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Aroma compounds derived from the thermal degradation of carotenoids in a cashew apple juice model

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ABSTRACT

The thermal degradation of carotenoids in cashew apple (*Anacardium occidentale* L.) juice leads to changes in the beverage colour, and possibly in the aroma and flavour, although the latter hypothesis has not yet been properly investigated. Thus the objective of this study was to investigate the formation of odour active volatiles derived from the thermal degradation of carotenoids in a cashew apple juice model. A carotenoid extract in an acidic aqueous medium was submitted to 60 and 90 °C for 1 and 2 h. The non-volatile compounds were identified by high performance liquid chromatography coupled with a photodiode array and mass spectrometry detectors (HPLC-PDA). The volatiles were isolated by headspace-solid phase micro-extraction, separated by gas chromatography, identified by mass spectrometry (SPME-GC–MS) and their odour significance assessed by GC-Olfactometry. Thirty-three odour active volatiles were identified in the heated system, amongst which 1,2,3,5-tetramethylbenzene, naphthalene and *p*-xylene. The results indicated that the volatiles formed from the thermal degradation of the carotenoids influence the aroma and flavour of thermally processed cashew apple products.

1. Introduction

During the concentration of cashew apple juice, the exotic and highly appreciated aroma and flavour of the beverage loses their sensory quality, decreasing juice acceptability amongst the consumers. Of the factors contributing to the undesirable changes occurring in the juice sensory attributes, the formation of aroma volatiles from the thermal degradation of carotenoids is a non-explored but feasible possibility.

A wide range of total carotenoid contents, from 8.2 to 197.8 μg/100 g, can be found in commercial cashew apple products, such as nectar, frozen pulp, concentrated juice and the ready-to-drink juice (Assunção & Mercadante, 2003). The major carotenoids present in the above-mentioned manufactured goods are all-*trans*-β-carotene and all-*trans*-β-cryptoxanthin, followed by α-carotene and *cis* isomers of β-carotene and β-cryptoxanthin; all of which undergo degradation during heat treatment.

Studies involving the formation of volatile compounds by the thermal degradation of carotenoids during heat treatment can be traced to the classical and pioneering work of Kuhn and Winterstein (1932), who identified toluene, *m*-xylene and 2,6-dimethylnaphthalene as the products of the thermal degradation of β-carotene. Later, Day and Erdman (1963) and Mader (1964) confirmed the findings of Kuhn and Winterstein (1932). According to Mader (1964), toluene, *m*-xylene and 2,6-dimethylnaphthalene, as well as *p*-xylene, are probably formed by cyclization of the polyene carotenoid chain, while the β-ionone ring is involved in the formation of ionene.

Even under mild thermal conditions, studies concerning the generation of volatile compounds by β-carotene degradation usually report the formation of toluene, *m*-xylene and 2,6-dimethylnaphthalene. Toluene and *m*-xylene were formed in oleoresins of marigold, tomato and paprika, after heating the products at 50, 100 and 150 °C (Rios, Fernández-García, Mínguez-Mosquera, & Pérez-Gálvez, 2008). The intramolecular cyclization activated by the thermal process was proposed as the main reaction mechanism involved in the formation of the above-mentioned volatiles, followed by an elimination reaction in the chain or a heterolytic fragmentation reaction. In this study, the identification of several methyl benzaldehydes in the marigold and paprika oleoresins, was attributed to carotenoid oxidation reactions. On the other hand, the presence of ketones, such as 6-methyl-5-hepten-2-one and 6-methyl-3,5-heptadien-2-one in the paprika and tomato oleoresins, was accredited to a mechanism of addition to the β-carotene chain at the 13,14 position, followed by heterolytic fragmentation, generating ketones that underwent further cleavage (Rios et al., 2008).

In a comprehensive investigation concerning the thermal degradation of β-carotene in an aqueous model solution in an atmosphere

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of oxygen, Kanasawud and Crouzet (1990) showed that after 3 h of heating at 97 °C, the following volatiles were formed: tridecane, decanal, β-cyclocitral, ionene, 4-ethylbenzaldehyde, naphthalene, β-damascone, β-ionone and several ketones, amongst others. Additional volatiles can be generated from further thermal degradation of some of the above-mentioned compounds. According to Ueno, Masuda, and Ho (2004), this is the case of citral, which, even under mild heating conditions (40 °C) in an acidic aqueous solution for 2 weeks, was easily degraded by a series of cyclization and oxidation reactions, generating a variety of secondary degradation volatiles, such as *p*-cresol, *p*-cymene, α-*p*-dimethylstyrene, *p*-methylacetophenone and 4-(2-hydroxy-2-propyl)benzaldehyde. Of these compounds, *p*-cresol and *p*-methylacetophenone were reported to be very potent off-odorants (Ueno et al., 2004).

The above-mentioned studies support the premise that aroma volatiles generated from the thermal degradation of cashew apple carotenoids during the heat treatment of the juice, might be at least partially responsible for the undesirable changes occurring in the aroma and flavour of the processed cashew apple juice. Nonetheless, up to now, this hypothesis has not been tested under controlled conditions.

Thus, the objective of the present work was to study the formation of volatile compounds derived from the thermal degradation of carotenoids in a cashew apple juice model, and to assess their odour significance by GC–Olfactometry. Up to now, the majority of the studies regarding the thermal degradation of carotenoids did not use this valuable technique to assess the odour significance of the volatile compounds formed.

2. Materials and methods

2.1. Samples and standards

Fifty kilogrammes of the red cashew apples (*Anacardium occidentale* L.) from the Petrolina region (Pernambuco State, Northeast region, Brazil) was acquired during their harvesting season (October, 2007). The cashew nut and part of the fruit peel were manually removed, and the resulting pulp homogenized and kept frozen at −35 °C until extraction of the carotenoids. The homogenized fresh pulp had a soluble solids content of 11 °Brix and pH value of 4.5.

The following standards 4-ethylbenzaldehyde, 4-hydroxy-4-methyl-2-pentanone, benzyl alcohol, carveol, 3-methyl-1-butanol, 2-methyl-1-propanol, 2-phenylethanol, perilaldehyde, ethyl hexanoate, ethyl butanoate, ethyl octanoate, naphthalene, ethyl decanoate, hexanal, ethyl cinnamate, ethyl hexanol, 2,4-dimethylbenzaldehyde, citronellol, myrcene, isoamyl acetate, γ-nonalactone, styrene, β-ionone, β-damascenone, β-cyclocitral, 5-hydroxymethyl-2-furaldehyde, octanal, 2-butanal, methyl hexanoate, linalool, methyl butanoate, geranyl acetate, 1-hexanol, 2,2,6-trimethylcyclohexanone, limonene, nonanal, α-terpinene, *p*-xylene, *o*-xylene, *p*-cresol, *p*-cymene, propyl acetate, 2,3-butanedione, ethyl isovalerate, ethyl-*trans*-caryophyllene and ethyl crotonoate were obtained from Sigma Aldrich (Steinheim, Germany). The paraffin homologues (C_7 to C_{30}) were obtained from Polyscience (Chicago, USA). Their identities were confirmed from their retention indices and comparison with the MS spectral database.

Standards of all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-β-cryptoxanthin, all-*trans*-β-carotene and all-*trans*-α-carotene, were donated by DSM Nutritional Products (Basel, Switzerland), with purities ranging from 95% to 99.9% as determined by HPLC-PDA.

2.2. Carotenoid extraction and analysis

The carotenoids were extracted with acetone, transferred to petroleum ether/diethyl ether, and saponified overnight at room temperature with 10% methanolic KOH followed by removal of the alkali. Due to the high oil content in the cashew apple peel, it was necessary to physically remove the oil as follows: prior to transfer to the ether, the carotenoid extract was placed in the freezer at −18 °C for 2 h, and then filtered using cold glassware and washed with cold acetone (De Rosso & Mercadante, 2007). The dried cashew apple carotenoid extract was then flushed with nitrogen (99.0%) and stored at −35 °C.

The carotenoid analyses were carried out by HPLC-PDA under the same experimental conditions described by De Rosso and Mercadante (2007). The carotenoids were identified considering the combined information obtained from the following parameters: elution order on the C_{30} HPLC column, co-chromatography with authentic standards, UV–visible spectrum characteristics (λ_{max}, spectral fine structure (% III/II), and *cis* peak intensity (% A_B/II)). The carotenoids were quantified using external calibration curves for all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-β-cryptoxanthin, all-*trans*-β-carotene, and all-*trans*-α-carotene with a minimum of five concentration levels. Total carotenoid content was calculated considering all identified peak areas.

2.3. Cashew apple juice model

The cashew apple juice model used in this study containing about 200 μg carotenoid, was dissolved in 5 mL of ethanol and mixed with 25 mL of Milli-Q water (Millipore, Billerica, USA) acidified at pH 3.8 with 0.5 M citric acid (juice model), as previously described by Zepka and Mercadante (2009).

The juice model-system was distributed amongst sealed glass tubes and heated at 60 °C and at 90 °C in water baths for 1 and 2 h at both temperatures. The tubes were then removed from the bath and immediately cooled under running water. A similar protocol was followed for the control system (blank), using 5 mL of ethanol and 25 mL of Milli-Q water acidified at pH 3.8 with 0.5 M citric acid.

2.4. Isolation of the volatile compounds

The volatile compounds were isolated using solid phase microextraction (SPME) with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Bellefonte, USA). The SPME fibre was inserted into the headspace of the vial containing the cashew apple juice system for 30 min at room temperature, with agitation provided by a magnetic stir bar. After this period, the fibre was removed from the vial and immediately desorbed into the injector of the GC equipment.

2.5. GC and GC–MS analysis

The volatile compounds were separated on DB-5 and DB-Wax fused silica capillary columns, both 30 m in length, 0.25 mm id and 0.25 μm film thickness (J & W, Folsom, USA) in a Varian model CP 3380 gas chromatograph. The splitless mode injector was maintained at 230 °C and the flame ionization detector (FID) at 250 °C. Hydrogen was the carrier gas at a flow rate of 1.5 mL/min. The oven temperature for the DB-5 column was set at 50 °C, held for 8 min, programmed to 260 °C at 4 °C/min, then to 280 °C at 20 °C/min and finally held at that temperature for 5 min. The initial oven temperature for the DB-Wax column was 40 °C for 15 min, followed by a linear increase at 4 °C/min to 210 °C, and held at this temperature for 17 min.

For identification, the volatile compounds were analysed using a Shimadzu (model QP 2010) GC with a mass spectrometer (MS), applying an electron-impact ionization voltage of 70 eV and using the same columns and oven conditions as described above for the GC-FID analysis, with the exception of using helium as the carrier gas. The volatile compounds were identified by a comparison of their MS spectra with those provided by the computerized library (NIST 2006 MS Library) and, when available, with those obtained from standards analysed under the same GC–MS conditions. In addition, to assist with the identification, each volatile linear retention index (LRI) was calculated using the retention times of a standard mixture of paraffin homologues prepared in hexane, and compared with the LRI values published