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# Physicochemical and Sensory Properties of Bovine Milk Treated by Different UV-C Dose: The Effect on Vitamin D₃, cholesterol, fatty acid, and formation of volatile compounds

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**ABSTRACT.** This study investigated the effect of different UV-C doses on physicochemical (pH, color, viscosity) and sensory properties, FFA, vitamin  $D_3$ , cholesterol, fatty acid composition, and oxidative volatile compounds formation. The physicochemical properties (pH, viscosity, and color) of milk were significantly affected by the application of UV-C and different UV-C doses (p < 0.05). The FFA of raw and pasteurized milk was determined as 0.053% and 0.10%, respectively. The FFA values significantly increased with the application of UV-C. The amount of cholesterol in UV-C-applied milk was in the range of 38.74-49.70 ppm. The cholesterol level was significantly reduced by the application effect of UV-C treatment at all dosages (p < 0.05). The amount of vitamin  $D_3$  of raw and pasteurized milk was found as 90.91 mg kg<sup>-1</sup> and 65.87 mg kg<sup>-1</sup>, respectively. The UV-C application at all dosages, with the exception of 98.4 J mL<sup>-1</sup>, significantly increased the amount of  $D_3$  (p < 0.05). UV-C application caused a significant change in the composition of fatty acid composition and this change varied according to applied UV-C dosage. The carbon disulfide and aldehyde formation rate increased and the sensory quality reduced as the UV-C dose increased.

Key words: Bovine Milk; UV-C application; cholesterol level; vitamin D<sub>3</sub>.

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## Introduction

Milk and dairy products are widely consumed globally due to their high content of carbohydrates, fats, proteins, vital amino acids, vitamins, and minerals. Milk is exposed to contamination in the processes from milking to the final product (Koca et al., 2018). The contamination of milk at any stage accelerates the microorganism and enzyme activity and decreases its shelf life due to its high nutritional content and water activity (Delorme et al., 2020; Koca et al., 2018). The milk should be subjected to any preservation methods to extend its shelf life and to inactivate spoilage and pathogenic microorganisms and enzymes.

Thermal treatments are commonly used in the dairy industry to increase the commercial shelf life of milk and dairy products. Thermal processes like pasteurization and sterilization deactivate microorganisms and enzymes, extending the shelf life of food goods (Koca et al., 2018). However, thermal applications can lead to negative consequences that cause quality loss in milk, such as damage to bioactive components, protein degradation, lipid oxidation, and negative effects on sensory properties such as color, taste, and flavor (Delorme et al., 2020; Moreno-Vilet et al., 2018). Moderate heat treatment results in high bioactive compounds retention while resulting in lower shelf life. It is a challenge for the milk industry to obtain milk with both high shelf life and higher sensory and nutritional quality. (Zhang et al., 2021). Researchers have focused on preservation methods that will provide microorganism and enzyme activation and cause the least change in the composition and sensory quality of milk. Recently, there has been an increasing trend toward the application of non-thermal preservation techniques such as ultrasound application, microfiltration, ultraviolet-C radiation (UVC), and high-pressure treatment as an alternative to the heat treatment due to the negative effects of heat treatment on milk quality (Zhang et al., 2021).

One of the main technologies studied on alternative heat treatment techniques is short-wavelength ultraviolet (UV-C) rays. UV-C radiation has numerous advantages, including inactivating a wide variety of pathogenic and spoilage microorganisms, thus minimizing the loss of nutritional and sensory quality (Martinez-Garcia et al., 2019). The European Food Safety Authority (EFSA) opinion on UV-C irradiated milk

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in the "Dietetic Products, Nutrition and Allergy Panel" are that the stated products are safe. The panel reported that the shelf life of UV-C irradiated pasteurized milk increased from 12 days to 21 days. (EFSA Panel on Dietetic Products & Allergies, 2016).

Studies on the UV-C processing in milk have mostly focused on; the microbiological quality of milk (Atik & Gumus, 2021; Bandla et al., 2012; Kasahara et al., 2015; Rossitto et al., 2012) and its effect on pathogen bacteria in milk (Atik & Gumus, 2021; Bhullar et al., 2017). In the studies conducted, the effectiveness of UV-C treatment in reducing the microbial load of different microorganisms in milk was researched. However, studies on the physicochemical properties of milk are limited. In the studies conducted, parameters such as; total fat, protein, moisture, ash, fatty acid composition (Cappozzo et al., 2015; Zhang et al., 2021), pH (Choudhary et al., 2011), viscosity (Orlowska et al., 2012), color (Hu et al., 2015) free fatty acidity, cholesterol (Cilliers et al., 2014) and vitamin D<sub>3</sub> (Kharitonov et al., 2019) were determined. In these studies, the effect of a certain dose of UV-C on some physicochemical and bioactive properties was investigated. Therefore, further studies are required to show the effect of different doses of UV-C on the physicochemical and bioactive properties of milk. A comprehensive physicochemical and bioactive characterization of UV-C applied milk samples in several doses was performed in our study. For this aim, the effect of different UV-C doses on physicochemical (pH, color, viscosity) and sensory properties, FFA, vitamin D<sub>3</sub>, cholesterol, fatty acid composition, and volatile oxidative formation was investigated.

## Materials and methods

#### Material

The milk was collected from four Simmental cattle at a small farm in Beyyazı Town, Afyonkarahisar Province. Milk samples were collected from the identical cows through milking under identical circumstances and promptly transported to the laboratory. Upon arrival at the laboratory, the samples were transferred into pristine glass bottles. The milk samples were cooled to temperatures of 4, 8, 11, 16, 17, 21, and 25 °C using a water bath and refrigerator. Each sample was subjected to UV-C radiation at various temperatures.

#### Method

#### **UV-C** treatment of milk

The UV-C reactor utilized in the research was fabricated by Defne Engineering Laboratory Equipment in Afyonkarahisar. The UV-C reactor comprised a 2 L stainless steel feeding system, a pump with peristaltic function, a stainless-steel column housing a UV-C lamp, and polyurethane connection tubing attached to the main body. The unit had a double-walled design to facilitate the circulation of cooling water, ensuring a consistent temperature in the column during the treatment process. The UV-C reactor was adapted from a previously constructed reactor by Shah et al., (2014) and is shown in Figure 1. The investigation utilized a Lightech GPH846T5L/HO/4 type UV-C lamp from Dunakeszi, Hungary. The lamp emitted radiation with a power of 18 watts at a wavelength of 253.7 nanometers. The UV-C reactor was activated 15 minutes before to the study's commencement to disinfect the column using UV-C radiation. The column's internal volume was found to be 150 mL. Consequently, prior to each application, the initial 200 mL of milk was discarded, and measurements were then collected.

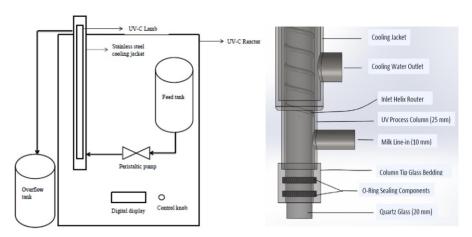


Figure 1. Flow-through UV light unit used for in the study.

#### **Cleaning of UV-C reactor**

Following the experiment, a CIP system was used to clean the column, feeding unit, and fasteners. The CIP procedure was implemented following the method described by Gopisetty et al. (2018) with certain adjustments. Following the protocol, 250 mL of sterile water, then 250 mL of 0.1 N NaOH, and finally another 250 mL of sterile water were pumped through at the maximum flow rate. The reactor pieces were reassembled after prewashing and then washed with 1 L of 0.1 N NaOH followed by 1 L of sterile water.

## Dosage calculation for UV-C radiation

The UV-C dosage applied while passing through the UV-C reactor was determined theoretically using Equation 1 as suggested by Cilliers et al. (2014). The factors included the milk flow rate across the column and the overall output power of the UV-C lamp, which was 18 watts.

Dose 
$$(J mL^{-1}) = Total \ UV-C \ output \ power \ (W) \ / \ Flow \ Rate \ (mL \ s^{-1})$$

$$W = J \ s^{-1}$$
(1)

#### **Reynolds Number Calculation (Re)**

The Re value was determined using Equation 2. (Bandla et al., 2012).

$$Re=(\rho \times V \times De)/\mu$$
 (2)

 $\rho$ : Milk density

μ: Viscosity

V: Fluid velocity

De: The diameter of the space between the two pipe

## Physicochemical analysis

The pH analysis was conducted using a digital pH meter (Isolab pH.mV.Temp model, Eschau GERMANY) calibrated with appropriate buffer solutions. The probe was dipped into 100-mL sample and the pH value was detected after it was stabilized. The viscosity of the milk samples was determined at the 0.5 mm gap level and at  $25^{\circ}$ C, equipped with a Peltier heating system, with stress and temperature control (Anton Paar, MCR 302, Austria). Color analysis of the samples was conducted with the HunterLab color measurement device. Three measurements were performed in each milk sample and L, a, and b and  $\Delta E$  values were determined. In determining the  $\Delta E$  value, equation 3 was used.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \tag{3}$$

#### Vitamin D<sub>3</sub> analysis

The vitamin D ( $D_3$ ) analysis was performed by an HPLC method using Shimadzu (Shimadzu Corp., Kyoto, Japan) Prominence Brand HPLC, a cooled autosampler (SIL 20ACHT) equipped with a 50  $\mu$ L injection loop (SIL-10ADvp), a vacuum membrane degasser (DGU-14A) and a diode-array detector (SPD-M20A) according to the method described by (Karppi et al., 2008). For the preparation of the samples for analysis, approximately 25 mL sample was taken and 30 mL of chloroform/methanol (2/1) was added to it. The extracted organic phase was evaporated and the remaining oil dissolved in 2 mL mobile phase was injected into the system.

Shimadzu's LC solution was utilized for instrument control, data gathering, and data processing. Chromatographic separations were performed using a C18 guard column (250×4.6 mm, 5 micron). The mobile phase was a combination of acetonitrile, methanol, and chloroform in a ratio of 60:25:15, respectively.

#### Cholesterol analysis

The cholesterol analysis of the samples was conducted according to the method specified by Oh et al., (2001). 1 milliliter of milk was moved to a test tube equipped with a Teflon-lined screw lid. Direct saponification was conducted using 1 mL of 10% potassium hydroxide (KOH) in ethanol (weight/volume) for 30 minutes at 70°C. The unsaponifiable portion was taken out using 5 mL of diethyl ether and 2 mL of distilled water. The diethyl ether extraction was conducted thrice, and the sample was thoroughly cleaned. A portion of the diethyl ether extract was moved to a 50 mL round bottom glass flask and evaporated to dryness using a rotating vacuum evaporator at 50°C. The substance was subsequently dissolved once more in 1 milliliter of

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methanol. 20  $\mu$ L of sample was injected directly into HPLC. For the analysis. Shimadzu (Shimadzu Corp., Kyoto, Japan) Prominence Brand HPLC, equipped with LC20AT pump, InertSustain C18 column (100×3.0 mm, 5 micron) and diode array detector (SPD-M20A).

## Free fatty acid value (FFA)

FFA value was determined by oil extraction and titration method according to the method of Gursoy et al., (2018). A 20 mL milk sample was combined with 200 mL of diethyl ether for 1 minute and then filtered using rough filter paper with a thickness of 0.18 mm and a weight of 82 g m<sup>-2</sup>. The diethyl ether-lipid extract was concentrated using a Scilogex rotary evaporator from the USA under vacuum at 40°C until it reached a final volume of around 1–3 mL for analysis. The lipid extracts were purged with nitrogen until drying and stored in glass bottles at -20°C for further analysis.

## Fatty acid composition

The fatty acid composition analysis was conducted by Agilent Brand (Agilent Technologies, Inc., Santa Clara, CA, USA) gas chromatography/mass spectroscopy (AGILENT 5975 C AGILENT 7890A GC ) using MSDCHEM software and DB WAX column (50\*0.20 mm, 0.20 µm). The methyl esters of fatty acids were synthesized using the procedure proposed by Yılmazer and Seçilmiş (2006). For this process, 200 µL of extracted oil was mixed with 1 ml of 1.5 M methanolic HCl and kept at 80°C for 2 hours. The injection volume was 1 µL. Detector and injector temperature was set to 240°C. The operating temperature was adjusted as it follows: The starting temperature of the oven is 80°C. After 4 minutes at 60°C, it was increased to 175°C with an increase of 13°C per minute. It was held at this temperature for 27 min. Then, with an increase of 4°C per minute, the temperature of 215°C was reached. It was held at this temperature for 15 minutes.

#### Volatile profile analysis

Oxidative aromatic compounds of samples were detected as it follows. First, solid phase microinjection (SPME) processes of the samples were carried out. 21 mL of milk sample was pipetted into 40 mL clear glass bottles equipped with Teflon septa (Supelco Inc.). A 75 µm carboxene poly(dimethyl siloxane) coated SPME fiber (Supelco) was positioned approximately 1 cm above the milk surface and subjected to magnetic stirring at 45°C for 22 minutes. Gas chromatography conditions: Volatile chemicals were released at the injector port of an Agilent 6890A gas chromatography system with a flame ionization detector. The injector temperature was set at 280°C, and all injections were performed using the split mode. A capillary column measuring 30 meters in length, with an inner diameter of 0.25 millimeters and a film thickness of 0.25 micrometers (DB-5ms; J&W Scientific, Folsom, CA) was used for the separation process. The linear flow rate of the helium carrier gas was set at 35 cm s<sup>-1</sup>. The oven temperature was increased from 35 to 180°C at a rate of 15°C min<sup>-1</sup> with 0.5 minutes of start, intermediate, and final holding durations, then increased from 180 to 260°C at a rate of 20°C min<sup>-1</sup>. Flame ionization detectors were kept at 300°C (van Aardt et al., 2005).

#### Sensory analysis

Sensory evaluations of the samples were conducted using the scoring test technique as stated by Altuğ and Elmacı (2005). For this purpose, 12 trained panelists between the ages of 25-40 were used. The panelists were trained on the sensory quality characteristics of milk samples for two weeks. In each tasting, only 6 examples were presented so that the panelist should not get tired. To help the panelists make more accurate evaluations during the tasting, the criteria to be taken into account while preparing the sensory evaluation scale of milk and its products are given in Table 1 and the evaluation chart for "raw milk" prepared by the German Agricultural Society (DLG) is given in Table 2.

#### Statistical analysis

Three replications of milk were produced from each point and three parallel measurements were taken from each replication. The mean as well as the standard deviation values of the results are provided. The study data were analyzed using SPSS version 24.0 statistical software. Group differences were assessed using Duncan's multiple comparison test (p < 0.05).

Table 1. Criteria to be taken into account while preparing the sensory evaluation scale of milk and its products (Anonymous 2012).

Quality	Score
Very good: Very compliant with the predetermined sensory standard	5
Good: compliant with the predetermined sensory standard	4
Less imperfect: Less imperfect according to predetermined sensory standard	3
Imperfect: Significantly imperfect according to predetermined sensory standard	2
Much imperfect: : Much imperfect according to predetermined sensory standard	1

Table 2. The evaluation chart for "raw milk" prepared by the German Agricultural Society (DLG) (Anonymous 2012).

Quality	Highest score
Odour	
Perfect	5
Imperfect	3
Taste	
Perfect	5
Tasteless	3
Bitter	3
Feedy	3
Oily	3
Metallic	3
Moldy	3
Malty	3
Fruity	3
Salty	3
Soapy	2
Fish oily	2
Rancid	2
Texture and appearance (Colour)	
Perfect	5
Deviations not considered as defects	4
Significant protein and fat particles	3
Hardened fat particles	2
Bloody	2
Dirty	2

## **Results and discussion**

#### The effect on pH and viscosity value

Table 3 showed the effect of different doses of UV-C and temperature on the physicochemical properties of milk. The pH value of raw milk was 6.62, while the pH value of pasteurized milk was found to be 6.43. The UV-C treatment did not significantly change the pH value of milk, with the exception of the 60 J mL $^{-1}$  UV-C application. As seen, 60 J mL $^{-1}$  UV-C application significantly reduced the pH value of milk (p < 0.05), and the pH value increased with the increase in UV-C dosage. The pH values of the milk samples treated with 60 J mL $^{-1}$  more UV-C dosage and the pH value of the raw milk sample were found to be insignificant. In a study where 16.822 mJ cm $^{-2}$  dose of UV-C was applied to raw milk, it was reported that the measurements of the pH of raw, unprocessed, and UV-C treated cows milk conducted at 24°C were in the range of 6.6 - 6.7 and these values remained in the normal range throughout a seven-day storage period (Bandla et al., 2012).

The viscosity of raw and pasteurized milk was determined as 2.58 mPa.s and 2.48 mPa.s, respectively. The effect of UV-C treatment on the viscosity value of milk was found to be significant (p < 0.05). The viscosity value decreased as the applied UV-C dose increased. However, it was determined that the difference between pasteurized milk and milk samples treated with a dose of 60 J mL<sup>-1</sup> UV-C was not significant (p > 0.05). In a study, the effect of UV-C application on milk quality parameters under different conditions was investigated. It was reported that the viscosity was not affected by UV-C application (Orlowska et al., 2013). Our viscosity results for the milk samples treated with higher UV-C dosage were contrary to the results of the study by Orlowska et al. (2013) , which mean that the viscosity was affected by UV-C treatment and it was decreased as the dose increased. The difference between literature could be explained by UV-C systems. In our study, the decrease in viscosity in samples treated with UV-C at high dosages can be explained by the instability of fat globules as increasing ultraviolet dosage.

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Table 3. pH value and viscosity results of milk samples treated with UV-C at different temperatures and flow rates.

	_		_				
Treatment	Dose (J mL <sup>-1</sup> )	рН	Viscosity (mPa.s)	L	a	b	ΔΕ
NC (Raw milk)	0	$6.62 \pm 0.07^{a}$	$2.58 \pm 0.07^{a}$	13.22 ± 2.04 <sup>a</sup>	$6.90 \pm 0.30^{g}$	$21.90 \pm 0.30^{a}$	-
PC (Pasteurized milk)	0	$6.43 \pm 0.02^{c}$	$2.48 \pm 0.05^{b}$	$12.89 \pm 0.78^{a}$	$5.92 \pm 1.29^{\rm f}$	$21.40\pm0.30^{ab}$	$1.15 \pm 0.12^{e}$
UV11a (18 mL min <sup>-1</sup> 25°C)		$6.59 \pm 0.11^{b}$	$2.39 \pm 0.04^{b}$	$10.55 \pm 0.45^{bc}$	$10.16 \pm 0.30^{e}$	$17.42 \pm 0.30^{\text{cde}}$	$6.15 \pm 2.30^{cd}$
UV11b (18 mL min <sup>-1</sup> 25°C)	(0)	$6.59 \pm 0.09^{b}$	$2.39 \pm 0.07^{\rm b}$	$10.50 \pm 0.38^{bc}$	$10.17 \pm 0.24^{\rm e}$	$17.42 \pm 0.12^{cde}$	$6.17 \pm 1.24^{cd}$
UV10a (18 mL min <sup>-1-1</sup> 1°C)	60	$6.59 \pm 0.06^{b}$	$2.46\pm0.08^{\rm b}$	$11.05 \pm 0.58^{b}$	$9.99 \pm 0.25^{e}$	$18.16 \pm 0.30^{bcd}$	$5.32 \pm 2.20^{d}$
UV10b (18 mL min <sup>-1</sup> 11°C)		$6.59 \pm 0.02^{b}$	$2.46 \pm 0.10^{b}$	$11.07 \pm 0.74^{b}$	9.98 ± 0.22 <sup>e</sup>	$18.57 \pm 0.30^{abc}$	$5.02 \pm 2.18^{d}$
UV9 (15 mL min <sup>-1</sup> 17°C)		6.61 ± 0.02 <sup>ab</sup>	$1.66 \pm 0.05^{fg}$	10.36 ± 1.18 <sup>bc</sup>	9.79 ± 0.14°	$17.03 \pm 0.30^{\text{cde}}$	$6.35 \pm 2.15^{cd}$
UV8a (15 mL min <sup>-1</sup> -4°C)	72	$6.60 \pm 0.10^{ab}$	$1.81 \pm 0.03^{cde}$	9.22 ± 1.67 <sup>cd</sup>	$10.01 \pm 0.48^{e}$	$15.26\pm0.30^{cde}$	$8.35 \pm 1.15^{bc}$
UV8b (15 mL min <sup>-1</sup> -4°C)		$6.60 \pm 0.08^{ab}$	$1.81 \pm 0.07^{cde}$	$9.22 \pm 3.04^{cd}$	$10.01 \pm 0.18^{e}$	$15.23 \pm 0.30^{e}$	$8.36 \pm 1.22^{bc}$
UV7 (12 mL min <sup>-1</sup> 25°C)	90	6.61 ± 0.01 <sup>ab</sup>	$1.75 \pm 0.06^{ef}$	$10.40 \pm 0.75^{bc}$	11.37 ± 0.74 <sup>cd</sup>	$17.26 \pm 0.30^{\text{cde}}$	$7.03 \pm 1.15^{cd}$
UV6 (11 mL min <sup>-1</sup> 16°C)	98.4	6.61 ± 0.11 <sup>ab</sup>	1.79 ± 0.05 <sup>de</sup>	11.28 ± 2.06 <sup>b</sup>	9.82 ± 1.89 <sup>e</sup>	$18.58 \pm 0.30^{abc}$	4.83 ± 1.19 <sup>d</sup>
UV5 (10 mL min <sup>-1</sup> 8°C)	107.8	$6.61 \pm 0.03^{ab}$	1.74 ± 0.11 <sup>ef</sup>	$8.39 \pm 0.77^{d}$	$12.09 \pm 0.64^{abc}$	14.04 ± 0.30°	10.59 ± 1.15 <sup>b</sup>
UV4 (8 mL min <sup>-1</sup> 21°C)	135.4	$6.60 \pm 0.03^{ab}$	1.61 ± 0.01 <sup>g</sup>	11.24 ± 0.18 <sup>b</sup>	$11.16 \pm 0.31^{d}$	$18.50 \pm 0.30^{abc}$	$5.80 \pm 2.43^{d}$
UV3a (5 mL min <sup>-1</sup> -25°C)		$6.63 \pm 0.02^{ab}$	1.51 ± 0.06 <sup>h</sup>	$8.34 \pm 0.77^{d}$	$12.18 \pm 0.36^{ab}$	10.64 ± 0.30 <sup>f</sup>	13.36 ± 0.11 <sup>a</sup>
UV3b (5 mL min <sup>-1</sup> -25°C)	216.0	$6.63 \pm 0.05^{ab}$	$1.51 \pm 0.03^{h}$	$8.34 \pm 0.23^{d}$	12.19 ± 1.59 <sup>ab</sup>	$10.64 \pm 0.30^{\rm f}$	$13.36 \pm 0.15^{a}$
UV2 (5 mL min <sup>-1</sup> 15°C)	216.9	$6.64 \pm 0.11^{ab}$	$1.90 \pm 0.05^{c}$	$8.56 \pm 0.41^{d}$	11.71 ± 0.29 <sup>bcd</sup>	$14.69 \pm 0.30^{de}$	9.84 ± 1.04 <sup>b</sup>
UV1a (5 mL min <sup>-1</sup> 4°C)		$6.63 \pm 0.08^{ab}$	$1.94 \pm 0.13^{cd}$	$8.40 \pm 1.07^{d}$	$12.58 \pm 0.43^{a}$	$14.08 \pm 0.30^{\rm e}$	10.80 ± 1.27 <sup>b</sup>
UV1b (5 mL min <sup>-1</sup> 4°C)		$6.63 \pm 0.02^{ab}$	1.94 ± 0.16 <sup>cd</sup>	$8.41 \pm 0.95^{d}$	$12.59 \pm 0.31^{a}$	$14.76 \pm 0.30^{de}$	10.32 ± 1.21 <sup>b</sup>

<sup>\*</sup> Different letters in the same column indicate statistical difference (p < 0.05).  $\pm$  : Standard deviation

L, a, b, and  $\Delta E$  color values of the irradiated and untreated milk samples are given in Table 3. The L value of the raw and pasteurized milk were 13.22 and 12.89, respectively. The L values of milk treated with different doses of UV-C varied between 8.34 and 11.28. The effect of UV-C application on the L value was found to be significant (p < 0.05), and the effect of different UV-C dosage on the L value was also found as significant (p > 0.05). The L value decreased with increasing UV-C dosage.

The a value of the raw and pasteurized milk were 6.90 and 5.92, respectively. The values of milk treated with different doses of UV-C irradiation varied between 9.79 and 12.59. The a value significantly increased with the application of UV-C treatment and the increase in a value continued as the UV-C dosage increased. The decreasing and increasing trend in L and a value, respectively, are attributed to the enzymatic and non-enzymatic reactions. The b value was determined as 21.90 in raw milk and 21.40 in pasteurized milk. The b values of UV-C irradiated milk were determined between 14.04-18.58. The UV-C treatment significantly reduced the b value, and the samples subjected to higher UV-C dosage showed the lowest b value. The reduction in b value by application of UV-C treatment could be explained by the pigment oxidation such as  $\beta$ -carotene and vitamins. The decreasing L and b and increasing a value was also reported from the previously published studies (Keklik et al., 2019). The data obtained in the study were in parallel with this study. All UV-C treated milk samples showed higher  $\Delta E$  value than the pausterized milk.  $\Delta E$  value indicated total color change and its value higher than 3 showed perceptible color differences. All milk samples treated UV-C showed perceptible color change.

## The effect of free fatty acid (FFA), cholesterol and vitamin D<sub>3</sub>

Table 4 showed the FFA, cholesterol, and vitamin  $D_3$  value of milk samples. The FFA of raw and pasteurized milk was determined as 0.053 and 0.10%, respectively. The FFA values significantly increased with the

application of UV-C. While the effect of UV-C dosage on FFA was significant (p < 0.05), the effect of different temperatures on FFA was insignificant (p > 0.05). The FFA content increased as UV-C dosage increased, indicating that a higher dosage of UV-C caused hydrolysis of triglycerides. The increase in FFA formation was also reported from other studies (Cappozzo et al., 2015; Cilliers et al., 2014). The increasing of the FFA could be related to demageging of fat globules during pumping of the milk in to UV-C system (Cilliers et al., 2014).

Table 4. Free fatty acidity (FFA), cholesterol and vitamin D3 values of UV-C treated raw milk samples at different temperature and flow rates.

T	Dose	FFA	Cholesterol	Vit. D <sub>3</sub>
Treatment	$(J mL^{-1})$	(%)	(ppm)	(mg kg <sup>-1</sup> )**
$NC_{(Raw milk)}$	0	$0.053 \pm 0.03^{e}$	$53.53 \pm 0.09^{a}$	90.91 ± 8.16 <sup>f</sup>
PC <sub>(Pasteurized milk)</sub>	0	$0.100 \pm 0.06^{cd}$	$55.70 \pm .10^{a}$	$65.87 \pm 1.10^{g}$
$UV11a_{(18 \text{ mL dk}^{-1} 25^{\circ}C)}$		$0.120 \pm 0.12^{bc}$	$49.59 \pm 0.83^{b}$	$151.41 \pm 4.08^{a}$
$UV11b_{(18mLdk^{-1}25^{0}C)}$	60	$0.120 \pm 0.012^{bc}$	$49.70 \pm 0.44^{b}$	$151.41 \pm 4.0^{8a}$
$UV10a_{(18 \text{ mL dk}^{-1} 11^{\circ}C)}$	60	$0.118 \pm 0.03^{bc}$	$48.73 \pm 0.25^{b}$	$145.91 \pm 2.06^{a}$
$UV10b_{(18 \text{ mL dk}^{-1} 11^{0}\text{C})}$		$0.118 \pm 0.03^{bc}$	$47.50 \pm 1.05^{b}$	$145.90 \pm 2.09^{a}$
UV9 <sub>(15 mL dk-1 17°C)</sub>		$0.092 \pm 0.03^{d}$	$49.45 \pm 0.27^{\rm b}$	$122.99 \pm 1.63^{bc}$
$UV8a_{(15 \text{ mL dk}^{-1}4^{\circ}C)}$	72	$0.091 \pm 0.01^{d}$	$46.14 \pm 0.16^{bc}$	$120.21 \pm 1.85$ bc
$UV8b_{(15 \text{ mL dk}^{-1} 4^{\circ}C)}$		$0.091 \pm 0.01^{d}$	$46.16 \pm 1.15^{bc}$	$120.20 \pm 1.15$ bc
$UV7_{(12 \text{ mL dk}^{-1} 25^{\circ}C)}$	90	$0.125 \pm 0.01^{abc}$	$46.56 \pm 0.18^{b}$	$100.79 \pm 2.44^{e}$
$UV6_{(11 \text{ mL dk}^{-1} 16^{\circ}C)}$	98.4	$0.092 \pm 0.02^{d}$	$46.08 \pm 0.20^{bc}$	$93.26 \pm 0.81^{\rm f}$
$UV5_{(10 \text{ mL dk}^{-1} 8^{\circ} C)}$	107.8	$0.126 \pm 0.01^{ab}$	$49.47 \pm 0.09^{b}$	$121.76 \pm 0.85$ bc
UV4 <sub>(8 mL dk<sup>-1</sup> 21°C)</sub>	135.4	$0.148 \pm 0.03^{a}$	$47.90 \pm 0.13^{b}$	$127.05 \pm 1.19^{b}$
UV3a <sub>(5 mL dk<sup>-1</sup> 25°C)</sub>		$0.144 \pm 0.05^{ab}$	$38.74 \pm 0.29^{d}$	$111.97 \pm 0.13^d$
$UV3b_{(5 \text{ mL dk}^{-1} 25^{\circ}C)}$		$0.144 \pm 0.02^{ab}$	$40.07 \pm 0.15^{d}$	$111.97 \pm 1.13^{d}$
$UV2_{(5 \text{ mL dk}^{-1} 15^{\circ}C)}$	216.9	$0.129 \pm 0.01^{ab}$	$39.97 \pm 1.12^{d}$	$117.44 \pm 1.45^{cd}$
$UV1a_{(5 \text{ mL dk}^{-1} 4^{\circ}C)}$		$0.118 \pm 0.01^{bc}$	$42.59 \pm 2.18^{cd}$	$103.59 \pm 2.01^{e}$
UV1b <sub>(5 mL dk-1 4°C)</sub>		$0.118 \pm 0.01^{bc}$	$42.57 \pm 0.24^{cd}$	103.59 ± 2.01e

<sup>\*</sup> Different letters in the same column indicate statistical difference (p < 0.05). ± : Standard deviation.

The cholesterol value of the raw and pasteurized milk was found as 53.53 ppm and 55.70 ppm, respectively. The amount of cholesterol in UV-C applied milk was in the range of 38.74-49.70 ppm. The cholesterol level significantly reduced by the application effect of UV-C treatment at all dosages (p < 0.05). The highest cholesterol level was detected at 60 J mL $^{-1}$  dose application and the lowest amount of cholesterol at 216.9 J mL $^{-1}$  dose application. The effect of temperature on the cholesterol value of milk was insignificant (p > 0.05). In a similar to our study, Cilliers et al. (2014) reported that UV application reduced cholesterol 35% and pasteurization application reduced cholesterol by 18%. Cholestrol can easily be oxidized by the effect of light and heat due to the it's unsaturated structure. The cholesterol oxidation product could occur due to the autooxidation of cholesterol under light and heat. The decreasing of cholesterol level by application UV-C could be explained by the oxidation of cholesterol into cholesterol oxidation products (Cilliers et al., 2014).

The highest vitamin  $D_3$  amount was detected at 18 mL min<sup>-1</sup> flow rate (60 J mL<sup>-1</sup> dose). The increasing UV-C dosage reduced vitamin  $D_3$  level. The temperature had no effect on the amount of  $D_3$  (p > 0.05). In a similar study investigating the effect of UV radiation on the physicochemical properties of milk, raw milk was exposed to radiation for 4 periods (5, 10, 15, and 25 minutes) and it was reported that the amount of vitamin  $D_3$  increased as the processing time increased. It has been reported that vitamin  $D_3$  content varies between 0.994<sup>-1</sup>.83 µg  $100g^{-1}$  depending on the processing time applied (Kharitonov et al., 2019). The overall outcome of our investigation revealed that applying high doses of UV-C led to a rise in the FFA value of the product, while simultaneously resulting in a fall in vitamin D levels. Based on these findings, the dosage used in UV-C application has a significant impact on the physicochemical characteristics of milk.

## The effect of fatty acid composition of milk

Table 5 showed the effect of different UV-C dosage and temperature applications on the saturated fatty acid composition of milk samples. In all applications, UV-C treatment caused a significant change in the percentage levels of all fatty acids compared to raw milk. With UV-C application, C18:0 amount increased, whereas C8:0 and C10:0 amount decreased significantly (p < 0.05) and C16:0 did not change significantly compared to raw milk (p > 0.05). The effect of different UV-C dosage on fatty acid composition was also found to be significant (p < 0.05). No clear trend was observed in the presence level of fatty acids with the application of different UV-C dosage. While high dosage application resulted in similar results to raw milk in major fatty acids of milk, medium and low dosage applications caused significant differences in the major fatty acid

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(C18:0, C18:1, C16:0). No significant difference was observed in terms of the level of the percentage of C16:0 and C18:0 of milk exposed to 216.9 J mL $^{-1}$  UV-C dosage and raw milk. The effect of different temperature applications on fatty acid composition was found to be significant for fatty acids in major amounts (C10:0 and C16:0) (p < 0.05), while the effect of different temperature applications on minor fatty acids (slightly above or below 1%) was found as insignificant (p > 0.05). It is possible to draw the conclusion that the application of UV-C induced a considerable change in the composition of saturated fatty acids in comparison to raw milk, and that this change varied according to the amount of UV-C that was applied.

Table 5. Saturated fatty acids results of raw and different doses of UV-C applied milk samples (%).

UV Dose	Butyric acid	Caproic acid	Caprylic acid	Nonanoic acid	Capric acid	Undecanoic	Lauric acid	Pentadecanoic	Palmitic acid	Heptadecanoic	Stearic acid
(J mL <sup>-1</sup> )	(C4:0)	(C6:0)	(C8:0)	(C9:0)	(C10:0)	acid (C11:0)	(C12:0)	acid (C15:0)	(C16:0)	acid (C17:0)	(C18:0)
0 (Raw)	$0.929 \pm 0.18^{c}$	$1.384 \pm 0.23^{d}$	$4.605 \pm 0.12^{a}$	$0.099 \pm 0.01^{a}$	$6.320 \pm 0.04^{a}$	$0.682 \pm 0.01^a$	$6.261 \pm 0.04^{ab}$	$0.528 \pm 0.04^{a}$	$22.965 \pm 0.13^{bcd}$	$0.807 \pm 0.02^{de}$	$6.467 \pm 0.03^{f}$
0 (Past.)	$0.704 \pm 0.11^{h}$	$2.460 \pm 0.13^a$	$2.054 \pm 0.05^{f}$	$0.065 \pm 0.03^{b}$	$4.159 \pm 0.12^{e}$	$0.566 \pm 0.30^a$	$5.309 \pm 0.34^{e}$	$0.277 \pm 0.02^{d}$	25.241 ± 0.11a	$0.876 \pm 0.05^{d}$	$7.048 \pm 0.18^{d}$
	$1.119 \pm 0.09^a$	$2.437 \pm 0.16^{a}$	$2.572 \pm 0.19^{cde}$	$0.056 \pm 0.03^{bc}$	$2.674 \pm 0.10^{g}$	$0.073 \pm 0.01^{b}$	$6.163 \pm 0.09^{abc}$	$0.355 \pm 0.01^{\circ}$	$22.434 \pm 0.01^{cd}$	$2.245 \pm 0.09^a$	$8.827 \pm 0.23^{b}$
60	$1.119 \pm 0.09^a$	$2.437 \pm 0.16^a$	$2.559 \pm 0.07^{\text{cde}}$	$0.056 \pm 0.03$ bc	$2.857 \pm 0.06^{g}$	$0.073 \pm 0.01^{b}$	$6.160 \pm 0.10^{abc}$	$0.358 \pm 0.09^{\circ}$	$22.202 \pm 0.28^d$	$2.273 \pm 0.05^{a}$	$8.820 \pm 0.15^{b}$
60	$1.128 \pm 0.14^a$	$2.435 \pm 0.25^{a}$	$2.826 \pm 0.12c^{de}$	$0.053 \pm 0.02^{bc}$	$2.729 \pm 0.05^{g}$	$0.082 \pm 0.03^{b}$	$6.506 \pm 0.16^{a}$	$0.341 \pm 0.02^{c}$	$23.883 \pm 1.31^{b}$	$2.129 \pm 0.04^{a}$	$9.068 \pm 0.24^{ab}$
	$1.128 \pm 0.14^{a}$	$2.435 \pm 0.24^{a}$	$2.835 \pm 0.15^{bcd}$	$0.053 \pm 0.02^{bc}$	$2.741 \pm 0.05^{g}$	$0.082 \pm 0.03^{b}$	$6.506 \pm 0.16^{a}$	$0.338 \pm 0.05^{\circ}$	$23.956 \pm 0.31^{b}$	$2.135 \pm 0.06^a$	$9.095 \pm 0.20^{a}$
	$0.739 \pm 0.16^{g}$	$2.183 \pm 0.08^{b}$	$2.957 \pm 0.04$ bc	$0.033 \pm 0.01^{\circ}$	$4.239 \pm 0.01^{\rm de}$	$0.112 \pm 0.01^{b}$	$5.723 \pm 0.16^{d}$	$0.418 \pm 0.05^{b}$	$23.790 \pm 0.30^{b}$	$0.636 \pm 0.11^{e}$	$6.474 \pm 0.15^{f}$
72	$0.742 \pm 0.18^{g}$	$2.150 \pm 0.17^{b}$	$2.869 \pm 0.11^{bcd}$	$0.032 \pm 0.05^{\circ}$	$4.549 \pm 0.05^{\circ}$	$0.117 \pm 0.03^{b}$	$5.767 \pm 0.06^{cd}$	$0.418 \pm 0.08^{b}$	$23.633 \pm 1.29$ <sup>bc</sup>	$0.737 \pm 0.09^{de}$	$6.370 \pm 0.06^{f}$
	$0.742 \pm 0.12^{g}$	$2.150 \pm 0.17^{b}$	$2.869 \pm 0.11^{bcd}$	$0.032 \pm 0.05^{\circ}$	$4.535 \pm 0.06^{\circ}$	$0.114 \pm 0.03^{b}$	$5.782 \pm 0.02^{cd}$	$0.415 \pm 0.02^{b}$	$23.097 \pm 0.10^{bcd}$	$0.730 \pm 0.07^{de}$	$6.673 \pm 0.05^{\mathrm{f}}$
90	$1.028 \pm 1.13^{b}$	$1.929 \pm 0.19^{\circ}$	$3.210 \pm 0.23^{b}$	$0.038 \pm 0.02^{bc}$	$4.339 \pm 0.24^{cd}$	$0.197 \pm 0.04^{b}$	$5.765 \pm 0.29^{cd}$	$0.165 \pm 0.01^{e}$	$18.532 \pm 0.89^{\text{f}}$	$1.571 \pm 0.36^{\circ}$	$7.020 \pm 0.02^{d}$
98,4	$1.030 \pm 1.11^{b}$	$1.728 \pm 0.18^{c}$	$2.433 \pm 0.25^{e}$	$0.032 \pm 0.04^{\circ}$	$3.881 \pm 0.06^{f}$	$0.167 \pm 0.05^{b}$	$5.247 \pm 0.24^{e}$	$0.245 \pm 0.01^{d}$	$20.483 \pm 0.58^{e}$	$1.859 \pm 0.22^{b}$	$8.015 \pm 0.20^{\circ}$
107,8	$0.930 \pm 1.01^{\circ}$	$1.814 \pm 0.09^{\circ}$	1.981 ± 1.23f	$0.038 \pm 0.01$ bc	$4.203 \pm 0.06^{e}$	$0.213 \pm 0.09^{b}$	$5.632 \pm 0.23^{de}$	$0.256 \pm 0.01^{d}$	$20.098 \pm 0.95^{e}$	$1.563 \pm 0.10^{\circ}$	$7.059 \pm 0.13^{d}$
135,4	$0.875 \pm 1.04^{d}$	$1.517 \pm 0.24^{d}$	$2.716 \pm 0.81^{cde}$	$0.035 \pm 0.03$ bc	$2.359 \pm 0.11^{h}$	$0.136 \pm 0.03^{b}$	$5.261 \pm 0.11^{e}$	$0.282 \pm 0.07^{d}$	$25.806 \pm 0.90^{a}$	$0.718 \pm 0.03^{de}$	$6.894 \pm 0.13^{d}$
	$0.785 \pm 1.14^{\text{f}}$	$2.427 \pm 0.16^a$	$2.747 \pm 0.27^{\text{cde}}$	$0.039 \pm 0.02^{bc}$	$4.267 \pm 0.04^{cd}$	$0.199 \pm 0.03^{b}$	$5.755 \pm 0.34^{cd}$	$0.257 \pm 0.01^{d}$	$22.984 \pm 0.88^{bcd}$	$0.751 \pm 0.06^{de}$	$6.607 \pm 0.19^{\rm ef}$
	$0.785 \pm 1.14^{\text{f}}$	$2.425 \pm 0.16^a$	$2.747 \pm 0.27^{\text{cde}}$	$0.040 \pm 0.04$ bc	$4.267 \pm 0.04^{cde}$	$0.199 \pm 0.03^{b}$	$5.750 \pm 0.34^{cd}$	$0.257 \pm 0.02^{d}$	$22.980 \pm 0.72^{bcd}$	$0.772 \pm 0.12^{de}$	$6.600 \pm 0.20^{\rm ef}$
216,9	$0.789 \pm 1.12^{f}$	$2.427 \pm 0.23^a$	$2.637 \pm 0.11^{cde}$	$0.044 \pm 0.05$ bc	$4.990 \pm 0.06^{b}$	$0.190 \pm 0.01^{b}$	$5.895 \pm 0.07^{bcd}$	$0.256 \pm 0.05^{d}$	$22.514 \pm 0.29^{cd}$	$0.738 \pm 0.01$ de	$6.841 \pm 0.07^{de}$
	$0.775 \pm 1.18^{f}$	$2.235 \pm 0.15^{\rm ab}$	$2.511 \pm 0.13^{de}$	$0.046 \pm 0.03$ bc	$4.499 \pm 0.40^{cd}$	$0.193 \pm 0.01^{b}$	$5.806 \pm 0.25^{cd}$	$0.280 \pm 0.03^{d}$	$22.473 \pm 0.37^{cd}$	$0.798 \pm 0.07$ de	$6.435 \pm 0.13^{f}$
	$0.775 \pm 1.18^{f}$	$2.235 \pm 0.13^{ab}$	$2.511 \pm 0.13^{de}$	$0.046 \pm 0.03^{bc}$	$4.560 \pm 0.53^{\circ}$	$0.193 \pm 001^{b}$	$5.806 \pm 0.25^{cd}$	$0.272 \pm 0.05^{d}$	$22.889 \pm 0.92^{bcd}$	$0.798 \pm 0.07^{de}$	$6.430 \pm 0.10^{f}$

\*Different letters in the same column indicate statistical difference (p < 0.05). ± : Standard deviation.

Table 6 presented the unsaturated fatty acids (%) of raw, pasteurized and UV-C treated milk samples. The effect of UV-C on unsaturated fatty acid composition was similar to that of saturated fatty acid composition. C14:1 and C16:1 amounts increased and decreased depending on the dose of radiation applied. Treatment of UV-C increased C18:1 amounts, which is one of the major unsaturated fatty acids and the level of C18:1 decreased with increasing UV-C dosage. The C18:1 values of the milk treated 135.4 and 216.9 J mL<sup>-1</sup> UV-C dosage and raw milk did not differ statistically (p > 0.05). The level of C18:2, C18:3n6 and C18:3n3 increased with the application of 60 J mL<sup>-1</sup> dose application and decreased with increasing dose of UV-C. There was an increase in all three fatty acids at 60 J mL<sup>-1</sup> dose application, and a proportional decrease occurred in other dosing applications. The decreasing of C18:2, C18:3n6, and C18:3n3 levels could be explained by lipid oxidation by increasing the UV-C dosage. These results are in agreement with the oxidation-induced volatile component analysis discussed in the next section.

Table 6. Unsaturated fatty acids results of raw and different doses of UV-C applied milk samples (%).

Dose (J mL <sup>-1</sup> )	Temperature (°C)	6-Nonanoic acid (C9:1)	Myristoleic acid (C14:1)	Metil 12-metil teradecanoat (C14:1)	Palmitoleic acid (C16:1)	Oleic acid (C18:1)	Lineloic acid (C18:2)	Gamma-linolenic acid (C18:3n6)	Linolenic acid (C18:3n3)
0 (Raw)		$0.074 \pm 0.01^{f}$	1.137 ± 0.01bcd	0.875 ± 0.01°	$0.785 \pm 0.04^{d}$	29.959 ± 0.11ef	1.209 ± 0.06 <sup>de</sup>	$0.821 \pm 0.02^{def}$	0.343 ± 0.01°
0 (Past.)		$0.090 \pm 0.01^{e}$	$1.127 \pm 0.19^{bcd}$	$0.603 \pm 0.07^{b}$	$0.740 \pm 0.15^{de}$	$32.883 \pm 0.38^{bcd}$	$1.737 \pm 0.58^{b}$	$0.950 \pm 0.05^{\circ}$	$0.211 \pm 0.01^{fg}$
	25	$0.098 \pm 0.02^{de}$	$0.128 \pm 0.01^{g}$	$0.252 \pm 0.04^{g}$	$1.708 \pm 0.03^{a}$	$32.950 \pm 0.09^{bcd}$	$2.007 \pm 0.03^{a}$	$1.169 \pm 0.03^{a}$	$0.652 \pm 0.02^a$
60	25	$0.099 \pm 0.03^{de}$	$0.126 \pm 0.02^{g}$	$0.256 \pm 0.06^{fg}$	$1.705 \pm 0.03^{a}$	$32.950 \pm 0.09^{bcd}$	$2.005 \pm 0.03^{a}$	$1.166 \pm 0.07^{a}$	$0.652 \pm 0.06^a$
60	11	$0.110 \pm 0.01^{d}$	$0.109 \pm 0.03^{g}$	$0.276 \pm 0.01^{efg}$	$1.207 \pm 0.07^{b}$	$33.148 \pm 0.99$ bc	$1.582 \pm 0.16$ bc	$1.177 \pm 0.13^{a}$	$0.617 \pm 0.03^{a}$
	11	$0.111 \pm 0.01^{d}$	$0.110 \pm 0.03^{g}$	$0.277 \pm 0.01^{efg}$	$1.211 \pm 0.05^{c}$	$33.248 \pm 1.10^{bc}$	$1.588 \pm 0.15$ bc	$1.172 \pm 0.15^{a}$	$0.619 \pm 0.03^{a}$
	17	$0.151 \pm 0.03^{\circ}$	$0.980 \pm 0.04^{e}$	$0.410 \pm 0.05^{\circ}$	$0.592 \pm 0.05^{ef}$	$31.163 \pm 0.82^{cde}$	$0.916 \pm 0.11^{e}$	$0.919 \pm 0.08^{cd}$	$0.242 \pm 0.013^{efg}$
72	4	$0.096 \pm 0.01^{de}$	$1.097 \pm 0.07^{d}$	$0.320 \pm 0.03^{cdefg}$	$0.600 \pm 0.01^{ef}$	$32.445 \pm 0.06^{bcd}$	$0.913 \pm 0.01^{e}$	$0.892 \pm 0.10^{cde}$	$0.262 \pm 0.05^{def}$
	4	$0.098. \pm 0.02^{de}$	$1.107 \pm 0.04^{cd}$	$0.298 \pm 0.01^{cdefg}$	$0.660 \pm 0.02^{def}$	$32.445 \pm 0.49^{bcd}$	$0.914 \pm 0.03^{e}$	$0.893 \pm 0.09^{cde}$	$0.260\pm0.06^{\rm def}$
90	25	$0.256 \pm 0.07^{a}$	$1.154 \pm 0.04^{bcd}$	$0.404 \pm 0.18^{cd}$	$1.191 \pm 0.09^{c}$	$34.413 \pm 1.15^{ab}$	$1.543 \pm 0.02^{bc}$	$1.073 \pm 0.14^{ab}$	$0.289 \pm 0.08^{cde}$
98,4	16	$0.100 \pm 0.02^{de}$	$0.752 \pm 0.05^{f}$	$0.391 \pm 0.03^{cde}$	$1.535 \pm 0.40^{b}$	$34.532 \pm 0.83$ ab	$1.446 \pm 0.10^{cd}$	$0.978 \pm 0.12^{bc}$	$0.468 \pm 0.06^{\circ}$
107,8	8	$0.102 \pm 0.03^{de}$	$0.897 \pm 0.03^{e}$	$0.391 \pm 0.01^{cde}$	$1.297 \pm 0.08^{\circ}$	36.201 ± 1.15 <sup>a</sup>	$1.388 \pm 0.05^{cd}$	$0.802 \pm 0.05^{ef}$	$0.461 \pm 0.05^{b}$
135,4	21	$0.234 \pm 0.01^{b}$	$1.289 \pm 0.17^{a}$	$0.529 \pm 0.06^{b}$	$0.502 \pm 0.01^{f}$	$30.159 \pm 1.25^{ef}$	$1.517 \pm 0.05^{e}$	$0.779 \pm 0.01^{f}$	$0.245 \pm 0.01^{efg}$
	25	$0.093 \pm 0.01^{de}$	$1.219 \pm 0.09^{abc}$	$0.376 \pm 0.02^{cdef}$	$0.636 \pm 0.03^{def}$	28.405 ± 0.85e	$0.790 \pm 0.02^{e}$	$0.744 \pm 0.07^{f}$	$0.197 \pm 0.02^{g}$
	25	$0.095 \pm 0.03^{de}$	$1.210 \pm 0.07^{abcd}$	$0.264 \pm 0.05^{fg}$	$0.630 \pm 0.04^{def}$	$28.705 \pm 0.73^{e}$	$0.790 \pm 0.02^{e}$	$0.759 \pm 0.05^{f}$	$0.193 \pm 0.02^{g}$
216,9	15	$0.094 \pm 0.07^{de}$	$1.235 \pm 0.13^{ab}$	$0.303 \pm 0.03^{\rm cdefg}$	$0.550 \pm 0.01^{f}$	$30.858 \pm 0.35^{de}$	$1.017 \pm 0.01^{f}$	$0.721 \pm 0.03^{f}$	$0.241 \pm 0.01^{efg}$
	4	$0.092 \pm 0.01^{de}$	$1.190 \pm 0.12^{abcd}$	$0.286\pm0.02^{\rm defg}$	$0.643 \pm 0.04^{def}$	$33.132 \pm 2.44$ bc	$0.983 \pm 0.07^{ef}$	$0.796 \pm 0.05^{cd}e$	$0.317 \pm 0.02^{cd}$
	4	$0.096 \pm 0.03^{de}$	$1.190 \pm 0.09^{abcd}$	$0.280\pm0.01^{\rm defg}$	$0.640 \pm 0.07^{bd}$	$33.130 \pm 2.40$ bc	$0.980 \pm 0.09^{ef}$	$0.791 \pm 0.07^{ef}$	$0.310 \pm 0.08^{cd}$

<sup>\*</sup> Different letters in the same column indicate statistical difference (p < 0.05). ± : Standard deviation.

Cilliers et al. (2014) reported that the UV-C treatment did not significantly change in fatty acid composition of milk. They also reported that the C4:0 amount decreased from 3.60 to 2.50% with application UV-C. In our study, the percentage level of the C4:0 for the UV-C treated milk at the highest dosage was lower

than the raw milk, similar to the results of the study by Cilliers et al. (2014), Matak et al. (2007) and Kharitonov et al. (2019) reported that UV-C application did not significantly change the fatty acid composition. The differences between our study and literature could be explained by the different UV-C dosage and UV-C system. In our study, an image similar to raw milk was detected in different UV-C dosages. In our study, the fatty acid profile of the milk samples subjected to high UV-C dosage was similar to the raw milk as in the previously published studies.

## The effect of volatile profile

The percentage results (%) of the volatile oxidative components of raw milk and UV-C treated are given in Table 7. As can be seen in the table, as the dose amount applied increased, the carbon disulfide rate increased. It was determined that new aroma compounds such as isobutyl aldehyde, 2N propyl 5 oxohexanal, 2,3,5,6 tetra-chloro-phenyl methyl sulfoxide, and 2-methyl pentanal were formed at a UV dose of 135.5 J mL<sup>-1</sup>. Carbon disulfide was determined as 0.48% in raw milk, 0.59% in pasteurized milk, and between 0.66-2.21% in different doses of UV-C radiation applied milk samples. As shown, the amount of carbon disulfide component increased as the dose of radiation increased. Increases in sulfur compounds with UV-C application have also been reported in other studies (Cilliers et al., 2014; Fernández et al., 2016). This increase in sulfur compounds was explained by direct and indirect oxidation of proteins.

Table 7. Results of oxidative aroma components of raw milk with and without UV-C applied at different temperatures and flow rates (%).

Dose (J mL <sup>-1</sup> )	Temperature (°C)	Carbon disulfide	3-methyl butanal	2-methyl butanal	Iso butyraldehyde	2-N-propyl-5- oxohexanal	2,3,5,6 tetra- chloro- phenyl methyl sulfoxide	2-methyl pentanal
0 (Raw)		$0.48 \pm 0.09^{i}$	-*	-	-	-	-	-
0 (Pasteurized)		$0.59 \pm 0.10^{1}$	-	-	-	-	-	-
	25	$0.82 \pm 0.02^{h}$	$0.07 \pm 0.02^{gh}$	$0.04 \pm 0.03^{cd}$	-	-	-	-
60	25	$0.80 \pm 0.06^{h}$	$0.05 \pm 0.05^{gh}$	$0.05 \pm 0.09^{cd}$	-	-	-	-
00	11	$0.66 \pm 0.07^{1}$	$0.06 \pm 0.09^{gh}$	-	-	-	-	-
	11	$0.66 \pm 0.02^{1}$	$0.03\pm0.05^{gh}$	-	-	-	-	-
	17	$1.03 \pm 0.03^{\rm f}$	$0.05 \pm 0.07^{gh}$	-	-	-	-	-
72	4	$1.00 \pm 0.05^{fg}$	-	-	-	-	-	-
	4	$0.98 \pm 0.02^{fg}$	-	-	-	-	-	-
90	25	$1.13 \pm 0.03^{e}$	$0.09 \pm 0.09^{g}$	$0.06 \pm 0.11^{cd}$	-	-	-	-
97,4	16	$0.92 \pm 0.07^{g}$	$0.21 \pm 0.03^{f}$	$0.09 \pm 0.08^{c}$	-	-	-	-
107,8	8	$1.44 \pm 0.12^{d}$	$0.20 \pm 0.05^{\rm f}$	$0.10 \pm 0.05^{c}$	-	-	-	-
135,4	21	$1.68 \pm 0.11^{c}$	$0.41 \pm 0.03^{e}$	$0.29 \pm 0.07^{a}$	0.07	0.02	-	0.05
	25	$2.21 \pm 0.09^{a}$	$2.18 \pm 0.14^{c}$	$0.26\pm0.12^ab$	-	-	1.73	-
	25	$2.18 \pm 0.09^{a}$	$2.10 \pm 0.12^{d}$	$0.23 \pm 0.02^{ab}$	-	-	1.68	-
216,9	15	$1.82 \pm 0.15^{b}$	$2.34 \pm 0.03^{b}$	$0.20 \pm 0.12^{b}$	-	-	-	-
	4	$1.53 \pm 0.08^{d}$	$3.03\pm0.07^a$	$0.25 \pm 0.05^{ab}$	-	-	-	-
	4	$1.50 \pm 0.09^{d}$	$3.00\pm0.10^a$	$0.25 \pm 0.02^{ab}$	-	-	-	-

<sup>\*</sup> Different letters in the same column indicate statistical difference (p < 0.05). ± : Standard deviation.

3-methyl butanal and 2-methyl butanal were formed in milk samples after UV-C application. In the first stages of oxidation, the first free radicals and then hydroperoxides are formed. In the later stages, volatile hydrocarbons such as aldehyde and ketone are formed, which cause sensory defects. (Menéndez-Carreño et al., 2008). Therefore, the formation of these components during the process is associated with oxidation. This component, which could not be detected in raw milk and pasteurized milk, was detected in the range of 0.03-3.03% in all samples except UV8a and UV8b coded samples (72 J mL-1 dose UV-C) after UV-C application. The level of 3-methyl butanal and 2-methyl butanal was increased by increasing UV-C dosage, indicating that higher UV-C dosage increased oxidation rate. Fernández et al. (2016) reported that the level of 3-methyl butanal and similar volatile compounds increased with application UV treatment. Matak et al. (2007) examined the effect of UV-C application on the chemical and sensory properties of goat milk in their study. Increases in pentanal, hexanal, and heptanal (in comparison to raw goat milk) concentrations were determined at doses after 1.3 mJ cm<sup>-2</sup> UV dose. There were differences in terms of detected aroma compounds between the results of this study and our results. Matak et al. (2005) reported that the applied UV dose

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increased the TBARs values twofold compared with raw milk. TBARs value is correlated with malonaldehyde, which is an oxidation product.

The finding of this study showed that exposure to UV-C has a notable impact on the formation of oxidative volatile compounds in milk. An appreciable rise in oxidative volatile compounds was noted, particularly at elevated UV-C dosages. The results indicate that the use of UV-C does not result in any detrimental impact to the chemical composition of milk at medium and low doses. However, it is not advisable to use very high doses of UV-C.

## Sensory analysis

The sensory properties of raw milk samples that were treated and untreated with UV-C at different doses are given in Table 8.

Table 8. Sensory properties of raw milk samples treated with and without UV-C at different temperatures and flow rates.

Uygulama	Dose (J mL <sup>-1</sup> )	Color	Smell	Taste
PC <sub>(Past. milk)</sub>	0	$4.7 \pm 0.16^{a}$	$4.5 \pm 0.58^{a}$	$4.8 \pm 0.52^{a}$
$UV11a_{(18 \text{ mL dk}^{-1} 25^{\circ}\text{C})}$		$4.4 \pm 0.46^a$	$2.8 \pm 0.81^{b}$	$2.5 \pm 0.57^{b}$
$UV11b_{(18 \text{ mL dk}^{-1} 25^{\circ}\text{C})}$	(0	$4.4 \pm 0.43^{a}$	$2.9 \pm 0.74^{b}$	$2.5 \pm 0.57^{b}$
$UV10a_{(18 \text{ mL dk}^{-1} 11^{\circ}\text{C})}$	60	$4.5\pm0.58^a$	$2.8 \pm 0.63^{b}$	$2.5 \pm 0.41^{b}$
$UV10b_{(18\ mL\ dk^{-1}\ 11^{o}C)}$		$4.5 \pm 0.58^{a}$	$3 \pm 0.56^{b}$	$2.7 \pm 0.30^{b}$
$UV9_{(15 \text{ mL dk}^{-1} 17^{\circ}C)}$		$4.3 \pm 0.31^{a}$	$2.5 \pm 0.57^{\rm b}$	$2.3 \pm 0.35^{b}$
$UV8a_{(15 \text{ mL dk}^{-1} 4^{\circ}C)}$	72	$4.3\pm0.32^a$	$2.5 \pm 0.57^{\rm b}$	$2.2 \pm 0.34^{\rm b}$
$UV8b_{(15\ mL\ dk^{-1}\ 4^{o}C)}$		$4.3. \pm 0.23^{a}$	$2.5 \pm 0.54^{b}$	$2.2 \pm 0.33^{b}$
$UV7_{(12 \text{ mL dk}^{-1} 25^{\circ}\text{C})}$	90	$4.3 \pm 0.17^{a}$	$2.3 \pm 0.12^{b}$	$2 \pm 0.16^{b}$
$UV6_{(11 \text{ mL dk}^{-1} 16^{\circ}\text{C})}$	98,4	$4\pm0.09^a$	$2.3 \pm 0.34^{b}$	$2.3 \pm 0.28^{b}$
$UV5_{(10 \text{ mL dk}^{-1} 8^{\circ}\text{C})}$	107,8	$4.2\pm0.23^a$	$2.3 \pm 0.25^{b}$	$2 \pm 0.15^{b}$
$UV4_{(8 \text{ mL dk}^{-1} 21^{\circ}\text{C})}$	135,4	$4.2\pm0.31^a$	$2.2 \pm 0.23^{b}$	$2 \pm 0.26^{b}$
$UV3a_{(5 \text{ mL dk}^{-1} 25^{\circ}C)}$		$3.9 \pm 0.041^{a}$	$2 \pm 0.15^{b}$	$1.6 \pm 0.31^{b}$
$UV3b_{(5\ mL\ dk^{-1}\ 25^{o}C)}$		$4\pm0.23^a$	$1.9 \pm 0.12^{\rm b}$	$1.6 \pm 0.25^{b}$
$UV2_{(5 \text{ mL dk}^{-1} 15^{\circ}\text{C})}$	216,9	$4\pm0.27^a$	$2 \pm 0.13^{b}$	$1.6 \pm 0.28^{b}$
$UV1a_{(5 \text{ mL dk}^{-1} 4^{\circ}C)}$		$4 \pm 0.11^{a}$	$2 \pm 0.18^{b}$	$1.5 \pm 0.19^{b}$
$UV1b_{(5 \text{ mL dk}^{-1} 4^{\circ}C)}$		$4 \pm 0.23^a$	$2 \pm 0.19^{b}$	$1.5 \pm 0.21^{b}$

<sup>\*</sup> Different letters in the same column indicate statistical difference (p < 0.05). ± : Standard deviation.

As seen in Table 8, the most admired sample was pasteurized milk in terms of color, odor, flavor and general taste, while the score given by the panelists on these criteria decreased as the applied UV-C dose increased. Although scores at low doses were close to control samples, samples with low doses also scored low in taste and odor. When the color evaluations of raw milk were examined, it was estimated that the scores were between 4.7-3.9. When the odor evaluations of milk samples were examined, it was estimated that the scores ranged from 4.5 to 1.9. According to the multiple comparison test results, the difference between the control group and the milk treated with UV-C was found to be significant (p < 0.05), while the difference between the groups treated with different doses of UV-C was found to be statistically insignificant (p > 0.05). These results showed that there was a change in the odor of milk with UV-C application. Bandla et al., (2012) applied 16,822 mJ cm<sup>-2</sup> dose of UV-C to raw milk and examined the change in sensory properties in the study they conducted. Because of the sensory test, it was reported that there was no change in the odor of the samples immediately after the application, but the odor of the samples changed from the first day of storage. These results partially show similarity to our results. When the taste scores of milk samples were examined, it was seen that they varied between 1.5 and 4.8. According to Duncan multiple comparison test results, the difference between the control group and the milk treated with UV-C was found to be significant (p < 0.05), while the difference between the groups treated with different doses of UV-C was found to be statistically insignificant (p > 0.05). When the general admiration scores of milk samples were examined, it was estimated that the scores vary between 1.8 and 4.8. The difference between the control group and the milk samples treated with 60 J mL<sup>-1</sup> dose of UV-C was found to be statistically insignificant (p < 0.05). For this reason, it was thought that the samples that were accepted in sensory meaning were the groups treated with 60 J mL<sup>-1</sup> dose UV-C. It has been determined that the factor affecting the general admiration was the flow rate. As the flow rate decreased, which means the UV-C radiation dose that the milk was exposed increased, and the general admiration scores decreased depending on the change in the aroma of the milk. The highest general admiration score was found as 4.2 in the 60 J mL<sup>-1</sup> dose of UV-C application, and the lowest general appreciation score as 1.8 in the application of 216.9 J mL<sup>-1</sup> dose of UV-C. Orlowska et al. (2013) examined the effect of UV-C application on milk quality parameters with different emission spectra, energy per pulse, and frequency (HIP-1: 31

J/pulse, 8 Hz; HIP-2: 344 J/pulse 0.75 Hz; HIP-3: 644 J/pulse, 0.5 Hz) and found a slight burning odor in HIP-1-treated samples. This study found comparable outcomes to ours.

## Conclusion

UV-C application did not significantly affected the pH value of milk while it had significant impacts on other physicochemical properties. Exposure to high amounts of UV-C radiation resulted in a notable rise in levels of free fatty acids (FFA), vitamin D3, oxidative volatile compounds, and overall color change values. Additionally, there was a drop in cholesterol content. It is not advisable to use high UV-C doses for preserving raw milk since it leads to a decline in sensory scores and a rise in free fatty acids, total color change, and oxidative volatile components as the UV-C doses increase.

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## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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