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Ascorbic acid and selected preservatives influence effectiveness of UV treatment of apple juice

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ABSTRACT

The influence of ascorbic acid, sodium benzoate, potassium sorbate, and sulfur dioxide on the effectiveness of UV pasteurization of apple juice and the effect of UV exposure on the stability of these compounds were evaluated. The concentration of ascorbic acid, total vitamin C, benzoate, sorbate, and sulfur dioxide, and the juices' physicochemical properties were determined. UV treatment consisted of multiple passes at a fixed dose of 14 mJ cm^{-2} per pass, achieved by adjusting the juice flow rate through the UV machine. Samples containing ascorbic acid were inoculated with *Escherichia coli* ATCC 25922 (10^7 CFU ml^{-1}) and analyzed for microbial reduction due to UV. The addition of ascorbic acid, sorbate, and benzoate significantly increased juices' absorption coefficients, which caused a reduction in the juice flow rate ($p < 0.05$) required to achieve the fixed UV dose. UV treatment had no significant effect on total vitamin C and benzoate concentrations ($p > 0.05$) but decreased sulfur dioxide, ascorbic acid, and particularly sorbate levels ($p < 0.05$). Increases in ascorbic acid concentration decreased inactivation of *E. coli* ($p < 0.0001$). Thus, additives than can either adversely influence UV efficiency or be degraded due to UV exposure should be added after UV treatment.

1. Introduction

Since the recognition of ultraviolet (UV) light treatment as an alternative to the thermal pasteurization of beverages (FDA, 2013a), the technology has become a viable nonthermal processing option for these products. The high efficiency of pathogen reduction (Basaran, Quintero-Ramos, Moake, Churey, & Worobo, 2004; Hanes et al., 2002; Oteiza, Peltzer, Gannuzzi, & Zaritzky, 2005; Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004), and the reduced loss of nutritional components accompanied by fewer unwanted physicochemical changes (Bhat, 2016; Islam et al., 2016; Caminiti et al., 2010; Tran & Farid, 2004) are some of the advantages that have attracted the attention of consumers, producers, and researchers towards this technology (Koutchma, Popović, Ros-Polski, & Popielarz, 2016). However, previous studies have suggested that UV applications might be limited for certain beverages due to the presence of compounds that strongly absorb UV light (Koutchma, Keller, Chirtel, & Parisi, 2004, 2007; Oteiza et al., 2005).

Research has shown that vitamin C, a naturally occurring and commonly added nutrient in juices, may dramatically decrease UV effectiveness by diminishing the inactivation rates of *E. coli* (Koutchma et al., 2004; Oteiza et al., 2005). Furthermore, this light-sensitive nutrient might be severely degraded during UV treatment (Bhat, 2016). Koutchma & Shmalts (2002) reported a destruction of vitamin C from 30 to 40% when apple juice was exposed to a 600 mJ cm^{-2} UV dose, and when exposed to a similar UV dosage, a degradation of 18% and 25% in orange and carrot juices, respectively. Tran and Farid (2004) revealed a vitamin C concentration decline of 17% in orange juice treated at a 100 mJ cm^{-2} UV dose. Contradictorily, no significant difference in ascorbic acid concentration was found when apple cider was treated for seven consecutive passes (accumulative dose of 98 mJ cm^{-2}) using a commercial UV apparatus, under a turbulent flow regime at a 14 mJ cm^{-2} UV dose per pass (Assatarakul, Churey, Manns, & Worobo, 2011; Dong et al., 2010).

The addition of preservatives is also thought to increase the absorptivity of beverages and consequently limit the performance

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of UV. Nevertheless, no published information regarding this effect is currently available. Considering that UV-treated beverages are not shelf-stable products, and that adding preservatives represents a viable hurdle approach to preserve their quality and extend its shelf life, it becomes relevant to evaluate if these compounds may adversely affect the application of UV. Furthermore, in the case of potassium sorbate, a preservative commonly used in beverages, Cigić, Plavec, Možinac, and Zupančič-Kralj (2001) found that, in water, this additive isomerizes under UV radiation after a 20 min exposure to a 50 W high-pressure mercury lamp, and that the resultant mixture of isomers had lower antimicrobial activity than the original *trans-trans* isomer. However, this phenomenon has not been studied in UV-treated juices or using commercial UV reactors yet.

Considering the relevance of understanding the effect of certain additives on the efficiency of UV, and the potential influence of UV on the stability of those compounds, this study sought to evaluate both effects in apple juice containing ascorbic acid and the most common antimicrobials used for beverage preservation: sodium benzoate, potassium sorbate and sulfur dioxide (Basaran-Akgul, Churey, Basaran, & Worobo, 2009).

2. Materials and methods

2.1. Reagents

1,4 dithiothreitol (DTT) was purchased from J.T. Baker (Center Valley, USA). L-(+)-ascorbic acid, stabilized metaphosphoric acid (MPA), high performance liquid chromatography (HPLC) grade acetonitrile, monobasic potassium phosphate, phosphoric acid, sodium benzoate, potassium sorbate, and sulfuric acid were obtained from Fisher Scientific (Hampton, USA). Trypticase soy broth (TSB) was purchased from BD Difco, Becton Dickinson (Sparks, USA).

2.2. Apple juice

Commercial apple juice concentrate (70 °Brix) was reconstituted with distilled water to about 12 °Brix. Reconstituted juice was pasteurized at 73.9 °C for 6 s in an UHT/HTST Lab-25 HV tubular heat exchanger (MicroThermics Inc., Raleigh, USA), to prevent the presence of background microbiota that may interfere with the microbiological analyses. Apple juice was kept refrigerated at 4 °C for up to two days until used.

2.3. UV machine

UV treatments were carried out in a *CiderSure* 3500 UV juice-processing unit (FPE Inc., Rochester, USA) at a wavelength of 254 nm and a fixed UV dose of 14 mJ cm^{-2}. This machine was validated to ensure a greater than 5-log reduction of *E. coli* O157:H7 and *Cryptosporidium parvum* in apple cider when operating under a turbulent flow regime and at a constant dose of 14 mJ cm^{-2} (Basaran et al., 2004; Hanes et al., 2002). The reactor comprises a stainless steel housing, three concentric inner quartz tubes, eight low-pressure mercury lamps, a positive displacement pump and two UVX-25 sensors (UVP, LLC, Upland, USA). Usaga, Worobo, Moraru, and Padilla-Zakour (2015) provide a thorough description of the machine. Beverages are pumped in a thin film through the system ensuring a turbulent flow regime (Re > 2200). The sensors measure UV transmittance through the juice every 50 ms. These values are relayed to the control panel and an algorithm, which ensures a constant UV dose exposure, is then used. The reactor has been programmed to automatically adjust the pump flow rate to achieve the fixed UV dose. For fluids with high UV absorption the juice flow rate through the system is automatically slowed down, while for products with lower UV absorption the flow rate is increased; ensuring a consistently delivered UV dose to the product (Usaga et al. 2015).

2.4. Sample preparation and UV processing

Apple juice containing various concentrations of either ascorbic acid (0–600 mg kg^{-1}), potassium sorbate (0–200 mg kg^{-1}), sodium benzoate (0–1000 mg kg^{-1}), or sulfur dioxide (0–280 mg kg^{-1}, corresponding to a concentration of free sulfur dioxide from 0 to 160 mg kg^{-1}) were treated at 14 mJ cm^{-2} fixed UV dose in a single-pass treatment. All concentrations comply with levels indicated by the U.S. Food and Drug Administration (FDA, 2013b).

Flow rates were determined volumetrically for all treatments by measuring the time required to collect a known volume of UV-treated juice. Samples before and after UV were collected in amber high-density polyethylene centrifuge tubes and stored at 4 °C until analyses were performed. For trials involving ascorbic acid and total vitamin C, samples were analyzed via HPLC immediately after the addition of ascorbic acid and after the application of the UV treatment, respectively.

Total vitamin C, ascorbic acid, sorbate, benzoate, and free and total sulfur dioxide concentrations, as well as the apparent absorption coefficient of all samples were determined before and after UV.

To evaluate if the bleaching effect caused by sulfur dioxide (Joslyn & Braverman, 1954) has a significant effect on the flow rate, the Hunter color parameters of the samples were measured before UV treatment.

To examine the potential degradative effect of UV on potassium sorbate, juice containing 100 mg kg^{-1} of the additive was subjected to 5 consecutive passes (cumulative dose up to 70 mJ cm^{-2}), and the residual sorbate concentration was measured after each pass.

To assess the effect of ascorbic acid concentration on the reduction of *E. coli* ATCC 25922, two independent batches of apple juice containing ascorbic acid between 0 and 600 mg kg^{-1} were inoculated at 10^7 CFU ml^{-1} and subjected to UV. Two treatments were performed: (1) fixed flow rate of 214.5 ml s^{-1} (corresponds to the maximum pumping capacity of the UV machine and gives the minimum time of UV exposure in the reactor), and (2) fixed UV dose of 14 mJ cm^{-2}, with automatic flow rate adjustment. *E. coli* counts before and after treatment were determined following the protocol detailed in section 2.8. All experimental trials were conducted in triplicate. Due to the well reported antimicrobial properties of sorbate, benzoate, and sulfur dioxide, as well as the difficulty of segregating the bacterial reduction caused by preservatives from that caused by UV exposure, the effect of these preservatives on inactivation of *E. coli* during UV treatment was not evaluated here.

2.5. Total vitamin C and ascorbic acid determination

Ascorbic acid (AA) and total vitamin C concentration, defined as the sum of ascorbic acid and its oxidized form dehydroascorbic acid (DHA), were determined via HPLC with a modified version of the protocol described by Margolis, Paule, and Ziegler (1990). For total vitamin C quantification, DHA was reduced to AA by the addition of DTT, and measured in conjunction with the native and residual AA present in the juice.

A 50 mmol potassium phosphate monobasic solution, adjusted to a pH of 2.8 via phosphoric acid, was used as mobile phase. A stock solution of ascorbic acid was used to prepare 7 standard solutions between 25 and 600 mg kg^{-1} in HPLC-grade water. One ml of each standard solution was diluted with 400 μl of 5 mg ml^{-1} DTT,

200 μl of 4 g·100 mL^{-1} MPA, and 400 μl of HPLC-grade acetonitrile; filtered into amber autosampler vials with a nylon syringe filter (13 mm × 0.45 μm pore size; Krackeler Scientific, Albany, USA), and analyzed by HPLC. The calibration standard curve was performed in triplicate and used to determine total vitamin C and ascorbic acid concentrations.

A volume of 20 μl of standard solutions and samples were injected onto a Thermo Scientific Aquasil C_{18} endcapped column (250 mm × 4 mm id, 5 μm particle size, 100 nm pore size; Thermo Scientific, Waltham, USA) and resolved at a 1 ml min^{-1} flow rate in an isocratic run for 20 min at ambient column temperature (22–25 °C) with a detection wavelength of 254 nm. One ml of each sample was diluted in a 2.2 ml vial with 400 μl of 5 mg ml^{-1} DTT; vials were capped and vortex-mixed for 15 s. After 1 h of storage at room temperature in dark conditions, 200 μl of a 4 g·100 mL^{-1} MPA solution and 400 μl of acetonitrile were added. Samples were vortex-mixed and centrifuged at 1000 × g for 30 min at 4 °C. The supernatant fluid was filtered as the standards and analyzed on an Agilent 1100 series HPLC (Santa Clara, USA).

The ascorbic acid quantification was performed following the same procedure indicated for total vitamin C except that the addition of DTT and the incubation time after the addition of the reagent were omitted.

2.6. Potassium sorbate and sodium benzoate determination

A 20 g·100 mL^{-1} HPLC-grade acetonitrile in a 0.005 mol L^{-1} sulfuric acid solution was used as mobile phase. For the calibration standard curve a combined stock solution of sorbate and benzoate at a concentration of 1000 mg kg^{-1} of each reagent were used for preparing 6 standard solutions between 2 and 100 mg kg^{-1} for each compound. Standards were filtered as the standards used for total vitamin C quantification. The calibration curve was performed in triplicate.

A Bio-Rad Aminex HPX-87H column fitted with a micro-guard cation H refill cartridge (Bio-Rad, Hercules, USA) was used at 0.6 ml min^{-1} flow rate in an isocratic elution over 30 min at a column temperature of 60 °C. For the potassium sorbate determination, a detection wavelength of 260 nm was used whereas 230 nm was selected for sodium benzoate analyses. After a 10-fold dilution of the samples using HPLC-grade water, juices were filtered as the standards and analyzed in an Agilent 1100 series HPLC.

2.7. Free and total sulfur dioxide determination

A multi-channel segmented-flow analyzer (FIA) was used to determine free and total sulfur dioxide (Barril, Clark, & Scollary, 2012). The system comprises a FIAstar™ 5000 (FOSS, Höganäs, Sweden) wine analyzer and an autosampler that operated by the SoFIA software (service pack 3). A sample of additive-free apple juice was used as the blank. All measurements were performed in triplicate.

2.8. Microbiological analysis

E. coli ATCC 25922, a non-pathogenic surrogate with similar UV sensitivity as *E. coli* O157:H7 (Quintero-Ramos et al., 2004), was obtained from the Food Microbiology Laboratory at the New York State Agricultural Experiment Station (Geneva, USA). A single colony was transferred into 5 ml of TSB and grown for 5 ± 1 h at 35 ± 2 °C and then transferred into 500 ml of TSB incubated overnight for 20 ± 2 h at 35 ± 2 °C on a rotatory platform shaker (New Brunswick Scientific Co., Edison, USA) at 250 rpm. Approximately 1.8 L of apple juice was inoculated with 20 ml of bacterial suspension at 10^7 CFU ml^{-1}. Juice was aseptically sampled and analyzed by standard plate counting before and after UV treatment. Serial dilutions were prepared using sterile 0.1 g·100 mL^{-1} peptone water. Each dilution was plated in duplicate on Trypticase soy agar. Plates were incubated for 20 ± 2 h at 35 ± 2 °C. The log reduction of *E. coli* was calculated as the difference between the log-transformed counts before and after UV.

2.9. Physicochemical characterization

The pH, total titratable acidity (grams of malic acid per 100 ml of juice), total soluble solids content (degrees Brix), turbidity, color, and the apparent absorption coefficient of the juice were measured. The pH was determined using a calibrated Accumet Basic AB15 pH meter (Fisher Scientific, Pittsburgh, USA). Total soluble solids were measured with a Leica Auto Abbe refractometer model 10500-802 (Leica Inc., Buffalo, USA). Total titratable acidity was measured using a G20 compact titrator (Mettler Toledo, Schwerzenbach, Switzerland). Turbidity measurements were performed in a HACH 2100P portable turbidimeter (Hach Company, Loveland, USA). Color parameters L′, a', and b' were determined using the reflectance-specular included (RSIN) mode in a Hunter UltraScan XE spectrocolorimeter (Hunter Lab Assoc., Reston, USA). The juice apparent absorption coefficient (α) was obtained following the protocol described by Koutchma et al. (2004), where α corresponds to the slope obtained from plotting the sample absorbance against the path length. After a 10-fold dilution in distilled water, the sample absorbance was measured at 254 mm with a UV-1800 spectrophotometer (Shimadzu Scientific Instruments, Columbia, USA) equipped with fused quartz cuvettes (NSG Precision Cells, INC., Farmingdale, USA). All physicochemical determinations were executed in triplicate.

2.10. Statistical analyses

Multiple linear regression analyses were used to assess the relationship between additives concentration and juice absorption coefficient, as well as the juice flow rate. This statistical analysis was also used to determine the effect of UV on the juice's absorption coefficients and additives concentration. The effect of sulfur dioxide on juice's color attributes, and the effects of UV dose on the stability of potassium sorbate, and the ascorbic acid concentration on the log reduction of *E. coli* were determined using analysis of variance

Table 1
Physicochemical characterization of reconstituted apple juices before the addition of additives (mean ± standard deviation, n = 3).

Additive	pH	Total soluble solids (°Brix)	Titratable acidity (g malic acid/100 mL)	Color			Turbidity (NTU)	Apparent absorption coefficient (mm^{-1})
				L′	a'	b'		
Ascorbic acid	3.61 ± 0.03	12.80 ± 0.20	0.40 ± 0.02	31.1 ± 0.3	5.3 ± 0.3	6.7 ± 0.5	18 ± 3	1.5 ± 0.2
Potassium sorbate	3.67 ± 0.01	12.93 ± 0.02	0.40 ± 0.02	30.7 ± 0.1	5.5 ± 0.2	7.1 ± 0.1	18 ± 1	2.00 ± 0.06
Sodium benzoate	3.56 ± 0.01	12.89 ± 0.04	0.375 ± 0.002	30.71 ± 0.01	5.4 ± 0.1	7.1 ± 0.3	26.5 ± 0.7	1.94 ± 0.04
Sulfur dioxide	3.30 ± 0.01	12.37 ± 0.03	0.63 ± 0.04	55.1 ± 0.2	4.69 ± 0.05	36.8 ± 0.03	7.0 ± 0.1	0.81 ± 0.07

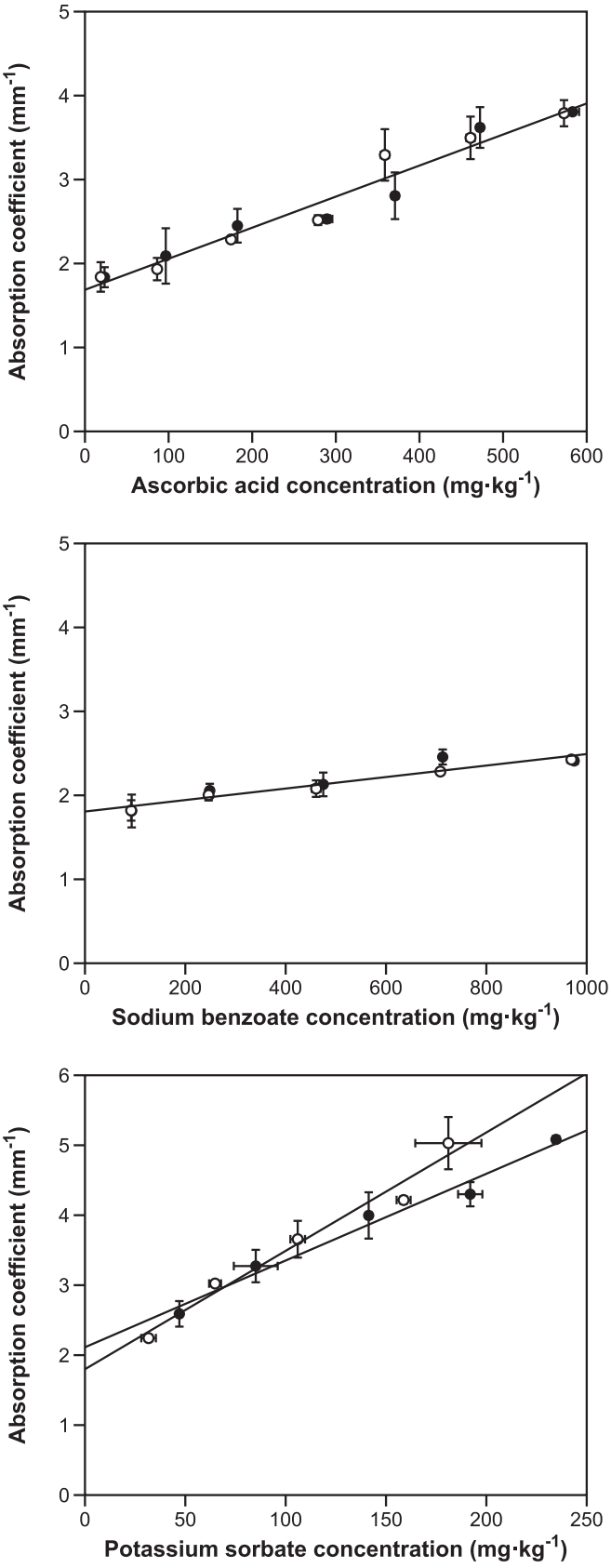

Fig. 1. Apparent absorption coefficient at 254 nm before (●) and after (○) UV treatment at 14 mJ cm^{-2} UV dose as a function of the concentrations of ascorbic acid (absorption coefficient = 0.0037 · concentration + 1.69, r^2 = 0.9083), sodium benzoate (absorption coefficient = 0.00068 · concentration + 1.81, r^2 = 0.8009) and potassium sorbate (before UV: absorption coefficient = 0.012 · concentration + 2.11, r^2 = 0.9378; after UV: absorption coefficient = 0.017 · concentration + 1.80, r^2 = 0.9470) (n = 3, error bars show standard deviation).

(ANOVA). Means were further compared using Tukey's honestly significant difference (HSD) test at a significance level of 0.05. Statistical analyses were performed using JMP® version 10 (SAS Institute, Cary, USA).

3. Results and discussion

3.1. Effect of additives on juice absorption coefficient and flow rate during UV processing

Table 1 shows the physicochemical characteristics of the apple juices used in the study. Since for each additive a new batch of juice was prepared, certain characteristics differ among batches. All replicates performed for each additive were executed using the same batch of juice and all batches had 11.5 °Brix, the minimum Brix level established by FDA for apple juice (FDA, 2015).

Linear relationships between ascorbic acid, sorbate, and benzoate concentrations, and the juices' apparent absorption coefficients were observed (Fig. 1). Only in the case of potassium sorbate a significant effect of UV on the relationship between concentration and absorption coefficient was observed. Accordingly, an increase in the concentrations of these three additives slowed down the juice flow rates during UV processing (Fig. 2). This is explained because the UV reactor was programmed to adjust the flow rate to ensure a constant UV dose. The flow rate of the juice through the machine was slowed down to compensate for the increase in the UV absorption coefficient due to additives addition.

A multiple linear regression analysis of the effect of the square root of the concentration of additives on the flow rate (Fig. 2) showed that increases in concentrations of sorbate and ascorbic acid caused a more pronounced decrease in the flow rate compared to sodium benzoate. Moreover, no significant differences in flow rate were observed for samples containing increasing concentrations of sorbate and ascorbic acid (p = 0.21). The resulting model presented a coefficient of determination (r^2) of 96%.

The juice's apparent absorption coefficient was not significantly altered by the addition of sulfur dioxide (p = 0.37) and therefore the flow rate was not affected by increasing levels of this preservative (p = 0.66). Differences among juices' absorption coefficients due to the addition of different compounds depend on the nature of each additive and its UV absorbing properties. Colored compounds, organic solutes, and suspended matter have been reported as UV absorbent (Koutchma, Forney, & Moraru, 2009). Absorption depends on the UV energy absorbance of photons by the reactant molecules, which has a direct relationship with the chemical bonds present in each molecule (Koutchma et al. 2009). Thus, differences in molecular bonds and bond energy, and the presence of aromatic rings (such as sodium benzoate) in the different additives tested explain the reported absorbance disparities.

Although the addition of sulfur dioxide did not affect the juice's absorption coefficient, the evaluated concentrations of free sulfur dioxide (0–160 mg kg^{-1}) caused a significant increase in lightness (p = 0.0001) and the b' color parameter (p = 0.02). Also, a significant detrimental effect on the color parameter a' (p = 0.0001) was found (Table 2). Likewise, Basaran-Akgul et al. (2009) observed a lightening of apple cider color due to the addition of sulfur dioxide and its bleaching action upon non-enzymatic browning pigments (Roberts & McWeeny, 1972). Even though sulfur dioxide leads to

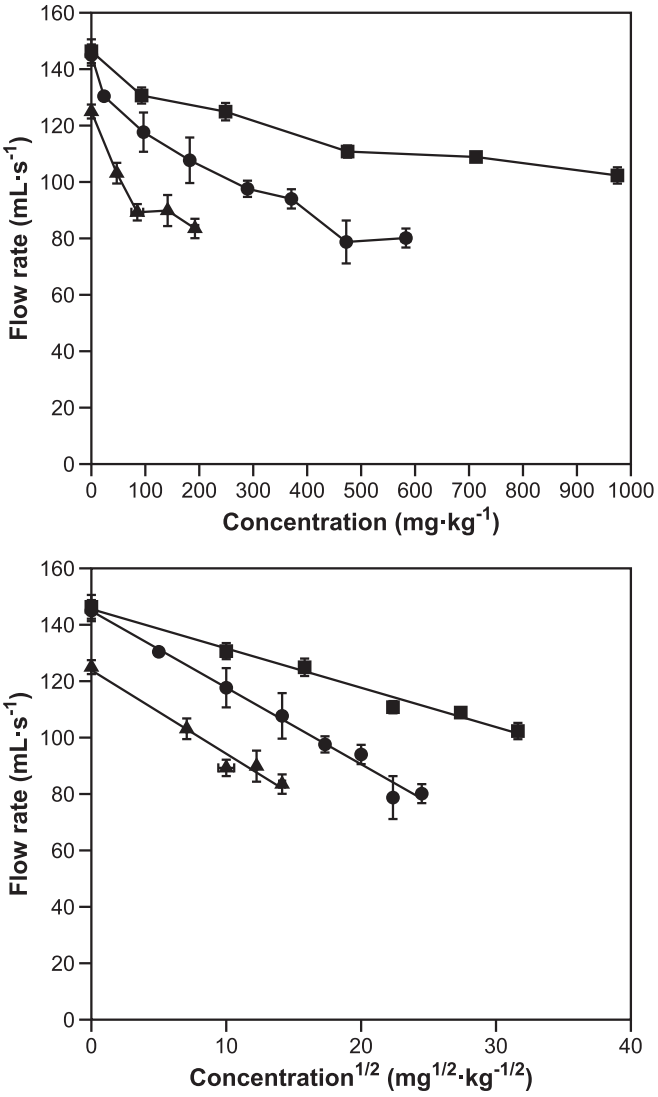

Fig. 2. Flow rate as a function of the concentration and the square root of the concentration of apple juice containing ascorbic acid (●, flow = −2.8 · concentration$^{1/2}$ + 144.7, r^2 = 0.9495), sodium benzoate (■, flow = −1.4 · concentration$^{1/2}$ + 145.5, r^2 = 0.9595) and potassium sorbate (▲, flow = −3.0 · concentration$^{1/2}$ + 123.5, r^2 = 0.9193) and treated with a 14 mJ cm^{-2} UV dose (n = 3, error bars show standard deviation).

significant changes in the visible spectrum, it did not impact the samples' UV absorption. This explains the lack of effect on flow rate for juices containing sulfur dioxide. Hence, color measurements do not represent the best physicochemical parameters to determine the feasibility of UV treatment of beverages; instead, the determination of absorption coefficient is recommended.

3.2. Effect of UV on the stability of the selected additives

The apparent absorption coefficients of samples with increasing concentrations of ascorbic acid, benzoate (Fig. 1), and sulfur dioxide were not significantly affected by the single-pass UV process ($p > 0.05$). Instead, changes were observed in juices containing potassium sorbate, where a marked effect of UV on the juices' absorption coefficients was observed – i.e. a significant increase ($p = 0.0003$) in the slope of the relationship between sorbate concentration and absorption coefficient after UV (Fig. 1). Total vitamin C and benzoate concentrations were not adversely affected by the UV treatment ($p > 0.05$). However, the concentrations of free sulfur dioxide and ascorbic acid slightly decreased after UV processing, as indicated by significant differences in the linear relationships between nominal and measured concentrations before and after UV (Fig. 3). Significantly different intercepts for ascorbic acid ($p = 0.0005$), and intercepts ($p = 0.0001$) and slopes ($p = 0.007$) for free sulfur dioxide were observed.

Table 2
Apple juice color parameters in samples containing increasing concentrations of free sulfur dioxide before UV radiation at 14 mJ cm^{-2} UV dose (mean ± standard deviation, n = 3).[a]

Free sulfur dioxide – nominal concentration (mg·kg^{-1})	L′	a′	b′
0	55.1^{c} ± 0.2	4.69^{a} ± 0.05	36.8^{b} ± 1
60	56.5^{ab} ± 0.3	3.60^{b} ± 0.04	37.4^{ab} ± 0.7
75	56.4^{b} ± 0.1	3.50^{c} ± 0.02	37.2^{ab} ± 0.5
110	56.8^{ab} ± 0.3	3.38^{d} ± 0.03	37.5^{ab} ± 1
130	57.3^{ab} ± 0.4	3.37^{d} ± 0.02	37.6^{ab} ± 0.2
160	57.4^{a} ± 0.4	3.27^{e} ± 0.03	37.7^{a} ± 1

[a] Means not followed by the same superscript within the columns are significantly different ($p < 0.05$).

Sulfur dioxide occurs in two forms in juices: free (inorganic forms, including SO_2 and HSO_3) and bound (fixed to organic compounds). Since the free fraction is responsible to prevent oxidation and microbial development (Santos, Nunes, Saraiva, & Coimbra, 2012), the effect of UV on this form was reported (Fig. 3). However, the total sulfur dioxide content was also determined and the same trend reported for the free form was observed (data not shown). Total sulfur dioxide concentration slightly decreased after UV when nominal and measured concentrations were compared, showing significantly different slopes ($p = 0.046$).

Chemical and biochemical degradation of ascorbic acid occurs through the pathway from ascorbic acid to DHA to diketogulonic acid, the first reaction being reversible and the second irreversible (Margolis et al., 1990). The reported differences between total vitamin C and ascorbic acid concentrations suggest that even though DHA is being produced via ascorbic acid degradation due to UV exposure, as this reaction is reversible, no significant differences are detected in terms of total vitamin C. Contrarily, Koutchma & Shmalts (2002), Tran and Farid (2004), and Tikekar, Anantheswaran, and LaBorde (2011) demonstrated that UV induces vitamin C degradation of 17–40%. Nevertheless, those studies evaluated considerably higher UV doses (600 mJ cm^{-2}, 100 mJ cm^{-2}, and 1.2–1.8 mW cm^{-2}, respectively), which may cause vitamin C degradation due to heat (indirectly caused by UV application) and other factors. Also, in the mentioned studies, the total vitamin C concentration was considered equivalent to the ascorbic acid concentration, and the concentration of DHA was not reported. Additionally, the degradation of ascorbic acid can be influenced by other juice physicochemical factors such as pH, organic acids concentration, and absorbance (Tikekar et al., 2011). All these factors likely contribute to the differences between previously reported results and the data shown here.

The most marked effect of UV on the stability of added preservatives was observed for potassium sorbate, as evidenced by significantly different slopes ($p < 0.0001$) and intercepts ($p = 0.0003$) of the linear relationships between nominal and measured concentrations before and after UV (Fig. 3). As seen, an important degradation of sorbate was produced after processing. Consequently, a derivative compound was found after UV (Fig. 4). This effect was UV-dose dependent (Fig. 5). The presence of this

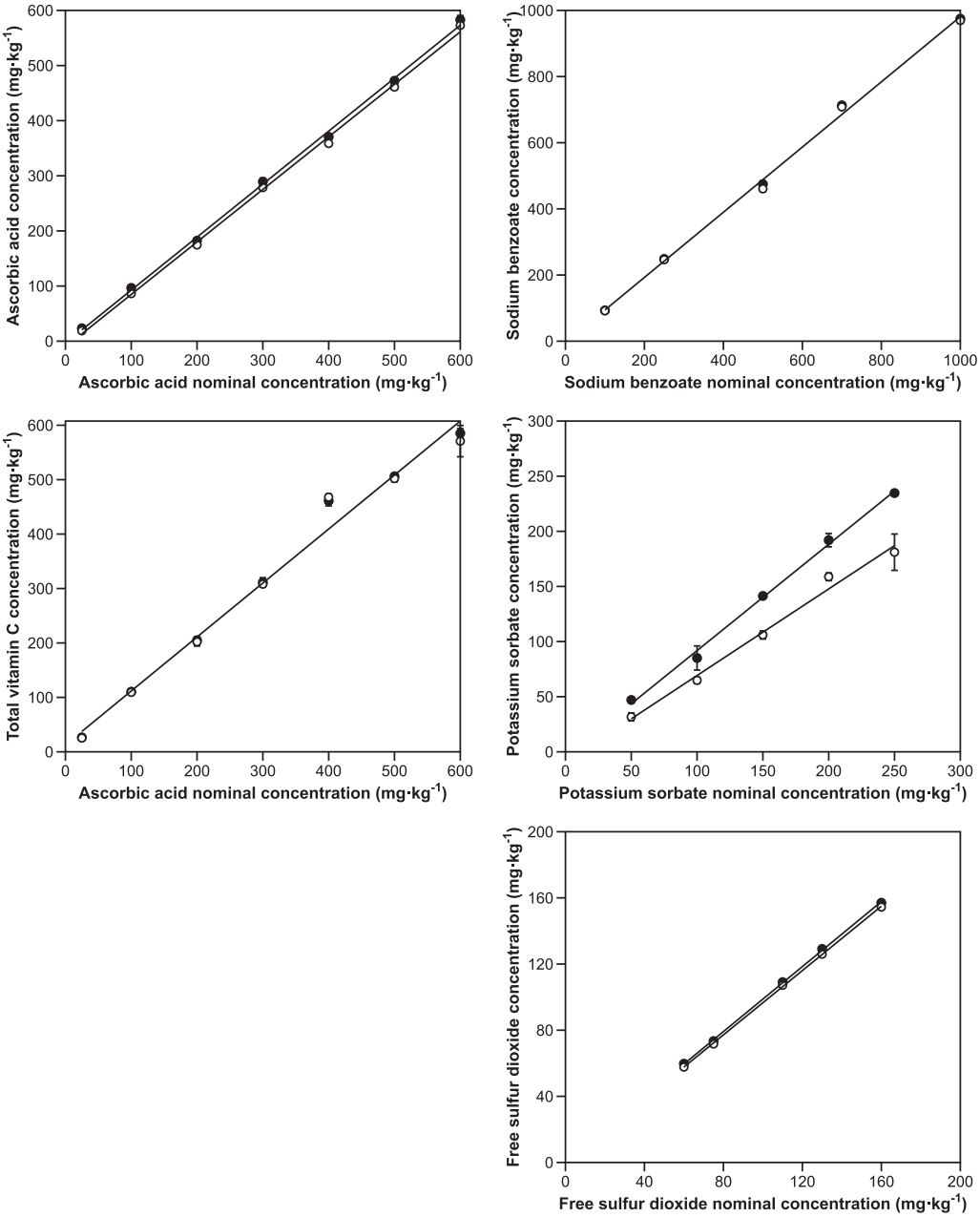

Fig. 3. Concentrations of selected additives in apple juice, before (●) and after (○) UV treatment at 14 mJ cm^{-2} UV dose: ascorbic acid (before UV: concentration = 0.96 · nominal concentration − 3.6, r^2 = 0.9983; after UV: concentration = 0.95 · nominal concentration − 10.6, r^2 = 0.9982), sodium benzoate (concentration = 0.99 · nominal concentration − 4.3, r^2 = 0.9973), total vitamin C (concentration = 0.99 · nominal ascorbic acid concentration − 12.6, r^2 = 0.9827), potassium sorbate (before UV: concentration = 0.96 · nominal concentration − 4.6, r^2 = 0.9922; after UV: concentration = 0.79 · nominal concentration − 9.3, r^2 = 0.9748), free sulfur dioxide (before UV: concentration = 0.98 · nominal concentration − 0.6, r^2 = 0.9994; after UV: concentration = 0.97 · nominal concentration - 0.7, r^2 = 0.9992) (n = 3, error bars show standard deviation).

UV-derivative was likely the cause for the significant effect of UV on the juice absorption coefficient (p = 0.0002) (Fig. 1). Cigić et al. (2001) reported that potassium sorbate isomerizes under UV, which affects its antimicrobial activity. In this study, the phenomenon was also observed when apple juice was exposed to UV at milder intensities and under shorter exposure times, using a commercial UV reactor, thus it could have unintended negative effects for commercially relevant applications. Though, further studies are needed to confirm the nature of the derivative compound and the impact of this degradation on the antimicrobial properties of sorbate.

3.3. Effect of ascorbic acid concentration on the log reduction of *E. coli*

Increasing the concentration of ascorbic acid led to a significant negative effect on the inactivation of *E. coli* when UV was applied at a constant flow rate of 214.5 ml s^{-1} ($p < 0.0001$) and at a fixed dose of 14 mJ cm^{-2} ($p < 0.0001$). The observed trends varied depending on the treatment (Fig. 6). When juices were treated at a fixed flow rate, results were in agreement with the findings reported by Koutchma et al. (2004), where the inactivation rate of *E. coli* decreased as the solution absorbance increased, and it was

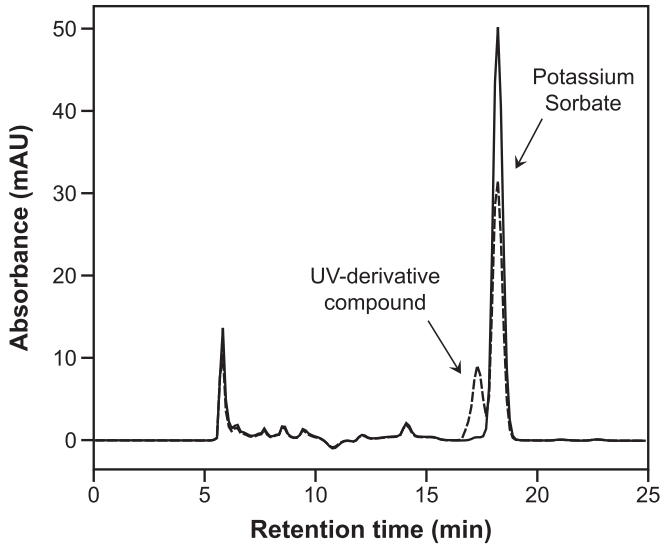

Fig. 4. Representative HPLC chromatogram (260 nm) for apple juice containing potassium sorbate at 100 mg kg^{-1} and treated at 14 mJ cm^{-2} UV dose, before (solid line) and after (dashed line) UV treatment.

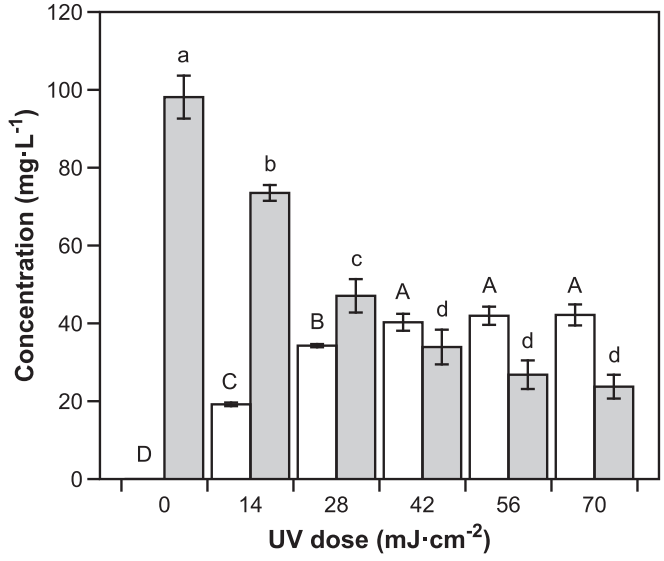

Fig. 5. Remaining concentration of potassium sorbate (grey bars) and the derivative UV product (white bars) as a function of UV exposure. Means with the same lower or uppercase letter are not significantly different (Tukey's test $p > 0.05$) (n = 3, error bars show standard deviation).

inversely proportional to the apparent absorption coefficient; suggesting that UV treatment of juices should not be applied at a fixed flow rate to achieve the greater than 5-log reduction of the pertinent pathogen (FDA, 2001), because at ascorbic acid concentrations above 100 mg kg^{-1}, the achieved reduction was lower than the required microbial inactivation. Instead, when UV treatment was performed at a fixed dose of 14 mJ cm^{-2}, a higher than 5-log reduction was achieved for samples containing up to 300 mg kg^{-1} ascorbic acid. In this study it was not possible to UV process juice with ascorbic acid concentrations exceeding 600 mg kg^{-1} because the pump was not able to adequately reduce the flow rate and therefore the machine automatically stopped since the validated dose of 14 mJ cm^{-2} was not achieved.

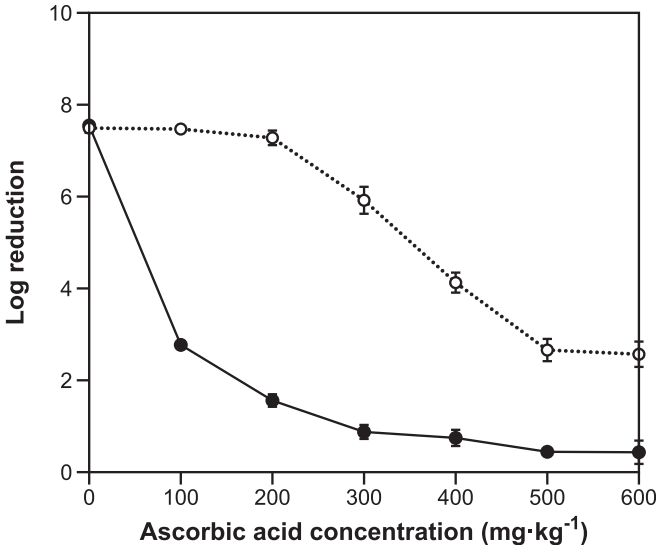

Fig. 6. Log reductions of *E. coli* ATCC 25922 in apple juice treated under fixed flow rate (●, 214.5 mL s^{-1}) and fixed UV dose (○, 14 mJ cm^{-2}) (n = 3, error bars show standard deviation).

4. Conclusions

Although the addition of certain additives represents a viable option to ensure the safety and extend the shelf life of UV-treated beverages, our study demonstrated that ascorbic acid, benzoate, and sorbate, additives commonly used by the juice industry, increase the juice's absorption coefficient and negatively interfere with the performance of UV. Furthermore, under the studied conditions, UV leads to a degradation of ascorbic acid, sulfur dioxide and potassium sorbate, while the addition of ascorbic acid adversely impaired the inactivation of *E. coli*. Therefore, additives that increase the absorption coefficient of liquid food products or are unfavorably affected by UV light should be added after UV treatment.

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