Quality changes of pasteurised orange juice during storage:

A kinetic study of specific parameters and their relation to colour instability

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Abstract

In view of understanding colour instability of pasteurised orange juice during storage, to the best of our knowledge, this study reports for the first time in a systematic and quantitative way on a range of changes in specific quality parameters as a function of time and as well as temperature (20 °C to 42 °C). A zero-order (°Brix, fructose, glucose), a first-order (vitamin C), a second-order (sucrose) and a fractional conversion model (oxygen) were selected to model the evolution of the parameters between parentheses. Activation energies ranged from 22 to 136 kJ mol^{-1}, HMF formation being the most temperature sensitive. High correlations were found between sugars, ascorbic acid, their degradation products (furfural and HMF) and total colour difference (ΔE^*). Based on PLS regression, the importance of the quality parameters for colour degradation was ranked relatively among each other: the acid-catalysed degradation of sugars and ascorbic acid degradation reactions appeared to be important for browning development in pasteurised orange juice during ambient storage.

Keywords: Orange juice; Quality; Colour; Kinetics; Storage; Multivariate data analysis

Chemical compounds studied in this article:

- ascorbic acid (PubChem CID: 54670067)
- fructose (PubChem CID: 5984)
- glucose (PubChem CID: 5793)
- sucrose (PubChem CID: 5988)
- furfural (PubChem CID: 7362)
- 5-hydroxymethylfurfural (PubChem CID: 237332)

Highlights

- Juice quality parameters linked to browning change due to storage time and temperature
- Ascorbic acid, sugars, HMF and furfural were highly correlated to browning
- Presence of oxygen and acidity may favour browning reactions
- Multiple pathways are important in the development of browning in stored orange juice
- Shelf-life can be extended by reducing oxygen and storage temperature

1 INTRODUCTION

Orange juice is known as the most popular fruit juice worldwide. Although its consumption is decreasing in developed countries, such as USA and Western Europe, in emerging countries, the consumption of juice, along with nectar and still drink, shows an increasing trend (Neves, Trombin, Lopes, Kalaki, & Milan, 2011). Orange juice is highly valued by consumers for its nutritional content, appealing colour and refreshing sweet and sour taste. Among different processing technologies, heat pasteurisation is the most common technique to extend the shelf-life of orange juice. The inactivation of spoilage microorganisms and thermally resistant endogenous enzymes (e.g. pectin methylesterase (PME)) can be achieved under temperature conditions at 90–98 °C for 10–60 seconds (Vervoort et al., 2011; Yeom, Zhang, & Chism, 2002). From microbial safety point of view, given the fact that orange juice is categorised as high acid food (pH < 4.6), intensively pasteurised orange juice is stable at room temperature (Silva & Gibbs, 2004). However, during storage, as the quality of most food products decreases, changes in sensorial and nutritional qualities of orange juice are the limiting factors determining the 'best before date' listed on the product.

An important quality loss of orange juice during shelf-life is the change in colour. It is the first visible sign which can negatively influence consumers' acceptance, thereby decreasing its commercial value (Manso, Oliveira, Oliveira, & Frías, 2001). It is known that colour change is an indicator for chemical and biochemical reactions (van Boekel, 2008). Depending on the extent of the reaction, changes in colour may occur as a result of the formation of brown pigments as well as the fading of carotenoids, the naturally occurring pigments of orange juice. Recently, a kinetic study reported on the carotenoid stability in orange juice and pointed out to their potentially limited contribution to colour change (Wibowo et al., 2015). In intensively pasteurised products, in which quality-degrading enzymes are inactivated, non-enzymatic browning reactions rather than enzymatic browning should be focussed on.

Authors have been suggesting different mechanisms for non-enzymatic browning in pasteurised citrus juice: (*i*) degradation of ascorbic acid (Kaanane, Kane, & Labuza, 1988; Roig, Bello, Rivera, & Kennedy, 1999; Solomon, Svanberg, & Sahlstrom, 1995), (*ii*) acid-catalysed degradation of sugars (Roig et al., 1999; Lee & Nagy, 1988b) and (*iii*) Maillard reactions between reducing sugars and amino acids (Bacigalupi et al., 2013; Lee & Nagy, 1988a). Nevertheless, due to the complexity of colour degradation, there is probably more than one mechanism involved. Moreover, interaction between different pathways or compounds and the sequence of occurring reactions could be important in explaining this browning phenomenon. Although some authors have reported on the kinetics of colour change and some of the markers for browning (ascorbic acid, 5-hydroxymethylfurfural or HMF, furfural, etc.) (Bacigalupi et al., 2013; Burdurlu, Koca, & Karadeniz, 2006; Kaanane et al., 1988; Van Bree et al., 2012), there is still a need for a systematic, comprehensive and quantitative study which investigates the change in a range specific quality parameters for colour change under a broad window of shelf-life time and temperature conditions. In addition, the potential of integrating information obtained from multiple responses and the potential to select significant response changes by the use of multivariate data analysis should not be forgotten (Grauwet, Vervoort, Colle, Van Loey, & Hendrickx, 2014).

The objective of the current research paper can be summarised as the study of chemical parameters linked to non-enzymatic browning of heat pasteurised orange juice as a function of storage. Changes in acids (section 3.1.1), sugars (section 3.1.2), oxygen (section 3.1.3), vitamin C (section 3.1.4), furfural and HMF (section 3.1.5) were quantitatively studied as a function of storage time and temperature (20 °C, 28 °C, 35 °C and 42 °C). Regarding to this, two steps were taken. First, to quantitatively describe changes of different parameters, a kinetic modelling approach was used for monitoring quality changes during storage as a

function of controlled time and temperature conditions (section 3.1). Secondly, to interpret the mutual importance of the selected quality parameters and their correlation to colour change, multivariate data analysis techniques were applied (section 3.2).

2 MATERIALS AND METHODS

2.1 Sample preparation

Frozen orange juice concentrate (*Citrus sinensis* (L.) Osbeck) (65 °Brix), purchased from a commercial juice producer in Brazil, was mixed with water at a ratio 1:5 (w/w) yielding 11.2 °$Brix_c$. The single strength juice was pasteurised in a tubular heat exchanger at 92 °C for 30 seconds. Under this processing condition, no residual pectin methylesterase (PME) activity was determined. Subsequently, juice was hot filled at 85 °C into 500 mL polyethylene terephthalate (PET) bottles and sealed by cap twist inversion. Finally, the bottles were cooled to ambient temperature by submerging them into a tank with circulating chlorinated water. Bottles were stored protected from light at 20 °C and 28 °C for 32 weeks, at 35 °C for 12 weeks and at 42 °C for 8 weeks, in T-controlled incubators (IPP500, Memmert, Schwabach, Germany). At a specific sampling time, bottles were randomly sampled from the incubator, transferred to smaller tubes ($\pm$30 mL) and stored at –80 °C. Each tube was thawed in a circulating water bath at 25 °C and homogenised prior to analysis.

2.2 Determination of the acidity

2.2.1 pH and titratable acidity

The orange juice pH was measured at 22 ± 1 °C using a pH meter (Meterlab PHM210, Radiometer Analytical, Villeurbanne, France), which was calibrated with calibration buffers of pH 4.0 and 7.0 (IUPAC, Radiometer Analytical, Villeurbanne, France). All measurements were done in triplicate. Titratable acidity, expressed in percent citric acid, was determined according to AOAC method 962.12 (AOAC, 1998). Analysis was done in triplicate by titrating 10 g of orange juice with 0.1 N NaOH to pH 8.2. The % citric acid was calculated as expressed by Equation 1.

$$\%\ \text{citric acid (w/w)} = \frac{\text{volume NaOH (ml)} \times 0.64}{\text{juice weight (g)}} \times 100 \quad (1)$$

2.2.2 Organic acid profile

Organic acids were extracted according to the method of Vervoort et al. (2011). 10 mL of orange juice was transferred into a Nalgene centrifuge tube (50 mL). 500 µL of each Carrez I (15% w/v $K_4[Fe(CN)_6]$) and Carrez II (30% w/v $ZnSO_4$) was added and the mixture was homogenised using a vortex mixer. After resting for 30 min at room temperature, samples were centrifuged at 24000*g* for 15 min at 4 °C (J2-HS centrifuge, Beckman, Brea, CA, US). The obtained supernatant was filtered through a 0.45 µm syringe filter (Chromafil A-45/25, Macherey-Nagel, Düren, Germany). 2 µL of the organic acids extract was injected into the reversed phase (RP) HPLC system (Agilent 1200 series, Diegem, Belgium) and separated on a Prevail Organic Acid column (250 mm × 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, IL) protected with a Prevail C_{18} guard cartridge (7.5 mm × 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, IL). Isocratic elution (25 mM potassium dihydrogen phosphate buffer pH 2.5) with a flow rate of 1 mL min^{-1} at 25 °C was used. The UV-DAD detector was set at 210 nm. All samples were analysed in triplicates. Identification was done by comparing the retention time and UV spectra with standard solutions of a wide range of organic acids in milli-Q water. Quantification was performed using calibration curves based on the peak area and known concentrations of injected standard solutions, with regression equation for citric acid ($y = 64.32x - 1.53$, $R^2 = 0.99$) and for malic acid ($y = 48.55x + 0.53$, $R^2 = 0.99$).

2.3 Sugar content determination

2.3.1 Total sugar content

The total sugar content, expressed in °Brix was measured in triplicate using a digital refractometer (RX-7000α, Atago, Tokyo, Japan) at 20 °C. The soluble solids (SS) content was determined and expressed in °$Brix_c$, after correction with acid correction factors (Kimball, 1991).

2.3.2 Sugar profile

Sugar extraction and chromatographic analysis were based on the procedure of Vervoort et al. (2011). The juices were clarified, centrifuged and filtered analogous to the organic acids analysis (2.2.2). A dilution (1/10) of the filtrate in milli-Q water was made prior to analysis in RP-HPLC system with evaporative light scattering detection (Alltech 3300 ELSD, Grace, Deerfield, IL). The injection volume was 5 µL. Separation was performed on a Prevail carbohydrate ES column (250 mm × 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, IL) coupled to a Prevail C_{18} guard cartridge. Isocratic elution (75% (v/v) acetonitrile/water) was applied at a flow rate of 1 mL min^{-1} at 30 °C. Sugar analyses were carried out in triplicate. Identification was performed by comparing of the retention times with glucose monohydrate, fructose and sucrose standard solutions, while the content of sugars was determined using calibration curves of standards, with regression equation for glucose (y = 579.56x - 1366.50, R^2 = 0.99), fructose (y = 700.23x - 1214.90, R^2 = 0.99) and sucrose (y = 700.355x - 1718.70, R^2 = 0.99).

2.4 Determination of the dissolved and headspace oxygen content

The dissolved and headspace oxygen concentrations were determined using non-invasive OxySense® 4000B systems (OxySense, Las Vegas, NV) which consisted of the instrument, a

reader-pen assembly with an infrared detector and the oxygen sensors (O_2xyDots®). OxyDots were attached inside the bottles (in triplicate for each temperature) prior to filling and sealing. This oxygen sensor, consisting of a metal organic fluorescent dye ($[Ru(dpp)_3](Cl)_2$), was illuminated by a blue LED from the OxySense fibre optic reader-pen. In the condition where no oxygen is detected, the fluorescent dye will emit red light, while in presence of oxygen, the fluorescence light from the dye is dynamically quenched. The fluorescence lifetime of the dye decreased in proportion to the oxygen partial pressure in the bottle, thus determined the oxygen concentration (Mills, 2005). Prior to analysis, calibration was done by entering the OxyDot calibration numbers into the system. The measurement of the dissolved oxygen and headspace oxygen content was performed five times for each bottle.

2.5 Vitamin C: ascorbic acid (AA) and dehydroascorbic acid (DHA) determination

Determination of vitamin C was based on a method described by Verbeyst, Bogaerts, Van der Plancken, Hendrickx, & Van Loey (2013) with minor modifications. 5 mL of orange juice was mixed with 15 mL extraction buffer (1% w/v meta-phosphoric acid with 0.5% oxalic acid adjusted to pH 2.0). The mixture was centrifuged at a speed of 24000*g* for 15 min at 4 °C. Subsequently, the supernatant was filtered through a 0.45 μm syringe filter and stored at –80 °C. 5 mL of the supernatant was adjusted to pH 3.5 with 1M HCl or 1N NaOH. To determine the AA content, 2 mL of phosphate buffer (20 mM NaH_2PO_4+1 mMNa_2EDTA, pH 3.5) was added to 1 mL of the pH-adjusted supernatant. For determination of DHA content, 2 mL of reducing agent (TCEP 2.5 mM tris (2-carboxyl-ethyl) phosphine in phosphate buffer, pH 3.5) was added to 1 mL of the pH-adjusted supernatant. The mixture was centrifuged at 19900*g* for 15 min at 23 °C (Microfuge 22R, Beckman Coulter). 1 mL of each mixture was filtered through a Chromafil A-45/25 prior to injection (25 μL) in the RP-HPLC/UVdetection (Dionex BioLC, Sunnyvale, CA). The stationary phase was prevail C_{18} (250 mm × 4.6 mm, 5

µm particle size and corresponding guard column Grace, Columbia, MD), while the mobile phase was 1 mM Na_2EDTA and 10 mM CH_3COONH_4 at 0.8 mL min^{-1} isocratic elution. Separation occurred at 25 °C and detection was performed at 245 nm.

To quantify the AA and DHA concentrations, calibration curves of an external standard solution of AA (99% Acros organics, Geel, Belgium) were used. A stock solution of 0.5% was prepared in extraction buffer. From this, a work solution (1/10 dilution) was prepared and diluted to different concentrations. The AA concentration of the stock solution was spectrophotometrically determined based on the Lambert-Beer equation ($A = \varepsilon \cdot c \cdot l$) with ε = 10.3 mM^{-1} cm^{-1} at 245 nm; 25 °C; pH 0.69. Extractions, chromatographic analyses and absorbance measurements were done in triplicate.

2.6 Furfural and 5-hydroxymethylfurfural (HMF) determination

Furfural and HMF content were determined according to the method of Lee, Rouseff, & Nagy (1986) with some modifications. 500 µL of each Carrez I and Carrez II was added to 10 mL of orange juice. After resting for 30 min, the mixture was centrifuged for 15 min at 24000g (4 °C). A C18 SPE pre-column (Sep-PAK Waters, Milford, MA) was preconditioned with 2 mL methanol and 5 mL 0.5% acetic acid. 1 mL of supernatant was transferred in a SPE column, then washed with 2 mL of milli-Q water. Furfural and HMF were eluted with 4.5 mL of ethyl acetate and dried with anhydrous sodium sulphate. The resulting extract was filtered through a 0.45 µm syringe filter into a 5 mL volumetric flask. 5 µL was injected in the HPLC system and the separation occurred at 25 °C in a Zorbax Eclipse XDB C18 column (150 × 4.6 mm, 5 µm particle size, Agilent technologies, Diegem, Belgium) coupled to a Prevail C18 guard cartridge. The mobile phase was a mixture of 10/90 (v/v) acetonitrile/water at 1 mL min^{-1} isocratic elution. Furfural was detected at 277 nm and HMF at 285 nm. All samples were analysed in triplicate. Identification and quantification were performed by comparing the retention time and spectra with furfural and HMF (1.5 mg mL^{-1}) standards in 10% methanol.

2.7 Data analysis

2.7.1 Kinetic data analysis

To quantify the influence of storage conditions on the quality parameters, changes in the property of interest during storage were modelled. Different kinetic models were evaluated to describe the detected changes: zero-order (Equation 2), first-order (Equation 3), second-order (Equation 4) and fractional conversion kinetic model (Equation 5), in which C is considered the parameter value at storage time t (weeks), C_0 is the initial value at the start of storage, C_∞ is the value of the stable fraction, which does not change over time and k is the (apparent) reaction rate constant (weeks^{-1}). Selected models should be considered as empirical models and should not be mechanistically interpreted. The temperature sensitivity of the degradation rate constants was investigated by the Arrhenius model (Equation 6), where E_a is the activation energy (kJ mol^{-1}), T is absolute temperature (K), k_{refT} is the reaction rate constant at reference temperature 293 K and R is the universal gas constant (8.3145 J mol^{-1} K^{-1}).

$$C = C_0 + kt \tag{2}$$

$$C = C_0 \exp(kt) \tag{3}$$

$$C = \frac{C_0}{1 + C_0\, kt} \tag{4}$$

$$C = C_\infty + (C_0 - C_\infty) \exp(kt) \tag{5}$$

$$k = k_{refT} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{6}$$

All kinetic parameters were estimated by non-linear regression analysis (SAS 9.3, Cary, NC). One-step regression was performed by incorporating Equation 6 into Equation 2, 3, 4 or 5. Model evaluation and selection were performed by examining R^2_{adj} (Equation 7) and by visual inspection of the parity plot (estimated values versus measured values) and the scatter plot

(residuals versus measured values). In Equation **7**, DF_{tot} and DF_{error} are degree of freedom of total and error, respectively and *SSQ* is the sum of squares.

$$R^2_{adj} = 1 - \left[\frac{(DF_{tot} - 1)(1 - \frac{SSQ_{regression}}{SSQ_{total}})}{DF_{error}} \right] \quad (7)$$

2.7.2 Multivariate data analysis

To understand the mutual importance of the quality parameters for colour degradation among each other, a multivariate data analysis was applied (Solo Version 6.5, 2011, Eigenvector Research, Wenatchee, WA). All data obtained on changes in acids (pH, titratable acidity and organic acid profile), sugars (total sugar content and sugar profile), oxygen (headspace and dissolved) content, vitamin C (ascorbic and dehydroascorbic acid) content, furfural and HMF content and total carotenoids content were considered as *X*-variables. *X*-variables were mean-centred and weighed by their standard deviation to give them equal variance. Partial least squares (PLS) regression analysis was carried out to determine the correlation between the specific quality parameters (*X*-variable) and the fitted ΔE^* values as a function of time (*Y*-variable). The ΔE^* was calculated from L^*, a^* and b^* parameters, in which L^* values represent lightness, range from 0 (black) to 100 (white), a^* values indicate greenness (-) to redness (+) and b^* values measure of blueness (-) to yellowness (+) of orange juice samples. ΔE^* indicates the magnitude of colour difference between stored and pasteurised juice before storage (week 0) and determines whether the change in colour is visually different. The fitted ΔE^* values and total carotenoid content were determined previously (Wibowo et al., 2015) and they were taken from the same samples as the current study.

Biplots (OriginPro 8, Origin Lab Corporation, Northampton, MA) were constructed as a graphically representation between scores plots and loadings plots, by dividing each score

through the maximal vector length of the original score plot matrix. Subsequently, to quantitatively rank the quality parameters' relevance for colour changes (ΔE^*) among each other and their mutual importance, Variable Identification (VID) coefficients were calculated. VIDs are the correlation coefficients between each original *X*-variable and predicted *Y*-variables (Grauwet et al., 2014).

3 RESULTS AND DISCUSSION

3.1 Kinetic evolution of the specific quality parameters linked to colour change

Based on literature, several factors are described to be linked with colour instability of orange juice during storage. In this paper, the studied quality parameters were acids (pH, titratable acidity and organic acid profile), sugars (total sugar content and sugar profile), oxygen (headspace and dissolved) content, vitamin C (ascorbic and dehydroascorbic acid) content, furfural and HMF content. To quantitatively study the evolution of these parameters as a function of storage time, kinetic modelling was applied (section 2.7.1). The effect of storage temperature was studied and modelled using the Arrhenius equation.

3.1.1 Orange juice acidity as affected by storage time and temperature

Acids may determine or influence some degradation reactions during prolonged storage. Kennedy et al. (1990) studied the influence of pH in the development of brown colour of orange juice during storage. After 4 days of storage at 38 °C, a slight brown colour was reported at pH 1.15 to 4.15 and yellow colour at pH 7. Also, the effect of pH in the formation of 5-Hydroxymethylfurfural (HMF), known as indicator for browning in citrus juices, was referred by Shallenberger & Mattick (1983), in which almost a 2-fold faster rate of HMF formation was found at pH 3 than at pH 4-6. Particular organic acids have been described to be of interest in literature, as it was suggested to contribute to the browning of orange juice (Roig et al., 1999; Shinoda, Komura, Homma, & Murata, 2005). Aside from their potential role in browning reactions, acids play a significant role in the taste of orange juice. Together with sugars, acids give a balance of sweetness and sourness which makes orange juice refreshing to consumers.

3.1.1.1 pH and titratable acidity

Before storage, the pH of orange juice samples was 3.72, while TA, expressed as citric acid, was 0.79%. During the entire storage, pH and titratable acidity did not change significantly or in other words, the acidity of the juice was stable as a function of the storage time and temperature conditions investigated. The pH values ranged from 3.68 to 3.74, whereas the TA values ranged from 0.77% to 0.82% citric acid during storage (data not shown). These values were relatively similar to the results found by previous researchers (Kelebek, Selli, Canbas, & Cabaroglu, 2009; Manso et al., 2001).

3.1.1.2 Organic acid profile

In this study, the organic acids identified and quantified were citric, malic and ascorbic acid. Among these, citric acid was found to be the major organic acid in orange juice. The initial concentration of citric acid was 7.85 g L^{-1} and malic acid was 1.78 g $L^{-1.}$ Ascorbic acid was quantified by a different method and is discussed in section 3.1.4. Similarly to changes in pH and TA, both citric and malic acid content were relatively stable during the 32-weeks of storage in the range of time and temperature conditions studied. The citric acid content ranged from 7.02 to 8.40 g L^{-1}, on the other hand, malic acid concentration ranged from 1.57 to 1.90 g L^{-1} (data not shown).

3.1.2 Orange juice sugar content as affected by storage time and temperature

Sugar is described as one of the contributing factors to browning in citrus juices as mentioned by several researchers (Lee et al., 1988a; Murata, Shinoda, & Homma, 2002; Roig et al., 1999). Sugars constitute the largest quantity of the total soluble solids in orange juice (Kelebek et al., 2009). During storage, sugars may break down producing carboxylic intermediates which further react to form brown polymers (Robertson & Samaniego, 1986).

One of the main decomposition products of sugars under acidic conditions is 5-hydroxymethylfurfural (HMF), which is discussed in section 3.1.5. In this work, changes in sugars were investigated as the total sugar content (°Brix) and as the sugar profile (glucose, fructose, and sucrose content). The relationship between °Brix and browning was investigated by Buedo, Elustondo, & Urbicain (2000). Likewise, a study on the type of sugars is relevant since different types of sugars are described to have a different reactivity for HMF formation (Lee et al., 1988b; Lee et al., 1988a).

3.1.2.1 Total sugar content

The total sugar content of orange juice can be expressed as °Brix. At the beginning of storage, the sugar content was 11.01° and at the end of storage, it varied between 11.19° and 11.38° depending on the storage temperature (data not shown). These results fall within the average sugar content of orange juice reported by previous investigators (Farnworth, Lagace, Couture, Yaylayan, & Stewart, 2001; Kelebek et al., 2009). Consequently, it was observed that there was a slight increase in °Brix during storage. A similar trend was also found by Farnworth et al. (2001) during refrigerated storage of orange juice for eight months. However, possible reasons for this trend were not mentioned by the authors. On the other hand, some studies found an insignificant change in the total sugar content during storage of orange juice (Cortés, Esteve, & Frígola, 2008) and lemon juice (Robertson et al., 1986). In some literature reports, °Brix is referred to as total soluble solids. However, since the acidity content of orange juice is relatively high, a correction of the Brix value can be put forward ($°Brix_c$). Furthermore, the °Brix values listed in various fruit juices' regulation may be determined according to $°Brix_c$ values (AIJN, 1996). In this work, an increase in soluble solids ($°Brix_c$) was also observed during storage, which could be explained by the formation of soluble degradation products as a function of time and temperature (data not shown).

3.1.2.2 Sugar profile

Fructose, glucose and sucrose were identified as the three major sugar compounds of orange juice, with the highest contribution of sucrose (46%), followed by fructose (31%) and glucose (23%), almost corresponding to a ratio of 2:1:1. It was calculated that the initial concentration of total sugar content was 65.29 g L^{-1}, which was relatively similar as the one found by Farnworth et al. (2001).

Figure 1 depicts the changes in fructose, glucose and sucrose concentrations during storage, wherein fructose and glucose concentrations increased, while sucrose concentration decreased. A similar trend was previously reported for orange juice, which could be attributed to sucrose hydrolysis in the presence of acids (Kennedy, Rivera, Lloyd, Warner, & Jumel, 1990; Lee et al., 1988a). For storage at 20 °C, the sucrose concentration decreased by 8% after 8 weeks, while, almost 70% of sucrose was hydrolysed at 42 °C. Indeed, Kennedy et al. (1990) found that sucrose inversion in acidic conditions was enhanced by an increase in storage temperature. Within 8 weeks of storage at 42 °C, fructose and glucose concentrations were increased almost twice than their initial concentrations. This considerable increase can be linked to the described increase in °Brix value during storage (section 3.1.2.1). It can be observed that the decrease of sucrose content did not coincide with the increase of fructose and glucose content. For example after 32 weeks at 20 °C, a decrease of sucrose (0.020 mole L^{-1}) was lower than an increase of fructose (0.034 mole L^{-1}) or glucose (0.028 mole L^{-1}). Hydrolysis of carbohydrates like orange fibres may be an explanation for this. Orange juice pulp is known to consist of pectins, cellulose, hemicellulose and lignin (Grigelmo-Miguel & Martín-Belloso, 1998).

The increase in fructose and glucose concentrations could be best modelled by a zero-order model, while a second-order kinetic model gave the best fit for the hydrolysis of sucrose. The estimated kinetic parameters are listed in **Table 1**. The range of estimated activation energies (E_a), as calculated by Arrhenius equation (Equation 6), for fructose, glucose and sucrose ranged from 88 to 109 kJ mol^{-1}.

3.1.3 Orange juice oxygen content as affected by storage time and temperature

The influence of oxygen on the quality of citrus juices during storage was investigated by many researchers (Kennedy, Rivera, Lloyd, Warner, & Jumel, 1992; Robertson et al., 1986; Solomon et al., 1995; Zerdin, Rooney, & Vermuë, 2003). During storage oxygen determines the oxidative degradation rate of compounds, such as ascorbic acid, which indirectly influences not only colour but also aroma of orange juice (Berlinet, Brat, Brillouet, & Ducruet, 2006). Oxygen can be present in the packed juice as dissolved oxygen, which is incorporated during preparation and processing, as well as headspace oxygen. In addition, it can enter the package from the environment through diffusion processes (Bacigalupi et al., 2013; García-Torres, Ponagandla, Rouseff, Goodrich-Schneider, & Reyes-De-Corcuera, 2009).

Figure 2 illustrates the changes in dissolved and headspace oxygen concentrations of orange juice during storage. At all temperatures, there was a large decrease in both headspace and dissolved oxygen concentrations during the first four weeks of storage, after which the concentrations became almost stable during the subsequent weeks. A sharp decrease of oxygen was probably due to its consumption by oxidative degradation reactions, such as ascorbic acid (AA) oxidation. As can be seen in section 3.1.4, a drop of AA concentration during storage was observed at all storage temperatures. A significant correlation between the level of dissolved oxygen and browning as a result of the oxidative degradation of AA was

shown by Solomon et al. (1995). Indeed, many investigator reported that oxygen is really important for the AA degradation and thereby browning of the juice (Robertson et al., 1986; Roig et al., 1999; Zerdin et al., 2003).

The constant oxygen concentration after prolonged storage (> 4 weeks) can probably be explained by a continuously diffusion of oxygen through the PET bottle which equals the oxygen consumption by oxidative degradation reactions. A similar trend in the evolution of dissolved oxygen concentration was observed by Roig et al. (1999). Also, Robertson and Samaniego (1986) reported the rapid disappearance of dissolved oxygen in lemon juice samples which reached a low level after 7 days of storage then remained relatively constant during the subsequent storage period.

The fractional conversion model described well the decreasing trend of dissolved (R^2_{adj} = 0.97) and headspace (R^2_{adj} = 0.99) oxygen concentrations as a function of storage. According to this model, the estimated initial concentration for dissolved oxygen was above 3000 ppb and over 5000 ppb for headspace oxygen, while the estimated end concentration (C_∞) were in the range of 60-140 ppb and 310-370 ppb for dissolved oxygen and headspace oxygen, respectively (data not shown). From the estimated rate constants (*k*-value), it can be seen that both dissolved and headspace oxygen decreased faster at higher temperatures. At all storage temperatures, it is likely that as the dissolved oxygen is consumed in the orange juice, it is replenished with the headspace oxygen to reach equilibrium. As long as the oxygen supply of the headspace lasts, this equilibrium can be kept (García-Torres et al., 2009). The temperature dependence of the rate constants was determined by the Arrhenius equation with an estimated activation energy (E_a) of 26 kJ mol^{-1} for dissolved oxygen and 22 kJ mol^{-1} for headspace oxygen. Polydera, Stoforos, & Taoukis (2003) reported a low activation energy (E_a

= 13–24 kJ mol^{-1}) for ascorbic acid degradation which was controlled by the oxygen diffusion process.

3.1.4 Orange juice vitamin C content as affected by storage time and temperature

There are two biological active forms of vitamin C, ascorbic acid (AA) and dehydroascorbic acid (DHA). Besides the previously observed correlation of AA degradation with an increase in non-enzymatic browning (Zerdin et al., 2003), the AA content is often used for the determination of orange juice's shelf-life (Lee & Coates, 1999; Polydera, Stoforos, & Taoukis, 2003).

Prior to storage, the AA content was 433 mg L^{-1} while the DHA content was 37 mg L^{-1}, which results in a total vitamin C content of 470 mg L^{-1}. This result is within the range of average vitamin C concentration in orange juice reported by Burdurlu et al. (2006), Solomon et al. (1995) and Vervoort et al. (2011). After 8 weeks of storage, the vitamin C retention varied from 68% at 20 °C to less than 26% when stored at 42 °C.

Figure 3 shows the changes in AA and DHA concentrations as a function of storage time and temperature. AA concentration was clearly affected by time as well as by temperature. More than 75% of AA decreased within 32 weeks at 20 °C, which was comparable to the results of 8 weeks at 42 °C of storage. This decrease in AA concentration can be explained by the aerobic degradation pathway as referred to by several researchers (Kennedy et al., 1992; Robertson et al., 1986; Zerdin et al., 2003). In the presence of oxygen, AA is reversibly oxidised to DHA. However, the decrease in AA concentration did not correspond to an increase in the concentration of DHA. The most possible explanation is that DHA is highly unstable and can further irreversibly hydrolyse to 2,3-diketogulonic acid (DKG) and other

breakdown products, such as 2-furoic acid, 3-hydroxy-2-pyrone, which is favoured by low pH conditions (Shinoda et al., 2005; Yuan & Chen, 1998).

The degradation of AA can be attributed largely to the oxygen that was dissolved in the juice and in the headspace in the early stages of storage. As discussed in section 3.1.3, headspace and dissolved oxygen concentrations showed a rapid initial decrease and became constant after four weeks of storage. This corresponds to the results of Polydera et al. (2003) who observed a decrease of AA at the beginning of storage due to reaction between AA and the dissolved oxygen. In addition, the rate of AA oxidation is dependent on the dissolved oxygen (Solomon et al., 1995; Zerdin et al., 2003) and headspace concentration (Van Bree et al., 2012). Recently, Bacigalupi et al. (2013) established a mathematical model to predict shelf-life of orange juice by taken into account factors such as oxygen diffusivity, solubility, permeability, thickness and surface of the packaging material. In this work, as AA concentration still decreased after the fast oxygen drop, the continuation of the aerobic oxidation of AA was mainly driven by the oxygen permeation through the PET bottles during storage (Berlinet et al., 2006). As both aerobic and anaerobic degradation pathways may occur simultaneously (Polydera et al., 2003), when oxygen is only present in a low amount, anaerobic AA degradation could be suggested to have taken place slowly as well.

In the present study, the degradation of AA was best described by a first-order kinetic model (R^2_{adj} = 0.99), which was in agreement with the results reported by several researchers (Burdurlu et al., 2006; Lee et al., 1999; Polydera et al., 2003). On the other hand, Kaanane, Kane, & Labuza (1988) reported a pseudo-zero order reaction, while a second-order kinetic model was suggested to fit the AA degradation when oxygen became limited (Robertson et al., 1986). Higher losses of AA were found at higher storage temperatures as presented in

Figure 3. The estimated activation energy of AA degradation was 45 kJ mol^{-1}, which was in accordance with the results reported by Burdurlu et al. (2006). In general, the changes of DHA showed a decreasing trend during storage, however since DHA content was varied, no model was selected to fit the data.

Since the nutritional quality in orange juice is primarily related to its AA content, the shelf-life of orange juice is often determined by its AA content, either by legislation or industrial practice. In the European Union, according to the Association of the Industry of Juices and Nectars (AIJN, 1996), the minimum AA content in orange juice should be 200 mg L^{-1} at the end of the shelf-life. Using the first-order kinetic model, an estimated shelf-life for each storage temperature was determined to be 16, 10, 7 and 4 weeks at 20 °C, 28 °C, 32 °C and 42 °C respectively. The shelf-life threshold is depicted in **Figure 3A** by a dashed line.

3.1.5 Orange juice furfural and 5-hydroxymethylfurfural (HMF) as affected by storage time and temperature

Furfural and 5-hydroxymethylfurfural (HMF) have been associated with several degradation reactions, including non-enzymatic browning of orange juice during storage (Lee et al., 1988a; Solomon et al., 1995; Robertson et al., 1986). Moreover, these compounds are recognised indicators for temperature abuse of processed and stored orange juice as well as quality deterioration in general. In the Code of Practice for the evaluation of fruit and vegetable juices, published by the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union (AIJN), HMF is indicated as one of the quality parameters with a maximum allowable limit of 10 mg L^{-1} for both direct and reconstituted orange juice (AIJN, 1996).

3.1.5.1 Furfural

The changes of furfural during storage are depicted in **Figure 4 A.** At the beginning of storage, no furfural was detected. Furfural was only formed when orange juice was stored at higher storage temperatures, above 20 °C. Based on a signal-to noise ratio (S/N) of 3:1, the limit of detection (LOD) of furfural was determined to be 0.035 mg L^{-1}. At 28 °C, 35 °C and 42 °C, furfural was accumulated from week 8, week 5 and week 2 on, respectively. An increase in furfural concentration at prolonged storage could be explained by the anaerobic ascorbic acid (AA) degradation reaction pathway. Indeed, a substantial decrease in headspace and dissolved oxygen concentration occurred after 2 weeks of storage (section 3.1.3) and a further decrease in AA concentration was observed (section 3.1.4). It is known that under anaerobic conditions, the direct cleavage of AA occurs by hydrolysis, which through decarboxylation and dehydration of intermediary products, further leads to the formation of furfural (Yuan et al., 1998).

In citrus juices, furfural can also be formed from sugars (Robertson et al., 1986; Shinoda et al., 2005). The concentrations of sucrose and AA, which are the main components for furfural formation, were decreasing at all storage temperatures (section 3.1.2.2 and 3.1.4). Based on these observation, it was expected to detect furfural in orange juice at all storage temperatures. However, furfural formation was detected only at storage temperatures higher than 20 °C. For storage temperatures of 28 °C, 35 °C and 42 °C, a clear increasing trend of furfural formation was observed. Similarly, a significant increase of furfural at higher temperatures was observed by Lee at al. (1988a), who reported more than 1 μg mL^{-1} furfural after 15 weeks at 30 °C, while at 10 °C, less than 0.05 μg mL^{-1} furfural.

The linear increase of furfural concentration as a function of time could be best modelled by a zero-order model. This is similar to the results of Robertson & Samaniego (1986) and Kaanane, Kane, & Labuza (1988) for citrus juices. At the beginning of storage, there was no furfural detected, which explains why the predicted lines did not fit well with measured values in the beginning of storage. Thus, the formation of furfural does not necessarily follow a zero-order model, it rather shows the best fit among models evaluated in this study (zero-, first- and second-order models). The effect of temperature was assessed using the Arrhenius equation, yielding an activation energy (E_a) of 110 kJ mol^{-1} for furfural formation.

3.1.5.2 5-hydroxymethylfurfural (HMF)

HMF is known as a degradation product of the hydrolysis of sugars under acidic conditions. It can be formed by enolisation and dehydration of glucose or fructose with fructose as the main precursor (Lee & Nagy, 1990). The Maillard reactions can also result in the accumulation of HMF as an intermediate. Its formation is affected by the type and concentration of sugars, quantity and structure of amino acids, pH, heating temperature and storage conditions (Lee et al., 1990; Lee et al., 1988a; Cortés et al., 2008).

As can be seen from **Figure 4 B**, HMF started to accumulate after 20 weeks at 20 °C, 8 weeks at 28 °C, 3 weeks at 35 °C and 4 days at 42 °C, respectively. A slow formation of HMF corresponded to the results of Lee & Nagy (1988b), who observed almost no HMF formation up to 15 weeks during storage at 20 °C, while significant quantities were produced after 3 weeks at 30 °C and 50 °C. At higher storage temperatures, there was a considerable increase of HMF. However, the HMF concentration of all stored orange juice samples was lower than the allowed maximum level of 10 mg L^{-1} as recommended by the AIJN. The highest concentration of HMF was 7.56 mg L^{-1}, which was reached after 8 weeks of storage at 42 °C. As previously mentioned, the rate of HMF formation is also dependent on the type and

concentration of sugars. Referring to the section 3.1.2.2, a decrease in sucrose, a precursor of fructose and glucose, concentration during storage was observed. Although it is well-known that decomposition of hexoses yields HMF, according to Lee et al. (1988a), the stoichiometric relationship between sugar loss and 5-HMF formation is difficult to explain because the observed accumulation of 5-HMF might result from equilibrium conditions between formation and degradation.

In this study, the formation of HMF during storage could be well-fitted by a zero-order model (R^2_{adj} = 0.96). Burdurlu et al. (2006) reported the HMF formation during storage of orange juice concentrate was described by a zero-order model, whereas a first-order was reported by Robertson & Samaniego (1986) for lemon juice. **Figure 4 B** shows the HMF formation was faster at higher storage temperatures. Orange juice stored at 42 °C had an HMF formation rate constant (*k*-value) approximately 3 times higher than the rate constant at 35 °C. The effect of temperature was modelled by the Arrhenius model. The estimated activation energy was 136 kJ mol^{-1}, which is lower than the values reported by Burdurlu et al. (2006) for citrus juice concentrates (181-334 kJ mol^{-1}).

3.2 Interpretation of the mutual importance of the selected quality parameters and their link to colour change

In this paper, a selection of quality parameters of orange juice were quantitatively studied as a function of storage time and temperature (section 3.1). Next, to gain insight into the importance of these parameters to colour change, the following steps were taken. In previous work, it was found that a distinctive colour degradation was found at prolonged storage and at higher temperatures, in which a bright yellow colour turned to brown. Total colour difference (ΔE^*) was chosen to determine the colour change objectively through CIELAB colour space. In accordance with the visual observation, it was found that ΔE^* increased during storage or

in other words the colour after storage was more perceivable than at the beginning of storage (Wibowo et al., 2015).

To study the mutual importance of the selected quality parameters for colour change, multivariate data analysis was performed. The PLS (partial least squares) models and their corresponding biplots were constructed to visualise the correlation between ΔE^* and different quality parameters, in which the studied parameters were considered as *X*-variables and the ΔE^* values, as *Y*-variables. Variable Identification (VID) coefficients were calculated to rank the importance of these parameters to colour change. Higher VID values can be interpreted as higher correlation between quality attributes change and colour change. In total, 15 quality parameters were taken into account for analysis (pH, TA, citric acid, malic acid, °Brix, fructose, glucose, sucrose, dissolved and headspace oxygen, ascorbic acid (AA), dehydroascorbic acid (DHA), furfural, HMF, and total carotenoids). In order to understand the mutual importance of all studied parameters, the change in total carotenoid content as reported in a previous study (Wibowo et al., 2015) was also included in the analysis.

The modelling was performed for all storage temperatures using two latent variables (LVs). The LVs are a linear combination of the original *X*-variables (parameters) for which the trend as a function of colour change (continuous *Y*-variable) is maximally explained. A relatively high percentage of the *Y*-variances explained by the first two LVs can be observed (98.7%, 99.3%, 97.5% and 98.7% for 20 °C, 28 °C, 35 °C and 42 °C, respectively). Due to a small difference among storage temperature of 28 °C, 35 °C and 42 °C, the respective biplots are only displayed only for 20 °C and 42 °C (**Figure 5**). The parameters' importance can be qualitatively evaluated depending on the location of the parameters on the bi-plot (inner and outer ellipses in the plot represent correlation coefficients of 70% and 100%). Moreover,

parameters which are situated in the same direction as the *Y*-vector are positively correlated with colour change, on the contrary, negative correlation was found if they are located at the opposite direction of the *Y*-vector. More quantitative insight on the mutual parameter's importance (i.e. correlation to) for colour change was provided by the VID coefficients. The higher the VID values, the higher the correlation between specific parameters and colour change. Since the ΔE^* value increased during storage, a positive VID coefficient also indicates an increase in value during storage, while a negative coefficient indicates a decrease. At 20 °C, six parameters had the highest absolute VID value: sucrose, fructose, glucose, °Brix, ascorbic acid and HMF, in decreasing order of VID. As the storage temperature increased to 42 °C, one additional parameter, furfural exhibited an important correlation with colour changes. In decreasing order of VID, they were fructose, glucose, sucrose, ascorbic acid, HMF, furfural and °Brix. Indeed, increasing storage temperature had influenced the furfural formation (section 3.1.5.1). The smaller absolute value of VID coefficients were shown by acids (TA, pH, citric and malic acid), total carotenoids, DHA and oxygen, which could imply their minor importance to colour instability. Concerning the correlation analysis, it should be taken into account that this analysis does not imply causality, rather it gives an idea which quality properties could be important to colour degradation. Due to the complexity of colour degradation reactions and possible interaction between different pathways, there is probably more than one responsible mechanism.

4 CONCLUSION

The evolution of quality parameters of heat treated orange juice as a function of storage time and temperature and the mutual importance of the selected parameters for colour change were studied in this research paper. Firstly, the kinetic evolution of the measured parameters, namely acids (pH, TA and organic acid profile), sugars (total sugar content and sugar profile), oxygen (headspace and dissolved oxygen) content, vitamin C (ascorbic and dehydroascorbic) content, furfural and HMF content, as a function of time was carefully assessed. Among the aforementioned parameters, the changes in acids during the entire storage were considered negligible. On the other hand, vitamin C, sugars, oxygen, furfural and HMF showed significant changes in concentration as a function of time and temperature. The acid nature of the juice may have caused the hydrolysis of sucrose resulting in an increase in fructose and glucose concentrations. While, a significant decrease of vitamin C content can be attributed to the ascorbic acid (AA) degradation reaction, which is known as a potential precursor of brown pigments in orange juice. Based on their high correlation with the colour changes, among many factors interplay, AA, sugar and their degradation products, furfural and HMF, are the most important factors for browning. Therefore, it can be concluded that there is more than one pathway in development of browning in orange juice, in which ascorbic acid and acid-catalysed degradation of sugar are important reactions in storage of pasteurised orange juice. Finally, in terms of colour stability, the shelf-life of orange juice will be extended by reducing the storage temperature and avoiding oxygen permeation through the packaging.

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References

AIJN (1996). *Code of practice for evaluation of fruit and vegetable juices*. Brussels: Association of Juices and Nectars from Fruits and Vegetables of European Union.

AOAC (1998). *Official methods of analysis of AOAC International (16th ed.).* Arlington, VA, USA: AOAC International.

Bacigalupi, C., Lemaistre, M. H., Boutroy, N., Bunel, C., Peyron, S., Guillard, V. et al. (2013). Changes in nutritional and sensory properties of orange juice packed in PET bottles: An experimental and modelling approach. *Food Chemistry, 141,* 3827-3836.

Berlinet, C., Brat, P., Brillouet, J. M., & Ducruet, V. (2006). Ascorbic acid, aroma compounds and browning of orange juices related to PET packaging materials and pH. *Journal of the Science of Food and Agriculture, 86,* 2206-2212.

Buedo, A. P., Elustondo, M. P., & Urbicain, M. J. (2000). Non-enzymatic browning of peach juice concentrate during storage. *Innovative Food Science & Emerging Technologies, 1,* 255-260.

Burdurlu, H. S., Koca, N., & Karadeniz, F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. *Journal of Food Engineering, 74,* 211-216.

Cortés, C., Esteve, M. J., & Frígola, A. (2008). Color of orange juice treated by high intensity pulsed electric fields during refrigerated storage and comparison with pasteurized juice. *Food Control, 19,* 151-158.

Farnworth, E. R., Lagace, M., Couture, R., Yaylayan, V., & Stewart, B. (2001). Thermal processing, storage conditions, and the composition and physical properties of orange juice. *Food Research International, 34,* 25-30.

García-Torres, R., Ponagandla, N. R., Rouseff, R. L., Goodrich-Schneider, R. M., & Reyes-De-Corcuera, J. I. (2009). Effects of dissolved oxygen in fruit juices and methods of removal. *Comprehensive Reviews in Food Science and Food Safety, 8*.

Grauwet, T., Vervoort, L., Colle, I., Van Loey, A., & Hendrickx, M. (2014). From fingerprinting to kinetics in evaluating food quality changes. *Trends in Biotechnology, 32*, 125-131.

Grigelmo-Miguel, N. & Martín-Belloso, O. (1998). Characterization of dietary fiber from orange juice extraction. *Food Research International, 31,* 355-361.

Kaanane, A., Kane, D., & Labuza, T. P. (1988). Time and temperature effect on stability of Moroccan processed orange juice during storage. *Journal of Food Science, 53,* 1470-1473.

Kelebek, H., Selli, S., Canbas, A., & Cabaroglu, T. (2009). HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. *Microchemical Journal, 91,* 187-192.

Kennedy, J. F., Rivera, Z. S., Lloyd, L. L., Warner, F. P., & Jumel, K. (1990). Studies on non-enzymic browning in orange juice using a model system based on freshly squeezed orange juice. *Journal of the Science of Food and Agriculture, 52,* 85-95.

Kennedy, J. F., Rivera, Z. S., Lloyd, L. L., Warner, F. P., & Jumel, K. (1992). L-ascorbic-acid stability in aseptically processed orange juice in Tetrabrik cartons and the effect of oxygen. *Food Chemistry, 45,* 327-331.

Kimball, D. (1991). *Citrus processing: Quality control and technology*. Springer Netherlands.

Lee, H. S. & Coates, G. A. (1999). Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study. *Food Chemistry, 65,* 165-168.

Lee, H. S. & Nagy, S. (1990). Relative reactivities of sugars in the formation of 5-Hydroxymethylfurfural in sugar-catalyst model systems. *Journal of Food Processing and Preservation, 14,* 171-178.

Lee, H. S. & Nagy, S. (1988a). Quality changes and nonenzymic browning intermediates in grapefruit juice during storage. *Journal of Food Science, 53,* 168-172.

Lee, H. S. & Nagy, S. (1988b). Relationship of sugar degradation to detrimental changes in citrus juice quality. *Food Technology, 42,* 91-94.

Lee, H. S., Rouseff, R. L., & Nagy, S. (1986). HPLC determination of furfural and 5-hydroxymethylfurfural in citrus juices. *Journal of Food Science, 51,* 1075-1076.

Manso, M. C., Oliveira, F. A. R., Oliveira, J. C., & Frías, J. M. (2001). Modelling ascorbic acid thermal degradation and browning in orange juice under aerobic conditions. *International Journal of Food Science & Technology, 36,* 303-312.

Mills, A. (2005). Oxygen indicators and intelligent inks for packaging food. *Chemical Society Reviews, 34,* 1003-1011.

Murata, M., Shinoda, Y., & Homma, S. (2002). Browning of model orange juice solution and changes in the components. *International Congress Series, 1245,* 459-460.

Neves, M. F., Trombin, V. G., Lopes, F. F., Kalaki, R., & Milan, P. (2011). *The orange juice business - A Brazilian perspective*. Wageningen: Wageningen Academic Publishers.

Polydera, A. C., Stoforos, N. G., & Taoukis, P. S. (2003). Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. *Journal of Food Engineering, 60,* 21-29.

Robertson, G. L. & Samaniego, C. M. L. (1986). Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *Journal of Food Science, 51,* 184-187.

Roig, M. G., Bello, J. F., Rivera, Z. S., & Kennedy, J. F. (1999). Studies on the occurrence of non-enzymatic browning during storage of citrus juice. *Food Research International, 32,* 609-619.

Shallenberger, R. S. & Mattick, L. R. (1983). Relative stability of glucose and fructose at different acid pH. *Food Chemistry, 12,* 159-165.

Shinoda, Y., Komura, H., Homma, S., & Murata, M. (2005). Browning of model orange juice solution: Factors affecting the formation of decomposition products. *Bioscience Biotechnology and Biochemistry, 69,* 2129-2137.

Silva, F. V. M. & Gibbs, P. (2004). Target selection in designing pasteurization processes for shelf-stable high-acid fruit products. *Critical Reviews in Food Science and Nutrition, 44,* 353-360.

Solomon, O., Svanberg, U., & Sahlstrom, A. (1995). Effect of oxygen and fluorescent light on the quality of orange juice during storage at 8°C. *Food Chemistry, 53,* 363-368.

van Boekel, M. A. J. S. (2008). Kinetic Modeling of Food Quality: A Critical Review. *Comprehensive Reviews in Food Science and Food Safety, 7,* 144-158.

Van Bree, I., Baetens, J. M., Samapundo, S., Devlieghere, F., Laleman, R., Vandekinderen, I. et al. (2012). Modelling the degradation kinetics of vitamin C in fruit juice in relation to the initial headspace oxygen concentration. *Food Chemistry, 134,* 207-214.

Verbeyst, L., Bogaerts, R., Van der Plancken, I., Hendrickx, M., & Van Loey, A. (2013). Modelling of vitamin C degradation during thermal and high-pressure treatments of red fruit. *Food Bioprocess Technol, 6,* 1015-1023.

Vervoort, L., Van der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M. et al. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part II: Impact on specific

chemical and biochemical quality parameters. *Innovative Food Science and Emerging Technologies, 12,* 466-477.

Wibowo, S., Vervoort, L., Tomic, J., Santiago, J. S., Lemmens, L., Panozzo, A. et al. (2015). Colour and carotenoid changes of pasteurised orange juice during storage. *Food Chemistry, 171,* 330-340.

Yeom, H. W., Zhang, Q. H., & Chism, G. W. (2002). Inactivation of pectin methyl esterase in orange juice by pulsed electric fields. *Journal of Food Science, 67,* 2154-2159.

Yuan, J. P. & Chen, F. (1998). Degradation of ascorbic acid in aqueous solution. *Journal of Agricultural and Food Chemistry, 46,* 5078-5082.

Zerdin, K., Rooney, M. L., & Vermuë, J. (2003). The vitamin C content of orange juice packed in an oxygen scavenger material. *Food Chemistry, 82,* 387-395.

Table 1. Estimated kinetic parameters based on the selected degradation kinetic model (20 °C as reference temperature) describing the changes during storage of quality parameters linked to colour change in the pasteurised orange juice. One-step non-linear regression was performed. Samples were stored at 20 °C, 28 °C, 35 °C and 42 °C.

Quality parameter	C_0 [1)]	C_∞ [1)]	k_{ref} [2)]	E_a (kJ mol^{-1})	R^2_{adj}
Zero-order model					
fructose	20.80 ± 0.14	-	0.29 ± 0.01	88.02 ± 1.79	0.98
glucose	15.91 ± 0.19	-	0.21 ± 0.01	96.27 ± 3.34	0.94
furfural	N/A[3)]	-	0.025 ± 0.002	110.31 ± 4.44	0.92
HMF	N/A	-	0.017 ± 0.002	136.20 ± 4.56	0.96
First-order model					
ascorbic acid	425.80 ± 4.52	-	-0.060 ± 0.002	44.59 ± 2.06	0.99
First-order fractional conversion model					
dissolved oxygen	3 490 ± 95	91 ± 40	-0.78 ± 0.06	25.56 ± 5.41	0.97
headspace oxygen	7 935 ± 297	344 ± 19	-0.85 ± 0.03	22.32 ± 1.16	0.99
Second-order model					
sucrose	31.43 ± 0.22	-	-0.0008 ± 3.1.10^{-5}	108.23 ± 2.33	0.99

± 95 % approximate confidence interval

[1)] Unit for sugars (g L^{-1}), oxygen (ppb), ascorbic acid, furfural and HMF (mg L^{-1})
[2)] Unit for fructose and glucose (g L^{-1} week^{-1}), furfural and HMF (mg L^{-1} week^{-1}), ascorbic acid and oxygen (week^{-1}) and sucrose (L g^{-1} week^{-1})
[3)] N/A is not applicable

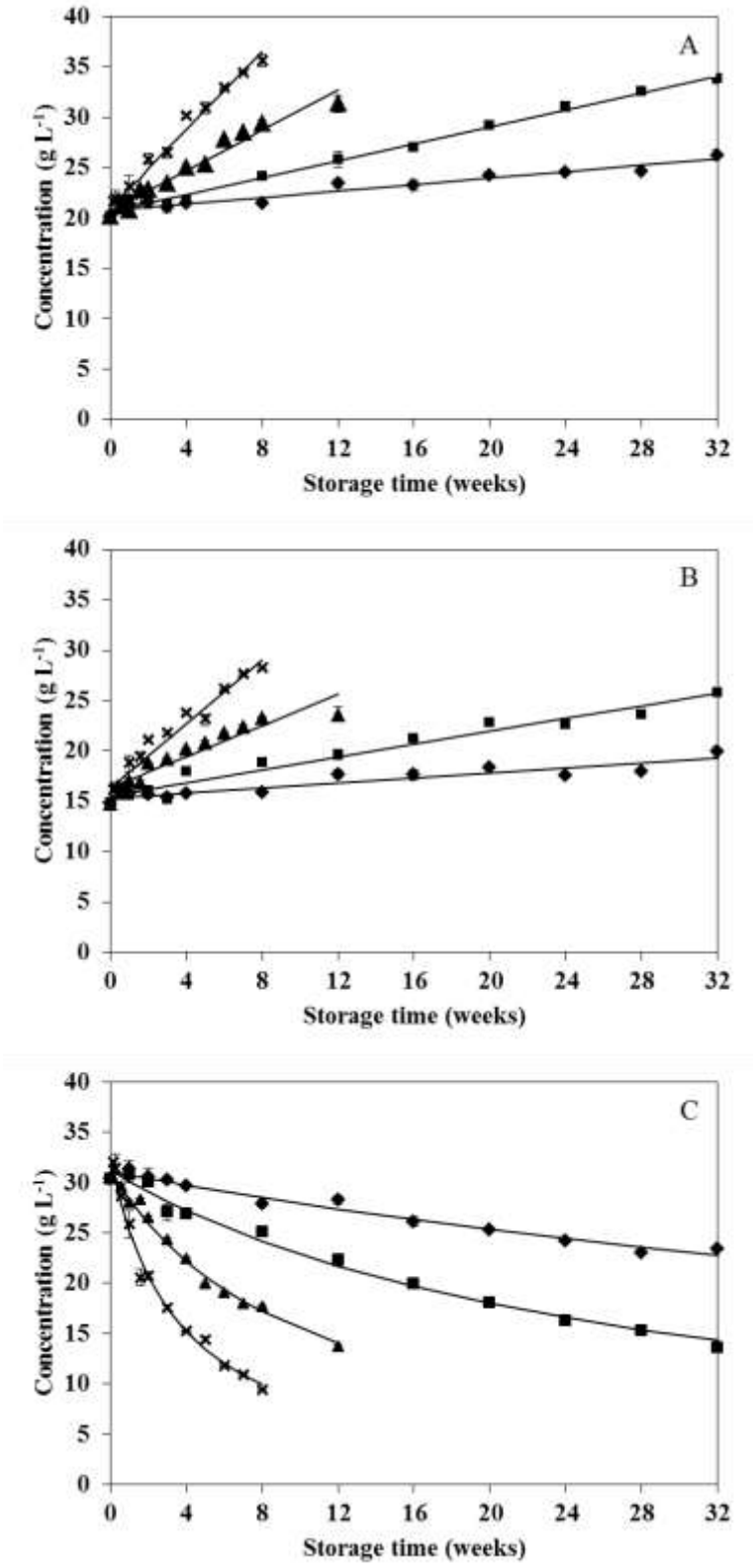


Figure 1. Fructose (A), glucose (B) and sucrose (C) concentrations during storage at 20 °C (◆), 28 °C (■), 35 °C (▲) and 42 °C (×). The full lines represent the fitted values by the zero-order model for fructose and glucose, and by the second-order model for sucrose.

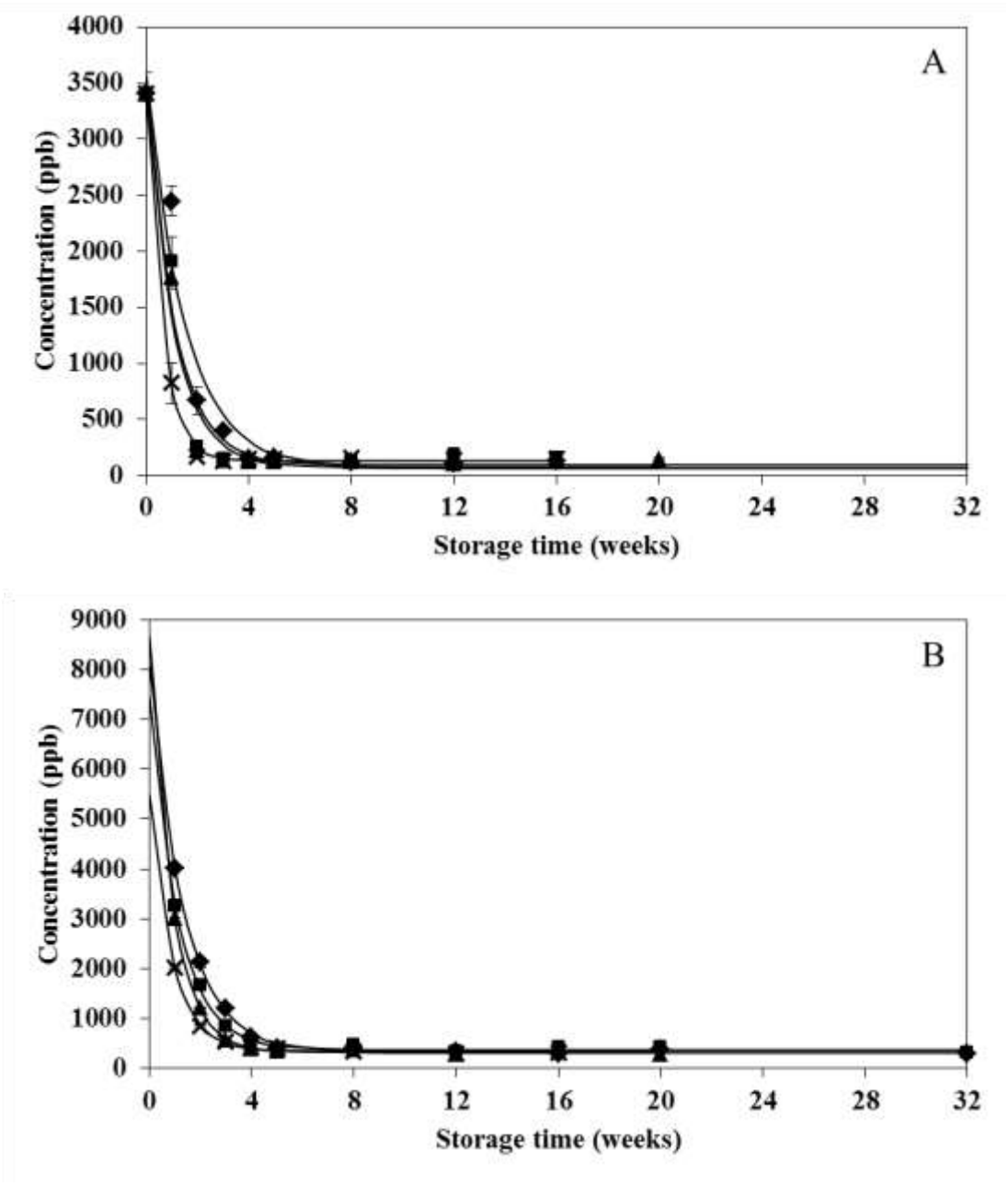


Figure 2. Dissolved (A) and headspace (B) oxygen concentration during storage at 20 °C (◆), 28 °C (■), 35 °C(▲) and 42 °C (×). The full lines represent the fitted values by the fractional conversion model.

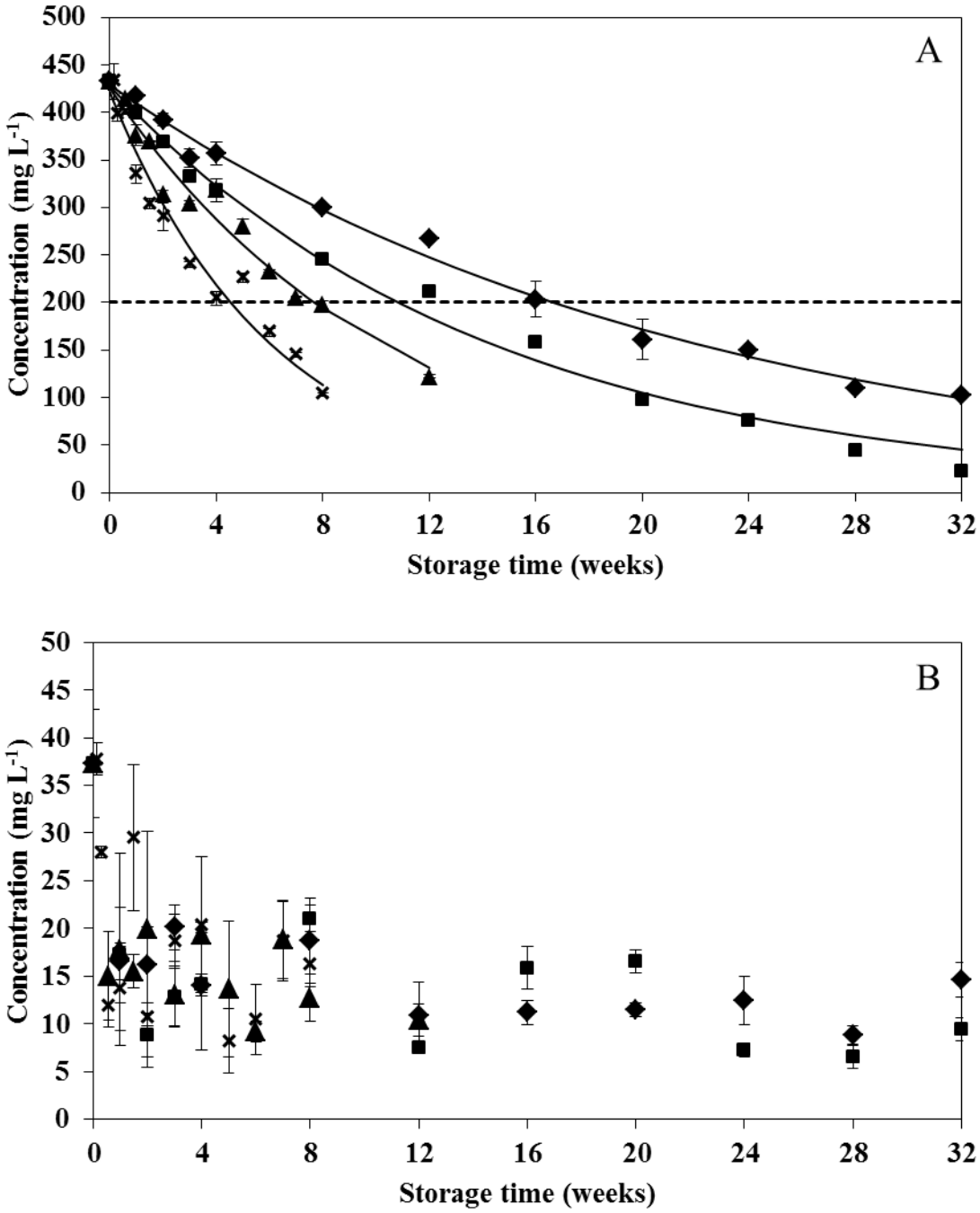


Figure 3. Ascorbic (A) and dehydroascorbic (B) acid concentration during storage at 20 °C (◆), 28 °C (■), 35 °C (▲) and 42 °C (×). The full lines represent the fitted values by the first-order model. The dashed horizontal line indicates the shelf-life threshold value of 200 mg L^{-1} AA.

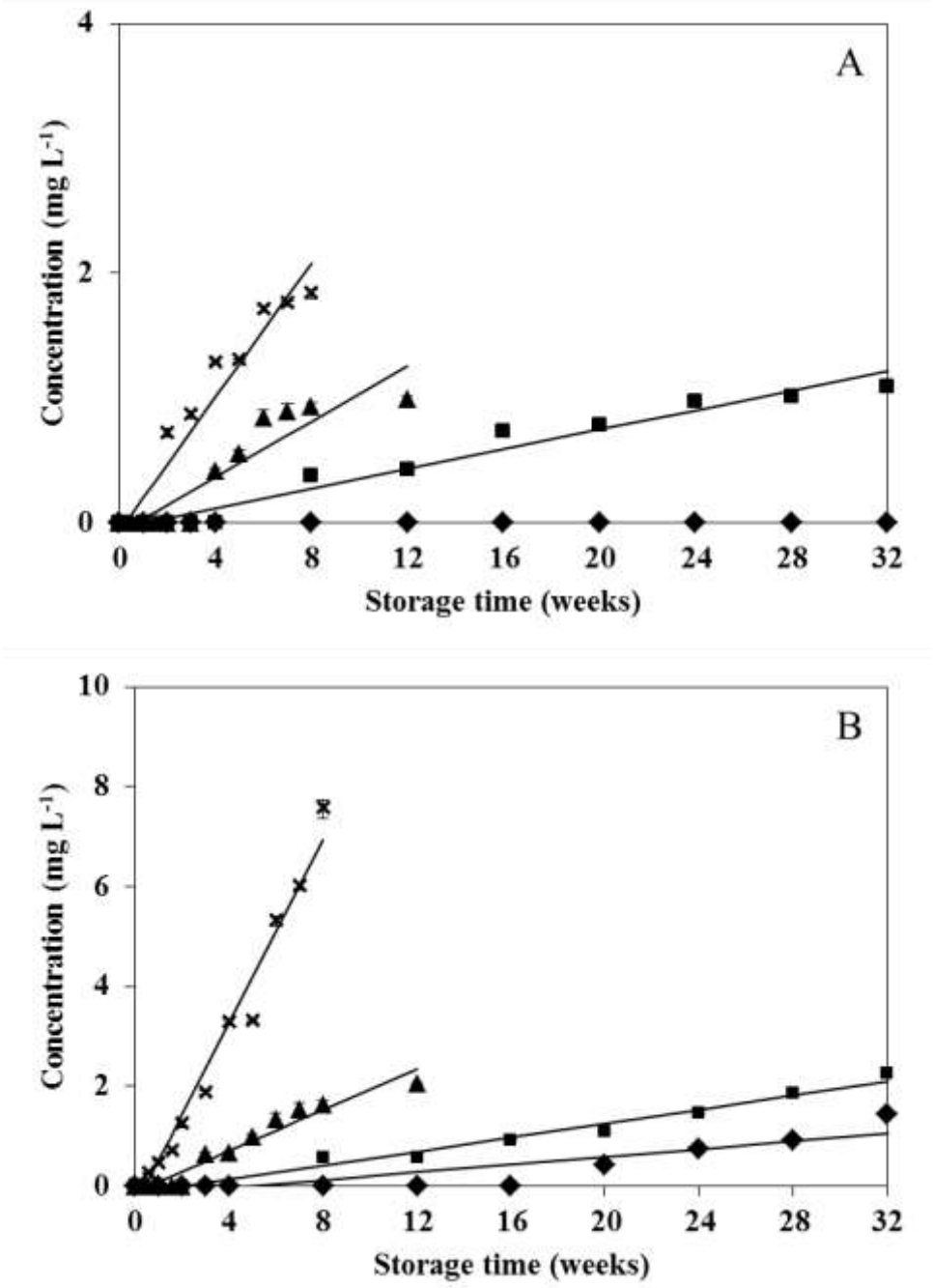
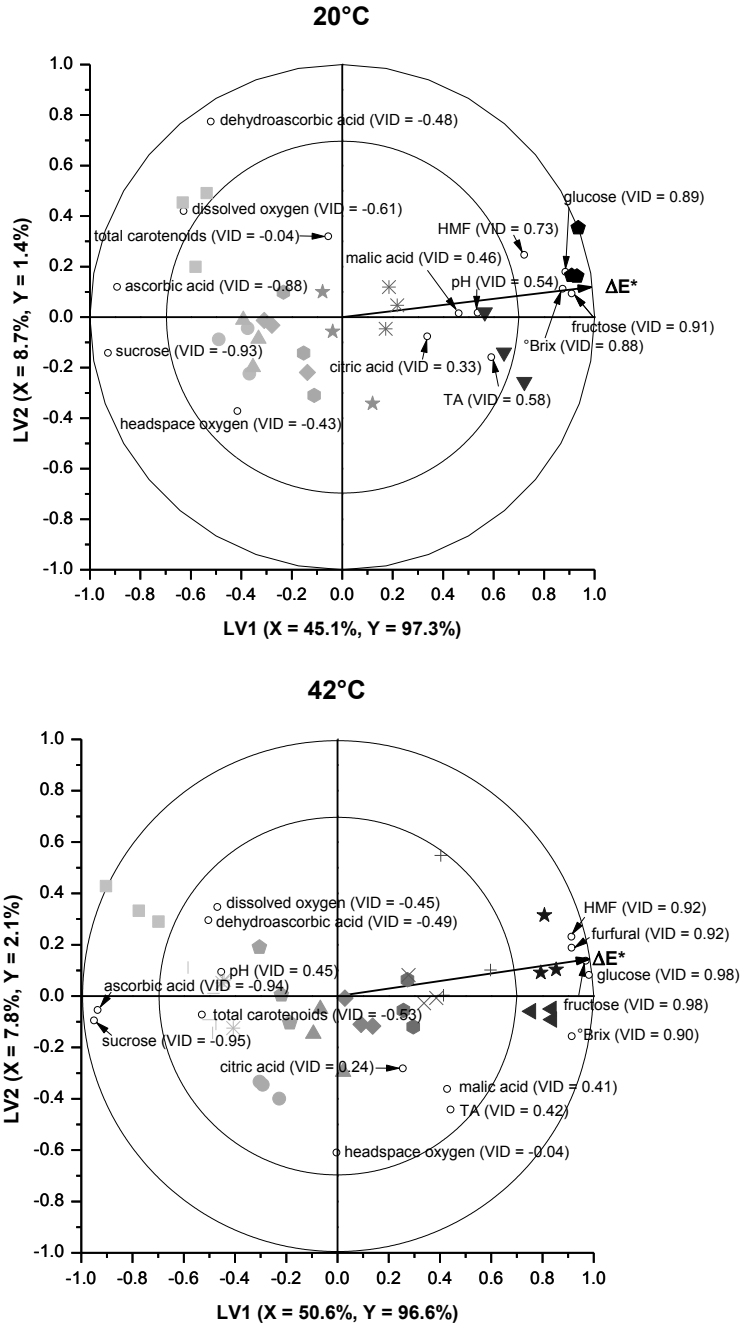


Figure 4. Furfural (A) and HMF (B) concentration during storage at 20 °C (◆), 28 °C (■), 35 °C (▲) and 42 °C (×). The full lines represent the fitted values by the zero-order model.



Storage time

■	week 0	●	week 1	⬢	week 4	★	week 8
▶	day 1	⬟	week 1.5	×	week 5	✳	week 16
✳	day 2	▲	week 2	+	week 6	▼	week 24
▼	day 4	◆	week 3	◀	week 7	⬟	week 32

Figure 5. PLS biplots visualising the correlation between colour changes and quality parameters of orange juice (objects represented by differently shaped symbols) at 20 and 42 °C. The open circles show different parameters of which the VID values are showed for each parameter. Positive VID coefficients indicate an increase in concentration or values during storage while negative coefficients denote a decrease. The correlation loadings for the continuous *Y*-variable (ΔE^*) are represented as a vector. The percentages of the variances in *X* and *Y* explained by each latent variable (LV1 and LV2) are indicated on the respective axes.