Modified Atmosphere Packaged Cheddar Cheese Shreds: Influence of Fluorescent Light Exposure and Gas Type on Color and Production of Volatile Compounds

Llori M. Colchin,† Sandra L. Owens,‡ Galina Lyubachevskaya,‡ Elizabeth Boyle-Roden,‡ Estelle Russek-Cohen,‡ and Scott A. Rankin*,‡

Sodexo Marriott Services, 9801 Washingtonian Boulevard, Gaithersburg, Maryland 20878, and Department of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20742-2311

The influences of fluorescent light exposure and packaging atmosphere on the headspace volatiles and color of Cheddar cheese shreds were evaluated using gas chromatography−mass spectrometry and spectrocolorimetry, respectively. Cheddar cheeses were packaged under atmospheres of 100% carbon dioxide or 100% nitrogen and stored at 4 °C under fluorescent light for 6 weeks. Cheeses stored under carbon dioxide contained higher concentrations of aldehydes and fatty acids and lower concentrations of alcohols and esters than cheeses stored under nitrogen. Carbon dioxide atmospheres potentiated light-induced oxidation in shredded Cheddar cheeses, as evidenced by aldehyde and fatty acid headspace volatiles measured following storage. Color bleaching occurred only in cheeses packaged under carbon dioxide and exposed to light. The shift in color is proposed to be due to an interaction between carbon dioxide and high-intensity light, leading to the oxidation of the pigment molecule, bixin. The results have significant implications for procedures used to handle and store pigmented cheeses to ensure desirable flavor and consumer acceptability.

Keywords: *Annatto; bixin; carbon dioxide; fluorescent light exposure; modified atmosphere packaging; shredded Cheddar cheese; volatile compounds*

INTRODUCTION

For purposes of convenience, many cheese varieties, including Cheddar, are available in a preshredded form in translucent modified atmosphere packaging. Even though flavor development in traditional Cheddar cheese has been well documented (*1*−*6*), little is known about the flavor chemistry of shredded cheese. The flavor and color quality of shredded cheese are likely to be significantly affected by processing and storage conditions that are different from traditional cheese storage conditions. The major difference is that the ripening process of shredded cheese, with significantly increased surface area, takes place under altered gas atmospheres and high-intensity fluorescent light, whereas traditional block cheese ripens in the dark under regular atmospheric conditions.

Modified atmosphere packaging is a commonly used method of food preservation that relies on the introduction of carbon dioxide (CO_2) and/or nitrogen (N_2). CO_2 extends the shelf life of cheese by inhibiting mold growth (*7*, *8*). However, CO_2 may also degrade many flavor compounds (*9*) and influence microorganisms essential for flavor development in cheese (*10*). N_2 acts as an inert filler gas and has no documented adverse influence on cheese flavor.

High-intensity light has been reported to have negative effects on the color stability of natural yellow cheeses (*11*−*13*). In addition, shredded Cheddar cheese stored under high-intensity fluorescent light may be susceptible to light-induced oxidation reactions that may affect flavor and color quality. The dramatic increase in surface area of shredded Cheddar cheese compared to that of traditional block cheese is likely to be an additional factor contributing to deleterious effects of high-intensity light.

A better understanding of the influence of storage and processing conditions on the flavor chemistry and color of shredded Cheddar cheese is necessary to maintain desirable flavor and consumer acceptability. The purpose of this study was to determine the effects of modified atmosphere packaging gas type and light exposure on the color and volatile compound profile of shredded Cheddar cheese following a 6-week storage period.

MATERIALS AND METHODS

Cheese Preparation. One-kilogram blocks of medium-aged Cheddar cheese (Tillamook County Creamery Association, Tillamook, OR) were stored in the dark at 4 ± 1 °C until sample preparation. The cheese block was shredded with a hand shredder. Ten 20-g samples of cheese shreds were packaged in low-oxygen permeable film under 100% CO_2 or 100% N_2. The oxygen transmission rate for the films was 2 cm^3/m^2/day for the forming layer and 4 cm^3/m^2/day for the nonforming film (Cryovac, Simpsonville, SC). Five packages of CO_2-treated and five packages of N_2-treated samples were randomly assigned to be stored under continuous fluorescent light (∼120 ft candles) or in the dark at 4 °C for 6 weeks. Thus, there were five packages in each of the four treatments: CO_2 packaged, stored under light; CO_2 packaged, stored in the dark; N_2 packaged, stored under light; N_2 packaged, stored in the dark.

Gas Composition of Package Headspace. The concentrations of O_2 and CO_2 were measured prior to analysis to

* Author to whom correspondence should be addressed [telephone (301) 405-4568; fax (301) 405-8831; e-mail rankins@wam.umd.edu].

† Sodexo Marriott Services.

‡ University of Maryland.

10.1021/jf0012392 CCC: $20.00
Published on Web 04/13/2001

Table 1. Quantification of Aldehydes Identified in Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions

compound	mean[a] (μg/g)			
	CO_2 light	CO_2 dark	N_2 light	N_2 dark
3-methylbutanal	0.066[a] (0.031)	0.059[a] (0.004)	nd[b]	nd
pentanal	0.064[a] (0.015)	0.028[a] (0.028)	nd	nd
hexanal	0.027[a] (0.163)	0.018[a] (0.012)	nd	nd
heptanal[c]	0.211 (0.018)	nd	nd	nd
(*E*)-2-hexenal	0.008[a] (0.003)	0.006[a] (0.011)	nd	nd
octanal	0.028[a] (0.001)	0.001[b] (0.003)	nd	nd
(*E*)-2-heptenal	0.024 (0.003)	nd	nd	nd
(*E*)-2-octenal	0.009 (0.001)	nd	nd	nd
2-propyl-2-heptenal	0.021 (0.004)	nd	nd	nd
decanal	nd	0.002[a] (0.003)	0.001[a] (0.002)	0.002[a] (0.002)
2,4-heptadienal	0.006 (0.002)	nd	nd	nd
(*E*)-2-nonenal	0.023[a] (0.001)	0.001[b] (<0.001)	nd	nd
2-butyl-2-octenal	0.018 (0.001)	nd	nd	nd
2,4-nonadienal	0.004 (0.002)	nd	nd	nd
total	0.739[a] (0.205)	0.116[b] (0.010)	0.001[c] (0.002)	0.002[c] (0.002)

[a] Means in a row with different superscript letters are different ($p < 0.05$) using LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). [b] Not detected. [c] Analysis of variance conducted on log transformed data.

verify packaging atmospheres for each package using a PBI Dansensor A/S (Combi Check 9800-1, Multivac Inc., Kansas City, MO). Gas concentration measurements were taken at the beginning, middle, and end of the packaging runs and averaged within gas type; packages were then randomized to storage treatments. Average gas concentrations for the CO_2-packaged cheeses were 2.69% O_2, 95.9% CO_2, and 1.46% N_2. Average concentrations for the N_2-packaged cheeses were 0.02% O_2, 1.95% CO_2, and 98.05% N_2.

Headspace Volatile Analysis. Extracts of volatile compounds were prepared by combining 10.0 g of shredded cheese with 40.0 mL of distilled water. Methyl butyrate was added as the internal standard at a concentration of 1.00 μg/g. Sample purge was conducted in a 35 ± 1 °C circulating water bath at a purge rate of 950 mL/min; total purge volume was 100 L. Nitrogen was used as the purge gas to limit oxidative reactions. Adsorbent traps (ORBO-32, Supelco, Bellefonte, PA) were eluted with distilled diethyl ether (Sigma-Aldrich, St. Louis, MO). The first 2 mL of solvent eluate was collected and concentrated under nitrogen to ~100 μL.

Separation of volatiles was performed using a gas chromatograph (model 6890, Hewlett-Packard; Avondale, PA) equipped with a 30 m × 0.25 mm (i.d.) capillary column (Stabilwax, film thickness = 0.5 mm; Restek Inc., Bellefonte, PA). The column flow rate was 1.3 mL/min. Helium was used as a carrier gas. The column temperature was programmed from 40 to 180 °C at 5 °C/min with a 5-min postinjection hold. The sample injection volume was 2 μL. The gas chromatograph was coupled with a Hewlett-Packard 5973 mass spectrometer. The ionization mode was electron impact, and the ionization voltage was 70 eV. Mass scan range was *m*/*z* 29−350. Compounds were identified by comparing their retention times with those of known standards and by matching their mass spectra with those of a published database.

Color Analysis. Color measurements were made with a dual-beam spectrophotometer (model CS-5, Applied Color Systems, Princeton, NJ). A GE cool white lamp (CWL) was used to determine whiteness (L^*), red/greenness (a^*), and yellow/blueness (b^*). Approximately 10 g of cheese shreds was compressed into a 50 × 50 × 10 mm optical cell. Reflectance values were obtained using a 25 mm view area aperture.

Statistical Analysis. Standardized peak areas were analyzed using analysis of variance (ANOVA) procedures of the General Linear Model Program (version 6.12, SAS Institute, Inc., Cary, NC, 1999). Data were treated as a completely randomized design and included CO_2 light, CO_2 dark, N_2 light, and N_2 dark as main effects. When treatment effects were significant ($p < 0.05$), differences between mean values were evaluated by least significant difference pairwise comparison tests. Colorimetry data were analyzed using the General Linear Model Program. Differences between treatments were determined using one-way ANOVA and least significant difference pairwise tests ($p < 0.05$). When necessary, data were log transformed to correct for heterogeneous variances.

RESULTS AND DISCUSSION

Headspace Volatiles. Aldehydes were the major constituent of the volatile fraction of shredded Cheddar cheese packaged under CO_2. They accounted for approximately 63 and 35% of the total volatiles in the CO_2-treated cheeses exposed to fluorescent light and in CO_2-treated cheeses stored in the dark, respectively. However, no aldehydes were detected in cheeses packaged under N_2, with the exception of very low concentrations of decanal (Table 1). The volatiles from cheeses packaged under CO_2 and exposed to fluorescent light contained >6 times the aldehyde concentration of cheeses packaged under CO_2 and stored in the dark. The compound present in the greatest quantity in CO_2-treated cheese exposed to fluorescent light was heptanal, accounting for ~29% of the total aldehydes. 3-Methylbutanal was the most abundant aldehyde present in the CO_2-treated cheeses that were stored in the dark, accounting for ~50% of the total aldehydes in this treatment group.

The aldehydes could be formed via two pathways: the oxidation of unsaturated fatty acids and amino acid degradation. Branched-chain aldehydes have been suggested to originate from amino acid degradation (*14*), and straight-chain aldehydes are formed through the oxidation of unsaturated fatty acids (*15*). The lack of aldehydes in the N_2-treated cheeses may suggest that neither of these pathways is favored in a nitrogen atmosphere. Branched-chain aldehydes also may originate from branched-chain fatty acids. Therefore, there is a possibility that the majority of aldehydes detected resulted from lipid oxidation. Detection of considerably higher concentrations of the aldehydes in cheeses exposed to light relative to cheeses kept in the dark also implies that light-induced lipid oxidation was the major pathway in the formation of the aldehydes.

Ketones were the major component of shredded Cheddar cheese packaged under N_2. Ketones represented 78% of the total volatiles identified in N_2-packaged cheese exposed to fluorescent light and 77% of the total volatiles identified in N_2-packaged cheese stored in the dark. However, in cheeses stored under CO_2, very low concentrations of ketones were detected.

Table 2. Quantification of Ketones Identified in Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions

compound	mean[a] (μg/g)			
	CO_2 light	CO_2 dark	N_2 light	N_2 dark
2-butanone	nd[b]	0.004 (0.001)	nd	nd
2-pentanone[c]	0.009^a (0.002)	0.011^a (0.002)	1.07^b (0.072)	0.851^b (0.16)
2-hexanone	nd	0.002^a (0.002)	0.045^b (0.015)	0.033^b (0.002)
2-heptanone[c]	0.022^a (0.006)	0.018^a (0.003)	1.39^b (0.18)	1.07^c (0.230)
3-octanone	nd	nd	0.010^a (0.003)	0.005^b (0.001)
2-octanone	nd	0.015^a (0.026)	0.029^a (0.005)	0.024^a (0.006)
3-hydroxy-2-butanone	0.038^a (0.006)	0.030^a (0.026)	nd	nd
2-nonanone[c]	0.003^a (0.006)	0.005^a (0.001)	0.521^b (0.100)	0.494^b (0.180)
8-nonen-2-one	nd	nd	0.054^a (0.012)	0.050^a (0.017)
2-decanone	nd	nd	0.003^a (0.001)	0.003^a (0.001)
2-undecanone	nd	nd	0.017^a (0.004)	0.016^a (0.004)
total	0.073^a (0.033)	0.085^a (0.004)	3.14^b (0.126)	2.55^b (0.492)

[a] Means in a row with different superscript letters are different ($p < 0.05$) using LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). [b] Not detected. [c] Analysis of variance conducted on log transformed data.

Table 3. Quantification of Alcohols Identified in Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions

compound	mean[a] (μg/g)			
	CO_2 light	CO_2 dark	N_2 light	N_2 dark
2-methyl-1-propanol[b]	nd[c]	nd	0.020^a (<0.001)	0.021^a (0.008)
2-pentanol	nd	nd	0.224^a (0.066)	0.209^a (0.046)
1-butanol	0.006^a (0.019)	0.009^a (0.004)	nd	nd
1-penten-3-ol[d]	0.073 (0.018)	nd	nd	nd
3-methyl-1-butanol	nd	nd	0.335^a (0.029)	0.261^a (0.054)
4-methyl-2-pentanol[d]	nd	nd	0.007^a (0.002)	0.006^a (0.005)
1-pentanol	0.006^a (0.019)	0.011^a (0.010)	nd	nd
3-methyl-3-buten-1-ol	nd	nd	nd	0.004 (0.001)
2-heptanol[b]	nd	nd	0.122^a (0.009)	0.102^a (0.102)
1-hexanol	0.041^a (0.001)	0.005^b (0.001)	0.012^b (0.001)	nd
2-octanol	nd	nd	0.003^a (0.001)	0.002^a (0.001)
1-octen-3-ol	0.026^a (0.001)	0.001^b (0.001)	0.002^b (0.001)	0.002^b (0.001)
2-nonanol	nd	nd	0.023^a (0.003)	0.027^a (0.004)
1-octanol	0.002^a (0.002)	nd	nd	nd
9-decen-2-ol	nd	nd	0.004^a (0.002)	0.005^a (0.001)
total	0.215^a (0.047)	0.026^a (0.009)	0.752^b (0.098)	0.640^b (0.075)

[a] Means in a row with different superscript letters are different ($p < 0.05$) using LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). [b] Analysis of variance conducted on log transformed data. [c] Not detected. [d] Tentatively identified.

The major ketones of importance were methyl ketones, 2-heptanone, 2-pentanone, and 2-nonanone (Table 2). The concentrations of 2-heptanone and 2-pentanone exceeded those of all other compounds detected for all treatments. In the cheeses packaged under N_2 and stored under light or dark conditions, 2-heptanone and 2-pentanone represented approximately 78 and 75%, respectively, of the total ketones.

In traditional Cheddar cheese, which is not a mold-ripened cheese, lipolysis is minimal, and lipolytic products including methyl ketones are present at very low concentrations. In ref *16* it was suggested that significant concentrations of 2-heptanone and 2-pentanone in non-mold-ripened cheeses are an indication of mold contamination. Thus, the high concentrations of methyl ketones present in the cheeses packaged under N_2 were most likely due to mold growth. Additionally, 8-nonen-2-one is a significant component in the flavor of blue and mold-ripened cheese. This compound was identified only in the cheeses packaged under N_2, further suggesting the presence of mold growth in these cheeses.

CO_2 is a powerful antimycotic agent, whereas N_2 acts as an inert filler gas. Thus, it was expected that compounds indicative of mold contamination were present in the N_2-treated samples and not in the CO_2-treated cheeses. None of the samples tested had any visual indications of mold growth.

The reducing conditions of Cheddar cheese favor the reduction of aldehydes and methyl ketones to their corresponding alcohols (*15, 17*). Alcohols represented the second largest class of volatile compounds in samples following both N_2 treatments as well as in those packaged under CO_2 and exposed to fluorescent light (Table 3). In cheeses packaged under CO_2 and stored in the dark, alcohols were present to a lesser extent, representing only 8% of the total volatiles. Significantly higher concentrations of alcohols were detected in N_2-treated cheeses than in CO_2-treated cheeses.

Among the alcohols, those present in the greatest concentration were 3-methyl-1-butanol, 2-pentanol, 2-heptanol, and 1-octen-3-ol. 3-Methyl-1-butanol is formed by the reduction of its corresponding aldehyde, which is produced from leucine. 1-Octen-3-ol is a product of the oxidation of linoleic and linolenic acid (*14*) and is a significant contributor to the flavor of cheese (*18*). The characteristic aroma of 1-octen-3-ol is described as mushroom-like (*14*).

Very low concentrations of esters were detected in cheeses analyzed in the present study (Table 4). However, even at very low concentrations, esters have been identified as major contributors to the flavor of Cheddar cheese (*19*). Due to their high volatility at ambient temperatures, esters can contribute significantly to the total aroma. Cheeses packaged under N_2 contained

Table 4. Quantification of Esters Identified in Aged Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions

compound	mean[a] (μg/g)			
	CO_2 light	CO_2 dark	N_2 light	N_2 dark
ethyl butanoate[b]	0.013[a] (0.001)	0.013[a] (0.002)	0.064[b] (0.026)	0.068[b] (0.022)
ethyl hexanoate	nd[c]	0.012[a] (0.002)	0.010[a] (0.0024)	0.008[a] (0.001)
total	0.013[a] (0.001)	0.024[a] (0.003)	0.074[b] (0.023)	0.076[b] (0.024)

[a] Means in a row with different superscript letters are different ($p < 0.05$) using LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). [b] Analysis of variance conducted on log transformed data. [c] Not detected.

Table 5. Quantification of Free Fatty Acids Identified in Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions

compound	mean[a] (μg/g)			
	CO_2 light	CO_2 dark	N_2 light	N_2 dark
acetic acid	0.016[a] (0.004)	0.007[b] (0.003)	0.006[b] (0.002)	0.004[b] (0.001)
butanoic acid	0.002[a] (0.002)	0.004[a] (0.001)	0.002[a] (0.001)	nd[b]
hexanoic acid	0.003[a] (0.002)	0.005[a] (0.002)	nd	nd
octanoic acid	0.003[a] (0.003)	0.005[a] (0.001)	nd	0.001[a] (0.002)
total	0.024[a] (0.004)	0.021[a] (0.007)	0.008[b] (0.002)	0.005[b] (0.001)

[a] Means in a row with different superscript letters are different ($p < 0.05$) using LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). [b] Not detected.

Table 6. Quantification of Sulfur Compounds Identified in Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions

compound	mean[a] (μg/g)			
	CO_2 light	CO_2 dark	N_2 light	N_2 dark
dimethyl disulfide	0.007[a] (0.005)	0.001[a] (0.001)	nd[b]	nd
dimethyl sulfoxide	nd	0.001[a] (0.001)	<0.001[a] (0.001)	nd
total	0.007[a] (0.004)	0.001[b] (0.001)	<0.000[b] (0.001)	nd

[a] Means in a row with different superscript letters are different ($P<0.05$) using LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). [b] Not detected.

significantly higher concentrations of esters than did cheeses packaged under CO_2.

The presence of higher concentrations of esters in cheeses stored under N_2 relative to those in cheeses stored under CO_2 may be due to the inhibitory effect of CO_2 toward the formation of some flavor compounds. CO_2, in addition to being inhibitory to spoilage microflora, decreases the concentration of many flavor compounds (*9*). Studies have shown that CO_2 has an inhibitory effect on fermentation bacteria that are essential for flavor development in cheeses (*10*). The mechanism of CO_2 action is not well understood. Researchers have suggested that CO_2 exerts its effects when the gaseous CO_2 dissolves into the liquid phase of the food tissue and is absorbed as carbonic acid (*20*, *21*).

Relatively low concentrations of volatile free fatty acids (FFA) were measured in the shredded cheeses analyzed in the present study (Table 5). The concentration of FFA in the cheeses packaged under N_2 was much less than that of the CO_2-packaged cheese. However, the concentrations of FFA were similar within each atmosphere, indicating that lipolysis was not affected by light. Low concentrations of FFA in cheese may be an indicator of insufficient maturation. In traditional Cheddar cheese, a gradual increase in FFA (C_2-C_{12}) occurs throughout 60 days of ripening (*17*).

FFA, aside from contributing to the background flavor of Cheddar cheese (*18*), also act as precursors to the development of flavor-impact compounds such as esters, methyl ketones, and alcohols. FFA can be derived from both microbial and endogenous lipase activities. The comparatively lower concentration of FFA detected in the N_2-packaged cheeses may be explained by a higher esterification rate as evidenced by the concomitantly higher concentration of ethyl esters.

Acetic acid was the most abundant of the volatile fatty acids detected. Acetic acid can be derived from the metabolism of carbohydrates and amino acids. The increased concentration of acetic acid in CO_2-treated cheese exposed to light suggests that these conditions may favor the growth of heterofermentative bacteria in shredded Cheddar cheese. The concentration of acetic acid was shown to increase during aging in traditional Cheddar cheese (*22*).

Sulfur compounds play an important role in the development of Cheddar cheese flavor (*23*). However, sulfur compounds were found in the lowest concentration of all the classes of volatile compounds measured (Table 6). No sulfur compounds were identified in cheeses packaged under N_2 and stored in the dark. Cheeses stored under the other three conditions contained very low concentrations of sulfur compounds, representing <1% of the total volatiles measured. Dimethyl disulfide and dimethyl sulfoxide were the only two sulfur-containing compounds present in the shredded cheese samples. Dimethyl disulfide was detected only in the cheeses packaged under CO_2, whereas dimethyl sulfoxide was detected in CO_2-packaged cheeses kept under light and N_2-packaged cheeses stored in the dark.

There are two possible explanations for the very low concentrations of sulfur compounds measured. The first explanation involves difficulties in the isolation of sulfur compounds due to their high volatility and instability that were reported by a number of researchers (*16*, *19*).

Table 7. Quantification of Cyclic Compounds Identified in Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions

compound	mean[a] (μg/g)			
	CO_2 light	CO_2 dark	N_2 light	N_2 dark
benzaldehyde[b]	0.010[a] (0.003)	0.002[b] (<0.001)	0.001[b] (0.001)	0.001[b] (<0.001)
cyclohexanone	nd[c]	nd	nd	0.001[a] (0.001)
2-ethylfuran	0.034[a] (0.040)	nd	nd	nd
(1-methylethyl)benzene[b]	0.049[a] (0.016)	0.052[a] (0.010)	0.046[a] (<0.001)	0.043[a] (0.012)
D-limonene	0.008 (0.005)	nd	nd	nd
total	0.102[a](0.034)	0.054[b] (0.010)	0.040[b] (<0.001)	0.046[b] (0.010)

[a] Means in a row with different superscript letters are different ($p < 0.05$) using LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). [b] Analysis of variance conducted on log transformed data. [c] Not detected.

Table 8. L^*, a^*, and b^* Color Values for Cheddar Cheese Packaged under CO_2 or N_2 and Stored under Light or Dark Conditions[a]

color	CO_2 light	CO_2 dark	N_2 light	N_2 dark
L^*	79.44[a] (0.953)	76.34[b] (1.02)	74.75[c] (0.774)	75.60[bc] (0.424)
a^*	2.34[a] (0.295)	7.62[b] (1.07)	7.76[b] (0.244)	8.30[b] (0.104)
b^*	21.66[a] (1.21)	41.00[b] (4.36)	43.20[b] (1.34)	44.67[b] (1.59)

[a] Means in a row with different superscript letters are different ($p < 0.05$) based on LSD pairwise mean comparisons. Values in parentheses denote standard deviation ($n = 3$). L^*, whiteness; a^*, red/greenness; and b^*, yellow/blueness.

The second explanation may be an insufficient maturation of the cheeses analyzed. An increase in sulfur concentrations has been correlated with proteolytic activities during the ripening process (*24*). Therefore, low amounts of sulfur compounds can be attributed to an inhibitory effect that N_2 may impose on proteolysis.

In the present study, the concentrations of cyclic compounds were similar following all of the cheese treatments with the exception of those cheeses packaged under CO_2 and exposed to fluorescent light (Table 7). These cheeses contained twice the total concentration of cyclic compounds of the other cheeses, due to the presence of two additional compounds, 2-ethylfuran and D-limonene, which were not present in cheeses stored under the other three conditions. The presence of 2-ethylfuran in CO_2-treated cheeses exposed to light may be attributed to degradation of thiamin present in Cheddar cheese. Limonene, which was present in the cheeses exposed to CO_2 and light, probably originated from degradation of bixin, the major pigment used to color most colored Cheddar cheeses. CO_2 in combination with light led to breakdown of bixin as evidenced by loss of color in the cheeses exposed to CO_2 and light. Bixin consists of isoprene units that may degrade to form limonene. Limonene was previously detected in Cheddar cheese and was associated with a characteristic citrus aroma (*15*). Benzaldehyde and 1-(methylethyl)benzene were the only cyclic compounds detected in all treatment groups. Benzaldehyde is derived from the degradation of phenylalanine (*25*). The presence of the highest concentration of benzaldehyde in the CO_2- and light-treated samples suggests that this reaction is favorable in these conditions.

Color Changes. Color data for shredded Cheddar cheeses packaged under N_2 and CO_2 and stored under light or dark conditions are presented in Table 8. L^* values were significantly higher in cheeses packaged under CO_2 and exposed to fluorescent light, whereas a^* and b^* values were significantly lower. The shredded cheeses packaged under CO_2 and exposed to fluorescent light experienced obvious color alterations during storage, shifting from the traditional orange of colored Cheddar cheese to a definite white hue. No significant differences in color were observed among the other treatment groups.

High-intensity light is known to be detrimental to the color stability of natural yellow cheeses (*11*, *13*). In ref *12* it was suggested that discoloration observed in yellow cheeses is related to lipid oxidation. A positive correlation between the rate of discoloration of Cheddar cheese and oxygen transmission rates of packaging films was observed in ref *13*.

One possible explanation for the color shift in the shredded Cheddar cheese packaged under CO_2 and exposed to fluorescent light is related to annatto, the pigment commonly used in the production of natural yellow cheeses such as Cheddar. Annatto extracts are obtained from the seeds of *Bixa orellana*. The carotenoid that is mainly responsible for the coloring properties of annatto is bixin, which contains numerous conjugated double bonds. Conjugated double bonds are the target of free radical molecules, leading to oxidation reactions in lipids and probably in the carotenoid, bixin. We propose that the color loss was due to bixin oxidation resulted from CO_2-derived free radical species generated under light exposure.

Conclusion. Even though visible signs of mold growth were not detected in the samples analyzed in the present study, alterations in volatile profiles, namely, high concentrations of methyl ketones, indicated that shredded Cheddar cheese packaged under N_2 was highly susceptible to molding. Packaging of shredded Cheddar cheese in a 100% CO_2 atmosphere had negative effects. The generation of important volatile compounds essential for the development of Cheddar cheese flavor was suppressed by CO_2 exposure. In addition, high concentrations of lipid oxidation products and bixin discoloration occurred in shredded Cheddar cheese packaged under 100% CO_2 and stored under fluorescent light.

For economic and functional purposes, actual industry practices may include a variety of CO_2 and N_2 gas blends. The present study showed that high CO_2 or N_2 concentrations potentiate undesirable changes during the storage of shredded Cheddar cheese. Additionally, processors may consider the use of packaging or displays designed to inhibit light-induced chemical reactions that potentially influence the flavor and appearance of the cheese product.

ACKNOWLEDGMENT

We express our appreciation to the Tillamook County Creamery Association for donation of cheese samples.

LITERATURE CITED

(1) Christensen, K. R.; Reineccius, G. A. Aroma extract dilution analysis of aged Cheddar cheese. *J. Food Sci.* **1995**, *60*, 218−220.

(2) Dunn, H. C.; Lindsay, R. C. Comparison of methods for the analysis of higher boiling flavor compounds in Cheddar cheese. *J. Dairy Sci.* **1985**, *68*, 2853−2858.

(3) Engels, W.; Visser, S. Isolation and comparative characterization of components that contribute to the flavor of different types of cheese. *Neth. Milk Dairy J.* **1994**, *48*, 127−140.

(4) Fox, P. F.; Singh, T. K.; McSweeney, P. L. H. Biogenesis of flavour compounds in cheese *Adv. Exp. Med. Biol.* **1995**, *367*, 59−98.

(5) Manning, D. J. Cheddar cheese flavor studies. I. Production of volatiles and development of flavor during ripening. *J. Dairy Res.* **1978**, *45*, 479−490.

(6) Urbach, G. Contribution of lactic acid bacteria to flavour compound formation. *Int. Dairy J.* **1995**, *5*, 877−903.

(7) Alves, R. M. V.; Sarantopoulos, C. I. G. de L.; Dender, A. G. F. van; Faria, J. de A. F. Stability of sliced mozzarella cheese in modified atmosphere packaging. *J. Food Prot.* **1996**, *59*, 838−844.

(8) Maniar, A. B.; Marcy, J. E.; Bishop, J. R.; Duncan, S. E. Modified atmosphere packaging to maintain direct-set cottage cheese quality. *J. Food Sci.* **1994,** *59*, 1305−1308, 1327.

(9) Drost, B. W. Fermentation and storage. *Proceedings of the 16th Congress of the European Brewing Convention*; Elsevier: Amsterdam, The Netherlands, 1977; pp 519−522.

(10) Dixon, N. M.; Kell, D. B. The inhibition by CO_2 of the growth and metabolism of microorganisms. *J. Appl. Bacteriol.* **1989**, *67*, 109−136.

(11) Deger, D.; Ashoor, S. H. Light-induced changes in taste, appearance, odor, and riboflavin content of cheese. *J. Dairy Sci.* **1987**, *70*, 1371−1376.

(12) Barnicoat, C. R. Cheese discoloration: oxidation of bixin in annatto-coloured cheeses promoted by sulphydryl compounds. *J. Dairy Res.* **1950**, *8*, 209−213.

(13) Hong, C. M.; Wendorff, W. L.; Bradley, R. L. Effects of packaging and lighting on pink discoloration and lipid oxidation of annatto-colored cheeses. *J. Dairy Sci.* **1995**, *78*, 1896−1902.

(14) Adda, J. Flavor of dairy products. In *Developments in Food Flavours*; Birch, G. G., Lindley, M. G., Eds.; Elsevier Applied Science Publishers: London, U.K., 1986; pp 151−172.

(15) Engels, W. J. M.; Dekker, R.; de Jong, C.; Neeter, R.; Visser, S. A comparative study of volatile compounds in the water-soluble fraction of various cheese types of ripened cheese. *Int. Dairy J.* **1997**, *7*, 255−263.

(16) Horwood, J. F. Headspace analysis of cheese. *Aust. J. Dairy Technol.* **1989**, *44*, 91−95.

(17) Fox, P. F.; Law, J.; McSweeney, P. L. H.; Wallace, J. Biochemistry of cheese ripening. In *Cheese: Chemistry, Physics, and Microbiology*; Fox, Q. F., Ed.; Chapman and Hall: London, U.K., 1993; Vol. 1, pp 389−438.

(18) Urbach, G. The flavour of milk and dairy products: II. Cheese: contribution of volatile compounds. *Int. J. Dairy Technol.* **1997**, *50*, 79−89.

(19) Arora, G.; Cormier, F.; Lee, B. Analysis of odor-active volatiles in Cheddar cheese headspace by multidimensional GC/MS/sniffing. *J. Agric. Food Chem.* **1995**, *43*, 748−752.

(20) Daniels, J. A.; Krishnamurthi, R.; Rizvi, S. S. H. A review of effects of carbon dioxide on microbial growth and food quality. *J. Food Prot.* **1985**, *48*, 532−537.

(21) Sears, D. F.; Eisenberg, R. M. A model representing a physiological role of CO_2 at the cell membrane. *J. Gen. Physiol.* **1961**, *44*, 869−886.

(22) Dimos, A.; Urbach, G. E.; Miller, A. J. Changes in flavor and volatiles of full-fat and reduced-fat Cheddar cheese during maturation. *Int. Dairy J.* **1996**, *6*, 981−995.

(23) Urbach, G. Relations between cheese flavour and chemical composition. *Int. Dairy J.* **1993**, *3*, 389−422.

(24) Barlow, G. T.; Lloyd, E. H.; Ranshaw, E. H.; Miller, A. J.; McCabe, G. P.; McCabe, L. Correlations and changes in flavour and chemical parameters of Cheddar cheese during maturation. *Aust. J. Dairy Technol.* **1989**, *44*, 7−18.

(25) Groot, M. N. N.; de Bont, J. A. M. Conversion of phenylalanine to benzaldehyde initiated by an aminotransferase in *Lactobacillus plantarum*. *Appl. Environ. Microbiol.* **1998**, *64*, 3009−3013.

Received for review October 12, 2000. Revised manuscript received February 8, 2001. Accepted February 22, 2001. We express our appreciation to the Maryland Agricultural Experiment Station, University of Maryland, for financial support.

JF0012392