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Effect of specific wavelengths on light-induced quality changes in Havarti cheese

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The effects of exposure of slices of Havarti cheeses to monochromatic light of wavelengths 366 nm, 405 nm, and 436 nm, respectively, were studied by tristimulus colorimetry, solid-phase microextraction gas chromatographic analysis of volatiles, and open-end fluorescence spectroscopy. Having determined the photon fluxes of the three wavelengths by ferrioxalate actinometry, it was possible to quantify the effects of light exposure in an absolute manner. For all analyses, the most severe effects were caused by visible light, leading to colour bleaching, change in hue, riboflavin degradation, and formation of the secondary oxidation products hexanal, 1-pentanol, and 1-hexanol. Apparent quantum yields for formation of hexanal and 1-pentanol were found to be insignificantly different for 405 nm and 436 nm exposures, having values of $(3–5).10^{-5}$ mol . einstein^{-1} and $(9–13).10^{-5}$ mol . einstein^{-1}, respectively. These compounds were not formed when exposed to 366 nm light. In contrast, 1-hexanol was formed when exposing cheese to all three wavelengths, resulting in apparent quantum yields of $(2–6).10^{-5}$ mol . einstein^{-1}. The results obtained are discussed in relation to the interplay between inherent product colorants, light sources, and transmission characteristics of the packaging materials.

Keywords: Photooxidation, quantum yields, wavelength effects, fluorescence spectroscopy, solid-phase microextraction GC/MS.

Fluorescent light in retail displays initiates oxidative processes and results in off-flavour formation and decreased nutritional value in dairy products (Bosset et al. 1995; Skibsted, 2000). Only absorbed light can initiate chemical reactions, and the more photons absorbed, the more molecules will be excited (Wayne & Wayne, 1996). The spectral distribution of the light source, the absorption spectra of sensitive components, and the wavelength dependence of quantum yields may be used for predicting photooxidative quality deterioration (Skibsted, 2000). In the long run, these factors may also be used to select the most appropriate packaging material for a given product and to develop new generations of fluorescent light tubes.

So far, the effect of specific wavelengths present in commercial fluorescent light has been insufficiently examined. Sattar et al. (1976, 1977) were among the first to address wavelength effects on light-induced oxidation of lipids, vitamin A, and β-carotene. Sattar et al. (1976) concluded, by monitoring peroxide values, that milk fat oxidation was affected by wavelengths shorter than 455 nm, indicating riboflavin-sensitising. Bosset et al. (1995) summarized that light in the low wavelength range of the visible spectrum (violet-blue light) is critical for products containing riboflavin.

Skibsted (2000) suggested the replacement of rate constants for light-induced processes in foods with quantum yields (proportional factor of light absorbed and rate of resulting chemical reaction). However, few experiments of relevance to dairy products, incorporating quantum yield determinations, have been reported. Hansen & Skibsted (2000) and Petersen et al. (1999) calculated apparent quantum yields in a model dairy spread system and Cheddar cheese colorant model systems, respectively.

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Quantum yields were determined for 366 nm, 405 nm, and 436 nm wavelengths in both studies, since these wavelengths are part of the mercury lamp line spectrum. Hansen & Skibsted (2000) stated that 366 nm was the most detrimental of the three with respect to the dairy spread system. They noted that this was most likely attributable to low light protection of the lipids offered by β-carotene at 366 nm, in contrast to its high absorbance at both 405 nm and 436 nm. Moreover, Petersen et al. (1999) noted that exposure to UV-light (313 nm and 366 nm) resulted in more definite photobleaching of annatto and β-carotene solutions than if exposed to visible light (436 nm).

Hence, most evaluations of photooxidative quality changes, incorporating calculations of quantum yields, have been performed on aqueous model systems. In contrast, the present study aimed at directly quantifying the deteriorative effects in cheese of monochromatic wavelengths emitted by the fluorescent tubes of dairy display cabinets. In accordance with the suggestion of Skibsted (2000), quantification of formation of volatile secondary oxidation products was included in the study. Moreover, as the formation of volatiles is closely linked to the concomitant photodegradation of the inherent colorants, riboflavin and β-carotene, this interplay was studied qualitatively employing tristimulus colorimetry and open-end fluorescence spectroscopy.

Materials and Methods

Packaging and storage of cheese

Sliced Havarti cheeses (38% fat) were obtained from Arla Foods amba (DK-8260 Viby J, Denmark) as part of its standard production. The cheeses were all withdrawn from the same batch of cheese in order to obtain homogeneous samples. The slices measured 12 × 12 cm and were 0·3 cm thick. Each sample (approx. 350 g 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 Raackmann, DK-8700 Horsens, Denmark). The oxygen transmission rate of the material was determined to be 40 $cm^3/m^2/24$ h/atm (23 °C, 0/50% RH), according to the manufacturer. No labels were attached to the packages. Cheeses were packaged in a modified atmosphere containing 25% CO_2 and 75% N_2 at a local Arla Foods dairy plant. The cheeses were stored in the dark at 0–2 °C prior to the photolysis experiments, which were all carried out within one month.

Gas composition

Prior to opening the cheese packages, gas composition was determined using a CheckMate 9000 gas analyser (PBI Dansensor, DK-4100 Ringsted, Denmark). Packages with O_2 contents above 0·1% and CO_2 contents below 20% were excluded from the experiment, as such variations could be caused by package leaks.

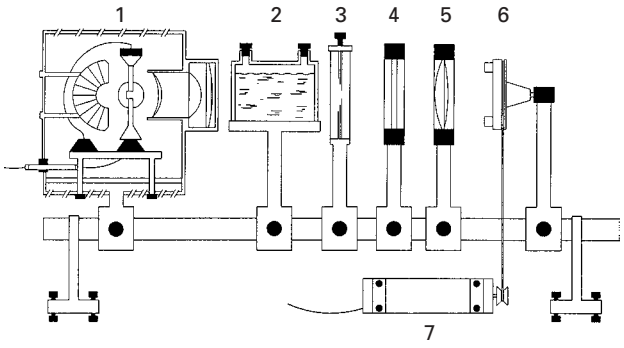

Fig. 1. Photolysis experimental set-up. (1) Hg lamp, (2) heat filter, (3) iris diaphragm, (4) interference filter, (5) light focusing lens, (6) rotating disc containing the cheese slice, and (7) motor.

Photolysis experiments

After having measured the gas composition, the middle slice of the nine slices in the package was withdrawn, cut into a circle, 9 cm in diameter, and sandwiched between two transparent, acrylic discs, which fit tightly together, thereby preventing any air influx and subsequent loss of moisture. The discs, embedding the cheese slice, were continuously rotated while being exposed to monochromatic light (wavelengths 366 nm, 405 nm, and 436 nm) selected from an Osram HBO 200/4 high pressure Hg lamp (line spectrum) (Osram, D-81543 Munich, Germany), mounted as a part of an optical train. The optical train also included a light condenser, a heat filter, an interference filter, and a light focusing lens (Fig. 1). All optical components were made of quartz (Linos, formerly Spindler und Hoyer, D-37081 Göttingen, Germany).

For each experiment, an individual cheese slice was exposed to a given wavelength for a given period of time, and following exposure, sampling took place. Samples stored in the dark were covered with aluminium foil and placed next to the photolysis set-up. Colour measurements were performed immediately after exposure, and fluorescence and secondary oxidation product determinations were carried out on the samples, which had been frozen immediately after exposure. Eight zero-samples (not exposed to light or storage) were withdrawn during the course of the photolysis experiment to ascertain that no pronounced changes took place for the duration of the experiment, e.g. due to ripening.

The experiments were carried out at room temperature (24 °C ± 2 °C), as cooling was not possible using the existing set-up. However, temperature is expected to have a low impact on photooxidative changes due to low activation energy levels (Turro, 1991).

Light fluxes (I_0, quanta . min^{-1}) at each of the three wavelengths were determined by ferrioxalate actinometry (Hatchard & Parker, 1956).

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The quantum yield is defined as

$$\Phi = \frac{\text{molecules reacted}}{\text{photons absorbed by reacting compound}} = \frac{\Delta C_i}{Q_i} \quad (1)$$

where ΔC_i is the change in concentration of the specific compound during a period of time resulting from the number of photons absorbed by this compound, Q_i, during the same period.

Apparent quantum yields based on total light absorption may be used for comparison with photoinduced processes in comparable systems:

$$\Phi_{app} = \frac{\text{moles reacted}}{\text{photons absorbed by cheese}} = \frac{\Delta C_i N_A m}{Q_{total}} \quad (2)$$

where ΔC_i is the change in concentration of the reacting compound determined by a specific chemical analysis, N_A is Avogadro's number, m is the mass of the illuminated cheese sample, and Q_{total}, the total number of photons absorbed by the system. Assuming no transmission and minimum reflection of light by the cheese, every photon emitted from the lamp is expected to be absorbed by the cheese. Hence, the intensity of light as determined by actinometry, I_0, can be used for calculation of number of photons absorbed, I_{abs}, during the time of light exposure, t

$$Q_{total} = I_{abs} t \approx I_0 t \quad (3)$$

Based on the above, the apparent quantum yields (Eq. 2) were calculated for the significant secondary oxidation products determined by solid-phase microextraction (SPME) GC/MS.

Characterisation of the product

Characterisation of the cheeses was carried out using standard methods to include: Total fat (International Dairy Federation, 1986), fatty acid composition (Jart, 1997), total protein (International Dairy Federation, 1993), total solids (International Dairy Federation, 1982), and ash (International Dairy Federation, 1964). Lipid extractions using the modified Folch extraction method (Mortensen et al. 2002b) were performed prior to fatty acid composition determinations.

Colour

The surface spectrum of the cheese slice was measured with a Cintra 40 spectrometer (GBC Scientific Equipment, Dandenong, Victoria, Australia) equipped with an integrating sphere detector.

The surface colour, i.e. CIELAB L*, a*, b* values (www.cie.co.at/cie/), of the cheese slices was measured with a Minolta tristimulus Chromometer CR-300 (Minolta Camera Co. Ltd., Osaka 564-8556, Japan). The measure of lightness (L* values, range 0–100) represents black to white (black is 0 and white is 100), the redness measurement (a* values) describes green (negative values) to red (positive values), and the yellowness measurement (b* values) represents blue (negative values) to yellow (positive values). The chromometer was standardized with a white standard plate. The results reported are averages of five measurements on the same slice of cheese exposed to the same treatment.

Odour

At the time of sampling, the analysts smelled the samples to qualitatively describe any odour changes.

Secondary oxidation products

Secondary oxidation products were determined by SPME GC/MS as described by Mortensen et al. (2002a).

Fluorescence measurements

Samples were mixed and squeezed into the sample holder of a BioView® spectrofluorometer (Delta Light & Optics, DK-2800 Lyngby, Denmark) measuring open-end (180° geometry) fluorescence, and using a pulsed Xenon lamp for excitation. Fluorescence landscapes were obtained with excitation and emission wavelength ranging from 270–550 nm and 310–590 nm, respectively. Bandwidth was 20 nm.

Data analysis

To correlate the volatile headspace profile with the other parameters describing the cheese, Partial Least Squares Regression (PLSR) analysis was applied, using the Unscrambler, version 7.5 (CAMO, N-0158 Oslo, Norway). The X variables were defined as the volatiles identified in the cheeses, i.e. 14 compounds. The design variables (Y variables) were defined on the basis of the experimental plan, i.e. exposure time (2, 4, 8, 12, and 24 h) and exposure conditions (dark storage, 366 nm, 405 nm, and 436 nm), thus using one dummy variable (value –1 or 1) for each of the wavelengths and for the light/dark design variable. Both X and Y variables were auto-scaled. Full cross validation and Jack-Knife were used to validate the calculated model (Martens & Martens, 2000). The optimal numbers of principal components were chosen at first minimum in RMSECV (root mean square error of cross validation). The above mentioned data analysis principles were also applied for the fluorescence data. Prior to data analysis, the three-dimensional data structure (samples × excitation wavelengths × emission wavelengths) was unfolded into a two-dimensional structure containing data including excitation and emission wavelengths for the individual samples. The colour measurement data means were compared by analysis of variance (ANOVA) using the statistical package, SPSS (Release 9.0, SPSS Inc. Chicago, IL 60606, USA).

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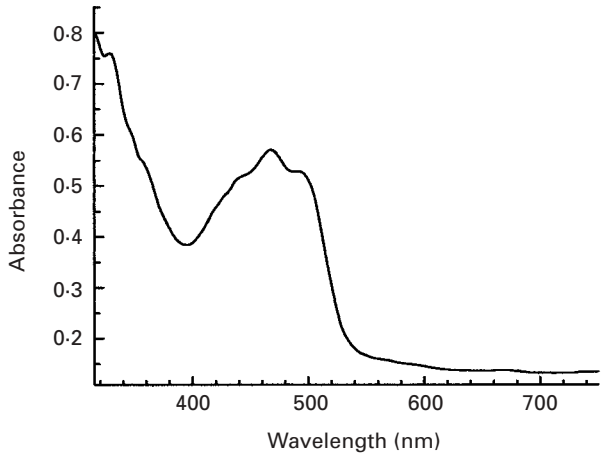

Fig. 2. Surface absorption spectrum of Havarti cheese measured with a Cintra 40 spectrometer (GBC Scientific Equipment, Dandenong, Victoria, Australia) equipped with an integrating sphere detector.

Results

Characterization of the product

The product contained 389 g fat/kg with the following fatty acid distribution: 665 g saturated fatty acids/kg, 305 g monounsaturated fatty acids/kg, and 30 g polyunsaturated fatty acids/kg (normalised values). Protein and dry matter contents were determined to be 192 g/kg and 640 g/kg, respectively. Ash content totalled 37 g/kg. The characterization indicated that the products were well within the expected gross composition range.

Colour

The absorption spectrum of the cheeses was obtained using a spectrometer equipped with an integrating sphere to compensate for diffuse reflection. The spectrum, shown in Fig. 2, results from the absorption spectra of the two major colorants in cheese, β-carotene and riboflavin. β-Carotene has an absorption maximum at approximately 460–470 nm neighboured at two characteristic shoulders (Britton, 1996), almost covering the visible absorption of riboflavin, which, however, dominates the near-ultraviolet spectrum. At wavelengths lower than approximately 330 nm, absorption is mainly attributable to aromatic amino acids.

The surface colour of the cheese slices was measured using tristimulus colorimetry during the irradiation period (Table 1). For each of the three irradiation wavelengths, the changes in the 3-dimensional L*, a*, b* colour space are shown in Fig. 3, and as may be seen the time traces show distinct differences in behaviour. L* values of the cheese slices decrease with time for all three irradiation wavelengths, whereas a change of hue for 405 nm and 436 nm is notable after 2–4 h resulting in a distinct bi-phasic time trace. In contrast, the colour changes upon irradiation with 366 nm light were less significant, as is evidenced from the L*, a*, b* coordinates, which after 24 h almost corresponded to those of the non-exposed

Table 1. Tristimulus (L*, a*, b*) coordinates of Havarti cheese slices at 24 °C during 24 h exposure to monochromatic light of wavelengths 366 nm, 405 nm, 436 nm, or storage in the dark

Values are means ± standard deviations for $n = 5$

Exposure time (h)	Dark storage	366 nm	405 nm	436 nm
L* values				
0	85·2 ± 1·5	85·2 ± 1·5	85·2 ± 1·5	85·2 ± 1·5
2	81·5 ± 0·3	81·5 ± 0·3	80·5 ± 0·6	80·9 ± 0·1
4	80·6 ± 1·7	80·6 ± 1·7	80·1 ± 0·0	80·2 ± 0·5
8	80·3 ± 0·9	80·3 ± 0·9	80·0 ± 0·0	79·0 ± 0·1
12	81·9 ± 2·2	81·9 ± 2·2	80·5 ± 0·9	79·7 ± 1·0
24	81·3 ± 0·3	81·3 ± 0·3	80·5 ± 0·4	80·1 ± 0·7
a* values				
0	−9·1 ± 0·3	−9·1 ± 0·3	−9·1 ± 0·3	−9·1 ± 0·3
2	−9·9 ± 0·0	−9·8 ± 0·1	−9·9 ± 0·1	−9·7 ± 0·1
4	−10·0 ± 0·1	−10·0 ± 0·1	−9·9 ± 0·1	−9·7 ± 0·1
8	−10·1 ± 0·1	−10·0 ± 0·1	−9·8 ± 0·1	−9·7 ± 0·0
12	−10·0 ± 0·1	−10·1 ± 0·0	−9·7 ± 0·0	−9·4 ± 0·0
24	−9·9 ± 0·0	−9·9 ± 0·4	−9·3 ± 0·2	−9·1 ± 0·1
b* values				
0	32·4 ± 0·8	32·4 ± 0·8	32·4 ± 0·8	32·4 ± 0·8
2	33·2 ± 0·5	33·6 ± 0·0	33·0 ± 0·2	31·8 ± 1·0
4	33·4 ± 0·2	33·5 ± 0·6	32·1 ± 0·8	31·6 ± 0·4
8	33·0 ± 0·3	33·2 ± 0·3	31·5 ± 0·4	30·7 ± 0·1
12	32·4 ± 0·6	33·2 ± 0·3	30·7 ± 0·2	29·2 ± 0·2
24	32·3 ± 0·3	32·6 ± 0·7	27·9 ± 1·2	27·3 ± 0·2

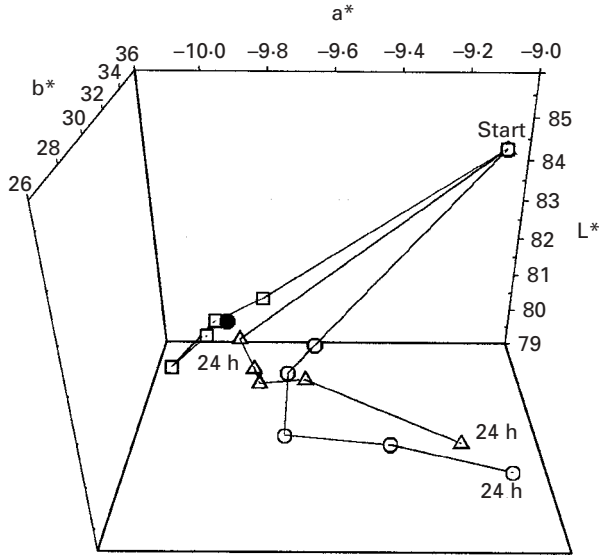

Fig. 3. Changes in tristimulus (L*, a*, b*) coordinates of Havarti cheese slices at 24 °C during 24 h exposure to monochromatic light of wavelengths 366 nm (□), 405 nm (△), or 436 nm (○), respectively. Starting coordinate is indicated, followed by results after 2, 4, 8, 12, and 24 h (end point indicated). Coordinates for cheeses protected against light during the whole exposure period are indicated by the filled circle.

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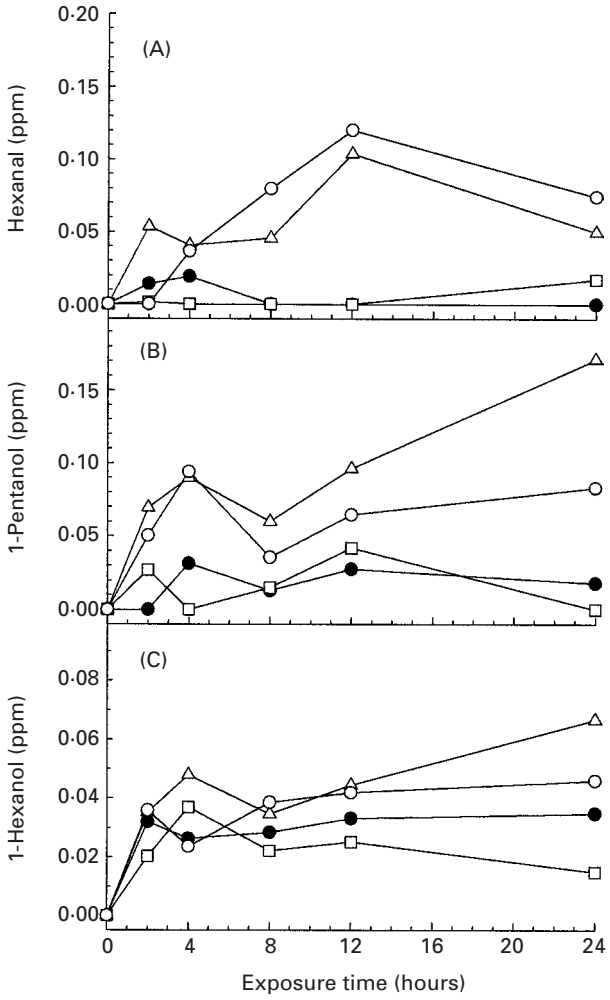

Fig. 4. Changes in contents of (A) Hexanal, (B) 1-Pentanol, and (C) 1-Hexanol of Havarti cheese slices at 24 °C during 24 h exposure to monochromatic light of wavelengths 366 nm (□), 405 nm (△), or 436 nm (○), respectively, or protected from light (●). Volatiles measured by SPME GC/MS, see materials and methods. Average coefficient of variation was 59%.

samples. Actually, the overall 24 h colour difference ($\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{\frac{1}{2}}$) was 4·0 for the cheese slices protected from light and 4·5 for that exposed to 366 nm light, whereas the corresponding changes for those exposed to 405 nm and 436 nm light were 6·6 and 7·2, respectively.

Odour

The simple, but indicative, odour evaluations of the exposed cheeses revealed that the cheeses exposed to 405 nm light were described as sweet and buttery in the interval of 0–4 h of exposure, but changed to nauseating and more acidic in the interval of 8–24 h. Samples exposed to 436 nm light changed from being described as sweet and buttery in the interval of 0–8 h to being described as technical/nauseating, an impression lasting until the end of the exposure period. Throughout the 24 h experiment, samples stored in the dark or exposed to 366 nm light retained their sweet and buttery odour, with sensory notes comparable to the non-exposed samples.

Table 2. Apparent quantum yields (Φ_{app}), based on the first 4 h of light exposure, for light-induced formation of volatiles in Havarti cheese slices. Cheese slices were exposed to average photon fluxes per minute of $2{\cdot}5 \pm 0{\cdot}3 \times 10^{17}$ at 366 nm, $2{\cdot}9 \pm 0{\cdot}3 \times 10^{17}$ at 405 nm, and $4{\cdot}4 \pm 0{\cdot}2 \times 10^{17}$ at 436 nm, respectively, as determined by ferrioxalate actinometry. Volatiles were quantified by SPME GC/MS

	Φ_{app}, 10^{-5} mol . einstein^{-1}		
	366 nm	405 nm	436 nm
Hexanal	0	5	3
1-Pentanol	0	13	9
1-Hexanol	5	6	2

Secondary oxidation products

The following components were identified by SPME GC/MS: dimethyl disulphide, hexanal, 2-heptanone, 1-pentanol, 3-hydroxy-2-butanone, 2-heptanol, 3-methyl-2-butene-1-ol, 1-hexanol, 2-nonanone, nonanal, 2-undecanone, butanoic acid, benzaldehyde, and hexanoic acid. PLSR with Jack-Knifing revealed that only the concentration of the secondary oxidation products, hexanal, 1-pentanol, and 1-hexanol, increased significantly when exposed to the specific wavelengths, as may be seen in Fig. 4.

Significant increases in concentrations of hexanal (Fig. 4A) and 1-pentanol (Fig. 4B) were determined for samples exposed to 405 nm and 436 nm light, being at least one order of magnitude higher than the concentrations in cheeses exposed to 366 nm light or protected from light. Although the concentration of 1-hexanol (Fig. 4C) increased for all samples, concentrations determined at the end of the exposure period were significantly higher for samples exposed to visible light. The qualitative results of the odour evaluations thus paralleled the GC headspace results of Fig. 4, indicating a very rapid quality deterioration rate when samples were exposed to monochromatic light of 405 and 436 nm, respectively.

Knowing the light fluxes at each of the three wavelengths of irradiation, the apparent quantum yields for formation of secondary oxidation products could be calculated. The determination of secondary oxidation products is rather uncertain (cf. legend to Fig. 4), and as these compounds are known to react with other compounds, the apparent photodegradation quantum yields were calculated based on initial rates of formation (Table 2).

Having compensated the effects of Fig. 4 for photon fluxes, it is notable from Table 2 that the most intense induction of volatiles was in fact caused by irradiation of cheese with 405 nm light.

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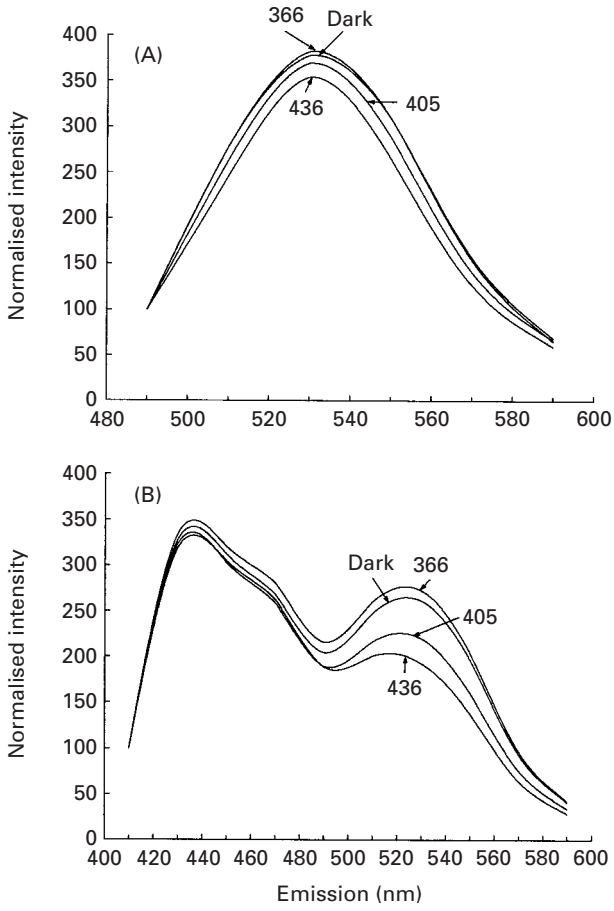

Fig. 5. Fluorescence spectra of Havarti cheese slices recorded after 12 h at 24 °C, when exposed to monochromatic light of wavelengths 366 nm, 405 nm, or 436 nm, respectively, or protected from light. Excitation wavelengths: (A) 450 nm and (B) 370 nm.

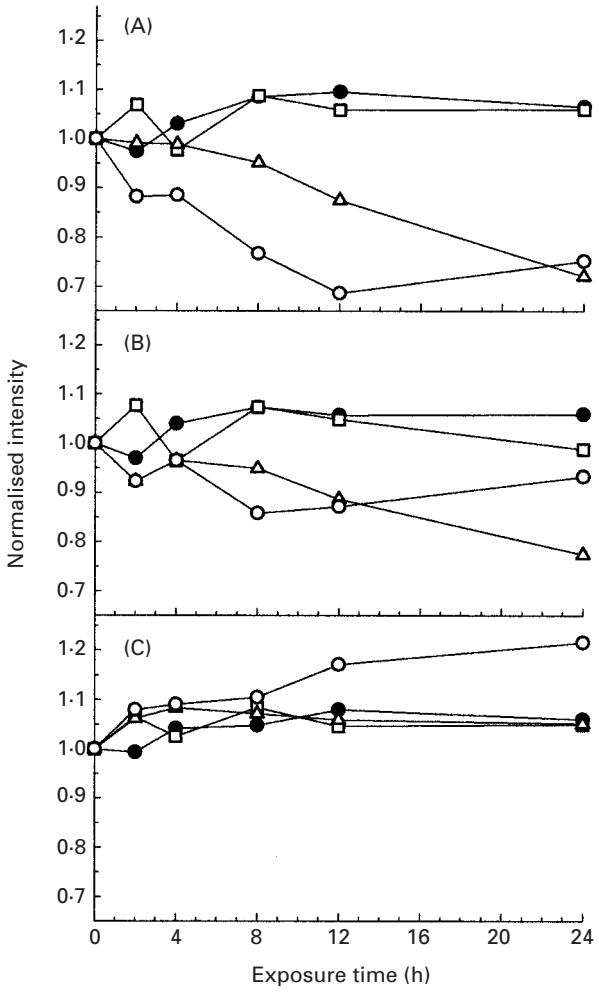

Fig. 6. Changes in fluorescence intensity of Havarti cheese slices at 24 °C during 24 h exposure to monochromatic light of wavelengths 366 nm (□), 405 nm (△), or 436 nm (○), respectively, or protected from light (●). (A) Excitation: 450 nm, emission: 530 nm, (B) excitation: 370 nm, emission 530 nm, and (C) excitation: 370 nm, emission: 430 nm. Average coefficient of variation for results: 4%.

Fluorescence measurements

Analysis of the fluorescence spectroscopy data set using multivariate statistical methods (by evaluating significant regression coefficients) revealed that excitation wavelengths 450 nm as well as 370 nm could be used to distinguish between the different exposure conditions.

As may be seen from Fig. 5A, excitation at 450 nm resulted in fluorescence with an emission maximum of approximately 530 nm. This emission can be ascribed to riboflavin, which, after light absorption through its $S_0 \rightarrow S_1$ transition, has an unstructured emission spectrum with a maximum of 536 nm in aqueous solution (Kotaki & Yagi, 1970). The exact wavelength of the emission maximum of the cheeses is likely to differ from this value due to polarity effects combined with absorption of emitted light by β-carotene (cf. Fig. 2). Upon excitation at 370 nm, emission spectra with two maxima of 435 nm and 530 nm, respectively, were recorded (Fig. 5B). As for Fig. 5A, the emission maximum of 530 nm, however, can be ascribed to riboflavin being excited through the $S_0 \rightarrow S_2$ transition of its absorption spectrum, whereas the violet emission, having a peak at 435 nm, is more difficult to assign.

Fig. 6A depicts the fluorescence intensity upon excitation at 450 nm, and as is apparent, the emission intensities after 12 h exposure are significantly lower for samples exposed to 405 nm and 436 nm light. The changes in intensity followed by excitation at 450 nm throughout the exposure period (Fig. 6A) show a decrease concurrently with exposure time progressing for samples exposed to monochromatic light of 405 nm and 436 nm, whereas samples stored in the dark and at 366 nm remain at a relatively constant level throughout exposure.

Fig. 6B pinpoints that the intensity of fluorescence, upon excitation at 370 nm, decreases in the 520–530 nm

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emission range after light exposure at 405 nm and 436 nm, whereas the intensity increases slightly for 12 h/436 nm-exposed samples in the 415–490 nm emission region. Taking into account the uncertainty of the fluorescence measurements, the changes in 530 nm emission intensity with time parallel the changes of Fig. 6A, thus indicating photodegradation of riboflavin. As is apparent from Fig. 6C, the fluorescence intensity of the 430 nm maximum increases when extending the 436 nm light exposure time, whereas samples stored in the dark, at 366 nm, or at 405 nm, remain at a relatively constant level during exposure.

Discussion

The present study contains, to the authors' knowledge, the first quantitative determination of the effects of light on cheese. Apparent quantum yields for formation of light-induced off-flavour products in cheeses were determined using a custom-built photolysis set-up (Fig. 1). In contrast to the majority of studies focusing solely on the effects of ultraviolet light, this study has investigated the effects of the 366–436 nm wavelength region, which is considered the most relevant spectral region with respect to formation of light-induced quality changes. Actually, cheeses are seldom exposed to high-intensity ultraviolet photons due to packaging in films, which do not allow for transmittance of wavelengths in the UV range (Bosset et al. 1995), and due to very low output in this wavelength region of fluorescent tubes used for display purposes. Photons of the ultraviolet wavelength applied in the present study may to some extent be transmitted through packaging films, and the 405 nm and 436 nm photons are readily transmitted through basically all non-yellow films. According to the Stark–Einstein law, only absorbed photons can lead to photochemical reactions (Wayne & Wayne, 1996), and in the current study, the conclusions regarding the effects of 405 nm and 436 nm on photooxidation were actually reversed after correction of photon fluxes. Hence, effects of illumination on the quality of cheeses are difficult to compare. This is ascribable to the usage of diverse light sources, where only light intensities are identified. However, spectral distribution patterns are crucial for fair comparisons.

Irrespective of light source, studies on cheese indicate photooxidative degradation of riboflavin (Deger & Ashoor, 1987; Marsh et al. 1994; Kristensen et al. 2000). These studies all applied different illumination conditions; however, white light (emitting wavelengths absorbed by riboflavin) was used in all studies. Hence, comparable results are expected. In accordance with these findings, non-destructive surface-fluorometric analyses give clear indications of riboflavin photodegradation (Figs. 5A and 5B). Considering the absorption spectrum of riboflavin, it is rather surprising that 366 nm light does not lead to significant photodegradation of riboflavin, as does irradiation at both 405 nm and 436 nm. The molecule absorbs all three wavelengths, and the molecular extinction coefficients are almost identical at 366 nm and 436 nm. However, 366 nm light is absorbed by a higher ($S_0 \rightarrow S_2$) energy band transition, than the one responsible for the 405 nm and 436 nm absorptions ($S_0 \rightarrow S_1$) (Wehry, 1993). Riboflavin has a high fluorescence quantum yield (0·24 in water; Kotaki & Yagi, 1970), and irrespective of excitation wavelength (Figs. 6A and 6B), fluorescence is emitted from the lowest excited singlet state. However, higher energy state excitation of riboflavin molecules at 366 nm apparently allows for a different deactivation route, most likely by energy transfer to β-carotene, thus returning to ground state without chemical breakdown. Different reaction mechanisms are also indicated by the changes in tristimulus parameters (L*, a*, b*), as depicted in Fig. 3, in which distinct biphasic changes are observed for cheese exposed to 405 nm and 436 nm photons in contrast to those exposed to 366 nm photons.

The decrease in fluorescence intensity of the 525 nm peak (excited at 380 nm) and increase in the 415–490 nm area upon light-exposure of both cream cheese and Jarlsberg cheese were ascribed to degradation of riboflavin and formation of its degradation compound, lumichrome (Wold et al. 2002). Likewise, lumichrome formation has been suggested by e.g. Parks & Allen (1977) and Fox & Thayer (1998). However, the red-side shoulder of the 435 nm emission maximum of Fig. 5B indicates the presence of another fluorophore with emission maximum of 475–480 nm. Moreover, it is notable that both fluorophores were present from the start of the experiment. Thus, the increase in fluorescence could simply be due to photodegradation of β-carotene, indicated by the decrease in b* value (Table 1; Fig. 3). β-Carotene very effectively absorbs the emission of fluorophores, e.g. riboflavin, in this wavelength range, due to its very high molar extinction coefficient ($>10^5\ \text{M}^{-1} \cdot \text{cm}^{-1}$, Britton, 1996), whereas it has a very low fluorescence quantum yield, i.e. approximately 10^{-5} (Jørgensen et al. 1992) and, thus, does not contribute to the fluorescence intensity. Given the ratio of fluorescence intensities between the two peaks, the unassigned fluorophore is expected to have a rather high fluorescence quantum yield and/or molecular extinction coefficient. Fluorescent compounds such as amino-carbonyl complexes formed during oxidation may influence the spectrum in the lower wavelength range, as shown by e.g. Stapelfeldt & Skibsted (1996), but can only be considered minor contributors, since such complexes have low fluorescence quantum yields. A non-riboflavin derived component could be retinol, which has emission maximum at approximately 330 nm upon excitation at 450 nm (Dufour & Riaublanc, 1997).

The formation of volatile secondary oxidation products was significantly different in Havarti cheeses exposed to monochromatic light of 405 nm and 436 nm *v.* stored in the dark or exposed to 366 nm (near-UV-region). The aldehydes detected may originate from degradation of

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unsaturated fatty acids (Arora et al. 1995; Engels et al. 1997) or from degradation of amino acids (Engels et al. 1997; McSweeney & Sousa, 2000; Yvon & Rijnen, 2001). The concentration of hexanal, the major aldehyde formed during degradation of linoleate (Kochar, 1996), has previously been found to increase in cheese during light exposure (Colchin et al. 2001). In the present study, hexanal contents increased during 0–12 h exposure to 405 nm and 436 nm light, and were followed by a decrease, which could be attributed to conversion into 1-hexanol and other degradation compounds. Notably, in contrast to the reaction patterns of secondary oxidation product formation in oils, the aldehydes formed in cheeses are reduced to the corresponding alcohols by a reaction pathway involving alcohol dehydrogenase (Engels et al. 1997). In accordance with the results of Lund et al. (2002) and Mortensen et al. (2002a), an increase in the formation of 1-pentanol and 1-hexanol during light-exposure at 405 nm and 436 nm was noted, whereas only minor increases were observed for samples exposed to 366 nm or stored in the dark.

The hexanal levels present in the exposed cheeses (405 nm and 436 nm), although low, are expected to impact on the sensory characteristics, as flavour thresholds range from 0·03–0·6 ppm depending upon solvents and methods of analysis (Kochar, 1996). The primary alcohols, such as 1-pentanol and 1-hexanol, are expected to have only minor impact on the total off-flavour sensation due to flavour thresholds significantly higher than those of the corresponding aldehydes (Kochar, 1996). Although the identified alcohols were present in small quantities, even below threshold values reported in simple systems, it is noteworthy that the analysts were able to detect sensory changes in the light-exposed samples. Odour evaluations and secondary oxidation product analyses (Fig. 4) pinpoint rapid transformation rates of fatty acids into hydroperoxides, which are subsequently converted to volatile compounds. This is in compliance with a previous study on Havarti cheese (Mortensen et al. 2002a).

The two colorants, riboflavin and β-carotene, play a key role in light-induced changes in dairy products, β-carotene being a light absorber and a well-known quencher of excited oxygen species (Mortensen et al. 2001) and riboflavin a sensitizer (Bradley & Min, 1992; Bosset et al. 1995). Accordingly, the balance between these two colorants to a great extent determines the light sensitivity of dairy products. The results of the present study are in some contrast to those reported by Hansen & Skibsted (2000) for a dairy spread model system which, however, due to its increased fat content has both a higher β-carotene/riboflavin ratio and a higher content of the inherent antioxidant, α-tocopherol. As these factors are known to be of utmost importance to light stability, the mechanisms and kinetics of photodegradation are likely to be different in dairy spreads and cheeses. Accordingly, future research in this area should focus on quantifying the effects of light by determination of quantum yields for degradation of both β-carotene and riboflavin and the resulting formation of their degradation products. Furthermore, quantum yield determinations of the formation of volatiles responsible for light-induced flavour should be calculated. Such studies would provide detailed information on the underlying mechanisms, which could ultimately be used to predict product stability from content of key compounds, spectral characteristics of the illumination source, and transmission characteristics of the packaging foil.

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