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Front-Face Fluorescence Measurement of Photosensitizers and Lipid Oxidation Products During the Photooxidation of Butter

A. Veberg,*†[1] E. Olsen,*† A. N. Nilsen,* and J. P. Wold*
*Matforsk AS, Norwegian Food Research Institute, Osloveien 1, NO-1430 Ås, Norway
†Norwegian University of Life Sciences, Department of Chemistry, Biotechnology, and Food Science, PO Box 5003, NO-1432 Ås, Norway

ABSTRACT

This paper shows that fluorescence spectroscopy can measure both degradation of photosensitizers and formation of lipid oxidation products in light-exposed butter. The photosensitizers were already notably degraded after 4 h of light exposure, whereas fluorescent lipid oxidation products were detected after 5 d. The fluorescence measurements were highly correlated with sensory assessments of acidic and rancid flavor. Photosensitizer degradation is therefore a promising indirect indicator of the onset of lipid oxidation in butter. Sensory analysis and measurement of peroxide value showed that the level of lipid oxidation was significantly higher for butter stored in air compared with butter stored in nitrogen (N_2). This might be explained by the formation of singlet oxygen from direct photooxidation and type II photosensitized oxidation. Addition of the singlet oxygen quencher β-carotene reduced the rancid flavor intensity in the air and N_2 packages from 9.0 to 4.9 and from 6.5 to 4.7, respectively. Results indicate that lipid oxidation in the butter stored in N_2 was mainly caused by type I photosensitized reactions, because addition of β-carotene had little effect on the rancid flavor intensity.
Key words: fluorescence spectroscopy, photooxidation, butter, photosensitizer

INTRODUCTION

In general, dairy products are susceptible to light and are exposed to light during processing and in grocery stores. Butter is particularly susceptible to photooxidation because of its high content of fatty acids, including some unsaturated ones (Bosset et al., 1995). Light induces the degradation of proteins, lipids, and vitamins, as well as the formation of off-flavors and changes in color. The most practical way to protect dairy products against such degradation is to exclude all kinds of light exposure.

Photooxidation can proceed either through direct photooxidation or by a photosensitizer. Direct photooxidation is due to free radicals produced by UV light and proceeds by normal free radical chain reactions (Frankel, 2005). Oxygen can also become activated in the presence of metals or metal complexes and initiate lipid oxidation by the formation of either free radicals or singlet oxygen ($^1\mathbf{O_2}$). Singlet oxygen is a very efficient oxidizing agent (Frankel, 2005).

Photooxidation by a photosensitizer can proceed through type I or type II reactions. These reactions often occur at the same time in a competitive manner. Type I reactions proceed through a free radical mechanism, and the reactions are most efficient at low oxygen concentrations (He et al., 1998). In type II reactions, the sensitizer reacts with oxygen to form $^1O_2$. These reactions are simpler and basically yield hydroperoxides (Spikes, 1989). Chain-breaking antioxidants will not inhibit these 2 types of photosensitized reactions. In contrast to type I reactions, type II reactions are affected by $^1O_2$ quenchers (Stratton and Liebler, 1997).

There are several methods to detect the photooxidation of dairy products, such as peroxide value (**PV**), sensory analysis, gas chromatography, and fluorescence spectroscopy. The first 3 methods either involve the use of relatively large amounts of solvents and are time-consuming, or they are expensive. Front-face fluorescence spectroscopy, on the other hand, is a rapid and nondestructive method that has been shown to provide strong correlations with sensory analysis for photooxidation in cheese (Wold et al., 2005) and sour cream (Wold et al., 2002a).

Fluorescence evaluation of the degree of photooxidation in dairy products has been done by measuring photosensitizer breakdown (Wold et al., 2005). This serves as an indirect measurement of the actual initiation of the oxidation process and can therefore indicate the onset of early lipid oxidation. In comparison, when evaluating autoxidation in meat by fluorescence, the formation of stable fluorescent lipid oxidation products is

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[1]Corresponding author: annette.veberg@matforsk.no

measured. These are mainly tertiary oxidation products and indicate somewhat more progressed oxidation. Previous studies have shown that the fluorescent lipid oxidation products in turkey and fish give an emission peak in the 470-nm region that increases with the degree of oxidation (Veberg et al., 2006a,b). This peak can be formed by the reaction of aldehydes with proteins or AA (Yamaki et al., 1992; Veberg et al., 2006b).

Recently, Wold et al. (2005) showed that naturally occurring porphyrins and chlorophylls play an important role as photosensitizers in dairy products. The degradation of these compounds showed higher correlation with sensory-measured lipid oxidation than did the degradation of riboflavin. Hansen and Skibsted (2000) stated that riboflavin had no effect on photooxidation in a dairy spread model system that was photodegraded. The $^1O_2$ quencher β-carotene protected riboflavin against photooxidation and the lipids against peroxidation.

Fluorescence spectroscopy has the interesting property of being able to measure both initiation of photooxidation and formation of tertiary oxidation products. Therefore, the aim of this study was to evaluate the photooxidation of butter using this method. To better understand the phenomena measured by fluorescence, the spectra were quantitatively compared with data from other analytical methods used for lipid oxidation: sensory analysis, headspace/GC-MS, and PV. We also wanted to investigate whether packaging in nitrogen (N_2) and air would have an effect on the photooxidation processes, and how the $^1O_2$ quencher β-carotene would influence these reactions.

MATERIALS AND METHODS

Dairy butter (TineSmør, ekte meierismør, 500-g packages) were obtained from Tine BA (Oslo, Norway), and all packages were from the same batch. Slices of butter (6.5 × 6.5 cm) with a thickness of 1 cm were wrapped in plastic film (Clingfilm, Toro, Norway), placed in amorphous polyethylene terephthalate-polyethylene trays (EKA AS, Hamar, Norway), and sealed with a laminate film based on oriented polyester, Biaxer 65 XX HFP AF (Wihuri Oy Wipak, Nastola, Finland; O_2 transmission rate of 5 cm^3/24 h at 23°C, 50% relative humidity) with a 511VG tray-sealing machine (Polimoon, Kristiansand, Norway). The gas in the packages was N_2 with traces of O_2 (0.03% ± 0.03). At the start of light exposure, small holes were made in the film of half the packages so that air could penetrate. Because effective wavelengths for the photooxidation of butter are in the near-UV and blue regions (Bosset et al., 1994), the packages were covered with a violet film (357 Royal Lavendel, Rosco Laboratories Inc., Stamford, CT) that transmits light mainly between 290 and 540 nm (maximum at 450 nm) and over 650 nm. Between 540 and 650 nm, less than 10% of the light is transmitted. The samples were stored for 0, 4, 8, 12, 24, 36, 48 (2 d), 72 (3 d), 120 (5 d), 168 (7 d), and 336 h (14 d) in an air or N_2 atmosphere under 2 broadband 575-W metal halide lamps (Osram HMI 575 W/SE, Osram, Munich, Germany), which have a relatively flat emission spectrum in the visible and near-UV region. The butter was exposed to a light intensity of 420 ± 20 lux, corresponding to 1.5 ± 0.1 W/m^2. This level is approximately the same as the amount of light dairy products are exposed to in grocery stores. The light intensity was measured by a lux meter (Lu-Ex02 digital lux meter, Ecom Rolf Nied GmbH, Assamstadt, Germany) and by a calibrated spectrometer (Apogee Spectroradiometer, Apogee Instruments Inc., Logan, UT), which was integrated in the 280 to 800 nm region. Because the intensity rates as well as the spectra of such lamps are dependent on the environmental temperature, intensity measurements were carried out in the refrigerated room where the storage experiment was performed. The 2 light sources were mounted on tripods approximately 2 m above the floor. The packages with butter were placed on the floor, where the temperature was 3 to 4°C. Sensory evaluation and fluorescence measurements were performed immediately after light exposure. The butter exposed for 14 d was evaluated only by fluorescence. Samples for PV, thiobarbituric acid-reactive substances (**TBARS**), and dynamic headspace/GC-MS analyses were packed in aluminum foil, vacuum-packed in plastic bags immediately after exposure, and stored at –80°C. Before analysis, the samples were tempered at room temperature for approximately 2 h, transferred to centrifuge tubes, and immersed in a water bath at 70°C until just melted. The butter was centrifuged for 5 min at 500 × *g* at 20°C, and the upper lipid phase was immediately used for the PV and dynamic headspace/GC-MS analyses. The butter was stored a second time at –80°C before TBARS analysis.

To investigate the effect of adding a $^1O_2$ quencher, dairy butter from a different batch (Tine BA) was melted in a water bath at 40°C and 600 mg of β-carotene/kg (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was added. The butter was stored in aluminum trays in a refrigerator at 4°C overnight. Slices of butter with a thickness of 1 cm were packed in N_2 or air, as described above, and exposed to light for 0, 3, or 7 d. Control samples (butter without β-carotene) stored in air and N_2 were also exposed to light for 7 d. All the samples were evaluated by sensory analysis and fluorescence spectroscopy separately from the main experiment.