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Application of Fluorescence Spectroscopy in the Evaluation of Light-Induced Oxidation in Cheese

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Light-induced oxidation of semihard cheese has been evaluated by fluorescence spectroscopy. The cheese was packaged in two packaging materials and exposed to different storage conditions, which included light/dark storage, oxygen availability, and storage time (0, 4, 7, 14, 21, 42, 70, or 84 days). Fluorescence excitation−emission matrices (EEM) were analyzed by PARAFAC, which gave an estimation of the pure excitation and emission spectra of the fluorophores and the concentrations of these. This analysis showed the presence of components such as tryptophan, tyrosine, vitamin A, fluorescent oxidation products, and riboflavin. Effects of packaging material, light or dark storage, and storage time were seen. However, there was no effect of the oxygen availability on the fluorescence measurements. The score values obtained by the PARAFAC models and chemical and physical measurements were analyzed together by principal component analysis (PCA). The loadings showed a separation of the variables into three groups; the first group was related to oxidation, the second group was related to the degradation of both riboflavin and vitamin A, and the third group was linked to the protein structure.

KEYWORDS: Fluorescence spectroscopy; semihard cheese; light-induced oxidation; chemometrics

INTRODUCTION

Cheeses exposed to light during processing, packaging, distribution, and retail are likely to undergo photooxidation. Light-induced oxidation causes the formation of off-flavors, color changes, and decreased nutritional value, which impair product quality and marketability. This has been shown in several studies, which have illustrated the effect of the storage conditions such as headspace volume, oxygen availability, and light intensity on the product (*1*−*7*). Typically, light-induced oxidation has been measured by using chemical measurements. These include the development of secondary lipid oxidation products (*5*, *6*, *8*), formation of primary lipid peroxides (*4*), decrease in riboflavin content (*1*, *2*, *4*, *8*), and variation in vitamin A content (*2*). Furthermore, color changes have been used to measure oxidation in dairy products exposed to light (*3*, *5*−*7*).

Recently, it has been shown that fluorescence spectroscopy can be used to describe light-induced oxidation of dairy products. For example, fluorescence spectroscopic data of yogurt have shown high correlation between fluorescence spectral data and the riboflavin content determined by chemical measurements (*9*, *10*). A decrease in fluorescence intensity of the maximum emission of riboflavin has also been illustrated for other dairy products such as sour cream, cream cheese, and semihard cheese (*6*, *11*, *12*). Furthermore, it has been indicated that fluorescence spectroscopy can be used to measure changes in vitamin A content (*13*), variation in protein structure (*9*, *10*, *13*), development of tertiary oxidation products (*11*, *12*, *14*), and decrease in the porphyrin content (*11*). These parameters can all be affected by light. Thus, fluorescence spectroscopy may give information about several factors of the light-induced oxidation mechanism.

This study examines how fluorescence spectroscopy can be used to evaluate the quality of semihard cheese (Danbo cheese) packaged in modified atmosphere and exposed to light for up to 84 days. The cheeses were stored in the light or dark with variations in oxygen availability provided by oxygen scavengers and the packaging materials as described by Holm et al. (*8*). The fluorescence spectroscopic measurements were analyzed with parallel factor decomposition (PARAFAC), which gave a chemical profile of the sample. The aim was to obtain information about several parameters that were sensitive to light and could be measured by fluorescence spectroscopy. Furthermore, principal component analysis (PCA) was used to compare the fluorescence spectroscopic data with chemical and physical measurements that were related to light-induced oxidation. These were described by Holm et al. (*8*).

MATERIALS AND METHODS

Packaging and Storage of Cheese. Danbo cheeses (165 g, 27% fat, 26% protein) were obtained from Arla Foods (Viby J., Denmark) as described by Holm et al. (*8*). The cheeses were packaged in poly-

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(lactic acid) (PLA) (Færch Plast, Holstebro, Denmark) or amorphous poly(ethylene terephthalate) (APET) (Færch Plast) trays and closed with PLA lids (biaxially oriented polyester film with an amorphous polyester heat seal layer) (Biophan, Trespaphan, Germany) or AF foil (DuPont Teilin Films, Luxembourg). Approximately 90% of the light in the visual region of the spectrum was transmitted through the lids. The cutoff wavelength for the PLA packages was 250 nm, and for the APET packages it was 325 nm (*8*). The dimensions of the cheeses in the packages were 3.5 × 8.5 × 6.0 cm.

The cheeses were packaged in modified atmosphere (30% carbon dioxide and 70% nitrogen, maximum 0.5% residual oxygen) and stored for 0, 4, 7, 14, 21, 42, 70, or 84 days (the durability of the cheeses is 84 days) in a display cabinet with measured temperature and relative humidity of approximately 4 °C and 45%, respectively. The storage conditions resemble real storage conditions. The samples were exposed to light from Phillips TLD 18W/830 New Generation fluorescent tubes (Philips, Eindhoven, The Netherlands) with an average intensity of 1400 lx at the surface of the cheese. The cheeses were rotated regularly to minimize temperature and light exposure differences. Half of the samples were stored under black plastic to hinder light exposure. Furthermore, an oxygen scavenger (Mitsubishi Gas Chemical Co., Tokyo, Japan) was included in half of the samples. Thus, the experimental design included eight types of samples (two types of packaging material × with or without oxygen scavenger × light or dark storage). Three packages were analyzed at each storage time for all eight combinations of storage parameters. A 4 mm thick slice was cut from the cheese surface facing the light source and used for analysis.

Fluorescence Measurements. The samples were measured on a Perkin-Elmer LS50 B spectrofluorometer equipped with a Front-Face accessory, which was placed in the light path at an angle of ~60°. A circular sample holder with a diameter of ~1 cm was used. For every sample, two excitation−emission matrices (EEM) were measured. One was obtained by measuring emission spectra from 280 to 600 nm with excitation every 10 nm from 260 to 360 nm. The slit widths were set to 7 nm for both excitation and emission, and a 1% attenuation filter was used. The other EEM was obtained by measuring emission spectra from 390 to 650 nm with excitation every 10 nm from 360 to 460 nm and slit widths of 5 nm for both excitation and emission. The two EEMs were obtained because some of the fluorophores with excitation maxima at very low wavelengths had intensities so high that fluorescence of fluorophores with excitation maxima at higher wavelengths could not be seen. Each measurement started with the highest excitation wavelength and ended with the lowest to minimize photodecomposition of the sample. All measurements were made at room temperature. The scan speed was set to 1500 nm/s.

Chemical and Physical Measurements. The chemical and physical measurements were made on the same samples as the fluorescence measurements. Color measurements were performed by measuring Hunter *L* (lightness), *a* (redness), and *b* values (yellowness) (BYK Gardner, CIE L*a*b system, Geretsried, Germany). Measurements were performed at five positions excluding white and wet spots, which were caused by condensation of water. Each measurement consisted of the average of three replicate instrumental measurements. Other measurements were performed as described by Holm et al. (*8*). The development of secondary lipid oxidation products was measured by headspace gas chromatography according to the method described by Shahidi and Pegg (*15*) and Holm et al. (*8*). Acetaldehyde, pentanal, hexanal, and octanal were used as indicators of the development of secondary oxidation products. Riboflavin was measured by the high-performance liquid chromatography (HPLC) method proposed by Holm et al. (*8*). Headspace gas composition was measured prior to the opening of the packages using a CheckMate 9000 gas analyzer (PBI Dansensor, Tingsted, Denmark) with a needle inserted through a septum placed on the packages. Moisture loss was determined by adding 2.00 g of cheese to 20 g of dried sand and drying the sample for ~16 h at 105 °C. The sample was weighed before and after drying.

Data Analysis. Principal component analysis (PCA) (*16*) was used to visualize and explore the multivariate data. By PCA the main systematic variation in data was extracted by principal components (PCs). The data were contained in a matrix **X** (each row holding the data from one sample and each column being one specific variable) and decomposed into a score matrix **T** and a loading matrix **P** (eq 1). The residual matrix, **E**, held the variation not described by the model.

$$\mathbf{X} = \mathbf{TP}^{\mathbf{T}} + \mathbf{E} \tag{1}$$

PARAFAC) (*17*) was used to model the fluorescence excitation−emission matrices (eq 2). The data were arranged in an $I \times J \times K$ so-called three-way array, where the first index (I) refers to the samples, the second (J) to the emission wavelengths, and the third (K) to the excitation wavelengths. By PARAFAC the data were decomposed into fewer components in a way similar to PCA, giving one set of scores (a_{if}) and two sets of loadings, $\mathbf{b}_f$ and $\mathbf{c}_f$. As for PCA the variation in data not described by the model was collected in the residuals, here as a three-way array (*18*).

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk} \tag{2}$$

$$(i = 1, ..., I; j = 1, ..., J; K = 1, ..., K)$$

Each PARAFAC component will be an estimate of the signal of a single fluorophore if the data are approximately low-rank trilinear and the correct number of components is used. Thus, the loadings are estimates of the pure emission and excitation spectra of each fluorescent compound in the sample. The scores can be interpreted as the relative concentration of each fluorophore.

RESULTS AND DISCUSSION

Raw Data. The fluorescence EEMs were measured in two intervals due to very high fluorescence intensity of fluorophores measured in the low excitation wavelength area. These fluorophores were measured with excitations from 260 to 360 nm (**Figure 1a**). The fluorescence EEM shows one clear peak with excitation and emission maxima located around 300 and 350 nm, respectively. Two shoulders are found with nearly the same emission maxima as the large peak and with higher and lower excitation maxima. An example of an EEM measured in the high excitation wavelength area is shown in **Figure 1b**. The excitation wavelengths vary from 360 to 460 nm. In this EEM two broad peaks are seen, indicating that at least two fluorophores are present. One of these peaks has an excitation maximum at 360−380 nm; the other has an excitation maximum around 450−460 nm. However, there is an overall high fluorescence intensity in the whole landscape showing that other fluorophores of lower intensity may be present as well.

Excitation from 260 to 360 nm. A PARAFAC model suggests three components [see Andersen and Bro (*19*) for details on choosing the number of components], which is in accordance with the initial visual interpretation of the landscapes. The model explains 99.7% of the variation in data. It is constrained with non-negativity in the scores mode and non-negativity and unimodality in the emission and excitation modes. The resolved peaks have excitation maxima around 270, 300, and 330 nm (**Figure 2**). The corresponding emission maxima are found at 350, 350, and 410 nm. Some initial hypotheses can be made to identify these PARAFAC components.

The fluorescence of proteins is due to fluorescence emission of the three aromatic amino acids phenylalanine, tyrosine, and tryptophan. Generally, tryptophan dominates the fluorescence emission due to a higher molar extinction coefficient and its presence in larger amounts (*20*). Tryptophan exhibits excitation and emission maxima in aqueous solution with pH 11 at 285 and 365 nm, respectively (*21*), and it has previously been indicated that tryptophan in cheese could be measured by fluorescence spectroscopy (*13*, *22*, *23*). The fluorescence properties of tryptophan depend on the polarity, pH, and local

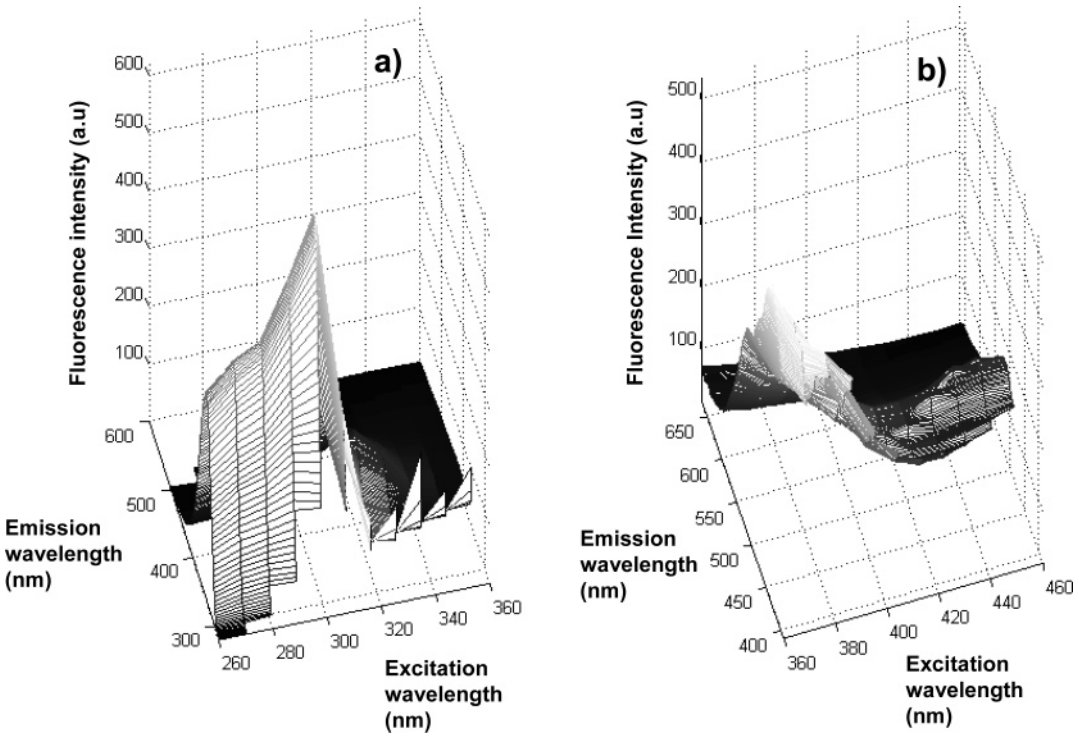

Figure 1. Raw excitation–emission matrices: (**a**) excitation, 260–360 nm; emission, 280–600 nm; (**b**) excitation, 360–460 nm; emission, 390–650 nm.

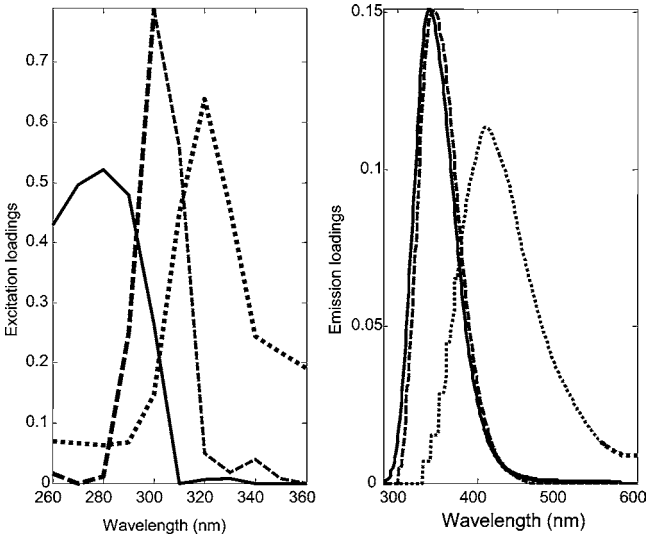

Figure 2. Excitation and emission loadings of the PARAFAC model with excitation from 260 to 360 nm: (solid line) component 1; (dashed line) component 2; (dotted line) component 3.

surroundings, which include interactions between tryptophan and proteins or between tryptophan and other constituents in the product (*24*, *25*). Therefore, component 2 with excitation and emission maxima at approximately 300 and 350 nm, respectively, may be attributed to the presence of tryptophan.

Christensen et al. (*13*) found for processed cheese a component with excitation and emission at 280 and 339 nm and suggested that the component was due to the presence of tryptophan but in a different environment than the tryptophan described by component 2. In **Figure 2** the estimated emission spectra of components 1 and 2 are similar, but the excitation spectra are different. Thus, component 1 does not represent tryptophan because only the emission spectra are sensitive to the local environment. The excitation maximum of component 1 corresponds to the excitation maximum of tyrosine given by the literature, which is seen at 275 nm (*24*). Light at 275 nm excites tyrosine, which in the excited state can transfer energy to tryptophan (*20*). After transferring energy to tryptophan in the excited state, the emission spectra estimated by the PARAFAC model will equal the emission spectra of tryptophan obtained by the chemometric modeling. Phenylalanine exhibits maximum excitation at 260 nm. Even though energy transfer to tryptophan can take place, the excitation maximum of component 1 varies from the excitation maximum of phenylalanine. Furthermore, phenylalanine is present in a lower concentration than tyrosine (*26*) and has a lower extinction coefficient (*27*). Nucleotides are also excited below 300 nm. However, their emission spectra are found at higher wavelengths than the excitation spectrum of tryptophan. Thus, energy transfer cannot take place. Furthermore, the nucleotides are present in very low concentrations.

Vitamin A has excitation maximum around 325 nm and emission maximum between 470 and 510 nm (*21*, *27*). Component 3 has excitation and emission maxima at 330 and 410 nm, respectively. Even though there are some differences in the wavelength positions, the component may describe the presence of vitamin A. Furthermore, Karoui and Dufour (*24*) measured excitation spectra of vitamin A with emission set at 410 nm. β-Carotene absorbs light between 400 and 500 nm and could absorb some of the light emitted by vitamin A. This will influence the estimated emission spectra and could explain why the emission maximum does not agree exactly with the values given in the literature. However, the concentration of β-carotene is much lower than the concentration of vitamin A, and absorption is much less sensitive than fluorescence. Thus, β-carotene may not have a significant influence on the fluorescence data.

Figure 3 shows average scores of the PARAFAC model as a function of the storage time of the investigated cheeses. Averages are taken over samples with the same combination of design parameters. Before the average was taken, the difference between the replicates was studied, and it was found

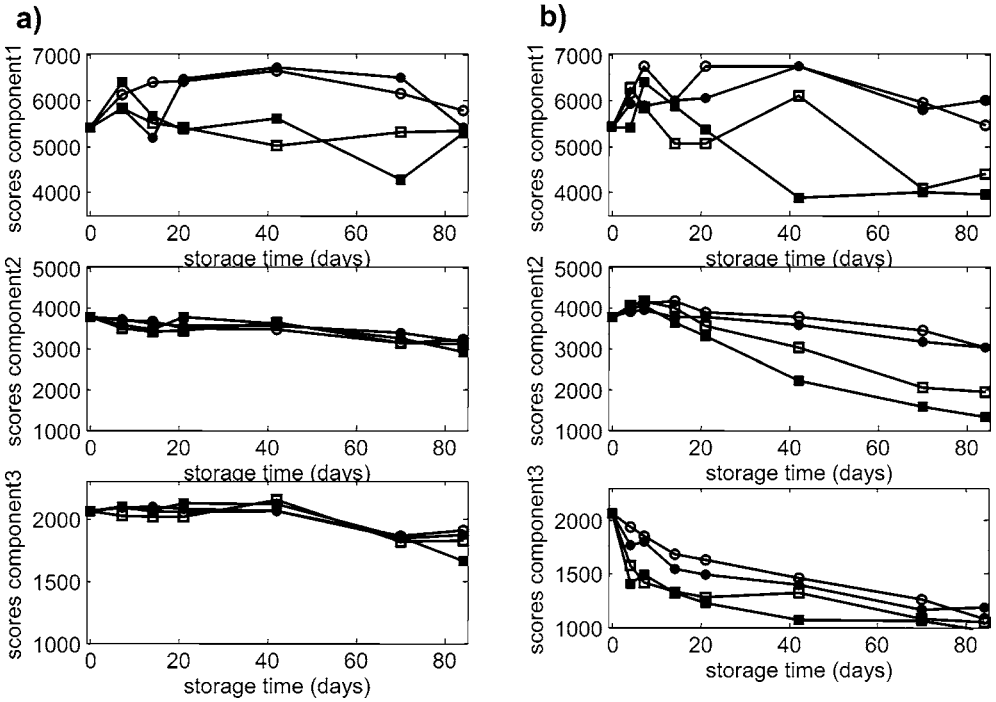

Figure 3. Scores of the PARAFAC model with excitation from 260 to 360 nm. The scores are averaged over (**a**) samples stored in the dark and (**b**) samples stored in the light: (□) PLA with oxygen absorber; (■) PLA without oxygen absorber; (○) APET with oxygen absorber: (●) APET without oxygen absorber.

that the variations were small. The scores of component 1 are lower for cheese packaged in PLA irrespective of storage in the light or dark. These score values decrease somewhat with storage time, especially for the cheeses that were exposed to light. A decrease in the score values or an increase as seen for samples packaged in APET after a few days of storage may not necessarily imply that the tyrosine content decreases or increases but rather that a change in the sample matrix influences the protein structure and thus the fluorescence properties of tyrosine. However, the exact changes in the proteins that result in changes in the fluorescence properties cannot be determined from the present data.

For light-exposed samples, the scores of component 2 increase slightly in the first few days. After 4 days of storage, the score values start decreasing, with the scores of samples packaged in PLA decreasing the most. The scores illustrate that structural protein changes that are sensitive to light take place especially when the cheeses are packaged in PLA. This is different from the scores describing tyrosine, which are influenced by the packaging material also for samples stored in the dark.

Variations in score values of PARAFAC models or PCAs corresponding to changes in protein structure have been shown for processed cheese (*13*) and yogurt (*9*, *10*). In the present study variations between the two packaging materials are seen also for samples stored in the dark. Thus, the variations may not only be an effect of light exposure. Transmissions of both oxygen and water vapor are higher for PLA compared with APET (*8*). The higher water vapor transmission rate of PLA could decrease the water content of the cheese, resulting in a change in the consistency. It is possible that these changes influence the protein structure in a way which can be identified by the variation in the fluorescence properties.

There is a fast decrease in the score values of component 3 for up to 4 days of storage for cheeses packaged in PLA and exposed to light. At longer storage time the scores find a constant level. When the cheeses are packaged in APET, the decrease is not as fast in the beginning of the storage period, but after 84 days of storage, the scores have reached the same level as for the samples packaged in PLA. The decrease in the score values of samples packaged in PLA and exposed to light indicates a fast decrease in the vitamin A content, which levels out after 4 days of storage. Vitamin A is sensitive to oxygen and light. This has been illustrated by measuring the vitamin A content chemically in light-exposed dairy products (*2*, *28*). The scores of component 3 of the cheeses stored in the dark do not change with storage time, indicating that no change in vitamin A content takes place in dark-stored samples. PLA transmits light above 250 nm, whereas APET transmits light only above ~325 nm (*8*). Vitamin A has maximal absorption at ~325 nm. Thus, more light can be absorbed by vitamin A in the PLA packages, which explains why more vitamin A is degraded in these packages. In addition to the degradation of vitamin A in the light-exposed samples, changes in the vitamin A fluorescence due to change of the lipid structure may take place as has been reported by Karoui and Dufour (*24*). Such changes could also be visualized as a variation in the score values obtained by the PARAFAC model and could have taken place in the dark-stored samples or due to the high water vapor transmission rate of PLA.

Excitation from 360 to 460 nm. The EEMs with excitations ranging from 360 to 460 nm could not as easily be fitted by a PARAFAC model. This was partly expected because no smooth unimodal peaks were seen, but rather an overall high fluorescence intensity with several small peaks obtained from many fluorophores in low concentration or with low quantum yield. Instead, two PARAFAC models were made; one containing the emissions from 400 to 500 nm, and the other containing the emissions from 500 to 580 nm. The PARAFAC models had the same excitation wavelengths (360−460 nm).

Figure 4 shows loadings and scores of samples stored in the light. With emissions measured from 400 to 500 nm a one-component model is found to be optimal. It explains 99.3% of the variation in data. An excitation maximum is seen at 360 nm or below and a shoulder at 380 nm. Emission maxima are

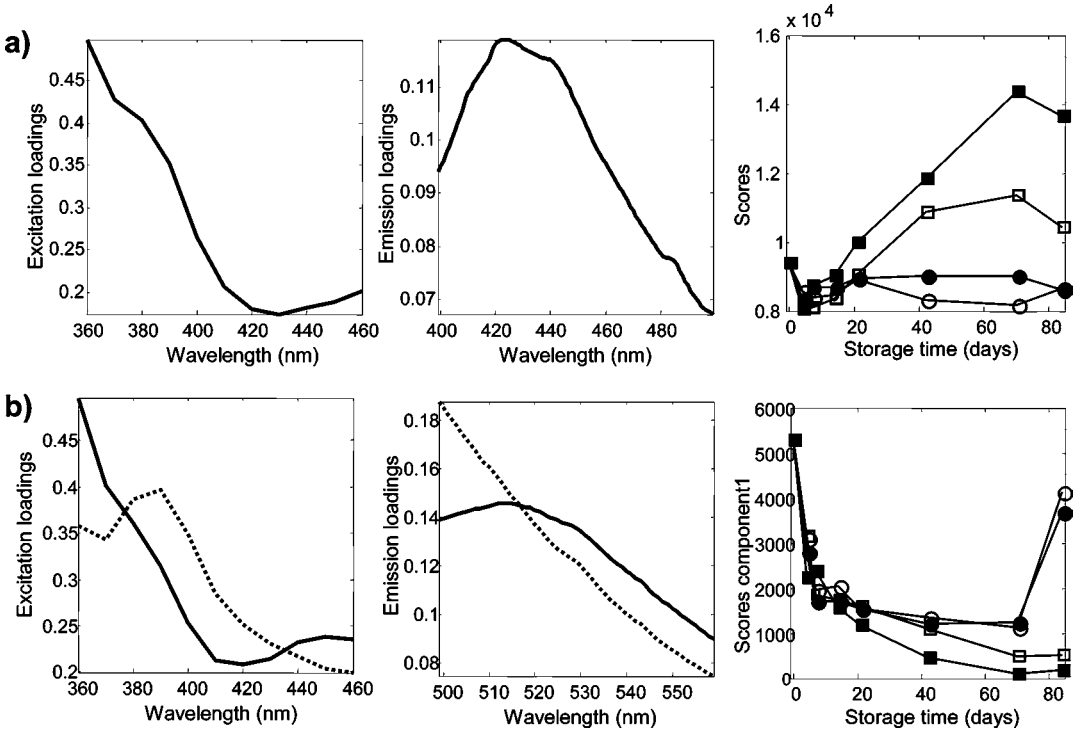

Figure 4. Loadings and scores of PARAFAC models made on data with excitation from 360 to 460 nm and emission (**a**) from 400 to 500 nm and (**b**) from 500 to 580 nm. Loadings: (solid line) component 1; (dotted line) component 2. Storage conditions: (□) PLA with oxygen absorber; (■) PLA without oxygen absorber; (○) APET with oxygen absorber; (●) APET without oxygen absorber. All samples were stored in the light. For **b**, only the scores of the first component are shown.

found between 410 and 440 nm. Also, a small shoulder is found at 480 nm. This fluorescence be due to the formation of fluorescent oxidation products, which are formed by the reaction between amino groups and aldehydes formed during the oxidation mechanism. These products exhibit fluorescence with excitation maxima at 340−370 nm and emission maxima at 400−470 nm (*29*, *30*). The presence of these products in light-exposed cheese has previously been indicated by Wold et al. (*12*). Typically, smooth, unimodal, and positive loadings are obtained from PARAFAC, reflecting the smoothness of the underlying pure spectra. This is not the case here, probably because the fluorescent oxidation products are a group of compounds with somewhat different fluorescence properties and the fluorescence intensity of the oxidation products may be low. Furthermore, the appearance of the loadings can be affected by absorption of some of the emitted light by β-carotene. The scores increase with storage time for cheeses packaged in PLA and exposed to light but do not change for cheeses stored in APET. This demonstrates that the development of fluorescent oxidation products takes place after longer storage in PLA but not for cheeses packaged in APET. The scores may also be influenced by β-carotene. When β-carotene is degraded by light exposure, it will absorb less of the light emitted by the fluorescent oxidation products. This will increase the score values and amplify the effect on these. However, with the present data and knowledge, it is not possible to separate the fluorescence properties of β-carotene and fluorescent oxidation products.

A two-component model is found to be the optimal PARAFAC model based on emissions from 500 to 580 nm. It explains 99.7% of the variation in data and is constrained with non-negativity in all three modes. The first component describes the presence of riboflavin. Riboflavin is commonly present in dairy products and is known to initiate photooxidation (*31*). Decreased contents have been shown by chemical measurements of riboflavin in dairy products exposed to light (*1*, *2*). Riboflavin has excitation maxima at 370 and 450 nm and an emission maximum around 520 nm (*32*). These peak maxima corresponds to the peak maxima or shoulders of component 1 (**Figure 4b**). The score values decrease rapidly for all samples exposed to light, indicating a fast decrease in the riboflavin content. After longer storage, the scores decrease more for samples stored in PLA than for samples stored in APET, displaying a difference between the two packaging materials. The increase after 84 days of storage of the APET samples was not found by the chemical determination of riboflavin, but a peak in the EEMs corresponding to riboflavin is seen. It is not possible to explain why this peak is present.

The second component describes fluorescent oxidation products. This is supported by the scores (not shown) and by comparing the loadings of this model with the loadings of the model with emission wavelengths from 400 to 500 nm.

Relationship between Fluorescence and Chemical Measurements. A PCA is made of the score values from all of the models described above and including chemical and physical measurements (all autoscaled to handle individual differences in scale). These include *L*, *a*, and *b* values, which denote the color of the cheese; hexanal, pentanal, octanal, and acetaldehyde contents, which are secondary oxidation products; water content in the cheeses; riboflavin content determined by the HPLC technique; and the amount of O_2 and CO_2 present in the packages prior to the opening. A detailed description of the results and conclusions of the chemical and physical measurements is given by Holm et al. (*8*).

Figure 5 shows loadings and scores of the first two PCs. These explain 49 and 22% of the variation in data, respectively. The loadings seem to be divided into three groups. One group has high values in PC1. This group consists of parameters describing oxidation such as the scores of the components from the PARAFAC models that describe fluorescent oxidation products and secondary oxidation products determined chemi-

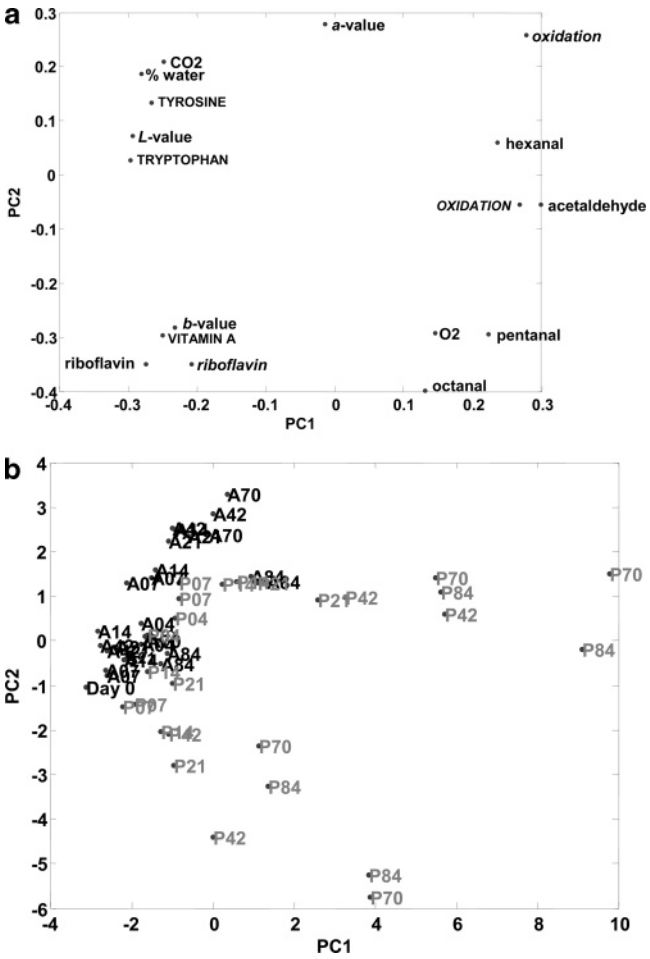

Figure 5. Loadings and scores of a PCA made on score values obtained by the PARAFAC models and chemical and physical parameters. (**a**) Loadings: PARAFAC model with excitations from 260 to 360 nm (roman capital letters), PARAFAC model with excitations from 360 to 460 nm and emissions from 400 to 500 nm (italic capital letters), PARAFAC model with excitations from 360 to 460 nm and emissions from 500 to 560 nm (italic lower case letters), and chemical and physical parameters (roman lower case letters). The chemical and physical parameters include *L*, *a*, and *b* values (color of the cheese); hexanal, pentanal, octanal, and acetaldehyde (secondary oxidation products) contents; water content; riboflavin content determined by HPLC; and O_2 and CO_2 present in the packages prior to the opening. (**b**) Scores: "A" denotes packaging in APET, and "P" denotes packaging in PLA. The numbers indicate storage time.

cally. Also, the oxygen level in the packages is found in this group. Samples stored for longer periods are placed closer to this group irrespective of storage in the light or dark. Furthermore, there is a difference between samples packaged in APET or in PLA in that PLA samples have higher score values in PC1. The loadings of this group are spread out along PC2. Factors such as octanal, pentanal, and O_2 have negative loadings in PC2. This corresponds to the score values of the samples stored in the dark. The other loadings of this group have positive PC2 score values, which are the same for the scores of samples stored in the light. A previous study showed high concentrations of the secondary oxidation products pentanal and octanal for dark-stored samples in PLA (*8*). Furthermore, high oxygen levels were seen in PLA packages without oxygen scavengers irrespective of storage in the light or dark. The high levels of these parameters also in the dark-stored samples may explain the negative loadings in PC2.

The second group has low loading values in both PC1 and PC2. It consists of parameters related to riboflavin as, for example, the chemically determined riboflavin content and the scores of the PARAFAC model that describe riboflavin. As shown in the previous model, riboflavin degrades by storage in the light irrespective of the packaging material and application of oxygen scavenger or not. This is supported by the score values (**Figure 5b**). Dark-stored samples have negative PC2 score values, illustrating that high riboflavin content and light-exposed samples have positive PC2 score values indicating low riboflavin content. Also, vitamin A and the *b* value, which describes the yellowness, are found in this group. The relationship between these parameters and riboflavin seems to be reasonable because the scores explaining vitamin A show the same tendencies as the scores representing riboflavin. Riboflavin is a yellow colorant. Thus, the decrease in yellow color corresponds well with the decrease in riboflavin content.

The negative loadings in PC1 of the parameters describing riboflavin and the positive loadings of parameters describing oxidation emphasize the fact that PC1 explains the degree of oxidation. One of the parameters that describes the degree of oxidation has high values in PC1 but is also oppositely correlated with the first component of the same model, which explains the presence of riboflavin. Lumichrome is formed by the degradation of riboflavin. It has emission maxima between 444 and 479 nm (*32*) and can be represented by this oxidation component. However, riboflavin is degraded for both packaging materials, but the formation of fluorescence in this wavelength area is seen only in cheeses packaged in PLA.

The third group contains parameters that describe the presence of tryptophan and tyrosine. Also, the *L* and *a* values (lightness and redness), percent water, and CO_2 content in the packages are found in this group. These parameters are correlated to samples stored for a shorter period, in the light and packaged in APET. There is a difference in the tyrosine component between samples packaged in the two materials irrespective of storage in the light or dark. This difference increases with increasing storage time. These score values are correlated to the *L* values, showing a relationship between protein structure and lightness. Furthermore, the water content in the samples is negatively correlated to the storage time in the PLA packages (*8*). The other parameters found in this group are also influenced by the design but not in exactly the same way. For example, the scores of the tryptophan component decrease after a lag phase, especially for light-exposed samples packaged in PLA. The *a* value increases somewhat in the beginning of the storage period for APET-packaged samples as the score values of the tyrosine component, but does not vary for samples packaged in PLA. The correlation between the components describing texture and some of the chemical and physical parameters shows that the protein structure is important for the quality of the cheese, which can be measured by parameters such as water content and color.

The loadings of O_2 and CO_2 are oppositely correlated, showing that samples with high O_2 level in the headspace have low CO_2 level. Because oxygen more easily permeates through PLA, it is expected that these packages contain larger oxygen levels in the headspace. This is supported by the score plot, which shows that samples packaged in PLA have higher PC1 score values irrespective of storage in the light or dark.

This study has shown that fluorescence spectroscopy can be used to give information about several parameters involved in lipid oxidation. Riboflavin absorbs light and initiates oxidation. Using fluorescence spectroscopy, this is measured by decreased

intensity at emissions of ~525 nm. The next step identified by fluorescence spectroscopy is degradation of vitamin A. In the present study, it is shown that vitamin A in cheese can be measured by excitation at 330 nm and emission at 410 nm. Vitamin A seems to be most sensitive when the samples are stored in a packaging material that transmits light which can be absorbed by vitamin A. After initiation by the light-sensitive compounds, several products are formed during lipid oxidation such as peroxides and carbonyl compounds. These products do not fluoresce and cannot be measured by fluorescence spectroscopy. In contrast, changes in protein structure can be measured by fluorescence spectroscopy. This is seen as changes in components describing tyrosine and tryptophan. In a later stage, fluorescent oxidation products are produced. These are stable products formed by reactions between secondary lipid oxidation products and amino acids. The formation is demonstrated by increased fluorescence emission between 420 and 480 nm. This is clearest for the packaging material that allows light which can be absorbed by light-sensitive compounds to pass through and which have higher water vapor and oxygen transmission rates. Thus, this study showed that in order to keep the optimal quality of the cheese it is necessary to use a packaging material with low water vapor and oxygen transmission rates and which excludes light that can be absorbed by vitamin A.

Riboflavin is known as an initiator of photooxidation in dairy products (*31*). It degrades in both packaging materials by light exposure. However, lipid oxidation only evolves in samples stored in one of the packaging materials. Thus, there may be other factors involved as, for example, initiation by vitamin A as explained above. Furthermore, porphyrins may initiate lipid oxidation as suggested by Wold et al. (*11*). Mortensen et al. (*6*) verified that parameters other than riboflavin can initiate oxidation. They showed a formation of secondary lipid oxidation products but no degradation of riboflavin when cheeses were exposed to yellow light. Another study showed decreased riboflavin content measured by fluorescence spectroscopy when the samples were exposed to light of 405 or 436 nm. The development of secondary oxidation products such as hexanal was seen for both illumination conditions. However, increased fluorescence at ~430 nm took place only when cheese was exposed to 436 nm light (*33*). This indicates that production of fluorescent oxidation products did not take place when the samples were illuminated with 405 nm light or that other factors influencing the fluorescence signal play a role.

The above illustrates that light-induced oxidation in dairy products is complex and depends on the storage conditions. Especially, exposure to light and storage in PLA seem to be important for the progression of lipid oxidation. Furthermore, it is illustrated that fluorescence can be used instead of laborious chemical and physical methods to measure light-induced quality changes in cheese.

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