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Effect of UV-C Radiation and Thermal Treatment on Volatile Compounds, Physicochemical, Microbiological and Phytochemical Parameters on Apple Juice (*Malus domestica*) with Raspberry (*Rubus idaleus* L.)

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Abstract: Volatile compounds contribute to aroma and flavor, these being the main sensory attributes in food acceptance. This work addresses the physicochemical, volatile compounds, polyphenols, and flavonoids content and, antioxidant activity of apple-raspberry (90/10%) juice treated by thermal and ultraviolet radiation (UV-C) alone or in combination with moderate heat-treatment. Nineteen volatile compounds were identified which experienced changes depending on the treatment. Compounds such as α-ionone and β-ionone, that contribute to raspberries characteristic aroma, were present in a greater concentration in the UV-C treatment and lower in the thermal treatment. Likewise, 2-methyl butyl acetate, which give a fruity-sweet aroma typical of apples was present in a greater concentration in the UV-C treatment. Regarding polyphenol content, control and combined treatment presented the greater concentrations. However, after twenty days of storage, control and combined treatment presented the lower flavonoid concentration. Nevertheless, at this time, treatments showed no variations in antioxidant activity. Yeast and mold and total aerobic mesophilic and psychrophilic counts were reduced in the heat and combined treatments. In conclusion, UV-C and moderate heat might successfully be used to process a stable apple-raspberry juice while maintaining its quality and safety.

Keywords: irradiation; juice; volatile compounds; phenolic

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1. Introduction

Currently, the demand for "super foods" is increasing due to their benefits in preventing diseases and the benefits these provide on consumers health [1]. This trend encourages the development and innovation of processes used in the transformation of food with the aim of maintaining or improving its nutrition al content. Many fruits and vegetables are recognized as a "super foods". Hence, fruit juices play a crucial role in a healthy diet, as they are a source of different bioactive compounds [2].

Apple juice is widely consumed due to its sensory and nutritional characteristics [3]. In addition, it is recognized for being an excellent source of antioxidant and phenolic compounds, such as chlorogenic acid, epicatechin, phloridzin, among others [4,5]. Besides, raspberry juice is highly appreciated due to its pleasant sensory attributes and its high nutritional value [6,7]. Raspberries have a wide variety of phytochemicals such as polyphenols,

flavonoids, anthocyanins, phenolic acids that help prevent chronic diseases [8–11]. Phenolic compounds present various health-promoting bioactivities such as: antioxidant, anticancer, antimutagenic, antimicrobial, anti-inflammatory and neuroprotective capacity [9,12–16]. Likewise, they favor the flavor and color attributes of fruits and vegetables [17]. However, these compounds are rapidly degraded, oxidized, or polymerized during fruit processing and storage. Therefore, total phenolic content is an indicator of fruit juice quality [18].

The combination of fruits in the production of juices results in a juice with a better nutraceutical and organoleptic profile that could be of interest to the consumer, due to its health benefits and sensory attributes [19–21].

However, it is also a challenge to maintain the nutritional quality of juices because the process traditionally used to obtain them and extend their shelf life have a negative impact on their nutritional and organoleptic properties [22]. Polyphenols contained in both, apples, and raspberries, can be degraded by thermal processing [23]. Additionally, the aroma and flavor compounds can be severely affected [24].

Likewise, the impact of processing and storage on juices volatile profile is of great importance. Flavor changes of fruit juices include evaporation of volatile compounds during thermal processing and bottling [25] or their lost due to Maillard reactions carried out during storage [26]. Given that, food preferences, acceptability, and consumption are directly influenced by flavor quality, so it is critical to comprehend the quality factors related to juice processing [27].

Due to the above, non-thermal technologies represent an option to obtain safe foods with a minimum of changes in their nutritional and sensory properties [28]. In this sense, ultraviolet radiation has multiple advantages relative to another non-thermal techniques such as not using chemicals or heat, there are no changes in color, taste, and smell, minimal operational costs and low energy usage are further benefits [29,30]. Another non-thermal technology to preserve food is the use of short-wave ultraviolet radiation (UV-C), either by itself or in conjunction with mild heat treatment [31–33].

The UV-C treatment of juices has been studied for its effectiveness in inactivating microorganisms and its impact on the juice quality. Research has shown that UV-C can be effectively used to reduce the number of spoilage and pathogenic bacteria, as well as yeasts and molds in different kinds of fruit juices [34]. Specifically, the effect of UV-C irradiation on the inactivation of spoilage microorganisms of orange juice has been investigated, with results indicating a reduction in microbial counts [35]. Additionally, the impact of UV-C treatment on the concentration of vitamin C in orange juice has been studied, showing a degradation of about 17% [36]. Furthermore, UV-C treatment did not significantly affect the pH values, titratable acidity, ascorbic acid content, and pectin methylesterase activity in both fresh and stored orange juice samples [37]. Overall, the research suggests that UV-C treatment can be a promising non-thermal method for reducing microorganisms in juices while preserving their quality.

A study applying UV-C alone and in combination with ultra-high-pressure homogenization (UHPH) in cloudy apple juice reported that UV-C technology did not inactive enzymes such as methylesterase and polyphenol oxidase. But, when UV-C was employed in combination with UHPH at 300 MPa, juice antioxidant activity and polyphenol content were incremented [38]. Thus, the use of UV-C in juice processing represents a key factor in their conservation since it has proven to be efficient in reducing fungi, spores, pathogens and other microorganisms, something that for example UHPH has not demonstrated effectiveness [38,39]. Additionally, UV-C irradiation applied to cranberry-flavored water showed no cytotoxic effects on healthy mice liver cells while *Escherichia coli* ATCC 25922 and *Salmonella enterica* serovar Typhimurium ATCC 13311 were inactivated by 5 $\log_{10}$ and, the concentration of anthocyanins did not change significantly [40].

Moreover, UV-Vis irradiation when applied to clarified nectarine juices at 25 °C 100 inactivate enzymes including polyphenol oxidase and peroxidase up to 60% and, when applied at 45 °C the enzymes were totally inactivated, although the irradiation process causes color changes, ascorbic acid and total sugar losses [41].

Otherwise, when UV-A (320 to 400 nm) and UV-B (280 to 320 nm) irradiation, alone or in combination, were applied to apple juice it was observed that the combined treatment was effective in inactivating *E. coli* O157:H7, *Salmonella Typhimurium*, and *L. monocytogenes* [42].

However, UV-C radiation efficacy when apply in juices and juice mixtures is affected by radiation penetration (the presence of particles can protect microorganisms from radiation). Therefore, factors such as UV-C dose, fluid, pH and the optical characteristics of liquids (color, turbidity, absorption coefficient) must be considered when applying radiation to achieve adequate elimination of microorganisms [43].

Meanwhile, the combination of UV-C light and mild heat has been shown to have a positive effect on the microbial quality and shelf-life of various juices. UV-C light and mild thermal treatments can reduce log cycles of mesophiles and molds/yeasts by 0.19 ± 0.03 and 0.25 ± 0.02, respectively, with an additive effect when combined [44]. In addition, UV-C light treatment, along with mild heat, can significantly decrease the surviving *E. coli* population in clear and turbid fruit juices after storage [45]. And, in a study on carrot juice, UV-H treatment (3.92 J/mL, 3.6 min, 60 °C) was found to better preserve the microbiological quality compared to longer (18.1 min) conventional thermal treatment [46]. In another study, the combined effect of UV-C radiation (39.6 J/L) and mild heat (65.0 ± 3.0 °C) resulted in the inactivation of aerobic mesophilic and yeast/mold microorganisms in grapefruit juice [31]. However, it is essential to note that the antioxidant capacity and ascorbic acid content in some juices, such as grapefruit juice, can be considerably reduced after treatment with UV-C light and mild heat [31]. Despite this, the treated juices did not show microbial growth during storage, indicating that the combined treatment can effectively ensure microbial quality [31]. Overall, the combination of UV-C light and mild heat shows promise in enhancing the safety and shelf life of juices, although the impact on specific quality parameters may vary depending on the type of juice and the treatment conditions.

Besides, the combination of UV-C and mild heat can affect volatile compounds in juices. Research has shown that UV-C treatment, combined with mild heat, can preserve the total phenol content in pineapple juice and reduce microbial load in fruit juices, including carrot, orange, and apple juices [34,47–49].

Additionally, UV-C treatment has been found to have a significant impact on the flavor and aroma of juices, with some studies indicating that non-thermal-treated juices were preferred over thermally treated ones [50].

However, the specific effects on volatile compounds may vary depending on the type of juice and the treatment conditions. Further research is needed to fully understand the impact of the combination of UV-C and mild heat on the volatile compounds in different types of juices since most of works conducted have focused on the safety or microbial quality of juices. Therefore, UV-C radiation, alone or in combination with moderate heat, could represent an alternative for processing apple and raspberry juice. Consequently, the objective of this study was to evaluate the impact of these processing technologies on the profile of volatile compounds, physicochemical and microbiological characteristics as well as the content of flavonoids and polyphenols of apple and raspberry juice.

2. Materials and Methods

2.1. Materials

Raspberries (cv. Heritage) were harvested during 2018, in Cuauhtémoc, Chihuahua, Mexico, in an experimental orchard situated at 28°24′45.1″ N, 106°52′54.9″ W and at 2060 altitude. After harvested raspberries were washed with tap water and frozen at −80 °C until processing (a month, which is how long the harvest period lasts). Raspberries were thawed and manually squeezed to extract the juice, which was then centrifuged for five minutes at 3500 rpm (IEC Thermos model CL3-R, Langenselbold, Germany). Then it was maintained at −80 °C until use (7 days). The apple juice (cv. Glory/Smothee) was donated by an apple producer company located in Cuauhtémoc, Chihuahua, Mexico. Apple juice was frozen at −5 °C until used (7 days).

2.2. Apple-Raspberry Juice Elaboration

First, the apple and raspberry juices were thawed in the refrigerator for 5 days. Once the apple juice was thawed, it was clarified by adding 22.5 µ/L of pectinase (Zympect) and letting it rest for 24 h, then it was decanted. The raspberry juice, once thawed, was centrifuged, and decanted. The apple (37.8 L)—raspberry (4.2 L) juice was prepared by mixing the juices in a proportion of 90% and 10% respectively.

2.3. Treatments and Measurements

All treatments were carried out in triplicate under a completely randomized design. And every measurement was conducted three times, for each repetition. Then results are expressed as mean ± standard error based on replicated trials (3) and replicated measurements (3).

2.3.1. Ultraviolet Radiation Treatment

The apple-raspberry juice was processed at 37.85 L per hour, with an exposure time of 9.94 s (UV dose of 9.68 mJ/cm^2 at 20 °C) in a commercial CiderSure 3500 flow UV unit. continuous (FPE Inc., Macedonia, NY, USA). The unit has eight mercury germicidal lamps and was operated at 254 nm. Irradiance was monitored every 50 m/s using two UVX-25 sensors (UVP, Inc., Upland, CA, USA). The unit was disinfected with a NaClO solution (200 ppm) and wash out with water. This procedure was conducted before and after each treatment. The average of each sensor's irradiance was considered to get the actual irradiance. The flow rate of each treatment was used to calculate the exposure periods. The following formula was used to get the UV dose:

$$\text{UV dose (mJ/cm}^2\text{)} = \text{irradiance} * \text{exposure time}$$

where, irradiance (µW/cm^{-2}), exposure time (s) [32].

2.3.2. Thermal Treatment

Using a continuous tubular pasteurizer (UHT/HTST unit, Micro Thermics, Raleigh, NC, USA), heat treatment was applied to the apple-raspberry juice, which was heated to 85 °C for six seconds.

2.3.3. Combined Treatment (Ultraviolet Radiation and Moderate Heat Treatment)

The combined treatment was carried out by subjecting the apple-raspberry juice to a UV dose of 9.68 mJ/cm^2, with an exposure time of 9.94 sec at 20 °C in a commercial CiderSure 3500 continuous flow UV unit (FPE Inc., Macedonia, NY, USA). Subsequently, the juice was subjected to moderate heat treatment at a temperature of 55 °C for 30 s in a continuous tubular pasteurizer (UHT/HTST unit, Micro Thermics, Raleigh, NC, USA).

2.4. Storage

All treatments were stored refrigerated at 4 °C and analyzed at 0, 5, 10, 15 and 20 days.

2.5. Physicochemical Analysis and Optical Properties

pH was measured according to AOAC method 981.12 [51], with a digital potentiometer (Hanna Instruments, model EDGE HI2020, Rhode Island, USA) An UV-Vis spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA) was employed to obtain the optical propierties of apple-raspberry juice (90–10%) [52]. The Lambert-Beer law equation was used to obtain the absorption coefficient (α) at 254 nm. Sample readings were taken using removable fused quartz cuvettes (FireflySci, Inc., New York, NY, USA) with path lengths of 0.1, 0.2, 0.5, and 1.0 mm. Using the slope of the linear equation, the path lengths of the cuvettes, the reciprocal of the absorption coefficient (1/α) and the penetration depth (λ), the absorption coefficient (cm^{-1}, logarithmic base 10) was calculated. A micro-turbidimeter 100 instrument (Micro 100 Hf, Scientific, Inc., Fort Myers, FL, USA) was utilized to measure

turbidity, and the resulting nephelometric turbidity unit (NTU) was used to express the results. A refractometer (Abbe, American Optical Corporation, New York, NY, USA) was used to measure soluble solids.

Color was measured with a Konica Minolta CR-400/410 colorimeter (Minolta Co., Osaka, Japan) previously calibrated and with the following parameters: Diffuse illumination/0° viewing angle, 1 s of measurement time and 3 s of minimum measure interval; 2 degrees closely matches CIE 1931 standard observers and C, D65 illuminant condition. Values of L* (luminosity), a* (green–red) and b* (blue–yellow) were obtained. Likewise, the a* and b* values were used to calculate the color differential during storage using the subsequent equation:

$$\Delta E = [(L^* - Lo)^2 + (a^* - ao)^2 + (b^* - bo)^2]^{\frac{1}{2}}$$

where, Lo, ao and bo were the raw juice color values (control sample). Reported results are the mean values of six determinations (n = 6) ± standard deviation.

2.6. Analysis of Volatile Compounds

Volatile compounds were evaluated using solid phase microextraction (SPME) in combination with mass spectrometry and gas chromatography [53].

2.7. Polyphenols, Flavonoids, and Antioxidant Activity Analysis

Polyphenols content was expressed as milligrams of gallic acid equivalents per gram of dry matter (mg eq GA/g dm) and determined by the Folin-Ciocalteu method [54]. The flavonoid content was expressed as equivalent milligrams of catechin per gram of dry matter (mg eq CAT/g dm) and determined according to Julián-Loaeza et al. [55]. The 2,2 diphenyl-1-picrylhydrazyl (DPPH) methodology was employed to evaluate antioxidant activity which was expressed in equivalent micromoles of Trolox per gram of dry matter (μmol eq Trolox/g dm) [56].

2.8. Microbiological Analysis

Before and after the applied treatments, samples were taken aseptically and analyzed by the serial pour-dilution methodology (1 mL) same day [57]. Aerobic mesophilic and psychrophilic count were done in plate count agar (PCA), coliforms in violet red bile agar (VRBA) and fungi and yeast in potato dextrose agar (PDA). Mesophiles and coliforms were incubated at 37 °C for 24 h, psychrophiles and at 4 °C for 11 days. And fungi and yeasts at 30 °C for 5 days.

2.9. Statistic Analysis

Treatments were developed under a completely randomized design. Data was analyzed using SAS software 9 (SAS Institute Inc, Cary, NC, USA) through an analysis of variance (ANOVA) and a Tukey's Tests was conducted when treatments showed differences. Each treatment was carried out on triplicate batches (n = 3).

3. Results y Discussion

3.1. Physicochemical Analysis and Optical Properties

The apple-raspberry juice (90–10%) had a pH of 3.57 ± 0.05 and a °Brix concentration of 11.08 ± 0.024. The absorption coefficient was 19.07 cm^{-1}, and the penetration depth was 0.053 cm, with a turbidity of 39.93 ± 0.38 NTU. Regarding color characteristics, parameters L*, a* and b* were 33.50 ± 0.20, 7.15 ± 0.02 and 5.06 ± 0.76, respectively.

3.2. Physicochemical Parameters

pH is one of the most important parameters that describe the stability of bioactive compounds in fruit juices [58]. The effect of processing on pH is shown in Figure 1. A general decrease on pH was observed ($p \leq 0.05$) from day 1 to 20 in all the treatments.

The decrease in the pH value in the control treatment was more pronounced ($p \leq 0.05$) compared to the rest of the treatments. However, on day 20, control and ultraviolet treatments showed no differences ($p > 0.05$). Meanwhile, the thermal treatment did not present changes ($p > 0.05$) in pH's values during storage. The decline in pH may be attributed to the increase in acidity derived from the metabolic activity of microorganisms during storage [59,60].

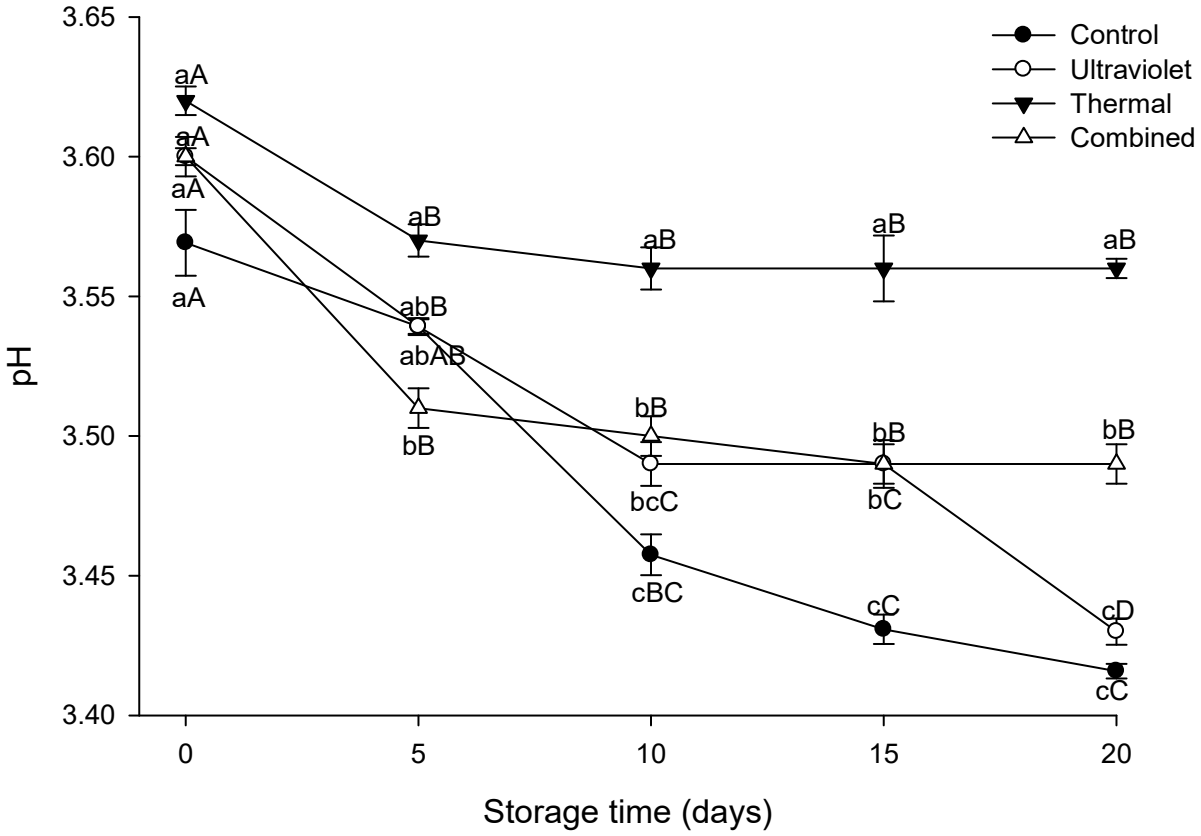

Figure 1. Effect of different treatments on pH. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, D different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

The behavior of the soluble solids is shown in Figure 2. The ultraviolet and combined treatments (ultraviolet/thermal) maintained the soluble solids without significant changes ($p > 0.05$) until day 15 of storage. On the other hand, the control and thermal treatments showed a reduction in this value ($p \leq 0.05$) from day 5 of storage. The control treatment showed the greatest decrease in soluble solids ($p \leq 0.05$). The decrease in total soluble solids during storage can be attributed to the microbial activity that causes sugar fermentation [61,62]. As mentioned previously, the control treatment was the one that presented the greatest decrease in soluble solids and was also the one that presented the highest count of aerobic mesophiles and psychrophiles as well as fungi and yeasts ($p \leq 0.05$).

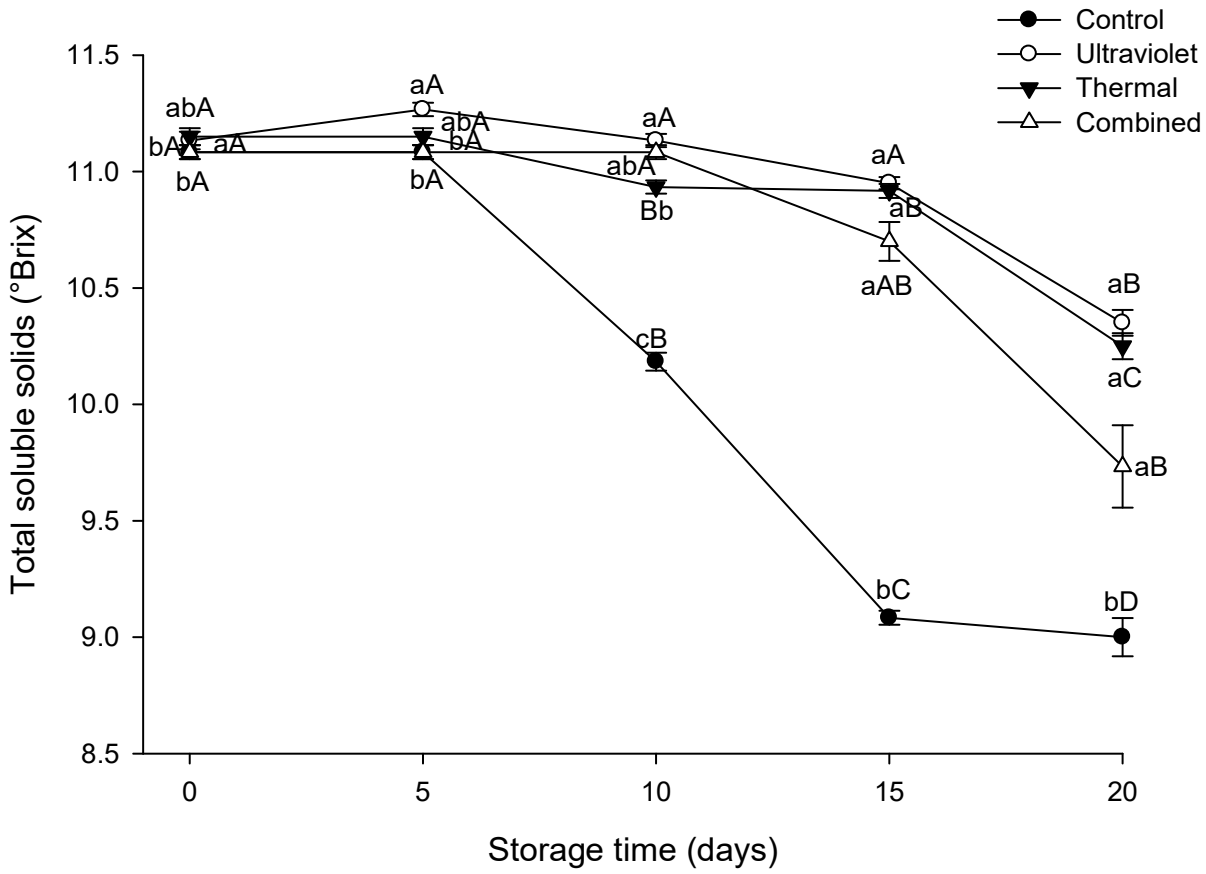

Figure 2. Effect of different treatments on soluble solids. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, D different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

In drinks and foods, color is among the most important attributes, since it is the first thing that consumers can observe and influences their choice [63]. Table 1 presents color results. In relation to L*, ultraviolet, thermal, and combined treatments were no different ($p > 0.05$) over time or among them. Further, control treatment presented a higher value of L* ($p \leq 0.05$) on days 0 and 20 compared to the rest of the treatments. Higher luminosity values refer to a brighter and more transparent juice, which may be due to the degradation of anthocyanins (main color compounds in raspberries) caused by storage time [64]. Instead, lower luminosity values can be attributed to the "swelling" a release of water from the cellulose matrix that happens after thermal treatment, and which also increases the viscosity of the juice. Likewise, high temperatures break cellular structures allowing pectin to escape, which contributes to increase the content of colloidal pectin in the juice and therefore a decrease in its clarity [65,66]. In relation to photodegradation reactions, these endorse the Maillard reaction between amino acids and reducing sugars, which cause darkening [67].

Table 1. Effect of different treatments on physicochemical properties of a blend of apple (*Malus domestica*) and raspberry (*Rubus idaleus* L.) juice during storage.

Parameters	Treatment	Storage Time (Days)				
		0	5	10	15	20
L*	Control	33.27 ± 0.05 aA	33.07 ± 0.22 aA	33.26 ± 0.46 aA	33.41 ± 0.36 aAB	34.46 ± 0.16 aB
	Ultraviolet	32.44 ± 0.17 bA	32.29 ± 0.19 aA	32.34 ± 0.05 aA	32.51 ± 0.17 aA	33.43 ± 1.01 aA
	Thermal	32.30 ± 0.12 bA	32.32 ± 0.37 aA	32.72 ± 0.28 aA	32.91 ± 0.11 aA	32.96 ± 0.16 aA
	Combined	32.24 ± 0.10 bA	32.44 ± 0.19 aA	32.29 ± 0.33 aA	32.72 ± 0.51 aA	32.98 ± 0.06 aA
a*	Control	7.62 ± 0.01 aA	7.40 ± 0.07 aA	7.57 ± 0.02 aA	6.75 ± 0.74 aA	6.43 ± 0.27 aA
	Ultraviolet	7.58 ± 0.16 aA	7.60 ± 0.05 aA	7.55 ± 0.14 aA	6.85 ± 0.27 aA	6.54 ± 0.42 aA
	Thermal	6.44 ± 0.06 bA	6.39 ± 0.10 bA	6.24 ± 0.19 bA	6.22 ± 0.39 aA	6.28 ± 0.02 aA
	Combined	6.78 ± 0.03 bA	6.58 ± 0.10 bA	6.51 ± 0.21 bA	6.51 ± 0.02 aA	6.53 ± 0.55 aA
b*	Control	4.22 ± 0.00 aA	4.43 ± 0.09 aB	5.54 ± 0.02 aC	5.55 ± 0.01 aC	5.57 ± 0.01 aC
	Ultraviolet	4.17 ± 0.19 aA	4.51 ± 0.05 aA	4.66 ± 0.08 bA	4.75 ± 0.56 bA	4.81 ± 0.18 bA
	Thermal	4.07 ± 0.01 aA	4.37 ± 0.16 aA	4.72 ± 0.04 bB	4.87 ± 0.07 bB	5.43 ± 0.01 aC
	Combined	4.12 ± 0.08 aA	4.69 ± 0.09 aB	4.74 ± 0.06 bB	5.19 ± 0.04 bC	5.25 ± 0.01 aC
ΔE	Control	-	-	-	-	-
	Ultraviolet	0.85 ± 0.14 aA	1.03 ± 0.20 abA	1.04 ± 0.01 aA	1.27 ± 0.30 aA	1.43 ± 0.51 aA
	Thermal	1.54 ± 0.03 bA	1.57 ± 0.32 bA	1.59 ± 0.08 aA	1.59 ± 0.35 aA	1.84 ± 0.03 aA
	Combined	1.33 ± 0.09 bA	1.41 ± 0.16 bA	1.58 ± 0.37 aA	1.61 ± 0.21 aA	1.62 ± 0.39 aA

Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). L* = luminosidad, a* = green (−) and red (+), b* = blue (−) and yellow (+). Values are expressed as mean ± standard error (n = 3). a, b different lowercase letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

In relation to the value of a*, the control and ultraviolet treatments presented higher values ($p \leq 0.05$) until day 10 of storage compared to the thermal and combined treatments. Subsequently, no significant differences ($p \geq 0.05$) were observed between treatments ($p \geq 0.05$). Regarding the value of b*, differences were found ($p \leq 0.05$) between the treatments until day 10 of storage where the control treatment showed a higher value ($p \leq 0.05$) compared to the rest of the treatments and continuing with this trend at day 15. However, at day 20 the control, thermal and combined treatments presented the highest values ($p \leq 0.05$) compared to the ultraviolet treatment. It is worth mentioning that, throughout the storage, the ultraviolet treatment was the one that maintained this parameter without change ($p > 0.05$). On the contrary, researchers observed a significant decrease ($p \leq 0.05$) in reddish-greenish (a*) and yellowish-bluish (b*) in apple juice exposed to ultraviolet light, they attribute the color changes of apple juice to the destruction of melanins and melanoidin, caused by exposure to ultraviolet light [67].

ΔE values below 2.7 indicate that color change is not perceptible to the human eye (none 0–0.7, slightly 0.7–2.5, remarkable 2.5–3.0, appreciable 3.0–6.0, considerable 6.0–12.0, and biggest 12.0) [68]. According to the values obtained for ΔE (Table 1), none of the treatments presented values above 2.7, which denotes that the color changes that occurred in the treatments will not be perceptible to the human eye.

3.3. Analysis of Volatile Compounds

Nineteen volatile compounds were identified (Table 2), which were grouped according to their chemical nature into aldehydes, alcohols, ionones, esters, terpenes, C13-norisoprenoids and others. In the control treatment, 14 compounds were identified, the five most abundant being hexanal; 1-hexanol; (1S,2S)-2-methylcyclopentan-1-ol; 2-methyl butyl acetate and β-ionone. In the thermal treatment, 17 compounds were identified where the five most abundant were hexanal; 1-hexanol; (3-hydroxy-2,4,4-trimethylpentyl)2-methylpropanoate; [2,2,4 trimethyl-3-(2-methylpropanoyloxy)pentyl] 2-methylpropanoate and 2-methyl butyl acetate. In the ultraviolet treatment, 14 volatile compounds were

identified, the most abundant being 2-methyl butyl acetate; 1-hexanol; hexanal; β-ionone and α-ionone. For its part, in the combined treatment, 15 volatile compounds were identified, the most abundant being (3-hydroxy-2,4,4-trimethylpentyl) 2-methylpropanoate; hexanal; [2,2,4Trimethyl-3-(2-methylpropanoyloxy)pentyl]2-methylpropanoate; α-ionone and 1-hexanol. It can be observed that the profile of the volatile compounds and their abundance experienced a change depending on the treatments applied, therefore, and because the odor threshold of olfactory perception of each volatile compound is different [69], it is deduced that the treatments presented a change in the overall flavor. Among the identified compounds, some that have been considered to have an impact on the aroma of raspberry, such as α-ionone, which has a floral and fruity odor; and, β-ionone, which is described as an aromatic and floral were present in a greater concentration in UV treatment and, a lower concentration in thermal treatment, although the differences were not significantly statistical [70]. Meanwhile, 2-methyl butyl acetate which give a fruity-sweet aroma in apples was present in a greater concentration in UV treatment ($p \leq 0.05$) [71]. Also, compounds such as L-α-Terpineol that only appear in samples that underwent heat treatment, were detected in thermal and combined treatments. This compound has been described as rancid or musty aroma and contributes significantly to the loss of quality of citrus juices [26].

Table 2. Effect of different treatments on volatile compounds of a blend of apple (*Malus domestica*) and raspberry (*Rubus idaleus* L.) juice during storage.

Aroma Compounds	Retention Time (min)	Diagnostic Ions (m/z)	Control	Thermal	Ultraviolet	Combined
Hexanal	18.17	56 (999) 44 (889) 41 (810) 57 (668) 43 (569)	17.16 ± 4.89 a	23.91 ± 4.47 a	8.59 ± 0.48 a	12.97 ± 3.93 a
2-Methylbutyl acetate	18.69	43 (999) 70 (289) 55 (143) 41 (128) 29 (117)	6.58 ± 2.65 a	7.37 ± 0.99 a	16.25 ± 1.17 b	2.64 ± 0.73 a
Butan-2-yl nitrite	23.70	43 (999) 57 (307) 41 (200) 45 (155) 44 (107)	3.85 ± 1.16 a	1.38 ± 0.68 a	n.d.	1.60 ± 0.62 a
Hexyl acetate	25.49	43 (999) 56 (308) 55 (180) 61 (178) 42 (161)	4.21 ± 0.93 a	n.d.	n.d.	1.35 ± 0.53 b
1-Hexanol	28.50	56 (999) 43 (831) 41 (590) 55 (569) 42 (534)	8.26 ± 3.69 a	11.44 ± 0.24 a	10.29 ± 2.22 a	5.86 ± 1.74 a
Nonanal	29.85	41 (999) 57 (997) 43 (695) 29 (692) 56 (607)	n.d.	1.57 ± 0.28 a	4.28 ± 1.62 a	1.47 ± 0.02 a
(1R, 2S) 2-Methylcyclopentanol	29.97	57 (999) 41 (490) 44 (380) 82 (330) 43 (320)	7.52 ± 3.93 a	3.88 ± 2.05 a	4.53 ± 1.30 a	n.d.
2-Methylhept-6-en-1-ol	31.72	95 (999) 41 (566) 69 (421) 45 (362) 55 (268)	2.50 ± 2.15 a	1.10 ± 0.52 a	n.d.	n.d.
2-Ethylhexan-1-ol	32.47	57 (999) 41 (454) 55 (366) 43 (287) 56 (271)	n.d.	2.40 ± 1.29 a	3.97 ± 0.34 a	n.d.
Decanal	32.81	43 (999) 41 (807) 57 (621) 55 (618) 44 (539)	n.d.	2.43 ± 0.06 a	n.d.	1.47 ± 0.35 b
Linalool	33.93	71 (999) 93 (610) 41 (571) 43 (486) 69 (486)	2.29 ± 1.24 a	1.43 ± 0.02 a	3.58 ± 0.71 a	1.38 ± 0.22 a
L-α-Terpineol	37.82	59 (999) 93 (580) 121 (470) 136 (430) 81 (370)	n.d.	0.43 ± 0.43 a	n.d.	1.55 ± 0.55 a
Geranyl vinyl ether	41.03	69 (999) 41 (667) 68 (265) 43 (209) 67 (147)	n.d.	1.37 ± 0.09 a	4.51 ± 0.79 b	n.d.
α-Ionone	41.46	121 (999) 93 (770) 43 (760) 136 (620) 77 (333)	4.63 ± 2.13 a	3.01 ± 0.42 a	6.83 ± 3.04 a	5.99 ± 0.89 a
(3-Hydroxy-2,4,4-trimethylpentyl) 2-methylpropanoate	41.63	71 (999) 56 (793) 89 (722) 43 (600) 41 (221)	4.53 ± 2.23 a	8.85 ± 0.24 ab	4.97 ± 0.06 a	13.69 ± 2.68 a

Table 2. *Cont.*

Aroma Compounds	Retention Time (min)	Diagnostic Ions (m/z)	Control	Thermal	Ultraviolet	Combined
2.2.4-Trimethyl-1,3-pentanediol diisoutyrate	42.10	71 (999) 43 (667) 56 (199) 41 (168) 83 (131)	4.46 ± 0.63 ab	7.79 ± 0.92 ac	3.49 ± 0.25 b	9.08 ± 1.22 c
α-Ionol	42.25	95 (999) 43 (656) 138 (400) 41 (158) 96 (135)	3.57 ± 0.71 a	2.81 ± 0.16 a	3.76 ± 0.40 a	5.21 ± 0.96 a
β-Ionone	43.35	177 (999) 43 (408) 91 (173) 135 (153) 178 (134)	5.89 ± 3.63 a	3.67 ± 0.27 a	5.23 ± 2.34 a	3.54 ± 1.77 a
1-Dodecanol	43.58	55 (999) 43 (901) 69 (858) 41 (854) 56 (770)	0.72 ± 1.02 a	1.24 ± 1.24 a	3.88 ± 1.19 a	5.16 ± 0.44 a

Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. n.d. not detected.

3.4. Content of total Polyphenols (CTP), Total Flavonoids (TF), Total Antioxidant Activity (TAA)

The effect of the treatments on the CTP is shown in Figure 3, where it is observed that the control and combined treatments, on day 0, were those that presented the highest concentrations ($p \leq 0.05$) of polyphenols, followed by the ultraviolet treatment. The heat treatment presented the lowest polyphenol content. The combined treatment had no significant changes ($p > 0.05$) until day 15 of storage. For its part, the control treatment showed a decrease in the polyphenol content from day 10. The ultraviolet and thermal treatments decreased the concentration of polyphenols from day 5, with the decrease in ultraviolet being greater. The generation of free radicals or radiation-induced degradation products is thought to be the source of the decline in antioxidants. For instance, radiation treatment of strawberries results in the breakdown of phenolic and acidic chemicals such as hydroxybenzoic acid, *p*-coumaric acid, gallic acid, and cinnamic acid [72].

Regarding the effect of the treatments on the TF (Figure 4). The combined and thermal treatments, on day 0, showed the lowest flavonoid content ($p \leq 0.05$). Therefore, the application of heat had a negative effect on these compounds. On the other hand, the application of ultraviolet radiation had no effect on the TF. This could be attributed to the fact that ultraviolet rays increase the activity of enzymes responsible for flavonoid biosynthesis [72]. However, there was a decrease in content during storage.

The antioxidant activity is shown in Figure 5. On day 0 control treatment presented the greatest ($p \leq 0.05$) antioxidant capacity. However, on day 20, this difference disappears ($p > 0.05$). The antioxidant activity in fruit juices decreases during storage due to various factors such as degradation of bioactive compounds, exposure to light, and changes in temperature. Several studies have shown that the antioxidant activity of fruit juices, including orange, mango, and *Momordica charantia* L., decreases over time, with significant degradation observed after a few days of storage [33,72].

This same behavior was observed in Aloe vera gel where the antioxidant activity was significantly reduced depending on the dose of ultraviolet rays applied [33]. Numerous investigations have demonstrated that antioxidant capability varies depending on the amount of radiation received. The increase in antioxidant capacity has been correlated with the content of antioxidant molecules, tissue browning and with enzymatic browning due to the enzyme polyphenol oxidase (PPO), whose activity can increase or decrease depending on the dose of UV rays administered [72].

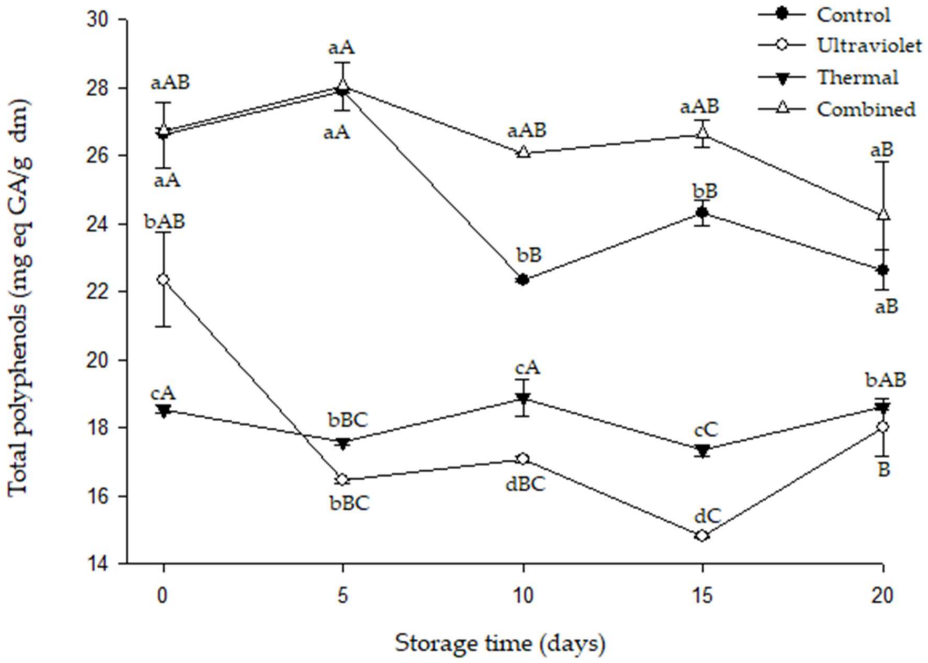

Figure 3. Effect of different treatments on total polyphenols. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, d, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

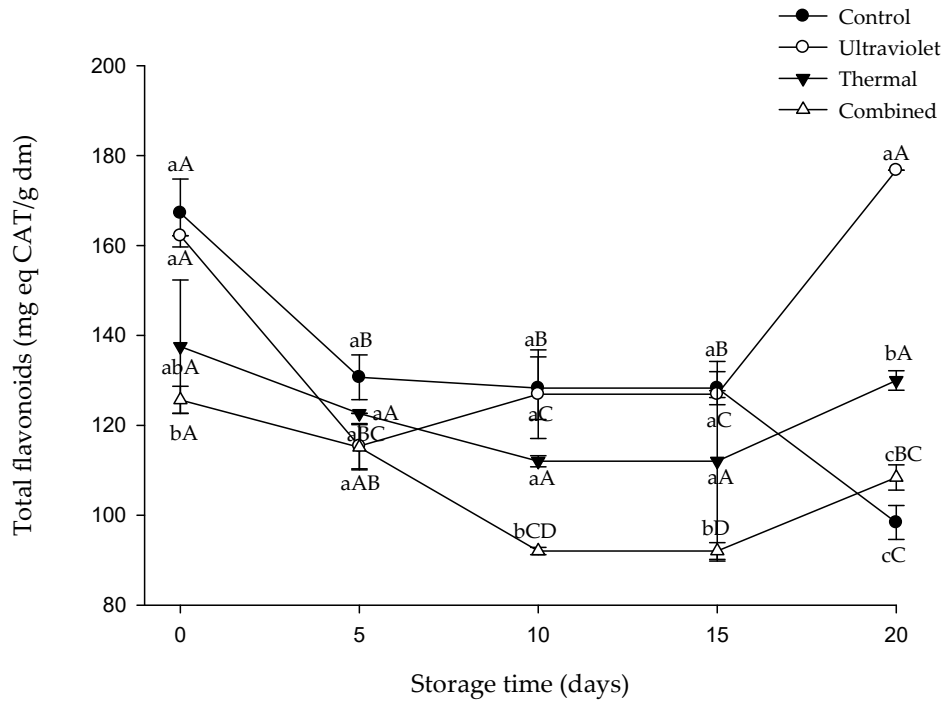

Figure 4. Effect of different treatments on total flavonoids. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, D, different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

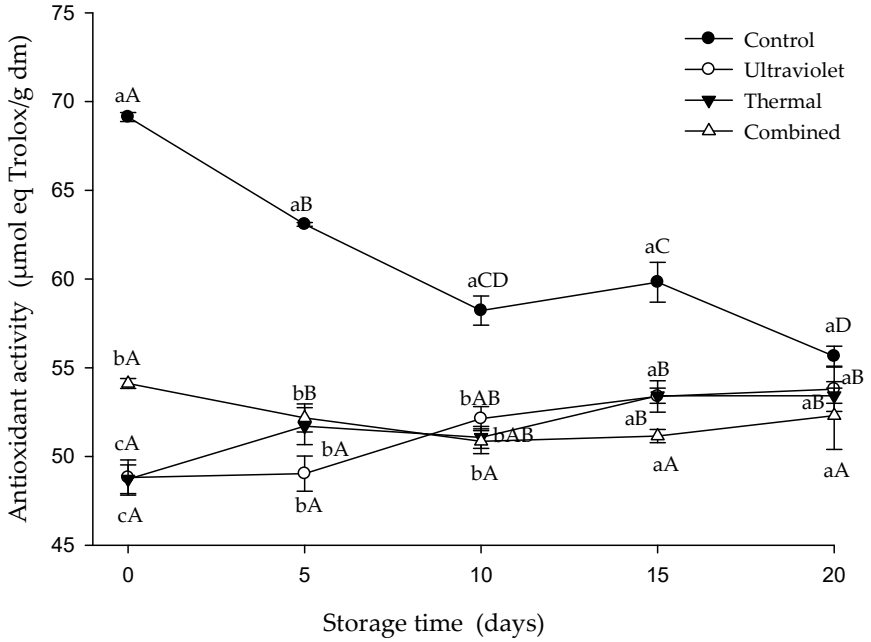

Figure 5. Effect of different treatments on antioxidant activity. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, D, different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

3.5. Microbiological Analysis

Figures 6–8 show microbiological results. The populations in unprocessed samples presented a growth range of between 1.11–6.75, 2.89–6.95, 1.08–6.83 Log CFU/mL, for TAM, TAP, HyL respectively. None of the treatments showed growth of total coliforms during storage.

In relation to the TAM, the thermal and combined treatments did not present growth of such organisms. The highest counts ($p \leq 0.05$) occurred in the control treatment followed by the ultraviolet treatment. Likewise, these two treatments presented an increase ($p \leq 0.05$) in the TAM count during storage.

Regarding the presence of TAP, the treatments presented a behavior like that presented in the TAM counts. The thermal and combined treatments did not present growth of TAP and the treatment that presented the highest counts ($p \leq 0.05$) was the control followed by ultraviolet. Likewise, the treatments showed an increase in TAP ($p \leq 0.05$) throughout the shelf life. Finally, the HyL count presented the same behavior as TAM and TAP.

The shelf life of raspberry apple juice mixture is 1 to 2 weeks. The ultraviolet treatment until day 15 presented TAM, TAP and HyL counts below 5 Log CFU/mL, which has been reported in other investigations as an acceptable microbial and sensory limit for fruit juice consumers [36,60].

The above agrees with what was reported in pineapple juice [61] and watermelon [59], where it was observed that juices treated with ultraviolet rays presented less growth of microorganisms during storage, this compared to untreated juice. The survival of HyL during storage is due to its morphology and structural composition. The resistance of microorganisms to ultraviolet treatments is largely determined by their ability to repair the damage caused to DNA caused by ultraviolet rays [29]. Molds causes deterioration of juices and affect flavor due to the presence of enzymes such as amylases, proteases, and pectinases [73]. In this study, the combination of ultraviolet irradiation (9.68 mJ/cm^2 UV-C) assisted with mild heat (55 °C) showed an efficiency equal to conventional thermal pasteurization, the same effect has been observed in carrot and orange juices with added with yerba mate (*Ilex paraguariensis*) [74], apple juice [75] and grape juice [76].

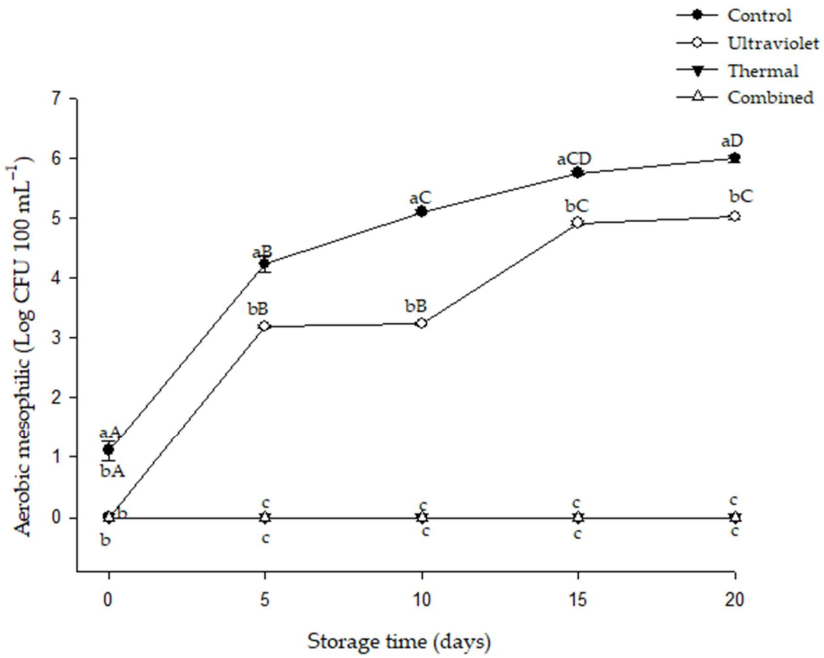

Figure 6. Effect of different treatments on aerobic mesophilic. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, D, different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

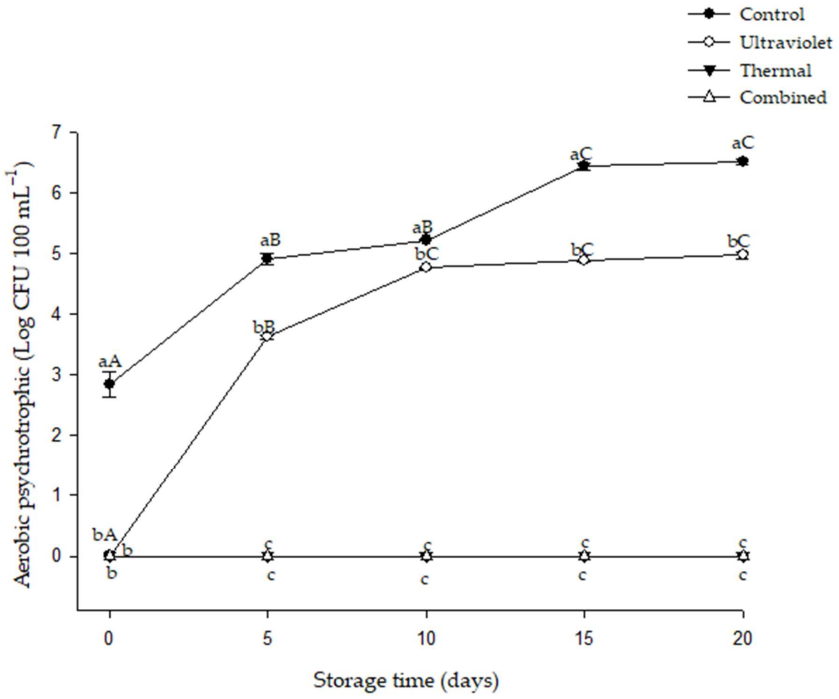

Figure 7. Effect of different treatments on aerobic psychrotrophic. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

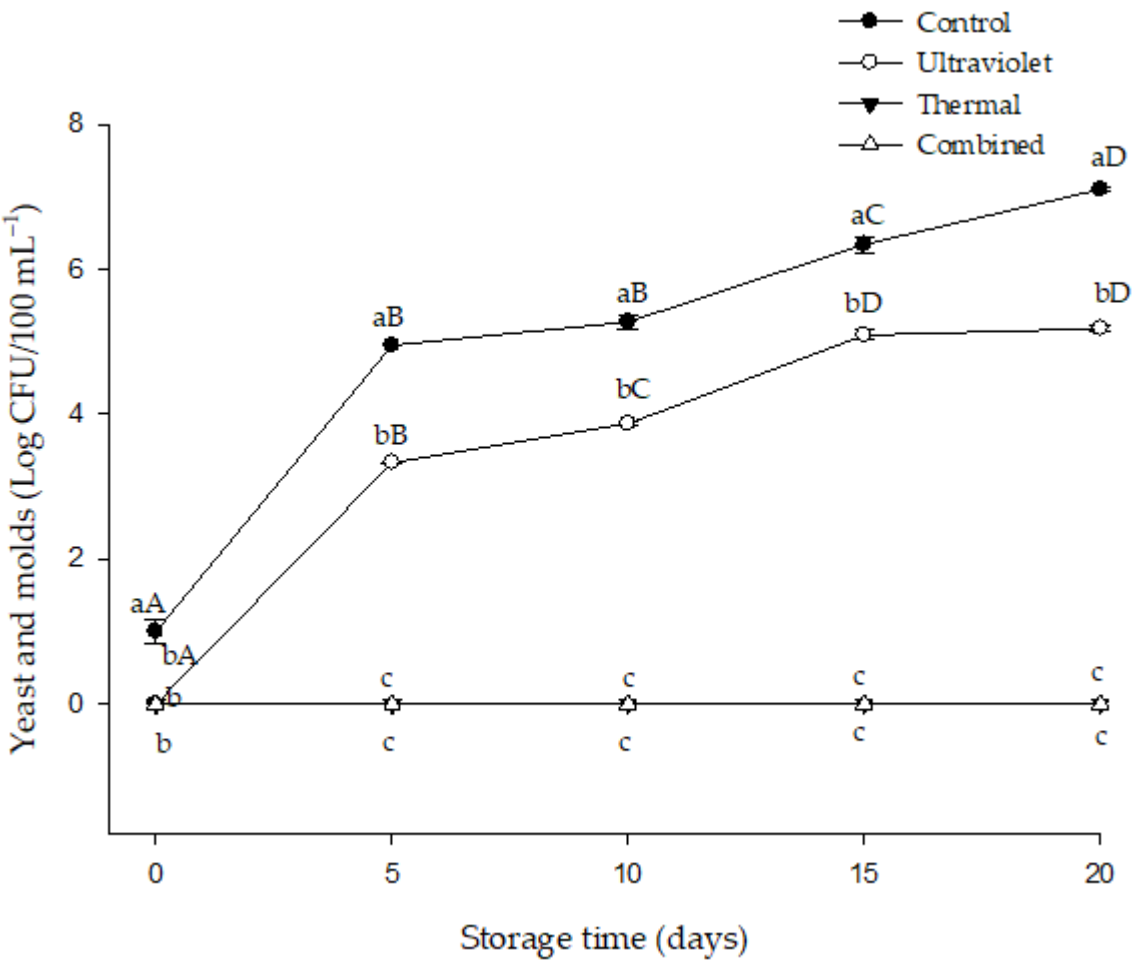

Figure 8. Effect of different treatments on yeast and molds. Control: apple-raspberry juice without treatment; Ultraviolet: apple-raspberry juice with ultraviolet treatment; Thermal: apple-raspberry juice with thermal treatment; Combined: apple-raspberry juice with combined treatment (Ultraviolet radiation and moderate heat treatment). Values are expressed as mean ± standard error (n = 3). a, b, c, different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments. A, B, C, D, different capital letters indicate significant differences ($p \leq 0.05$) during storage for treatment.

4. Conclusions

The study evaluated the effect of the application of UV radiation, heat, and the combination of both treatments on the physicochemical and microbiological quality of apple-raspberry juice. The combined treatment showed greater retention of bioactive compounds, such as polyphenols, and the presence or growth of mesophiles, psychrophiles, fungi and yeasts were not observed. From the above, it is concluded that the use of UV radiation and heat is a treatment with potential use in the production of apple-raspberry juice. Although, regarding the differences presented in the volatile compounds between the treatments, it is recommended to carry out sensory studies to know if these changes are perceived by the consumer. It's important to note that the specific effects of UV treatment and heat in juice elaboration will depend on various factors, including the type of juice, the initial microbial load, the UV intensity and exposure time, and the temperature and duration of the heat treatment. Careful optimization is necessary to achieve the desired results while ensuring the safety and quality of the final product. Keep in mind that UV treatment and heat pasteurization are just two of many possible techniques for juice elaboration. The choice of method depends on the specific goals of the juice manufacturer and the characteristics of the juice being processed.

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