INHIBITION OF LIGHT-INDUCED OFF-FLAVOUR DEVELOPMENT BY SINGLET OXYGEN QUENCHERS IN CLOUDY APPLE JUICE

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Abstract

Beverage companies sometimes receive reports that cloudy fruit juices have developed a “metallic flavour” when they are exposed to light, which results in consumer complaints. These degradations have been shown to be caused by photooxidation, in which singlet oxygen played an essential role. In the present study, aqueous antioxidants, such as crocin, crocetin, and norbixin, have been investigated. The development of seven off-flavour compounds was monitored in cloudy apple juices from concentrates stored in glass bottles under fluorescent light (3000 lx, 8 °C). It was found that crocin, known to be a singlet oxygen quencher and a food colouring additive, was effective in inhibiting the development of light-induced off-flavour.

Introduction

Cloudy apple juice develops a metallic flavour when it is exposed to light (1). Off-flavour induced by fluorescent lights in reconstituted cloudy apple juice was analysed by SPME-GC-MS and seven volatile compounds; pentanal, 2-methyl-1-penten-3-one, hexanal, 1-octen-3-one, (E)-2-heptenal, 6-methyl-5-hepten-2-one, and (E)-2-octenal, were found to increase significantly in the cloudy apple juice after light exposure and this could contribute to the off-flavour (2). The current approach to avoiding the development of light-induced off-flavour in beverages is through the choice of packaging materials. Opaque cans, bottles and aluminium-paper cartons inhibit photooxidation by protecting juice from light. In addition, glass bottles are impermeable to oxygen (3) and effective in inhibiting photooxidation in spite of light transparency. When the initial dissolved oxygen in cloudy apple juice is low and the headspace is small enough that little oxygen is present in the bottle, the formation of off-flavours in the juice is low because of the limited availability of oxygen (4). However, these approaches are relatively passive ways to inhibit off-flavour formation and the effect of other relevant factors should be studied. Singlet oxygen may play a role in the formation of lipid peroxides in the presence of light. Many compounds including synthetic and natural components are reported to act as singlet oxygen quenchers, but most are insoluble in water (e.g. canthaxanthin, β-carotene, lycopene, and zeaxanthin). Three singlet oxygen quenchers were chosen and their effect on the photooxidation of cloudy apple juice has been evaluated.

Experimental
Samples. Apple juice concentrates (40° Brix), to which ascorbic acid had been added for the inhibition of browning in juice, were reconstituted to 12° Brix. The samples were stored in glass bottles with 30% air headspace, exposed to 3000 lx fluorescent lights and kept at 8 °C for 40 h in a cold storage room. Simultaneously, equivalent samples were stored in the dark as a reference control. The singlet oxygen quenchers used in the study were water soluble and dissolved by adding them directly into the reconstituted apple juice.

Analysis of volatiles. Twenty grams of cloudy apple juice, an internal standard (750 ng bromobenzene) and a stirring bar were placed in a 40 mL vial and capped with a PTFE septum. Solid phase microextraction (SPME) was performed with a 75 μm carboxen/ polydimethylsiloxane (PDMS) fibre. The SPME fibre was exposed to the sample headspace at 35 °C for 45 min. Each sample was analysed in triplicate. A Hewlett-Packard 6890 Series GC coupled to a 5973 Series MSD was used for analysis. The column was a 60 m INNOWAX column (i.d. 0.25 mm, 0.25 μm, Agilent, Inc., USA). The oven temperature was kept at 40 °C for 2 min and programmed to increase at 4 °C per minute. The final temperature was 240 °C and was held for a further 10 min. The temperature of the injector was 250 °C. The GC Linear Retention Indices (LRI) of the isolated compounds were determined externally with a mixture of hydrocarbons (C6-C25) which were run under the same conditions. The relative peak areas of off-flavour compounds were estimated by comparing the peak areas in the selected ion chromatogram (m/z 44 for pentanal, m/z 69 for 2-methyl-1-penten-3-one, m/z 56 for hexanal, m/z 70 for 1-octen-3-one, m/z 83 for (E)-2-heptenal, m/z 108 for 6-methyl-5-hepten-2-one, and m/z 83 for (E)-2-octenal) with that of the internal standard (m/z 158), and the proportion of each compound (%) was calculated relative to the amount present in the light-exposed control after 40 h.

Results and Discussion

Crocin (crocetin di-gentiobiose ester) is one of the carotenoids extracted from Saffron (Crocus sativus L.). It is well soluble in water and can be commercially obtained as a chemical reagent (Sigma-Aldrich K.K., Japan). According to Manitto et al. (5), the rate constant for quenching singlet oxygen in water is $1.8 \times 10^9$ M$^{-1}$s$^{-1}$. By adding crocin (25-250 mg/l) to the reconstituted apple juice, the formation of off-flavour compounds was reduced significantly compared to the light-exposed control (Figure 1). The apparent amounts of alkanals in the light-exposed crocin-added-sample (250 mg/l) were 74-80% of those in the control. However, the initially existing concentrations of pentanal and hexanal were 33% and 34%, respectively, and when this is taken into account the increased amounts following light-exposure were calculated to be approximately 41-47%. Therefore, the effect on these volatiles is similar to that of other lipid oxidation products (1-octen-3-one, (E)-2-heptenal, and (E)-2-octenal), since these 3 compounds were formed at approximately 49-55% of the concentration in the light-exposed control. However, the effect of crocin on the formation of 2-methyl-1-penten-3-one and 6-methyl-5-hepten-2-one was different. Although crocin inhibited their formation significantly when compared with the light-exposed control, the relative amounts were 69-78% of those in the control which was higher than those of the other off-flavour compounds.

Crocin is also extracted from Saffron and obtained from Sigma-Aldrich Japan K.K. Its rate constant for quenching singlet oxygen in water is higher than that of crocin at $2.5-5.5 \times 10^9$ M$^{-1}$s$^{-1}$ (5). It is less soluble than crocin and up to 100 mg/l crocetin could be dissolved (or suspended) in the juice. The addition of a low
concentration (25-50 mg/l) of crocetin resulted in increasing the formation of off-flavours except for pentanal, and it seems crocetin acted as a pro-oxidant.

Norbixin (9'-cis-6,6'-diapocarotene-6,6'-dioic acid) is extracted from the seeds of the annatto tree (Bixa orellana), and is a well-known colour additive for foods. The quenching rate constant in water is $2.3 \times 10^{10}$ M$^{-1}$s$^{-1}$ (6). The annatto extract was obtained from Saneigen FFI K.K. (Japan). The results with norbixin were similar to those of crocetin. Apparently it acts as a pro-oxidant for the lipid degradation and the formation of 6-methyl-5-hepten-2-one. The formation of hexanal was not changed significantly by the addition of norbixin.

Thus it has been shown that crocin has a remarkable inhibitory effect on light-induced off-flavour development in cloudy apple juice. However, when crocin was dissolved in cloudy apple juice, its colour became orange-yellowish and was obviously different from the natural appearance of apple juice. Therefore, it is not practical to use crocin for inhibiting the photooxidation of cloudy apple juice. It was also shown that not all aqueous singlet oxygen quenchers were effective inhibitors. Further investigation into the mechanism seems necessary.

References