CHANGES IN AROMATIC CHEMISTRY AND SENSORY QUALITY OF MILK DUE TO LIGHT WAVELENGTH

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Dissertation submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

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(ABSTRACT)

Gas chromatography (GC) and gas chromatography olfactometry (GCO) was used to
determine the effect of specific light wavelengths on light oxidation in milk. The most
damaging wavelengths to milk quality appear to be the UV (200-400 and 395 nm) and
short visible (463 nm) wavelengths. However, exposure to 610 nm also appears to be
damaging.

GC and GCO were also used to look at the efficacy of film over-wraps made from
iridescent films. Single-layer over-wraps were not as effective in reducing light
oxidation as multi-layer film over-wraps. Single-layer over-wrap treatments had higher
numbers of odor-active compounds than multi-layer over-wrap treatments with a number
of odor-active compounds detected consistently in single-layer over-wrap treatments but
not in the multi-layer over-wrap treatments. Concentrations of volatile compounds were
slightly lower in the multilayer treatments.

Multi-layer film over-wrap treatments were tested for light oxidation flavor intensity with
a balanced incomplete block multi-sample difference test using a ranking system and a
trained panel. Packaging over-wraps limited the production of light oxidation flavor in
milk over time but not to the same degree as the complete light block. Blocking all
visible riboflavin excitation wavelengths was better at reducing light oxidation flavor
than blocking only a single visible excitation wavelength.

A method to determine light oxidation in oil using Fourier Transform Infrared (FTIR)
spectroscopy was established and preliminary data is prese

ACKNOWLEDGEMENTS

My first exposure to the Virginia Tech Department of Food Science and Technology (FST) was in 1989 when I interviewed with Dr. Merle Pierson to be his laboratory technician. I remember sitting in Dr. Pierson's office, discussing his trips to Israel and eating ice cream that Walter Hartman had made. I remember thinking it was so cool to be eating ice cream at an interview! I had no idea at the time what food science was or what the FST Dept. was all about. I just knew that I needed a job and the one I was interviewing for sounded interesting.

Little did I know that that interview would be the beginning of a long journey with FST that would culminate in being awarded the Doctorate of Philosophy. It didn't take long for me to realize after I was hired that I absolutely loved the place—I loved the work and I loved the people that I worked with. I loved the interdisciplinarity of the work and the fact that so much of the research had a truly practical application. When I started taking classes for the master's degree, for the first time, what I was learning made sense to me and I understood why I needed to know what was being taught and why it was important.

I also loved FST because of how I was treated by the members of the department. Unlike many departments on campus that I had worked in before, I wasn't treated as "just a technician". I was treated with respect and made to feel like a collaborator, with worthy and important ideas. I think this, above all else, was what made me yearn to come back to the department after being laid off in 1991 due to budget cuts. I spent over a decade doing other things, but always had in the back of my mind this idea that one day I would go to FST. I finally did in 2003, when I joined the department as a PhD student.

As a student, I feel like I have truly been blessed. I worked on a project that I enjoyed, I learned more than I could have imagined, I have had opportunities beyond my wildest dreams and I've had a mentor that was not only a good role model but also a good friend. I don't think that I would have had as positive an experience in graduate school if I had worked under anyone other than Susan Duncan. She has always understood my need to

balance family life with school life and supported me in all of my endeavors. Sue, thank you for all you have given me—you've been a teacher, a mentor and a friend and for that I truly thank you.

I also want to thank my committee: Dr. Joe Marcy, Dr. Sean O'Keefe, Dr. Susan Sims and Dr. Tom Ward. Although we may not have had many formal meetings, the informal discussions have been invaluable. Not only did we talk about my project, we also discussed science, faculty life, and life in general.

I want to thank NSF and the MILES program for making my life easy financially and for exposing me to interdisciplinary research and learning. It hasn't always been easy. There have been times when I think my colleagues and I are on different planets, but this is the way of the future and the benefits certainly outweigh the problems. What I have learned in this program has definitely helped me at the PDT and I'm sure it will continue to do so. I would also like to thank Dairy Management Incorporated (DMI), the Center for Advanced Processing and Packaging Studies (CAPPS), and the Department of Food Science and Technology for all their financial support.

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About six months ago, I overheard Ellie, my youngest, telling her Girl Scout leader that mom had "a little paper to write". Well, I have now finished that "little" paper and I present it to you below!

ATTRIBUTIONS

Dr. Susan E. Duncan

Dr. Duncan served as the committee chair for this dissertation research. She is a professor in the Department of Food Science and Technology at Virginia Tech conducting research on the chemical and physico-chemical measures of multiphase biological systems, such as dairy systems, including oxidative mechanisms, emulsion stability, sensory quality, and interactions with synthetic and natural macromolecular molecules. Dr. Duncan is also director of the Macromolecular Interfaces with Life Sciences (MILES) Integrative Graduate Education and Research Traineeship (IGERT) Program in which the candidate was working. Dr. Duncan gave advice for the entire project but gave special assistance with the design and implementation of the sensory research.

Dr. Joseph E. Marcy

Dr. Marcy served as committee member for this dissertation research. He is a professor in the Department of Food science and Technology at Virginia Tech and is the interim department head of the Human Nutrition, Foods and Exercise department. His research areas include food chemistry and interactions of foods and packaging. Dr. Marcy gave advice on the food packaging portion of this research.

Dr. Sean O'Keefe

Dr. O'Keefe served as a committee member for this dissertation research. He is an associate professor in the Department of Food Science and Technology and his area of research includes flavor chemistry, improving quality of peanuts by selecting varieties with modified fatty acid composition, control of maturity and other agronomic factors,

and use of novel processing methods. Dr. O'Keefe gave assistance with the analytical evaluation of milk.

Dr. Thomas Ward

Dr. Ward served as a committee member for this dissertation research. He is a professor in the Department of Chemistry at Virginia Tech. His area of research is physical chemistry and gave invaluable advice on using Fourier Transform Infrared (FTIR) spectroscopy.

Dr. Susan R. Nielson-Sims

Dr. Sims served as a committee member for this dissertation research. She is a scientist with Eastman Chemical Company working with Polyethylene Terephthalate (PET) films. Dr. Sims gave advice on packaging materials.

TABLE OF CONTENTS   **PAGE**

CHAPTER III
EFFECT OF SPECIFIC WAVELENGTHS ON VOLATILE CHEMISTRY
AND PRODUCTION OF AROMA-ACTIVE COMPOUNDS IN MILK..................50

CHAPTER VI
CONTROLLING LIGHT OXIDATION FLAVOR IN MILK BY
BLOCKING RIBOFLAVIN EXCITATION WAVELENGTHS

LIST OF FIGURES   **PAGE**

Chapter IV

Chapter V

Chapter VI

Chapter VII

LIST OF TABLES   PAGE

Chapter VI

Chapter VII

Chapter I

Introduction

Many foods and beverages are sensitive to light and this light sensitivity is exacerbated by the practice of displaying food, packaged in transparent and/or translucent materials, under high intensity fluorescent light. Transparent and translucent packaging allows light to reach the food, making it susceptible to photo-oxidation. For many foods, sensory quality can decline considerably even when there is only a small amount of oxidation (Rosenthal 1992).

Even though photo-oxidation can be detrimental to the flavor and nutrition of food, the practice of displaying food in "see through" material continues because of marketing policies, consumer preference, and sales (Young 2002). Consumers prefer foods and beverages to be packaged in clear containers where they can see the product (Sattar and deMan 1976; Rosenthal 1992; Cladman and others 1998; Chapman 2002; Doyle 2004).

Light oxidized flavor in milk, which arises due to photo-oxidation, is widespread and possibly responsible for declining milk sales (Barnard 1973; Bray and others 1977; Heer and others1995). High quality milk has a bland, slightly sweet flavor that leaves a clean, pleasant aftertaste (Bodyfelt and others 1988). The bland flavor can be altered by even low levels of volatile compounds resulting from photo-oxidation reactions. Light oxidized milk is described as having a "wet cardboard" flavor and this defect has become more common due to the increased use of transparent/translucent packaging. Barnard (1973) tracked the increase in consumer complaints for light oxidized flavor in milk from 1967, where it accounted for only 6.7% of consumer complaints, to 1970, where it accounted for 24% of complaints. The increase in oxidized flavor coincided with the introduction, and increased use, of blow molded plastic milk containers. Over 86% of the blow molded plastic containers tested had light oxidized flavor (Barnard 1973). White and Bulthaus (1982) found that 53% of samples from plastic containers were found to have moderate to strong light oxidized flavor.

1

A consumer sensory study in 1977 found that greater than 73% (n=2000) of panelists were able to detect differences between good quality milk and light oxidized milk (Bray and others 1977). However, approximately 20% of the panelists preferred light oxidized milk to good quality milk. These panelists indicated that the oxidized milk was sweeter, creamier, more flavorful and/or more familiar in flavor than the good quality milk (Bray and others 1977). By 1995, Heer and others (1995) found that 15 out of 24 panelists (62.5%) could not detect light oxidized flavor. Both Bray and others (1977) and Heer and others (1995) hypothesized that the reason for this lack of detection may have been due to consumers becoming conditioned to light oxidized flavor because of its increased incidence.

For panelists that could detect oxidized flavor, there was a significant difference in acceptability of light oxidized milk due to gender. Females rated light oxidized milk significantly lower than males (Bray and others 1977; Heer and others 1995). There was also a significant difference in milk preference due to age. Middle school aged children had significantly lower (P<0.0001) preference for light oxidized milk than both college aged and adult consumers (Heer and others 1995). Another study found that 34.5% of teenagers could detect light oxidized flavor in milk exposed to 2000 lux fluorescent lighting within ½ hour of exposure. By three hours of exposure, 70.7% detected the off-flavor (Chapman 2002). Teens indicated that they did not like the flavor of light oxidized milk. The average exposure time of milk in retail cases is 8 hrs at an average of 2000 lux light intensity (Chapman 2002). It is likely, then, that much of the milk teens are served is light oxidized and they will be reluctant to drink it.

Decreased milk consumption in teenage years can have lifelong health implications. Milk consumption is extremely important in maintaining healthy bones and teeth and in reducing the risk of chronic diseases such as osteoporosis because it is an excellent source of both calcium and vitamin D. One cup of milk contains approximately 300 mg of calcium (slightly less than 1/3 of the dietary reference intake (DRI) for adult males and females ≤ 50 yrs of age) (Sizer and Whitney 2005). Milk also contains vitamin D, which

2

is important for proper calcium absorption. Unfortunately, many Americans, especially females over the age of 9-12, do not consume adequate (1300 mg/day ages 9-18, 1000 mg/day adults ≤ 50 yr) amounts of calcium (Groff and Gropper 1999; http://teamnutrition.usda.gov/Resources/changing.html). As girls age, they consume less milk and do not consume the recommended 3 servings of dairy per day (Fiorito and others 2006). According to the National Health and Nutrition Examination Survey III, only 19% of females aged 9-19 consumed adequate amounts of calcium (Figure 1.1) (http://teamnutrition.usda.gov/Resources/changing.html). This percentage decreased considerably by 2001. Data presented to the 2001 NIH Consensus Development Panel on Osteoporosis showed that only 25% of boys and 10% aged 9-17 yr ingested the recommended daily requirement for calcium (Anonymous 2004). The childhood and early adult years are extremely important for adding bone mass and reducing the risk of osteoporosis, since the majority of increase in bone density occurs before one reaches age 30 (Sizer and Whitney 2005). It is essential, therefore, that the quality of milk remain as high as possible in order to encourage milk consumption, especially in children and young adults.

Figure 1.1. Percentage of U.S. youth who met dietary recommendations for calcium intake by age and sex between 1988-1994 (adapted from The Changing Scene, http://teamnutrition.usda.gov/Resources/changing.html).
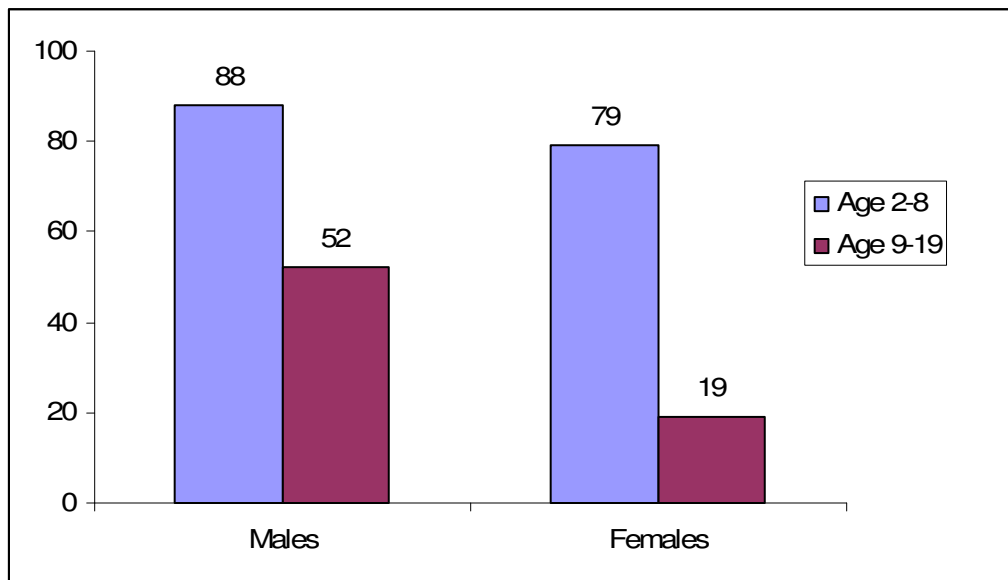
Source: National Health and Nutrition Examination Survey III, CDC

3

Maintenance of food quality during storage depends considerably on packaging (Sattar and deMan 1976; Bekbolet 1990). Packaging materials may be able to inhibit or delay reactions that cause light oxidation flavor in milk. It is relatively simple and inexpensive for milk processors to use high-density polyethylene (HDPE) jugs with light absorbing compounds, such as titanium dioxide, to make the jug opaque and protect milk against photo-oxidation. However, marketing objectives often influence packaging material selection because, as mentioned above, consumers want to see the food inside (Sattar and deMan 1976; Doyle 2004). The identification of novel packaging materials that protect milk quality and enhance marketing and sales of fluid milk is needed.

The Child Nutrition and WIC Reauthorization Act of 2004 required schools participating in the USDA's Child Nutrition program to implement wellness policies by 2006-2007. These policies must include plans for access and consumption of all foods available on school grounds during the school day, including plans for "competitive" food sales (ie-foods sold in competition with the National School Lunch Program (NSLP)) (Swanson 2006). Competitive foods can be divided into two categories: foods with minimal nutritional value, such as soft drinks, water ices, chewing gum and candy, and other foods. The sale of soft drinks has been particularly prominent in both the popular press and scientific literature as being linked to childhood obesity (Malik and others 2006). Soft drink consumption has also been linked to a reduction in calcium intake. Yen and Lin (2002) found that between the years of 1977 and 1996, for every 1 oz decrease in milk consumption there was a 4.2 oz increase in soft drink consumption in children aged 2 to 17. This resulted in a net decrease of 34 mg of calcium and a net increase of 31 calories. This replacement of milk as a beverage increased girls' risk for osteoporosis (Ballew and others 2000; Cavadini and others 2000). In response many school districts have been replacing soda beverage machines with healthy vending machines which carry single serve milk. Consumption of single-serve milk has been one section of the fluid milk industry that has been increasing in recent years, outpacing all other product categories and increasing by 45% during a 3-year period from 2000 to 2003 (Sloan 2005). Milk in these vending machines is expected to have a 60-day (or longer) shelf life (Anonymous 2002), yet still be packaged in translucent materials, such as PET, and

4

stored under light. Milk flavor quality must be maintained in order for school aged children to increase their consumption of milk and improve their health and well being. It is imperative that the quality of milk sold in vending machines remain high. Package material selection to avoid product photo-oxidation and attract youthful buyers is needed.

The overall objectives of this study were:

1. To determine the specific light wavelengths that are most damaging to milk quality, using analytical and sensory techniques, so that packaging materials might be designed to block wavelengths most detrimental to milk quality;

2. To determine the efficacy of unique multilayer polymers, that could be used as decorative over-wraps on single serve bottles, in inhibiting light oxidation in milk using analytical and sensory techniques. These films block specific light wavelengths while still allowing the consumer to "see" the product inside; and

3. To develop a method for monitoring the chemical changes occurring in milk during photo-oxidation in real time using fourier transform infra-red (FTIR) spectroscopy.

5

Chapter II
Literature Review

Whole milk is comprised of approximately 87% water, 3.25% fat and 4% protein. The remaining 5.75% consists of lactose, minerals and other solids. Triacylglycerides make up the majority (97-98%) of fat in milk, while the remaining 2-3% is comprised of small amounts of di- and mono-acylglycerides, free fatty acids, free cholesterol, esterified cholesterol, phospholipids, and glycolipids. Approximately 70% of fatty acids in milk are saturated. The remaining fatty acids, include monounsaturated (~27%, mostly palmitoleic and oleic acid), polyunsaturated (~4%, mostly linoleic acid) and minor components, such as phospholipids, glycolipids, sterols and fat soluble vitamins (A, D and E) (Fox 1995).

Fat globules are encapsulated by phospholipids and protein which increases their stability in the hydrophilic environment (Elling and others 1996). Approximately 40-60% of phospholipids at the milkfat droplet surface are unsaturated, with one third of them being polyunsaturated (Elling and others 1996; Deeth 1997). Unsaturated fatty acids are more susceptible to oxidation than saturated fatty acids (Bekbolet 1990; deMan 1990). Homogenization disrupts native milk lipid globules, creating smaller globules and reducing globule size from about 3 µm to approximately 0.8 µm. This increases the surface area by as much as 10 times (Jensen 2002) increasing the susceptibility of the milk to oxidation (Bradley 1980).

Milk contains a number of photosensitizers, most notably riboflavin (Sattar and deMan 1976; Dimick 1982; Bekbolet 1990; Skibsted 2000), but also porphoryns and chlorins (Wold and others 2005). Riboflavin is found in the whey portion of milk at an average concentration of 1.75 mg/L (Dimick 1982). Porphoryns and chlorins are found in much lower concentrations but have been shown to contribute to photo-oxidation in milk and dairy products (Wold and others 2005). Photosensitizers absorb light of specific wavelengths and initiate free radical oxidation reactions. These oxidation reactions ultimately lead to the production of off-odor and flavor compounds.

The purpose of this literature review is to explore the chemistry of oxidation, the implications of oxidation reactions in milk, and the possible ways to inhibit oxidation reactions through the blocking of specific wavelengths by packaging materials.

Overview of Oxidation Chemistry

Proteins and lipids in milk are susceptible to oxidation and can produce off-flavor compounds. Oxidative degradation of these macromolecules ultimately yields volatile flavor compounds affecting milk quality. Proteins appear to be oxidized and produce off-flavor compounds faster than lipids (Aurand and others 1966; Allen and Parks 1975; Dimick 1982; Davies and Dean 1997; Ostdal and others 2000). The first off-flavors that appear in milk are due to oxidation of protein and have a burnt feather or burnt protein flavor. This flavor dissipates within a few days and is replaced by a tallowy or wet cardboard flavor, which is produced by the oxidation of lipids (Aurand and others 1966).

Milk is typically stored at temperatures between 2-5º C, which slows the rate of chemical reactions. However, temperature alone is not enough to protect unsaturated lipids from oxidation (Madhavi and others 1996). Bonds in unsaturated lipids can react with oxygen to form primary, secondary and tertiary oxidation products. These products can produce off-flavors and possibly cause the milk to become unpalatable if concentrations become excessive (deMan 1990).

Lipid Oxidation Chemistry

Oxidation reactions proceed under two major mechanisms: autoxidation, in which molecular oxygen reacts with organic substrates through a free radical mechanism or through singlet oxygen attack on unsaturated fatty acids (Frankel 1980). Free radical reactions proceed, in general, through three basic steps: initiation, propagation and termination.

Initiation

Initiation (1) commences when an initiator such as heat, light, or a metal abstracts hydrogen from the lipid, producing a free radical. Typically, an allylic hydrogen is abstracted because the bond dissociation energy for these hydrogens is lower than for those of the double bond (~80 kJ vs ~ 100 kJ) (personal communication, Jim Tanko). However, in the presence of singlet oxygen, oxidation proceeds by the "ene" reaction, with addition directly to the double bond (Frankel 1980)

$$\text{(1) RH} \xrightarrow{\text{(hv, M, }\Delta\text{)}} \text{R* + H*}$$
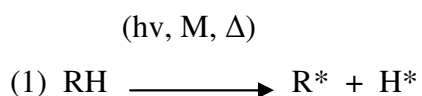
A peroxy free radical (ROO*) forms upon addition of oxygen to the radical (R*) (2).

$$\text{(2) R* + O}_2 \longrightarrow \text{ROO*}$$
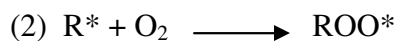
Propagation

This peroxy radical is extremely reactive and will attack points of unsaturation in nearby molecules, such as other lipid molecules, leading to propagation of the free radical chain reaction (3).

$$\text{(3) ROO* + RH} \longrightarrow \text{ROOH + R*}$$
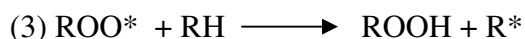
The newly formed radical reacts by a chain reaction mechanism to form other free radicals. Propagation reactions are shown in reactions (3) and (4).

$$\text{(4) R* + O}_2 \longrightarrow \text{ROO*}$$
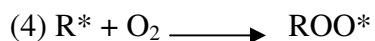
Propagation will continue until no substrate is available or a termination reaction occurs (deMan 1990).

Termination

Termination occurs when free radicals react with themselves and yield non-reactive products (5, 6, 7).

(5) $R* + R* \longrightarrow R_2$

(6) $R* + ROO* \longrightarrow ROOR$

(7) $nROO* \longrightarrow (ROO)n$
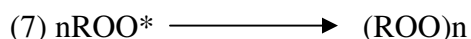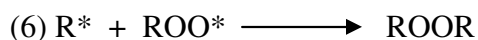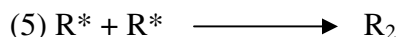
Production of Primary Oxidation Products

Primary oxidation products are hydroperoxides that are first formed during propagation. Where the hydroperoxide forms depends on the susceptibility of the allylic hydrogen. In oleate, hydrogen abstraction occurs on both carbons 8 and 11 to form radical intermediates. These radical intermediates react with oxygen to form hydroperoxides on carbons 8, 9, 10 and 11, with the C8 and C11 hydroperoxides being slightly more common (Figure 2.1). Hydrogen abstraction on linoleate occurs on carbon 11 with oxygen addition to carbons 9 and 13. A mixture of conjugated 9 and 13 diene-hydroperoxides are formed with slightly higher amounts of 9 diene-hydroperoxide being formed (Figure 2.2). In linolenate, two pentadienyl radicals are formed by abstraction of hydrogen from the two methylene carbons (C11 and C14) which react with oxygen to produce a mixture of conjugated diene-triene 9, 12, 13, and 16-hydroperoxides (Figure 2.3). The 9 and 16 hydroperoxides form in significantly higher quantities than the 12 and 13 hydroperoxides, possibly due to regioselectivity, steric hinderance, higher decomposition of the 12 and 13 hydroperoxides, or cyclization of these compounds (Frankel 1980).
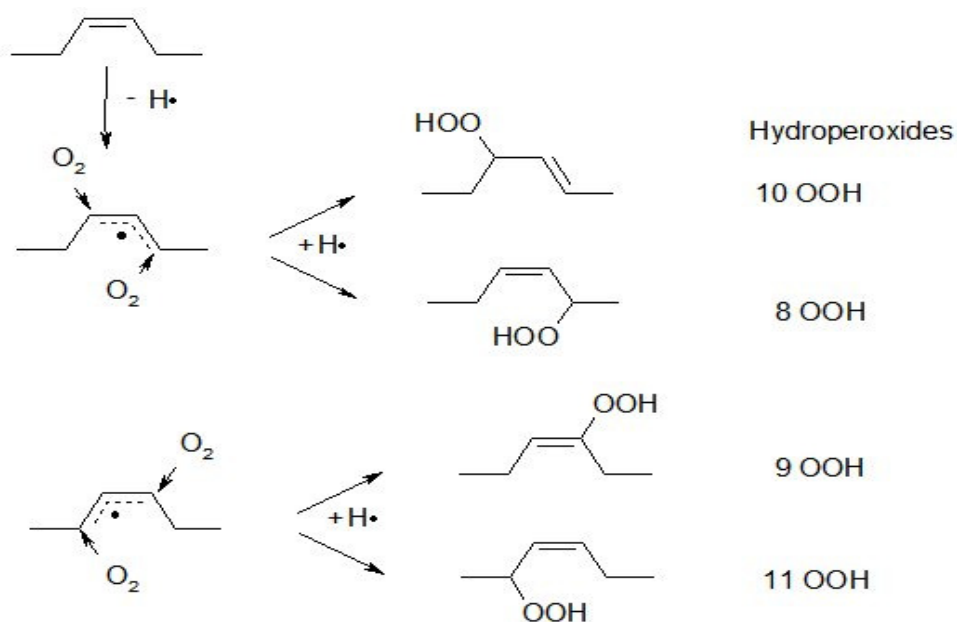
Figure 2.1. Mechanism of oleate autooxidation (Reprinted from Frankel EN. 1980. Lipid Oxidation. Prog. Lipid Res. 19: 1-22, with permission from Elsevier)

13-Hydroperoxide   9-Hydroperoxide
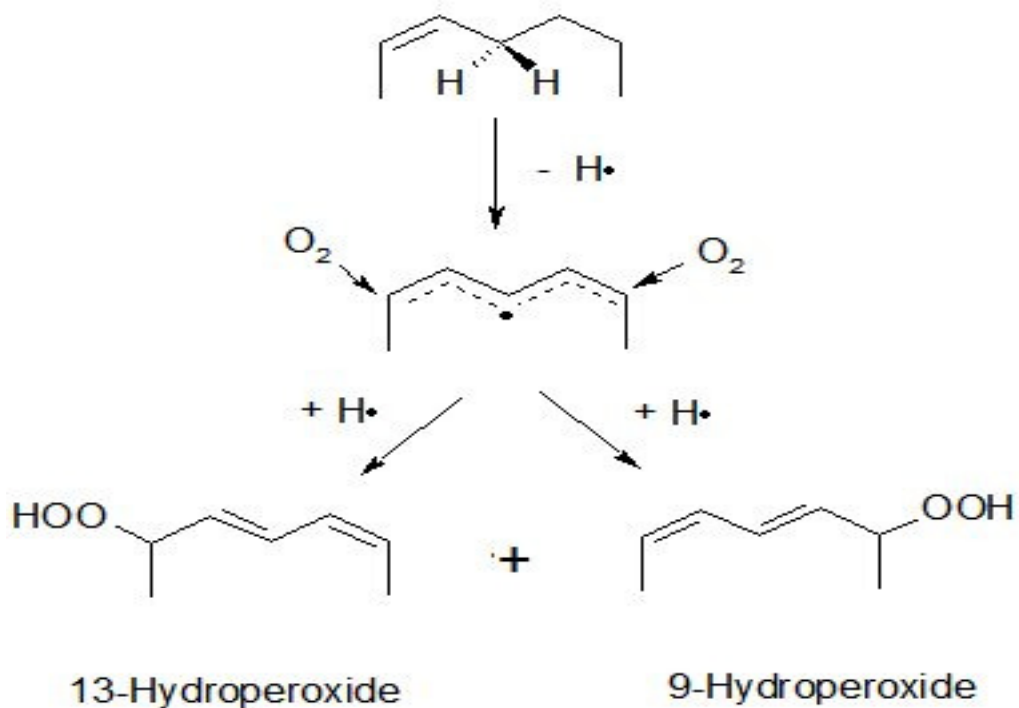
Figure 2.2. Mechanism of linoleate autooxidation (Reprinted from Frankel EN. 1980. Lipid Oxidation. Prog. Lipid Res. 19: 1-22, with permission from Elsevier)
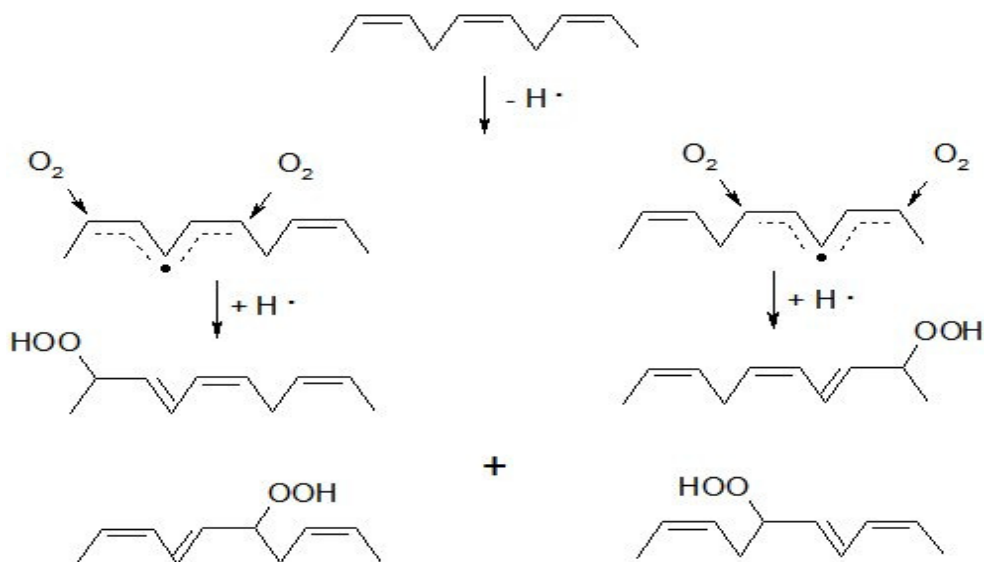
Hydroperoxides

Figure 2.3. Mechanism of linolenate autooxidation (Reprinted from Frankel EN. 1980. Lipid Oxidation. Prog. Lipid Res. 19: 1-22, with permission from Elsevier)

Production of Secondary and Tertiary Oxidation Products

Secondary oxidation occurs when hydroperoxides, which are relatively unstable, decompose to form aldehydes and minor constituents such as hydrocarbons, free fatty acids, esters, ketones, lactones, and furans as well as cis/trans isomerizations. These secondary oxidation products contribute to odor and flavor characteristics associated with oxidation. Aldehydes can be oxidized further to tertiary products such as carboxylic acids which also cause odor and flavor problems (deMan 1990).

The first step in decomposition to form secondary oxidation products involves the formation of alkoxy and hydroxy free radicals from hydroperoxides (8).

$$(8) \ R - CH(OOH) - R \longrightarrow R - \underset{\underset{O*}{|}}{CH} - R \ + \ OH*$$
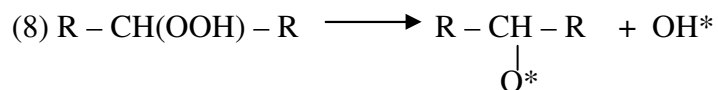
The alkoxy radical then decomposes to form aldehydes (9).

11

$$(9)\ R - CH - R \longrightarrow R* + RCHO$$
$$\overset{|}{O*}$$
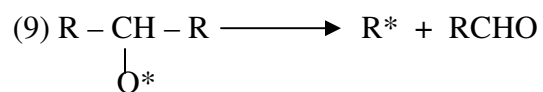
During alkoxy radical decomposition, a covalent bond in the radical is cleaved. Cleavage can occur on either side of the free radical and, depending on which bond is cleaved, a short chain volatile aldehyde or a nonvolatile aldehyde (one that is attached to the glyceride portion of the triacylglyceride) is formed (deMan 1990).

The alkoxy radical can also abstract hydrogen from another lipid molecule to form an alcohol and a new free radical (10).

$$(10)\ R - CH - R + RH \longrightarrow R - CH - R + R*$$
$$\overset{|}{O*} \qquad\qquad\qquad \overset{|}{OH}$$
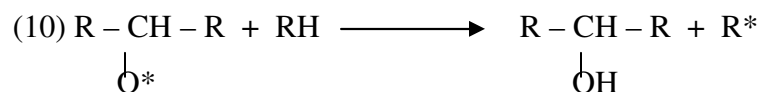
This new radical may participate further in propagation reactions.
Ketones are formed when alkoxy radicals react with other free radicals (11).

$$(11)\ R - CH - R + R* \longrightarrow R - C - R + RH$$
$$\overset{|}{O*} \qquad\qquad\qquad \overset{\|}{O}$$
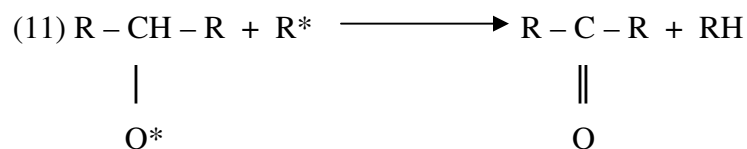
Protein Oxidation Chemistry

Proteins, peptides and amino acids are susceptible to free radical oxidation and this oxidation occurs relatively quickly (Aurand and others 1966; Allen and Parks 1975; Dimick 1982; Davies and Dean 1997; Ostdal and others 2000). Protein oxidation can be initiated by heat, light, metal, certain food additives, and the products of enzymatic and non-enzymatic browning (Macrae and others 1993). Significant consequences of protein oxidation are polymerization and hydrolysis (Bekbolet 1990) due to cross linking and breakdown of the primary structure of the protein (Dean and others 1997).

12

The amino acids cysteine, methionine, tryptophan and histidine, found in casein and lactalbumin, have been implicated in off-flavor production in milk and dairy products (Allen and Parks 1975; Sattar and deMan 1976; deMan 1990). Allen and Parks (1975) found that methional was produced within 10-15 min when skim milk was exposed to direct sunlight. Methional production was correlated to an increase in broth or potato flavor which changed to cabbage, burnt feather flavor upon further exposure to sunlight. Singleton and others (1963) found that tryptophan and riboflavin formed a complex upon exposure to light which produced flavor typical to protein oxidation in milk.

Amino acids can also undergo substantial oxidation when exposed to peroxidizing lipids. Methionine, cysteine, histidine and lysine have been implicated in this type of oxidation and compounds formed include imidazole, lactic acid, methionine sulfoxide, hydrogen sulfide, and diaminopentane (Macrae and others 1993; Jadhav and others 1996).

Overview of Photo-Oxidation

Photo-oxidation reactions are initiated by light. However, the compounds being oxidized, such as lipids, typically do not directly absorb light >220 nm (Hamilton and others 1997). Many foods, however, contain compounds called photosensitizers which do absorb light, both UV and visible, and can either initiate free radical reactions through direct contact with the substrate or produce singlet oxygen. This section will review the distinct aspects of photo-oxidation. An excellent review of photo-oxidation of milk and dairy products was provided by Bekbolet (1990).

Light Absorption in Foods

Light energy must first be absorbed by a chromophore for a photochemical reaction to occur. A chromophore consists of chemical bonds and configurations of atoms in a molecule that absorbs light. Groups absorbing light in chromophores usually contain pi

13

electron groups and/or hetero atoms that have nonbonding valence shell electron pairs (www.cem.msu.edu/~reusch/VirtTxtJml/Spectrpy/UV-Vis/spectrum.htm). When a chromophore absorbs light, excitation of the molecule containing the chromophore occurs. During excitation, the energy from ultraviolet (UV) light, and in some cases visible light, causes valence electrons to be elevated from their ground state orbital into a higher, more energetic, orbital (Rosenthal 1992).

Absorption of light is quantized (it can only vary by discrete values), so only particular wavelengths, or energies, can be absorbed by specific compounds. The specific wavelengths that are absorbed are determined by the particular chromophore (a chemical group capable of selective light absorption resulting in coloration of certain organic compounds) in the compound. For each quantum of energy absorbed, only one molecule is excited. Quantum yield (Φ) is defined as the ratio between the number of molecules undergoing change and the number of quanta absorbed. It can range from zero to infinity (Φ = molecules reacted/photons absorbed by reacting compound = $\Delta C_i/Q_i$ where ΔC_i = the change in concentration of the compound, and Q_i = the number of photons absorbed by the compound) (Mortensen and others, 2003). In free radical chain reactions, the quantum yield can be several orders of magnitude because, although one quanta initiates only one reaction, propagation can produce numerous reactions (Rosenthal 1992).

Light absorption of compounds can be quantified through the Beer-Lambert Law:
$$\log (I_o/I) = \kappa cb/\ln/10 = \varepsilon cb$$
> I and I_o is the intensity of the transmitted light and the incident light, respectively
> κ = molar constant of proportionality
> c = concentration of the absorber (mole/l)
> b is the depth of the absorber (optical path (cm) and
> ε = extinction coefficient ($M^{-1}cm^{-1}$).

The Beer-Lambert law applies only to homogeneous materials. Food, however, is heterogeneous and light will not only be absorbed, but will also be scattered, reflected, and transmitted. Scattering of light intensifies absorption by several orders of magnitude

and increases with both decreasing wavelength and decreasing particle size (Rosenthal 1992).

Sensitization

Molecules in their excited state react in three different ways: 1) the electron in the higher energy anti-bonding orbital (higher excited-state orbital) re-enters the original bonding orbital, releasing energy in the form of light or heat; 2) the molecule reacts chemically, especially through substrate hydrogen abstraction or through addition to a double bond; or 3) the molecule transfers its energy to another molecule, a process called sensitization (Rosenthal 1992). Sensitization is a common pathway for the initiation of photo-oxidation in milk through the excitation of molecules such as riboflavin.

Molecules in their stable ground states have all electrons spin-paired. Excitation causes electrons to be raised to a higher energy level but the direction of spin of the excited electron is not changed. At this point, the molecule is said to be in the singlet state and is very short-lived—usually less than 10 nanoseconds. Molecules in the singlet state can undergo fluorescence or decay with subsequent emission of light or heat. They can also undergo a process called intersystem crossing. In intersystem crossing, the electron in the highest-occupied molecular orbital relocates to the lowest-unoccupied molecular orbital and undergoes a spin flip to make the system spin-parallel. The molecule is now said to be in its triplet state and is much longer lived than the singlet state—microseconds to seconds.   A molecule in the triplet state can undergo phosphorescence or degradation with subsequent emission of light or heat, or it can react with other molecules in the food system, such as oxygen (Figure 2.4) (Rosenthal 1992; Kagan 1993).

15
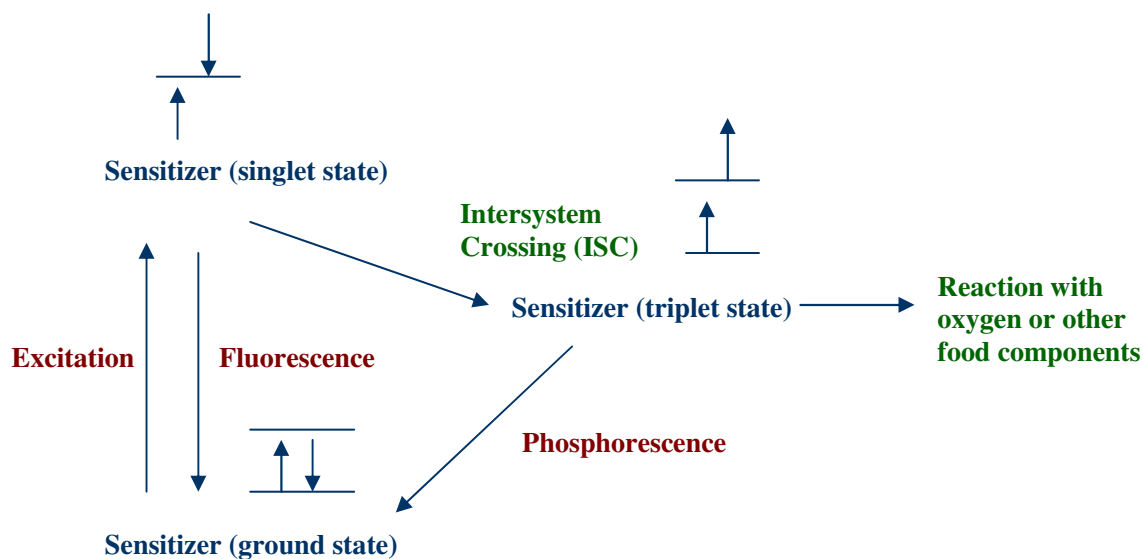
Figure 2.4. Reactions involving excited molecules (Reprinted from Kagan J. 1993. Organic Photochemistry: Principles and Applications. New York: Academic Press Ltd. 234 p.

Natural pigments found in foods that commonly act as photochemical initiators are flavonoids, riboflavin (vitamin B_2), chlorophyll, heme, and vitamin K. Riboflavin (Figure 2.5) is one of the most studied sensitizers. Riboflavin is found in high concentrations in the whey fraction of milk and increases the susceptibility of milk to photo-oxidation (Sattar and others 1976; Bekbolet 1990). Riboflavin can exist in three oxidation states—fully oxidized, radical, and fully reduced. At each oxidation state, there are three conjugate acid-bases (Figure 2.6). Due to the pH of milk (~7.0), riboflavin has three stable forms that have absorption maxima within the visible range at 400nm, 446 nm, and 570 nm (Kyte 1995).
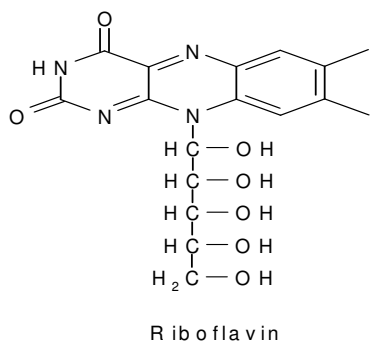
Figure 2.5. Structure of riboflavin
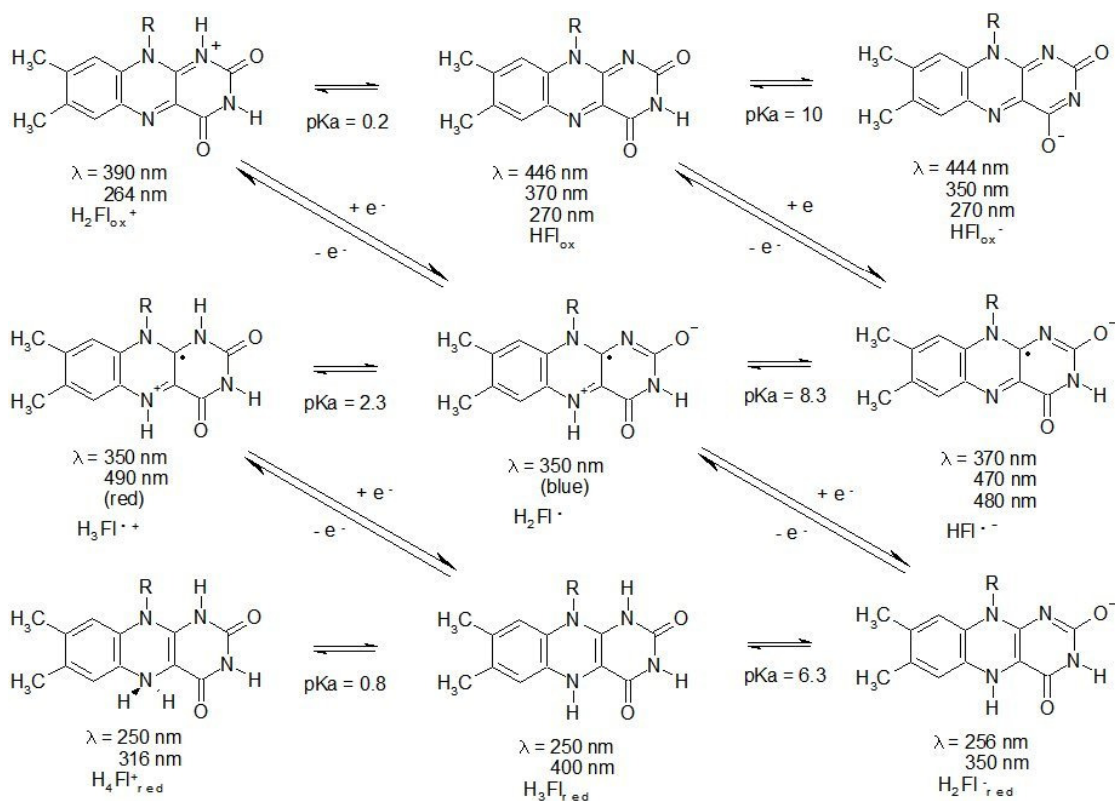
Figure 2.6. Oxidation states and conjugate acid-bases of riboflavin (Copyright 1995 from Mechanisms in Protein Chemistry by Kyte J. Reproduced with permission from Taylor & Francis Group, LLC., http://www.taylorandfrancis.com)

Dairy products also contain porphyrin and chlorin compounds, specifically protoporpyrin, hematoporphyrin (Figure 2.7), and chlorophyll a and b (Figure 2.8), which also act as photosensitizers. Cream and milk have been shown to have measurable amounts of chlorophyll a and b, but only very small amounts of protoporphyrin (Wold and others 2005). Porphyrins and chlorins produce singlet oxygen upon exposure to light (Bekbolet 1990). Wold and others (2005) found that the degradation of these compounds correlated better than riboflavin to the sensory attributes (acidic flavor, sun flavor, and oxidized odor) in cheese. Kristensen and others (2002) also found that riboflavin degradation did not correlate to the introduction of off flavor in Havarti cheese.

17
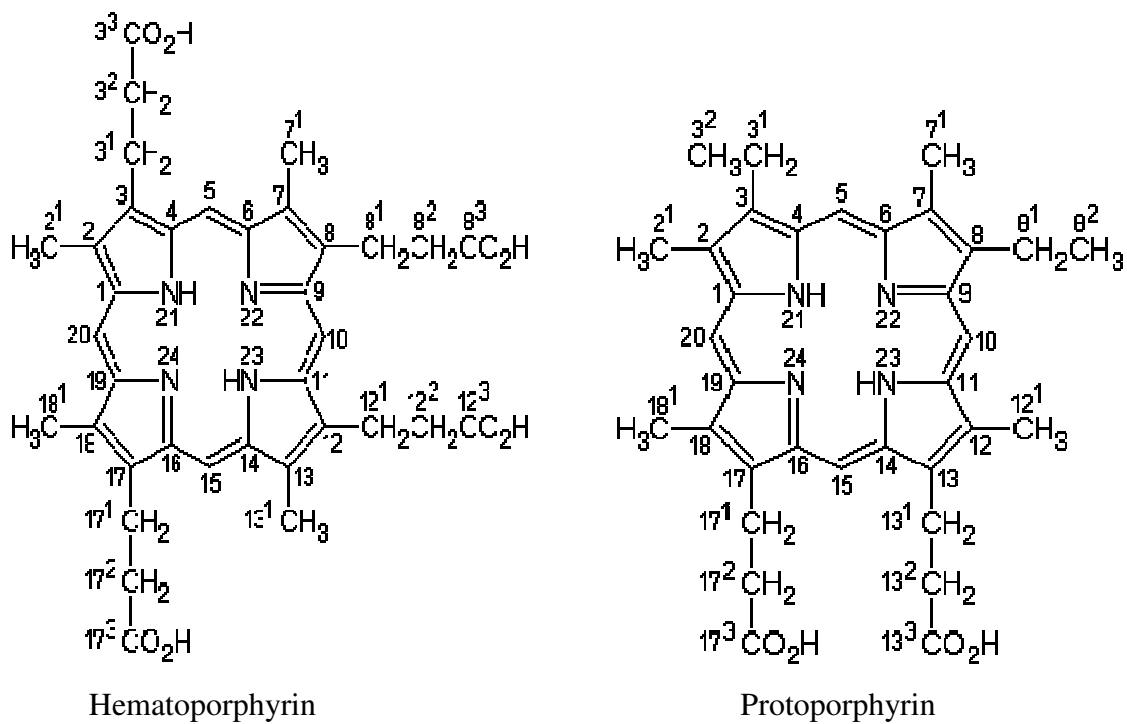
Figure 2.7. Structures of hematoporphyrin and protoporphyrin

Chlorophyll a   Chlorophyll b
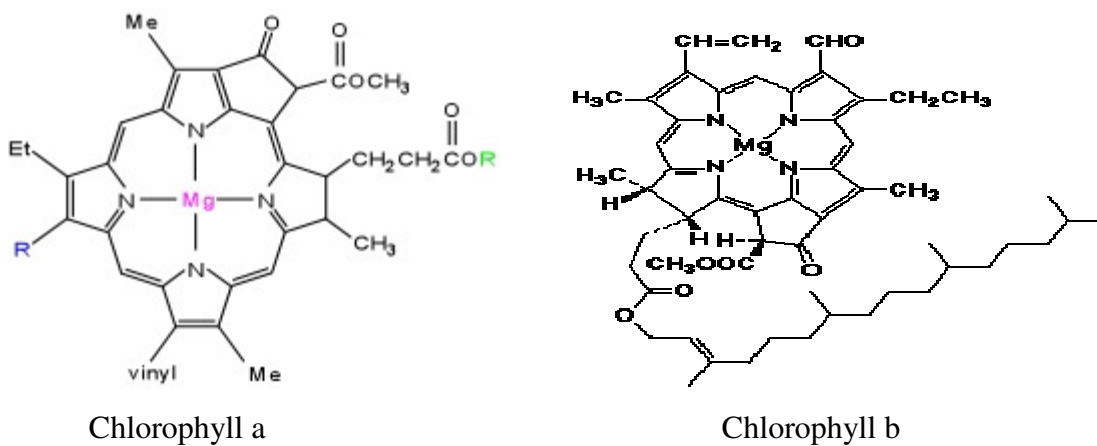
Figure 2.8. Structures of chlorophyll a and b

Mechanisms of Photo-Oxidation: Type I and Type II Reactions

Photo-oxidation initiated by a photosensitizer can proceed by one of two mechanisms depending on the conditions that are present at the time of the reaction. The triplet state

of the sensitizer reacts with lipids to form free radicals either by the abstraction of an electron or a hydrogen (Type I reaction) or by reacting directly with molecular oxygen to form singlet oxygen (Type II reaction) (Lennersten and Lingnert 2000; Kristensen and others 2002; Viljanen and others 2002). Figure 2.9 diagrams these mechanisms.
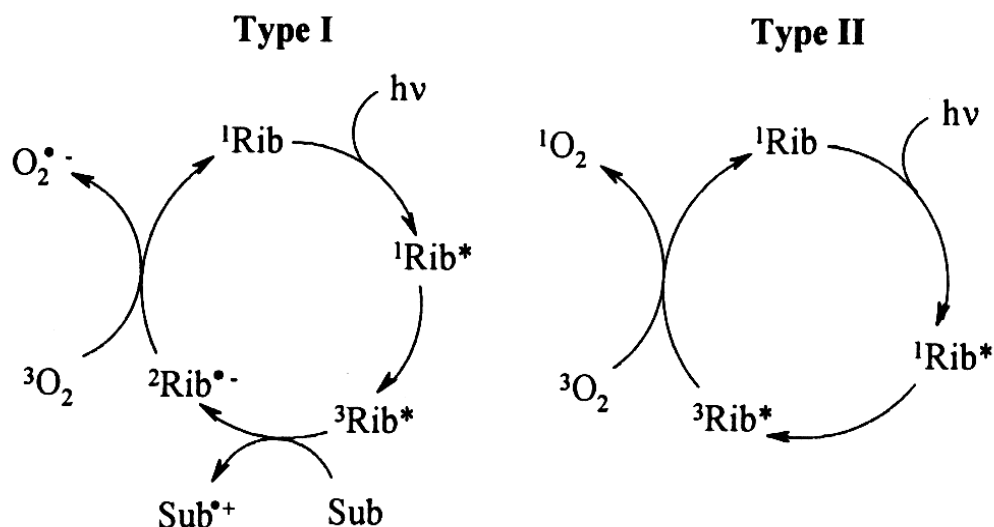
Figure 2.9. Type I and Type II mechanisms of photo-oxidation with riboflavin as the sensitizer (Reprinted with permission from Kristensen D, Kroger-Ohlsen MV, Skibsted LH. 2002. Radical formation in dairy products: Prediction of oxidative stability based on electron spin resonance spectroscopy. In: Morello MJ, Shahidi F, Ho CT, editors. Free Radicals in Food: Chemistry, Nutrition, and Health Effects. Washington, D.C.: American Chemical Society. 356 p. Copyright 2002 American Chemical Society)

In Type I photo-oxidation the sensitizer becomes excited to its singlet state and undergoes intersystem crossing to form a more stable triplet state. The triplet sensitizer extracts an electron directly from a substrate, such as a lipid, which becomes oxidized. Reduced riboflavin ($^2Rib^{*-}$) can react with molecular oxygen to form the super-oxide anion radical (O_2^{*-}) which can continue free radical reactions.

In Type II photo-oxidation, just like in Type I photo-oxidation, the sensitizer is excited by light to its singlet state, which undergoes intersystem crossing to form the more stable triplet state. The triplet sensitizer then reacts with molecular oxygen to form singlet oxygen. Singlet oxygen is highly reactive and forms hydroperoxides from unsaturated

19

lipids through a pathway that does not include the production of free radicals. Secondary oxidation products are then formed by free radical side reactions (deMan 1990). Type II photooxidation proceeds by four main reactions (Rosenthal 1992):

1. "ene" type reactions in which lipids with two or more double bonds form allyl peroxides. In these reactions the double bond shifts to a position adjacent to the original double bond.
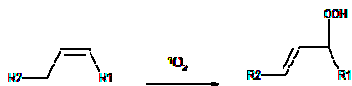
2. the [4 + 2] Diels-Alder type reaction in which peroxides are formed by the addition to a diene or a heterocyclic. The products of this reaction are an endoperoxide of varying stability.
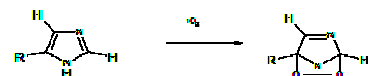
3. Singlet oxygen reacts with electron rich olefins or enamines to form dioxetane. These compounds are unstable and cleave to form carbonyls.
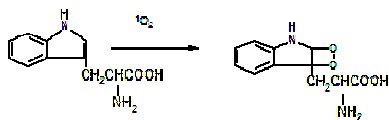
4. Compounds with heteroatoms, such as sulfur or nitrogen, react with singlet oxygen to form sulfoxides or nitroxides.
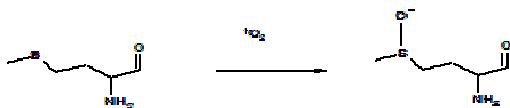
Destruction of Riboflavin by Light

When riboflavin becomes excited it undergoes one of several transformations: 1). the excited electron re-enters the original bonding orbital, giving off energy in the form of light or heat; 2). the molecule abstracts a hydrogen from the substrate; or 3). it transfers its energy to another molecule through sensitization (Rosenthal 1992). Theoretically, once these transformations occur, riboflavin goes back to its ground state and is available

to be excited again. In reality, however, some riboflavin molecules are destroyed during these reactions and are no longer available for further free radical initiation. Degradation compounds of riboflavin include lumiflavin and lumichrome (Huang and others 2006).

The degradation of riboflavin is correlated to an increase in light oxidized flavor in milk. Packaging material, wavelength of light exposure, intensity of light, time of exposure, and temperature all affect the rate of riboflavin degradation.   The rate of riboflavin destruction was found to be proportional to the amount of light coming through the container and the wavelength of light (Herreid and others 1952; Sattar and others 1977b). Maniere and Dimick (1975) showed that this rate was greater when riboflavin was in its free form and unassociated with proteins or fat in milk. Riboflavin destruction was also found to be inversely related to fat content; as fat decreased, loss increased. This is because lower fat content allows more light to penetrate into the milk due to reduction in light scattering (Senyk and Shipe 1981).

Many investigators found that a complete light block was best at preventing against riboflavin degradation (Singh and others 1975; Senyk and Shipe 1981; Bekbolet 1990). Others found that blocking UV wavelengths only did not protect against riboflavin destruction (Fanelli and others 1985) but several authors found that blocking wavelengths between ~400-500 nm did protect against riboflavin destruction (Fukumoto and Nakashima 1975; Sattar and others 1977b; Bradley 1980; Senyk and Shipe 1981). The major absorption maximum for riboflavin is 446 nm although it absorbs other wavelengths as well (Kyte 1995).

Packaging material is an important factor in the protection of riboflavin from photo-degradation in milk (Hoskin and Dimick 1979). Packaging materials that blocked all light were found to be best at protecting riboflavin from degradation. A number of authors found that tinting packaging materials yellow, amber, green or red was effective in either fully or partially reducing riboflavin degradation (Fukimoto and Nakashima 1975; Singh and others 1975; Sattar and others 1977b; Luquet and others 1977; Hoskin and Dimick 1979; Fanelli and others 1985).

21

Table A1 (appendix) gives a summary of the literature for riboflavin degradation in milk and cheese, indicating packaging type, color of packaging material, wavelength blocked, type of product, light intensity, time and temperature of exposure, amount of or loss of riboflavin and sensory characteristics of the product.

Effect of Photo-Oxidation on Milk Quality

Nutritional Quality

Other vitamins in milk besides riboflavin, such as vitamin A, D and C, are affected by exposure to light. This section will review the effect of light on these vitamins

Vitamin A

Vitamin A destruction is dependent on fat content and degradation increases as fat content decreases (deMan 1990; Senyk and Shipe1981). This is due to the fact that in higher fat milks there is less penetration of light into the milk. Added Vitamin A was found to be more susceptible to light destruction than native Vitamin A (deMan 1990).

The wavelength of light that milk is exposed to has an effect on the retention of Vitamin A. Many investigators found that UV light contributes to vitamin A degradation (Satter and others 1977a, 1977b; Fanelli and others 1985; Cladman and others 1998; Mestadagh and others 2005).

Many investigators found that milk packaged in paperboard cartons had less Vitamin A destruction than milk packaged in transluscent material (Senyk and Shipe 1981; Farrer 1983; Haisman and others 1992; Marchetti and Forti 2001). Several authors, however, found no difference in vitamin A retention between translucent and opaque containers (Goussault and others 1978; Al-Zawawi and Caldwill 1993). Vitamin A does not appear to be degraded by the presence of oxygen (Mestdagh and others 2005).

Table A2 (appendix) gives a summary of the literature for Vitamin A degradation in milk indicating packaging type, color of packaging material, wavelength blocked, type of product, light intensity, time and temperature of exposure, amount of or loss of riboflavin and sensory characteristics of the product.

Ascorbic Acid, Folic Acid, Thiamine, and Vitamins A, D and E

Oxidation of ascorbic acid to dehydroascorbic acid is accelerated by exposure to light and was correlated to light intensity and time of exposure (Haisman and others 1992). However, protecting milk from light exposure, while it reduced the loss of Vitamin C, did not completely protect against loss. Exposure of thiamine, and Vitamins A, D and E are variable in their degradation upon exposure to light.

Table A3 (appendix) gives a summary of the literature for degradation of these vitamins in milk indicating packaging type, color of packaging material, wavelength blocked, type of product, light intensity, time and temperature of exposure, amount of or loss of riboflavin and sensory characteristics of the product.

Overview of Volatile Chemistry

Exposure to light is a well-known cause of flavor and nutritional degradation in milk (Bekbolet 1990). A number of factors, including light source, light intensity, light wavelength, exposure time, and storage temperature affect the rate of light oxidation in foods. Packaging material also affects oxidation rate by controlling the intensity and wavelength of the light that impinges upon the food (Hoskin and Dimick 1979; Bekbolet 1990). Three factors, light source and intensity, light wavelength and packaging material will be discussed in the context of volatile chemistry, odor and flavor.

23

Effect of Light Source and Intensity on Volatile Chemistry, Odor and Flavor

Both natural and artificial sources of light can cause light oxidation. Sunlight has been shown to have the strongest oxidation effect, while incandescent light the weakest (Koo and Kim 1971). Exposure to fluorescent lighting plays a major role in the production of oxidized flavor in milk because the majority of milk today is sold through supermarkets and retail establishments and can remain on retail shelves for ten to twenty-one days after processing (Hoskin and Dimick 1979; Anonymous 2001). Milk may be exposed to light between 750 to 6460 lux for 24 hrs a day during the distribution and marketing period (Chapman and others 2002).   The average light intensity on retail shelves is 2000 lux and the average exposure time is 8 hrs (Chapman 2002).

Dairy display cases are commonly lighted by fluorescent sources. Fluorescent bulbs are typically filled with argon and a small amount of mercury. The mercury vaporizes when electricity flows through the lamp and ultraviolet (UV) light is produced. The UV light hits a layer of phosphor coated on the inside of the bulb which produces various frequencies of visible light. The frequencies of light (both visible and UV) that are transmitted depend on the type of fluorescent lighting used. Lamps that produce colors, as well as several types of "white" lamps, are available. White lamps fall into one of three categories: Warm White, Cool White and Daylight. All of these lamps contain at least one phosphor which produces light between 647-700 nm, 491-575 nm and 424-491nm. The ratio of the phosphors determines what type of light is being perceived. Warm white lamps produce more red and orange light, cool white lamps have more green light, while daylight lamps have a blue tint and mimic daylight. Grocery stores typically use cool white lamps but sometimes use warm white lamps (in bakery and meat departments) to improve the look of their products (www.nemesis.lonestar.org). Cool white fluorescent lighting is not the best type to use with photosensitive foods because they have a large emission band of blue-green light (444 nm) which is very close to the major absorption band of riboflavin (Bosset and others 1995).

Light intensity also plays a role in the rate of off-flavor development (Hansen and others 1975). Whited and others (2002) found that exposure of whole and reduced fat milk to 1000 ± 5% lux light had significantly less oxidized flavor (detected by a trained sensory panel) compared to milk exposed to 2000 ± 5% lux light for the same amount of time and temperature (16 hrs at 6° C).

Volatile Chemistry Due to Lipid Oxidation

Milk lipids are comprised mostly of saturated fatty acids, but do contain unsaturated fatty acids such as oleic, linoleic and linolenic acid. Primary oxidation leads to the production of hydroperoxides which decompose into secondary oxidation products. Table 2.1 gives the expected decomposition products for oleate, linoleate and linolenate hydroperoxides (Frankel 1980).   The rate of lipid oxidation is dependent on the composition of the fat, with degree of unsaturation having a huge effect on rate. Relative rates of oxidation for stearic, oleic, linoleic and linolenic acids are 1:100:1200:2500. This increase in rate due to increase in unsaturation is most likely due to resonance stabilization (deMan 1990).

Table 2.1. Expected decomposition products from oleate, linoleate, and linolenate depending on the position of the alkoxy radical (adapted from Frankel 1980)

Fatty Acid	Alkoxy Radical Position	Expected Decomposition Products
Oleate	C8	decanal, methyl-9-oxooctanoate, 2-undecaenal, methyl-heptanoate
	C9	nonanal, methyl-9-oxonanoate, 2-decanal, methyl octanoate
	C10	octane, 1-octanol, methyl10-oxo-8-decenoate, nonanal, methyl-9-oxononanoate
	C11	heptane, 1-heptanol, methyl 11-oxo-9-undecenoate, octanal, methyl-10-oxodecanoate
Linoleate	C9	3-nonenal, methyl-9-oxononanoate, 2,4-decadienal, methyl-oxtanoate
	C10	2-octene, 2-octene-1-ol, methyl-10-oxo-8-decenoate, 3-nonenal, methyl-9-undecenoate
	C12	hexanal, methyl-12-oxo-9-dodecenoate, 2-heptenal, methyl-9-undecenoate
	C13	pentane, 1-pentanol, methyl-13-oxo-9,11-tridecadienoate, hexanal, methyl-12-oxo-9-dodecenoate
Linolenate	C9	3,6-nonadienal, methyl-9-oxononanoate, 2,4,7-decatrienal, methyl-octanoate
	C10	2,5-octadiene, 2,5-octadien-1-ol, methyl-10-oxo-8-decanoate, 3,6-nonadienal, methyl-9-oxononanoate
	C12	3-hexenal, methyl-12-oxo-9-dodecenoate, 2,4-heptadienal, methyl-9-undecenoate
	C13	2-penetene, 2-penten-1-ol, methyl-13-oxo-9,11-tridecadienoate, 3-hexenal, methyl-12-oxo-9-dodecenoate
	C15	propional, methyl-15-oxo-9,12-pentadecadienoate, 2-butenal, methyl-9,12-butadecadienoate
	C16	ethane, ethanol, methyl-16-oxo-9,12,14-hexadecatrienoate, propional, methyl-15-oxo-9,12-pentadecadienoate

Compounds such as acetaldehyde, methyl sulfide, dimethyl disulfide, propanal, n-pentanal, and n-hexanal, heptanal, nonanal, 3-methyl butanal, 2-methyl propanal, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 1-octene-3-one, 2-nonanone.have been found in milk exposed to light (Mehta and Bassett 1978; Bekbolet 1990; Rysstad and others, 1998; van Aardt and others, 2001; Mestdagh and others 2005).

Effect of Volatile Chemistry on Sensory Perception

Exposure of milk to light causes aldehydes and a variety of other minor products, such as ketones, to be produced. These compounds are the primary causative agents of off-odor

and flavor in milk. Off-odor and flavor falls into two main categories: activated flavor and oxidized flavor. Activated flavor arises through the oxidation of proteins and imparts a flavor described as burnt feathers, burnt protein, scorched, cabbage and mushroom flavor. Oxidized flavor arises from the oxidation of lipids and has been described as tasting like wet cardboard, or having a metallic, tallowy and oily flavor (Hansen and others 1975). Many researchers refer to flavor due to photo-oxidation, regardless of source, as light oxidized flavor. A schematic of the relationship of oxidation mechanism to food component and flavor is shown in Figure 2.10.
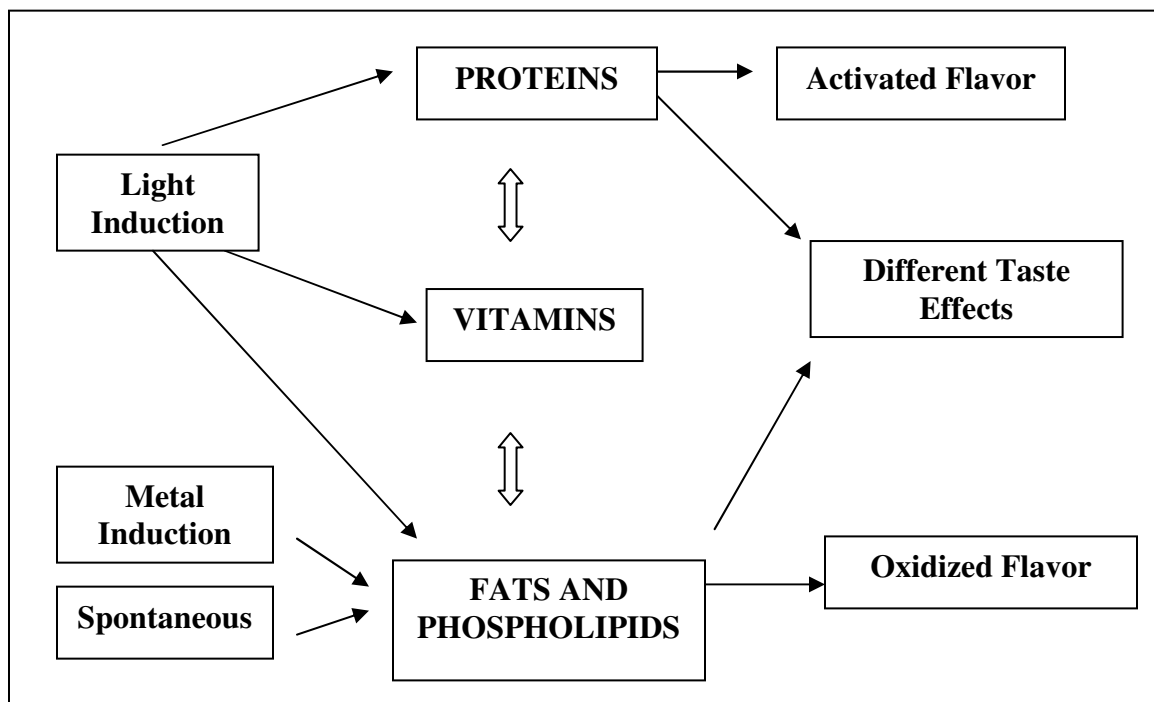
Figure 2.10. Schematic of oxidation initiation interactions with food components resulting in flavor effects (Copyright 1976 from adapted from Sattar A, deMan JM. 1975. Photooxidation of milk and milk products: A review. CRC Crit Rev Food Sci Nutr 7: 13-37. Reproduced by permission of Taylor & Francis Group, LLC., http://www.taylorand francis.com)

Proteins oxidize first, within the first few hours to days of light exposure, but light activated flavor dissipates within a few days and is replaced by light oxidized flavor originating from lipid oxidation. The flavor threshold for light oxidation in reduced fat milk (2%) packaged in HDPE and exposed to fluorescent lighting of 2000 ± 5 % lux and stored at 6° C by a trained sensory panel was estimated to be between 15 and 30 minutes.

27

Untrained consumers were able to detect differences between light exposed and control milk, exposed to 2000 lux fluorescent light, between 54 minutes and 2 hrs using a difference from control method (Chapman and others 2002). Heer and others (1995) found the threshold level for detection of light oxidized flavor to be 2 hr and 40 min. Whited and others (2002) found that a trained sensory panel could determine slight oxidized flavor development in both reduced fat and whole milk after 4 hrs of exposure to $2000 \pm 5\%$ lux light at $6\,^\circ$C and strong oxidized flavor after 16 hrs. The amount of fat in the product affected the rate of flavor development. Non-fat milk showed only moderate light oxidized flavor development.

Sensory Impact Due to Protein Oxidation

The amino acids cysteine, methionine, tryptophan and histidine, found in casein and lactalbumin, have been implicated in off-flavor production in milk (Allen and Parks 1975; Sattar and deMan 1975; deMan 1990). Much of the research has shown that oxidation of methionine causes sunlight flavor in milk. Patton (1954) was the first to determine that methionine was the parent compound of sunlight flavor. This amino acid, in the presence of riboflavin and light, is converted to methional (β-methyl-mercaptopropionaldehyde) (Harper and Brown 1964; Allen and Parks 1975; Allen and Joseph 1985). The reaction mechanism is similar to the photo-oxidation of lipids in the presence of riboflavin. Riboflavin transforms to its triplet state upon absorption of light. Triplet riboflavin then oxidizes methionine to methional, and the riboflavin itself becomes reduced. Reduced riboflavin is subsequently reoxidized to a flavin radical in the presence of oxygen. The oxygen is reduced to a superoxide radical which can further react with protein. Allen and Parks (1975) found that methional gave a broth-like or potato flavor to the milk that changed to a cabbage, burnt feather odor when further exposed to sunlight. Dimick (1982) found that sunlight flavor could be detected even at concentrations as low as 50 ppb. Several investigators, however, did not find methional in light oxidized milk. Dimick and Kilara (1983) determined that methionine sulfoxide, formed from methionine in the presence of light, riboflavin, protein and oxygen,

28

produced sunlight flavor. Foote (1976) found that singlet oxygen produced methionine sulfoxide from methionine.

A number of studies found the production of mercaptan and dimethyl sulfide increased in light oxidized milk (Balance 1961; Harper and Brown 1964). These compounds were produced from the oxidation of cysteine (Harper and Brown 1964). Balance (1961) determined that methional broke down into methyl mercaptan (cabbage odor) and dimethyl disulfide (onion, cabbage odor). Forss (1979) felt that sunlight flavor was due to the formation of methanethiol, dimethyl sulfide and dimethyl disulfide. Jung and others (1998) found that cysteine produced a hydrogen sulfide odor when exposed to light, but methionine produced dimethyl disulfide which has an onion or cabbage odor. The authors concluded that dimethyl disulfide formation was produced through singlet oxygen oxidation of methionine. Dimick (1982) found that methional flavor changed to methyl mercaptan flavor upon further light exposure.

Sensory Impact Due to Lipid Oxidation

Forss and others (1955) found acetone, ethanal, n-hexanal, and C4 to C11 monounsaturated aldehydes in light exposed milk. Forss and others (1955) and Stark and Forss (1962) found that specific compounds gave specific flavors: n-hexanal, n-heptanal, 2-hexenal, and 2-heptanone gave an oily flavor, while n-heptanal, n-octanal, n-nonanal, 2 heptanone, 2-heptenal and 2-nonenal gave a tallowy flavor. Pentanal and the C5-C10 alkenals gave a painty flavor in butter. Day and Lillard (1960) concluded that oxidized flavor came about due to a combination of compounds and that combinations of carbonyl compounds below threshold levels were additive and were able to give rise to off flavor.

Rysstad and others (1998) found no differences in concentrations of a number of different compounds in milk (pentanal, hexanal, heptanal, nonanal, 2-butanone, 2-pentanone, 2-heptanone, 2-nonanone, DMDS) for all packaging materials tested (X board (non-foil, paperbased barrier carton), PE board (polyethylene paperboard) and aluminum foil covered paperboard (standard for long shelf-life aseptic packaging). All concentrations

29

were below 10 µg/kg and all compounds were below threshold levels. However, milk packaged in PE board had light oxidized flavor after 24 weeks storage at 6 C in the dark. Milk packaged in PE board and stored at room temperature showed light oxidized flavor within 6 weeks. The PE board transmitted ~ 0.8% of 400 nm light and ~6% of 500 nm light, well below the recommended levels by the International Dairy Federation (IDF) (Bossett and others 1995).

Whited and others (2002) found that light oxidized flavor increased linearly over time when milk was exposed to 2000 lux fluorescent lighting for up to 16 hrs. Light oxidized flavor intensity was least in skim milk and highest in whole milk, although there was no significant difference in intensity between the types of milk overall. Olsen and Ashoor (1987) found similar results.

Van Aardt and others (2001) found that amber pigmented PET, which blocks light below 450 nm and partially blocks light between 450 nm and 700 nm, had light oxidation flavor similar to light protected samples. PET with UV blockers had less oxidation flavor at 7 days than glass, clear PET and HDPE. Hexanal and DMDS increased least in amber PET and most in HDPE. Pentanal increased in HDPE but not in the PET containers. There were higher concentrations of acetaldehyde in PET containers exposed to light, although there was slightly less acetlaldehyde in the PET-UV treatment than in the other PET containers. Acetaldehyde is a degradation product of PET.

Effect of Light Wavelength on Volatile Chemistry, Odor and Flavor

Exposure to both ultraviolet (UV) radiation and visible light has been found to cause oxidation in lipids and proteins and cause the degradation of vitamins and colorants in foods (Bosset and others 1995). Degradation of lipids and proteins can produce compounds that cause off-odor and off-flavors in food.

UV wavelengths, which have higher energy than visible wavelengths, are typically more damaging to food quality than visible wavelengths (Bekbolet 1990; Hansen and Skibsted 2000). Hansen and Skibsted (2000) found that lipid peroxide concentration increased in a water-in-oil emulsion of rapeseed oil as wavelength decreased from 435 nm to 405 nm and 366 nm. Lennersten and Lingnert (2000) found that lipid oxidation, as measured by hexanal concentration, increased in mayonnaise upon exposure to 365 nm, 405 nm and 435 nm wavelengths, with the lower wavelength causing the highest production of hexanal. Hexanal did not increase when exposed to 405 and 435 nm until the yellowness (b) value had stabilized, indicating that beta carotene needed to be completely degraded before oxidation proceeded. Satter and others (1976) similarly found that a decrease in beta carotene corresponded to an increase in peroxide value.

UV light, however, is not the only type of light causing lipid oxidation. Visible light can also cause deterioration of milk and dairy product quality (Bradley 1983). A study done by Gorgern (2003) determined that both a UV and a visible light barrier are needed to preserve milk quality from light deterioration. Hansen and others (1975) and Bradley (1983) reported that light below 500 nm needed to be blocked in order to reduce the production of light oxidized flavor in milk. However, Lennersten and Lingnert (2000) found that exposure of mayonnaise to wavelengths above 470 nm did not cause oxidation. Hansen and others (1975) looked at the effect that differently colored plastic shields had on the oxidation of milk packaged in polyethylene (PE) containers and exposed to 200 ft candles (2152 lux) of light. Yellow and dark green filters prevented the development of light oxidized flavor in milk (32 hr exposure) to a greater extent than pink, light green and smoky shields. The yellow and dark green shields absorbed light between 400-500 nm.

Effect of Packaging Material on Volatile Chemistry, Odor and Flavor

Packaging material plays an important role in the protection of foods against oxidation. The primary plastic packaging materials used for refrigerated milk products are HDPE and polyethylene terephthalate (PET) (Anonymous 2002).

Many investigators have found that light oxidation flavor is eliminated when milk is packaged in opaque materials (Hoskin and Dimick 1979; Schroeder and others 1985; Deger and Ashoor 1987; Hoskin 1988; Haisman and others 1992; Mestdagh and others 2005). Dimick (1973) found that milk packaged in fiberboard gave protection from the production of light activated flavor for up to 48 hrs on exposure to 100 ft candles (1076 lux) fluorescent light. Milk packaged in plastic and glass containers, which allowed light to be transmitted, had off-flavor development in as little as 12 hr of exposure, with sensory assessment based on a 9-point hedonic scale and a multiple comparison test. Zygoura and others (2004) found that both clear and pigmented (2% TiO_2) PET had significantly higher lipid oxidation than paperboard and 3 layer pigmented co-extruded HDPE and monolayer pigmented HDPE between days 3 and 7 (end of test). Oxygen permeability of the packaging material did not affect oxidation, but this could have been due to large headspace. Sensory evaluation showed that milk packaged in clear bottles were much less acceptable than milk packaged in pigmented bottles.

Pigmentation of the packaging material can also reduce light oxidation flavor depending on the intensity of the pigmentation and the color. Van Aardt and others (2001) looked at the effect of light exposure on oxidation in milk packaged in pigmented and UV block polyethylene terephthalate (PET). After three weeks of exposure, milk in amber PET had significantly less hexanal production (a common marker of lipid oxidation) than clear PET with UV block. The amber PET container completely blocked light between 300 and 400 nm and partially blocked light between 400 and 700 nm. UV block PET blocked light between 300 and 350 nm but transmitted most light between 400 and 700 nm. In a study that compared green pigmented PET containers to clear PET containing UV blockers, it was found that green PET protected against photo-oxidation better than clear UV block PET (Cladman and others 1998).

Christy and others (1981) studied the effect of light (1600 lux) on the production of light oxidized flavor in milk packaged in either clear polyethylene (PE) pouches or pouches with a pigmented over-wrap. The over-wrap allowed less than 3% of UV and visible light to be transmitted. Milk packaged in the clear PE pouch exhibited light oxidized off-flavor within 24 hrs of exposure, while the over-wrapped pouch did not exhibit any off-

flavor, even after 10 days exposure to light.   In a study by Rysstad and others (1998), sensory analysis found no differences in flavor of milk stored at 6 C between the cartons until week 24 where the PE board container was found to be significantly more oxidized than milk in the other containers. Milk stored at room temperature had significant light induced off flavor after 6 week storage in PE board containers. PE boards had ~0.4% light transmission at 400 nm and ~6% light transmission at 500 nm. All packaging material had light transmission below IDF recommendations (2% at 400 nm and 8% at 500 nm) yet still showed significant light oxidation flavor (Rysstad and others 1998).

Simon and Hansen (2001) found that milk packaged in oxygen barrier board (EVOH and foil) deteriorated much more slowly than milk packaged in standard or juice boards. The foil-lined board had the added benefit of a light block. Inhibition of oxygen permeation into a package does not solely protect against degradation. Milk packaged in HDPE with a carbon black layer (light barrier) and no oxygen barrier was shown to have better protection against light oxidation than HDPE with an EVOH oxygen barrier but no light barrier (Gorgern 2003).

Lennersten and Lignert (2000) studied the effect of light and packaging material on lipid oxidation in a water in oil system (mayonnaise). Samples of mayonnaise stored in PET had the highest hexanal concentration after 41 days of storage compared to samples stored in polyethelene naphthalate (PEN) and a copolymer of PET/PEN. The increase in hexanal concentration was thought to be due to increased transmittance of light of 365 nm by PET compared to PEN and PET/PEN copolymer, which did not transmit this wavelength. No significant differences in hexanal concentration were found between the PEN polymer and the PET/PEN copolymer after 41 days storage. However, hexanal concentration did increase in these samples, indicating that visible light affects production of hexanal but not to the same degree as UV light. Mayonnaise stored in the dark showed no increase in hexanal concentration during 100 days of storage.

New polymer materials, with unique optical properties, have recently come on the market, of which iridescent and pearlescent films are examples. Iridescent films have a unique appearance of shifting color which is dependent upon the viewing angle. They

33

work through light interference rather than the use of dyes and pigments. These films are translucent, satisfying the major consumer demand of being able to "see" the product within the package, and can be engineered to block specific light wavelengths, including those that are most damaging to milk quality. They can be laminated to complexly shaped containers and could easily be used as film over-wraps for single-serve milk containers. They also provide an upscale look that appeals to consumers and could increase market share. In an independent survey, >50% of respondents said that iridescent film packaging made the product more distinctive and unique than existing packaging

(www.idspackaging.com/Common/Paper/Paper_180/Building%20Brand%20Equity1.htm).

Traditional and Novel Analytical Methods for Detection of Oxidation

Chemical, spectrometric, chromatographic and sensory methods are all employed to measure primary and secondary oxidation in foods. Chemical methods include iodine value, saponification number, 2-thiobarbituric acid (TBA/TBARS) number, peroxide value and anisidine value. Spectrometric methods include UV/VIS absorption, fluorometric techniques, electron spin resonance (ESR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy. Chromatographic methods include gas chromatography (GC), gas chromatography mass spectrometry (GCMS) and high performance liquid chromatography (HPLC). Sensory evaluation includes a variety of difference or descriptive tests and gas chromatography olfactometry (GCO). This literature review will focus on the spectrometric techniques of GC, GCMS, and GCO as well as FTIR.

Gas Chromatography (GC) and Extraction Methods

Gas chromatography (GC) is one of the most widely employed analytical techniques used today for separating and quantifying volatile compounds. Volatile components are separated through the use of a stationary phase, which has a very large surface area, and a gas phase that permeates through the stationary phase. Volatile compounds are separated

34

because of their differing affinities to the stationary phase and the gas phase. Use of capillary columns and sensitive detectors such as flame ionization detectors (FID), thermal conductivity detectors (TCD) and electron capture detectors (ECD) allows for the detection of very low concentrations of compounds (McNair and Miller 1997).

Volatile flavor components in milk generally are found in very low concentrations and typically require a concentration and extraction step before analysis. Several methods are employed for extracting and concentrating volatiles. These methods typically fall into one of five different categories: 1). Direct extraction where an organic solvent is used to extract compounds from either a liquid or a solid, 2). super critical fluid extraction, 3). steam distillation or "stripping" followed by concentration with either a solvent or cryoconcentration, 4). vacuum distillation or 5). Static and dynamic headspace methods (Curioni and Bosset 2002). All of these extraction methods have drawbacks including long time periods for extraction, cost, the use of elevated temperatures which can change the volatile profile and/or can lead to the production of artifacts and loss of highly volatile compounds, and bias in the concentration of certain compounds (Frank and others 2004).

Static headspace is the simplest and least expensive method for concentrating volatiles (Yang and Peppard 1994; Chin and others 1996). Solid phase microextraction (SPME) is a type of static headspace extraction and is gaining in popularity due to its ease and simplicity. Some of the advantages of SPME is that it is a simple and fast analytical procedure, it produces clean extracts, it is a solventless method and it can be automated (Takekawa and others 1998). SPME concentrates both volatile and non-volatile compounds from either liquid or headspace. Once compounds are adsorbed onto the SPME fiber, they can be desorbed, separated, and analyzed using GC, GCMS or HPLC. By adjusting the type of coating and the coating thickness on the fused silica fiber, polar or non-polar components can be extracted preferentially. Equilibrium is established among the compounds in the sample, headspace and the polymer coating on the fiber (Anonymous 1998) Marsili (1999) used SPME to extract volatile oxidation compounds from light-exposed milk which were then separated and identified using GCMS. Yang and Peppard (1994) were able to get a very low detection limit (0.1 –10 ppb) in the

analysis of flavor compounds in food and beverages using SPME. Van Aardt and others (2001) successfully used SPME-GC to monitor photo-oxidation in milk.

One of the drawbacks of using SPME is that because it preferentially extracts specific compounds, extraction is not universal. For instance, Carboxen/PDMS has been found to be best at extracting low molecular weight compounds (C2-C12). This fiber has small pores (10 Å) in which these molecules adsorb into. DVB/Carboxen/PDMS fibers are better at extracting higher molecular weight compounds (C6-C15) due to the larger (17 Å) pores (Perkins and others 2005). The use of carboxen/PDMS fibers in the aroma analysis of cheeses was found to work very well, picking up many trace compounds (Frank and others 2004). However, the authors found displacement of compounds occurred. 2-Nonanone and ethyl octanoate displaced 2-pentanone, 2-hexanone and 2-heptanone. Butanoic and hexanoic acid, 2-heptanol, methyl butanol and acetic acid were also displaced to some degree. There was little or no displacement for trace and medium concentration headspace components. Another disadvantage to the use of SPME is the limited life of the fiber. Fabre and others (2002) found huge differences between sensitivity of fibers of the same type. In a study on the effect of milk proteins on flavor compounds, they found that, of the three PDMS/DVB fibers they used, one gave peak areas that was two times greater than the other two fibers for all compounds tested. Fibers were also found to work optimally for only 30 uses (Perkins and others 2005)

Gas Chromatography Mass Spectrometry (GCMS)

Gas chromatography mass spectrometry (GCMS) is a method in which GC is coupled with mass spectroscopy (MS). In MS, the detector identifies and quantifies unknown compounds through ionization of sample molecules by a number of different methods, the most common being with an electron beam. Ions are then either repelled or attracted to the charged lenses of the mass analyzer where they are separated by their mass to charge ratio by either a magnetic or electrical field. The detector counts the ions and generates a mass spectrum. The ratio of ion abundance to the mass to charge ratio is unique for each compound. Spectra are matched to similar spectra in a data library and

identified (McNair and Miller 1997). Quantification can be achieved through the use of a standard curve.

Ionization in mass spectrometry instruments can be achieved through a number of different techniques including electron impact (EI), electrospray (ES), atmospheric pressure chemical ionization (APCI), and matrix assisted laser desorption ionization (MALDI). Chemical and thermal techniques, glow discharge, inductively coupled plasma (ICP) and fast bombardment can also be used for ionization. Mass analyzers employed include magnetic selectors, electric selectors, quadripoles, time of flight, and ion-trap devices (Ibanez and Cifuentes 2001).

Mass spectrometry can be coupled to other analytical or separation techniques such as GC, HPLC, LC, IR and nuclear magnetic resonance (NMR). These techniques have been used in the food industry to monitor the stability of food components, identify volatile components, and monitor contaminants and degradation products. The most common coupled technique is GCMS and this technique is increasing in use as the price of equipment goes down (Ibanez and Cifuentes 2001).

Gas Chromatography Olfactometry (GCO)

Gas chromatography olfactometry (GCO) is a method that uses a GC to separate volatile compounds and a human detector to assess the odor activity of the separated compounds. A portion of the volatile stream is shunted to a humidified sniffer port where sensory panelists "sniff" the compounds coming off the column and describe the odors and rate their intensity. It is estimated that only a small portion of the volatile compounds in food contribute to the aroma (van Ruth 2001) and in many cases the most abundant volatiles have little odor impact (Curioni and Bosset 2002).

GCO complements GC or GC/MS by determining which compounds are odor active. It is estimated that volatiles must be present at $> 10^{-5}$ g/L in order to be detected by GC and GC/MS. The concentration of volatiles in the headspace above a food product ranges from 10^{-11} to 10^{-4} g/L. Thus, only the most abundant volatiles will be detected (Friedrich

and Acree 1998). However, for some compounds, the human nose can detect odors with as little as 10^{-19} moles. Therefore, GCO can be much more sensitive than other GC techniques (Curioni and Bosset 2002).

Although sensitivity can be very high, there are a number of drawbacks with using GCO and the majority of these drawbacks are related to the use of humans as detectors (Friedrich and Acree 1998; Curioni and Bosset 2002). Human sniffers may have differing sensitivities to specific odors, they must be prescreened for their sensitivity and for specific anosmias, and they need to be trained. GCO is very time intensive and inattention by the evaluator or fatigue can cause inconsistency and non-reproducability. Often, it is difficult to compare results from one laboratory with another because of the use of different odor descriptors, different odor intensity evaluation techniques, different extraction methods and the use of different food products (Curioni and Bosset 2002).

Analysis of Photo-Oxidation Using Fourier Transform Infrared (FTIR) Spectroscopy

The use of FTIR to study oxidation, especially oxidation of oils, is increasing. However, there is a paucity of published literature dealing with this technique in studying photo-oxidation of foods. FTIR is an analytical tool that may help to elucidate the kinetics and sequencing of photo-oxidation reactions that occur in milk and will add to the body of information that has already been determined through the use of other analytical techniques.

FTIR is a very sensitive analytical technique that uses vibrational energy, produced by chemical bonding, to detect the presence of functional groups within a compound. It has a very low signal to noise ratio, high resolution and has the advantage of being non-destructive, highly sensitive and reproducible. A large amount of spectral information can be obtained in a very short period of time. Use of attenuated total reflectance (ATR) in conjunction with a flow through cell, allows for the measurement of oxidation in aqueous systems over time without sample disturbance.

38

For compounds where analyte concentration is high, aqueous solutions directly in contact with the ATR crystal can be monitored. This technique has been successfully used to monitor the kinetics and production of new species in bioreactor systems (Doak and Phillips 1999; Alberti and others 1986). In order to monitor compounds with low concentrations, which is the situation with photo-oxidation of milk, techniques must be developed to enhance sensitivity and reduce interference due to water. One such method is solid phase ATR. In this method, the ATR crystal is coated with a polymer(s) that extracts and concentrates compounds of interest. In a recent study by Acha and others (2000), polymer coated ATR crystals were used successfully to monitor low levels of chlorinated hydrocarbons. Use of polyisobutylene and polyethylene coatings gave sensitivities for chlorinated hydrocarbons in the low parts per million range.

FTIR is not commonly used in food studies, mostly because of the complexity of food matrices, but research into its application is increasing. FTIR is becoming an important tool in the detection of food adulteration and in food quality control (Lai and others 1994; Kemsley and others 1996; Ding and others 2000; Gangidi and other, 2003). It also has been used for the characterization of foods and some researchers have correlated FTIR data with classical wet chemical values for oxidation in lipids (Li and others 1996; Downey and others 1997).

Several authors have looked at thermal oxidation of oils using FTIR (van de Voort and others 1994; Guillen and Cabo 1997, 2000, 2004; Ruiz and others 2001; Innawong and others 2004).   No literature has been found looking at photo-oxidation of lipids using FTIR. We feel that, once a method is developed, FTIR will be an excellent research tool to study the effect that light wavelength has on the oxidation of milk or model milk.

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40

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Chapter III

Effect of Specific Wavelengths on Volatile Chemistry and Production of Aroma-Active Compounds in Milk

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Abstract

The effect that specific light wavelengths have on the quality of milk was elucidated. Milk was exposed for 7 hrs to light of 200-400 nm, and wavebands (50 nm) with intensity peaks at 395, 463, 516, 567 or 610 nm or to full light or no light and tested for the production of volatile and odor-active compounds using gas chromatography (GC) and gas chromatography olfactometry (GCO). In general, exposure to UV wavelengths (200-400 and 395 nm) and full light produced the highest amounts of volatile compounds. Hexanal, pentanal, and four unidentified compounds (with retention times of 2.49, 3.43, 7.69, and 9.04 min) were produced in high concentrations when exposed to 200-400 nm and full light, and to some degree 395 nm light. Riboflavin absorbs UV light of 250, 270 and 370 nm and visible light of 400, 463 and 570 nm when at neutral pH. It is suspected that photo-oxidation in milk exposed to 200-400 and 395 nm wavelengths and to full light was through riboflavin sensitization.

Pentanal and the unidentified compound with a retention time of 3.429 were also produced in high concentrations when exposed to 610 nm light. The 610 nm filter allows 45% transmission of 570 nm wavelength and, therefore, it is possible that production of pentanal and the unidentified compound when exposed to this filter was through riboflavin sensitization. However, there was very little production of these compounds when exposed to the 567 nm filter, which allows 69% transmission of 570 nm. Therefore, it is likely that some other sensitizer besides riboflavin is responsible for production of these compounds.

A number of aroma-active compounds were produced in milk exposed to specific wavelengths. In general, there was more aroma-activity by lower molecular weight compounds when milk was exposed to longer visible wavelengths (516, 567, and 610 nm) and by higher molecular weight compounds when milk was exposed to shorter visible and UV wavelengths (200-400, 395 and 463 nm). Aroma-active compounds produced by exposure to longer visible wavelengths showed only slight intensities, while

51

those produced by exposure to shorter visible and UV wavelengths showed slight to medium intensities.

Introduction

The effect of light wavelength on the quality of milk and other dairy products has been studied by a number of investigators (Hansen and others 1975; Bradley 1983; Bekbolet 1990; Bosset and others 1995; Hansen and Skibsted 2000; Lennersten and Lingnert 2000; Gorgern 2003; Wold and others 2005, 2006). However, Mortensen and others (2004) feel that this topic has not been fully explored and should be a priority topic for research. The majority of investigators have focused on the effect that specific light wavelengths have on riboflavin photo-sensitization. Many have concluded that milk quality is affected mostly by light of short wavelengths (violet-blue) below 455 nm (Sattar and others 1976; Bosset and others 1995; Mortensen and others 2003; Wold and others 2005, 2006), suggesting that riboflavin plays a role in off-odor and flavor production. Josephson (1946), however, found that all wavelengths below 620 nm must be blocked in order to prevent photo-chemical changes that result in sunlight flavor in milk. Sattar and others (1976) found that wavelengths above 595 nm contributed to light oxidation flavor in milk. Recently, Wold and others (2005, 2006) indicated that wavelengths between 600 and 750 nm affect the quality of milk and other dairy products. They determined that chlorins and porphyrins, specifically chlorophyll a and b and protoporphyrin and hematoporphyrin, contributed to off-odor and flavor production in dairy products.

Riboflavin, found in high concentration in the whey fraction of milk, acts as a photosensitizer and can initiate oxidation reactions that lead to off-flavor production (Bekbolet 1990; Sattar and others 1976). Riboflavin absorbs light of specific wavelengths, principally 250, 270, 370, 400, 446 and 570 nm when in foods with a neutral pH (Kyte 1995), and is converted to its singlet excited state by those wavelengths. Once excited, riboflavin can either undergo fluorescence, returning to the ground state and giving off its extra energy as light, or it can undergo intersystem crossing and form a much more stable triplet state. The triplet state of riboflavin can then react with molecular oxygen to form singlet oxygen (Type II mechanism), which readily reacts with compounds within the milk to form free radicals, or react directly with a lipid molecule to form peroxides (Type I mechanism). These peroxides are relatively unstable and quickly

53

degrade into free radicals. Ultimately, the free radicals initiate oxidation reactions which result in the production of off-odor and flavor compounds (Rosenthal 1992; Kagan 1993). Off-odor and flavor compounds produced from sensitized oxidation of milk include pentanal, hexanal, 1-hexanol, heptanal, 1-heptanol, 1-octene-3-ol, nonanal, 2-nonanone, dimethylsulfone, dimethyl disulfide, benzothiazole and decalactone (Moio and others 1994; Marsili 1999; Van Aardt and others 2005). Cadwallader and Howard (1998) found dimethyl sulfide (with a canned corn odor), 2-methylpropanal (dark chocolate odor), pentanal (sour cut grass odor), hexanal (green cut grass odor), dimethyldisulfide (cooked cabbage odor) and 1-octene-3-one (earthy, mushroom odor) to be the prominent odor active compounds in light oxidized milk, while van Aardt and others (2005) found hexanal (green grass odor), 2-heptanone (cereal, roasted grain odor), n-heptanal (green, fish oil odor), 1-octene-3-ol (mushroom odor), octanal (citrus odor) and nonanal (soapy, floral odor) to be the major aroma-active compounds produced.

Porphoryns, such as hemoglobin, and chlorins, such as chlorophyll, also act as photosensitizers but are much less studied than riboflavin in dairy products. These compounds were found in very low concentrations in dairy products by Wold and others (2005). The degradation of these compounds correlated better to sensory attributes of oxidized odor, sunlight flavor and acidic flavor in Norvegia cheese than did the degradation of riboflavin. The porphyrins and chlorins have major absorption peaks in the UV and blue/violet visible bands, similar to riboflavin, but also absorb throughout the rest of the visible spectrum, including wavelengths above 600 nm.

Food quality during storage depends considerably on packaging (Sattar and deMan 1976; Bekbolet 1990) and packaging materials may inhibit or delay detrimental oxidation reactions. It is relatively simple and inexpensive for milk processors to use high-density polyethylene (HDPE) that has compounds, such as titanium dioxide, mixed into the polymer to make the package opaque and protect against photo-oxidation. However, marketing objectives often influence packaging material selection because consumers want to see the food inside (Sattar and deMan 1976; Rosenthal 1992; Cladman and others 1998; Doyle 2004). It is of great interest to the dairy industry to design a package that

54

will reduce the transmission of light wavelengths that specifically initiate oxidation reactions leading to flavor defects, yet still be translucent so that consumers can see the product inside. In order to achieve this goal, one must first determine the effect that specific wavelengths have on milk quality and determine which are most damaging.

Therefore the objective of this study was to determine the effect that specific light wavelengths had on the quality of milk by monitoring the production of volatile and odor-active compounds using gas chromatography (GC) and gas chromatography olfactometry (GCO).

Materials and Methods

Milk Processing and Packaging

Raw milk was obtained from the Virginia Tech dairy farm and processed in the Food Science and Technology dairy pilot plant within 24 hrs of collection. Milk was held at 4º C until processing. Before pasteurizing, milk was pre-warmed (55ºC) and separated into cream and skim milk using a pilot plant separator (Model 1G, 6400 rpm, Bonanza Industries, Inc., Calgary, Canada). Cream was added back to the skim milk and standardized to 2.0 ± 0.1% milkfat using the Babcock method (AOAC 995.18 (Fat in Cream) and AOAC 989.04 (Fat in Milk)). Milk (2%) was homogenized in a two stage homogenizer (10,339 kpa (1500 psi)—first stage; 3,446 kpa (500 psi)—second stage) (Type DX, Cherry Burrel Corp., Delavan, Wisconsin) and ultra high temperature (UHT) pasteurized at 131.1º C (268º F) for 2 sec (UHT/HTST Lab-25 DH pasteurizer, MicroThermics, Raleigh, NC).

Pasteurized milk was collected under a laminar flow hood (Atmos-Tech Industries, Ocean, NJ) in 2 L sterile glass Erlenmeyer flasks covered in aluminum foil to eliminate transmission of light and stored for up to 2 wks at 4º C until use.

Milk Exposure to Specific Light Wavelengths

<u>Photo-Reactor Set Up</u> – A Thermo Oriel Photo-Reactor (Model 66902 Universal Arc Lamp Housing, Model 66910 Power Supply, Thermo Oriel Instruments, Strattford CT) was used to expose milk to specific light wavelengths (Figure 3.1). A 350 W mercury lamp shone through narrow band filters permitting light of 200-400, 395, 463, 516, 567, and 610 nm through to the sample. The full light treatment did not use any filter. Filters had an approximate 50 nm band width, in which the peak transmission was at the wavelength specified and the majority of the total light was within the 50 nm band width (Figure 3.2).

56
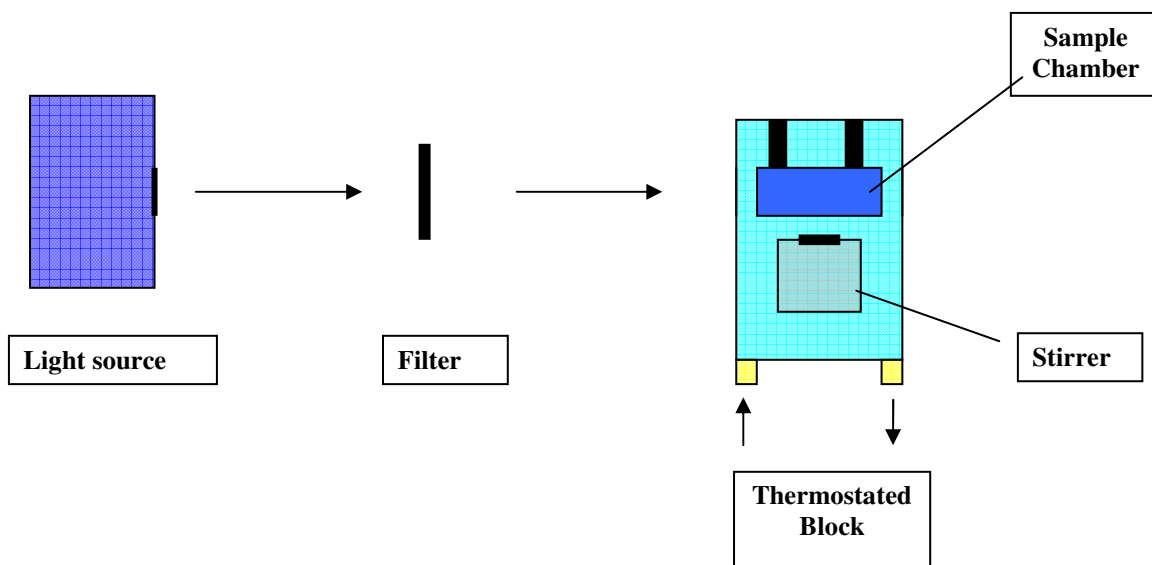
Figure 3.1. Light exposure set up using a Thermo Oriel Photo-Reactor with a thermostated block to maintain sample temperature.
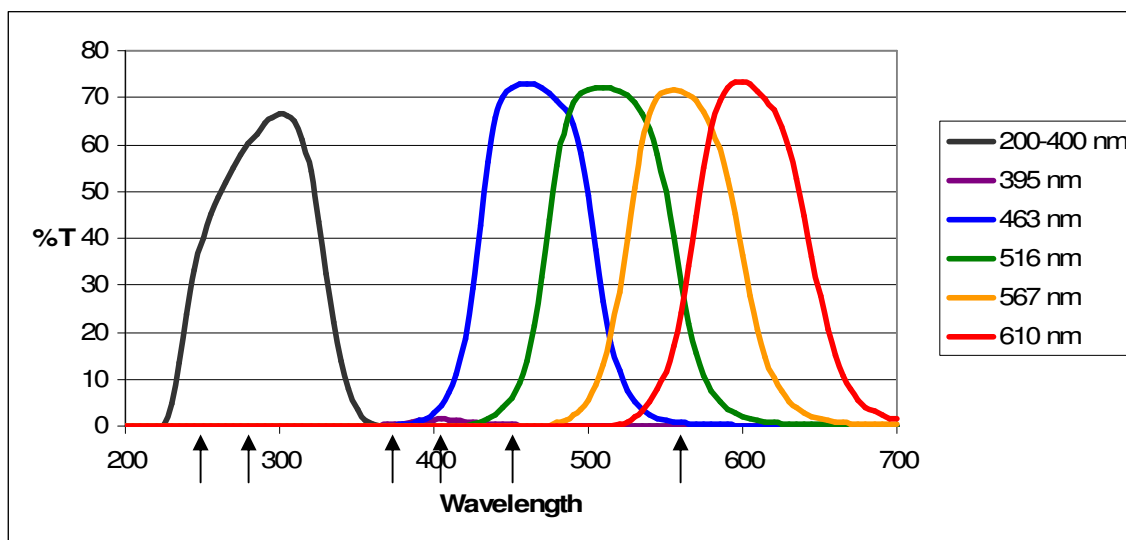
Figure 3.2. Wavelengths and % transmission for filters used in the Thermo Oriel Photo-Reactor equipped with a 350 W Hg lamp. Arrows show absorption maxima for riboflavin (250, 270, 370, 400, 446 and 570 nm).

Filtered light struck the sample (12 ml) contained in a cylindrical 15 ml quartz crystal vial (Fisherbrand, 50 mm cylindrical cell) capped with rubber septa and situated within a thermostated aluminum block. The sample was stirred constantly at low rpm throughout the experiment. The aluminum block was kept at constant temperature by pumping a 50% solution of antifreeze continuously through the block. A bath circulator (Masterline, Model 2095, Marietta OH) kept the antifreeze solution at constant temperature. Antifreeze was pumped through Tygon tubing to the aluminum block and back to the circulator. The milk sample was held at $10 \pm 1^{\circ}$ C; temperature was monitored using an Omega HH81 Digital Thermometer (Omega Engineering, Stamford CT) with a K type thermocouple with a stainless steel sheath (Model KMQSS-0206-12, Omega Engineering, Stamford CT). Dry air was blown onto the quartz crystal vial face to prevent condensation, using an air pump (TetraTec AP80 Air Pump, Tetra, Blacksburg, VA) in which air was circulated through anhydrous calcium sulfate (Drierite, W A Hammond Company, Xenia, OH).

Sample Exposure and Collection – Samples were exposed to selected wavelengths of light (200-400, 395, 463, 516, 567, and 610 nm, full light) for 7 hrs. Milk was drawn into a hypodermic syringe for transfer to sample vials (8 ml) containing 1 g NaCl and a magnetic stir bar. An aliquot (4 mls) was added to each of three vials per treatment and capped with Teflon septa. No filter was used for the full light exposure treatment and a light-protected treatment (control) consisted of stirring the milk under experimental conditions with no exposure to light. Samples were held at 4° C (not longer than 24 hrs) until analyzed. Table 3.1 shows the amount of light, in mW, that impinged on the face of the quartz crystal vial for each filter. Light energy readings were determined using a Radiant Power Energy Meter (Model 70260, Thermo Oriel Instruments, Strattford, CT).

Table 3.1. Light intensities, in mW, for each filter used in the experiment (n = 5).

Peak Transmission Wavelength of Filter (nm)	mW x ± sd
200-400	$0.301^d \pm 0.006$
395	$0.079^e \pm 0.007$
463	$0.561^c \pm 0.002$
516	$0.573^c \pm 0.002$
567	$0.951^b \pm 0.001$
610	$0.571^c \pm 0.005$
Full Light	$8.113^a \pm 0.014$

[a, b, c, d, e] Means followed by the same letter are not significantly different at the P<0.05 level experimentwise using Tukey's HSD

Lipid Oxidation Analysis Using Headspace Gas Chromatography

Volatile compounds were extracted and concentrated using a 75 µm carboxen-polydimethyl siloxane (PDMS) solid phase microextraction (SPME) fiber (Supelco, Bellefonte, PA). Vials were heated to 45º C on an RCT basic (IKA Werke, Wilmington, NC) heater with an ETS-D4 Fuzzy Controller (IKA Werke, Wilmington, NC) while being stirred. The SPME fiber was exposed to milk headspace for 22 min during heating.

Volatile products adsorbed on the SPME fiber were desorbed and analyzed using an HP 5890 Series II Plus GC (Hewlett Packard, Palo Alto, CA) with a flame ionization detector (FID). An RTX-5 (crossbond 5% diphenyl-95% dimethyl polysiloxane, Restek Corp., USA) capillary column (30 m x 0.320 mm id x 1.00 µm film thickness) was used to separate the volatiles. Helium gas flow was 1.80 ml/min. The injector temperature was 280º C, detector temperature 300º C, and the program was run in splitless mode. Initial run temperature was 35º C, which was held for 0.5 min. The temperature was then ramped to 180º C at a rate of 15º C/min and held for 0.5 min. The temperature was ramped again to 260º C at a rate of 20º C/min and held for 0.5 min. Total run time was 15.17 min. Chromatograms were plotted using HP ChemStation software (Rev. A.05.02[273], Hewlett Packard, Palo Alto, CA). Area and height of rejection were set at 2000 counts each.

59

Volatiles were tentatively identified using an HP 6890 GCMS with a 5973 Mass Selective Detector (Hewlett Packard, Palo Alto, CA). Separation on the GCMS was completed on an HP-5 capillary column (15 m x .025 mm i.d. x 0.25 µm film thickness, Hewlett Packard, Palo Alto, CA). The temperature program was the same as described above for the HP 5890 Series II Plus. Identification was further confirmed using external standards run on the HP 5890 Series II Plus and through Kovats index (KI) number. An alkane series was run using the same temperature program as was used to separate volatile compounds from milk. Retention Index was plotted vs log V_n (log of retention time) and curve fit. KI was then compared to published KI's and odor descriptors to identify the compound (www.flavornet.org). Concentrations were determined using standard curves.

Odor-active Compound Analysis Using Gas Chromatography Olfactometry (GCO)

Training. Six panelists (5 females, 1 male), consisting of graduate students and staff from the Food Science and Technology department at Virginia Tech, were trained in the recognition of aromas and odors common to beer and milk. Training consisted of using Beer Aroma Recognition standards and Beer Taint reagents (Brewing Research International, UK) to develop a memory and lexicon for aromas (Table 3.2). Panelists were tested for ability to identify aromas/intensity during training by performing blind aroma recognition analyses of the standards and reagents. All panelists were able to correctly recognize at least 70% of the aromas and odors. Panelists trained for a total of two hours.

Table 3.2. Beer Aroma standards/reagents[1] used for training sensory panelists for the recognition of off-odors in milk.

Beer Aroma Recognition Standards	Beer Taint Recognition Reagents
Sweetcorn	Dimethyl sulfide (DMS)
Malty	Phenolic
Late hop	Medicinal
Rose floral	Musty
Lemon floral	Diacetyl
Citrus	Rancid
Spicy	Cheesy
Grassy	Cardboard
Banana	Catty
Pineapple	Papery
Phenolic	Cooked vegetable
Butterscotch	Onion

[1] Brewing Research International (BRi), Nutfield, UK

Sensory Testing. Three panelists, selected for their availability and ability, evaluated aromas/odors in milk exposed to specific wavelengths. A GCO sniff port (ODO II, SGE International, Ringwood, Australia) attached to an HP 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with an FID was used to carry the aromas of the separated compounds to the panelists. Panelists evaluated both the type and intensity of the odors. Intensity was evaluated on a scale from 1-5 where 1 meant "slight" and 5 meant "very strong".

GC-O samples were adsorbed onto a 75 µm carboxen-PDMS SPME fiber as described previously. A DB-5 ms capillary column (30 m x 0.25 mm id x 0.25 µm film thickness, J & W Scientific, Agilent Technologies, USA) was used to separate the volatile compounds. Helium was used as the carrier gas at a flow rate of 32 cm/sec. The temperature program was the same as described previously. Integration of peaks was done using an HP 3396A Integrator (Hewlett Packard, Palo Alto, CA).

Three replications of this experiment were performed and intensity data reported as slight, moderate or high. Average ratings were converted to category data of slight (1-2) denoted by a +, medium (3) denoted by ++, and strong (4-5) denoted by +++.

Statistical Analysis

Means were calculated and standard least squares and Tukey's HSD were performed on the gas chromatography data to determine significant differences in volatile oxidation compounds between treatments. These analyses were performed using JMP 5.1 Statistical Discovery Software (SAS Institute Inc, 1989-2004).

Energy emission for all filters was less than 1.0 mW (Table 3.1). Direct comparisons to the protective or damaging effects of exposure to 463, 516 and 610 nm were able to be made because energy emissions for these filters were similar. Inferences could be made about the other wavelengths. Because energy emission was significantly different for several of the wavelength filters, it is possible that energy intensity, in addition to wavelength, was responsible for the differences seen. Variation in energy among wavelengths when using filters was reported by Wold and others (2005) in their evaluation of Norvegia cheese.

Volatile Compound Analysis Using Gas Chromatography

Changes in concentration of hexanal, pentanal, and four unidentified compounds (RT 2.49 min; RT of 3.43 min; RT 7.69 min; RT 9.04 min) occurred due to exposure to different wavelength filters as compared to the light-protected control milk. Variation in volatile chemistry between replications was observed and was possibly due to analytical sensitivity as well as seasonal influence on fatty acid chemistry of the raw milk.

Hexanal (Figure 3.3) concentration in milk was highest when exposed to UV and short visible wavelengths, and was similar to the increase when exposed to full light (Figure 3). This increase was significantly higher in milk exposed to 200-400 nm than in milk exposed to longer visible wavelengths (516, 567 and 610 nm) and the light protected sample. The 200-400 nm filter had the second lowest energy output, significantly lower than output for the 516, 567 and 610 nm filters   Therefore, it can be inferred that exposure to UV light (200-400 nm) is more important in the production of hexanal than exposure to the longer visible wavelengths (516, 567 and 610 nm). Concentration of hexanal was similar in milk exposed to full light, 200-400 nm, 395 nm and 463 nm. This is not surprising since these wavelength exposures include riboflavin excitation wavelengths (250, 270, 370, 400, and 446 nm), which have been implicated in the

production of light oxidation flavor in milk (Fritsch and Gale 1977; Warner and others 1978; Robards and others 1988; Andersson and Lingnert 1998; Lennersten and Lingnert, 2000). It is noteworthy that hexanal concentration was not significantly different in milk when exposed to 395 nm than when exposed to 200-400 nm because the 395 nm filter emits a significantly lower amount of energy than the 200-400 nm filter. One can infer from this that exposure to 395 nm is also important in the production of hexanal. Riboflavin is also excited by 570 nm when in foods with a neutral pH (Kyte 1995). However, exposure to 570 nm did not produce large amounts of hexanal.
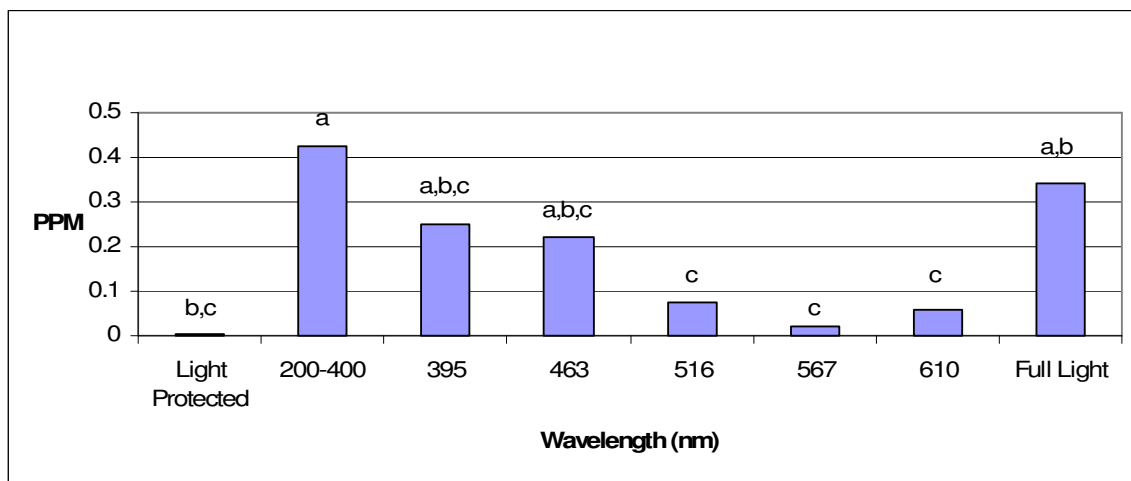
Figure 3.3. Hexanal concentration in 2% UHT milk as a function of wavelength exposure (7 hrs at 10º C at various mW (see Table 1).
[a, b, c]Means followed by the same letter are not significantly different at the $P<0.05$ level experimentwise using Tukey's HSD

A number of investigators have shown an increase in hexanal production when lipids were exposed to UV and short visible wavelengths. Sattar and deMan (1976) reported that light below 455 nm was damaging to milk quality. Lennersten and Lingnert (2000) found a rapid increase in hexanal concentration in mayonnaise exposed to blue light with emission peaks at 365 nm, 405 nm, 435 nm and between 410 and 470 nm. This increase was slower when mayonnaise was exposed to all three peaks than when exposed to 365 nm alone. The authors also found that there were higher amounts, and a faster rate of development, of hexanal in mayonnaise stored in PET, which allows ~ 40% transmittance of 365 nm, as compared to PET/Polyethylene napthalate (PEN) copolymer and PEN

64

alone, which only allows ~1% transmission of 365 nm. Our data are similar to Lennersten and Lingnert's (2000), with higher increases in hexanal when milk was exposed to UV wavelengths (200-400 and 395 nm) than when exposed to 463 nm. Mortensen and others (2003), however, looking at the effect of wavelength on Havarti cheese, found opposite results; hexanal increased significantly more when exposed to 405 and 436 nm, than when exposed to 366 nm.

Statistically, there were few differences in pentanal concentration as affected by wavelength. Even though the pentanal concentration in the light-protected and light-exposed controls were not statistically different, pentanal concentration increased in milk when exposed to UV wavelengths (200-400 nm) and when exposed to light of 610 nm and full light (Figure 3.4). Exposure to 200-400 nm produced significantly higher amounts of pentanal than exposure to 516 nm, but there were no significant differences in pentanal concentration for any other wavelength exposure. Since the 516 nm filter emits significantly higher energy than the 200-400 nm filter, one can infer that exposure to 200-400 nm is more important in the production of pentanal than exposure to 516 nm.
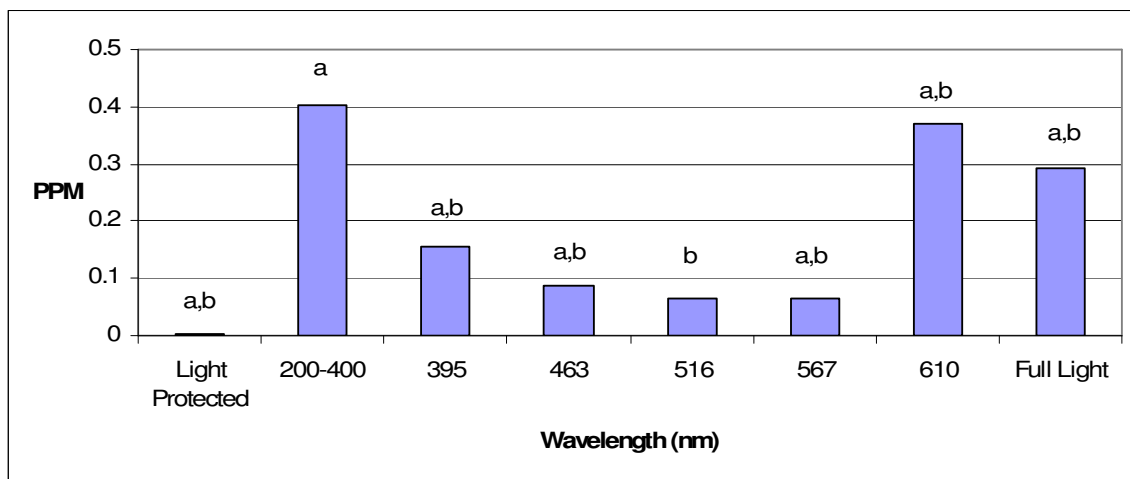
Figure 3.4. Pentanal concentration as a function of wavelength exposure (7 hrs at 10º C at various mW (see Table 1)
[a, b]Means followed by the same letter are not significantly different at the $P < 0.05$ level experimentwise using Tukey's HSD

Exposure of milk to 610 nm may cause higher production of pentanal than exposure to 463 and 516 nm, although the difference was not statistically significant. Because energy

emissions for these three wavelengths (463, 516 and 610 nm) are similar, a direct comparison as to the protective or detrimental nature of these wavelengths can be made. It is interesting to note that exposure to 463 nm, which encompasses the major absorption maxima for riboflavin (446 nm), did not produce as much pentanal as exposure to 610 nm. The 610 nm filter allows a moderate amount of transmission of 570 nm (45%) which is also an absorption maximum for riboflavin indicating that this compound could have been produced through riboflavin sensitization. However, there was little production of pentanal when exposed to the 567 nm filter, which allows in significantly higher amounts of 570 nm (69%). It is possible that another sensitizer that absorbs around 610 nm may be present in milk causing the production of pentanal. Wold and others (2005) found that protoporphyrin, hematoporphyrin and chlorophyll a and b were present in dairy products in very small amounts and contributed significantly to the oxidation of Norvegia cheese. Chlorophyll a has absorption maxima at 410, 430 and 662 nm (Rebeiz and others 1972); chlorophyll b at 453 and 642 nm (Rebeiz and others 1972); protoporphyrin at 404, 502, 536, 576 and 633 nm (Rebeiz and others 1972); and hematoporphyrin at 402, 500, 532, 569, 596 and 623 nm (Granick and others 1952). Thus, it is possible these compounds, especially the porphyrins, are responsible for photosensitization leading to pentanal production in milk since the 610 nm filter encompases excitation wavelengths for these compounds (623 and 633 nm). However, porphyrins also absorb wavelengths close to 567 nm and we did not see an increased production of pentanal when milk was exposed to this wavelength.

There were no significant differences in 1-octene-3-ol concentration when milk was exposed to any wavelength (Figure 3.5). However, 1-octene-3-ol odor (mushroom) appeared to be strongest when milk was exposed to 395 nm followed by 567 nm light. Because the energy emission for the 395 nm filter is so much lower than the other filters, it can be inferred that exposure to 395 nm is important in the production of 1-octene-3-ol.

66
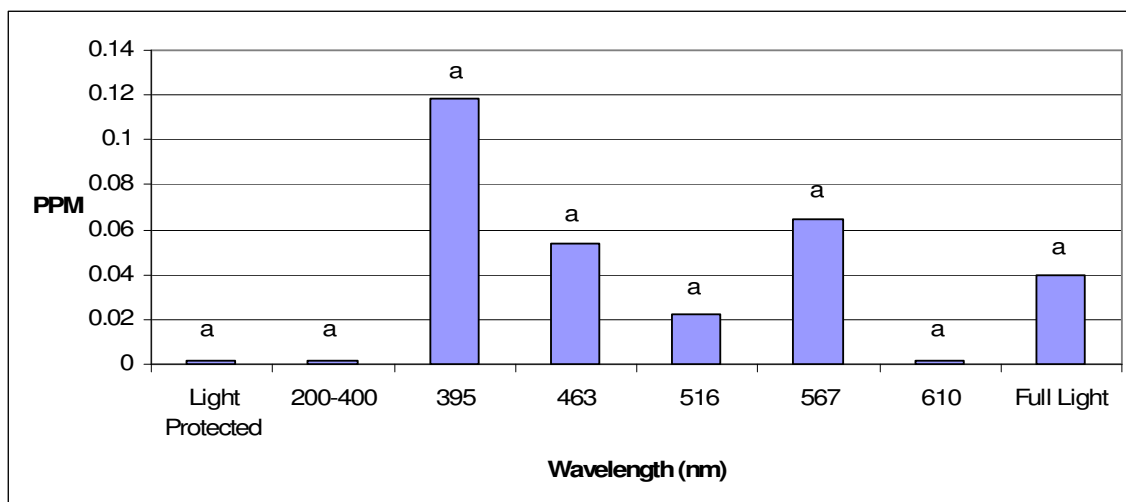
Figure 3.5. 1-Octene-3-ol concentration as a function of wavelength exposure (7 hrs at 10° C at various mW (see Table 1)
[a]Means followed by the same letter are not significantly different at the P<0.05 level experimentwise using Tukey's HSD

Several compounds which were unidentified, demonstrated differences in concentration as a function of wavelength exposure. An unidentified compound (retention time (RT) of 3.43 min, Figure 3.6) shows a similar pattern to pentanal, with production of this compound being significantly higher when exposed to 200-400 nm than when exposed to 463, 516 or 567 nm. Concentrations of this compound were similar when milk was exposed to 200-400, 395 and 610 nm and to full light. Similar conclusions can be made to those that were made for the production of pentanal: exposure to 463, 516 and 567 nm appears to be much less important in the production of this compound than the UV wavelengths (200-400 nm, 395 nm) and exposure to 610 nm is more important than exposure to 463 and 516 nm.
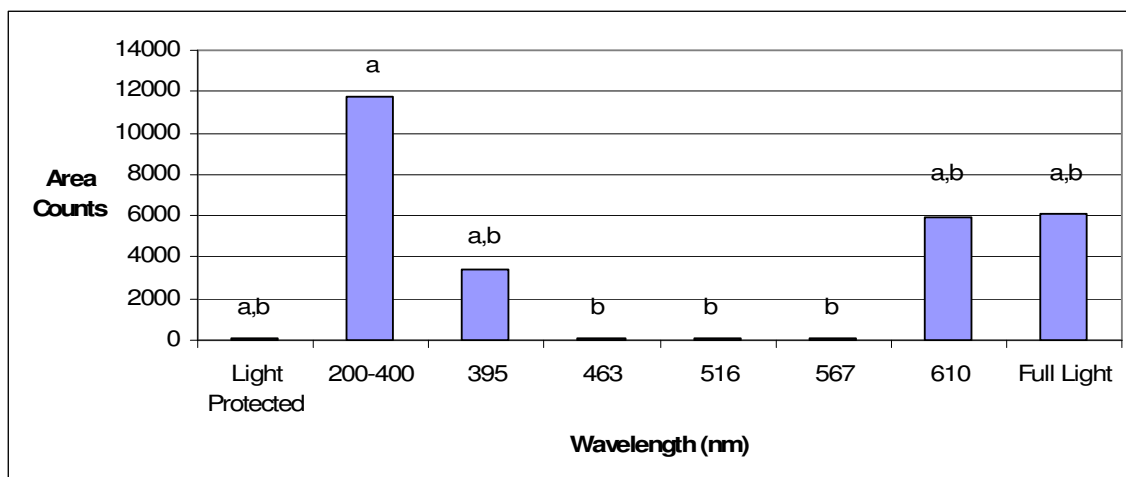
Figure 3.6. Average area counts of an unidentified compound (RT 3.429) due to wavelength exposure (7 hrs at 10º C at various mW (see Table 1)
[a, b]Means followed by the same letter are not significantly different at the $P<0.05$ level experimentwise using Tukey's HSD

Another unidentified compound (RT of 2.49, Figure 3.7), had highest concentrations ($P<0.05$) when exposed full light but also had higher concentrations when exposed to UV wavelengths (200-400 nm) than when exposed to other wavelengths. Two other unidentified compounds (Figure 3.8 and 3.9) showed a similar pattern, where the highest increases occurred when the milk was exposed to either UV (200-400 and 395 nm) or full light. Again, because the energy emission of the 200-400 nm filter was less than any other, except for the 395 nm filter, and exposure to these wavelengths produced high concentrations of these compounds, one can infer that the UV wavelengths are the most important for the production of these compounds.
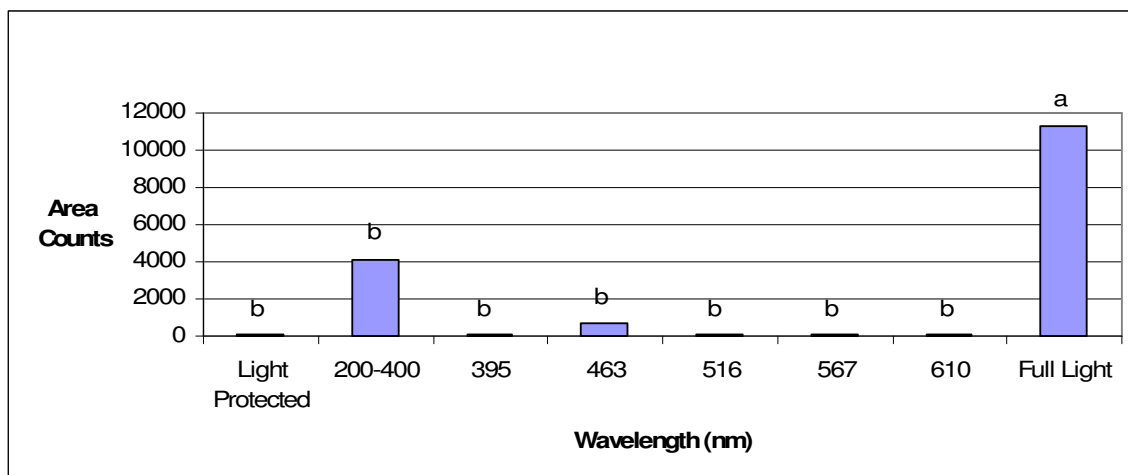
Figure 3.7. Average area counts of an unidentified compound with an RT of 2.490 due to wavelength exposure (7 hrs at 10º C at various mW (see Table 1)
[a, b]Means followed by the same letter are not significantly different at the $P<0.05$ level experimentwise using Tukey's HSD
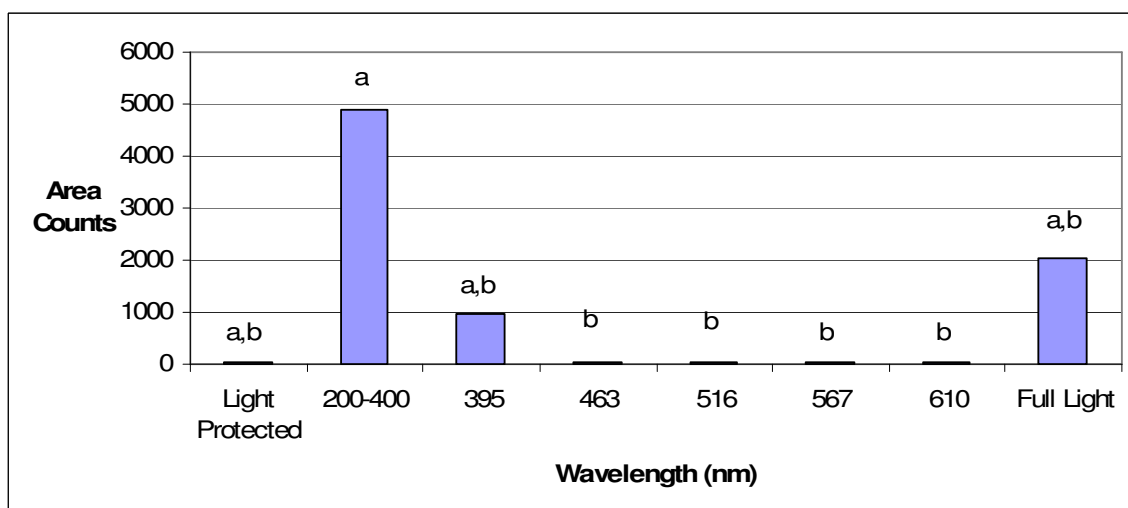
Figure 3.8. Average area counts of an unidentified compound (RT 7.691) due to wavelength exposure (7 hrs at 10º C at various mW (see Table 1)
[a, b]Means followed by the same letter are not significantly different at the $P<0.05$ level experimentwise using Tukey's HSD

69
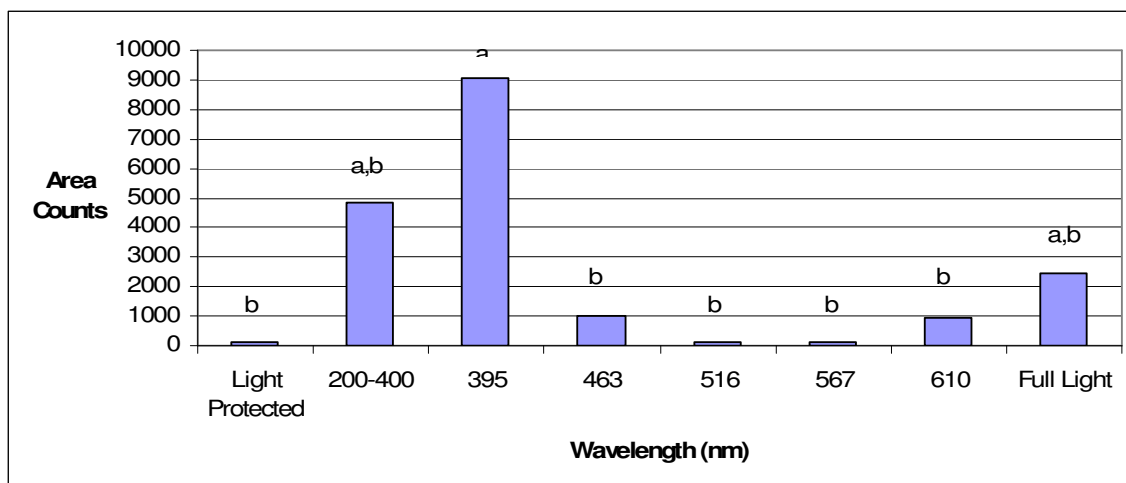
Figure 3.9. Average area counts of an unidentified compound (RT 9.040) due to wavelength exposure (7 hrs at 10° C at various mW (see Table 1)
[a, b]Means followed by the same letter are not significantly different at the P<0.05 level experimentwise using Tukey's HSD

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The oxidation compounds that we monitored in these experiments are presumed to contribute to oxidation flavor and odor. Wold and others (2005) found that oxidized odor was significantly higher in cheese that had light exposure through transparent, violet and blue films than cheese that had light exposure through red, yellow, green, and orange films and in a light protected sample. Oxidized odor in cheese was also not significantly different when exposed through red film than through violet or blue film. In a subsequent experiment, Wold and others (2006) found that green filters gave the least oxidation in Norvegia cheese, followed by yellow and orange. Hansen and others (1975) also found that green and yellow light produced the least amount of light oxidation in milk. These results are similar to our findings. We found that full light, and light through 200-400, 395, 463, and 610 nm filters (corresponding to their transparent, violet, blue and red films) produced the highest amount of oxidation compounds while 516 and 567 nm filters (corresponding to their yellow, green and orange films) produced the least amount of oxidation products.

70

Gas Chromatography Olfactometry

Odors generally indicate the presence of similar flavors. Day and Lillard (1960) concluded that oxidized flavor came about due to a combination of compounds and that combinations of carbonyl compounds below threshold levels were additive and able to give rise to an overall off flavor. This may explain how our treatments had different light oxidation odor intensities, yet did not have significantly different concentrations of volatile compounds.

Table 3.3 shows the average intensity of aroma-active compounds that were detected for each wavelength exposure. Overall, it appears that lower molecular weight aroma-active compounds were detected more often when milk was exposed to longer visible wavelengths (516 to 610 nm and full light) and the higher molecular weight aroma-active compounds were detected more often when the milk was exposed to the UV and shorter visible wavelengths (200-463 nm and full light). This finding however, may be a function of energy intensity, in addition to wavelength exposure, since the longer wavelength filters had, in general, higher energy emission than the UV and shorter visible wavelength filters. It also appears that the higher molecular weight compounds had, in general, stronger aroma intensities in milk than the lower molecular weight compounds exposed to shorter wavelengths.

Table 3.3. Aroma active compounds detected in milk when exposed to specific wavelengths showing RT (retention time), compound, aroma, and odor intensity at each wavelength tested.

			Wavelength							
RT	Compound	Aroma	Light Protected	200-400 nm	395 nm	463 nm	516 nm	567 nm	610 nm	Full Light
1.19-1.74	Unknown	Manure/Gas	+	+	+	+	+	+	+	+
1.81-1.98		Acetic Acid/ Chemical	+				+	+	+	+
2.10-2.22		Sweet/Floral					+	+	+	+
2.32-2.53		Grainy/Musty	+				+	+	+	+
2.94		Floral/Spicy							+	+
3.30	Pentanal	Grassy					+	+		
4.12-4.19		Metal/Plastic						+	+	+
4.47-4.50	Hexanal	Grassy					+	+	+	+
4.68		Rancid/ Cheesy		+		++	+			
5.52-5.84		Roasted Grain		+		+	+			+
5.98-6.18	2-Heptanone	Cereal/ Roasted Grain		++	++	++	+			+++
6.73-6.93	1-Octene-3-ol	Mushrooms		+		++	+	+	+	+++
8.36		Burned Cookies/ Caramel	++	+	+					

+ = slight intensity, ++ = moderate intensity, +++ = high intensity

Exposure of milk to full light gave the highest intensities of aroma overall, but this may be due to the higher energy intensity of the full light treatment rather than an effect of the light wavelengths themselves. Moderate aroma intensities were found in milk exposed to UV and the shorter visible wavelengths (200-400, 395 and 463 nm), while only slight aroma intensities were found in milk that was exposed to the longer visible wavelengths (516, 567 and 610 nm).

72

van Aardt and others (2005) found similar odor-active compounds (hexanal, 2-heptanone, n-heptanal, 1-octene-3-ol, octanal and nonanal) in light oxidized milk treated with antioxidants. However, they also detected dimethyl disulfide (cooked milk aroma) which we did not. Dimethyl sulfide and dimethyl disulfide were reported to have very low odor intensities in cheeses (Frank and others 2004). It is possible that this compound was present, but at extremely low concentrations, and was not detected by the panelists. Cadwallader and Howard (1998) found hexanal (green, cut grass odor), 1-octene-3-one and 1-nonene-3-one (mushroom odor) and 1-hexene-3-one (plastic odor) in milk exposed to 2200 lux light for 48 hrs. In a review by Freidrich and Acree (1998), raw cow's milk was found to have the odor-active compounds ethyl butanoate (fruity, sweet), ethyl hexanoate (fruity, pineapple), heptanal (green, sweet), indole (fecal, putrid, musty, floral in high dilution), nonanal (sweet, floral), 1-octene-3-ol (mushroom-like) and dimethylsulphone (sulfurous). Upon heating, the odor-active profile of the milk changed and four unique compounds arose: hexanal (green, cut grass), 2-nonanone (grassy-herbal, green-fruity), benzothiazole (quinoline, rubbery) and δ-decalactone (coconut).

Only one compound, with an RT of 1.19-1.79 min, was detected in all wavelengths tested. This compound had an odor of manure or gas, and odor intensity ratings for all wavelength exposures was slight. This compound is the unidentified compound, described above, with an RT of 2.49 min (Figure 3.5). Although the concentration of this compound was significantly higher when exposed to full light, the odor intensity remained the same for all concentrations. It is possible that even though the concentration was significantly higher for the full light exposure than other wavelength exposures, there needs to be larger differences in concentration for panelists to rate odor intensities differently.

Pentanal, which has a sour grass aroma, was only detected at slight odor intensity in milk that was exposed to 516 and 567 nm. Interestingly, these two wavelengths produced significantly less pentanal than the 200-400 nm (Figure 3.4) in which no pentanal aroma was detected. The human nose is considerably more sensitive than most analytical techniques and can have a detection limit as low as 1×10^{-19} moles for some compounds

73

(Curioni and Bosset 2002). It is possible that the odor of pentanal is grassy when at low concentration but changes in sensory character somewhat when at higher concentrations. Pentanal odor in cheddar cheese has been described as being chemical-like, rather than green (Curioni and Bosset 2002). Therefore, even though this compound might have been present in the samples exposed to other wavelengths, the odor may have changed enough to recieve a different descriptor and was not recognized as coming from pentanal.

Hexanal, which also has a grassy aroma, was detected at slight intensity in milk exposed to the longer visible wavelengths (516, 567 and 610 nm) and full light, but was not detected when exposed to the shorter wavelengths (200-400, 395 or 463 nm). Hexanal concentration (Figure 3.3), however, was significantly lower when exposed to 516, 567 and 610 nm than when exposed to 200-400 nm and full light. The threshold limit for hexanal is reported as being 0.339 ppm in milk (Norton 2003). The concentration for hexanal at its highest in this experiment was ~ 0.42 ppm when milk was exposed to 200-400 nm. Having concentrations so close to their threshold limit may explain the inconsistent results. A number of other investigators found hexanal to be a common odor-active compound in light exposed milk (Cadwallader and Howard 1998; van Aardt and others 2003).

Odor analysis was done using a different gas chromatograph than for the volatile compound analysis (HP 5890A gas chromatograph with a DB-5 ms capillary column (30 m x 0.25 mm id x 0.25 µm film thickness) vs. HP 5890 Series II Plus GC with an RTX-5 capillary column (30 m x 0.320 mm id x 1.00 µm film thickness). Although the column types (DB-5 vs RTX-5) are equivalent, the GCO column had a much lower thickness of film (0.25 µm vs 1.0 µm). As a general rule, the thinner the film thickness, the quicker the volatile compounds are eluted. This explains why the retention times were shorter when using the GCO chromatograph compared to the chromatograph used for the volatile data. Samples used for GCO evaluation, also, were not the same as those used for SPME-GC analysis. We know from the volatile compound analysis that concentrations of the compounds produced by exposure to specific light wavelengths were low and in many cases there was no significant difference in concentration amongst treatments. The

74

use of different GCs and columns, different samples and low concentrations of volatiles with few statistically significant differences may explain some of the discrepancies that were seen between the volatile analyses and the GCO analyses.

Conclusions

Exposure to UV light appeared to be the most damaging to milk quality, producing higher amounts of hexanal, pentanal and several unidentified compounds than when exposed to longer wavelengths. These compounds were also produced in high amounts when exposed to 395 nm, although concentrations were less than when exposed to 200-400 nm, except for one unidentified compound (RT of 9.04 min). It is significant that high concentrations of these compounds were produced upon exposure to 395 nm because this filter had substantially lower energy emission than any other filter. Because riboflavin has absorption maxima (250, 270, 370 and 400 nm) encompassed by these filters, it is likely that oxidation occurred due to riboflavin photo-sensitization.

Two compounds, pentanal and an unidentified compound (retention time of 3.43 min), showed increased production when exposed to 610 nm. It is possible that production of these compounds was initiated by some photosensitizer other than riboflavin. These results support the conclusions of Wold and others (2005, 2006) who determined that chlorins and porphyrins, found in low amounts in dairy products, were partially responsible for photo-oxidation in Norvegia cheese. It is possible that hematoporphyrin and/or protoporphyrin, which absorb light of 623 and 633 nm, respectively, were responsible for production of these two compounds.

In general, panelists detected aroma-activity by low molecular weight compounds when milk was exposed to longer visible wavelengths (516, 567 and 610 nm) and by higher molecular weight compounds when milk was exposed to shorter visible (463 and 395 nm) and UV (200-400 nm) wavelengths.

Because energy emissions for the filters were significantly different, we are unable to say if the effects were solely due to wavelength or were due, at least in part, to light intensity.

Acknowledgements

77

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Chapter IV

**Reduction of Photo-oxidation in Milk Using Novel Polymer Materials to Block
Riboflavin Excitation Wavelengths-Single Layer Films**

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Abstract

The efficacy of film over-wraps, made from single layers of iridescent film, to reduce the production of light oxidation in milk was tested. Film over-wraps blocked 446 nm, 550 nm, 570 nm and/or UV wavelengths. Solid phase microextraction gas chromatography (SPME-GC) and gas chromatography olfactometry (GCO) were used to assess the production of volatile and odor-active compounds. Single layer film over-wraps did not reduce the production of volatile and odor-active compounds in milk and, in fact, produced slightly higher numbers of odor-active compounds than milk packaged with no over-wrap. Although no treatment appeared to be effective at reducing light oxidation, the 570 nm block treatment (blocks transmission of 570 nm but allows transmission of 400 and 446 nm) was the least effective of the treatments tested. The light protected sample had very few odor-active compounds documenting minimal change in volatile chemistry due to photooxidation. The 570 nm block treatment appeared to peak in number of odor-active compounds on day 7 upon which numbers dropped. The 446 and 550 nm block treatments, the light-exposed treatment and the light-protected control treatment did not show this trend.

Key Words: gas chromatography (GC), gas chromatography olfactometry (GCO), milk

Introduction

Milk proteins and lipids are susceptible to oxidation reactions which can negatively affect odor and flavor due to volatile compound production which reduces shelf life. Oxidation can be catalyzed by metals, light, and heat or may occur spontaneously. Riboflavin, found at an average concentration of 1.75 ppm in the whey portion of milk (Dimick 1973), is implicated in oxidation because it acts as a photosensitizer, initiating free radical reactions when exposed to light of 250, 270, 370 nm, 400, 446 and 570 nm (Kyte 1995). The major abosorption maximum for riboflavin excitation is 446 nm (Satter and others 1976; Bosset and others 1995; Mortensen and others 2003). Once light is absorbed, riboflavin is converted to its singlet excited state. Singlet riboflavin can undergo intersystem crossing, forming a relatively stable triplet state. The triplet state of riboflavin reacts with either molecular oxygen to form singlet oxygen (Type II mechanism), which readily reacts with compounds within the milk to form free radicals, or directly with a lipid molecule to form peroxides (Type I mechanism) (Rosenthal 1992; Kagan 1993).

Initially, hydroperoxides are formed which do not impact odor and flavor. These peroxides are unstable and degrade into secondary oxidation products, such as aldehydes, ketones and other minor constituents (hydrocarbons, free fatty acids, esters, lactones, and furans), which do impact odor and flavor (deMan 1990). Secondary oxidation products, reported in the literature, that are formed as a result of photo-oxidation in milk include pentanal, hexanal, 1-hexanol, heptanal, 1-heptanol, 1-octene-3-ol, nonanal, 2-nonanone, dimethylsulfone, dimethyl disulfide, benzothiazole and decalactone (Moio and others 1994; Marsili 1999; van Aardt and others 2003, 2005).

Milk in the US is commonly packaged in either high density polyethylene (HDPE) or polyethylene terephthalate (PET) (Anonymous 2001). HDPE is a translucent polymer which transmits up to 62% of light wavelengths between 300-700 nm. PET, a packaging material frequently utilized for single-serve milk products, is a clear polymer which transmits up to 75-85% of visible light. Most milk today is stored under fluorescent

83

lighting (mean of 2000 lux) for an average of 8 hrs in dairy retail display cases before it is sold (Chapman 2002). Single-serve milk, typically, has much longer exposure times (Anonymous 2002). Chapman (2002) found that 34.5% of consumers could detect light oxidation flavor in milk packaged in translucent material after only 30 min exposure to 2000 lux fluorescent lighting and 70.7% could detect this flavor after 3 hrs exposure. Clearly, photo-oxidation in milk is widespread and it is thought that light oxidation flavor may be responsible for declining milk sales (Barnard 1973; Bray and others 1977; Heer and others 1995). Even though exposure of milk to light is detrimental to its quality, the practice of packaging it in translucent materials continues because consumers want to "see" the product they are buying (Sattar and deMan 1975; Rosenthal 1992; Cladman and others 1998; Doyle 2004) and packaging is a major driver of retail sales (Young 2002).

Two important factors that affect the development of light-oxidized flavor in milk are light wavelength and packaging material. The effect of light wavelength has been studied by a number of investigators (Hansen and others 1975; Bradley 1983; Bekbolet 1990; Bosset and others 1995; Hansen and Skibsted 2000; Lennersten and Lingnert 2000; Gorgern 2003; Mortensen and others 2004). Many investigators have shown that visible light of low wavelength, between 365nm (black light) and 500 nm (green light), causes a significant increase in light oxidation in milk (Herreid and Ruskin 1952; Sattar and others 1976; Hoskin and Dimick 1979; Bosset and others 1995; Nielson 1999; Hansen and Skibsted 2000; Lennersten and Lingnert 2000; Van Aardt and others 2001). Wold and others (2006), however, found wavelengths above 600 nm were also important in the production of oxidized odor and flavor in Norvegia cheese. This conclusion is similar to the findings of Sattar and others (1976) and Josephson (1946) who indicated that light between 595-620 nm also needed to be blocked in order to reduce light oxidation flavor in milk.

Packaging material can have a protective effect on milk quality through blocking or reducing the transmission of certain light wavelengths. This protection is based on material thickness, processing conditions and material coloration (Mortenson and others 2004). It is important, then, to develop packaging materials that will enable a consumer

84

to "see" the product yet block the most damaging wavelengths to milk quality. New polymer materials, with unique optical properties, such as iridescent and pearlescent films, have recently come on the market and may have a protective effect when used as package over-wraps; satisfying the demand for a "see through" appearance while protecting against sensory deterioration by photo-oxidation. Iridescent films block wavelengths through light interference rather than the use of dyes and pigments. They are translucent and can be engineered to block specific light wavelengths, including those that are most damaging to milk quality. They can be laminated to complex shaped containers and could easily be used as film over-wraps for single-serve milk containers. They also provide an upscale look that appeals to consumers and could potentially increase market share—in an independent survey, >50% of respondents said that iridescent film packaging made the product more distinctive and unique than existing packaging www.idspackaging.comCommon/Paper/Paper_180/Building%20Brand%20Equity1.htm.

The objectives of this study were:

1. To determine the efficacy of film over-wrap treatments, made by wrapping one layer of iridescent film with different optical properties around glass vials, in reducing light oxidation in 2% milk as determined by gas chromatography (GC) and gas chromatography olfactometry (GCO).

2. To determine the effect that visible excitation wavelengths of riboflavin (400, 446 and 570 nm) have on the production of volatile and odor-active compounds in 2% milk.

Materials and Methods

Milk Processing and Packaging

Milk was processed following the procedure reported in Chapter III, Effect of Specific Wavelengths on Volatile Chemistry and Production of Aroma-Active Compounds in Milk, in the Milk Processing and Packaging section (pg 56).

Pasteurized milk was collected under a laminar flow hood (Atmos-Tech Industries, Ocean, NJ) in 2 L sterile glass Erlenmeyer flasks covered in aluminum foil so as to eliminate transmission of light. Milk (25 ml) was added to sterile 40 ml borosilicate glass vials containing magnetic stirrers and capped with screw lids fitted with Teflon septa. Vials were over-wrapped with one of four film treatments (UV, 446, 550 or 570 nm block treatments depending on the experiment) or wrapped in aluminum foil (light-protected control) or left unwrapped (light-exposed control).

Film Treatments

Milk samples were randomly assigned to treatments made from a single layer of packaging material over-wraps (Aurora® Standard Films, 9231 series, Engelhard Corporation, Iselin, NJ) (Table 4.1). These over-wraps blocked between 50 and 90% of specific wavelengths (Figure 4.1). A light-protected treatment, made by wrapping vials with aluminum foil, blocked all UV and visible wavelengths, while a light-exposed treatment allowed between 50-75% transmission of wavelengths between 300 and 340 nm and 80% of wavelengths above 340 nm. All experimental treatments, except for the light-protecting control, allowed for good visibility of the milk inside the container.

Table 4.1. Primary wavelengths blocked by packaging film treatments[1]

Packaging Over-wrap Treatment[1]	# of Layers of Film	Major Wavelengths Blocked
446 nm Block	1	425-480 nm
550 nm Block	1	520-600 nm
570 nm Block	1	560-660 nm
Light Protected	NA	All visible and UV
Light Exposed	NA	No visible, UV below ~300 nm

[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ
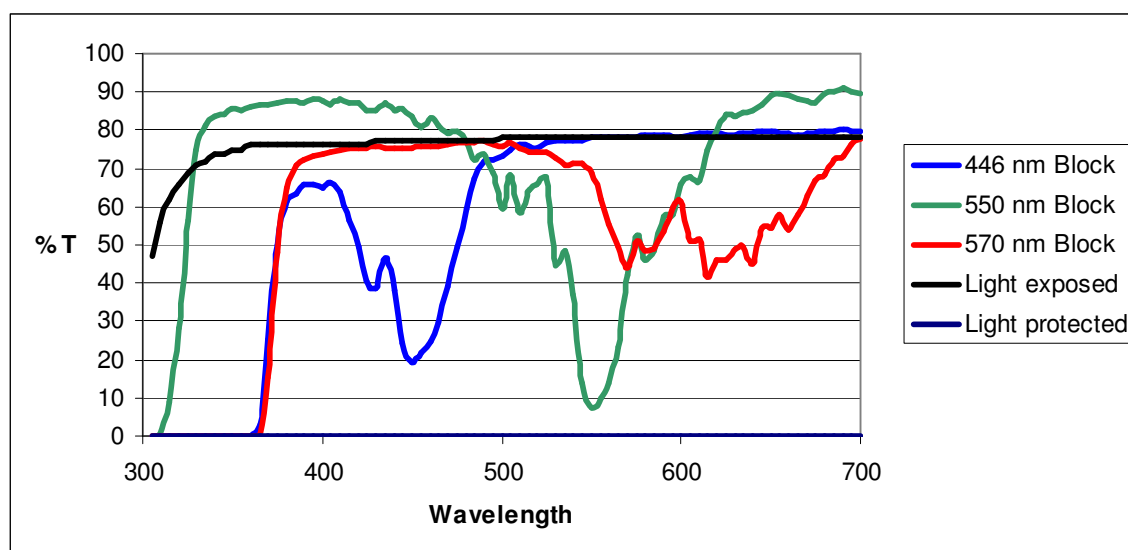
Figure 4.1.   % Light transmission of wavelengths between 300 and 700 nm for packaging treatments[1]
[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ

Milk Storage Under Fluorescent Lighting

Vials were randomly arranged upright under 900-1100 lux fluorescent lighting in a 4º C walk-in cooler (Tonka, Hopkins, Minn.) for up to 35 days. Lighting consisted of two Phillips 30-34 W cool white fluorescent bulbs per light fixture with four fixtures total being used in the experiment. Light intensity was tested at the beginning and end of each experiment using a light meter (Extech Instrument Corp., Walthaun, MA).

Microbiological Analysis

One vial was randomly pulled and tested for microbial count on each day of testing. Total plate count (TPC) and coliform count (FC), using standard methods for Petrifilm™ (3M, St. Paul, MN) were completed on selected sample bottles prior to any additional testing (Marshall 1983). Testing was done to ensure that on no day did microbial growth exceed the limit for milk (100,000 cfu/ml TPC) and possibly contribute to the off-flavor of the milk. Samples were tested in duplicate at both undiluted and ten-fold dilutions. Only one sample was tested on each sampling day and the treatment that was sampled was randomly picked.

Lipid Oxidation Analysis Using Headspace Gas Chromatography

Volatile oxidation compounds were analyzed according to the procedure reported in the Lipid Oxidation Analysis Using Headspace Gas Chromatography section in Chapter III (pg 59). Newly pasteurized milk was tested for volatile oxidation products on day 0 and three vials of each treatment were randomly pulled from the cooler and analyzed on testing days 4, 7, 10, 14, 21, 28 and 35. Three vials were pulled on days 7, 14, and 35 for the first replication, days 7, 17 and 28 for the second replication and days 7, 14 and 28 for the third replication of the experiment to test for odor-active compounds. During the second replication samples were pulled on day 17 rather than day 14 because the GCO was not working properly on day 14 and samples could not be run until it was fixed. The last sample for GCO analysis for replications 2 and 3 were pulled on day 28 rather than day 35.

During replication 2 and 3, an extra treatment, the 550 nm block, was added. This treatment blocked wavelengths that were slightly lower and to a greater degree than the 570 nm block treatment.

Odor-active Compound Analysis Using Gas Chromatography Olfactometry (GCO)

Training. Six panelists (5 females, 1 male), consisting of graduate students and staff from the Food Science and Technology department at Virginia Tech, were trained in the recognition of aromas and odors common to beer and milk.   Training consisted of using Beer Aroma Recognition standards and Beer Taint reagents (Brewing Research International, UK) to develop a memory and lexicon for aromas (Table 4.2). Panelists were tested for ability during training by performing blind aroma recognition analyses of the standards and reagents. All panelists were able to correctly recognize at least 70% of the aromas and odors. Panelists trained for a total of two hours.

Table 4.2. Beer Aroma standards/reagents[1] used for training sensory panelists for the recognition of off-odors in milk

Beer Aroma Recognition Standards	Beer Taint Recognition Reagents
Sweetcorn	Dimethyl sulfide (DMS)
Malty	Phenolic
Late hop	Medicinal
Rose floral	Musty
Lemon floral	Diacetyl
Citrus	Rancid
Spicy	Cheesy
Grssy	Cardboard
Banana	Catty
Pineapple	Papery
Phenolic	Cooked vegetable
Butterscotch	Onion

[1] Brewing Research International (BRi), Nutfield, UK

Sensory Testing. Three panelists, selected for their availability and ability, evaluated aromas/odors on days 7, 14, and 35 for replication 1, days 7, 17 and 28 for replication 2 and days 7, 14 and 28 for replication 3. A GCO sniff port (ODO II, SGE International, Ringwood, Austrailia) attached to an HP 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a FID was used to carry the aromas of the separated compounds to the panelists. Panelists evaluated both the type and intensity of the odors. Intensity was evaluated on a scale from 1-5 where 1 meant "slight" and 5 meant "very

89

strong".   Average ratings were converted to category data of slight (1-2) denoted by a +, medium (3) denoted by ++, and strong (4-5) denoted by +++. An odor was reported if two out of three panelists detected it.

GC-O samples were adsorbed onto a 75 µm carboxen-PDMS SPME fiber as described in the Lipid Oxidation Analysis Using Headspace Gas Chromatography section. A DB-5 ms capillary column (30 m x 0.25 mm id x 0.25 µm film thickness, J & W Scientific, Folsom CA) was used to separate the volatile compounds. Helium was used as the carrier gas at a flow rate of 32 cm/sec. The temperature program was the same as described previously. Integration of peaks was done using an HP 3396A Integrator (Hewlett Packard, Palo Alto, CA).

Statistical Analysis

Standard least squares and Tukey's HSD were performed on the gas chromatography data to determine significant differences in volatile oxidation compounds among treatments. These analyses were performed using JMP 5.1 Statistical Discovery Software (SAS Institute Inc, 1989-2004).

Microbiological Analysis

UHT pasteurized milk was of high microbial quality throughout the experimental shelf-life. The production of volatiles and off-odors observed in these experiments were attributed to chemical changes and not to microbial growth.

Volatile Compound Changes

Heptanal (Figure 4.2) and three unidentified compounds (RT 2.49 (this compound gives a flatulence odor) (Figure 4.3), RT 11.93 (Figure 4.4) and RT 13.34 min (Figure 4.5)) showed initial increases in concentration but as the course of the experiment continued, decreased in concentration. In most, but not all, cases the peak concentration occurred somewhere between days 21 and 28 upon which concentration started to decrease.
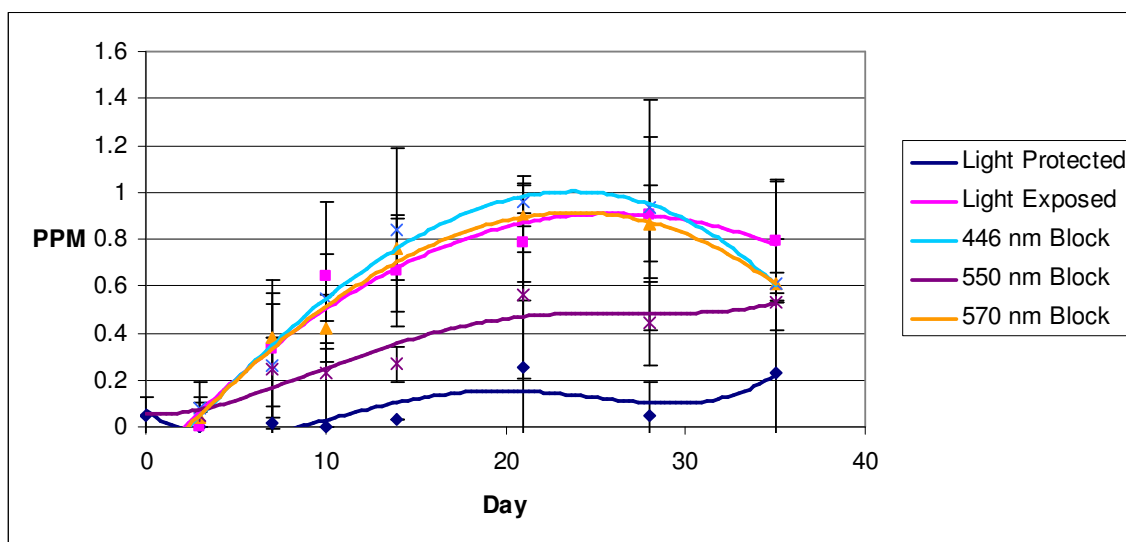

Figure 4.2. Concentration of heptanal ± standard deviations in milk exposed to 900-1100 lux fluorescent light over 35 days at 4º C.
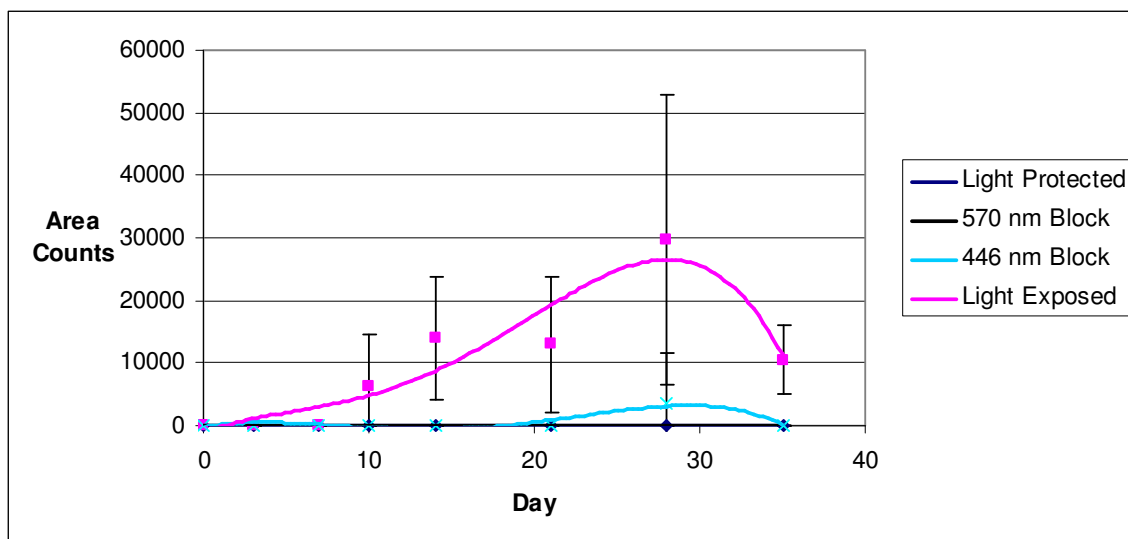
Figure 4.3. Average area counts ± standard deviations of unidentified compound with a retention time of 2.49 min. in milk exposed to 900-1100 lux fluorescent light over 35 days at 4º C.
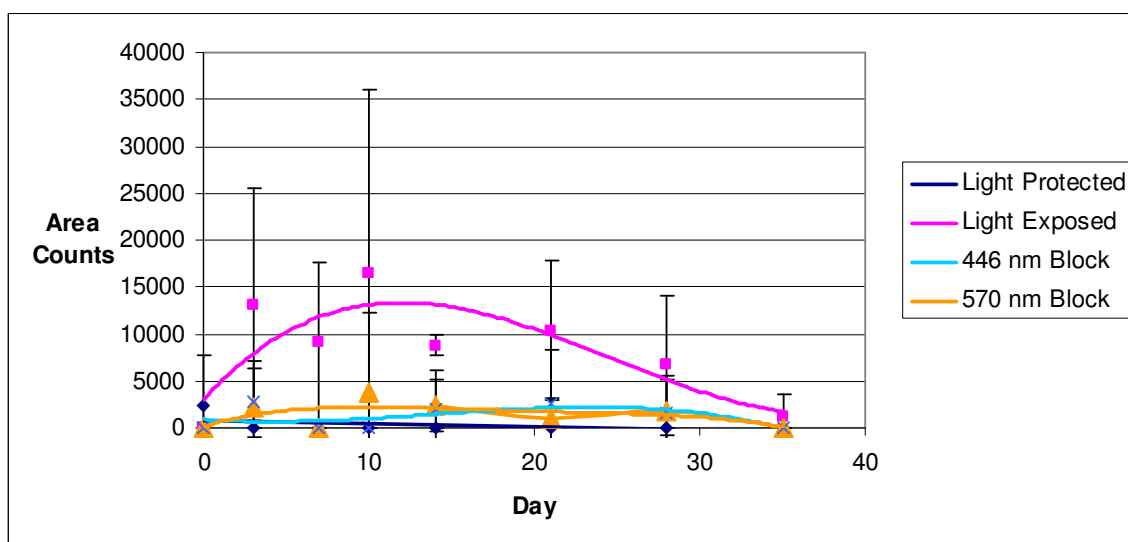
Figure 4.4. Average area counts ± standard deviations of unidentified compound with a retention time of 11.93 min. in milk exposed to 900-1100 lux fluorescent light over 35 days at 4º C.

92
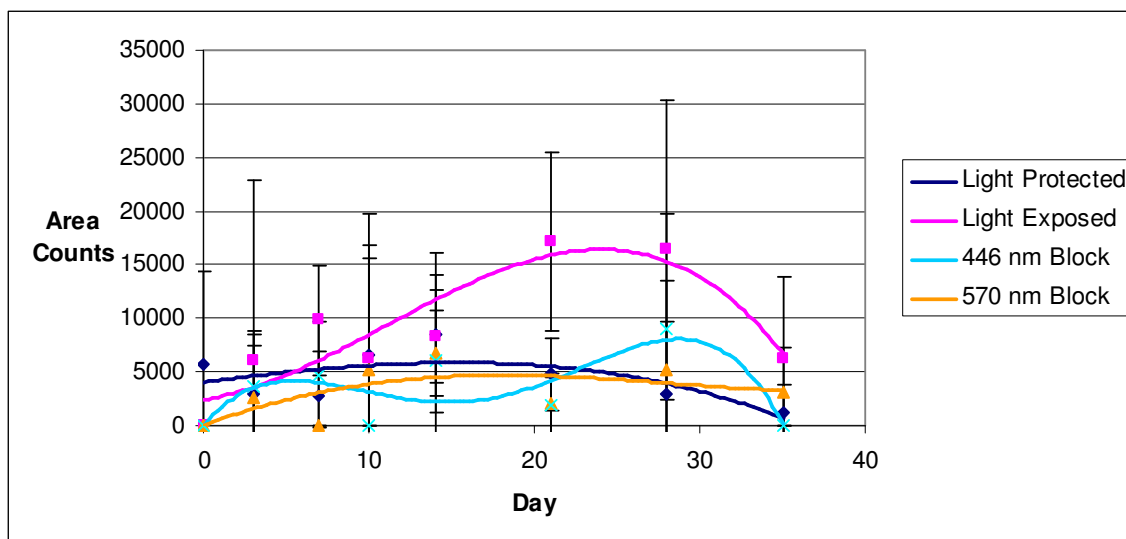
Figure 4.5. Average area counts ± standard deviations of unidentified compound with a retention time of 13.39 min. in milk exposed to 900-1100 lux fluorescent light over 35 days at 4º C.

 Production of pentanal (Figure 4.6) and hexanal (Figure 4.7) did not show this trend of increasing and then decreasing over time. These compounds showed a steady increase in concentration over the 35 day period of the experiment. There were no significant differences in pentanal concentration for any treatment except for the light-protected treatment. In general, though, the 550 nm block treatment reduced concentration of pentanal compared to the other treatments.   Pentanal was shown to be produced upon exposure to UV and short visible wavelengths as well as 610 nm (Chapter III). It is somewhat surprising that the lowest amount of pentanal was produced in the 550 nm block treatment because this treatment allows exposure of UV and the short visible wavelengths (~320-510 nm). Perhaps a sensitizer other than riboflavin which absorbs light between ~520 and 560 nm, the wavelengths the 550 nm block treatment reduces, is responsible for the initiation of pentanal production.

93
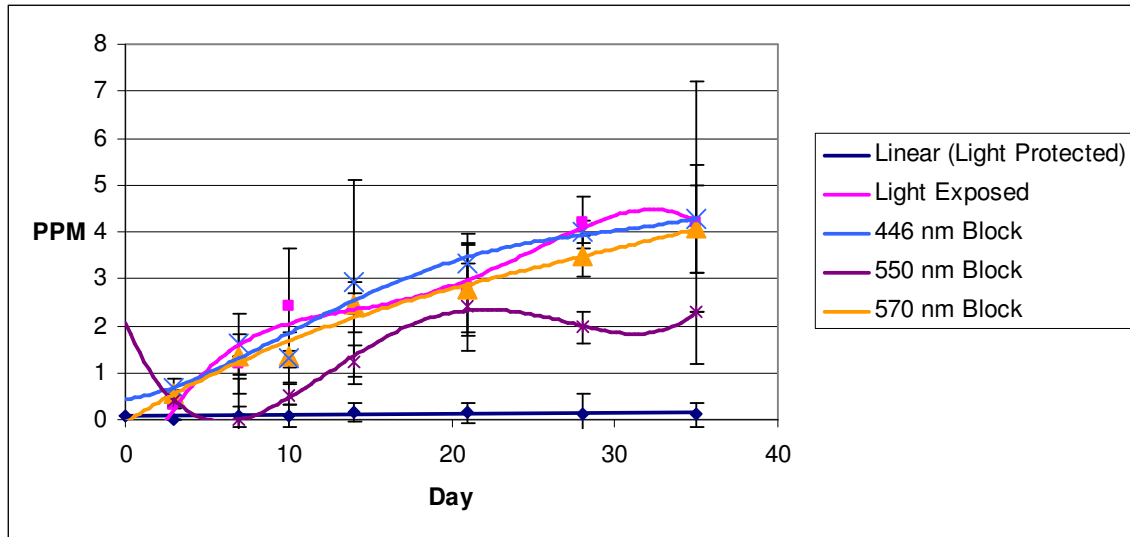
Figure 4.6. Concentration (ppm) of pentanal in milk exposed to 900-1100 lux fluorescent light for 35 days at 4º C.

Hexanal (Figure 4.7) showed an increase in concentration over time with no significant differences between film over-wrap treatments, except for the light-protected control treatment which, in general, protected against production of this compound.
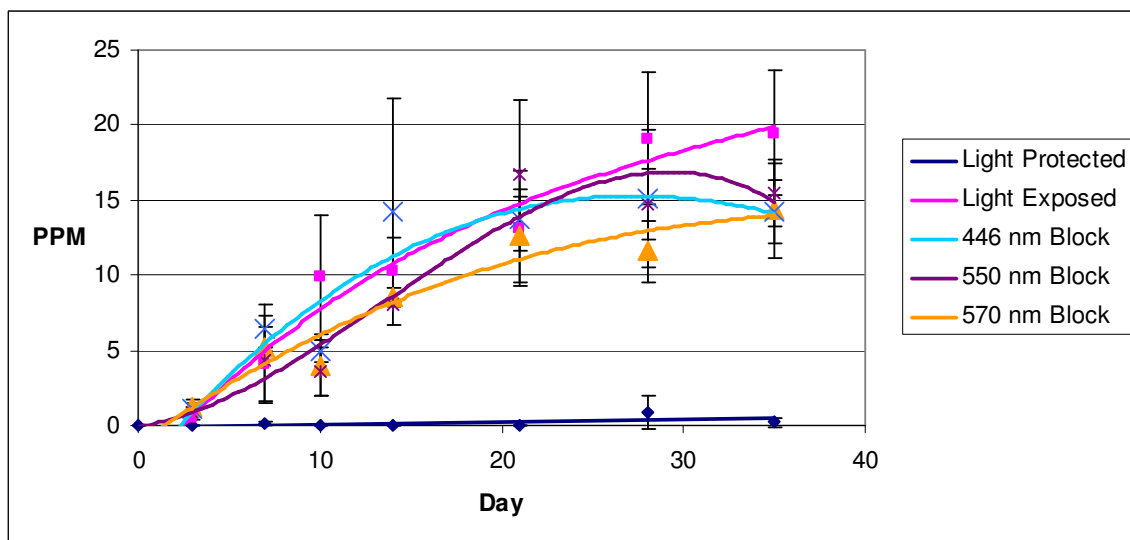

Figure 4.7. Concentration (ppm) of hexanal in milk with exposed to 900-1100 lux fluorescent lighting over a 35 day period at 4º C.

94

Pentanal, hexanal and heptanal are common oxidation compounds found in milk (Mehta and Bassett 1978; Farrer 1983; Bekbolet 1990; Cadwallader and Howard 1998; Rysstad and others 1998; van Aardt and others 2001; Mestdagh and others 2005). Generally, there was no significant difference in the concentration of these compounds among the over-wrap treatments (446, 550 and 570 nm block treatments and the light-exposed control treatment). However, the light-protected control treatment completely stopped or significantly slowed production of these compounds. One exception is the unidentified compound with a retention time of 2.469 min. This compound was not significantly produced by any of the treatments, except for the light-exposed control treatment. However, this compound, which has an odor of flatulence, was detected in all treatments on all days, except for a few days in the light-protected control treatment (See Gas Chromatography Olfactometry section below).

Gas Chromatography Olfactometry

The 570 nm block treatment, in general, produced the highest numbers of odor-active compounds than all other treatments throughout the experiment. The number of compounds produced in milk with this treatment was much higher than all other treatments on day 7 but dropped to being about the same number as the 446 and 550 nm block treatments and the light-exposed treatment by day 14-17. Odor intensities also tended to be strongest on day 7, dropping considerably in overall intensity by day 28-35 (See Tables 4.3-4.5). It is unknown why there was a decrease in production and intensity of odor-active compounds, but one explanation may be that as the samples continued to be exposed to light, odor-active compounds were further degraded into non-odor-active compounds. The 446 and 550nm block treatments and the light-exposed treatment did not increase in numbers of odor-active compounds until day 28-35 and that increase was minimal. Throughout the shelf-life, the light protected sample had very few odor-active compounds, documenting minimal change in volatile chemistry due to photooxidation. Numbers of odor-active compounds steadily increased in the light-protected treatment over time.

95

Table 4.3. Retention time, compound identification, aroma and intensities of odor-active compounds in UHT milk exposed to 900-1100 lux fluorescent light on day 7 of refrigerated storage (4º C)

RT	Compound	Aroma	446 nm Block	570 nm Block	550 nm Block	Light Protected	Light Exposed
2.03-2.33	Unknown	Flatulence	+	++	++	+	+
2.64-2.81	2-Propanone	Sweet/Caramel	++	+	—	—	—
3.13-3.17		Spice/Pepper	+	+	++	++	—
3.51-3.63	Pentanal	Grass/Green	+	+	+	—	+
4.27-4.64		Musty	—	+	—	—	—
4.75-4.82	Hexanal	Grass	++	++	++	+	++
4.89-5.07		Roasted Grain	++	++	++	—	++
5.63-5.64		Grass/Perfume	—	+	—	—	+
5.64-5.78		Nuts/Roasted	++	++	++	+	+
5.98-6.07		Cardboard/Chemical	+	++	+++	—	—
6.11-6.16		Grass	—	+	—	—	++
6.12-6.45		Roasted Grain	++	+++	++	+	++
6.99-7.08		Musty	+++	++	++	—	—
7.07-7.22	1-Octene-3-ol	Mushrooms	+++	+++	+++	—	++
7.56-7.64		Rotten Grapefruit	+	+	+	—	+
8.17-8.57		Carmalized Sugar/Chemical	—	+	—	—	—
8.54-8.62		Musty/Billy Goat	+	++	+	—	++
9.22-9.23		Green/Grass	—	+++	—	—	—
9.16-9.40		Chemical	—	++	++	—	++

+ = slight intensity, ++ = moderate intensity, +++ = high intensity

Table 4.4. . Retention time, compound identification, aroma and intensities of odor-active compounds in UHT milk exposed to 900-1100 lux fluorescent light on day 14-17 of refrigerated storage (4º C)

RT	Compound	Odor	446 nm Block	570 nm Block	550 nm Block	Light Protected	Light Exposed
1.85-2.38		Flatulence	++	++	++	+	+
2.33-2.40		Chemical	+	—	—	—	—
2.64-2.81	2-Propanone	Sweet/Caramel	—	+	—	—	—
3.13-3.35		Spicy/Pepper	+	+	++	+	+
3.48-3.55	Pentanal	Green/Grass	++	—	—	+	+
4.53-4.80	Hexanal	Grass	+	++	++	+	++
4.85-4.9		Roasted Grain/grape nuts	++	+	+	—	++
5.45-5.64		Grass/Perfume	—	—	—	+	—
5.64-5.72		Grape Nuts/Vitamins	++	++	++	+	++
5.98-6.07		Cardboard/ Chemical	++	+	+	+	—
6.10-6.37		Roasted Grain	++	++	++	—	+
6.99-7.08		Musty	+	++	++	—	++
7.01-7.22	1-Octene-3-ol	Mushrooms/ Musty	++	+++	+++	+	+++
7.59-7.65		Rotten Grapefruit/ Chicken Manure	+	++	++	—	++
7.98		Chicken Manure	—	—	+	—	—
8.05-8.57		Caramelized Sugar/Sweet	—	+	—	—	+
8.54-8.62		Musty/Billy Goat	—	—	+	—	—
9.10-9.11		Chemical	—	+	—	—	—
9.24-9.30		Green/Grass	++	++	+	—	+++

+ = slight intensity, ++ = moderate intensity, +++ = high intensity
NP=Not Performed

97

Table 4.5. . Retention time, compound identification, aroma and intensities of odor-active compounds in UHT milk exposed to 900-1100 lux fluorescent light on day 28-35 of refrigerated storage (4º C)

RT	Compound	Odor	446 nm Block	570 nm Block	550 nm Block	Light Protected	Light Exposed
1.79-2.25	Unidentified	Flatulentce	++	++	+	+	++
2.44-2.62		Roasted Grain	+	+	+	—	—
3.41-3.45		Spice/Pepper	+	+	+	—	+
3.65-3.72	Pentanal	Grass/Green	++	+	+	—	+
4.16-4.91	Hexanal	Grass/Floral	+++	++	+++	+	+
4.99-5.09		Roasted Grain	++	+	+	—	++
5.46-5.96		Grape Nuts/Roasted Grain	++	++	++	+	+
5.80-6.15		Cardboard/ Chemical	++	+	++	+	+
6.02-6.26		Roasted Grain	+	++	++	+	+
6.57-6.68		Roasted grain	—	—	—	+	—
6.79-7.12		Cardboard/Musty	++	—	++	—	++
7.15-7.21	1-Octene-3-ol	Mushrooms	++	++	++	++	+
7.33-7.40		Earthy	—	—	—	+	—
7.34-7.41		Rotten Grapefruit	++	+	++	—	+
7.41-7.80		Chicken Manure	++	++	++	—	++
8.06-8.12		Citrus/Floral/Caramel	—	+	—	+	—
8.58-8.94		Musty/Billy Goat	++	++	+	—	++
9.10-9.26		Musty	—	+	—	—	—
9.04-9.32		Chemical	++	++	—	—	+

+ = slight intensity, ++ = moderate intensity, +++ = high intensity

These results suggest that the 570 nm block treatment, which blocks ~ 60% of wavelengths between 560 and 660nm, but allows ~80% transmission of wavelengths between 360 and 520 nm, is the most detrimental to milk quality of all the treatments tested. Results from our study on the effect of exposure to specific light wavelengths did indicate that exposure to UV and short visible wavelengths (Chapter III) produced higher amounts of volatile compounds, overall, and support the findings from this experiment. However, it is interesting that the light-exposed treatment, at least on day 7, had significantly lower numbers of odor-active compounds than the 570 nm block treatment, yet allows approximately the same percent transmission of UV and short visible wavelengths as the 570 nm block treatment.

The light-exposed treatment also tended to have lower or equal numbers of odor-active compounds compared to the other film over-wrap treatments (446 and 550 nm block treatment). It is unknown as to why this treatment would consistently produce approximately equal numbers of odor-active compounds to the 446 and 550 nm block film over-wrap treatments because the milk in this treatment was exposed to all wavelengths and the percent transmission of these wavelengths was either higher or similar to that of the over-wrap treatments. One possible explanation is that light that is scattered off of the milk in the film over-wrap treatments may be reflected back into the milk causing an increase in light exposure, whereas light scattered from milk in the light-exposed treatment goes off into the environment (Figure 4.8).
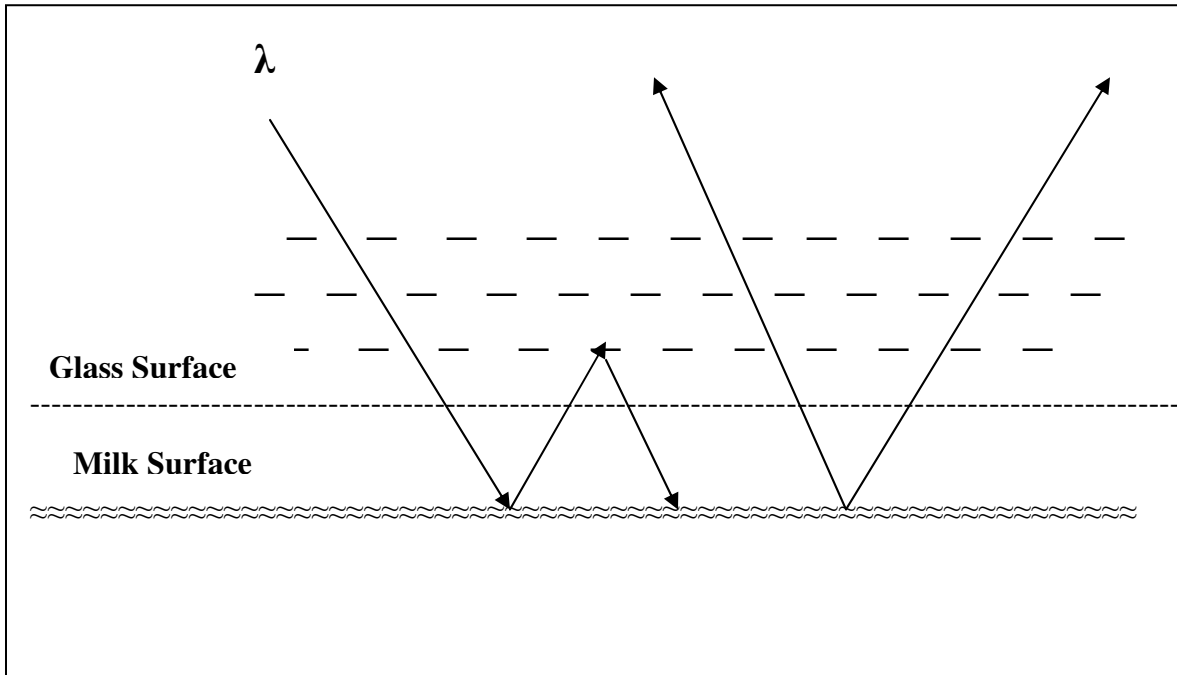
Figure 4.8. Possible explanation for increased production of odor-active compounds in film over-wrap treatments compared to light-exposed control treatment. Light wavelength is scattered off of milk surface and is reflected back onto surface again.

The unidentified compound with a flatulence odor was consistently detected on all days and in all treatments throughout the experiment. There were slightly higher intensities (medium compared to slight) for this compound in the 570 and 550 nm block treatment on day 7. By day 14-17, the 446 nm block treatment matched the 570 and 550 nm block intensity and by day 28-35, the light-exposed control had increased to medium intensity. Interestly, this compound showed consistently higher concentrations in the light-exposed treatment when tested by GC (Figure 4.3), similar to results found in Chapter III, yet did not show equal intensity to other treatments until the end of the shelf-life period. It is possible, however, that because these intensity ratings are somewhat subjective, the overall intensity was similar for all treatments.

Propanone, which has a sweet or caramel aroma, was found in only the 446 and 570 nm block treatments. Over time this odor dissipated and was not detected in any treatments

100

by day 28-35. This is generally a positive aroma and it makes sense that it would be reduced in intensity and amount over the course of the experiment.

The odor of pentanal (green grass) was detected, in general, in all treatments except the light-protected treatment on all days. The 446 nm treatment, overall, appeared to have slightly higher odor-intensity for this compound than the other treatments. Pentanal was found to be produced in high amounts when exposed to light of 610 nm and it was speculated that a photosensitizer other than riboflavin was responsible for the production of this compound (Chapter III). The 446 nm treatment allows ~ 80% transmission of light above 510 nm. However, the light-exposed treatment also allows high transmission of these wavelengths as well, but showed lower overall intensity of pentanal odor.

Hexanal (grass odor) was detected in all treatments on all days of the experiment. In general, intensities increased over the course of the shelf-life. This followed the same trend seen in the volatile GC data.

One-octene-3-ol (mushroom odor) was also detected in all treatments in all days of the experiment, except for the light-protected treatment on day 7. This compound was not detected by GC analysis. One-octene-3-ol has an odor threshold of 34 ppb in camembert cheese (Curioni and Bosset 2002) and it is likely that concentrations were below the detection limit for the GC, but not for the human nose.

An unidentified compound with a spice or pepper aroma (RT 3.13-3.17) was detected, in general, in all treatments on all days of the experiment. The intensity of this aroma decreased somewhat over time.

Several unidentified compounds had a roasted grain aroma (RT 4.85-5.10 min; 5.46-5.96 min; 6.12-6.45 min). These compounds were detected, in general, in all treatments, although detection in the light-protected treatment was variable. Intensities generally stayed the same or decreased over time.

Compounds with a musty odor (RT 6.99-7.08 min), a rotten grapefruit odor (RT 7.07-7.22 min) and one with a green grass odor (RT 9.22-9.23 min) were detected, in general, in the treatments (446, 550 and 570 nm block and the light-exposed treatment) but not in the light protected samples. The musty and green grass odor decreased in intensity over the duration of the experiment, while the rotten grapefruit odor generally increased.

Conclusion

Film over-wrap treatments made from a single layer of different types of iridescent films did not reduce the production of volatile compounds and odor-active compounds in 2% UHT milk held at 4 C and exposed to 900-1100 lux fluorescent lighting. Concentration of volatiles and number and intensity of odor-active compounds was generally higher or equal in the film over-wrap treatments than in the light-exposed control treatment. Although no treatment appeared to be effective at reducing light oxidation, the 570 nm block treatment (blocks transmission of 570 nm but allows transmission of 400 and 446 nm) was the least effective of the treatments tested, while the 446 and 550 nm block treatments and the light-exposed treatment were about equal in efficacy for reducing the production of odor-active compounds. The light protected sample had very few odor-active compounds indicating minimal change in volatile chemistry due to photooxidation. The 570 nm block treatment appeared to peak in number of odor-active compounds on day 7 upon which numbers dropped. The 446 and 550 nm block treatments, the light-exposed treatment and the light-protected control treatment did not show this trend.

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105

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Chapter V

Reduction of Photo-oxidation in Milk Using Novel Polymer Materials to Block Riboflavin Excitation Wavelengths-Multilayer Films

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Abstract

The efficacy of film over-wraps, made from multi-layers of iridescent film, to reduce the production of light oxidation in milk was tested. Packaging over-wraps were designed to block either a single visible riboflavin excitation wavelength (400, 446 or 570 nm) or all three visible riboflavin excitation wavelengths. Over-wraps blocked all UV riboflavin excitation wavelengths. Solid phase microextraction gas chromatography (SPME-GC) and gas chromatography olfactometry (GCO) were used to assess the production of volatile and odor-active compounds. Riboflavin degradation was determined using high performance liquid chromatography (HPLC). Packaging over-wraps reduced production of volatile and odor-active compounds in milk compared to milk with no over-wrap, but not to the extent that the complete light block (foil wrap) did. Volatile compounds that increased over time (28 days) included pentanal, hexanal, 1-octene-3-ol and an unidentified compound with a retention time of 5.79 min. Odor-active compounds that were apparent in all treatments, except the light-protected treatment, included hexanal (grassy odor), 1-octene-3-ol (mushroom odor), an unidentified compound with a retention time of 6.52 min (mushroom/musty odor) and another unidentified compound with a retention time of 2.05 min (flatulence odor). In general, no film treatment was more efficient at reducing oxidation than any other treatment. However, blocking the 400 nm wavelength appeared to be slightly better at reducing hexanal production than blocking 446 or 570 nm or all three wavelengths. Riboflavin was degraded over time in all treatments except the light-protected treatment. Only minor differences occurred in the amount of degradation among treatments.

Key Words: gas chromatography (GC), gas chromatography olfactometry (GCO), riboflavin, hexanal, 1-octene-3-ol, milk

Introduction

Milk proteins and lipids are susceptible to oxidation reactions which can negatively affect flavor and odor and reduce shelf life. Oxidation can be catalyzed by metals, light, and heat or may occur spontaneously. Initially, hydroperoxides are formed which do not impact odor and flavor. These peroxides are unstable and degrade into secondary oxidation products, such as aldehydes, ketones and other minor constituents (hydrocarbons, free fatty acids, esters, lactones, and furans), which do impact odor and flavor (deMan 1990). Secondary oxidation products formed as a result of photo-oxidation in milk include pentanal, hexanal, 1-hexanol, heptanal, 1-heptanol, 1-octene-3-ol, nonanal, 2-nonanone, dimethylsulfone, dimethyl disulfide, benzothiazole and D-decalactone (Moio and others 1994; Marsili 1999; van Aardt and others 2005a). Because high quality milk flavor is bland, providing limited masking of off-flavor compounds, only small amounts of these compounds are needed to negatively affect flavor.

Milk is commonly packaged in either high density polyethylene (HDPE) or polyethylene terephthalate (PET). HDPE is a translucent polymer which transmits up to 62% of light wavelengths between 300-700 nm. Polyethylene terephthalate (PET), a packaging material frequently utilized for single-serve milk products, is a clear polymer which transmits up to 75-85% of visible light. Most milk today is stored under fluorescent lighting (mean of 2000 lux) for an average of 8 hrs in dairy retail display cases before it is sold. Single-serve UHT milk, typically, has much longer exposure times, with shelf life expectancy longer than 60 days (Anonymous 2002). Chapman (2002) found that 34.5% of consumers could detect light oxidation flavor in milk packaged in translucent material after only 30 min exposure to 2000 lux fluorescent lighting and 70.7% could detect this flavor after 3 hrs exposure. Clearly, photo-oxidation in milk is widespread and it is thought that it may be responsible for declining milk sales (Barnard 1973; Bray and others 1977; Heer and others 1995). Even though exposure of milk to light is detrimental to its quality, the practice of packaging it in translucent materials continues because consumers want to "see" the product they are buying (Sattar and deMan 1975; Rosenthal

1992;   Cladman and others 1998; Doyle 2004) and packaging is a major driver of retail sales (Young 2002).

The presence of photosensitizers in milk facilitates photo-oxidation (Sattar and others 1976; Bekbolet 1990). Riboflavin, found in high concentration in the whey fraction of milk, is a photosensitizer and initiates oxidation reactions when exposed to a number of different wavelengths; principally 250, 270, 370, 400, 446 and 570 nm when in foods with a neutral pH (Kyte 1995). Porphyrin and chlorin compounds, contained in dairy products in minute amounts, also act as photosensitizers and contribute to the production of off-flavor compounds (Wold and others 2005). The first off-flavor that appears in milk is due to oxidation of protein and has a burnt feather or burnt protein flavor. This flavor dissipates within a few days to be replaced by a tallowy or wet cardboard flavor, produced upon the oxidation of lipids (Aurand and others 1966).

The effect of light wavelength on the production of light oxidation flavor has been studied by a number of investigators (Hansen and others 1975; Bradley 1983; Bekbolet 1990; Bosset and others 1995; Hansen and Skibsted 2000; Lennersten and Lingnert 2000; Gorgern 2003), although Mortensen and others (2004) feel that this topic has not been studied enough and should be a priority topic for research. Sattar and others (1976) found that lipid oxidation in milk was affected by wavelengths shorter than 455 nm, indicating riboflavin sensitization, since the major absorption maxima for riboflavin is 446 nm. Bosset and others (1995) concluded that light of low wavelength (violet-blue; ~ 390-490 nm) negatively affected the flavor of products containing riboflavin. Mortensen and others (2003) determined that exposure to light wavelengths of 405 and 436 nm was significantly more damaging to quality than exposure to 366 nm in Havarti cheese. Josephson (1946), however, indicated that all wavelengths below 620 nm must be blocked in order to prevent photo-chemical changes that result in sunlight flavor in milk and Wold and others (2005) found that porphyrins and chlorins, which absorb wavelengths throughout the visible spectrum, significantly contributed to photo-oxidation in dairy products. In a study using colored films, Wold and others (2006) found that green light was least detrimental to Norvegia cheese quality, followed by yellow, orange,

red, blue, white, and violet, with violet being most detrimental to cheese flavor and odor. Interestingly, there was no statistical difference between sensory scores in cheese exposed to blue or red light.

Packaging material can have a protective effect on milk quality through blocking or reducing the transmission of certain light wavelengths. This protection is based on material thickness, processing conditions and material coloration (Mortenson and others 2004). Opaque packaging materials will block both UV and visible light, but consumers prefer to "see" the food they are buying (Sattar and deMan 1975; Rosenthal 1992; Cladman and others 1998; Doyle 2004). It is important, then, to develop packaging materials that will enable a consumer to "see" the product yet block the most damaging wavelengths to milk quality. New polymer materials, with unique optical properties, such as iridescent and pearlescent films, have recently come on the market and may have a protective effect when used as package over-wraps; satisfying the demand for a "see through" appearance while protecting against sensory deterioration by photo-oxidation. Iridescent films block wavelengths through light interference rather than the use of dyes and pigments. They are translucent and can be engineered to block specific light wavelengths, including those that are most damaging to milk quality. They can be laminated to complex shaped containers and could easily be used as film over-wraps for single-serve milk containers. They provide an upscale look that appeals to consumers and could potentially increase market share—in an independent survey, >50% of respondents said that iridescent film packaging made the product more distinctive and unique than existing packaging
(www.idspackaging.comCommon/Paper/Paper_180/Building%20Brand%20Equity1.htm).

The objectives of this study were:
1. To determine the efficacy of film over-wrap treatments, made from iridescent films with different optical properties, in reducing light oxidation in 2% milk as determined by gas chromatography (GC) and gas chromatography olfactometry (GCO).
2. To determine the effect that visible excitation wavelengths of riboflavin (400, 446

and 570 nm) have on the production of volatile and odor-active compounds in 2% milk.

3. To determine the effect that visible excitation wavelengths of riboflavin have on riboflavin concentration.

<div align="center">**Materials and Methods**</div>

Milk Processing and Packaging

Milk was processed following the procedure reported in Chapter III, Effect of Specific Wavelengths on Volatile Chemistry and Production of Aroma-Active Compounds in Milk in the Milk Processing and Packaging section (pg 56).

UHT pasteurized milk was collected under a laminar flow hood (Atmos-Tech Industries, Ocean, NJ) in 2 L sterile glass Erlenmeyer flasks covered in aluminum foil so as to eliminate transmission of light. Milk (25 ml) was added to sterile 40 ml borosilicate glass vials containing flea magnetic stirrers and capped with screw lids fitted with Teflon septa. Vials were over-wrapped with one of six film treatments.

The complete experiment, from milk collection through 28 d of shelf life, was replicated three times with evaluation of all parameters occurring as described in the following sections. For each replication (n=3), 18 vials were over-wrapped with each film treatment (n=6) for a total of 54 vials per treatment.

Packaging Treatments

Six packaging treatments, designed to block either a single visible riboflavin excitation wavelength or all visible riboflavin excitation wavelengths, were used in this study (Table 5.1). Excitation wavelengths in the UV range (250, 270 and 370) were blocked by the packaging material so only the effect of visible wavelengths were tested in this experiment. Packaging treatments were created using film over-wraps made from iridescent film (Aurora® Standard Films, 9231 series, Engelhard Corporation, Iselin, NJ). The over-wrap films were transparent with a slight iridescent hue to them. They are made of 226 layers of micro-thin polymers and reduce light transmission through interference rather than absorption. Four different materials were used, either singly or in

combination, to provide wavelength control in the 400, 446, and 570 nm wavelength regions. Two control treatments were used for comparison. A light-protected treatment, made by wrapping the vials with aluminum foil, blocked all UV and visible wavelengths, while a light-exposed treatment allowed between 50-75% transmission of wavelengths between 300 and 340 nm and 80% of wavelengths above 340 nm. Figure 5.1 shows the wavelength spectra from 300 nm to 700 nm for all treatments.

Table 5.1. Makeup of the packaging treatments[1] used in study, the number of layers of film used in each treatment and the major wavelengths that each treatment blocked.

Packaging Over-wrap Treatment	Treatment Composition[1]	# of Layers of Film	Major Wavelengths Blocked
400 nm block	9231 Blue-Violet	4	385-415 nm
446 nm block	9231 Blue-Green	4	425-520 nm
570 nm block	9231 Red-Green	4	520-580 nm
Broad spectrum	2 layers 9231 Blue-Violet 1 layer 9231 Blue-Green 1 layer 9231 Red-Red	4	370-460 nm, 525-580 nm
Light Protected	Aluminum Foil	NA	All visible and UV
Light Exposed	No film or Foil	NA	No visible, UV below ~300 nm

[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ
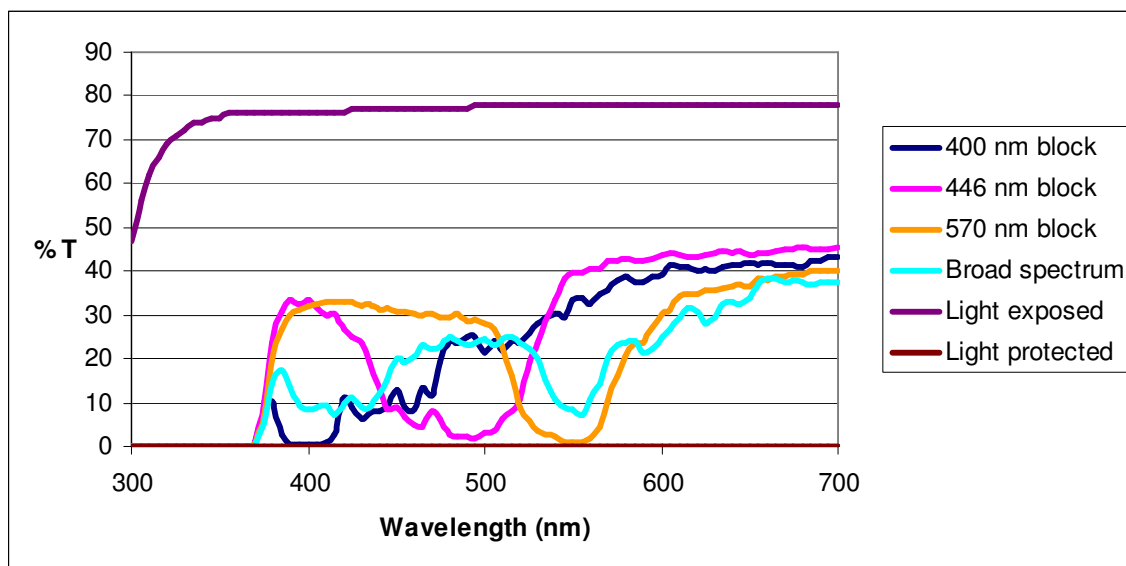
Figure 5.1. % Light transmission of wavelengths between 300 and 700 nm for
packaging treatments[1]
[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ

Packaging material blocked 100% of the UV riboflavin excitation wavelengths of 250
and 270 nm. However, it did allow a small amount (< 0.6%) of transmission of 370 nm.
Because this transmission was so low, we considered this wavelength to be sufficiently
blocked and did not contribute to oxidation. Therefore, only the visible riboflavin
wavelengths (400, 446, and 570 nm) were responsible for photo-oxidation in this
experiment. Three treatments blocked a single visible excitation wavelength for
riboflavin: the 400, 446 and 570 nm block treatments. A broad spectrum treatment
blocked all three wavelengths. However, the broad spectrum treatment did not block the
individual riboflavin excitation wavelengths to the same degree as the single wavelength
block treatments. Table 5.2 and Figure 5.2 show the % transmission for the visible
riboflavin excitation wavelengths for the packaging treatments.

116

Table 5.2. % Transmittance ± SEM (n=5) for packaging treatments[1] blocking a single visible riboflavin excitation wavelength (400, 446 and 570 nm block) or all visible riboflavin excitation wavelengths (Broad spectrum).

Treatment	% Transmittance			
	370 nm	400 nm	446 nm	570 nm
400 nm block	0.5 ± 0.1a	0.7 ± 0.1b	8.2 ± 1.4c	37.1 ± 1.4a
446 nm block	0.6 ± 0.1a	21.6 ± 4.8a	3.6 ± 1.1c	37.4 ± 3.7a
570 nm block	0.4 ± 0.1a,b	31.2 ± 2.2a	32.1 ± 1.8a	5.6 ± 1.9c
Broad spectrum	0.1 ± 0.1b,c	9.7 ± 3.9b	19.1 ± 3.4b	17.8 ± 4.1b

[a,b,c] Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD
[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ
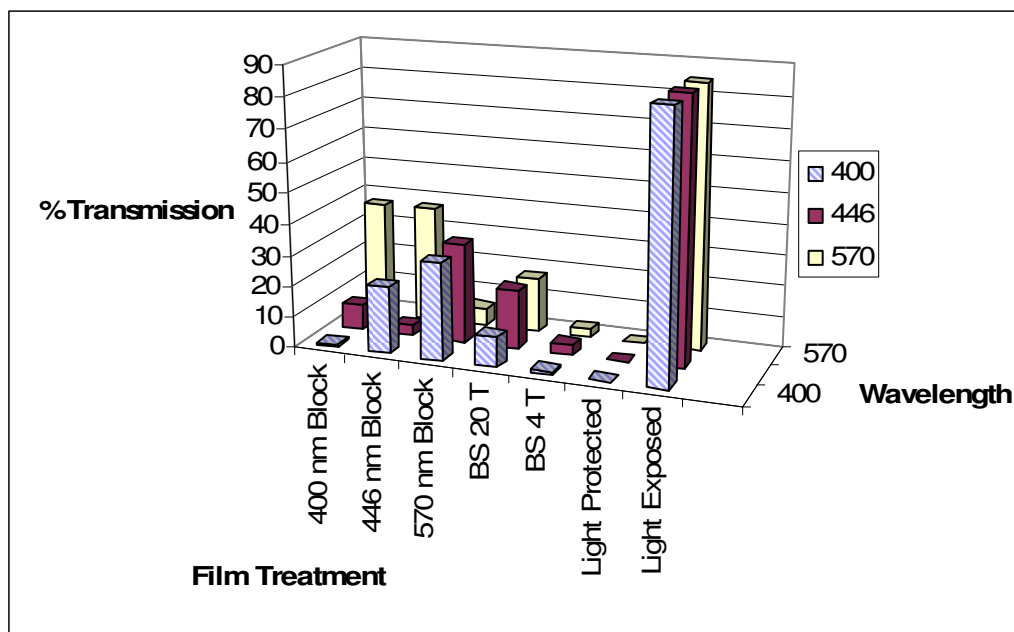

Figure 5.2. % Transmission of visible riboflavin excitation wavelengths (400, 446 and 570 nm) for each packaging treatment[1]
[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ
Packaging treatments (Aurora Standard films, 9231 series) were tested for percent light

Film treatments (Aurora® Standard films 9231 series, Engelhard Corp., Iselin NJ) were tested for percent light transmittance using a Shimadzu UV-2101PC UV-VIS scanning spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). The spectrophotometer was set with a 2.0 nm slit width, scanning from 300-700 nm with a sampling interval of 1.0 nm.

Characterization of Milk

Measurement of water/total solids (AOAC 990.20-Solids (Total) in Milk), ash (AOAC 930.30-Ash of Dried Milk), crude protein (AOAC 991.22-Protein Nitrogen Content in Milk) and fat (AOAC 995.18 Babcock method-Fat in Cream and AOAC 989.04 Babcock method-Fat in Milk) were conducted on UHT pasteurized milk (AOAC 1990). Samples were tested in triplicate. Water/total solids and ash samples were dried in a drying oven (Blue M Electric Company, Model OY-490A-2, Blue Island, Illinois) at 120º C for 4 hrs and ash samples were further heated in a muffle furnace at 500º C for up to 24 hrs (S/B Lindberg, Watertown, Wisconsin). Protein analysis was done using a mini keldjhal system (Rapid Still II, Labconco Corp., Kansas City).

Microbiological Analysis

Microbial analysis was performed by the procedure described in Chapter IV Reduction of Photo-oxidation in Milk Using Novel Polymer Materials to Block Riboflavin Excitation Wavelengths-Single Layer Films (pg 87).

Milk Storage Under Fluorescent Lighting

Vials were randomly arranged under 854 ± 161 lux fluorescent lighting in a 4º C walk-in cooler (Tonka, Hopkins, Minn.) for up to 28 days. Bottles were laid horizontally under lights, exposing approximately 20 cm^2 (3.09 in^2) of surface area to the light. Lighting consisted of two Phillips cool white fluorescent bulbs (30-34 W) per light fixture with four fixtures total being used in the experiment. There were no significant differences in light intensity for any light fixture (Appendix B1). Light intensity was tested using a light meter (Extech Instrument Corp., Walthaun, MA) at the beginning and end of each experimental replication.

Freshly pasteurized milk was analyzed on day 0 for volatile oxidation products, riboflavin concentration, and microbial count. Three vials of each of the six treatments were randomly pulled from the cooler on days 3, 7, 10, 14, 21, and 28 and analyzed for

volatile oxidation products, riboflavin concentration and microbial count. Three vials of each of the six treatments were randomly pulled on days 7, 14, 21 and 28 for determination of odor active compounds, using GCO, by a trained sensory panel.

Lipid Oxidation Analysis Using Headspace Gas Chromatography

Volatile oxidation compounds were analyzed according to the procedure reported in the Lipid Oxidation Analysis Using Headspace Gas Chromatography section in Chapter III (pg 59).

Odor-active Compound Analysis Using Gas Chromatography Olfactometry (GCO)

Training. Eight panelists (7 females, 1 male), consisting of graduate students and staff from the Food Science and Technology (FST) department and graduate students from the Human Nutrition, Foods and Exercise (HNFE) department and Biological Systems Engineering (BSE) department at Virginia Tech, were trained in the recognition of aromas and odors common to beer and milk.   Training consisted of using Beer Aroma Recognition standards and Beer Taint reagents (Brewing Research International, UK) to develop a memory and lexicon for these aromas (Table 5.3). Panelists were tested for ability during training by performing blind aroma recognition analyses of the standards and reagents. All panelists were able to correctly recognize and name at least 70% of the aromas and odors. Panelists trained for a total of six hours.

Table 5.3. Beer Aroma standards/reagents[1] used for training sensory panelists for the recognition of off-odors in milk

Beer Aroma Recognition Standards	Beer Taint Recognition Reagents
Sweetcorn	Dimethyl sulfide (DMS)
Malty	Phenolic
Late hop	Medicinal
Rose floral	Musty
Lemon floral	Diacetyl
Citrus	Rancid
Spicy	Cheesy
Grssy	Cardboard
Banana	Catty
Pineapple	Papery
Phenolic	Cooked vegetable
Butterscotch	Onion

[1] Brewing Research International (BRi), Nutfield, UK

Sensory Testing-Three panelists, selected for their availability and ability, evaluated aromas/odors on days 7, 14, 21 and 28 of the experiment. A GCO sniff port (ODO II, SGE International, Ringwood, Australia) attached to an HP 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a FID was used to carry the aromas of the separated compounds to the panelists. Panelists evaluated both the type and intensity of the odors. Intensity was evaluated on a scale from 1 to 5 where 1 meant "slight" and 5 meant "very strong". Average ratings were converted to category data of slight (1-2) denoted by a +, medium (3) denoted by ++, and strong (4-5) denoted by +++. An odor was reported if two out of three panelists detected it.

GCO samples were adsorbed onto a 75 µm Carboxen-PDMS SPME fiber as described in the previous section. A DB-5 ms capillary column (30 m x 0.25 mm id x 0.25 µm film thickness; J & W Scientific, Agilent Technologies, USA) was used to separate the volatile compounds. Helium was used as the carrier gas at a flow rate of 32 cm/sec. Temperature program was the same as described previously. Integration of peaks was done using an HP 3396A Integrator (Hewlett Packard, Palo Alto, CA).

120

Three replications of the GCO analysis were performed and data is reported as the combination of three replications. Average ratings were converted to category data of slight (1-2) denoted by a +, medium (3) denoted by ++, and strong (4-5) denoted by +++.

Riboflavin Analysis

Riboflavin was analyzed on an Agilent 1100 series High Performance Liquid Chromatograph (HPLC) (Agilent Technologies) equipped with HP ChemStation Software (Rev. A.09.03[1417], Hewlett Packard, Palo Alto, CA) following a modified method used by Toyosaki and others (1988). The HPLC was equipped with a Waters Spherisorb reversed phase C8 analytical column (4.6 x 10 mm, 5 µm id, Waters Corporation, Milford, MA) and the absorption wavelength was set at 447 nm (Huang and others, 2004). Solvent phase consisted of a water-methanol (80:20) mixture with 50 ml/L acetic acid added and solvent flow rate was 1 ml/min. A 10 µl sample was injected.

Samples were prepared by adding milk (1 ml) to 2% acetic acid (1 ml) at pH 3.17 and centrifuged at 15,000 rpm (22,870-27,821 g) in an SM-24 rotor (Dupont Instruments, Wilmington, DE) in a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Dupont Instruments, Wilmington, DE) for 60 min. The supernatant was filtered through a 0.2 µm filter into amber 2 ml vials with rubber septa. Standards were made from riboflavin (Sigma Chemical, USA) and deionized water and prepared in the same way as the samples and analyzed at the same time as the samples.

Statistical Analysis

Means with standard error of the mean and/or standard deviation were calculated for light intensity of the light banks and for proximate analysis of milk. Means were determined for the GCO data. Standard least squares and Tukey's HSD were performed on the gas chromatography data to determine significant differences in volatile oxidation compounds among treatments. These analyses were performed using JMP 5.1 Statistical Discovery Software (SAS Institute Inc, 1989-2004).

121

Results and Discussion

Characterization of Milk

UHT pasteurized milk for all three replications met compositional and microbial quality criteria (Table B2) and were within the range of published values (Saxelin and others 2003).

Lipid Oxidation

Several compounds, pentanal, hexanal, 1-octene-3-ol and an unidentified compound with a retention time of 5.793 increased in concentration over time. Other identified and unidentified compounds, such as 2-butanone, were detected but did not change over time and are therefore not addressed in this paper. Hexanal, pentanal and 1-octene-3-ol have been used by several investigators as a measure of lipid oxidation (Min and Schweizer 1983; Kim and others 2003).

Hexanal concentration was significantly higher in the light-exposed treatment and the packaging treatments than the light-protected treatment by day 3 and this finding remained true through day 28 (Fig. 5.3). Table B3 (appendix) shows the concentrations of hexanal ± standard deviations for all treatments from day 3-28 and indicates significant differences for all days and treatments. The packaging treatments (400, 446, 570 nm block treatments and the broad spectrum treatment) were not significantly different from one another in hexanal concentration throughout the experiment. However, several treatments were significantly lower in hexanal concentration than the light-exposed treatment on several days of the experiment (Table B3). The 400 nm block treatment appeared to control conditions to limit hexanal production, secondary to the light-protected treatment. This treatment blocked light of 400 nm, but allowed transmission of 446 and 570 nm. These results are in agreement with Mortensen and others (2003) who found that hexanal increased significantly in Havarti cheese when exposed to 366, 405, and 436 nm and that the highest increases were when the cheese was exposed to 405 nm. This is also in agreement with work in our laboratory which

122

found that UV light between 200 and 400 nm produced significantly higher amounts of hexanal than exposure to 516, 567 and 610 nm and, although not statistically significant, 395 and 463 nm and full light.
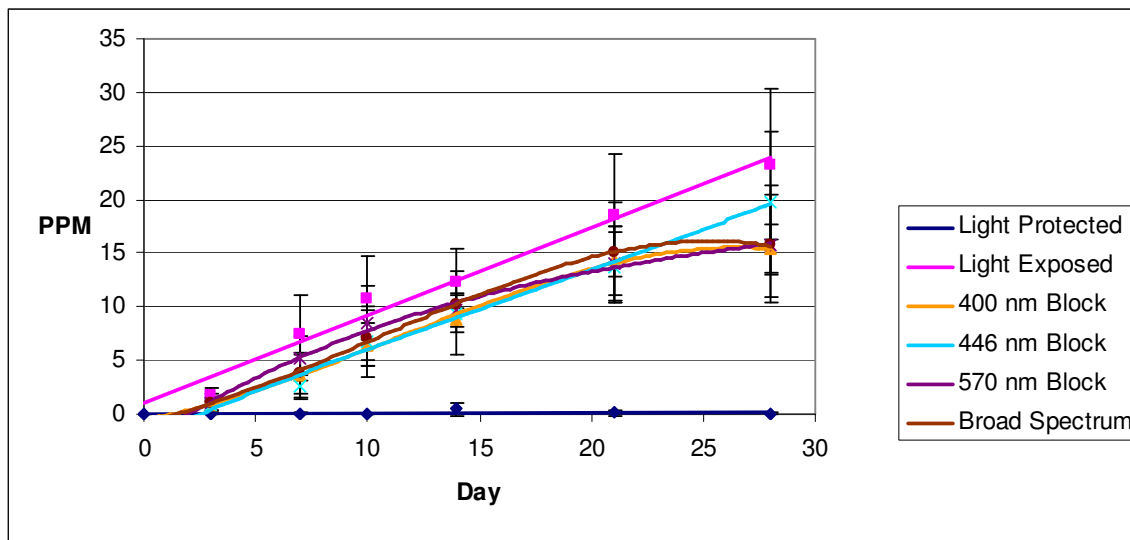

Figure 5.3. Hexanal concentration ± standard deviation in 2% milk held at 4º C after 0, 1, 4, 7, 10, 14 , 21 and 28 days exposure to 854 ± 16 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and one that blocked all riboflavin excitation wavelengths (broad spectrum).

Pentanal was detected in all treatments except the light-protected control. On all days, except for day 14, the light-exposed treatment had a significantly higher concentration of pentanal than the light-protected treatment (Fig. 5.4, Table B4). The film treatments (400, 446, 570 nm block treatments and the broad spectrum treatment) were not significantly different from each other in pentanal concentration, the light-exposed treatment or the light-protected treatment.   Concentrations were slightly lower than when milk was over-wrapped with a single layer of iridescent film. One exception occurred on day 7 when the 570 nm block treatment had a significantly higher pentanal concentration than the light-protected treatment. Our laboratory found that exposure of milk to light of 610 nm produced higher amounts, although not statistically significant, of pentanal than exposure to full light and 395, 463, 516, and 567nm. All film over-wrap treatments allow in substantial amounts of this wavelength, between 30 and 45% transmission. Therefore

123

it is not surprising that this compound was produced in relatively equal amounts in all treatments. Pentanal odor was only detected on day 7 in the 446 nm block treatment.
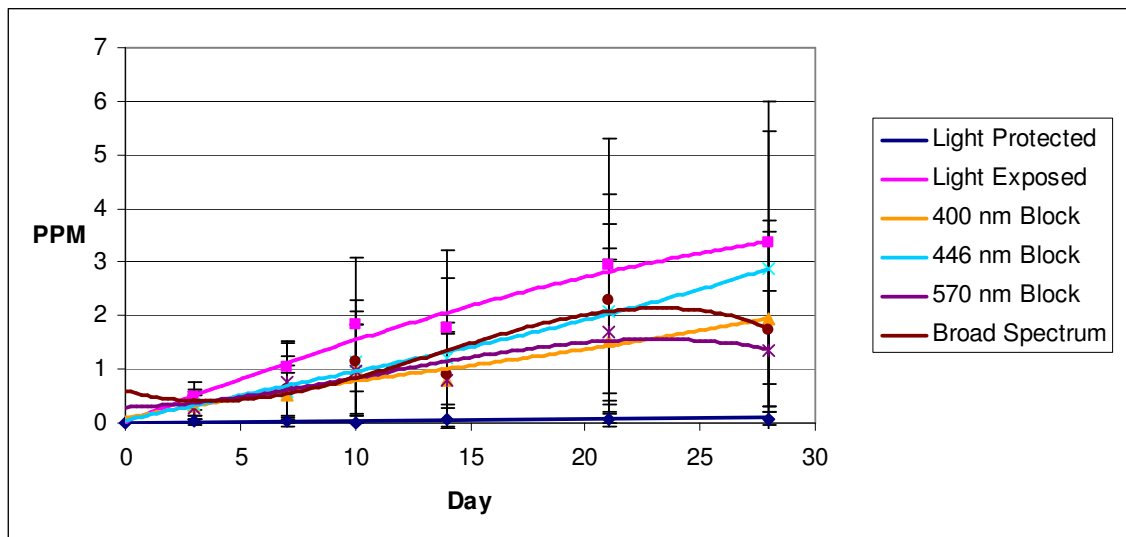

Figure 5.4. Pentanal concentration ± standard deviation in 2% milk held at 4° C after 0, 1, 4, 7, 10, 14 , 21 and 28 days exposure to 854 ± 16 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and one that blocked all riboflavin excitation wavelengths (broad spectrum).

One-octene-3-ol is a compound resulting from oxidation of linoleic acid (Lee and Min 2003). On all days, except for day 21, the light-exposed treatment had a significantly higher concentration of this compound than the light-protected treatment. The film treatments (400, 446, 570 nm block and the broad spectrum treatment) had similar concentrations of this compound throughout the experiment and no one treatment appeared to protect better against production of 1-octene-3-ol than any other (Fig. 5.5, Table B5). This is in agreement with data from our laboratory that found no significant differences in 1-octene-3-ol concentration regardless the wavelength of exposure.
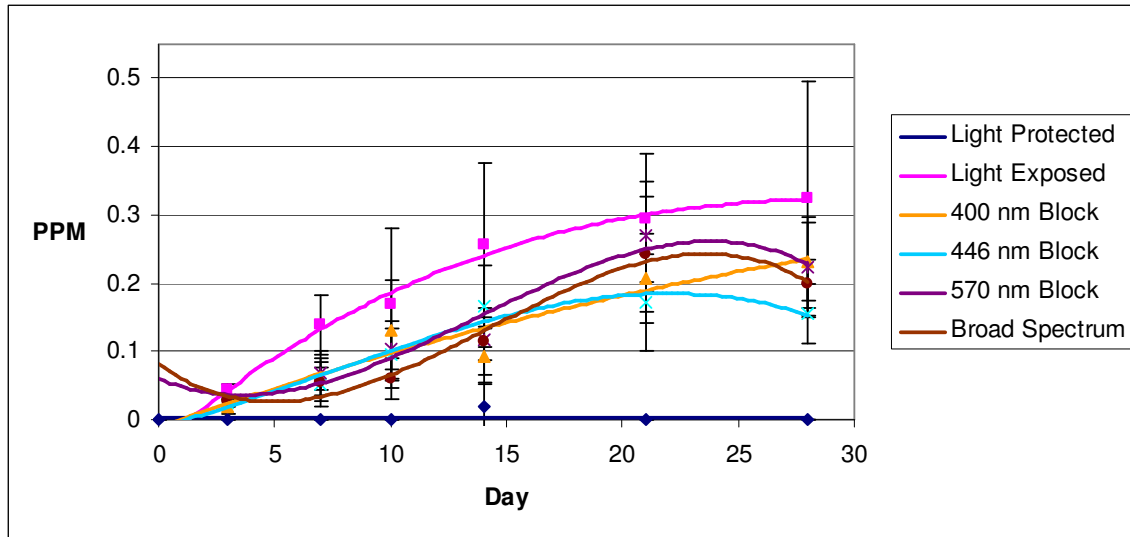
Figure 5.5. 1-Octene-3-ol concentration ± standard deviation in 2% milk held at 4º C after 0, 1, 4, 7, 10, 14 , 21 and 28 days exposure to 854 ± 16 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and one that blocked all riboflavin excitation wavelengths (broad spectrum).

A compound with a retention time of 5.8 min was not identified, but did demonstrate increased peak area counts over time (Fig. 5.6, Table B6). Significant differences in area counts for this compound occurred after 7 days of light exposure. From day 7 through 28, the light-exposed treatment had significantly higher concentrations than the light-protected treatment for this compound, except on day 10.   The film treatments (400, 446, 570 nm block treatments and the broad spectrum treatment) were all similar to one another on all days of the experiment. The 446 and 570 nm block treatment were significantly lower in concentration than the light-exposed treatment for this compound on day 7 and 21, while the 400 nm block and broad spectrum treatments were only significantly lower than the light-exposed treatment for this compound on day 21. No one treatment appeared to protect against production of this compound more than any other.

125
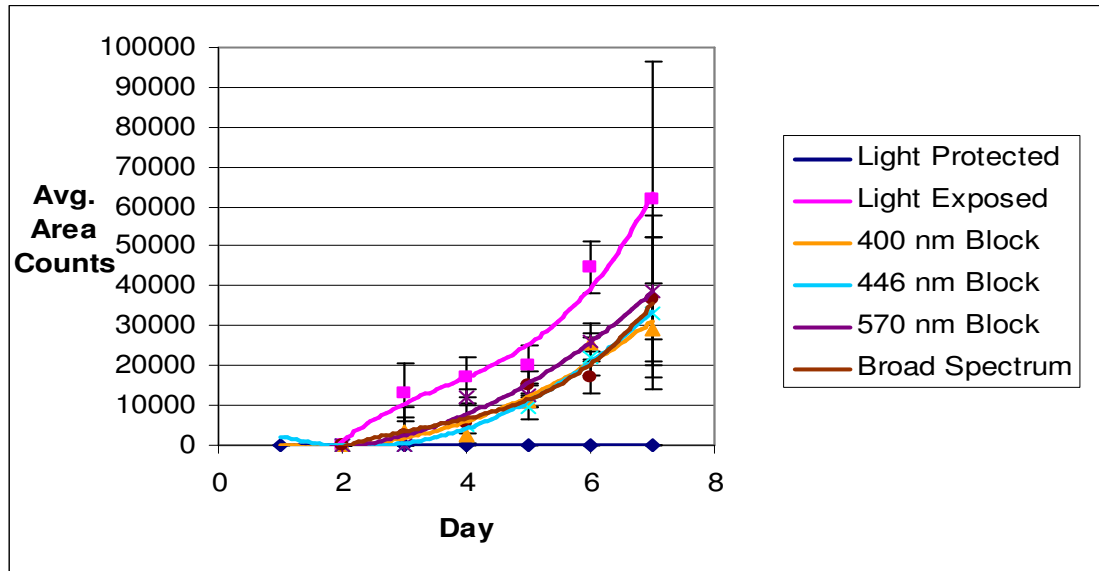
Figure 5.6. Average area counts ± standard deviation for an unidentified compound with a retention time of 5.793 in 2% milk held at 4º C after 0, 1, 4, 7, 10, 14 , 21 and 28 days exposure to 854 ± 16 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and one that blocked all riboflavin excitation wavelengths (broad spectrum).

One striking difference between this experiment, using multi-layer film over-wraps, and the experiment using single layer film over-wraps, is that we are not seeing the increase in concentration followed by a decrease trend that was seen in the single layer treatments. It is possible that the reason for this is that because the % transmission of light is substantially reduced for the multi-layer film treatments compared to the single layer film treatments and this reduction is delaying the natural progression of oxidation. Concentrations of volatile compounds were less than in the single layer film over-wrap experiments, however, not tremendously so.

Production of Odor-Active Compounds

GCO was used to identify and rate the intensities of odor-active compounds that contributed to light oxidation in milk. Tables 5.3-5.4 show the compounds, their retention times, aromas, and aroma intensities, that were prominent in the six treatments on days 7 and 21 of the experiment. The aromas reported in these tables were ones that

were detected by at least 2 out of 3 panelists for each replication (n=3). Aromas reported for days 14 and 28 can be found in the appendix (B7-8).

Table 5.4. Retention time, compound identification, aroma and average intensities of odor-active compounds in milk exposed to 854 ± 16 lux fluorescent light for the combined replications (n=3) for all treatments on day 7 of the experiment.

RT	Compound	Aroma	Wavelength					
			400 nm Block	446 nm Block	570 nm Block	Broad spectrum	Light Exposed	Light Protected
2.05-2.53		Flatulance	+	+	+	+	+	+
3.42-3.78	Pentanal	Green	—	+	—	—	—	—
3.58-3.95		Cooked/ Baked/ Sweet	+	—	+	—	—	+
4.75-5.36	Hexanal	Grass	++	++	++	+	++	
5.82-6.13	2-Heptanone	Roasted Grain/ Baked/ Musty	—	++	—	—	—	+
6.03-6.07		Greens	—	—	—	+	—	—
6.07-6.52		Musty/ Cardboard/ Mushroom	+++	—	+	—	+	—
6.45-6.92		Cardboard/ Musty	+	—	—	+	+	—
6.47-6.52	Heptanal	Grass	—	++	—	—	—	—
6.49-6.93		Roasted Grain	—	—	—	+	—	+
6.71-6.79	1-heptanol	Green/ Floral	—	—	—	—	+	—
7.09-7.78	1-Octene-3-ol	Mushrooms	+	—	+	—	+	—
7.16-7.35		Baked/ Cookies/ Cooked	+	—	—	—	—	+
8.21-8.64	Nonanal	Sugar/ Cooked/ Cookies	—	—	—	+	—	—
8.87-9.25		Sweet/ Baked Cookies	—	—	+	—	—	—
8.95-9.39		Chemical	—	—	—	—	+	—
9.64-9.87		Grass/ Floral	—	—	—	—	+	—
11.00-11.35		Musty/ Cardboard	—	+	—	—	—	—

+ = slight intensity, ++ = moderate intensity, +++ = high intensity

Table 5.5. Retention time, compound identification, aroma and average intensities of odor-active compounds in milk exposed to 854 ± 16 lux fluorescent light for the combined replications (n=3) for all treatments on day 21 of the experiment.

RT	Compound	Aroma	Wavelength					
			400 nm Block	446 nm Block	570 nm Block	Broad spectrum	Light Exposed	Light Protected
1.82-2.17		Flatulance	+	+	+	+	+	—
2.06-2.27		Astringent/Sharp/Acetone	+	—	+	—	+	+
2.90-2.97		Musty	—	—	—	—	+	—
3.42-3.65		Roasted	—	—	—	—	++	—
4.37-5.16	Hexanal	Green Grass	+++	++	++	++	++	—
4.82-4.87		Baked Bread/Roasted	+	—	—	—	—	—
5.54-5.94		Floral/Sweet Candy	—	—	—	—	—	+
5.70-6.05		Earthy/Mushroom	++	++	++	—	++	—
6.11-6.46	2-Heptanone	Roasted Grain	—	—	—	—	++	—
6.10-6.30		Earthy/Mushroom	—	++	++	+++	—	—
6.24-6.27		Roasted Grain	—	—	—	+	—	—
6.63-6.76		Roasted Grain	—	—	+	—	—	—
6.57-6.79		Musty	—	—	++	++	—	—
6.84-6.99		Earthy/Mushroom	++	++	++	—	++	—
6.99-7.13		Sweet/Baked	—	—	—	—	—	+
7.28-7.63	1-octene-3-ol	Mushrooms	++	+	+	++	++	—
7.59-7.80		Mushrooms	—	—	—	++	—	—
7.99-8.27	Nonanal	Baked Cookies/Cotton Candy	—	—	—	—	++	—
8.33-8.53		Baked	—	—	—	—	—	+
8.55-8.72		Baked Cookies/Sweet Candy	—	—	—	—	+	—
8.59-8.95		Musty	+	+	+	—	+	—
8.78-9.33		Baked/Caramel	—	—	—	—	—	+
9.05-9.16		Baked Cookies	+	—	—	—	—	—
9.08-9.18		Chemical/Musty	—	++	—	++	—	—
9.10-9.13		Astringent/Sharp	—	—	—	—	++	—
9.29-9.41		Baked/Roasted	—	—	—	—	+	—
9.47-9.85		Musty	+	+	+	—	++	—

+ = slight intensity, ++ = moderate intensity, +++ = high intensity

The light-exposed treatment, which allowed in significantly higher amounts of both visible and UV wavelengths than any other treatment, produced a higher number of odor-active compounds than all other treatments on days 7 and 21. However, on day 14, both the 570 nm and 400 nm block treatment, and on day 28, the 570 nm block and broad spectrum treatment, showed slightly higher numbers of odor-active compounds than the light-exposed treatment. This was consistent with the results we found for the single layer film over-wrap treatments, where the 570 nm block showed higher numbers of odor-active compounds than the other treatments. It also makes sense that the light-exposed treatment had higher numbers of odor-active compounds in this experiment on several days since the multi-layer film treatments allow in significantly lower transmission of light than the single layer film treatments. Thus it was expected that the light-exposed treatment, which allows in between 50-75% transmission of wavelengths between 300 and 340 nm and 80% of wavelengths above 340 nm would have more odor-active compounds. What was not consistent with the single layer film treatment findings, however, was that in this experiment, the 446 nm block treatment did not become highest in numbers of odor-active compound by the end of the experiment. Possibly, by reducing transmission of the major riboflavin absorption maximum so drastically, we have further delayed production of these compounds by this treatment and we did not see as much production of odor-active compounds. On all days, the light-protected treatment had the lowest number of odor-active compounds except for on day 14 where the broad spectrum treatment had an equal amount of odor-active compounds as the light-protected treatment. Interestingly, numbers of odor-active compounds decreased from day 21 to day 28. It is unknown why this occurred, but this trend was also seen with the single layer film over-wrap treatments as well. Possible explanations are that the compounds produced after 28 days of exposure to light became higher in molecular weight and the carboxen/PDMS SPME fiber may not have adsorbed these compounds as well as lower molecular weight compounds or the odor active compounds produced throughout the experiment degraded into non-odor-active compounds.

In general, intensity of odor-active compounds was low on day 7 and increased for compounds with increased duration of light exposure. The light-protected sample

generally had low odor intensity throughout the study and several compounds were described as having pleasant odors, such as sweet and baked. Odor-active compounds in the light-exposed and film treatments, in general, had higher odor intensities and had more compounds described as unpleasant odors than the light-protected sample.

Odor-active compounds detected in our experiment included pentanal, hexanal, 2-heptanone, heptanal, 1-heptanol, 1-octene-3-ol and nonanal. van Aardt and others (2005) found similar odor-active compounds (hexanal, 2-heptanone, n-heptanal, 1-octene-3-ol, octanal and nonanal) in light oxidized milk treated with antioxidants. However, van Aardt and others (2005b) also detected dimethyl disulfide (cooked milk aroma) which we did not. Possibly, this compound had dissipated by our first day of testing (day 7). Dimethyl disulfide is produced from protein oxidation, which occurs initially in milk, and dissipates within several days. van Aardt and others (2005b) tested for odor-active compounds after only 10 hrs exposure to 1100-1300 lux light, while we first tested for odor-active compounds on day 7. Cadwallader and Howard (1998) found hexanal (green, cut grass odor), 1-octene-3-one and 1-nonene-3-one (mushroom odor) and 1-hexene-3-one (plastic odor) in milk exposed to 2200 lux light for 48 hrs. In a review by Freidrich and Acree (1998), raw cow's milk was found to have the odor-active compounds ethyl butanoate (fruity, sweet), ethyl hexanoate (fruity, pineapple), heptanal (green, sweet), indole (fecal, putrid, musty, floral in high dilution), nonanal (sweet, floral), 1-octene-3-ol (mushroom-like) and dimethylsulphone (sulfurous).   Upon heating, the odor-active profile of the milk changed and four unique compounds arose: hexanal (green, cut grass), 2-nonanone (grassy-herbal, green-fruity), benzothiazole (quinoline, rubbery) and δ-decalactone (coconut). It is quite interesting that common odors that were detected on almost all days of the single layer film over-wrap experiments and were typically at medium or high intensities, such as "roasted grain", "grape nuts", "cardboard" and "musty", were not noticed at all or only sporadically in the multi-layer film over-wrap treatments. Apparently, the reduction in overall transmission of wavelengths (ie-percent reduction of transmission) and/or reduction in transmission of specific wavelengths reduced the amounts of these compounds being formed.

130

Three odor-active compounds showed up in all, or a majority of, treatments. In most cases these compounds were not detected in the light-protected treatment and there did not seem to be substantial differences in aroma intensity among the other treatments. These compounds included an unidentified compound that had a relative retention time of ~ 2.05 min and smelled of flatulence, hexanal which had a grassy or green odor, and 1-octene-3-ol which had an odor of mushrooms. These findings are in accordance with the results from the single layer film over-wrap study. Hexanal and 1-octene-3-ol were common odor-active compounds found in all of the single layer film over-wrap treatments and either were not found, or were lower in intensity, in the light-protected treatment. The unidentified compound with the flatulence odor did not seem to increase in intensity over the testing period nor was it consistently higher in intensity for any single treatment. These results are similar to those of the single layer film study, which is interesting because there was significantly higher percent transmission of light in the single layer treatments, yet no difference in the odor intensity or distribution for this compound was seen between the single layer and the multi-layer study.

Hexanal, which is used as an indicator of lipid oxidation (Min and Schweizer 1983) and has a grassy aroma, was found in all samples on all days, except for the light-protected treatment on days 7 and 21. Hexanal is a common odor-active compound in light exposed milk (Cadwallader and Howard 1998; Van Aardt and others 2005b). In general, the intensity of the grassy aroma was similar for all treatments, except in the light-protected treatment which was much less intense, and increased from day 7 to 21. By day 28, the intensity of the hexanal odor decreased somewhat in all samples except for the light-exposed treatment, in which it increased. These findings did not correlate with our concentration data for hexanal. The light-exposed treatment had significantly higher concentrations of hexanal than several film treatments on several days. However, there were no real differences in the intensity of hexanal odor for these treatments. One explanation for this could be that concentrations well over the threshold limit smell relatively similar even though they may have different concentrations. The threshold limit for hexanal is reported as being 0.339 ppm in milk (Norton 2003). Concentrations

131

of hexanal in our samples were substantially higher (Table C3) than this threshold yet were slightly lower than those produced in the single layer film over-wrap study.

One-octene-3-ol is a common odor-active compound in light exposed milk (Cadwallader and Howard 1998; van Aardt and others 2005b). This aroma increased slightly in intensity throughout the experiment in all treatments except in the light-protected control treatment, where it was generally not detected. Even though the light-exposed treatment was significantly higher in 1-octene-3-ol concentration than the light-protected treatment on all days of the experiment, the odor of the light-exposed treatment was not substantially different in intensity than any other treatment. On day 28, 1-octene-3-ol odor was not even detected for the light-exposed treatment, but the concentration for this treatment ($0.323^a \pm 0.171$ ppm) was likely above the sensory threshold on that day. One explanation for this could be that concentrations well over the threshold limit smell relatively similar even though they may have different concentrations. One-octene-3-ol has an odor threshold of 34 ppb in camembert cheese (Curioni and Bosset 2002). The concentration of 1-octene-3-ol in our samples was approximately ten times higher than the threshold level. However, this does not explain why no odor of 1-octene-3-ol was detected in the light-exposed sample on day 28 of the experiment. Possibly, this aroma was masked by another aroma coming off the column near this compound, or the odor changed as concentration increased and was not recognized as being from 1-octene-3-ol.

Riboflavin Analysis

Riboflavin concentration decreased over the 28 day period of the experiment in all treatments exposed to light, while the light-protected treatment did not change significantly (Fig. 5.7). These findings are in accordance with those reported by a number of investigators (Dimick 1973; Hoskin and Dimick 1979; Christy and others 1981; Hoskin 1988, Moyssiadi and others 2004).

132
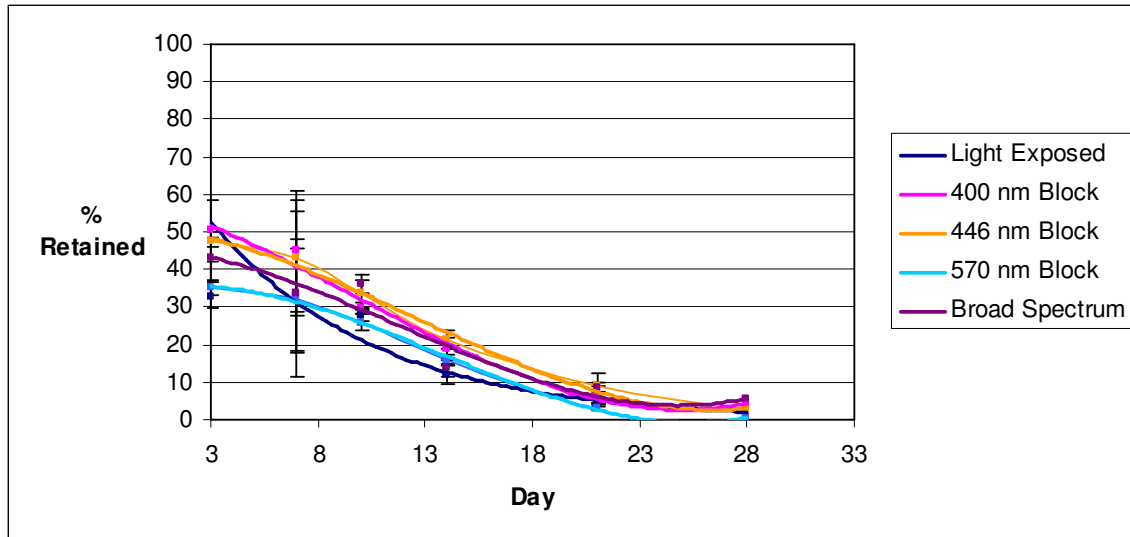
Figure 5.7. % retention ± standard deviation for riboflavin in 2% milk held at 4º C after 1, 4, 7, 10, 14, 21and 28 days exposure to 854 ± 16 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and one that blocked all riboflavin excitation wavelengths (broad spectrum).

No statistical differences were found in riboflavin loss between the light-exposed and the packaging treatments (400, 446, 570 nm block and broad spectrum treatment). By day 3, the light-exposed and packaging treatments had a loss of between 52.6 and 67.5% in total riboflavin. By the end of the experiment (day 28), there was a loss of between 94.7 and 97.9% in total riboflavin for the light-exposed and packaging treatments. These results are different from other investigators (Fukumoto and Nakashima 1975; Sattar and others 1977; Bradley 1980; Senyk and Shipe 1981) who found that blocking wavelengths between ~400 and 500 nm protected against riboflavin destruction. Gold or yellow pigment, which blocks light from 400-480 nm, partially protected riboflavin from destruction (Luquet and others 1977; Senyk and Shipe 1981) and Singh and others (1975) found yellow pigmented packaging material had the same protection as paperboard packaging against light oxidation flavor in milk. Sattar and others (1977) found that amber bottles gave complete protection against riboflavin destruction. Fukumoto and Nakashima (1975) determined the effect of colored filters on the degradation of riboflavin. When no filter was used there was a 60% destruction of riboflavin. Use of a

blue filter (400-520 nm) showed 20% destruction, a brown filter (500 nm) showed 30% destruction, and a red filter (>550 nm) showed 10% destruction.

It was expected that the 400 and 446 nm block treatments, as well as the broad spectrum treatment, would have less riboflavin destruction than the 570 nm block treatment and the light-exposed treatment. However, the treatments in our experiment did not reduce riboflavin destruction compared to the light-exposed treatment. Apparently, enough destructive wavelengths were able to go through the packaging material and cause riboflavin degradation in all treatments except the light-protected treatment. These results are consistent with the volatile compound production data. Riboflavin destruction is a measure of oxidation and there were no significant differences among treatments for both riboflavin degradation and volatile compound production.

Conclusions

GC analysis showed that the film over-wrap treatments reduced the production of volatile compounds in 2% UHT milk held at 4 C and exposed to 854 ± 161 lux fluorescent lighting, but this reduction was generally not significant. GCO analysis showed that the number of odor-active compounds produced in the film over-wrap treatments was generally the same, or even a little higher, than the light-exposed treatment. There was no difference in the rate of riboflavin degradation among the treatments and the light-exposed control treatment. Over-wrap treatments were not as effective as a complete light block in reducing the production of these compounds and it does not appear that any treatment reduced photo-oxidation more than any other.

135

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Chapter VI

Controlling Light Oxidation Flavor in Milk by Blocking Riboflavin Excitation Wavelengths Through Interference

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Abstract

Iridescent packaging materials that obstructed riboflavin excitation wavelengths (400, 446, and 570 nm) were evaluated for effectiveness in controlling light oxidized flavor in milk. Milk was packaged in glass bottles with film over-wraps that interfered at a single excitation wavelength (3 treatments) and at all excitation wavelengths (2 treatments). A balanced incomplete block multi-sample difference test using a ranking system and a trained panel was used for evaluation of light oxidation flavor intensity. Chemical analysis consisted of volatile compound evaluation using gas chromatography and riboflavin degradation using fluorescent spectroscopy. Packaging over-wraps limited the production of light oxidation flavor in milk over time but not to the same degree as the complete light block. Blocking all visible riboflavin excitation wavelengths was better at reducing light oxidation flavor than blocking only a single visible excitation wavelength. UV excitation wavelengths were blocked in all treatments. Riboflavin degraded over time in all treatments except the light-protected control treatment and only minor differences in the amount of degradation among the light-exposed control and over-wrap treatments was observed. Hexanal production was significantly higher in the light-exposed control treatment compared to the light-protected control treatment from day 7 to 21 but was only sporadically significantly higher in the 570 nm and 400 nm block treatments. Pentanal, heptanal, and an unidentified compound of low molecular weight also increased in concentration over time, but there were no significant differences in concentration among the packaging over-wrap treatments for these compounds.

Key Words: Photo-oxidation, Packaging, Riboflavin, Sensory, Milk, Hexanal, Pentanal, Heptanal

141

Introduction

Two important factors that affect the development of light-oxidized flavor in milk are light wavelength and packaging material. The effect of light wavelength has been studied by a number of investigators (Hansen and others 1975; Bradley 1983; Bekbolet 1990; Bosset and others 1995; Hansen and Skibsted 2000; Lennersten and Lingnert 2000; Gorgern 2003), although Mortensen and others (2004) feel that this topic has not been adequately considered and should be a priority topic for research. Many investigators have shown that visible light of low wavelength, between 365nm and 500 nm, causes a significant increase in light oxidation in milk (Herreid and Ruskin 1952; Bradfield and Duthie 1965; Sattar and others 1976a; Hoskin and Dimick 1979; Bosset and others 1995; Nielson 1999; Hansen and Skibsted 2000; Lennersten and Lingnert 2000; Van Aardt and others 2001). Riboflavin, found in high concentrations in the whey fraction of milk, is implicated in this oxidation because it acts as a photosensitizer and can initiate oxidation reactions (Sattar and others 1977; Bekbolet 1990). This flavin compound becomes excited when exposed to a number of different light wavelengths, principally wavelengths of 250, 270, 370, 400, 446 and 570 nm when in foods with a neutral pH (Kyte 1995). Josephson (1946), however, indicated that all wavelengths below 620 nm must be blocked in order to prevent photo-chemical changes that result in sunlight flavor in milk. Wold and others (2005) found that porphyrins and chlorins, which absorb wavelengths above 600 nm, significantly affected the production of light oxidation flavor in dairy products. Wold and others (2006) also found that exposure of cheese to both blue light (400-500 nm) and red light (>600 nm) gave similar results for oxidized odor and flavor.

Packaging material can have a protective effect on milk quality through blocking or reducing the transmission of certain light wavelengths. This protection is based on material thickness, processing conditions and material coloration (Mortenson and others 2004). The International Dairy Federation (IDF) recommends that light transmittance of a package should not exceed 2% for 400 nm wavelength and 8% for 500 nm wavelength (Bossett and others 1995; Rysstad and others 1998; Mestdagh and others 2005). Milk is

commonly packaged in high density polyethylene (HDPE), a translucent polymer which transmits up to 62% of light wavelengths between 300-700 nm. Polyethylene terephthalate (PET), a packaging material often utilized for single-serve milk products, is a clear polymer which transmits up to 75-85% of visible light. Ultra-violet (UV) absorbers can be added to polymer packaging materials, such as PET, to block UV wavelengths without affecting the clarity of the package and provide some protective effect against light oxidized flavor development (van Aardt and others 2001). The use of UV absorbers, however, does not affect the transmission of visible wavelengths which can have a damaging effect on milk.

Single-serve bottles for vending machine sales are typically made from PET and partially or completely covered in an opaque over-wrap. This opaque over-wrap reduces light transmission of all wavelengths and allows milk quality to remain high through a long shelf-life (60 days or longer) (Anonymous 2002). However, because consumers prefer to "see" the food they are buying (Sattar and deMan 1976b; Rosenthal 1992; Cladman and others 1998; Doyle 2004), marketing forces pressure companies to package even photo-labile foods, such as milk, in transparent materials which do not protect against quality deterioration through photo-oxidation. Therefore it is important to develop packaging materials that will enable a consumer to "see" the product within, yet block the most damaging wavelengths.

New polymer materials, with unique optical properties, have recently come on the market, of which iridescent and pearlescent films are examples. Iridescent films have a unique appearance of shifting color which is dependent upon the viewing angle. They work through light interference rather than the use of dyes and pigments. These films are translucent, satisfying the major consumer demand of being able to "see" the product within the package, and can be engineered to block specific light wavelengths, including those that are most damaging to milk quality. They can be laminated to complexly shaped containers and could easily be used as film over-wraps for single-serve milk containers. They also provide an upscale look that appeals to consumers and could increase market share. In an independent survey, >50% of respondents said that iridescent film packaging made the product more distinctive and unique than existing

143

packaging
(www.idspackaging.com/Common/Paper/Paper_180/Building%20Brand%20Equity1.htm).

The objectives of this study were:

1. To determine the efficacy of film over-wrap treatments made from iridescent films with different optical properties in reducing light oxidation flavor in 2% milk.
2. To determine the effect that the visible excitation wavelengths of riboflavin (400, 446 and 570 nm) have on the sensory properties of light exposed 2% milk.
3. To determine the effect that over-wrap treatments have on the production of secondary oxidation products as determined by the concentration of volatiles using solid phase micro-extraction-gas chromatography (SPME-GC).

144

Materials and Methods

Milk Processing and Packaging

Milk was processed following the procedure reported in Chapter III, Effect of Specific Wavelengths on Volatile Chemistry and Production of Aroma-Active Compounds in Milk in the Milk Processing and Packaging section (pg 56).

UHT processed milk (620 ml) was collected in 1 L sterile glass bottles under a laminar flow hood (Atmos-Tech Industries, Ocean, NJ) and capped with sterile aluminum foil and a screw-on lid. The complete experiment, from milk collection through 21 d of shelf-life, was replicated three times with evaluation of all parameters occurring as described in the following sections. For all replications of the experiment (n=3), seven bottles were over-wrapped with each film treatment (n=7) for a total of 49 bottles per experimental replication.

Packaging Treatments

Milk samples were randomly assigned to the seven packaging treatments, designed to block specific excitation wavelengths of riboflavin at different levels and combinations. Packaging treatments were created using film over-wraps (Aurora® Standard Films, 9231 series, Engelhard Corporation, Iselin, NJ) covering the glass surface. The over-wrap materials were transparent, with a slight iridescent hue to them (Table 6.1). Films are made of 226 layers of polymer and reduce light transmission through interference rather than absorption.

Table 6.1. Reflection color, transmission color and the visual appearance of Aurora[®] Standard Films[1] used to make packaging treatments.

Reflection/Color	Transmission Color	Visual
Red/Red	Blue	Sheer coppery tones
Red/Green	Blue/Pink	Pink-green shimmer
Blue/Green	Pink/Yellow	Blue-green highlighted with pink and yellow
Blue/Violet	Yellow	Hint of blue in a transparent violet

[1]Englelhard, Corp., Iselin, NJ (website)
(http://www.specialchem4polymers.com/sf/Engelhard/index.aspx?id=standardfilms)

Four film materials were used, either singly or in combination, to provide wavelength control in the 400, 446, and 570 nm regions either independently or for all three regions (Table 6.2). The 400 nm block treatment blocks > 99% of 400 nm, but only 92% of 446 and 63% of 570 nm. The 446 nm block treatment blocks > 96% of 446 nm but only 78% of 400 nm and 63% of 570 nm. The 570 nm block treatment blocks > 94% of 570 nm but only 69% of 400 nm and 68% of 446 nm. Two treatments blocked all three visible riboflavin excitation wavelengths. One blocked greater than 80% of all three wavelengths (the broad spectrum treatment with < 20% transmission of 400, 446 and 570 nm wavelengths, BS 20 T) and the other blocked greater than 96% of all three wavelengths (the broad spectrum treatment with < 4% transmission of 400, 446 and 570 nm wavelengths, BS 4 T). Film over-wrap treatments blocked all UV wavelengths below 370 nm so there was no excitation of riboflavin by UV wavelengths. A light-protected control treatment, which blocked all visible and UV wavelengths, was created by wrapping sample bottles with aluminum foil. A light-exposed control treatment, which blocked 100% of wavelengths < 300 nm, 25-50% of wavelengths between 300-340 nm, and 20% of wavelengths above 340 nm, represented the light barrier properties attributed to the glass packaging.

Table 6.2. Makeup of the packaging treatments[1] used in study, the number of layers of film used in each treatment and the major wavelengths that each treatment blocked.

Treatment Name	Treatment[1]	# of layers of film	Major Wavelengths blocked
400 nm block	9231 Blue-Violet	4	385-415 nm
446 nm block	9231 Blue-Green	4	425-520 nm
570 nm block	9231 Red-Green	4	520-580 nm
BS 20 T	2 layers 9231 Blue-Violet 1 layer 9231 Blue-Green 1 layer 9231 Red-Red	4	370-460 nm, 525-580 nm
BS 4 T	4 layers 9231 Blue-Violet 2 layers 9231 Blue-Green 2 layers 9231 Red-Red	8	370-460 nm, 525-580 nm
Light-Protected	Aluminum Foil	NA	All visible and UV
Light-Exposed	No film or Foil	NA	No visible, UV below ~300 nm

[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ
BS 20 T = broad spectrum treatment with less than 20% transmission of 370, 400, 446 and 570 nm. BS 4 T = broad spectrum treatment with less than 5% transmission of 370, 400, 446 and 570 nm

Figure 6.1 shows the percent transmission of all packaging treatments for wavelengths between 300 and 700 nm. Figure 6.2 shows the percent transmission of each of the three visible riboflavin excitation wavelengths (400, 446 and 570 nm) transmitted by the packaging treatments.
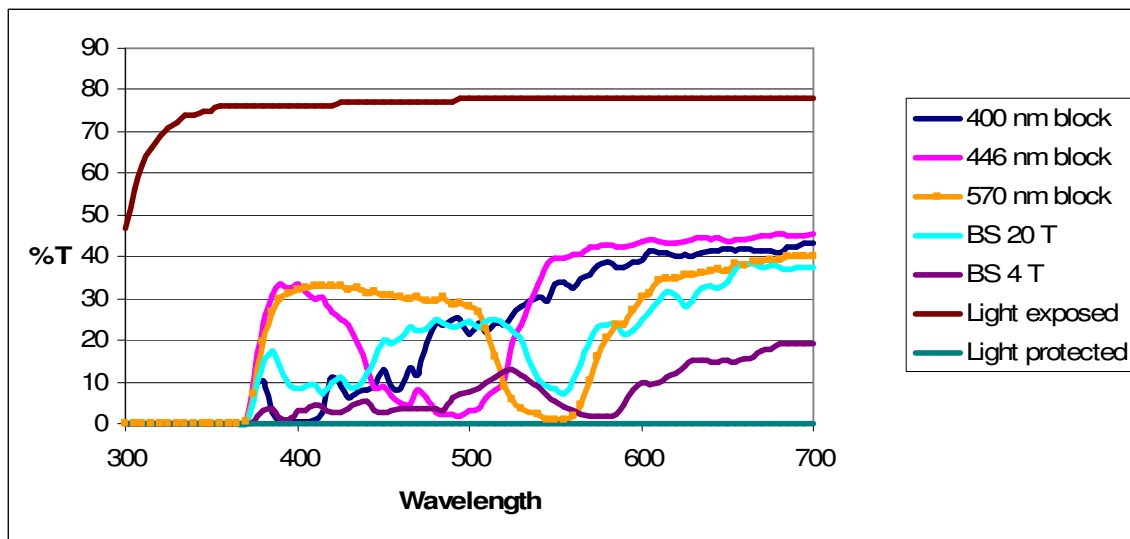
Figure 6.1. % Light transmission of wavelengths between 300 and 700 nm for packaging treatments[1]

[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ
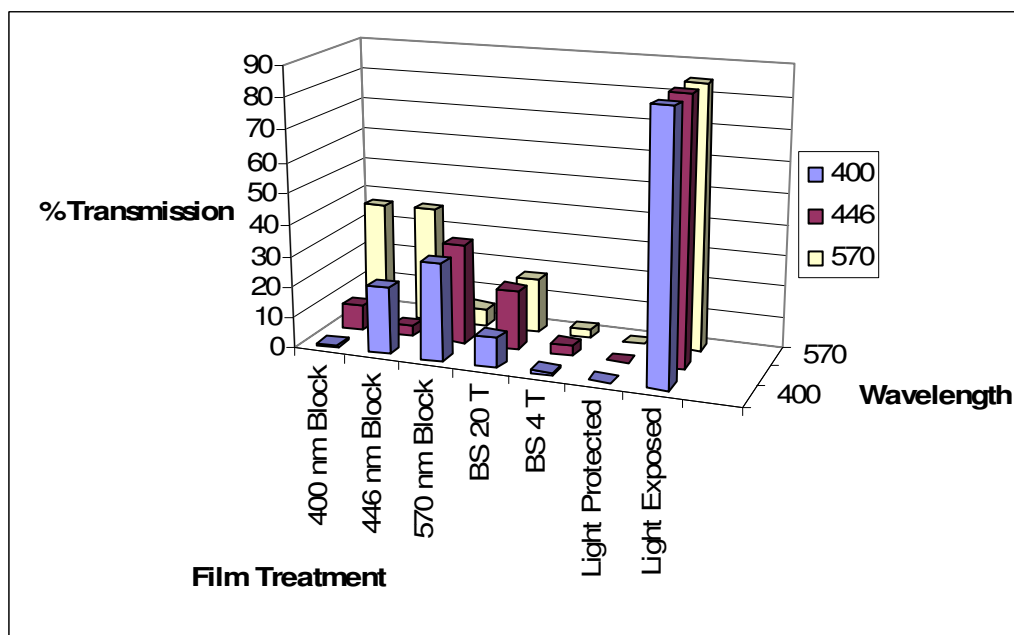
Figure 6.2. % Transmission of visible riboflavin excitation wavelengths (400, 446 and 570 nm) for each packaging treatment[1]

[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ

148

Film treatments (Aurora® Standard films 9231 series, Engelhard Corp., Iselin NJ) were tested for percent light transmittance using a Shimadzu UV-2101PC UV-VIS scanning spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Measurements were recorded for wavelengths of 370, 400, 446 and 570 nm. These wavelengths were chosen because at neutral pH, riboflavin absorbs and is excited by these wavelengths (Kyte 1995). The spectrophotometer was set with a 2.0 nm slit width, scanning from 300 to 700 nm with a sampling interval of 1.0 nm. Table 6.3 verifies the light transmittance for each packaging treatment at the visible wavelengths associated with riboflavin excitation.

Table 6.3. % Transmittance ± SEM (n=5) for packaging treatments[1] blocking a single visible riboflavin excitation wavelength (400, 446 and 570 nm block) or all visible riboflavin excitation wavelengths (4-layer combination and 8-layer combination).

Treatment	% Transmittance			
	370 nm	400 nm	446 nm	570 nm
400 nm block	$0.5^a \pm 0.1$	$0.7^b \pm 0.1$	$8.2^c \pm 1.4$	$37.1^a \pm 1.4$
446 nm block	$0.6^a \pm 0.1$	$21.6^a \pm 4.8$	$3.6^c \pm 1.1$	$37.4^a \pm 3.7$
570 nm block	$0.4^{a,b} \pm 0.1$	$31.2^a \pm 2.2$	$32.1^a \pm 1.8$	$5.6^c \pm 1.9$
BS 20 T	$0.1^{b,c} \pm 0.1$	$9.7^b \pm 3.9$	$19.1^b \pm 3.4$	$17.8^b \pm 4.1$
BS 4 T	$0.0^c \pm 0.0$	$0.8^b \pm 0.3$	$3.3^c \pm 0.5$	$2.6^c \pm 0.9$

[a,b,c] Means followed by the same letter are not significantly different at the P= 0.05 level experimentwise using Tukey's HSD
[1] Treatments were made from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ

Characterization of Milk

Proximate analysis of milk was done according to the procedure described in Chapter V, Reduction of Photo-oxidation in Milk Using Novel Polymer Materials to Block Riboflavin Excitation Wavelengths-Multi-layer films, Characterization of Milk section (pg 115).

Milk Storage Under Fluorescent Lighting

Bottles were randomly arranged under fluorescent lighting in a 4º C walk-in cooler
(Tonka, Hopkins, MN) for up to 21 days. Bottles were laid horizontally under lights,
exposing approximately 146.77 cm^2 (22.75 in^2) of surface area to the light. Air volume
in the headspace was 360 ml. Lighting consisted of two cool white fluorescent bulbs (30-
34 W) per light fixture with four fixtures total being used in the experiment. The overall
average intensity of light exposure at the bottle surface was 1521 ± 24 lux. There was no
significant difference in light intensity between light fixtures (Table C1). Light intensity
was tested using a light meter (Extech Instrument Corp., Walthaun, MA) at the beginning
and end of each experimental replication.

Freshly pasteurized milk was analyzed on day 0 for volatile oxidation products and
microbial count. One bottle from each treatment was randomly sampled on days 1, 4, 7,
10, 14, and 21 and analyzed for light oxidation flavor intensity, volatile oxidation
products, riboflavin concentration, and microbial quality.

Microbiological Analysis

Microbial analysis was performed by the procedure described in Chapter IV Reduction of
Photo-oxidation in Milk Using Novel Polymer Materials to Block Riboflavin Excitation
Wavelengths-Single Layer Films (pg 56).

Lipid Oxidation Using Headspace Analysis Gas Chromatography

Volatile oxidation compounds were analyzed according to the procedure reported in the
Lipid Oxidation Analysis Using Headspace Gas Chromatography section in Chapter IV
(pg 59)

150

Riboflavin Analysis

Riboflavin concentration in milk was analyzed using the fluorometric method (AOAC Method 970.65) and was measured on a Shimadzu RF-1501 spectrofluorophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Standards and calibration curves were made according to AOAC Method 970.65 (AOAC 1990). Riboflavin was obtained from Sigma Chemical (St. Louis, MO) and stored in the dark.

Sensory Analysis

Sensory Panelist Training. A triangle test for difference was used to screen for panelists who were able to discriminate light oxidation flavor in milk. Panelists (n=30) were given three triangle tests and asked to determine the "different" sample. All tests used UHT milk (Parmalat, Parma, Italy) bought from a local grocery store and samples consisted of a comparison of light-exposed (12, 24, or 48 hrs) and unexposed milk. Light exposure intensity was 1521 ± 24 lux. Panelists who were correct in 2 of 3 triangle tests (n=22) were asked to undergo further training for the sensory panel for this experiment. Panelists were trained individually for approximately 1 ½ hours over a 2 week period in the determination of light oxidation flavor and intensity in milk. Training consisted of learning a lexicon of descriptors for light oxidation flavor, light oxidation flavor recognition, and ranking of the intensity of light oxidation flavor in milk that had been oxidized under 1521 ± 24 lux light for varying time spans (2 to 48 hrs). Panelist performance was evaluated through ranking of light oxidation intensity. Panelists selected to continue on the panel had at least a 75% correct response rate in ranking order.

Sensory Analysis of Treatments. A balanced incomplete block (BIB) design multi-sample difference test was used for the sensory analysis. Samples were presented according to design 11.7 of Cochran and Cox (SIMS 2000, Sensory Information Management System, Sensory Computer Systems, LLC). Three samples were presented simultaneously to each panelist, and panelists were asked to rank samples in order of

151

increasing light oxidation flavor, where 1 indicated the lowest intensity and 3 indicated the highest intensity. Panelists were instructed to make a definite decision, even when light oxidation intensity was similar. A total of fourteen panelists were used for the test and each panelist ranked 3 sets of three samples (total of 9 samples) on days 1, 4, 7, 10, 14 and 21 of each of three experiments. In the rare event that there were not enough trained panelists to complete the sensory panel, persons who had previous experience in the detection of light oxidation flavor in milk, but had not been specifically trained for this experiment, were used.

On each testing day, ~ 25 ml of sample was poured into a 40 ml plastic cup, labeled with a 3-digit code for sample identification, capped and stored at 4º C. Milk was presented to the panelists at refrigeration temperature. Panelists were seated in individual booths, lighted with white light, and given unsalted crackers and filtered tap water to be used between sample sets to cleanse the palate. Panelists were instructed to wait for 1 minute between sample sets to avoid sensory fatigue. All panelists completed human consent forms for each day of testing (Figures C1 and C2). This research was reviewed and approved by the Institutional Review Board (IRB) at Virginia Tech.

Statistical Analysis

Sensory Data. The rank sums for all three replications were combined and T values determined using a Friedman-type test statistic ($T = [12/\rho\lambda t(k+1)] \Sigma R^2 - 3(k+1)\rho r^2/\lambda$ where ρ= the number of repetitions of the fundamental BIB design, λ= the number of times all pairs are evaluated in a single BIB repetition, t= total number of samples, k = number of samples each panelist evaluated in a single BIB repetition, and r = number of times a sample was evaluated in a single BIB repetition). A T value exceeding the upper 5% critical value for X^2 with (t-1) = 6 degrees of freedom ($X^2_{6, 0.05}$ = 12.60) indicated significant differences in the data set. A nonparametric analog of Fisher's LSD for rank sums ($LSD_{rank} = t_{\alpha/2, \infty} \sqrt{\rho(k+1)(rk-r+\lambda)/6}$) was used to determine which treatments were significantly different from one another (Meilgaard and others 1999).

152

Other. Means with standard error of the mean were calculated for light intensity of the light banks, proximate analysis for each of the three replications and for microbiological data. Standard least squares and Tukey's HSD were performed on the gas chromatography data to determine significant differences in volatile oxidation compounds between treatments. These analyses were performed using JMP 5.1 Statistical Discovery Software (SAS Institute Inc, 1989-2004).

Results and Discussion

Characterization of Milk

UHT pasteurized milk used for all three experimental replications met compositional and microbial quality criteria (Tables C2 and C3) and were within the range of published values (Saxelin and others 2003).

Analysis of Light Oxidation Flavor in Milk

<u>Combined Replications.</u> Significant differences in light oxidation flavor was found on all sampling days, except day 1, with T values exceeding the upper 5% critical value for X^2 with 6 degrees of freedom. Table 6.4 shows the overall ranking in light oxidation flavor for combined data for all sampling days. By combining the rank sums for all treatments for all days and replications, the overall ranking order for treatments was Light Exposed[a] >570[b]>400[c]>446[d]>BS 20 T[d,e]>BS 4 T[e]>Light Protected[f] (treatments with different letters indicate significantly different intensity of light oxidation flavor using a nonparametric analog to Fisher's LSD at the P<0.05 level).

Table 6.4. Ranking of packaging treatments, from most oxidized to least oxidized, for light oxidation flavor for days 1-21 for all three replications combined.

				Day		
Overall Rank	**1**	**4**	**7**	**10**	**14**	**21**[†]
	NS	LE[a]	LE[a]	LE[a]	LE[a]	LE[a]
Most oxidized	NS	570[b]	570[b]	400[b]	570[b]	570[a,b]
↑	NS	BS 20 T[b]	446[b]	570[c]	400[c]	446[a,b]
	NS	400[b,c]	BS 20 T[b]	446[c]	BS 4 T[c]	400[a,b]
↓	NS	BS 4 T[b,c]	400[b]	BS 20 T[c,d]	BS 20 T[c,d]	BS 4 T[b,c]
Least oxidized	NS	446[c]	BS 4 T[b]	BS 4 T[d]	446[d]	BS 20 T[c]
	NS	LP[c]	LP[c]	LP[e]	LP[e]	LP[d]

[a,b,c,d]Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD
[†]Day 21 combined only replications 1 and 2 due to a sampling error in replication 3.

NS=not significant, LE=light-exposed, LP=light-protected, 400=400 nm block treatment, 446=446 nm block treatment, 570=570 nm block treatment, BS 20 T= Broad Spectrum treatment with < 20% riboflavin excitation wavelengths, BS 4 T = Broad Spectrum treatment with < 4% transmission of riboflavin excitation wavelengths.

Milk in the light-protected control treatment, which was over-wrapped with aluminum foil and blocked all visible and UV wavelengths, was significantly less light oxidized, as expected, than all other treatments from day 7 through day 21. This supports evidence from other studies that a complete barrier to light is most effective in controlling light-induced flavor in milk (Kristoffersen and others 1964; Hellerup-Nielsen 1973; Hong and others 1995).

Milk in the light-exposed control treatment, which allowed 75-78% transmission of light wavelengths from 340-700 nm, 50-75% of wavelengths from 300-340 nm, and completely blocked light < 300 nm, was higher in light oxidized flavor than all other treatments from day 4 through 14; thereafter milk in the single wavelength block treatments (570, 446, and 400 nm block treatments) increased sufficiently in light oxidized flavor so as not to be distinguishable from the light-exposed control by day 21.

155

Percent light transmission of these packaging treatments was below 6% for the particular wavelength that the treatment blocked but between 8 and 37% for the other riboflavin excitation wavelengths (Table 6.3).

In general, the single excitation wavelength block treatments and the broad spectrum treatments were intermediate in light oxidation flavor between the light-exposed treatment and the light-protected treatment. The single wavelength block treatments tended to rank higher in light oxidized flavor, overall, than the broad spectrum treatments. Milk in the 570 nm block treatment was consistently more oxidized than all other packaging treatments, but was still discernable from the light-exposed sample from day 4 through 14. This finding is somewhat in agreement with the findings from the multi-layer film study (Chapter V) that showed higher amounts of odor-active compounds being produced in the 570 nm block treatment but is different from the single layer film study (Chapter IV) that showed that the film treatments had higher amounts of odor-active compounds than the light-exposed control treatment. In general, compounds that have odor also have taste. Milk in the 400 and 446 nm block treatments varied from day to day in rank for light oxidation flavor. On all days, the 400 nm block treatment was either similar or significantly higher in light oxidized flavor than the 446 nm block treatment. These findings are in agreement with a number of investigators (Herreid and Ruskin 1952; Bradfield and Duthie 1965, Hoskin and Dimick 1979; Nielsen 1999; Van Aardt and others 2001) who found that blocking wavelengths between 380 and 500 nm gave greater protection against light oxidation flavor than blocking other wavelengths. It also indicates that blocking 446 nm is important in reducing light oxidation flavor, since both the 400 and 570 nm block treatments, which transmit this wavelength, tended to be higher in light oxidation flavor than the 446 nm block treatment. This finding is in agreement with the results from our single layer film study (Chapter IV) that found a delay in the production of odor-active compounds for the 446 nm block treatment.

The broad spectrum treatments that blocked riboflavin excitation wavelengths to less than 20% or 4% transmission were, in general, lower in light oxidation flavor than the single wavelength block treatments. Even though all riboflavin excitation wavelengths were

blocked, these treatments did not protect against the production of light oxidation flavor as well as the light-protected treatment, which blocked all visible and UV wavelengths. One explanation may be that other wavelengths, besides those that excite riboflavin, excite other sensitizers involved in the production of light oxidation flavor. Wold and others (2005, 2006) found that chlorophyll a and b, hematoporphyrin and protoporphyrin acted as sensitizers in photo-oxidation of cheese and contributed significantly to the production of sunlight flavor and oxidation odor. An alternative explanation may be that transmission of the blocked wavelengths in these treatments, while low, may still be sufficient to produce photo-oxidation reactions and result in light induced flavor.

Interestingly, on no day was there a significant difference in light oxidation flavor between the broad spectrum treatment with less than 4% transmission of riboflavin excitation wavelengths (BS 4 T) and the broad spectrum treatment with less than 20% transmission of riboflavin excitation wavelengths (BS 20 T). This was surprising because there was statistically higher light transmission in the BS 20 T treatment than in the BS 4 T treatment for 446 nm and 570 nm. The BS 20 T treatment also had higher transmission of 400 nm than the BS 4 T treatment, but the difference was not statistically significant (Table 6.3). For both of these treatments, even though all riboflavin excitation wavelengths were blocked, even to <4% transmission, light oxidation flavor intensity was significantly higher than the light- protected treatment, which blocked 100% of all UV and visible wavelengths.

A ranking system was used in this research to measure light oxidation off-flavor, rather than a rating system. The use of ranking provides a relative order of oxidation intensity allowing a measurement of protective efficiency. However, this method gives less information than a rating system because the interval in oxidation intensity of each treatment cannot be determined. Ranking is best used when the panelists are moderately trained and we felt that this was the best method to use based on the length of our study. Because we did not use a rating scale, we are unable to describe the intensity of light oxidation flavor in each treatment in relation to the light-exposed or light-protected control treatments.

157

Panelists were asked to note any other flavor descriptors associated with the treatments to elucidate possible changes in flavor due to wavelength exposure. On several days during the experiment, panelists described bitter off-flavors. This bitter flavor showed up only in the single block treatments during the latter part of the experiment: the 400 nm block on day 21 of replication 2 and 3, the 446 nm block on day 21 of replication 3, and the 570 nm block on day 14 of replication 3. Sour and spoiled were other descriptors for this flavor in the 570 nm block treatment. It is hypothesized that the bitter off-flavor is due to protein oxidation. Bitter flavor in cheese is associated with the accumulation of hydrophobic peptides that occur due to an imbalance between proteolysis and peptidolysis (Fallico and others 2005).   Further research into this phenomenon and the effect that light wavelength has on the production ofbitter off flavor is ongoing.

Light Transmission of Packaging Materials for Protection Against Light Oxidation Flavor. The International Dairy Federation (IDF) states that light transmittance of packaging material should not exceed 2% at 400 nm and 8 % at 500 nm in order to maintain optimal milk flavor (Bosset and others 1995; Rysstad and others 1998; Mestdagh and others 2005). A number of investigators (Coleman 1976; Hoskin and Dimick 1979; Christy and others 1981) found that reducing transmission of wavelengths between 400-500 nm to only 1-3% total transmission produced no off-flavor development in milk. Our BS 4 T treatment had 0.8 ± 0.3 % transmission at 400 nm, 3.3 ± 0.5 % transmission at 446 nm, and 4.78 ± 1.4 % transmission at 500 nm—satisfying IDF specifications and being only slightly higher in transmission of these wavelengths than investigators that had no off-flavor production (Coleman 1976; Hoskin and Dimick 1979; Christy and others, 1981). Thus, the BS 4 T treatment should have had sensory characteristics similar to the light-protected treatment. However, our results showed that on all days (7 through 14), the light-protected treatment was significantly lower in light oxidation flavor than the BS 4 T treatment. These results suggest that the limits of light transmission set by IDF are not adequate to stop the production of light oxidation flavor in milk. Rysstad and others (1998) found similar results. UHT milk packaged in polyethylene coated paperboard, which allowed ~0.4% transmission of 400 nm and ~6% transmission of 500 nm wavelengths, meeting IDF specifications, showed light oxidized

158

flavor after 6 weeks exposure to light at room temperature. Milk packaged in X type boards and aluminum foil packaging, which allowed virtually no light transmission between 400 and 500 nm, did not have any off-flavor production. Light wavelengths above or equal to 446 nm may play an important role in the induction of light oxidation flavor in milk based on evidence in this study. Christy and others (1981) had percent transmission rates below 3% and showed no off-flavor production while our 8-layer film combination treatment exceeded 3% transmission between 446 and 500 nm and did show off-flavor production. Riboflavin absorbs most strongly at 446 nm. On the other hand, wavelengths above 500 nm or between ~370 and ~390 nm, may also be important in the production of light oxidation flavor in milk through the excitation of photosensitizers other than riboflavin.

The BS 20 T treatment allowed 9.7 ± 3.9 % transmission at 400 nm, 19.1 ± 3.4 % transmission at 446 nm, and 27.6 ± 0.5% transmission at 500 nm; much higher than the 8-layer film combination treatment. The BS 20 T treatment had higher transmission of 400 nm than the BS 4 T treatment, but the difference was not statistically significant. Even with these large differences in percent transmission between the two film over-wraps, there was no significant difference in light oxidation flavor intensity between these two treatments on any day of the experiment. One can conclude, therefore, that there may be other photosensitizers in milk besides riboflavin that contribute to light oxidation flavor. Wold and others (2005) identified other compounds in addition to riboflavin that contributed to light-induced oxidation of cheese. Chlorins and porphyrins, specifically chlorophyll a and b, protoporphyrin, and hematoporphyrin, become excited upon exposure to wavelengths in the UV and violet/blue region, but also throughout the visible spectrum (642-662 nm for chlorins and 500-633 for porphyrins) and contributed significantly to light induced oxidation in Norvegia cheese. These compounds are observed in most, if not all, dairy products (Wold and others 2005). Both the BS 20 T and BS 4 T treatments allow ≥ 10% transmission of wavelengths between ~ 510 and 540 nm and ~600 and 700 nm and ≥ 5% transmission of wavelengths between ~370 and 390 nm. Chlorophyll a has absorption maxima at 410, 430 and 662 nm (Rebeiz and others 1972); chlorophyll b at 453 and 642 nm (Rebeiz and others 1972); protoporphyrin at 404,

159

502, 536, 576 and 633 nm (Rebeiz and others 1972; and hematoporphyrin at 402, 500, 532, 569, 596 and 623 nm (Granick and others 1952). The degradation of chlorins and porphyrins correlated better than riboflavin degradation to sun flavor and acidic flavor in Norvegia cheese as reported by Wold and others (2005). Sattar and others (1976) found that fat oxidation was affected mostly by wavelengths shorter than 455 nm indicating riboflavin activity and wavelengths above 595 nm indicating the activity of unidentified blue-green components.

Protection Against Light Oxidation Flavor by Colored Films. The iridescent films used in this study reduce light transmission through interference rather than absorption (www.specialchem4polymers.com/sf/Engelhard/index.aspx?id=standardfilms). Visually there is a slight hue that changes as the angle of incidence changes. Colored packaging materials, which absorb specific light wavelengths, have been used to study the effect of wavelength on light oxidation flavor and odor. Pigmented glass or PET materials have been used in a number of research studies for protection against light oxidation flavor in milk. Herreid and Ruskin (1952) found that ruby glass bottles were most protective against sunlight flavor, followed by amber, paper and clear glass bottles. Ruby glass blocks 100% of light below 600 nm. Hendrickx and deMoor (1962) found that yellow, red and brown-black containers offered protection from light oxidation in milk. Hoskin and Dimick (1979) found that yellow polycarbonate containers, which blocked light between 380-480 nm, gave intermediate protection against light oxidation flavor production in milk, between fiberboard containers and clear glass, HDPE, and clear polycarbonate. Milk in the tinted polycarbonate containers had a significantly lower (P<0.05) hedonic rating than the control milk and milk packaged in fiberboard after 24 to 48 hrs, while milk packaged in clear containers (polycarbonate, HDPE and glass) had significantly lower hedonic ratings after only 12 hrs light exposure.

van Aardt and others (2001) found that milk packaged in amber colored polyethylene terephthalate (PET), which blocks wavelengths below 450 nm, was significantly lower in light oxidation flavor than milk packaged in glass, HDPE, clear PET, and clear PET with a UV blocker when held at 4 C and exposed to light of 1100-1300 lux for up to 18 days.

Clear PET with a UV blocker had significantly less light oxidation flavor than milk packaged in glass, HDPE and clear PET, but not amber PET, after 7 days exposure but not after 18 days. Cladman and others (1998) found that whole and 2% milk packaged in green PET had the lowest amount of oxidation, as determined by increases in conjugated dienes, over an 18 day period when exposed to four 60 W cool white fluorescent lights, compared to clear PET, and LDPE pouches and jugs. Oxidation was significantly lower ($P<0.05$) in whole milk packaged in the green PET after 14 days and after 10 and 18 days in 2% milk. Hansen and others (1975) looked at the effect that differently colored plastic shields had on the oxidation of milk packaged in polyethylene (PE) containers and exposed to 200 ft candles (2152 lux) of light. Yellow and dark green filters prevented the development of light oxidized flavor in milk (32 hr exposure at 4º C) to a greater extent than pink, light green and smoky shields. The yellow and dark green shields absorbed light between 400-500 nm. Wold and others (2006) found similar results to Hansen and others (1975) with the exposure of Norvegia cheese having least degradation in flavor when exposed to light and covered with a green filter. Degradation increased with use of yellow, orange, red, white and violet (most degradation) filters.

The 400 and 446 nm block treatments blocked wavelengths between 400 and 500 nm. Although we did see as a general trend that blocking these wavelengths reduced light oxidation flavor intensity, we found evidence that blocking wavelengths from 400 to 600 nm (BS 20 T and BS 4 T treatments) did a better job at reducing off-flavor production. This is similar to results of other investigators (Herried and Ruskin 1952; Hendrickx and deMoor 1962; Hansen and others 1975; Sattar and others 1976; Hoskin and Dimick 1979; Cladman and others 1998; van Aardt and others 2001; Wold and others 2006). However, blocking these wavelengths was not as effective as blocking all wavelengths (a complete light block as seen in our light-protected control treatment).

Lipid Oxidation

<u>Headspace Analysis Using GC-SPME</u>. A number of different compounds, mostly saturated aldehydes, were produced in milk upon exposure to fluorescent light. Farrer (1983) also found mainly saturated aldehydes as oxidation by-products. Identified compounds included pentanal, hexanal, heptanal, octanal and nonanal. Only hexanal

161

showed significant differences in concentration as a result of packaging treatment (Table C4). The light-exposed treatment was significantly higher in hexanal concentration than the light-protected treatment from day 7 to day 21 with a change in concentration from 0.07 ppm to about 0.58 ppm. The light-protected treatment consistently had measured hexanal concentrations of about 0.05 ppm or less. These concentrations are substantially lower than concentrations reported in Chapters IV and V, and is likely due to the reduced surface to volume ratio of the 1 liter bottles used in this experiment compared to the surface to volume ratio in the 40 ml vials used in both of the film study experiments. Hexanal concentration in film protected treatments demonstrated increased hexanal concentrations over time but generally had concentrations less than the light-exposed control. These concentrations were generally not statistically separated from either control. Figure 6.3 shows increases in hexanal concentration over time. Off-flavor compounds produced from sensitized oxidation of milk identified in the literature include acetaldehyde, methyl sulfide, dimethyl disulfide, propanal, n-pentanal, n-hexanal, heptanal, nonanal, 3-methyl butanal, 2-methyl propanal, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, and 2-nonanone (Mehta and Bassett 1978; Bekbolet 1990; Cadwallader and Howard 1998; Rysstad and others 1998; van Aardt and others 2001; Mestdagh and others 2005).
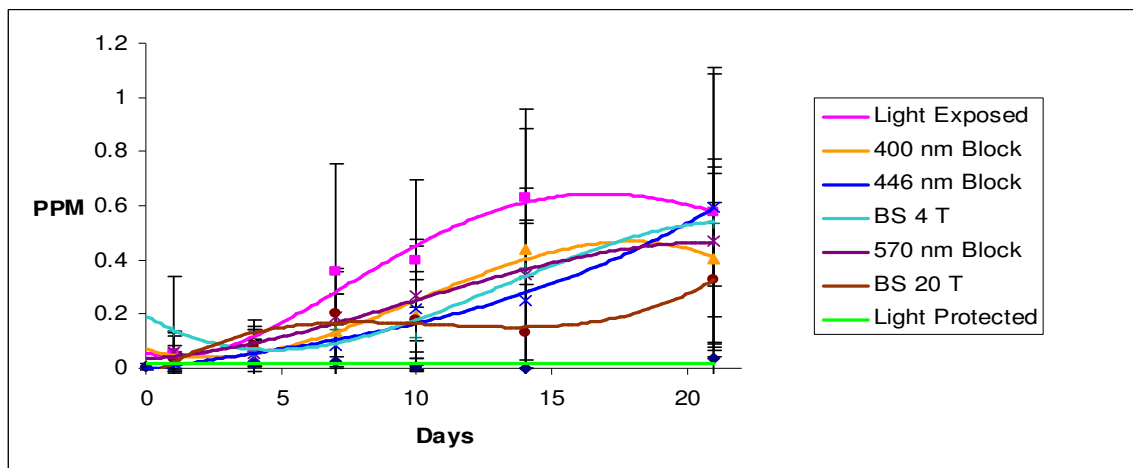
Figure 6.3. Hexanal concentration ± standard deviation in 2% milk held at 4º C after 0, 1, 4, 7, 10, 14 and 21 days exposure to 1521 ± 24 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and all riboflavin excitation wavelength block treatments (BS 20 T and BS 4 T treatments).

162

Hexanal is reported to be a good measure of lipid oxidation, correlating well with sensory evaluation results (Fritsch and Gale 1977; Warner and others 1978; Robards and others 1988; Anderssen and Lingnert 1998; Lennersten and Lingnert, 2000). Hexanal concentration generally correlated to our sensory evaluation results in that the light-protected treatment had the lowest hexanal concentration and the lowest intensity of light oxidation flavor, while the light-exposed treatment had the highest concentration of hexanal, on most days, and was highest in light oxidation flavor intensity. Also, the BS 20 T and BS 4 T treatments, which were generally lowest in light oxidation flavor intensity were not significantly different in hexanal concentration than the light-protected treatment on several days. However, hexanal concentration did not correlate closely enough to explain the differences in light oxidation flavor of the film over-wrap treatments completely. Hedegaard and others (2006) also found that hexanal concentration did not correlate completely with sensory characteristics. They did not see significant differences in hexanal concentration in milks that had very different sensory characteristics.

A number of other compounds, specifically pentanal, heptanal and an unidentified compound (figures 6.4-6.6, Table C5-7) showed increasing concentrations over time but did not show significant differences between treatments. Mestdagh and others (2005) found that even though pentanal and dimethyl disulphide (DMDS) did not increase significantly in transparent PET with UV blocker, the taste panel could taste off-flavor in this treatment. Day and Lillard (1960) concluded that oxidized flavor came about due to a combination of compounds and that combinations of carbonyl compounds below threshold levels were additive and able to give rise to an overall off flavor.   This may explain how our treatments had different light oxidation flavor intensities, yet did not have significantly different concentrations of volatile compounds.
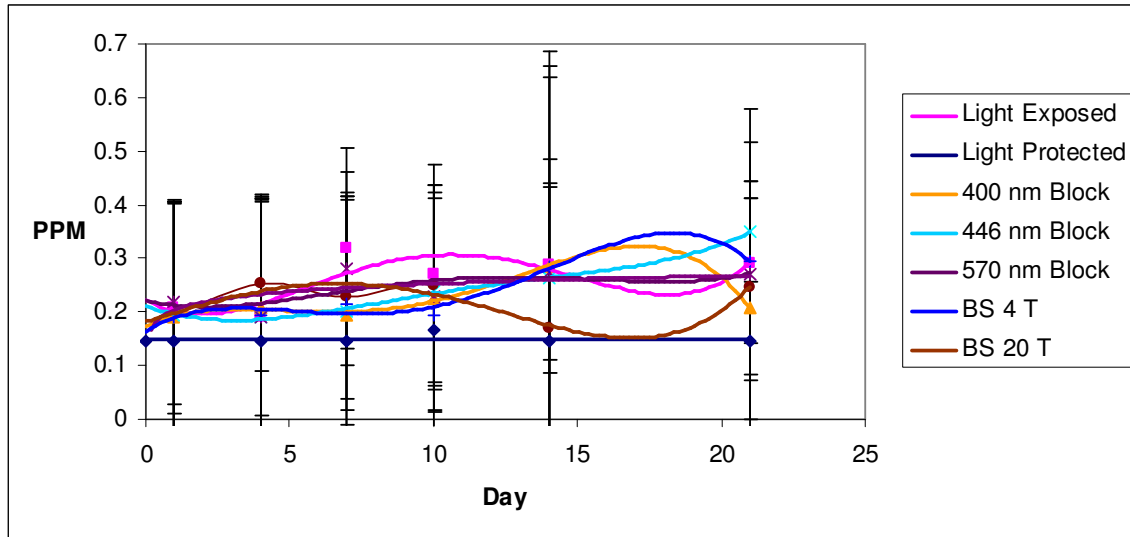
Figure 6.4. Pentanal concentration ± standard deviation in 2% milk held at 4º C after 0, 1, 4, 7, 10, 14 and 21 days exposure to 1521 ± 24 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and all riboflavin excitation wavelength block treatments (BS 20 T and BS 4 T treatments).
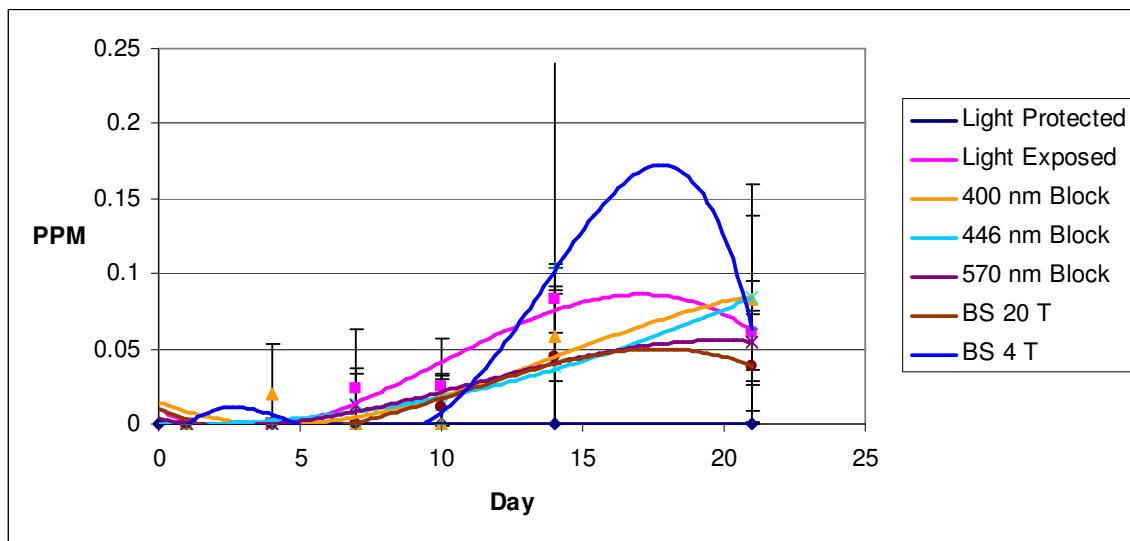
Figure 6.5. Heptanal concentration ± standard deviation in 2% milk held at 4º C after 0, 1, 4, 7, 10, 14 and 21 days exposure to 1521 ± 24 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and all riboflavin excitation wavelength block treatments (BS 20 T and BS 4 T treatments).

164
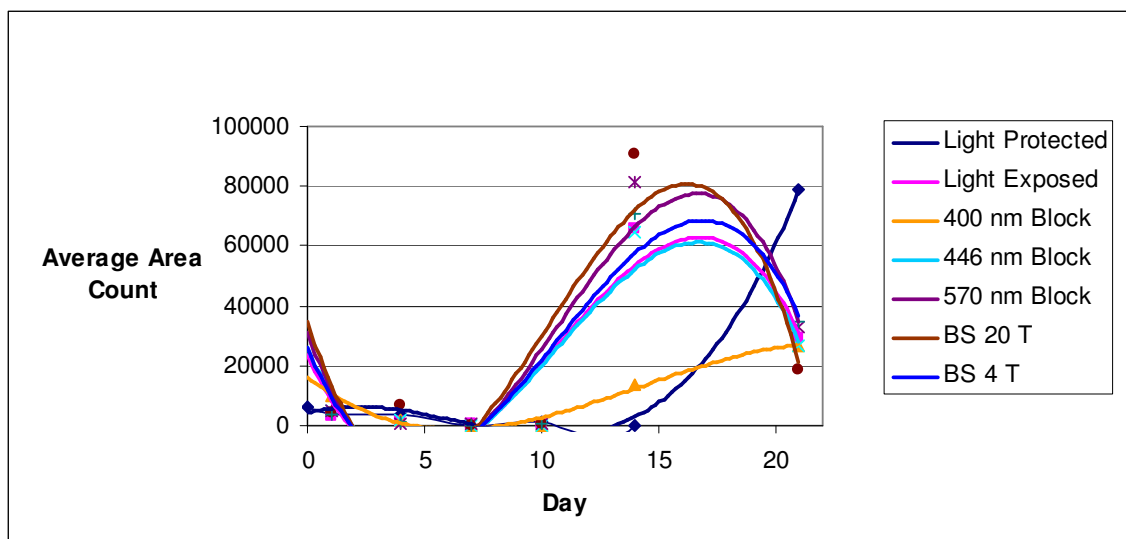
Figure 6.6. Average area counts for unidentified compound in 2% milk with a retention time of 3.3 min held at 4º C after 0, 1, 4, 7, 10, 14 and 21 days exposure to 1521 ± 24 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and all riboflavin excitation wavelength block treatments (BS 20 T and BS 4 T treatments).

Volatile Compound Production Due to Wavelength Exposure. Lennersten and Lingnert (2000) found that there was a rapid increase in hexanal concentration for mayonnaise exposed to blue light with emission peaks at 365 nm, 405 nm, 435 nm and between 410 and 470 nm, but this increase was slower than when exposed to 365 nm alone. The authors also found that there were higher amounts, and a faster rate of development, of hexanal in mayonnaise stored in PET, which allows ~ 40% transmittance of 365 nm, as compared to PET/Polyethylene napthalate (PEN) copolymer and PEN alone, which only allows ~1% transmission of 365 nm. Mortensen and others (2003) found that only hexanal, 1-pentanol and 1-hexanol increased significantly in Havarti cheese when exposed to 366, 405, and 436 nm. Hexanal and 1-pentanol were increased significantly more when exposed to 405 and 436 nm, than when exposed to 366 nm. Mortensen and others (2003) also found that the highest concentration of volatiles produced was caused by exposure to 405 nm light. Our research did not show this trend. The 446 and 570 nm block treatments, which allow transmission of 400 nm light, did not show a greater amount of hexanal production than the 400 nm block treatment.

165

Riboflavin

Figure 6.7 shows the degradation of riboflavin for each packaging treatment throughout the 21 day period of the experiment. The light-protected control was significantly higher in riboflavin concentration throughout the experiment and did not have any significant degradation. This finding is in accordance with a number of investigators (Dimick 1973; Hoskin and Dimick 1979; Christy and others 1981; Hoskin 1988; Moyssiadi and others 2004). The concentration of riboflavin in milk not exposed to light is similar to reported average values (1.75 ppm) (Dimick 1973).
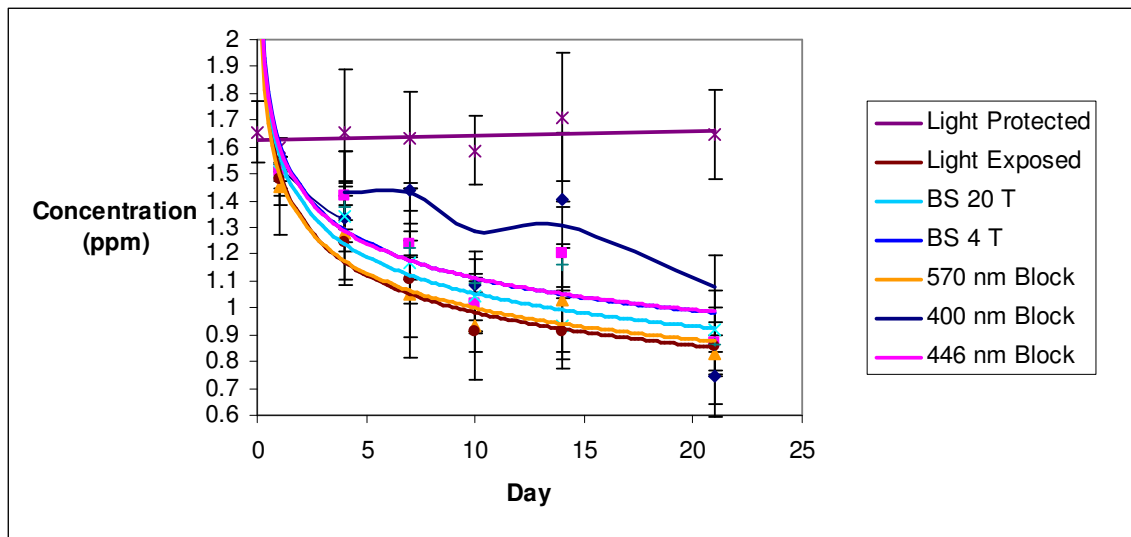
Figure 6.7. Riboflavin concentration (ppm) ± standard deviation in 2% milk held at 4° C after 0, 1, 4, 7, 10, 14 and 21 days exposure to 1521 ± 24 lux fluorescent lighting. Treatments included light-protected and light-exposed controls, single riboflavin excitation wavelength block treatments (400, 446, and 570 nm block treatments) and all riboflavin excitation wavelength block treatments (BS 20 T and BS 4 T treatments).

The light-exposed control and packaging treatments showed significant (up to 55%) riboflavin degradation over time. This amount of degradation, however, was less than the degradation found in the multi-layer film study (Chapter V). It is likely that there was less degradation due to the reduced surface to volume ratio of the 1 liter bottles used in this experiment compared to the higher surface to volume ratio in the 40 ml vials used in the multi-layer film study. There were no significant differences among the light-

exposed control and the packaging over-wrap treatments (400, 446, 570 nm block treatments and the BS 20 T and BS 4 T treatments) on days 1 and 4. However, the 400 nm block treatment, which allows in light at 446 and 570 nm, was significantly higher in riboflavin concentration than most of the other treatments on days 7-14, but was the lowest in riboflavin concentration by day 21. This is in contrast to other investigators who found that exposure to 450 nm wavelengths was the most destructive to riboflavin (Singleton and others 1963; Sattar and others 1977; Fanelli anad others 1985). On day 7 the 400 nm block treatment was significantly lower in riboflavin concentration than the light- protected control and significantly higher than all the other treatments except for the 4-layer combination treatment. By day 10, the 400 nm block treatment was significantly lower in riboflavin concentration than the light-protected control but was significantly higher than the 570 nm block and the light-exposed control treatment. On day 14, the 400 nm block treatment was not significantly lower than the light-protected control, nor any of the other treatments except for the light-exposed control treatment. By day 21, however, the 400 nm block treatment was lowest in riboflavin concentration.

Several investigators found that red films/plastic shields which absorb light between 400-500 nm limited destruction of riboflavin (Singleton and others 1963; Sattar and others 1977). Our data did not show any benefit to blocking those particular wavelengths, except for blocking the 400 nm wavelength, and blocking that wavelength was not consistently better at protecting against riboflavin destruction than the other treatments.

Conclusion

Use of packaging materials that provide optical clarity through wavelength interference, rather than absorption, as over-wraps can provide a new avenue for value-added packaging. These packaging materials may be optimized to block specific wavelengths that will protect milk flavor and allow for increased shelf-life.   However, it is still unclear which wavelengths and the degree of transmission reduction of these wavelengths that is needed to fully protect against light oxidation flavor production. Sensory evaluation showed that packaging over-wraps made from iridescent film reduced the perception of light oxidized flavor in milk but were not as efficient as the light-protected treatment in reducing the production of light oxidation flavor. Blocking 446 nm produced less light oxidation flavor than blocking 400 nm or 570 nm and blocking all riboflavin excitation wavelengths (BS 20 T and BS 4 T treatments) produced less light oxidation flavor than blocking only one excitation wavelength. Blocking transmission of all riboflavin excitation wavelengths at the levels suggested by IDF was not sufficient to completely protect against the production of light oxidation flavor suggesting the presence of a photosensitizer other than riboflavin in the milk. Our findings give support to those of Wold and others (2005) who found that chlorins and porphyrins significantly affected odor and flavor in cheese and dairy products.
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168

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170

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Chapter VII

Measurement of the Photooxidation of Oleic Acid by Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FTIR) Spectroscopy

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Abstract

A method for observing photooxidation in a model dairy system was developed using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Oleic acid with added riboflavin (1.75 ppm) was exposed to light (50W, halogen bulb) for up to 48 hrs. Preliminary data indicated that this method had the sensitivity to measure small changes associated with primary and secondary photooxidation of oil. Spectral changes associated with hydroxyl, carbonyl and double bond functional groups were monitored and correlated with the rise in peroxides and saturated and unsaturated aldehydes and ketones, respectively. Spectral bands associated with the C-H functional group decreased in intensity and were associated with peroxidation and loss of the cis double bond functional group.

Key Words: Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), Photooxidation, Oleic Acid

175

Introduction

FTIR Theory

Infrared radiation is found on the electromagnetic spectrum between 0.77 µm and 1,000 µm and is generally divided into three regions: near (0.77 to 2.5 µm), mid (2.5 to 50.0 µm), and far (50.0 to 1,000 µm). When using infrared spectroscopy, wavelengths are usually reported in wavenumbers, which are defined as

$$v \ (cm^{-1}) = 1/[\lambda \ (in \ cm)] \tag{1}$$

where v is the wavenumber and λ corresponds to the wavelength. Thus, wavenumbers corresponding to the infrared spectrum range from 10 to 2900 cm^{-1}. Wavenumber is proportional to both frequency and energy through the equation

$$E = hv = hc/\lambda \tag{2}$$

where h = Plank's constant (6.63 x 10^{-34} J*s), v = wavenumber, c = speed of light (3.0 x 10^{10} cm*s^{-1}), and λ = wavelength (Skoog, Holler and Nieman 1998).

Infrared spectroscopy is a technique that detects the absorption of infrared light associated with molecular vibrational modes and can be used to identify the presence of functional groups in a compound. In Fourier Transform Infrared (FTIR) spectroscopy (Figure 7.1), infrared light is directed through a beam-splitter which splits the beam in half. One half of the light is sent to a fixed mirror, while the other half is directed onto a moving mirror. These two light beams reflect off the mirrors and recombine again at the beam-splitter. When the light paths recombine, an interferogram is formed due to the difference in path lengths, resulting in both constructive and destructive interference. The recombined light then passes through the sample, which absorbs specific wavelengths characteristic for functional groups in that sample. A sensitive detector is used to monitor the amount of transmitted light and a signal representing the variation in

176

energy (intensity) versus time for all wavelengths can be monitored. A Fourier transform can be used to change intensity versus time spectrum into an intensity versus frequency spectrum for all points in the interferogram (Skoog, Holler and Nieman 1998).
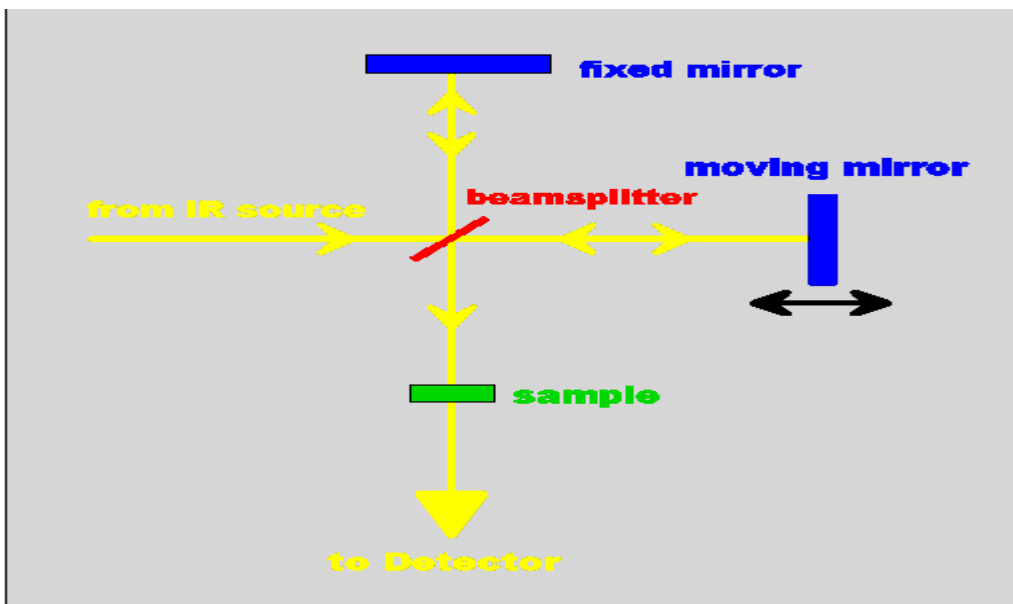
Figure 7.1. Schematic of light path in FTIR spectroscopy.

The absorption of infrared light involves the principle of simple harmonic motion. Vibrational modes within a molecule can potentially vibrate with light absorption as long as the temperature is higher than absolute zero. Atoms with smaller masses vibrate at a higher frequency than atoms with higher masses. Stronger chemical bonds vibrate at higher frequencies than weaker chemical bonds. Because every atom has a different mass and each type of bond (single, double or triple) has a different strength, each unique combination of atoms and bonds will vibrate at a different frequency. When a molecule absorbs infrared irradiation, each combination of atoms and bonds absorbs a frequency of light that is characteristic for that combination and the molecule as a whole. Common vibrational modes that absorb infrared radiation include stretching, bending, twisting, rocking and wagging. Sensitive detectors can then be used to monitor the infrared throughput and the frequencies of light absorbed. All frequencies are monitored

simultaneously and the Fourier transform algorithm is used to give a spectrum within seconds (Skoog, Holler and Nieman 1998).

Not all types of bonds will absorb IR radiation. Bonds with no net dipole moment or have no change in dipole moment when the distance between the atoms changes, will not absorb IR radiation and are called IR-inactive. Examples of molecules with IR-inactive bonds are O_2 and N_2. Bonds that do absorb IR radiation will produce a change in dipole moment when the distance between the atoms is changed. These bonds are called IR-active molecules and examples is the carbonyl group (C=O) and C-H modes (Skoog, Holler and Nieman 1998).

FTIR is a very sensitive analytical technique. Unlike other spectroscopic techniques, there is no monochromator, so all of the source light energy reaches the sample. There are also very few optical elements that reduce throughput. This results in a very low signal to noise ratio and is called the Jaquinot or throughput advantage. Many scans are collected for signal averaging and this improves the signal to noise ratio. FTIR instruments are highly accurate and precise in their wavelengths and tend to have high resolution. Beam path length determines the resolution of the instrument, which in turn is determined by the moving mirror. As the light path lengthens, the resolution becomes higher. Using the FTIR technique, all wavelengths can be detected and analyzed simultaneously which decreases the typical run time (Skoog, Holler and Nieman 1998).

FTIR spectra of organic compounds are found in two main regions: the functional group region, which is found between 4000 and 1500 cm^{-1}, and the fingerprint region, found between 1500 and 400 cm^{-1}. The functional group region peaks are characteristic of various types of functional groups. This region can be used to determine if a specific functional group is present and tends to be the most useful analytically. In the fingerprint region, peaks come about due to deformations of the molecule. These deformations may produce a single peak that is specific for a particular molecular symmetry or several peaks, which arise from multiple bonds deforming simultaneously. Compounds can be

identified using information gained from both regions and the spectrum can be compared to a reference spectrum to confirm compound identification.

Specific functional groups typically give peaks in a narrow range. Table 7.1 gives a summary of the frequencies specific to typical functional groups.

Table 7.1. General absorption frequencies for specific functional groups.

Functional Group	Region on Spectrum
C-H	3000-3100 cm^{-1} (sp^2)
	2800-3000 cm^{-1} (sp^3)
C=O	1600-1800 cm^{-1}
	Acids: 1650-1700 cm^{-1}
	Esters: 1740-1750 cm^{-1}
	Aldehydes: 1720-1750 cm^{-1}
	Ketones: 1720-1750 cm^{-1}
	Amides: 1650-1715 cm^{-1}
O-H (alcohol)	3300-3600 cm^{-1}
	Monomeric forms: sharp
	H-bonding leads to broadening
O-H (acids)	2400-3000 cm^{-1}
	Very broad, medium intensity
C≡C	2200-2100 cm^{-1}
C≡N	Usually weak; maybe not visible in internal alkynes.
	Nitriles are quite strong.
C-O	1200-1300 cm^{-1}
	Often difficult to assign, depending on fingerprint region.
N-H	3400 cm^{-1}
	Usually sharper than O-H.

IR can also be coupled to other techniques including gas chromatography and mass spectrometry, and these techniques are called hyphenated techniques. Coupling to GC allows for the separation of compounds before determining the structural characteristics by IR.

179

Attenuated Total Reflectance (ATR) Spectroscopy

Attenuated total reflectance spectroscopy is one of the most widely used infrared techniques used today, especially for the analysis of thick absorbing samples were transmission cannot be used. With this technique, the infrared beam is directed into a crystal of relatively high refractive index that is in contact with a sample of lower refractive index. If the angle of incidence is greater than the critical angle, the beam is internally reflected producing an evanescent wave that propagates into the sample. Interaction of infrared absorbing groups in the sample with this evanescent wave results in signal loss that is monitored by the spectrophotometer detector. Figure 7.2 below shows a schematic representation of the ATR technique for a single reflection.
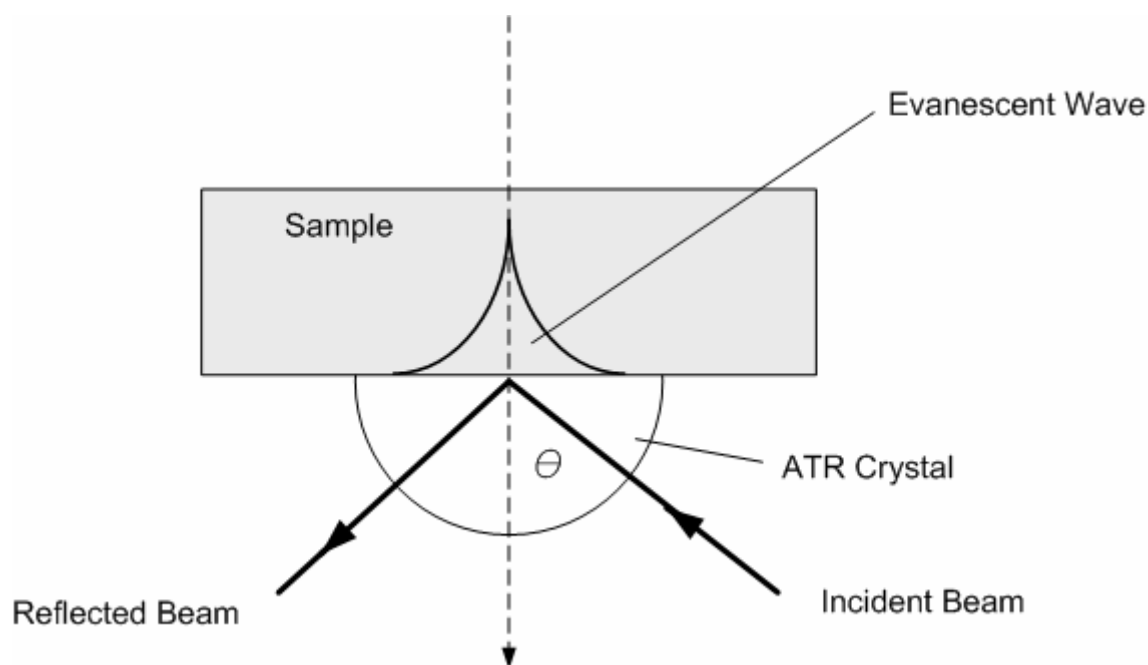
Figure 7.2. Schematic of light reflection and evanescent wave propagation for ATR.

The critical angle can be calculated using the following relationship

$$\theta_C = \sin^{-1}\left(\frac{n_2}{n_1}\right)$$

(4)

180

where n_1 is the refractive index of the ATR crystal and n_2 is the refractive index of the sample. As shown in Figure 2 above, the evanescent wave decays exponentially into the sample and the sampling depth is defined as the distance for the field intensity to decay to 1/e of its value at the crystal surface. This depth can be can be calculated using the following relationship

$$d_P = \frac{\lambda}{2\pi\left(n_1^2 \sin^2\theta - n_2^2\right)^{\frac{1}{2}}} \tag{5}$$

where λ is the infrared wavelength and θ is the incidence angle. Using ZnSe ($n_1 = 2.42$) and an incidence angle of 45°C ($\theta_c = 38.5°$ for ZnSe), the sampling depth at 1000 cm^{-1} for a sample with $n_2 = 1.5$ would be 2 μm, showing the advantage of using the ATR technique for the analysis of very thick samples.

Oxidation of Lipids

Oxidation is a common reaction involving lipids and leads to the development of off-odors and flavors in food. A number of oxidation products are formed, the main ones being hydroperoxides, alcohols and aldehydes. Minor products formed are hydrocarbons, free fatty acids, esters, ketones, lactones, and furans as well as cis/trans isomerizations. The rate of oxidation and the type of products being produced depends heavily on the source, the fatty acid composition, and the degree of unsaturation of the lipid. Other factors that affect the rate of oxidation include the presence of metal ions, antioxidants, and the length of time that the lipid has endured the oxidative stress (deMan 1990).

FTIR in Food Studies

FTIR is not commonly used in food studies, although its use is beginning to increase. It is becoming an important tool in the detection of food adulteration and in food quality control (Lai and others 1994; Kemsley and others 1996; Downey and others 1997; Ding and others 2000; Paradkar and others 2002; Gangidi and others, 2003). It can also be

used for the characterization of foods and some researchers have correlated FTIR data with classical wet chemical values for oxidation including iodine value, saponification number, 2 thiobarbituric acid (TBA) number, peroxide value and anisidine value (Li and others 1996; Downey and others 1997).   FTIR has also been used to determine moisture content, free fatty acid content, cis and trans double bond content and solid fat content. There are several standardized methods (IUPAC, AOCS, and AOAC) for the quantification of trans double bonds using FTIR (Guillen and Cabo 1997). Van de Voort and others (1994) have shown a high correlation ($R^2 = 0.9991$) between IR predicted iodine values and chemically determined values.

FTIR spectroscopy can potentially be used to follow the changes that occur in lipids as they oxidize. Several authors have looked at thermal oxidation of oils using FTIR (van de Voort and others 1994; Guillen and Cabo 1997, 2000, 2004; Ruiz and others 2001; Innawong and others 2004).   Few studies have looked at photooxidation of oils. However, a recent paper used FTIR look at TiO_2 photo-catalysis in a phosphatidyl-ethanolamine lipid bilayer exposed to UVA light (Kiwi and Nadtochenko 2004). Another looked at the effect of UV irradiation on oxidation in corn oil and the synergistic effect of heat and light on the kinetics of oxidation (Vlachos and others 2006). We feel that, once a method is developed, ATR-FTIR will be an excellent research tool to study the effect that light wavelength has on the oxidation of milk or model milk. FTIR has the advantage of being non-destructive, highly sensitive, and yields spectral information that can be used to monitor the complex chemical changes occurring.

FTIR Determination of Lipid Oxidation

Guillen and Cabo (2000) looked at the use of FTIR to determine the degree of oxidation, and oxidative stability, in edible oils. Thirteen edible oils of differing saturations were heated at 70º C to a high viscosity in a convection oven. They found that the oxidation process followed a general trend for all oils but small changes were noted due to the differences in composition.

182

Several bands (peaks) shifted as the oxidation proceeded through the first, second and third stages of oxidation and the rate of this shift was used to determine the oxidative stability of the oil. The first stage of oxidation was defined as the period in which no major changes occurred in the IR spectrum of the oil. This period lasted from several days to several weeks under the conditions studied depending on the composition of the oil. It was found that oils with a high concentration of oleic acyl groups have longer first stages than oils with more linoleic and linolenic acyl groups. The second stage of oxidation was correlated to the production of hydroperoxides and was defined as the interval of primary oxidation. This stage ended when the concentration of hydroperoxides decreased. Typically, the second stage occurred for two to five days for most oils. However, olive and virgin olive oils had second stages lasting for twenty and twenty-six days, respectively. The third stage of oxidation began when secondary oxidation products such as alcohols, aldehydes and ketones were formed. The products of this stage of oxidation were the cause of off-flavor and off-odor in oils and other food commodities (Guillen and Cabo 2000).

Guillen and Cabo (2004) also investigated the stability of pork fat treated with liquid smoke flavorings. They found that the timing of the appearance/disappearance of different frequency bands correlated with oxidative stability in the pork fat. Liquid smoke showed antioxidant properties in the pork adipose tissue.

Vlachos and others (2006) studied chemical changes in corn and extra virgin olive oil heated for 30 min at various temperatures (130-275º C) using FTIR. As temperature increased, the C=O region at 1746 cm^{-1} widened. This widening was due to an increase in carbonyl groups from saturated aldehydes and other secondary oxidation compounds.

The formation of hydroperoxides was determined by FTIR in a mayonnaise-like oil in water emulsion heated at 60º C for up to 12 days. Emulsions were made from soybean oil (SBO), or blends of SBO and palm kernel oil (PKO). Blends consisted of SBO:PKO ratios of 90:10, 80:20, 70:30 and 60:40. It was found that all samples increased in

oxidation throughout the heating period, but the emulsions with PKO increased less quickly. Blends with both 30 and 40% PKO had similar amounts of oxidation. In this study the authors found a decrease in the O-H stretching region (3600-3020 cm^{-1}) over time. This was attributed to water evaporation. The O-H stretching from hydroperoxide formation was masked by the water peaks. An increase in the 1746 cm^{-1} band indicated an increase in the formation of aldehydes and ketones over time (Nor Hayati and others 2005).

Innawong and others (2004) found that FTIR could be used to differentiate between acceptable, marginal and unacceptable frying oils using principal component analysis of the whole infrared spectra (4000-850 cm^{-1}). Correlation of absorbance at 3300 cm^{-1} and free fatty acid content and absorbance at 3471 cm^{-1} and peroxide value was 0.84-0.94 and 0.90 to 0.97 respectively.
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FTIR Determination of Lipid Photo-Oxidation

Vlachos and others (2006) looked at the oxidation of corn oil (5 ml) exposed to UV light (220-440 nm) of 1.6 J/cm^2 for 1, 2 and 3 hrs. In addition, the oil exposed to 3 hrs light was subsequently heated to 270º C to determine the synergistic effect of exposure to both heat and light on oxidation. It was found that as exposure increased, the cis-olefinic double bonds decreased and disappeared completely by 3 hrs. It was also found that the C=O region at ~ 1746 cm^{-1} (due to the ester carbonyl functional group of the triacylglyceride) widened as light exposure continued. Widening of this band was due to the production of saturated aldehyde functional groups and other secondary oxidation products (aborbance of ~ 1728 cm^{-1}) which overlapped the 1746 cm^{-1} band. Irradiating corn oil for 3 hr was equivalent to heating corn oil at 220º C for 30 min. Heating to 270º C for 30 min after exposure to UV light for 3 hrs produced significant increases in the production of carbonyl groups compared to irradiation only, producing almost twice the amount of carbonyls as heating alone. This indicates possible stability problems in oil used for cooking that has also been previously exposed to light (Vlachos and others 2006).

184

Kiwi and Nadtochenko (2004) looked at the TiO_2 photo-catalyzed oxidation of phosphatidyl ethanolamine. A layer of lipid films was cast onto a TiO_2 film and irradiated with UVA (366 nm) light. The authors found an increase in the O-H stretching region (3500-3000 cm^{-1}) by 2.5 minutes due to the formation of hydroperoxides, alcohols and carboxylic groups. By 15 min this peak had diminished somewhat and was completely gone by 120 min exposure. The band that correlated to cis double bonds (3008 cm^{-1}) decreased steadily over the entire 165 min exposure period. Changes in the absorption profile between 1750 and 1500 cm^{-1} was seen and was due to production of carbonyl groups, phosphate groups, and $-CH_2$ groups. Consistent increases in a number of bands between 1800 and 1500 cm^{-1} were attributed to the formation of saturated and unsaturated aldehydes and ketones formed from the breakdown of hydroperoxides or endoperoxides.

In a study on the production of conjugated linoleic acid (CLA) by photoisomerization, soybean oil was exposed to both UV and visible light for a total of 144 hrs at 22-25° C in the presence of 0.15% iodine. High amounts (24%) of CLA were produced. Typical daily intake of CLA through natural food sources is only ~ 10% of the suggested recommended value. Dairy products, which are relatively high in CLA, only average 0.6%. Interestingly, there was no production of peroxides, as determined by FTIR, throughout the 144 hr period, nor was there any production of hexanal, as determined by gas chromatography mass spectrometry (Jain and Proctor 2006).

185

Materials and Methods

Materials

Oleic acid (250 ml) (NF/FCC, Fisher Scientific) with added riboflavin (1.75 ppm) was exposed to light (50W, halogen bulb) for up to 48 hrs. Samples were stirred with a magnetic stirrer for 1 hr prior to exposure to light to ensure the riboflavin was dispersed uniformly. The sample container was covered with aluminum foil to eliminate light exposure before the experiment began and to limit light loss during the experiment. Fatty acid analysis results are shown in Table 7.2.

Table 7.2. Fatty acid analysis of Oleic Acid (NF/FCC, Fisher Scientific)

Fatty Acid*	% Area
Myristic acid (14:0)	3.7
Myristoleic Acid (14:1 ω5)	1.1
Palmitic Acid (16:0)	4.3
Palmioleic Acid (16:1 ω7)	5.8
Unknown Acid	1.1
Margaric Acid (17:0)	1.8
Stearic Acid (18:0)	0.6
Oleic Acid (18:1 ω9)	78.3
Linoleic Acid (18:2 ω6)	3.2

* Traces of Linolenic (18:3 ω3), Arachidic Acid (20:0), and Gadoleic Acid (20:1) were also found.

Fatty Acid Determination

Oleic acid (30 mg) was mixed in 2 mls sulfuric acid/methanol (H_2SO_4-MeOH) solution (5% concentrated sulfuric acid in methanol) and heated in boiling water for 60 minutes under nitrogen. After cooling, water (2 ml) and isooctane (2 ml) were added to the H_2SO_4-MeOH solution and mixed. After settling, the aqueous phase was removed and the organic phase was washed with distilled water (2 ml). The aqueous phase was

removed again and the sample was treated with sodium sulfate to remove trace amounts of water.

Replicate samples (1 µl) were injected into a Hewlett Packard 5890 gas chromatograph with a model 3393A integrator (Hewlett Packard, Palo Alto, CA). A DB-225 column (30m x 0.25mm, 0.25 µm film, J&W Scientific, Folsom CA) was operated isothermally at 220º C. Injector and detector (flame ionization detector) temperatures were held at 275º C. Helium carrier gas had a linear flow velocity of 40 cm/sec and the split ratio was 1:50. Fatty acids were identified by retention times using standards and Canola oil as references. Results are reported as normalized area percentages (closely matching weight percent).

Irradiation System

The photooxidation reaction system (Figure 7.3) consisted of a jacketed 300 ml borosilicate glass reaction vessel. This vessel was situated on top of a magnetic stir plate (Fisher Scientific) and samples were stirred using a Teflon coated magnetic stirrer. The vessel was cooled at a constant temperature of 25º C using a circulating thermostated water bath (Model 900, Allied Fisher Scientific).

An immersion well made from quartz glass was fitted into the reaction vessel and housed a 50 W light bulb emitting light between 300-700 nm. The lamp was operated at 12 V and 4 amps using a Tenma Laboratory DC power supply unit. Cooling air was continuously blown into the immersion well to dissipate heat produced by the bulb. The bulb was situated below the oil surface during the entire experimental period. The reactor vessel was wrapped in aluminum foil to eliminate any outside light and limit light loss during the experiment. Headspace was kept to a minimum.

Samples were taken at various times throughout the experiment by drawing approximately 2 ml of oleic acid from the reaction vessel into a glass syringe. Samples were immediately analyzed by FTIR using a liquid flow-through cell.
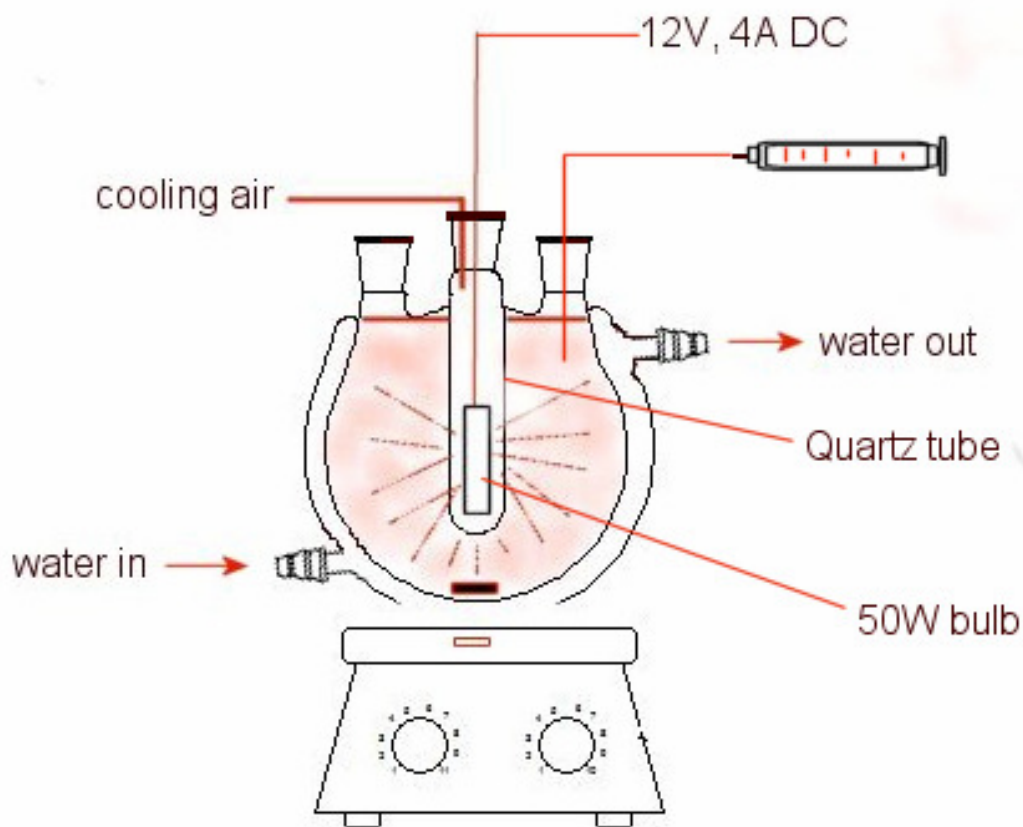
Figure 7.3. Photooxidation Reactor System.

FTIR Measurement

Oxidation products were monitored using attenuated total reflectance infrared spectroscopy. Spectra for all samples were collected using a Digilab FTS175 spectrophotometer (Canton, MA) equipped with a Harrick Seagull™ single reflection accessory (Figure 7.4) and Mercury-Cadmium-Telluride (MCT) detector. A hemispherical zinc selenide crystal with liquid flow cell (Figure 7.5) was used employing an incidence angle of 45°. Scans were taken at $4cm^{-1}$ resolution and 256 scans were co-added. Samples were introduced into the flow cell using a syringe and the flow cell was purged with un-oxidized oil before each analysis and this spectrum used as the

background. Using a refractive index of 1.5 for oleic acid, the sampling depth (3dp) for analysis was calculated to be approximately 3 um at 2000 cm^{-1}. Spectra were analyzed using WinIR (Digilab, Canton MA) software.
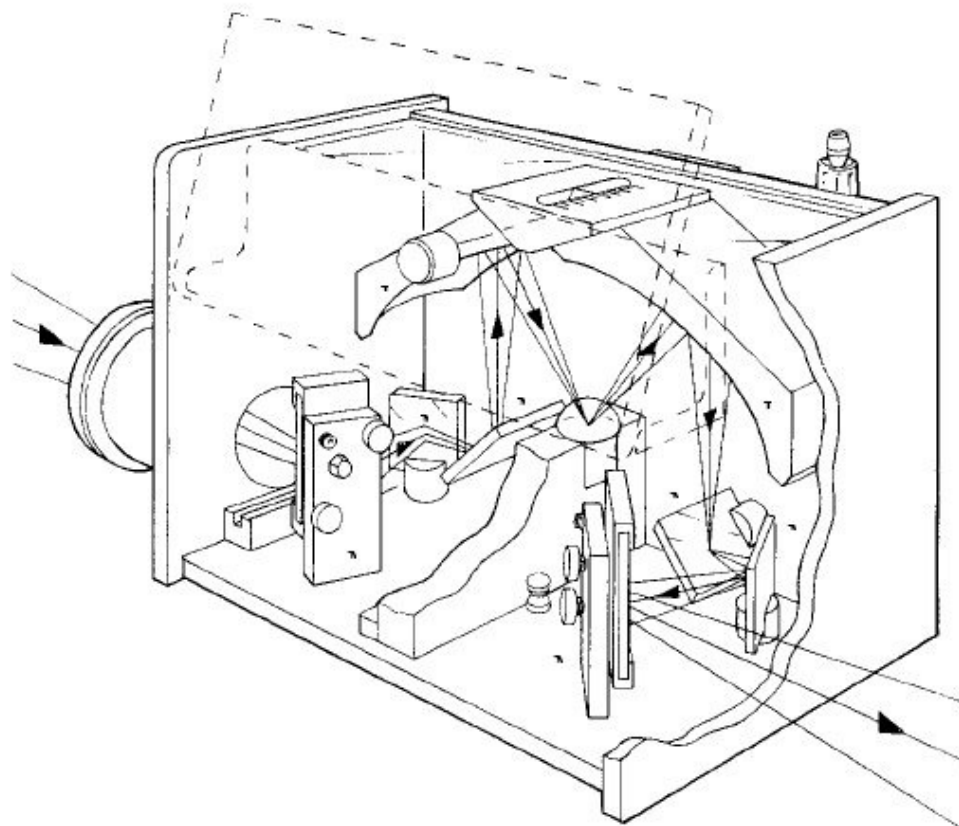
Figure 7.4. Harrick Seagull™ single reflection accessory.
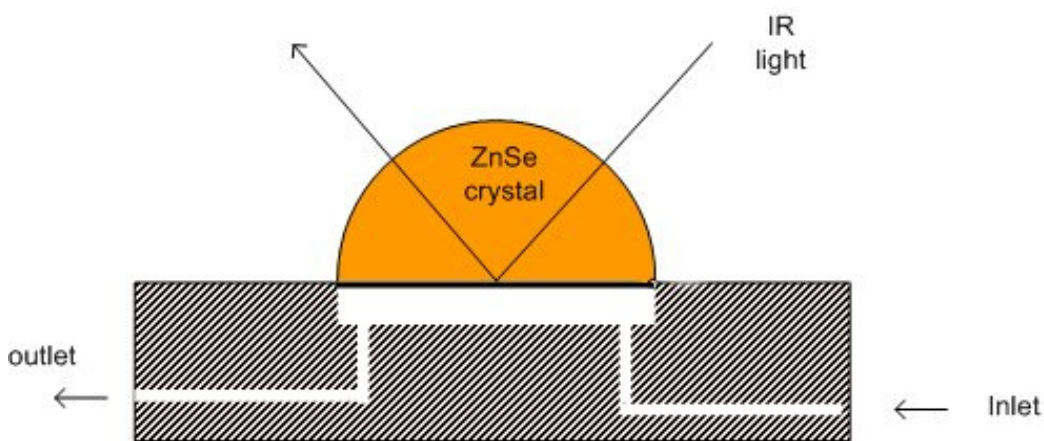
Figure 7.5. Hemispherical zinc selenide crystal with flow through cell.

189

Results and Discussion

Figure 7.6 shows the background spectrum for oleic acid with peak assignment.
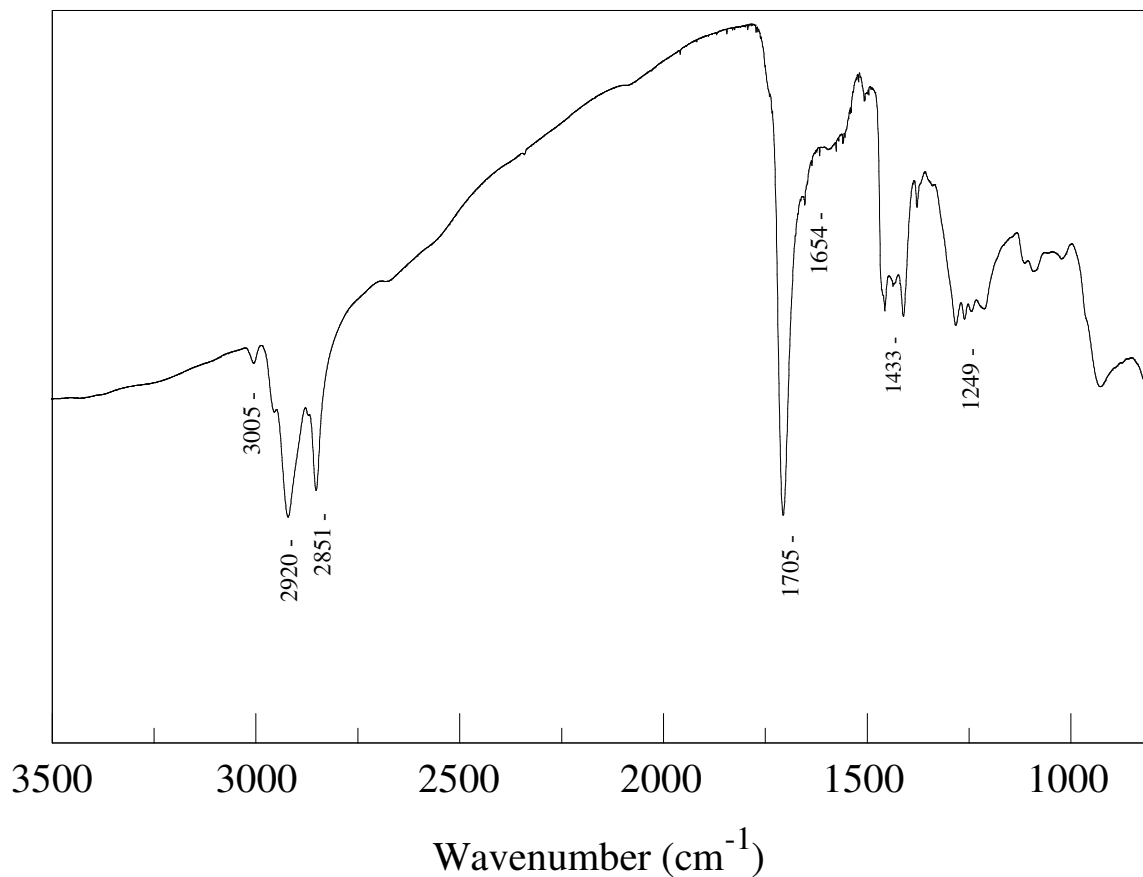
Figure 7.6. Background spectrum for oleic acid.

Figures 7.7 and 7.8 illustrate the infrared spectra for the photooxidation of oleic acid over a 48 hr period. All spectra are ratioed to the unoxidized oil at the same temperature. Changes in the infrared spectrum included increases and decreases in peak intensity and the bands that decreased over time are noted in Figure 7.7, while bands that increased are noted in Figure 7.8.
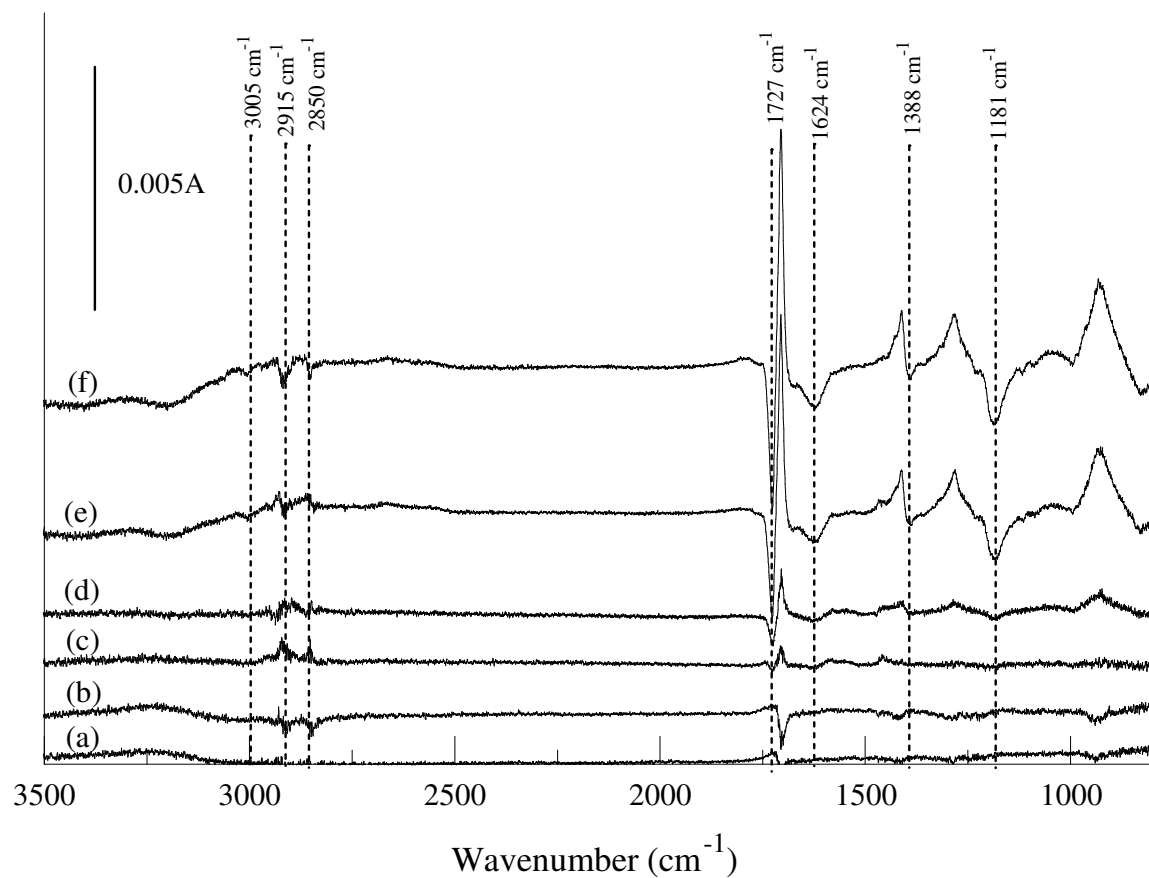
Figure 7.7. ATR-FTIR spectrum at 2 hrs (a), 4 hrs (b), 8 hrs (c), 12 hrs (d), 24 hrs (e) and 48 hrs (f) for oleic acid with added riboflavin (1.75 ppm) held at 25º C and exposed to a 50 W broad spectrum light (300-700 nm). Decreasing bands are marked.
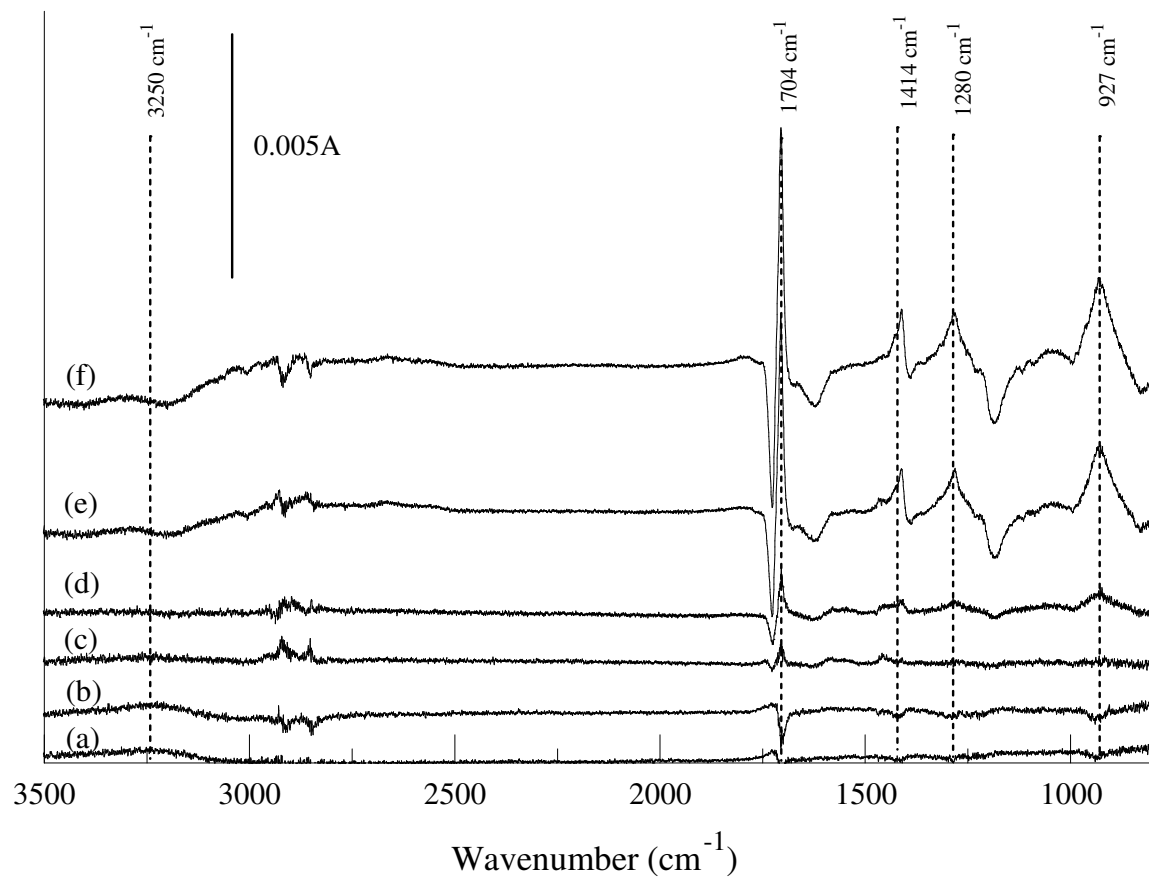
Figure 7.8. ATR-FTIR spectrum at 2 hrs (a), 4 hrs (b), 8 hrs (c), 12 hrs (d), 24 hrs (e) and 48 hrs (f) for oleic acid with added riboflavin (1.75 ppm) held at 25º C and exposed to a 50 W broad spectrum light (300-700 nm). Increasing bands are marked.

The decrease in the band at 3005 cm^{-1} at long times corresponds to a decrease in cis double bonds in the oil. This decrease was not unexpected and a number of authors report a similar decay (Guillen and Cabo 1997; Kiwi and Nadtochenko 2004; Vlachos and others 2006). This decrease corresponds to an increase in secondary oxidation products which eliminated the double bond during cleavage of the alkoxy radical.

The decay in two bands (2915 and 2850 cm^{-1}) corresponded to the asymmetric and symmetric C-H stretching bands, respectively, and was most likely due to peroxidation. This decay is seen within 4 hrs of initiation of irradiation and corresponded with an increase in the O-H band located at ~ 3250 cm^{-1}. Several other authors have also reported a decrease in these two bands as oxidation increased (Guillen and Cabo 1997; Kiwi and Nadtochenko 2004).

By far, the most pronounced spectral change was located in the 1727-1704 cm^{-1} region which corresponds to the carbonyl functional group (Figure 7.9). This region is clearly changing shape during oxidation as indicated by the emerging derivative shaped peak. Clearly changes in peak width or a shift to lower average frequencies would lead to this type of spectral feature associated with peak broadening. A number of authors observed peak broadening due to an increase in carbonyl groups from saturated aldehydes and other secondary oxidation compounds (Guillen and Cabo 2000; Vlachos and others 2006). Nor Hayati and others (2005) and Kiwi and Nadtochenko (2004) attributed the appearance of a 1705 cm^{-1} peak to the formation of ketones. Vlachos and others (2006), however, assigned 1715 cm^{-1} as the maximum absorbance band for ketones and Kiwi and Nadtchenko (2004) assigned 1697 cm^{-1} as hexenal. Because our system was different from both Hayati and others (2005) and Kiwi and Nadtochenko (2004) the changes in this carbonyl region cannot be assigned. Nonetheless, it is clear that the spectral changes in the carbonyl region were due to the increase in secondary oxidation products. In future studies, standards will be added to the model system, including saturated and unsaturated aldehydes and ketones, to determine wavenumber assignments. Figure 7.9 shows a more detailed analysis of the carbonyl peak. Absorbance increases begin at approximately 8 hrs and continue until the end of the experiment.

193
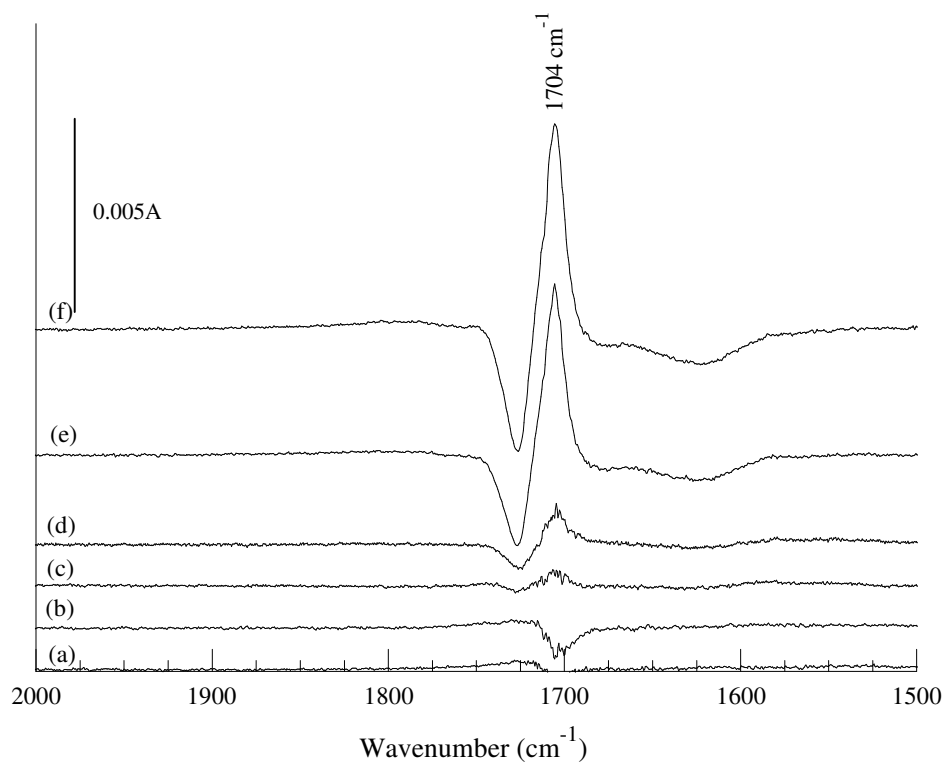
Figure 7.9. ATR-FTIR spectrum showing increase in 1704 cm^{-1} band at 2 hrs (a), 4 hrs (b), 8 hrs (c), 12 hrs (d), 24 hrs (e) and 48 hrs (f) for oleic acid with added riboflavin (1.75 ppm) held at 25º C and exposed to a 50 W broad spectrum light (300-700 nm).

The kinetics of oxidation were examined by monitoring the peak intensity at 1704 cm^{-1} with time as shown in Figure 7.10. The peak increase showed typical first order kinetics with a rate constant of 0.032 min^{-1}.

194
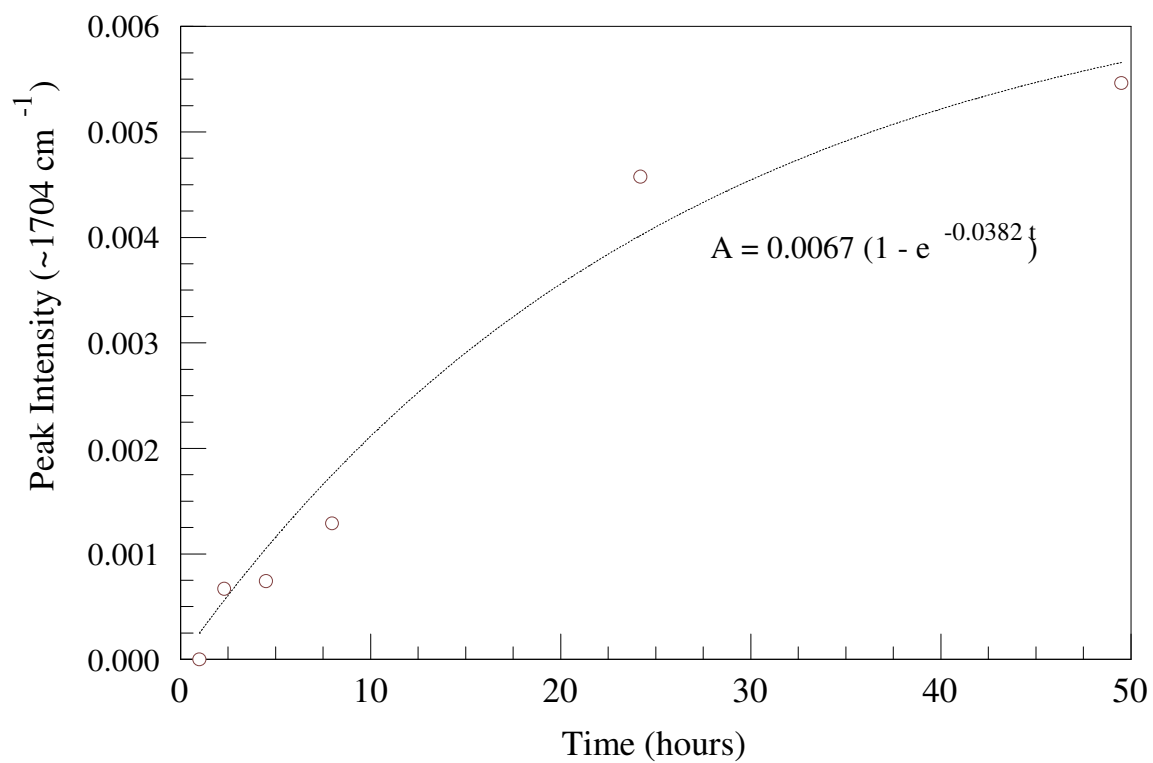
Figure 7.10. Increase in peak intensity at 1704 cm^{-1} over time. Increase follows first order kinetics and is described by the equation A = 0.0067(1-e$^{-0.032t}$).

Conclusion

A method for observing photooxidation in a model dairy system, made of oleic acid and riboflavin, was developed using ATR-FTIR. Preliminary data indicates that ATR-FTIR had the sensitivity to measure small changes associated with primary and secondary oxidation of oleic acid over a period of two days. Changes that occurred in the oleic acid due to photooxidation followed classical oxidation theory, showing peroxidation occurring relatively quickly with secondary oxidation beginning to appear after approximately eight hours exposure to light. This method will be used to compare the kinetics of photooxidation in the model dairy system that is protected by the packaging over-wrap treatments described in Chapter VI to an unprotected system.

Acknowledgements

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197

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198

Appendix Table A1: Summary of Literature Review for Riboflavin Degradation

Packaging Material	Color of Packaging Material	Wavelength Blocked	Product	Light Intensity	Time of Exposure	Temp.	Riboflavin Concentration/ Riboflavin Loss	Sensory Change	Reference
HDPE	Transparent		Milk				60% loss		Fukumoto and Nakashima (1975)
HDPE	Blue	(400-520 nm)					20% loss		
HDPE	Brown	(500 nm)					30% loss		
HDPE	Red	(>550 nm)					10% loss		
PE	Transparent		Whole milk	150, 300 and 450 ft-c	48 hr	1.7-10º C	150 ft-c: 3% 300 ft-c: 10% 450 ft-c: 15%		Singh and others (1975)
PE	Yellow	(~400-480 nm)					150 ft-c: 0% 300 ft-c: 3.5% 450 ft-c: 2%		
Paperboard	Opaque						150 ft-c: 2% 300 ft-c: 2% 450 ft-c: 2%		
Glass	Transparent						150 ft-c: 4% 300 ft-c: 11% 450 ft-c: 15%		
Uncovered Petri dishes		350 nm 415 nm 465 nm 465 nm 485 nm 520 nm 535 nm 560 nm 570 nm 959 nm Dark control	Water	500 ft-c	5 hr	15º C	15.79 µg/L 20.05 µg/L 35.17 µg/L 40.53 µg/L 46.91 µg/L 49.58 µg/L 50.00 µg/L 50.00 µg/L 50.00 µg/L 50.00 µg/L 50.00 µg/L		Sattar and others (1977b)
	Gold/Yellow	400-480 nm					Partial Protection		Luquet and others (1977)

Package	Appearance	Light transmission	Milk	Light intensity	Time	Temperature	Loss	Sensory	Reference
TetraBrik	Opaque		Milk	2000 ± 100 lux	5 days	4° C	Full protection		Eberhard and Gallmann (1978)
PE/Cardboard (no headspace)	Opaque		Milk		6 days	6° C	Full protection		Goussault and others (1978)
PE (air in headspace)	Transparent						Slight loss		
HDPE	Transparent	40-90% between 400-750nm	Whole milk	1076 ± 50 lux	72 hrs	7 ± 1° C	10% loss	2.6 on 9pt. scale 2.5-2.9 on 9 pt. scale	Hoskin and Dimick 1979
Clear PC	Transparent	No light block < 500 nm					13% loss	3.4-4.7 on 9 pt. scale	
Tinted PC	Yellow						6% loss	2.6 on 9 pt. scale	
Glass	Transparent	No light block					27% loss	5.6-6.4 on 9 pt. scale	
Fiberboard	Opaque	100% between 400-750 nm					10% loss		
Fiber "Vitagold" (PC with gold tint)	Opaque Gold/Yellow	All 400-480 nm	Skim, 1%, 2% and Whole Milk	2000 lumens/M^2	24 hr	5 ± 1° C	2% loss in skim 1% loss in skim		Senyk and Shipe (1981)
Glass	Transparent						9% loss in skim		
Polycarbonate	Transparent						16% loss in skim		
PE (returnable)	Somewhat Transparent						15% loss in skim		
PE (throw-away)	Somewhat Transparent						14% loss in skim		
Paperboard	Opaque		Milk	800 µW	8 hr 24 hr 48 hr 72 hr	3° C	2.5 % loss 5 % loss 10 % loss 13 % loss		Farrer (1983)
HDPE w/TiO$_2$	Opaque				8 hr 24 hr 48 hr 72 hr		10% loss 24 % loss 31 % loss 33 % loss		
HDPE	Transparent				8 hr 24 hr 48 hr 72 hr		8 % loss 24 % loss 34 % loss 37 % loss		

Material	Color	Wavelength	Food	Light intensity	Time	Temp	Result	Sensory	Reference
HDPE with:			Milk	190 ± 10 lumens	16 hr	1.5 ± 1.5º C			Fanelli and others (1985)
FD&C Yellow #5 (0.05%)	Yellow	< ~ 500 nm					71% loss		
(0.3%)	Yellow						0.5% loss		
Quinacridone Red (2.89 g/L)	Red	< ~ 575 nm					71% loss		
Ultramarine Blue (0.962 g/L)	Blue	< ~ 500					75% loss		
HDPE/Cyasorb 531	Transparent	< ~ 350 nm					70% loss		
HDPE/Tinuvin 326	Transparent	< ~ 375 nm					70% loss		
HDPE/Tinuvin 622	Transparent						57% loss		
PE 2 pint cartons	White/opaque		Whole milk	4000 lux	90 hrs	7º C	20: ~1.4 µg/ml 40: ~1.3 µg/ml 65: ~1.1 µg/ml 90: ~1.0 µg/ml	Dislike very much by 17 hrs.	Schroeder and others 1985
HDPE 4 pint bottles	White/opaque						20: 1.5 µg/ml 40: 1.5 µg/ml 65: 1.5 µg/ml 90: 1.5 µg/ml	Dislike moderately by 17 hrs.	
			Cheddar cheese		12 days	5-10º C		9 pt Hedonic	Deger and Ashoor 1987
PE	Transparent			538 lux			Thin slice: 23.8 ± 7.9% loss	5.6 ± 1.9	
							Intermediate: 28.6 ± 7.2 % loss	5.6 ± 1.6	
							Thick: 8.8 ± 3.4% loss	5.6 ± 1.8	
				1614 lux			Thin: 38.6 ± 7.7% loss	5.6 ± 1.9	
							Intermediate: 33.4 ± 6.8% loss	5.4 ± 1.8	
							Thick: 27.6 ± 3.6% loss	5.9 ± 1.9	
				5380 lux			Thin: 44 ± 5.6 % loss	5.9 ± 1.7	
							Intermediate: 41.6 ± 6.9% loss	5.4 ± 1.8	
							Thick: 40.2 ± 1.2% loss	4.9 ± 2.2	

					Time			
		Whole milk	4300 ± 100 lux	6 ± 0.5 C			On a 9 pt Hedonic Scale	Hoskin (1988)
PC	Transparent				0	0.839 µg/ml	6.11-6.69	
					12	0.905 µg/ml	4.57-4.85	
					24	0.829 µg/ml	4.02-4.33	
					48	0.828 µg/ml	3.61-3.72	
					72	0.730 µg/ml	3.31-4.00	
					0	0.788 µg/ml	5.97-6.55	
Aluminum over HDPE Top shield					12	0.834 µg/ml	5.39-6.07	
					24	0.849 µg/ml	3.77-4.14	
					48	0.834 µg/ml	3.73-4.26	
					72	0.779 µg/ml	3.38-3.97	
					0	0.857 µg/ml	6.17-6.23	
Bottom shield					12	0.832 µg/ml	5.98-6.08	
					24	0.932 µg/ml	4.74-5.42	
					48	0.866 µg/ml	4.77-5.00	
					72	0.844 µg/ml	3.82-5.01	
Oriented Polypropylene over HDPE Top shield	~25-35% T between 380-700 nm				0	0.770 µg/ml	6.03-6.78	
					12	0.836 µg/ml	5.43-5.95	
					24	0.899 µg/ml	3.98-4.78	
					48	0.829 µg/ml	3.81-4.24	
					72	0.792 µg/ml	2.99-4.31	
Bottom shield					0	0.821 µg/ml	6.33-6.99	
					12	0.872 µg/ml	5.16-5.32	
					24	0.918 µg/ml	3.96-4.88	
					48	0.854 µg/ml	3.94-4.22	
					72	0.844 µg/ml	3.68-4.36	

Package	Opacity	Transmission	Milk	Light	Time	Temp	Result 1	Result 2	Reference
			Milk	Fluorescent light = 1600 lux Daylight = 12,000 lux	Variable times (see Riboflavin loss section)	5° C		7 pt scale for light off flavor where 1 – none, 7 = intense	Haisman, and others 1992
Fiberboard carton (1 liter)	Opaque						72 hr dark: 2.2 3 hr daylight: 2.2 6 hr: 2.3 24 hr: 2.2 72 hr: 2.3	72 hr dark: 2.7 3 hr daylight: 3.5 6 hr: 2.0 24 hr: 3.4 72 hr: 2.7	
HDPE bottles (2 liter)	Transparent						72 hr dark: 2.4 3 hr daylight: 2.1 6 hr: 2.3 24 hr: 2.2 72 hr: 2.1	72 hr dark: 2.3 3 hr daylight: 5.9 6 hr: 3.1 24 hr: 5.3 72 hr: 5.3	
Paperboard	Opaque		Whole milk		60 days		15 % loss		Al-Zawawi and Caldwell (1993)
Plastic	Transparent		Whole milk		60 days		16 % loss		
Paperboard	Opaque		Skim milk		60 days		15 % loss		
TetraBrik TetraRex PET	Opaque Opaque Transparent		Milk	Neon lighting	Up to 12 days	1-4° C	2-3 % loss 2-3 % loss 3-5 % loss	Poorer quality in PET	Marchetti and Forti (2001)
3 layer PET (white/black/white)	Opaque	<0.5% T of 200-800 nm	Milk, 1.3% fat	2500 lux	2 months	18-25° C	Full protection against loss	Full protection against LOF	Mestdagh (2005)
PET with oxygen scavenger	Transparent	~70-80% T between ~350 and 800 nm. ~ 0% T below 350 nm					100% loss, ~ 0.7 µg/100 g loss/day	SD in LOF (P< 0.05) by 2 days	
PET with UV absorber	Transparent	0.2-0.5% T between 400-800 nm. 0% < 400 nm					100% loss, ~ 0.7 µg/100 g loss/day	SD in LOF(P< 0.05) by 2 days	

Appendix Table A2: Summary of Literature Review for Vitamin A Degradation

Packaging Material	Color of Packaging Material	Wavelength Blocked	Product	Light Intensity	Time of Exposure	Temp.	Vitamin A Concentration/ Loss	Sensory Change	Reference
HDPE HDPE HDPE HDPE	Transparent Blue Brown Red	 (400-520 nm) (500 nm) (>550 nm)	Milk				60% loss 20% loss 30% loss 10% loss		Fukumoto and Nakashima (1975)
Petri Plates	Transparent	350 nm 415 nm 455 nm 465 nm 485 nm 520 nm 535 nm 560 nm 570 nm 595 nm Dark control	Vitamin A in chloroform	500 ft-c	77 hr	25° C	1.20 µg/g 5.46 µg/g 6.29 µg/g 6.29 µg/g 6.26 µg/g 6.27 µg/g 6.29 µg/g 6.29 µg/g 6.38 µg/g 6.37 µg/g 6.42 µg/g		Sattar and others (1977b)
	Gold/Yellow	400-480 nm					Partial Protection		Luquet and others (1977)
TetraBrik	Opaque		Milk	2000 ± 100 lux	5 days	4° C	Full protection		Eberhard and Gallmann (1978)

Package	Appearance	Wavelength	Milk type	Light intensity	Time	Temperature	Loss	Sensory	Reference
Fiber "Vitagold" (PC with gold tint)	Opaque Gold/Yellow	All 400-480 nm	Skim, 1%, 2% and Whole Milk	2000 lumens/M^2	24 hr	5 ± 1° C	2% loss in skim		Senyk and Shipe (1981)
Glass	Transparent						1% loss in skim		
Polycarbonate	Transparent						9% loss in skim		
PE (returnable)	Somewhat Transparent						16% loss in skim		
							15% loss in skim		
PE (throw-away)	Somewhat Transparent						14% loss in skim		
Paperboard	Opaque	0% at 400 nm 0.5% at 500 nm	Milk	800 µW	16 hr 24 hr 48 hr 72 hr	3° C	-- 5 % loss 11 % loss 12 % loss		Farrer (1983)
HDPE w/TiO$_2$	Opaque	3% at 400 nn 11% at 500 nm			16 hr 24 hr 48 hr 72 hr		32% loss 37 % loss 54 % loss 58% loss		
HDPE	Transparent	44.5% at 400 nm 49% at 500 nm			16 hr 24 hr 48 hr 72 hr		32 % loss 37 % loss 54 % loss 58 % loss		
HDPE with: FD&C Yellow #5			Milk	190 ± 10 lumens	16 hr	1.5 ± 1.5° C			Fanelli and others (1985)
(0.05%)	Yellow	< ~ 500 nm					71% loss		
(0.3%)	Yellow						0.5% loss		
Quinacridone Red (2.89 g/L)	Red	< ~ 575 nm					71% loss		
Ultramarine Blue (0.962 g/L)	Blue	< ~ 500					75% loss		
HDPE/Cyasorb 531	Transparent	< ~ 350 nm					70% loss		
HDPE/Tinuvin 326	Transparent	< ~ 375 nm					70% loss		
HDPE/Tinuvin 622	Transparent						57% loss		
PE 2 pint cartons	White/opaque		Whole milk	4000 lux	90 hrs	7° C	Full protection	Dislike very much by 17 hrs.	Schroeder and others 1985
HDPE 4 pint bottles	White/opaque						15% loss	Dislike moderately by 17 hrs.	

		Milk	Fluorescent light = 1600 lux	Variable times (see Riboflavin loss section)	5° C	Concentrations in µg/l	7 pt scale for light off flavor where 1 – none, 7 = intense	Haisman, and others 1992
Fiberboard carton (1 liter)	Opaque		Daylight = 12,000 lux			72 hr dark: 191 3 hr daylight:170 6 hr: 160 24 hr: 147 72 hr: 173	72 hr dark: 2.7 3 hr daylight: 3.5 6 hr: 2.0 24 hr: 3.4 72 hr: 2.7	
HDPE bottles (2 liter)	Transparent					72 hr dark: 167 3 hr daylight: 132 6 hr: 184 24 hr: 147 72 hr: 122	72 hr dark: 2.3 3 hr daylight: 5.9 6 hr: 3.1 24 hr: 5.3 72 hr: 5.3	
Paperboard	Opaque	Whole milk		6 hrs	23° C	7 % loss		Al-Zawawi and Caldwell (1993)
Plastic	Transparent	Whole milk		60 days		10 % loss		
Paperboard	Opaque	Skim milk		60 days		20 % loss		
HDPE	Transparent	Whole and skim milk	½ average light intensity of front row/top shelf in supermarket	18 days	4° C	Whole: ~80% 2%: ~70%		Cladman and others 1998
LDPE	Transparent					Whole: ~58% 2%: ~52%		
PET	Transparent					Whole: ~50% 2%: ~71.5%		
PET	Green	~ 510 nm				Whole: ~47% 2%: ~52%		
TetraBrik	Opaque	Milk	Neon lighting	Up to 12 days	1-4° C	2-3 % loss	Poorer quality in PET	Marchetti and Forti (2001)
TetraRex	Opaque					2-3 % loss		
PET	Transparent					3-5 % loss		

HDPE	Transparent		Whole milk Reduced fat Skim milk	1000 lux 2000 lux	16 hrs	6º	2000 lux exposure: Reduced: 29% loss Skim: 49% loss 1000 lux exposure: Whole: 0% loss Reduced: 24% loss Skim: 32% loss		Whited and others 2002
3 layer PET (white/black/white) PET with oxygen scavenger	Opaque Transparent	<0.5% T of 200-800 nm ~70-80% T between ~350 and 800 nm. ~ 0% T below 350 nm	Milk, 1.3% fat	2500 lux	2 months	18-25º C	Full protection against loss 71% loss	Full protection against LOF SD in LOF (P< 0.05) by 2 days	Mestdagh (2005)
PET with UV absorber	Transparent	0.2-0.5% T between 400-800 nm. 0% < 400 nm					61% loss	SD in LOF(P< 0.05) by 2 days	

Appendix Table A3: Summary of Literature Review for Other Vitamin Degradation

Packaging Material	Color of Packaging Material	Wavelength Blocked/ Exposed to	Product	Light Intensity	Time of Exposure	Temp.	Vitamin Concentration/ Loss	Sensory Change	Reference
Petri Plates exposed to light	Transparent		Ascorbic Acid in water	500 ft-c	0 min 30 min 60 min 90 min 120 min 150 min	15º C	40.00 mg/100 ml 39.54 mg/100 ml 39.50 mg/100 ml 39.54 mg/100 ml 39.32 mg/100 ml 39.54 mg/100 ml		Sattar and others (1977c)
Petri Plates not exposed to light					0 min 30 min 60 min 90 min 120 min 150 min		40.00 mg/100 ml 39.35 mg/100 ml 39.54 mg/100 ml 39.32 mg/100 ml 39.20 mg/100 ml 39.54 mg/100 ml		
PE/Cardboard (no headspace)	Opaque		Acorbic Acid in Milk		6 days	6º C	9% loss		Goussault and others (1978)
PE (air in headspace)	Transparent						56% loss		
Paperboard	Opaque		Ascorbic Acid in Milk	800 µW	8 hr 24 hr 48 hr 72 hr	3º C	9 % loss 14 % loss 29 % loss 43 % loss		Farrer (1983)
HDPE w/TiO$_2$	Opaque				8 hr 24 hr 48 hr 72 hr		13% loss 40 % loss 79 % loss 100 % loss		
HDPE	Transparent				8 hr 24 hr 48 hr 72 hr		16 % loss 53 % loss 98 % loss 100 % loss		

Tetra Brik	Opaque	Ascorbic Acid	2000 ± 100 lux	12 hrs 5 days	4º C	~0% loss ~0% loss		Eberhard and Gallman 1991
Plastic Tubes	Transparent			12 hrs 5 days		50% loss 100% loss		
		Ascorbic Acid in Milk	Fluorescent light = 1600 lux Daylight = 12,000 lux	Variable times (see Riboflavin loss section)	5º C	Content in mg/l	7 pt scale for light off flavor where 1 – none, 7 = intense	Haisman, and others 1992
Fiberboard carton (1 liter)	Opaque					72 hr dark: 10.0 3 hr daylight: 9.7 6 hr: 12.9 24 hr: 11.3 72 hr: 8.5	72 hr dark: 2.7 3 hr daylight: 3.5 6 hr: 2.0 24 hr: 3.4 72 hr: 2.7	
HDPE bottles (2 liter)	Transparent					72 hr dark: 10.5 3 hr daylight: 0.5 6 hr: 8.9 24 hr: 1.4 72 hr: 0.5	72 hr dark: 2.3 3 hr daylight: 5.9 6 hr: 3.1 24 hr: 5.3 72 hr: 5.3	
TetraBrik TetraRex PET	Opaque Opaque Transparent	Ascorbic Acid in Milk	Neon lighting	Up to 12 days	1-4º C	2 % loss 7 % loss 13 % loss		Marchetti and Forti (2001)

3 layered HDPE (without oxygen barrier)	Opaque		Ascorbic Acid		3 days 1 month 4 months		35% loss 99% loss --		Gilgeum and Birlouez-Aragon 2005
6 layered HDPE (with oxygen barrier)	Opaque				3 days 1 month 4 months		35% loss 51% loss 75% loss		
			Thiamine in Milk	Fluorescent light = 1600 lux Daylight = 12,000 lux	Variable times (see Riboflavin loss section)	5º C	Content in µg/l	7 pt scale for light off flavor where 1 – none, 7 = intense	Haisman, and others 1992
Fiberboard carton (1 liter)	Opaque						72 hr dark: 314 3 hr daylight: 320 6 hr: 319 24 hr: 308 72 hr: 320	72 hr dark: 2.7 3 hr daylight: 3.5 6 hr: 2.0 24 hr: 3.4 72 hr: 2.7	
HDPE bottles (2 liter)	Transparent						72 hr dark: 297 3 hr daylight: 297 6 hr: 314 24 hr: 313 72 hr: 308	72 hr dark: 2.3 3 hr daylight: 5.9 6 hr: 3.1 24 hr: 5.3 72 hr: 5.3	
Petri Plates	Transparent	350 nm 415 nm 455 nm 465 nm 485 nm 520 nm 535 nm 560 nm 570 nm 595 nm Dark control	B carotene in Hexane	500 ft-c	77 hr	25º C	1.12 µg/g 2.23 µg/g 2.97 µg/g 3.51 µg/g 3.54 µg/g 3.59 µg/g 3.59 µg/g 3.54 µg/g 3.58 µg/g 3.57 µg/g 3.65 µg/g		Sattar and others (1977b)

Table B1. Mean light intensity readings ± SD (lux) for light fixtures used in study

Position	Right, First Row	Right, First Little Row	Right, Second Row	Left, First Row	Left, First Little Row	Left, Second Little Row
Mean ± SD	$805^a \pm 190$	$896^a \pm 10$	$848^a \pm 175$	$901^a \pm 150$	$887^a \pm 9$	$886^a \pm 8$

[a]Means followed by the same letter are not significantly different at the 0.05 level experimentwise using Tukey's HSD (Sall, Lehman, and Creighton, 2005)

Table B2. Means and standard errors of the mean for proximate analyses of milk used for each of the three test replications.

Replication	% Water ± SEM	% Total Solids ± SEM	% Ash ± SEM	% Crude Protein ± SEM	% Fat	Number of Samples
1	88.7 ± 0.03^a	11.3 ± 0.03^a	0.65 ± 0.01^a	3.561 ± 0.06^a	2.1	3
2	88.8 ± 0.00^a	11.2 ± 0.00^a	0.62 ± 0.01^a	3.50 ± 0.10^a	2.05	3
3	88.3 ± 0.28^a	11.7 ± 0.28^a	0.40 ± 0.08^b	3.58 ± 0.14^a	2.1	3

[a]Means followed by the same letter are not significantly different at the 0.05 level experimentwise using Tukey's HSD (Sall, Lehman, and Creighton, 2005)

Table B3. Mean hexanal concentration ± standard deviation (ppm) from three experimental replications for all treatments on all sampling days of the experiment for milk exposed to 854 ± 16 lux fluorescent light.

	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28
Light Protected	$0.02^c \pm$ 0.05	$0.03^c \pm$ 0.06	$0.00^c \pm$ 0.00	$0.46^c \pm$ 0.06	$0.09^b \pm$ 0.19	$0.05^c \pm$ 0.10
400 nm Block	$0.85^b \pm$ 0.54	$3.62^b \pm$ 2.03	$6.46^b \pm$ 1.98	$8.80^b \pm$ 1.15	$14.30^a \pm$ 3.16	$15.37^b \pm$ 2.30
446 nm Block	$0.81^b \pm$ 0.38	$2.53^{b,c} \pm$ 1.16	$6.71^b \pm$ 3.28	$9.44^{a,b} \pm$ 3.90	$13.76^a \pm$ 3.30	$19.74^{a,b} \pm$ 6.65
570 nm Block	$1.12^{a,b} \pm$ 0.36	$5.17^{a,b} \pm$ 2.06	$8.47^{a,b} \pm$ 3.47	$9.68^{a,b} \pm$ 1.57	$13.99^a \pm$ 3.42	$15.83^b \pm$ 5.48
Broad spectrum	$1.04^b \pm$ 0.82	$3.77^b \pm$ 1.85	$7.07^{a,b} \pm$ 2.59	$10.19^{a,b} \pm$ 0.97	$15.09^a \pm$ 4.64	$15.69^b \pm$ 4.75
Light Exposed	$1.81^a \pm$ 0.58	$7.45^a \pm$ 3.59	$10.82^a \pm$ 3.84	$12.35^a \pm$ 3.16	$18.50^a \pm$ 5.70	$23.23^a \pm$ 7.01

[a,b,c] Means followed by the same letter are not significantly different at theP=0.05 level experimentwise using Tukey's HSD (Sall, Lehman, and Creighton, 2005)

Table B4. Mean pentanal concentration ± standard deviation (ppm) from three experimental replications for all treatments on all sampling days of the experiment for milk exposed to 854 ± 16 lux fluorescent light.

	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28
Light Protected	$0.05^b \pm$ 0.10	$0.03^b \pm$ 0.09	$0.00^b \pm$ 0.00	$0.08^a \pm$ 0.20	$0.08^b \pm$ 0.15	$0.08^b \pm$ 0.13
400 nm Block	$0.32^{a,b} \pm$ 0.26	$0.53^{a,b} \pm$ 0.42	$0.99^{a,b} \pm$ 0.84	$0.81^a \pm$ 0.90	$1.52^{a,b} \pm$ 1.52	$1.94^{a,b} \pm$ 1.62
446 nm Block	$0.35^{a,b} \pm$ 0.28	$0.57^{a,b} \pm$ 0.50	$1.16^{a,b} \pm$ 1.12	$1.21^a \pm$ 1.51	$2.06^{a,b} \pm$ 1.63	$2.87^{a,b} \pm$ 2.57
570 nm Block	$0.29^{a,b} \pm$ 0.22	$0.77^a \pm$ 0.76	$0.98^{a,b} \pm$ 0.86	$0.80^a \pm$ 0.88	$1.71^{a,b} \pm$ 1.55	$1.34^{a,b} \pm$ 1.12
Broad spectrum	$0.33^{a,b} \pm$ 0.21	$0.63^{a,b} \pm$ 0.61	$1.13^{a,b} \pm$ 0.97	$0.89^a \pm$ 0.98	$2.30^{a,b} \pm$ 1.95	$1.73^{a,b} \pm$ 2.06
Light Exposed	$0.49^a \pm$ 0.27	$1.04^a \pm$ 0.46	$1.84^a \pm$ 1.25	$1.78^a \pm$ 1.43	$2.93^a \pm$ 2.38	$3.36^a \pm$ 2.63

[a,b,c] Means followed by the same letter are not significantly different at theP=0.05 level experimentwise using Tukey's HSD (Sall, Lehman, and Creighton, 2005)

Table B5. Mean 1-octene-3-ol concentration ± standard deviation (ppm) from three experimental replications for all treatments on all sampling days of the experiment for milk exposed to 854 ± 16 lux fluorescent light.

	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28
Light Protected	$0^c \pm 0$	$0^c \pm 0$	$0^c \pm 0$	$0^c \pm 0$	$0^a \pm 0$	$0^c \pm 0$
400 nm Block	$0.019^b \pm 0.010$	$0.063^b \pm 0.020$	$0.132^{a,b} \pm 0.072$	$0.093^{b,c} \pm 0.041$	$0.207^a \pm 0.065$	$0.232^{a,b} \pm 0.057$
446 nm Block	$0.026^b \pm 0.067$	$0.052^b \pm 0.024$	$0.095^{a,b,c} \pm 0.049$	$0.166^{a,b} \pm 0.060$	$0.171^a \pm 0.072$	$0.156^b \pm 0.044$
570 nm Block	$0.028^b \pm 0.005$	$0.069^b \pm 0.033$	$0.105^{a,b,c} \pm 0.030$	$0.118^{a,b,c} \pm 0.032$	$0.268^a \pm 0.079$	$0.223^{a,b} \pm 0.075$
Broad spectrum	$0.026^b \pm 0.008$	$0.055^b \pm 0.036$	$0.059^{b,c} \pm 0.030$	$0.114^{a,b,c} \pm 0.048$	$0.242^a \pm 0.085$	$0.199^{a,b} \pm 0.035$
Light Exposed	$0.043^a \pm 0.085$	$0.140^a \pm 0.044$	$0.168^a \pm 0.112$	$0.255^a \pm 0.119$	$0.295^a \pm 0.093$	$0.323^a \pm 0.171$

[a,b,c] Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD (Sall, Lehman, and Creighton, 2005)

Table B6. Average area counts ± standard deviations for an unknown treatment with a retention time of 5.793 from all three experimental replications for all treatments on all days of the experiment for milk exposed to 854 ± 16 lux fluorescent light.

	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28
Light Protected	$0^a \pm 0$	$0^b \pm 0$	$0^a \pm 0$	$0^b \pm 0$	$0^c \pm 0$	$0^b \pm 0$
400 nm Block	$0^a \pm 0$	$3490^{a,b} \pm 6045$	$2561^a \pm 4436$	$11285^{a,b} \pm 1843$	$25733^b \pm 2309$	$28937^{a,b} \pm 11759$
446 nm Block	$0^a \pm 0$	$0^b \pm 0$	$5057^a \pm 4397$	$9453^{a,b} \pm 9598$	$22306^b \pm 12217$	$33243^{a,b} \pm 11716$
570 nm Block	$0^a \pm 0$	$0^b \pm 0$	$12080^a \pm 2072$	$12765^{a,b} \pm 3004$	$26150^b \pm 4624$	$38853^{a,b} \pm 18968$
Broad spectrum	$0^a \pm 0$	$2554^{a,b} \pm 4423$	$5759^a \pm 5043$	$15250^a \pm 3253$	$17130^{b,c} \pm 4169$	$36643^{a,b} \pm 15403$
Light Exposed	$0^a \pm 0$	$13296^a \pm 7227$	$17113^a \pm 5221$	$19923^a \pm 4992$	$44933^a \pm 6516$	$61606^a \pm 35070$

[a,b,c] Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD (Sall, Lehman, and Creighton, 2005)

Table B7. Retention time, compound identification, aroma and average intensities of odor-active compounds in milk exposed to 854 ± 16 lux fluorescent light for the combined replications (n=3) for all treatments on day 14 of the experiment.

RT	Compound	Aroma	Wavelength					
			400 nm Block	446 nm Block	570 nm Block	Broad spectrum	Light Exposed	Light Protected
1.89-2.29		Flatulence	+	+	+	++	+	—
2.29-2.65		Astringent	—	—	—	—	—	+
3.19-3.30		Flatulence	—	—	—	+	—	—
3.58-4.07		Cooked/ Baked/ Sweet	—	—	—	—	+	—
3.67-3.99		Musty	—	+	—	—	—	—
4.46-5.17	Hexanal	Grass	++	++	++	++	++	++
5.03-5.08		Cooked/ Baked Bread	—	—	+	—	—	—
5.22-5.91		Greens	—	—	+	+	—	—
5.82-6.13	2-Heptanone	Roasted Grain/ Baked/ Musty	++	—	—	++	+++	++
6.07-6.52		Musty/ Cardboard/ Mushroom	—	—	++	—	—	—
6.45-6.92		Cardboard/ Musty/ Mushroom	—	—	++	—	—	++
6.47-6.52	Heptanal	Grass	+	—	—	—	—	—
6.59-6.95		Musty	+++	+++	—	—	—	—
6.71-6.79	1-heptanol	Green/ Floral	—	—	—	—	++	—
6.81-7.19	1-octene-3-ol	Mushrooms /Musty	++	+	—	++	++	+
8.21-8.64	Nonanal	Sugar/ Cooked/ Cookies	—	—	++	—	++	—
8.62-8.93		Cigarette Smoke	+	—	—	—	—	—
8.87-9.25		Sweet/ Baked Cookies	—	—	—	—	—	+
9.23-9.25		Musty	—	—	++	—	—	—
9.50-9.97		Musty	+	+	—	—	—	—
9.64-9.87		Grass/ Floral	+	—	—	—	—	—
11.52-11.69		Cooked	—	+	—	—	—	—

+ = slight intensity, ++ = moderate intensity, +++ = high intensity

Table B8. Retention time, compound identification, aroma and average intensities of odor-active compounds in milk exposed to 854 ± 16 lux fluorescent light for the combined replications (n=3) for all treatments on day 28 of the experiment.

RT	Compound	Aroma	Wavelength					
			400 nm Block	446 nm Block	570 nm Block	Broad spectrum	Light Exposed	Light Protected
1.99-2.01		Flatulance	+	+	+	+	+	—
2.27		Acetone	+	—	—	—	—	—
2.69-2.74		Flatulance	—	—	—	—	+	—
2.90		Acetone	—	—	—	—	++	—
3.14-3.73		Baked/Roasted	—	—	—	++	+	—
3.63-3.79		Astringent	—	—	+	—	—	—
4.44-4.85	Hexanal	Grass	++	++	++	++	+++	+
5.10-5.22		Baked	—	—	+	—	—	—
5.51-5.82		Baked Bread/ Roasted Nuts	—	—	—	+	—	—
5.97-6.10		Mushroom/Musty	+++	++	+++	+++	+++	+
5.99-6.19	2-Heptanone	Baked Bread/ Roasted	—	—	—	+++	—	—
6.15-6.34		Mushroom/Earthy	—	—	+++	—	—	—
6.51-6.92		Baked Bad Smell	—	—	+++	—	—	—
6.78-7.19	1-Octene-3-ol	Musty/Mushroom	++	++	+++	++	—	—
7.19-7.33		Ashtray	—	—	++	—	—	—
7.71-8.10	Nonanal	Cotton Candy/Sugar	—	+++	++	+	—	—
7.85-8.50		Musty	—	—	+	—	+	—
8.77-9.25		Candy/Sweet	—	—	—	++	—	+++
9.17-9.21		Green	—	+	—	—	—	—
9.20-9.50		Sugar	+	—	—	—	—	+++
10.00-10.40		Medicinal	—	—	—	—	+	—

+ = slight intensity, ++ = moderate intensity, +++ = high intensity

216

Table C1. Means with standard errors measured in lux (mean ± SE (lux) and number of readings taken (N) for light intensity for each light fixture[1] used in study.

Light Fixture	Mean ± SE (lux)	N
1	1560 ± 64[a]	6
2	1528 ± 30[a]	5
3	1531 ± 33[a]	6
4	1472 ± 53[a]	7

*Means followed by the same letter are not significantly different at the 0.05 level experimentwise using Tukey's HSD

[1]Each light fixture consisted of two 30-36W cool white fluorescent bulbs

Table C2. Means and standard errors of the mean for proximate analyses of milk used for each of the three test replications.

Replication	% Water ± SEM	% Total Solids ± SEM	% Ash ± SEM	% Crude Protein ± SEM	% Fat ± SEM	Number of Readings (N)
1	88.6[a] ± 0.06	11.4[a] ± 0.06	0.47[a] ± 0.03	3.55[a] ± 0.16	1.99[a] ± 0.04	N=4 for % fat, N=3 for all other calculations
2	88.7[a] ± 0.03	11.3[a] ± 0.03	0.52[a] ± 0.05	3.68[a] ± 0.07	1.98[a] ± 0.03	N=4 for % fat, N=3 for all other calculations
3	87.4[a] ± 0.51	12.6[a] ± 0.51	0.48[a] ± 0.04	3.67[a] ± 0.02	2.10[a] ±0.04	N=4 for % fat, N=3 for all other calculations

* Means foll.owed by the same letter are not significantly different at the 0.05 level experimentwise using Tukey's HSD

Table C3. Average total plate counts (TPC) and coliform count (FC) in cfu/ml for each replication by day.

		Average Bacterial Count						
Replication	Media	Day 0	Day 1	Day 4	Day 7	Day 10	Day 14	Day 21
1	TPC	0	0	0	0	0	0	0
	FC	0	0	0	0	0	0	0
2	TPC	0	0	0	0	1	0	0
	FC	0	0	0	0	0	0	0
3	TPC	0	107	0	836	1920	0	254
	FC	0	52	0	396	865	0	102

218

Table C4. Average concentrations (ppm) of hexanal on days 0, 1, 4, 7, 10, 14 and 28 ± standard deviation for each of the packaging treatments.

Treatment	Day						
	0	**1**	**4**	**7**	**10**	**14**	**21**
400 nm Block	NP	$0.030^a \pm$ 0.029	$0.092^a \pm$ 0.088	$0.137^{a,b} \pm$ 0.134	$0.194^{a,b,c} \pm$ 0.160	$0.437^{a,b} \pm$ 0.100	$0.405^{a,b} \pm$ 0.315
446 nm Block	NP	$0.019^a \pm$ 0.033	$0.040^a \pm$ 0.069	$0.082^{a,b} \pm$ 0.077	$0.219^{a,b,c} \pm$ 0.232	$0.248^{a,b,c} \pm$ 0.299	$0.597^a \pm$ 0.517
570 nm Block	NP	$0.057^a \pm$ 0.077	$0.051^a \pm$ 0.089	$0.191^{a,b} \pm$ 0.174	$0.266^{a,b} \pm$ 0.207	$0.348^{a,b,c} \pm$ 0.318	$0.468^{a,b} \pm$ 0.276
BS 20 T	NP	$0.039^a \pm$ 0.045	$0.081^a \pm$ 0.072	$0.201^{a,b} \pm$ 0.159	$0.181^{a,b,c} \pm$ 0.144	$0.132^{b,c} \pm$ 0.179	$0.239^{a,b} \pm$ 0.337
BS 4 T	NP	$0.139^a \pm$ 0.201	$0.060^a \pm$ 0.041	$0.145^{a,b} \pm$ 0.126	$0.111^{b,c} \pm$ 0.114	$0.610^{a,b,c} \pm$ 0.591	$0.536^a \pm$ 0.235
Light Protected	$0.006^a \pm$ 0.011	$0.047^a \pm$ 0.082	$0.020^a \pm$ 0.034	$0.041^b \pm$ 0.072	$0.006^c \pm$ 0.011	ND^c	$0.035^b \pm$ 0.060
Light Exposed	NP	$0.053^a \pm$ 0.064	$0.074^a \pm$ 0.068	$0.357^a \pm$ 0.040	$0.397^a \pm$ 0.030	$0.628^a \pm$ 0.026	$0.576^a \pm$ 0.508

[a,b,c]Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD
NP=not performed (GC analysis was only done on the light protected sample on day 0)
ND=not detected

Table C5. Average concentrations (ppm) of pentanal on days 0, 1, 4, 7, 10, 14 and 28 ± standard deviation for each of the packaging treatments.

Treatment	Day						
	0	1	4	7	10	14	21
400 nm Block	NP	0.191[a] ± 0.212	0.208[a] ± 0.200	0.195[a] ± 0.222	0.225[a] ± 0.212	0.286[a] ± 0.198	0.208[a] ± 0.206
446 nm Block	NP	0.195[a] ± 0.209	0.190[a] ± 0.223	0.200[a] ± 0.210	0.240[a] ± 0.183	0.263[a] ± 0.424	0.349[a] ± 0.094
570 nm Block	NP	0.218[a] ± 0.190[a]	0.192[a] ± 0.223	0.281[a] ± 0.181	0.228[a] ± 0.209	0.272[a] ± 0.161	0.272[a] ± 0.306
BS 20 T	NP	0.195[a] ± 0.209	0.252[a] ± 0.162	0.230[a] ± 0.191	0.250[a] ± 0.186	0.171[a] ± 0.269	0.247[a] ± 0.164
BS 4 T	NP	0.192[a] ± 0.213	0.195[a] ± 0.223	0.216[a] ± 0.198	0.195[a] ± 0.217	0.284[a] ± 0.355	0.294[a] ± 0.221
Light Protected	0..145[a] ± 0..252	0.145[a] ± 0.252	0..145[a] ± 0.252	0.145[a] ± 0.252	0.161[a] ± 0.261	0.145[a] ± 0.252	0..145[a] ± 0.252
Light Exposed	NP	0.207[a] ± 0.198	0.189[a] ± 0.217	0.319[a] ± 0.187	0.271[a] ± 0.203	0.289[a] ± 0.369	0.292[a] ± 0.150

[a,b,c]Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD
NP=not performed (GC analysis was only done on the light protected sample on day 0)
ND=not detected

Table C6. Average concentrations (ppm) of heptanal on days 0, 1, 4, 7, 10, 14 and 28 ± standard deviation for each of the packaging treatments.

Treatment	Day						
	0	1	4	7	10	14	21
400 nm Block	NP	$0.000^a \pm$ 0.000	$0.020^a \pm$ 0.034	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.058^a \pm$ 0.029	$0.083^a \pm$ 0.055
446 nm Block	NP	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.014^a \pm$ 0.024	$0.012^a \pm$ 0.021	$0.038^a \pm$ 0.066	$0.084^a \pm$ 0.075
570 nm Block	NP	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.012^a \pm$ 0.021	$0.015^a \pm$ 0.017	$0.043^a \pm$ 0.048	$0.054^a \pm$ 0.018
BS 20 T	NP	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.011^a \pm$ 0.019	$0.044^a \pm$ 0.045	$0.038^a \pm$ 0.037
BS 4 T	NP	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.104^a \pm$ 0.136	$0.063^a \pm$ 0.046
Light Protected	$0.000 \pm$ 0.000^a	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000
Light Exposed	NP	$0.000^a \pm$ 0.000	$0.000^a \pm$ 0.000	$0.023^a \pm$ 0.040	$0.025^a \pm$ 0.032	$0.083^a \pm$ 0.023	$0.061^a \pm$ 0.035

[a,b,c]Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD
NP=not performed (GC analysis was only done on the light protected sample on day 0)
ND=not detected

221

Table C7. Average area counts of an unknown compound with a average retention time of 3.302 on days 0, 1, 4, 7, 10, 14 and 28 for each of the packaging treatments.

Treatment	Day						
	0	1	4	7	10	14	21
400 nm Block	NP	10248	1740	0	137	13791	26878
446 nm Block	NP	4785	1833	0	0	64459	26546
570 nm Block	NP	5113	902	762	409	81500	32951
BS < 20% T	NP	4558	6795	0	361	90833	18403
BS < 4% T	NP	3762	1552	0	526	70711	34880
Light Protected	6396	3828	3537	0	1042	0	79053
Light Exposed	NP	2916	1269	313	372	65963	29107

[a,b,c]Means followed by the same letter are not significantly different at the P=0.05 level experimentwise using Tukey's HSD
NP=not performed (GC analysis was only done on the light protected sample on day 0)
ND=not detected

222

Figure C1. Sample IRB Informed Consent Form

Title: Effect of Film Over-wraps on the Production of Light Oxidation in 2% Milk
Principal Investigator: Susan E. Duncan

I. THE PURPOSE OF THIS PROJECT
You are invited to participate on a sensory evaluation panel that is looking at the effect of film overwraps on the sensory quality of UHT milk over a 21 day storage period.

II. PROCEDURES
There will be 6 sessions over a period of 3 weeks involving about 10 minutes at each session. You will be presented with 3 sets of 3 samples at each session. As a panelist, it is critical to the project that you attend each session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergy, list them in the following space:

III. BENEFITS/RISKS OF THE PROJECT
Your participation in the project will provide the following information that may be helpful. We would like to determine the effect that iridescent film overwraps have on the sensory quality of milk, specifically on the production of light oxidized flavor. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY
The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION
For participation in the project, you will receive candy for each session completed.

VI. FREEDOM TO WITHDRAW
It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH
This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology

223

VIII.   SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require 6 sessions lasting 10 minutes each period for three weeks.

_____

Signature/Date

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

Address: _____

Phone: _____

- -(tear off)- -

IX.   SUBJECT'S PERMISSION (provide tear off for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study which will require 6 sessions lasting ten minutes each period for three weeks.

_____

Signature

Should I have any questions about this research or its conduct, I should contact:

Janet B. Webster (540) 230-6760
Investigator/Phone

Dr. Susan E. Duncan (540) 231-8675
Faculty/Phone

Dr. Moore (540) 231-6077
Chair, IRB/Phone for Research Division

224

Figure C2. Sample Scoresheet for ranking data for film study.

Ranking Test

Panelist Number: _____

Panelist Name: _____
Date: _____
Type of Sample: _____UHT pasteurized 2% milk_____

Instructions

1. You have received three sets of 3 samples. Note the sample code on each sample.
2. Taste the first set of 3 samples and rank them from least to most in light oxidized flavor.
3. Write a 1 for least oxidized under the code of the sample that is least oxidized. Write a 3 for most oxidized under the code of the sample that is most oxidized. Write a 2 for the sample that is intermediate under the code for that sample.
4. If you cannot decide on the order, make your best guess.
5. Wait 1 minute and eat a cracker and sip some water.
6. Taste the second set of three samples and rank them according to the instructions above.
7. Repeat steps 5 and 6 for the third sample set.

Ranking Test 1

Code #   _____   _____   _____

Rank   _____   _____   _____

Ranking Test 2

Code #   _____   _____   _____

Rank   _____   _____   _____

Ranking Test 3

Code #   _____   _____   _____

Rank   _____   _____   _____

225

<center>**Janet Brown Webster**</center>

PERSONAL INFORMATION

Address: Proposal Development Team
             1880 Pratt Drive, Room 2003
             Virginia Tech, Blacksburg VA 24061
Telephone: (540) 231-6806 (W), (540) 230-6760 (Cell)
Email: jbwebste@vt.edu

EDUCATION

2002-present   Ph.D (in progress), Department of Food Science and Technology
                    Virginia Tech, Blacksburg, VA
                    4.0 GPA in doctorate, 3.98 overall graduate
1994   M.S. in Food Science and Technology, Virginia Tech, Blacksburg, VA
1984   B. S. in Biology, Virginia Tech, Blacksburg, VA
                        Virginia Teaching Certificate-Grades 6-12, Biology endorsement

PROFESSIONAL EXPERIENCE

1997-2004   *Adjunct Faculty*
                    Radford University, Dept. of Chemistry and Physics,
                    Department of Foods and Nutrition

1997-2004   *Instructor (SAT, GRE and MCAT)*
                    The Princeton Review
1993-1994   *Research Associate*
                    Virginia Tech, Dept. of Food Science and Technology
1991-1993   *Laboratory Specialist, Electron Microscopy Laboratory*
                    Virginia Tech, Dept. of Biology
1989-1991   *Laboratory Specialist, Food Microbiology*
                    Virginia Tech, Dept. of Food Science and Technology
1988-1989   *Assistant Laboratory Manager*
                    Olver Inc., Environmental Engineering
1985-1987   *Volunteer, Community Development*
                    United States Peace Corps, Papua New Guinea
1984-1985   *Laboratory Specialist, Water Chemistry Laboratory*
                    Virginia Tech, Dept. of Biology

AWARDS AND HONORS

<u>National:</u>

2006   Manfred Kroger Oral Competition, Institute of Food Science (IFT),
                    Orlando FL, July 2006
2005   National Milk Producers Federation (NMPF) National Dairy Leadership
                    Scholarship Program-$2,000
2005   V. Duane Rath Fellowship, International Association of Food
                    Industry Suppliers (IAFIS)-$10,000
2004   National Food Processors Association (NFPA) Graduate Student
                    Scholarship Award-$3,000

2004-2006	National Science Foundation, Macromolecular Interfaces with Life Sciences-Integrative Graduate Education and Research Traineeship (NSF MILES-IGERT) Fellowship-Tuition and stipend for 21 months- $73,000 total value
2003	Third place overall, 82nd Annual Dairy Products Evaluation Contest, Chicago (graduate student division)
1991	Institute of Food Technologists (IFT) Graduate Student Paper Competition, Semi-finalist

Regional/Local:

2006	Virginia Tech Graduate School, Graduate Student of the Month (February)
2005	Virginia Tech Graduate Research Travel Grant Award to present at IFT National Meeting, New Orleans LA-$262
2005	Food Science and Technology Faculty Award for Outstanding Graduate Student-$250
2004	Virginia Tech Graduate Research Travel Grant Award to present at IFT National Meeting, Las Vegas, NV-$250
1991	Gamma Sigma Delta (Agriculture Honor Society)

PROFESSIONAL MEMBERSHIPS

2004-present	American Dairy Science Association (ADSA)
2003-present	American Chemical Society (ACS)
1990-present	Institute of Food Technologists (IFT)

SERVICE

2005-2006	Chairman of Fund Raising Committee, Dublin Middle School Parent Teacher Organization
2000-2004	Radford University Pre-Health Advisory Committee
2000-present	Board of Directors, Camp Timber Ridge Science Camp
2002-2005	Girl Scout Leader, Skyline Council, Brownie Troop 89
2005-present	Girl Scout Assistant Leader, Skyline Council, Junior Troop 619
2004-present	Secretary, Pulaski County Wolves AAU Basketball Club
2001-2003	Chairman of Fund Raising Committee, Riverlawn Elementary School Parent Teacher Organization

TEACHING EXPERIENCE

2006	*HNFE 1004: Foods and Nutrition (in fulfillment of professional teaching internship)*
	Virginia Tech, Dept. of Human Nutrition, Foods and Exercise Topics covered in this introductory course included digestion, proteins, lipids, carbohydrates, energy metabolism, vitamins and nutraceuticals, and food safety.
	Course enrollment: ~500 (100 majors in one section, 400 non-majors in second section). Teaching responsibility: 50%

2004-2005	*FST 2104/4974: Dairy Products Sensory Evaluation* Virginia Tech, Dept. of Food Science and Technology Teaching responsibilities included training students in sensory evaluation of milk, butter, yogurt, cottage cheese, cheddar cheese, and ice cream and coaching students in the 83rd and 84th Annual Collegiate Dairy Products Evaluation Competition Course enrollment: 8-12. Teaching responsibility: 50%

1997-2004	*CHEM/BIOL 47 & 472: Biochemistry*
	  Radford University, Dept. of Chemistry and Physics
	  Topics covered in this senior level, year-long course included proteins, lipids, carbohydrates, enzyme kinetics, energy metabolism, and anabolic and catabolic metabolic mechanisms.
	Course enrollment: ~ 40. Teaching responsibility: 100%

1999-2003	*FDSN 414 & 415: Advanced Nutrition and Biochemistry*
	  Radford University, Dept. of Foods and Nutrition
	    Topics covered in this senior level, year-long course included digestion and absorption, proteins, lipids, carbohydrates, energy metabolism, anabolic and catabolic metabolism, vitamins, minerals, and phytochemicals and their metabolic mechanisms.
	Course enrollment: ~12. Teaching responsibility: 100%

2001-2002	*CHEM 101: General Chemistry and General Chemistry Laboratory*
	  Radford University, Dept. of Chemistry and Physics
	  Topics covered in the first semester of this freshman course included atoms, molecules and ions, stochiometry, chemical reactions, solution stochiometry, gases, thermochemistry, atomic structure and periodicity, and bonding and orbitals
	Course enrollment: ~ 25. Teaching responsibility: 100%

1991-1993	FST 4604: Food Microbiology Laborat *Virginia Tech, Dept. of Food Science and Technology* Teaching responsibilities included introducing students to laboratory methods in the detection and identification of microorganisms in food and their effect on food safety. Course enrollment: ~ 25. Teaching responsibility: 50%

PUBLICATIONS

Arany CB, Hackney CR, Duncan SE, Kator H, Eigel WN, **Webster JB**. 1995. Improved recovery of *Bifidobacterium* from water and dairy products. J. Food Prot 58(10): 1142-6.

Linton RH**, Webster JB**, Pierson MD, Bishop JR, Hackney CR. 1992. The effect of sub-lethal heat shock and growth atmosphere on the heat resistance of *Listeria monocytogenes* Scott A. J. Food Prot 55(2): 84-7.

Webster JB, Pierson MD, Flick GJ. 1991. Heat tolerance of spoilage organisms in pasteurized crabmeat. Proceedings of the 15th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas.

PRESENTATIONS

Janet B. Webster, Susan E. Duncan, Joseph E. Marcy, Sean F. O'Keefe, Thomas Ward, and Susan Nielsen-Sims. 2005.   Protection against light oxidation in milk by iridescent film. MILES Industry Showcase, Blacksburg, VA (oral presentation) and Macromolecular Interfaces Institute, Blacksburg, VA, (poster presentation)

Janet B. Webster, Susan E. Duncan, Joseph E. Marcy, and Sean F. O'Keefe. 2005. Effect of light wavelength on photo-oxidation in milk. MILES Industry Showcase, Blacksburg, VA, (poster presentation) and Macromolecular Interfaces Institute, Blacksburg, VA (poster presentation)

Janet B. Webster, Susan E. Duncan, Joseph E. Marcy, and Sean F. O'Keefe. 2005. Photo-protective efficiency of iridescent films for milk and orange juice. Institute of Food Technologists Annual Meeting, New Orleans, LA, (oral presentation)
Janet B. Webster, Susan E. Duncan, Joseph E. Marcy, and Sean F. O'Keefe. 2005. Effect of light wavelength on photo-oxidation in milk. Institute of Food Technologists Annual Meeting, New Orleans, LA, (oral presentation)

Janet B. Webster, Susan E. Duncan, Joseph E. Marcy, Sean F. O'Keefe, Thomas Ward, Susan N.Sims, and H. Francis Webster. 2005. Changes in aromatic chemistry and sensory quality of milk due to light wavelength. National Science Foundation IGERT Annual Meeting, Arlington, VA, (poster presentation)

Janet B. Webster, Susan E. Duncan, Joseph E. Marcy. 2004. Sensory issues in plastics. Institute of Food Technologists Annual Meeting, Las Vegas, NV, (Invited Presentation)

Susan E. Duncan, **Janet B. Webster**, Heather W. Clarkson. 2003. Photoprotective efficiency of tunable polymeric films for food systems. Center for Applied Packaging and Performance Studies (CAPPS), Davis California, Nov. 2003 (oral presentation)

GRANT PROPOSALS

Janet B. Webster. 2005. Virginia Tech Graduate School. Professorial Teaching Internships for MILES IGERT Fellowship Candidates. $8,573 (tuition and stipend for Spring Semester, 2006).

Janet B. Webster. 2005. Virginia Tech Graduate Research Development Grant. Effect of Specific Light Wavelengths on Oxidation in Milk. $375.00

Janet B. Webster. 2003. Virginia Tech Graduate Research Development Grant. Development of a Novel Technique to Monitor Light Oxidation in Milk Using Fourier Transform Infrared (FTIR) Spectroscopy. $375.00.

EXPERIENCE IN FEDERAL GRANT WRITING

Susan E. Duncan, Ph.D, R.D., H. Francis Webster, Ph.D.. Influence of specific light wavelengths on the chemical and sensory quality of a model beverage and juice with added bioactive compounds. USDA-CREES-NRI Competitive Grants Program: 71.1-Improving Food Quality and Value. Submitted December, 2004. Reviewed but not accepted, June, 2005. This grant proposal was written and submitted as part of my written preliminary examination.

Lactic Acid Fermentation to increase bioavailability of flavonoids. Co-principle investigators: Susan E. Duncan, Ph.D., R.D., Janet W. Rankin, Ph.D, Kumar Malakarjunan, Ph.D., Sean F. O'Keefe, Ph.D. This grant was written in NIH format as a multidisciplinary class project. Team members included Janet B. Webster (Food Science and Technology), Abby Turpyn (Human Foods, Nutrition and Exercise), Tameshia Ballard (Biological Systems Engineering), and Pinar Omur (Civil Engineering).
Parts of this grant were excerpted and submitted to General Mills, Bell Institute of Health and Nutrition as a letter of intent. The grant submission was entitled "In vitro bioavailability and antioxidant assessment of flavonoids enhanced by fermentation with probiotic and lactic acid bacteria." Co-investigators were Susan E. Duncan, Ph.D., R.D., Yong Woo Lee, Ph.D., and Craig Thatcher, Ph.D, D.V.M. Submitted November, 2005.