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Light Wavelength Effects on a Lutein-Fortified Model Colloidal Beverage

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ABSTRACT: The effect of light on a model colloidal beverage system containing whey protein, lutein, and limonene was investigated. Changes in volatile chemistry were evaluated under accelerated conditions (12 h, 25 °C) at selected wavelengths regions (395, 463, 516, 567, and 610 nm absorbance maxima) using a photochemical reactor. The most damaging wavelengths to lutein stability were UV (200−400 nm) and 463 nm wavelengths. Hexanal formation was highest in the control beverage when exposed to full spectrum light and UV (200−400 nm) wavelengths. Hexanal also was formed in the lutein-fortified beverage under full spectrum light and UV (200−400 nm) wavelengths but to a significantly lesser degree. Limonene degraded significantly under all treatment conditions, with most degradation occurring during full spectrum light exposure. Lutein fortification did not completely protect limonene from degradation.

KEYWORDS: lutein, limonene, oxidation, light, functional food, carotenoids, flavor

INTRODUCTION

Colloidal systems such as dairy-based beverages, infant formulas, and some nutrient-enriched drinks are very common in the beverage market. Functional beverages that impart health benefits beyond basic nutrition are increasingly emerging in the market place.[1,2] The definition of a functional food is not legally established, and the description varies by scientific organization, but the addition of bioactive substances to optimize health benefits broadly meets most accepted definitions.[3,4] However, incorporating bioactive ingredients, such as vitamins, minerals, proteins, and phospholipids, is a challenge to the food industry.[2,5] Some bioactive compounds, such as riboflavin, function as photosensitizers; some may be rapidly degraded by photochemical reactions.[6] Knowledge of the photochemical response of bioactive compounds is important because we hypothesize that specific light barrier packaging can be developed to reduce off-flavor formation and protect functional food ingredients from photo-oxidation,

Artificial fluorescent light, commonly used in beverage display cases in food service and retail establishments, emits a broad spectrum of ultraviolet and visible light.[7] Ultraviolet and visible light can cause photochemical reactions in food systems, leading to molecular excitation of susceptible compounds.[7] Molecular excitation is caused by photon energy, which is directly related to light wavelengths.[8]

When a ground state molecule is promoted to an excited state through light absorption, energy release can occur in several ways. Excited state molecules can release energy through heat, transfer of energy to other molecules, or emission of photons, also known as fluorescence.[9] Transfer of energy to other molecules can cause damage and also lead to off-flavor formation, color changes, and other negative sensory characteristics. For example, lipid oxidation results in the production of hydroperoxides, which upon decomposition, result in the formation of aldehydes, ketones, lactones, esters, and furans. These secondary products lead to off-flavors and rancidity in oxidized beverages.[10,11]

Compounds susceptible to light-induced oxidation include unsaturated fatty acids, such as ω-6 and ω-3 fatty acids, phospholipids, and flavor compounds such as limonene. Limonene is the main odor constituent of citrus and is found in high concentration in orange oil, which is a main flavor component in orange-flavored beverages. Limonene oxidation results in off-flavor and aroma formation and the production of degradation products such as carveol, carvone, and *p*-cymene. Limonene and its oxidation products have been detected using gas chromatography (GC) with solid-phase microextraction (SPME).[12]

Although light-barrier packaging significantly reduces off-flavor formation by protecting nutrients from photo-oxidation,[11,13,14] consumers prefer to see the product. Thus, antioxidant fortification may lead to a reduction in oxidized components due to light exposure, reducing the need for full barrier light-protective packaging. Specifically, antioxidants may delay oxidation of light-susceptible compounds during initial stages when hydroperoxide formation is increasing slowly.[15] The efficiency of an antioxidant is based on the structure of the molecule, the structure of the molecule being oxidized, and the conditions under which oxidation is occurring.[10] Carotenoids act as quenchers of oxidation by acting as an acceptor of energy from other excited state molecules and then releasing the energy in the form of heat.[16−18] The quenching rate increases as the number of double bonds is increased in the antioxidant.[18]

Lutein and zeaxanthin are carotenoids in the xanthophyll family that possibly function as antioxidants in the human eye.[19,20] Both are found in high density in the macular region of the human retina. Lutein and zeaxanthin are suspected to act as a shield from short-wavelength radiation that could damage the unsaturated fatty acid components of photoreceptors.[19,21] Lutein

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and zeaxanthin also have been shown to have passive light filtering characteristics as they absorb blue light at an absorbance maximum of 450 nm.[21] Lutein and zeaxanthin may have optimal wavelengths at which they provide protection but, conversely, degradation may occur more rapidly at some specific wavelength regions. However, this is not well-known.

On the basis of their antioxidant chemistry, lutein and zeaxanthin are potential food additives and may be considered for antioxidant use to protect other food components from light oxidation.[18] Boon et al.[5] suggest that degradation of carotenoids, such as lutein and zeaxanthin, should be better understood so that the stability of these compounds can be optimized and delivery systems of carotenoids can be developed for use in food systems.

Lutein for use as a food ingredient or dietary supplement is typically extracted from marigolds.[22,23] Marigold pigment is primarily composed of lutein and, to a lesser extent, its isomer zeaxanthin (3−6%).[24] It is difficult to separate lutein and zeaxanthin in the extraction process, so many lutein sources contain small amounts of zeaxanthin. Therefore, when lutein is discussed in this paper, it must be recognized that zeaxanthin is also present. Lutein is lipid soluble and has been shown to degrade under full light illumination and high storage temperatures (35 °C).[25] Zulueta et al.[26] found that lutein was not stable in an orange juice−milk beverage system when treated with pulsed electric fields. Haila et al.[27] showed that lutein behaves as a prooxident when added to triglycerides purified from rapeseed oil. Hydroperoxides were formed at various concentrations of lutein in the dark and light. However, lutein combined with α-tocopherol inhibited peroxide formation in rapeseed oil. This contrasts with a study that showed that lutein alone quenched singlet oxygen and reduced peroxide formation in the chlorophyll-sensitized photo-oxidation of soybean oil.[18]

The effects of light on foods and colloidal beverages such as milk have been reviewed.[6,28] There is relatively little published research on the effects of light wavelength on carotenoid structures in foods and beverages and the implications to volatile chemistry, yet it is recognized that these structures are responsive to light.[5] Very few studies have determined if lutein is effective as an antioxidant in a food system at specific wavelengths of light. The objective of this study was to determine lutein's stability and protective effect on aroma-active flavor compounds in a colloidal beverage system exposed to specific light wavelengths. We also wanted to determine if lutein functions as an antioxidant and protects photosensitive molecules, such as the limonene in this system.

MATERIALS AND METHODS

Beverage System Processing. A model colloidal beverage system (MCBS), with and without lutein, was formulated from whey protein, lecithin, orange-flavored instant beverage mix, and water. Whey protein isolate (Provon 292 Instantized, Glanbia, Monroe, WI) (146.52 g; 2 mg riboflavin/g whey protein) was hydrated in filtered water (10 L) for 15 min under agitation. Lecithin granules (Annie Kay's Whole Foods Dietary Supplements, 7.2−7.35 g/Tbsp, 96−98% phosphatides) (7.32 g) were heated in 400 mL of filtered water until dispersed and allowed to cool. Lecithin was added as an emulsifier to enable lutein to remain dispersed. The lutein extract source contained 21.9% lutein and 2.25% zeaxanthin, of which 99.15% was free xanthophylls, with tricalcium phosphate as the carrier (Pharmline, Florida, NY). Lutein extract powder (0.44 g) was mixed into the dissolved lecithin mixture to formulate the MCBS with lutein but was not added to the control system. The lecithin mixture was stirred into the hydrated whey protein. Orange-flavored instant beverage mix (Gatorade, Gatorade Co., Chicago, IL) (615.4 g) was incorporated slowly into the whey and lecithin solution using a hand-held mixer.

Both (lutein, no lutein) beverage systems were homogenized on a two-stage homogenizer (Type DX, Cherry Burrel Corp., Delavan, WI) at 10.2 MPa (first stage, 1500 psi) and 3.4 MPa (second stage, 500 psi) and pasteurized at 90.6−92.2 °C for 2 s using a UHT tubular heat exchanger (UHT/HTST Lab 25 HV, Microthermics Inc., Raleigh, NC). After heat treatment, the beverage was cooled to <25 °C. The beverage (800 mL) was collected in seven sterile 1 L glass bottles per treatment. This process was replicated three times.

Table 1. Light Intensities for Each Wavelength Filter Treatment[a]

peak transmission wavelength of filter (nm)	light intensity (mW, $x \pm$ SD)
full light	8.113 a ± 0.014
UV (200−400)	0.3101 d ± 0.006
463	0.561 c ± 0.002
516	0.573 c ± 0.002
567	0.951 b ± 0.001
610	0.571 c ± 0.005

[a] Means ($n = 3$) followed by the same letter are not significantly different at the $p < 0.05$ level. Statistical significance was determined using Tukey's HSD.

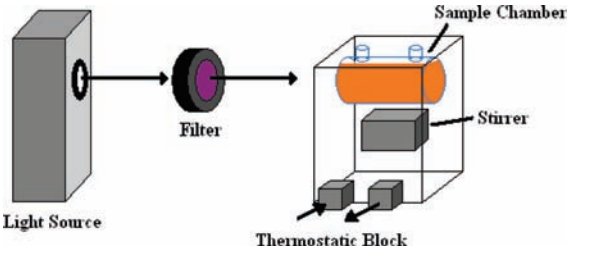

Figure 1. Photochemical reactor (Thermo Oriel, Stratford, CT) setup. A 350 W mercury lamp transmitted light through 50 nm bandwidth wavelength filters onto the sample chamber. A thermostated aluminum block maintained sample temperature at 25 °C.

Preparation of Samples for Specific Light Wavelengths. Beverage systems, with and without lutein, were exposed to specific (50 nm) light wavelength bands using a Thermo Oriel Photo-Reactor with a 350 W mercury lamp (model 66902 Universal Arc Lamp Housing, model 66910 Power Supply, Thermo Oriel Instruments, Stratford, CT) with specific light filters (Figure 1) as described by Webster et al.[29] The specific light wavelength filters included ultraviolet (UV) 200−400 nm and very narrow bandwidth (50 nm) light with maximum absorbance peaks at 463, 516, 567, and 610 nm (Table 1). A full spectrum light treatment was also studied. The beverage system (13 mL) and a stir bar were placed in a 15 mL quartz crystal vial (Fisherbrand, 50 mm cylindrical cell) and capped with a rubber septum. The vial was inserted into a cooling block set to maintain sample temperature at 25 ± 1 °C and stirred continuously for 12 h. A constant temperature for the sample was created by continuously pumping a 50% solution of antifreeze through the cooling block. A stainless steel thermocouple (Omega HH81 digital thermometer, Omega Engineering, Stamford, CT) was inserted into the rubber septum to monitor sample temperature. Light was transmitted from the 350 W mercury lamp through the wavelength filter and onto the crystal vial face. Transmitted light energy was measured using an Oriel energy meter (Radiant Power Energy Meter, model 70260, Thermo Oriel Instruments, Stratford, CT). A light-protected control

exposed to the same temperature and stirring conditions was prepared for comparative analysis.

Volatile Headspace Analysis Using Gas Chromatography. After light treatment, triplicate samples (3 mL) were transferred, using a hypodermic syringe, to borosilicate glass vials (8 mL) containing 0.75 g of NaCl and fitted with Teflon septa. Samples were stirred and heated at 45 °C on an RCT basic heater with an ETS-D4 Fuzzy Controller (IKA Werke, Wilmington, NC) for 22 min while a 75 μm carboxen polydimethyl siloxane (PDMS) coated SPME fiber (Supelco, Bellefonte, PA) was exposed to the headspace to adsorb volatile compounds. The fiber was positioned approximately 1 cm above the model beverage system surface. PDMS fibers are effective at separating limonene[12] and hexanal peaks.[14]

Volatile compounds on the SPME fiber were desorbed into the injector port of an HP 5980 series II gas chromatograph (GC) with flame ionization detector (FID) (Hewlett-Packard Co., Palo Alto, CA). Injector temperature was set to 280 °C and detector temperature to 300 °C, and the program was run in splitless mode. Volatiles were separated using an RTX-5 capillary column (crossbond 5% diphenyl−95% dimethyl polysiloxane 30 m × 0.32 mm i.d. × 1.00 μm film thickness, Restek Corp.) with helium carrier gas at a flow rate of 1.50 mL/min. A temperature ramp was used starting with 15 °C/min for 0.50 min and then 20 °C/min for 5.50 min. HP ChemStation software (Rrev. A.05.02[273], Hewlett-Packard, Palo Alto, CA) was used to plot and integrate the chromatograms.

Identification of hexanal (Aldrich) and limonene (Acros Organics) compounds was confirmed using external standards following the same methodology as above. Concentrations were determined using standard curves.

High-Performance Liquid Chromatography (HPLC) Analysis of Lutein. HPLC extraction was conducted following the method of Katchek et al.[30] After exposure to wavelength treatment for 12 h, extractions were carried out in an ice bath under minimal light. Model beverage (2.5 mL), magnesium carbonate (0.3 g), tetrahydrofuran (4.0 mL), and a stir bar were placed into a 50 mL Erlenmeyer flask wrapped in aluminum foil. β-Carotene (4000 μg/mL) internal standard was added to the mixture at 10 μL. Sample was blended for 20 min and then filtered through a Whatman no. 1 filter paper on a 250 mL Buchner funnel wrapped in aluminum foil. Components were partitioned into dichloromethane (~13 mL) and salt water (~8 mL) in a separatory funnel covered in aluminum foil.

After the components had remained undisturbed for 10 min, the lower organic layer was removed. The organic layer was washed with salt water (24 mL). The organic layer containing carotenoids was dried over anhydrous sodium sulfate (powder) and filtered through a Whatman no. 42 filter paper on a Buchner funnel wrapped in aluminum foil. The organic layer was brought to volume in a 25 mL volumetric flask with dichloromethane. The solution was then filtered through a 0.45 μm filter into a 12 × 32 mm amber crimp-top vial. The vial was flushed with nitrogen gas and sealed with an 11 mm aluminum seal with a PTFE/butyl rubber septum. Vials were stored at 0 °C until HPLC analysis.

Dichloromethane extracts were placed in an autosampler and analyzed on a Waters 2695 HPLC system with a Waters 2487 dual wavelength (Waters Corp., Milford, MA) using a Luna 5u C18 reversed-phase column (250 × 4.6 mm) (Phenomenex). The mobile phase consisted of 75% acetonitrile/15% methanol/5% hexane/5% dichloromethane at a flow rate of 1 mL/min.

Lutein and internal standard (β-carotene) were quantified (mg/mL of beverage system) by comparing peak area counts to standard curves created from 0, 0.2, 0.4, 0.8, and 1.0 mg/mL standards of lutein and β-carotene in dichloromethane.

Analytical Statistical Analysis. GC and HPLC results were analyzed using SAS statistical software (SAS, Cary, NC) Proc GLM Factorial ANOVA. The main effects were light levels (463, 516, 567, and 610 nm, full light, no light), lutein levels (lutein, no lutein), and replication.[1−3] Two- and three-way interactions were tested as well. Mean separation was determined using LS means with a $p = 0.05$.

RESULTS AND DISCUSSION

In this study, we isolated narrow wavelength regions of light to evaluate the effects of these wavelength regions on lutein and the volatile chemistry that resulted from a colloidal beverage system. Caution must be used in making comparisons of wavelength effect on aroma-active volatile components. In our laboratory, variation in light energy due to wavelength filters was determined according to the method of Webster et al.[29] Similar results were determined according to the method of Wold et al.[31] In our laboratory, Webster et al.[29] found significant energy differences between wavelengths studied (Table 1). The highest energy output (8.1 mW) was noted with full light exposure. The lowest energy output (0.31 mW) was found with the UV (200−400 nm) filter. Overall energy emission measures were <1.0 mW for all filters used.[29] In this study, conditions for the photochemical reactor were identical to those of Webster et al.[29] Data that are presented for lutein, limonene, and hexanal concentrations are shown with observed concentrations. Therefore, direct comparison of observed concentrations for volatile compounds can be made only between 463, 516, and 610 nm wavelengths due to similar energy measurements at ~0.5 mW ($p < 0.05$), whereas only inferences can be made about other wavelengths. Normalized concentrations were calculated and reported per milliwatt of energy.

It was hypothesized that lutein concentration would decrease when exposed to full spectrum light and 463 nm specific wavelength. Lutein has been shown to degrade under full light illumination and high storage temperatures.[32] Experimental conditions (25 °C with 12 h stirring) alone caused a 23% decrease in lutein concentration compared to the original concentration (data not shown). Lutein concentration, measured prior to heating and stirring, was significantly higher than all other treatments including the light-protected (no light) treatment. Stirring may have incorporated more oxygen into the system from the sample headspace.[33]

Exposure to full broad spectrum light, UV, and 463 nm wavelength regions of light significantly decreased ($p < .05$) lutein concentration below all other treatments (Figure 2). Full broad spectrum light treatment had the highest energy output (8.1 mW) and is substantially higher than normal light exposure conditions in a retail setting, so it was expected to cause significant damage to the lutein molecule. It can be inferred that UV light (200−400 nm) is very important in the degradation of lutein because the 200−400 nm filter had the lowest energy output of all treatments. When normalized per milliwatt of energy, lutein degradation was significantly decreased only with full light. When the wavelengths of equal light energy were compared, lutein concentration was decreased at 567 nm. Variation in light energy due to wavelength filters was determined by Webster et al.[29] and Wold et al.[31] in fluid milk and Norvegia cheese, respectively. Webster et al.[29] found significant light intensity (lux) difference between wavelengths studied. In our study, the highest intensity and energy output (13086 ± 590.12 lx; 8.1 mW) were noted with full light exposure; the lowest intensity and energy output (84.8 ± 4.15 lx; 0.08 mW) were observed with the 395 nm filter. Direct comparison between wavelengths could only be made for observed concentrations between wavelengths

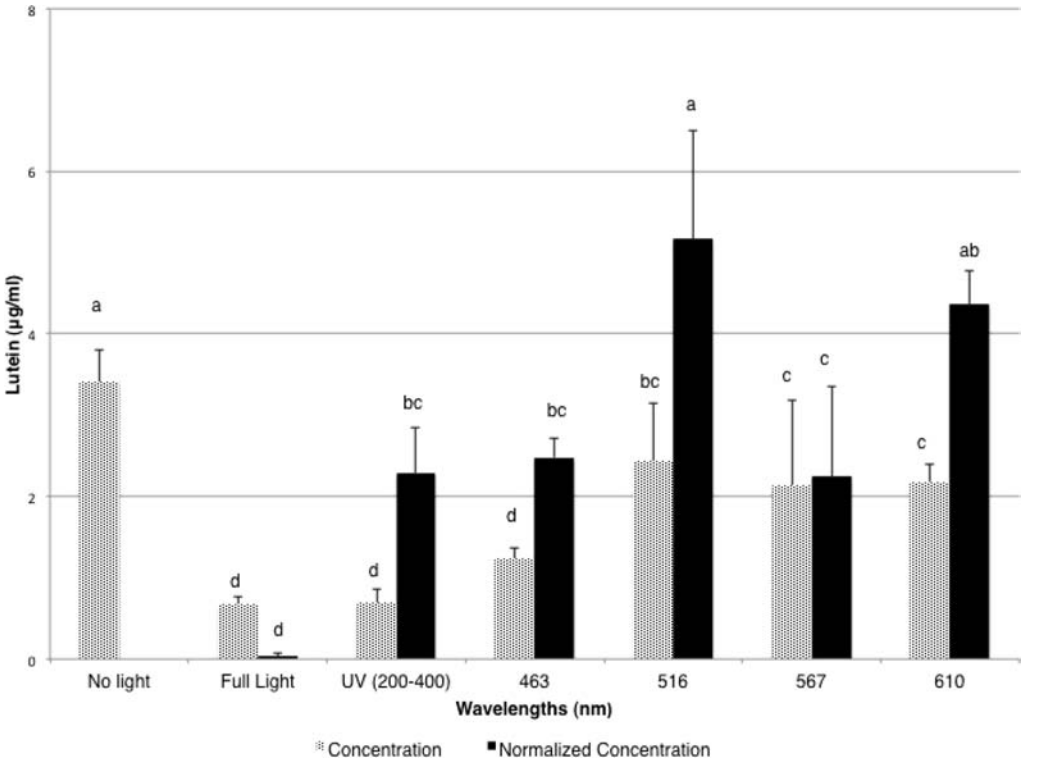

Figure 2. Effect of light wavelength exposure (25 °C, 12 h continuous stirring) on lutein concentration in a model colloidal beverage system. No light + stirring control sample was held at 25 °C for 12 h with continuous stirring. Wavelength values represent a 50 nm bandwidth filter with peak absorbance at the identified wavelength. Light energy is shown in Table 1. Normalized data (μg/mL/mW) are shown due to differences in light energy for each wavelength. Values with the same letter within observed or normalized data are not significantly different at the $p = 0.05$ level (three replications). Statistical analysis was conducted using one-way ANOVA with Tukey's HSD to determine differences among treatments. Statistics were completed separately for observed concentrations and normalized calculations.

of 463, 516, and 610 nm due to similar energy measurements at ~0.5 mW ($p < 0.05$), whereas inferences could be made about other wavelengths. Overall energy emission measures were <1.0 mW for all filters used.

The stability of lutein to light exposure and storage has been previously studied in tomato juice.[32] Lin et al.[25] found that although *all-E*-lutein in tomato juice degraded even in dark storage conditions, greater degradation occurred under full light illumination. *all-E*-Lutein degraded more at room (25 °C) and higher (35 °C) storage temperatures compared to refrigerated (4 °C) temperatures.

The decrease in observed lutein concentration when exposed to 463 nm light wavelengths can be attributed to lutein and zeaxanthin's absorption of blue light wavelengths at 450 nm. Considering that lutein received less or equal energy at 463, it may be that energy at wavelength 463 nm is very efficient at delivering photons. Because light energy levels were similar for 463, 516, and 610 nm filters, it can be concluded that 463 nm wavelengths caused significantly more damage to lutein than 516 and 610 nm wavelengths. This appears to be confirmed with normalized calculations. Junghans et al.[34] reported that lutein and zeaxanthin, which filter blue light, diminished the fluorescence yield in liposomes to about 40% of the control at 1.4 nmol carotenoid/mg phospholipid. Excitation by absorption of blue light can lead to degradation of these carotenoid molecules.

Overall, the results of HPLC quantification of lutein in a beverage system exposed to various wavelength treatments suggests that lutein does degrade during 12 h of full light exposure at 25 °C. In addition to degradation under ultraviolet and low visible wavelengths, the 567 nm wavelength region is an important contributor to lutein degradation. The main food industry implication of these findings is that functional beverages fortified with lutein that are not light protected may undergo lutein degradation, resulting in a product with substantially less lutein than initially fortified.

Volatiles in the MCBS were affected by light as well as indicated by changes in concentration. Two peaks on the gas chromatogram, corresponding to hexanal and limonene, were affected by specific wavelength treatment and lutein fortification.

Observed hexanal concentrations were significantly higher in the full light and UV-exposed (200−400 nm) lutein-fortified and no lutein beverages compared to the other light wavelength treatments (Figure 3). This trend was seen only with UV light in normalized calculations. In food systems, breakdown of polyunsaturated fatty acids has been shown to form hexanal, most frequently detected during lipid oxidation.[35] It can be inferred that UV light (200−400 nm) is important in hexanal formation because the 200−400 nm filter had the second lowest energy output of all treatments. All other treatments (no light + stirring, 463, 516, 567, and 610 nm wavelength exposures) had relatively similar hexanal concentrations (Figure 3). Hexanal was present in the beverage system at low concentrations; its presence is attributed to formulation and processing conditions, as evident in the pretreatment and light-protected control samples. No significant differences ($p > 0.05$) in hexanal concentration were found after exposure to 463, 516, and 610 nm narrow-band

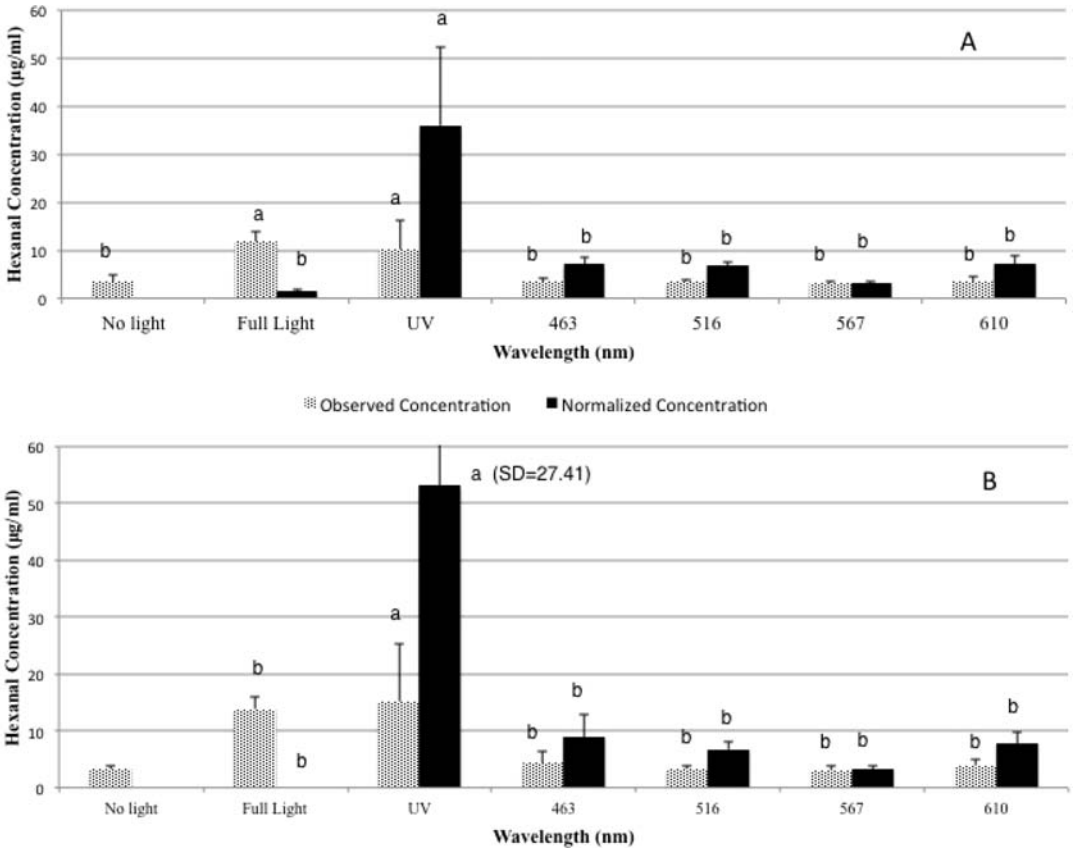

Figure 3. Effect of light wavelength exposure (25 °C, 12 h continuous stirring) on hexanal concentration (μg/mL) in lutein-fortified (A) and no lutein added (B) model colloidal beverage systems. No light + stirring control sample was held at 25 °C for 12 h with continuous stirring. Wavelength values represent a 50 nm bandwidth filter with peak absorbance at the identified wavelength. Light energy for each wavelength is shown in Table 1. Normalized data (μg/mL/mW) are shown due to differences in light energy for each wavelength. Values with the same letter within observed or normalized data are not significantly different at the $p = 0.05$ level (three replications). Statistical analysis was conducted using one-way ANOVA with Tukey's HSD to determine differences among treatments. Statistics were completed separately for observed concentrations and normalized calculations.

wavelengths, suggesting that these wavelengths did not affect hexanal concentration. This may mean these wavelengths do not cause photo-oxidative damage to the phospholipids (lecithin) in the beverage system. Hexanal concentrations at 463, 516, and 610 nm can be directly compared because light energy levels were equivalent. The low energy at UV, compared to the other wavelength regions, must be considered. It is possible that hexanal concentrations may be even higher if the energy level of this wavelength band was equivalent.

A low concentration of hexanal in the lutein-fortified beverage at some wavelength regions could mean that lutein reduced the degree at which the degradative compound was formed. The effect of light wavelength on hexanal production in the control beverage without lutein followed the same pattern as observed in the MCBS with lutein. Hexanal concentration was significantly higher after full light and UV (200−400 nm) wavelength exposure in the control MCBS without lutein compared to all other control MCBS light exposure treatments (Figure 3).

Hexanal concentrations for the lutein-fortified and control beverages at each wavelength treatment were compared to determine if lutein fortification affected hexanal production. Significant differences were found only when beverages with and without lutein were compared at the full light and UV (200−400 nm) wavelength treatments (Figure 3). Hexanal concentration was significantly lower for the lutein-fortified beverage compared to the no light + stirring beverage when exposed to UV (200−400 nm) wavelengths (10.22 and 15.2 μg/mL, respectively) and full light (11.79 and 13.85 μg/mL, respectively). A reduction in hexanal concentration during UV and full light exposure could mean that lutein is inhibiting photochemical reactions that result in the formation of hexanal at these wavelengths. This trend was seen in normalized calculations.

Limonene was the major peak observed in the gas chromatogram and was found in all beverage samples. Orange oil is a common flavoring for orange-flavored mixes used to make the beverage system. Orange oil is composed primarily of limonene, which contributes a pleasant citrus flavor. Limonene has been shown to degrade during light exposure,[36] so it was of interest to determine the effects of specific light wavelength treatments and lutein fortification on relative limonene concentration in the beverage system.

Mean limonene concentrations were compared among wavelength treatments for lutein-fortified and also between the lutein-fortified and unfortified control beverages at each wavelength. It was hypothesized that the limonene concentration would decrease due to light exposure. The no light + stirring control was significantly higher in limonene concentration than all other wavelength treatments (Figure 4).

Observed limonene concentrations were not significantly different at 463, 516, and 610 nm and can be directly compared

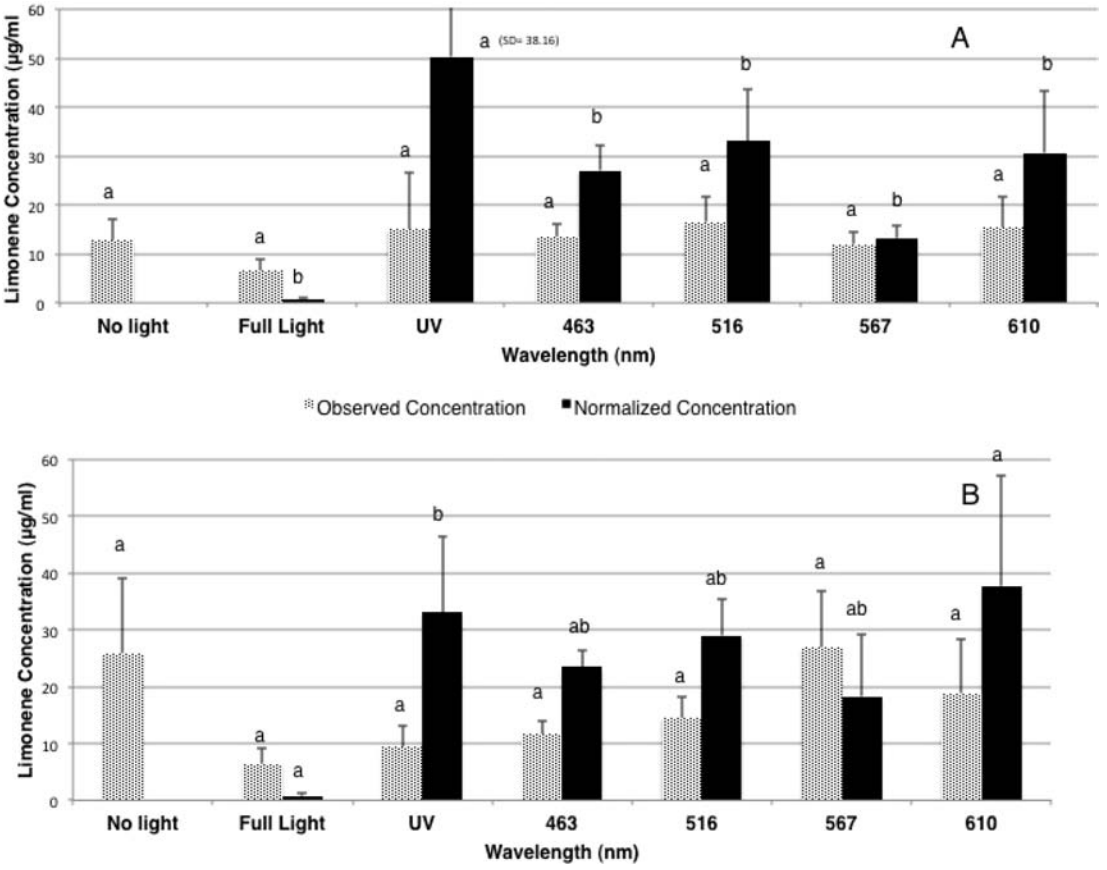

Figure 4. Effect of light wavelength exposure (25 °C, 12 h continuous stirring) on limonene concentration (μg/mL) in lutein-fortified (A) and no lutein added (B) model colloidal beverage systems. No light + stirring control sample was held at 25 °C for 12 h with continuous stirring. Wavelength values represent a 50 nm bandwidth filter with peak absorbance at the identified wavelength. Light energy is shown in Table 1 for each wavelength. Normalized data (μg/mL/mW) are shown due to differences in light energy for each wavelength. Values with the same letter within observed or normalized data are not significantly different at the $p = 0.05$ level (three replications). Statistical analysis was conducted using one-way ANOVA with Tukey's HSD to determine differences among treatments. Statistics were completed separately for observed concentrations and normalized calculations.

because light energy levels were equivalent (Figure 4). It can be inferred that under wavelength treatment conditions (12 h, 25 °C) limonene concentration declined. Although not statistically significant, limonene concentration under full light conditions was lower than all other treatments, including the light-protected sample. A wavelength of 610 nm appeared to have the least effect on limonene degradation, from normalizing calculations. This calculation does not consider the efficiency of delivering photons to the system.

Mean limonene concentrations were also examined in the fortified beverage at the same wavelength treatments as above. Limonene concentrations in light + stirring were significantly higher ($p < .05$) than the full light-exposed control sample (Figure 4). Overall, limonene concentration did not decline significantly with exposure to various wavelength regions. A comparison based on wavelength treatment for the lutein-fortified and unfortified beverages was also examined. Overall, changes in limonene concentration did not suggest lutein has a protective effect for limonene against photo-oxidation. This may imply that lutein fortification does not inhibit reactions that cause limonene degradation. Lutein's antioxidant function toward other molecules may be dependent on the structure and location within the complex matrices of the beverage system. These results must also consider that the intensity of all wavelength regions tested were higher than most lighting sources in commercial display cases and overhead lighting.

Some studies have shown that lutein is effective as an antioxidant in other systems. Sujak et al.[21] added lutein and zeaxanthin to liposomes containing egg yolk phosphatidylcholine and dipalmitoylphosphatidycholine (DPPC). Upon exposure to UV light, the liposomes that contained lutein had lower rates of UV oxidative damage. Broniowska et al.[37] found that free lutein in a lipid membrane system has a moderate effect against lipid peroxidation in liposomes incubated with 2,2′-azobis(2,4-dimethylvaleronitrile). However, when spin labeled, the antioxidant activity of lutein was enhanced. Not all studies demonstrate that lutein is effective as an antioxidant in a membrane system. Cantrell et al.[16] added lutein and other carotenoids to DPPC liposomes and found that, although lutein does quench singlet oxygen when exposed to 532 nm laser excitation of rose bengal, it was not nearly as effective as other carotenoids. Lutein may be more effective as an antioxidant when incorporated within a micelle or membrane system rather than free in the food system. Perhaps there is a need for lutein to work synergistically with other carotenoids or antioxidant compounds to functional optimally. Boon et al.[5] suggested that lutein and other carotenoids should be studied as functional food components added in an emulsified form or within some type of nanostructure. Further

studies are needed to elucidate lutein's antioxidant potential at a lipid−water interface or in synergy with other antioxidants.

Exposure to full broad spectrum light and specific wavelengths negatively affects colloidal beverage systems with whey proteins and phospholipids. Susceptibility can be attributed to photooxidation of lipid, protein, and flavor components. Lutein provided some, although limited, protection of phospholipids and proteins at ultraviolet (200−400 nm) wavelengths under conditions of high-intensity light exposure. Lutein fortification reduces hexanal off-flavor formation in a colloidal beverage system, suggesting positive implications toward its use as a photoprotective agent. However, lutein fortification did not inhibit degradation of limonene, the major flavoring component of the system during light exposure. Lutein degradation is a potential problem in light-exposed functional beverages, indicating that appropriate packaging or synergistic antioxidants are needed to protect this molecule if added for eye health functionality.

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Notes

Safety. Use of the photoreactor requires sunglasses and gloves to protect against UV radiation.

ABBREVIATIONS USED

MCBS, model colloidal beverage system; HPLC, high-performance liquid chromatrography; GC, gas chromatography; SPME, solid-phase microextraction; PDMS, polydimethyl siloxane; FID, flame ionization detector; DPPC, dipalmitoyl phosphatidylcholine.

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