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Light-Induced Oxidation in Semihard Cheeses. Evaluation of Methods Used To Determine Levels of Oxidation

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Light-induced oxidation in Havarti cheese (38% fat) stored in the dark and exposed to fluorescent light was evaluated by an array of chemical, physical, and spectroscopic methods. Light-induced changes were noticeable already after short exposure times (<12 h). A clear differentiation between samples stored in the dark and samples exposed to 1000 lx fluorescent light was obtained by means of the following methods: color measurements (*a** values), peroxide value determinations, and evaluations of volatile oxidation products by solid-phase microextraction gas chromatography (SPME-GC). The expected changes in peroxide values in relation to storage time were not evident. Measuring free radicals by electron spin resonance spectrometry could not be done to distinguish between samples, possibly due to the conversion of radicals during sample preparation. However, significant light-exposure effects on secondary oxidation products, detected by SPME-GC, were noted for 1-pentanol, 1-hexanol, nonanal, and benzaldehyde.

KEYWORDS: Havarti cheese; lipid oxidation; light-induced oxidation; analytical procedures; photooxidation; color; electron spin resonance spectrometry; peroxide value; solid-phase microextraction gas chromatography−mass spectrometry

INTRODUCTION

Fluorescent light from retail displays and sunlight trigger oxidation of cheeses. This may lead to formation of, for example, off-flavors (protein and lipid oxidation), discoloration, loss of nutrients (primarily riboflavin), and formation of potentially toxic compounds (*1*). This will in turn result in decreased sales appeal. During the past decade, marketing of cheese in transparent retail packages has grown dramatically. At the same time store business hours have increased, and the packaging materials have been down-gauged. The net result is that photooxidative changes in the more light-sensitive dairy products are likely to take place. The review by Mortensen et al. (*2*) gives an introduction to light-induced changes in cheeses and elaborates on possible preventive measures.

Suitable methods for evaluating oxidative changes often vary from one product to the other. The peroxide value (POV) method has been applied to many dairy products, probably due to the existence of a rather simple official method issued by the International Dairy Federation (*3*). This method was initially aimed at peroxide determinations of butter oils but has subsequently been used for extracted cheese fats (*4*, *5*). Peroxide value experiments on extracted cheese fats (*4*, *5*) revealed no differences in POVs in either light-exposed or in dark-stored samples. However, light-induced differences were evident in the study by Kristensen et al. (*4*) when sensory analysis was performed only a few days after the experiment had begun. Hence, one may suspect that changes in POVs take place soon after light exposure has been initiated. This study reports POV measurements during the first 24 h of light exposure. Additionally, the following parameters were included in the evaluation of light-induced oxidation: color, free radicals [electron spin resonance spectrometry (ESR)], odor, and formation of secondary oxidation products [solid-phase microextraction gas chromatography−mass spectrometry (SPME-GC-MS)]. The additional analyses were included to evaluate other potential methods for photooxidation studies of Havarti cheese as well as other semihard cheeses. It was outside the scope of the experiment to develop new methods or improve existing methods.

MATERIALS AND METHODS

Packaging and Storage of Cheese. Sliced Havarti cheeses (38% fat) were obtained from Arla Foods amba (Viby J, Denmark) as part of its standard production. The cheeses were all withdrawn from the same batch of cheese to obtain homogeneous samples. The slices measured 12 × 12 cm and were ∼3 mm thick. Each sample (∼350 g of sliced cheese), corresponding to nine slices, was flow-packaged in conventional packaging materials consisting of oriented polyamide/linear low-density polyethylene (OPA/LLDPE) (Amcor Flexibles Europe, Horsens, Denmark). The oxygen transmission rate of the material was determined to be 40 cm^3/m^2 (24 h, 23 °C, 0/50% relative

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humidity), according to the manufacturer. No labels were attached to the packages. The light transmission of the packaging material was determined using a Cintra 40 spectrometer (GBC Scientific Equipment, Dandenong, Victoria, Australia) equipped with an integrating sphere detector. Only very limited amounts of light were transmitted through the packaging material at wavelengths shorter than 230 nm (<1%). Wavelengths in the range 230−290 nm were increasingly transmitted; at the latter wavelength, >89% of the light was transmitted, and at wavelengths above 290 nm, transmission remained constant at 91 ± 2%.

Cheeses were packaged in a modified atmosphere consisting of approximately 25% CO_2 and 75% N_2 at a local dairy plant. The sliced cheeses were stored at 3−4 °C in a display counter under conditions similar to those in retail stores. Samples were exposed 24 h per day to light from Philips TLD 18W/830 New Generation (Philips, Eindhoven, The Netherlands) fluorescent tubes with a measured light intensity of 1000 lx at the surface of the cheese. Half the packages were covered with black plastic to protect the cheeses from light. The samples were rotated regularly to minimize possible temperature differences in the display cabinets. Chemical and color measurements were performed after 0, 2, 4, 6, 8, 10, 12, 16, and 24 h. Secondary oxidation product analyses were carried out after 0, 12, 24, 168 (1 week), 338 (2 weeks), and 506 h (3 weeks), respectively. Two packages stored in the dark and two packages exposed to fluorescent light were withdrawn at the respective times of analysis. Only the top cheese slices of the packages were used for analysis, in view of the top slices representing the worst-case scenario.

Characterization of the Cheeses. Characterization of the cheeses was carried out using standard methods to include total fat (*6*), fatty acid composition (*7*), total protein (*8*), total solids (*9*), and ash (*10*).

Lipid Extractions. Lipid extractions using the modified Folch extraction method described in Mortensen et al. (*5*) were applied.

Color. Surface color of the top slice in the cheese package was measured by a Minolta tristimulus Chromometer CR-300 (Minolta Camera Co. Ltd., Osaka, Japan) using CIELAB *Lab* values (www.cie.co.at/cie/). The measure of lightness (L^*, range = 0−100) represents black to white, the redness measurements (a^*) describe green to red, and the yellowness measurement (b^*) represents blue to yellow. The chromometer was standardized using a white standard plate. The results reported are averages of five measurements on cheeses exposed to identical treatment. The measurement of the lightness (L^* value) may be reported separately, but a^* and b^* values are merely coordinates that indirectly reflect hue and chroma (*11*). As such, they are difficult to interpret separately, and oftentimes the hue angle is used to overcome this obstacle. Hue angle is defined as $[\tan b^*/a^*]^{-1}$, that is, the angle between the hypotenuse and 0° on the a^* axis, and thus provides information about the overall color change.

Gas Composition. Prior to opening the packages, gas composition, expressed as percent O_2 and percent CO_2, was determined using a CheckMate 9900 gas analyzer (PBI Dansensor, Ringsted, Denmark). Samples with oxygen contents >1% and carbon dioxide contents <20% were excluded from the experiment, as these variations could be attributed to package leaks.

Peroxide Values. The colorimetric, official reference method issued by the International Dairy Federation (IDF) (*3*)/Shantha and Decker (*12*) was used employing an HP 8453 UV−vis spectrophotometer (Hewlett-Packard, Palo Alto, CA) for measurements of A_{500}. Results, expressed as milliequivalents of oxygen per kilogram of lipid, are means of triplicate determinations.

Formation of Free Radicals. ESR measurements were used to monitor formation of radicals in the Havarti cheese according to the procedure previously described by Kristensen et al. (*4*) with minor modifications, which are described below. The ESR measurements were carried out with a JEOL FR30 spectrometer (JEOL, Tokyo, Japan). Typical instrument parameters were as follows: microwave power, 4 mW; center field, 335.598 mT; sweep width, 5 mT; sweep time, 8 min; modulation width, 0.4 mT; time constant, 1 s. Relative signal intensity was measured using a manganese internal standard.

Odor. At the time of sampling, the analysts smelled the samples in order to qualitatively describe any odor changes.

Secondary Oxidation Products. The contents of secondary oxidation products were determined after 0, 12, and 24 h and 1, 2, and 3 weeks. The quantification is based on the addition of 10 μL of internal standard (1.135 g of 5-methyl-2-hexanone/1000 mL of rapeseed oil). The following SPME-GC-MS method was used: 2 g of sample and the internal standard were weighed in a 10 mL vial and frozen until analysis. Prior to analysis, the samples were thawed at 60 °C for 30 min. Subsequently, the SPME fiber (75 μm Carboxen/PDMS, Supelco, Bellefonte, PA) was inserted into the headspace of the vial for 20 min at 60 °C. The fiber was manually inserted into an HP GC 5890 series 11 gas chromatograph equipped with an MS detector 5971 series (Hewlett-Packard). The analyses included the following parameters: HP FFAP 25 m × 0.2 mm × 0.30 ìm (Hewlett-Packard) column; injection temperature, 250 °C; helium carrier gas flow, 0.4 mL/min; splitless; purge time, 2 min; temperature program, initial temperature of 50 °C for 5 min, increased to 80 °C at 6 °C/min, followed by an increase to 210 °C at 10 °C/min. The following mass spectrometry parameters were applied: 0−1.6 min, scan m/z 41−300 (2.84 scan/s); 1.6−31 min, scan m/z 33−300 (5.24 scan/s).

The Carboxen/PDMS fiber was selected on the basis of initial experiments (not published) with this fiber used for the detection of polar and nonpolar low molecular weight volatile compounds. Furthermore, Marsili (*13*) found that the PDMS fiber was suitable for evaluations of light-induced oxidation in milk. Temperature and time combinations with respect to the preconditioning of samples were evaluated prior to establishment of the applied SPME method. Response factors were not included in the experiment because a semiquantitative method was applied. Hence, the results reported are not "true" parts per million values but internal standard equivalents. However, response factors close to 1 are expected for 1-hexanol, 1-pentanol, and nonanal, which resemble the internal standard, 5-methyl-2-hexanone.

Data Analysis. Principal component analysis (PCA) with full cross-validation was used to explore color, POV, and ESR data. Variables were autoscaled (mean centered and standardized by 1/SD) prior to analysis.

To correlate the volatile headspace profile with the other parameters describing the cheese, partial least-squares regression (PLSR) analysis was employed using the software program, the Unscrambler, version 7.5 (CAMO, Oslo, Norway). The X variables were defined as the volatiles identified in the cheeses, that is, 15 compounds. The design variables were defined on the basis of the experimental plan, that is, storage time (12 and 24 h, in addition to 1, 2, and 3 weeks) and light/darkness, the latter using a dummy variable (0 for samples stored in the dark and 1 for light-exposed samples). Both X and Y variables were autoscaled. Full cross-validation and Jack-Knife were used to validate the calculated model. The optimal numbers of principal components were chosen at first minimum in root-mean-square error of cross-validation (RMSECV).

POV means were compared by analysis of variance (ANOVA) using the statistical package SPSS (release 9.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Characterization of the Product. The product contained 39.8% total fat with the following fatty acid distribution: saturated fatty acids, 61.4%; monounsaturated fatty acids, 34.9%; and polyunsaturated fatty acids, 4.0%. Protein and dry matter contents were determined to be 19.2 and 64.0%, respectively. Ash content totaled 3.7%. Thus, the characterization revealed that the products were well within the expected gross composition range for this cheese type.

Preliminary Explorative Data Analysis. To fully explore the data, PCA was performed and included the following parameters: L^*, a^*, b^* values as well as POVs and ESR results. The PCA results are illustrated in **Figure 1**.

From the score plot in **Figure 1**, it is obvious that light exposure shifts the samples toward higher values of principal component (PC) 1 and PC 2. Furthermore, the loading plot reveals that this is synonymous with higher peroxide and a^* (redness) values.

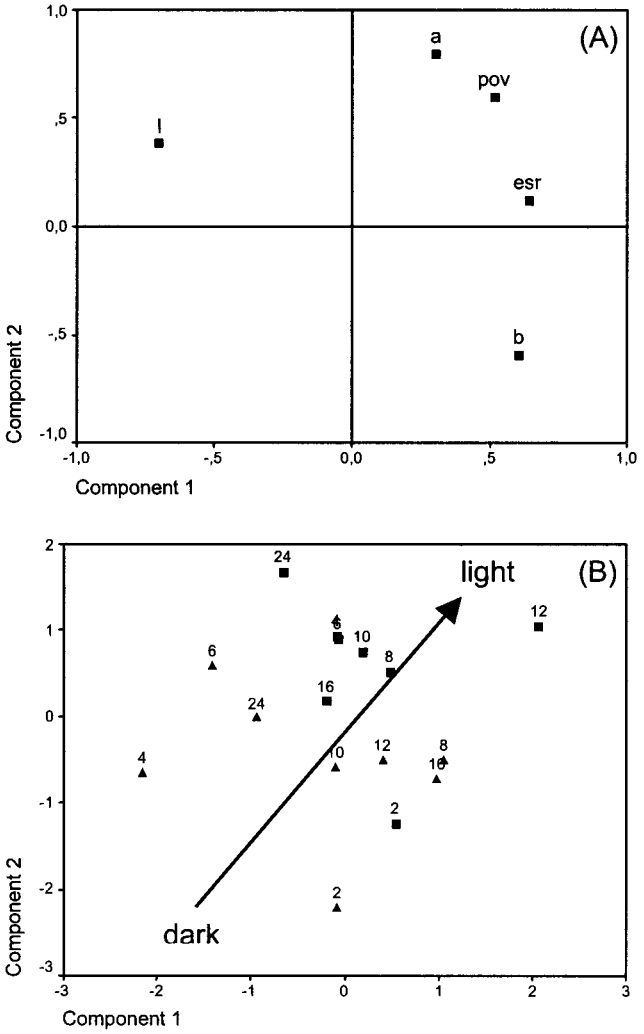

Figure 1. Preliminary PCA of selected variables: *L**, *a**, *b**, peroxide values, and free radicals determined by ESR with (A) loading plot and (B) score plot [cheeses stored at (■) 1000 lx and (▲) in the dark; numbers refer to hours of storage]. The variances explained by the first and second principal components were 32 and 30%, respectively.

Orthogonal to this dark/light axis is an axis with variations in *L** and *b** and partly also in ESR values. This indicates that these three variables are unaffected by light exposure. The score plot in **Figure 1** reveals no systematic variation due to storage time.

Color. Color measurements may be used as a rapid method for evaluating color bleaching. Color bleaching, which is an indirect effect of light-induced oxidation, depends on fluorescent light exposure (both intensity and time) (*4*, *13*−*15*). A clear separation of samples ($p < 0.05$) stored in the dark versus exposed to light was noted for *a** (redness) values as is seen in **Figure 2**. The *L** (lightness) and *b** (yellowness) parameters could not be used to distinguish light from dark-stored samples (refer to **Figure 1**). No significant effect of storage time was found.

Kristensen et al. (*4*) also performed color measurements on Havarti cheese. They found no significant effects of light exposure on *L** or *b** values, whereas *a** values increased significantly during light exposure; thus, the results are in compliance with ref *4*. Increase in redness has previously been attributed to a phenoloxidase reaction, which leads to the formation of melanin compounds (*17*). However, unpublished

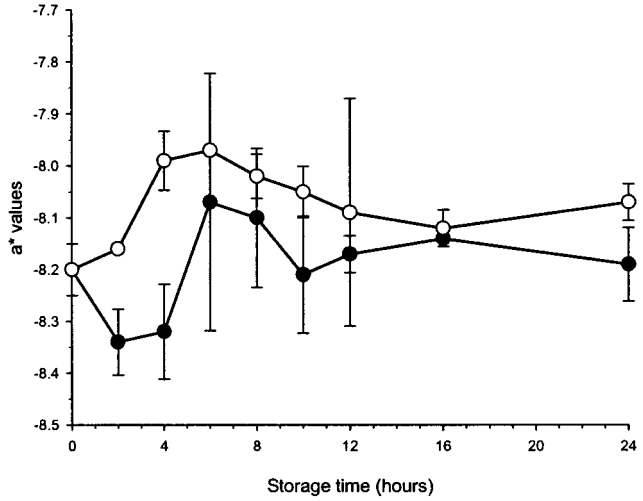

Figure 2. Changes in redness (*a** values) for Havarti cheeses stored for 0–24 h at 3–4 °C in the dark (●) and at 1000 lx (○).

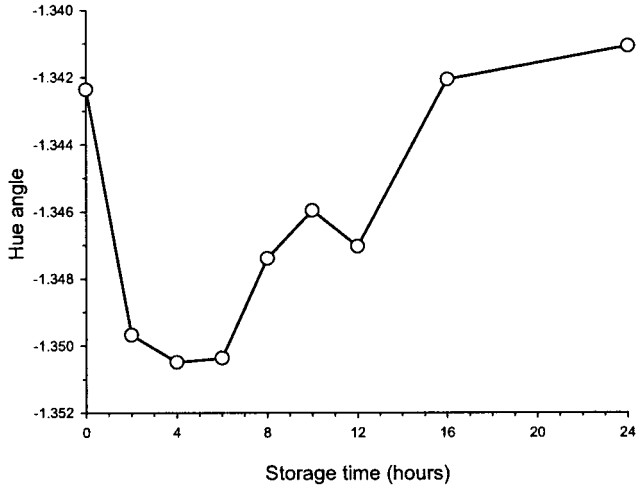

Figure 3. Changes in hue angles (arctan *b**/*a**) for Havarti cheeses stored for 0–24 h at 3–4 °C at 1000 lx (○).

results reveal that *a** values do indeed increase during light-exposed storage.

Hue angles, which depict the overall color changes, are shown in **Figure 3**. As is seen in **Figure 3**, a clear differentiation of color changes into two stages during light exposure is evident. The first stage may be caused by degradation of β-carotene (acting as an inner filter), as suggested by Hansen and Skibsted (*18*), followed by a hue change attributable to the formation of shorter, conjugated chain breakdown products and the appearance of riboflavin, which have been screened off by partial overlap with the absorption spectrum of β-carotene. These changes may be accompanied by oxidative changes ascribed to the destruction of the free radical scavenger β-carotene (*19*) and exposure of the inherent singlet oxygen sensitizer riboflavin.

Gas Composition. Figure 4 depicts the headspace oxygen concentration in samples exposed to light and stored in the dark. Although low, the oxygen levels reported are sufficient for oxidative processes to occur. A significant effect of light exposure and storage time was noted ($p < 0.05$), resulting in lower oxygen contents in the light-exposed samples. This may result from the consumption of oxygen during oxidation. Furthermore, the oxygen content decreased concurrently with storage time progression, most likely due to oxidative reactions and microbial activity.

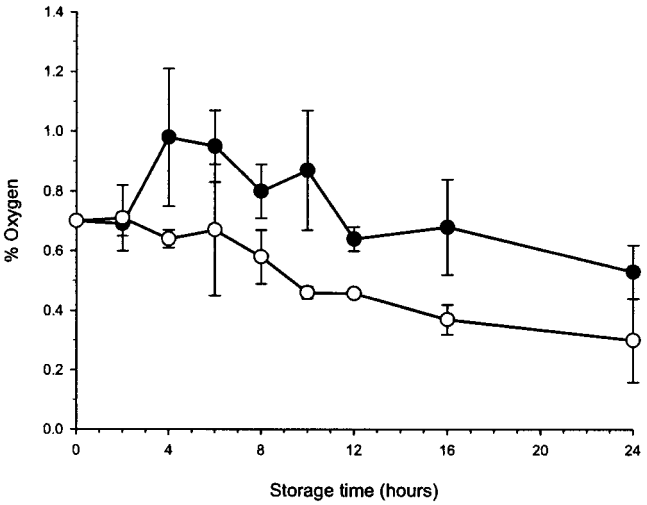

Figure 4. Oxygen contents in headspace measured throughout 0–24 h of storage at 3–4 °C using a CheckMate gas analyzer (PBI Dansensor, Ringsted, Denmark). Samples were stored in the dark (●) or at 1000 lx (○).

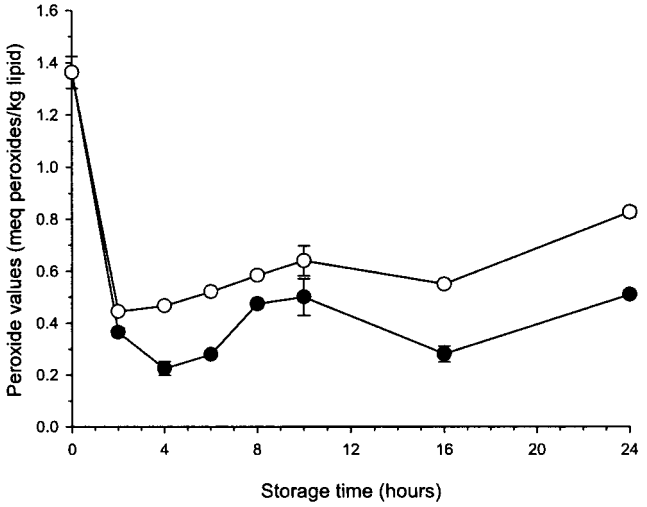

Figure 5. Changes in peroxide values in Havarti cheeses stored for 0–24 h at 3–4 °C in the dark (●) or at 1000 lx (○).

No significant changes in carbon dioxide due to respiration were observed during storage in the dark or at 1000 lx, and consequently results are not shown.

Peroxide Value. Figure 5 depicts the development in POVs during storage. A sharp decrease in POVs was observed within the first 4 h, followed by a slightly increased level of POVs. Apparently, POV development in light-exposed samples stabilized at higher levels than did the values of samples stored in the dark ($p < 0.05$). Stabilization was attained as early as within 2 h of storage. The development in peroxides resembles that found by Mortensen et al. (*5*).

Additionally, the POVs are in the same range as found earlier for Havarti cheeses (*4*). The POV measurements did not reveal the classical picture of hydroperoxides being formed and subsequently being broken down into numerous reaction products (see e.g. ref *20*). One reason may be that the formation of peroxides had taken place already during processing. Additionally, the turnover rate of hydroperoxide degradation may be so rapid that the changes are not detectable when using the applied methodology. It is notable, however, that the change of POV over time is similar to the changes in hue angle depicted in **Figure 3**, indicating a link between color changes and initial decrease in POV followed by the gradual increase in POVs toward the end of the storage period. PCA also introduced the correlation between POVs and *a** values (**Figure 1**).

To conclude, peroxide determinations could be used to separate dark-stored samples from light-exposed ones. However, the expected changes in relation to storage time were not evident, and the differences between dark-stored and light-exposed samples were slight, although significant. Hence, the POV method, applied here, cannot be used as a reliable method for the determination of primary oxidation products in light-exposed Havarti cheese.

Formation of Free Radicals. ESR methodology is used to detect free radicals in order to measure initial changes in foods. ESR measurements have been used for numerous dairy applications, for example, processed cheese (*21*), raw milk (*22*), milk powder (*23*, *24*), and Havarti cheese (*4*). The method applied produced contradictory results, not resembling those of Kristensen et al. (*4*) (results not shown).

Part of the variations in the ESR results may be attributable to the formation and disintegration of radicals during the freeze-drying process. Thus, at the time of measuring, the free radical contents may be decreasing with the resulting increase in the concentrations of secondary oxidation products. However, improving methodology may be a solution. This also explains the suitability of the method for dairy products, which are more fluid in nature (*22*−*24*) and do not require freeze-drying prior to analysis.

Odor. The simple, but indicative, odor evaluations of the stored cheeses revealed that the cheeses stored in light were characterized as sweet and buttery in the interval of 0−6 h of storage, which changed to nauseating and sweet from 6 to 12 h. After 12 h of light-exposed storage, a change into a less technical/nauseating and a more acidic odor was noted. This impression lasted until the end of the storage period (24 h). The samples stored in the dark retained their sweet and buttery odor throughout the storage experiment. The qualitative results of the odor evaluations, paralleling the results of **Figures 2**, **3**, and **5**, indicated a very rapid quality deterioration rate due to photooxidation.

Secondary Oxidation Products. SPME is a simple, solvent-free, inexpensive method for sampling, extracting, and concentrating volatile components from foods (*25*, *26*). The method has proven to be suitable for the determination of volatile secondary oxidation products, especially when limited sample amounts are available. The following components were identified by SPME-GC-MS: dimethyl disulfide, hexanal, 2-heptanone, 1-pentanol, 3-hydroxy-2-butanone, 2-heptanol, 3-methyl-2-butene-1-ol, 1-hexanol, 2-nonanone, nonanal, 2,4-heptadienal, 2-undecanone, butanoic acid, benzaldehyde, and hexanoic acid. PLSR with Jack-Knifing revealed that the concentration of the following secondary oxidation products increased significantly during light exposure: 1-pentanol, 1-hexanol, nonanal, and benzaldehyde. The development in 1-pentanol, 1-hexanol, nonanal, and benzaldehyde is depicted in **Figure 6**. The content of volatile free fatty acids increases during storage (results not shown) due to a weak lipolytic activity in the Havarti cheese.

The aliphatic aldehydes, for example, nonanal, are the most important volatile breakdown products because they are major contributors to unpleasant odors and flavors of food products (*27*). Nonanal has previously been detected in similar cheeses such as Danbo (*28*) and Havarti (*29*). Lund et al. (*29*) observed an increase of nonanal levels during light exposure only.

Benzaldehyde has been identified in cheeses such as Danbo (*28*) and Cheddar (*30*, *31*). McSweeney and Sousa (*32*) and

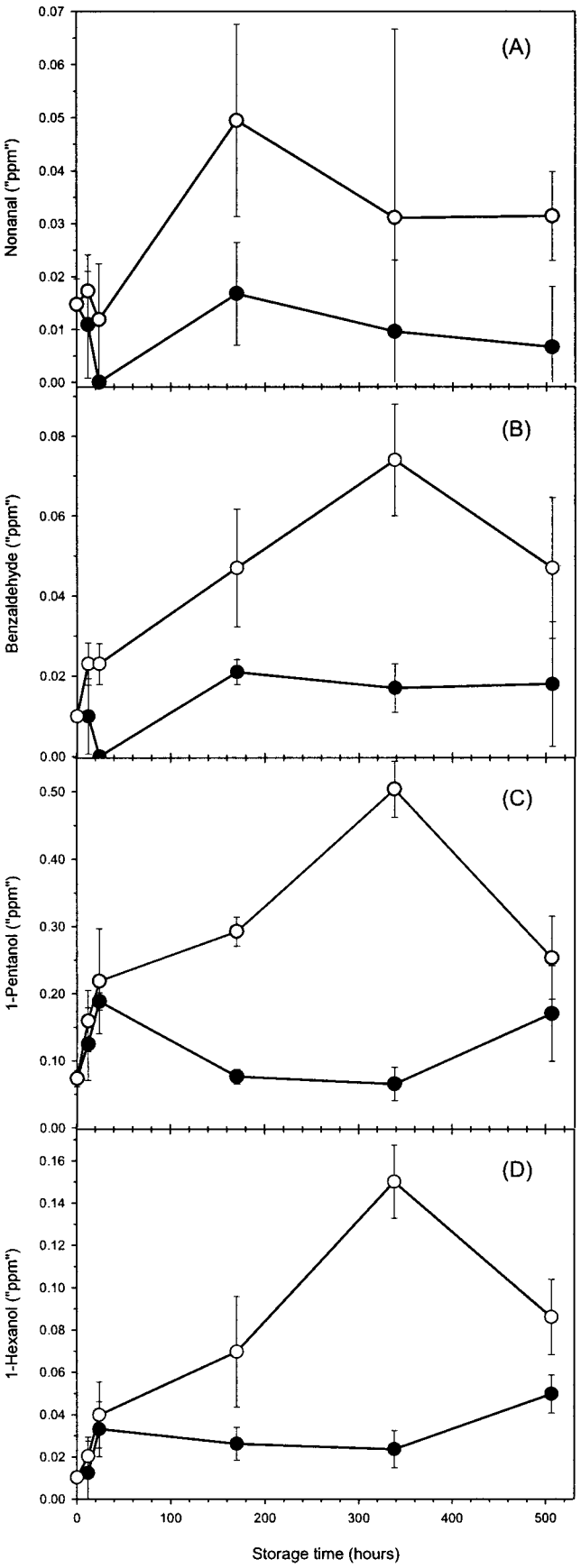

Figure 6. Formation of (A) nonanal, (B) benzaldehyde, (C) 1-pentanol, and (D) 1-hexanol in Havarti cheeses stored for 0–3 weeks at 3–4 °C in the dark (●) or at 1000 lx (○). Values are semiquantitative and based on internal standard equivalents.

Yvon and Rijnen (*33*) note that benzaldehyde may be formed from phenylalanine, which is most likely converted enzymatically into phenyl pyruvate, followed by splitting into benzaldehyde and phenylethanol.

The formation of primary alcohols in cheese is mainly caused by the reduction of the corresponding aldehydes and follows a reaction pathway involving alcohol dehydrogenase (*34*, J. Sørensen and L. Vognsen, Arla Foods, unpublished results). Aldehydes may originate from the degradation of unsaturated fatty acids (*34*−*37*) or from the degradation of amino acids (*32*−*34*). Primary alcohols, such as 1-pentanol and 1-hexanol, make only a minor contribution to the off-flavors produced by oxidation due to significantly higher flavor thresholds than those of the corresponding aldehydes (*27*). However, flavor may be affected indirectly, because the alcohols may form esters with fatty acids (*32*, *38*). In accordance with the results of Lund et al. (*29*), an increase in the formation of 1-pentanol during light-exposed storage was noted. This alcohol has also been detected in similar cheeses, such as Cheddar (*38*, *39*) and Danbo (*28*). 1-Hexanol contents increased significantly during light exposure. 1-Hexanol has previously been reported to be a flavor component in Danbo cheeses (*28*). Although the identified components were present in small quantities, even below threshold values reported in simple systems, the analysts were able to detect changes in the light-exposed samples. However, analysis of the aroma of dairy products is deemed to be very complex due to the heterogeneous nature of milk (*40*). Synergistic or antagonistic effects of the volatiles may be noted, and active enzyme systems or interactions with other components (*41*) render aroma analysis a complicated task. Instrumental analysis of aroma compounds should be consolidated by initial sensory evaluations, because compounds present at concentrations below the detection level may be major contributors to the off-flavor development.

Marsili (*13*) evaluated SPME-GC versus dynamic headspace performance in a study on the analysis of light-induced lipid oxidation products in milk and reported the method to have great potential within analysis of secondary oxidation products, being effective in detecting low levels of flavor compounds in foods and beverages. Furthermore, it was concluded that SPME was more accurate than dynamic headspace in measuring the quantity of lipid oxidation products in milk that had been exposed to fluorescent light.

In this study, SPME-GC proved to be a suitable methodology for the detection of volatile secondary oxidation products and for differentiating between Havarti cheeses exposed to fluorescent light or stored in the dark. The results are well in keeping with the indicative odor evaluations performed by the analysts.

To conclude, quality changes in light-exposed samples apparently took place already after only a few hours of storage, as indicated by both odor evaluations and chemical analyses. Color measurements (a^* values), POVs, and SPME-GC-MS proved to be suitable for the differentiation of dark-stored and light-exposed Havarti cheeses. L^* and b^* values could not be used to separate exposed from nonexposed samples. Measuring the free radicals by ESR could not be used to distinguish between samples. Further optimization of the methodology may be a solution. Peroxide determinations could be used to separate dark-stored from light-exposed samples. However, the expected changes in relation to storage time were not evident.

ABBREVIATIONS USED

ANOVA, analysis of variance; ESR, electron spin resonance spectroscopy; OPA/LLDPE, oriented polyamide/linear low-

density polyethylene; PC, principal component; PCA, principal component analysis; PLSR, partial least-squares regression; POV, peroxide value; RMSECV, root-mean-square error of cross-validation; SPME, solid-phase microextraction.

SAFETY

Chloroform is a mutagen agent; hence, precaution must be taken when determining peroxide values.

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