JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

REVIEWS

Fluorescence Spectroscopy: A Rapid Tool for Analyzing Dairy Products

CHARLOTTE MØLLER ANDERSEN*,† AND GRITH MORTENSEN‡

Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK-Frederiksberg C, Denmark, and Danish Dairy Board, The Danish Dairy Research Foundation, Frederiks Allé 22, DK-8000 Aarhus C, Denmark

This paper gives a critical evaluation of the use of fluorescence spectroscopy for measuring chemical and physical changes in dairy products caused by processing and storage. Fluorescence spectroscopy is able to determine various properties of foods without use of chemicals and time-consuming sample preparation. This is shown by examples where the measurement of a given chemical parameter has been appropriately described and validated, as well as situations showing potential applications, but where further research and validation is required. The interpretation of fluorescence spectroscopic data is complex due to absorbance by other molecular groups, changes caused by variation in the sample matrix, etc. It is illustrated how advanced data analytical techniques are required to obtain optimal interpretation of the data. Even though the review focuses on examples from the dairy industry, the principles are broader and can be applied to other fields of food and agricultural research.

KEYWORDS: Fluorescence spectroscopy; dairy products; chemometrics; advanced data analysis

INTRODUCTION

During the last 10 years, fluorescence spectroscopy has provided important information about chemical and physical properties as well as changes in several types of complex food products (*1–4*). The increased use has been facilitated by improved instruments and new data analytical techniques such as multivariate and even multiway chemometric data analysis (*5, 6*). Fluorescence landscapes are obtained when emission spectra are measured at several excitation wavelengths. Such fluorescence landscapes can, ideally, be decomposed by multiway techniques such as PARAFAC, enabling identification of the underlying fluorescent phenomena. Other advantages are that fluorescence spectroscopy is fast, nondestructive, selective, and sensitive. Fluorescence spectroscopy possesses a great potential for on-line or at-line applications. However, most industrial use requires proper instrument standardization and validation against classical techniques such as sensory parameters or chemical measurements.

When measuring fluorescence on food samples, chemical compounds occurring naturally within the sample matrix induce fluorescence emission. In dairy products, it is primarily riboflavin, vitamin A, aromatic amino acids, Maillard reaction products, NADH, porphyrins, chlorophylls, and lipid oxidation products that lead to fluorescence emission. **Figure 1** visualizes the excitation and emission maxima of the most important fluorophores. Each fluorescent molecule has a characteristic excitation and emission spectrum, which can be used to separate and identify molecules as well as to differentiate between substitutions and conformations of the same molecule.

Traditionally, fluorescence has been applied to clear solutions with known fluorophores. The measurements are carried out using an angle of 90° between the sample and the excitation light. In such situations, and when the concentration is below a certain level, the measured intensity is proportional to the concentration and follows Lambert–Beer's law. Scatter, quenching, and inner filter effects destroy this relationship when the concentration is high or when the sample is turbid or solid. Instead, front-face fluorescence spectroscopy can be applied. It measures fluorescence emitted only from the sample surface, which reduces the influence of nonfluorescence disturbances. In front-face fluorescence, the angle between the sample and the light beam is changed to, for instance, 60°. Almost all dairy applications cited here have been analyzed in this way.

This review gives a critical evaluation of the use of fluorescence spectroscopy in relation to quality changes of dairy products. The paper discusses examples where the use of fluorescence spectroscopy for measuring a given quality parameter has been properly validated as well as situations, which show promising possibilities but where further investigations are required. The paper is made up as follows: (1) evaluation of

* Corresponding author. E-mail: cma@life.ku.dk. Phone: +45 3533 3542. Fax: +45 3533 3245.
† University of Copenhagen.
‡ The Danish Dairy Research Foundation.

10.1021/jf072025o CCC: $40.75
Published on Web 01/04/2008

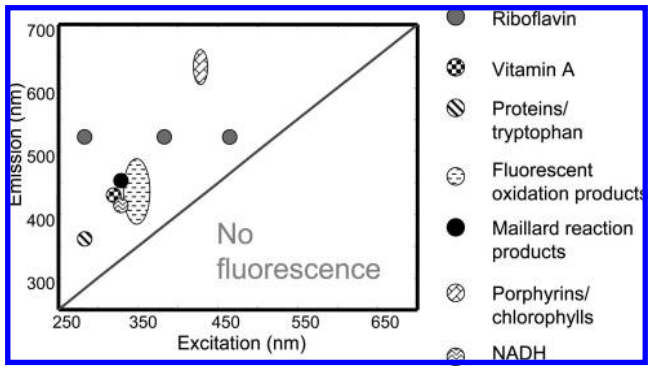

Figure 1. Excitation and emission maxima of fluorophores present in dairy products (*4, 7, 8*).

processing-induced changes in dairy products, (2) light-induced oxidation, and (3) perspectives in relation to future research and development and implementation within the dairy industry.

PROCESSING-INDUCED CHANGES

Overall Product Structure. Fluorescence spectroscopy can give valuable information about small changes in protein and lipid structure due to high sensitivity to the molecular environment. **Table 1** cites the literature describing processing-induced changes on the overall dairy product structure. Intrinsic fluorescence of milk proteins is caused by the three aromatic amino acids: tryptophan, tyrosine, and phenylalanine. Of these three amino acids, tryptophan dominates the fluorescence emission due to its large extinction coefficient. It is the fluorescence of this amino acid that provides information about protein structure. The fluorescence depends on how tryptophan is exposed in the three-dimensional configuration of the proteins.

A number of studies use emission spectra from 305 to 400 nm to explain changes in protein structure as an effect of chemical composition (*15, 25*), processing conditions (*9, 13, 26, 28*), and geographical origin and time of season (*17, 18, 21, 22*). It is in this wavelength area that tryptophan emits fluorescence (*29*). The spectra showed variations in peak maximum and wavelength position depending on the experimental parameters and illustrated that fluorescence spectroscopy could distinguish between cheeses with different structures at the molecular level.

A shift in the peak maximum toward lower wavelengths indicates exposure of tryptophan to more hydrophobic surroundings, and a shift toward longer wavelengths suggests relatively increased exposure of tryptophan toward more hydrophilic surroundings. Examples of these shifts are given by Herbert et al. (*27*) and Lopez and Dufour (*30*). The emission maximum of tryptophan changed to lower wavelengths during coagulation of milk (*27*). This change in the emission spectra was explained by changes in casein micelle structure induced by decreased pH. In contrast, a shift of tryptophan fluorescence toward higher wavelengths was noted by Lopez and Dufour (*30*), who measured fluorescence emission during acidification of reconstituted milk.

PARAFAC (*6, 31*) has been used to study protein fluorescence in dairy products. It is a multiway decomposition method, which has been shown to give reliable descriptions of fluorescence spectroscopic data (*32*). Such data can be approximated to a trilinear data structure when, for several samples, emission spectra are measured for a number of excitation wavelengths. The PARAFAC model can be written as

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}$$

where $i = 1, \ldots, I$; $j = 1, \ldots, J$; and $k = 1, \ldots, K$. PARAFAC resembles principal component analysis (PCA), but while PCA works on a two-dimensional matrix, PARAFAC is able to model higher order data structures. It gives one set of scores (a_{if}) and two sets of loadings (b_f and c_f). As for PCA the variation in data not described by the model is collected in the residuals (e_{ijk}). Unlike PCA, the components from PARAFAC models estimate emission and excitation profiles of the underlying fluorescent phenomena as well as the relative concentrations. Ideally, PARAFAC estimates the pure spectra of the fluorophores. However, in complex samples such as dairy products it may not be possible due to covariation, nontrilinearities, etc. The PARAFAC methodology has been applied with respect to cream cheese (*12*), semihard cheese (*33*), and processed cheese (*34*). Two components with emission maxima corresponding to those of tryptophan were found. It was suggested that one of these explained the presence of tryptophan. The other component described tyrosine. The estimated excitation spectra of this component resembled fluorescence of tyrosine given by the literature (*29*), and probably tyrosine in the excited state transferred energy to tryptophan as illustrated by the similar emission properties of the two components. However, this is only a tentative assignment since no verification of the compounds was made. The relative concentrations of these components described the experimental conditions such as storage time, product composition, packaging material, and temperature.

The above-mentioned studies reveal changes in the apparent concentrations of the two amino acids. However, the studies do not explain if the amino acids are simply degraded or if the variation is due to more subtle changes in protein structure. Furthermore, these are the only studies trying to identify tyrosine in dairy products. Thus, further studies using PARAFAC for modeling fluorescence landscapes and including reference measurements are required to give a thorough understanding of the variation in protein and amino acid composition as an effect of storage or processing.

Excitation spectra measured from 250 to 350 nm with emission set to 410 nm were obtained in several studies (*13, 14, 17, 19–21, 23, 24, 28*). These spectra were suggested to represent vitamin A, and it was stated that the fluorescence properties of vitamin A provided information about the physical state of triglycerides and protein–lipid interactions. The emission at 410 nm is a rather low emission wavelength compared with the emission maximum of 470 or 480 nm found for vitamin A dissolved in ethanol or isooctane (*4, 7*). Furthermore, the excitation maximum of vitamin A in solution was identified at 325 or 346 nm (*4, 7*). This large difference in emission and excitation maxima of vitamin A in dairy products and vitamin A in pure solution has not been questioned and requires verification using traditional chemical analyses. Thus, there is still research to be done to obtain a detailed knowledge of the fluorescence properties of vitamin A in dairy products and the changes of these properties in relation to the processing conditions.

Effects of Heat Treatments. Several fluorescence indicators have been shown to give information about the degree of heat treatment such as advanced Maillard reaction products and tryptophan fluorescence. Furthermore, fluorescence spectro-

Table 1. Fluorescence Spectroscopy Studies Illustrating Overall Product Structure of Various Dairy Products

product	parameters	wavelengths[a]	reference measurements	ref
soft cheese	samples from the central and the external zone of three cheese products	ex at 290 nm and em at 305–400 nm, ex at 380 nm and em at 400–640 nm, ex at 250–350 nm and em at 410 nm	none	*9*
raclette cheese	samples from the center and the rind of cheeses from summer and winter	ex at 380 nm and em at 400–640 nm	none	*10*
nonfat dry milk	samples from three manufacturers exposed to different temperatures for up to 8 weeks	ex at 290 nm and em at 305–450 nm, ex at 360 nm and em at 380–480 nm, ex at 380 nm and em at 400–590 nm, ex at 250–350 nm and em at 410 nm	none	*11*
cream cheese	designed experiment with variation in fat, salt, and pH	ex from 260 to 360 nm and em from 280 to 600 nm	none	*12*
soft cheese	three different products	ex at 290 nm and em at 305–400 nm, ex at 380 nm and em at 400–640 nm, ex at 250–350 nm and em at 410 nm	fat, dry matter, pH, total nitrogen, and water-soluble nitrogen	*13*
raclette cheese	four products with different physicochemical composition of the starting material; studied during ripening (2, 30, and 60 days)	ex at 290 nm and em at 305–400 nm, ex at 380 nm and em at 400–640 nm, ex at 250–350 nm and em at 410 nm	none	*14*
ice cream	produced from different types of fat, emulsifier, and protein	ex at 290 nm and em at 310–360 nm	none	*15*
whey	samples obtained from mammals with different heat treatments and cream content	fluorescence landscapes; exact wavelength areas not given	none	*16*
Emmental cheese	cheeses of different ages and geographic origin made from raw milk or thermized milk	ex at 290 nm and em at 305–400 nm	fat, total and water-soluble nitrogen, sodium chloride, and pH	*17*
milk	milk produced in different seasons and at different geographical regions (altitudes)	ex at 250, 290, and 380 nm with em at 280–480, 305–400, and 400–640 nm, respectively, and ex at 250–350 nm with em at 410 nm	none	*18*
Jura hard cheese, Swiss Gruyere, L'Evitaz	hard cheeses with different dry matter and fat contents produced with milk from different regions in Jura; hard cheeses collected from production sites at different altitudes	ex at 250 and 290 nm with em at 280–480 and 305–400 nm, respectively, and ex at 250–350 nm with em at 410 nm	none	*19*
soft cheese	cheeses with variation in dry matter and fat produced with milk from regions in Jura; analyzed before salting and after 30 days of ripening	ex at 290 nm with em at 305–400 nm and ex at 250–350 nm with em at 410 nm	rheology (stress/stain curves), pH, dry matter, fat, and total and water-soluble nitrogen	*20*
Emmental cheese	cheeses from six countries produced during winter and summer with different ripening times	ex at 290 nm, em at 305–400 nm	none	*21*
Emmental cheese	cheese from six countries produced during summer with different ripening times	ex at 290 nm and em at 305–400 nm, ex at 250–350 nm and em at 410 nm	total nitrogen, water-soluble nitrogen, nonprotein nitrogen, and pH	*22*
soft cheese	slices from the exterior to the interior of three cheeses	ex at 290 nm and em at 305–400 nm, ex at 250–350 nm and em at 410 nm	rheology (G' and G''), pH, dry matter, total water-soluble nitrogen	*23*
Emmental, Comté, and raclette cheese	cheeses ripened for 10 weeks, 5 months and 8 weeks. Analyzed at 5, 10 15, 20, 25, 30, 40, 50 and 60 °C	ex at 290 nm and em at 305–400 nm, ex at 250–350 nm and em at 410 nm	rheology (G' and G''), pH, dry matter, fat, and total and water-soluble nitrogen	*24*
soft cheese	cheese produced with different starter cultures and an ultrafiltrated cheese, studied during the ripening period	ex at 290 nm and em at 305–400 nm, ex at 260–350 nm and em at 410 nm	pH, dry matter, fat content, total nitrogen, and texture sensory profiles	*25*
soft cheese	cheese produced with different starter cultures and an ultrafiltrated cheese, studied during ripening	ex at 290 nm and em at 305–400 nm, ex at 260–350 nm and em at 410 nm	none	*26*
milk	milk coagulated with GDL, rennet, or a combination studied during coagulation	ex at 290 nm and emission at 305–400 nm	rheology (G'), pH	*27*
milk	designed experiment with two homogenization levels and two heat treatment levels	ex at 290, 321, and 371 nm and em at 305–400, 350–500, and 400–600 nm, respectively, ex at 260–350 and 250–450 nm with em at 410 and 466 nm	β- and κ-caseins, α-caseins, α-lactalbumin, and β-lactoglobulin	*28*

[a] em and ex denote the emission and excitation wavelengths measured, respectively.

Table 2. Fluorescence Spectroscopy Studies Illustrating Effects of Heat Treatment

product	parameters	wavelengths[a]	reference measurements	ref
milk	exposed to different heat treatments	ex at 340 nm with em at 440 nm, ex at 290 nm with em at 340 nm	several measures of heat treatment	*35*
milk	raw skim milk exposed to different heat treatments	ex at 290 nm and em at 305–450 nm, ex at 360 nm and em at 380–600 nm	hydroxymethylfurfural	*36*
whey	samples obtained from mammals with different heat treatments and cream content	fluorescence landscapes; exact wavelength areas not given	none	*16*
milk	milk exposed to different heat treatments	ex at 250 and 380 nm with em at 280–480 and 380–600 nm, respectively, and ex at 290–490 nm with em at 518 nm	β-lactoglobulin, α-lactalbumin, IgG, BSA, lactoferrin, and alkaline phosphatase	*37*
milk	milk with two fat levels exposed to different heat treatments for two different time periods	ex at 290 nm and em at 340 nm, ex at 330 nm and em at 420 nm	protein concentration, lactulose and furosine	*38*
milk	overheated and normally heated half-cream UHT milk and pasteurized milk mixed to give a range of heat treatments	ex at 290 and 360 nm with em at 305–450 and 380–600 nm, respectively, and ex at 250–420 nm with em at 440 nm	furosine and lactulose	*39*
milk	commercial milk products exposed to different heat treatments	ex at 290 nm and em at 340 nm, ex at 330 nm and em at 420 nm	soluble protein, β-lactoglobulin, lactulose and furosine	*40*
milk	designed experiment with two homogenization levels and two heat treatment levels	ex at 290, 321, and 371 nm and em at 305–400, 350–500, and 400–600 nm, respectively, and ex at 260–350 and 250–450 nm with em at 410 and 466 nm	β- and κ-caseins, α-caseins, α-lactalbumin, and β-lactoglobulin	*28*
milk	raw milk exposed to different heat treatments for different time periods	ex at 340 nm and em at 415 nm	none	*41*

[a] em and ex denote the emission and excitation wavelengths measured, respectively.

scopic data were correlated to lactulose and furosine that are formed during heat treatment of milk. **Table 2** sums up the literature.

Advanced Maillard reaction products such as pyrrole and imidazole derivatives are fluorescent and are formed as intermediary products during heating. The development of these products in milk and model systems was measured by univariate fluorescence measurements using excitations at 340, 350, or 360 nm and emissions at 415 or 440 nm (*36, 38, 40–42*). The samples underwent different heat treatments for a number of time periods. It was clear that the fluorescence intensity increased with more severe heat treatment, illustrating the formation of Maillard reaction products. The possibility of measuring the fluorescence of advanced Maillard products led to the introduction of the so-called FAST (*f*luorescence of *a*dvanced Maillard products and *s*oluble *t*ryptophan) index. It is a measurement of the fluorescence of advanced Maillard products (ex 330 nm/em 420 nm) divided by the tryptophan fluorescence (ex 290 nm/em 340 nm) of the pH 4.6 soluble supernatant (*40*). However, the method requires a chemical sample preparation, which excludes some of the advantages of fluorescence spectroscopy.

Other studies have used model systems to show that the univariate fluorescence measurements depend on the chemical composition (*42, 43*). The actual fluorescence signal is influenced by parameters such as polarity, pH, temperature, and instrumental artifacts. The peak maximum varies depending on the sample material, and the instrument and the applicability of univariate measurements as a general tool for measuring heat treatment can be questioned. This is supported by Feinberg et al. (*35*), who showed that the use of the univariate fluorescence measurements did not give a thorough measurement of the heat treatment of commercial milk samples. The univariate fluorescence measurements should be combined with other chemically determined tracers to give a relevant measurement of the degree of heat treatment.

Tryptophan fluorescence of milk was found to decrease with increasing heat treatment (*28, 38, 39*). This is supported by the finding that fluorescence emission at 340 nm was correlated to the content of native β-lactoglobulin (*38, 44*). However, solutions of β-lactoglobulin showed both increasing and decreasing intensities with heat treatment (*44–46*), illustrating the need for further studies to fully understand the effect of heat on dairy proteins.

Lactulose and furosine are often used as indicators of heat treatment of milk (see ref *47*). Correlation of the concentration of these compounds to fluorescence spectroscopic data gave contradictory results. In a study of milk heat treatment, Kulmyrzaev and Dufour (*39*) found high correlations between lactulose/furosine and tryptophan spectra. The milk was given a UHT (*u*ltra*h*igh *t*emperature) or pasteurization treatment. On the other hand, Birlouez-Aragon et al. (*38*) only found high correlations for UHT-treated milk. For pasteurized or sterilized milk, there was no correlation to the lactulose or furosine content. The different results may be due to variation in sample preparation and measurement method between the two experiments. Lactulose and furosine are nonfluorescent and are not measured by fluorescence. Thus, the high correlations found are due to an indirect relationship because the lactulose/furosine concentration changes together with the change in tryptophan fluorescence.

Kulmyrzaev et al. (*37*) used emission spectra measured from 380 to 600 nm (excitation at 360 nm) and excitation spectra measured from 290 to 400 nm (emission at 518 nm) to predict alkaline phosphatase activity and the concentration of native β-lactoglobulin, which were used as indicators of heat treatment. It was stated that these fluorescence spectra contained information about NADH and FADH. They obtained correlations of 0.67−0.8 between the measured and predicted values, which indicates that there is some relationship between the suggested fluorescence of NADH/FADH and the alkaline phosphatase activity/native β-lactoglobulin content. However, alkaline phosphatase and β-lactoglobulin are proteins and exhibit tryptophan fluorescence. Tryptophan does not emit fluorescence at the emission wavelengths measured and will only be half-covered by the excitation spectra. Thus, the reasonable predictions must be due to some indirect relationship. The concentration of the fluorophores, phosphatase activity, and the native β-lactoglobulin content are influenced similarly by the heat treatment.

Overall, the studies cited show that there are several wavelength areas that give information about the degree of

Table 3. Fluorescence Spectroscopy Studies Illustrating Changes of Dairy Products during Ripening

product	parameters	wavelengths[a]	reference measurements	ref
raclette cheese	four products with different physicochemical composition of the starting material; studied during ripening (2, 30, and 60 days)	ex at 290 nm and em at 305–400 nm, ex at 380 nm and em at 400–640 nm, ex at 250–350 nm and em at 410 nm	none	*13*
raclette cheese	samples taken from three stages of ripening	ex at 290 nm and em at 305–400 nm, ex at 380 nm and em at 400–640 nm	rheological measurements	*11*
soft cheese	cheeses with different dry matter and fat contents produced with milk from different regions in Jura; analyzed before salting and after 30 days of ripening	ex at 290 nm with em at 305–400 nm and em at 250–350 nm with ex at 410 nm	rheology (stress/stain curves), pH, dry matter, fat, and total and water-soluble nitrogen	*20*
cheese	cheeses with variations in fat, protein, and moisture, analyzed after different ripening periods	ex at 290 nm with em at 305–400 nm	none	*48*
soft cheese	cheese produced with different starter cultures and an ultrafiltrated cheese, studied during the ripening period	ex at 290 nm and em at 305–400 nm, ex at 260–350 nm and em at 410 nm	pH, dry matter, fat content, and total nitrogen, texture sensory profiles	*25*
semihard cheese	16 cheeses studied during ripening	ex at 290 nm and em at 305–400 nm	pH, moisture, fat, protein, salt, and lactate	*49*
cheese	experimental design with variations in fat, protein, and moisture, analyzed after different ripening periods	ex at 260−350 nm and em at 410 nm	none	*50*
soft cheese	cheese produced with different starter cultures and an ultrafiltrated cheese, studied during the ripening period	ex at 290 nm and em at 305–400 nm, ex at 260–350 nm and em at 410 nm	none	*26*

[a] em and ex denote the emission and excitation wavelengths measured, respectively.

Table 4. Fluorescence Spectroscopy Studies Illustrating the Relationship between Fluorescence and the Texture of Dairy Products

product	parameters	wavelengths[a]	reference measurements	ref
raclette cheese	samples taken from three stages of ripening	ex at 290 nm and em at 305–400 nm, ex at 380 nm and em at 400–640 nm	rheological measurements	*51*
cream cheese	designed experiment with variations in fat, pH, and salt level	ex from 260 to 360 nm and em from 280 to 600 nm	rheology (G')	*52*
processed cheese	processed cheese spreads from three manufacturers	ex at 290 nm and em at 305–400 nm	fat, moisture, and protein, meltability	*53*
soft cheese	cheese produced with different starter cultures and an ultrafiltrated cheese, studied during the ripening period	ex at 290 nm and em at 305–400 nm, ex at 260–350 nm and em at 410 nm	pH, dry matter, fat content, and total nitrogen, texture sensory profiles	*25*
salers cheese	cheeses with a large range of textures	ex at 290 nm and em at 305–400 nm, ex at 260–350 nm and em at 410 nm	sensory determined texture parameters	*54*

[a] em and ex denote the emission and excitation wavelengths measured, respectively.

heating even though none of them give unambiguous information about heat treatment. An advantage of fluorescence spectroscopy is that a number of the parameters can be measured at the same time when collecting a fluorescence landscape. Combining measurements at several wavelength areas could give more precise information about the degree of thermal treatment since several parameters involved at different stages during heating can be measured simultaneously. The results are promising but require further investigation to give a full understanding of the relationship between fluorescence spectroscopy and heat treatment of dairy products and to correlate the findings with already established methods used to determine the extent of heat treatment.

Changes during Cheese Ripening. Chemical and physical changes in fats and proteins take place during cheese ripening. These changes have been illustrated in a number of studies (**Table 3**). For example, tryptophan fluorescence shifted to longer wavelength during ripening (*13, 25*). This variation was supported by principal component analyses (*25, 26, 48*) and was suggested to describe exposure of tryptophan to the aqueous phase caused by proteolysis and increasing pH levels.

Excitation spectra of semihard cheeses measured between 260 and 350 nm (emission at 410 nm) showed a maximum at 322 nm and two shoulders at 295 and 305 nm. The peak at 322 nm increased with the ripening time, whereas the 295 nm shoulder decreased (*50*). Other studies have shown similar variations in excitation spectra of soft cheese (*14, 20*). The spectra were assigned to vitamin A, and the variations were attributed to changes in the molecular environment of vitamin A, solvent viscosity, and the physical state of triglycerides in the fat globules during ripening. However, the assignment of the peaks can be questioned since no verification was made.

Karoui et al. (*14*) investigated emission spectra between 400 and 640 nm (excitation at 380 nm) during ripening of raclette cheese. Several components were suggested to influence the emission spectra such as riboflavin, lumiflavin, vitamin A, oxidation products, and β-carotene. However, no conclusion of how these compounds vary in relation to ripening was given, and the possibility of improving the understanding of the ripening process from these spectra is not clear.

Relationship between Fluorescence and Texture. The texture of dairy products is attributable to the concentration of protein and fat, protein structure, physical state of fat, salt, and pH. Some of these parameters may be identified by fluorescence spectroscopy. **Table 4** lists studies using fluorescence spectroscopy for measuring texture in dairy products.

Intrinsic protein fluorescence may give a valid measure of texture. This is illustrated by Karoui et al. (*24*), who noted a shift in the emission peak from 330 to 340 nm when melting hard and semihard cheeses by increasing the temperature from

Table 5. Fluorescence Spectroscopic Studies of Light-Induced Oxidation in Dairy Products

product	parameters	wavelengths[a]	reference measurements	ref
butter	exposure to light for up to 14 days with storage in air or N_2; β-carotene added to some of the samples	ex at 382 nm and em from 410 to 750 nm	sensory, volatile compounds, PV and TBARS	*55*
semihard cheese	exposure to light of 366, 436, and 546 nm for 4 or 24 h	fluorescence landscape (ex 260–360 nm, em 360–600 nm) and ex 410 nm, em 580–770 nm	volatile compounds determined by GC-MS	*60*
Jarlsberg cheese, Gouda-like Norvegia cheese	storage in light or dark, covered with plastic films with different spectral properties, UV filter	ex 380 nm, em 400–750 nm	sensory analysis	*56*
butter	storage in air or low O_2 atmospheres (<0.05%); exposure to violet, green, or red light	fluorescence landscape (ex 350–452 nm, em 580–720 nm); nonexposed butter (a, ex 250–500 nm, em 530 and 670 nm; b, ex 380 nm, em 450–720 nm)	sensory analysis	*57*
Havarti cheese	storage in different MAP packaging, with variations in temperature, light source, and light intensity	ex 380 nm, em 400–640 nm	none	*61*
yogurt	storage under different light and packaging conditions	fluorescence landscape (ex 270–550 nm, em 310–590 nm)	riboflavin	*62, 63*
Gouda-like Norvegia cheese and other dairy products	exposed to light and covered with differently colored films	ex 380 nm, em 400–640 nm	sensory analysis	*58*
semihard cheese (Danbo)	storage in MAP packaging	fluorescence landscape (ex 260–460 nm, em 280–680 nm)	several chemical and physical oxidation parameters	*33*
Havarti cheese	storage in different MAP packaging, with variations in temperature, light source, and light intensity	ex 370 and 450 nm, em 530 nm	chemical and physical oxidation parameters	*64*
Havarti cheese	exposed to monochromatic light at 366, 405, or 436 nm	ex 370 and 450 nm, em 530 nm	chemical and physical oxidation parameters	*65*
processed cheese	storage in light or dark and at different temperatures	fluorescence landscape (ex 240–360 nm, em 275–475 nm)	none	*34*
sour cream, goat cream, and semihard cheese	storage in light or dark with or without air	ex 380 nm, em 400–640 nm	sensory analysis	*59*

[a] em and ex denote the emission and excitation wavelengths measured, respectively.

5 to 60 °C. The possibility of predicting meltability of processed cheese from fluorescence spectroscopic data was illustrated by Garimella Purna et al. (*53*). They obtained high correlations between fluorescence and meltability determined by dynamic stress rheometry.

Other texture parameters have also been predicted from fluorescence spectroscopy. For example, prediction of G' (elastic modulus) in low-fat cream cheese, semihard cheese, and soft cheese was illustrated (*51, 52*). However, high correlations were also found between moisture or protein and the rheological measurements. Thus, the possible prediction of texture parameters from fluorescence spectroscopy is likely due to an indirect measure of protein concentration. This should be investigated further by predicting texture from fluorescence measurements for samples with the same chemical composition, but with variation in other process parameters such as heat treatment or homogenization conditions.

Fluorescence emissions have been correlated to texture determined by sensory measurements. Squared correlation coefficients between measured texture of soft cheese and texture predicted from the fluorescence spectra varied between 0.22 and 0.86 (*25*). The largest correlations were seen for pastiness, texture length, and dry/watery ratio. Lebecque et al. (*54*) obtained squared correlation coefficients below 0.3 when predicting sensory parameters such as elasticity, firmness, and adhesivity from tryptophan emission spectra with respect to salers cheese. The correlation coefficients were somewhat higher using excitation spectra between 260 and 400 nm (with emission set to 410 nm). The poor correlations were ascribed to low variation in the samples. The possibility of measuring sensory parameters by an instrumental method such as fluorescence is interesting, since the sensory data indicate how the product is assessed by the consumers. Furthermore, sensory measurements are expensive. Replacement of these measurements with a cheaper instrumental method could be an advantage.

LIGHT-INDUCED CHANGES IN DAIRY PRODUCTS DURING STORAGE

Cheeses exposed to light are likely to undergo photooxidation, resulting in formation of off-flavors, color changes, and loss of nutritional value, which rapidly impair product quality and marketability. For example, it has been shown that fluorescence emission spectra of light-exposed dairy samples could predict sensory measured oxidation (*55–58*). Many studies have been carried out in the attempt to uncover the underlying chemical changes associated with such photooxidation, but there are still many unanswered questions. In the following, applications of fluorescence spectroscopy for measuring light-induced oxidation are exemplified. **Table 5** sums up the literature cited.

Initiation of Light-Induced Oxidation. Riboflavin has been known as the initiator of light-induced oxidation in dairy products (*66*). It is a highly fluorescent molecule with excitation maxima at 270, 370, and 450 nm and emission maximum in the range 525–531 nm (*67*). Several studies have shown fluorescence of riboflavin in dairy products and decreased content with storage in light by visually comparing the known fluorescence properties of riboflavin with the fluorescence spectra (*56, 57, 59, 61, 64, 65*).

A direct correlation between the fluorescence data and the chemically determined riboflavin content is shown by Christensen et al. (*62*) and Becker et al. (*63*). Becker et al. (*63*) correlated unfolded fluorescence landscapes to the chemically measured riboflavin content in yogurt by multivariate partial

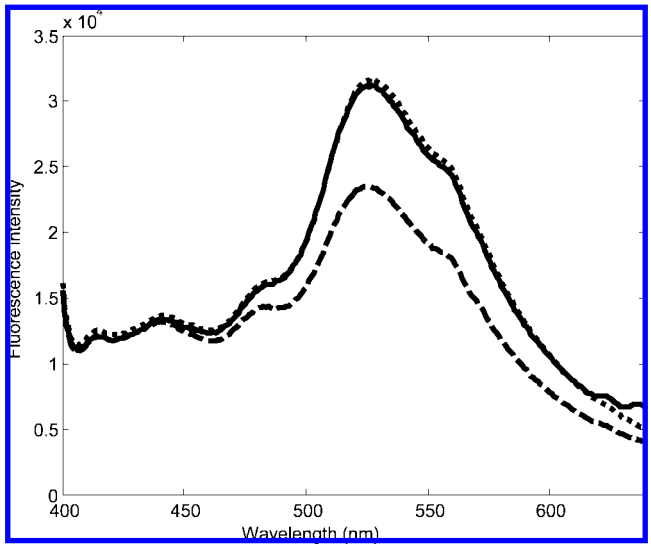

Figure 2. Emission of Havarti cheese (excitation 380 nm) exposed to yellow light (dotted), white light (dashed), or kept in dark (solid) for 72 h. The large peak around 520 nm represents riboflavin, which is degraded by white light but not by yellow light. Above 600 nm two small peaks describe porphyrin and chlorophyll in the dark-stored samples. These compounds are degraded by both yellow and white light.

least-squares (PLS) regression. This gave a prediction error of 0.0092 mg of riboflavin/100 g of yogurt (the measured content varied between 0.02 and 0.18 mg of riboflavin/100 g of yogurt).

Mortensen et al. (*64*) and Wold et al. (*56–58*) noted the development of secondary oxidation products and sensory parameters describing oxidation without degradation of riboflavin. Another study showed that progression of lipid oxidation did not take place, even though riboflavin was degraded (*33*). This has led to the hypothesis that light-induced oxidation can be initiated by another route than degradation of riboflavin.

Visual inspection of emission spectra revealed that peaks with emission maxima above 600 nm decreased in intensity during storage in light and air (*59*). Further studies suggested that these peaks were due to degradation of porphyrins and chlorophylls (*56–58*). They are a complex group of compounds with approximately similar structure and with excitation maxima at 400–410 nm and emission maxima at 600–650 nm (*8*). Degradation of porphyrins and chlorophylls could be an alternative route for initiation of light-induced oxidation in dairy products. This is supported by high (approximately 0.9) correlations between fluorescence spectra and sensory properties measured on Jarlsberg cheese (*56*). A number of studies have shown that the degradation of photosensitizers depends differently on the illumination wavelength (*56, 57, 60*) (**Figure 2**). This explains why the progression of lipid oxidation varies between studies.

The possibility of measuring riboflavin, porphyrins, and chlorophylls suggests that fluorescence can be used as an early marker of lipid oxidation. In order to do so, it is important to clarify under which circumstances the different initiation mechanisms take place and to make proper validations.

Progression of Light-Induced Oxidation. The emission area from 400 to 500 nm is complex because many compounds relevant with respect to light-induced oxidation emit fluorescence. This concerns the degradation products of riboflavin, lumichrome, and lumiflavin (*67*), as well as vitamin A (*7*) and stable oxidation products (*68*). Furthermore, Maillard reaction products and compounds, which absorb light in the range 400–500 nm, may influence the emission spectra. Several studies have noted changes in fluorescence in this wavelength area as a function of storage conditions and light exposure (*33, 34, 55, 59, 61*). Some of these papers provide educated guesses of the compounds measured, but clear proof of the exact mechanisms is still lacking.

Lumichrome and lumiflavin are fluorescent compounds with emission maxima in the ranges 444–479 nm and 516–522 nm, respectively. The excitation maxima are approximately the same as the excitation maxima of riboflavin (*67*). By PARAFAC decomposition of fluorescence landscapes, Christensen et al. (*62*) found a component with two excitation maxima between 330 and 450 nm and an emission maximum around 450 nm. They suggested that this component stemmed from lumichrome. This was supported by score values, which were oppositely correlated to the score values of riboflavin. Also, Wold et al. (*59*) suggested that fluorescence of lumichrome made up part of the emission around 450 nm.

Vitamin A is a fluorescent carotenoid with excitation and emission maxima in pure solution around 325 and 470 nm, respectively (*7*). It is light-sensitive. Thus, the concentration would decrease with exposure to light as it occurs during retail storage. Christensen et al. (*34*) and Andersen et al. (*33*) found a variation in the relative content of a fluorescent compound with excitation and emission maxima close to those of vitamin A. The relative concentration decreased with increased storage in light. However, fluorescence spectra have not yet been correlated to the actual content of vitamin A, and the results have to be verified.

Fluorescent oxidation products are conjugated Schiff bases formed by reaction between secondary oxidation products (aldehydes and ketones) and amino groups of, for example, proteins. They exhibit fluorescence with excitation maxima at 340–370 nm and emission maxima at 400–470 nm (*68*). Preliminary experiments illustrate that formation of fluorescent oxidation products takes place in dairy products. In a model system resembling dairy products, reactions between β-lactoglobulin and aldehydes gave rise to fluorescence with excitation and emission maxima of 350 and 410 nm, respectively (*69*). Wold et al. (*59*) and Christensen et al. (*34*) ascribed fluorescence with emission between 410 and 460 nm partly to be caused by fluorescent oxidation products. Veberg et al. (*55*) estimated the fluorescence emission spectrum of lipid oxidation products by multivariate curve resolution and found increased content with exposure to light and air. Fluorescent oxidation products are formed from a number of different aldehydes or ketones produced during oxidation and different amino groups of the proteins. This gives rise to a number of compounds with somewhat different excitation and emission maxima, making it difficult to obtain proper estimates of the spectral profiles of such compounds.

Structural protein changes, which are sensitive to light, have been shown (*33, 34, 62, 63*). Light-induced oxidation may result in protein oxidation, which affects the protein structure and thereby the fluorescence properties. However, the structural changes that take place during protein oxidation cannot be explained from any of the studies. It would be interesting to investigate this subject further.

The above-mentioned studies illustrate that fluorescence spectroscopy is a powerful technique for measuring several aspects of light-induced oxidation. Fluorescence can measure a number of oxidation parameters at the same time. These are shown in **Figure 3**, which gives a schematic representation of the succession of light-induced oxidation in relation to the application of fluorescence spectroscopy. Initiation takes place

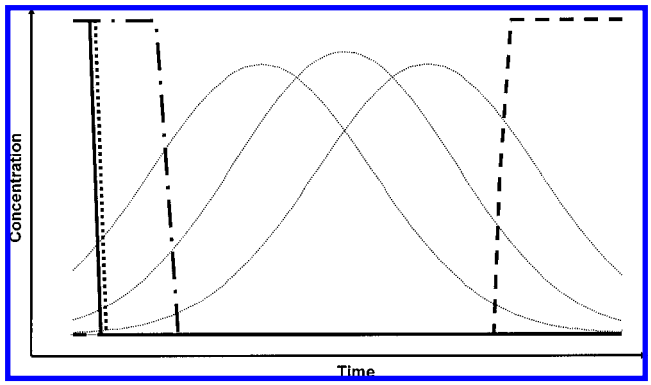

Figure 3. Schematic representation of the light-induced oxidation mechanism. The lines represent riboflavin (solid), porphyrins/chlorophylls (dotted), vitamin A (dash-dotted), fluorescent oxidation products (dashed), and intermediary secondary oxidation products (thin lines).

by light absorption of riboflavin, chlorophylls, and/or porphyrins, resulting in decreased content of these compounds. Studies have shown reduced vitamin A content with exposure of dairy products to light (*70, 71*), which has also been indicated by fluorescence measurements even though it is not yet verified. These initiation steps are followed by a number of reactions involving formation and degradation of various compounds that are not fluorescent. At the end of the reaction mechanism, stable fluorescence compounds are formed as illustrated in **Figure 3**.

There is still research to be done in relation to light-induced oxidation. Some parameters such as vitamin A, stable oxidation products, and degradation products of riboflavin have yet to be verified and calibrated using standardized chemical methods. The relationship between light-induced oxidation and protein structure should be clarified. Furthermore, the initiation step should be investigated further to identify the importance of riboflavin, porphyrins, and chlorophylls, and investigation should be done to evaluate how fluorescence can be used for reliable quantitative modeling of photooxidation.

PERSPECTIVES IN RELATION TO FUTURE RESEARCH AND DEVELOPMENT AND INDUSTRIAL IMPLEMENTATION

Fluorescence spectroscopy has a potential for on-line industrial quality control, as it is possible to measure compounds related to the process conditions fast and nondestructively. For example, degradation of riboflavin, porphyrins, and chlorophylls can give an indication of light exposure during processing, and advanced Maillard reaction products or protein denaturation can give information about the heat treatment. Most likely, fluorescence spectroscopy can be used for several other parameters in product development, process control, raw material determination, etc., but research is still required before it can be used for routine measurements within the industry. Furthermore, fluorescence gives information about more than one parameter in one measurement, whereas traditional analytical chemical measurements typically provide information about one parameter at a time. One drawback of fluorescence spectroscopy is that the signal is not measured relative to an internal light beam. The measurement conditions and method have to be optimized and calibrations performed for each individual product and application. Standardized protocols for these calibrations are required.

Other factors influencing the fluorescence signals are quenching, scatter, and inner filter effects (absorption of the excitation or emitted light by other molecules in the sample matrix). These are parameters that destroy the proportionality between the signal and the concentration, but which also make fluorescence unique. By careful development of the measuring method and interpretation and modeling of the data, these effects can be handled and can provide valuable information about the sample matrix.

Fluorescence spectroscopic data are complex when measured on whole food samples. The recorded signal is made up of the signal from all of the contributing fluorophores. Each of these has its own excitation and emission spectra. Thus, the fluorescence for one sample consists of a number of overlapping signals. This complexity of the measurements opens up new possibilities for analysis and interpretation. PARAFAC modeling enables identification of the underlying structure in the spectral data, giving estimates of the excitation and emission spectra of the fluorescent phenomena in the samples as well as the relative concentration of these. The three-way data structure together with unique decomposition methods such as PARAFAC enables second-order calibration. Second-order calibration entails accurate predictions of the relevant compounds even in the presence of known and unknown interferents (*72*). Thus, if prediction of a certain compound has been shown for a group of samples, the compound can also be predicted from new samples even if they contain somewhat different fluorophores. The three-way data may also be extended. For example, time, temperature, etc. could be added, making up a fourth dimension.

Fluorescence imaging of dairy products has only been scarcely investigated. However, it may have potential for future applications. In imaging, a fluorescence measurement, spectrum, or landscape is obtained at each pixel, giving information about the variation in fluorescence of inhomogeneous sample matrices or on fluorescence development within a sample.

Identification of the various fluorophores making up the fluorescence signal in a given application is necessary for the acceptance of fluorescence spectroscopy within the dairy industry. More work is needed to obtain such interpretations of the very complex spectroscopic measurements in general. Among others, such interpretations should also allow for handling some of the artifacts that are likely to occur in fluorescence spectroscopy. There may be compounds in the sample which do not fluoresce but absorb light. For example, the absorption of light by β-carotene affects the measured fluorescence of vitamin A, fluorescent oxidation products, lumichrome, and Maillard reaction products due to the absorption of light at wavelengths corresponding to fluorescence emission of the other compounds. Furthermore, the fluorescence properties of a specific chemical compound depend on the solvent polarity, interactions with other molecules, etc. These interactions are not fully understood, and more experiments as well as more adequate data analytical tools are required to interpret the relationship between the different molecular structures and the fluorescence properties.

To conclude, fluorescence spectroscopy may provide valuable information about several parameters such as light-induced oxidation, product structure, geographical origin, heat treatment, homogenization, milk coagulation, and changes during ripening. The scientific attainments in combination with the fact that equipment prices are dropping as well as the commercialization of portable, highly sensitive fluorescence spectrophotometers make fluorescence spectroscopy a promising tool for future quality determination and process control as well an efficient and versatile research tool with respect to a wide range of dairy products.

ACKNOWLEDGMENT

Jakob Christensen, Rasmus Bro, and John Sørensen are gratefully acknowledged for valuable comments during the preparation of the manuscript. Gilda Kischinowski is thanked for proofreading the paper.

LITERATURE CITED

(1) Novales, B.; Bertrand, D.; Devaux, M.-F.; Robert, P.; Sire, A. Multispectral fluorescence imaging for the identification of food products. *J. Sci. Food Agric.* **1996**, *71*, 376–382.

(2) Munck, L.; Norgaard, L.; Engelsen, S. B.; Bro, R.; Andersson, C. A. Chemometrics in food science—a demonstration of the feasibility of a highly exploratory, inductive evaluation strategy of fundamental scientific significance. *Chemom. Intell. Lab. Syst.* **1998**, *44*, 31–60.

(3) Zandomeneghi, M.; Carbonaro, L.; Caffarta, C. Fluorescence of vegetable oils: olive oils. *J. Agric. Food Chem.* **2005**, *53*, 759–766.

(4) Christensen, J.; Norgaard, L.; Bro, R.; Engelsen, S. B. Multivariate autofluorescence of intact food systems. *Chem. Rev.* **2006**, *106*, 1979–1994.

(5) Martens, H.; Næs, T. *Multivariate calibration*; John Wiley & Sons: Chichester, U.K., 1989.

(6) Bro, R. PARAFAC. Tutorial and applications. *Chemom. Intell. Lab. Syst.* **1997**, *38*, 149–171.

(7) Duggan, D. E.; Bowman, R. L.; Bradie, B. B.; Udenfriend, S. A spectrometric study of compounds of biological interest. *Arch. Biochem. Biophys.* **1957**, *68*, 1–14.

(8) Wolfbeis, O. S. The fluorescence of organic natural products. In *Molecular luminescence spectroscopy: Methods & Applications—Part 1*; Schulman, S. G., Ed.; John Wiley & Sons: New York, 1985; pp167–370.

(9) Karoui, R.; Dufour, E.; Schoonheydt, R.; De Baerdemaeker, J. Characterisation of soft cheese with chemometric tools: Effect of the manufacturing process and sampling zone. *Food Chem.* **2007**, *100*, 632–642.

(10) Karoui, R.; Dufour, E.; De Baerdemaeker, J. Front face fluorecence spectroscopy coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout ripening. *Food Chem.* **2007**, *101*, 1305–1314.

(11) Liu, X.; Metzger, L. E. Application of fluorescence spectroscopy for monitoring changes in nonfat dry milk during storage. *J. Dairy Sci.* **2007**, *90*, 24–37.

(12) Andersen, C. M.; Vierick, N. Spectroscopic study of low- and nonfat cream cheeses—relationships and method differences,in preparation.

(13) Karoui, R.; Mouazen, A. M.; Dufour, E.; Schoonheydt, R.; De Baerdemaeker, J. Utilisation of front-face fluorescence spectroscopy for the determination of some selected chemical parameters in soft cheeses. *Lait* **2006**, *86*, 155–169.

(14) Karoui, R.; Dufour, E.; De Baerdemaeker, J. Common components and specific weights analysis: A tool for monitoring the molecular structure of semi-hard cheese throughout ripening. *Anal. Chim. Acta* **2006**, *572*, 125–133.

(15) Granger, D.; Da Costa, J. P.; Toutain, J.; Barey, P.; Cansell, M. Mapping of ice cream formulation using front-face fluorescence spectroscopy. *Int. Dairy J.* **2006**, *16*, 489–496.

(16) Pulgarín, J. A. M.; Molina, A. A.; Pardo, M. T. A. Fluorescence characteristics of several whey samples subjected to different treatments and conditions. *Anal. Chim. Acta* **2005**, *536*, 153–158.

(17) Karoui, R.; Dufour, E.; Pillonel, L.; Schaller, E.; Picque, D.; Cattenoz, T.; Bosset, J.-O. The potential of combined infrared and fluorescence spectroscopies as a method of determination of the gerographic origin of emmental cheeses. *Int. Dairy J.* **2005**, *15*, 287–298.

(18) Karoui, R.; Martin, B.; Dufour, E. Potentiality of front-face spectroscopy to determine the geographical origin of milks from the Haute-Loire department (France). *Lait* **2005**, *85*, 223–226.

(19) Karoui, R.; Bosset, J.-O.; Mazerolles, G.; Kulmyrzaev, A.; Dufour, E. Monitoring the geographic origin of both experimental French Jura hard cheeses and Swiss Gruyére and Létivaz PDO cheeses using mid-infrared and fluorescence spectrscopies: a preliminary investigation. *Int. Dairy J.* **2005**, *15*, 275–286.

(20) Kulmyrzaev, A.; Dufour, E.; Noël, Y.; Hanafi, M.; Karoui, R.; Qannari, E. M.; Mazerolles, G. Investigation at the molecular level of soft cheese quality and ripening by infrared and fluorescence spectroscopies and chemometrics—relationships with rheology properties. *Int. Dairy J.* **2005**, *15*, 669–678.

(21) Karoui, R.; Dufour, E.; Pillonel, L.; Picque, D.; Cattenoz, T.; Bosset, J.-O. 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. *Eur. Food Res. Technol.* **2004**, *219*, 184–189.

(22) Karoui, R.; Dufour, E.; Pillonel, L.; Picque, D.; Cattenoz, T.; Bosset, J.-O. Fluorescence and infrared spectroscopies: a tool for the determination of the geographic origin of Emmental cheeses manufactured during summer. *Lait* **2004**, *84*, 359–374.

(23) Karoui, R.; Dufour, E. Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheese. *Int. Dairy J.* **2003**, *13*, 973–985.

(24) Karoui, R.; Laquet, A.; Dufour, E. Fluorescence spectroscopy: A tool for the investigation of cheese melting—Correlation with rheological characteristics. *Lait* **2003**, *83*, 251–264.

(25) Dufour, E.; Devaux, M. F.; Fortier, P.; Herbert, S. Delienation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy—relationship with texture. *Int. Dairy J.* **2001**, *11*, 465–473.

(26) Herbert, S.; Mouhous Riou, N.; Devaux, M. F.; Riaublanc, A.; Bouchet, B.; Gallant, D. J.; Dufour, E. Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. *Lait* **2000**, *80*, 621–634.

(27) Herbert, S.; Riaublanc, A.; Bouchet, B.; Gallant, D. J.; Dufour, E. Fluorescence spectroscopy investigation of acid- or rennet-induced coagulation of milk. *J. Dairy Sci.* **1999**, *82*, 2056–2062.

(28) Dufour, E.; Riaublanc, A. Potentiality of spectroscopic methods for the characterization of dairy products. *Lait* **1997**, *77*, 657–670.

(29) Lackowicz, J. R. *Principles of fluorescence spectroscopy*, 2nd ed.; Kluwer Academic/Plenum: New York, 1999.

(30) Lopez, C.; Dufour, E. The composition of the milk fat globule surface alters the structural characteristics of the coagulum. *J. Colloid Interface Sci.* **2001**, *233*, 241–249.

(31) Harshman, R. A. Foundations of the PARAFAC procedure: model and conditions for an "explanatory" multi-mode factor analysis. *UCLA Working Papers in Phonetics* **1970**, *16*, 1–84.

(32) Andersen, C. M.; Bro, R. Practical aspects of PARAFAC modelling of fluorescence excitation-emission data. *J. Chemom.* **2003**, *17*, 200–215.

(33) Andersen, C. M.; Vishart, M.; Holm, V. K. Application of fluorescence spectroscopy for evaluating light-induced oxidation in dairy products. *J. Agric. Food Chem.* **2005**, *53*, 9985–9992.

(34) Christensen, J.; Povlsen, V. T.; Sørensen, J. Application of fluorescence spectroscopy and chemometrics in the evaluation of processed cheese during storage. *J. Dairy Sci.* **2003**, *86*, 1101–1107.

(35) Feinberg, M.; Dupont, D.; Efstathiou, T.; Louâpre, V.; Guyonnet, J.-P. Evaluation of tracers for the authentication of thermal treatments of milks. *Food Chem.* **2006**, *98*, 188–194.

(36) Schamberger, G. P.; Labuza, T. P. Evaluation of front-face fluorescence for assessing thermal processing of milk. *J. Food Sci.* **2006**, *71*, C69–C74.

(37) Kulmyrzaev, A.; Levieux, D.; Dufour, E. Front-face fluorescence spectroscopy allows the characterization of mild heat treatments applied to milk. Relations with the denaturation of milk proteins. *J. Agric. Food Chem.* **2005**, *53*, 502–507.

(38) Birlouez-Aragon, I.; Sabat, P.; Gouti, N. A new method for discriminating milk heat treatment. *Int. Dairy J.* **2002**, *12*, 59–67.

(39) Kulmyrzaev, A.; Dufour, E. Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy. *Lait* **2002**, *82*, 725–735.
(40) Birlouez-Aragon, I.; Nicolas, M.; Meatais, A.; Marchond, N.; Grenier, J.; Calvo, D. A rapid fluorimetric method to estimate the heat treatment of liquid milk. *Int. Dairy J.* **1998**, *8*, 771–777.
(41) Morales, F. J.; van Boekel, M. A. J. S. A study on advanced Maillard reaction in heated casein/sugar solutions: Fluorescence accumulation. *Int. Dairy J.* **1997**, *7*, 675–683.
(42) Morales, F. J.; Romero, C.; Jiménez-Pérez, S. Fluorescence associated with Maillard reaction in milk and milk-resembling systems. *Food Chem.* **1996**, *57*, 423–428.
(43) Matiacevich, S. B.; Buera, M. P. A critical evaluation of fluorescenceas a potential marker for the Maillard reaction. *Food Chem.* **2006**, *95*, 423–430.
(44) Elshereef, R.; Budman, H.; Moresoli, C.; Legge, R. L. Fluorescence spectroscopy as a tool for monitoring solubility and aggregation behaviour of β-lactoglobulin after heat treatment. *Biotechnol. Bioeng.* **2006**, *95*, 863–865.
(45) Manderson, G. A.; Haardman, M. J.; Creamer, L. K. Effect of heat treatment on bovine β-lactoglobulin A, B, and C explored using thiol availability and fluorescence. *J. Agric. Food Chem.* **1999**, *47*, 3617–3627.
(46) Renard, D.; Lefebvre, J.; Griffin, M. C. A.; Griffin, W. G. Effects of pH and salt environment on the association of β-lactoglobulin revealed by intrinsic fluorescence studies. *Int. J. Biol. Macromol.* **1998**, *22*, 41–49.
(47) Mendoza, M. R.; Olano, A.; Villamiel, M. Chemical indicators of heat treatment in fortified and special milks. *J. Agric. Food Chem.* **2005**, *53*, 2995–2999.
(48) Mazerolles, G.; Devaux, M. F.; Dufour, E.; Qannari, E. M.; Courcoux, Ph. Chemometric methods for the coupling of spectroscopic techniques and for the extraction of the relevant information contained in the spectral data tables. *Chemom. Intell. Lab. Syst.* **2002**, *63*, 57–68.
(49) Mazerolles, G.; Devaux, M.-F.; Duboz, G.; Duployer, M.-H.; Mouhious Riou, N.; Dufour, E. Infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening. *Lait* **2001**, *81*, 509–527.
(50) Dufour, E.; Mazerolles, G.; Devaux, M. F.; Duboz, G.; Duployer, M. H.; Mouhous Riou, N. Phase transition of triglycerides during semi-hard cheese ripening. *Int. Dairy J.* **2000**, *10*, 81–93.
(51) Karoui, R.; Dufour, E. Prediction of the rheoloogy parameters of ripened semi-hard cheeses using fluorescence spectra in the UV and visible ranges recorded at a young stage. *Int. Dairy J.* **2006**, *16*, 1490–1497.
(52) Janhøj, T.; Andersen, C. M.; Viereck, N.; Frøst, M. B.; Ipsen, R.; Edrud, S. Sensory, rheological and spectroscopic characterization of low-fat cream cheese. In *Proceedings of the 4th International Symposium on Food Rheology and Structure*, Feb 19−20, 2006, Zürich, pp 383–387.
(53) Garimella Purna, S. K.; Prow, L. A.; Metzger, L. E. Utilization of front-face fluorescence spectroscopy for analysis of process cheese functionality. *J. Dairy Sci.* **2005**, *88*, 470–477.
(54) Lebecque, A.; Laguet, A.; Devaux, M. F.; Dufour, E. Delineation of the texture of salers cheese by sensory analysis and physical methods. *Lait* **2001**, *81*, 609–623.
(55) Veberg, A.; Olsen, E.; Nilsen, A. N.; Wold, J. P. Front-face fluorescence measurement of photosensitizers and lipid oxidation products during photooxidation of butter. *J. Dairy Sci.* **2007**, *90*, 2189–2199.
(56) Wold, J. P.; Veberg, A.; Lundby, F.; Nilsen, A. N.; Moan, J. Influence of storage time and color of light on photooxidation in cheese: A study based on sensory analysis and fluorescence spectroscopy. *Int. Dairy J.* **2006**, *16*, 1218–1226.
(57) Wold, J. P.; Bro, R.; Veberg, A.; Lundby, F.; Nielsen, A. N.; Moan, J. Active photosensitizers in butter detected by fluorescence spectroscopy and multivariate curve resolution. *J. Agric. Food Chem.* **2006**, *54*, 10197–10204.
(58) Wold, J. P.; Veberg, A.; Nilsen, A.; Iani, V.; Juzenas, P.; Moan, J. The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. *Int. Dairy J.* **2005**, *15*, 343–353.
(59) Wold, J. P.; Jørgensen, K.; Lundby, F. Nondestructive measurement of light-induced oxidation in dairy products by fluorescence spectroscopy and imaging. *J. Dairy Sci.* **2002**, *85*, 1693–1704.
(60) Andersen, C. M.; Andersen, L. T.; Hansen, A. M.; Skibsted, L. H.; Petersen, M. A. Light-induced changes of naturally occuring photosensitizer in cheese. Effect of specific wavelengths and exposure time, submitted for publication.
(61) Andersen, C. M.; Wold, J. P.; Mortensen, G. Light-induced changes in semi-hard cheese determined by fluorescence spectroscopy and chemometrics. *Int. Dairy J.* **2006**, *16*, 1483–1489.
(62) Christensen, J.; Miquel Becker, E.; Frederiksen, C. S. Fluorescence spectroscopy and PARAFAC analysis in yogurt. *Chemom. Intell. Lab. Syst.* **2005**, *75*, 201–208.
(63) Becker, E. M.; Christensen, J.; Frederiksen, C. S.; Haugaard, V. K. Front-face fluorescence spectroscopy and chemometrics in analysis of yoghurt—Rapid analysis of riboflavin. *J. Dairy Sci.* **2003**, *86*, 2508–2515.
(64) Mortensen, G.; Sørensen, J.; Stapelfeldt, H. Effect of modified atmosphere packaging and storage conditions on photooxidation of sliced havarti cheese. *Eur. Food Res. Technol.* **2003**, *216*, 93–98.
(65) Mortensen, G.; Sørensen, J.; Danielsen, B.; Stapelfeldt, H. Effect of specific wavelengths on light-induced quality changes in havarti cheese. *J. Dairy Res.* **2003**, *70*, 413–421.
(66) Skibsted, L. H. Light induced changes in dairy products. In *Packaging of milk products*,IDF Bulletin 346; International Dairy Federation: Brussels, Belgium, 2000; pp 3–9.
(67) Fox, J. B.; Thayer, D. W. Radical oxidation of riboflavin. *Int. J. Vit. Res.* **1998**, *68*, 174–180.
(68) Kikugawa, K.; Beppu, M. Involvement of lipid oxidation products in the formation of fluorescent and cross-linked proteins. *Chem. Phys. Lipids* **1987**, *44*, 277–296.
(69) Stapelfeldt, H.; Skibsted, L. H. Modification of β-lactoglobulin by aliphatic aldehydes in aqueous solution. *J. Dairy Res.* **1994**, *61*, 209–219.
(70) Whited, L. J.; Hammond, B. H.; Chapman, K. W.; Boor, K. J. Vitamin A degradation and light-oxidized flavor defects in milk. *J. Dairy Sci.* **2002**, *85*, 351–354.
(71) Marsh, R.; Kajda, P.; Ryley, J. The effect of light on the vitamin B_2 and the vitamin A content of cheese. *Nahrung* **1994**, *5*, 527–532.
(72) Booksh, K.; Kowalski, B. Theory of analytical chemistry. *Anal. Chem.* **1994**, *66*, 782A–791A.

Received for review July 6, 2007. Revised manuscript received November 5, 2007. Accepted November 6, 2007. This work is part of the FØTEK program supported by the Danish Dairy Research Foundation (Danish Dairy Board) and the Directorate for Food, Fisheries and Agri Business.

JF072025O