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Light-induced formation of free radicals in cream cheese

Signe Westermann, Dagmar A. Brüggemann, Karsten Olsen, Leif H. Skibsted *

Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

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ABSTRACT

Radicals were found, by electron spin resonance (ESR) spectroscopy, to accumulate in cream cheese (26% fat, 7% protein) and more significantly in low fat cream cheese (17% fat, 11% protein) upon light exposure. The decay of radicals following illumination (875 lux, with a strong UV-component for up to 80 min) followed first-order kinetics with a half-life at room temperature of around 0.5 h both for cream cheese and low fat cream cheese. The surprisingly long-lived radicals had a broad structureless ESR-spectrum (*g*-value of 2.006) which, for partly desiccated cream cheese, changed towards a nitrogen-centred ESR powder spectrum (*g*-value of 2.0014) typical for immobilised protein-based radicals. The protein oxidation product, dimethyl disulphide, and the lipid oxidation products, hexanal and 2-butanone, were detected by GC-analysis in higher concentrations in the outer 1 mm layer than in the second layer (of 1 mm thickness) of the product, in agreement with absorption of 99% of the UV-light intensity in the outer 1 mm layer. The low fat cream cheese had higher levels of both lipid and protein oxidation products, in agreement with the higher steady state concentration of radicals and confirming the role of proteins in oxidative changes also for lipids in cheese. The low fat cream cheese was initially more oxidised than was the cream cheese, as seen from the peroxide values, and oxidation products in lipid droplets could be visualised in three dimensions by confocal laser scanning microscopy, using the fluorescent probe C_{11}-Bodipy (581/591).

1. Introduction

Light-induced oxidation is important for quality and shelf-life of many foods, including dairy products. Riboflavin and chlorophyll degradation products act as photosensitisers and may initiate formation of radicals and other reactive species, resulting in formation of lipid and protein oxidation products and off-flavours in the product. Light exposure of dairy products may accordingly decrease the content of unsaturated fatty acids and vitamins, including riboflavin and α-tocopherol, in effect decreasing the nutritional value of the product (Mortensen, Bertelsen, Mortensen, & Stapelfeldt, 2004). The initial stage of oxidation involves formation of radicals, and detection and quantification of radicals may be used for prediction of further oxidative damage. Radicals can be detected by ESR spectroscopy, and ESR spectroscopy may accordingly be developed to become a valuable technique for prediction of oxidative stability of dairy products and other sensitive foods. ESR spectroscopy has previously been used to follow formation of radicals in whole milk powder during storage (Stapelfeldt, Nielsen, Jensen, & Skibsted, 1999; Thomsen, Lauridsen, Skibsted, & Risbo, 2005), and to determine lag-phase for oxidation in caprine milk and in dairy cream (Kondyli, Andersen, & Skibsted, 2005; Westermann, Møller, & Skibsted, 2008). Progression of oxidation in food is normally quantified by the peroxide value as a measurement of lipid hydroperoxides as the primary oxidation products or by the TBARS-value to measure aldehydes and ketones as secondary lipid oxidation products. Hexanal, dimethyl disulphide and 2-butanone are among the most important oxidation products serving as indicators for damaging light exposure to dairy products (Andersson & Lingnert, 1998; Juric, Bertelsen, Mortensen, & Petersen, 2003; Kim & Morr, 1996). Upon light exposure of cheese, fluorescence (caused by riboflavin) declines as oxidation is initiated, but other compounds absorbing light at higher wavelengths may also contribute to oxidation by acting as photosensitisers. Among these are chlorophyll and porphyrins, both naturally occurring in dairy products, as shown by front face fluorescence spectroscopy of the product surface (Andersen, Wold, & Mortensen, 2006; Wold et al., 2005).

Confocal laser scanning microscopy is a well known technique for investigation of structural properties of dairy products (Lopez, Camier, & Gassi, 2007). Fluorescent dyes have recently been introduced to detect oxidation in living cells upon light exposure and to determine antioxidant capacity in cell cultures, as well as in liposome systems. Among these, the fluorescent probe C_{11}-Bodipy (581/591) seems to be very useful for the microscopic detection of oxidation in two or three dimensions in biological systems (Drummen, van Liebergen, Op den Kamp, & Post, 2002; Pap et al.,

* Corresponding author. Tel.: +45 35 28 32 21; fax: +45 35 28 33 44.
E-mail address: ls@life.ku.dk (L.H. Skibsted).

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1999). C_{11}-Bodipy (581/591) is a fatty acid analogue, resembling phosphatidyl choline, located at the interface between the lipid and water phase in biological membranes. C_{11}-Bodipy (581/591) is used as a ratio probe for which the fluorescence emission maximum changes from 595 nm towards 520 nm upon oxidation of the conjugate double bond in the tail of the probe (Drummen, Gadella, Post, & Brouwers, 2004).

During the past two decades, the number of fat-reduced dairy products has continuously increased. Such products seem to be more vulnerable to light-induced oxidation than are the original products, and it has been found that light penetrates skim milk deeper than it does whole milk, with effective depths of 1.2 and 1.0 cm, respectively, as determined for light with a wavelength of 436 nm (Allen & Parks, 1979). Although lipid oxidation in model cream cheeses was found to dominate over protein oxidation, even in low fat products, the importance of protein oxidation seems, to increase when the content of lipids decreases (Andersen, Lund, et al., 2006).

The aim of the present study was to investigate the formation and the decay of radicals formed by light exposure of cream cheese and low fat cream cheese, by direct ESR spectroscopy, in relation to light penetration and to migration of oxidation in the cheese. Confocal microscopy, with labelling with C_{11}-Bodipy (581/591), was carried out in order to detect and monitor the spatial distribution of primary lipid oxidation products. Furthermore, formation of volatile protein oxidation products and secondary lipid oxidation products, in two layers of the products, were determined. The combination of these techniques should make it possible to monitor the oxidation initiated at the surface and moving towards the product interior.

2. Materials and methods

2.1. Materials

Cream cheeses containing 26% or 17% of fat were provided by Arla Foods amba (Viby J, Denmark). The protein contents were 7.5% and 11% for the cream cheese and the low fat cream cheese, respectively. 4-Oxo-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and $FeCl_3 \cdot 4H_2O$ were purchased from Sigma–Aldrich (Steinheim, Germany), $CaCl_2 \cdot 2H_2O$ from AppliChem GmbH (Darmstadt, Germany), $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ from Fluka (Steinheim, Germany), and NH_4SCN from Merck (Darmstadt, Germany). All solvents used were of HPLC-grade and from either Bie and Berntsen (Rødovre, Denmark) or Lab-Scan (Dublin, Ireland). The fluorescence probe, 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (C_{11}-Bodipy (581/591)), was from Molecular Probes (Invitrogen, Carlsbad, CA, USA). Deionised water (Milli Q-water purification system, Millipore Corp., Bedford, MA, USA) was used for all aqueous solutions.

2.2. Light exposure

All samples of cream cheese were irradiated using a UV-lamp (Osram HNS 15 W OFR FLH1, Osram, Munich, Germany) with a maximal intensity at 253.7 nm and a total intensity of 875 lux, measured by an Illumination Meter IM/1 (Topcan, Toyko, Japan). The same light source was used for light exposure of all cream cheese samples for both ESR measurements, microscopy, and for chemical analysis.

2.3. ESR spectrometry

Light exposure and ESR measurements of cream cheese were divided into three separate series of experiments: (i) development of radicals during 80 min of illumination, (ii) decay of radicals formed after 80 min of light exposure, and (iii) spectral characterisation of radicals after prolonged illumination for 180 min. During series (i), the samples were moved from the light source to the ESR cativity for measurements and back to the light for further light exposure (each in double determinations). During series (ii), the samples were kept in constant darkness inside the ESR cativity during measurements following illumination (each in triple determinations).

The developments or decays of radicals were followed by ESR measurements, using a Magnettech MiniScope MS 200 (Magnetech Ltd., Berlin, Germany) and a tissue cell (30×5 mm). The instrument settings for all measurements, directly on cream cheese ((i), (ii), and (iii)), were as follows: centre field 336.40 mT, sweep width 9.5 mT, sweep-time 30 s, number of sweep 2, data points 4096, modulation amplitude 0.20 mT, and microwave power 10 dB. The instrument was tuned by autotunings between samples. In order to monitor the decay of radicals formed after 80 min of light exposure in series (ii), the instrument was automatically recording spectra every 10 min during 180 min (Kinetics Capillary 3.07, Magnettech Ltd., Berlin, Germany). For quantification of radical concentration, the area beneath the ESR-signal was calculated using the programme WINEPR (Bruker, Karlsruhe, Germany), and converted to relative concentrations. The conversion between ESR-signal and concentration of radicals was based on the observed linearity of standard curves for TEMPO radicals. A stock solution of 1.00 mM TEMPO dissolved in Milli Q-water was prepared. Stock solution and water in a total amount of 100 μl were added to 5.0 g of cream cheese during stirring. ESR-spectra, recorded in a series of varying concentrations for both cream cheeses, were each carried out as double determinations. Between determinations, the signal of the empty tissue cell was measured in order to correct for any radicals present in the cell. Attempts were not made to express the concentration of radicals in the cheese on a molar basis. The instrument settings used for preparing the TEMPO standard curve were: centre field 336.2 mT, sweep width 5.70 mT, sweep-time 30 s, number of sweeps 2, data points 4096, modulation amplitude 0.20 mT, and microwave power 10 dB.

2.4. Light transmittance

Light transmittance (200–700 nm) of layers of cream cheese of 1 mm thickness was measured in Teflon holders (1, 2, 3, and 4 mm) separated by thin plates of quartz glass with a UV–Vis spectrophotometer (Cintra 40 – UV–Vis spectrophotometer, GCB Scientific Equipment, Victoria, Australia).

2.5. Aroma analysis

Cream cheese in a Teflon holder was illuminated with UV-light for 15 or 30 min at room temperature before aroma collection on a Tenax-TA trap while placed in a water bath at 30 °C for 20 min and purged with nitrogen (100 ml/min N_2). The analysis (each in duplicate) was carried out on separate layers, on accurately weighed samples of illuminated cream cheese. The volatiles trapped were desorbed using an automatic thermal desorption unit (ADT 400, Perkin–Elmer, Norwalk, US). Main desorption was carried out by heating the trap to 250 °C with a flow of helium as carrier gas (60 ml/min) for 15 min. The stripped volatiles were trapped in a Tenax TX cold trap and held at 5 °C, followed by heating to 300 °C for 4 min for rapid transfer of volatiles to a gas chromatograph–mass spectrophotometer through a heated (225 °C) transfer line (GC–MS, G1800A GCD system, Hewlett–Packard, Palo Alto, CA, US). Separation of volatiles was carried out on a DB-Wax capillary column with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 μm. Column temperature programme was as follows: 10 min at 45 °C, from 45 °C to 240 °C at 6 °C each

minute and finally 10 min at 240 °C. The mass spectra were compared to the NIST Mass Spectral Search Program for identification. The 'relative amounts' reported are the area of the peak in the total ion chromatogram (TIC).

2.6. Microscopy

Cream cheese samples were labelled with C_{11}-Bodipy (581/591) for microscopy. Upon preparing the samples, 2 μl of C_{11}-Bodipy (581/591) stock solution (5.04×10^{-4} M in ethanol) were placed on a cover slip filling a circle with a diameter of approximately 1 cm. Cream cheese was applied on top of the C_{11}-Bodipy (581/591) after evaporation of the ethanol, and care was taken to avoid damage of the texture. An object glass was fitted on top of the cream cheese and the samples were incubated in darkness at 5 °C overnight.

The samples were visualised with a confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany). Channel 1 (oxidised state) was excited at 488 nm and the emission was recorded from 490 to 542 nm; channel 2 (non-oxidised state) was excited at 543 nm while the emission spectrum ranged from 547 to 624 nm. An oil immersion objective (×40, N.A. 1.25) was used and image resolution was set to 1024 × 1024 pixels. For image formation, both channels were recorded simultaneously and each represented line was an average of two repeated scans. The initial settings of the acousto optical tuneable filter (AOFT) and photomultiplier parameters were defined on non-illuminated samples in a way that the fluorescence intensity of channel 2 was at an optimum according to the Leica glow over/under standards. For channel 1, the gain settings were determined as follows: AOFT 56% and 750 V and zero offset. The same settings were used for all samples. For three-dimensional imaging, upper and lower ends were defined accordingly by using the appearance/respective disappearance of the fluorescence signal. The number of optical sections recorded for one stack was defined according to the Nyquist theorem. Stacks were visualised as a maximum intensity projection. Quantification of fluorescence intensity was performed using LeicaLite software (LeicaMicrosystems, Wetzlar, Germany).

2.7. Peroxide value

For determination of peroxide values, fat was extracted from 2.5 g of cream cheese. The extraction was carried out according to Folch, Lees, and Stanely (1957), using the 2:1 chloroform–methanol (v/v) method. Measurements of peroxide values and preparation of a standard curve were performed accordingly to IDF standard method 74A:1991 (Shantha & Decker, 1994). The peroxide values in the fat were calculated as meqiv/kg lipid. All measurements were carried out as double determinations.

3. Results

Exposure of cream cheese to light-induced formation of free radicals in the product, which could be detected by ESR spectroscopy. The radicals were sufficiently long-lived for quantification and the accumulation of radicals in cream cheese and in low fat cream cheese, as detected by ESR spectroscopy, is shown in Fig. 1A. The radicals were, (also by direct ESR spectroscopy), found to decay rather slowly, apparently in a first-order reaction, as may seen in Fig. 1B. From the exponential decay curves, half-lives for the light-induced radicals were calculated for both cream cheese and for low fat cream cheese, as shown by an example for cream cheese in Fig. 1B. While each exponential decay curve gave an adequate description of the decay kinetics, variations between individual experiments were large, probably due to variation in the

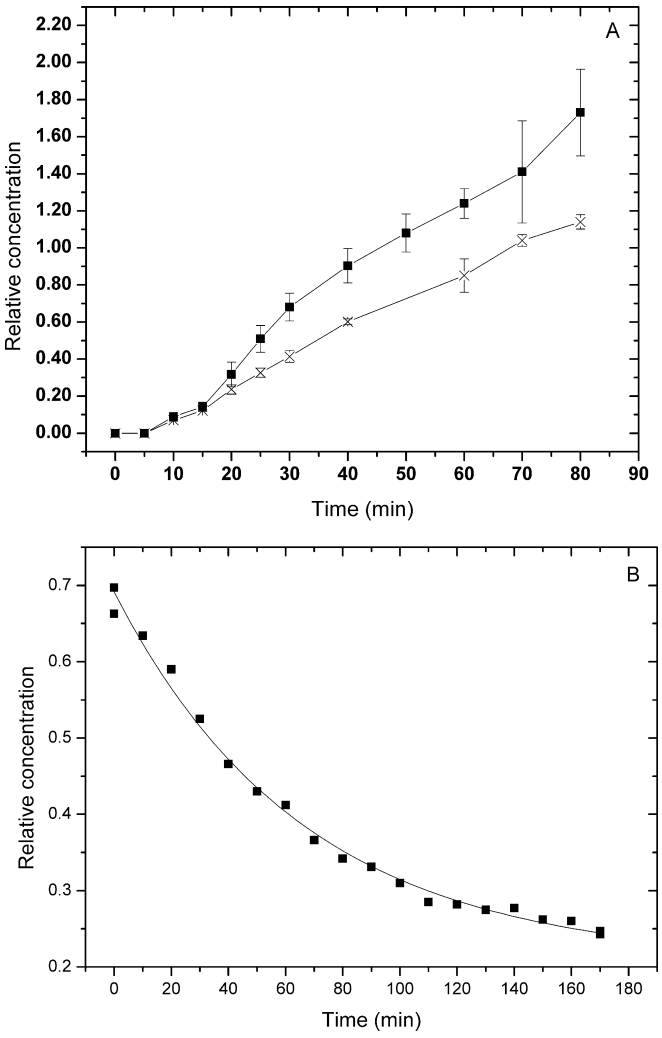

Fig. 1. Formation (A) of free radicals in cream cheese (■) and low fat cream cheese (*x*) during 80 min of light exposure, and an example of decay (B) of free radicals in cream cheese after 80 min of continuous light exposure at room temperature. The decay could be described by the exponential expression $\text{Conc} = a \cdot \exp(-k \cdot t) + b$ for which a half-life of 46 ± 3 min was calculated.

cheese. The half-life was approximately 0.5 h, with a non-significant tendency to longer half-lives for the low fat cream cheese. This quantitative analysis on a relative concentration scale was justified by the demonstration of linearity between the concentration of added TEMPO, a semi-stable radical, to cream cheese or low fat cream cheese and the double integrated ESR-spectrum, as shown in Fig. 2. A conversion of the relative concentration of light-induced radicals in the two types of cream cheese, as based on the integrated ESR-spectra to absolute concentrations, was, however, not attempted, due to the many uncertainties of effects of the semi-solid matrix of the cheeses on the radicals, for example seen as a difference between the standard for TEMPO in cream cheese and low fat cream cheese in Fig. 2. From Fig. 1A, it is further seen that, while the induction periods for radical formation in the two types of cream cheese are similar, the radical accumulation is more significant in the low fat cheese. The higher concentration of radicals detected in low fat cream cheese seems to be related to the slower decay of radicals in low fat cream cheese, in effect increasing the steady state concentration.

Prolonged exposure of cream cheese for 180 min at ambient conditions led to a change in the observed ESR-spectrum. After 180 min of light exposure, the ESR-spectrum changes, as shown

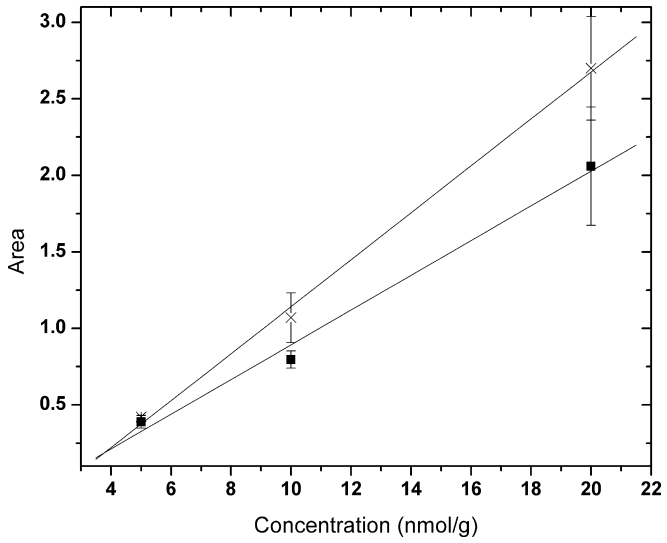

Fig. 2. Free radical standard curve for TEMPO in cream cheese (■) or low fat cream cheese (x). Relative area under ESR-spectrum obtained by double integration versus concentration of added TEMPO for measurements at 21 °C.

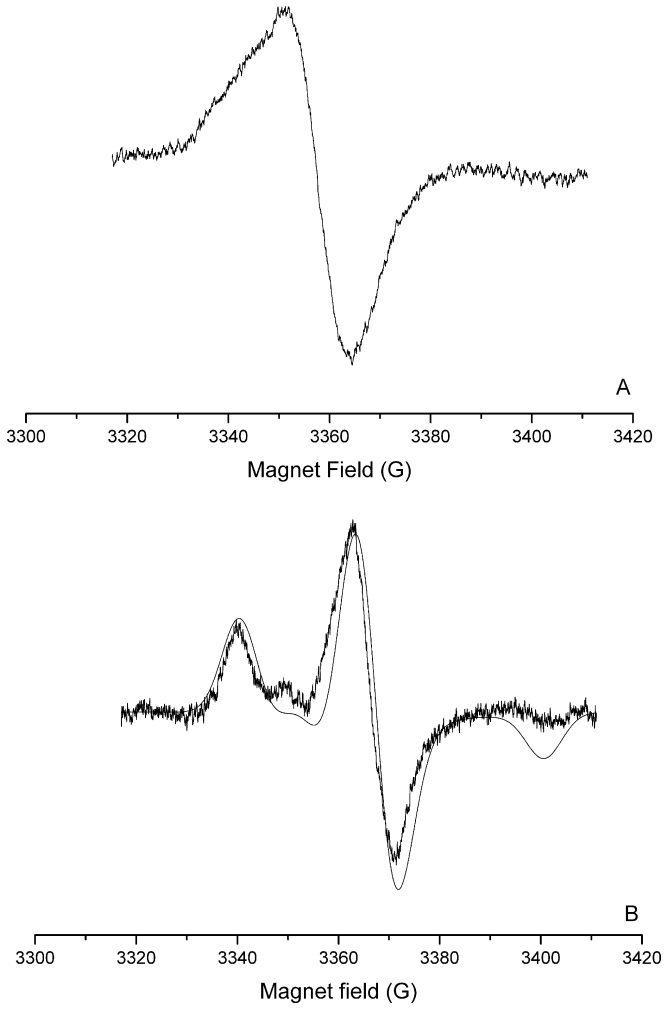

Fig. 3. ESR-spectrum of low fat cream cheese exposed to light for 80 min (A) and of partly desiccated low fat cream cheese following exposure to light for 180 min (B). The *g*-values are 2.006 and 2.0014 for the spectra in (A) and (B), respectively. Simulated powder spectrum for the partly desiccated low fat cream cheese is shown in spectrum (B). A coupling constant A'_{zz} of 30 G results from the simulation.

in Fig. 3A. The change from the spectrum of Fig. 3A towards the spectrum of Fig. 3B is accompanied by a shift in the *g*-values, found to be 2.006 and 2.0014 for 80 and 180 min of illumination, respectively. The difference in *g*-value indicates that the natures of radicals present in the samples are unlikely to be the same and the change with time towards a more structured ESR-spectrum indicates immobility of the radicals (powder spectrum). In order to investigate this spectral change during the 180 min of light exposure, a simulation of the spectrum was carried out, revealing a coupling constant A'_{zz} of 30 G with a distance between the outer peaks of 61 G, as shown in Fig. 3B. Given the appearance of the spectrum and the value of the coupling constant in relation to the distance between the peaks, the spectrum possesses the properties characteristic of a immobilised nitrogen-centred radical. The immobilisation of the radical is due to a partial desiccation of the cream cheese during prolonged illumination which indirectly helped to identify the nature of the radicals.

Measurement of light transmittance in the wavelength region from 250 to 700 nm through cream cheese and through low fat cream cheese (1, 2, 3, and 4 mm) showed that most light was absorbed in the first outer mm, as may be seen in Fig. 4. Around

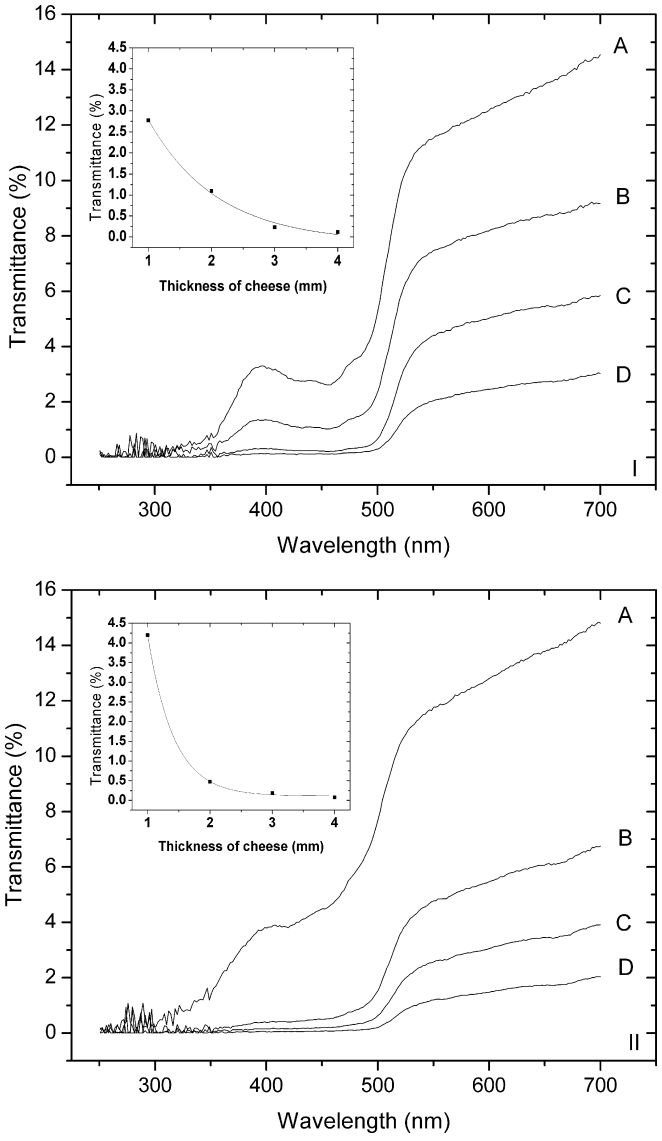

Fig. 4. Light transmission for layers of cream cheese (I) and low fat cream cheese (II): (A) 1 mm, (B) 2 mm, (C) 3 mm, and (D) 4 mm. Insert: transmittance at 450 nm.

450 nm a lower transmittance was observed which correlates with the wavelength at which light is absorbed by riboflavin (Borle, Sieber, & Bosset, 2001). A difference in transmittance between the two cream cheeses was observed, particularly around 450 nm. At this wavelength the transmittances were 5% and 3% in low fat cream cheese and in cream cheese, respectively, indicating that the second mm layer receives 20 and 30 times less light than does the first mm. At 600–700 nm, the second mm of cheese receives 7–5 times less light than does the first mm. The transmittance inwards in the cream cheeses seems to decay exponentially and transmittance of light through the first mm did not exceed 15% at any wavelength in the spectral region of relevance, with only a small difference between the two kinds of cream cheeses. Microscopy of the cheese provided further insight of the light penetration into the product. Using confocal laser scanning microscopy, with three-dimensional imaging, a fluorescence signal could be obtained at a depth of 10 μm, which is illustrated by Fig. 5. In order to determine whether this was due to limited diffusion of the probe into the cheese or to optical limitations, the samples were scanned simultaneously in confocal reflection microscopy at 633 nm, yielding no differences in signal depth. Scanning in the *xy*-direction revealed fully labelled lipid droplets in cream cheese in channel 2 (non-oxidised) after labelling with C_{11}-Bodipy (581/591), which is shown in Fig. 6 (channel 2). Furthermore, the figure shows the oxidised stage of the probe (channel 1).

Between and within different samples, local differences in fluorescence intensities could be observed, due to inhomogeneous distribution of the probe, making the calibration of the microscopy settings difficult. Many non-illuminated samples already showed strong fluorescence signals of the oxidised probe. Upon repeated image recording of the samples, a considerable loss of fluorescence signal, in both channels, was observed. A systematic investigation of the behaviour of the fluorescence probe, after repeated laser beam exposure, showed significant differences in the decay of the fluorescence intensity of the two channels (Fig. 7). While the decay in channel 1 followed a first-order exponential function, the decay in channel 2 appeared linear. Using confocal laser scanning microscopy, some changes in the oxidative status of the lipids were evident but a further quantitative description was not

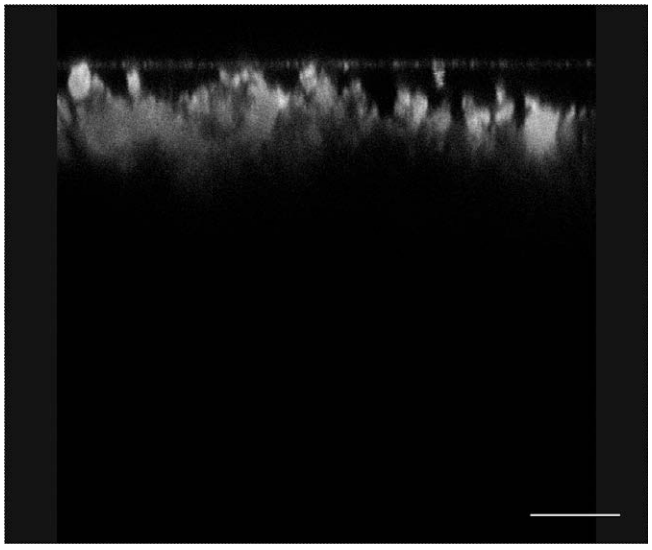

Fig. 5. Image recording of working depth in the cream cheese superimposing channel 1 (oxidised probe) and channel 2 (non-oxidised probe). The bar equals 10 μm.

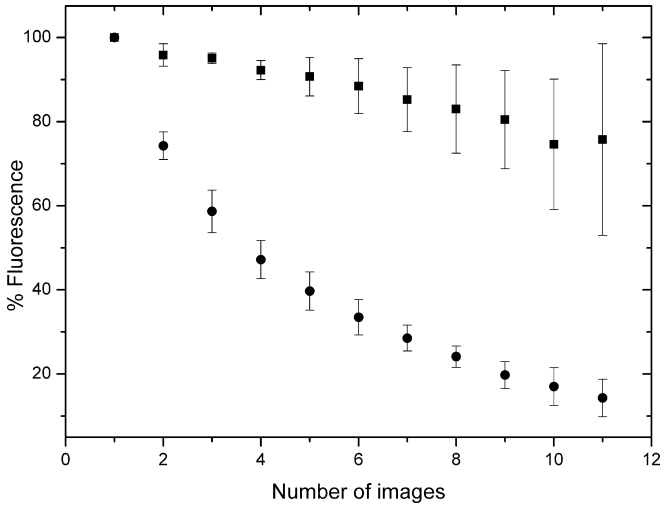

Fig. 7. Decay of fluorescence for the oxidised (●) and the non-oxidised (■) forms of the probe during image recording due to photobleaching of the two forms of the probe in cream cheese.

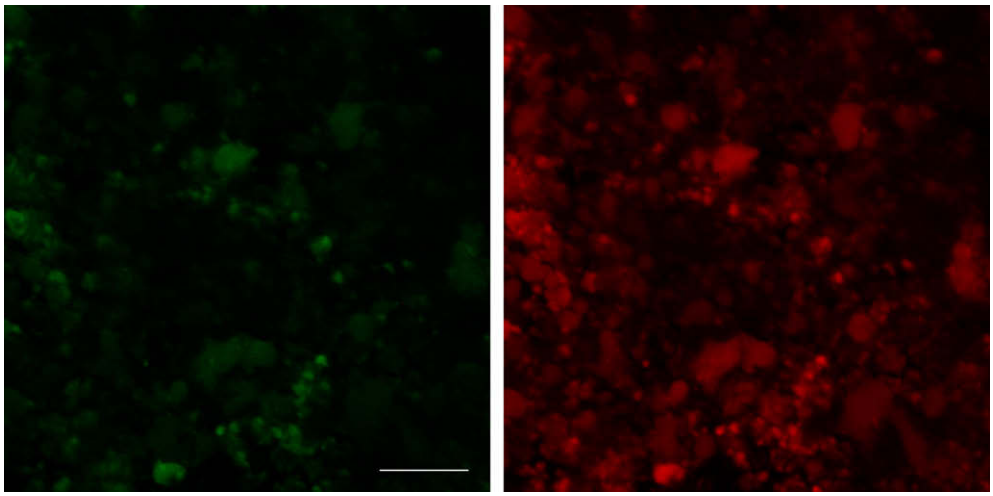

Fig. 6. Two-dimensional images of oxidised (from channel 1) and non-oxidised (from channel 2) forms of the probe recorded simultaneously showing the labelling of the lipids in cream cheese for recording in the upper 10 μm layer. The bar equals 10 μm.

possible due to bleaching of the probe. However, it was found that the low fat cheese, in general, was more oxidised than was the full fat product, as evidenced by a relative higher intensity of channel 1 (results not shown).

The peroxide numbers in the two cream cheese products were 0.48 ± 0.17 and 1.46 ± 0.05 meqiv/kg lipid in cream cheese and in low fat cream cheese, respectively, indicating some lipid oxidation. The measured level of lipid peroxides, which is within the normal range for processed dairy products, confirms the oxidation detected by confocal laser scanning microscopy prior to illumination of the two types of cream cheese.

Upon light exposure, such oxidative changes will affect the aroma profile of the cheese. Relative changes of concentration of selected aroma compounds in cream cheese after light exposure, as determined by GC, are shown in Fig. 8. From the figure it is seen that the first 1 mm of cream cheese is subjected to most of the changes (especially compared to the second mm), in agreement with the light absorption. Dimethyl disulphide is found only in the upper layer of samples exposed to light and in almost equal amounts, regardless of time of illumination. 2-Butanone, on the contrary, shows a higher formation after 30 min than after 15 min of light exposure. The content of 2-butanone in the first mm of the two types of cream cheese is significantly different from the content in the second mm of the samples and from samples kept in darkness. Moreover, the figure shows the same development for hexanal as seen for 2-butanone. Overall the differences between two layers of cream cheese were more distinct than the differences between illumination times.

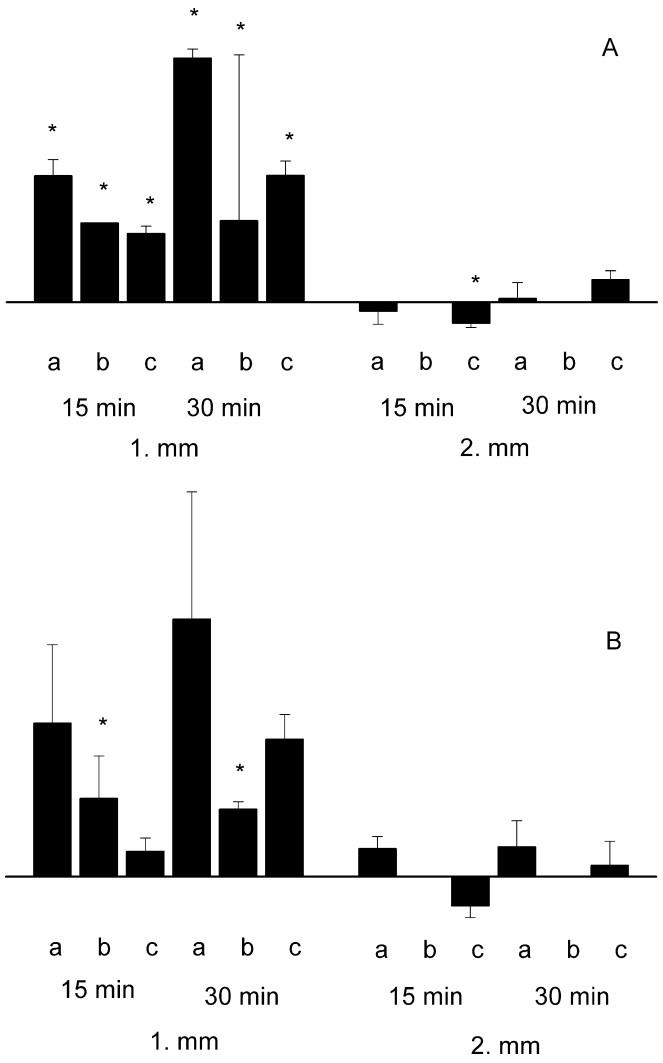

Fig. 8. Effect of light exposure on formation of oxidation products in cream cheese and low fat cream cheese. Difference between illuminated samples and non-illuminated samples is presented as relative amount of 2-butanone (a), dimethyl disulphide (b), and hexanal (c) in cream cheese (A) and low fat cream cheese (B) exposed to light for 15 and 30 min in two layers of each 1 mm as analysed by GC–MS and given with standard deviations. A significant difference between the illuminated and the non-illuminated samples is marked with ∗. The quantity of aroma compounds cannot be compared directly for different aroma compounds. Negative values for illuminated samples indicate that the illuminated sample had lower contents of volatiles than did the non-illuminated sample. Values for non-illuminated samples are not shown.

4. Discussion

Upon light exposure, free radicals have (by direct ESR spectroscopy) been shown to be formed in cream cheese. The relative concentrations of free radicals were based on the area beneath the signals over the actual concentration range, as based on the linearity of the standard curves observed for TEMPO radicals in each of the two cream cheeses.

Radicals in food systems, which have a life time of sufficient length to be measured by direct ESR spectroscopy, are often nitrogen-centred radicals and the spectrum appearing after 180 min of illumination shows the features of a typical nitrogen-centred radical. Oxidation measured by direct ESR spectroscopy (e.g., formation of radicals), however, does not hold information about the origin of the radicals in lipids or proteins and such mechanistic information will depend on analysis of oxidation products. Dairy products are very sensitive to light exposure, and off-flavours were detected after as little as 15–20 min of exposure to 2000 lux of fluorescent light (Chapman, Whited, & Boor, 2002). Specific aroma compounds are related to either protein or lipid oxidation; detection of such specific compounds may provide further knowledge about the oxidation taking place in the actual product. Formation of dimethyl disulphide is an indicator of protein oxidation and more specifically of methionine degradation (Jung, Yoon, Lee, & Min, 1998). Presence of hexanal is regarded as a marker for oxidation of linoleic acid in triglycerides or phospholipids (Pan, Ushio, & Ohshima, 2005; Yang, Lee, Lee, & Lee, 2007).

Aroma analysis of different layers of a dairy product, as established for two layers of cream cheese in the present study, provides information for an understanding of the impact of light when penetrating the dairy product. The difference in aroma profile between the two layers of cream cheese was found to be consistent with the measured light penetration through the two types of cream cheese. Light-induced formation of aroma compounds can, with the present experimental design, be solely caused by the penetration of light inwards in the cream cheese as plates of quarts glass separating the two layers of cream cheese prevent migration of radicals and aroma compounds from one layer to the other. The measurements of light transmittance showed that 99% of the UV-light and around 95% of the light at 450 nm is absorbed by the first mm of cheese and nearly no light is transmitted through the second mm (for 450 nm light), most likely due to the presence of riboflavin. Transmittance through the first mm of the low fat cream cheese was slightly higher than through the cream cheese, which is in agreement with earlier findings of light transmittance through milk with two different fat contents (Allen & Parks, 1979). The fact that approximately 20% of the light is transmitted in the spectral region 630–670 nm to deeper regions of the cheese makes it possible for other photosensitisers present in the cream cheese to be active in the interior of the product. It has been found that protoporphyrin and degradation products of chlorines (chlorophyll) are efficient photosensitisers, absorbing light at 635 and

665 nm, respectively, and both could be present in the actual cream cheese (Bekbolet, 1990; Wold et al., 2005). Since a larger amount of light is transmitted through the first mm of cream cheese, in the spectral region 600–700 nm, protoporphyrins and chlorophyll degradation products will become more important as photosensitisers for the second mm and inwards in the cream cheese than in the outer first mm. The second mm of cream cheese received 20–30 times less light of 450 nm than did the first mm while, at wavelengths between 600 and 700 nm, the second mm received 5–7 times less light than did the first mm. Furthermore, protoporphyrin and chlorophyll will be located inside the lipid droplets and will consequently have easier access to the lipids than will riboflavin. However, determination of the three volatiles in cream cheese showed (for all samples) formation only in the outer first mm with no significant formation in the second mm after 15 min or 30 min of light exposure, indicating a more important role of riboflavin than of the other photosensitisers.

Cream cheese labelled with C_{11}-Bodipy (581/591) made it possible to detect lipid oxidation taking place in the cream cheese by confocal laser scanning microscopy. C_{11}-Bodipy (581/591) reacts with a wide range of reactive oxygen species accumulating, in the product in contrast to direct ESR spectroscopy, which measures the presence of free radicals in a steady state concentration at the moment of measurement. C_{11}-Bodipy (581/591) is capable of, not only detecting newly formed oxidation products, but also oxidation, which has already taken place (Aitken, Wingate, De Iuliis, & McLaughlin, 2007). The fact that C_{11}-Bodipy (581/591) shows oxidation on non-illuminated cream cheese is in accordance with the peroxide values showing presence of peroxides in non-treated cream cheese. Compared with previously reported peroxide values, determined for semi hard cheese (0.1–1.4 meqiv/kg), the peroxide values cannot be regarded as unusually high, and the values are within the normal range, although the values found for low fat cream cheese are slightly elevated (Mortensen, Sørensen, & Stapelfeldt, 2002).

This is the first study utilising C_{11}-Bodipy (581/591) in a non-membrane lipid system. From the staining pattern of the lipid droplets in two-dimensional images, it can be concluded that C_{11}-Bodipy (581/591) is not only located in the interface between lipid and water phase, but also in the interior of the neutral lipid droplets. For the purpose of looking at lipid droplets, these became more distinct by being fully labelled with the probe. Though it was possible to identify lipid oxidation in cream cheese by confocal laser scanning microscopy, it was not possible to record oxidation depths deeper than 10 μm. The combined effects of scattering, refraction, absorption, and spherical aberrations are responsible for the loss of signal because recording of back-scattered light in the confocal reflection mode is independent of the presence of a fluorophore. The major difficulty, when employing detection of lipid oxidation in cream cheese by labelling with C_{11}-Bodipy (581/591), was accordingly identified as the sample preparation.

During the recording of microscopic images, a significant loss of fluorescence intensity from the probe was observed. C_{11}-Bodipy (581/591) has been widely used in cell cultures and liposome systems and has, for these systems been, described as both thermally and photochemically stable (Naguib, 1998). Our conclusion, based on simultaneous image recording accompanied by image analysis, shows that loss of fluorescence in channel 1 (the oxidised probe) follows first-order exponential decay and the loss in channel 2 shows linear properties. The pattern of channel 1 is a characteristic of photobleaching (Diaspro, Chirico, Usai, Ramoino, & Dobruki, 2006). Photobleaching is a well known phenomenon in fluorescence microscopy, describing a permanent photochemical destruction of the fluorophores in excited triplet or singlet state (Song, Hennink, Young, & Tanke, 1995). The loss of fluorescence in channel 2 is probably due to both photobleaching and a photoreaction oxidising the probe. In an actual type of cheese, the probe could accordingly only be used to detect the spatial distribution of oxidation, as seen in Fig. 6, but not for kinetic studies of progression of photoinduced oxidation.

Low fat products have become more and more popular. Studies have shown a difference in oxidative stability between low fat products and the original products regarding both protein and lipid oxidation (Andersen et al., 2006). Radicals were found, in the present study, to be formed in higher yields upon light exposure of the low fat cream cheese than in the cream cheese. Once formed, the radicals follow the same pattern of decay for both types of cream cheeses; however, a higher steady state concentration of radicals will induce more oxidation. More light is transmitted through the second and the third mm of cream cheese than of the low fat cream cheese; however, the changes in volatiles formed upon similar light exposure do not differ significantly between the two types of cream cheese. The low fat cream cheese had the highest content of protein but exposure to light did not result in formation of significantly more dimethyl disulphide.

In conclusion, formation of radicals in cream cheese and low fat cream cheese, as a result of light exposure, can be followed by direct ESR spectroscopy, showing surprisingly long-lived radicals which are involved in lipid and protein oxidation. GC-analysis of off-flavours correlated with measurements of light transmittance and the outer 1 mm layer is the most important regarding light-induced oxidation in cream cheese. Confocal laser scanning microscopy, using a probe sensitive to free radicals, has been used for imaging of spatial distribution of lipid oxidation in cream cheeses, but its use for kinetic studies awaits further developments.

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References

Aitken, R. J., Wingate, J. K., De Iuliis, G. N., & McLaughlin, E. A. (2007). Analysis of lipid peroxidation in human spermatozoa using BODIPY C-11. *Molecular Human Reproduction, 13*(4), 203–211.

Allen, C., & Parks, O. W. (1979). Photodegradation of riboflavin in milks exposed to fluorescent light. *Journal of Dairy Science, 62*(9), 1377–1379.

Andersen, C. M., Wold, J. P., & Mortensen, G. (2006). Light-induced changes in semi-hard cheese determined by fluorescence spectroscopy and chemometrics. *International Dairy Journal, 16*(12), 1483–1489.

Andersen, L. T., Lund, M. N., Glyager, R., Jensen, S. B., Mortensen, G., & Skibsted, L. H. (2006). Light-induced deterioration of reduced-fat cream cheese. Relative importance of protein and lipid oxidation. *Milchwissenschaft, 62*(2), 162–165.

Andersson, K., & Lingnert, H. (1998). Influence of oxygen concentration and light on the oxidative stability of cream powder. *Lebensmittel-Wissenschaft und – Technologie, 31*, 169–176.

Bekbolet, M. (1990). Light effects on food. *Journal of Food Protection, 53*(5), 430–440.

Borle, F., Sieber, R., & Bosset, J. O. (2001). Photo-oxidation and photoprotection of foods with particular reference to dairy products. An update of a review article (1993–2000). *Sciences des Aliments, 21*, 571–590.

Chapman, K. W., Whited, L. J., & Boor, K. J. (2002). Sensory threshold of light-oxidized flavor defects in milk. *Journal of Food Science, 67*(7), 2770–2773.

Diaspro, A., Chirico, G., Usai, C., Ramoino, P., & Dobruki, J. (2006). Photobleaching. In J. B. Pawley (Ed.), *Handbook of biological confocal microscopy* (3rd ed., pp. 690–702). New York, USA: Springer.

Drummen, G. P. C., Gadella, B. M., Post, J. A., & Brouwers, J. F. (2004). Mass spectrometric characterization of the oxidation of the fluorescent lipid peroxidation reporter molecule C11-BODIPY581/591. *Free Radical Biology and Medicine, 36*(12), 1635–1644.

Drummen, G. P. C., van Liebergen, L. C. M., Op den Kamp, J. A. F., & Post, J. A. (2002). C11-BODIPY581/591, an oxidation-sensitive fluorescent lipid peroxidation probe: (micro)spectroscopic characterization and validation of methodology. *Free Radical Biology and Medicine, 33*(4), 473–490.

Folch, J., Lees, M., & Stanely, H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*(1), 497–509.

Jung, M. Y., Yoon, S. H., Lee, H. O., & Min, D. B. (1998). Singlet oxygen and ascorbic acid effects on dimethyl disulfide and off-flavor in skim milk exposed to light. *Journal of Food Science, 63*(3), 408–412.

Juric, M., Bertelsen, G., Mortensen, G., & Petersen, M. A. (2003). Light-induced colour and aroma changes in sliced, modified atmosphere packaged semi-hard cheeses. *International Dairy Journal, 13*, 239–249.

Kim, Y. D., & Morr, C. V. (1996). Dynamic headspace analysis of light activated flavor in milk. *International Dairy Journal, 6*, 185–193.

Kondyli, E., Andersen, M. L., & Skibsted, L. H. (2005). Evaluation of the oxidative stability of goat milk by electron spin resonance spectroscopy (ESR). *Milchwissenschaft, 60*(3), 271–274.

Lopez, C., Camier, B., & Gassi, J. Y. (2007). Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy. *International Dairy Journal, 17*(3), 235–247.

Mortensen, G., Sørensen, J., & Stapelfeldt, H. (2002). Comparison of peroxide value methods used for semihard cheeses. *Journal of Agricultural and Food Chemistry, 50*, 5007–5011.

Mortensen, G., Bertelsen, G., Mortensen, B. K., & Stapelfeldt, H. (2004). Light-induced changes in packaged cheeses – A review. *International Dairy Journal, 14*(2), 85–102.

Naguib, Y. M. A. (1998). A fluorometric method for measurement of peroxyl radical scavenging activities of lipophilic antioxidants. *Analytical Biochemistry, 265*(2), 290–298.

Pan, X. Q., Ushio, H., & Ohshima, T. (2005). Comparison of volatile compounds formed by autoxidation and photosensitized oxidation of cod liver oil in emulsion systems. *Fisheries Science, 71*(3), 639–647.

Pap, E. H. W., Drummen, G. P. C., Winter, V. J., Kooij, T. W. A., Rijken, P., Wirtz, K. W. A., et al. (1999). Ratio-fluorescence microscopy of lipid oxidation in living cells using C11-BODIPY581/591. *FEBS Letters, 453*(3), 278–282.

Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC International, 77*(2), 421–424.

Song, L. L., Hennink, E. J., Young, I. T., & Tanke, H. J. (1995). Photobleaching kinetics of fluorescein in quantitative fluorescence microscopy. *Biophysical Journal, 68*(6), 2588–2600.

Stapelfeldt, H., Nielsen, K. N., Jensen, S. K., & Skibsted, L. H. (1999). Free radical formation in freeze-dried raw milk in relation to its alpha-tocopherol level. *Journal of Dairy Research, 66*(3), 461–466.

Thomsen, M. K., Lauridsen, L., Skibsted, L. H., & Risbo, J. (2005). Two types of radicals in whole milk powder. Effect of lactose crystallization, lipid oxidation, and browning reactions. *Journal of Agricultural and Food Chemistry, 53*(5), 1805–1811.

Westermann, S., Møller, J. K. S., & Skibsted, L. H. (2008). Effect of light exposure on radical formation in dairy cream during subsequent dark storage. *Milchwissenschaft*.

Wold, J. P., Veberg, A., Nielsen, A., Iani, V., Nuzenas, P., & Moan, J. (2005). The role of naturally occurring chlorophyll and prophyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. *International Dairy Journal, 15*, 343–353.

Yang, S., Lee, J., Lee, J., & Lee, J. (2007). Effects of riboflavin-photosensitization on the formation of volatiles in linoleic acid model systems with sodium azide or D_2O. *Food Chemistry, 105*(4), 1375–1381.