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Fiber optic fluorescence as non-invasive tool to monitor the ripening process of cheeses: *Torta del casar* and *Queso de la Serena*

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ABSTRACT

In this work, the use of excitation-emission fluorescence matrices (EEMs) has been addressed to differentiate two kinds of cheeses with protected designations of origin (PDO), "Torta del Casar" and "Queso de la Serena". The fluorescence signals were recorded directly on cheese samples during the ripening process, by using a fiber optic assembled to the fluorescence spectrometer, and the data were subjected to analysis by the multivariate algorithm parallel factor analysis (PARAFAC). PARAFAC analysis identified the presence of three fluorophores in both cheeses. The scores of the two first components, attributed to the presence of tryptophane, decreased as the ripening time increased. However, the third component, related to retinol (vitamin A), remains almost constant along the ripening period. EEMs of the samples of the two types of cheese were similar in the initial days of the maturation periods. However, when only the samples from day 36 onwards were considered, it was possible to differentiate them with high precision by PARAFAC in combination with linear discriminant analysis (LDA).

In addition, a model was built for the quantification of vitamin A and α-tocopherol (vitamin E), by using the EEMs combined with the multivariate unfolded partial least squares (UPLS) algorithm.

1. Introduction

Extremadura, a region located in the southwest of Spain, is the cradle of two soft cheeses of recognized international prestige. Both cheeses are crafted from raw ewe's milk. One of them, known as "Torta del Casar", is produced in the northern region of Extremadura, while the other, "Queso de la Serena", is crafted in the southeast of the region. They are marketed under the Registry of the Protected Designation of Origin (PDO) "Torta del Casar" (Casar de Cáceres, Cáceres, Spain), and "Queso de la Serena" (Castuera, Badajoz, Spain) in accordance with Regulations (EC) 1491/2003 and 1107/96, respectively. Both cheeses belong to the soft to semi-soft Spanish variety, made exclusively from raw Merino ewe milk. Throughout their maturation process, only a vegetable coagulant, specifically rennet extracted from Cynara cardunculus, is employed, and no additional starter culture is added. The use of Merino ewe raw milk and vegetable rennet gives these cheeses their peculiar characteristics in terms of flavour and texture. They are consumed with a minimum maturation period of 60 days from the date of moulding, but they may maturate for up to 4 months. The texture of these cheeses is more or less soft depending on their maturity. The firmness and consistency of these cheeses decreased along ripening while adhesiveness increased (Delgado, Rodriguez-Pinilla, González-Crespo, Ramírez, & Roa, 2010).

During the maturation process, the fats and proteins of cheese are subjected to physical and chemical changes, which determine the sensory properties of the final product. On-line monitoring of the changes in the composition of the cheese constituents, especially proteins, and fats, will help understand the ripening process and can be useful to characterize and discriminate between different cheeses.

Fluorescence spectroscopy, being a sensitive, rapid, selective, and non-invasive analytical methodology, can be a good technique for monitoring the structural evolution of foods at a molecular level. In the case of cheeses, intrinsic fluorophores have been identified such as retinol (vitamin A) and aromatic amino acids of proteins. Vitamin A is in both, the core, and the membrane of the fat globule (Dufour et al., 2000) and, due to its conjugated double bonds, presents adequate fluorescence. Aromatic amino acids of proteins are also fluorescence compounds that can be used to study protein structure and protein interactions with hydrophobic molecules or ions.

Fluorescence spectroscopy has been applied with different purposes in different types of cheeses. For instance, changes in the vitamin A fluorescence spectra during ripening of semihard cheeses were studied allowing the discrimination between 16 cheeses by using principal

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component analysis (PCA) (Dufour et al., 2000). A similar study was performed by (Herbert et al., 2000) but, in this case, in addition to vitamin A, the tryptophan fluorescence spectra were also used. Both spectra, in combination with PCA and factorial discriminant analysis, enabled the discrimination of 8 soft cheeses (Herbert et al., 2000). Fluorescence spectra in this spectroscopic region were also used for monitoring the oxidation of semi-hard cheeses throughout ripening (Karoui, Dufour, & De Baerdemaeker, 2007) or differentiate cheeses submitted to different manufacturing processes (Karoui, Dufour, Schoonheydt, & Baerdemaeker, 2007).

Tryptophan fluorescence spectrum was also used in other studies to investigate changes during the maturation process, for the characterization of cheeses, or the discrimination between different types (Dufour et al., 2001; Mazerolles et al., 2001), such as to discriminate between Emmental cheeses originating from different European countries (Karoui et al., 2004).

Moreover, the coupling of fluorescence spectra and infrared spectra was used to study the modifications of proteins intervening during cheese ripening. The authors proposed two complementary chemometric methods to manage the whole information provided by both spectroscopic techniques (Mazerolles et al., 2002).

All the articles described previously are based on the use of emission or excitation spectra in combination with first-order chemometric algorithms, such as PCA or discriminant analysis. However, the excitation-emission matrices (EEMs) have also been used, in combination with Parallel Factor analysis (PARAFAC), with different purposes in cheese samples, such us the evaluation of the stability of processed cheeses during storage (Christensen et al., 2003).

Fluorescence spectroscopy has also been used with quantification purposes in cheese samples. In this context, the meltability and the viscoelastic behaviour of hard and semi-hard cheeses were evaluate using protein tryptophan emission spectrum and vitamin A excitation spectrum (Karoui et al., 2003). In the same way, tryptophan, riboflavin, and vitamin A fluorescence spectra were used to predict some chemical parameters, as fat, dry matter, pH, total nitrogen, and water-soluble nitrogen contents, in soft cheeses (Karoui et al., 2006), and EEMs were used to quantify the maturation time, proteolysis index and total free amino acids content (Kokawa et al., 2015).

Given the utility of fluorescence spectroscopy in characterizing cheese samples, excitation-emission matrices, in combination with chemometric algorithms, have been used in this study for different purposes. Firstly, the aim was to identify the fluorophores present in these types of cheese and examine their evolution throughout the maturation process. Secondly, the focus was on discriminating between both types of cheeses and, finally, the quantification of vitamin A and α-tocopherol (vitamin E).

2. Materials and methods

2.1. Reagents, solvents, and standards

Retinol (vitamin A) and α-tocopherol (vitamin E) were purchased from Sigma-Aldrich (Stenheim, Germany). Potassium hydroxide (KOH), HPLC grade ethanol, n-hexane, ethyl acetate, and methanol were obtained from Panreac (Barcelona, Spain). Sodium chloride (NaCl) was acquired from Scharlau (Barcelona, Spain). Tert-butylhydroquinone was provided by Merck (Barcelona, Spain).

Ultrapure water was obtained through a Milli-Q purification system from Millipore (Bedford, USA).

2.2. Cheese samples

Cheeses samples were purchased from two dairies belonging to the two Protected Designations of Origin (PDO) of Extremadura. One of them belongs to the "Torta del Casar" PDO and the other belongs to the "Queso de la Serena" PDO.

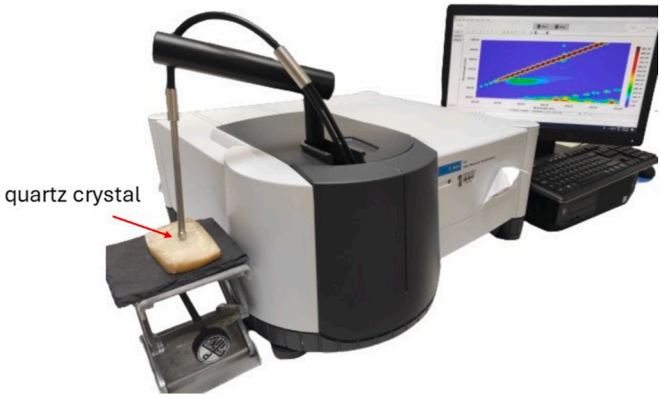

Fig. 1. Experimental setup for excitation-emission fluorescence matrix collection.

For each type of soft cheese, a batch was randomly selected, and three samples were taken each week along the maturation process, until day 60, when it is considered that this type of cheese can be commercialised. In total, 30 samples of each type of cheese were analyzed.

2.3. Excitation-emission matrices collection

All spectrofluorimetric measures were acquired with a fiber optic assembly for the Cary Eclipse fluorescence spectrometer (Agilent Technologies, Madrid, Spain). The equipment was connected to a PC microcomputer via an IEEE 488 (GPIB) serial interface. The Cary Eclipse 1.2.2.0 software was used for data acquisition.

The cheeses were analyzed on the same day of arrival, cutting slices approximately 1 cm thick. A quartz crystal was placed on these to avoid direct contact of the fiber with the soft paste. Fig. 1 shows the experimental setup.

EEMs were recorded as a set of fluorescence emission spectra over a range of excitation wavelengths under normal laboratory illumination. The excitation wavelengths ranged from 200 to 500 nm (each 10 nm). At each excitation wavelength, the emission spectra were recorded from 250 to 550 nm (each 1 nm). Two EEMs were collected for each cheese sample and three different cheeses of each type were analyzed each week.

The slits of excitation and emission monochromators were set at 5 nm and the photomultiplier tube sensitivity was 650 V. The scan rate was set at 600 nm min^{-1}.

2.4. Chemometric analysis

Data analysis was carried out in MatLab R2016B (The MathWorks Inc., USA). The Rayleigh dispersion correction was made employing the EEM_corr routine (Chiappini, Alcaraz, Goicoechea, & Olivieri, 2019), freely downloaded from https://fbcb.web1.unl.edu.ar/laboratorios/ladaq/download/. PARAFAC and U-PLS calculations were performed using the graphical interface MVC2 (Chiappini et al., 2023), available at http://www.iquir-conicet.gov.ar/descargas/mvc2.rar. U-PLS regression models were constructed to predict the vitamin A and vitamin E concentrations from the fluorescence data.

2.5. Extraction and liquid chromatographic analysis of vitamin A and vitamin E

The amount of Vitamin A and Vitamin E in the cheese samples was determined by reversed phase liquid chromatography (LC) following previously proposed methods (Manzi et al., 1996; Panfili et al., 1994).

For each sample, 0.5 g of the center of the cheese were subjected to alkaline digestion in a centrifuge tube with 2 mL of KOH (60 g in 100 mL

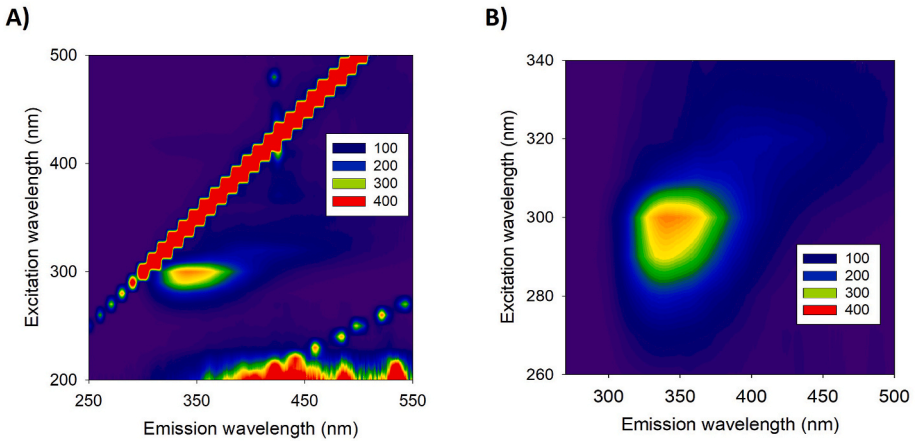

Fig. 2. Fluorescence contour plots of the EEMs obtained with the fiber-optic assembly of the spectrofluorometer, in a commercial cheese sample. A) Contour plot in the entire wavelength range. B) Contour plot in the selected wavelength range and with Rayleigh scattering correction.

of water), 2 mL of ethanol (99.9%), and 1 mL of NaCl (1 g in 100 mL of water). In addition, 5 mL of *tert*-butylhydroquinone (6 g in 100 mL of ethanol) were also added as an antioxidant. The tubes were placed in a water bath at 70 °C for 30 min. After the suspension was cooled, 5 mL of the NaCl solution were added to prevent emulsification**.** The suspension was then extracted twice with 10 mL of n-hexane/ethyl acetate (9:1, mL: mL). The supernatants were mixed and evaporated to dryness with a rotatory evaporator at 30 °C. The residue was dissolved in 1 mL of methanol and filtered with a 0.20 μm PTFE filter before the injection in the chromatographic system.

LC analysis was carried out on an Agilent 1260 Infinity Quaternary LC system (Agilent Technologies) equipped with an online degasser, quaternary pump (G1311B), column oven compartment (G1316 A), autosampler (G1329B), UV–vis diode-array detector (G1315D) and fluorescence detector (G1321B). The ChemStation software was used to treat data and control the instrument. The analytical column was a Zorbax Eclipse XDB-C18 analytical column (100 × 4.6 mm; 3.5 μm; Agilent Technologies) thermostated at 20 °C. The injection volume was 20 μL and the elution was in isocratic mode with 100% of methanol at a flow rate of 1 mL min^{-1}. The detection was performed in the

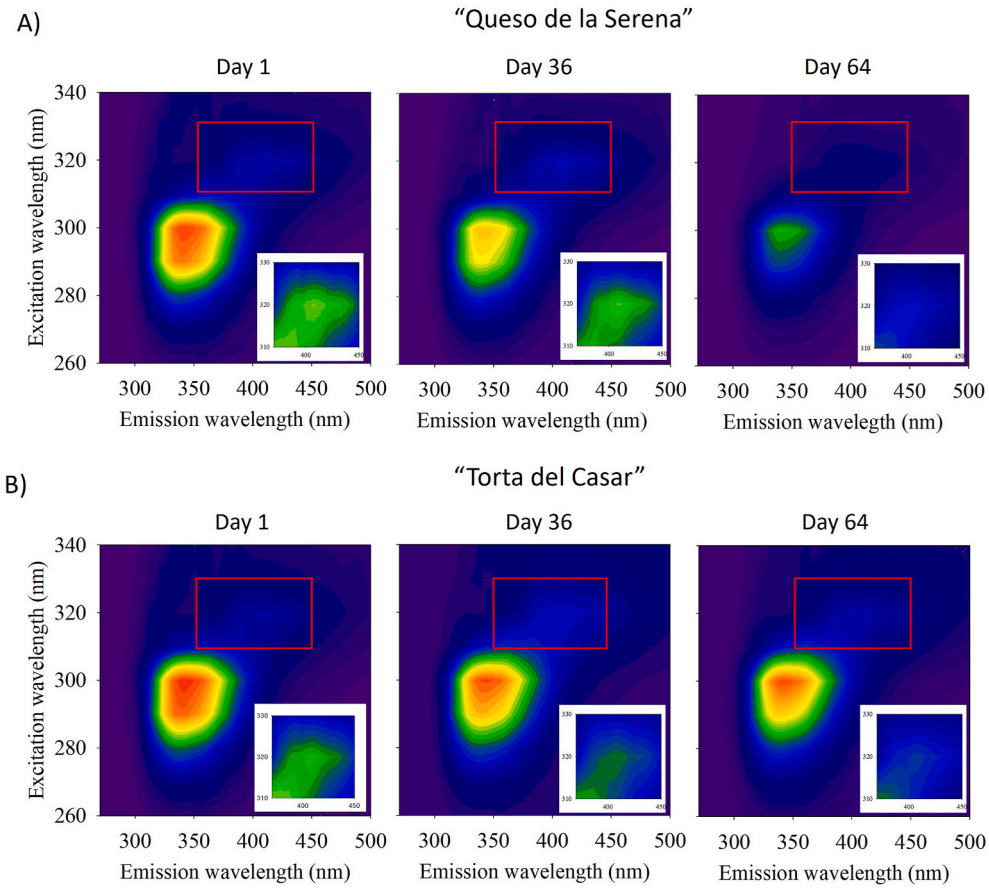

Fig. 3. Fluorescence contour plots of the EEMs obtained with a fiber-optic from samples corresponding to the first, middle and last days of the ripening period. A) "Queso de la Serena" and B) "Torta del Casar".

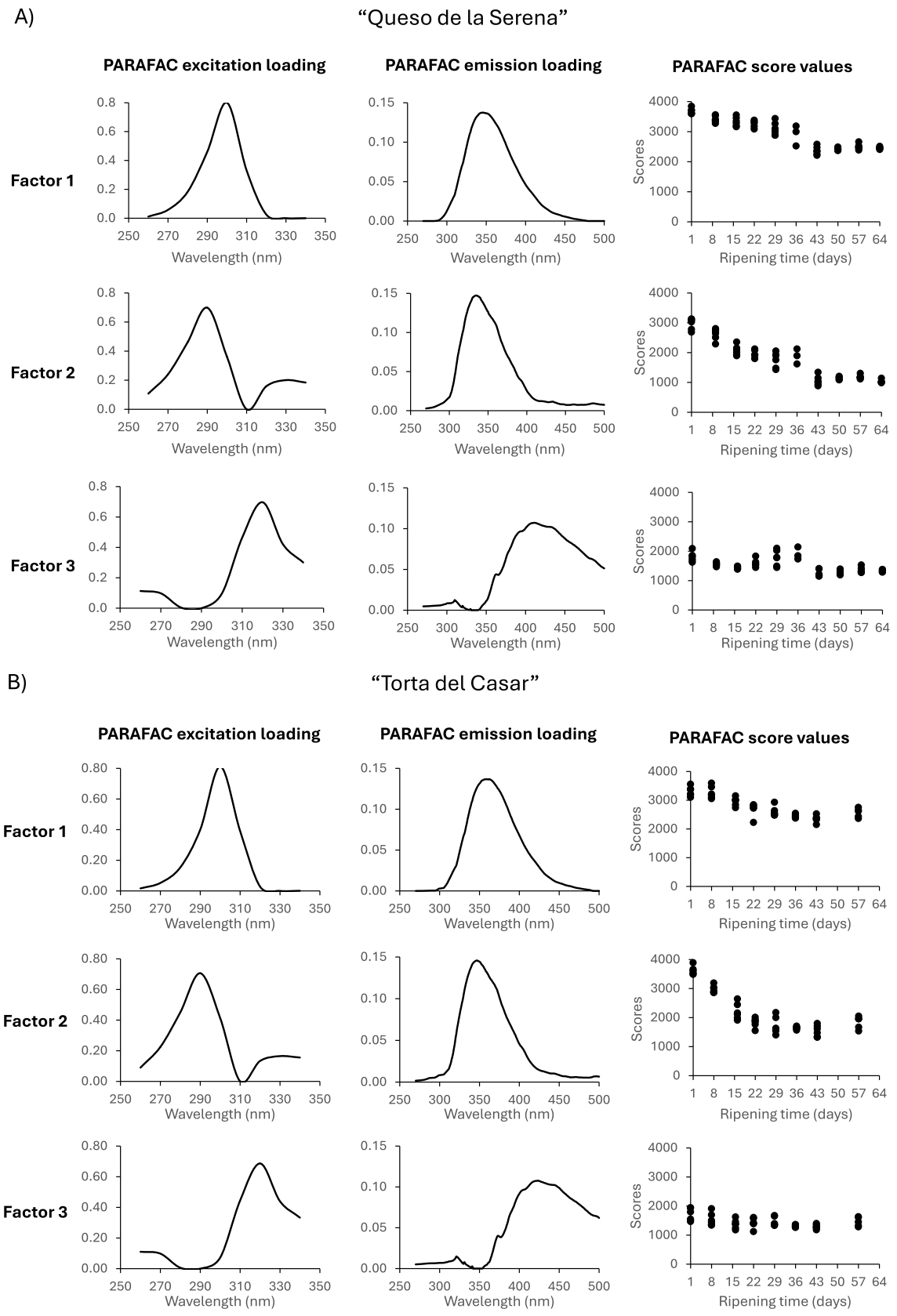

Fig. 4. Scores, and excitation and emission loadings of the three PARAFAC factors of the two types of cheeses. A) "Queso de la Serena"; B) "Torta del Casar".

fluorescence detector, being the excitation wavelength at 325 nm and the emission wavelength at 475, until 3 min of elution, and then changing the excitation wavelength to 280 nm and the emission wavelength to 325 nm.

External standard calibration, based on peak areas, was used for quantification of retinol and α-tocopherol in the cheeses.

3. Results and discussion

3.1. Optimization of measurement conditions. Fluorescence of the cheeses

To obtain reproducible and comparable fluorescence signals, it was first necessary to perform some experiments to optimize the measurement conditions in the spectrofluorometer. For these studies, a commercial sample of Torta del Casar cheese was used.

For the first experiment, the cheese sample was cut in half and a slice was obtained from the center. The EEMs were obtained by placing the optical fiber directly on the surface of the cheese in a wide range of excitation (200–500 nm, each 10 nm) and emission wavelengths (250–550 nm, each 1 nm), setting the excitation and emission slits at 5 nm and the photomultiplier tube sensitivity at 600 V.

It was demonstrated that the intensity of fluorescence signals was very influenced by the distance between the tip of the probe and the cheese surface. This results in a lack of reproducibility in consecutive measurements of the same sample due to the soft texture of this type of cheese, making it difficult to maintain the same distance in different measurements. To solve this problem, a quartz crystal was placed between the probe tip and the cheese sample. Under these modified conditions, the reproducibility of the measurements was verified, obtaining highly satisfactory results.

Another aspect to optimize was the selection of the specific location on the cheese for recording excitation-emission matrices. To achieve this, EEMs were recorded at different points along the cheese slice. The results showed that all the obtained EEMs presented a similar fluorescent pattern, but the intensity of the signals decreased as we moved away from the center towards the outside of the cheese slice. This decrease in intensity is likely attributed to the fact that in soft cheeses the maturation takes place from the rind towards the center. Consequently, the center of the cheese was chosen as the optimal point for conducting fluorescence measurements.

The instrumental parameters were also optimized. In the initial whole matrix (Fig. 2A), a fluorescent area containing relevant information can be observed with excitation/emission maxima at 300/350 nm, respectively. For the subsequent studies, fixed wavelength ranges were set from 260 to 340 nm (each 10 nm) in excitation and from 270 to 500 nm (each 1 nm) in emission. The slits and the photomultiplier tube sensitivity were maintained at their previous settings (5 nm for the excitation and emission slits, and 600 V).

As depicted in Fig. 2A, Rayleigh scattering can interfere in our area of interest. To address this, the EEM_corr routine was employed after acquiring the EEMs to eliminate Rayleigh's scattering. The interpolation option was applied to minimize alterations to the matrix. A 10 nm width was removed in both the first (Ry1) and second (Ry2) level Rayleigh scattering correction. Fig. 2B illustrates the EEMs in the selected area with Rayleigh's scattering successfully removed.

3.2. Effect of ripening on the fluorescence properties of the studied cheeses

To obtain the fluorescence fingerprints of the two types of cheeses, EEMs were collected with the fiber-optic assembly of the spectrofluorometer each week along the maturation period (up to day 64). EEMs were obtained under optimized conditions. In Fig. 3, EEMs of samples taken on the first day (day 1), a middle day (day 36), and the last day (day 64) of the ripening period are presented for both cheese types (A: "Torta del Casar"; B: "Queso de la Serena").

As can be observed, the fluorescence shape is similar in both types of cheeses throughout the ripening period. Two different zones can be distinguished: a highly intense zone with excitation between 270 and 310 nm and emission between 300 and 400 nm, and a second fluorescence zone, less intense, placed between 310 and 330 nm of excitation and 370 and 450 nm of emission, which was amplified in the insert of Fig. 3 for better visualization. These fluorescence fingerprints were similar to those obtained in previous works on other types of soft cheeses (Christensen et al., 2003; Dufour et al., 2001).

Concerning fluorescence intensity, it undergoes changes over the course of ripening in both zones. The intensity of the first zone decreases as the maturation time progresses. In "Queso de la Serena" cheeses, the decrease in fluorescence intensity is gradual, resulting in a much lower intensity at the end of the maturation compared to fresh cheeses. However, in "Torta del Casar" cheeses, it appears that the fluorescence intensity of this area initially decreases but then stabilizes. Concerning the second fluorescence zone, it also decreases with increasing ripening time, but the decline is more gradual than in the first zone.

3.3. PARAFAC analysis. Qualitative study about the maturation process of the cheeses

To obtain more information about the compounds responsible of the fluorescence of these cheeses and their evolution throughout the maturation period, PARAFAC analysis was performed (Bro, 1997).

The PARAFAC algorithm was applied independently in the two types of cheeses. Data were arranged in a 3D array with dimensions 60 × 220 x 9 (number of samples (3 samples x 10 weeks x 2 repetitions) x number of emission wavelengths x number of excitation wavelengths). PARAFAC was performed with one, two, three, and four components to select the optimal number of factors. Non-negative constraints were applied in all modes considering that neither the concentration nor the spectral values can be negative. The selection of the optimal number of factors or components was performed by applying the core consistency diagnosis (Bro & Kiers, 2003), the residual fit analysis (Muñoz de la Peña et al., 2003), and the physiognomy of the loadings. The core consistency criterion takes as the optimal value the largest tested value for which the core consistency is higher than 50 %. On the other hand, the analysis of residuals considers the optimal number of factors as the one at which the value of the residual fit becomes approximately constant. From these considerations, three factors were selected as optimal number by both methods in the two types of cheeses. This indicates that three different fluorophores are present in the cheese samples whose excitation and emission loading profiles are shown in Fig. 4.

No significant differences are observed between the loading profiles obtained for both types of cheese. The first and second factors present excitation/emission maxima at 300/346 nm and 290/336 nm, respectively. These loading profiles can be attributed to tryptophan (Christensen et al., 2003). The fluorescence properties of protein-bound amino acids can be influenced by the protein's structure. Therefore, these two loadings can correspond to different forms of tryptophan due to their inclusion in distinct protein structures (Lakowicz, 2006). With respect to the third component, it shows an excitation maximum at 320 nm and an emission maximum at 410 nm, and it can be assigned to vitamin A (Christensen et al., 2003).

On the other hand, Fig. 4 also illustrates the evolution of the scores of the three factors of PARAFAC along the maturation process in both types of cheeses. As can be seen, the progression is similar in both types of cheeses. The scores of the first and second components exhibit a decrease until day 36, after which they remain constant. In the case of the third component, score values remain practically constant throughout the maturation process. This indicates that the presence of vitamin A is not altered during the maturation process in both types of cheesses.

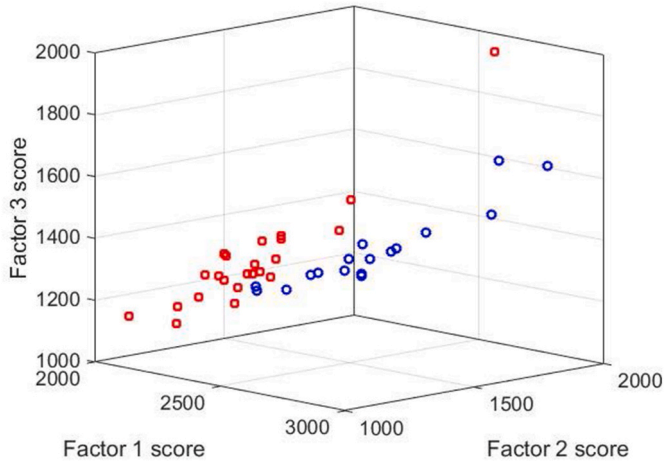

Fig. 5. PARAFAC scores for the discrimination between the two types of cheese, considering only samples from day 36. "Quesos de la Serena" (red) and "Torta del Casar" (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Confusion matrix for the cross validation obtained through the PARAFAC-LDA model for the discrimination of the two types of cheese. The values are expressed in number of samples.

Real type of cheese	Predicted type of cheese	
	"Queso de la Serena"	"Torta del Casar"
"Queso de la Serena"	**23**	0
"Torta del Casar"	1	**16**

Diagonal bold contains the percentage of correct assignments.

3.4. Discrimination between both types of cheese

Product authentication is an issue of growing importance within the food industry. Regulatory authorities, food processors, and consumers all have an interest in ensuring that food products are correctly labelled. These two types of cheese are very similar, in appearance and organoleptically, and could become seemingly alike, leading to confusion. For that, with the aim of studying if both types of cheeses can be discriminated by means of the fluorescence signal, the scores of the three PARAFAC components were plotted in a 3D graph. When samples from all the maturation times were included, the scores of the samples belonging to both types of cheese were overlapping, making it difficult to distinguish the two groups. However, when only the scores of the samples belonging to longer maturation times (from day 36 of maturation) were represented, two groups can be distinguished based on the type of cheese, as can be observed in Fig. 5.

In view of the separation obtained by considering the samples from day 36 onwards, a classification model was constructed by applying the LDA algorithm to the PARAFAC scores. Table 1 shows the results of the venetian blinds cross validation in the application of PARAFAC-LDA, expressed as a confusion matrix. As can be seen, almost all the samples were well classified, with a percentage of correct prediction of 97.5 %. Considering these results, we can conclude that, utilizing the fluorescent information obtained, the two types of cheese are similar in the early stages of the maturation period. However, for the more mature samples, both types of cheese can be distinguished with great accuracy.

3.5. Total retinol (vitamin A) and α-tocopherol (vitamin E) determination

Based on the above results, the possibility of quantifying the amount of total retinol and α-tocopherol in cheeses using EEMs combined with multivariate algorithms was addressed. For this purpose, the content of these vitamins in the cheese samples was analyzed using the LC method described in section 2.5. After that, U-PLS was applied to perform quantitative models between the EEMs and the experimental data obtained. A model was built for each type of cheese and vitamin. For each model, samples were randomly divided into two sets, the calibration set (70% of the samples) and the validation set (30% of the samples). The U-PLS models were built using the calibration samples. First, the optimum number of components was selected through cross validation, following the Haaland and Thomas criterion (Haaland & Thomas, 1988). The optimal number of components is indicated when a PRESS value that is not statistically different to the minimum PRESS value (F-ratio probability falling below 0.75) is achieved. The optimum number of latent variables was five for all models. Following this, predictions were performed with the validation samples.

The obtained results of the U-PLS models for the cross validation and the validation of both types of cheese are shown in Table 2. Concerning the accuracy of the developed U-PLS models, good results were obtained for all the data sets. The R2 values were higher than 0.85 and 0.73 in cross validation and validation, respectively. Additionally, low values for REMSECV, RMSEP, and REP were obtained, further indicating the robust performance of the models.

4. Conclusions

The present study demonstrated that fluorescence spectroscopy, combined with multiway algorithms, offers a powerful tool to monitor soft cheeses such as "Torta del Casar" PDO and "Queso de la Serena" PDO. The advantage of using fluorescence signals as sample fingerprints is related to their high selectivity. In addition, obtaining the EEMs with

Table 2
U-PLS parameters obtained for the cross validation and validation in both types of cheese.

		Torta del Casar		Queso de la Serena	
		retinol	α-tocopherol	retinol	α-tocopherol
	Components	5	5	5	5
cross validation	**R^2**	0.86	0.85	0.95	0.90
	RMSECV	33.5	2.3	16.2	9.9
	REP (%)	3.6	2.6	7.5	10.2
	LOD	13.9	1.0	2.2	0.5
	LOQ	41.5	3.0	6.7	1.4
validation	**R^2**	0.73	0.84	0.94	0.86
	RMSEP	73.3	6.3	23.9	10.1
	REP (%)	7.9	6.9	11.0	10.5

R2: coefficient of determination; RMSECV: root mean squares error of the cross validation; REP: relative error of prediction; LOD: limit of detection; LOQ: limit of quantification.

an optical fiber avoids sample treatment and allows on-line monitoring of the samples for proper quality control.

The EEMs in combination with PARAFAC showed that both types of cheeses are similar in the first stages of the ripening period. However, when only samples from day 36 were considered, PARAFAC-LDA allowed the classification of both types of cheeses with a high percentage of correct prediction. This indicates that this methodology could be a good option to be used as an authentication method for the final product. **For future works, it would be appropriate to select commercial samples from more industries of each denomination of origin and establish a more solid model for the discrimination of both types of cheese.**

Furthermore, it was demonstrated that EEMs in combination with U-PLS could be a viable alternative to the traditional method using HPLC, for the determination of vitamin A and E in cheeses. This method proves to be simpler, less expensive, and solvent-free.

CRediT authorship contribution statement

E. Martín-Tornero: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **I. Durán-Merás:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **A. Muñoz de la Peña:** Writing – review & editing, Supervision, Methodology, Conceptualization. **T. Galeano-Díaz:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Bro, R. (1997). PARAFAC. Tutorial and applications. *Chemometrics and Intelligent Laboratory Systems, 38*(2), 149–171. https://doi.org/10.1016/S0169-7439(97)00032-4

Bro, R., & Kiers, H. A. L. (2003). A new efficient method for determining the number of components in PARAFAC models. *Journal of Chemometrics, 17*, 274–286. https://doi.org/10.1002/cem.801

Chiappini, F. A., Alcaraz, M. R., Goicoechea, H. C., & Olivieri, A. C. (2019). A graphical user interface as a new tool for scattering correction in fluorescence data. *Chemometrics and Intelligent Laboratory Systems, 193*, 103810.

Chiappini, F. A., Muñoz de la Peña, A., Goicoechea, H. C., & Olivieri, A. C. (2023). An upgrade of MVC2, a MATLAB graphical user interface for second-order multivariate calibration: Beyond trilinear models. *Chemometrics and Intelligent Laboratory Systems, 237*, Article 104814. https://doi.org/10.1016/j.chemolab.2023.104814

Christensen, J., Povlsen, V. T., & Sørensen, J. (2003). Application of fluorescence spectroscopy and chemometrics in the evaluation of processed cheese during storage. *Journal of Dairy Science, 86*(4), 1101–1107. https://doi.org/10.3168/jds.S0022-0302(03)73692-3

Delgado, F. J., Rodriguez-Pinilla, J., González-Crespo, J., Ramírez, R., & Roa, I. (2010). Proteolysis and texture changes of a Spanish soft cheese ('Torta del Casar') manufactured with raw ewe milk and vegetable rennet during ripening. *International Journal of Food Science and Technology, 45*, 512–519.

Dufour, E., Devaux, M. F., Fortier, P., Herbert, S., & Typicit, U. R. (2001). Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy-relationship with texture. *International Dairy Journal, 11*, 465–473.

Dufour, E., Mazerolles, G., Devaux, M. F., Duboz, G., Duployer, M. H., & Riou, N. M. (2000). Phase transition of triglycerides during semi-hard cheese ripening. *International Dairy Journal, 10*, 81–93.

Haaland, D. M., & Thomas, E. V. (1988). Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Analytical Chemistry, 60*, 1193–1202.

Herbert, S., Riou, N. M., Devaux, M. F., Riaublanc, A., Bouchet, B., Gallant, D. J., Dufour, É., & Herbert, S. (2000). Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. *Dairy Science & Technology, 80*, 621–634.

Karoui, R., Dufour, É., & De Baerdemaeker, J. (2007). Front face fluorescence spectroscopy coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout ripening. *Food Chemistry, 101*(3), 1305–1314. https://doi.org/10.1016/j.foodchem.2006.01.028

Karoui, R., Dufour, É., Pillonel, L., Picque, D., Cattenoz, T., & Bosset, J. O. (2004). Determining the geographic origin of Emmental cheeses produced during winter and summer using a technique based on the concatenation of MIR and fluorescence spectroscopic data. *European Food Research and Technology, 219*(2), 184–189. https://doi.org/10.1007/s00217-004-0936-z

Karoui, R., Dufour, E., Schoonheydt, R., & Baerdemaeker, J. De (2007). Characterisation of soft cheese by front face fluorescence spectroscopy coupled with chemometric tools: Effect of the manufacturing process and sampling zone. *Food Chemistry, 100*(2), 632–642. https://doi.org/10.1016/j.foodchem.2005.09.082

Karoui, R., Laguet, A., & Dufour, É. (2003). Fluorescence spectroscopy: A tool for the investigation of cheese melting - correlation with rheological characteristics. *Le Lait, 83*(3), 251–264. https://doi.org/10.1051/lait:2003014

Karoui, R., Mouazen, A. M., Dufour, E., Schoonheydt, R., & De Baerpemaeker, J. (2006). Utilisation of front-face fluorescence spectroscopy for the determination of some selected chemical parameters in soft cheeses. *Le Lait, 86*(2), 155–169. https://doi.org/10.1051/lait:2005047

Kokawa, M., Ikegami, S., Chiba, A., Koishihara, H., Trivittayasil, V., Tsuta, M., Fujita, K., & Sugiyama, J. (2015). Measuring cheese maturation with the fluorescence fingerprint. *Food Science and Technology Research, 21*(4), 549–555. https://doi.org/10.3136/fstr.21.549

Lakowicz, J. R. (2006). Protein fluorescence. In *Principles of fluorescence spectroscopy* (3rd ed., pp. 446–485). Kluwer Academic/plenum.

Manzi, P., Panfili, G., & Pizzoferrato, L. (1996). Normal and Reversed-Phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products. *Chromatographia, 43*, 89–93.

Mazerolles, G., Devaux, M.-F., Duboz, G., Duployer, M.-H., Riou, N. M., & Dufour, É. (2001). Infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening. *Dairy Science & Technology, 81*, 509–527.

Mazerolles, G., Devaux, M. F., Dufour, E., Qannari, E. M., & Courcoux, P. (2002). Chemometric methods for the coupling of spectroscopic techniques and for the extraction of the relevant information contained in the spectral data tables. *Chemometrics and Intelligent Laboratory Systems, 63*, 57–68. www.elsevier.com/locate/chemometrics.

Muñoz de la Peña, A., Mansilla Espinosa, A., Gonzalez Gómez, D., Olivieri, A. C., & Goicoechea, H. C. (2003). Interference-free analysis using three-way fluorescence data and the parallel factor model. Determination of fluoroquinolone antibiotics in. *Analytical Chemistry, 75*(11), 2640–2646.

Panfili, G., Manzi, P., & Pizzoferrato, L. (1994). High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses. *Analyst, 169*, 1161–1165.