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Generation of reactive oxidative species from thermal treatment of sugar solutions

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ABSTRACT

Sugars, prominently fructose, have been shown to accelerate the degradation of food components during thermal treatment. Yet, the mechanism by which this occurs is not well understood. Fructose and glucose have been reported to undergo autoxidation to generate reactive oxidative species (ROS) under physiological conditions; however, information on ROS generation during thermal treatment is limited. We observed that hydrogen peroxide was generated during thermal treatment (up to 70 °C) of aqueous solutions of fructose and glucose (up to 10% w/v), with significantly higher concentrations observed in fructose solutions. The rate of generation of hydrogen peroxide increased with temperature, pH, oxygen concentration and the presence of phosphate buffer. Singlet oxygen was also detected in fructose and glucose solutions prepared in phosphate buffer. Results of this study indicated that fructose and glucose undergo oxidation during thermal treatment resulting in generation of ROS that may have deleterious effects on food components.

1. Introduction

Thermal processing is the most widely used method of food preservation (Awuah, Ramaswamy, & Economides, 2007). However, it also results in quality losses due to undesirable chemical degradation reactions. Fructose and glucose are the most abundant simple sugars in many fruits and vegetables, and have been shown to accelerate the degradation of food components such as anthocyanin in currant, elderberry, strawberries, and blood orange juice and ascorbic acid in aqueous systems during thermal treatment (Cao et al., 2009; Hubbermann, Heins, Stöckmann, & Schwarz, 2006; Markakis, 1982; Rojas & Gerschenson, 1997, 2001; Rubinskiene, Viskelis, Jasutiene, Viskeliene, & Bobinas, 2005). One study reported that fructose (360 g/L) and glucose (270 g/L) accelerated ascorbic acid degradation in an aqueous model system (pH 3.5) that mimics concentrated fruit juices at treatment temperatures of 70, 80 or 90 °C, and fructose showed a more pronounced effect than glucose (Rojas & Gerschenson, 2001). Another study showed that anthocyanin degradation was accelerated by glucose, sucrose and fructose in a model blood orange juice system (pH 3.5). Fructose, which caused a 67% degradation of anthocyanins, had a more pronounced effect than glucose (~59% degradation) and sucrose (~45% degradation) at 90 °C after 3 h (Cao et al., 2009). Previous studies have attributed these effects to the formation of degradation products such as furfural at high temperature. However, a clear understanding of the mechanisms underlying these deleterious effects is still lacking.

Simple sugars such as glucose and fructose have been shown to undergo slow, metal-catalyzed oxidative degradation processes, with the simultaneous generation of ROS (superoxide, hydrogen peroxide, hydroxyl radicals) under physiological conditions (Hunt, Dean, & Wolff, 1988; Lawrence, Mavi, & Meral, 2008; Thornalley & Stern, 1984; Thornalley, Wolff, Crabbe, & Stern, 1984; Wehmeier & Mooradian, 1994; Wolff & Dean, 1987). These studies showed that monosaccharides such as fructose and glucose could be a source of oxidative stress. They are prone to reduce transition metal and molecular oxygen, generating hydrogen peroxide, reactive intermediates, such as the hydroxyl radical, and ketoaldehydes. Based on these previous studies, it is possible that sugars can generate ROS in food systems during thermal treatment as well, which can have a deleterious effect on food components. However, most of the studies reporting on the generation of ROS from sugars were carried out at room temperature or under physiological conditions. Studies on the generation of ROS from sugars at elevated temperature are still limited.

The objective of this study was to investigate whether ROS were generated from sugars such as glucose, fructose and sucrose at ele-

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vated temperatures, and to determine the factors that impact the rate of generation of these species. To test this hypothesis, the generation of hydrogen peroxide from thermal treatment of sugar solutions was evaluated. The generation of singlet oxygen during thermal treatment of sugar solutions was also investigated because singlet oxygen is typically produced from a reaction between the triplet state oxygen (the most abundant form of oxygen) and another excited triplet state molecule through triplet–triplet annihilation mechanism (Min & Boff, 2002). Singlet oxygen is frequently observed when other oxidative free radicals are also detected. Given the ability of singlet oxygen to rapidly oxidize food components such as lipids, amino acids and vitamins at a rate significantly higher than the triplet state oxygen (Min & Boff, 2002), it is important to characterize its generation in thermally processed foods. However, scarce information is currently available regarding generation of singlet oxygen during thermal treatment of sugar solutions. Additionally, the effect of factors such as sugar type (fructose, glucose or sucrose), pH (5–7.5), temperature (24–70 °C), the presence of phosphate buffer and the presence of oxygen on the generation of ROS was also investigated.

2. Materials and methods

2.1. Materials

Fructose, glucose, sucrose, hydrochloric acid, sodium hydroxide, methanol, xylenol orange sodium salt, ferrous sulfate, ferric chloride, sorbitol and sulfuric acid were obtained from Sigma–Aldrich™ (St. Louis, MO, USA) and Fisher Scientific™ (Pittsburgh, PA, USA). Sodium phosphate monobasic monohydrate crystal and sodium phosphate dibasic 7-hydrate crystal were obtained from JT Baker® (Aston, PA). Singlet Oxygen Sensor Green (SOSG) was ordered from Life Technologies™ (Carlsbad, CA).

2.2. Sample preparation and thermal treatment

Sugar solutions were prepared by dissolving fructose, glucose or sucrose separately in distilled water or 100 mM phosphate buffer with a final concentration of 10% w/v at pH 6.7. An aliquot (10 mL) of each test solution was placed in a 15 mL glass test tube and heated in a water bath (Isotemp 205, Fisher Scientific™, USA) at 50 °C or 70 °C for up to 40 min (the time required for the solution to reach the set temperature was approximately 3 min). Samples were obtained periodically for quantification of ROS species.

2.3. Quantification of hydrogen peroxide

Generation of hydrogen peroxide from thermal treatment of sugar was investigated by ferrous ion oxidation xylenol orange (FOX) method with some modifications (Jiang, Woollard, & Wolff, 1990). This method was selected due to its high sensitivity and reproducibility (Cao et al., 2009; Jiang et al., 1990). It is based on the ability of hydrogen peroxide to convert ferrous ions into ferric ions which can form a complex with xylenol orange (XO), the concentration of which is determined using spectrophotometry (Bou, Codony, Tres, Decker, & Guardiola, 2008). Assay solution (100 μL) containing 1 mM xylenol orange, 2.5 mM ferrous sulfate, 1 M sorbitol, and 250 mM sulfuric acid was added to 700 μL samples of sugar (fructose, glucose or sucrose) solution prepared in distilled water or phosphate buffer. After 30 min of incubation at room temperature, the absorbance of solutions was measured at 560 nm with a UV–Vis plate-reader (Molecular Devices, Sunnyvale, CA). Hydrogen peroxide concentrations were determined based on an external standard curve (Supporting information, Fig. S1).

To investigate the role that oxygen played in hydrogen peroxide generation, sugar solutions were bubbled with oxygen while heating at 70 °C for 20 min. Hydrogen peroxide production was measured by the FOX method at intervals of 4 min. Distilled water heated with oxygenation as well as sugar solution heated in the presence of atmospheric oxygen (without oxygenation) were used as controls.

2.4. Dissolved oxygen analysis

Oxygen consumption during the thermal treatment of sugar solutions was measured using a dissolved oxygen meter (Orion 4 Star™, Thermo Scientific™, USA). A 50 mL polypropylene test tube was filled with sugar (fructose or glucose) solutions prepared in either water or phosphate buffer, and heated in a water bath at 50 °C or 70 °C. At an interval of 10 min, samples were cooled down to about 40 °C, and dissolved oxygen was measured. After each measurement, the test tube was immediately covered and put back into the water bath. All oxygen consumption values were calculated using Eq. (1)

$$\text{Oxygen consumption} = (C_0 - C_t)_{\text{sample}} - (C_0 - C_t)_{\text{blank}} \quad (1)$$

where C_0 = dissolved oxygen concentration at time t = 0 min and C_t = dissolved oxygen concentration after 't' minute thermal treatment.

2.5. Detection of singlet oxygen

Singlet Oxygen Sensor Green (SOSG) reagent® was used as a singlet oxygen detector. It is a highly selective probe for detecting singlet oxygen that produces fluorescent SOSG endoperoxides (SOSG-EP) upon reaction with singlet oxygen (Lin et al., 2013). Stock solution of 5 mM concentration of SOSG was prepared by dissolving the reagent in methanol. The test solution, consisting of 10% w/v fructose, glucose or sucrose in 100 mM phosphate buffer (pH 6.7) and approximately 5 μM SOSG was prepared. Next, 5 mL of the test solution was transferred into a test tube and placed either in a water bath (Isotemp 205, Fisher Scientific™, USA) set at 50 °C or 70 °C, or incubated at room temperature (24 °C). The fluorescence of control and thermally treated samples was measured in a Gemini XPS fluorescence micro-plate reader (Molecular Devices, Sunnyvale, CA) with excitation and emission wavelengths of 504 nm and 525 nm respectively.

2.6. Statistical analysis

All the experiments were performed in triplicates. Statistical t-test from Microsoft® Excel 2010® was used to determine significant differences between treatments. The level of significance was set at $p < 0.05$.

3. Results

3.1. Generation of hydrogen peroxide from thermal treatment of sugar solutions

3.1.1. Effect of sugar type and temperature

Based on the previous studies performed in physiological conditions, we hypothesized that hydrogen peroxide was likely to be one of the ROS generated from thermal treatment of sugar solutions. Fig. 1(a) shows the concentration of hydrogen peroxide generated in solutions of fructose or glucose (10% w/v) prepared in distilled water during thermal treatment at 50 °C for 40 min. No hydrogen peroxide was detected in sucrose solution under the same condition (Fig. S2). In contrast, hydrogen peroxide was