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Assessment of the action spectrum for photooxidation in full fat bovine milk

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ABSTRACT

The action spectrum for photooxidation in full fat bovine milk was measured. Samples of milk with air or argon in headspace were exposed to narrow wavelength bands of light in the range 400–700 nm. Photooxidation in terms of off-flavors was measured by a sensory panel, volatile compounds by headspace solid phase micro extraction (SPME–GC–MS), and photobleaching of photosensitizers in milk (riboflavin, protoporphyrin IX and a chlorophyllic compound) by front face fluorescence spectroscopy. The action spectrum deviated significantly from the absorption spectrum of milk. Significant oxidation was induced by wavelengths around 400 nm and 500–650 nm in milk with air in headspace. Argon in headspace gave significant oxidation also at 700 nm. It is suggested that protoporphyrin IX and chlorophyll are responsible for oxidation induced by wavelengths >500 nm, and that also riboflavin is contributing from 400 to 500 nm.

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1. Introduction

Light induced oxidation is one of the main factors limiting shelf life of milk. Exposure to visible light leads to off-flavors related to oxidation of proteins and lipids due to excitation of photosensitizers among which riboflavin has been recognized to play a major role (Bradley, Lee, & Min, 2003). Riboflavin and beta-carotene are the two most prominent light absorbers in milk. They are present in full fat cow milk (typically 3.5% fat) at the approximate concentrations 141 μg/100 g and 20 μg/100 g, respectively (Lindmark-Månsson, Fondén, & Petterson, 2003), and consequently they absorb light at about the same level in the violet and blue region (400–500 nm) of the visible spectrum (Airado-Rodríguez, Intawiwat, Skaret, & Wold, 2011). Of the two absorbers, only riboflavin is a photosensitizer contributing to photochemical reactions leading to photooxidation. Beta-carotene absorbs light in the same region as riboflavin, and it has therefore been suggested to protect against photooxidation since less light then reaches riboflavin (Airado-Rodríguez et al., 2011; Skibsted, 2000). Beta-carotene also works as a quencher of the highly reactive singlet oxygen (Foote, 1968).

During the recent years it has been reported that naturally occurring residues of tetrapyrroles in milk play an important role in photooxidation of dairy products. This was first reported for cheese and butter (Wold et al., 2005) and later for milk (Airado-Rodríguez et al., 2011; Intawiwat et al., 2010). The exact identification of these tetrapyrroles remains, but protoporphyrin IX (PpIX) is one certain photosensitizer with notable contribution. In addition, there are at least four more photoactive compounds, most likely chlorophyll derivatives (Wold et al., 2006). The concentrations of some of these compounds have been tentatively determined in butter by front face fluorescence spectroscopy (Wold & Lundby, 2007) and are very low (0.02 ppm for PpIX). The compounds are fat soluble and when the concentrations for fat in butter are used for milk with 3.5% fat, the concentrations are in the range 0.8 ppb, about 250 times less than the concentration of riboflavin. All tetrapyrroles absorb strongly in the violet region (the Soret band), and then weaker in the blue to red region. Since riboflavin is not photoactive for wavelengths longer than about 500 nm, photooxidation in milk induced by longer wavelengths has so far been ascribed to these tetrapyrroles (Airado-Rodríguez et al., 2011).

Riboflavin is typically a type I photo sensitizer, thus generating radicals either by abstraction of an H-atom or donation of an electron through a direct reaction with double bonds in proteins and lipids (Foote, 1968, 1976; Huvaere, Cardoso, Homem-de-Mello, Westermann, & Skibsted, 2010), whereas e.g. chlorophylls act primarily as type II sensitizer with the generation of the highly reactive singlet oxygen as a result (Foote, 1968). Singlet oxygen has also been detected after riboflavin induced photooxidation in skim

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milk (Bradley, Lee, & Min, 2003), indicating that type I and II photoreactions are competing with each other. Abundance of oxygen might favor photoreactions of type II, while low concentrations of oxygen can lead to domination of type I reactions. For milk, this is relevant to consider since it can be packed with different levels of oxygen in headspace. The two reaction types might result in different oxidation products, and thereby different volatile compounds and sensory off-flavors (Airado-Rodríguez et al., 2011; Dalsgaard et al., 2010; Huvaere et al., 2011; Lee & Min, 2009).

An action spectrum is defined as the efficiency with which electromagnetic radiation produces a photochemical reaction plotted as a function of the wavelength of the radiation. The action spectrum of a material is usually quite similar to its absorption spectrum, but not always. It depends on the absorption spectrum of the photoactive compounds, but will also be influenced by other absorbing compounds, light scattering properties, as well as how the photoactive compounds are distributed in the microstructure of the material. The action spectrum can be used as a basis to explain the underlying photoreactions and to develop antioxidants and packaging materials with optimal protective properties.

The objective of the work presented in this article was to experimentally obtain the action spectrum in the visible range for photooxidation in full fat bovine milk. As a response for photooxidation we used sensory analysis and headspace SPME–GC–MS. Photobleaching of the photosensitizers riboflavin, protoporphyrin IX and a chlorophyllic compound was monitored by front face fluorescence spectroscopy. The results are presented followed by a discussion considering factors such as the effects of different light absorbing compounds, light scattering properties, and likely photoreactions in the microstructure of milk.

2. Materials and methods

2.1. Overview

Three different light exposure experiments were conducted. In the first, milk samples in different atmospheres were exposed to two broad regions of the visible spectrum (blue and orange). These samples were then analyzed by SPME–GC–MS. In the second and third experiment milk samples were exposed to light of narrower wavelength bands separated by 50 nm. After light exposure, milk samples were analyzed by SPME GC–MS, profiled by the sensory panel and analyzed by front face fluorescence spectroscopy.

2.2. Samples and light exposure conditions

Commercially produced, homogenized, pasteurized bovine milk with 3.5% fat content, packed in gable-top cartons, was obtained from a local dairy company (Tine, Oslo, Norway). The milk for each experiment was obtained from a single batch and stored at 4 °C in the dark before being repacked in plastic trays. Milk from all cartons was mixed before samples were made.

0.4 L milk was filled in transparent, high-density polyethylene (HDPE) trays ($5 \times 8.5 \times 13$ cm) (Promens AS, Kristiansand, Norway). A magnet for stirring was put into each tray. Each of these trays was placed in the middle of black polyethylene trays ($14.5 \times 20.5 \times 7.5$ cm) that were sealed with a top web consisting of PET/PE/ethylene vinyl alcohol/PE (Wipak) using a 511VG tray-sealing machine (Polimoon, Kristiansand, Norway). The surface of the milk samples was 117 cm^2. Two broadband 575 W metal Halide lamps (Osram HMI 575 W/SE, Osram, Munchen, Germany), which have a relatively flat emission spectrum in the visible region, were used as light source. The light intensity was measured and adjusted according to a calibrated spectrometer (Apogee Spectroradiometer, Apogee Instruments Inc., Roseville, CA). All light intensity adjustments and light exposure experiments were carried out in a cold-storage chamber at 4 °C.

In the first experiment (exposure to blue and orange light) the milk samples were packed with air, Ar or N_2 in headspace. The packages were covered with two types of colored plastic filters; a blue filter transmitting light between 375 and 550 nm (“69 Super Brilliant Blue”, manufactured by Rosco, Stamford, CT), and an orange filter transmitting light from about 530–750 nm (Orange transparent film based on PET (Ciba Specialty Inc., Basel, Switzerland). The filters were thoroughly described by Airado-Rodríguez et al. (2011). Two samples were covered with blue, two with orange and two samples were stored in the dark. This was done for samples in Ar, N_2 and air, a total number of samples of 18. Light intensity at surface of exposed samples was 1.6 W/m^2. Exposure time was 20 h. These samples were analyzed for volatile oxidation products by SPME–GC–MS. The colored plastic filters allowed light exposure of the entire surface of the milk samples.

In the second experiment the gas in the headspace was air or argon. The sealed black trays were covered on top with black carton with a 5 cm diameter circular whole in the middle. Over this hole, optical filters were placed to generate light of different wavelengths.

Circular ($D = 5$ cm) interference filters with bandwidth 40 nm and center wavelengths at 400, 450, 500, 550, 600, 650 and 700 nm (Filter set 03IFS008, Melles Griot, CA, USA) were used. Forty nm bandwidth means that a filter transmits a band of 40 nm around the center wavelength. For instance the 500 nm filter transmits light in the region 480–520 nm. The filters transmitted about the same share of light, and the combination of the exposure lamps and filters resulted in a light intensity at the milk surface of approximately 1.0 W/m^2. With this setup only 20 cm^2 of the sample surface was exposed. During storage time, the milk was stirred every 6 h to circulate the milk. The exposure time was 22 h. The samples were analyzed by the sensory panel and fluorescence spectra were measured immediately after light exposure, while samples for SPME–GC–MS were frozen at −80 °C and shipped at dry ice overnight and stored again at −80° C until analysis.

The storage experiment was run over two days. First day the following samples were run (number indicates wavelength, capital letter indicates atmosphere, Argon/aiR): 400A, 450R, 500A, 550R, 600A, 650R, 700A, control in darkA, control in darkR. Second day: 400R, 450A, 500R, 550A, 600R, 650A, 700R, control in darkA, control in darkR. In addition the first day, we made two controls in the dark and one light exposed argon sample under orange plastic filter (see specification above) for training of the sensory panel.

A third similar light exposure experiment similar to the second was repeated after two months, but exposure time was increased to 72 h, and only samples with argon in headspace were included. These samples were analyzed with SPME–GC–MS and front face fluorescence spectroscopy. Samples stored in air were not measured due to limited resources.

2.3. Sensory analysis

The milk samples were evaluated by a trained sensory panel at Nofima AS (Ås, Norway) using a modified quantitative method as described in ISO standard 6564 (ISO, 1985). The panel consisted of ten trained people. The panelists were selected and trained according to the recommendations in ISO standard 8586-1 (ISO, 1993). The sensory laboratory was designed according to guidelines in ISO standard 8589 (ISO, 1988) with separate booths and electronic data registration (CSA, Compusense Five, version 4.80, Guelph, ON, Canada). Prior to the assessments, the panel went through a training session with three samples, two fresh controls stored in the dark and one sample exposed to 650 nm light for 20 h, to agree on the definition of each attribute and variation in

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