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# Study of Light-Induced Volatile Compounds in Goat's Milk Cheese

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Light-induced volatile compounds in goat cheese were studied by a combination of solid phase microextraction (SPME)–gas chromatography (GC)–mass spectrometry (MS), headspace oxygen depletion, and sensory evaluation. Samples stored under fluorescent light for 2 days at 30 °C had 90% more volatile compounds and 4 times more headspace oxygen depletion than samples stored in the dark at 30 °C. The volatiles 1-heptanol, heptanal, nonanal, and 2-decenal were formed and increased only in the light-stored samples, which may be formed from singlet oxygen oxidation of unsaturated fatty acids. Sensory evaluation showed that samples stored under light had significantly more off-flavor than samples stored in the dark at 30 °C (P < 0.05), and 1-heptanol, heptanal, nonanal, and 2-decenal increased the goat cheese off-flavor significantly (P < 0.05).

# KEYWORDS: Goat cheese; light-induced volatile compounds; singlet oxygen; off-flavor

# INTRODUCTION

Goat's milk and goat's milk products have been widely consumed in Europe (1), and the demand for goat's milk products has increased in the United States among ethnic groups, gourmets, and consumers of health and diet foods (2). Goat's milk cheese has good nutritional value due to the significantly higher contents of short-chain, medium-chain, and polyunsaturated fatty acids compared with cow's milk and cow's milk cheese, and whey protein in goat's milk cheese was reported to enhance the nutritional value (3). It was reported that the consumption of goat's milk reduced the level of cholesterol in the small intestine (4).

Cheese flavor comes from microbial, enzymatic, and chemical transformations of milk components during ripening. Proteolysis and lipolysis are the two most important reactions during cheese manufacturing. Lipolysis can produce free fatty acids such as hexanoic, octanoic, nonanoic, and decanoic acids and branched fatty acids including 4-methyloctanoic and the 4-ethyloctanoic acids. These fatty acids can give the characteristic goaty flavors to goat's milk cheese (5, 6).

Cheeses are often displayed on the shelves with fluorescent light in the markets of United States. The harmful effects of light on the formation of off-flavor in cheese have been reported in the literature. Light accelerated the production of the rancid and sour off-flavor, which influenced negatively the flavor quality in cheese (7). High-intensity light can cause changes in the taste, flavor, and color of yellow cheese (8), and 2-thiobarbituric acid values, which represent the degree of oxidation, increased in the cheese exposed to fluorescent light (9). Light can deteriorate the quality of cheese packaged in transparent film or plastic containers (10). However, the mechanisms of the photo-oxidation on the formation of light-induced volatile compounds and the effects of light-induced volatile compounds on the flavor quality of goat's milk cheese have not been reported even as the demand for goat's milk cheese in the United State continuously increases and fluorescent light is a primary display light source in the markets.

The objectives of this work were to study the effects of fluorescent light on the formation of headspace volatile compounds, headspace oxygen depletion, and flavor quality in goat's milk cheese by a combination of SPME-GC-MS and sensory evaluation.

# MATERIALS AND METHODS

**Materials.** Blocks of goat's milk cheese were purchased from a local grocery store (Wild Oats, Columbus, OH) and stored in a -10 °C freezer room. A manual SPME fiber holder unit, 65  $\mu$ m poly-(dimethylsiloxane)/divinylbenzene (PDMS/DVB), serum bottles, Teflon-coated rubber septa, and aluminum caps were purchased from Supelco (Bellefonte, PA). 1-Heptanol, heptanal, nonanal, 2-decenal, and other chemicals were purchased from Aldrich (St. Louis, MO).

Sample Preparation for Light and Dark Storage. All samples were prepared in a -10 °C cold room in the Department of Food Science at The Ohio State University. The 0.5 cm surface of goat's milk cheese was removed to reduce the oxidation effects from the surface. Goat's milk cheese was cut with a cheese cutter into small pieces 0.1 cm long to increase surface area, and 20.0 g of prepared goat cheese was put into a 100-mL serum bottle. The sample bottles were sealed airtightly with aluminum caps and Teflon-coated rubber septa. Sample bottles were placed at a distance of 10 cm from fluorescent lamps in a light box at 30 °C for 6 days. The light source was four Sylvania fluorescent lamps (General Electric, Cleveland, OH)

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with 1500 lx light intensity. Sample bottles were also wrapped with aluminum foil and designated the samples stored in the dark. One set of samples was put in a 60 °C oven in the dark for 6 days to study the temperature effects on the headspace volatile compounds. Headspace volatile compounds and headspace oxygen content of samples were measured at 0, 0.5, 1, 2, 4, and 6 days. Samples were prepared in triplicate at each sampling point.

Headspace Volatile Compound Analysis by SPME. Sample bottles were taken from the light box at each sampling time. Sample bottles were shaken for 1 min and placed in a 40 °C water bath for 30 min to achieve equilibrium of volatile compounds between the headspace and sample matrix. The 65  $\mu$ m PDMS/DVB trapped the headspace volatile compounds in sample bottles for 30 min at 40 °C in a water bath. The volatile compounds isolated by PDMS/DVB were desorbed at the injector port of GC with a flame ionization detector.

**Headspace Oxygen Analysis.** The headspace oxygen of goat's milk cheese was determined by injecting 100  $\mu$ L of headspace gas of samples into a GC equipped with a thermal conductivity detector. A column (1.8 m × 0.32 cm) packed with 60/80 molecular sieve 13X (Alltech Associates Inc., Deerfield, IL) was used. The flow rate of hydrogen was 20 mL/min. The temperatures of the oven, injector, and thermal conductivity detector were 40, 120, and 150 °C, respectively.

Sensory Evaluation of Samples Stored under Light and in the Dark. Goat's milk cheese samples stored at 30 °C under fluorescent light or in the dark for 2 days were evaluated using overall difference tests (11). Twelve trained panelists (faculty and graduate students) ranging in age from 25 to 40 years from the Department of Food Science and Technology at The Ohio State University participated in this study. The panelists were trained to develop a scaling technique, evaluation process, and panel confidence by recognizing goat's milk cheese reference samples and reaching an agreement in an open session approach with fellow panel members. Sample bottles were taken from the light box and placed for 30 min at 25 °C for the equilibrium of flavor in the bottles. The panelists were seated in individual booths in the sensory facility of the Department of Food Science and Technology, and each panelist evaluated a control sample and samples stored at 30 °C under light or in the dark for 2 days at each sitting. The samples were coded with three-digit random numbers, and panelists were allowed to choose randomly the order of sampling. The panel members inhaled directly at the outlet of the sample bottles for the evalutation of intensity of off-flavor after releasing the rubber septum and aluminum cap. The panelists were instructed to wait at least 1 min before evaluating the next sample. The panelists were asked to mark the intensity of off-flavor on the line scale and to describe the off-flavor. A nine-point line scale was used  $(1 = no \text{ off-flavor}; 5 = mild \text{$ flavor; 9 = extreme off-flavor).

Sensory Evaluation of Samples with the Addition of Light-Induced Volatile Compounds. 1-Heptanol, heptanal, nonanal, and 2-decenal were prepared in distilled water to obtain a concentration of 10  $\mu$ g/mL, respectively. Each solution of 1-heptanol, heptanal, nonanal, and 2-decenal was mixed with control goat's milk cheese samples to obtain similar electronic counts of 1-heptanol, heptanal, nonanal, and 2-decenal in goat's milk cheese stored under light for 6 days, which were 0.3, 1.4, 1.7, and 1.5 (1 × 10<sup>4</sup>), respectively.

The concentrations of 1-heptanol, heptanal, nonanal, and 2-decenal in goat's milk cheese stored under light for 6 days were mixed and were put in control goat's milk cheese samples, and the flavor of the samples was evaluated using overall difference tests (11). The same 12 trained panelists evaluated the samples. Sample bottles were kept for 30 min at 25 °C for the equilibrium of flavor in the bottles. Each panelist evaluated a control sample and a sample with the addition of light-induced volatile compounds at each sitting. The sensory procedure was the same as described previously. The panelists were asked to mark the intensity of off-flavor on the line scale and to describe the offflavor. The nine-point line scale was used (1 = no off-flavor; 5 = mild off-flavor; 9 = extreme off-flavor).

Gas Chromatography for the Analysis of Headspace Volatile Compounds. A Hewlett-Packard 5890 gas chromatograph was equipped with a 0.75 mm i.d. glass injection liner, a flame ionization detector, and a 30 m  $\times$  0.25 mm i.d., 1.0  $\mu$ m film, DB-5, from J&W (Folsom, CA). The oven temperature was held at 40 °C for 2 min and increased

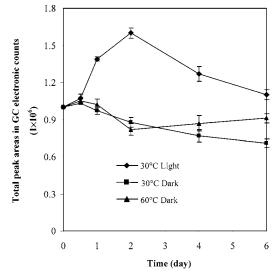


Figure 1. Total peak areas of goat's milk cheese stored under light and in the dark at 30 °C or in the dark at 60 °C for 6 days by SPME.

from 40 to 160 °C at the rate of 6 °C/min and from 160 to 210 °C at 8 °C/min. The temperatures of the injector and detector were 250 and 300 °C, respectively. The flow rate of nitrogen carrier gas was 1.0 mL/min. The isolated volatile compounds in the solid phase of SPME were desorbed at 250 °C for 2 min.

Identification of Volatile Compounds. A Hewlett-Packard 5971A mass selective detector equipped with a Hewlett-Packard 59822B ionization gauge controller was used. All mass spectra were obtained at 70 eV and 220 °C of ion source temperature. Identification of compounds was made by the combination of NIST mass spectra and gas chromatographic retention times of standard compounds. Helium carrier gas at 0.9 mL/min and an HP-5 column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m thick) from Agilent Technologies (Palo Alto, CA) were used. The GC conditions for GC-MS were the same as the gas chromatographic analysis conditions of headspace volatile compounds described previously.

Statistical Analysis. One-way analysis of variance, Tukey's multiplecomparisons method, and a general linear model were used to analyze the data. A *P* value of  $\leq 0.05$  was considered to be significant. All statistical analyses were conducted with Minitab 12.1 (Minitab Inc., State College, PA).

#### **RESULTS AND DISCUSSION**

Light Effects on the Headspace Volatile Compounds in Goat's Milk Cheese. Total peak areas of goat's milk cheese stored at 30 °C under light, in the dark, and at 60 °C in the dark for 6 days are shown in Figure 1. Total volatile compounds in samples stored in the dark at 30 and 60 °C were not significantly different from 0 to 4 days of storage (P > 0.05). Total peak areas from the samples stored under light at 30 °C were significantly higher than those of samples stored at 30 or 60 °C in the dark from 1 to 6 days (P < 0.05). Samples stored under fluorescent light for 2 days at 30 °C had 90% more volatile compounds than samples stored in the dark at 30 °C. Total peak areas from the light-stored samples at 30 °C for 0.5, 1, 2, 4, and 6 days increased by 5, 40, 60, 30, and 10% compared with those of 0 day samples, respectively. The decrease in total peak areas of light-stored samples after 2 days may be due to the increasing ratio of nonvolatile compound formations to volatile compound formations. The higher total peak areas in the samples stored under light than that in samples stored in the dark at 30 °C may be due to the presence of photosensitizers such as riboflavin in goat's milk cheese. Riboflavin can work as a photosensitizer, which can accelerate the oxidation reactions of unsaturated fatty acids, amino acids, and vitamins (12).

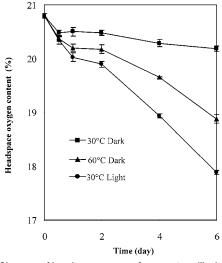


Figure 2. Changes of headspace oxygen from goat's milk cheese stored under light and in the dark at 30  $^{\circ}$ C or in the dark at 60  $^{\circ}$ C for 6 days.

Changes of Headspace Oxygen in Goat's Milk Cheese. The changes of headspace oxygen in goat's milk cheese stored under light and in the dark at 30 °C or in the dark at 60 °C for 6 days are shown in Figure 2. As storage time increased from 0 to 6 days, headspace oxygen decreased in all samples. Headspace oxygen in the samples stored under light at 30 °C was significantly different from that in the dark-stored samples at 30 °C from 1 to 6 days of storage (P < 0.05). Headspace oxygen content in samples stored in the dark at 60 °C was significantly different from that in samples stored in the dark at 30 °C from 1 to 6 days of storage. The depletion of headspace oxygen content in samples stored in the dark at 60 °C may be due to nonenzymatic browning reactions of amino acids, lactose, or lipid oxidation products. The depletion of headspace oxygen content in samples stored in the dark at 30 °C may be due to the enzymatic reactions in goat cheese. Headspace oxygen from samples stored in the dark or under light at 30 °C for 6 days decreased by 3.0 and 14.0%, respectively. The difference in the headspace oxygen between the samples stored under light and in the dark is due to the effect of photo-oxidation on the goat's milk cheese components. Li et al. (13) reported that singlet oxygen oxidation was responsible for the depletion of headspace oxygen in the light-stored samples containing photosensitizers such as riboflavin. Riboflavin is a well-known photosensitizer in milk, which can generate singlet oxygen from triplet oxygen under light. It is reported that riboflavin plays an important role in the light-induced oxidation in dairy products (14). The concentration of riboflavin in goat's milk cheese was reported as  $0.4 \,\mu g/100$  g of cheese, whereas goat's milk contains 1.2-1.7 mg/L depending on the analysis methods and samples (15, 16).

**Formation of Light-Induced Volatile Compounds in Goat's Milk Cheese.** Identified headspace volatile compounds in goat's milk cheese stored under light and in the dark at 30 °C or in the dark at 60 °C are shown in **Table 1**. 1-Heptanol, heptanal, nonanal, and 2-decenal were detected in the goat's milk cheese stored at 30 °C under light but not in the samples stored in the dark at 30 °C. Ethyl acetate and 2-phenylethylacetic acid, which were formed in both dark- and light-stored samples at 30 °C, may come from the enzymatic reactions of proteolysis and lipolysis. Le Quere et al. (6) reported the presence of 2-phenylethylacetic acid in goat's milk cheese. The peak area changes of light-induced volatile compounds in goat's milk cheese stored under light at 30 °C for 6 days are shown in

Table 1. Identified Headspace Volatile Compounds in Goat's Milk Cheese Stored for 2 Days at 30  $^\circ\text{C}$  in the Dark and Light or at 60  $^\circ\text{C}$  in the Dark

			30 °C		60 °C
RT <sup>a</sup>	volatile compound	0 days	dark	light	dark
1.80	ethanol <sup>GC,MSf</sup>	8.21 <sup>b</sup>	4.82	4.95	4.02
2.51	ethyl acetate <sup>GC,MS</sup>	nd <sup>c</sup>	8.01	7.82	n.d.
2.91	3-methylbutanal <sup>d GC,MS</sup>	nd	nd	nd	1.52
3.33	2-pentanone <sup>GC,MS</sup>	nd	nd	nd	1.12
3.91	acetic acid <sup>GC,MS</sup>	1.89	6.19	8.62	3.41
4.31	dimethyl disulfideGC,MS	nd	nd	nd	2.52
6.13	butanoic acid <sup>GC,MS</sup>	1.22	1.37	1.58	1.15
6.35	furfural <sup>MS</sup>	nd	nd	nd	0.45
7.95	2-heptanone <sup>GC,MS</sup>	nd	nd	nd	3.97
8.19	5-methyl-2-hexanone <sup>MS</sup>	nd	nd	nd	0.81
8.28	heptanal <sup>e GC,MS</sup>	nd	nd	0.78	n.d.
10.49	1-heptanol <sup>GC,MS</sup>	nd	nd	0.25	n.d.
11.04	hexanoic acid <sup>GC,MS</sup>	4.87	5.27	5.84	7.18
11.85	limonene <sup>GC,MS</sup>	4.01	4.22	4.18	3.89
13.87	nonanal <sup>GC,MS</sup>	nd	nd	1.07	0.41
15.83	octanoic acid <sup>GC,MS</sup>	2.57	2.58	2.94	3.72
17.01	2-decenal <sup>GC,MS</sup>	nd	nd	0.82	n.d.
17.89	2-phenylethylacetic acid <sup>MS</sup>	nd	7.17	6.81	1.25
18.75	2-undecanone <sup>GC,MS</sup>	nd	nd	nd	0.45
20.53	decanoic acid <sup>GC,MS</sup>	1.52	1.55	1.84	3.58
26.82	$\gamma$ -dodecalactone <sup>MS</sup>	nd	nd	nd	0.35

<sup>*a*</sup> RT, retention time in the total ion chromatograms from SPME-GC-MS. <sup>*b*</sup> Mean value of peak area of a compound in ion count  $(1 \times 10^7)$  by SPME-GC-MS (n = 3). <sup>*c*</sup> nd, not detected. <sup>*d*</sup> Boldface type represents compounds found only in the samples stored at 60 °C. <sup>*e*</sup> Italic type represents compounds found only in the light-stored samples at 30 °C. <sup>*f*</sup> Superscript GC,MS represents a volatile identified by both GC retention time of a standard compound and GC-MS. Superscript MS represents a volatile identified by GC-MS only.

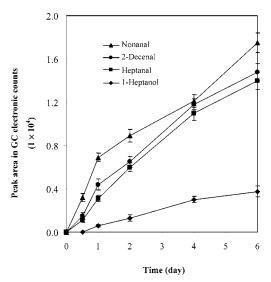
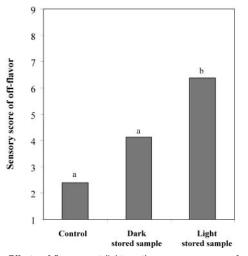


Figure 3. Peak area changes of 1-heptanol, heptanal, nonanal, and 2-decenal in goat's milk cheese stored under light at 30 °C for 6 days.

**Figure 3.** 1-Heptanol, heptanal, nonanal, and 2-decenal in samples stored under light at 30 °C increased significantly in peak area as the light exposure time increased from 1 to 6 days (P < 0.05). The headspace oxygen in sample bottles stored under light at 30 °C may be used for the formation of light-induced volatile compounds.

Light-induced volatile compounds may come from singlet oxygen oxidation of unsaturated fatty acids. The free fatty acid content of goat's milk cheese used in this study was analyzed as described in Martin-Hernandez et al. (17) except *n*-heptanoic acid was used as internal standard instead of *n*-nonanoic acid. Palmitic, stearic, oleic, and linoleic acids in goat's milk cheese



**Figure 4**. Effects of fluorescent light on the sensory scores of off-flavor from goat's milk cheese stored at 30 °C. Bars with different superscripts are significantly different (P < 0.05).

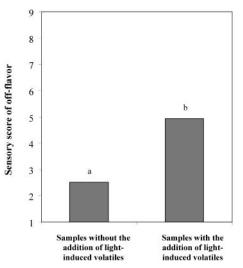
were 22, 10, 18, and 2%, respectively. The contents of oleic acid in goat's milk cheese are higher than linoleic acids by 9 times. Nonanal and 2-decenal, which are the decomposition products of oleic acid (18), can be detected first due to the high contents of oleic acid in goat's milk cheese. To produce nonanal and 2-decenal, hydroperoxides should be formed at positions C10 and C9 of oleic acid, respectively (18). Singlet oxygen can form hydroperoxide at positions 9 and 10 in oleic acid, whereas triplet oxygen can form hydroperoxide at positions 8, 9, 10, and 11 in oleic acid (18). Both singlet and triplet oxygen oxidation can form nonanal and 2-decenal from oleic acid. The reaction rate of singlet oxygen with oleate was 30000 times higher than that of triplet oxygen (19). Considering the absence of nonanal and 2-decenal in the samples stored in the dark at 30 °C, singlet oxygen oxidation plays a more important role than triplet oxygen oxidation in the formation of nonanal and 2-decenal in goat's milk cheese stored under light at 30 °C.

Heptanal and 1-heptanol were reported in the thermally decomposed methyl oleate hydroperoxides from photosensitized oxidation and autoxidation (18). Lercker et al. (20) reported that heptanal and 1-heptanol were not identified from the thermal decomposition of purified C9 and C10 hydroperoxides. Singlet oxygen oxidation forms C9 and C10 hydroperoxides from oleic acid, which can form the C11 hydroperoxide by isomerization (20). Grosch (21) and Ho and Chen (22) proposed the formation of 1-heptanol from C11 oleic acid hydroperoxide.

Furfural and  $\gamma$ -dodecalactone were detected in the goat's milk cheese stored for 2 days at 60 °C in the dark only. Furfurals and lactones are the products of lipid oxidation and nonenzymatic browning reactions at the elevated temperature (23).

Sensory Evaluation of Samples Stored under Light and in the Dark. Sensory scores of goat's milk cheese stored under fluorescent light or in the dark for 2 days at 30 °C are shown in **Figure 4**. The sensory scores of off-flavor from the samples stored under light were significantly higher than those from samples stored in the dark and control samples (P < 0.05). Sensory scores of off-flavor from samples stored in the dark at 30 °C were not significantly different from control samples (P > 0.05). Sensory panelists described the off-flavor from the samples stored under light as oxidized, fatty, acidic, and pungent.

This result suggests that light-induced volatile compounds produce more off-flavor and reduce the flavor quality of goat cheese. Kristensen et al. (7) reported that light-induced volatile compounds increased rancid and sour off-flavor in cheese.



**Figure 5.** Sensory scores of off-flavor from goat's milk cheese with or without the addition of light-induced volatile compounds. Bars with different superscripts are significantly different (P < 0.05).

Sensory Evaluation of Samples with the Addition of Light-Induced Volatile Compounds. Sensory scores of off-flavor from goat's milk cheese samples with or without the addition of 1-heptanol, heptanal, nonanal, and 2-decenal are shown in Figure 5. The sensory scores of off-flavor from a sample with the light-induced volatile compounds were significantly different from those of samples without the light-induced volatile compounds (P < 0.05). Sensory panelists described the sample with the light-induced volatile compounds as rotten, soapy, rancid, and cheesy. The sensory characteristics of 1-heptanol, heptanal, nonanal, and 2-decenal are reported as chemical, fatty, soapy, and tallowy, respectively (24). The light-induced volatile compounds are responsible for the increases of the off-flavor in goat's milk cheese stored under light.

In conclusion, fluorescent light can increase the headspace volatile compounds including 1-heptanol, heptanal, nonanal, and 2-decenal, which increase the off-flavor in goat's milk cheese. These light-induced volatile compounds can be formed from singlet oxygen oxidation of oleic acid in goat's milk cheese. The formation of light-induced volatile compounds should be prevented to protect flavor quality of any type of cheese displayed under fluorescent lighting on shelves in markets by packaging the cheese in light-impermeable material or removing headspace oxygen.

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