

CHAPTER V

Effects of UV Light on Chemical Indicators of Goat's Milk

MATAK, K.E.¹, SUMNER, S.S.¹, DUNCAN, S.E.¹, HOVINGH, E.²,
WOROBO, R.W.³, HACKNEY, C.R.⁴, PIERSON, M.D.¹

¹Virginia Tech, Department of Food Science and Technology, Blacksburg, VA, 24061.

²The Pennsylvania State University, Department of Veterinary Science,
University Park, Pennsylvania, 16802.

³Cornell University, Department of Food Science & Technology,
New York State Agricultural Experiment Station,
Geneva, New York, 14456.

⁴West Virginia University, Davis College of Agriculture, Forestry, and Consumer Sciences,
Morgantown, West Virginia, 26506.

ABSTRACT

Organoleptic and chemical consequences of goat's milk treated with UV technology were assessed. Goat's milk samples were processed 12 consecutive times using a UV fluid processor for a cumulative exposure time of 18 sec and targeted UV dose of 15.8 +/- 1.6 mJ/cm². Olfactory studies were conducted and a highly significant difference was determined between the odor of raw goat's milk and UV irradiated milk ($p < 0.05$). The extent of oxidation and hydrolytic rancidity was measured by thiobarbituric acid reactive substances (TBARS) and acid degree values (ADVs). Results indicated that as the UV dose increased, there was a significant increase in TBARS values and ADVs of the milk samples ($p < 0.05$). Milk samples were processed using the UV processor under the same conditions as previously described but without exposure to the UV source to determine if lipase activity and agitation from pumping contributed to the off-odors. The ADVs from these samples increased at the same rate as the UV irradiated samples; however, olfactory studies indicated that the increase of free fatty acids (FFA) was not enough to cause detectable off-odors in the milk ($p < 0.05$). Solid phase microextraction and gas chromatography (SPME-GC) was utilized to quantify the production of volatile compounds that were formed during UV processing. The formation of pentanal, hexanal and heptanal was identified after as little as 1.3 mJ/cm² UV dose. Peak areas were measured and analyzed after 7.8 mJ/cm² and 15.6 mJ/cm² and increased significantly as UV dose increased ($p < 0.05$). The chemical analyses supported the findings from the olfactory studies. The outcome of this research showed that UV irradiation at the wavelength 254 nm, was detrimental to certain chemical properties of fluid milk. The properties that were perceived as negative in fluid milk may be considered an attribute in certain types of cheese and future studies in the cheese production sector should be considered. Other applications for this technology could be for use in developing countries where milk is not typically processed because of the high costs of thermal pasteurization. On-farm applications for the treatment of replacement milk could also be considered.

Key words: goat's milk, oxidation, UV irradiation, solid-phase microextraction, thiobarbituric acid reactive substances (TBARS), acid degree values (ADVs)

INTRODUCTION

A recent phenomenon in the dairy processing sector is the introduction of small-scale producers that process dairy commodities on-farm to sell at high-end restaurants, farmer's markets and natural foods stores. In the US, dairy goat farmers are small in number, but cheeses made from goat's milk have seen an increase in popularity and consumption per capita (Park, 1990). The market for goat's milk products is expanding and the consumer trend towards "fresher" and more "natural" food choices has created a niche market for minimally processed and raw dairy foods (Reed and Grivetti, 2000). Consumer preference for raw dairy products is linked with perceived superior organoleptic characteristics which cannot be obtained when the milk is heat-treated (Buchin *et al.*, 1998). Grappin and Beuvier (1997) found that cheeses made with raw milk versus those made with pasteurized milk developed a stronger flavor and ripened faster. These differences were attributed to the preservation of heat sensitive enzymes and indigenous microbiota. While thermal pasteurization of milk has been proven to be effective at reducing pathogenic bacteria and controlling lipase activity, UV irradiation is an alternative method that is being studied which would be less costly for producers working with smaller volumes and may have less perceived effects on organoleptic properties.

It has been well reported that both UV and visible light, with wavelengths between 280 nm and 700 nm, are key factors in the creation of flavor defects and malodors in milk (Azzara and Cambell, 1992; Bekbölet, 1990; Borle *et al.*, 2001; Cadwallader and Howard, 1998; Dimick, 1982; Frankel, 1980; Frankel, 1991; Min and Boff, 2002). Historical studies that looked at the potential of UV irradiation for vitamin D enrichment at the specific germicidal wavelength of 254 nm did not report negative sensory data (Burton, 1951; Capstick *et al.*, 1949; Caseiro *et al.*, 1975). Matak (2004) determined that UV processing was effective as a non-thermal process for the reduction of certain bacterial pathogens in goat's milk. The target pathogen reduction was achieved when the flow rate of the milk through the UV processing unit was increased to the point where the milk was exposed to the UV source in a state of turbulent flow. The development of off-odors after processing was apparent by personal observations so the current study was conducted to determine the nature

of the off-odors; i.e., if these defects were catalyzed by UV exposure, lipase activity brought about by turbulence and agitation in the system, or both.

MATERIALS AND METHODS

Milk

Fresh goat's milk was purchased from a commercial dairy and the gross composition of the milk (total fat, protein, total solids) was evaluated to verify consistency among replications. An infrared analyzer (Infrared Analyzer 115 Vac, Denver Instrument Company, Arvado, CO) was used to determine moisture content and total solids. The modified Babcock procedure was used to determine percent milkfat (Marshall, 1993). The Bradford method (Bio-Rad protein assay, Bio-Rad, Hercules, CA) (using a spectrophotometer {Spectronic 20 Colorimeter, Bausch & Lomb Inc., Rochester, NY}) was used to determine protein content. Milk was collected, transported and stored in a covered 5 gal cooler (Igloo Products Corp., Houston Texas, USA) and temperature was maintained at 4°C.

UV Irradiation

Milk samples were exposed through an ultraviolet fluid processor 12 consecutive times for an exposure time of approximately 18 sec and targeted UV dose of 15.8 +/- 1.6 mJ/cm² (CiderSure 3500A, FPE Inc., Rochester, NY). This UV dose was in the range that was shown to be effective at achieving a 5-log reduction in the pathogen *Listeria monocytogenes* in fresh goat's milk (Matak, 2004). The UV apparatus was designed to allow fluids to flow in thin films through a quartz tube encased in a stainless steel outer housing. Eight germicidal, low-pressure mercury lamps situated in the inner housing along the same axis guarantee uniform UV exposure throughout the milk. UV dose was calculated by multiplying exposure time by irradiance. The machine was run at 75% capacity (a flow rate of 567 L/h) so that comparisons could be made with findings from previous studies. This flow rate corresponds to a Reynolds Number of 5187 which represents turbulent flow (Fellows, 1997). The lamps generate approximately 1.3 mJ/cm² UV dose at exposure times

between 1 and 2 sec at this flow rate measured using two UVX-25 sensors (UVP Inc., CA). Aliquots of the milk were taken after 4, 6, 8 and 12 passes through the processor for further chemical analyses. The approximate UV dose for each of these samples was 5.2, 7.8, 10.4, and 15.6 mJ/cm², respectively. Agitation caused by the act of pumping the milk through the UV apparatus 12 times could have adverse effects on milk quality due to lipase activity, therefore, the milk was passed through the UV apparatus 12 times with the UV lights turned off (12 pass no UV) and the results of the following sensory and chemical analyses were compared to the results of UV treated milk. Milk samples were maintained at 4°C during processing.

Fatty Acid Profile

Fatty acid analyses were conducted at the Dairy Forage Lab of Virginia Tech. Composition analyses were conducted on fresh goat's milk, goat's milk that had been subjected to agitation (12 pass no UV), and goat's milk that had been irradiated with a UV dose of 15.6 mJ/cm² (12 pass). The milk samples were frozen and maintained at -80°C until analyses were conducted. The procedure for extraction and methylation were as follows: milk was thawed, warmed, and gently mixed to provide a uniform sample. One-ml milk samples were weighed into 50-ml extraction tubes. Lipid was extracted using modified Folch procedure (Folch *et al.*, 1957). Lipid residue was weighed after drying at 40-45°C under a stream of nitrogen. Fatty acids were transesterified to methyl esters with 0.5N NaOH in methanol and 14% BF₃ (Park and Goins, 1994). Undecenoic acid (Nu-Check Prep) was added prior to methylation as an internal standard.

Chromatographic analysis was conducted on prepared samples. All samples were analyzed on a 6890N gas chromatograph with a 7683 autoinjector, split/splitless capillary injector and flame ionization detector (Agilent Technologies, USA). Ultrapure H₂ was used as the carrier gas with gas velocity set at 30 cm/sec, flow rate at 1.5 ml/min, injection volume 0.5 ul, and split ratio 100:1. A Chrompack CP-Sil 88 100m x .25mm id capillary column (Varian, Inc., USA) was used to separate fatty acids and methyl esters. Temperature program for separations began at 70°C, held for 1 min, increased to 100 at 5°C/min, held for 3 min,

increased to 175°C at 10°C/min, held for 45 min, increased to 220°C at 5°C/min and held for 15 min. Total runtime was 86.5 minutes. Temperatures for injector and detector were 250°C and 300°C, respectively. A customized mixture of pure methyl ester standards as described by Loor and Herbein (2003) was used to identify peaks and determine individual response factors. Data were integrated and quantified using a Chem DataStation (Agilent Technologies, USA).

Olfactory Evaluation of Milk Samples

A triangle test (Meilgaard *et al.*, 1999) was administered to determine whether a significant difference in odor existed between three treatment groups. The three treatment groups assessed were: fresh goat's milk (control), milk after 12 passes through the UV processor with ultraviolet exposure (12 pass UV), and milk after 12 passes through the UV processor without ultraviolet exposure (12 pass no UV). Samples were poured into 20-ml plastic cups, fitted with plastic lids and stored at 4°C until sensory testing was conducted. Immediately after processing, samples were poured into 20-ml plastic cups, fitted with plastic lids and stored at 4°C until sensory testing was conducted. Samples were tested within 6 h of treatment. Approximately 23 volunteers over the age of 18 years were recruited from the Food Science and Technology Department to serve on each panel session. Three triangle tests were conducted: 1) control samples against 12 pass UV; 2) control samples against 12 pass no UV; and 3) 12 pass UV against 12 pass no UV. Testing was conducted in individual booths in the sensory laboratory within the Food Science and Technology Department. The panelists were asked to complete a consent form prior to testing (Human Subject's Consent Form – Appendix A). Each panelist was verbally reminded not to drink the samples but to smell them only. Two sets of three samples were presented and the panelists were informed that in each set two of the samples are the same and that one was different. Panelists were instructed to determine the different smelling sample in each group of three and asked to describe any off-odors associated with the unique sample (Scorecard – Appendix B).

Chemical Analyses

The extent of oxidation as a result of processing with and without UV irradiation was assessed chemically using three different methods: thiobarbituric acid reactive substances (TBARS) test, acid degree values (ADV), and solid-phase microextraction with gas chromatography (SPME-GC). All samples were tested concurrently within 6 h following UV exposure.

Malondialdehyde is a secondary product of oxidation. The TBARS test measures the malondialdehyde in the sample and the results are reported as mg malondialdehyde per liter sample (Appendix C). Results of this test are based on the color reaction of lipid peroxidation products and thiobarbituric acid; the color absorbance is read spectrophotometrically at 532 nm. Samples were tested in duplicate (control, 4 pass UV, 6 pass UV, 8 pass UV, 12 pass UV and 12 pass no UV) using a modified version of the TBARS test described by Spanier and Traylor (1991). One ml of each sample and 4 ml of Solution I (0.375% thiobarbituric acid, 0.506% SDS, and 9.37% acetic acid) were pipetted into disposable centrifuge tubes and vortexed. Tubes were capped and incubated in a 95°C water bath for 60 min. After cooling the tubes, 5 ml of Solution II (15:1 n-butanol and pyridine) were added and samples were vortexed. Samples were centrifuged at room temperature (25°C) at 3000 rpm for 15 min. Absorbancy was measured at 532 nm and the TBARS value calculated by multiplying the slope of the standard curve and the absorbance of the sample.

ADVs are classified as a standard method for indication of hydrolytic rancidity and used as a measurement of free fatty acids in milk fat recovered from an extraction method and titration. These values, coupled with sensory evaluation, can be used as an indicator of rancid off-flavors in milk. The procedure for measuring ADVs is described by Marshall (1993). Samples were tested in duplicate (control, 4 pass UV, 6 pass UV, 8 pass UV, 12 pass UV and 12 pass no UV).

SPME-GC is an extraction and analytical technique used to detect low concentrations of flavor compounds in foods and beverages. SPME fibers (75 μm CarboxenTM-PDMS), manual holder assemblies, Viton septum, caps, micro stirring bars and 40 ml glass bottles were purchased from Supelco, Inc. (Bellefonte, PA. USA). Prior to use, the fibers were conditioned in a GC injection port at 280°C for 1 h. Volatile compounds associated with light oxidation, hexanal, heptanal, pentanal and methyl sulfide were purchased from Sigma Chemical (St. Louis, MO. USA) and were used as standards for identification purposes. Volatile compounds were identified by retention index comparisons. Three treatment groups were assessed: fresh goat's milk (control), milk after 12 passes through the UV processor with ultraviolet exposure (12 pass UV), and milk after 12 passes through the UV processor without ultraviolet exposure (12 pass no UV). The samples were prepared immediately after processing by pipetting 25 ml of each milk group (control, 12 pass UV and 12 pass no UV) into 40 ml glass bottles that were covered with aluminum foil to minimize further light exposure. The bottles were fitted with teflon-coated septa and held at 4°C until analyses were conducted.

Volatile analysis was completed in triplicate for each treatment within 6 h of processing. The 75 μm carboxen polydimethyl siloxane (PDMS) coated SPME fiber was inserted through the septum of the sample bottles and positioned approximately 1 cm above the surface of the milk to allow maximum exposure to the milk headspace. The fibers were exposed for 22 min at 45°C with magnetic stirring. When exposure was complete, the SPME fiber was retracted and the fiber assembly unit was withdrawn from the septum for further analysis. The loaded SPME fiber unit was injected into the injector port of a Hewlett Packard gas chromatograph (model 5890 Series II Plus, Hewlett Packard, Avondale, PA). Volatile compounds were separated using a 30 m x 0.32 mm, 1.05 μm , Rtx-S capillary column (Restek Corp., USA) and helium as the carrier gas with flow rates of 1.8 ml/min. The temperature of the injector was 280°C and the temperature of the flame ionization detector was 300°C. The temperature program was 30 sec at 35°C, 15°C per min to 180°C, hold 30 sec, 20°C per min to 260°C, hold 30 sec. Data were integrated and quantified using a Chem DataStation (Agilent Technologies).

Statistical Analysis

The data for each of the olfactory triangle tests were analyzed by the number of “correct” responses versus the total number of responses (**Table 1**). The difference was considered significant at an α -risk of 5% (probability $p < 0.05$). Type II error (rejecting the null hypothesis (H_0) when it is actually true) was reduced by choosing a low β value ($\beta = 0.05$). With the proportion of discrimination (p_d) set at 50%, the critical number of correct responses for significance is 27 out of 48. Significant differences were determined by comparing responses using Table 8 in Meilgaard *et al.* (1999). One sensory test was conducted for each treatment pair (control against 12 pass UV; control against 12 pass no UV; and 12 pass UV against 12 pass no UV). One test was administered per repetition.

The mean \pm standard deviation (SD) TBARS value and ADV for each treatment were calculated and data were analyzed by one-way analysis of variance and Tukey’s Honestly Significant Difference (HSD) was used to compare means. Control and “12 pass no UV samples” were compared as described above. Linear regression was conducted on the data from both tests for each repetition to explain the relationship between values and UV processing. Data were analyzed using Jmp In (Version 4.04, SAS Institute, 2001) software.

Volatile compound levels were determined by calculated means \pm standard deviation (SD) of gas chromatogram area counts from triplicate observations during the same replication. Data were analyzed by one-way analysis of variance and Tukey’s Honestly Significant Difference (HSD) test was used to determine statistical differences of volatile concentrations between treated and untreated milk.

RESULTS AND DISCUSSION

Olfactory Evaluation

The odor and flavor of fresh, high quality milk has a light, almost bland taste, but with a characteristic sweet and salty flavor from lactose and milk salts (Walstra and Jenness, 1984). Many other compounds are present in undetectable amounts; they independently and synergistically contribute to the odor and flavor of milk. The odor of milk is delicate and minor variations in chemical composition could render it unacceptable by consumers; therefore, olfactory testing was conducted to determine if changes in goat's milk aroma were evident after exposure to a UV dose shown to reduce certain bacterial pathogens in experimentally inoculated goat's milk. The critical number of correct responses for significance was 27 out of 48. The results of the triangle tests are contained in **Table 1**. The odor of fresh goat's milk and milk that had been exposed to UV light was different ($p < 0.05$) as was milk that was pumped through the machine but not exposed to the UV source and the UV treated milk ($p < 0.05$). The difference between the odor of the samples was highly detectible and the differences were significant at $p < 0.001$. No perceivable differences were determined (18 correct responses of 48 judgments) between the aroma of fresh goat's milk and milk that had been agitated without UV exposure. There were no negative descriptors provided for agitated or fresh goat's milk; however, UV treated samples were described by many panelists as "manure," "stinky," "barnyard," and "goaty." Common off-flavors typically associated with light oxidation in cow's milk are described as acidic, astringent, unclean, cowy, bitter, cooked, feedy, weedy, fruity, foreign, lacks freshness, malty, rancid, salty and oxidized (Ogden, 1993).

Chemical Analyses

The observed sensory changes in aroma may be indicative of lipid oxidation and or hydrolytic rancidity in the UV processed milk. Analytical assessment to further elucidate the chemical changes contributing to the change in odor included TBARS, acid degree values, analysis of fatty acids by gas chromatography for total fatty acids and headspace volatiles.

The composition of the milk averaged approximately 92% moisture and 8% solids for each repetition. The mean fat content was $4.1 \pm .09\%$ and the average protein content was $2.9 \pm 0.03\%$. The fatty acid profile for fresh goat's milk (control), milk that was pumped through the machine but not exposed to the UV source (NoUV), and UV processed milk (UV) is contained in **Table 2**. For most fatty acids, the profile was not significantly altered by UV treatment. There was a significant change in the conjugated linoleic acids (CLA) after UV processing ($p < 0.05$). Compared to the fresh goat's milk, the UV treated goat's milk showed a 52% decrease of CLA *c9, t11 18:2* and a 1050% increase of other *t/t* CLA. These results are not surprising because fatty acids are known to oxidize at different rates; for example, linoleic acid oxidizes 64 times faster than oleic acid (Hamilton, 1994). Timmons *et al.* (2001) reported that as concentrations of unsaturated fatty acids in milk increase, particularly of PUFA $C_{18:2}$ and $C_{18:3}$, milk becomes more susceptible to oxidation. This may be a contributing factor to the production of off-odors in the UV treated milk.

TBARS values increased as UV dose increased in goat's milk samples ($p < 0.05$) ($R^2 = 0.93, 0.97, \text{ and } 0.95$ for repetitions 1, 2 and 3, respectively) (**Figure 1**). The slope of the line for each repetition was the same; however combination of the data from the three repetitions resulted in a low R-squared value, or low predictive power. This may be attributed to natural variations in the milk between milkings caused by lactation stage and season. TBARS were measured on milk that was processed with no UV exposure and the results indicated that the agitation of milk, alone, did not have a significant effect on TBARS values (**Table 3**). The TBARS values ranged from 0.31 ± 0.09 in the fresh milk to 0.58 ± 0.11 in UV irradiated milk. These trends are consistent with those reported by van Aardt (2003) who found that milk exposed to light for 10 h had significantly greater TBARS values than milk that was protected from light exposure.

The TBARS test is a method used to assess the extent of lipid peroxidation in a product by measurement of the oxidation end product malondialdehyde in the sample. Compounds other than malondialdehyde may react with the thiobarbituric acid producing color reactions by other secondary oxidation products; therefore the test can only give a general measurement of lipid peroxidation (Frankel, 1998). Autoxidation proceeds by a three stage

radical process: initiation, propagation and termination. During the initiation phase, a free radical (R^{\cdot}) and hydrogen radical (H^{\cdot}) are formed by the dissociation of the hydrogen molecule (H) from the fatty acid moiety (R) resulting from initiators such as light, oxygen, transition metals, radiation, heat and other free radicals (Wong and Kitts, 2001). The cycle is propagated because the hydroperoxides are very reactive and are able to react with other fatty acids to create additional reactive compounds. Termination occurs when the peroxides are broken down further into aldehydes and ketones that are not reactive, but highly volatile and typically responsible for the off flavors indicative of lipid oxidation. Agitation from the pumping action of the machine would not catalyze this reaction. The results imply that since there was no increase in TBARS values in non-UV treated samples, that the UV light exposure was a significant element for increasing the production of oxidation bi-products.

Acid degree values were used as a possible indication of hydrolytic rancidity. Initial ADVs were significantly less than the values after approximately 15.6 mJ/cm^2 UV dose ($p < 0.05$) ($R^2 = 0.73, 0.70, \text{ and } 0.79$ for repetitions 1, 2, and 3, respectively) (**Figure 2**). **Table 3** shows that as passes through the UV unit increased (as agitation increased), ADVs increased regardless of UV exposure. The final ADVs for 12 pass UV and 12 pass no UV samples were significantly different than the corresponding control samples as determined by Tukey's HSD ($p < 0.05$).

Historically, an ADV of $>1.0 \text{ meq/100g}$ in cow's milk is indicative of rancid off-flavors. In this study the ADVs were very significantly lower than this number ($p < 0.05$). The highest ADV reported was $0.51 \pm 0.05 \text{ meq/100g}$ for milk treated with approximately 15.6 mJ/cm^2 UV dose. Duncan and Christen (1991) conducted a study that evaluated the relationship between ADVs and rancid off-flavors. It was determined that short-chained fatty acids (C4 to C8) did not enter the fat phase recovered by the ADV procedure in quantities comparable with medium- (C10 to C16) or long-chain (C18:0 to C18:1) fatty acids. Their results implied that the ADV procedure did not measure the fatty acids responsible for rancid flavor (C4 to C12) at the same rate as the longer chained fatty acids (Duncan and Christen, 1991). Relative to cow's milk, goat's milk has a higher level of short-chain fatty acids; therefore, there may have been limitations of the ADV analysis to measure

a true rancidity score. Regardless, the results of this study show that the ADV values were not great enough to imply rancidity; this was consistent with sensory analysis that showed no significant difference in the odor of fresh goat's milk and milk that had been subjected to agitation.

Hydrolytic rancidity is caused by enzyme activity on milk fat globules ruptured by agitation and foaming, homogenization, temperature activation and freezing. Hydrolytic changes were likely catalyzed by lipase action on triglycerides in response to agitation and pumping through the machine. Lipase enzymes may be inherent in the milk or from bacterial sources. Inherent goat's milk lipases are distributed equally between the cream and serum phases and only a minute amount is found in the casein; total activity is lower than in cow's milk (Deeth and Fitz-Gerald, 1995). The temperature of milk is very important during agitation because lipase activity is greatest between 37°C and 40°C and least at cold storage temperatures below 5°C (Deeth and Fitz-Gerald, 1995). The processing temperature was maintained at or below 4°C and slowed lipase activity, and the turbulent flow induced by the pumping of the milk through the CiderSure 3500 UV apparatus was not enough to cause perceivable off-odors in the milk in the first 6 h after processing. No shelf-life study was conducted to see if off-odors developed after time. However, UV irradiation or the combination of turbulent flow and UV irradiation did affect the sensory properties of the milk. Human sensory test results showed that there were no perceived olfactory differences between fresh goat's milk samples and the milk samples that were processed without UV exposure even though the ADVs were significantly higher. This is consistent with the conclusions of Duncan and Christen (1991) that ADVs should not be used as a sole determinant of rancidity.

Gas chromatograms of headspace volatile compounds in fresh goat's milk and goat's milk that has been treated with approximate UV doses of 7.8 and 15.6 mJ/cm² are shown in **Figure 3**. The chromatogram of fresh goat's milk exhibited a large peak after a retention time (RT) of approximately 2.92 min and then a much smaller peak after approximately 3.15 min which were identified by comparison to literature as acetone and n-butanone, respectively (van Aardt *et al.*, 2001; Marsili, 1999a). These peaks were consistent for each

treatment and there were no statistical difference in peak area for each treatment. The formation of other volatile peaks was displayed on the chromatogram after a UV dose of 7.8 mJ/cm². Pentanal, hexanal and heptanal were identified after exposure to UV but methyl sulfide was not identified.

The chromatograms for fresh goat's milk and goat's milk samples processed without UV were very similar (**Figure 4**). A concern was that mechanical agitation from the pumping mechanism through the UV apparatus would cause hydrolytic rancidity which would result in negative off-flavors. Compounds associated with this type of flavor defect were not detected. There were two volatile compounds detected (acetone and n-butanone) and the concentrations of both were not statistically different between treatments ($p > 0.05$). Pentanal, hexanal, heptanal and methyl sulfide were not detected. These results indicate that light exposure was an important element for the production of these volatile compounds. With SPME-GC, Lee (2002) found that the formation of pentanal, hexanal, heptanal and dimethyl disulfide occurred only when milk was stored under lighted conditions.

Table 4 quantifies the mean value +/- SD of headspace volatile compound peak formation. Acetone (RT 2.92) and n-butanone (RT 3.15) were the only volatile compounds detected in fresh goat's milk samples and milk samples that were processed in the UV apparatus without exposure to UV irradiation (0 mJ/cm²). A very slight increase in peak area was detected after exposure to both treatments of UV irradiation; however, this difference was not considered statistically significant ($p > 0.05$). Other volatile peaks were present after UV exposure; among those identified were pentanal (RT 4.46), hexanal (RT 6.09) and heptanal (RT 8.35). There was a statistically significant difference between the peak areas of pentanal, hexanal and heptanal after 7.8 mJ/cm² and 15.6 mJ/cm² UV dose, respectively. The peak areas (1×10^4) after 7.8 mJ/cm² were 0.91 ± 0.21 , 2.03 ± 0.22 , and 0.60 ± 0.07 for pentanal, hexanal and heptanal, respectively. The peak area increased significantly after 15.6 mJ/cm² of UV exposure for pentanal, hexanal and heptanal to 1.76 ± 0.38 , 3.07 ± 0.27 , and 1.38 ± 0.32 , respectively. A preliminary study indicated that these compounds were formed in goat's milk exposed to as little as 1.3 mJ/cm² UV dose. Lee (2002) found that milk stored at 4°C under florescent light displayed pentanal and hexanal formation before 2 h and

heptanal formation in fewer than 4 h. It was also reported that fat content had an effect on the formation of these volatile compounds, as fat content increased from 0.5 to 1.0, 2.0 and 3.4%, there was a significant increase in formation of the volatile compounds. The peak areas for each compound in photosensitized whole milk (8 h under florescent light at 4°C) were consistent with those reported in this current study (Lee, 2002). These compounds were not present in the samples that received zero light exposure.

CONCLUSIONS

The pursuit to find alternative processing technologies to replace traditional thermal methods may begin with the assessment of safety parameters; however, when developing novel technologies, sensory properties and consumer acceptance must also be considered in the viability assessment. Even though UV treatment was effective for the reduction of the bacterial pathogen *Listeria monocytogenes* in goat's milk, this present study indicated that UV irradiation at the wavelength 254 nm was detrimental to aroma and certain chemical properties of the raw milk. The properties that were perceived as negative in fluid milk may be considered an attribute in certain types of cheese and future studies in the cheese production sector should be considered. Other applications for this technology could be for use in developing countries where milk is not typically processed because of the high costs of thermal pasteurization. On-farm applications for the treatment of replacement milk should also be considered.

ACKNOWLEDGEMENTS

This work was funded by USDA-CSREES #2001-5110-11363. We would like to thank Phil Hartman of FPE, Inc. for the time and effort put into the maintenance of the CiderSure 3500. Thanks to Wendy Wark and Joseph Herbein for their work on the fatty acid analyses.

REFERENCES

1. Azzara, C.D., and L.B. Campbell. 1992. Off-flavors of dairy products. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands. pp 329-374.
2. Bekbölet, M. 1990. Light effects on food. *J. Food Prot.* 53(5):430-440.
3. Borle, F., R. Sieber and J.O Bosset. 2001. Photo-oxidation and photoprotection of foods, with particular reference to dairy products: An update of a review article (1993-2000). *Sciences des Aliments.* 21:571-590.
4. Buchin, S., V. Delague, G. Duboz, J.L. Berdague, E. Beuvier, S. Pochet, and R. Grappin. 1998. Influence of pasteurization and fat composition of milk on the volatile compounds and flavor characteristics of a semi-hard cheese. *J. Dairy Sci.* 81:3097-3108.
5. Burton, H. 1951. Ultra-violet irradiation of milk. *Dairy Sci. Abstr.* 13(3):229-244
6. Cadwallader, K.R. and C.L. Howard. 1998. Analysis of aroma-activated components of light activated milk. In *Flavor Analysis: Developments in Isolation and Characterization*; ACS Symposium Series 705; American Chemical Society: Washington, DC, 1998; p 343-358.
7. Capstick, E., H.A. Hall, and F.K. Neave. 1946. *Further Developments in Dairying in Germany.* Final Report No. 770. British Intelligence Objectives Sub-Committee Trip No. 2144.
8. Caserio, G., M.A. Bianchi, G. Beretta, and I. Dragoni. 1975. Studies on the ripening of 'Grana Padano' cheese manufactured from milk irradiated with UV light. *Eur. J. Appl Microbiol.* 1(3):247-257.
9. deMan, J. 1999. *Principles of Food Chemistry, 3rd Edition.* Aspen Publishing Company. Gaithersburg, MD.
10. Deeth, H.C. and C.H. Fitz-Gerald. 1995. Lipolytic enzymes and hydrolytic rancidity in milk and milk products. pp 308. In Fox, P.F. 1995. *Advanced Dairy Chemistry Volume 2: Lipids.* Chapman & Hall. London, UK.
11. Dimick, P.S. 1982. Photochemical effects on flavor and nutrients of fluid milk. *Can. Inst. Food Sci. Technol. J.* 15(4):247-256.

12. Duncan, S.E. and G.L. Christen. 1991. Sensory detection and recovery by acid degree value of fatty acids added to milk. *J. Dairy Sci.* 74:2855-2859.
13. Fellows, P.J. 1997. *Food Processing Technology: Principles and Practice*. Woodhead Publishing Limited. Cambridge, England.
14. Fennema, O.R. 1996. *Food Chemistry, 3rd Edition*. MerceL Dekker, Inc. New York.
15. Folch J., M. Lees, and G.H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol Chem.* 226:497-502
16. Frankel, E.N. 1980. Lipid Oxidation. *Prog. Lipid Res.* 19:1-22.
17. Frankel, E.N. 1991. Recent advances in lipid oxidation. *J. Sci. Food Agric.* 54:495-511.
18. Frankel, E.N. 1998. Antioxidants. *In Lipid Oxidation*. The Oily Press Ltd., Dundee, Scotland. pp. 129-166.
19. Gonzalez-Cordova, A.F. and B. Vallejo-Cordova. 2003. Detection and prediction of hydrolytic rancidity in milk by multiple regression analysis of short-chain free fatty acids determined by solid phase microextraction gas chromatography and quantitative flavor intensity assessment. *J. Agric. Food Chem.* 51:7127-7131.
20. Grappin, R. and R. and Beuvier, E. 1997. Possible implication of milk pasteurization on the manufacture and sensory quality of ripened cheese. *Int. Dairy J.* 7:751-761.
21. Hamilton, R.J. 1994. The chemistry of rancidity in foods. *In* Allen, J.C. and R.J. Hamilton. *Rancidity in Foods 3rd Edition*. Blackie Academic & Professional. London, U.K. pp 1-21.
22. Kim, G.Y., J.H. Less, and D.B. Min. 2003. Study of light-induced volatile compounds in goat's milk cheese. *J. Agric. Food Chem.* 51: 1405-1409.
23. Lee, J.H. 2002. Photooxidation and photosensitized oxidation of linoleic acid, milk and lard. Doctoral dissertation. The Ohio State University, Department of Food Science and Nutrition.
24. Loor, J.J. and J.H. Herbein. 2003. Reduced fatty acid synthesis and desaturation due to exogenous *trans*¹⁰, *cis*¹²-CLA in cows fed oleic or linoleic oil. *J. Dairy Sci.* 86:1354-1369
25. Marshall, R.T. (ed.). 1993. *Standard Methods for the Examination of Dairy Products*. 16th ed. American Public Health Association, Washington, DC.

26. Marsili, R.T. 1999a. Comparison of solid-phase microextraction and dynamic headspace methods for the gas chromatographic-mass spectrometric analysis of light-induced lipid oxidation products in milk. *J. Chromatogr. Sci.* 37:17-23.
27. Marsili, R.T. 1999b. SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J. Agric. Food Chem.* 47(2): 648-654.
28. Matak, K.E. 2004. *Effects of UV Irradiation for the Reduction of Bacterial Pathogens and Chemical Indicators in Milk*. Doctoral Dissertation. Virginia Tech. Chapter IV.
29. Meilgaard, M., G.V. Civille, and B.T. Carr. 1999. *Sensory Evaluation Techniques*. 3rd ed. CRC Press, Inc., Boca Raton, LA.
30. Min, D.B., and J.M. Boff. 2002. Chemistry and reaction of singlet oxygen in foods. *Comprehensive Reviews in Food Science and Food Safety*. Institute of Food Technologists. 1:58
31. Ogden, L.V. 1993. Sensory Evaluation of Dairy Products. In Hui, Y.H. 1993. *Dairy Science and Technology Handbook*. VCH Publishers, Inc. New York, NY. pp. 157-276.
32. Østdal, H., M.J. Bjerrum, J.A. Pedersen, and H.J. Andersen. 2000. Lactoperoxidase-induced protein oxidation in milk. *J. Agric. Food Chem.* 48(9):3939-44.
33. Reed, B.A. and L.E. Grivetti. 2000. Controlling on-farm inventories of bulk-tank raw milk—An opportunity to protect public health. *J. Dairy Sci.* 83:2988-2991.
34. Park, Y.W. 1990. Nutrient profiles of commercial goat milk cheeses manufactured in the United States. *J. Dairy Sci.* 73:3059-3067.
35. Park, P.W., and R.E. Goins. 1994. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *J. Food Sci.* 59:1262–1266.
36. Spanier, A.M., and R.D. Traylor. 1991. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. *J. Muscle Foods.* 2:165-176.
37. Timmons, J.S. , W.P. Weiss, D.L. Palmquist, and W.J. Harper. Relationships among dietary roasted soybeans, milk components, and spontaneous oxidized flavor of milk. *J. Dairy Sci.* 84:2440–2449
38. van Aardt, M. 2003. *Controlled Release of Antioxidants via Biodegradable Polymer Films into Milk and Dry Milk Products*. Doctoral Dissertation. Virginia Tech.

39. van Aardt, M., S.E. Duncan, D. Bourne, J.E. Marcy, T.E. Long, C.R. Hackney, and C. Heisey. 2001. Flavor threshold for acetaldehyde in milk, chocolate milk, and spring water using solid phase microextraction gas chromatography for quantification. *J. Agric. Food. Chem.* 49:1377-1381.
40. Wong, P.Y.Y. and D.D. Kitts. 2001. Factors influencing ultraviolet and electron beam irradiation-induced free radical damage of ascorbic acid. *Food Chemistry.* 74:75-84.
41. Walstra, P. and Jenness, R. 1984. *Dairy Chemistry and Physics.* Wiley and Sons, New York.
42. Wong, P.Y.Y. and D.D. Kitts. 2001. Factors influencing ultraviolet and electron beam irradiation-induced free radical damage of ascorbic acid. *Food Chemistry.* 74:75-84.

Table 1. Olfactory triangle test responses for UV irradiated raw goat milk (12 pass UV), pumped raw goat milk (12 pass no UV), and untreated fresh goat milk (control)

Treatment	# of Correct Responses¹	Result²
Control vs. 12 pass UV	42	highly significant
Control vs. 12 pass no UV	18	not significant
12 pass UV vs. 12 pass no UV	42	highly significant

¹ n = 48 observations

² critical response number = 27; $\alpha = 0.05$; $\beta = 0.05$ and $p_d = 50\%$.

Table 2. Average fatty acid profile of fresh goat milk (control); milk that was pumped through the machine but not exposed to the UV source (No UV); and UV processed milk (UV). Values are expressed as a percentage of total fatty acid. (Dairy Forage Lab, Virginia Tech)

Fatty Acid	Control	No UV	UV
4:0	1.76	1.85	1.87
6:0	2.05	2.09	2.10
8:0	3.05	3.08	3.08
10:0	10.75	10.71	10.66
12:0	5.11	5.07	5.05
14:0	9.93	9.87	9.89
c9 14:1	0.14	0.14	0.14
15:0	0.79	0.78	0.79
16:0	23.13	23.10	23.17
trans 16:1 isomers			
t9 16:1	0.69	0.69	0.69
t11 16:1	0.13	0.11	0.11
cis 16:1 isomers			
c9 16:1	0.88	0.88	0.87
c11 16:1	0.05	0.04	0.03
17:0	0.70	0.70	0.70
18:0	10.78	10.79	10.82
coeluted 18:1 isomers			
t6 & t7 18:1	0.36	0.36	0.36
t12 & c7 18:1	0.28	0.28	0.28
t13 & c6 18:1	0.72	0.74	0.73
trans 18:1 isomers			
t4 18:1	0.02	0.03	0.02
t5 18:1	0.03	0.03	0.03
t9 18:1	0.39	0.39	0.39
t10 18:1	0.65	0.64	0.65
t11 18:1	3.15	3.16	3.16
t14 18:1	0.18	0.20	0.17
t16 18:1	0.29	0.29	0.30
cis 18:1 isomers			
c9 18:1	18.63	18.59	18.68
c11 18:1	0.57	0.57	0.57
c12 18:1	0.49	0.49	0.50
c13 18:1	0.11	0.12	0.11
c15 18:1	0.13	0.13	0.13
non-conjugated 18:2 isomers			
t9,t12 18:2n6	0.01	0.02	0.02
c9,t12 18:2n6	0.08	0.09	0.08
t9,c12 18:2n6	0.04	0.04	0.04
c9,c12 18:2n6	1.60	1.59	1.60

Fatty Acid	Control	No UV	UV 1
conjugated 18:2 isomers			
c9,t11 18:2	1.25	1.23	0.64
c9,c11 18:2	0.00	0.00	0.08
t11,t13 18:2	0.02	0.02	0.01
other t/t CLA	0.04	0.04	0.42
c6,c9,c12 18:3n6	0.04	0.04	0.04
c9,c12,c15 18:3n3	0.30	0.29	0.30
20:0	0.17	0.16	0.17
c11 20:1	0.08	0.07	0.08
c11,c14 20:2n6	0.04	0.04	0.03
c8,c11,c14 20:3n6	0.03	0.03	0.03
c5,c8,c11,c14 20:4n6	0.24	0.24	0.24
c5,c8,c11,c14,c17 20:5n3	0.01	0.01	0.01
c13 22:1	0.01	0.01	0.01
c7,c10,c13,c16 22:4n6	0.03	0.03	0.03
c4,c7,c10,c13,c16,c22 22:5n6	0.01	0.01	0.01
c7,c10,c13,c16,c19 22:5n3	0.09	0.09	0.09
c4,c7,c10,c13,c16,c19 22:6n3	0.03	0.03	0.02
24:0	0.03	0.04	0.04
c15 24:1	0.01	0.00	0.00
Total ug FA	18996	20510	19330
mg solvent extracted lipid	35.40	35.33	35.97
Total ug FA/mg extracted lipid	536	586	537

Table 3: Thiobarbituric acid-reactive substances (TBARS) values (mean \pm SD) and Acid Degree Values (ADV) (mean \pm SD) of fresh goat's milk in response to increasing doses of UV light.

Approximate UV Dose (mJ/cm ²)	Number of Passes	Malondialdehyde (mg/L)	ADV (meq/100g)
	X \pm SD	X \pm SD	X \pm SD
0.0	0	0.31 \pm 0.09 ^a	0.18 \pm 0.05 ^a
0.0	12	0.34 \pm 0.11 ^a	0.36 \pm 0.15 ^b
5.2	4	0.37 \pm 0.11 ^a	0.29 \pm 0.05 ^a
7.8	6	0.44 \pm 0.10 ^{a,b}	0.36 \pm 0.12 ^b
10.4	8	0.50 \pm 0.12 ^b	0.31 \pm 0.11 ^{a,b}
15.6	12	0.58 \pm 0.11 ^b	0.40 \pm 0.09 ^b

^{a, b} Values designated with the same letter within a column are not significantly different ($p < 0.05$) as determined by Tukey's HSD test.

Table 4: Mean value of peak area (area counts) \pm SD (1×10^4) of headspace volatile compounds in goat's milk formed as a result of UV irradiation.

RT ^a	Volatile Compound ^b	Fresh Goat's Milk	UV Dose ^c		
			0 mJ/cm ²	7.8 mJ/cm ²	15.6 mJ/cm ²
2.44	ni ^d	nd ^e	nd	1.78 \pm 0.80	2.46 \pm 1.1
2.92	<i>acetone</i> ^f	14.6 \pm 4.9	14.7 \pm 4.8	18.1 \pm 4.0	17.2 \pm 4.8
3.15	<i>n-butanone</i>	0.52 \pm 0.17	0.49 \pm 0.19	0.51 \pm 0.09	0.57 \pm 0.19
3.73	ni	nd	nd	1.57 \pm 0.15	2.17 \pm 0.16
3.81	ni	nd	nd	1.41 \pm 0.19	2.72 \pm 0.98
4.46	pentanal	nd	nd	0.91 \pm 0.21	1.76 \pm 0.38^g
4.58	ni	nd	nd	1.12 \pm 0.07	2.11 \pm 0.65
4.95	ni	nd	nd	0.49 \pm 0.10	1.00 \pm 0.31
5.92	ni	nd	nd	2.18 \pm 0.21	2.74 \pm 0.36
6.09	hexanal	nd	nd	2.03 \pm 0.22	3.07 \pm 0.27
6.29	ni	nd	nd	1.08 \pm 0.28	2.27 \pm 0.52
8.35	heptanal	nd	nd	0.60 \pm 0.07	1.38 \pm 0.32

^a RT, retention time in min.

^b Compounds were identified by retention times using external standards.

^c Milk passed through a Cidersure 3500 UV processor 6 or 12 consecutive times to achieve desired UV dose. At dose 0 mJ/cm², milk was passed 12 times through the UV apparatus with the UV lights turned off.

^d ni, not identified

^e nd, not detected

^f Volatile compounds designated with italics type have been identified by comparison to literature.

^g Values designated with boldface type within a row are significantly different ($p < 0.05$) as determined by Tukey's HSD test.

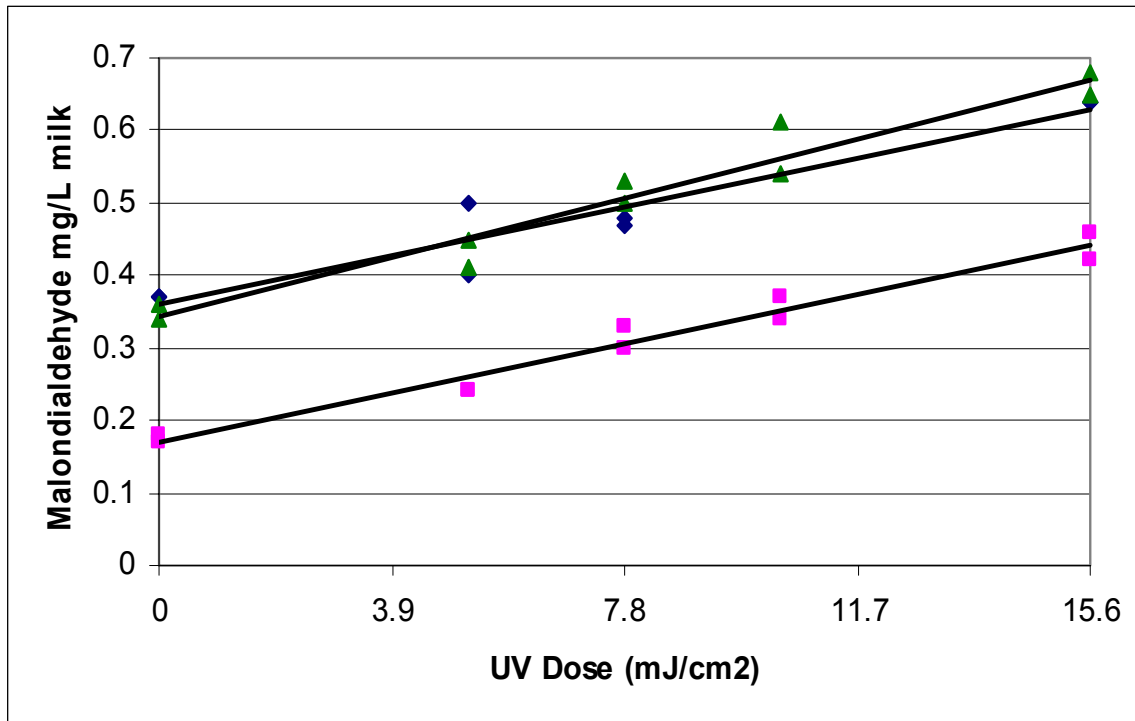


Figure 1: Thiobarbituric acid-reactive substances values of fresh goat milk in response to UV light. The means for each UV dose were calculated per repetition (n=3) and analysis of variance showed that malondialdehyde formation increased as UV dose increased ($p < 0.05$). Repetition 1 (♦) $R^2 = 0.93$, Repetition 2 (▲) $R^2 = 0.97$, Repetition 3 (■) $R^2 = 0.95$.

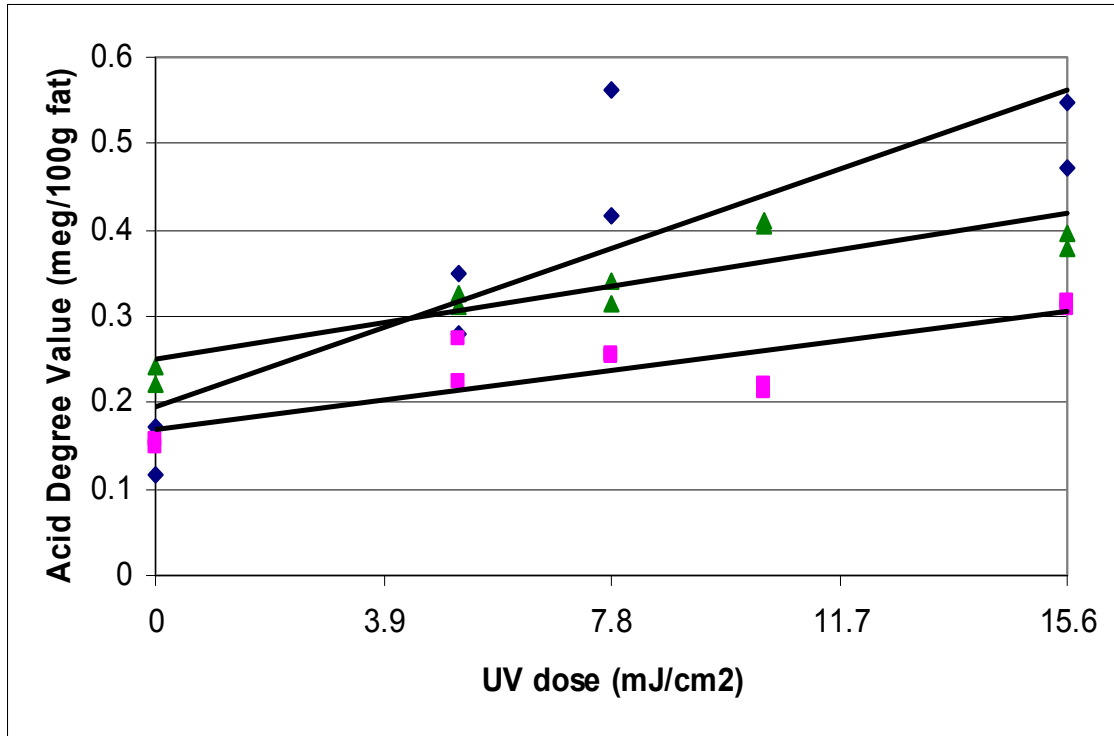


Figure 2: Acid degree values of fresh goat milk in response to UV light. The means for each UV dose were calculated per repetition (n=3) and analysis of variance showed that values increased as UV dose increased ($p < 0.05$). Repetition 1 (♦) $R^2 = 0.73$, Repetition 2 (▲) $R^2 = 0.70$, Repetition 3 (■) $R^2 = 0.79$.

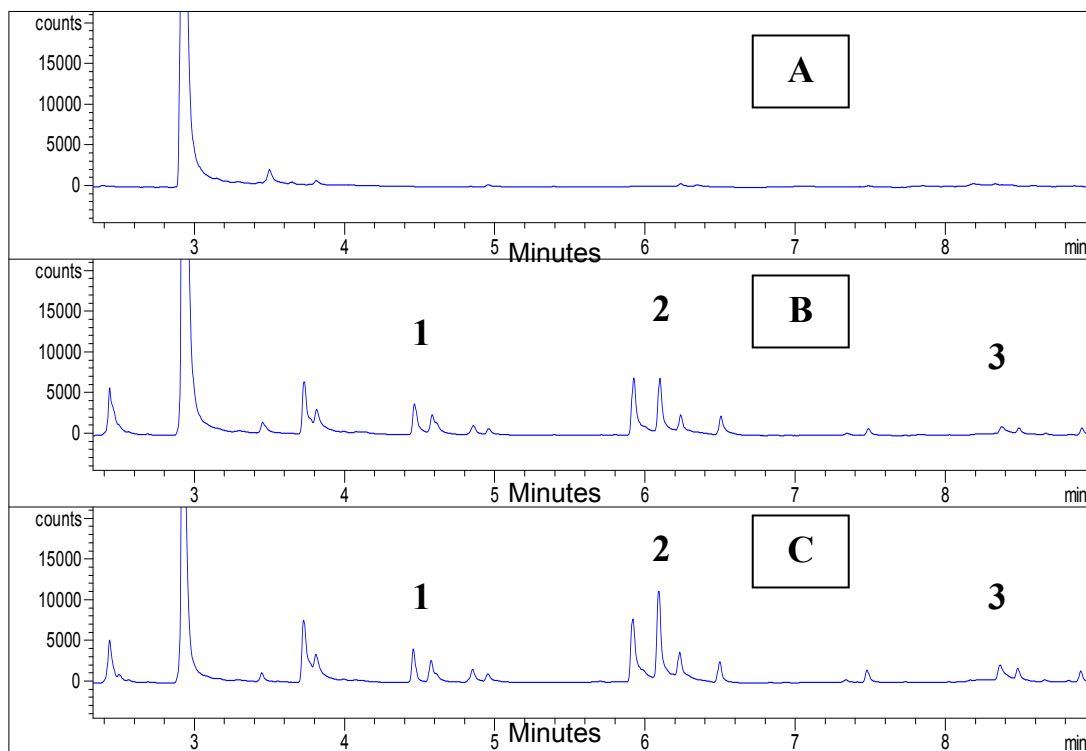


Figure 3: The production of headspace volatile compounds were detected by solid-phase microextraction and gas chromatography of (A) fresh goat milk, (B) goat milk exposed to a UV dose of approximately 7.8 mJ/cm^2 , and (C) goat milk exposed to a UV dose of approximately 15.6 mJ/cm^2 . 1: pentanal, 2: hexanal, and 3: heptanal. Compounds identified by retention time comparisons to external standards.

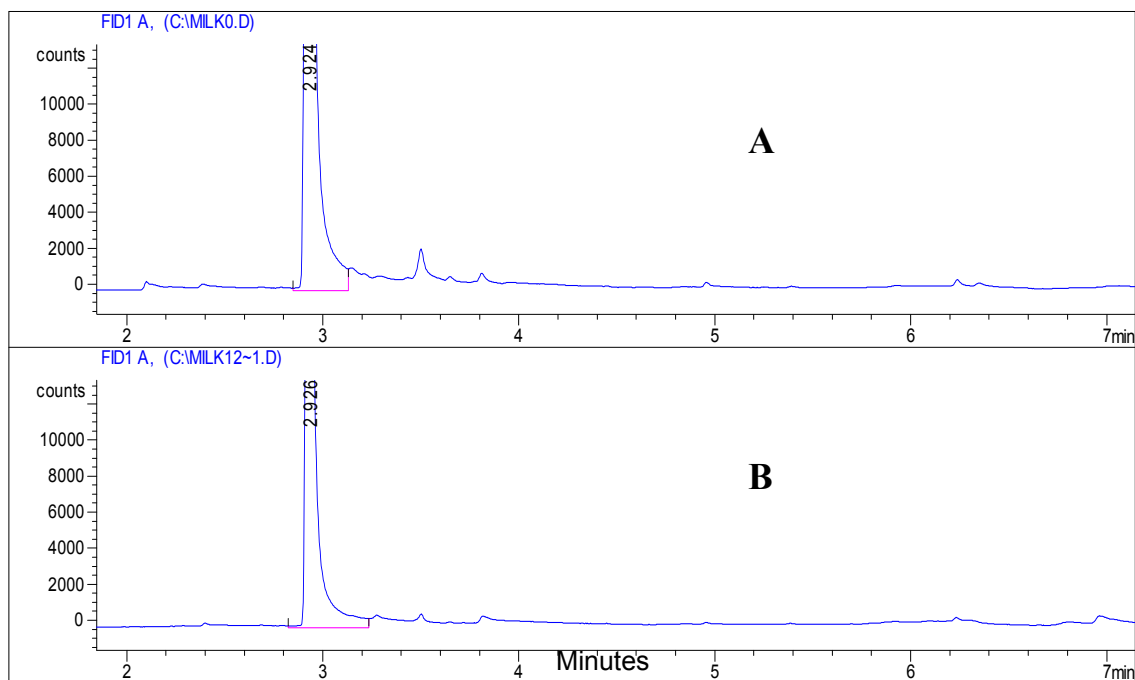


Figure 4: Comparison of gas chromatographs of (A) fresh goat milk and (B) goat milk passed through a UV processor 12 times with the lights turned off.

APPENDIX A: Human Subjects Forms for Olfactory Evaluation

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants in Research Projects Involving Human Subjects

Title of Project **Effect of Ultraviolet Light on Goat's Milk**

Investigator Kristen Matak

I. Purpose of this Research/Project

The purpose of this study is to determine whether off-odors are detectable in goat's milk samples that are treated with ultraviolet light versus unprocessed milk.

II. Procedures

There will be 3 sessions over a period of 4 weeks involving about 15 minutes at each session. You will be presented with 2 sets of 3 samples and asked to identify the odd sample by smelling each in the order presented. As a panelist, it is critical for you complete your session.

The research will be conducted in the sensory kitchen at the Food Science and Technology Department. Amber bottles will be used to camouflage possible color differences between samples.

III. Benefits/Risks

Your participation in the project will provide information about the effect of UV exposure on oxidation of milk samples and its possible usage as an alternative to thermal pasteurization. You will have access to the results of the panel at the completion of the project.

There are no identifiable risks associated with smelling milk samples. Please use sanitizer to wash hands when finished sampling.

IV. Extent of Anonymity and Confidentiality

The results of your performance are strictly confidential. Individual panelists will not be referred to in any publications or reviews.

V. Compensation

There is no monetary compensation for participation in this project. However, a candy treat will be offered at the end of the session.

VI. Freedom to Withdraw

If after becoming familiar with the sensory project you chose not to participate, you may withdraw without penalty. It is essential to the success of the sensory panel that you complete each session, however, there may be circumstances under which that you may chose not to complete your session, you may also withdraw at any time without penalty.

VII. Approval of Research

This research has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subject’s review of the Department of Food Science and Technology.

VIII. Subject's Responsibilities

I know of no reason that I cannot participate in this study that requires me to smell 9 milk samples in one session.

Signature/date

Please provide an address and phone number so investigator may reach you in case of emergency of schedule changes.

------(tear off)-----

X. Subject's Permission

I have read and understand the Informed Consent and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

_____Date_____

Subject signature

Should I have any pertinent questions about this research or its conduct, and research subjects' rights, and whom to contact in the event of a research-related injury to the subject, I may contact:

- | | |
|------------------------------|-----------------------|
| Kristen Matak (Investigator) | Phone: (540) 231-9518 |
| Susan S. Sumner (Faculty) | Phone: (540) 231-5280 |
| David M. Moore (Chair, IRB) | Phone: (540) 231-4991 |

APPENDIX B: Sensory Scorecard

EFFECT OF ULTRAVIOLET LIGHT ON GOAT'S MILK

Name _____

Instructions:

DO NOT TASTE THE PRODUCTS.

Please complete the human subjects consent form before starting sensory analysis.

You will be presented with 2 three-sample sets. Two of the three samples in a set are the same and one is different. Please **SMELL** each sample in the order presented, from left to right.

Circle the number of the sample in each set of three that smells "different".

Use provided sanitizer located in the kitchen to wash hands when finished.

Set 1:

Describe odor of odd sample: _____

Set 2:

Describe odor of odd sample: _____

Thank you for your participation!

APPENDIX C

THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) DETERMINATION FOR MILK

Modified by Hengjian Wang

June, 2004

REAGENTS

1. Solution I. 0.375% TBA (thiobarbituric acid), 0.596% SDS, and 9.370% acetic acid. Dissolve 1.875 g of TBA, 2.53 G of SDS in distilled water, add 59.5 ml of 80% acetic acid (47.5 ml for 99.9%) and bring up to 500 ml. Adjust pH to 3.4 with High concentrated sodium hydroxide (NaHO). Need 4.0 ml per sample.
2. Solution II. Mix n-butanol and pyridine at the ratio of 15:1. Make fresh daily under a hood. Need 5.0 ml per sample.
3. 5.0 mM MDA (TMP) stock solution. Transfer 0.829 ml of TMP (99% tetramethoxypropane, Sigma Chemical Company) standard solution into a 1000 ml dilution flask, add distilled water up to 1000 ml mark. Mix gently.

PREPARATION OF STANDARD SOLUTIONS

1. Pipet 1.0 ml of 5.0 mM TMP stock solution into a 50 ml volumetric flask and then fill the flask to the mark with distilled water. The TMP concentration of this standard working solution is 0.10 mM.
2. Estimated TBARS values of samples are **high**, therefore pipet 0.0 (blank), 2.5, 5.0, 7.5 and 10.0 ml of 0.10 mM standard TMP solution (above step) and transfer into 100 ml volumetric flasks, respectively. If estimated TBARS values were **medium**, pipet 0.0 (blank), 1.25, 2.0, 3.75, and 5.0 ml of 0.10 mM standard solution. If estimated TBARS values of samples were **low**, pipet 0.0 (blank), 0.25, 0.50, 0.75, and 1.0 ml of 0.10 mM standard TMP solution.

3. Bring the standard solution up to the mark (100 ml) with distilled water. Mix gently.
TMP concentrations are:
 - a. high: 0.0, 2.5, 5.0, 7.5, and 10.0 μM
 - b. medium: 0.0 (blank), 1.25, 2.0, 3.75, and 5.0 μM
 - c. low: 0.0 (blank), 0.25, 0.50, 0.75, and 1.0 μM

TBARS PROCEDURE

Sample preparation and absorbance measurement

1. Mix sample.
2. Pipet 1.0 ml of samples and each of the standard solutions into a 15 ml disposable centrifuge tube. Add 4.0 ml solution I into each tube and vortex until well mixed. Cap the tubes and incubate the mixture in a 95°C water bath for 60 min.
3. Cool tubes in tap water and ice water. Add 5.0 ml of solution II to each tube and mix on vortex mixer for 10 seconds under hood.
4. Centrifuge the solution at room temperature (25°C) at 3000 rpm (1600 G) for 15 min. Pipet about 2.5-3.0 ml organic solution (top layer, do not disturb bottom layer) to cuvette.
5. Read absorbance of the organic solutions at 532 nm under hood within 1 hr.

CALCULATION OF TBARS VALUE

1. Calculate TBARS value for standard TMP solutions.

The TBARS value of each standard TMP solution (C_i) is calculated as following:

$$\begin{aligned} C_i &= V_i \times C_o \times 10^{-6} \text{ mol} \times (72.3 \times 10^3) \text{ mg/mol} / 10^{-1} \text{ L} \\ &= 7.23 \times 10^{-1} \times V_i \times C_o \text{ mg/L} \end{aligned}$$

where V_i = Volume (ml) of working solution in each standard TMP solution and C_o = TMP concentration (mM) of the working solution, 0.1 mM for this procedure.

2. Conduct regression on absorbance and TBARS of the standard TMP solutions.

Determine K_s for the following equation:

$$\text{TBARS of the standard solution } C_i = K_s \times A_s \times D$$

where A_s = absorbance of each standard solution.

3. Calculate TBARS value for samples:

$$\text{Sample TBARS} = K_s \times A_i \times D$$

where A_i = absorbance of the sample

D = dilution rate. For milk, it is undiluted (1)

Reference:

Spanier, A.M., and R.D. Traylor. 1991. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. *J. Muscle Foods*. 2:165-176. Modified by Hengjian Wang
June, 2004

APPENDIX D

Fatty Acid Profile

Fatty acid analyses were conducted at the Dairy Forage Lab of Virginia Tech. Composition analyses were conducted on fresh goat's milk, goat's milk that had been subjected to agitation (12 pass no UV), and goat's milk that had been irradiated with a UV dose of 15.6 mJ/cm² (12 pass). The milk were frozen and maintained at -80°C until analyses were conducted. The procedure for extraction and methylation were as follows: milk was thawed, warmed, and gently mixed to provide a uniform sample. One-mL milk samples were weighed into 50-mL extraction tubes. Lipid was extracted using modified Folch procedure (Folch *et al.*, 1957). Lipid residue was weighed after drying at 40-45°C under a stream of nitrogen. Fatty acids were transesterified to methyl esters with 0.5N NaOH in methanol and 14% BF₃ (Park and Goins, 1994). Undecenoic acid (Nu-Check Prep) was added prior to methylation as an internal standard.

Chromatographic analysis was conducted on prepared samples. All samples were analyzed on a 6890N gas chromatograph with a 7683 autoinjector, split/splitless capillary injector and flame ionization detector (Agilent Technologies, USA). Ultrapure H₂ was used as the carrier gas with gas velocity set at 30 cm/sec, flow rate at 1.5 ml/min, injection volume 0.5 ul, and split ratio 100:1. A Chrompack CP-Sil 88 100m x .25mm id capillary column (Varian, Inc., USA) was used to separate FAMES. Temperature program for separations began at 70°C, held for 1 min, increased to 100 at 5°C/min, held for 3 min, increased to 175°C at 10°C/min, held for 45 min, increased to 220°C at 5°C/min and held for 15 min. Total runtime was 86.5 minutes. Temperatures for injector and detector were 250°C and 300°C, respectively. A customized mixture of pure methyl ester standards as described by Loor and Herbein (2003) was used to identify peaks and determine individual response factors. Data were integrated and quantified using a Chem DataStation (Agilent Technologies, USA).

The fatty acid profile of fresh goat's milk, processed but not irradiated, and milk processed through the UV apparatus is contained in the following tables. For most fatty

acids, the profile was not significantly altered by UV treatment. There was a significant change in the conjugated linoleic acids (CLA) after UV processing ($p < 0.05$) (Table 6b). Compared to the fresh goat's milk, the UV treated goat's milk showed a 52% decrease of CLA *c9, t11* 18:2 and a 1050% increase of other *t/t* CLA. These results are not surprising because fatty acids are known to oxidize at different rates; for example, linoleic acid oxidizes 64 times faster than oleic acid (Hamilton, 1994). Timmons *et al.* (2001) reported that as concentrations of unsaturated fatty acids in milk increase, particularly of PUFA C_{18:2} and C_{18:3}, milk becomes more susceptible to oxidation. This is likely to contribute to the production of off-flavors in the UV treated milk.

References

Folch J., M. Lees, and G.H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol Chem.* 226:497-502

Loor, J.J. and J.H. Herbein. 2003. Reduced fatty acid synthesis and desaturation due to exogenous *trans*10, *cis*12-CLA in cows fed oleic or linoleic oil. *J. Dairy Sci.* 86:1354-1369

FA as percent of total FA Fatty Acid	Control 1	Control 2	No UV 1	No UV 2	UV 1	UV 2
4:0	1.66	1.87	1.82	1.89	1.87	1.87
6:0	1.95	2.10	2.06	2.12	2.09	2.10
8:0	3.01	3.09	3.06	3.10	3.07	3.09
10:0	10.77	10.72	10.67	10.75	10.62	10.69
12:0	5.14	5.07	5.05	5.08	5.04	5.06
14:0	10.00	9.86	9.84	9.89	9.86	9.91
c9 14:1	0.14	0.14	0.14	0.14	0.14	0.14
15:0	0.79	0.78	0.78	0.78	0.78	0.79
16:0	23.24	23.01	23.06	23.14	23.12	23.21
trans 16:1 isomers						
t9 16:1	0.69	0.69	0.69	0.68	0.69	0.68
t11 16:1	0.13	0.13	0.12	0.10	0.11	0.11
cis 16:1 isomers						
c9 16:1	0.68	1.08	1.08	0.68	1.06	0.68
c11 16:1	0.05	0.04	0.03	0.05	0.03	0.03
17:0	0.70	0.69	0.70	0.70	0.70	0.70
18:0	10.83	10.73	10.79	10.79	10.80	10.84
coeluted 18:1 isomers						
t6 & t7 18:1	0.36	0.36	0.36	0.36	0.36	0.36
t12 & c7 18:1	0.28	0.28	0.28	0.28	0.28	0.28
t13 & c6 18:1	0.71	0.73	0.73	0.74	0.73	0.73
trans 18:1 isomers						
t4 18:1	0.02	0.02	0.02	0.03	0.02	0.02
t5 18:1	0.03	0.03	0.03	0.04	0.03	0.03
t9 18:1	0.39	0.39	0.39	0.39	0.39	0.39
t10 18:1	0.65	0.64	0.64	0.64	0.65	0.64
t11 18:1	3.16	3.13	3.15	3.16	3.15	3.16
t14 18:1	0.18	0.17	0.22	0.17	0.17	0.17
t16 18:1	0.29	0.29	0.29	0.29	0.30	0.29
cis 18:1 isomers						
c9 18:1	18.72	18.54	18.56	18.62	18.66	18.69
c11 18:1	0.57	0.56	0.57	0.57	0.56	0.57
c12 18:1	0.49	0.48	0.49	0.49	0.49	0.50
c13 18:1	0.11	0.11	0.11	0.12	0.11	0.10
c15 18:1	0.12	0.13	0.13	0.13	0.13	0.13
non-conjugated 18:2 isomers						
t9,t12 18:2n6	0.00	0.02	0.02	0.02	0.01	0.02
c9,t12 18:2n6	0.07	0.08	0.09	0.08	0.07	0.08
t9,c12 18:2n6	0.04	0.04	0.04	0.04	0.04	0.04
c9,c12 18:2n6	1.60	1.59	1.59	1.59	1.60	1.60

Dairy Forage Lab, Virginia Tech

Duplicate fatty acid profiles of fresh goat's milk (con1, con2), goat's milk that has been mechanically agitated (noUV1, noUV2), and goat's milk that has been processed at a UV dose of 15.6 mJ/cm² (UV1, UV2).

FA as percent of total FA						
<i>conjugated</i> 18:2 isomers	Control 1	Control 2	No UV 1	No UV 2	UV 1	UV 2
c9,t11 18:2	1.25	1.24	1.23	1.23	0.64	0.64
c9,c11 18:2	0.00	0.00	0.00	0.00	0.08	0.08
t11,t13 18:2	0.02	0.02	0.02	0.02	0.01	0.01
other <i>t/t</i> CLA	0.04	0.04	0.04	0.04	0.42	0.42
c6,c9,c12 18:3n6	0.04	0.05	0.04	0.04	0.04	0.05
c9,c12,c15 18:3n3	0.30	0.30	0.29	0.29	0.30	0.30
20:0	0.17	0.16	0.16	0.16	0.17	0.16
c11 20:1	0.08	0.08	0.07	0.07	0.08	0.08
c11,c14 20:2n6	0.04	0.04	0.04	0.04	0.03	0.04
c8,c11,c14 20:3n6	0.03	0.03	0.03	0.02	0.03	0.03
c5,c8,c11,c14 20:4n6	0.24	0.24	0.24	0.24	0.24	0.24
c5,c8,c11,c14,c17 20:5n3	0.01	0.01	0.01	0.01	0.01	0.01
c13 22:1	0.01	0.01	0.01	0.00	0.01	0.01
c7,c10,c13,c16 22:4n6	0.03	0.03	0.03	0.03	0.03	0.03
c4,c7,c10,c13,c16,c22 22:5n6	0.01	0.01	0.01	0.01	0.01	0.00
c7,c10,c13,c16,c19 22:5n3	0.09	0.09	0.09	0.09	0.09	0.09
c4,c7,c10,c13,c16,c19 22:6n3	0.03	0.03	0.03	0.03	0.02	0.03
24:0	0.03	0.03	0.04	0.03	0.04	0.03
c15 24:1	0.01	0.00	0.00	0.00	0.00	0.00
Total ug FA	18895.515	19098.413	22862.040	18159.836	19514.230	19146.099
mg solvent extracted lipid	35.45	35.34	36.16	34.50	35.97	35.97
Total ug FA/mg extracted lipid	533.019	540.419	632.247	526.372	542.514	532.280

Dairy Forage Lab, Virginia Tech

Duplicate fatty acid profile of fresh goat's milk (con1, con2), goat's milk that has been mechanically agitated (noUV1, noUV2), and goat's milk that has been processed at a UV dose of 15.6 mJ/cm² (UV1, UV2).

VITAE

Kristen Matak was born and raised in New Jersey. She graduated from Vernon Township High School in 1992. A Bachelors of Science in Human Nutrition and Foods was earned in 1996 at West Virginia University and a Masters of Science in Food Science and Technology was earned in 1999 from Virginia Tech. After completion of the Master's degree, Kristen worked as a dairy safety consultant for the Safe Quality Foods 2000 project for AgWest in Western Australia. After returning to the United States, Kristen took a position at USDA's Cooperative State Research Education and Extension Service as a Program Specialist of the food safety competitive grants program. After two years, Kristen returned to Virginia Tech to complete her graduate studies and earn a Doctorate of Philosophy in Food Science and Technology. She has accepted a faculty position at her alma mater WVU in the Human Nutrition and Foods department.