ARTICLE

Understanding and measuring photooxidation in dairy products by fluorescence spectroscopy

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Light is known to initiate oxidation processes resulting in discolouration and formation of off-flavours in foods. Milk and milk products are particularly sensitive to light and the photo-initiated reactions affect not only the sensory quality but may also lead to formation of toxic compounds in certain products and to degradation of nutrients. Dairy products are often exposed to light during retail storage and display. This exposure can affect the quality of the products, especially when they are packed in transparent films or containers. Efforts are, therefore, being made to design protective packaging materials and light sources with minimal adverse effects. In order to optimise packaging and display conditions, it is necessary to have detailed knowledge of the presence and properties of the photosensitisers in the product

Riboflavin has, until recently, been regarded as the active photosensitiser in dairy products.[1] When riboflavin is exposed to ultraviolet (UV) radiation or visible light up to about 500 nm (Figure 1), it can initiate photooxidation. The violet and blue parts of visible light have, therefore, been regarded as the most harmful, whereas yellow and red light should be harmless. However, several studies had shown that the photodegradation of riboflavin in dairy products does not correlate well with the formation of oxidation, and some studies indicated that dairy products are oxidised after exposure to red light also. This suggested that other photosensitisers than riboflavin were active.

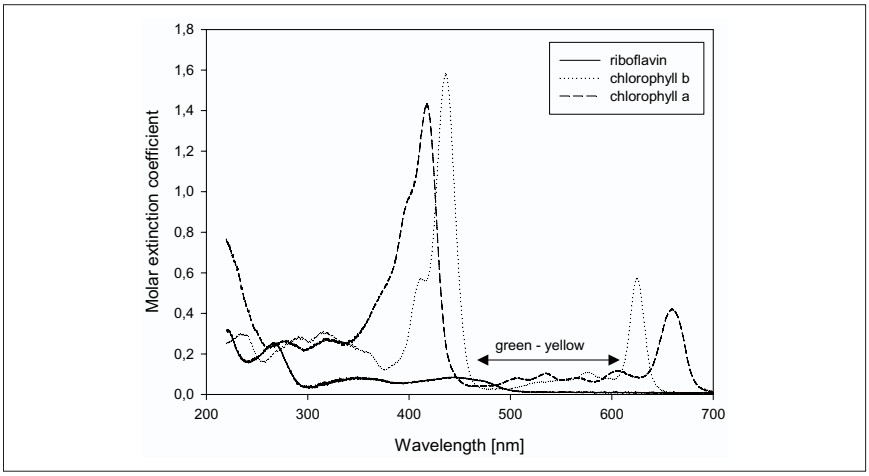

Figure 1. Absorption spectra for riboflavin, chlorophyll a and b.

Four years ago, we used front face fluorescence spectroscopy to monitor the photodegradation of riboflavin directly in intact cheese. Riboflavin has a strong fluorescence emission peak at about 530 nm. In the same spectra, we discovered two weak peaks around 620 and 635 nm. The photodegradation of these peaks did, to our surprise, correspond very well to a sensory evaluation of photooxidation.[2] We then discovered that the emission spectrum between 600 and 750 nm revealed at least four light-sensitive substances. In close collaboration with the Photo Dynamic Therapy group at The Norwegian Radium Hospital, we identified that the peaks had originated from protoporphyrin, hematoporphyrin and, possibly, chlorophyll a and b, all powerful photosensitisers.[3]

Front face fluorescence spectroscopy

Recently, front face fluorescence spectroscopy, in combination with multivariate statistical methods, has been more commonly used for studying quality parameters of complex food samples such as dairy products, meat and fish, oils, sugar, fruit and vegetables, to mention a few.[4] Fluorophores such as riboflavin, chlorophyll, tryptophan, tyrosin, phenylalanin, NADH, collagenous connective tissue and various oxidation products can be detected and quantified non-destructively. Challenges are interfering phenomena like quenching, re-absorption and spectral overlap, but these can likely be modelled and accounted for by careful multivariate calibration.

Instrumentation for front face fluorescence is not complicated and should

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not be very expensive. The main components are a stable excitation light source with a specified narrow bandwidth output, a spectrograph and a CCD detector. A cut-off filter in front of the spectrograph is required to suppress the excitation light. Systems like this can be put together in different ways, depending on the desired applications. For instance, systems can be designed to measure large surfaces, to do point measurements based on fibre-optics, or even for multispectral imaging.

Most of our work related to photooxidation has been performed with a system consisting of a 300 W Xenon light source, a 10 nm bandwidth excitation filter at 380 nm and an imaging spectrograph (Acton SP-150, Acton Research Corp., Acton, MA, USA) connected to a sensitive CCD-camera (Roper Scientific NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ, USA). The light is directed onto the samples at an angle of about 45° and a cut-off filter at 400 nm is used. Emission spectra from a circular sample surface of 5 cm in diameter are measured and typical exposure time per sample is 1 s. Simple rearrangement of the system, including a photographic lens, enables multispectral imaging of the samples.

Photooxidation

Photooxidation in foods can occur if a photosensitiser is present. Photosensitisers in food include, among others, chlorophyll, hemeproteins, porphyrins and riboflavin. These compounds serve as photosensitisers by absorbing visible light or UV radiation to become electronically excited. The excited states can induce oxidation through Type I or Type II photoreactions. Type I reactions proceed through a free radical mechanism and the sensitiser itself is normally degraded. In Type II reactions, the sensitiser reacts with oxygen to form singlet oxygen, which is highly reactive. During the latter reaction, the sensitiser is not degraded but might subsequently be attacked and degraded by the created singlet oxygen. In a given system, both Type I and Type II reactions can take place simultaneously in a competitive fashion but, at low oxygen concentrations, Type I reactions are most efficient. Most photosensitisers are photolabile, and it has been shown that the photoactive fraction of photosensitisers is often identical with the fluorophore; that is, when there is no longer any fluorescence, the sensitiser is deactivated. It is, therefore, reasonable to believe that the photodegradation of the sensitisers will, in some way, correspond to initiation and formation of oxidation and subsequent quality degradation.

The absorption properties of the photosensitisers define the spectral regions where they are active. Figure 1 shows that riboflavin is active in the UV, violet and blue regions up to about 500 nm. Chlorophyll is active in the same regions, but also in the red. Absorption of porphyrins (not shown) resembles that of chlorophyll, with a major peak around 410 nm (the Soret band), a prominent peak in the red but also minor peaks throughout the green and yellow part of the visible.

Improved understanding of photooxidation in dairy products

To get an impression of the role of the different photosensitisers in dairy products, we conducted an experiment where Swiss-like cheese was exposed to different colours: violet, blue, green, yellow, orange and red, as well as white light.[5] Some samples were stored in the dark for control. The different coloured lights were created by covering the samples with coloured plastic films manufactured by Rosco (Rosco, Stamford, CT, USA). The light intensity ($W m^{-2}$) was adjusted to the same level for each colour, so that the effects could be compared. After 36 h of light exposure, the samples were sensory evaluated by a trained sensory panel and fluorescence measurements were performed.

Figure 2 shows the average sensory scores for "oxidised flavour". Violet and blue light gave the worst quality degradation, but also green, orange, yellow and red light induced significant oxidation. The diagram can be regarded as a very crude action spectrum for photooxidation in cheese and the shape is much more similar to the absorption spectra of chlorophyll and porphyrins than to that of riboflavin. This alone was a clear indication that riboflavin could not be the only active photosensitiser in cheese. Figure 3 shows fluorescence spectra from the light-exposed cheese samples. They show that riboflavin, as expected, was degraded by violet, blue and partly green light but negligibly by yellow, orange and red light. On the other hand, the porphyrins and chloropyll-like compounds were strongly degraded by violet and red light and least degraded by green light. The fluorescence spectra gave a causal explanation for the sensory responses. Photodegradation of the porphyrins and chlorophyll corresponded well with the sensory scores. Based on these data alone, it is difficult to decide whether one or more of the

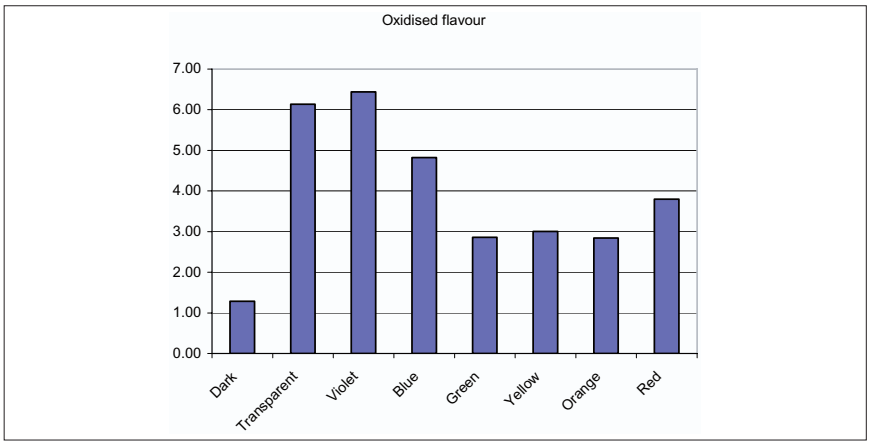

Figure 2. Sensory scores for oxidised flavour measured on Swiss-like cheese exposed to light of seven different colours and darkness.

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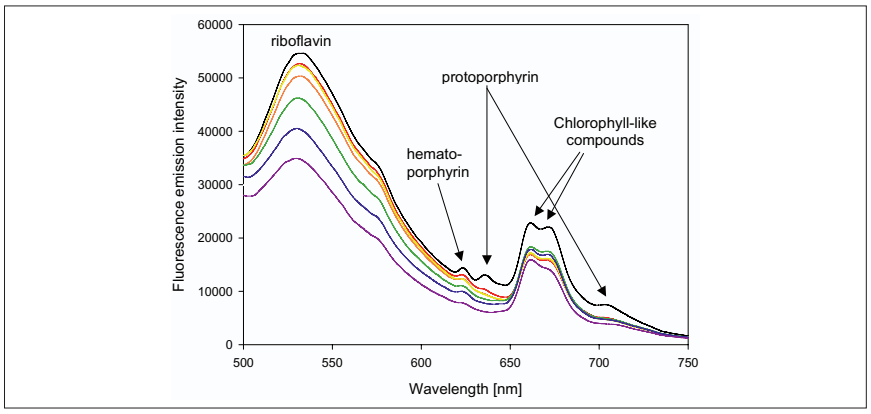

Figure 3. Fluorescence emission spectra from Swiss-like cheese samples stored under light of different colours. The colours of the spectra refer to the light colour used for each storage condition. The spectrum from cheese stored in the dark is black. The spectrum from cheese with transparent filter (white light) is grey.

photosensitisers are active. Probably, they are all active, but to differing degrees. A quantitative approach, where the fluorescence spectra are related to the sensory responses by partial least squares regression (PLSR) shows that modelling the emission region from 600 nm to 750 nm gives high correlations (>0.9) with sensory measured attributes. Figure 4 shows the regression coefficients for a regression model for "acidic flavour" based on this spectral region. In general, the variables of high absolute value contribute mostly to explain the variance in the sensory score. The spectrum of coefficients very closely resembles the emission spectrum of protoporphyrin with peaks at 635 and 705 nm. This indicates that protoporphyrin is one of the more active sensitisers and on-going work confirms this.

The porphyrins and chlorophyll-like substances seem to be present naturally in all dairy products: milk, cream, cheese, butter, yoghurt etc. The concentrations are very low, at approximately 0.01 ppm; this is perhaps the reason why these substances have not been detected earlier. The findings related to photooxidation described above have been confirmed also for butter and sour cream. This means, unfortunately, that the entire visible region is harmful to dairy products, not only the violet and blue. Violet, blue and red light is the most harmful, while green and yellow light give the least adverse effects. Consequently, new considerations on effective packaging and light sources for dairy products can be made based on the absorption properties of the different photosensitisers.

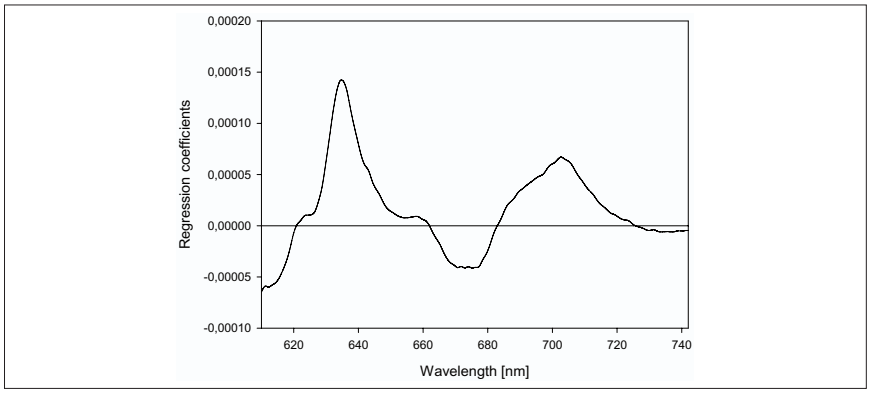

Figure 4. Regression coefficients for PLSR model between fluorescence spectra and sensory measured acidic flavour.

Rapid quantification of photooxidation

Great efforts have been made to develop reliable instrumental methods to measure photooxidation in dairy products. The most sensitive, reliable and relevant method is to use a sensory panel of trained judges. A sensory panel is useful for small experiments, but is expensive, time-consuming and impractical for routine measurements. The common established instrumental method is based on measuring the peroxide value but it is destructive and time-consuming. Front face fluorescence spectroscopy is intuitively an interesting option, since it has the ability to measure rapidly the degradation of the photosensitisers in intact products. The degree of degradation correlates well with sensory analysis of photooxidation, even after a short exposure to light. Actually, fluorescence spectroscopy seems to be more sensitive to photoinduced changes than a trained sensory panel, which is reasonable since the method measures the actual initialisation of the oxidation processes. Figure 5 shows how fluorescence spectroscopy can be used to follow the degradation of photosensitisers in cheese during exposure to a commercial display light source. Notable changes can be measured after 30 min of exposure.

In several light exposure studies on cheese, butter and sour cream, successful PLS calibrations between fluorescence spectra and sensory analysis have been obtained (correlations above 0.9). Based on these results, the sensitivity and accuracy of front face fluorescence is a substitute for a sensory panel. Accordingly, the technique can be used as a non-destructive and effective method for both quantitative and qualitative evaluation of photooxidation in dairy products related to light source, light intensity, storage time and packaging materials.

Summary

Front face fluorescence spectroscopy enabled detection of natural contents of porphyrins and chlorophyll-like components in dairy products. These molecules are powerful photosensitisers, so the discovery greatly improves our

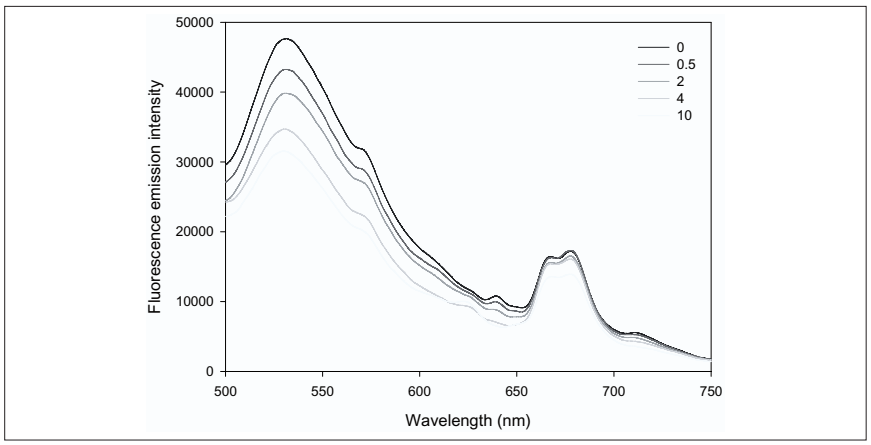

Figure 5. Fluorescence emission spectra from Swiss-like cheese exposed to light from commercial fluorescent light tube. Light exposure time varied from 0 h to 10 h.

understanding of the main causes of photooxidation in dairy products. Front face fluorescence spectroscopy is also a very promising method for rapid and non-destructive quantitative evaluation of sensory properties connected to photooxidation in dairy products. The method enables direct monitoring of the degradation of several photosensitisers, the precursor of oxidative processes, and thereby offers rich opportunities for efficient evaluation of factors affecting photooxidation, such as exposure time, temperature, packing materials and light sources.

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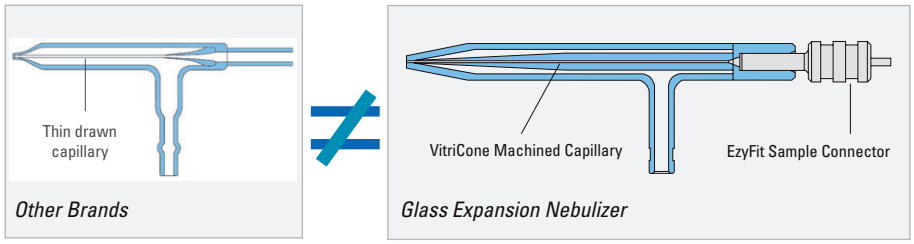

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