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Development of a portable spectrofluorometer for measuring the quality of cheese

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Abstract – The determination of some chemical parameters of Saint-Nectaire cheese samples (fat, dry matter (DM), pH, total protein (TP) and soluble protein (SP)) was investigated using fluorescence spectroscopy. A total of 12 cheeses (with a ripening time of 1 month) produced using different manufacturing processes and ripened in different conditions – farmhouse Saint-Nectaire ripened at a farmhouse ($n = 3$), farmhouse Saint-Nectaire ripened at a dairy plant ($n = 3$), Saint-Nectaire “Laitier” ripened at a dairy plant ($n = 3$) and Savaron ($n = 3$) ripened at a dairy plant – were analyzed by laboratory and portable spectrofluorometers developed at the Foodstuffs Unit of ENITA Clermont-Ferrand. Partial least squares (PLS) regression with the leave-one-out cross-validation technique was used to perform calibration models. The best results for DM ($R^2 = 0.91$; ratio of standard deviation to root mean square error of prediction (RPD) = 3.37), TP ($R^2 = 0.86$; RPD = 2.71), fat ($R^2 = 0.83$; RPD = 2.42) and SP ($R^2 = 0.65$; RPD = 1.66) were obtained with the portable fluorometer, with the excitation wavelength set at 380 nm. Regarding the prediction of pH value, the best results were also obtained with the portable fluorometer with excitation set at 280 nm ($R^2 = 0.74$; RPD = 1.98). It could be concluded that the portable spectrofluorometer could be used as a suitable technique for the prediction of DM, TP and fat. The SP and pH could also be estimated, but with much lower precision.

Saint-Nectaire cheese / portable spectrofluorometer / laboratory spectrofluorometer / calibration model / partial least squares method

摘要 – 利用便携式荧光分光光度计测定干酪质量。本文使用荧光分光光度计测定了12个不同加工工艺和成熟条件下生产的(成熟期为1个月) Saint-Nectaire 干酪的脂肪、干物质含量(DM)、pH值、总蛋白(TP)和可溶性蛋白(SP)。其中3例 Saint-Nectaire 干酪是在农家成熟, 3例 Saint-Nectaire 干酪是在乳品厂成熟, 3例 Laitier Saint-Nectaire 干酪是在乳品厂成熟, 3例 Savaron 干酪是在乳品厂成熟, 样品分别经实验室用荧光分光光度计和 ENITA Clermont-Ferrand 食品原料公司研制的便携式荧光分光光度计分析。采用留一法交互校验法建立了偏最小二乘(PLS)回归的校正模型。使用便携式荧光分光光度计, 在380 nm 激发波长处测定了 DM ($R^2 = 0.91$, 标准差与预测误差均方根的比值(RPD) = 3.37), TP ($R^2 = 0.86$; RPD = 2.71), 脂肪 ($R^2 = 0.83$; RPD = 2.4) 和 SP ($R^2 = 0.65$; RPD = 1.66) 的最佳实验结果。对于 pH 值的预测, 在 280 nm 的激发波长下也获得了最佳结果 ($R^2 = 0.74$; RPD = 1.98)。由此可见便携式荧光分光光度计可以有效地预测 DM, TP 和脂肪含量。也可以预测 SP 和 pH 值, 但精确度较低。

Saint-Nectaire 干酪 / 便携式荧光光度计 / 实验室用荧光光度计 / 校正模型 / 偏最小二乘法

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Résumé – Développement d'un spectrofluorimètre portatif pour mesurer la qualité des fromages. La mesure de quelques paramètres chimiques de fromages de Saint-Nectaire (matière grasse, extrait sec, pH, protéines totales et protéines solubles) a été déterminée par spectroscopie de fluorescence. Au total, 12 fromages de Saint-Nectaire (ayant un temps d'affinage de 1 mois) ont été fabriqués en utilisant différents procédés de fabrication et conditions d'affinage – Saint-Nectaire fermier affiné dans une ferme ($n = 3$), Saint-Nectaire fermier affiné dans une laiterie ($n = 3$), Saint-Nectaire "Laitier" affiné dans une laiterie ($n = 3$) et Savaron ($n = 3$) affiné dans une laiterie – ont été analysés par un spectrofluorimètre du laboratoire et le spectrofluorimètre portatif développé au sein de l'Unité de Recherche « Typicité des Produits Alimentaires » (UR TPA) de l'ENITA de Clermont-Ferrand. La méthode des moindres carrés partiels avec validation croisée a été utilisée pour développer des modèles de calibrage, et ce pour tous les paramètres chimiques. Les meilleurs résultats pour l'extrait sec ($R^2 = 0,91$; RPD = 3,37), les protéines totales ($R^2 = 0,86$; RPD = 2,71), la matière grasse ($R^2 = 0,83$; RPD = 2,42) et les protéines solubles ($R^2 = 0,65$; RPD = 1,66) ont été obtenus avec le spectrofluorimètre portatif, après excitation à 380 nm. Concernant la détermination de la valeur du pH, les meilleurs résultats ont été obtenus également avec le spectrofluorimètre portatif après excitation à 280 nm ($R^2 = 0,74$; RPD = 1,98). Le spectrofluorimètre portatif peut être considéré comme une technique appropriée pour la détermination de l'extrait sec et des teneurs en protéines totales et en matière grasse. Les teneurs en protéines solubles et le pH peuvent être également prédits, mais avec une précision beaucoup plus faible.

Saint-Nectaire / spectrofluorimètre portatif / spectrofluorimètre de laboratoire / modèle de calibration / méthode des moindres carrés partiels

1. INTRODUCTION

Rapid screening techniques for determining quality characteristics of cheeses are of great interest for both industry and consumers. The dairy industry, like the food processing industry in general, has come under increasing pressure to deliver products of high and constant quality into the market place. The chemical determination of cheeses is a very important task, which is classically undertaken by different physico-chemical methods to determine pH value, fat and calcium contents, nitrogen fractions, etc. This approach suffers from a number of disadvantages, namely, the ever-increasing range of analytes, which must be included in any test procedure and the limited knowledge of the range of each constituent in normal lots of the substance. In addition, the above-mentioned methods require sophisticated analytical equipment and skilled operators; they are also time-consuming and need both the purchase and disposal of chemical reagents. For all these reasons, there is a continuing demand for new, rapid and relatively cheaper methods for direct

quality measurements in food and food ingredients.

Nowadays, there is a need for the cheese processing industry to have tools available for real-time control of production lines to check whether in-process material, during a given processing step, meets the necessary compositional or functional specifications to reach a predetermined quality standard in the final product. In this context, fluorescence spectroscopy could be considered as fast, relatively low-cost and provides a great deal of information with only one test [5]. It is sensitive, non-destructive, rapid, environmentally friendly and non-invasive, making it suitable for at-line or on-line process control and appropriate for process control.

Several papers have demonstrated the ability of fluorescence spectroscopy as a rapid technique for the determination of the quality of different dairy products. Indeed, Christensen et al. [3] used this technique to predict riboflavin content in 42 yogurt samples, obtaining successful results and underlining the potential of fluorescence spectroscopy to be utilized as a rapid method for the determination of riboflavin

content in dairy products. This technique has been also utilized for the determination of: (1) lactulose and furosine in milks [20] and (2) some chemical parameters in different soft cheese varieties in both the external and central zones [18]. Karoui et al. [10] and Purna et al. [23] have also used front-face fluorescence spectroscopy as a non-destructive technique to determine the cheese melting point of semi-hard cheeses produced during summer and autumn periods and commercial cheese samples, respectively. The authors pointed out the ability of fluorescence spectroscopy to predict cheese melting point as well as some chemical parameters.

However, all these studies were performed on cheese samples produced at the industrial level and by using standard laboratory-based spectrofluorometers [4, 7–18, 21, 27]. No research using this technique has been performed on farmhouse cheeses such as Saint-Nectaire.

The aim of the present study is to examine the feasibility of laboratory and portable spectrofluorometers, comprising a light-emitting diode (LED) for excitation, a spectrometer, a fiber optic and an integrated PC with a touch-screen for recording and evaluating data sets, for the determination of some chemical parameters (pH, fat, dry matter (DM), total protein (TP) and soluble protein (SP)) of 12 French semi-hard cheeses belonging to four brands. For a given chemical parameter, an optimal calibration model developed using laboratory and portable spectrofluorometers and partial least squares (PLS) regression was selected.

2. MATERIALS AND METHODS

2.1. Cheese samples

Twelve different Saint-Nectaire cheeses belonging to four brands, obtained directly from different farms and dairies,

were available in the laboratory after less than 1 h. Farmhouse Saint-Nectaire ($n = 3$) ripened at the farmhouse, Farmhouse Saint-Nectaire ($n = 3$) ripened at the dairy plant, Saint-Nectaire “Laitier” ($n = 3$) ripened at the dairy plant and Savaron ($n = 3$) ripened at the dairy plant were produced using different manufacturing processes and ripening conditions with a ripening time of 1 month. Farmhouse cheeses were produced with raw milk, while the other investigated cheeses were made with pasteurized milk. Each cheese was cut into two symmetric parts according to its median line. One section was used for chemical analyses, the other for fluorescence spectroscopic measurements. The acquisitions of fluorescence spectra by the two apparatus were determined on different fresh cheese slices.

2.2. Physico-chemical analyses

The determination of pH, DM, fat, TP and SP contents of the analyzed cheeses was as described by Bouton et al. [2]. All cheese samples were kept at $-20\text{ }^{\circ}\text{C}$ for approximately 7 months until analysis. All the analyses were carried out in triplicate.

2.3. Laboratory spectrofluorometer

For each fresh cheese, three slices (2 cm length, 1 cm width and 0.2 cm thickness) were obtained using a controlled thickness wire cutter. Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorometer (Spex-Jobin Yvon, Longjumeau, France). The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation and depolarization phenomena were minimized. Cheese samples were mounted between two quartz slides and spectra were recorded at $20\text{ }^{\circ}\text{C}$. The emission spectra (305–400 nm), (330–500 nm) and (400–640 nm) were recorded with the excitation wavelengths set at 280 nm, 320 nm

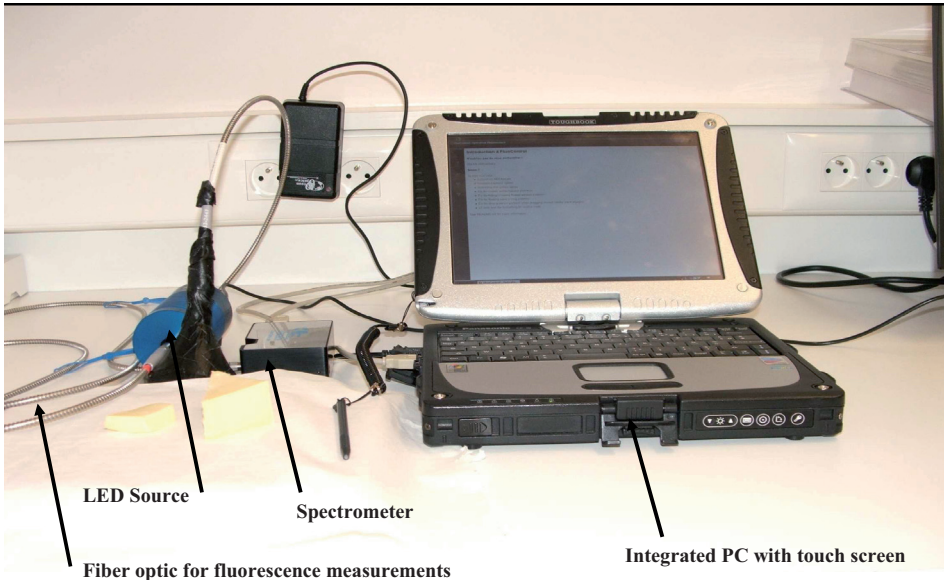


Figure 1. Integrated portable spectrofluorometer.

and 380 nm, respectively. All the spectra were corrected for instrumental distortions in excitation using a rhodamine cell in the reference channel. Analyses were done in triplicate for each cheese sample.

2.4. Portable spectrofluorometer

For each fresh cheese, three slices (2 cm length, 1 cm width and 0.2 cm thickness) were obtained using a controlled thickness wire cutter. The portable apparatus consisted of a commercially available compact device for fluorescence analysis. As shown in Figure 1 the portable spectrofluorometer is composed of a light-emitting diode (LED) source, a spectrometer, a fiber optic and an integrated PC with a touch-screen for recording and evaluating data sets. All the instruments were purchased from IDIL Fibres Optiques (Lannion, France).

The emission spectra (307–460 nm), (346–600 nm) and (410–705 nm) were recorded with the excitation wavelengths

set at 280 nm, 320 nm and 380 nm, respectively. All spectra were corrected by subtracting the dark for each measurement. The dark was acquired with no light source.

2.5. Principal component analysis (PCA)

In order to determine the variability in the physico-chemical parameters and the effect of the manufacturing process and ripening conditions for the investigated cheeses, principal component analysis (PCA) was used. PCA transforms the original variables (chemical parameters) into new axes called principal components (PCs), which are orthogonal, so that the data sets presented on these axes are uncorrelated with each other. This statistical multivariate treatment was earlier used to differentiate between different cheese samples according to their manufacturing processes, ripening stages and geographical origins [4, 7, 10], reducing the dimension

to two or three PCs, keeping most of the original information found in the data set. The PCA was performed on the chemical parameters by using StatBoxPro[®] software (version 5.0, Paris, France).

2.6. Partial least squares (PLS) regression

The objective of this task was to perform a statistical model between the processed spectra and cheese properties (chemical parameters). Because the number of the wavelengths in the different fluorescence spectra was much larger than the number of cheese samples in the data set, it was necessary to use chemometric tools to extract information from the data set. Several statistical modeling techniques can be adopted for proper calibration performance, such as linear and nonlinear multiple regression analysis, principal component regression (PCR) and PLS regression. In the present study, PLS regression was used in order to predict some chemical parameters (pH, fat, DM, TP and SP) of the investigated cheese samples from the fluorescence spectral data sets. For each excitation wavelength and for each chemical parameter, two different models (using laboratory or portable spectrofluorometers) were developed utilizing fluorescence spectra. As the number of observations was small, the regression models were validated by a leave-one-out cross-validation. This cross-validation enables the dimensions of the predictive model to be chosen. The optimum number of latent variables (LV) was chosen by the software automatically. PLS regression was performed using Unscrambler[®] software (version 7.8, Camo Process AS, Norway).

In order to compare the different established models for a given parameter, the values of the root mean square error of prediction (RMSEP) of the calibration set

were considered. The performance of the models was quantified by determining the R^2 for predicted versus measured compositions in cross-validation and the ratio of standard deviation (SD) of the data set to the RMSEP. In fact, R^2 indicates the percentage of the variance in the Y variable that is accounted for by the X variable. Values of R^2 comprised between 0.50 and 0.65 indicate that more than 50% of the variance in Y is accounted for by variance X ; thus, differentiation between only high and low values can be observed. A value for R^2 from 0.66–0.81 and 0.82–0.90 indicates approximate and good prediction, respectively. Calibration models with a value for R^2 above 0.91 are considered to be excellent [26]. The ratio of the SD to the RMSEP, called the ratio of prediction to deviation (RPD), is the factor by which the prediction accuracy increased compared with the use of the mean composition for all samples. This ratio is desired to be larger than 5, but at least 3, for a good calibration [26]. A RPD ratio less than 2 indicates poor predictions and the model cannot be used for further prediction. Practical utility of the calibrations can also be assessed using the range error ratio (RER) [22]. This ratio is calculated by dividing the range of a given constituent by the prediction error for that constituent and, while susceptible to the presence of extreme values at both ends of the range, it is a useful indicator of the practical utility of a predictive model.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical measurements

The results for fat, DM, pH, TP and SP of the analyzed cheeses are reported in Table I. The obtained results were in agreement with previous findings [1, 25].

In order to determine the effect of the manufacturing process and ripening conditions on the chemical parameters of

Table I. Physico-chemical composition of Saint-Nectaire cheese samples used for the PLS-cross-validation model^a.

Cheese samples	Physico-chemical composition					
		DM (g·100 g ⁻¹)	Fat (g·100 g ⁻¹)	pH	TP (g·100 g ⁻¹)	SP (g·100 g ⁻¹)
Farmhouse Saint-Nectaire produced with raw milk and ripened at the farmhouse (<i>n</i> = 3)	Mean	55.138	30.389	5.716	22.226	1.934
	SD	1.132	0.953	0.205	0.445	1.128
Farmhouse Saint-Nectaire produced with raw milk and ripened at the dairy plant (<i>n</i> = 3)	Mean	55.874	32.444	5.659	21.648	2.995
	SD	3.839	2.715	0.109	0.505	0.412
Saint-Nectaire "Laitier" produced with pasteurized milk and ripened at the dairy plant (<i>n</i> = 3)	Mean	56.868	30.056	5.811	23.750	2.814
	SD	0.808	0.527	0.239	0.366	1.430
Savaron produced with pasteurized milk and ripened at the dairy plant (<i>n</i> = 3)	Mean	56.658	27.389	5.873	25.110	4.019
	SD	4.078	2.346	0.237	0.700	0.906
Samples used for the calibration (<i>n</i> = 12)	Minimum	53.270	25.500	5.480	20.850	1.230
	Maximum	60.940	36.000	6.070	25.980	4.800
	Mean	56.130	30.070	5.760	23.170	2.960
	SD	2.840	2.560	0.210	1.450	1.170

^a Abbreviations: DM, dry matter; TP, total protein; SP, soluble protein; SD, standard deviation.

the investigated cheeses, PCA was applied to the normalized data sets. The normalization consists of dividing each column by the corresponding standard deviation. The obtained results (data not shown) showed a good separation between farmhouse cheeses (ripened at the farmhouse or the dairy plant), which presented mostly negative score values according to PC1, and Saint-Nectaire Laitier and Savaron (ripened at the dairy plant), which exhibited mostly positive score values according to the PC1.

3.2. Fluorescence spectra of different cheese samples

Typical fluorescence spectra recorded with laboratory and portable spectroflu-

orometer instruments are shown in Figures 2–4.

Fluorescence spectra obtained with excitation set at 280 nm using a laboratory spectrofluorometer exhibited a maximum located at about 345 nm (Fig. 2a) varying slightly between cheese samples, while spectra recorded using a portable spectrofluorometer exhibited a maximum located around 345 nm and a slight one observed at 440 nm (Fig. 2b). This latter peak was attributed to fluorescence of the Maillard-reaction products, which present a maximum emission at 440 nm [20]. As shown in Figure 2b, there was a good discrimination between farmhouse cheeses and the others, which was not observed for cheese samples obtained by using the laboratory spectrofluorometer. The obtained results partially confirmed the findings of

Karoui et al. [9, 13], reporting that a maximum located around 345 nm was observed for different cheese varieties and was attributed to tryptophan residues. The difference observed between the shapes of the spectra recorded on the investigated cheese samples could be due to the different molecular environments of tryptophan residues. One of the main conclusions of this study was that the portable spectrofluorometer could be considered as more sensitive than the laboratory spectrofluorometer. This could be due to the high number of measurement points collected with the portable spectrofluorometer (increment ~ 0.3 nm) compared with the laboratory spectrofluorometer (increment 1 nm).

Regarding the emission spectra acquired with excitation wavelength set at 320 nm, a maximum located around 410 nm was observed for cheese samples scanned by the laboratory spectrofluorometer (Fig. 3a). Considering spectra collected using the portable spectrofluorometer, maxima located around 405, 410, 422, 442, 488 and 510 nm were observed for all the cheese samples (Fig. 3b). The band located around 410 nm has, previously, been attributed to vitamin A, as pointed out by several findings [4, 8]. The difference observed between the spectra scanned on cheese samples could be related to the modification in the physical state of triglycerides in the fat globule during the ripening stage. Indeed, Dufour et al. [4] reported that the structure of triglyceride acyl chains in fat globules is modified during the ripening stage of semi-hard cheese. Again, a good differentiation between farmhouse cheeses and the other cheese samples was found with the portable spectrofluorometer.

Finally, the emission spectra scanned with excitation set at 380 nm using the laboratory and the portable spectrofluorometer(s) showed three spectral regions (Figs. 4a and 4b): the broad peak around

523 nm (observed with the two instruments) is due to riboflavin compounds, as suggested previously by several authors [21, 24]. The second region located between 410 and 480 nm shows fluorescence from stable oxidation products formed by aldehydes and amino acids [19]; lumichrome, a photo breakdown product from riboflavin, as well as β -carotene, exhibits fluorescence in the 410–480 nm [6], and could influence the shape of the fluorescence spectra. The last region observed, essentially, on cheese samples recorded with the laboratory spectrofluorometer is from 600 to 640 nm, characteristic of porphyrin and chlorin compounds as has been reported by Wold et al. [27]. As we can notice, a good differentiation between farmhouse cheeses ripened at the farmhouse and dairy plant and the other cheese samples ripened at the dairy plant was observed with the portable spectrofluorometer.

The emission spectra of the investigated cheese samples are due to several fluorescent compounds occurring in the various cheese samples in different concentrations and different environments, leading to different spectral features of cheeses. In addition, as the number of point measurements (wavelengths) is higher than the number of cheese samples, it is essential to use multivariate statistical analysis to predict chemical parameters.

3.3. Partial least squares regression

Table I indicates the minimum, maximum, mean and standard deviation (SD) values of the investigated chemical parameters performed on cheese samples.

The best cross-validation results for the three different excitation wavelengths are presented in Table II. For all the investigated parameters, the optimum number of latent variables (LV) varies between 1 and 15.

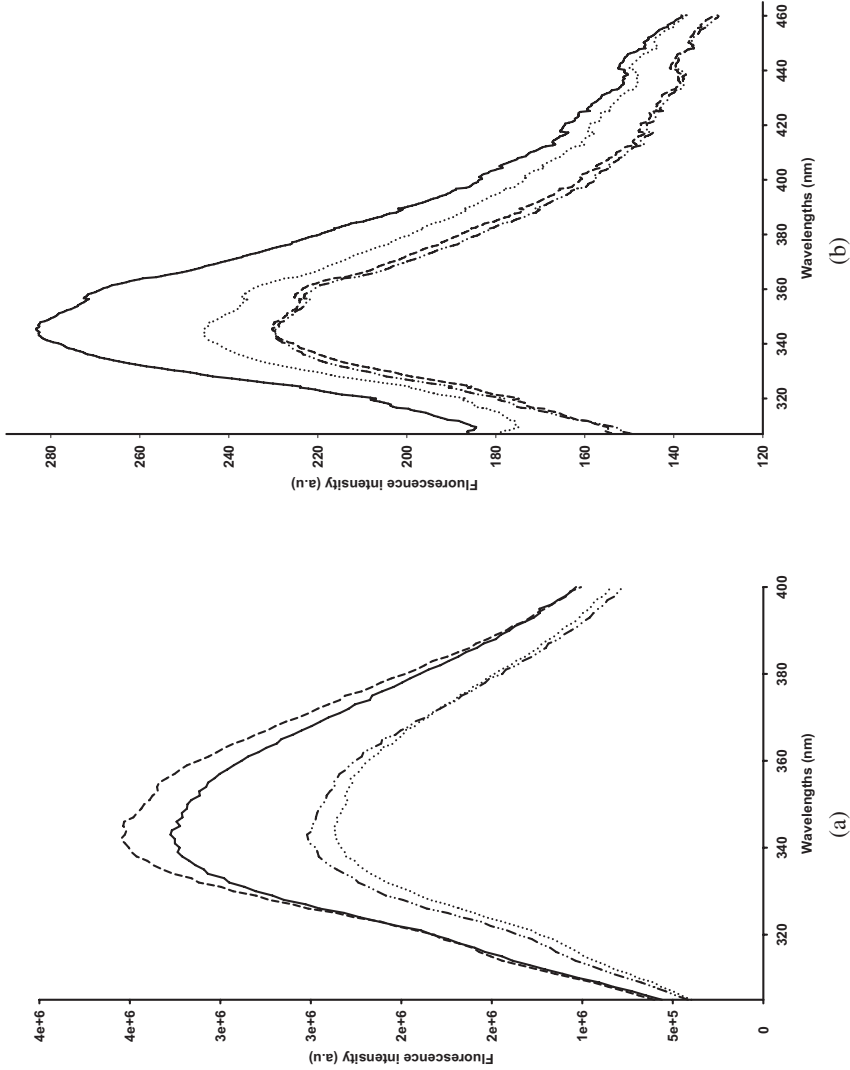


Figure 2. Fluorescence spectra recorded with excitation set at 280 nm using laboratory (a) and portable (b) spectrofluorimeters on farmhouse Saint-Nectaire ripened at the farmhouse (—), farmhouse Saint-Nectaire ripened at the dairy plant (· · ·), Saint-Nectaire “Laitier” ripened at the dairy plant (---) and Savaron ripened at the dairy plant (— · — · ·).

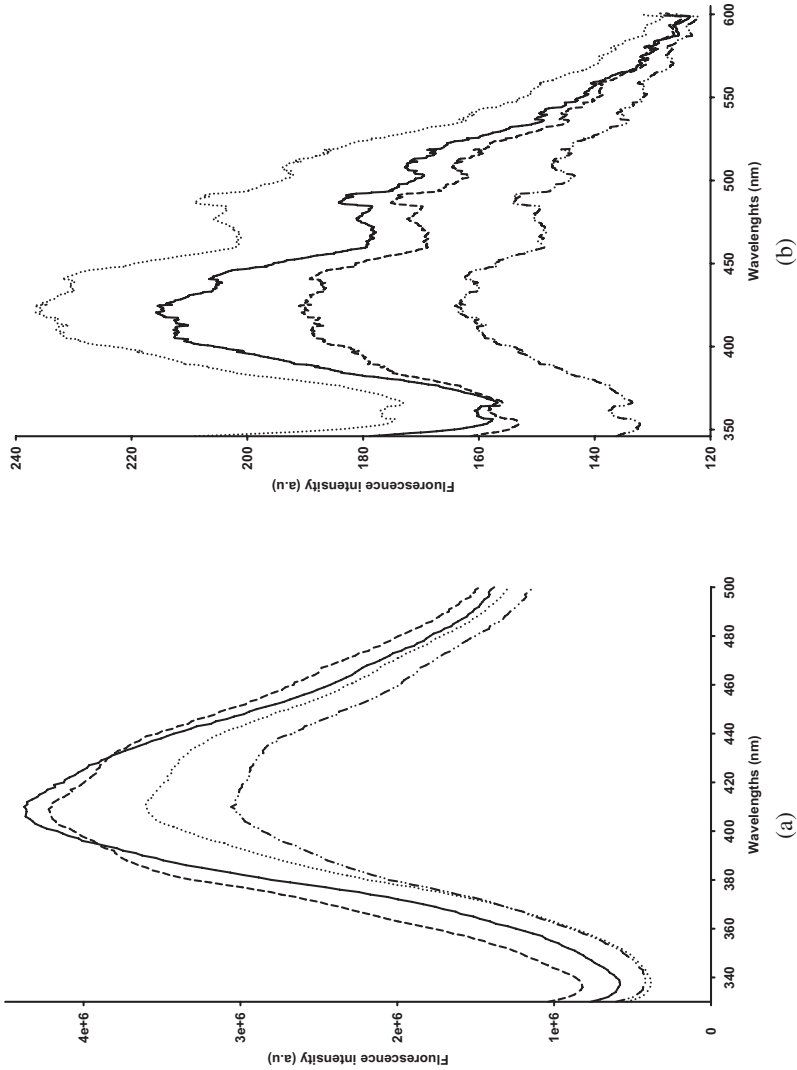


Figure 3. Fluorescence spectra recorded with excitation set at 320 nm using laboratory (a) and portable (b) spectrofluorimeters on farmhouse Saint-Nectaire ripened at the farmhouse (—), farmhouse Saint-Nectaire ripened at the dairy plant (···), Saint-Nectaire “Laitier” ripened at the dairy plant (---) and Savaron ripened at the dairy plant (—·—·).

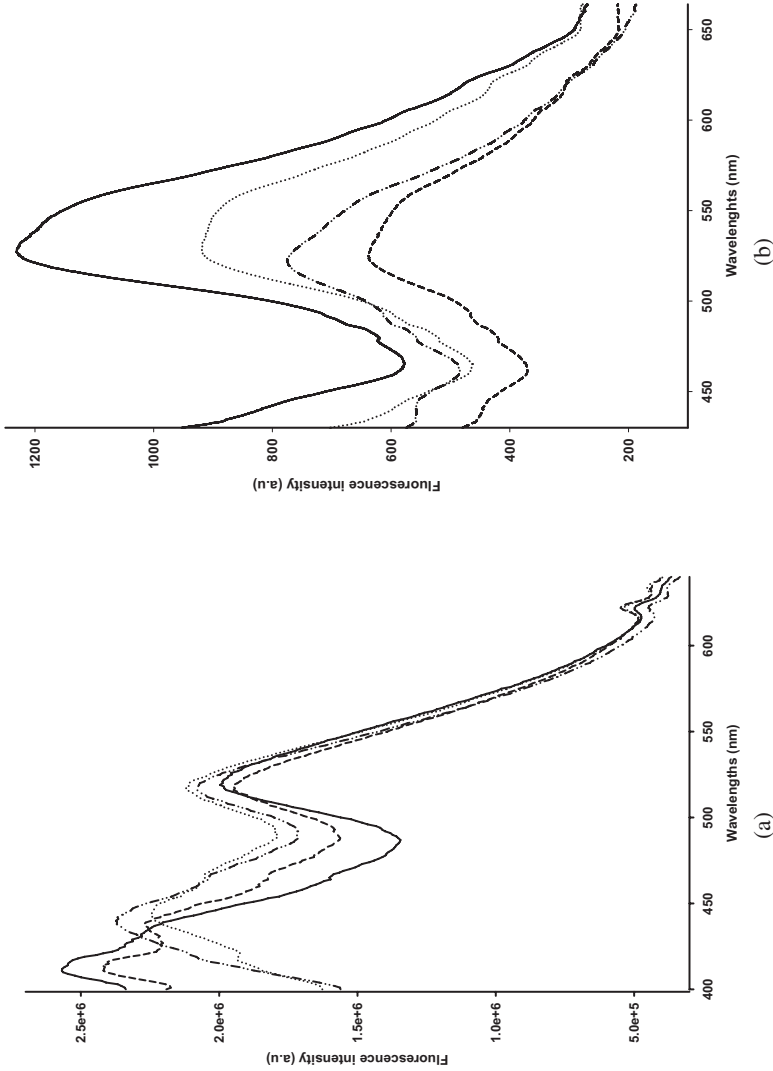


Figure 4. Fluorescence spectra recorded with excitation set at 380 nm using laboratory set (a) and portable (b) spectrofluorimeters on farmhouse Saint-Nectaire ripened at the farmhouse (—), farmhouse Saint-Nectaire ripened at the dairy plant (...), Saint-Nectaire “Laitier” ripened at the dairy plant (---) and Savaron ripened at the dairy plant (-.-.-).

Table II. Validation results of the different excitation wavelengths: 280, 320 and 380 nm models developed with the PLS-cross-validation regression coefficient using the laboratory and portable spectrofluorometers^a.

Compositional parameter	Excitation wavelength (nm)	LV	R ²	Slope	RMSEP	RPD	RER
pH	280: Laboratory	4	0.25	0.35	0.19	1.14	3.15
	Portable	15	0.74	0.81	0.11	1.98	5.44
	320: Laboratory	7	0.38	0.47	0.17	1.27	3.49
	Portable	8	0.71	0.81	0.12	1.83	5.06
	380: Laboratory	1	0.01	0.03	0.22	0.98	2.69
	Portable	13	0.51	0.67	0.15	1.39	3.83
Fat (g·100 g ⁻¹)	280: Laboratory	5	0.62	0.70	1.59	1.61	6.62
	Portable	7	0.80	0.82	1.12	2.29	9.40
	320: Laboratory	5	0.80	0.85	1.14	2.24	9.21
	Portable	4	0.75	0.78	1.27	2.02	8.29
	380: Laboratory	9	0.76	0.79	1.23	2.07	8.50
	Portable	11	0.83	0.88	1.06	2.42	9.93
DM (g·100 g ⁻¹)	280: Laboratory	3	0.29	0.36	2.40	1.19	3.20
	Portable	8	0.59	0.66	1.81	1.57	4.23
	320: Laboratory	8	0.75	0.78	1.41	2.02	5.44
	Portable	11	0.71	0.83	1.57	1.81	4.88
	380: Laboratory	13	0.71	0.84	1.57	1.81	4.89
	Portable	14	0.91	0.92	0.85	3.37	9.08
TP (g·100 g ⁻¹)	280: Laboratory	5	0.69	0.76	0.80	1.81	6.42
	Portable	12	0.77	0.77	0.69	2.11	7.45
	320: Laboratory	7	0.68	0.74	0.81	1.78	6.30
	Portable	11	0.67	0.77	0.84	1.73	6.10
	380: Laboratory	10	0.71	0.82	0.80	1.82	6.42
	Portable	13	0.86	0.88	0.53	2.71	9.59
SP (g·100 g ⁻¹)	280: Laboratory	5	0.52	0.60	0.80	1.45	4.45
	Portable	10	0.52	0.68	0.83	1.40	4.29
	320: Laboratory	5	0.27	0.38	1.01	1.15	3.54
	Portable	8	0.65	0.76	0.70	1.67	5.13
	380: Laboratory	1	0.19	0.21	1.04	1.12	3.44
	Portable	11	0.65	0.77	0.70	1.66	5.09

^a Abbreviations: DM, dry matter; TP, total protein; SP, soluble protein; LV, latent variables; R², determination coefficient; RMSEP, root mean square error of prediction; RPD, ratio of prediction deviation (standard deviation/RMSEP); RER, range error ratio ((maximum – minimum)/RMSEP).

3.3.1. Prediction of chemical parameters with the laboratory spectrofluorometer

The best results for pH, fat and DM were obtained with the excitation wavelength set at 320 nm. Quite similar results were obtained with the three excitation wavelengths for TP, while the excitation

wavelength set at 280 nm gave the best results for the prediction of SP. From the obtained results, it was concluded that measurements of fat, DM and TP could be considered as approximate by using the excitation wavelength set at 320 nm or 380 nm. Measurements of SP could be used only for differentiating between low

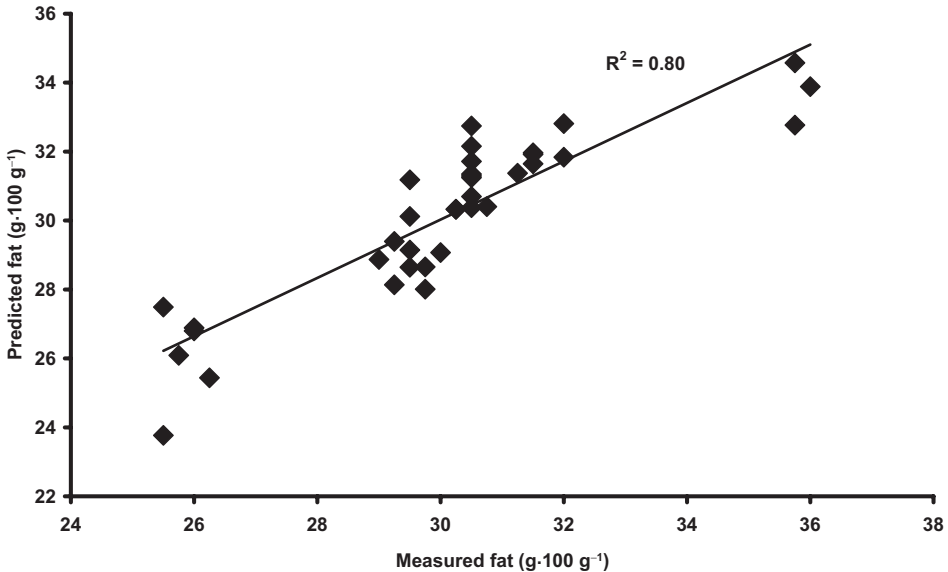


Figure 5. Partial least squares (PLS) prediction models: actual versus predicted value plots for cross-validation prediction of a training set of emission spectra scanned with excitation set at 320 nm using a laboratory spectrofluorometer for fat.

and high values by using only the excitation wavelength set at 280 nm, since the other two excitation wavelengths (320 nm and 380 nm) gave unsuccessful results. Also, unsuccessful prediction of the pH value was observed, whatever the considered excitation wavelength. Using the excitation wavelength set at 320 nm, the best results for fat and DM were obtained after applying range normalization to the fluorescence spectra ($R^2 = 0.80$; RPD = 2.24; RER = 9.21; Fig. 5) and ($R^2 = 0.75$; RPD = 2.02; RER = 5.44), respectively (Tab. II). For the prediction of TP, the best result was obtained with the excitation wavelength set at 380 nm (Tab. II). The obtained results confirmed previous findings reporting that the best results for the prediction of fat and DM of 15 soft cheeses, produced by using different mesophilic starter cultures, were obtained with the vitamin A spectra [16]. The quite standard deviation of these two parameters (2.56 and 2.84 for fat and DM, respectively) observed in the present study

was also reported in the research of Karoui et al. [16] on soft cheese samples (2.19 and 2.27 for fat and DM, respectively).

Regarding the prediction of the pH value, the results obtained in the present study were less successful than those obtained by Karoui et al. [16] on 15 different soft cheeses. Indeed, the authors pointed out that the determination of the pH value by fluorescence techniques could be considered as approximate, while in the present study an unsuccessful result was obtained. One explanation could arise from the fact that a small range in the variation of the pH value was observed in the present study (standard deviation of 0.21 against 0.39 in the work of Karoui et al. [16]).

Concerning the prediction of TP, similar results to those observed with the investigation of Karoui et al. [16] was found, while the poorest results were obtained in the present study for SP compared with those observed by Karoui et al. [16] on 15 different soft cheeses. Again, this could

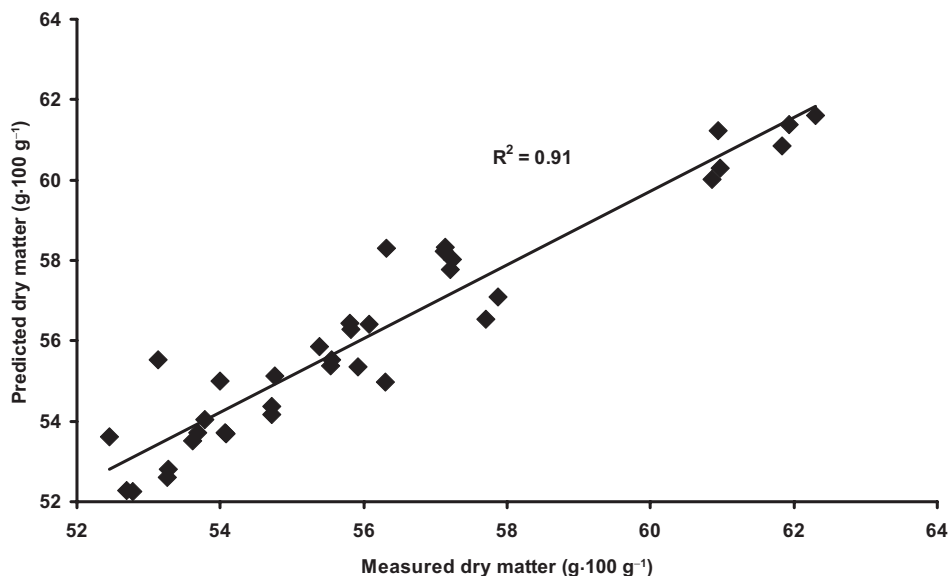


Figure 6. Partial least squares (PLS) prediction models: actual versus predicted value plots for cross-validation prediction of a training set of emission spectra scanned with excitation set at 380 nm using a portable spectrofluorometer for dry matter.

be explained by the low variation in the values of SP in the investigated cheeses. Thus, a high number of cheese samples presenting large variation in SP should be investigated in light of these findings.

3.3.2. Prediction of chemical parameters with the portable spectrofluorometer

The best results for fat, DM and TP were obtained with the excitation wavelength set at 380 nm, while similar results were obtained for SP with the excitation wavelength set at 320 nm and 380 nm, and quite similar results were obtained for pH values by using the excitation wavelength set at 280 nm and 320 nm.

Based on the calibration data set, measurement of all the investigated chemical parameters with excitation at 380 nm were obtained after applying mean normalization to the fluorescence spectra data sets. Prediction of DM (Fig. 6) could be

considered as excellent, while measurements of fat and TP could be considered as good by using the same excitation wavelength set at 380 nm (Tab. II). The determination of SP and pH with the excitation wavelength set at 380 nm ($R^2 = 0.65$; RPD = 1.66; RER = 5.09) and 280 nm ($R^2 = 0.74$; RPD = 1.98; RER = 5.44), respectively, could be considered as approximate. From the obtained results it can be concluded that the portable spectrofluorometer could be used successfully for measuring DM, fat and TP for the investigated cheeses. One of the main conclusions of this study was that the portable spectrofluorometer gave similar/better results than the laboratory spectrofluorometer. This could be a benefit for on-line measurement of such chemical parameters at the industrial level using the portable spectrofluorometer. It could also be an alternative method to the reference ones used to determine fat and TP, taking into account that the Kjeldahl and Gerber methods used

for determining these parameters, respectively, in such cheeses are very expensive and need a long time.

In order to have an interpretation at the molecular level for the prediction of some chemical parameters, the regression coefficients of the PLS models were studied. Since the best results were obtained with the portable spectrofluorometer, only regression coefficients obtained with this apparatus were studied by using the excitation wavelength set at 380 nm, except for the pH parameter where an excitation wavelength of 320 nm was considered. The regression coefficients of fat, DM, TP, SP and pH are shown in Figures 7a, 7b, 7c, 7d and 7e, respectively.

The regression coefficient of fat indicates that 410–550 nm was the most important spectral region (Fig. 7a). The 560–705 nm spectral region also showed some modifications but less marked than the former one. Three peaks located at 419, 461 and 533 nm were correlated positively, while two other peaks located at 432 and 523 nm were negatively correlated with the fat. The peak located at 523 nm was attributed to riboflavin as reported by Wold et al. [27].

Regarding the regression coefficient of DM (Fig. 7b), two spectral regions located between 410–470 nm and 580–652 nm were interesting due to some correlation. Using only these two spectral regions, similar results to the model developed with the entire wavelength range (data not shown) was found. The regression coefficient of TP showed a strong positive peak located at 614 nm (Fig. 7c), while that of SP showed two strong negative peaks located at 470 and 624 nm and a positive peak located at 485 nm (Fig. 7d).

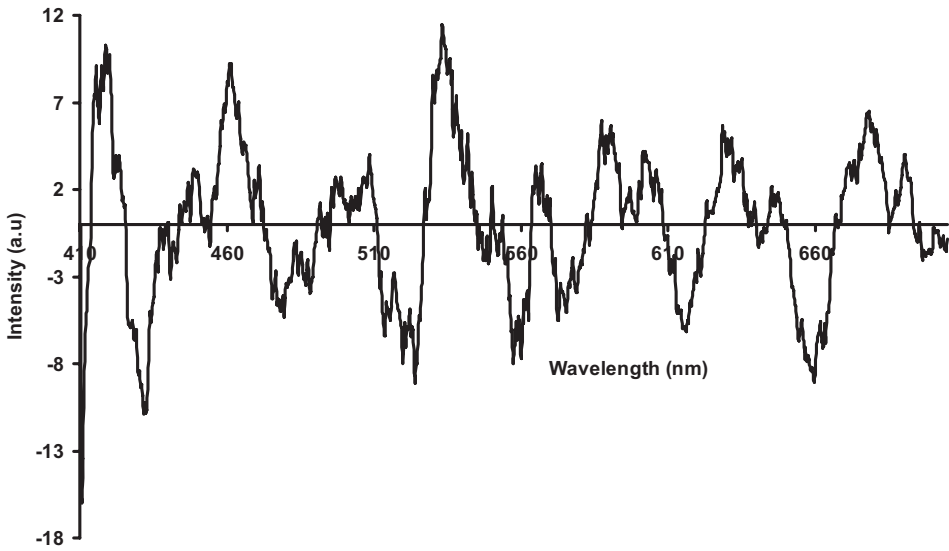
The regression coefficient of pH indicates a strong negative peak around 410 nm, which could be due to vitamin A compound. This negative correlation indicates that a decrease in the maximum emission intensity around 410 nm occurred

when the pH increased (Fig. 7e). This could be explained by the sensitive characteristic of vitamin A. Indeed, as vitamin A is present in the core and the membrane of fat globules, the modifications of pH could modify the ionization state as well as the hydration of some components such as proteins and phospholipids. These changes could induce some modifications in the environment of the fat globules and, as a consequence, in the membrane, that would affect the shape of vitamin A spectra [18].

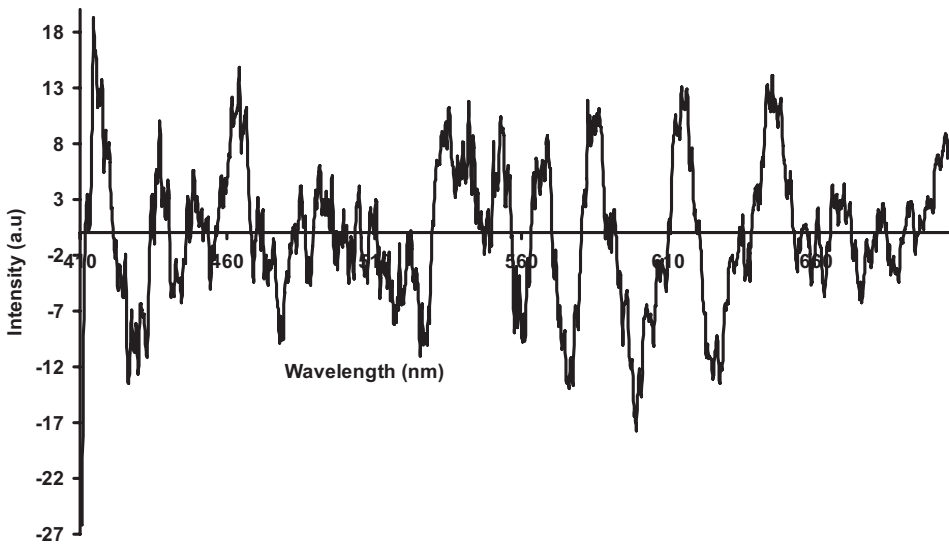
4. CONCLUSION

This preliminary work demonstrated that it was possible to determine with excellent and good predictions DM and fat contents, respectively, of the investigated semi-hard cheeses by using portable spectrofluorometer, with the excitation wavelength set at 380 nm. Using the laboratory spectrofluorometer with the excitation wavelength set at 320 nm/380 nm, the determination of DM and TP could be considered as approximate. Similar results were obtained with the portable spectrofluorometer with the excitation wavelength sets at 380 nm and 280 nm for the prediction of SP and pH, respectively.

The portable spectrofluorometer, comprising a light-emitting diode (LED) for excitation, a spectrometer, a fiber optic and an integrated PC with a touch-screen for recording and evaluating data sets, demonstrated its accuracy and effectiveness in predicting DM and fat of 12 French semi-hard cheeses belonging to four brands by using partial least squares (PLS) regression. Simple to use, this portable spectrofluorometer provides a fast and accurate analysis in 1 s, while 2 min is needed to acquire the same spectrum using the standard laboratory-based spectrofluorometer. Although we are still limited to testing the portable spectrofluorometer in dairy plants, which will be realized in the next five months, the results illustrated in the present



(a)



(b)

Figure 7. Regression coefficient distribution over the spectra scanned on Saint-Nectaire cheeses with excitation set at 380 nm using a portable spectrofluorometer for fat (a), dry matter (b), total protein (c) and soluble protein (d), and 320 nm for pH (e).

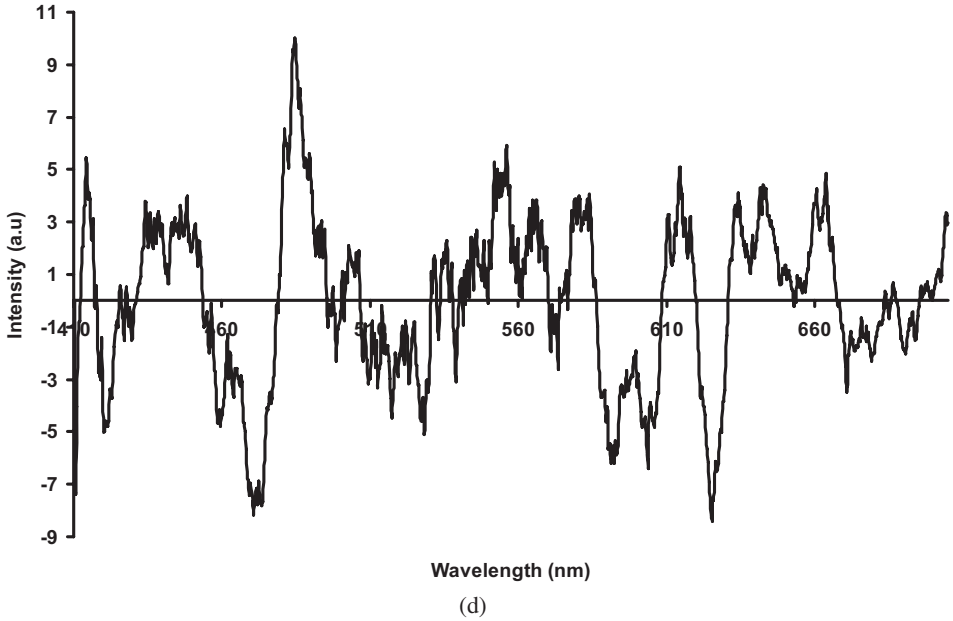
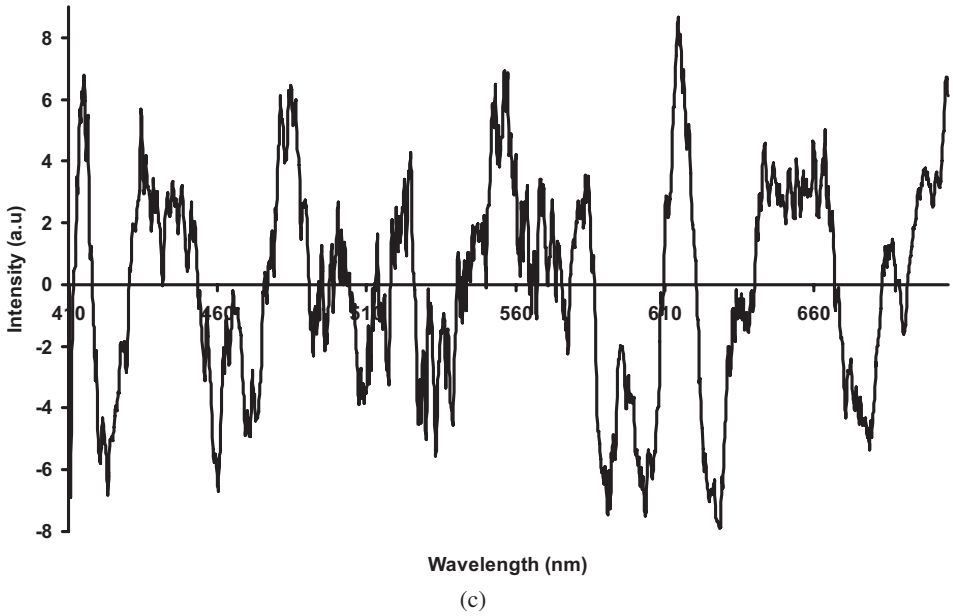
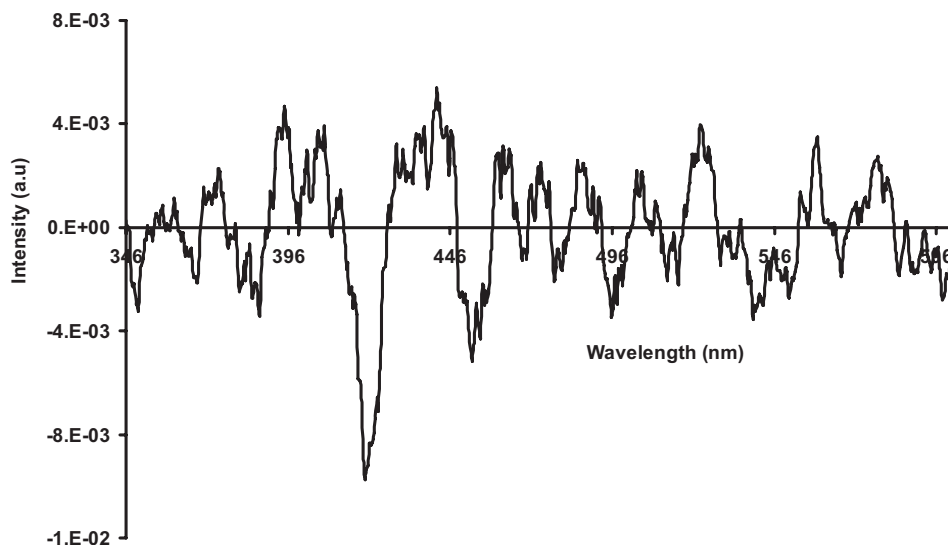


Figure 7. Continued.



(e)

Figure 7. Continued.

study, among others, appear to be very encouraging in considering this prototype as the starting point for a new low-cost technology and a rapid tool for the evaluation of dairy products as an on-line technique.

The simplicity of the new portable spectrofluorometer offers rich opportunities for efficient characterization of cheese products among others at a relatively low cost. In addition, the portable spectrofluorometer has the potential of dramatically reducing the analytical time when looking at physico-chemical measurements.

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