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Generation of oxidative species from ultraviolet light induced photolysis of fructose

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ABSTRACT

Fructose has shown significant reactivity during ultraviolet light (UV, 254 nm) processing of fruit juices that can adversely affect product quality. The present study demonstrates that this reactivity of fructose is due to the oxidative nature of products formed from UV induced photolysis of fructose. This was accomplished using fluorescein, a fluorescent dye that loses fluorescence intensity upon reaction with oxidative species. Fructose caused a concentration dependent decay of fluorescence from fluorescein only in presence of UV, indicating oxidative nature of photolysis products of fructose. The transient oxidative species including free radicals and not one of the final photolysis products, furan, were responsible for fluorescence decay. Addition of an antioxidant and removal of oxygen from solution lowered the rate of fluorescence decay, suggesting strategies that can be employed to lower the deleterious effects of fructose on products. The understanding developed can be used to optimise UV processing of juices.

1. Introduction

Ultraviolet light (UV) processing of juices has emerged as an attractive alternative to heat pasteurisation, due to its effectiveness in inactivating bacteria and enhanced retention of flavour and nutritional attributes (Koutchma, Keller, Chirtel, & Parisi, 2004; Pala & Toklucu, 2013; Tran & Farid, 2004). It is a relatively cost-effective method, performed by exposing the juice product to 254 nm UV light. Absorption of UV light by the DNA results in cross-linking between the pyrimidine nucleoside bases (thymine and cytosine) within the DNA strand, which leads to the cell death (Donahue, Canitez, & Bushway, 2004). Previous studies have established that the effectiveness of UV processing in inactivating microorganisms is significantly affected by processing variables such as UV intensity, flow patterns and juice properties such as its absorbance coefficient and turbidity (Donahue et al., 2004; Guerrero-Beltrán & Barbosa-Cánovas, 2004; Keyser, Műller, Cilliers, Nel, & Gouws, 2008; Koutchma et al., 2004).

Fructose, a sugar commonly found in fruits and their juices such as apples (5.9%), mangoes (4.68%) oranges (2.25%) and tomatoes (3.56%) (USDA, 2011), has demonstrated a significant reactivity upon exposure to 254 nm UV light. Previous studies have reported the UV exposure of fructose resulted in the formation of furan in a model system and fruit juice (Bule et al., 2010; Fan & Geveke, 2007). Another study showed that UV exposure to a 30% fructose solution resulted in significant changes in the pH and colour of the fructose solution (Orlowska et al., 2013). They attributed these changes to possible photochemical reactions of fructose upon UV exposure. In previous studies, we demonstrated that UV induced degradation rates of patulin and ascorbic acid in a model apple juice system significantly increased in the presence of fructose (Tikekar, Anantheswaran, & LaBorde, in press; Tikekar, Anantheswaran, Elias, & LaBorde, 2011; Tikekar, Anantheswaran, & LaBorde, 2011). The phenomenon of UV induced reactivity was specific to fructose and sugars such as glucose and sucrose did not show a significant reactivity under UV light (Fan & Geveke, 2007; Tikekar et al., in press; Tikekar, Anantheswaran, & LaBorde, 2011). This unique reactivity of fructose upon exposure to UV light has not been extensively studied. To the best of authors' knowledge, only one prior study by Triantaphylides and others investigated the mechanism of fructose reactivity under UV light and its potential degradation products (Triantaphylides, Schuchmann, & von Sonntag, 1982). The authors in this previous study attributed the unique reactivity of fructose under UV light to its relatively more reactive chain conformation which amounts to 0.8% of total fructose in solution. They suggested that upon UV exposure, fructose

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molecules in chain conformation undergo photolysis, leading to the formation of hydroxyalkylacyl and hydroxyalkyl radicals via a Norrish type 1 reaction. These free radicals subsequently react with atmospheric oxygen to produce a number of free radicals including peroxyl radicals (Triantaphylides et al., 1982). In previous studies, we hypothesised that due to their oxidative nature, these free radicals maybe responsible for the observed rate accelerating effect of fructose on the UV induced degradation of patulin and ascorbic acid (Tikekar et al., in press; Tikekar, Anantheswaran, & LaBorde, 2011). Glucose did not show this UV reactivity due to its more stable ring conformation resulting in only 0.024% glucose being present in the chain form (Binkley & Binkley, 1998). The reactivity of photolysis products of fructose can have significant implications in product quality, since it involves formation of free radicals that can be oxidative and non-specific in their reactions. These free radicals can oxidise vitamins, health-benefitting antioxidants and aroma compounds, thereby affecting the nutritional and sensory properties of the product. Therefore, there is a need to characterise the nature of fructose reactivity under UV light.

The objective of this study was to demonstrate the oxidative nature of UV induced photolysis products of fructose using fluorescein as a model fluorescent reporter dye that loses its fluorescence intensity upon reaction with oxidative species including free radicals such as hydroxyl and peroxyl radicals (Ou et al., 2002; Ou, Hampsch-Woodill, & Prior, 2001). Factors such as presence of oxygen and antioxidant on oxidative properties of photolysis products of fructose were also evaluated. The results of this study will enhance understanding of the impact of fructose on the quality of UV processed juice products and aid in development of strategies to minimise its impact.

2. Materials and methods

2.1. Materials

Fructose, sucrose, glucose, sodium salt of fluorescein, furan, ascorbic acid, 30% (w/w hydrogen peroxide were obtained from Sigma Aldrich (St. Louis, MO). Monosodium phosphate monohydrate and disodium phosphate, heptahydrate were obtained from JT Baker® (Aston, PA).

2.2. UV processing unit

A batch-UV processing unit (Spectronics Spectrolinker XL-1500 UV Crosslinker, Westbury, NY) was used for all the experiments. The apparatus consisted of 5 UV lamps (254 nm, 15 W, Spectronics Corporation, Westbury, NY) that generated UV intensity of approximately 20 mW/cm^2 at the surface of exposure mounted within a shielded box (46.4 × 15.9 × 31.8 cm) Variation in incident intensity was minimised by allowing the lamps to warm up for at least 15 min prior to treatments.

2.3. Experimental design and fluorescence measurement

Test solution consisted of approximately 1 μM fluorescein solution prepared in deionized water (pH 6.3) or 100 mM buffer at pH 6. Effect of various compounds on the rate of decay of fluorescence from fluorescein was investigated by dissolving these compounds individually in the fluorescein solution and exposing it to the UV light. Treatments were carried out by adding 10.0 ml solution into an uncovered glass petri dish and exposing it to UV radiation for various amounts of time (0–12 min) in the UV processing unit. The samples in petri-dish were stirred to achieve uniform exposure of sample to UV light. Whenever stored outside the UV chamber, samples were covered with aluminium foil to minimise interaction with ambient light. Ambient room temperature (20–22 °C) was used for all experiments. To measure the fluorescence intensity of the solution, at each time interval, 100 μl of the sample was pipetted from the petri dish into a well of 96-well plate optimised for fluorescence measurement. Fluorescence was measured in a Gemini XPS fluorescence micro-plate reader (Molecular Devices, Sunnyvale, CA) with an excitation and emission wavelengths of 485 nm and 510 nm, respectively. All the fluorescence values were normalised using Eq. (1):

$$\text{Relative fluorescence intensity} = \frac{100 \times I_t}{I_0} \quad (1)$$

where, I_0 = fluorescence intensity at time t = 0 min and I_t = fluorescence intensity after 't' minute of UV exposure.

2.4. Effect of sugars and their decomposition products

To examine the effect of sugars such as fructose, glucose and sucrose on the fluorescence decay rate of fluorescein upon exposure to UV light, each of the sugars was separately dissolved in 1 μM fluorescein solution at the level of 263 mM for sucrose (9% w/v) and 500 mM of glucose and fructose (9% w/v). These solutions were subsequently exposed to UV light for up to 12 min. To investigate the role of concentration of fructose on fluorescence decay rate fluorescein, 10, 20, 100, 300 and 500 mM fructose was dissolved in 1 μM fluorescein solution and exposed to UV light. Previous studies have shown that UV exposure of fructose formed furan (Bule et al., 2010; Fan & Geveke, 2007). To investigate its role on fluoresce decay rate, in a separate experiment furan was added to fluorescein solution at 33, 66 and 132 μM levels prior to UV exposure.

2.5. Effect of antioxidant and oxygen

To investigate the effect of added antioxidant on the rate of fluorescence decay, ascorbic acid (AA) was added at concentrations of 25 and 50 μM to 1 μM fluorescein solution containing 20 mM fructose prepared in 100 mM phosphate buffer (pH 6). The solutions were prepared in phosphate buffer to minimise pH changes after addition of ascorbic acid.

To investigate the role of dissolved oxygen on the generation of oxidative species from photolysis of fructose, experiments were performed in absence of atmospheric oxygen. Quartz cuvettes were filled with 1 μM fluorescein solution containing 500 mM fructose and exposed to nitrogen for 5 min and immediately sealed. These sealed quartz cuvettes were subsequently exposed to UV light for 60 s and fluorescence of solution was measured. The control for this experiment consisted of 1 μM fluorescein solution containing 20 mM fructose filled in quartz cuvettes exposed to UV light without prior nitrogen purging.

2.6. Quantitative comparison of oxidative effect of UV exposed fructose with a known oxidiser

The oxidative effect of fructose on fluorescein was quantitatively compared with hydrogen peroxide, a compound known to produce oxidative species upon exposure to UV light (Nienow, Bezares-Cruz, Poyer, Hua, & Jafvert, 2008; Pereira, Weinberg, Linden, & Singer, 2007). Hydrogen peroxide was added to 1 μM fluorescein solution to a final concentration of 294 μM (0.001% w/v). This solution was subsequently exposed to UV light and fluorescence of the sample was measured at an interval of 10 s. Fructose was added at a level of 100 mM to 1 μM fluorescein solution and the experiment was performed in a similar manner. The plot of % relative fluorescence against time was plotted against the duration of UV