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# Wavelength Dependence of Light-Induced Lipid Oxidation and Naturally Occurring Photosensitizers in Cheese

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Degradation of the potential photosensitizers, riboflavin, chlorophyll, and porphyrin, in Danbo cheese by monochromatic light of wavelength 366, 436, or 546 nm was studied. Three cheeses were investigated, two conventional (16% fat and 25% fat) and one "organic" (25% fat). The effect of illumination was measured by fluorescence spectroscopy and analyzed using multiway and multivariate data analysis. Riboflavin was found to degrade only by 436 nm light, whereas chlorophylls and porphyrins also were influenced by 436 and 546 nm light. The organic cheese had the largest chlorophyll content both before and after similar light exposure, and no change in chlorophyll of this cheese was observed for any of the illumination wavelengths. Upon light exposure of the cheeses, volatile compounds were formed, as analyzed by gas chromatography–mass spectrometry (GC-MS). The relative concentrations of methyl butanoate, 1-pentanol, benzaldehyde, 2-butanone, 2-heptanone, and butyl acetate were found to weakly correlate with the surface fluorescence intensity. 1-Pentanol and the ketones are secondary lipid oxidation products, consistent with a chemical coupling between photosensitizer degradation and formation of volatile lipid oxidation products.

**KEYWORDS: Fluorescence; light-induced oxidation; photosensitizers; GC-MS; chemometrics**

## INTRODUCTION

Cheeses are exposed to light during processing, packaging, distribution, and retail storage, resulting in deterioration of the product quality. Light-induced oxidation of lipids, proteins, and vitamins becomes most significant when cheeses are packed in transparent packaging material and exposed to light during retail display. These oxidation processes lead to off-flavor formation, discoloration, nutrient loss, and formation of toxic compounds (*1*). Efforts have been made to produce packaging materials that are more protective against the effects of light by excluding specific wavelength regions.

Photosensitizers, such as riboflavin, chlorophyll, and porphyrin, are naturally present in dairy products. They easily absorb light due to a conjugated system of double bonds (*2*). This leads to the formation of excited triplet states, long-lived enough to react and initiate oxidation. Knowledge about the wavelength region in which the photosensitizers absorb light can help in the design of packaging materials and the establishment of storage conditions that minimize oxidative degradation.

Riboflavin has for a long time been recognized as the initiator of light-induced oxidation in dairy products. It is degraded during initiation of light-induced oxidation (*1*). However, studies have shown that lipid oxidation occurs in Havarti cheese during exposure to yellow light without degradation of riboflavin (*3, 4*). Riboflavin is not acting as a photosensitizer and is not degraded by yellow light because it does not absorb light at these wavelengths. It was accordingly concluded that oxidation must be initiated by another mechanism. On the basis of fluorescence emission spectra, Wold et al. (*5*) suggested that the presence of porphyrins and chlorophylls could be an alternative route for the initiation of lipid oxidation as these compounds together with riboflavin could act as photosensitizers and initiate light induced oxidation. Chlorophyll- and porphyrin-photosensitized oxidation were later verified for other dairy products such as butter and cheese (*6, 7*).

The present study explores how exposure of Danbo cheese to light of three specific wavelengths, 366, 436, and 546 nm, influences the surface fluorescence of the products and how changes in riboflavin, porphyrin, and chlorophyll can be followed. Danbo cheese is used as an example of a semihard cheese. The same trends are expected in other types of similar cheeses. The wavelengths of light to which the cheeses were exposed were chosen to match the absorption of the three potential photosensitizers. Riboflavin has maximum absorption at 366 and 436 nm but does not absorb at 546 nm. Porphyrins and chlorophylls absorb light corresponding to a varying absorptivity throughout the whole visual spectral region. A second investigation was made of the relationship between

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fluorescence spectroscopic data and the estimated relative concentrations of volatile compounds found by gas chromatography–mass spectrometry (GC-MS). The purpose is to evaluate if any changes in the composition of volatile compounds are correlated to changes in the concentration of the photosensitizers.

## MATERIALS AND METHODS

**Sample Preparation.** Three cheeses of approximately 9 kg were obtained from Arla Foods (Taulov, Denmark): a Danbo 30+ (16% fat), a Danbo 45+ (25% fat), and an "organic" Danbo 45+ (25% fat). The cheeses were cut into slices of 10 cm × 10 cm × 3 mm and packed under vacuum using a film with a low oxygen transmission rate with three slices stacked in each bag. The cheeses were stored in the dark at 5 °C until experimental use. Only the slices in the center were used for further investigations.

**Light Exposure Experiments.** The cheese slices were cut into half-circles, 9 cm in diameter, and mounted on a rotating disk to cover half of the rotating disk. The disk contained two cheese samples weighing in total approximately 18 g during each light exposure experiment. The two slices were randomly selected from the three cheeses. The cheese slices were irradiated for 4 or 24 h and continuously rotated during light exposure.

The samples were exposed to monochromatic light of 366, 436, or 546 nm selected from an Osram HBO 200/4 high-pressure Hg lamp (line spectrum) (Osram, Munich, Germany), mounted as part of an optical train, also including a light condenser, a heat filter, an interference filter, and a light focusing lens. All optical components were made of quartz (Linos, Spindler and Hoyer, Göttingen, Germany). The temperature was 24 ± 2 °C during light exposure. Slices from the three cheeses were packed in foil and kept at similar temperature for 4 or 24 h to simulate dark-stored samples.

Immediately after light exposure, each sample was divided in two and placed in two Petri dishes, one for fluorescence measurements and one for GC-MS measurements. The Petri dishes were packed in aluminum foil and frozen at −40 °C until further analysis.

**Fluorescence Spectroscopy.** All fluorescence measurements were performed within a few days. The samples were moved to a refrigerator (5 °C) the day before measurement to secure a slow defrosting. Front-face fluorescence was measured on the side of the cheese facing the light with a Perkin-Elmer LS50B spectrophotometer (Beaconsfield, Buckinghamshire, U.K.) using a front-face accessory. The cheese samples with a diameter of 1 cm were placed directly on the quartz plate and measured at an angle of approximately 60°. Fluorescence measurements were obtained as fluorescence landscapes or emission spectra in two wavelength intervals: (i) fluorescence landscapes with excitation from 360 to 460 nm (intervals of 10 nm) and emission from 500 to 560 nm (intervals of 1 nm), and (ii) fluorescence emission spectra with excitation set to 410 nm and emission measured from 580 to 770 nm. Slit widths were 5 nm for both excitation and emission. The scan speed was set to 1500 nm/min. All measurements were made at room temperature. For the fluorescence landscapes, each measurement started with the highest excitation wavelength and ended with the lowest to minimize photodecomposition of the sample.

**GC-MS.** All analyses were carried out in duplicate. A homogenized suspension was produced from 4.5 g of shredded cheese and 15 mL of volatile-free tap water added to 0.25 mL of 4-methyl-1-pentanol (50 mg $L^{-1}$) as internal standard. Volatile compounds were collected on Tenax-TA traps using a dynamic headspace technique. The traps contained 250 mg of Tenax-TA with mesh size 68/80 and a density of 0.37 g $mL^{-1}$ (Buchem bv, Apeldoorn, The Netherlands). The cheese suspension was equilibrated to 30 °C in a circulating water bath and then purged with nitrogen (200 mL $min^{-1}$) for 60 min. An automatic thermal desorption unit (ATD 400, Perkin-Elmer, Norwalk, CT) was used to desorb the trapped volatiles. The thermal desorption process was carried out by heating the trap to 250 °C with a flow (60 mL $min^{-1}$) of carrier gas (He) for 15.0 min (primary desorption). The stripped volatiles were trapped in a Tenax TA cold trap (5 °C), which was subsequently heated at 300 °C (secondary desorption). This allowed for rapid transfer of volatiles to a GC-MS (G1800A GCD System, Hewlett-Packard, Palo Alto, CA) through a heated (225 °C) line. Separation of volatiles was carried out on a DB-Wax capillary column (30 m long and 0.25 mm i.d.). The column flow rate was 1.0 mL $min^{-1}$ using helium as a carrier gas. The column temperature program was as follows: 10 min at 45 °C, from 45 to 240 °C at 6 °C $min^{-1}$, and finally 10 min at 240 °C. The GC was equipped with a mass spectrometric detector operating at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. Volatile compounds were identified by matching their mass spectra with those of a commercial database (Wiley275.L, HP product G1035A). The software program GCD Plus ChemStation G1074B (version A.01.00, Hewlett-Packard, Palo Alto, CA) was used for data analysis. Relative area (peak area of the volatile compound divided by the peak area of internal standard) was used as a measure of concentration.

**Actinometry.** Light intensities for 366 and 436 nm were determined by ferrioxalate actinometry (*8*) and that for 546 nm was determined by Reinecke salt actinometry (*9*) prior to each series of experiments. Average light intensities were calculated.

**Data Analysis.** Parallel factor analysis (PARAFAC) (*10, 11*) was applied to find the underlying fluorophores in the fluorescence landscapes and the relationship between these and the experimental design. PARAFAC decomposes the data into fewer components. Each PARAFAC component is an estimate of the signal of one fluorophore if the data are approximately low-rank trilinear and the correct number of components is chosen. The model gives one set of scores and two sets of loadings, one for excitation and one for emission, where the loadings are estimates of the pure emission and excitation spectra and the scores are estimates of the relative concentration of the fluorescent compounds.

The effect of the experimental design on the fluorescence emission spectra was found by discriminant partial least-squares regression (DPLSR). DPLSR analyzes the qualitative main effects of the experimental design ($Y$) on the fluorescence emission spectra ($X$), where the experimental design is represented by indicator variables (0 or 1). PLS performs a simultaneous decomposition of $X$ and $Y$ in such a way that the information in $Y$ is directly used as a guide for the decomposition of $X$.

The relative areas of volatile compounds found by GC-MS were predicted by partial least-squares regression (PLS) or multiway partial least-squares regression (NPLS) and compared with the experimental values. NPLS regression is an extension of PLS regression but handles $X$-variables that have more than two dimensions (*12*). The models were made from average fluorescence spectra and average GC-MS areas. This gave a total of 24 samples. All models were validated by full cross-validation.

## RESULTS AND DISCUSSION

**Determination of Light Intensity.** The three illumination wavelengths have different light intensities. Previous studies have shown that the light intensity has a considerable influence on light-induced oxidation in dairy products (*13, 14*). For the experiments in the present study, the average light intensity at 366 nm is found to be $1.52 \times 10^{16}$ quanta/s, at 436 nm it is $3.13 \times 10^{16}$ quanta/s, and at 546 nm it is $1.71 \times 10^{16}$ quanta/s. These values are comparable to the intensity used for model studies (*15*). The light used in retail trade is not monochromatic, and a direct comparison is not possible. Day to day variation was approximately 5%.

**Fluorescence.** A PARAFAC model of the fluorescence landscapes is estimated to evaluate the effect of the experimental design on the spectral measurements of the cheese slices. A two-component model is found to give the best fit. Component 1 explains the presence of riboflavin with excitation maxima at 390 and 450 nm and an emission maximum between 520 and 530 nm (**Figure 1**) corresponding to the known fluorescence properties of riboflavin (*16*). The second component is due to fluorescence of tryptophan or tyrosine, which both have fluorescence maxima at lower wavelengths than measured in the present study.

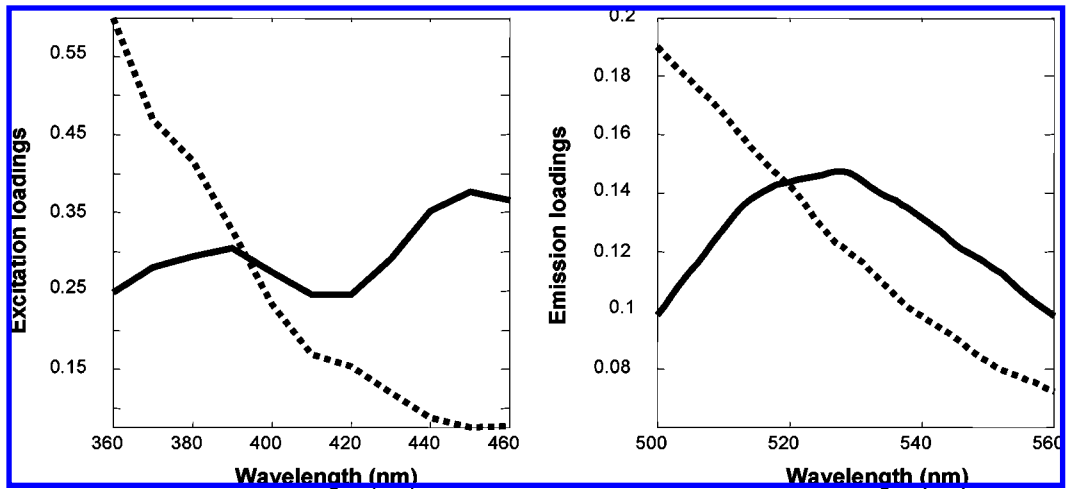


**Figure 1.** Loadings of a PARAFAC model made with excitation from 360 to 460 nm and emission from 500 to 560 nm. The peak maxima in the loadings of component 1 (solid) correspond to the fluorescence maxima of riboflavin. Component 2 (dotted) is a compound with emission maximum below 360 nm (tryptophan or tyrosine).

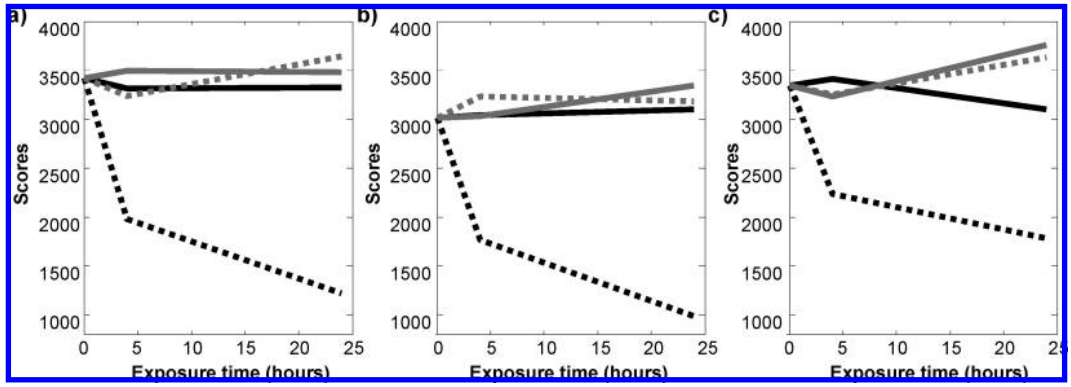


**Figure 2.** Scores of component 1 versus exposure time: (**a**) 16% fat; (**b**) 25% fat; (**c**) organic 25% fat. The PARAFAC model is made with excitation from 360 to 460 nm and emission from 500 to 560 nm. The lines indicate exposure to 366 nm (black solid), exposure to 436 nm (black dotted), exposure to 546 nm (gray solid), and dark (gray dotted).

The scores of the PARAFAC model give information about the relative concentrations of the compounds. The size of the scores can be used to compare the concentrations of the compounds identified by the model. **Figure 2** shows the scores of component 1, which decreases with exposure to light of 436 nm, showing that riboflavin concentration decreases with time of light exposure. Riboflavin is degraded fastest for the conventional cheese and especially for the low-fat product. All cheeses have approximately the same initial riboflavin concentration. Thus, compounds in the organic cheese seem to protect riboflavin against degradation. For example, $\beta$-carotene could diminish the photo-oxidation of riboflavin as suggested by Hansen and Skibsted (*15*).

For all cheeses, there is a fast decrease in the riboflavin content within the first 4 h of exposure to light of 436 nm. Between 4 and 24 h of light exposure, there is a smaller decrease in the riboflavin content. However, because the scores give the relative concentrations, the present data do not show how much riboflavin is left in the samples. There is no change in the scores of samples exposed to light of 366 or 546 nm as well as of samples not exposed to light. Riboflavin does not absorb light at 546 nm, and no degradation of riboflavin is expected at this wavelength. Riboflavin absorbs light at 366 nm, but it seems exposure to this wavelength does not lead to degradation. The difference in effect on riboflavin degradation following exposure to light of 436 and 366 nm was also observed for Havarti cheese (*17*). It was suggested that the different effect of the two wavelengths is due to the higher energy at 366 nm, which makes another deactivation route from higher energy excited states of riboflavin possible. For example, energy transfer to $\beta$-carotene may take place and riboflavin will return to the ground state without chemical breakdown. Furthermore, the light intensity in our experimental setup is almost twice as high for the 436 nm light as for the 366 nm light. The extinction coefficient of riboflavin is somewhat larger at 436 nm than at 366 nm as seen from the estimated excitation spectrum in **Figure 1**, which is an approximation of the absorption spectrum of riboflavin.

Porphyrins and chlorophylls are fluorescent molecules found in low concentrations in dairy products with excitation maxima around 400 nm and emission maxima above 600 nm (*18*). Recently, it has been shown that these compounds in addition to riboflavin can initiate oxidation in dairy products upon exposure to light (*5*–*7*). **Figure 3a** shows emission spectra (580–780 nm) of the cheeses not exposed to light. The organic cheese deviates from the two other cheeses in having higher fluorescence intensity. Especially, a double peak in the emission spectrum at approximately 660 and 670 nm is very large due to larger chlorophyll content in the organic cheese. Chlorophyll *a* has been found to have emission maximum at 671 nm in another cheese product (*5*). The compound that gives rise to the other part of the double peak was first suggested to be chlorophyll *b*, but this suggestion has later been questioned (*7*).

In addition to the peak caused by chlorophyll, the three cheeses show peaks at approximately 620, 640, and 730 nm.

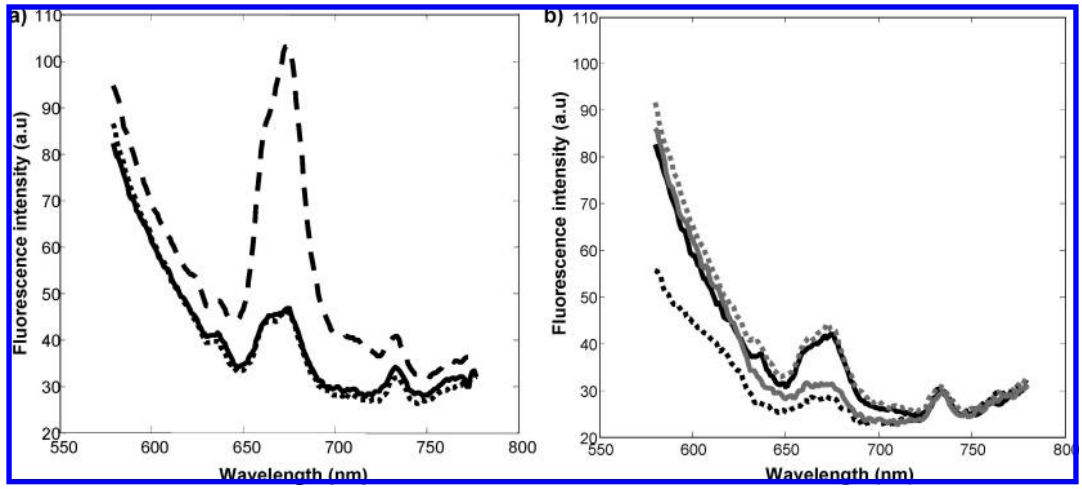


**Figure 3.** Emission spectra of (**a**) samples not exposed to light (excitation at 410 nm) and (**b**) Danbo 16% exposed to 366 nm (black solid), 436 nm (black dotted), and 546 nm (gray solid) and stored in the dark (gray dotted) for 24 h.

Wold et al. (*5*) swabbed solutions of various porphyrins on Jarlsberg cheese. They found peak maxima of hematoporphyrin at 620 nm, uroporphyrin at 618 nm, and protoporphyrin at 635 and 705 nm. We assign accordingly the shoulder at 620 nm in the spectra in **Figure 3** to be due to either hematoporphyrin or uroporphyrin and the peak at 640 nm to protoporphyrin, although no emission peak at 705 nm is identified. The peak at 730 nm is caused by an instrumental artifact. There is no visual difference between the fluorescence spectra of the conventional cheeses with 16% fat and with 25% fat, indicating a similar content of porphyrins and chlorophylls in these cheeses. These compounds are lipid soluble and a higher content could be expected in the product with higher fat content. **Figure 3b** shows the emission spectra of the cheese with 16% fat after light exposure or dark storage for 24 h. The spectrum obtained after exposure to 366 nm light is similar to the emission spectrum of the dark-stored sample, indicating no effect of 366 nm light on the content of porphyrins and chlorophylls. The samples exposed to light of 436 or 546 nm show an overall decreased intensity. Especially, the chlorophyll peak between 650 and 700 nm has decreased. Furthermore, the tail at low wavelengths has lower intensity when exposed to 436 nm light. Because riboflavin has emission maximum between 520 and 530 nm, this is due to riboflavin degradation and supports the previously shown results.

The effect of cheese type and illumination wavelength on the emission spectra is investigated by discriminant partial least-squares regression (DPLSR). Fluorescence emission spectra measured between 580 and 780 nm are contained in the *X* matrix and a matrix containing zeros and ones describing the experimental design is held in *Y*. The loadings shown in **Figure 4** are rather complex. However, the most important information can be related to peaks in the fluorescence spectra. Components 1 and 2 describe mainly chlorophyll but are oppositely correlated. The chlorophyll peak has negative loadings in component 2. This component also has high positive loadings below 630 nm, which may be due to fluorescence emission by riboflavin. The reason why chlorophyll and riboflavin are negatively correlated in component 2 cannot be explained. The loadings of component 1 are positive and have an offset, indicating either an effect of an overall peak or instrumental artifacts. Component 3 explains hematoporphyrin and/or uroporphyrin, and component 4 describes protoporphyrin with negative loadings and has positive loadings in the emission wavelengths of chlorophyll. The loadings of component 4 have variation below 650 nm and could be influenced by riboflavin as well, indicating covariation between some of the photosentizers. The loadings of component 4 are rather noisy, but they seem to be important due to the appearance of the peaks, which can be explained, and a systematic variation of the scores.

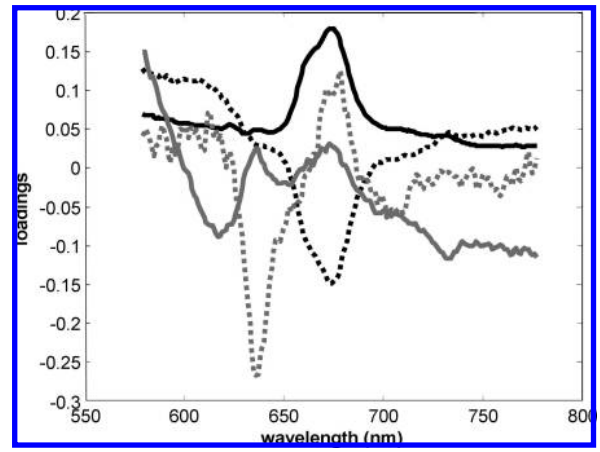


**Figure 4.** Loadings of a DPLSR made with emission spectra obtained after excitation at 410 nm; the lines indicate PC1 (black solid), PC2 (black dotted), PC3 (gray solid), and PC4 (gray dotted). The spectral data are used as *X*-variables and the design as *Y*-variables. The four components explain 82.8, 12.5, 4.0, and 0.2% of the variation in the *X*-variables, respectively.

**Figure 5** shows scores of the DPLSR. The high component 1 scores and low component 2 scores of the organic cheese illustrate a high content of chlorophyll (**Figure 5a**), but there is no separation in the scores within the organic cheese according to the light exposure conditions. The chlorophyll content of this cheese may be so high that the small change that takes place during light exposure cannot be measured by the current method. Furthermore, the cheese may contain other pro- and antioxidants that inhibit the degradation of chlorophyll.

The conventional cheeses show an effect of exposure to light of 436 and 546 nm (**Figure 5c−f**), supporting the appearance of the emission spectra in **Figure 3b**. For these cheeses, light of 546 nm results in low component 1 scores and high component 2 scores compared with the cheeses not exposed to light, which illustrates reduced chlorophyll content with light exposure. This does not support the findings by Wold et al. (*5*–*7*), who found that chlorophyll in cheese and butter was primarily degraded by light above 600 nm but also of light below 500 nm. Exposure of the conventional cheeses to 436 nm results

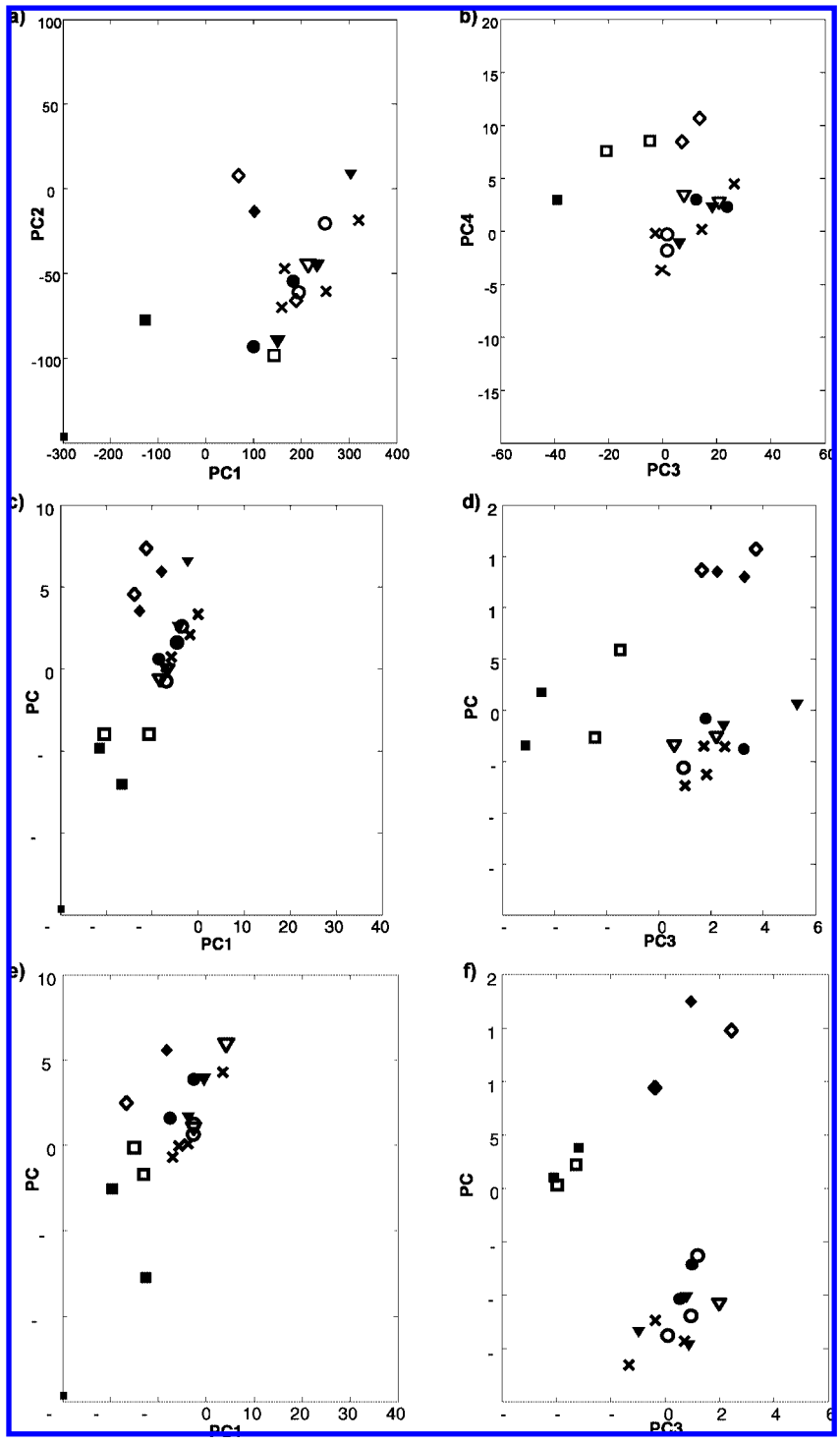


**Figure 5.** Scores of (**a**) PC1 and PC2 and (**b**) PC3 and PC4 for a DPLSR made with emission spectra obtained after excitation at 410 nm as *X*-variables. The samples are denoted by circles (366 nm), squares (436 nm), diamonds (546 nm), and triangles (dark). Samples not exposed to light are denoted by crosses, samples exposed for 4 h are denoted by open symbols, and samples exposed for 24 h are denoted by solid symbols. Organic cheeses are marked gray, and conventional cheeses are shown as black. The fat content is given as numbers.

in low scores in both components. The change is largest for longer exposure time. The low scores of component 1 indicate decreased chlorophyll content, whereas the low scores in component 2 indicate increased chlorophyll content. However, the variation in the loadings of component 2 below 650 nm may hinder the expected degradation of chlorophyll to be visualized. The scores do not show a variation between the conventional cheeses with different fat contents, which was expected due to the lipophilic properties of chlorophyll and porphyrins.

Chlorophyll absorbs light at all three wavelengths in use in the experiment but to varying degrees (*19*). However, the absorbance at the three wavelengths cannot be related to the detected variation in chlorophyll. Furthermore, the light intensity at 436 nm is twice as high as the intensity at 366 and 546 nm, but this difference does not seem to have a large effect on the degradation of chlorophyll.

Exposure to 546 nm gives high scores in component 4 for all three cheeses (**Figure 5b**,**d**,**f**). The conventional cheeses also show increasing component 4 scores of samples exposed to 436

**Table 1.** Prediction of GC-MS Data by Partial Least-Squares Regression (PLS) or Multiway Partial Least-Squares Regression (NPLS) from Fluorescence Emission Spectra or Fluorescence Landscapes, Respectively[a]

| | #PC | $R^2$ | RMSECV |
|---|---|---|---|
| Fluorescence Landscapes of Riboflavin | | | |
| methyl butanoate | 2 | 0.41 | 0.004 (0.000–0.004) |
| 1-pentanol | 4 | 0.62 | 0.002 (0.001–0.012) |
| benzaldehyde | 4 | 0.50 | 0.027 (0.004–0.157) |
| Fluorescence Emission Spectra of Porphyrins/Chlorophylls | | | |
| 2-butanone | 4 | 0.48 | 0.373 (0.053–3.341) |
| methyl butanoate | 1 | 0.40 | 0.004 (0.000–0.004) |
| butyl acetate | 2 | 0.41 | 0.012 (0.000–0.008) |
| 2-heptanone | 1 | 0.36 | 0.005 (0.024–0.048) |

[a] The predictions are shown by the number of components in the models (#PC), the squared correlation coefficients ($R^2$), and the root mean squared error of cross validation (RMSECV). The measured range is given in parentheses.

nm, reflecting decreased protoporphyrin content in these cheeses. This is partly supported by the findings of Wold et al. (*7*), which indicate that protoporphyrin is degraded by all wavelengths in the visual spectrum but fastest with light between 500 and 550 nm. Illumination with 436 nm light results in low scores in component 3, which indicates low hematoporphyrin/uroporphyrin content. Wold et al. (*5–7*) showed that hematoporphyrin/uroporphyrin was mostly influenced by light below 500 nm and could not distinguish between the effects of 366 and 436 nm light. The cheeses not exposed to light vary in component 4 with the organic cheese having the highest scores and the conventional cheese with 25% fat having the lowest. This variation can not be visualized after exposure to either 436 or 546 nm light, showing that photooxidation has led to similar contents of porphyrins in all three cheeses.

The scores of samples exposed to light of 366 nm cannot be separated from samples not exposed to light (**Figure 5**) for any of the components. Thus, chlorophyll and porphyrin are not degraded by exposure to light of this wavelength.

A comparison of the results obtained in the present study with results of other studies is not straightforward. Some products that are formed during oxidation are further degraded at more advanced stages of oxidation. These reactions may take place at different rates depending on the experimental conditions. Overall, the present study, however, shows that exposure to 366 nm is least destructive with respect to the photoinitiation of oxidation by riboflavin and chlorophyll/porphyrin. Riboflavin is degraded by 436 nm light, and porphyrins/chlorophylls are degraded by both 436 and 546 nm light. Small differences are seen between illumination for 4 or 24 h, indicating that the changes in photosensitizer concentration take place during the first period or even faster and that further reactions occur to a lesser degree. However, the results do not show if all photosensitizer is degraded or if there is still some left.

**Prediction of Volatile Compounds by Fluorescence Spectroscopy.** GC-MS has previously been shown to give relevant information about volatiles, which correlated with the results of a sensory evaluation of light-induced oxidation in cheese (*20*). In the present study, the relationship between fluorescence of the photosensitizers in the cheese and the information obtained from GC-MS has been evaluated. The GC-MS data give a tentative identification of 47 volatile compounds originating from the cheese. The relative concentrations of the 47 compounds are further predicted from the fluorescence spectroscopic data by PLS or NPLS regression and compared with the result from the chemical analyses. The best predictions are shown in **Table 1** and illustrate that there is some relationship between

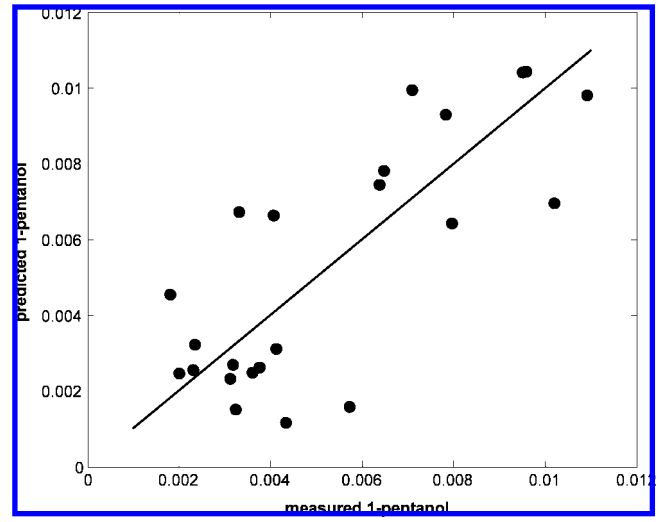


**Figure 6.** Relative content of 1-pentanol predicted from the NPLS model versus the relative 1-pentanol content estimated from GC-MS.

the fluorescence spectral data and the distribution of the volatile compounds as estimated by GC-MS, even though the correlation coefficients and the error estimates are not impressive.

The prediction of 1-pentanol performs best using the fluorescence landscapes of riboflavin. The correlation between measured and predicted values is 0.79, the error estimate reasonably low, and the number of components not unreasonably high. **Figure 6** shows a linear relationship between the predicted 1-pentanol as a relative content versus the measured values, although some scattering is evident. The loadings of component 1 of the NPLS model have an appearance similar to the fluorescence properties of riboflavin (not shown), providing evidence for a related variation in the content of the two compounds. 1-Pentanol has earlier been found to increase with light exposure of cheese, especially in presence of air (*3, 17, 21*). It is formed by degradation of pentanal produced during the oxidation of linoleate, and 1-pentanol can accordingly be used as an indicator of lipid oxidation.

The PLS regressions using the emission spectra of porphyrins and chlorophylls as independent variables show reasonable predictions of 2-butanone and 2-heptanone when the correlation between measured and predicted values and the error estimates are considered. Both compounds are secondary products from lipid oxidation (*22*). For example, 2-heptanone was found to increase during the storage of cream powder in light and oxygen (*23*). Furthermore, 2-heptanone was found to develop during prolonged storage of semihard cheese exposed to light (*20*). **Figure 7** shows the regression coefficients of the PLS models. A peak corresponding to the emission maximum of chlorophyll is obtained when 2-heptanone is predicted, indicating that the chlorophyll content correlates positively with the content of 2-heptanone. However, the positive correlation illustrates that 2-heptanone is not formed as chlorophyll is degraded but rather degrades together with chlorophyll. The model of methyl butanoate gives regression coefficients similar to the regression coefficients obtained when 2-heptanone is predicted. The contents of these volatile compounds correlate and have the same relationship to the variation in chlorophyll content. The two compounds may degrade due to various complex reactions or through evaporation. This degradation of 2-heptanone is higher than the formation through lipid oxidation.

The regression coefficients obtained for the prediction of 2-butanone show a negative peak at wavelengths corresponding to the emission of protoporphyrin. Thus, there is an opposite

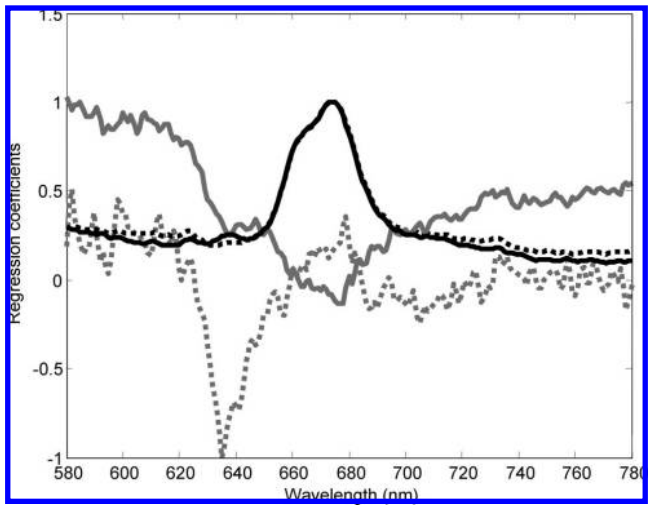


**Figure 7.** Regression coefficients for PLS regression models. Fluorescence emission spectra with excitation at 410 nm are used for predicting the content of 2-heptanone (black solid), methyl butanoate (black dotted), butyl acetate (gray solid), and 2-butanone (gray dotted).

correlation between protoporphyrin and 2-butanone. 2-Butanone develops as lipid oxidation progresses, and protoporphyrin disappears during the action as a photosensitizer.

Prediction of butyl acetate gives regression coefficients that differ from the emission spectra of both porphyrin and chlorophyll. The degradation of porphyrin and chlorophyll has little relationship to the changes in the concentration of butyl acetate. Other variations in the emission spectra give rise to the relationship between fluorescence emission and butyl acetate. These variations have not been exploited yet. Both butyl acetate and methyl butanoate are present in newly produced cheese, have as esters a fruity aroma, and are important flavor compounds of the products (*24*). The reason why the concentrations of these esters are related to light-induced oxidation parameters measured by fluorescence spectroscopy should be investigated further. Aldehydes are usually regarded as the main secondary lipid oxidation products. For example, hexanal was used as an indicator of lipid oxidation in studies evaluating the effect of light on a cream cheese model system (*25*) and on cream powder (*23*). In the present study, only the content of benzaldehyde was found to correlate with fluorescence of the photosensitizers.

The possibility of predicting the relative content of volatile compounds from fluorescence measurements is promising, although the correlation coefficients are relatively low and the error estimates in some cases are large. The relatively poor predictions observed in the present study could be caused by the low sample amounts used. Due to the experimental setup, it was only possible to use 4.5 g cheese, whereas in an earlier study as much as 75 g was used (*20*). Moreover, the samples were kept at −40 °C prior to GC-MS measurements, and further chemical reactions may have taken place during freeze storage and thawing, interfering with the effect of light. Various products are formed and degraded in cascades of reactions during oxidation, and it is possible that some of the volatile compounds have already undergone further reactions during illumination or during the storage period. The models are based on average samples, and only 24 samples are used in each model, which could probably be improved by an increased sample number. Furthermore, none of the volatile compounds that can be predicted by the models exhibit fluorescence, and the predictive ability is accordingly due to an indirect relationship. Notably, the predictive power of the model is rather impressive provided that fluorescence spectroscopic measurements give information about the photosensitizers involved in the initiation step, whereas the secondary lipid oxidation products are formed in a later stage in the lipid oxidation mechanism.

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