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Effects of ultraviolet-light emitting diodes (UV-LEDs) on microbial inactivation and quality attributes of mixed beverage made from blend of carrot, carob, ginger, grape and lemon juice

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ABSTRACT

Efficacy of ultraviolet light-emitting diodes (UV-LEDs) with peak and coupled emissions at 280, 365 and 280/365 nm on inactivation of *E. coli* K12 in mixed beverage (MB) was investigated. MB comprised of 31.6% carrot, 44.3% carob, 8.7% grape, 10.2% ginger, and 5.2% lemon juice. The impact of UV-LEDs on some physicochemical and phytochemical properties of MB was compared to that of heat treatment (70 °C, 120 s). While, UV-LED irradiation using coupled 280/365 nm for 40 min resulted in the highest inactivation of *E. coli* K12 (>4 log) out of tested wavelengths, the number of mesophilic bacteria (TAC), and yeast and molds (YM) in mixed beverage were reduced by 2.59 log CFU/mL (from 5.69 log CFU/mL of initial load), and 0.17 log CFU/mL (from 3.28 log CFU/mL of the initial load), respectively. Although, the color parameters slightly changed after irradiation, the color of MB did not show visual difference ($\Delta E = 0.94$) compared to untreated samples. UV-LED treatment caused a significant increase in total phenolic compound (1.75-fold) and antioxidant capacity (4.60 fold) compared to heat-treated samples ($p < 0.05$). UV-LED treatment caused a decrease in carotenoid content (71.3%) lower than that of heat-treated samples (88.9%), indicating that UV-LED irradiation preserved the total carotenoid content better than the heat treatment.

Industrial relevance: Light-emitting diodes (LEDs) are new sources of ultraviolet light utilized for non-thermal processing of foods. In this study, a static bench top unit was designed to investigate the efficacy of UV-LEDs with different treatment times and peak emissions by considering the inactivation of *E. coli* K12 in newly formulated mixed drink (MB). UV-LED irradiation of MB using coupled 280/365 nm for 40 min provided the highest microbial inactivation and preserved bioactive compounds better than the heat treatment. It can be proposed as an effective method for the processing of fruit juices which is rich in bioactive constituents.

1. Introduction

The demand of juice blends and beverages has increased due to growing interest in health and well-being in recent years (Das, Goud, & Das, 2019). Fruit juices are rich sources of minerals, vitamins and various other bioactive compounds and may contribute to a healthy diet and healthy life (Bevilacqua et al., 2018). The market for fruit juice continues to expand. New juice drinks are now also emerging with enhanced nutritional properties. Blending fruit and vegetable juices in order to develop new beverages became popular since it is a simple and cheap method to enhance the fruit juice functionality (Ferrario, Schenk, García Carrillo, & Guerrero, 2018).

In this study, a mixed beverage formulation was developed by taking varying nutritional properties of different fruits and vegetables such as carrot, lemon, grape, ginger and carob. Carrot is an important vegetable in human diet due to its composition of bioactive compounds such as phenolic acids, especially carotenoids (Hernández-Carranza et al., 2016). The phenolic acid prevents the DNA damage in the cell by quenching the free radicals. Consequently, it prevents unwanted mutations and cancer. Besides, the vitamins in the lemon fruit have strong antioxidant properties and prevent oxidative DNA damage (Paoloni-Giacobino, Grimble, & Pichard, 2003). Ginger has an immunomodulatory effect due to the effects of gingerol and phenolic substances (Arablou & Aryaeian, 2018). Grape fruit is a rich source of flavonoids with high antioxidant properties and they have an anti-inflammatory action in the cell (Georgiev, Ananga, & Tsolova, 2014). Carob is also rich in

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vitamins, minerals, and antioxidants. Thus it has protective properties against lung cancer and cardiovascular diseases (Durazzo et al., 2014). However, the mixed beverage (MB) produced from freshly squeezed carrot, lemon, grape, ginger and carob juices has a very short shelf-life (2–3 days).

The alternative non-thermal treatments are sought by fruit juice processors in order to maintain the quality of the juice products while preserving their nutritional value (Tahiri, Makhlouf, Paquin, & Fliss, 2006). Ultraviolet (UV) irradiation technology is one of these method and has been widely investigated in view of possible commercial applications for food products (Koutchma, 2009). Low or medium pressure mercury vapor lamps are used in this technology. However, these lamps contain mercury known to have toxic effects on the human body and the environment (Mori et al., 2007). Recently, the use of light-emitting diodes (LEDs) has become prominent as an ultraviolet light source. UV-LEDs are created using non-toxic semiconductor material which allows to emit in different wavelengths (Song, Mohseni, & Taghipour, 2016). The color of the emitted light depends on the band gap energy of the material of semiconductor (Anbazhagi, Shilpa, & Munthikote, 2018). Besides, it is possible to use the combination of different UV-LEDs emitting light at different wavelengths (Akgün & Ünlütürk, 2017).

UV-LEDs have been widely investigated for water treatment. However, there are limited number of studies related to the use of UV-LEDs for fruit juice pasteurization (Akgün & Ünlütürk, 2017; Lian et al., 2010; Xiang, Fan, Zhang, Ma, & Liu, 2020; Cheng et al., 2020). More research is needed on the applicability of this technology for juice pasteurization. The germicidal capabilities of ultraviolet light-emitting diodes at single and multiple wavelengths must be also investigated thoroughly. Besides, the effect of the UV-LEDs on quality attributes of juice and bioactive compounds needs to be explored in detail.

Therefore, the present study aimed to evaluate the effect of single (280 and 365 nm) and multiple wavelengths (280/365 nm) on the microbial quality and physicochemical and phytochemical attributes of newly formulated mixed beverage (MB). Microbial inactivation efficiency of UV-LEDs was tested by inoculating *E. coli* K12 in mixed beverage. Moreover, the effect of UV-LED irradiations on natural biota, some physicochemical attributes, e.g. color, total soluble solid, pH, and phytochemical properties, e.g. total phenolic content (TPC), total antioxidant capacity (TAC) and total carotene content of mixed beverage was investigated.

2. Material and methods

2.1. Fruit juice formulation and sensory evaluation

The mixed beverage was prepared using carrot, carob, ginger and lemon juices. Carrot (*Daucus carota*), carob (*Ceratonia silique*), ginger *(Zingiberaceae),* lemon *(Citrus limon*) and grape (*Vitis vinifera* L. *cv. Sultana*) were purchased from a local supermarket in Izmir, Turkey.

Juice from carrot, ginger, grape, lemon and carob (after blanching) was obtained using a household tabletop juice extractor (Arçelik Roboligo, Istanbul, Turkey). Mixed beverage (MB) comprised of 31.6% carrot juice, 44.3% carob juice, 8.7% grape juice, 10.2% ginger juice, and 5.2% lemon juice. pH of the beverage was adjusted to 4.0 by adding lemon juice. This was the most preferred formulation in the preliminary sensory tests. Mint leaf (ML) and lemon peel (LP) were added to improve the aroma of the juice mixture. An alternative juice mixture without mint leaf and lemon peel were also prepared for comparison. Sensorial assessment was performed to determine the consumer acceptability of the mint and lemon peel in formulated beverages.

Sensory evaluation was carried out with 20 semi-trained panelists. The panelists were between the ages ranging from 20 to 42. All panelists were familiar with the sensorial attributes of all ingredients used in the mixed beverage formulation and acquainted with the scoring technique. Consumer acceptance test was applied to determine mint and lemon peel preferences. Based on 5-point hedonic scale, the panelists evaluated the attributes - color, odor, aroma, taste, mouthfeel, and overall acceptability. The results of sensory evaluation were assessed using analysis of variance (ANOVA) and Tukey's pairwise comparison test ($p < 0.05$). Internal consistency test was applied to calculate Cronbach's alpha (CA) coefficient using Minitab 16 (Minitab Inc., State College, PA, USA). CA measures the similarity between evaluation profiles from different panelists. An evaluation profile corresponds to assessments made by a panelist on a given attribute, over all products (Pinto, Fogliatto, & Qannari, 2014). The formulation which was scored the highest in sensory evaluation was used in microbial study.

2.2. Bacterial strain and sample inoculation

In this study, *E. coli* K12 (ATCC 25253) which is a surrogate of *E. coli* O157: H7 was selected as a pertinent pathogen and inoculated into mixed beverage (MB) (Koutchma, Keller, Chirtel, & Parisi, 2004). *E. coli* K12 (ATCC 25253) was cultured from −80 °C lyophilized vials and enriched according to the manufacturer instruction. Then, the cells were adapted to pH 4.0 following the procedure described by Kaya and Unluturk (2019). After this process, stock cultures of viable acid adapted cells were prepared by transferring them into vials containing glycerol and stored at −80 °C until used.

MB was pasteurized at 70 °C for 120 s by means of a continuous flow pasteurizer as described in Akgün and Ünlütürk (2017) to eliminate any background microbiota prior to UV-C treatment. Then, 0.1- mL from acid-adapted stock culture was inoculated into a test tube containing 10-mL Nutrient Broth (NB) for enrichment, and incubated overnight at 37 °C. Then 1 μL from this culture medium was taken and inoculated into 3-mL of MB to obtain a final microbial concentration of 6–7 $\log_{10}$ CFU/mL.

2.3. UV-LED treatment

The inoculated MB samples were treated using a static UV-LED unit. Details of the experimental device was presented in Akgün and Ünlütürk (2017). Briefly, UV-LED unit was designed with four UV-LEDs. These LEDs could be used together or separately, and the electronic circuit was connected to a customized four channel power supply. Each UV-LED was operated with a constant forward current of 20 mA. A total of four UV-LED lamps (8.33 mm diameter, SETI Sensor Electronic Technology Inc., Columbia, SC, USA), two of which emit light at 280 nm and the others at 365 nm, were used in this study. The forward voltage and optical output power of each UV-LED at 20 mA was 5.8 and 5.50 V, and 0.60 and 0.40 mW for the 280 and 365 nm UV-LEDs, respectively.

The inoculated sample (3-mL) was placed in a 55-mm sterilized Petri dish. UV-LEDs were fixed facedown, 1 cm below the UV source. The medium depth was fixed at 0.15 cm in order to avoid edge effects caused by stirring and provide homogenous light distribution (Unluturk, Atılgan, Baysal, & Unluturk, 2010). The samples were exposed to 280, 365 nm and wavelength combination of 280/365 nm for 10, 20, 30, 40, 60, 80, and 100 min at room temperature (25 °C).

UV dose was calculated from the product of incident UV intensity and exposure time. Incident UV intensity emitted by each LED was measured by ferrioxalate actinometry as described in Akgün and Ünlütürk (2017).

2.4. Microbial enumeration

Enumeration of the viable microorganisms in UV-LED treated MB samples from different dilutions prepared with 0.1% peptone water were surface-plated in duplicate on Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany) containing 0.1% dihydrostreptomycin. All plates were incubated at 37 °C for 24 h and then counted. The background biota (total aerobic bacteria (TAC), total coliform (TC), yeast and mold counts (YMC)) of the MB samples was checked by surface-plating on Plate Count Agar (PCA), Violet Red Bile Agar (VRBA), and Potato

Dextrose Agar (PDA) acidified to pH 4.0 with 10% tartaric acid (Merck, Darmstadt, Germany), respectively. The PCA, VRBA and PDA plates were incubated at 30 °C for 3 days,37 °C for 24 h and 25 °C for 5 days, respectively (Hakguder, 2009).

2.5. Physicochemical properties of mixed beverage

The total soluble solids (TSS), pH, color, turbidity and absorption coefficient of mixed beverage were measured before and after UV-LED treatment. Untreated control (CMB) and heat-treated (HMB) samples at 70 °C for 120 s were also used as a negative and positive control, respectively. Soluble solid content (°Brix) was measured by a bench-top refractometer (Mettler-Toledo RE40D, AEA Investors Inc., ABD), and pH was determined using a benchtop pH meter (HANNA Instruments, USA) at room temperature (25 °C). The color of MB samples was determined by Konica Minolta CR 400 Chromameter (Konica Inc., Japan) in Hunter L* (brightness-darkness), a* (redness-greenness) and b*(yellowness-blueness) color scales. Total color change (ΔE) and the browning index (BI) of the samples were calculated by the following Eqs. (1, 2).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$$BI = \frac{100\left(\frac{a^* + 1.75L}{5.645L + a - 3.012b^*}\right) - 0.31}{0.17} \quad (2)$$

Turbidity measured by a turbidimeter (Model 2100AN IS, HACH Company, USA) was expressed as the Nephelometric Turbidity Unit (NTU). Absorption coefficient of the beverage was determined using Shimadzu UV–Visible Spectrophotometer (UV- 2450, Japan) at 280 and 365 nm. Different dilution factors were applied (1:10, 1:25, 1:50, 1:100, 1:250, 1:500, and 1:1000). The absorption coefficient (cm^{-1}) was estimated from the slope of the graph of absorbance versus concentration.

2.6. Phytochemical properties of mixed beverage

The total phenolic content of treated and untreated MB samples was determined by using Folin-Ciocalteu method described by Pala and Toklucu (2011). MB samples were firstly diluted in different ratios with a mixture of MeOH: H2O (3:2). Three-hundred μL of diluted MB mixed with 1.5-mL of Folin–Ciocalteu reagent in a glass tube followed by the addition of 1.2-mL of sodium carbonate (75 g/L). The mixture was thoroughly mixed by using vortex mixer (ZX3, VELP Scientifica S.r.l., Usmate, Italy) and allowed to stand for a further 90 min in dark and the absorbance was measured at 765 nm using a UV–Visible spectrophotometer (Shimadzu, UV-2450, Japan). The comparison was made with a calibrated curve made of different gallic acid (Sigma-Aldrich, Germany) concentrations and TPC values were expressed as mg gallic acid equivalents (GAE) per L.

Free-radical scavenging activity (RSA) of treated and untreated MB samples was evaluated by the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method described by Odriozola-Serrano, Soliva-Fortuny, and Martin-Belloso (2009). A volume of 3.9-mL DPPH solution (0.025 g/L) was added to 0.01-mL MB and vortexed. A control tube (blank) was prepared by 0.01-mL deionized water. All samples including controls were kept in dark for 60 min at room temperature. Then, absorbance values were recorded at 515 nm. Methanol was used as blank to zero the spectrophotometer. Antioxidant capacities of the samples were then calculated in percentage by means of Eq. 3;

$$\text{DPPH inhibition } (\%) = 100 - \frac{As}{Ab} \times 100 \quad (3)$$

where A_s and A_b indicate absorbance values of sample and blank, respectively.

The amount of carotenoids in treated and untreated MB samples was determined according to the spectrophotometric method described by Castro-López et al. (2016) with slight modifications. A MB sample of 0.01-mL was added to 5-mL of a solution (acetone, hexane, ethanol 1:2:1) supplemented with 0.1% ascorbic acid to prevent the oxidation of samples. After incubation of the mixture at 37 °C for 30 min in dark, it was centrifuged at 6000 rpm (7647 *g*) for 5 min at 4 °C. Absorbance of the mixture was recorded at 450 nm using hexane as blank. The results were expressed as mg β-carotene /L using the calibration curve prepared with the same standard.

2.7. Statistical analysis

All the experiments were performed in triplicate. The experimental data were evaluated using Minitab 17 (Minitab Inc., State College, PA, ABD) for analysis of variance (ANOVA). The means of data were compared by Tukey's pairwise comparison test at a 95% confidence interval ($p \leq 0.05$). The correlation between the color parameters and color indexes was studied by means of Pearson's correlation matrix.

3. Results and discussion

3.1. Sensory evaluation of the beverage

Mixed beverage with pH 4.0 was developed based on the sensorial assessment of two recipes prepared with or without mint and lemon peels. The average of sensory acceptance scores of MB samples varied from 2.9 ± 1.07 ("neither like nor dislike") to 3.75 ± 1.07 ("liked") (Fig. 1). The overall quality, odor, mouthfeel, taste and aroma attributes of MB samples with added mint and lemon peels were scored much higher than those prepared without the mint and lemon peels. However, there was no significant difference ($p > 0.05$) between the color and odor attributes of two formulations. Additionally, acceptability index (AI) of two formulations was calculated according to the method described by Oliveira, Marques, Kwiatkowski, Monteiro & Clemente, (2013). Oliveira, Marques, Kwiatkowski, Monteiro, and Clemente (2013) indicated that the scores of AI must be equal or higher than 70%. AI of both MB formulations was above the 70% whereas MB samples with mint and lemon peels had a higher acceptance index score (75%) than those prepared without mint and peels (72.5%).

Cronbach's alpha (CA) is a numerical coefficient of reliability and can be used to describe the reliability of factors extracted from scales (Reynaldo & Santos, 1999). According to Nunnally and Bernstein (1994), CA values greater than 0.7 are considered acceptable, indicating

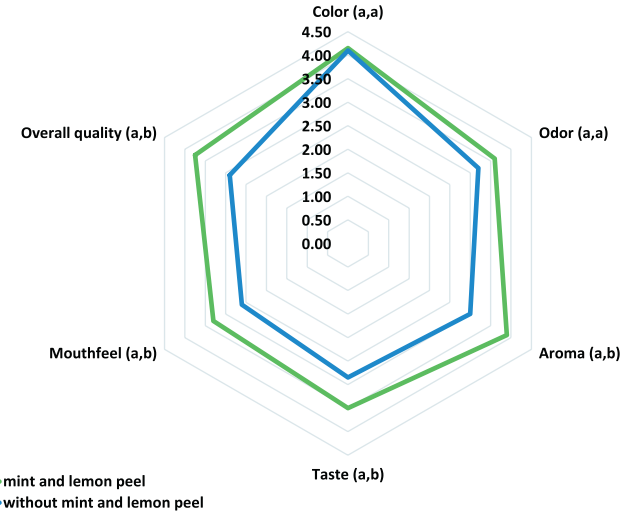

Fig. 1. Sensory assessment of newly formulated mixed beverages. The different small-case-letters in parenthesis indicate the significant differences among different formulations (mint and lemon peel, without mint and lemon peel) based on Tukey pairwise comparison test ($p < 0.05$).

panelists presenting similar evaluation profiles. Since CA coefficient based on the internal consistency test was calculated as 0.9, panel results are considered satisfactory. It was found that the MB formulation, which was prepared with the addition of mint and lemon peels and had a pH of 4.0, was highly preferred product. This formula of the mixed beverage was used throughout the study.

3.2. Effect of single and multiple wavelengths of UV-LEDs on the inactivation of E. coli K12

E. coli K12 inactivation in MB exposed to UV-LED irradiation at 280, 365 and 280/365 nm for 10–100 min was investigated. The population of *E. coli* K12 reduced by 1.38 log CFU/mL at 217.20 mJ/cm^2 and 3.4 log CFU/mL at 77.16 mJ/cm^2 when MB treated with UV-A (365 nm) and UV-C LED (280 nm) for 100 min, respectively. It was found that *E. coli* K12 exhibited very low UV sensitivity to 365 nm although the applied UV dose was appreciable higher than that of 280 nm (Table 1). The log reductions in *E. coli* K12 obtained with the application at 365 nm for 10 and 20 min were negligible (<0.3 log CFU/ml). UVA induces cellular membrane damage and growth delay indirectly by producing hydroxyl and oxygen radicals in the cell. However, this process takes more time than the direct damage produced by UV-C radiation (Chatterley & Linden, 2010). Because, the living organism synthesize a number of antioxidant as a defense mechanism to prevent it from entering the autocatalytic phase under oxidative stress Song, K., Taghipour, F & Mohseni, M. (2018). Similarly, Hinds, Charoux, Akhter, O'Donnell, and Tiwari (2019) achieved no significant reduction in the number of *Bacillus subtilis* in nutrient broth and peptone buffer saline solutions exposed to 365 nm UV-LEDs (0.17 ± 0.21–0.69 ± 0.4 $\log_{10}$ CFU/mL).

In contrast, UV treatment at 280 and 280/365 nm resulted in higher logarithmic reductions. The reduction of *E. coli* K12 count was 3.73 ± 0.13 log CFU/mL and 3.55 ± 0.35 log CFU/mL at wavelengths of 280 nm (46.30 mJ/cm^2) and 280/365 nm, respectively, after exposure of MB to UV light for 60 min. The inactivation level at a combined emission of 280/365 nm was similar to that of obtained at 280 nm ($p > 0.05$). Besides, the inactivation effect of 40 min and 60 min exposure times at 280 and 280/365 nm wavelengths were not significantly different from each other and prolonging the treatment period over 40 min did not have a significant effect on the inactivation of *E. coli* K12 in MB.

Although the highest inactivation (4.05 ± 0.07 log CFU/mL) was achieved using combination wavelengths, i.e. 280/365 nm and 100 min exposure time, 5 log reduction could not be achieved. However, this study demonstrated that utilization of multiple wavelengths has a great potential for increasing inactivation efficiency and reducing the treatment time. This is in accordance with several previous findings. Green et al. (2018) stated that combining 259 and 289 nm UV-LED wavelengths had a synergistic germicidal effect against certain microorganisms and can be used to optimize UV treatment. Similarly, Akgün and Ünlütürk (2017) reported the highest inactivation of *E. coli* K12 as 2 log CFU/mL in cloudy apple juice (CAJ) exposed to 280 and 280/365 nm for 40 min. They also found that the inactivation efficiencies of 40 min and 50 min exposure times were not significantly different from each other ($p > 0.05$). Moreover, the use of 280 and 280/365 nm LEDs for the treatment of MB resulted in higher *E. coli* K12 inactivation compared to the values reported for CAJ by Akgün and Ünlütürk (2017). The reason might be attributed to the absorption coefficient and turbidity known to be important factors affecting the UV efficiency (Koutchma, 2009). MB had lower turbidity and absorption coefficient compared to CAJ (24.71 cm $^{-1}$, 908.5 NTU). Additionally, CAJ had color compounds, organic matter, and suspended solids; therefore, UV efficiency was lower than MB (Akgün & Ünlütürk, 2017; Falguera, Pagan, & Garza, 2011). Thus, it was concluded that the inactivation efficiency of UV-LEDs was highly influenced by the physicochemical characteristics of treatment medium. For instance, Xiang, Fan, Zhnag, reported that UV-C LED irradiation reduced the population of *Zygosaccharomyces rouxii* in sterile saline and apple juice by 4.87 log CFU/mL at 40 mJ/cm^2 and 4.86 log CFU/mL at 800 mJ/cm^2, respectively. Apple juice contains suspended particles, organic materials and color compounds. Presence of these particles and compounds decreased the transmission of UV light reducing the microbial inactivation efficiency. Thus, apple juice was required to be exposed to higher dose of UV-C LED irradiation than sterile saline in order to achieve equal reduction in the population of *Z. rouxii*.

The main outcome of this study was that the microbial inactivation efficiencies of 280 and 280/365 nm were not significantly different. In order to choose the best UV processing condition between 280 or 280 / 365 nm, the inactivation mechanisms of these wavelengths have been considered. It is known that UV-A radiation has a better penetration property but poor efficiency in inducing DNA damage. It can damage DNA via indirect photosensitizing reactions resulting in the formation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) (Rastogi, Richa, Kumar, Tyagi, & Sinha, 2010). By this means, cell membrane and other cellular components can be damaged, and the protein synthesis can be inhibited (Chatterley & Linden, 2010). On the other hand, UV-C light is directly absorbed by DNA of microorganisms and causes the formation of cyclobutane pyrimidine dimers on the same DNA strand between two adjacent nucleotides leading to inhibition of transcription and replication and eventually death of the cell (Koutchma, 2009). DNA damage caused by UV-C irradiation can be repaired by photolyase enzymes resulting in photoreactivation in microorganisms, while UV-A rays damages these repair enzymes causing the cell membrane damage to be irreversible. Chevremont, Farnet, Sergent, Coulomb, and Boudenne (2012) stated that the combined emission at 280 and 365 nm increased the effectiveness of microbial inactivation via the inhibition of the reactivation of the microorganism caused by UV-C light. Thus, in subsequent analyzes, it was decided to employ 40 min 280/365 nm UV-LED irradiation. Besides, prolonging the UV irradiation longer than 40 min caused the color compounds to degrade. The color of juice turned brownish. Similarly, Akgün and Ünlütürk (2017) reported that the color compounds of the apple juice adversely affected by increasing the exposure time.

Table 1
Logarithmic reductions of *E. coli* K12 in MB exposed to UV-LED irradiation at different wavelengths and exposure times.

Time (min)	UV Dose (mJ/cm^2)		Logarithmic reductions (log10 CFU/mL)		
	280 nm UV-LED lamp	365 nm UV-LED Lamp	280 nm	365 nm	280/365 nm
10	7.716	21.720	1.35 ± 0.10^{dA}	0.21 ± 0.07^{dC}	0.73 ± 0.07^{dB}
20	15.432	43.440	2.2 ± 0.03^{cA}	0.24 ± 0.10^{cdC}	1.56 ± 0.20^{cB}
30	23.148	65.160	3.14 ± 0.20^{bA}	0.44 ± 0.01^{cdB}	2.96 ± 0.09^{bA}
40	30.864	86.880	3.44 ± 0.18^{abA}	0.47 ± 0.01^{cB}	3.34 ± 0.10^{bA}
60	46.296	130.320	3.73 ± 0.13^{aA}	0.96 ± 0.09^{bB}	3.55 ± 0.35^{abA}
80	61.728	173.760	3.7 ± 0.06^{aA}	0.95 ± 0.07^{bB}	3.44 ± 0.16^{abA}
100	77.160	217.200	3.4 ± 0.02^{abB}	1.38 ± 0.0^{aC}	4.05 ± 0.07^{aA}

A-B: Values within each exposure time followed by the same letter are not significantly different ($p > 0.05$).
a-b: Values within each wavelength and their combination followed by the same letter are not significantly different ($P > 0.05$).

3.3. Effect of UV-LEDs on natural Flora

The number of mesophilic bacteria (TAC), and yeast and molds (YM) in mixed beverage were reduced by 2.59 log CFU/mL (from 5.69 log CFU/mL of initial load), and 0.17 log CFU/mL (from 3.28 ± log CFU/mL of the initial load), respectively, after subjecting the juice to UV-LED irradiation at 280/365 nm for 40 min. Higher numbers of coliform bacteria were inactivated under the same conditions (4.09 log CFU/mL of initial load) compared to TAC and YM. After the treatment, the number of coliform bacteria, (1.48 ± 0.67 $\log_{10}$ CFU/mL) was below 2 $\log_{10}$ CFU/mL, i.e. the recommended microbiological standard for fruit juices (Kaya, Yıldız, & Ünlütürk, 2015; Rashed et al., 2013).

It is known that the yeast and molds are more resistant to UV irradiation than bacteria since they have larger sizes, thicker cell wall and lower content of thymine or cytosine basis in their genomes (Tran & Farid, 2004). Mesophilic bacteria and coliform bacteria found in the natural flora of MB might be competing with yeasts to get sufficient amount of UV light. Consequently, bacteria might have a higher chance to be inactivated when exposed to UV-A and UV-C irradiation. Because bacteria are smaller than yeasts/molds; this causes easier UV passage. Additionally, bacteria have different cell wall constructions, and existence of higher levels of pyrimidine in bacteria DNA increases the chance of cross linkage of neighboring thymine and cytosine (Torkamani & Niakousari, 2011). The findings of this study are compatible with others. Many studies showed that natural foodborne flora was more resistant to UV light than inoculated microorganisms (Koutchma et al., 2004; Linden & Darby, 1998).

3.4. Impact of treatments on the physicochemical properties of MB

pH of mixed beverage samples did not change significantly by the effect of the treatments ($p > 0.05$) (Table 2). This finding was in agreement with other studies reporting no significant changes in pH after UV-C treatment of several fruit juice products (Hernández-Carranza et al., 2016; Kaya et al., 2015; Pala & Toklucu, 2011; Pala &Toklucu 2013). On the contrary, change in the absorption coefficients of UMB and HMB samples was significant ($p < 0,05$). The absorption coefficient or percentage permeability of the beverage is a critical factor for UV irradiation since it is directly related to the performance of the UV-LED treatment (Koutchma, Popovic, Ros-polski, & Popielarz, 2016). In this study, the absorption coefficient of untreated mixed beverage was measured as 22.32 cm^{-1} at 280 nm and 4.71 cm^{-1} at 365 nm. After UV-LED and heat treatment, these values slightly increased and the highest increase was observed in samples treated with UV-LED. This was consistent with the results published by Unluturk and Atilgan (2015) and Müller, Noack, Greiner, Stahl, and Posten (2014) who reported an increase in absorption coefficient of apple and grape juices after UV-C and UV-B irradiation. This was probably caused by the degradation of color compounds, e.g. melanin and melanoidins, by UV-LED treatment, resulting in an increase in turbid structure. Similarly, Ibarz, Pagán, Panadés, and Garza (2005) reported an increase in absorption coefficient of juice due to degradation of melanoidins after long exposure of the fruit juice samples to UV-C light. Additionally, the total soluble solids of samples were significantly increased after UV treatment. This might be attributed to the evaporation of water from the juice samples during prolonged UV treatment. Juice became more concentrated and an increase in TSS content was observed (Falguera, Garvín, Garza, Pagán, & Ibarz, 2014).

Table 2
Physicochemical properties of untreated (CFB), heat-treated (HFB) and UV-LED treated (UFB) mixed beverages.

		CFB	HFB	UFB
pH		4.01 ± 0.08^{a}	3.99 ± 0.1^{a}	3.99 ± 0.08^{a}
Brix (%)		7.8 ± 1.94^{b}	7.85 ± 1.89^{b}	8.83 ± 1.69^{a}
Color	L*	28.91 ± 0.21^{a}	29.31 ± 0.25^{a}	29.35 ± 0.57^{a}
	a*	-0.17 ± 0.02^{b}	-0.39 ± 0.25^{c}	-0.09 ± 0.3^{a}
	b*	4.32 ± 0.02^{b}	4.1 ± 0.58^{c}	5.21 ± 1.49^{a}
	ΔE		0.36	0.94
Absorption coefficient (cm^{-1})	280 nm	22.32^{c}	24.47^{b}	27.77^{a}
	365 nm	4.71^{c}	5.62^{b}	6.47^{a}
Turbidity (NTU)		891		
BI		15.25 ± 0.04^{b}	13.71 ± 0.03^{c}	18.87 ± 0.02^{a}

Results were represented as "means ± standard error". The least significant difference was determined by Tukey pairwise nonparametric comparison test. Means that do not share the same letter represents significantly different results ($p < 0.05$).
Abbreviations: L* (brightness-darkness), a* (redness-greenness), b* (yellowness-blueness), BI (Browning index), ΔE (total color difference), NTU (Nephelometric Turbidity Units), BI (Browning Index).

Unlike L* values, a* and b* values were significantly affected by UV-LED irradiation and heat treatment. BI and ΔE values of UV treated samples were higher compared to that of heat-treated samples. This was probably caused by the oxidation of certain phytochemicals present in MB samples (carotenoids, ascorbic acids, polyphenols, etc.) during long exposure to UV light (Riganakos, Karabagias, Gertzou, & Stahl, 2017). Additionally, the residual enzyme activities could be another reason of an increase observed in BI and ΔE values. Similarly, Akgün and Unlütürk, (2017) pointed out the residual activity of the polyphenoloxidase (PPO) in apple juice samples after UV-LED and heat treatments. They reported higher residual PPO activity in UV-LED treated samples than that of thermal treated ones. At this point, it was also noteworthy to look at the total phenolic content of UV treated MB samples. The total phenolic content of UV treated samples were the highest, compared to those of untreated and heat-treated samples (Fig. 2). This might indicate that high amount of substrate is available to be used by PPO enzyme in enzymatic browning reaction which may lead to the high amount of browning product, i.e., melanin (Rocha & Morais, 2001). Also, an increase in the amount of total phenolic component may result in high photo-oxidation and high levels of browning (Müller et al., 2014). As a result, these may explain the observation of higher a * and b * values in UV-treated MB samples. On the other hand, the thermal treatment led to a decrease in a* and b* values of the MB samples. Since the enzymes causing browning were vulnerable to heat at temperatures >50 °C, the PPO activity and Maillard reaction intermediates and products may not cause a significant change in these color parameters. As a result, pigment degradation in heat-treated mixed beverage (HMB) samples was probably more important than the formation of non-enzymatic browning reaction intermediates and end products (Rattanathanalerk, Chiewchan, & Srichumpoung, 2005).

The total color changes (ΔE) was 0.38 and 0.94 for heat and UV-LED treated samples, respectively. As ΔE was less than 2, it was concluded that no noticeable changes were observed between the untreated beverage, the heat-treated beverage and the UV-LED treated beverage (Hernández-Carranza et al., 2016).

Pearson correlation coefficients for color parameters, i.e., L*, a*, b* and BI, were calculated and presented in Table 3. Measured values of between 0.8 and 1.0 and − 0.8 to −1 indicated a strong positive correlation and a strong negative correlation, respectively (Profillidis & Botzoris, 2019). Thus, strong correlation was found between color parameters according to this classification. However, there was a linear but negative correlation between L* (brightness- darkness) and a* (−0.98), and also L* and b* (−0.89), and L* and BI (−0.94). The negative correlation between L* and BI value showed that higher degree of browning gave rise to lower L* value. Therefore, as could be expected the correlation indicates an increase in occurrence of brown pigments (Rocha & Morais, 2001). Also, a* and b* showed a strong correlation with BI values supporting the relationship between these parameters.

3.5. Effect of treatments on the phytochemical attributes of MB

Since, the beverage was a rich source of bioactive compounds and

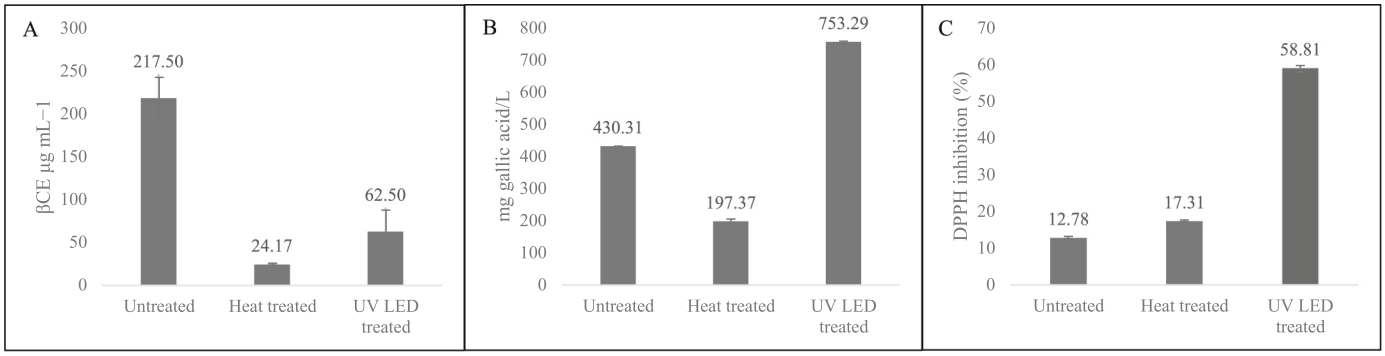

Fig. 2. Changes in the total bioactive content of MB subjected to UV and heat treatments (A) total carotene content, (B) total phenolic compounds, (C) total antioxidant activity.

Table 3
Correlation matrix for *CIELAB* (*L**, *a**, *b**) values, and Browning index.

	L*	a*	b*	BI
L*	–			
a*	−0.98	–		
b*	−0.89	0.95	–	
BI	−0.94	0.98	0.99	–

Abbreviations: L* (brightness-darkness), a* (redness-greenness), b* (yellowness-blueness), BI (Browning index).

exhibited a powerful antioxidant activity, it was noteworthy to study the change in total carotenoids, total phenolic components and antioxidant activity after UV-LED treatment (Fig. 2).

MB subjected to UV-LEDs exhibited a significant decrease in carotenoid content (Fig. 2a). In contrast, Santhirasegaram, Razali, George, and Somasundram (2015) reported a significant increase in carotenoid content of Chokanan mango subjected to UV-C (254 nm) irradiation. They claimed that the reason for this could be the alteration in carotene-binding proteins resulting in an increase in the free carotenoid content. In addition, it was assumed that the increase in the carotenoid content in Chokanan mango could be due to the effective inactivation of oxidative enzymes causing carotenoid loss. In the present study, a decrease in total carotenoid content might be attributed to the lack of oxidative enzyme inactivation. In addition to that, many natural molecules are highly sensitive to UV-A radiations because it induces the oxidation of these molecules. UV irradiation causing the transformation of reactive intermediates is a strong oxidizer. Carotenoid molecules can be oxidized in two ways; directly or indirectly by photoreactants (photosensitized) (Chevremont, Farnet, Coulomb, & Boudenne, 2012). Thus, the dramatic loss in carotenoid could be caused by the reactive oxygen species which are induced by UV-A irradiation. However, a decrease in carotenoid content of mixed beverage subjected to heat treatment was more significant than that of UV treated samples. The results are in line with those studies reporting a decrease in carotenoid content after thermal treatment of carrot, Chokanan mango, and cashew apple juice (Santhirasegaram et al., 2015; Zhang et al., 2016). It is important to point out that, UV-LED treatment caused a decrease in carotenoid content (71.3%) lower than that of heat-treated samples (88.9%). Better retention of carotenoids during the UV-LED treatment could be caused by increased antioxidant capacity. Owing to that, reactive oxygen species could be scavenged and carotene stability was retained (Santhirasegaram et al., 2015).

Fig. 2b illustrates the changes in the total phenolic content of MB samples after UV-LED and heat treatment. UV-LED treatment caused a significant increase in MB samples compared to that of control sample. Untreated MB had TPC of 430.31 ± 1.33 mg/L while TPCs of samples treated with heat and UV-LED irradiation were 197.37 ± 7.25 and 753.29 mg GA/L ± 5.44, respectively. While heat treatment reduced the TPC of MB, UV-LED treatment increased TPC content of the MB by almost 1.75 fold. Similarly, it was reported that thermal pasteurization at 90 °C for 30 min caused considerable amount of loss of TPC in apple juice (Aguillar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon & Martin-Belloso, 2007). Additionally, Zhang et al. (2016) reported a significant decrease in total phenolic components of heat-treated carrot juice. However, many studies reported an increase in total phenolic content of UV treated fruit juices such as starfruit, pear, and carrot juices (Bhat, Ameran, Voon, Karim, & Tze, 2011; Falguera et al., 2014; Hernández-Carranza et al., 2016). In contrast, Pala and Toklucu (2013) reported no significant change in the total phenolic content of orange juice subjected to UV-C radiation. Also, they found no change in the total phenolic component of pomegranate juice after UV irradiation (Pala & Toklucu, 2011). Noci, Riener, Cronin, Morgan, and Lyng (2008) reported a significant decrease in the total phenolic component of apple juice after UV-C treatment. However, they did not observe a significant change in total phenolic content after thermal pasteurization of apple juice.

The differences in the TPC content of fruit juices after UV and heat treatments have been linked to some pretreatments that affect the phenolic content. It was assumed that some of these processes increased their extractions, while others caused an impairment of some phenolic molecules (Falguera, Garza, Pagán, Garvín, & Ibarz, 2013). Additionally, the increase in UV-LED treated samples could be attributed to the breakdown of polyphenols (complex phenolic components) into smaller phenolic components. Therefore, Folin-Ciocalteau regent interacted with smaller phenolic components and resulted in higher TPC values (Falguera et al., 2014).

The total antioxidant activity of the mixed beverages measured in terms of the percentage reduction of the DPPH. The results indicated that UV-LED irradiation increased DPPH inhibition by a 4.60 fold (Fig. 2c). While heat treatment caused a slight increase in (17.31%), UV-LED irradiation increased DPPH inhibition from 12.78% to 58.81%. The DPPH assay is based on the scavenging capacity of specific antioxidants of stable free radical 2,2-diphenyl-1-picrylhydrazyl and the reduction in DPPH causing the loss of color. Therefore, this increase could be due to the amount of high phenolic content of the irradiated functional beverage. Since phenolic compounds have the ability to scavenge the free radicals, available phenolic components may contribute to high antioxidant activity. Likewise, earlier studies found a significant increase in the antioxidant capacity of star fruit and Chokanan mango juices after UV treatment (Bhat et al., 2011; Santhirasegaram et al., 2015). However, several studies reported no significant alteration in the antioxidant activity of carrot-orange, pomegranate, and orange juices after the UV-C irradiation (Ferrario et al., 2018; Hernández-Carranza et al., 2016; Pala & Toklucu, 2011; Pala & Toklucu, 2013).

It was evident that antioxidant activities of UV-treated MB samples followed a similar trend with the TPC. This could be attributed to the contribution of phenolic compounds to the antioxidant activity of fruit

juices (Gil, Toma, Hess-pierce, Holcroft, & Kader, 2000). It was also noticeable to indicate that heat treatment at 72 °C for 120 s was able to maintain the initial antioxidant activity of untreated MB with a non-significant difference. Since the mixed beverage was formulated to take advantage of different fruit and vegetable antioxidants and ingredients, the result showed that the beverage has functional properties. Thus, despite the decrease in total carotene, UV-LED irradiation seems to be a reasonable technology in cases where the bioactive constituents need to be preserved.

4. Conclusion

In this study, efficacy of Ultraviolet light-emitting diodes (UV-LEDs) with peak and coupled emissions at 280, 365 and 280/365 nm on inactivation of *E. coli* K12 in Mixed Beverage (MB) was investigated. UV treatment at 280 and 280/365 nm resulted in higher logarithmic reductions than that one's treated at 365 nm and the inactivation efficiencies of 280 and 280/365 nm were not significantly different. This study showed that the combination of UVA/UVC wavelengths has a similar inactivation potential when compared to UVC wavelength. On the other hand, the UV-LEDs have a minimum effect on the physical properties. UV-LEDs irradiation lead to an increase in amount of TPC and total antioxidant activity and also it retained the total carotene content of MB better than the heat treatment. As a result, functional ingredients that a consumer intends to take advantage of for health benefits are well protected without harming any quality parameter of the MB. It is concluded that UV-LED irradiation can be a reasonable technology and may have a potential to become an economic and proper method for the processing of fruit juices which is rich in bioactive constituents. On the other hand, by using higher number of UV-LEDs or combining UV-LEDs with other non-thermal preservation techniques may possibly increase the microbial efficiency. More research is required on this subject.

Author statement

The authors declare that there are no conflicts of interest related to this article.

Declaration of Competing Interest

None.

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