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Antioxidant Properties of Green Tea Extract Protect Reduced Fat Soft Cheese against Oxidation Induced by Light Exposure

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ABSTRACT: The effect of two different antioxidants, EDTA and green tea extract (GTE), used individually or in combination, on the light-induced oxidation of reduced fat soft cheeses (0.2 and 6% fat) was investigated. In samples with 0.2% fat, lipid hydroperoxides as primary lipid oxidation products were not detected, but their interference was suggested from the formation of secondary lipid oxidation products such as hexanal and heptanal. The occurrence of these oxidation markers was inhibited by spiking with 50 ppm EDTA or 750 ppm GTE, or a combination of the two prior to irradiation. In contrast, addition of 50 ppm EDTA to samples with 6% fat was ineffective, but 750 ppm GTE (alone or in combination with EDTA) strongly reduced levels of hexanal and heptanal. Accumulation of primary lipid hydroperoxides was not affected by GTE, hence antioxidative activity was ascribed to scavenging of hexanal and heptanal precursors. These radical intermediates result from hydroperoxide disintegration, and subsequent scavenging by GTE, which acts as a radical sink, corroborates the intense signal observed by electron paramagnetic resonance (EPR) spectroscopy.

KEYWORDS: soft cheese, oxidation, antioxidants, irradiation, off-flavors, green tea

INTRODUCTION

Quality of food products inevitably changes during storage because exposure to heat, enzymes, transition metal ions, oxygen, and light eventually causes degradation or formation of flavor-active compounds. The role of light-induced oxidation has become more important since packaging trends followed consumer preference for transparent materials, although fluorescent light, to which foods are mainly exposed during shelf life, is harmless for most constituents. However, naturally occurring photosensitizers such as flavins and chlorophylls, which play pivotal roles in the (photo)chemistry of various biological systems, readily absorb visible light and convert into potentially reactive excited states. Dairy products, for example, contain the nutritionally valuable riboflavin (RF, also known as vitamin B_2),[1] which includes the photoactive isoalloxazine chromophore. Its main absorption bands around 375 and 450 nm allow excitation by blue light with formation of the corresponding singlet excited state, followed by intersystem crossing to produce the longer-lived triplet state (^{3}RF*).[2] This strongly oxidizing species readily accepts an electron from suitable donors (type I photooxidation) or transfers its excess triplet energy to produce singlet oxygen ($^1O_2$), a reactive electrophilic intermediate that readily interacts with electron-rich molecular sites (type II photooxidation).[3] As such, riboflavin has been identified as responsible for the development of flavor changes in various light-exposed foods or beverages.[4−10] Unlike flavins, porphyrins such as chlorophyll derivatives also absorb red light and are typically involved in type II photoreactions.[11] Their presence in dairy products, which is influenced by cow feeding patterns, largely expands the range of damaging wavelengths in the visible part of the spectrum[9,12] and, accordingly, complicates efforts to reduce light sensitivity.

Proteins, containing several sites of high electron density, and unsaturated lipids are prone to light-induced oxidations,[5,13,14] and both degradation pathways have been associated with characteristic off-flavors.[15,16] In dairy products such as soft cheese, lipid oxidation was found to be the major mechanism, although importance of protein oxidation increased with decreasing lipid content.[17,18] Progress of these degradation mechanisms may be significantly accelerated due to light penetration through translucent packaging materials,[19,20] leading to considerable shortening of the predicted shelf life. To avoid such premature spoiling, it was investigated whether adding sacrificial compounds with direct or indirect antioxidative properties to the cheese matrix was effective in protecting against light-induced changes. For that purpose, formation of typical off-flavor compounds, monitored by gas chromatographic (GC) analysis with mass spectrometric (MS) detection, was evaluated in cheese treated with antioxidants and compared to results from reference (i.e., untreated) cheese samples. Furthermore, electron paramagnetic resonance (EPR) spectroscopy was applied to monitor possible intervention of radical species in the light-induced degradation process.

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MATERIALS AND METHODS

Chemicals. Soft cheeses (0.2 and 6% fat content), which are commercially available on the English and German market, were obtained from Arla Foods amba (Holstebro, Denmark), while natamycin (Natamax) came from Danisco A/S (Grindsted, Denmark). Iron(III) chloride hexahydrate (97%), hexanal (98%), heptanal (>92%), and dimethyl disulfide (98%) were obtained from Sigma Aldrich Chemie GmbH (Steinheim, Germany), and sodium acetate, pentanal (97%), and 4-methyl-2-pentanone (99%) came from Fluka Chemie GmbH (Buchs, Switzerland). Solvents acetonitrile, chloroform, and methanol (all HPLC grade), as well as heptane (99%), were from Rathburn Chemicals (Walkerburn, Scotland), while NH_4SCN (99%) was from VWR BHD Prolabo (Leuven, Belgium). Other chemicals were obtained from Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid calcium disodium salt (EDTA $CaNa_2 \cdot H_2O$) (>98%) was from Akzo Nobel Functional Chemicals bv (Amersfoort, The Netherlands). Green tea extract (80%, containing 20% flavan-3-ols) was obtained from Danisco (Brabrand, Denmark).

Sample Preparation. The soft cheeses were mixed with 50 ppm EDTA (~0.12 μmol/g soft cheese), 750 ppm green tea extract (giving a total catechin content of ~150 ppm in the cheese), or a combination of both, using a mixer cooker emulsifier (UMC5, Stephan, Hameln, Germany). The soft cheeses were heated to 70−75 °C using jacket heating (95 °C) while mixing for approximately 11−14 min. Finally, the cheeses were mixed for 1 min at 20,000 N/m^2. Reference cheese was treated by an identical procedure but without the addition of antioxidants. Soft cheeses were subsequently transferred to 250 mL plastic containers (DUMA, Apodan Nordic, Denmark) and stored at 5 °C until further use. Prior to irradiation experiments, soft cheeses were transferred into the lids (Ø = 9 mm) of sterile Petri dishes (Frisenette, Knebel, Denmark), treated with 5 g/L natamycin (Natamax) and packed either in vacuum or with air, respectively, in vacuum packs consisting of a gastight layer of 70 μm polyamid and 20 μm polyethylene (PM Pack Service, Horsens, Denmark).

Irradiation Experiments. To characterize degradation products resulting from light exposure, cheeses were simultaneously incubated at 15 °C under fluorescent light (400−600 nm) generated by a TL-D 90 de Luxe Pro (18W/965 SLV) light source from Philips (Eindhoven, The Netherlands). This simulated the conditions used in retail, and an intensity of 1800 lx was measured at the surface of the cheese samples. For control experiments, cheese was wrapped in aluminum foil to avoid light exposure and incubated in parallel. After incubation for 0, 1, 2, 3, and 7 days, samples were homogenized and analyzed for lipid hydroperoxides and volatile compounds. Irradiation of samples to be analyzed for radical species is discussed in Electron Paramagnetic Resonance (EPR) Spectroscopy.

Lipid Hydroperoxides. For measuring the presence of lipid hydroperoxides in cheese, sample preparation was performed according to Dalsgaard et al.[21,22] One gram of cream cheese was dispersed in 5 mL of demineralized water and mixed by Ultraturax for 45 s. Lipid hydroperoxides were extracted into a 10 mL methanol:chloroform (1:1, v/v) mixture. Samples were mixed for 30 s using a whirlmixer and finally centrifuged for 10 min at 1000*g*. Then 1 mL of the chloroform phase was transferred into chloroform-rinsed glasses and mixed with 1 mL of iron(II) thiocyanate solution according to the IDF standard (74A:1991) modified by Østdal et al.[23] Absorbance was measured at 500 nm with 700 nm used as background subtraction on an HP-8453 diode array spectrophotometer from Agilent Technologies (Palo Alto, CA, USA), and quantification was based on external standards using a calibration curve made with the concentrations 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, and 20.0 μg/mL of iron(III). The samples were diluted properly to be within the range of the standard curve. Samples were analyzed in triplicate.

Analysis of Volatiles. Characteristic volatile compounds, including pentanal, hexanal, and heptanal, were identified and quantified by headspace GC−MS analysis according to a slightly modified literature procedure,[21,22] while dimethyl disulfide was monitored but not detected. Thus, 1 mL of deionized (18.2 MΩ cm) and filtered water (0.22 μm) was mixed with 1 mL of internal standard solution (100 ng/mL 4-methyl-2-pentanone) and transferred to a 12.5 mL vial containing 2 g of light-exposed cheese (or a nonexposed control) that was sealed with Teflon-coated lids prior to incubation (30 min at 37 °C). The headspace was analyzed for volatile compounds using a Carboxen/PDMS SPME fiber from Supelco (Bellefonte, PA, USA) with a film thickness of 30 μm, which was incubated at 60 °C for 30 min in the headspace of each sample. Desorption of the sample from the fiber was performed into the inlet of a GC 154ON from Agilent Technologies (Santa Clara, CA, USA) equipped with a Zebron ZB-5ht column from Phenomenex (Torrance, CA, USA) and coated with a nonmetal 5% phenyl 95% dimethylpolysiloxane phase with the following dimensions: 0.25 mm i.d.; 0.25 μm film thickness; 30 m length. Helium was used as carrier gas with constant flow of 1.2 mL/min at a pressure of 100 N/m. The splitless injector was kept at 250 °C. An SPME injection sleeve linear from Supelco with an inner diameter of 0.75 mm was applied. The column temperature was programmed to stay at 40 °C for 4 min followed by an increase from 40 to 120 °C with a rate of 5 °C/min, a hold time of 5 min, and a subsequent temperature gradient from 120 to 300 °C at a rate of 20 °C/min. Mass spectral analysis was performed in selected ion monitoring, according to the ions determined by the use of a standard for each compound according to Dalsgaard,[21,22] on a quadrupole MSD 5975 (Agilent Technologies) keeping the quadrupole temperature at 150 °C and applying a fragmentation voltage of 70 eV. The ion source temperature was 230 °C, and the interface was at 280 °C. Quantification was performed relatively using external standard curves with appropriate concentrations of each analyte to avoid day to day variation on individual compounds. The standards were prepared as aqueous solutions from analyte stocks in methanol. Samples were measured in duplicate.

Electron Paramagnetic Resonance (EPR) Spectroscopy. For monitoring radical formation by EPR, cheese samples were placed in plastic trays, covered by polyethylene foil, and subsequently exposed to fluorescent light in a photoreactor equipped with cool white lamps (4 × 15 W reaching approximately 1000 lx) (Philips) for a 7 day period. All samples were exposed simultaneously to filter out fluctuations in photon output. Control samples were protected from light by aluminum foil, but were exposed to identical conditions otherwise. As a result of prolonged irradiation, samples had solidified, which allowed homogenization in a coffee grinder. The resulting powder was freeze-dried (48 h at −50 °C) while being protected from light, followed by transfer of a known quantity (~300 to 600 mg) to a 5 mm inner diameter EPR tube. Sample height was measured to allow correction for variations in powder density before the tube was placed in the cavity of an ECS 106 spectrometer (Bruker, Karlsruhe, Germany). Spins were measured with the following instrument settings: sweep width, 80 G; modulation amplitude, 2.5 G; modulation frequency, 100 kHz; conversion time, 40.96 ms; time constant, 20.48 ms; microwave power, 10 mW; center field, 3480 G. All samples were measured at room temperature.

Statistical Analysis. Statistical analysis was performed using the Generalized Linear Models (GLM) procedure of SAS version 9.2 (SAS Institute, INC., Cary, NC, USA). In order to obtain normality, the data were transformed by the log(10) function. The LS-Means were calculated and differences regarded as significant at minimum 95% level ($p < 0.05$). Differences were classified by the Ryan−Einot−Gabriel−Welsch (REGW) multiple range test (SAS Institute).

RESULTS

Determination of Oxidation Products. Light-induced changes in foods generally involve a complex set of chemical reactions, which eventually result in the formation of several

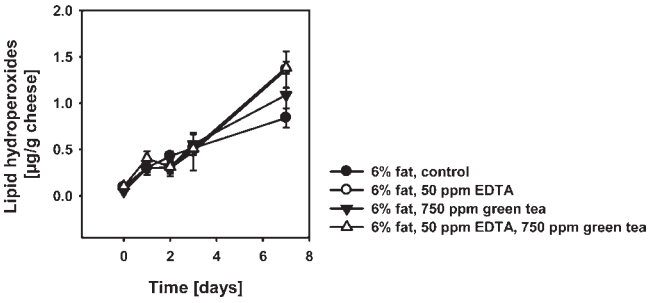

Figure 1. Accumulation of lipid hydroperoxides in cream cheese with 6% fat. Untreated control cheese, as well as cheeses with 50 ppm EDTA (~0.12 μmol per g soft cheese), 750 ppm green tea extract (GTE) (giving a total catechin content of ~150 ppm in cheese), and a combination of 50 ppm EDTA and 750 ppm GTE, respectively, were exposed to fluorescent light (1800 lx) for 0−7 days at 10 °C.

flavor-active volatiles that alter organoleptic perception. Among these, oxidation products, and particularly those derived from lipids, are known to give rise to pungent off-flavors. The prevalence of lipids in dairy compounds, even in low-fat derivatives, therefore rationalizes investigation of their oxidation products, including typical aldehyde markers such as pentanal, hexanal, and heptanal and their lipid hydroperoxide precursors. The latter are primary oxidation products, and their presence is used to estimate oxidation progress. Spectrophotometrical detection in 6%-fat samples showed that accumulation was significant ($p < 0.001$) from the first day of light exposure and levels increased with prolonged irradiation (Figure 1). Interestingly, their formation was not significantly affected by addition of EDTA or green tea extract (GTE), despite known antioxidative properties of these products. Cheese containing only 0.2% fat responded very differently as hydroperoxides were not detected as such (data not shown), although this could be due to the method detection limit (0.05 μg/g).

Aldehydes, which are secondary reaction products, were determined by SPME-GC−MS and characterized by comparing retention times and ions in fragmentation patterns of mass spectra with authentic reference materials. For 6%-fat cheeses, levels of hexanal (Figure 2A) and heptanal (Figure 2B) increased significantly ($p < 0.001$) during light exposure, especially within the first 3 days. Formation of light-induced oxidation products was subsequently evaluated in samples treated with the above-mentioned antioxidants prior to light exposure. Addition of 50 ppm EDTA failed to inhibit oxidation in 6%-fat cheese, but was apparently very efficient when applied to samples with only 0.2% fat (Figures 3A and 3B). The presence of 750 ppm green tea extract (GTE), alone or in combination with EDTA, strongly reduced hexanal and heptanal formation in both cheeses; no significant increase was observed between dark- and light-exposed 6%-fat samples, while very-low-fat samples (0.2%) showed lower levels ($p < 0.05$) of both aldehydes compared to samples with (only) EDTA added. Pentanal was not detected in the 0.2%-fat cheese, but its formation increased significantly ($p < 0.001$) during light exposure of the 6%-fat sample. However, unlike hexanal and heptanal, it was not influenced by the presence of GTE (Figure 2C).

Although dimethyl disulfide (DMDS), as a typical marker for protein oxidation, was previously detected in light-exposed low-fat cheese samples,[22] its formation could not be detected after irradiation of the current samples.

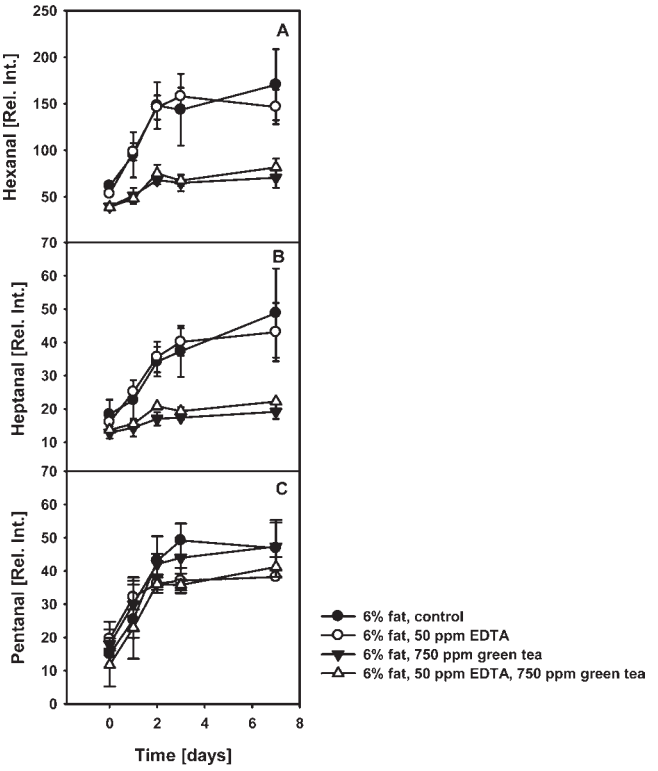

Figure 2. Accumulation of hexanal (A), heptanal (B), and pentanal (C) in soft cheese with 6% fat. Untreated control cheese and cheeses with 50 ppm EDTA (~0.12 μmol/g soft cheese), 750 ppm green tea extract (GTE) (giving a total catechin content of ~150 ppm in cheese), and a combination of 50 ppm EDTA and 750 ppm GTE, respectively, were exposed to fluorescent light (1800 lx) for 0−7 days at 10 °C.

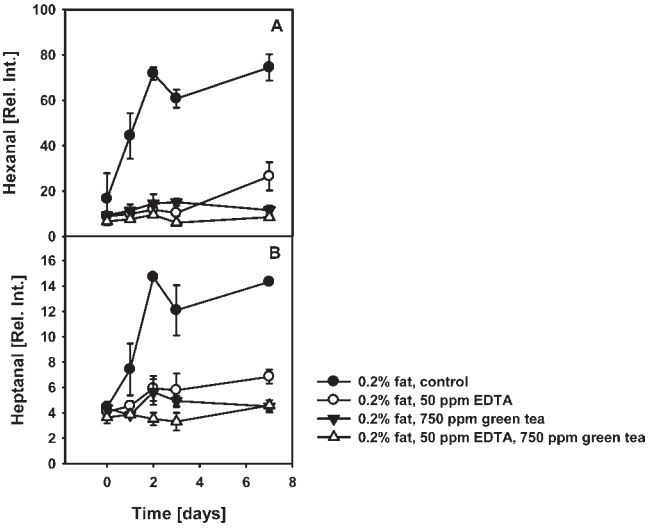

Figure 3. Accumulation of hexanal (A) and heptanal (B) in soft cheese with 0.2% fat. Untreated control cheese and cheeses with 50 ppm EDTA (~0.12 μmol/g soft cheese), 750 ppm green tea extract (GTE) (giving a total catechin content of ~150 ppm in cheese), and a combination of 50 ppm EDTA and 750 ppm GTE, respectively, were exposed to fluorescent light (1800 lx) for 0−7 days at 10 °C.

Exposure of soft cheese to UV irradiation was previously demonstrated to generate radical species,[24] but it was unclear

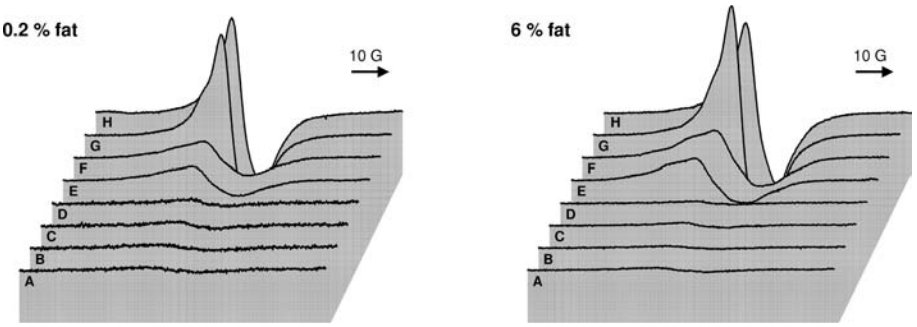

Figure 4. Electron paramagnetic resonance spectra of 0.2%-fat (left panel) and 6%-fat soft cheese (right panel; scale on both panels is identical). Traces represent measurements of dark control samples (A−D) and samples exposed to fluorescent light (E−H) of cheese without additives (traces A and E); cheese with 50 ppm EDTA (∼0.12 μmol/g soft cheese) added (traces B and F); cheese with 750 ppm green tea extract added (giving a total catechin content of ∼150 ppm in cheese) (C and G); and cheese with 50 ppm EDTA and 750 ppm green tea extract added (D and H).

whether fluorescent light induced similar reactivity. Soft cheese was therefore exposed to fluorescent light under atmospheric conditions for 7 days, and signals of radical formation were compared to those observed in the corresponding dark samples. After prolonged exposure in the reactor, samples appeared to have cured and, after milling, the resulting homogeneous powder was analyzed by EPR spectroscopy. However, instability of the microwave detection, presumably due to randomly dispersed residual water, hampered detection and, accordingly, an additional freeze-drying step prior to analysis was introduced.[25] The resulting spectra strongly supported the involvement of a light-induced radical mechanism. Although the signal was relatively broad (peak to peak width, $\Delta H_{pp} \sim 13.4$ G; $g \sim 2.0084$) and structureless and thus failed to provide mechanistic details as such (Figure 4), it was concluded that fat content (0.2% or 6%) or the presence of EDTA only had a minor influence on radical formation. However, a strong and sharper signal ($\Delta H_{pp} \sim 8.1$ G) with $g \sim 2.0075$ arose when samples were treated with GTE or a mix of GTE and EDTA followed by light irradiation.

DISCUSSION

Similarly as shown for other foods, the presence of sensitizing molecules like riboflavin and chlorophyll in soft cheese is suspected to trigger oxidative degradation during visible-light exposure. Considering the constituents of reduced fat soft cheese, proteins and lipids (the latter which make up to 6% weight fraction of the current samples) are feasible targets and ensuing disintegration pathways may result in formation of unwanted flavor-active compounds. These reactions often involve radical precursors, of which insight into structures is very informative in view of mechanistic interpretation of the photooxidation process. The fact that only an $^{\bullet}$OH−DMPO spin adduct was assigned in previous spin trapping experiments, in combination with the observation that light exposure for longer times led to secondary reactions degrading the spin adduct, led us to prefer the use of freeze-dried samples with direct detection of radical species.[25] However, applying EPR spectroscopy directly to irradiated cheese samples produced signals that lacked (hyper)fine patterns, which compromised characterization of the observed species. Still, as differences in fat content did not affect EPR signals, detection of radicals derived from lipids or endogenous, lipophilic antioxidants (such as vitamin E) was refuted. Instead, formation of a protein-derived radical was suggested, as, indeed, the relatively broad shape reminded of a large, motion-restricted species. The g factor concurred with a previously reported protein radical observed in UV-exposed soft cheese, although the different shape of the signal suggested another radical was detected.[24] Signal shape and g value however correlated better with a previously identified tyrosyl radical that was generated by casein oxidation[26] and that acted as precursor for dityrosine cross-linking.[21] Moreover, in a recent report, it was found that line width of the observed species nicely correlated with a *o*-semiquinone radical that was suggested to be formed in riboflavin mediated photo-oxidation of milk proteins as a consequence of further oxidation of DOPA, a Tyr-derived oxidation product.[27]

Despite the lack of detectable lipid-derived radical species, it is known that lipids degrade by radical reactions as well. Moreover, lipid oxidation was found to dominate in soft cheese, even in low-fat products, although the importance of protein oxidation increases with decreasing lipid content due to changes in particle size and surface area.[17] Formation of hydroperoxides (LOOH), the primary oxidation products, is possibly initiated by type I photooxidation[28] or, alternatively, by nonradical addition of $^1O_2$ in a so-called type II mechanism mediated by excited flavin or porphyrin species.[3] The resulting peroxides are decomposed in the presence of suitable reaction partners such as proteins, transition metal ions, and heme compounds[29,30] and give rise to precursors of a variety of secondary oxidation products. Particularly the Fenton-like reaction, in which transition metals account for reductive cleavage of the peroxide bond, is well-documented and suggests the interference of a lipid-derived alkoxyl radical ($LO^{\bullet}$). Subsequent β-scission eventually gives hexanal and heptanal, both of which were found in irradiated low-fat (0.2%) cheese despite the lack of detectable hydroperoxide precursor. On the other hand, LOOH were readily observed in fattier soft cheese (6%) which, to a large extent, is due to higher substrate (i.e., lipid) concentration. The fattier matrix also protects lipophilic hydroperoxides against exposure to metal ions[29] thus preventing Fenton-like peroxide disintegration. The former suggests that under these conditions hexanal and heptanal were formed from (thermal or photochemical) processes that do not involve intervention of transition metals, which is confirmed by the lack of protection exerted by the chelating EDTA .

According to EPR analyses, EDTA also failed to affect protein radical formation, an early event upon light exposure of dairy products.[31] On the contrary, addition of green tea extract, a rich source of catechins (particularly epigallocatechin-3-gallate), changed spectra dramatically. The appearance of an intense, sharp EPR signal was associated with formation of stabilized semiquinone

radicals, which have a typical line width of around 10 G with reported g values of 2.00023, 2.0022, and 2.0075 (the latter of which correlates well with the value found for the GTE-derived species).[32−34] Most likely, catechin derivatives acted as hydrogen or electron donors and stability of the resulting phenolic radicals accounted for chain breaking activity. This effectively lowered hexanal and heptanal levels, but formation of lipid hydroperoxide remained unaffected. The latter finding suggests that, in the lipid phase where oxygen and lipophilic sensitizers like chlorophylls prevail, occurrence of a nonradical type II mechanism is more likely responsible for LOOH formation. Radical scavenging properties of phenolic compounds still effectively counter $LO^{\bullet}$, which thus inhibits conversion of hydroperoxides to secondary oxidation products (*vide supra*) and prevents formation of typical aldehyde off-flavors. Pentanal formation was however unaffected by the presence of green tea compounds because its formation differs from the hexanal and heptanal pathway.[21,22,35]

It was thus concluded that the presence of GTE effectively reduced formation of lipid-derived aldehydes in light-exposed reduced fat soft cheese, possibly resulting in improved flavor stability. Furthermore, insights in the complex set of mechanisms that account for light-induced quality changes in soft cheese have been further elaborated. Results corroborated the notion that both lipids and proteins suffer from oxidative changes, although degradation reactions presumably follow independent pathways. However, as reactions were initiated by mixed mechanisms (including type I and II photooxidations), the radical scavenging properties of GTE were only partly effective and complementary action of physical quenchers of excited states may be considered to offer a more comprehensive protection. Carotenes, for example, quench singlet oxygen, the active species in type II photooxidation, while they also exert an inner-filter effect to deflect light from being absorbed by sensitizers. Future research in this matter, in combination with new developments in packaging technology, will allow the flavor stability and shelf life of dairy products, such as soft cheese and its low-fat alternatives, to be significantly improved.

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REFERENCES

(1) Borle, F.; Sieber, R.; Bosset, J. O. Photo-oxidation and photoprotection of foods, with particular reference to dairy products—An update of a review article (1993−2000). *Sci. Aliments* **2001**, *21* (6), 571–590.

(2) Heelis, P. F. The photophysical and photochemical properties of flavins(isoalloxazines). *Chem. Soc. Rev.* **1982**, *11* (1), 15–39.

(3) Choe, E.; Min, D. B. Chemistry and reactions of reactive oxygen species in foods. *Crit. Rev. Food Sci. Nutr.* **2006**, *46* (1), 1–22.

(4) Huvaere, K.; Andersen, M. L.; Skibsted, L. H.; Heyerick, A.; De Keukeleire, D. Photooxidative degradation of beer bittering principles: A key step on the route to lightstruck flavor formation in beer. *J. Agric. Food Chem.* **2005**, *53* (5), 1489–1494.

(5) Dalsgaard, T. K.; Otzen, D.; Nielsen, J. H.; Larsen, L. B. Changes in Structures of Milk Proteins upon Photo-oxidation. *J. Agric. Food Chem.* **2007**, *55* (26), 10968–10976.

(6) Huang, R.; Hyun, J. K.; Min, D. B. Photosensitizing effect of riboflavin, lumiflavin, and lumichrome on the generation of volatiles in soy milk. *J. Agric. Food Chem.* **2006**, *54* (6), 2359–2364.

(7) Shiota, M.; Ikeda, N.; Konishi, H.; Yoshioka, T. Photooxidative stability of ice cream prepared from milk fat. *J. Food Sci.* **2002**, *67* (3), 1200–1207.

(8) Mattivi, F.; Monetti, A.; Vrhovsek, U.; Tonon, D.; Andres-Lacueva, C. High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light. *J. Chromatogr., A* **2000**, *888* (1−2), 121–127.

(9) Wold, J. P.; Bro, R.; Veberg, A.; Lundby, F.; Nilsen, A. N.; Moan, J. Active photosensitizers in butter detected by fluorescence spectroscopy and multivariate curve resolution. *J. Agric. Food Chem.* **2006**, *54* (26), 10197–10204.

(10) Mortensen, G.; Bertelsen, G.; Mortensen, B. K.; Stapelfeldt, H. Light-induced changes in packaged cheeses—a review. *Int. Dairy J.* **2004**, *14* (2), 85–102.

(11) Thron, M.; Eichner, K.; Ziegleder, G. The Influence of Light of Different Wavelengths on Chlorophyll-Containing Foods. *LWT—Food Sci. Technol.* **2001**, *34* (8), 542–548.

(12) Wold, J. P.; Veberg, A.; Nilsen, A.; Iani, V.; Juzenas, P.; Moan, J. The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. *Int. Dairy J.* **2005**, *15* (4), 343–353.

(13) Cardoso, D. R.; Franco, D. W.; Olsen, K.; Andersen, M. L.; Skibsted, L. H. Reactivity of bovine whey proteins, peptides, and amino acids toward triplet riboflavin as studied by laser flash photolysis. *J. Agric. Food Chem.* **2004**, *52* (21), 6602–6606.

(14) Choe, E.; Min, D. B. Mechanisms and factors for edible oil oxidation. *Compr. Rev. Food Sci. Food Saf.* **2006**, *5* (4), 169–186.

(15) Huvaere, K.; Andersen, M. L.; Storme, M.; Van Bocxlaer, J.; Skibsted, L. H.; De Keukeleire, D. Flavin-induced photodecomposition of sulfur-containing amino acids is decisive in the formation of beer lightstruck flavor. *Photochem. Photobiol. Sci.* **2006**, *5* (10), 961–969.

(16) Kim, G. Y.; Lee, J. H.; Min, D. B. Study of light-induced volatile compounds in goat's milk cheese. *J. Agric. Food Chem.* **2003**, *51* (5), 1405–1409.

(17) Andersen, L. T.; Lund, M. N.; Glyager, R.; Jensen, S. B.; Mortensen, G.; Skibsted, L. H. Light-induced deterioration of reduced-fat cream cheese. Relative importance of protein and lipid oxidation. *Milchwissenschaft* **2006**, *61* (2), 162–165.

(18) Mestdagh, F.; Kerkaert, B.; Cucu, T.; De Meulenaer, B. Interaction between whey proteins and lipids during light-induced oxidation. *Food Chem.* **2011**, *126* (3), 1190–1197.

(19) Alves, R. M. V.; Van Dender, A. G. F.; Jaime, S. B. M.; Moreno, I.; Pereira, B. C. Effect of light and packages on stability of spreadable processed cheese. *Int. Dairy J.* **2007**, *17* (4), 365–373.

(20) Pettersen, M. K.; Eie, T.; Nilsson, A. Oxidative stability of cream cheese stored in thermoformed trays as affected by packaging material, drawing depth and light. *Int. Dairy J.* **2005**, *15* (4), 355–362.

(21) Dalsgaard, T. K.; Sørensen, J.; Bakman, M.; Vognsen, L.; Nebel, C.; Albrechtsen, R.; Nielsen, J. H. Light-induced protein and lipid oxidation in cheese: Dependence on fat content and packaging conditions. *Dairy Sci. Technol.* **2010**, *90* (5), 565–577.

(22) Dalsgaard, T. K.; Sørensen, J.; Bakman, M.; Vognsen, L.; Nebel, C.; Albrechtsen, R.; Nielsen, J. H. Light-induced protein and lipid oxidation in cheese: whey as antioxidant. *Dairy Sci. Technol.* **2011**, *91* (2), 171–183.

(23) Østdal, H.; Andersen, H. J.; Nielsen, J. H. Antioxidative activity of urate in bovine milk. *J. Agric. Food Chem.* **2000**, *48* (11), 5588–5592.

(24) Westermann, S.; Bruggemann, D. A.; Olsen, K.; Skibsted, L. H. Light-induced formation of free radicals in cream cheese. *Food Chem.* **2009**, *116* (4), 974–981.

(25) Kristensen, D.; Skibsted, L. H. Comparison of three methods based on electron spin resonance spectrometry for evaluation of oxidative stability of processed cheese. *J. Agric. Food Chem.* **1999**, *47* (8), 3099–3104.

(26) Steffensen, C.; Mattinen, M. L.; Andersen, H.; Kruus, K.; Buchert, J.; Nielsen, J. Cross-linking of tyrosine-containing peptides by hydrogen peroxide-activated Coprinus Cinereus peroxidase. *Eur. Food Res. Technol.* **2008**, *227* (1), 57–67.

(27) Dalsgaard, T. K.; Nielsen, J. H.; Brown, B. E.; Stadler, N.; Davies, M. J. Dityrosine, 3,4-dihydroxyphenylalanine (DOPA), and radical formation from tyrosine residues on milk proteins with globular and flexible structures as a result of riboflavin-mediated photo-oxidation. *J. Agric. Food Chem.* **2011**, *57* (14), 7939–7947.

(28) Huvaere, K.; Cardoso, D. R.; Homem-de-Mello, P.; Westermann, S.; Skibsted, L. H. Light-Induced Oxidation of Unsaturated Lipids as Sensitized by Flavins. *J. Phys. Chem. B* **2010**, *114* (16), 5583–5593.

(29) Gardner, H. W. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. *J. Agric. Food Chem.* **1975**, *23* (2), 129–136.

(30) Schaich, K. M. Free radical initiation in proteins and amino acids by ionizing and ultraviolet radiations and lipid oxidation—part III: free radical transfer from oxidizing lipids. *Crit. Rev. Food Sci. Nutr.* **1980**, *13* (3), 189–244.

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

(32) Merdy, P.; Guillon, E.; Dumonceau, J.; Aplincourt, M. Characterisation of a wheat straw cell wall residue by various techniques: A comparative study with a synthetic and an extracted lignin. *Anal. Chim. Acta* **2002**, *459* (1), 133–142.

(33) Morsy, M. A.; Khaled, M. M. Novel EPR characterization of the antioxidant activity of tea leaves. *Spectrochim. Acta, Part A* **2002**, *58* (6), 1271–1277.

(34) Polovka, M.; Brezova, V.; Stasko, A. Antioxidant properties of tea investigated by EPR spectroscopy. *Biophys. Chem.* **2003**, *106* (1), 39–56.

(35) Lee, J. H.; Min, D. B. Changes of headspace volatiles in milk with riboflavin photosensitization. *J. Food Sci.* **2009**, *74* (7), C563–C568.