using the AFNOR method (*18*) and the Heiss butyrometric method (*19*), respectively. Total sodium chloride content was measured using a chloride analyzer (Model 926, Corning, Halstead, Essex, United Kingdom), and pH was determined using a 763 multi-Calimatic pHmeter (Bioblock Scientific, Illkirch, France). Retinol (vitamin A), α-tocopherol (vitamin E), β-carotene, and xanthophyll (lutein and zeaxanthin) contents were simultaneously measured by HPLC using an UV−visible photodiode-array detector after saponification and hexane extraction adapted from Lyan et al. (*20*). as previously described (*6*). Calcium, magnesium, and zinc contents were determined by atomic absorption spectrophotometry and potassium content by atomic emission spectrophotometry (*21*). The total antioxidant capacity was assessed using the ferric reducing/antioxidant power (FRAP) assay adapted from Benzie and Strain (*22*) as previously described by Lucas et al. (*6*). Color was measured using a portable Minolta CM-2002 spectrocolorimeter (Minolta Camera Co, Osaka, Japan). The CIELAB color system (*23*) was chosen to numerically describe the color parameters: L^* expresses the brightness on a numerical scale from 0 (black) to 100 (white), a^* represents a position between red (positive values) and green (negative values), and b^* designates a position between yellow (positive values) and blue (negative values). All the reference analyses were performed in duplicate.

Visible-Near-Infrared Spectroscopy. Approximately 20 g of grated fresh cheese or crushed freeze-dried cheese was placed in a 50 mm diameter ring cup and scanned in reflectance mode at 2 nm intervals from 400 to 2498 nm using a Foss NIRSystems model 6500 scanning VIS/NIR spectrometer (Foss NIRSystems, Silver Spring, MD, USA) controlled by ISIscan software, version 2.21 (Infrasoft International, Port Matilda, PA, USA). Each spectrum was time averaged from 32 scans. The reflectance (R) values were converted into absorbance (A) values using the formula $A = \log(1/R)$.

Calibrations and Statistics. Calibrations were developed using WinISI III, version 1.60 (Infrasoft International, Port Matilda, PA, USA). The samples were divided into calibration ($n = 362$) and validation ($n = 83$) sets. Samples provided by 60 farmhouses were included in the calibration set, whereas samples obtained from 14 farmers randomly chosen between the farmers were used to validate the models. All cheese types were represented in both, validation and calibration sets. The modified partial least-squares (MPLS) regression method (*24*) was used to obtain NIR equations for all the studied parameters. To optimize the accuracy of calibration, the data were subjected to different combinations of scattering corrections (no correction, detrending, standard normal variate + detrending, and multiplicative scatter correction) and mathematical pretreatments of the data (first and second derivatives applied on different gaps 1,4,4,1; 1,8,8,1; 2,5,5,1; and 2,10,10,1; where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in the first smoothing, and the fourth is the number of data points in the second smoothing). The best treatment was selected for each constituent on the basis of the highest coefficient of determination of cross-validation (R^2CV) and the lowest standard error of cross-validation (SECV). Assessment of the calibration model was performed first, by cross-validation. The set of calibration samples was first randomized and then divided into four groups. Finally, each group was validated using a calibration developed on the other samples. In the calibration process, samples were considered outliers if the residual between the reference method and the predicted value was larger than 2.5-fold of the standard error of cross-validation (SECV). On completion of calibration, the model was applied to the validation set. Samples from the validation set with values out of the calibration range were considered outliers and excluded from the validation set. The accuracy of calibrations was established on the basis of the coefficient of determination in cross-validation (R^2CV) and external validation (R^2V), the SECV and standard error of prediction (SEP), and the ratio of standard deviation of reference data to SECV or SEP, namely, the residual predictive deviation (RPDCV or RPDV respectively). A RPD value greater than 3 was considered adequate for analytical purposes (*25*). Similarly, quantifications were considered as poor, approximate, good, and excellent for an R^2 value under 0.66, between 0.66 and 0.81, between 0.82 and 0.90, and above 0.91, respectively (*26*).

Table 1. Chemical and Physical Composition of Fresh Cheeses within Calibration and Validation Sets[a]

	calibration set ($n = 357$)			validation set ($n = 83$)		
	range	mean	SD	range	mean	SD
Gross Composition						
dry matter (g/kg)	367−684	562	82.64	359−655	553	92.26
fat (g/kg)	190−363	289	42.78	190−350	286	47.75
pH	5.00−6.39	5.54	0.28	4.98−6.18	5.53	0.29
Vitamins						
retinol (mg/kg)	0.71−2.67	1.56	0.39	0.83−2.59	1.43	0.34
α-Tocopherol (mg/kg)	0.25−3.98	1.74	0.80	0.62−3.92	1.81	0.85
Carotenoids						
β-Carotene (mg/kg)	0.00−2.55	0.80	0.70	0.00−2.44	0.77	0.72
xanthophylls (μg/kg)	10.0−160.0	70.0	36.0	10.0−150.0	64.8	37.9
Minerals						
sodium chloride (g/kg)	6.20−28.0	15.67	4.83	9.40−26.70	15.93	5.17
calcium (g/kg)	0.71−11.12	5.87	3.19	0.78−9.79	5.86	3.13
potassium (g/kg)	0.65−1.92	1.15	0.30	0.65−1.85	1.15	0.28
magnesium (g/kg)	0.11−0.41	0.25	0.07	0.13−0.41	0.25	0.06
zinc (mg/kg)	3.97−57.00	28.36	15.64	4.06−48.40	28.65	15.42
TAC (mol Fe^{2+}/kg)	2.09−14.40	7.16	2.51	2.21−13.84	7.08	2.68
Color Parameters						
brightness (L^*)	65.80−81.50	72.99	3.99	66.6−80.3	72.87	4.09
redness (a^*)	−0.66−4.90	1.02	0.99	−0.57−3.64	1.05	1.04
yellowness (b^*)	8.00−17.20	12.12	2.16	8.00−17.00	11.87	2.37

[a] Abbreviations: SD, standard deviation; TAC, total antioxidant capacity.

RESULTS AND DISCUSSION

Chemical and Physical Composition. The ranges, mean values and standard deviations of the chemical and physical composition of fresh and freeze-dried cheeses within the calibration and validation sets are shown in **Tables 1** and **2**, respectively. The variation in composition of the samples of fresh cheese covered most of the variability reported in the literature for cow's and goat's milk pressed cheeses. The range and mean values for each parameter were similar within calibration and validation sets. The relative standard deviation

Table 2. Chemical and Physical Composition of Freeze-Dried Cheeses within Calibration and Validation Sets[a]

	calibration set (n = 357)			validation set (n = 83)		
	range	mean	SD	range	mean	SD
Gross Composition						
fat (g/kg)	377−570	499	27.83	438−560	497	24.66
pH	5.02−6.39	5.55	0.28	5.03−6.18	5.54	0.29
Vitamins						
retinol (mg/kg)	1.39−4.80	2.73	0.70	1.62−4.42	2.52	0.55
α-Tocopherol (mg/kg)	0.44−6.66	3.05	1.31	0.95−6.09	3.13	1.31
Carotenoids						
β-carotene (mg/kg)	0.00−3.78	1.25	1.09	0.00−3.69	1.15	1.08
xanthophylls (μg/kg)	20.0−270.0	118	56.0	30.0−240.0	109.1	56.0
Minerals						
sodium chloride (g/kg)	11.10−44.30	26.85	6.40	14.00−44.20	27.92	7.47
calcium (g/kg)	1.40−17.90	9.61	4.63	1.70−17.90	9.77	4.61
potassium (g/kg)	1.07−11.80	2.21	1.34	1.07−15.0	2.57	2.14
magnesium (g/kg)	0.22−0.63	0.43	0.08	0.23−0.55	0.42	0.06
zinc (mg/kg)	8.60−86.60	43.93	22.31	10.20−77.2	45.17	22.38
TAC (mol Fe^{2+}/kg)	4.60−25.50	12.44	3.92	5.60−20.0	11.96	3.58
Color Parameters						
brightness (L^*)	75.68−86.04	81.42	1.62	77.32−85.71	81.28	1.62
redness (a^*)	−0.74−3.78	1.11	1.05	−0.55−3.31	1.18	1.00
yellowness (b^*)	12.24−21.74	16.34	1.68	13.07−21.22	16.29	1.72

[a] Abbreviations: SD, standard deviation; TAC, total antioxidant capacity.

of repeatability for the reference method of gross composition was 0.9, 2.4, and 0.4% for dry matter, fat, and pH, and 7 and 4% for carotenoids (β-carotene and xanthophyll), and vitamins determinations. The repeatability of mineral determinations and TAC were 2 and 12%, respectively. Finally, the repeatability for the color determination was 0.25, 2.8, and 0.6% for L^*, a^*, and b^* parameters, respectively.

Cheese Spectra. Averaged absorbance and first derivative of VIS/NIR spectra for fresh and freeze-dried cheeses are presented in **Figure 1**. Maxima in the averaged spectra are located at 464, 1208, 1728, 1762, 1940, 2308, and 2348 nm for both fresh and freeze-dried cheeses, at 982 and 1456 nm for only fresh cheeses, and at 926, 1502, 2056, and 2174 nm for only freeze-dried cheeses. The absorption bands at 982, 1456, and 1940 nm have been attributed to water (*27*), which explains why they are present in the spectra of fresh cheeses and absent or smaller in those of freeze-dried cheeses. These bands originate from the second overtone of the O−H stretch (982 nm), the first overtone of the O−H stretch (1456 nm), and the combination band of the asymmetric and scissor stretch O−H vibrations (1940 nm). Absorbance at 926, 1208, 1728, 1762, 2308, and 2348 nm is related to the lipid and arises from the third overtone of a C−H stretch (926 nm), the second overtone of C−H stretch (1208 nm), the first overtone of a C−H stretch (1728 and 1762 nm), and combination bands arising from C−H stretch and deformation in a CH_2 group (2308 and 2348 nm) in the lipid molecules (*8, 11*). Absorption at 1502, 2056, and 2174 nm are associated with specific groups in protein (*27*), i.e., N−H stretch (1502 nm), N−H stretch, and amide II (2056 nm), amide I, and amide III (2174 nm). Within the visible range, the absorption band at 462 nm was probably related to carotenoids, known to have strong absorption in this spectral region (*28*).

Calibration and Validation Models. In total, 20 calibration models were developed for each parameter using the whole wavelength range. The statistical summary of the best calibration and prediction by cross-validation and external validation for dry matter (except for freeze-dried cheeses), fat, pH, vitamins, carotenoids, minerals, total antioxidant capacity, and color are shown in **Table 3** for fresh cheeses and in **Table 4** for freeze-dried cheeses. Independent calibrations were also performed for cow's milk and goat's milk cheeses, but, as the precision of the prediction was not improved for most of the parameters (data not shown), we decided to consider cow's and goat's milk cheeses together.

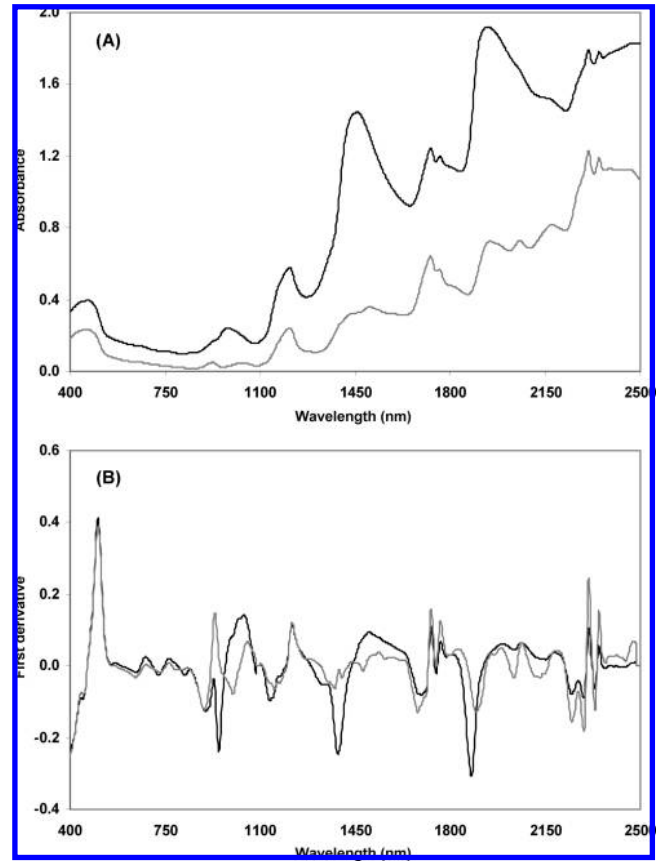

Figure 1. Averaged absorbance (**A**) and first derivative (**B**) of visible and near-infrared spectra for fresh cheeses (black) and freeze-dried cheeses (gray).

Fat and dry matter models in fresh cheeses are considered adequate for quality control (*13*). According to their coefficients of determination in cross-validation (R^2CV) of 0.98 and 0.96 and residual predictive values in cross-validation (RPDCV) of 6.69 and 4.78 for dry matter and fat, respectively, we can state that they provided a similar degree of accuracy (**Table 3**). Similarly, fat in freeze-dried cheeses were associated with R^2CV of 0.88 and RPDCV of 2.90 (**Table 4**). However, these results have not been confirmed by the statistics obtained in external validation. The variability, estimated by the coefficient of variation (CV) (SD*100/mean), in freeze-dried cheese calibration population (CV of 6 for fat) was lower than that in fresh cheese calibration population (CV of 15). This lower variability can explain the poor results obtained for fat in freeze-dried cheeses in relation to fresh cheese. Our results for dry matter and fat in fresh cheeses are consistent with previous findings, reporting good predictions of moisture and fat in Cheddar and Emmental cheeses (*8, 10*). Results obtained for the dry matter were confirmed by those obtained in the external validation (**Tables 3** and **4**) in which the linear relationship between the measured and predicted values for dry matter and fat is illustrated in **Figure 2**. The better statistics obtained for fresh cheeses in relation to freeze-dried cheeses allow us to conclude that freeze-drying can be avoided for the prediction of fat.

Table 3. Calibration and Validation Statistics for Physical and Chemical Composition of Fresh Cheeses[a]

	calibration set						validation set				
	math	N	T	SECV	R^2CV	RPDCV	N	SEP	bias	R^2V	RPDV
Gross Composition											
dry matter	MSC 2,10,10,1	346	4	12.36	0.98	6.69	81	15.40	0.60	0.97	5.99
fat	MSC 2,5,5,1	351	4	8.95	0.96	4.78	83	14.85	0.78	0.90	3.22
pH	Det 2,10,10,1	343	10	0.14	0.76	2.00	83	0.18	0.01	0.60	1.61
Vitamins											
retinol	SNV+Det 1,4,4,1	340	3	0.35	0.22	1.11	82	0.34	−0.01	0.19	1.02
α-tocopherol	none 1,4,4,1	345	6	0.58	0.47	1.38	82	0.67	0.18	0.43	1.26
Carotenoids											
β-carotene	Det 2,5,5,1	347	6	0.20	0.91	3.50	82	0.21	−0.04	0.92	3.43
xanthophylls	SNV+Det 1,4,4,1	348	3	27.0	0.45	1.33	81	24.0	−0.04	0.60	1.58
Minerals											
sodium chloride	Det 1,4,4,1	332	9	1.68	0.88	2.88	80	1.76	−0.27	0.89	2.94
calcium	SNV+Det 1,4,4,1	321	10	0.70	0.95	4.56	77	0.68	−0.03	0.95	4.62
potassium	Det 2,5,5,1	327	8	0.14	0.79	2.14	77	0.14	0.00	0.78	2.06
magnesium	SNV+Det 1,4,4,1	324	9	0.03	0.79	2.33	77	0.03	−0.00	0.74	1.93
zinc	None 1,4,4,1	320	9	3.61	0.95	4.33	77	4.11	0.07	0.93	3.75
TAC	SNV+Det 1,4,4,1	347	11	1.60	0.59	1.57	83	1.64	0.08	0.62	1.63
Color Parameters											
brightness (L^*)	SNV+Det 1,4,4,1	346	11	0.72	0.97	5.54	82	0.84	−0.23	0.96	4.88
redness (a^*)	Det 1,4,4,1	349	8	0.17	0.97	5.82	83	0.20	0.00	0.96	5.23
yellowness (b^*)	SNV+Det 1,4,4,1	349	10	0.66	0.91	3.27	81	0.63	−0.14	0.93	3.73

[a] Abbreviations: Det, detrending; math, mathematical treatment; MSC, multiplicative scatter correction; N, number of samples used to develop the model; none, no scatter correction; R^2CV, coefficient of determination in cross-validation; RPDCV, ratio of standard deviation of reference data in calibration set to standard error of cross-validation; RPDV, ratio of standard deviation of reference data in validation set to standard error of prediction; R^2V, coefficient of determination in external validation; SEP, standard error of prediction; SECV, standard error of cross-validation; SNV, standard normal variate; Det, detrending; T, number of components used to perform the calibration model; TAC, total antioxidant capacity.

Table 4. Calibration and Validation Statistics for Physical and Chemical Composition of Freeze-Dried Cheeses[a]

	calibration set						validation set				
	math	N	T	SECV	R^2CV	RPDCV	N	SEP	bias	R^2V	RPDV
Gross Composition											
fat	SNV+Det 2,10,10,1	345	3	9.61	0.88	2.90	81	18.15	−0.56	0.49	1.36
pH	SNV+Det 1,4,4,1	339	14	0.15	0.73	1.87	82	0.16	−0.00	0.68	1.77
Vitamins											
retinol	SNV+Det 1,4,4,1	341	1	0.63	0.18	1.11	80	0.57	−0.20	0.11	0.97
α-tocopherol	SNV+Det 1,4,4,1	347	2	1.08	0.32	1.21	81	1.13	0.14	0.27	1.16
Carotenoids											
β-carotene	Det 1,4,4,1	338	9	0.21	0.87	5.19	80	0.46	−0.25	0.88	2.3
xanthophylls	SNV+Det 1,4,4,1	348	10	50.2	0.32	1.12	81	47.0	−0.00	0.32	1.19
Minerals											
sodium chloride	SNV+Det 2,5,5,1	328	7	2.61	0.83	2.45	80	3.17	−0.20	0.82	2.36
calcium	SNV+Det 1,4,4,1	340	8	1.47	0.90	3.15	83	1.99	0.46	0.82	2.32
potassium	MSC 2,10,10,1	332	13	0.66	0.75	2.03	78	1.33	0.21	0.64	1.61
magnesium	SNV+Det 1,4,4,1	347	9	0.05	0.56	1.60	82	0.05	0.00	0.48	1.33
zinc	MSC 2,5,5,1	345	7	5.63	0.94	3.97	82	7.43	1.46	0.89	3.01
TAC	SNV+Det 1,4,4,1	343	4	2.93	0.45	1.34	83	2.93	−0.07	0.33	1.22
Color Parameters											
brightness (L^*)	Det 2,5,5,1	335	9	0.74	0.79	2.19	83	0.95	−0.16	0.68	1.70
redness (a^*)	none 1,8,8,1	336	9	0.22	0.96	4.77	80	0.37	0.02	0.88	2.71
yellowness (b^*)	none 1,8,8,1	333	9	0.51	0.91	3.29	83	0.84	0.00	0.79	2.05

[a] Abbreviations: Det, detrending; math, mathematical treatment; MSC, multiplicative scatter correction; N, number of samples used to develop the model; none, no scatter correction; R^2CV, coefficient of determination in cross-validation; RPDCV,: ratio of standard deviation of reference data in calibration set to standard error of cross-validation; RPDV, ratio of standard deviation of reference data in validation set to standard error of prediction; R^2V, coefficient of determination in external validation; SEP, standard error of prediction; SECV, standard error of cross-validation; SNV, standard normal variate; Det, detrending; T, number of components used to perform the calibration model; TAC, total antioxidant capacity.

Models obtained for pH predictions were not suitable for practical application with R^2CV < 0.77 and a RPDCV ≤ 2.00 for both fresh and freeze-dried cheeses. However, for pH prediction, we obtained slightly better results than those reported by Karoui et al. (*10*) who found an R^2 of 0.43 and a RPD of 1.32 in cross-validation but in a cheese population where pH ranges from 5.49 to 5.95 instead of 5.00 to 6.39 in the case of the present study.

Among the vitamins and carotenoids, only β-carotene shows a good predictive ability (*29*), with R^2CV and RPDCV values ranging from 0.91 to 0.87 and from 3.50 to 5.19, respectively, for fresh and freeze-dried cheeses (**Tables 3**

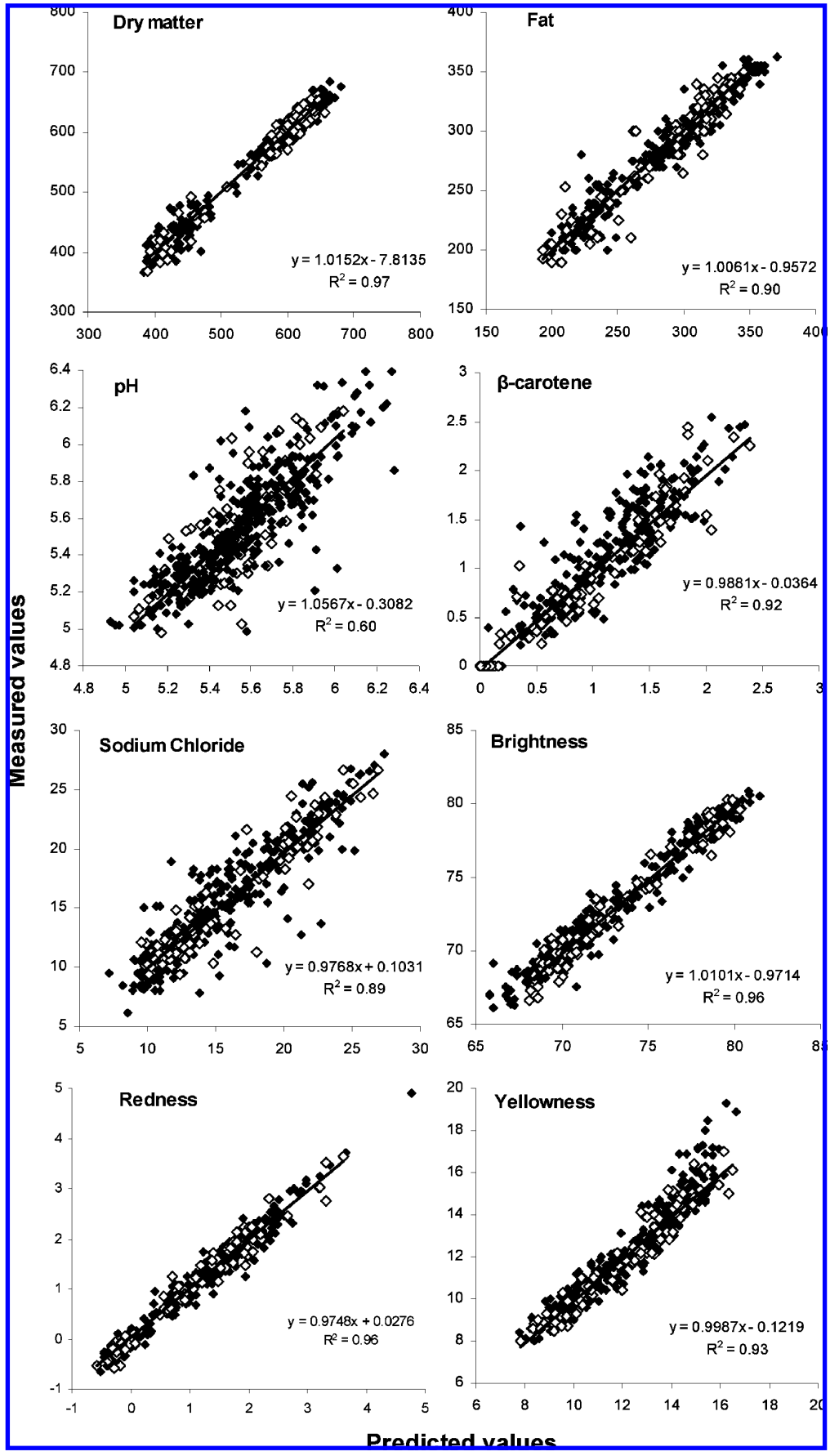

Figure 2. Linear regression plot of measured versus predicted values within the validation set for dry matter ($g \cdot kg^{-1}$), fat ($g \cdot kg^{-1}$), pH, β-carotene ($mg \cdot kg^{-1}$), sodium chloride ($g \cdot kg^{-1}$), brightness, redness, and yellowness in fresh cheeses.

Table 5. Correlation Coefficients for the Studied Parameters of Fresh Cheeses in the Calibration Set

	DM	fat	pH	retinol	α-tocopherol	β-carotene	xantophylls	NaCl	Ca	K	Mg	Zn	TAC	*L**	*a**
fat	0.94														
pH	−0.12	−0.08													
Retinol	0.14	0.20	0.02												
α-tocopherol	0.28	0.24	0.00	0.28											
β-carotene	0.71	0.60	−0.10	0.17	0.53										
xantophylls	0.52	0.45	−0.22	0.19	0.50	0.63									
NaCl	0.52	0.39	−0.17	0.09	0.29	0.56	0.45								
Ca	0.84	0.75	−0.04	−0.01	0.10	0.59	0.35	0.44							
K	−0.41	−0.37	0.23	−0.14	−0.25	−0.41	−0.39	−0.29	−0.14						
Mg	0.63	0.55	0.09	−0.06	0.01	0.43	0.17	0.29	0.88	0.23					
Zn	0.84	0.77	−0.02	0.02	0.07	0.55	0.32	0.37	0.95	−0.13	0.85				
TAC	0.52	0.42	0.15	0.15	0.38	0.59	0.35	0.44	0.42	−0.29	0.31	0.39			
*L**	−0.81	−0.70	0.09	−0.10	−0.38	−0.81	−0.54	−0.52	−0.71	0.45	−0.53	−0.69	−0.60		
*a**	0.68	0.58	−0.17	0.09	0.36	0.80	0.55	0.40	0.59	−0.40	0.43	0.57	0.52	−0.83	
*b**	0.63	0.54	−0.13	0.11	0.46	0.84	0.51	0.41	0.54	−0.37	0.38	0.51	0.52	−0.77	0.84

and **4**). The method of preparation of the samples did not differ significantly, but more robust results in terms of RPD were found for fresh cheeses (RPDV = 3.43). The linear relationship between the measured and predicted values for the β-carotene content of fresh cheeses within the calibration and validation sets is shown in **Figure 2**. To our knowledge, no previous work has studied the VIS/NIRS as a tool to predict the carotenoid content of cheese. However, good predictions have been obtained with maize (*30*), wheat (*31*), or virgin olive oil (*15*).

Retinol, α-tocopherol, and xanthophyll were poorly predicted as shown by R^2CV values lower than 0.48 and RPDCV values lower than 1.39 in all cases (**Tables 3** and **4**). Ayerdi et al. (*32*) could not accurately predict the α-tocopherol content in sunflower seeds. These poor results could be explained by an absence of interactions between the near-infrared radiations and these micronutrients, the extremely low variability in composition within the samples, the extremely small concentration of some components (case for xanthophylls) to give a signal in the NIR spectra, and/or analytical errors, as may be the case for xanthophylls where the measurement by the reference method is lacking in accuracy because of its low concentration in cheese. Concerning the ability of VIS/NIR spectroscopy to predict minor components in food, several reports have found successful relationships between NIRS signal and components present in low concentration (*16*). In this study, good predictions were also obtained for other components present only in low concentration (Zn, β-carotene). In addition, the freeze-drying had no significant influence on the accuracy in predicting carotenoids and vitamins in cheese (**Tables 3** and **4**). Similarly, the total antioxidant capacity of cheese, assessed by the FRAP, which partially depends on the carotenoid and vitamin contents, was unsuccessfully predicted (**Tables 3** and **4**). However, no high correlation was found between this parameter and the carotenoid and vitamin contents (**Table 5**). This last result contrasts with previous findings reporting the approximate prediction of total antioxidant capacity (*33*) on other products (green tea), while total antioxidant capacity was assessed by another method (ABTS radical cation decolorization assay).

An excellent prediction model for quality control was obtained for calcium, (R^2CV ≥ 0.95, RPDCV ≥ 4.56, R^2V ≥ 0.95, RPDV ≥ 4.62) in fresh cheeses (**Table 3**). Coefficients of determination in cross-validation higher than 0.80, and RPDCV ratios ≥2.45 were found for sodium chloride and zinc in both fresh and freeze-dried cheese. However, results were slightly poorer (**Tables 3** and **4**) when external validation results were analyzed, although RPD values in the validation set for Zn was always higher than 3. These results contrast with previous findings, reporting unsuccessful prediction of the sodium chloride content of cheese using NIRS (*10*). Although no absorbance occurs in the NIR region, sodium chloride can change the spectrum of water in the infrared overtone region and consequently be indirectly estimated by NIRS (*34*). For instance, sodium chloride can cause a wavelength shift in the absorption band of water from 1795 to 1806 nm (*34*), hence perhaps the strong contribution of this spectral region to the prediction of the sodium chloride content. However, this argument is not valid for freeze-dried cheeses when water is removed.

No models with predictive ability were obtained for potassium and magnesium in both fresh and freeze-dried cheeses with R^2 and RPD values lower than 0.79 and 2.33, respectively (**Tables 3** and **4**). These poor predictions are not surprising as NIR radiations do not interact with inorganic compounds. However, indirect predictions of minerals could be obtained because they are closely related to the organic fraction in milk (*35*). As a result of milk fermentation, the decrease in milk pH renders soluble a part of minerals during the cheese-making process (*36*), which could explain the poor predictions.

Successful prediction models of the color parameters (brightness, redness, and yellowness) of fresh cheeses were obtained within both the calibration and validation sets (R^2CV ≥ 0.91; RPDCV ≥ 3.27; R^2V ≥ 0.93; RPDV ≥ 3.73) (**Table 3**). Linear relationships between the measured and predicted values for brightness, redness, and yellowness of fresh cheeses within the validation set are shown in **Figure 2**. Whereas good predictions were also observed for redness and yellowness parameters of freeze-dried cheeses within the calibration set (R^2CV ≥ 0.91; RPDCV ≥ 3.29), the prediction of color parameters within the validation set was approximate (R^2V ≤ 0.68; RPDV ≤ 1.70) (**Table 4**). Previous work has evaluated NIRS to predict the color parameters of foods. The prediction of color by VIS/NIRS was good for pork (*37*) and wine (*38*), but poor for freeze-dried egg yolk (*39*). Our results showed that VIS/NIRS is also suitable for predicting the color of cheese. In our case, the use of wavelengths of the visible segment and the relationship between carotene and color (**Tables 3**, **4**, and **5**) could have helped to obtain these good models for quality control of the color parameters.

The correlation coefficients observed between the parameters evaluated in fresh cheeses are shown in **Table 5**. Strong correlation was found between the dry matter and fat determinations. The Ca, Mg, and Zn contents were also highly correlated. However, these correlations were lower than the cross-validation correlations between NIRS and the evaluated parameters (**Table 3**). As a consequence, the predicted

concentrations of the parameters evaluated seems more due to the real NIR absorbances than to the cross-correlation between different parameters.

To our knowledge, no previous study has investigated the ability of VIS/NIRS to predict vitamins, carotenoids, minerals, and color in cheese. The results of this study showed that VIS/NIRS can be used to accurately predict dry matter, fat, β-carotene, calcium, sodium chloride, zinc, brightness, redness, and yellowness in cheese. However, VIS/NIRS is not suitable for the quantitative determination of pH, xanthophyll, retinol, α-tocopherol, potassium, magnesium, and total antioxidant capacity in cheese, particularly in our data set. Removing water from cheese before VIS/NIRS analysis did not improve the accuracy of quantification. Consequently, the freeze-drying of cheese before VIS/NIRS analysis is not necessary, which makes the VIS/NIRS analysis more economical and fast. Future work is needed in order to extend the results of this study to other cheese-making technologies. More research should be done in order to try to increase the variability or reduce the error of the reference method of several parameters, particularly potassium, magnesium, and sodium chloride. For retinol, α-tocopherol, and xanthophylls, VIS/NIRS analysis is not accurate enough for its use in practical conditions.

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