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Note

Fluorescence spectroscopy: A tool for the investigation of cheese melting – Correlation with rheological characteristics

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Abstract – Trends in the texture and structure as a function of temperature were determined for 2 hard cheeses (Comté and Emmental) and 1 semi-hard cheese (Raclette) using dynamic testing rheology and front-face fluorescence spectroscopy. The storage modulus (G'), the loss modulus (G") and the complex viscosity (η*) decreased while strain and tan δ increased as the temperature increased from 5 °C to 60 °C. Protein tryptophan emission spectra and vitamin A excitation spectra were recorded on cheese samples at 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 °C. For each cheese, the data sets containing fluorescence spectra and rheology data were evaluated using principal component analysis and factorial discriminant analysis. It was shown that the maps defined by principal components 1 and 2 discriminated cheese samples as a function of temperature whatever the data (dynamic testing rheology data or fluorescence spectra). In addition, the melting temperature of fats for the three cheeses determined from the dynamic rheology data and the vitamin A fluorescence spectra gave similar results, i.e., 30, 32 and 31 °C for Emmental, Comté and Raclette cheeses, respectively. Canonical correlation analysis was applied to cheese dynamic rheological measurements and tryptophan and vitamin A fluorescence spectral collections in order to measure the link between the groups of variables measured on the same samples. The rheology and fluorescence groups of variables were found to be highly correlated since the squared canonical coefficients for canonical variables 1 and 2 were higher than 0.94 and 0.49, respectively.

Cheese / structure / texture / melting / fluorescence / rheology

Résumé – **La spectroscopie de fluorescence frontale : un outil pour l'étude de la fonte des fromages – Corrélations avec les données rhéologiques.** L'évolution de la texture et de la structure de deux fromages à pâte pressée cuite (Comté et Emmental) et un fromage à pâte pressée non cuite (Raclette) en fonction de la température a été déterminée par des mesures rhéologiques (test d'oscillation) et par spectroscopie de fluorescence frontale. Le module d'élasticité (G'), le module de perte (G") et la viscosité complexe (η*) diminuent lorsqu'on fait varier la température entre 5 et 60 °C. Cependant, on note un accroissement de la déformation et de la tangente de l'angle de perte, tan δ, dans la même gamme de température. Les spectres d'émission des tryptophanes de protéines et les spectres d'excitation de la vitamine A ont également été enregistrés sur des échantillons de fromages à 5, 10, 15, 20, 25, 30, 35, 40, 50 et 60 °C. Pour chaque type de fromage étudié, les données spectrales (tryptophanes et vitamine A) et les données rhéologiques ont été

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analysées par analyse en composantes principales et analyse factorielle discriminante, respectivement. La carte factorielle définie par les 2 premières composantes principales a permis de suivre l'évolution des caractéristiques du fromage en fonction de la température appliquée, et ce pour les 2 techniques utilisées. En outre, la température de fusion de la matière grasse des trois types de fromage, déterminée à partir des données rhéologiques et de fluorescence frontale, donne des résultats similaires, soit respectivement, 30, 32 et 31 °C pour l'Emmental, le Comté et la Raclette. L'application de l'analyse canonique des corrélations aux mesures rhéologiques et aux collections spectrales de fluorescence des tryptophanes et de vitamine A a permis de mesurer le lien entre les 2 groupes de variables mesurées sur les mêmes échantillons et a mis en évidence de très fortes corrélations. En effet, les coefficients canoniques au carré pour les variables canoniques 1 et 2 étaient respectivement supérieurs à 0,94 et 0,49.

Fromage / structure / texture / point de fusion / fluorescence / rhéologie

1. INTRODUCTION

Textural properties play a key role in consumer acceptance of cheese [1, 7, 19, 34]. The rheological characterisation of cheeses is important as a means of determining body and texture for quality and identity as a function of composition, processing techniques and storage conditions. It is generally assumed that at room temperature and for a given manufacturing process, milk proteins contribute to firmness and milk fats provide smoothness to cheese: the higher the fat content, the softer the cheese [22]. Moreover, cheese texture may change with the physical state of the fats, depending on temperature [12]. Concomitant with this is a need to characterise cheese behaviour during heating, the change in viscosity and the nature of protein-protein and protein-lipid interactions at elevated temperature. Meltability is one of the most important physical properties of cheese at high temperatures. The melting property of cheese is important not only for texture, but also for the mixing property of cheese in food preparation. It is affected by various technological factors of cheese manufacture such as fat and moisture contents, chymosin level and proteolysis [23, 29].

There are many methods available to study the phenomenon of cheese meltability [2, 25]. Some methods are based on measuring the change in the diameter or height of a cylindrical cheese sample after heating it in an oven [2]. All these methods are empirical and have a low repeatability. However, dynamic testing methods offer very rapid results with minimal chemical and physical changes [22]. Dynamic low amplitude strain testing refers to the application of a continuously changing stress or strain [30, 33]. This testing procedure is the most common dynamic method for the study of the viscoelastic behaviour of food [3, 6, 8, 18, 30, 31].

The melting point of fats and protein-protein and protein-lipid interactions in cheeses can also be determined by applying front-face fluorescence spectroscopy, which is a sensitive, rapid and non-invasive analytical technique [14, 17]. Indeed, front-face fluorescence spectroscopy can provide information on the presence of fluorescent molecules and their environment, [14, 28]. Thus, the fluorescence of milk proteins has been used to monitor the structure changes in proteins, and their physico-chemical environment, at two different ripening times [17]. Furthermore, it is possible to monitor the environmental changes of vitamin A trapped in fat globules and the interactions between fat globules and protein. Indeed, due to its conjugated double bonds, vitamin A is a good fluorescent probe with excitation and emission wavelengths at about 330 and 410 nm, respectively [10, 11].

The purpose of this study was to evaluate the meltability and the viscoelastic behaviour of 2 hard cheeses and 1 semi-hard cheese using two methods – fluorescence spectroscopy and dynamic testing rheology, in order to discriminate between these cheeses by applying principal component analysis (PCA) and factorial discriminant analysis (FDA) to the whole fluorescence spectra and rheological data. The study serves to show the interest of fluorescence spectroscopy for the investigation of cheese melting and the correlation with rheological characteristics. Indeed, until now information about the relations between rheological measurements and fluorescence spectral data has been lacking. Another objective was to gain some insight into the structure of the cheese matrix and into the relation between the cheese matrix structure and cheese texture using canonical correlation analysis (CCA).

2. MATERIALS AND METHODS

2.1. Cheese samples

Three different marketed cheeses, 2 hard cheeses (Emmental and Comté) and one semi-hard cheese (Raclette), were purchased in a local supermarket. They were manufactured from raw milk and their duration of ripening was 10 weeks, 5 months and 8 weeks, respectively.

Slices were cut off in the middle of the cheese height 20 mm from the rind for physico-chemical, rheological and spectroscopic analysis.

2.2. Physico-chemical analysis

The determination of pH, dry matter, fat content, total nitrogen and water-soluble nitrogen as protein content for the three cheeses was as described by Bouton et al. [5]. All the analyses were done in duplicate. The ripening coefficient was determined as follows:

Ripening coefficient = 100*[water-soluble nitrogen ($g \cdot 100\ g^{-1}$)/total nitrogen ($g \cdot 100\ g^{-1}$)].

2.3. Dynamic oscillatory experiments

Cheeses were sliced into thin disks (2 mm thick and 20 mm diameter) with a cheese slicer and the sliced samples were placed into plastic bags to prevent dehydration and stored at 5 °C until analysis. The dynamic oscillatory experiments were performed with a rheometer (CP 20, TA Instrument, Guyancourt, France) with plate geometry of 20 mm diameter. Oscillation experiments were performed in the linear viscoelastic region by applying a constant force of 0.5 N and a constant frequency of 1 Hz. All the experiments were carried out at temperatures ranging between 5 and 60 °C by applying a Peltier plate that provided very accurate and rapid temperature control. In order to select a test condition applicable to all cheese samples, the effect of the duration of the temperature ramp was first investigated for three different times (20 min, 30 min and 45 min). From the results of this preliminary study, a temperature ramp of 30 min was selected, since it allowed an equilibration of the thin cheese samples, to obtain reproducible results and a discrimination of the different cheeses investigated.

The data obtained included the two components of shear modulus G*, i.e., the elastic component G' (storage modulus) and the viscous component G" (loss modulus). The complex viscosity (η^*), $\tan\delta$ and the strain were also measured. Data were recorded every 0.4 °C between 5 and 60 °C (number of points = 136). For each cheese, five curves were recorded using different samples. Any slippage was detected for all the experiments. Plots of log (η^*) versus temperature were obtained for the investigated cheeses. They showed two distinct linear regions and the intersection point

was identified as the melting point of fats in the cheese.

2.4. Fluorescence spectroscopy

Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France). The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation and depolarisation phenomena were minimised. The spectrofluorimeter was equipped with a thermostated cell and the temperature was controlled by a Haake temperature controller (Haake, Champlan, France). Slices of 2 cm length, 2 cm width and 0.2 cm thickness were cut off in the middle of the cheese height 20 mm from the rind. Spectra of cheese samples (2 cm × 1 cm × 0.2 cm) mounted between two quartz slides were recorded at 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 °C. For each cheese, three kinetics were performed using different samples, and for each temperature three spectra were recorded. The sample is illuminated by the photons of excitation (light beam: ~ 3 mm in height × ~ 0.3 mm width) in its centre, limiting the dehydration of the sample. Between the acquisition of spectra at 2 different temperatures, the temperature of the cheese sample was equilibrated for 10 min. The emission spectra of tryptophan residues 305–400 nm (increment: 0.5 nm, which corresponded to 192 points of measurement) were recorded with the excitation wavelength set at 290 nm and the excitation spectra of vitamin A 250–350 nm (increment: 1 nm, which corresponded to 100 points of measurement) were recorded with the emission wavelength set at 410 nm. All spectra were corrected for instrumental distortions in excitation using a rhodamine cell in the reference channel.

30 spectra of tryptophan residues and 30 spectra of vitamin A were collected for each cheese.

2.5. Mathematical treatment of data

2.5.1. Principal component analysis

In order to reduce scattering effects, the fluorescence spectra were normalised by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter [4]. PCA was applied to the rheological data and to the normalised spectra in order to investigate changes in the data [15, 16]. This statistical multivariate treatment makes it possible to draw similarity maps of the samples and to get spectral patterns [4, 20]. While the similarity maps allow the comparison of the spectra in such a way that two neighbouring points represent two similar spectra, the spectral patterns exhibit the absorption bands that explain the similarities observed on the maps.

2.5.2. Factorial discriminant analysis

The ability of the data to describe the 3 kinds of cheeses was investigated by applying discriminant analysis to the first 10 principal components of the PCA performed on the rheological data. A group was created for each type of cheese. From the first 10 principal components selected, factorial discriminant analysis (FDA) assessed new synthetic variables called discriminant factors, which were not correlated and allowed the best separation of the qualitative groups. Similarity maps can be drawn, in analogy to those for PCA.

2.5.3. Correlation between two sets of data

The fluorescence spectral data and the rheological data were further analysed using canonical correlation analysis (CCA), a multivariate treatment that describes the correlation between two sets of data [32]. CCA was carried out with the Statgraphics Plus Program (Statistical Graphics Corp., Englewood Cliffs, NJ, USA).

Table I. Physico-chemical composition (mean and standard deviation) of the investigated cheeses.

Parameters	Mean	SD*	CV*
Emmental cheese			
pH	5.90	0.00	0.00
Fat (%)	30.13	0.18	0.59
Dry matter (%)	64.30	0.27	0.42
Fat in dry matter (%)	46.85	0.08	0.17
TN* ($g \cdot 100\ g^{-1}$)	4.14	0.02	0.56
WSN*/TN (%)	15.25	0.30	1.97
Comté cheese			
pH	5.91	0.01	0.12
Fat (%)	33.75	0.00	0.00
Dry matter (%)	65.06	0.06	0.09
Fat in dry matter (%)	51.88	0.05	0.09
TN* ($g \cdot 100\ g^{-1}$)	4.16	0.07	1.70
WSN*/TN (%)	22.72	0.45	2.00
Raclette cheese			
pH	5.88	0.01	0.12
Fat (%)	29.50	0.00	0.00
Dry matter (%)	57.65	0.07	0.13
Fat in dry matter (%)	51.17	0.06	0.13
TN* ($g \cdot 100\ g^{-1}$)	3.41	0.06	1.66
WSN*/TN (%)	30.48	1.92	6.31

* SD: standard deviation; CV: 100×(SD/mean); TN: total nitrogen; WSN: water-soluble nitrogen.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical characterisation of cheeses

The results for pH, fat, fat in dry matter, total nitrogen and water-soluble nitrogen determinations for the 3 cheeses are reported in Table I. On the one hand, the fat content in Comté cheese was higher than in Emmental and Raclette cheeses. The results of a Student test showed that a significant difference was observed between fat content and fat in dry matter of the three cheeses ($P < 0.05$). On the other hand, no significant difference was observed for the pH of the three cheeses. The Raclette cheese had a lower dry matter than Comté and Emmental cheeses, which exhibited similar values for this parameter. This difference may be explained by the fact that hard cheeses undergo cooking (53–55 °C) for 30 to 60 min during work in the tank, in order to carry out a more extensive drainage to reach a final dry extract from 60 to 65%, which is in agreement with our results. The ripening coefficient of the Raclette cheese (30.48% ± 1.92) was drastically higher than those of Emmental (15.25% ± 0.3) and Comté (22.72% ± 0.45) cheeses. This can be explained by the differences related to the milk composition, manufacturing process and ripening parameters, which have an effect on the nature and the intensity of proteolysis [9, 21].

3.2. Dynamic rheological measurements

For the 3 cheeses, G', G'' (Tab. II) and η^* (data not shown) decreased, but $\tan\delta$ and strain increased (data not shown) when the temperature increased. This result was in agreement with Rosenberg et al. [30] who report that G' is affected by the temperature, the frequency and the cheese age. This can be attributed to the effect of temperature on protein-protein and protein-water interactions [13, 30]. In addition, the decrease in the temperature induces an increase in the yield of milk fat in the solid state and, as a consequence, results in an increase in G' and G'' [13, 27]. From Table II, it appears that during the increase in temperature, Emmental cheese presented a less marked character of the "liquid type" since it is the firmest and the most rubbery cheese. Indeed, whatever the temperature, Emmental cheese exhibited the highest values of G' and G'', whereas Comté cheese showed the lowest ones at 20, 25, 30, 35, 40, 50 and 60 °C (Tab. II). These differences may be explained by the

Table II. Rheological parameters (G', G'') measured for the three cheeses at 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 °C.

	G'(Pa)					
	Emmental		Comté		Raclette	
Temperature (°C)	Mean	SD*	Mean	SD*	Mean	SD*
5	159.46 10^3	27.71 10^3	120.18 10^3	15.90 10^3	103.10 10^3	9.99 10^3
10	146.28 10^3	13.54 10^3	107.77 10^3	19.39 10^3	102.52 10^3	11.75 10^3
15	87.48 10^3	9.12 10^3	68.68 10^3	12.46 10^3	76.58 10^3	7.26 10^3
20	56.68 10^3	5.69 10^3	47.19 10^3	10.14 10^3	56.20 10^3	4.21 10^3
25	39.66 10^3	3.76 10^3	33.03 10^3	8.54 10^3	39.08 10^3	3.32 10^3
30	27.55 10^3	2.12 10^3	21.80 10^3	7.08 10^3	26.36 10^3	2.34 10^3
35	17.37 10^3	1.25 10^3	12.83 10^3	5.13 10^3	16.35 10^3	1.18 10^3
40	11.52 10^3	0.66 10^3	7.60 10^3	3.53 10^3	10.06 10^3	0.70 10^3
50	4.63 10^3	0.71 10^3	1.57 10^3	0.85 10^3	3.12 10^3	0.39 10^3
60	1.35 10^3	0.18 10^3	0.36 10^3	0.09 10^3	1.03 10^3	0.13 10^3
	G''(Pa)					
	Emmental		Comté		Raclette	
Temperature (°C)	Mean	SD*	Mean	SD*	Mean	SD*
5	54.60 10^3	8.67 10^3	40.67 10^3	2.85 10^3	32.96 10^3	2.81 10^3
10	49.66 10^3	5.20 10^3	35.67 10^3	4.39 10^3	31.32 10^3	2.98 10^3
15	32.30 10^3	3.84 10^3	24.50 10^3	2.72 10^3	24.09 10^3	1.55 10^3
20	21.56 10^3	2.46 10^3	17.39 10^3	2.52 10^3	18.08 10^3	0.89 10^3
25	15.25 10^3	1.58 10^3	12.60 10^3	2.40 10^3	13.16 10^3	0.85 10^3
30	11.03 10^3	0.99 10^3	8.91 10^3	2.31 10^3	9.33 10^3	0.65 10^3
35	7.59 10^3	0.63 10^3	5.91 10^3	2.03 10^3	6.28 10^3	0.41 10^3
40	5.48 10^3	0.41 10^3	3.96 10^3	1.61 10^3	4.38 10^3	0.29 10^3
50	3.15 10^3	0.37 10^3	1.19 10^3	0.54 10^3	2.12 10^3	0.26 10^3
60	1.46 10^3	0.12 10^3	0.44 10^3	0.10 10^3	1.02 10^3	0.12 10^3

SD*: standard deviation.

characteristics of the protein network and the gross composition of the cheese. The lowest values of G' and G'' of Comté cheese at high temperatures could be related to the amount of fat. Indeed, at high temperatures, the stiffness decreases with the amount of fat [35].

Delta equal to 45° of angle ($\tan \delta = 1$) indicates the temperature where the viscous properties prevail, i.e., the cheese starts to melt [13]. For Comté cheese, this temperature was observed at 55 °C, while temperatures of 58 and 60 °C were obtained for Emmental and Raclette

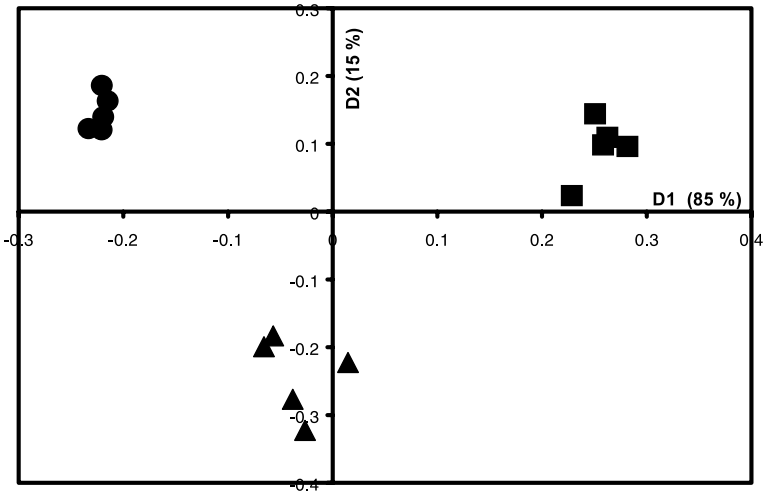

Figure 1 : Discriminant analysis similarity map determined by discriminant functions 1 and 2 for the rheological data (G', G", tan δ, strain and η*) of the three cheeses at a temperature ranging between 5 and 60 °C: (▲): Raclette; (●): Comté; (■): Emmental.

cheeses, respectively (data not shown). This result is in agreement with [13] reporting that the melting point of Emmental cheese is in the 55–60 °C temperature range.

In a second step, PCA was applied to the rheology data (G', G", tan δ, strain and η*) of Emmental cheese in order to investigate changes in the data in the 5–60 °C temperature range. The data matrix included 5 variables (rheological data) and 136 objects (temperatures). The PCA results showed that the first two principal components took into account 99.7% of the total variance with a large predominance of principal component 1 (85.1%), discriminating the samples as a function of temperature (data not shown). The rheological values recorded at low temperature had positive scores according to the principal component 1, whereas negative scores were observed for the ones recorded at high temperatures. Similar results were obtained on the two other cheeses.

Finally, the rheological data recorded during the temperature increase for the three cheeses (5 repetitions of each type of cheese) were pooled in one matrix and this table was analysed in a first step by PCA and then FDA was performed on the first 10 principal components of the PCA. The data matrix for FDA included 15 objects and 10 variables (the first 10 principal components). The first two discriminant factors took into account 85% and 15% of the total variance, respectively, and the map defined by discriminant factors 1 and 2 showed that the data were well suited for the discrimination of the 3 cheeses (Fig. 1). A discrimination of samples of Emmental cheese from Raclette and Comté cheeses was essentially observed according to the discriminant factor 1. Emmental cheeses, exhibiting the highest values of G' (Tab. II), were on the positive side. The discriminant factor 2 mainly discriminated Comté and Emmental cheeses from Raclette cheese.

3.3. Evolution of tryptophan and vitamin A fluorescence properties

The tryptophan emission spectra of the three commercial cheeses presented a maximum located at about 330 nm, and it

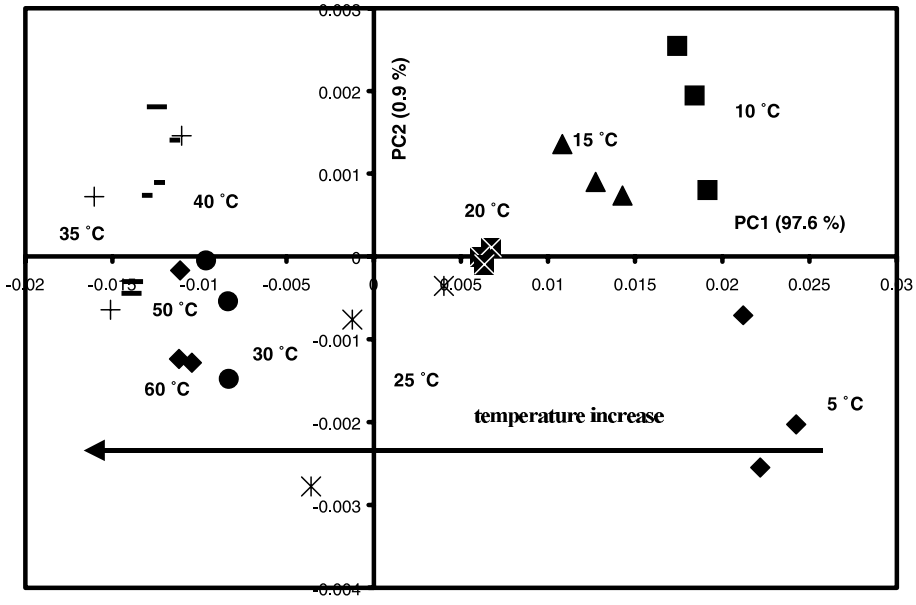

Figure 2. Principal component analysis similarity map determined by principal components 1 and 2 for tryptophan spectra of Emmental cheese at different temperatures.

appeared that the shape of the fluorescence spectra of tryptophan at 60 °C was larger than those at 5 °C and 30 °C, whatever the cheese. In addition, the maximum emission shifted with temperature, i.e., the maxima located at 330 nm and 340 nm were observed at 5 °C and 60 °C, respectively.

The excitation spectra of vitamin A were characterised by a maximum located at 322 nm and two shoulders at 305 and 295 nm. The shape of the spectra changed drastically with the increase of temperature (data not shown). The *F* 322 nm/*F* 295 nm ratio exhibited differences from one cheese to another, but it decreased with the increase in temperature for all the investigated cheeses. It is explained by the decrease in the viscosity of triglycerides with the temperature increase. Indeed, it has been shown that the fluorescent properties of fluorophores are very sensitive to the changes in the solvent viscosity [10–12].

As the fluorescence spectra of the three cheeses exhibited slight differences, univariate analysis was not really appropriate for the study of large data sets. Multivariate analysis techniques, such as PCA, FDA and CCA make it possible to extract information related to the structural changes in cheeses from the fluorescence spectra [32].

First, PCA was applied to the set (30 objects and 192 variables) of tryptophan fluorescence spectra recorded on Emmental cheese. The first two principal components accounted for 98.5% of the total variance with a large predominance of the principal component 1 (97.6%). It appeared that spectra recorded at temperatures below 20 °C exhibited positive scores according to the first principal component, whereas those recorded at 25, 30, 35, 40, 50 and 60 °C exhibited negative scores (Fig. 2). Although samples were close to each other at 30, 35, 40, 50 and 60 °C, a different trend was observed for the tryptophan spectra recorded at 5, 10, 15, 20 and 25 °C. These differences could be explained by the change in the structure of

proteins, the physical state of triglycerides and protein-lipid interactions.

Spectral pattern 1 (data not shown) indicated that the shape of fluorescence spectra was larger at high temperatures than at low temperatures. It agrees with a broader diversity of the environments of tryptophan residues in the cheese matrix at high temperatures. These spectral differences have to be related to the changes in protein-protein and protein-lipid interactions and to the different network structures resulting from cheese melting [15, 17, 28]. Similar results were obtained following the analysis of Comté and Raclette cheese spectra recorded in the 5–60 °C temperature range.

Secondly, PCA was applied to the set (30 objects and 100 variables) of vitamin A fluorescence spectra recorded on Emmental cheese, and the map defined by principal components 1 and 2 took into account 98.6% and 0.9% of the total variance (data not shown), respectively, and discriminated the samples according to temperature. The trend observed for vitamin A spectra is close to that observed for tryptophan data. The principal component 1 that explained most of the total inertia of the spectral data allowed the discrimination of the spectra according to temperature. The examination of spectral pattern 1 (data not shown) showed an opposition between a positive peak at 275 nm and a negative peak at 324 nm. This pattern has been reported previously [12] and agrees with the change in the physical state of triglycerides. Similar results were obtained following the analysis of the vitamin A fluorescence spectra of Comté and Raclette cheeses in the 5–60 °C temperature range. It suggests that vitamin A is a valuable probe for investigating the melting of cheeses in general, and of the fats in cheeses in particular.

Finally, the tryptophan fluorescence spectra recorded between 5 and 60 °C for the 3 cheeses were pooled in one matrix including 90 objects and 192 variables. A similar matrix (90 objects and 100 variables) was built from the vitamin A spectra. Considering the maps defined by the principal components 1 and 2 of the PCA performed on the two data sets, a better discrimination of the three cheeses was observed for the tryptophan fluorescence data. The map defined by the principal components 1 and 2 took into account 86.2% and 12.8% of the total variance, respectively, and showed quite a good discrimination of Comté cheese from Raclette and Emmental cheeses according to the principal component 1 (Fig. 3). In addition, a discrimination of the spectra of Emmental cheese at 5, 10, 15, 20 and 25 °C from those of Comté cheese was observed according to the second principal component. The examination of the spectral pattern 1 indicated that the shape of fluorescence spectra was larger for the spectra with negative scores than those with positive scores. It agrees with a broader diversity of the environments of tryptophan residues in the cheese matrix at high temperatures. It was concluded that the increase in the temperature induced specific modifications in the shape of the fluorescence spectra [10] and that tryptophan and vitamin A fluorescence spectra allowed discrimination between Raclette, Comté and Emmental cheeses during melting. These results clearly indicate that the structures of the cheeses at a molecular level as measured by fluorescence are related to the manufacturing processes and the melting properties of the 3 investigated cheeses.

3.4. Determination of the melting temperatures of fats in the 3 cheeses from the rheology and fluorescence data

Table III reports the melting points of the fats in the three cheeses derived from rheology data and fluorescence spectra. Considering the rheology data, a significant difference was observed between the data obtained from Emmental cheese and the two other cheeses at a level effect of 5%.

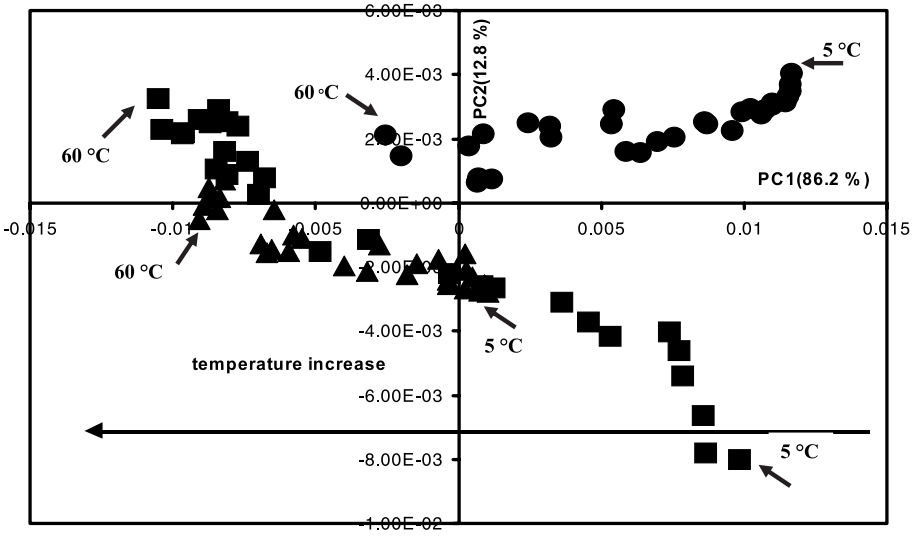

Figure 3. Principal component analysis similarity map determined by principal components 1 and 2 for the tryptophan spectra of the three cheeses at different temperatures: (▲): Raclette; (●): Comté; (■): Emmental.

Considering fluorescence, it has been reported that the melting temperature of fats in a model emulsion can be derived by plotting *F* 322 nm/*F* 295 nm versus temperature [26]. From the spectral data of vitamin A, typical plots of the *F* 322 nm/*F* 295 nm ratio versus temperature were drawn for each cheese (Fig. 4). The plots showed two distinct linear regions and the intersection point was identified as the melting point of fats in the cheese [26]. No significant difference was observed between the data obtained with the two methods. Indeed, the application of the Student test did not show difference between the results obtained with the two methods at a level effect of 5%.

Considering the investigated temperature range, melting temperatures obtained with the two methods allow mainly the investigation of fat melting in cheese. The similar melting temperatures derived from rheological and fluorescence data also suggest that there was a relation between the structure of the cheese at the molecular level as investigated by fluorescence spectroscopy, and the texture determined using dynamic testing rheology measurements.

Table III. Evaluation of the melting temperatures of fat in cheeses using rheological data and fluorescence spectra.

	Melting temperature (°C)					
	Rheological data			Fluorescence data		
Cheese	Mean	SD*	CV*	Mean	SD*	CV*
Emmental	29.45	0.20	0.69	30.41	0.77	2.53
Comté	31.20	0.62	2.00	32.44	1.35	4.16
Raclette	31.79	1.22	3.85	31.27	0.31	1.00

* SD: standard deviation. CV=100×(SD/mean). Reported melting temperatures are the means of five measurements (rheology) or three measurements (fluorescence).

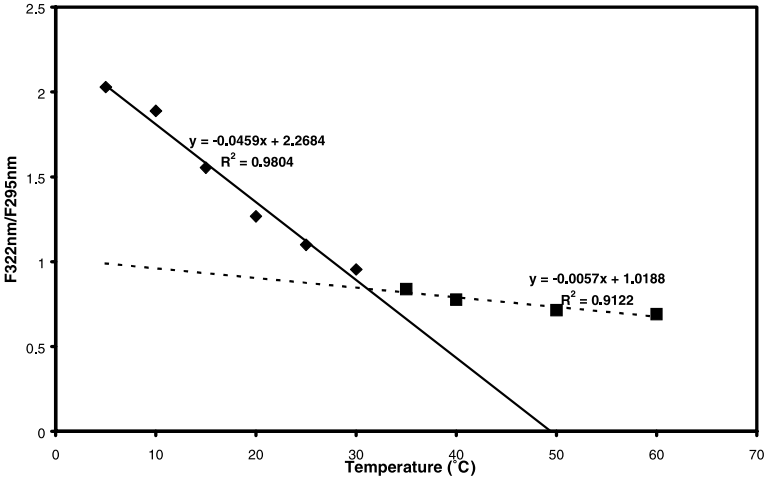

Figure 4. Determination of the melting point of Emmental cheese from the fluorescence spectra of vitamin A.

3.5. Canonical correlation analysis of fluorescence and rheological data sets

The possible correlations between the rheological data (G', G", tan δ, strain and η*) and the fluorescence spectra (tryptophan and vitamin A) were investigated in order to get a better insight into the relationships between the characteristics at macroscopic and molecular levels of the investigated cheeses. For the CCA analysis, the rheological parameters and the fluorescence spectra recorded at the same temperatures, i.e., 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 °C were considered. It has been successfully applied to comparing fluorescence and mid-infrared spectra of semi-hard cheeses [12] and rheology data and sensory data of Salers cheese [24]. In both cases, the authors were able to provide relevant similarity maps of the samples that were not immediately found by principal component analysis. From the presented data, it appeared that tryptophan fluorescence spectra and vitamin A spectra were highly correlated with the rheological data. Considering the fluorescence spectra of tryptophan and rheology data, the first two pairs of canonical variates were correlated, with their squared canonical correlation coefficients were equal to 0.94 and 0.60. CCA applied to the fluorescence spectra of vitamin A and rheology data showed that the first two pairs of canonical variates were correlated since that their squared canonical correlation coefficients equal to 0.97 and 0.49. Since the *P*-values of the first two pairs of canonical variates were lower than 0.05, the considered data sets had statistically significant correlations at the 95% confidence level (data not shown). It indicated that a common description of the samples was possible both from the fluorescence and rheological data. The similarity maps for the CCA analysis performed on vitamin A spectral data and the rheological data are shown in Figures 5A-B. These figures show that the first canonical variates discriminated the data according to temperature. Indeed, the data recorded at temperatures below 20 °C had positive scores according to the first canonical variate. Similar results were obtained for the CCA analysis performed on the tryptophan spectral data and the rheological data (data not shown).

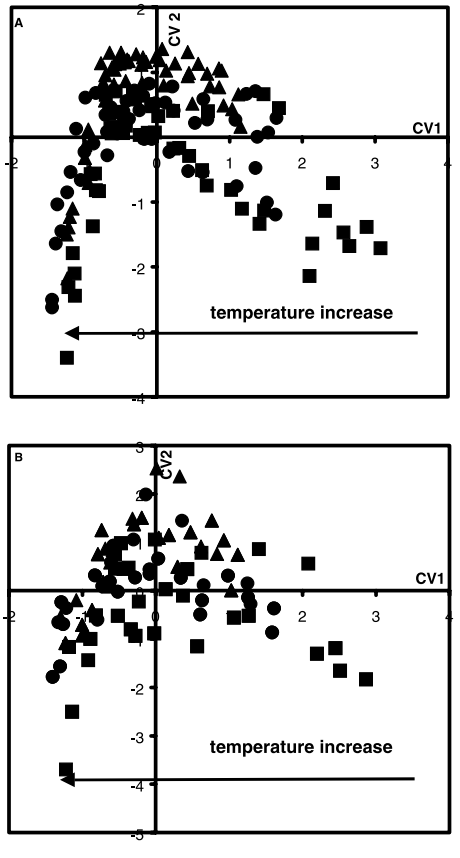

Figure 5. CCA applied to the vitamin A spectral data and the rheology data. (A) Similarity map defined by the canonical variates 1 and 2 for the rheology data. (B) Similarity map defined by canonical variates 1 and 2 for the vitamin A fluorescence spectral data: (▲): Raclette; (●): Comté; (■): Emmental.

As the two methods allowed the discrimination of the three cheeses during melting, it is suggested that the changes in the shape of fluorescence spectra were related to the changes in the cheese matrix structure induced by the increase of temperature. Indeed, during the temperature increase, the interactions between the protein network and the fat globules varied, depending on the temperature. Fusion of the triglycerides during the increase in temperature altered simultaneously the shape of vitamin A spectra and of tryptophan spectra. Our data strongly suggest that the structure and the interactions of micelles and fat globules, as investigated by fluorescence spectroscopy, determined the cheese texture. So, the phenomena observed at the molecular and macroscopic levels were related to the changes in the texture of the cheeses during melting.

4. CONCLUSION

This study shows that dynamic testing rheology measurements and fluorescence spectroscopy can be useful for monitoring the change in the texture and in the structures at the macroscopic and molecular levels, and for the determination of the melting point of cheeses and cheese fats during a temperature increase. The tryptophan and vitamin A fluorescence spectra of cheeses were fingerprints that allowed identification of the investigated cheeses. Rheological parameters such as the storage modulus (G'), loss modulus (G''), tan δ and the strain could be used to explain structural characteristics and changes in the 5–60 °C temperature range. Applying CCA to the rheological data and fluorescence spectra can provide different information related to the physical state of the fats, the structure of the cheese matrix and the change in the texture of cheeses during an increase in the temperature. The understanding of cheese rheological properties as a function of temperature is very important, since cheese is more and more often used as an ingredient for the manufacturing of elaborate food products.

Spectroscopic methods have been used for a long time to determine the chemical composition of foodstuffs. It is shown in this study that quality parameters of cheeses, such as rheology attributes, may be derived from fluorescence data. Fluorescence has the same potential to address problems in food science as in biochemical

science, because the scientific questions requiring answers are closely related.

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