

Quality Attributes of Black Mulberry (*Morus Nigra L.*) Juice Treated with Ultraviolet Radiation

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Received: 01 January 2021; Accepted: 28 June 2021; Published: 05 July 2021

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Abstract

After exposing the black mulberry juice to ultraviolet light with the intensity of 5, 10 and 20 kJ/m², their quality analysis have been performed for 4 days at +4 °C and +25 °C. Within the current study the antioxidant activity, Total Phenolic Content (TPC), microbial charges and pH values of the samples have also been investigated. In the UV light exposed samples there existed no meaningful difference in the % DPPH radical scavenging activity level, a small amount of decrease has been detected in the TPC creation process. While the storage time worsens the DPPH activity, it affects the TPC positively. Microbial studies showed reduction in total mesophile aerobic microorganisms, yeasts and mould counts about by 1-log cycle on UV treatments. This is the first report on the effects of UV radiation on black mulberry.

Keywords: UVC treatment; Black mulberry; Quality; Fruit juice

Introduction

The genus of mulberry (*Morus* spp.), belonging to the Moraceae family, is tropical and subtropical species. East, West and South East Asia, South Europe, South of North America, Northwest of South America and some parts of Africa are areas of distribution of this species [1]. The mulberry production in Turkey is important and exhibit significant diversity of wild species in Anatolia [2]. Today, mulberry fruit is frequently used as fresh and processed and has a high nutritional value.

UV radiation is a small part of the electromagnetic spectrum, which comprises radio waves, microwaves, infrared, visible, X-rays and gamma rays [3]. Ultraviolet-C is known as germicidal against the microorganisms such as bacteria, viruses, protozoa, yeast, mold and most. The most pronounce effect has been detected at the wavelengths of 250 to 270 nm. Therefore, the wavelength of 254 nm is generally selected to disinfect the surfaces, water and fruit juices [4,5].

UV light treatment is promising in that it is low cost, non-thermal and newly developing technology and frequently used in microbial security of fruit juices and in protection of the food properties [6-9].

There are several studies in the literature on the elongating the shelf life of the fruit juices treated with UV light. In the current study it is aimed to find out the elongation effect of shelf life by the

application of UV light for the black mulberry juice. Throughout the process it is also aimed to evaluate the microbial charge pH value, DPPH scavenging activity and total phenolic content of the samples being researched.

Material and Methods

Black mulberry samples (5 kg) were collected from the Serdivan Bahçelievler neighborhood in June. Black berries of 2.5-4 cm in size and similar maturity were crushed by a domestic juicer (Arzum). Juice was immediately filtered on a double layer cheese cloth to remove seeds and pulp from it.

Solvents and Reagents

All the solvents used in the present study were procured from Sigma Aldrich (Laborchemikalien GmbH, 30926, Seelze, Germany) and R&M Chemicals (Essex, UK). Peptone water (buffer), Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Folin-Ciocalteu, Gallic Acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Sodium Carbonate, Ascorbic Acid were procured from MERCK (Darmstadt, Germany).

UV Treatment

As UVC light source 4 units of low pressured mercury lamps Puritec HNS 8W G5 (Osram, Germany) have been employed. Light intensity (*E₀*) is defined to be the ratio of the optical power of light source (*Q*) to the area (*S*) of application and has the unit of W/m².

$$E_o = Q/S$$

The light intensities of the lamps have been measured to be 25 W/m² using PRC-Krochmann radiometer and PRC-Krochmann radiometer header 121116-3 at the distance of 5 cm. Optical dose (H_o), which is the absorbed energy amount of UV light in unit area, is calculated by the formula:

$$H_o = \int E_o.t dt$$

and has the unit of J/m². These dose calculations have been fulfilled where each lamp was placed 5 cm apart to the centre of petri and being on top of the setup (Figure 1).

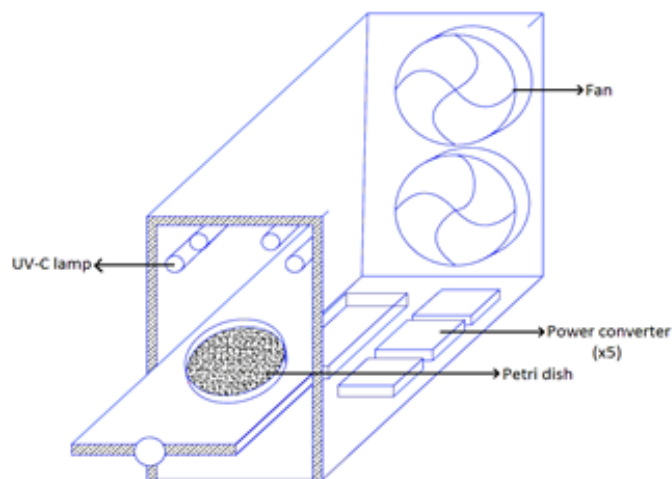


Figure 1: UV application setup.

After transferring the 10 mL of prepared fruit juice samples into the sterile petri containers (15x 90 mm) they are exposed to UV light in a laminar flow. During the process to eliminate the heat produced a fan is added to the system. Average values of optical doses of 5, 10 and 20 kJ/m² have been applied for all experiment groups.

Total Phenolic Content (TPC)

The total phenolic content was determined by Folin-Ciocalteu procedure as described with minor modifications [10]. The 100 μ L of Fresh juice sample diluted 1/10 with 50% ethanol was mixed with 200 μ L of Folin-Ciocalteu (50%) and was kept waiting for 2 mins. Then, 1 mL of 2% sodium carbonate solution was added and shaken well. The mixture was kept in a dark place for 1 hour. The absorbance of the mixture was measured at 760 nm by using a spectrophotometer (Shimadzu UV mini-1240). The total phenolic content values were determined from a calibration curve prepared with a series of gallic acid standards (50, 100, 200, 300, 400 mg/L). The results were expressed as mg of GAE/100 g.

Antioxidant Activity (DPPH assay)

The modified Blois method was used for the antioxidant activity determination [11]. In short, 1 ml of 0.004% solution of DPPH radical in ethanol was mixed with 1 mL Fresh juice sample diluted 1/100 with 50% ethanol. These solutions were kept in dark place for 30 mins and the optical density was then measured at 517 nm using a spectrophotometer and 50% ethanol was used for the blank. The following equation was employed to evaluate the % DPPH radical scavenging activity: % DPPH radical scavenging = (control absorbance - extract absorbance) / control

absorbance) x 100.

Microbial Analysis (aerobic plate count, yeast and mould counts)

UV treated and non-treated juice were analysed for the status of total mesophile aerobic microorganisms, yeast and mould counts. A series of decimal dilutions (10⁻¹ -10⁻⁵) was prepared with 0.1% (w/v) peptone water. Further, one millilitre of decimal dilution of the sample was pipetted into Petri dish and the total plate counts were enumerated using the pour plate method. The plates (NA) were incubated at 37 \pm 1 $^{\circ}$ C for 48 h.

The total yeast and moulds were enumerated by pour plate method using potato dextrose agar (PDA) media. To inhibit the growth of others microbes, 10% tartaric acid was added into PDA agar (final pH 3.5-3.7). The plates were incubated at 25 $^{\circ}$ C for 5-7 days in the incubator. The results were expressed as log colony-forming units (cfu) per millilitre of juice (n=5).

Statistical Analysis

Data were analyzed using SPSS software. Values were expressed as means \pm standard deviations.

Results and Discussion

pH and acidity is known to be the most reliable indicator to evaluate the general properties of the product and can have the effect to elongate the shelf life during the production process of the fruit juices. pH variation of black mulberry juice with respect to the UV dose and the temperature for 5 days is given in Table 1. The control groups exposed to

UV dose and the fruit juices exposed to 5, 10 and 20 kJ/m² doses have shown no any difference. The storage temperature is seen to be effective

on pH values and it has been found that the pH value decreases for both temperatures while the storage time increases.

Table 1: Effects of UV treatment on pH.

Days	4°C				25 °C			
	Control	Dose (kJ/m ²)			Control	Dose (kJ/m ²)		
		5	10	20		5	10	20
1	4	4	4.1	4.1	3.7	3,6	3.6	3.6
2	4	4	4	4	3.5	3.5	3.5	3.5
3	3.9	3.9	3.9	3.9	3.4	3.4	3.4	3.4
4	3.8	3.8	3.8	3.8	3.3	3.4	3.4	3.4
5	3.5	3.5	3.5	3.5	3.2	3.2	3.3	3.3

Consumers think that the acidic fruits or fruit juices are microbiologically safe since they have lower pH values. However the current reports states that presence of the pathogens like *Alicyclobacillus acidoterrestris*, *Bacillus spp.*, *Escherichia coli* O157: H7, *Staphylococcus aureus* ve *Salmonella spp.* within the foods causes several diseases. The acidic pH can also grow very rapidly in mold and yeast. Black mulberry has an acidic pH value such as various fruits (approximately 3.5) and the mold and yeast is responsible for the microbial decomposition/deterioration in the fruits (Ercilli and Orhan, 2007). The first day total live and yeast-mold value for the UV exposed/treated samples is given in Figure 2.

The number of microorganisms during the storage period is found to increase in all samples. Similar increase tendency has been observed by other researchers [12,13].

It has been observed that the number of yeast and mold and microorganisms slowly decreases by increasing the doses. As expected, lower number of microorganisms grow at +4°C with respect to the room temperature. The samples stored at +4°C respond well to the UV treatment as well.

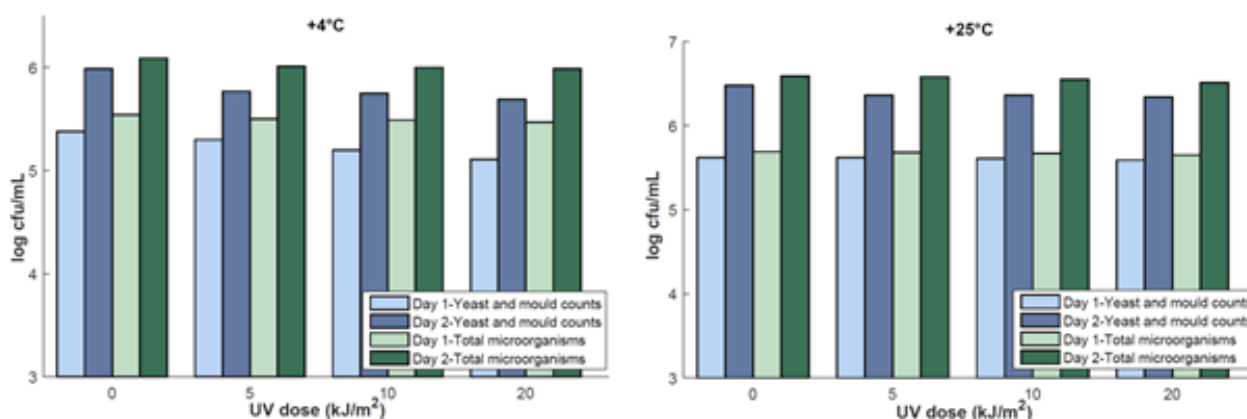


Figure 2: 1st and 2nd days microbial charge effect of samples exposed to UV-C.

Owing to the presence of various antioxidant constituents in fruits, very large number of methods have been developed to measure the antioxidant activity/capacity. In the current study DPPH free radical scavenging test has been employed to evaluate the antioxidant capacity of tomatoe juice. Increase in storage time decreases the DPPH scavenging radical level [14]. Similarly, Bo Jiang et al., reported that DPPH sweeping activity decreases with increasing storage time of heat treated black mulberry [15]. In general, the control group has shown higher

DPPH scavenging value than the samples exposed to UV light for both temperatures (Figure 3). The doses applied have shown no any effect on DPPH radical scavenging activity level. The dose of 10 kJ/m² has higher DPPH scavenging level than others. Similar results have been reached by López-Rubira et al., [16] and they found that the antioxidant capacity of pomegranate seeds did not change much after UV-C treatment. Same was observed in tropical fruits that were exposed to UV light.

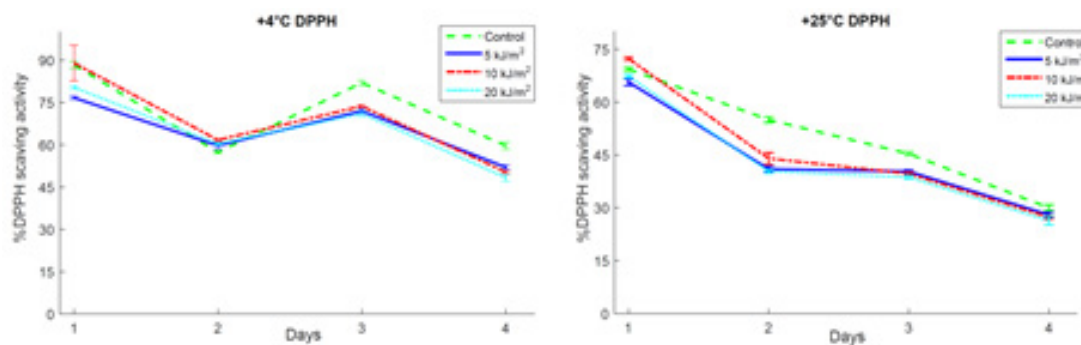


Figure 3: Effect of UV treatment on % DPPH radical scavenging activity.

Plants are rich of natural bioactive components in secondary metabolites and antioxidants. Phenolic constituents show resistance to pathogens and predators and these are secondary metabolites which have a great role in pigmentation, growing and reproduction of the plants. They have been shown to provide anti-allergic, anti-inflammatory, antioxidant, antiviral, hepatoprotective and anticarcinogenic activities [17,18].

The TPC content is shown to decrease when the amount of the dose is increased in one day after UV treatment at +4°C. While the storage time is increased it has been observed that the TPC amount at 20 kJ/m² has shown rapid increase with respect to other groups (Figure 4). In literature the phenolic constituents of the fruit juices exposed to UV light have

shown no any difference. There are several reports as well mentioning about that the effect of the treatment of UV-C light on phenolic content and antioxidant capacity of other fruit juices. For example Noci et al., has concluded that the antioxidant capacity of fruit juices exposed to UV-C light has not been effected whereas its total phenolic content was decreased to a certain extent with respect to fresh fruit juices [19].

Temperature has no effect on TPC value for the first day, while the increase in storage time at room temperature raises the TPC amount. Depending on temperature, increase in microbial charge and maturity are thought to cause an increase in total phenolic content level. In literature it has been shown that the microbial fermentation can increase the phenolic content [20-22].

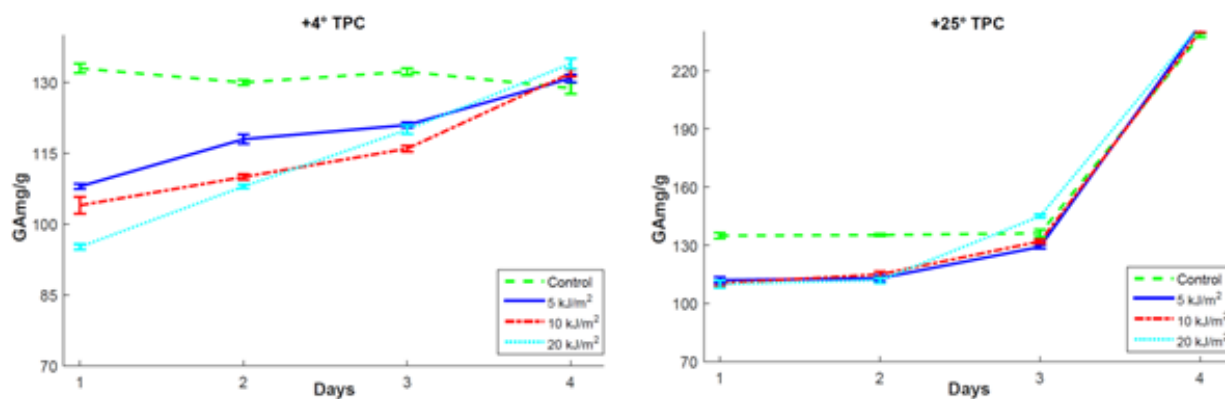


Figure 4: Total phenolic content variations.

UV values applied in different doses of black mulberry juice, which was studied for the first time in the literature, did not significantly affect DPPH scavenging activity ($p < 0.05$) and pH values. It was observed that the increase in UV dose reduced the amount of aerobic microorganism and yeast-mold in the total mesophyll. As a further study one may look at the effect of over-dose application or combined treatments to increase the UV-C effectivity.

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Citation: Kenan TUNÇ. "Quality Attributes of Black Mulberry (*Morus Nigra* L.) Juice Treated with Ultraviolet Radiation." *J Clin Nutr Heal* (2021);2: 001-009