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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,