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Sensorial and analytical profiling of orange juice and apple juice: Development and validation of shelf-life prediction models

Sabrina Kotter-Seel

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LIST OF ABBREVIATIONS

A.I.J.N.	European Fruit Juice Association
AEDA	aroma extract dilution analysis
AF	acceleration factor
ANOVA	analysis of variance
AsA	ascorbic acid
ASLT	accelerated shelf-life testing
c	concentration
CAM	citric acid monohydrate
CIS	cold injection system
CoP	Code of Practice
CTS	cryo trap system
d	day *[unit]*
DOD	degree of difference
E_a	activation energy
ED	Euclidean distance
EI	electron ionisation
FC	*(juice)* from concentrate
FD factor	flavour dilution factor
FID	flame ionisation detector
FTNF	from the named fruit
GC-MS	gas chromatography/mass spectrometry
GC-TCD	gas chromatography/thermal conductivity detection
H_0	null hypothesis
5-HMF	5-hydroxymethylfurfural
HP	high pressure
HSD	honestly significant test *(Tukey's HSD test)*
ISTD	internal standard
k(T)	reaction rate
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
MD	mean deviation from the median
MDGC/MS	multi-dimensional gas chromatography/mass spectrometry
MFA	multiple factor analysis
mo	months *[unit]*
MS	mean squares
MSD	mass selective detector
NFC	*(juice)* not from concentrate
OAV	odour activity value
PC	principal component
PCA	principal component analysis
PDMS	polydimethylsiloxane
PEF	pulsed electric field

PLS	partial least square
PME	pectin methylesterase
PTV	programmed temperature vaporisers
QDA	quantitative descriptive analysis
(R)	reference sample
RI	retention index
RMSE	root mean squared error
RT	room temperature
SAFE	solvent assisted flavour evaporation
SBSE	stir bar sorptive extraction
SDE	simultaneous-distillation-extraction
SIM	selected ion monitoring
SS	sum of squares
TDU	thermal desorption unit
(V)	validation sample
VdF	Verband der deutschen Fruchtsaft-Industrie e.V.
wk	week *[unit]*

GENERAL REMARKS

For a clear distinction of the used time specification, the following abbreviations/terms are used in this thesis: If the specific measurement time point is meant, the abbreviations, e.g. “3 wk”, are used. If the duration of a storage is meant, terms such as “three weeks” are used.

1 INTRODUCTION AND OBJECTIVES

In modern product development processes, newly developed products have to be tested in terms of their analytical and sensorial stability throughout the whole shelf-life partly lasting up to one year or more. A real-time storage at ambient conditions until reaching the best before date is not efficient considering the required time and commercial resources. Therefore, shelf-life studies under accelerated storage conditions are preferred to quickly obtain an estimation of the product stability. This so-called accelerated shelf-life testing (ASLT), which is typically performed by exposing the product to elevated temperatures and/or light, has become a central step in the usual product development procedure to estimate and to ensure the product stability over the minimal product shelf-life by extrapolating the findings to real-time conditions at ambient temperatures.

This study focused on fruit juices with 100 % fruit juice content containing flavouring from the named fruit (FTNF). The selected juice matrices were orange juice and apple juice. They are the most consumed fruit juices being manufactured from one type of fruit (A.I.J.N., 2016), and their compositions of volatiles are very different.
In the past, a considerable amount of data has been published on the key volatiles of orange juice (Averbeck & Schieberle, 2009; Buettner & Schieberle, 2001; Seideneck & Schieberle, 2011) and apple juice (Hey et al., 2007; Nikfardjam & Maier, 2010, 2011; Steinhaus et al., 2005). Also the molecular changes including the degradation pathways and the sensorial deterioration have been assessed during normal aging and accelerated storage for orange juice (Bielig et al., 1972; Marcotte et al., 1998; Moshonas & Shaw, 1989, 2000; Petersen et al., 1998; Tatum et al., 1975) and to a lesser extent for apple juice (Wolter, 2011; You et al., 1994). However, these studies were not intended for the application of ASLT and a subsequent development of prediction models.
Whilst for apple juice no literature data regarding storage-related prediction models were available, there are some studies for orange juice with the aim of establishing predictive statements on the basis of an ASLT approach. Petersen et al. (1998) conducted shelf-life testing with normally stored and accelerated stored orange juice and collected data for the volatile components and the sensorial impressions at the same time for a small-sized data set. The most recent investigations with the aim of fingerprinting the volatile fraction in the context of ASLT were done by Wibowo et al. (2015a) for orange juices. However, they did not pursue an integrated approach for analytical and sensorial results and even stated as conclusion that exactly this combination must be the next step.

The objective of this work was the development and establishment of science-based prediction models regarding the stability and shelf-life of orange juice and apple juice. Different stress tests should be compared with real-time storage conditions, and both analytical and sensorial aspects should be taken into account in a holistic approach. In order to achieve this goal, the ASLT was performed by collecting the sensorial profiles by quantitative descriptive analysis (QDA) and by monitoring the kinetic behaviour of volatiles by an untargeted profiling.

For the necessary evaluation and interpretation of this comprehensive set of sensorial and analytical data, an adequate strategy was newly developed in this study. As initial steps, the sensorial data and the kinetics of the volatiles were investigated independently by different complementary univariate and multivariate statistical methods with structure-discovering and structure-confirming purposes. In addition, the kinetic behaviours of the volatiles were followed and expressed by values based on the Arrhenius approach. For the final holistic prediction model, it was a very important and a particular feature of this study to combine the sensory-related and volatiles-related data for receiving evidences for an appropriate or an inappropriate use of different stress test systems in comparison to the real-time storage.

2 BACKGROUND

2.1 Accelerated shelf-life testing (ASLT)

The main objective of an ASLT approach is to predict the shelf-life of a product within a short timeframe by using accelerated storage conditions and by extrapolating the results to real-time conditions. The shelf-life of foods is defined in Directive 2000/13/EC of the European Parliament and of the Council (EU, 2000) as “the date until which the foodstuff retains its specific properties when properly stored”. During the development of pharmaceuticals, the chemical stability is a major focus and decisive for the end of the shelf-life. In the food industry also the sensorial aspects besides the chemical and microbiological composition have to be taken into consideration.

2.1.1 Practical application - accelerated storage conditions

Within ASLT studies the quality of the product can be assessed by the measurement of chemical and physical changes of the product, by sensory evaluation, and by assessment of the microbiological stability. The decrease in quality is mainly influenced by compositional factors like pH-value, water activity, catalysts or reaction inhibitors, and environ-mental factors like temperature, relative humidity, or pressure (Singh & Cadwallader, 2003). The mostly applied acceleration method is the increase of the storage temperature with the effect of influencing the reaction rate k(T) in order to demonstrate the product stability and to estimate the shelf-life by extrapolation tools. Besides temperature as acceleration tool, the exposure to light can promote reactions in photosensitive products, whereby factors like the light spectrum, the light intensity, and the exposure time have to be taken into account (Manzocco et al., 2011). Depending on the field of application also chemicals (e.g. in microbiology), moisture or increased relative humidity (e.g. in dehydrated products), mechanical agitation like centrifuge tests or shaking tests (e.g. for emulsions), or various gas compositions (e.g. oxygen-pressure chamber) are applicable acceleration methods. A combination of several methods for speeding up the degradation kinetics is also possible, e.g. the concomitant use of increased temperature and light exposure or non-isothermal conditions and step stress methodology conditions.
In general, performing accelerated tests requires the consideration of potential distortions of the kinetic behaviour. For example, abused temperatures in both directions (elevated and depressed) can cause a phase transition, by which a certain reaction could be accelerated or slowed down. With increasing temperature the oxygen solubility decreases and thus especially oxidative degradation pathways can be influenced. Another example is that at high temperatures proteins start to denature and the accelerated results cannot be extrapolated to lower temperatures. However, not only increased temperatures can lead to problems in extrapolation but also the decrease can implicate storage defects like non-homogenous distribution of components between frozen and unfrozen phases (Singh & Cadwallader, 2003). As a consequence, results from such affected acceleration methods cannot be readily used for extrapolation to real-time

conditions. Therefore, the practical applicability of acceleration conditions has to be well-considered.

2.1.2 Evaluation strategies

The idea behind ASLT approaches is the establishment of predictive factors between the real-time and accelerated storages in order to shorten the shelf-life tests by using accelerated conditions. These predictive statements for biochemical processes are generally based on the assumption that the reactions occurring during the normal storage are solely accelerated but not changed in their nature under accelerated conditions. Therefore, the kinetic behaviour can be expressed by the Arrhenius equation which shows the dependency of the reaction on the storage temperature (details in chapter 2.7). This time- and temperature-dependent correlation was used in several studies for estimating the microbiological growth or the kinetic behaviour of single product ingredients especially in the context of loss of nutritional quality (Gómez-Alonso et al., 2004; Jha & Patel, 2014). Deduced from the van 't Hoff equation and the Arrhenius equation it is commonly assumed for most chemical reactions that the correlation between two reaction rates k(T) for temperatures differing by 10 °C can be modelled by a factor of 2 to 3. But it has to be kept in mind that there are also reactions not showing an Arrhenius behaviour or even a time- and temperature-dependent behaviour (Waterman & Adami, 2005). One reason can be that the degradation or formation pathway consists of multiple reaction steps, whereby the single reaction steps indeed show Arrhenius behaviour, but the cumulative reagent-product-pathway does not. The existence of multiple pathways leading to the same product may also influence the Arrhenius behaviour because each pathway has its own activation energy (E_a); thus one reaction pathway may dominate at another temperature than the other pathways.
The shelf-life prediction for the sensorial properties is often based on the acceptability of the product quality by the consumer. The subsequent statistical evaluation is performed by the Weibull hazard analysis technique or the survival analysis, both dealing with the probability of rejecting the product by the consumers (Cardelli & Labuza, 2001; Fu & Labuza, 1993; Hough & Garitta, 2012; Hough et al., 2006; Hough et al., 2003). For the Weibull hazard analysis the number of panelists is increased for each subsequent measurement time point and additionally after the declaration of unacceptability of the product by at least 50 % of the panelists. This study design allows to start with a small and practical panel size (Bili & Taoukis, 1998). In contrast, the panel size in connection with the survival analysis remains constant and the probability of rejection is calculated thereof. Another form of compiling sensorial data is the quantitative descriptive analysis (QDA) technique in which pre-defined descriptors are quantitatively evaluated and which requires a trained panel. The evaluation of those quantitative data sets is diverse and ranges from univariate kinetic models like an Arrhenius model and multivariate methods like the principal component analysis (PCA) (Córdova et al., 2011; Klimczak & MałEcka, 2011; Pedro & Ferreira, 2006; Saavedra et al., 2013) to regression analyses like partial least square (PLS) regressions (Upadhyay & Mishra, 2015).

In the present study, the aim of the sensorial assessments was not the rejection of the product by questioning the acceptability, but the determination of the product quality by evaluating several descriptors.

2.1.3 Uncertainty of measurement

The extrapolation of the results of accelerated storage to real-time storage contains errors due to variability in various factors like uncertainty of the calibration, imprecision of analytical methods (repeatability and reproducibility), and sample uncertainty (lot-to-lot variability, sample non-homogeneity, chemical reactions, or storage). The cumulative variability is also known as uncertainty of measurement (Magari, 2007). The accuracy of extrapolated data can be improved by an appropriate study design. The use of an accurate analytical procedure and calibrator and an adequate number of replicates and lots can reduce the uncertainty of measurement and increase the predictions quality. The quality is also determined by number and range of accelerating conditions. Magari (2007) showed that the prediction of one year of shelf-life has approximately an error of one month. Corradini and Peleg (2007) pointed out that the reliability of prediction is the higher, the closer the accelerated conditions are to real-time storage.
Remini et al. (2015) expressed the goodness-of-fit between the predicted values ($X_{predicted}$) and the experimental data ($X_{experimental}$) as the root mean squared error (RMSE) for all the measured data. The better the prediction model fits to the experimental data, the lower is the standard deviation expressed as RMSE between prediction and reality.

$$RMSE = \sqrt{\frac{1}{n} * \sum_{i=1}^{n} (X_{i\,predicted} - X_{i\,experimental})^2}$$

n	number of measured data
$X_{predicted}$	predicted mean value
$X_{experimental}$	experimental determined value

The standard deviation and thus the RMSE can only be calculated for mean values. However, the application of the mean value is susceptible to outliers, whereas the median value is a location parameter dividing the data set into two parts and being itself the value in the mid. When using the median value ($\tilde{X}$) and not the mean value, the mean deviation from the median (MD) can be used as estimation for goodness-of-fit.

$$MD = \frac{1}{n} * \sum_{i=1}^{n} (\left|\tilde{X}_{experimental} - \tilde{X}_{predicted}\right|)$$

n	number of measured data
$\tilde{X}_{predicted}$	predicted median value
$\tilde{X}_{experimental}$	experimental determined value

2.2 Market analysis

In 2015, the non-alcoholic beverage segment (without bottled water) in Europe was composed of 56 % carbonates and 26 % juices or juice based drinks (Euromonitor International Ltd). The remaining shares were split between squashes/sirups, flavoured water, tea or coffee drinks, energy drinks, and sport drinks. Since the focus of the present study was on beverages with flavours from the named fruit (FTNF), the relevant market figures for the juice segment are briefly shown in the following chapter. The European Fruit Juice Association (A.I.J.N.) commissioned the Canadean Ltd. as specialist for the beverage market and published the annual market report with detailed market figures for the juice and juice based (nectars) segment. The following market data for the year 2015 have been taken from the liquid fruit market report 2016 (A.I.J.N., 2016), unless otherwise stated. In 2015 the European population (510 million) in 28 countries (without Norway, Switzerland, and Turkey) consumed 9.6 billion litres of fruit juices and nectars, of which 6.1 billion litres represented fruit juices (100 % juice content), which included 69 % juices being reconstituted from concentrate. From 1989 to 2003, the annual consumption of fruit juices and nectars increased steadily and remained at the same level until 2009. Since 2009, the annual consumption of fruit juices-based beverages decreased by 14 % in general and in particular for fruit juices. Almost three-quarter (70 %) of the European fruit juice and nectars market (6.7 billion litres) was covered by five countries with Germany as the main consumer (25 %), followed by France, UK, Spain, and Poland. The high consumption of fruit juices and nectars in Germany is also evident in the figures for the per capita consumption of 29.4 litres in 2015 in comparison to 18.9 litres as average in the EU. This German figure is consistent with the stated per capita consumption by the Verband der deutschen Fruchtsaft-Industrie e.V. (VdF) of 33.0 l based on fruit and vegetable juices and nectars (preliminary figures for the year 2015).

Across the 28 European countries, orange taste showed the biggest share with 36.7 % followed by apple taste with 15.1 % as single variety flavour for fruit juices and nectars in the year 2015. However, the preferred taste varied between the different countries. Whilst for example in UK 56.5 % belonged to the orange taste, in Italy only 16.3 % of the juice based beverages had an orange taste profile. The largest proportion (approximately seven eights) of consumed orange juice in the EU was imported from non-European countries. In contrast, the apple juices both as not from concentrate and from concentrate were mainly supplied by EU sourcing/intra-EU trade, whereby Poland, Italy, Spain, and Germany – in descending order - were the leading producers in 2013.

The predominant packaging of the juices in the year 2015 was carton with 61.2 % followed by plastic (29.2 %) and glass bottles (8.6 %). Germany as Europe's leading juice market showed a deviant allocation in packaging material with the highest share in plastic with 50.1 % followed by carton with 39.7 % and glass with 9.3 %.

2.3 Legal requirements for juices

The Directive 2012/12/EU of the European Parliament and the Council (EU, 2012) regulates the statutory requirements for fruit juices and certain similar products, mainly regarding the production, the composition, and the labelling of the products. The directive was transferred into national law with the German fruit juice and soft drink regulation of 24.05.2004 with amendment of 21.05.2012. For the implementation into national law a transition period of 18 months was established and the legal force of the amendment was set for 28 October 2013.
The directive 2012/12/EU defined fruit juices not from concentrate (NFC) as “the fermentable but unfermented product” obtained from the sound, ripe, fresh, or chilled fruit with “the characteristic colour, flavour, and taste typical of the juice” of the used fruit. For fruit juice from concentrate (FC) the physically removed water content during concentration has to be replaced and the “essential physical, chemical, organoleptic and nutritional characteristics of an average type of juice” has to be restored. In both, NFC fruit juices and FC fruit juices, the addition of restoration flavour, pulp, and cells is authorised. The revision of the regulations led also to the establishment of minimum Brix levels for reconstituted fruit juices in annex V of the directive. For apple juice and orange juice a minimum brix level of 11.2°Brix was determined. The amendment also included a new article (Art. 7) that should enable the Commission to adopt modifications of the annex via a simplified procedure. With the amendment the technical progress should be adapted and international standards like the Codex General standard for fruit juices and nectars (Codex Stan 247-2005) of the Codex Alimentarius Commission and the Code of Practice (CoP) of the European Fruit Juice Association (A.I.J.N.) should be intensified included “to bring the annexes to this Directive into line with developments in relevant international standards” (Art. 7). The CoP of the A.I.J.N. presents reference guidelines for the evaluation of quality and authenticity of different fruit juices and is also accepted by the National Fruit Juice Associations, the national food inspections, and fruit processors. The CoP is divided into two sections, where the first section includes absolutely necessary quality requirements and the second section contains further criteria for the interpretation of juice quality and authenticity.

2.4 Orange Juice

2.4.1 Juice production

Orange juice is produced from the matured sweet orange fruit of the species *Citrus sinensis*. The production of orange juice has been reviewed by Perez-Cacho and Rouseff (2008a). The juice production starts with the mechanical squeezing (extraction) of the whole fruit whereby the degree of squeezing influences the share of peel oil (flavedo = orange coloured layer) entering into the juice and thus the composition of the volatiles. During the so-called finishing step the solid particles are removed from the liquid juice. Depending on the degree of finishing, the pulp is contained in smaller or larger amounts influencing the volatiles due to association of hydrocarbons mainly with the pulp and

esters, alcohols, and aldehydes with the liquid serum. For the production of orange juice concentrates – since in Europe the majority of orange juice is reconstituted from concentrate (69 %; A.I.J.N., 2016) – the water is removed in an evaporator under vacuum and the carried along volatiles are collected. The obtained aroma mixture is added back to the orange juice concentrate when producing and rediluting to a final orange juice.
The quality and the stability of orange juice are impacted by microbial growth and enzymatic activity. Therefore, orange juices are subjected to pasteurisation for inactivation of spoilage microorganisms and endogenous enzymes like pectin methylesterase (PME). In addition to the conventional thermal processing (pasteurisation), high pressure (HP), and pulsed electric field (PEF) received important attention especially in the field of searching for mild pasteurisation techniques. Since the consumers attach importance to the nutritional value, to the sensorial profile, and also to an appealing colour of orange juice, most of the studies connect the success of a technique to the ascorbic acid (AsA) content and the browning index besides the inactivation of microorganisms and enzymatic activity. Vervoort et al. (2011) detected no significant differences between thermal processing, HP, and PEF for sugars, organic acid, bitter compounds, carotenoids, furfural, 5-hydroxymethylfurfural (5-HMF), and also not for AsA, whilst other authors stated the retaining of higher AsA amounts and lower browning indices for PEF and HP in comparison to the conventional heat pasteurisation (Polydera et al., 2004; Yeom et al., 2000). Also the sonication yielded a lower AsA degradation than the thermal process (Tiwari et al., 2009). The difference between the studies was the temperature. It is undisputed that higher temperature leads to faster AsA degradation. Whilst the purpose of Vervoort et al. (2011) was the equivalent degree of microbial inactivation between the three methods leading to an temperature of 72 °C for heat pasteurisation, the other mentioned authors used temperatures between 80 °C and 98 °C, which lead to a higher AsA deterioration.

2.4.2 Flavour profile

The flavour profile of orange juices is composed of volatiles from different chemical classes and can primarily be attributed to aldehydes, esters, alcohols, ketones, and terpene hydrocarbons (Perez-Cacho & Rouseff, 2008a). The determination of the key flavour components was carried out with different methods. Hinterholzer and Schieberle (1998) and Averbeck and Schieberle (2009) isolated the flavour components by solvent assisted flavour evaporation (SAFE), analysed the extracts by aroma extract dilution analysis (AEDA) and calculated flavour dilution (FD) factors, which could give indications of the importance of substances to the aroma profile. Plotto et al. (2004, 2008) dealt with the contribution of different flavour components to the profile by using the determination of the odour activity values (OAV), which evaluate the contributions of volatile components to the overall aroma by including the individual thresholds. They pointed out that the odour thresholds partly differ strongly depending whether they were determined in water or in orange juice matrix, which caused differences in OAV by factor 10 or some 100. Buettner and Schieberle (2001) combined the determination of FD factors and OAV in one study. The mentioned investigations demonstrated that the most aroma-active components in orange juice are the aldehydes acetaldehyde, hexanal,

octanal, nonanal, decanal, and (Z)-3-hexenal, the esters (S)-ethyl 2-methylbutyrate, ethyl butyrate, ethyl 2-methylpropanoate, and ethyl hexanoate, the monoterpene hydrocarbons myrcene, (R)-α-pinene, and (R)-limonene, and the monoterpene alcohol (S)-linalool (Averbeck & Schieberle, 2009, 2011; Buettner & Schieberle, 2001; Plotto et al., 2008; Plotto et al., 2004). These findings were confirmed by several flavour reconstitution experiments (Averbeck & Schieberle, 2009; Buettner & Schieberle, 2001). In the context of the identification of the key flavour volatiles, Plotto *et al.* (2004) highlighted that OAVs are for sure indications for the influence of single components on the overall flavour, but that the OAV approach cannot predict the perception in a mixture of different components. Additionally, there are lot of research studies dealing with the screening of volatile components in freshly squeezed orange juice and orange juice from concentrate (Moshonas & Shaw, 1994; Nisperos-Carriedo & Shaw, 1990; Seideneck & Schieberle, 2011).

2.4.3 Changes in the volatiles profile during storage

The profile of orange juice volatiles is changing during the storage of the product up to its indicated shelf-life. These changes may result from both the decrease of volatile components being important for the orange flavour and the increase of storage-related reaction products. The most important factor for the changes during storage in the composition of volatiles is the storage temperature besides time, light exposure, or oxygen content. The quantitatively largest degradation product in orange juice is alpha-terpineol (stale, musty, or piney-like impression), whose concentration is increased by higher temperatures and longer storage durations. Alpha-terpineol is predominantly formed by an acid-catalysed hydration reaction from limonene (Askar et al., 1973; Clark & Chamblee, 1992; Haleva-Toledo et al., 1999; Petersen et al., 1998), which it itself the main volatile in orange juices (Averbeck & Schieberle, 2009; Berlinet et al., 2005; Buettner & Schieberle, 2001), and by a Wagner-Meerwein rearrangement from linalool (Askar et al., 1973). Despite of the by far highest amount of limonene, linalool was stated as the most potent odorant in the group of the terpenes due to its low threshold and the corresponding higher OAV. Besides α-terpineol, 4-vinylguaiacol is also mentioned as indicator for thermal exposure of orange juices. 4-Vinylguaiacol with its undesirable flavour impressions, being described as “old fruit” and “rotten” (Tatum et al., 1975), is formed from the odourless precursor ferulic acid and increases in a time- and temperature-dependent way (Averbeck & Schieberle, 2011; Klimczak & MałEcka, 2011; Lee & Nagy, 1990; Peleg et al., 1992).

Besides the temperature-dependent reactions, also oxidative reactions can impair the profile of orange juice volatiles. The most discussed oxidatively formed volatile is carvone which seems to increase in connection with thermal exposure. With its caraway-like or minty flavour – depending on the configuration – and its increase at forced storage, it was considered as off-flavour (Kirchner & Miller, 1957; Perez-Cacho & Rouseff, 2008b). But, since Averbeck and Schieberle (2011) showed that the carvone concentration remained nearly constant in orange juice during storage, the contribution of carvone to an off-flavour perception and the use as storage index was challenged. In the sensorial view, Petersen et al. (1998) showed a correlation between oxidised

perceptions with components like α-terpineol, β-terpineol, 2-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, acetic acid, or carveol. Berlinet et al. (2005) investigated the influence of different packaging materials (glass containers and different PET bottle qualities) during real-time storage and concluded that the PET material and the associated permeability of oxygen showed no correlation with the kinetic behaviour of the volatile components. Furthermore, they studied the influence of the packaging material on the absorption of certain volatiles into the packaging material with the result that only limonene and beta-myrcene, the two quantitatively dominating terpenes in orange juice, were absorbed with approximately 0.3 % of the initial concentration. These findings were in agreement with van Willige et al. (2003), who detected decanal in low quantities in addition to the two mentioned terpenes.

At the same time as volatiles with potentially negative sensorial impacts are formed, a decrease of odour-active components contributing to the orange flavour may lead to reduced fruitiness and storage notes. In general, decreases of key flavour components like octanal, linalool, limonene, myrcene, or ethyl butyrate were observed during storage of orange juice at real-time conditions and at accelerated temperatures (Averbeck & Schieberle, 2011; Berlinet et al., 2005; Petersen et al., 1998) and were connected with a deterioration of the sensorial properties.

During the aging of orange juice, also non-volatile components like ascorbic acid (AsA) influence the profile of orange juice. AsA has been shown to contribute to the formation of e.g. furfural, 2-furoic acid, and 3-hydroxy-2-pyrone in the course of non-enzymatic browning in aqueous model solutions and in orange juice under acidic conditions (Murata et al., 2002; Roig et al., 1999; Shinoda et al., 2005; Tatum et al., 1969). Especially the loss of AsA and the connected browning of orange juice is often used as criterion for the shelf-life prediction of orange juice (Esteve et al., 2005; Tiwari et al., 2009). The degradation of AsA by an oxidative reaction explained the much better retention of the AsA content in glass containers compared to different PET materials during a storage period of nine months due to the prevention of oxygen permission through the packaging material (Berlinet et al., 2006). The occurrence of Maillard reaction or Strecker degradation can also increase the formation of off-flavours and non-enzymatic browning. Two examples for the occurrence of a Maillard reaction are furaneol® (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone) and ethylfuraneol (2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone), showing both a caramel, burnt sugar, or sweet flavour perception (Averbeck & Schieberle, 2011; Mottram, 2007; Perez-Cacho & Rouseff, 2008b; Tatum et al., 1975).

2.4.4 Existing shelf-life prediction models

Most shelf life investigations for orange juices regarding analytical aspects referred to the degradation kinetics of AsA as nutritional value during storage (Esteve et al., 2005; Klimczak et al., 2007; Ros-Chumillas et al., 2007; Wibowo et al., 2015c). The AsA degradation was stated as the reason for browning of orange juices (Shinoda et al., 2004), whereas the colour issue, also called “browning” index, was often of additional interest in those studies (Wibowo et al., 2015b). The enhanced retention of AsA from the beginning of the juice production by techniques like sonication, PEF, or HP in comparison

to the conventional heat pasteurisation was often the subject of analytical shelf-life predictions (Agcam et al., 2014; Kaanane et al., 1988; Polydera et al., 2005; Tiwari et al., 2009).

Besides the studies regarding the stability of AsA, there were many investigations regarding the composition of volatiles of orange juice at single measurement time points at normal storage and even at accelerated storages (Averbeck & Schieberle, 2009, 2011; Buettner & Schieberle, 2001). However, these studies mainly focused on the key flavour components and were mostly not conducted with the purpose of developing predictive statements in the context of ASLT.

Klimczak and MałEcka (2011) investigated the sensorial profile of orange juices by QDA at different storage temperatures (18 °C, 28 °C, 38 °C) after 2, 4, 6, and 12 mo and combined the results to the 4-vinylguaiacol concentration as indicator substance for aging. They performed several PCAs for the single temperature conditions and concluded that prolonged storage and storage at higher temperatures caused a decrease of positive sensorial attributes and an increase of undesirable impressions like “old fruit”, “fermented”, and “rotten” notes. They found that the 4-vinylguaiacol content was closely related to these negative impressions and stated that only some particular volatile components were responsible for the diminishing quality of orange juice. The authors observed first significant quality changes by ANOVA after 2 mo at 28 °C. Orange juices after 12 mo at 28 °C or after 2 mo at 38 °C were of a quality unfit for drinking. In conclusion, they stated that the storage at 18 °C for 12 mo corresponded to the storage at 28 °C for 2 mo, even though it was unclear on what basis this statement had been made.

Another study was conducted by Petersen et al. (1998) who investigated the sensorial and volatiles-related changes in normal stored (5 °C and 20 °C) and accelerated stored (30 °C) orange juices. The sensorial comparability of the samples was deduced from PC1 of the PLS regression on the basis of the four descriptors “orange odour”, “orange taste”, “oxidised odour”, and “oxidised taste”. For the volatiles data set eleven volatile components were selected. For connecting the sensorial and the volatiles-related data set, the first PC of the PLS regression was evaluated and resulted in the statement that octanal, nonanal, linalool, and alpha-pinene were correlated to the typical orange aroma and alpha-terpineol, beta-terpineol, 2-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, acetic acid, carveol and furfural to the oxidised and bitter impressions. The final predictions were drawn on the basis of the sensorial results, which were validated by the incorporation of the theoretical volatile values. The authors predicted certain comparability between samples after 6 mo at 20 °C and after 5 d at 50 °C or 13 d at 40 °C. However, the sensorial evaluations of the last two measurement time points at the storage at 20 °C (6 mo and 9 mo) and even the last three measurement time points at 50 °C (9 d, 13 d, and 19 d) were reversed on the PC1-axis representing 60 % of the total variability and being used as the only basis for this estimation. Unfortunately, the applied experimental test design, using a limited number of measurement time points during the real-time storages (five sensorial evaluations after 0.5, 1, 3, 6, and 9 mo at 5 °C and 20 °C) and the accelerated storages (0, 2, 5, 9, 13, and 19 d at 30 °C, 40 °C, and 50 °C), cannot provide a closer monitoring with intermediate assessments in order to explain the positions in reverse order on PC1. Thus, it could not be ensured that the