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MICROBIAL AND PHYSICOCHEMICAL PROPERTIES OF UV-C PROCESSED *Aloe vera* GEL BLENDS AT DIFFERENT pHs USING A CONTINUOUS FLOW UV SYSTEM

PROPIEDADES MICROBIOLÓGICAS Y FISICOQUÍMICAS DE MEZCLAS DE GEL DE *Aloe vera* PROCESADAS CON UV-C A DIFERENTES pHs EN UN SISTEMA UV DE FLUJO CONTINUO

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Abstract

UV-C irradiation doses (12.8, 24.2, 35.8, and 54.6 mJ·cm^{-2}) on the physicochemical and microbiological properties of 10% *Aloe vera* gel at different pHs (3.5, 4.5, and 5.5) were evaluated. An unprocessed treatment (UP) and a short (45 s) thermal treatment (TT) at 90 °C were used as controls. The irradiation doses and pH levels affected ($p < 0.05$) the elimination of coliforms and aerobic mesophilic microorganisms with the same efficiency as TT. Molds and yeasts were successfully eliminated at doses ≥ 24.2 mJ·cm^{-2} regardless of pH. Reducing sugars, total polyphenols, and aloin A content decreased as pH increased ($p < 0.05$). Although, the antioxidant activity was significantly reduced at doses ≥ 35.8 mJ·cm^{-2}. TT and UV-C treatments affected the a^* color parameter ($p < 0.05$), resulting the UV-C with the highest values. In both treatments, a reddish color was present at pH ≥ 4.5. The UV-C treatment resulted in minimal changes in most physicochemical properties, compared to UP treatment. However, TT significantly affected most physicochemical properties of *A. vera* gel blends. These results suggest that dose of 24.2 mJ·cm^{-2} in a continuous flow UV system is a non-thermal alternative for the stabilization of 10% *A. vera* gel blends at pH of 3.5.

Keywords: UV-C irradiation, *Aloe vera*, acemannan, polyphenols, aloin A.

Resumen

Se evaluaron las dosis de irradiación UV-C (12.8, 24.2, 35.8 y 54.6 mJ·cm^{-2}) sobre las propiedades fisicoquímicas y microbiológicas de gel de *Aloe vera* al 10% a diferentes pHs (3.5, 4.5 y 5.5). Con respecto a un tratamiento sin proceso (UP) y un tratamiento térmico (TT) (45 s; 90 °C) como controles. De acuerdo a los resultados obtenidos, las dosis y los niveles de pH afectaron ($p < 0.05$) la eliminación de coliformes y mesófilos aerobios con la misma eficiencia que el TT. Los hongos y levaduras se eliminaron a dosis ≥24.2 mJ·cm^{-2} independientemente del pH. Los azúcares reductores, polifenoles totales y el contenido de aloína A disminuyeron conforme aumentó el pH ($p < 0.05$), mientras que la actividad antioxidante se redujo significativamente a dosis ≥35.8 mJ·cm^{-2}. Los tratamientos TT y UV-C afectaron el parámetro de color a^* ($p < 0.05$), resultando el UV-C con los valores más altos; en ambos tratamientos, se presentó una coloración rojiza a pH ≥ 4.5. El tratamiento con UV-C mostró cambios mínimos en la mayoría de las propiedades fisicoquímicas, en comparación con el tratamiento UP. Sin embargo, el TT afectó significativamente la mayoría de las propiedades fisicoquímicas de las mezclas de gel de *A. vera*. Estos resultados sugieren que la dosis de 24.2 mJ·cm^{-2} en un sistema UV de flujo continuo es una alternativa no térmica para la estabilización de mezclas de gel de *A. vera* al 10% a pH de 3.5.

Palabras clave: Irradiación UV-C, *Aloe vera*, acemanano, polifenoles, aloína A.

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1 Introduction

Aloe vera (*Aloe barbadensis* Miller) is a perennial plant of the Liliaceae family with turgid green, elongated, and pointed leaves joined at the stem in a rosette pattern. The leaves are formed by a thick, shiny epidermis (skin) and an internal clear mucilaginous substance referred to as a gel. The gel represents approximately 70-80% of the weight of the whole leaf (Femenia *et al*., 1999; Domínguez-Fernández *et al*., 2012) and consists of approximately 98.5% to 99.5% water. The solid component of the gel contains more than 200 different compounds, including a combination of polysaccharides and acetylated derivatives such as acemannan, glycoproteins, phenolic anthraquinones, flavonoids, flavanols, low molecular weight substances, enzymes, minerals, essential and non-essential amino acids, sterols, saponins and vitamins (Rodríguez *et al*., 2010). A range of biological activities have been attributed to these compounds; therefore, *A. vera* gel is increasingly being used in food products (Rodriguez-Gonzalez *et al*., 2011; Ray *et al*., 2013). However, anthraquinone content should be regulated because an excessive dose or its consumption for large periods of time has negative effects on consumer health. The International Aloe Science Council (IASC) has established a maximum allowable amount of 10 ppm of aloin in products for oral consumption (IASC, 2006).

The effective use of *A. vera* gel in beverages is limited by its poor stability; once it is extracted, it may become contaminated with spoilage microorganisms or it can be oxidized, losing much of its biological activity and affecting the overall quality and shelf-life of the end product (He *et al*., 2005). *Aloe vera* gel is processed using heating, pasteurization, and/or dehydration technologies. However, thermal processing can cause chemical and physical changes that lead to the loss of the natural organoleptic properties (Ling *et al*., 2015) and a reduction in content and bioavailability of bioactive compounds (Patras *et al*., 2010; Rawson *et al*., 2011; González-Cruz *et al*., 2018). The main bioactive compound of *A. vera* gel is acemannan, which is structurally affected by thermal processing, leading to considerable changes not only in its physicochemical properties, but also in its physiological and pharmacological properties (Femenia *et al*., 2003; Minjares-Fuentes *et al*., 2016). Thus, the development of mild treatments is needed for gel stabilization that also preserves the gel's natural properties. Emerging technologies have been evaluated to replace or supplement conventional technologies in the food processing industry. Ultraviolet light (UV-C) irradiation is an alternative food preservation technology that may serve as a substitute for thermal processing (Worobo, 1999) or can be used in combination for safe processing. UV-C treatment is a cost-effective non-thermal technology for liquid food pasteurization. The Food and Drug Administration in the United States has recognized it as an alternative to heat treatments (FDA, 2013), which is effective in fruit juices against pathogens such as *Escherichia coli* O157:H7 and *Cryptosporidium parvum* (Hanes *et al*., 2002; Quintero-Ramos *et al*., 2004; Pala and Toklucu 2013). Additionally, some reports show that juices treated with UV radiation tend to maintain their nutritional and sensory qualities (Pala and Toklucu 2013). Furthermore, UV treatment has other multiple advantages, such as a lack of reported toxic effects, no formation of toxic residues during treatment, and very low energy consumption compared to other non-thermal pasteurization processes (Lopez-Malo and Palou, 2005; Gayan *et al*., 2014).

UV-C is a short wavelength radiation, which is germicidal for bacterial in the 260-265 nm range, since it corresponds to the peak UV absorption by bacterial DNA (Gayan *et al*., 2014), but has minor effects on the inactivation of fungi and yeasts (Usaga *et al*., 2016). Even though the effectiveness of UV-C light for the inactivation of microorganisms has been demonstrated, the treatment efficacy is dependent on the physical, optical and chemical properties of the liquid (Koutchma, 2009), impacting that efficacy of the treatment. Therefore, these factors must be optimized to establish adequate conditions for the process (Koutchma, 2009; Gopisetty *et al*., 2018). As *A. vera* is used in the formulation of food and pharmaceutical products (Domínguez-Fernández *et al*., 2012), it is important to preserve it without causing physicochemical changes that alter the quality and sensory characteristics. The use of UV-C radiation under appropriate conditions could represent an alternative for the stabilization of *A. vera* gel; however, data regarding this approach are scarce and the impact of such treatment on the microbiological and physicochemical characteristics of *A. vera* is not well understood. The present study evaluates the effect of different UV-C doses of irradiation at different pH values on the physicochemical and microbiological properties of a 10% *A. vera* gel blend.

2 Materials and methods

2.1 Samples

Four-year-old *A. vera* plants (*A. barbadensis* Miller) were used as the raw material to carry out the experiments. They were obtained from the greenhouse of the Chemistry Department of the University of Chihuahua (Chihuahua, Chihuahua, Mexico). Homogenous leaves were selected according to size (40-50 cm in length), ripeness, and color. The leaves were washed with tap water, disinfected by immersion in a sodium hypochlorite solution (200 ppm) for 5 min, and rinsed with distilled water. Afterwards, the acibar (a yellow-colored liquid that comes from the leaves) was extracted by cutting the leaves at the base and allowing them to drain vertically for 1 h. The cuticle of the Aloe leaves was carefully separated from its gel using a scalpel-shaped knife. The gel fillets were washed thoroughly with distilled water to remove slime from their surfaces, triturated in a blender (Phillips Electric Blender, Mexico) and filtered. Finally, the Aloe gel was stored at 4 ºC for 1 h or less prior to treatments.

2.2 UV-C continuous flow system

A CiderSure 3500 commercial UV processing unit (FPE Inc., Macedon, NY, USA) was used in this study. The unit comprises a stainless-steel outer housing and three chambered, inner quartz tubes connected in series. Ten percent *A. vera* gel was pumped as a thin film between the outer steel housing and the inner quartz tubing by a positive displacement; four-vane pumps allowed a variable flow rate. Eight germicidal low-pressure mercury lamps are the UV light sources that irradiate the passing fluid; they are concentrically placed within the interior of the quartz stainless steel cylinder with a gap of 0.08 cm. The UV unit operated at an irradiation peak of 254 nm. Two-light UVX-25 sensors (UVP, Inc., Upland, CA, USA) at the top and bottom of the cylinder measured the emitted UV energy every 50 ms. UV doses were varied by adjusting the flow rate (10-40 GPH) through the UV unit. Prior to and following each treatment, the UV unit was sanitized with 200 ppm hypochlorite solution and rinsed with water.

2.3 Aloe vera gel treatments

The *A. vera* gel was separated into three batches, diluted with water to 10%, and adjusted to different pHs (3.5, 4.5, and 5.5). Each of the batches was subjected to UV-C irradiation doses (UV) of 12.8, 24.2, 35.8, and 54.6 mJ·cm^{-2}, with residence times of 2.6, 4.9, 7.4, and 11.2 s, respectively; each experiment was performed in duplicate. A thermal treatment (TT) control was performed using a continuous tubular pasteurizer (UHT/HTST unit, Micro Thermics, Raleigh, NC, USA) where the gel samples at different pH levels were treated at 90 ºC for 45 s (He *et al*., 2005). The physicochemical properties and microbial characteristics of processed (TT and UV) and unprocessed (UP) samples were analyzed immediately.

2.4 UV irradiance measurements

The measurement of incident UV intensity on the liquid was done at a wavelength of 254 nm with two UVX-25 sensors (UVP, Inc.) calibrated by the National Bureau of Standard Lamp. The coefficient of absorption of the sample was calculated by Beer's law at the sample depth (0.0403 cm-1). The result was then multiplied by the sensor placement factor (supplied by the manufacturer) to obtain the actual intensity. Exposure times were determined from the flow rate for each test. The UV dose was calculated by Equation 1 (Quintero-Ramos *et al*., 2004):

$$UV\ \ dose(mJ \cdot cm^{-2}) = Irradiance \times Exposure\ \ time \quad (1)$$

2.5 Optical properties

Optical properties of the *A. vera* gel blend diluted to 10% were measured according to methods described by Koutchma *et al*. (2004). Absorbance was measured at 254 nm using a spectrophotometer (PerkinElmer model Lambda 25 UV/VIS, Waltham, MA, USA.). Sample solutions were evaluated using matched demountable fused quartz cuvettes (FireflySci, Inc., NY, USA.) with path lengths of 0.1, 0.2, 0.5, and 1.0 mm. The absorption coefficient (with log base 10) of the sample solution was determined from the slope of the absorbance *vs*. path length (cm). The turbidity was measured using a micro Turbidimeter 100 (Scientific Inc, Fort Myers FL, USA.) and the results are expressed as nephelometric turbidity unit (NTU). The soluble solids content was measured using an Abbe

refractometer (American Optical Corporation, NY, USA.). All measurements were done in triplicate and mean values with standard deviation (SD) are reported.

2.6 Microbiological analyses

Microbiological analyses of the 10% *A. vera* blend gel with and without treatment were performed using the serial dilution-pour plate method as per APHA. *Aloe vera* blend samples were collected aseptically before and after UV and thermal treatment and analyzed within 10 min. When necessary, the samples were diluted with a phosphate buffer. Aliquots of appropriate dilutions of one mL of the inoculum was pour plated into different media plates. For total aerobic mesophile counts (TAM), the samples were pour plated on plate count agar (PCA; Difco, Detroit, MI, USA.) and incubated at 37 ºC for 24 h. For total coliforms (TC), violet red bile agar (VRBA, Difco) was used, and plates were incubated at 37 °C for 18 to 24 h. Yeasts and molds (YM) were plated on potato dextrose agar (PDA, Difco) and incubated for 5 days at 30 ºC. All microbiological analyses were performed in triplicate. After incubation, colony forming units (CFU) were enumerated, and triplicate counts were averaged and expressed as log (base 10) CFU·mL^{-1} ± standard deviation (SD).

2.7 Color characteristics

The color of reference (UP) and processed (P) 10% *A. vera* blend samples was measured with a Konica Minolta CR-400/410 colorimeter (Minolta Co., Osaka, Japan), which was calibrated with a plate with X = 94.9, y = 0.3185 and x = 0.3124 values. The color parameter values of lightness (L^*), greenness/redness (-/+ a^*), and blueness/yellowness (-/+ b^*) were directly recorded for each treatment. The total color difference (ΔE) or the change between treated and untreated samples was calculated using Equation 2.

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \qquad (2)$$

where L_0^*, a_0^*, and b_0^* represent the values of the reference sample, and L^*, a^* and b^* represent the measured values corresponding to a processed sample. All parameters were measured four times in triplicate of each treatment and the mean values with the standard deviation (SD) were reported.

2.8 Analytical methods

The pH was measured in accordance with the AOAC (1998) method 981.12. The soluble solids contents were measured using an Abbe refractometer (American Optical Corporation). Total sugars (TS) were determined following the phenol-sulphuric acid method (Dubois *et al*., 1956) and reducing sugars (RS) by the colorimetric method of Miller (1959). Glucose was used as a standard in both cases. Results were expressed as mg·g^{-1} dry mass (d.m.) of the 10% *A. vera* gel blend. The total phenolic content (TP) was estimated using the Folin-Ciocalteu method as described by Singleton *et al*. (1999) with some modifications. Gallic acid was used as a standard for the calibration curve, deionized water was the solvent, and the results were expressed as mg gallic acid equivalent (GAE)·g^{-1} d.m. of the 10% *A. vera* gel blend. The total antioxidant activity (TAA) of the samples was estimated using the free radical method of Brand-Williams *et al*. (1995). The free radical used in this study was 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), and the results were expressed as mM of Trolox equivalents (mM TE·g^{-1} d.m.) of the 10% *A. vera* gel blend. All determinations were made using a spectrophotometer (Perkin Elmer model Lambda 25 UV/VIS). All parameters were measured in triplicate, and the mean values with SD were reported.

2.9 Determination of the aloin A content

Aloin A content was analyzed using a Thermo Scientific Dionex Ultimate 3000 UHPLC system (Sunnyvale, CA, USA) equipped with a diode array detector (DAD Varian ProStar model 410, Palo Alto, CA) and auto sampler. The analytical column was a Dionex Acclaim^{TM} 120-C18 (4.6 × 150 mm, 5 μm). The separation was carried out using water/acetonitrile 90:10% v/v in isocratic mode. Acetic acid (0.1% v/v) was added to the mobile phase to minimize peak tailing. The temperature, flow rate, and injection loop were 50 °C, 1.0 mL·min^{-1}, and 25 μL, respectively. The detection was made at 297 nm. The sample was filtered through a 0.45 μm membrane filter before injection (Bozzi *et al*., 2007). All solvents were HPLC grade (Merck, Darmstadt, Germany), an aloin A standard of high purity was purchased from Sigma-Aldrich (St. Louis, MO, USA). All measurements were done in triplicate, and the aloin A content in the 10% *A. vera* gel blend was expressed as μg·g^{-1} d.m. Mean values with SD were reported.

2.10 Experimental design and statistical analysis

A statistical design consisting of a 4 × 3 completely randomized factorial experiment was employed to determine the influences of UV-C dose (12.8, 24.2, 35.8, and 54.6 mJ·cm^{-2}) and pH (3.5, 4.5, and 5.5) on the 10% *A. vera* gel blend. Additionally, unprocessed treatment (UP) and thermal treatment (TT) at 90 °C for 45 s functioned as controls. The data obtained from the various experimental analyses were subjected to an analysis of variance (ANOVA). In addition, a contrast analysis of the mean differences between treatments was performed. Significance was defined as $p < 0.05$ using Minitab version 16 software (Minitab, 2010, State College, PA) and Microsoft Excel software version 16 (Microsoft Office Professional Plus 2016, Redmond, Washington, U.S.).

3 Results and discussion

3.1 Physicochemical characterization of the Aloe vera gel

The 10% *A. vera* gel blend had a pH of 6.15. The TP content was 0.795 ± 0.061 mg GAE·g^{-1} d.m., which is consistent with levels reported by Loots *et al*. (2007). Specifically, the aloin A content was 0.76 ± 0.014 μg·g^{-1} d.m., which is comparable to values reported by Lee *et al*. (1999). On the other hand, the antioxidant activity was measured as 0.79 ± 0.056 mM TE·g^{-1} d.m., a value slightly less than that previously reported (1.2 to 2.5 mM TE·g^{-1} d.m.) by Lee *et al*. (2012). This variation in TAA has been attributed to factors such as the age and temperature of the plant's development, the season in which the plant was harvested, conditions in which the gel was extracted and even the length of the stalk (Minjares-Fuentes and Femenia, 2017). The 10% *A. vera* gel blend contained 28.57 ± 0.43 mg·g^{-1} d.m. of reducing sugars and 54.60 ± 1.83 mg·g^{-1} d.m. of total sugars, values consistent with those reported by other researchers (Bozzi *et al*., 2007; Femenia *et al*., 1999). The high TS content compared with the RS content confirms that complex sugars or polysaccharides with a high molecular weight are the main component of the solids in *A. vera* gel (Minjares-Fuentes and Femenia, 2017). The absorption coefficient of the 10% *A. vera* gel blend was low (0.493 cm^{-1}), while the turbidity value was 21.50 ± 0.91 NTU indicating a relatively low suspended solids content. These values of both optical properties could favor the irradiation process of this gel blend.

The UV-C irradiation dose and pH value had a significant effect ($p < 0.05$) on the linear and interaction effects, causing the complete elimination of TAM, TC, and YM, indicating that this UV-C method is as efficient as TT at any of the pHs evaluated. These results of TAM and TC are consistent with other reports demonstrating that UV-C treatment at low doses (14 mJ·cm^{-2}) in apple juice was sufficient to inactivate microorganisms classified within the TC (Quintero-Ramos *et al*., 2004; Usaga *et al*., 2016). Irradiation treatments with 4.5 and 5.5 pH values proved to be effective with the YM group, whose initial load was 2.98 ± 0.08 and 2.81 ± 0.07 Log CFU·mL^{-1}, respectively; no growth of these microorganisms was observed. Still, survival of the YM microbial group was observed at a pH of 3.5 and 12.8 ± 0.18 mJ·cm^{-2} irradiation dose (0.95 ± 0.09 log CFU·mL^{-1}). Yeast and mold survival was observed in apple and carrot juice treated with UV-C irradiation (Gouema *et al*., 2015; Usaga *et al*., 2016; Riganakos *et al*., 2017); this was attributed to mold and yeast morphological characteristics (larger size and thickness of the cell wall) that induce light scattering, thereby reducing the effectiveness of the UV-C treatment.

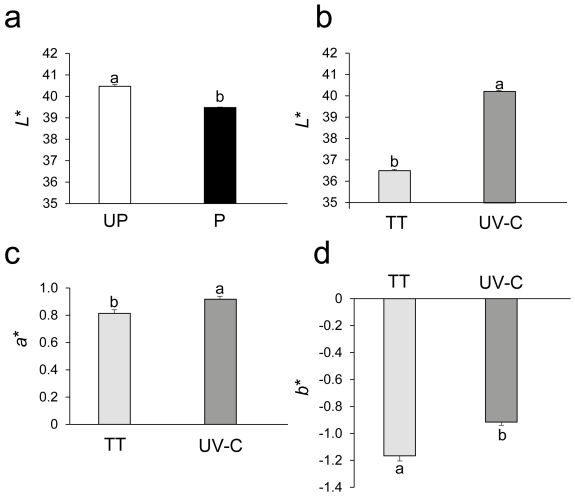

Fig. 1. Effect of treatments on color parameters of 10% *Aloe vera* gel blends. a) Comparison of the L^* parameter between a) processed (P) and unprocessed (UP) treatments and b) TT and UV-C treatments, c) UV-C and TT effect on the a^* parameter, d) UV-C and TT effect on the b^* parameter. For each figure panel, different letters denote significant differences at $p < 0.05$ by contrast test.

Table 1. Native microbiota changes of 10% *Aloe vera* gel blends associated with different pH values and treatments.

Treatment	TC	TAM	YM
	Log CFU·mL^{-1}*		
UP/3.5	2.47 ± 0.13	2.89 ± 0.16	2.95 ± 0.24
UP/4.5	3.01 ± 0.10	3.06 ± 0.14	2.98 ± 0.08
UP/5.5	3.02 ± 0.60	3.15 ± 0.27	2.81 ± 0.07
D1/3.5	ND	ND	0.95 ± 0.09
D1/4.5	ND	ND	ND
D1/5.5	ND	ND	ND
D2/3.5	ND	ND	ND
D2/4.5	ND	ND	ND
D2/5.5	ND	ND	ND
D3/3.5	ND	ND	ND
D3/4.5	ND	ND	ND
D3/5.5	ND	ND	ND
D4/3.5	ND	ND	ND
D4/4.5	ND	ND	ND
D4/5.5	ND	ND	ND
TT/3.5	ND	ND	ND
TT/4.5	ND	ND	ND
TT/5.5	ND	ND	ND

*All values are expressed as means ± standard deviation (n = 3). TC, Total coliforms; TAM, Total aerobic mesophiles; YM, Yeasts and molds; ND, Not detected; UP, Unprocessed treatment; D1, 12.8 ± 0.18 mJ·cm^{-2}; D2, 24.2 ± 0.12 mJ·cm^{-2}; D3, 35.8 ± 0.36 mJ·cm^{-2}; D4, 54.6 ± 0.39 mJ·cm^{-2}; TT, Thermal treatment (90 °C, 45 s).

In addition, another factor that influences survival is the lower thiamine and cytosine content in their genome, (Oteiza *et al*., 2009; Gouema *et al*., 2015). Complete inactivation of YM in the 10% *A. vera* gel blend was achieved at UV-C irradiation dose of ≥ 24.2 ± 0.12 mJ·cm^{-2}, irrespective of the pH value.

3.2 Color parameters

The effect of the treatments applied to the 10% *A. vera* gel blends on the L^*, a^* and b^* color parameters as well as the change in color, ΔE, at different pH is shown in Table 2. For each treatment, the pH of the blend did not demonstrate significant effects on the L^* parameter (Table 2). However, the processing of the blends (TT and UV-C) resulted in a decreased ($p < 0.05$) L^* parameter (luminosity) compared with the UP blends (Fig. 1a), causing the darkening observed in the blend. However, for the UV-C irradiation treatments, the doses applied did not cause significant differences ($p > 0.05$), in the linear and interaction effects. The luminosity values of the blends were higher for the irradiated treatment (UV-C) and significantly different ($p < 0.05$) from those for the thermal treatment (TT), resulting in low L^* values (darker blends) (Fig. 1b). This darkening of *A. vera* gel has been reported by other authors; this change has been attributed to non-enzyme-related darkening reactions during thermal pasteurization (Minjares-Fuentes and Femenia, 2017).

Regarding the a^* color parameter (greenish-reddish hues), this was significantly affected ($p < 0.05$) by the blend's pH for both treatments (TT and UV-C) (Table 3). In addition, we observed that gel blends treated with UV-C yielded the highest values for the a^* parameter compared with the TT (Fig. 1c), which resulted in a significant decrease ($p < 0.05$) at a pH of 5.5 (Table 2). The b^* color parameter did not show significant ($p > 0.05$) changes due to pH or irradiation dose. Nonetheless, it was observed that the lowest b^* parameter (darker blends) corresponded to the TT in comparison with the UV-C treatments (Fig. 1d). The effect of the irradiation dose on the b^* parameter reported by Caminiti *et al*. (2010) in apple juice; did not observe significant changes despite the use of UV-C doses > 26550 mJ·cm^{-2}. The changes in the a^* and b^* parameters could have been generated by the polyphenolic compounds present in the 10% *A. vera* gel, which at pH > 4 can be oxidized faster, condensing into compounds that are initially pink colored but later turn brown when the oxidation level is higher (Chang *et al*., 2006).

Table 2. Color parameters of the 10% *Aloe vera* gel blends associated with different pH values and treatments.

Treatments	L^*	a^*	b^*	ΔE
UP/3.5	41.35 ± 0.40^{a}	0.85 ± 0.01^{ab}	-0.88 ± 0.07^{ab}	—
UP/4.5	40.63 ± 0.06^{a}	0.96 ± 0.01^{ab}	-1.05 ± 0.002^{ab}	—
UP/5.5	39.38 ± 0.02^{a}	0.94 ± 0.02^{ab}	-1.13 ± 0.002^{ab}	—
D1/3.5	40.04 ± 0.17^{a}	0.85 ± 0.07^{ab}	-0.95 ± 0.08^{ab}	1.25 ± 0.74^{bc}
D1/4.5	40.74 ± 0.10^{a}	1.01 ± 0.01^{a}	-0.87 ± 0.02^{ab}	0.99 ± 0.19^{bc}
D1/5.5	40.18 ± 0.34^{a}	0.83 ± 0.05^{ab}	-0.93 ± 0.17^{ab}	1.00 ± 0.77^{bc}
D2/3.5	39.97 ± 0.24^{a}	0.93 ± 0.12^{ab}	-0.91 ± 0.01^{ab}	1.34 ± 0.75^{bc}
D2/4.5	40.49 ± 0.30^{a}	0.97 ± 0.01^{a}	-0.97 ± 0.02^{ab}	0.82 ± 0.27^{c}
D2/5.5	40.06 ± 0.02^{a}	0.90 ± 0.08^{ab}	-0.88 ± 0.12^{ab}	0.53 ± 0.46^{c}
D3/3.5	39.51 ± 0.20^{a}	0.94 ± 0.07^{ab}	-0.82 ± 0.17^{a}	1.71 ± 0.74^{bc}
D3/4.5	40.91 ± 1.17^{a}	0.90 ± 0.06^{ab}	-0.90 ± 0.16^{ab}	1.09 ± 0.62^{bc}
D3/5.5	41.27 ± 0.26^{a}	0.82 ± 0.08^{ab}	-0.91 ± 0.08^{ab}	1.02 ± 0.60^{bc}
D4/3.5	39.48 ± 1.62^{a}	0.86 ± 0.08^{ab}	-0.97 ± 0.01^{ab}	1.43 ± 0.80^{bc}
D4/4.5	40.24 ± 0.33^{a}	0.88 ± 0.02^{ab}	-0.99 ± 0.02^{ab}	0.91 ± 0.49^{c}
D4/5.5	39.71 ± 0.03^{a}	1.04 ± 0.03^{a}	-0.85 ± 0.04^{ab}	0.95 ± 0.47^{c}
TT/3.5	36.62 ± 0.71^{b}	0.82 ± 0.01^{ab}	-1.22 ± 0.02^{b}	4.50 ± 0.67^{a}
TT/4.5	36.09 ± 0.84^{b}	0.87 ± 0.01^{ab}	-1.07 ± 0.07^{ab}	4.63 ± 1.08^{a}
TT/5.5	36.76 ± 0.50^{b}	0.74 ± 0.02^{b}	-1.19 ± 0.13^{ab}	2.58 ± 0.32^{b}

*All values are expressed as means ± standard deviation (n = 3). Different letters per column indicate significant differences by means of an ANOVA by Tukey test ($p < 0.05$). UP, Unprocessed treatment; D1, 12.8 ± 0.18 mJ·cm^{-2}; D2, 24.2 ± 0.12 mJ·cm^{-2}; D3, 35.8 ± 0.36 mJ·cm^{-2}; D4, 54.6 ± 0.39 mJ·cm^{-2}; TT, Thermal treatment (90 °C, 45 s).

These results suggest that the characteristic color of the gel can be preserved more effectively when the pH is kept at 3.5. A ΔE value above 2.4 shows if a color change is visible (Caminiti *et al*., 2010). Color changes were not noticeable in the 10% *A. vera* gel blend treated with UV-C at the different pH values tested (Table 2). Similar results were observed for UV-C irradiated apple juice by Caminiti *et al*. (2010) and Usaga *et al*. (2017); they reported that irradiation doses lower than 5000 mJ·cm^2 did not show significant changes in the apple juice color.

However, in other materials as edible films irradiated with UV-C, López-Díaz *et al*. (2018), reported changes in color with doses of 1555.2 J·m^2. The 10% *A. vera* gel blends processed with the TT yielded higher values, showing a visible color change. This undesirable change in color that occurs during the thermal pasteurization has been attributed to a Maillard reaction or caramelization, or a condensation of the phenolic compounds into their oxidized forms (Chang *et al*., 2006; Ding *et al*. 2014). In this case, the polyphenols present in the diluted *A. vera* gel, including aloin, could be responsible for the color change; this will be addressed in greater detail in subsequent sections (3.5 and 3.6).

3.3 Reducing sugars (RS)

The irradiation doses either alone or in combination with pH did not cause significant ($p > 0.05$) changes in the RS content. Nonetheless, the pH alone decreased ($p < 0.05$) the RS content of the blends (Table 3). Low pH values lead to a hydrolysis of sugars (L'homme *et al*., 2003), thus increasing the RS content. The TT of the *A. vera* gel blend yielded the highest RS content (34.56 ± 2.63 mg·g^{-1} d.m.) compared with the UV-C treatment (Fig. 2a).

Even though the exposure time of the TT was short (45 s), the temperature used, 90 °C, appears to be the important factor associated with the increased RS content. It has been reported that thermal treatments with temperatures higher than 80 °C degrade polysaccharides with a high molecular weight as well as pectins (Femenia *et al*., 2003; Chang *et al*., 2006; Rodríguez-González *et al*., 2011), leading to the liberation of the monomeric units, which in turn produces an increase in RS content. In addition, it has been reported that the biological activity of *A. vera* gel decreases when it is subjected to temperatures > 65 ºC for less than 15 min (Ramachandra and Rao, 2008).

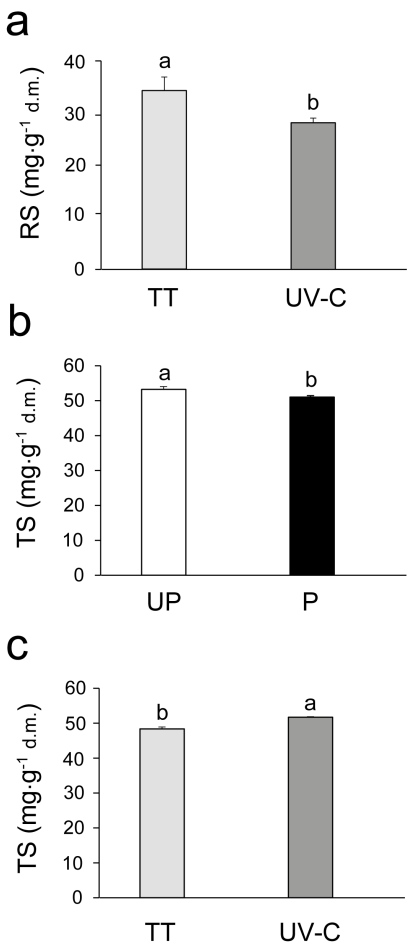

Fig. 2. Reducing sugars (RS) and total sugars (TS) content in the 10% *Aloe vera* gel blends. a) Effect of the thermal (TT) and UV-C irradiation treatments on RS, b) Comparison of TS content in processed (P) and unprocessed (UP) treatments, c) Comparison of TS content from TT and UV-C treatments. For each figure panel, different letters denote significant differences at $p < 0.05$ by contrast test.

3.4 Total sugars (TS)

The TS content of the 10% *A. vera* blends was affected by the pH alone (Table 3), meanwhile irradiation dose by itself and in interaction with the pH did not show significant effects (Table 3). The processing (TT and UV-C) caused a significant decrease ($p < 0.05$) in the TS content compared to the UP blend (Fig. 2b). The high TS content found in the blends of all treatments can be related to high content of complex carbohydrates in the 10% *A. vera* gel. This result is consistent with other reports (Bozzi *et al.*, 2007, Femenia *et al.*, 1999). The TT of the *A. vera* gel blend caused a significant decrease in the TS content compared with the UV-C treatment (Fig. 2c), where the irradiation dose and pH did not significantly affect the TS content. It is plausible that the heating process promoted the thermal degradation of polysaccharides in the *A. vera* gel blend. Similar findings were reported by Chang *et al.* (2016), where the maximum thermal stability of the polysaccharides in *A. vera* gel was achieved around 70 ºC, although this finding was dependent on the processing time.

3.5 Total polyphenols (TP)

The total polyphenol content (TP) in the 10% *A. vera* gel blends was affected ($p < 0.05$) by the pH value (Table 3). For the UV-C irradiation treatments, the irradiation dose and the interaction effects with pH did not show significant effects. However, the TP content decreased significantly when they were processed (TT and UV-C) compared with the UP (Fig. 3a). Nevertheless, the phenolic content is consistent with that reported by other studies for *A. barbadensis* (Loots *et al.*, 2007), where 0.792 mg GAE·g^{-1} of dry mass of gel was obtained. The irradiation treatment did not significantly affect the phenolic content and yielded the highest concentrations compared with the TT.

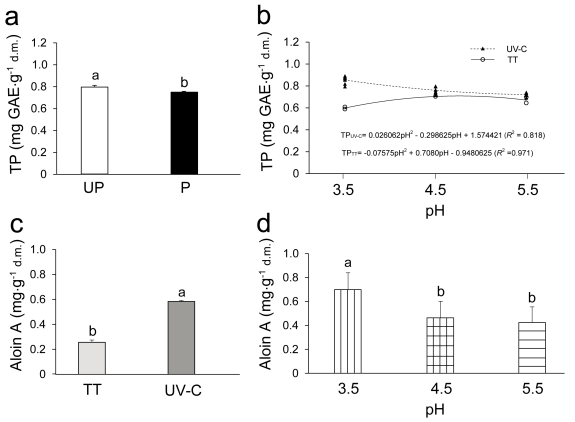

Fig. 3. Total polyphenols (TP) and aloin A content in the 10% *Aloe vera* gel blends. a) Comparison of TP of processed (P) and unprocessed (UP) treatments, b) Effect of pH on TP in UV-C and TT, c) Aloin A content in TT vs UV-C, d) Effect of pH on aloin A content. For each figure panel, different letters denote significant differences at $p < 0.05$ by contrast test. Mean differences of d) was done by Tukey test at $p < 0.05$.

Table 3. Contrasts analysis of treatment effects on the physicochemical properties of 10% *Aloe vera* gel blends at different pH values.

Source	DF	Sum of Square							
		RS	TP	Aloin A	TS	TAA	L^*	a^*	b^*
Model	17	198.27*	0.188*	1.102*	121.146	0.758*	82.721*	0.186*	0.473*
UP *vs*. P	1	5.4775*	7.8×10^{-3}*	0.029*	24.440*	0.486*	4.788*	3.3×10^{-3}	0.0168
TT *vs*. UV-C	1	183.3348*	0.071*	0.507*	53.766*	0.083*	66.74*	0.049*	0.298*
pH	1	2.4978*	0.061*	0.453*	20.681*	0.016*	0.028	2.8×10^{-4}	3.8×10^{-3}
pH2	1	0.0499	6.6×10^{-4}	0.077*	0.391	1.5×10^{-4}	0.841	0.025*	9.5×10^{-5}
pH *UP *vs*. P	1	0.5379	2.3×10^{-3}	2.89×10^{-3}	10.541	1.2×10^{-4}	4.951*	7.4×10^{-3}	0.065*
pH2* UP *vs*. P	1	0.0133	4.6×10^{-4}	1.62×10^{-3}	0.038	1.8×10^{-3}	4.06×10^{-3}	1.7×10^{-4}	2.8×10^{-3}
pH *TT *vs*. UV-C	1	0.1496	0.026*	1.28×10^{-3}	1.766	1.2×10^{-3}	0.143	4.9×10^{-3}	1.03×10^{-4}
pH2* TT *vs*. UV-C	1	0.3842	0.011*	2.92×10^{-4}	0.195	2.7×10^{-3}	1.465*	2.4×10^{-3}	0.031
D	3	1.709	4.2×10^{-3}	0.0161	3.043	0.162*	1.788	0.01	0.011
pH*D	6	4.1195	2.5×10^{-3}	0.0124	6.281	3.4×10^{-3}	1.969	0.082*	0.043
Error	18	5.8236	0.0103	0.0433	84.375	0.0349	5.611	0.058	0.172

*Significance level at $p < 0.05$. UP, Unprocessed treatment; P, Processed treatment; TT, Thermal treatment; UV-C, UV treatment; D: UV dose; RS, Reducing sugar; TP, Total polyphenols; TS, Total sugars; TAA, Total antioxidant activity;

L^*, Lightness (−/+); a^*, Greenness/redness (−/+); b^*: Blueness/yellowness (−/+).

Similar results were reported for pomegranate juice (Pala and Toklucu, 2011), apple juice (Caminiti *et al*., 2010; Islam *et al*., 2016), orange juice (Pala and Toklucu, 2013) carrot-orange juice blends (Caminiti *et al*., 2012) and a carrot-orange juice blend with added yerba mate (Ferrario *et al*., 2018) when subjected to UV irradiation. The stability of TP in the irradiated food liquid matrices has been attributed to the short time spans of exposure, which prevent photooxidation of these compounds. Figure 3b shows that the TT at pH 3.5 decreased the TP content compared with the UV-C treatment, with an opposite concave tendency with an asymptotic behavior at a pH of 4.5. It has been reported that high temperatures employed in thermal pasteurization, such as that used in this study (90 ºC for 45 s), cause the degradation of polyphenols due to their reduced stability at temperatures >70 ºC (Chang *et al*., 2006; Ding *et al*., 2014). It has been reported that some polyphenols show reduced stability at low pH (Friedman and Jürgens, 2000), so that this effect combined with thermal processing causes a greater degradation of the TP compared to the UV-C treatment at pH 3.5. The asymptotic tendency found at pH 4.5 resembles the results presented by Pala and Toklucu (2013), who did not find significant changes in the TP content at pH 4.0 in orange juice processed by thermal and UV-C treatment.

3.6 Aloin A content

The processing of the 10% *A. vera* gel blend with both treatments (TT and UV-C) caused a slight decrease ($p < 0.05$) in the aloin A content compared with the UP treatment (Table 3). The TT led to a decreased ($p < 0.05$) level of aloin A as compared with the UV-C treatments (Fig. 3c). Mainly, these changes likely occurred because pH-related effects, since the impact of irradiation doses on their linear and interaction effects was not significant ($p > 0.05$). The variation in aloin A content of the gel at different pH values showed a significant decrease ($p < 0.05$) at pH > 3.5 (Fig. 3d). The effect of pH on aloin stability was reported by Ding *et al*. (2014), who evaluated the effect of different pH values (2.0-8.0) on aloin stability; they observed a higher stability at acidic conditions (pH < 3) and a noticeable decrease in its content with at higher pH values (pH > 5). A higher pH could enhance the condensation of aloin into aloe emodin or some other oxidized form, resulting in a decrease in aloin content (Chang *et al*., 2006). Regarding the TT, it was observed that the aloin A content decreased approximately 50% compared to UV-C treatments (Fig. 3c), which is attributed to the low stability of aloin at high processing temperatures. Similar findings were reported by Chang *et al*. (2006) and Ding *et al*. (2014).

3.7 Total antioxidant activity (TAA)

Figure 4a shows the significant effect of processed treatments (TT and UV-C) on the TAA compared to the UP treatment 10% *A. vera* gel blend. The thermal process caused a significant decrease ($p < 0.05$) in the TAA as compared with the UV-C irradiation treatments (Fig. 4b), which resulted in higher TAA values. The UV-C irradiation dose and pH of the 10% *A. vera* gel blends had a significant effect ($p < 0.05$) (Table 3).

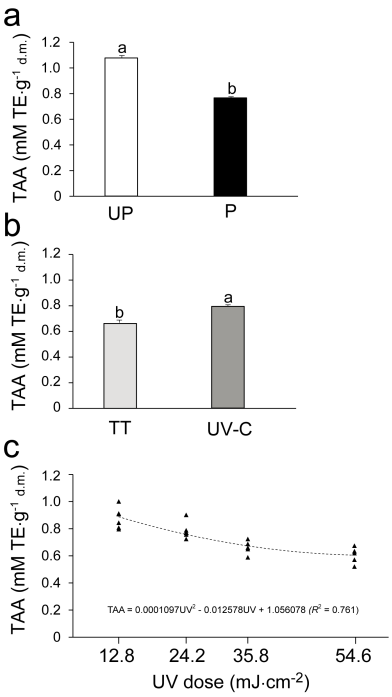

Fig. 4. Total antioxidant activity (TAA) of the 10% *Aloe vera* gel blends. a) Comparison of TAA for processed (P) and unprocessed (UP) treatments, b) Comparison of TAA for thermal (TT) and UV-C treatments, c) Modeling of TAA at different UV doses. For each figure panel, different letters denote significant differences at $p < 0.05$.

This behavior is similar to that observed under the UV-C treatments for aloin A and total polyphenols, compounds that affect the functional properties of the *A. vera* gel (Ray *et al*., 2013). Fig. 4c, shows the tendency of the TAA at different UV-C doses at any pH´s evaluated, observing a visible decrease at doses > 24.2 mJ·cm^{-2}. The best result of TAA observed for the irradiation treatments was 25% lower than that obtained for the UP treatment. Similar results were presented by Caminiti *et al*. (2010) on irradiated apple juice, where a decrease in the TAA was observed when the irradiation dose was increased. This decrease could be due to a combination of ultraviolet light and oxygen, producing free radicals that are reduced by antioxidant compounds, which in turn results in a decreased TAA in the samples (Cvetkovic *et al*., 2011). Thermal treatment decreased the antioxidant activity almost 38% as compared with the UP treatments and approximately 15% compared to the UV-C treatments (Fig. 4a, b). Such decrease in the TAA associated with thermal technologies has been reported by Hendrawati (2015) and Saberian *et al*. (2013), who observed a decrease in antioxidant compounds in both spray dried and pasteurized *A. vera* gel samples. This decrease has been attributed to the low stability of the antioxidant compounds when subjected to temperatures >70 °C (Chang *et al*., 2006; Ding *et al*., 2014).

Conclusions

The application of UV-C treatment to 10% *A. vera* blends, at all the pH values studied, eliminated the TC and TAM microorganisms with the same efficiency as TT. Molds and yeasts survived at low UV-C irradiation doses, but their elimination was achieved at 24.2 mJ·cm^{-2} at all evaluated pH values. In addition, at a dose of 24.2 mJ·cm^{-2}, minimal changes were observed in the physicochemical properties of the 10% *A. vera* gel blend at any pH, compared with the TT.

The aloin A content was affected by the UV-C treatment, whereas the TT decreased the aloin A content by approximately 50%. However, neither treatment was able to reduce the aloin A content below the maximum level allowed. Overall, our results suggest that a pH of 3.5 and a 24.2 mJ·cm^{-2} dose achieve an optimal inactivation of the native microbial load while best maintaining the physicochemical properties of the 10% *A. vera* gel blend. Therefore, UV-C continuous flow technology could be utilized for the stabilization of the gel in 10% blends for its use in pasteurized beverages containing *A. vera*, while preserving its functional properties.

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Conflict of interest

None declared.

Nomenclature

UV	Ultraviolet light
UV-C	Short wavelenght ultraviolet light
UP	Unprocessed
P	Processed
TT	Thermal treatment
RS	Reducing sugars, $mg \cdot g^{-1}$
TS	Total sugars, $mg \cdot g^{-1}$.
TAA	Total antioxidant activity, mM TE$\cdot g^{-1}$ d.m.
TP	Total polyphenols, mg GAE$\cdot g^{-1}$ d.m.
d.m.	Dry mass
GAE	Gallic acid equivalents
TE	Trolox equivalents
ANOVA	Analysis of variance
SD	Standard deviation
NTU	Nephelometric turbidity unit
TAM	Total aerobic mesophiles, log CFU$\cdot mL^{-1}$
TC	Total coliforms, log CFU$\cdot mL^{-1}$
YM	Yeast and molds, log CFU$\cdot mL^{-1}$
ΔE	Total color difference
UHT	Ultra high temperature
HTST	High temperature short time

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