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Study of chemical changes in pasteurised orange juice during shelf-life: A fingerprinting-kinetics evaluation of the volatile fraction

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ABSTRACT

The current work used fingerprinting-kinetics for the first time to monitor shelf-life changes in a low-pH, pasteurised, shelf-stable product, more particular in orange juice. Orange juice samples were stored as a function of time at four different storage temperatures (20, 28, 35 and 42 °C). To obtain insight into chemical changes in the volatile food fraction, samples were fingerprinted with headspace GC–MS. The objectives of this work were twofold: (*i*) to identify major chemical changes of pasteurised orange juice during shelf-life and (*ii*) to study the kinetics of selected shelf-life compounds in the context of accelerated shelf-life testing (ASLT). At 20 °C, changes in terpenes and a decrease in aldehydes were observed. Oxides and sulphur compounds increased and esters decreased at increased storage temperatures (at 28 °C and above). Concerning ASLT, four volatile compounds had clear temperature and time dependent kinetics within the investigated temperature range.

1. Introduction

The commercial importance of citrus fruits has been recognised for many decades. The global citrus fruit production showed an increasing trend, with the main leading producers being Brazil, China and the United States. Among citrus juices, orange juice is the most widely consumed. According to the United States Department of Agriculture (USDA), its global consumption reached nearly two million metric tons in 2014, which was mainly driven by the United States and the EU (USDA, 2014). Various processing techniques are developed to achieve shelf-stable orange juice: one of the most commonly used methods in food industry being pasteurisation by heat. Nevertheless, orange juice quality changes over time, either during processing or storage. When orange juice is processed with the aim of obtaining a shelf-stable product, its product shelf-life is determined by a best-before date, which is limited due to physical and (bio) chemical reactions. The number and type of degradation reactions and their interactions make the quantitative study of quality degradation of orange juice during processing and storage highly complex.

Numerous studies have been aimed at understanding quality changes of orange juice during storage (Bacigalupi et al., 2013; Berlinet, Brat, Brillouet, & Ducruet, 2006; Roig, Bello, Rivera, & Kennedy, 1999; Esteve, Frigola, Rodrigo, & Rodrigo, 2005). In order to obtain insight into colour instability of pasteurised orange juice, our previous works investigated changes in a wide range of a priori selected quality parameters (e.g., acids, sugars, oxygen, vitamin C, furfural and HMF) linked to colour changes as a function of storage time and temperature. It was demonstrated that due to limited contribution of carotenoid degradation reactions, other mechanisms play important roles in browning of orange juice. Furthermore, several quality parameters were selected as potential markers for colour changes: ascorbic acid, sugars and their degradation products, furfural and HMF (Wibowo et al., 2015b; Wibowo et al., 2015a). This strategy is known as a targeted analytical approach, in which specific chemical compounds (e.g., ascorbic acid) or quality attributes (e.g., colour) are focused on (Grauwet, Vervoort, Colle, Van Loey, & Hendrickx, 2014). However, investigating responses related to a particular chemical reaction or characteristic selected at the starting point of the study could result in overlooking unexpected effects.

Over the past years, there has been a growing interest in applying untargeted fingerprinting as a methodological approach to gain insight into the reaction complexity of a food system (Picariello, Mamone, Addeo, & Ferranti, 2012). Fingerprinting is defined as a more unbiased and hypothesis-free methodology that considers as many compounds as possible in a particular food fraction (liquid or headspace) compared

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to the commonly used targeted approaches. Without fixating on a specifically known compound, it allows an initial fast screening to detect differences among samples. This approach has been suggested to investigate chemical reactions which are influenced by either processing or shelf-life (Grauwet et al., 2014; Vervoort et al., 2012). Advanced analytical methods such as separation techniques by liquid chromatography (LC), gas chromatography (GC) coupled to a mass spectrometry (MS) or nuclear magnetic resonance (NMR)-based detector for compound identification are indispensable analytical tools used in fingerprinting research (Cevallos-Cevallos, Reyes-De-Corcuera, Etxeberria, Danyluk, & Rodrick, 2009; Wishart, 2008).

Unfortunately, today, it is not possible to fingerprint the whole chemical composition of a food matrix using one particular analytical method. Therefore, from an analytical point of view, untargeted analysis can be quite challenging because of the diversity of the compounds studied. The use of GC–MS is particularly advantageous, since it is a robust method which allows preliminary compound identification by the availability of mass spectral libraries. Volatile compounds such as aldehydes, esters, terpenes, alcohols, and ketones are often linked to a range of process- and storage-induced chemical reactions (Kebede et al., 2014; Mahattanatawee, Rouseff, Valim, & Naim, 2004; Perez-Cacho & Rouseff, 2008a). Being regularly degradation products of major food components such as sugar, fat, and nutrients, volatiles can be approached not only for understanding chemical changes in the volatile fraction itself, but also as a witness for what is happening in other food fractions (e.g., liquid fraction). In addition, changes in volatile compounds contribute to the perceived aroma and are important for the overall sensorial quality of food products.

Applying headspace GC–MS fingerprinting of the volatile fraction as an untargeted method to obtain insight in chemical changes induced by processing has been a subject of study in our research group (Vervoort et al., 2012; Aganovic et al., 2014; Kebede et al., 2014). Recently, our first work was published on the use of fingerprinting integrated with kinetics in the context of the evaluation of quality changes of a sterilised vegetable-based puree as a function of (accelerated) storage (Kebede et al., 2015). The current work will apply this untargeted analytical and engineering toolbox of fingerprinting-kinetics for the first time for monitoring shelf-life changes in a low-pH, pasteurised, shelf-stable product, more particular in orange juice. The objective of this work was to identify major chemical changes of pasteurised orange juice during shelf-life. Orange juice was not only stored at classical ambient storage temperatures but also under increased temperature conditions in order to study the kinetics of selected shelf-life markers in the context of accelerated shelf-life testing (ASLT).

2. Materials and methods

2.1. Sample processing and shelf-life study

Single strength orange juice (11.2 corrected °Brix, pH 3.7, titratable acidity 0.8%) was prepared by reconstituting frozen Brazilian orange concentrate (*Citrus sinensis* (L.) Osbeck) (65 °Brix) with water (1:5, w/w). The 200 L of orange juice mixture was pasteurised at 92 °C for 30 s. Subsequently, hot filling at 85 °C into 500 mL polyethylene terephthalate (PET) bottles, sealing by cap twist inversion and submerging bottles into a water tank to reach ambient temperature were applied. A shelf-life study was conducted at 20 and 28 °C for a total of 32 weeks, at 35 °C for 12 weeks and at 42 °C for 8 weeks. 75 bottles were stored per each incubator (IPP500, Memmert, Schwabach, Germany) protected from light at a specific storage temperature. These orange juice bottles were also used for characterisation in colour, pH, titratable acidity, organic acid profile, °Brix, sugar profile, oxygen, vitamin C, furfural, 5-hydroxymethylfurfural (HMF) and carotenoids (Wibowo et al., 2015b; Wibowo et al., 2015a). At a particular sampling moment, three bottles were randomly taken per storage temperature. Juices were uniformly mixed and divided into smaller tubes (±30 mL), frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.2. Headspace GC–MS analysis

Frozen tubes were thawed in a circulating water bath at 25 °C. After thawing, juice inside the tube was homogenised using a vortex mixer. 3 mL juice and 2 mL saturated NaCl solution were pipetted into an amber glass vial (10 mL, VWR International, Radnor, PA, USA). The vials were tightly closed using screw-caps with silicone septum seal (GRACE, Columbia, MD, USA), homogenised and placed in the cooling tray of the autosampler which was maintained at 10 °C. All headspace analyses were conducted on a gas chromatography (GC) system (7890 N, Agilent technologies, Diegem, Belgium) coupled to a mass selective detector (MSD) (5977 N, Agilent Technologies, Diegem, Belgium) and equipped with a CombiPAL autosampler (CTC analytics, Zwingen, Switzerland). Aiming at detection of as many volatiles as possible, an HS–SPME–GC–MS method (e.g., SPME fibre type and GC and MS parameters, etc.) was optimised beforehand. With the aim of selecting a fibre that enables the extraction of a wide range of volatilisable compounds, a comparison between five types of SPME fibre coatings (PDMS, CAR/PDMS, PDMS/DVB, PA, and DVB/CAR/PDMS) was performed. Based on the observations of the total peak area and the number of peaks (a high area and a high number), a fibre with carboxen/polydimethylsiloxane (CAR/PDMS) coating was chosen for the headspace SPME analysis (data not shown). Samples were equilibrated for 20 min at 30 °C under agitation at 500 rpm. Volatiles were extracted using a SPME fibre coated with 85 μm CAR/PDMS (StableFlex, Supelco, Bellefonte, PA, USA) at 30 °C for 10 min. Each fibre was conditioned according to the manufacturer's guidelines before its first use. After the extraction step, the fibre was inserted into the heated GC injection port (230 °C) for 2 min where the volatile compounds were desorbed. Subsequently, the fibre was thermally cleaned for 5 min at 300 °C in the conditioning station of the autosampler. Injection of the samples to the GC column was performed in split-mode with a split-ratio of 1/10. The separation was carried out on a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Santa Clara, CA, USA) using helium as carrier gas at a constant flow of 1.5 mL min^{-1}. The oven temperature was programmed at an initial temperature of 40 °C, which was maintained for 2 min, after which it was elevated to 160 °C at a rate of 4 °C min^{-1}, then ramped to 300 °C at 20 °C min^{-1} and kept constant for 2 min at 300 °C before cooling back to 40 °C. The mass spectra were obtained by electron ionisation (EI mode) at 70 eV with a scanning range of 35 to 400 m/z. The ion source and quadrupole temperatures were 230 and 150 °C, respectively. A new fibre was used for each storage temperature. During analysis, the samples were randomised as a function of storage time per storage temperature. Possible fibre degradation was monitored by analysis of a reference sample (pasteurised orange juice), every 14 injections. The peak area of the reference samples was investigated as a function of time to monitor the performance of the SPME fibre and the GC–MS. In this work, deviation of the peak area of the reference samples was less than 5% during the analysis, which shows limited fibre degradation and a good performance of the instrument (results not shown).

2.3. Data analysis

2.3.1. Data pre-processing and multivariate analysis

GC–MS has long been the method of choice for identifying volatile compounds in complex mixtures. This method can fail, however, when acquired spectra are “contaminated” with extraneous mass spectral peaks, as commonly arise from co-eluting compounds and ionisation chamber contaminants. These extraneous peaks can pose a serious problem for automated identification methods where they can cause identifications to be missed by reducing the spectrum comparison factor below some pre-set identification threshold. Automated Mass