Analysis of the Storage Stability of Grape and Apple Juices in Terms of Antioxidative Capacity and Their Polyphenols, Hydroxymethylfurfural and Ascorbic Acid Content

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Dissertation

Analysis of the Storage Stability of Grape and Apple Juices in Terms of Antioxidative Capacity and Their Polyphenols, Hydroxymethylfurfural and Ascorbic Acid Content

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Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn.

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Meinen Eltern

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Abstract

The aim of this work is to determine the storage stability of commercial grape and apple juices. Two storage studies are performed to provide extensive knowledge about the changes of the selected juices under different storage conditions. The analysis of fruit juice stability is of certain interest not only for the juice production but also for the development of new materials and composites for food packaging.

Deterioration of each juice is observed during one year of storage (i.e. usual period of shelf-life). Sensory quality as well as antioxidative capacity and concentrations of single juice compounds (especially antioxidants like polyphenols or ascorbic acid) are determined to clarify whether the antioxidative status of a juice can provide useful information concerning its storage stability.

In addition to the optimisation of the HPLC analysis of several juice compounds a new test is developed to determine the antioxidative capacity of a juice via electrochemical reactions of antioxidants. The *voltammographic analysis of the reducing potential* (VARP) assay uses the coulometric electrode array detector (CEAD) without prior HPLC separation.

The resulting voltammogram shows the ability of the sample to reduce other molecules and thus, provides an indication of the antioxidative capacity of the sample. It is shown that this fast, simple and sensitive method enables the detection of minor changes of the antioxidative status of the stored juice.

In conclusion, the storage studies demonstrate which parameters are mainly influenced by storage time, temperature and oxygen permeation of the packaging. They also point out how to ensure adequate storage stability.

Furthermore, for fruit juices with considerable amounts of antioxidants strong correlations are revealed between VARP data and sensory evaluations under different storage conditions. These findings indicate that antioxidative capacity might be associated with sensory color and taste evaluation.

In contrast, a juice with low amounts of antioxidants is less affected by storage temperature and an oxygen permeable packaging. The results indicate that such a juice might obtain an adequate storage stability when filled in

packaging with considerable oxygen permeability. The use of this packaging could reduce the production costs since the aluminium foil accounts for a considerable share.

Zusammenfassung

Ziel dieser Arbeit ist es, die Lagerstabilität handelsüblicher Trauben- und Apfelsäfte zu bestimmen. Zwei Lagerungsstudien werden durchgeführt, um umfassende Erkenntnisse über die Veränderungen ausgewählter Säfte unter verschiedenen Lagerungsbedingungen zu gewinnen. Die Stabilitätsuntersuchung von Fruchtsäften ist nicht nur für die Saftherstellung von gewissem Interesse, sondern auch für die Entwicklung von neuen Materialien und Verbundstoffen für die Verpackung von Lebensmitteln.

Die Veränderung jedes Saftes wird im Lagerungsverlauf eines Jahres beobachtet (dies entspricht dem üblichen Haltbarkeitszeitraum). Die sensorische Qualität, sowie die antioxidative Kapazität und die Konzentrationen einzelner Saftbestandteile (insbesondere Antioxidantien wie Polyphenole oder Ascorbinsäure) werden bestimmt, um zu klären, ob mit Hilfe des antioxidativen Status eines Saftes eine Aussage über seine Lagerungsstabilität getroffen werden kann.

Zusätzlich zur Optimierung der HPLC Analyse einiger Saftbestandteile wird ein neues Verfahren entwickelt, um die antioxidative Kapazität eines Saftes mit Hilfe der elektrochemischen Reaktionen seiner Antioxidantien zu bestimmen. Die *voltammographische Analyse des reduzierenden Potentials* (VARP) verwendet den coulometrischen Elektrodenarraydetektor (CEAD) ohne eine vorhergehende HPLC-Trennung durchzuführen.

Das resultierende Voltammogramm zeigt die Fähigkeit einer Probe, andere Moleküle zu reduzieren und gibt damit einen Hinweis auf die antioxidative Kapazität der Probe. Es wird gezeigt, dass diese schnelle, einfache und sensitive Methode geeignet ist, um bereits kleine Veränderungen des antioxidativen Status des gelagerten Saftes festzustellen.

Schließlich zeigen die Lagerungsstudien auf, welche Parameter hauptsächlich von der Lagerungszeit und -temperatur, sowie von der Sauerstoffdurchlässigkeit der Verpackung beeinflusst werden. Zudem stellen sie dar, wie eine angemessene Lagerstabilität gewährleistet werden kann.

Darüber hinaus werden für Fruchtsäfte mit einem hohen Gehalt an Antioxidantien starke Korrelationen zwischen VARP-Daten und sensorischer Beurteilung unter verschiedenen Lagerungsbedingungen offenbart. Diese Erkenntnisse deuten darauf hin, dass die antioxidative Kapazität mit der sensorischen Farb- und Geschmacksbewertung verknüpft sein könnte.

Im Gegensatz dazu wird ein Saft mit geringem Gehalt an Antioxidantien durch Lagerungstemperatur und eine sauerstoffdurchlässige Verpackung weniger beeinflusst. Die Ergebnisse zeigen, dass ein solcher Saft eine ausreichende Lagerstabilität aufweisen könnte, wenn er in einer Verpackung mit erhöhter Sauerstoffdurchlässigkeit abgefüllt wird. Die Benutzung dieser Verpackung könnte die Produktionskosten senken, da die Aluminiumfolie hieran einen beträchtlichen Anteil hat.

Publications

Parts of this thesis have been published before:

Posters

Brachmann S., Papagiannopoulos M., Galensa R.: *Einflüsse auf die Lagerstabilität von Trauben- und Apfelsäften: Veränderungen der antioxidativen Kapazität sowie des Gehaltes an Polyphenolen, HMF und Vitamin C*
35th Deutscher Lebensmittelchemikertag, Dresden (Germany), 18–20th September 2006
Abstract see: Lebensmittelchemie 61 (2007) 119-120

Brachmann S., Rodrigues R. B., Marx F., Stehle P., Galensa R.: *Antioxidanzien in Fruchtsäften - Vergleich neuer Messverfahren zur Bestimmung der antioxidativen Kapazität*
43rd Scientific Congress of the Deutsche Gesellschaft für Ernährung, Stuttgart-Hohenheim (Germany), 9–10th March 2006
Abstract see: Proc. Germ. Nutr. Soc. 8 (2006) 34

Brachmann, S., Rodrigues, R.B., Lichtenthäler, R., Marx, F., Hiller, K., Galensa, R.: *Comparison of New Techniques to Estimate the Antioxidative Capacity of Fruit Juices and Dietary Supplements*
34th Deutscher Lebensmittelchemikertag and Euro Food Chem XIII, Hamburg (Germany), 13–23th September 2005
Abstract see: Lebensmittelchemie 60 (2006) 63

Oral Presentation

Brachmann S., Galensa R.: *Untersuchung der Lagerstabilität von Trauben- und Apfelsäften anhand ihrer antioxidativen Kapazität sowie ihres Gehaltes an Polyphenolen und Vitamin C.* Regionaltagung NRW der Lebensmittelchemischen Gesellschaft, Wuppertal (Germany), 9th March 2005
Abstract see: Lebensmittelchemie 59 (2005) 115

1. Introduction

1.1. Aim of this Work

The stability of fruit juices during storage is of certain interest not only for the producers but also for the packaging industry. Materials for food packaging are developed to comply with different requirements (e.g. physical characteristics of the food, usual transportation or storage conditions, consumer habits and claims). Thus, research for new materials includes several shelf-life tests which nowadays mainly consist of periodical sensory analyses and sometimes, microbiological incubation tests.

However, these tests are both time-consuming and rather cost-intensive. Furthermore, a consistent evaluation by a sensory panel might be impaired within the course of a storage study (e.g. due to different composition of the panel). Therefore, objectively measurable parameters help to further substantiate the sensory ratings and thus, might improve shelf-life tests.

In the context of this research project fruit juices are investigated during storage in many respects. In addition to the determination of sensory changes the research focuses on oxidative reactions which occur in the juice. Due to their high reactivity it is likely that antioxidants undergo oxidative degradation prior to other compounds and thus, lower the antioxidative capacity of the juice.

Consequently, commercial fruit juices are selected for the storage studies due to their contents of antioxidative compounds. A red grape and two different apple juices cover a wide range of antioxidants, i.e. different polyphenols and/or ascorbic acid. During storage, numerous parameters including antioxidative capacity and several single compounds are analysed periodically over a usual period of shelf-life to clarify whether the antioxidative status of a juice provides useful information concerning its storage stability.

For determination of the antioxidative capacity a new test is developed on basis of electrochemical reactions of antioxidants. The method uses the coulometric electrode array detector (CEAD) without prior HPLC separation. Antioxidants are oxidised at low potentials and thus, the resulting voltammogram provides an indication of the antioxidative capacity of the sample.

The research project consists of two phases. Within the first phase the analytical methods for the determination of several juice compounds are optimised. Furthermore, the first study is conducted to elucidate the alterations of the selected juices under usual storage conditions.

Due to preceding results the storage study of the second phase is designed to accelerate deterioration of the juices. In addition to the regular conditions, the juices are filled in packaging with considerable oxygen permeability and samples are stored at two different temperatures.

The results show which parameters are mainly influenced by storage temperature and oxygen permeation of the packaging. They also demonstrate which storage conditions are necessary to yield adequate storage stability for each juice. Furthermore, the findings reveal possible correlations between sensory juice quality and its antioxidative characteristics.

1.2. Packaging of Fruit Juices

1.2.1. The Laminated Composite Packaging

In Germany 76 % of fruit juices and nectars are filled in laminated composite packages [74]. A schematic set-up of a commercial aluminium-coated composite made by the SIG Combibloc company is shown in figure 1.1. The packaging material consists of three different components: carton, aluminium and low density polyethylene (LDPE).

With 75 % of the total weight the carton fulfils the function as carrier material and it gives the package the necessary stability. On both sides the carton is coated with a thin LDPE layer. The one on the outside protects the carton against moisture and further environmental influences, the interior coating improves the adhesion between the carton and the following aluminium layer. This 6.5 μm thin foil protects the juice against light exposure, oxygen diffusion and aroma leakage. As another liquid barrier an inner LDPE layer protects the aluminium against corrosion by the filling. By the use of this set-up a composite packaging of 1.0 L content weights 28.5 g. [63]

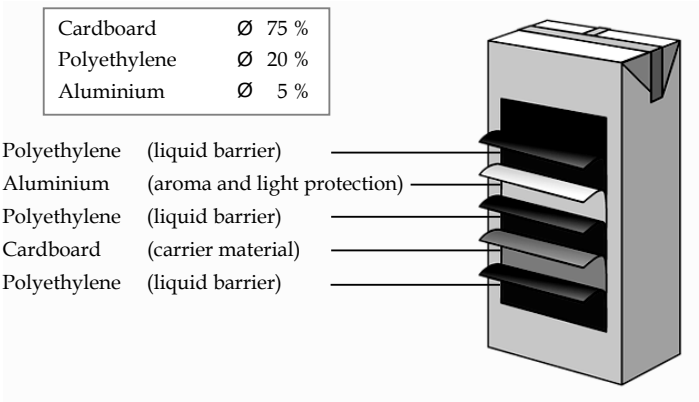


Figure 1.1.: Set-up of a laminated composite packaging [63]

In addition to this regular packaging the juice was also filled into a further kind of package for the second storage study. For this packaging the aluminium foil is replaced by a polyamide coating. Adhesion between carton

and polyamide is ensured by an LDPE layer. Thin LDPE coatings on the outside and on the inside of the package complete this composite. It contains no aluminium and thus, is more susceptible to oxygen diffusion as well as to aroma loss and alterations due to light exposure. The filling process for both kind of packaging does not differ basically and is described in the following.

1.2.2. The Aseptic Filling Process

Fruit juices are filled in laminated composite packages by an aseptic filling process. As its special feature the cold sterile product is filled under aseptic conditions in sterile containers. [28]

The figure 1.2 illustrates the Combibloc system of the filling procedure that uses a ready-made sleeve with a sealed longitudinal seam. Directly before filling it is opened and its base is sealed. Hydrogen peroxide sterilises the packaging material and is removed afterwards by drying. To avoid its flooding the juice is filled step by step and subsequently the foam is steamed out with water vapour. After the top is sealed and formed the juice packaging is ready for conveying. [63]

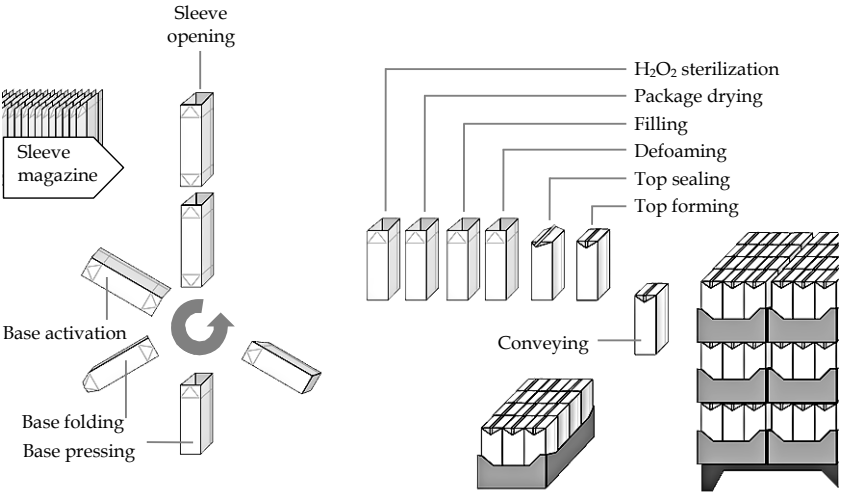


Figure 1.2.: Juice filling system [63]

1.3. Antioxidants in Grape and Apple Juices

The selection of the fruit juices for the storage studies is due to their contents of antioxidative compounds. As few juices as possible shall cover a wide range of antioxidants especially in the group of polyphenols and ascorbic acid. Thereupon a filtered apple juice without ascorbic acid enrichment is chosen as a juice with a low antioxidative capacity. A naturally cloudy apple juice with ascorbic acid (from acerola juice concentrate or synthetic) combines this powerful antioxidant with the typical apple polyphenols that causes a high antioxidative potential. And a red grape juice containing anthocyanins as red pigments with antioxidative value but no ascorbic acid completes the composition of juices with different levels of specific antioxidants.

This section presents the antioxidants found in apple and grape juices in terms of their general functions, structure and typical reactions. Additionally, it focusses on prevalent analytical methods for their quantification in juice samples.

1.3.1. Ascorbic Acid

Ascorbic acid is an antioxidant taking part in hydroxylation reactions and providing reduction equivalents to intra and extra cellular processes. Thus, it fulfils various biochemical functions both in the human and the plant organism. Due to two chiral centers four stereoisomers of ascorbic acid exist. The human cells utilise only L-ascorbic acid (i.e. vitamin C) and its oxidised form the L-dehydroascorbic acid (DHAA) due to the easy reversibility of the oxidation. Therefore, also the concentration of DHAA in the juice is of certain interest. However, the oxidised molecule has no antioxidative activity and it cannot protect the juice against further oxidation. Whereas the other isomers which are inactive in the human organism, fulfil the same antioxidative effect in the juice as vitamin C. [17]

1.3.1.1. Reactions of Ascorbic Acid

The degradation reactions of ascorbic acid can proceed the aerobic or anaerobic route (see figure 1.3). The reversible oxidation to DHAA occurs under aerobic conditions. In aqueous solution, it exists as a hydratised hemiketal. DHAA also undergoes a spontaneous conversion to 2,3-diketogulonic acid formed by the irreversible opening of the lactone ring. Also the anaerobic degradation of ascorbic acid leads to 2,3-diketogulonic acid and in the following decar-

boxylation to xylosone and 4-desoxypentosone. Further reaction products are ethylglyoxal, reductones, furfural and furancarboxylic acid. Ascorbic acid degradation is influenced not only by the oxygen content but also by pH, temperature and the presence of metallic ions. Under anaerobic conditions, the ascorbic acid content decreases slowly and only at pH 2 - 4. [8]

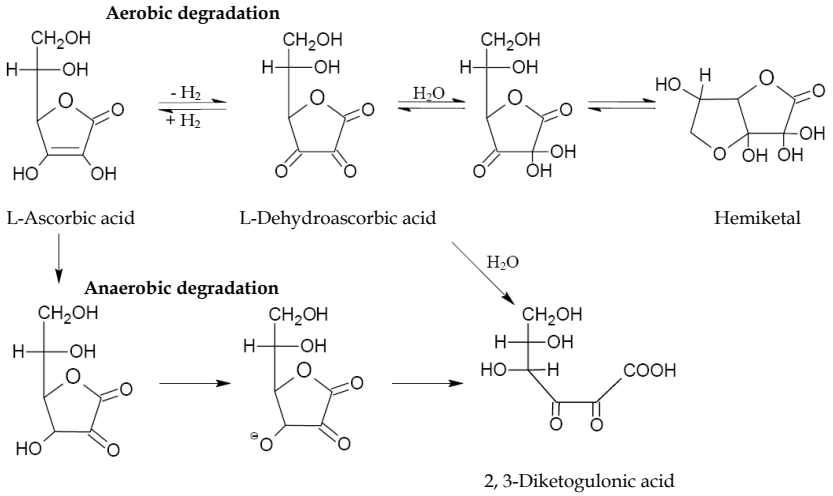


Figure 1.3.: Aerobic and anaerobic degradation reactions of ascorbic acid [8]

Ascorbic acid is an antioxidant that decreases the degradation of polyphenols by suppressing enzymatic browning (refer to section 1.3.2.2 on page 9). Furthermore, ascorbic acid accelerates the non-enzymatic browning process as it and its aforementioned degradation products undergo the maillard reaction in the presence of amino acids. [8]

In addition, Sawamura *et al.* (1994) shows that DHAA forms brown degradation products itself in the absence of other reaction partners. One of the brown molecules was identified as 3,4-dihydroxy-5-methyl-2(5H)-furanone. Its formation is promoted in absence of oxygen. [62]

Thus, the alteration of the ascorbic acid content in fruit juice during storage influences the juice quality not only regarding its vitamin status but also in terms of its color changes.

1.3.1.2. Analysis of Ascorbic Acid

In the past, ascorbic acid in aqueous samples was usually quantified by using the titration method with Tilman solution (i.e. 2,6-dichlorphenolindophenol). [24, 35,31] Since HPLC is a widely-used analytical technique various methods have been developed which quantify ascorbic acid by using RP-HPLC most commonly with UV detection systems. [79, 36, 42]

Though DHAA has no potential to further protect the juice against oxidation, its content in the juice is of certain interest as it can still be utilised by the human organism. To quantify DHAA the molecule is usually reduced to ascorbic acid in a first step by reducing agents like D,L-homocysteine [16], L-cysteine [30] or dithiothreitol [46, 61]. Subsequently, the sample is analysed for its total ascorbic acid content and the difference between this and the amount of ascorbic acid measured without reducing DHAA in advance is the amount of DHAA in the sample.

A further method published applies a derivatisation reaction of DHAA with 1,2-phenylenediamine prior to injection and a subsequent HPLC analysis of both ascorbic acid and derivatised DHAA. [70, 82] In contrast, a post-column derivatisation reaction of DHAA and ascorbic acid following an HPLC separation allows to quantify both compounds simultaneously. [32, 33, 34] Another approach to determine DHAA and ascorbic acid in a simultaneous analysis resulted in a combination of two different HPLC columns, a reversed-phase column and an ion exchange column. The latter should avoid the co-elution of DHAA and oxalic acid. [51]

1.3.2. Phenolic Compounds

Phenols are prevalent compounds of the secondary plant metabolism and fulfil a variety of protective functions in the flora mainly due to their antioxidative potential. Though they are minor compounds in plants phenols contribute to the color and taste of fruits and fruit juices. Due to their antioxidative capacity phenols are also capable to stabilise the color and taste characteristics in the plant product.

1.3.2.1. Structure of Phenolic Compounds

Due to the number of carbon atoms in their basic structure phenolic compounds can be divided into three main groups:

- phenol carbonic acids
- flavonoids
- low molecular phenols

The latter belong to the group of volatile flavourings but are not further elucidated in the following. The term polyphenol is used for phenolic compounds containing at least two hydroxylic groups.

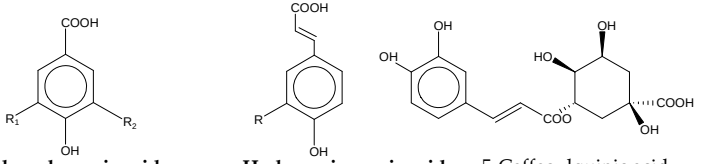
Phenol carbonic acids can be subdivided into hydroxy benzoic acids (C6-C1) and hydroxy cinnamic acids (C6-C3). As the most extensive group, the flavonoids can be classified into different sub-classes. Especially occurring in fruits, these are flavones, flavonols, flavanones, flavan-3-ols (also known as catechins which are the basic units of oligomeric proanthocyanidins), dihydrochalcones and anthocyanins (glycosidic bound anthocyanidins as the predominant form in plants). [8,27,59] Their basic structure and typical examples are given in figure 1.4.

1.3.2.2. Reactions of Phenolic Compounds

In the unprocessed fruit phenolic compounds are found mainly inside the cell and thus, protected against exterior influences (e.g. oxygen). More precisely, the anthocyanins are situated mainly in the grape paring. During juice processing they are released from the paring and are dissolved in the juice.

In general, after cell destruction by juice straining phenols are susceptible to oxidation following an enzymatic or a non-enzymatic pathway. Due to diverse influences the concentration of phenols can change not only during the juice production but also during its storage period. [67,68]

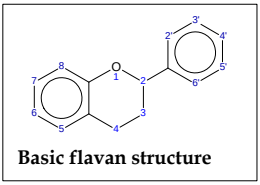
The first and speed-controlling step of the enzymatic reaction oxidises phenolic compounds to o-quinones and is catalysed by polyphenol oxidases (PPO). O-quinones are highly reactive molecules that undergo various reactions with either carbohydrates, proteins or other phenols. [58] In the juice, the formation of protein-phenol complexes adds to haze development depending on molecular size and degree of polymerisation of the phenolic compounds. Therefore,



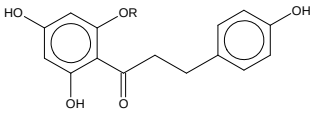
Hydroxybenzoic acids
Gallic acid (R_1: OH, R_2: OH)

Hydroxycinnamic acids
Caffeic acid (R: OH)

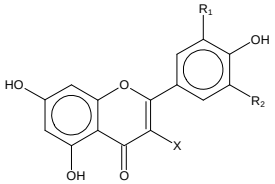
5-Caffeoylquinic acid
"Chlorogenic acid"



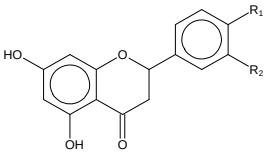
Basic flavan structure



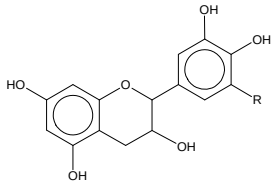
Dihydrochalcones
Phloridzin (R: ß-D-glucopyranoside)



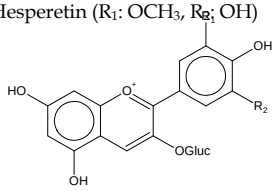
Flavones (X: H) **Flavonols** (X: OH)
e.g. flavonol: Quercetin (R_1: OH, R_2: H)



Flavanones
Naringenin (R_1: OH, R_2: H)
Hesperetin (R_1: OCH_3, R_2: OH)



Flavan-3-ols (Catechins)
Catechin (R H; trans C_2-C_3)
Epicatechin (R: H; cis C_2-C_3)



Anthocyanins
Cya-3-gluc (R_1: OH, R_2: H)
Del-3-gluc (R_1: OH, R_2: OH)
Mal-3-gluc (R_1: OCH_3, R_2: OCH_3)
Peo-3-gluc (R_1: OCH_3, R_2: H)
Pet-3-gluc (R_1: OH, R_2: OCH_3)

Figure 1.4.: Basic structures and typical examples of phenolic compounds [8]

phenols particularly proanthocyanidins are often removed by addition of flocculants (e.g. gelatin, diatomite, caseinate or polyvinylpolypyrrolidon) to avoid haze formation in clear juices. [20, 69, 8]

Another reason for the removal of phenolic compounds from commercial juices is their influence on the juice color. It is caused by the formation of more stable polymeric pigments from anthocyanin monomers [12, 77, 76]. In general, this polymerisation reaction is called enzymatic browning, is catalysed by PPO and leads to the formation of brown pigments. The process is decelerated by deoxygenation, PPO deactivation due to heating or by adding antioxidants like ascorbic acid and sulfite that reduce o-quinones to polyphenols. [8]

Recent fabrication conditions (e.g. pasteurisation or clarification) allow to reduce adverse influences of phenols and their degradation products on commercial juices. Thus, it is reasonable to conclude that in pasteurized or clarified fruit juices enzymatic browning usually does not occur. [27]

1.3.2.3. Polyphenols in Apple and Grape Juice

The phenolic compounds identified in the apple and grape juices that were used for these storage studies contained at least two hydroxylic groups. Thus, in general these compounds are named as polyphenols in the following.

The amounts of polyphenols found in apples and grapes vary with their cultivar and their degree of ripeness. And their content in the juice is also influenced by the manufacturing process used. [26, 67, 68] While the concentrations of single polyphenols strongly depend on the present conditions, there are some polyphenolic compounds identified as typically for apple and grape, respectively (see table 1.1).

1.3.2.4. Analysis of Polyphenols

In the past, polyphenols were often not specified separately but determined as total polyphenols by the Folin-Ciocalteau reaction. [64] Due to interferences by other juice compounds this colorimetric method usually overestimates the polyphenolic content of biological samples. This is mainly caused by the presence of endiols or reductones that occur as intermediates of the maillard reaction or during the degradation of polyphenols or ascorbic acid. [67, 68]

Thus, the use of chromatographic separation systems coupled with a suitable detector is considered to yield more specific results. The most common analytical system used for the determination of polyphenols is RP-HPLC with UV-DAD [18, 67, 68].

polyphenol class	apple juice	red grape juice
phenol carbonic acids	chlorogenic acid	gallic acid
		caffeic acid
flavan-3-ols	catechin	catechin
	epicatechin	epicatechin
procyanidines	B2	
dihydrochalcones	phloridzin	
flavonols		rutin
anthocyanins		malvidin-3-glucoside
		peonidin-3-glucoside
		cyanidin-3-glucoside
		petunidin-3-glucoside
		delphinidin-3-glucoside

Table 1.1.: Typical polyphenols in commercial apple and red grape juice [26, 67, 68, 49, 81]

As the analyte passes the UV detector without any chemical process this detection system can be coupled with another detector to optimize the analysis. Depending on the required information the use of an additional mass spectrometric detector helps to identify unknown substances [22, 37, 53, 55, 54], whereas the electrochemical detection is useful to quantify analytes at very low concentrations. The use of coulometric electrode array detection is particularly suitable for the determination of polyphenols due to their high susceptibility to oxidation [4, 3, 21, 83, 84].

1.4. Hydroxymethylfurfural (HMF)

As one of the degradation products of sugars during the maillard reaction, HMF is an important parameter to measure the quality of fruit juices. Due to heat treatment and increased dry matter juice concentrates are more susceptible to non-enzymatic browning. [27]

As the accumulation of HMF in the juice is described as autocatalytic reaction its concentration at the beginning of a storage period may have an influence of its further increase [6, 73]. Thus, a juice made from concentrate - especially in case of a heat treatment during concentration - probably has a higher HMF formation rate during storage than a low-temperature-treated juice.

Whether this parameter is suitable to observe juice quality and storage stability is miscellaneously discussed in the literature. HMF is considered to cause boiled taste with a threshold value of 5000 ppb [5, 19]. However, the correlation between HMF formation and taste evaluation during storage is not yet approved [56].

1.4.1. Analysis of HMF

HMF is usually determined by photometric measurement of the pigment formed by a specific reaction of HMF with barbituric acid and toluidine [6,15] but also an HPLC method with UV detection is used [41,57]. The simultaneous determination of HMF and polyphenols was developed during this project using an HPLC system with a coupled UV-DAD and CEAD. Though HMF cannot be detected by the CEAD due to its oxidation stability, its absorption maximum is at 280 nm and thus, in the same range as many polyphenols.

2. Materials

2.1. Samples

2.1.1. First Storage Study

NCA juice Apple-acerola juice, naturally cloudy, 100% juice from concentrate, containing 3 % acerola juice from concentrate as natural source of ascorbic acid (as vitamin C), filled in 1.5 L packages.

FA juice Apple juice, filtered, 100% juice from concentrate, filled in 1.5 L packages.

RG juice Red grape juice, filtered, 100% direct juice, filled in 1 L packages.

The juices were filled in commercial laminated carton packages with aluminium layer (illustrated in figure 1.1 on page 4) and stored at room temperature in the dark for one year. The temperature was controlled periodically and did not exceed the range from 16 °C to 24 °C. At regular intervals two packages of each juice were sampled and the A and B specimens were stored at -30 °C until analysed.

A part of the juice samples were stored in air-conditioned heating cabinets at either 20 °C or 30 °C in the laboratory of the packaging company SIG combibloc. Though the storage conditions of the room without air-conditioning differ slightly from the one in the 20 °C heating cabinet at the laboratory of SIG combibloc, both kind of samples are further indicated as LT samples (i.e. low temperature storage).

In opposite, the specimens taken from the juice stored at 30 °C are defined as HT samples (i.e. high temperature storage). During this study SIG combibloc provided two HT samples for the complete analysis of all tested parameters, i.e. after 11 and 46 weeks of storage.

2.1.2. Second Storage Study

NCA juice Apple juice, naturally cloudy, added ascorbic acid, 100% juice from concentrate, filled in 1 L packages.

FA juice Apple juice, filtered, 100% juice from concentrate, filled in 1 L packages.

RG juice Red grape juice, filtered, 100% juice from concentrate, filled in 1 L packages.

For the second storage study the juices were filled in two different laminated carton packages and stored for one year. Beside the regular aluminium laminated composite which was already used in the first storage study, a further packaging material was applied. It consisted of an LDPE coated carton with a polyamide layer on the inside of the package. This aluminium free packaging material permitted a higher oxygen permeability than the aluminium coated composite. The two packaging materials were indicated as follows:

ALC Aluminium laminated composite

PAC Polyamide composite, aluminium free

At regular intervals two packages of each juice in each packaging were sampled and the A and B specimens were stored at -30 °C until analysed (B specimens only available for LT stored juices). This time the juice samples were stored in an air-conditioned room of the IEL laboratory at room temperature (between 16 °C and 24 °C). At the laboratory of SIG combibloc the juice samples were again stored in air-conditioned heating cabinets at either 20 °C or 30 °C. According to the first storage study, the samples stored at room temperature as well as the ones in the heating cabinet at SIG combibloc are indicated as LT samples; samples stored at 30 °C are defined as HT samples.

2.2. Chemicals

2.2.1. Solvents

Acetonitrile	Ultra Gradient HPLC Grade, Mallinckrot Baker (Deventer, The Netherlands)
UHQ Water	provided by an Elgastat UHQ-II, Elga (Bucks, UK)

2.2.2. Standard Compounds

L-(+)-Ascorbic acid	p.a., Merck (Darmstadt, Germany)
Dehydro ascorbic acid	no. 261556, EC-no. 207-720-6, Aldrich (Steinheim, Germany)
Gallic acid	p.a., Merck (Darmstadt, Germany)

HMF	5-(hydroxymethyl)-furfural, 99 %, Sigma-Aldrich (Steinheim, Germany)
Chlorogenic acid	purum (97 %), no. 25700, Fluka (Buchs, Switzerland)
(+)-Catechin	hydrate, 98%, no. C-1251, Sigma (Steinheim, Germany)
Caffeic acid	purum, no. 26858, Serva Feinbiochemica (Heidelberg, Germany)
(-)-Epicatechin	EEC-no. 207-710-1, Sigma (Steinheim, Germany)
p-Coumaric acid	99 %, no. 17613, Serva (Heidelberg, Germany)
Ferulic acid	EEC-no. 214-490-0, Sigma (Steinheim, Germany)
Rutin	Quercetin-3-O-rutinoside, hydrate, >95 %, EC-no. 205-814-1, Sigma (Steinheim, Germany)
Phloridzin	Phloretin-2'-β-glucoside, dihydrate, Serva Feinbiochemica (Heidelberg, Germany)
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%, no. 21894, Acros Organics (Geel, Belgium)

2.2.3. Further Chemicals

o-Phosphoric acid	p.a., 85%, Roth (Karlsruhe, Germany)
Sodium dihydrogen phosphate	p.a., monohydrate, Merck (Darmstadt, Germany)
Oxalic acid	dihydrate, Merck (Darmstadt, Germany)
Natrium hydroxide	p.a., Merck (Darmstadt, Germany)
ABAP	2,2'-azobis(2-methylpropionamidine) dichloride, 98 %, no. 40156, Acros Organics (Geel, Belgium)

DTPA	diethylenetriaminepentaacetic acid, purified, no. D-6518, Sigma (Steinheim, Germany)
KMBA	(α-keto-γ-methiolbutyric acid) sodium salt, no. K-6000, Sigma (Steinheim, Germany)
tri-Potassium phosphate	(K_3PO_4) trihydrate, extra pure, no. 105102, Merck (Darmstadt, Germany)
tri-Fluor acetic acid	(TFA) for synthesis ($>$ 99 %), EC-no. 200-929-3, Merck-Schuchardt (Hohenbrunn, Germany)

2.3. Consumables

Membrane filters	regenerated cellulose, RC 58, 0.2 μm, $\emptyset$ 50 mm, Schleicher & Schuell (Dassel, Germany)
	PTFE, 0.2 μm, $\emptyset$ 47 mm, Alltech (Deerfield, Il, USA), distributed by Restek (Bad Homburg v.d.H., Germany)
Syringe filters	polyester (PET), CHROMAFIL, 0.2 μm and 0.45 μm, $\emptyset$ 15 mm, Macherey-Nagel (Düren, Germany)

2.4. Analytical Equipment

2.4.1. HPLC-UV-CEAD

All devices by ESA (Chelmsford, Ma, USA), if not otherwise indicated.

Pumps	Two 580 Solvent Delivery Modules
Degasser	Degasys DG-1310, Uniflows (Tokyo, Japan)
Mixer	M 800, Kontron (Neufahrn, Germany)
Pulsation damper	Art. 14-0177
Autosampler	540 with 20 μL injection loop and tray-cooling (set at 4 °C)
Column oven	Mistral, housing HPLC column and electrodes, set at 30 °C
Detector I	System Gold, Diode Array Detector 168, Beckman Coulter (Unterschleißheim, Germany) Analogue interface for CoulArray
Software	Gold 7.11, Beckman Coulter (Unterschleißheim, Germany)
Detector II	CoulArray 5600 with eight electrodes
Software	CoulArrayWin v1.02

Table 2.1.: HPLC system for quantification of polyphenols, HMF and ascorbic acid in juice samples

2.4.2. HPLC-UV-Vis I

Pump	600 Multisolvent Delivery System, Waters (Eschborn, Germany)
Degasser	Degasys 1310, Uniflows (Tokyo, Japan)
Detector	LC 55 B UV-Vis detector, Perkin-Elmer (Norwalk, USA)
Software	EZChrom Elite v2.8 (Scientific Software Inc.)

Table 2.2.: HPLC-UV-Vis system I for quantification of anthocyanins in the first storage study

2.4.3. HPLC-UV-Vis II

All devices by Beckman Coulter (Unterschleißheim, Germany), if not otherwise indicated.

Pump	Beckman System Gold, programmable solvent module 125
Degasser	Degasys DG-1210, Uniflows (Tokyo, Japan)
Autosampler	LC-Triathlon for Beckman no. 507, Spark Holland Inc. (Emmen, Netherlands)
UV/Vis detection	Beckman System Gold; scanning detector module 167
Software	Beckman 32 Karat™ Software

Table 2.3.: HPLC-UV-Vis system II for quantification of anthocyanins in the second storage study

2.4.4. HPLC-UV-MS

All devices by Dionex (Germering, Germany), if not otherwise indicated.

Pump	P-580 A HPG
Degasser	Degasys DG-1310, Uniflows (Tokyo, Japan)
Autosampler	ASI-100 T
Column oven	STH-585 set at 35 °C
UV detection	UVD-340 S, equipped with a capillary cell
Software	Chromeleon version 6.20 Build 531
Mass spectrometer	LCQ classic ion-trap, Thermo Finnigan (Egelsbach, Germany)
Ion source	Electrospray interface (ESI) with metal needle kit
Sheath liquid	System Gold Solvent Module 116, Beckmann Coulter (Unterschleißheim, Germany)
Software	Xcalibur Software v1.2, Thermo Finnigan (Egelsbach, Germany)

Table 2.4.: HPLC-MS system for identification of compounds in juice samples

2.4.5. GC-System with CombiPAL Autosampler

Instrument	GC-17A, Shimadzu (Duisburg, Germany)
Detector	Flame ionisation detector (FID)
Software	EZChrom Elite v2.8, Scientific Software (Pleasanton, USA)
Autosampler	CombiPAL, CTC Analytics (Zwingen, Switzerland)
Syringe	1mL Headspace syringe
Incubator	Agitator with 6 heatable positions and interval shaking
Software	PAL Cycle Composer v1.5, CTC Analytics (Zwingen, Switzerland)

Table 2.5.: GC system for ethylene quantification within TOSC assay

2.4.6. VARP-System

All devices by ESA (Chelmsford, Ma, USA), if not otherwise indicated.

Pumps	Two 580 Solvent Delivery Modules
Degasser	Degasys DG-1310, Uniflows (Tokyo, Japan)
Mixer	M 800, Kontron (Neufahrn, Germany)
Pulsation damper	Art. 14-0177
Autosampler	540 with 20 μL injection loop and tray-cooling (set at 4 °C)
Column oven	Mistral, housing electrodes, set at 30 °C
Detector	CoulArray 5600 with eight electrodes
Software	CoulArrayWin v1.02

Table 2.6.: VARP-system for measuring antioxidative capacity

3. Methods

3.1. Analysis of Phenols and HMF

3.1.1. Quantification by HPLC-UV-CEAD

Prior to HPLC analysis for quantification of HMF and phenolic compounds, the juice samples were filtrated through a 0.2 μm PET syringe filter. The NCA juice of the first storage study was diluted with UHQ water (1+1, v+v) before filtration. The NCA juice of the second storage study was centrifuged for 10 min at 10,000 U/min before filtration.

To quantify the content of HMF and of different phenols except anthocyanins the juice samples were separated by HPLC and the analytes were detected by UV detection and/or coulometric electrode array detection. External standards were used and calibration curves were plotted for each standard compound on the basis of UV peak area or sum of cluster peak areas for CEAD (i.e. sum of peak area of dominant, pre-dominant and/or post-dominant channel). All samples were analysed in duplicate. The results are presented graphically showing mean values ± SD.

The basic HPLC parameters are displayed in table 3.1. The optimised gradient elution programs for the different juices of the first and second storage study are presented in appendix chapter B.

HPLC parameters	
Instrument	HPLC-UV-CEAD system (refer to table 2.1)
Guard column	RP-18 Security Guard, 4 mm x 3 mm i. d., Phenomenex (Aschaffenburg, Germany)
Analytical column	Aqua RP-18, 150 mm x 4.6 mm i. d., particle size 3 μm, Phenomenex (Aschaffenburg, Germany)
Injection volume	20 μL
Mobile phase A	0.02 M NaH_2PO_4, set at pH 3.4 with phosphoric acid
Mobile phase B	Acetonitrile + 0.02 M NaH_2PO_4, pH 3.4 (2+1) (v+v)
Flow	0.7 mL/min
Gradient type	Linear
UV-scan range	200-400 nm
Voltage coulometric cells	8 electrodes set at 0–700 mV in steps of 100 mV, maintained at 30 °C

Table 3.1.: Quantification of phenols and HMF by HPLC-UV-CEAD

3.2. Analysis of Anthocyanins in RG Juice

3.2.1. Identification by HPLC-MS

Prior to anthocyanin analysis, the undiluted RG juice was filtered through a 0.45 μm syringe PET filter. After separation by high-performance liquid chromatography the anthocyanins were detected by UV-Vis diode array detection and multi step-mass spectrometric fragmentation. The analytical parameters are displayed in table 3.2. The identification was made by comparison of the samples mass fragmentation patterns with these from literature. [49, 78, 81]

3.2.2. Quantification by HPLC-UV-Vis

3.2.2.1. First Storage Study

For quantification of anthocyanins in RG juice of the first storage study, the undiluted RG juice was filtered through a 0.45 μm syringe PET filter prior to HPLC analysis. After separation by high-performance liquid chromatography the anthocyanins were detected by UV-Vis detection at 525 nm.

Cyanidin-3-glucoside was used as external standard, a calibration curve was plotted on the basis of peak area and all anthocyanins were quantified as cyanidin-3-glucoside. Samples were analysed in duplicate. The results are presented graphically showing mean values ± SD. The analytical parameters are displayed in table 3.3 on page 27.

3.2.2.2. Second Storage Study

For quantification of anthocyanins in RG juice of the second storage study, the undiluted RG juice was filtered through a 0.45 μm syringe PET filter prior to HPLC analysis. After separation by high-performance liquid chromatography the anthocyanins were detected by UV-Vis detection at 520 nm.

Malvidin-3-glucoside was used as external standard, a calibration curve was plotted on the basis of peak area and all anthocyanins were quantified as malvidin-3-glucoside. Samples were analysed in duplicate and the results are presented graphically showing mean values. The analytical parameters are displayed in table 3.4 on page 28.

HPLC parameters	
Instrument	HPLC-UV-MS system (refer to table 2.4)
Guard column	RP-18 Security Guard, 4 mm x 2 mm i. d., Phenomenex (Aschaffenburg, Germany)
Analytical column	Synergy 4u Fusion RP 80A, 150 mm x 2 mm i. d., 20 °C, Phenomenex (Aschaffenburg, Germany)
Injection volume	5 μL
Mobile phase A	0.2 % TFA in UHQ water and acetonitrile (95+5) (v+v)
Mobile phase B	0.2 % TFA in UHQ water and acetonitrile (55+45) (v+v)
Flow	0.2 mL/min
Gradient elution program	
Gradient type	Linear
0 min	0 % B
60 min	75 % B
Washing step	10 min with 100 % B
Re-equilibrating	10 min with 0 % B
UV-scan range	200-595 nm
Comparison to MS data	Track at 520 nm
MS-System	
Ionisation	ESI positive
Ionisation enhancement	Addition of 0.1 mL/min methanol
Source voltage	3.0 kV
Sheath gas flow	90
Auxillary gas flow	5
Capillary voltage	10 V
Capillary temperature	200 °C
First octapole offset	-5 V
Interoctapole lens	-30 V
Second octapole offset	-10 V
Trap DC offset	-10 V

Table 3.2.: Identification of anthocyanins in RG juice by HPLC-MS

HPLC parameters	
Instrument	HPLC-UV-Vis I system (refer to table 2.2)
Guard column	RP-18 Security Guard, 4 mm x 3 mm i. d., Phenomenex (Aschaffenburg, Germany)
Analytical column	MAX-RP 80A, 4 μm C_{18}, 150 mm x 4.6 mm i. d., Phenomenex (Aschaffenburg, Germany)
Injection volume	20 μL
Mobile phase A	2 % formic acid in UHQ water (v+v)
Mobile phase B	2 % formic acid in acetonitrile (v+v)
Flow	0.8 mL/min
Gradient elution program	
Gradient type	Linear
0 min	0 % B
40 min	30 % B
60 min	90 % B
Washing step	10 min with 100 % B
Re-equilibrating	20 min with 0 % B
UV-Vis wavelength	525 nm

Table 3.3.: Quantification of anthocyanins in RG juice of the first storage study

3.3. Analysis of Ascorbic Acid in NCA juice

3.3.1. Optimised Serial Analysis by HPLC-UV-CEAD

For the determination of ascorbic acid in NCA juice a conventional HPLC-UV method was modified to obtain an accelerated analysis. Fast isocratic elution of ascorbic acid at aqueous conditions (retention time: $t_R = 2.1$ min) enables up to twelve serial injections of diluted juice samples and standards before a washing step becomes necessary. Due to matrix components juice samples should be queued at the end of the series.

Prior to HPLC analysis, the juice samples are diluted if necessary to obtain an ascorbic acid concentration within the linear range (0.5 to 80 mg/L for 5 μL injection) using 2 % oxalic acid. Oxalic acid was used to stabilise ascorbic acid. If samples were analysed undiluted they were centrifuged for 10 min at 5,000 U/min prior to analysis. After separation by high-performance liquid chromatography ascorbic acid was detected by UV-Vis detection at 243 nm and by CEAD at 400 mV.

HPLC parameters	
Instrument	HPLC-UV-Vis II system (refer to table 2.3)
Analytical column	Aqua 3u C18 125A , 3 μm C_{18}, 150 mm x 4.6 mm i. d., Phenomenex (Aschaffenburg, Germany)
Injection volume	20 μL
Mobile phase A	0.2 % TFA in UHQ water (v+v)
Mobile phase B	0.2 % TFA in acetonitrile (v+v)
Flow	1.0 mL/min
Gradient elution program	
Gradient type	Linear
0 min	5 % B
60 min	35 % B
70 min	100 % B
Washing step	10 min with 100 % B
Re-equilibrating	10 min with 5 % B
UV-Vis wavelength	520 nm

Table 3.4.: Quantification of anthocyanins in RG juice of the second storage study

L-ascorbic acid dissolved in 2 % oxalic acid was used as external standard and a calibration curve was plotted on the basis of UV peak area and on basis of sum of cluster peak areas for CEAD (i.e. sum of peak area of dominant (no. 1) and post-dominant channel (no. 2)). Diluted samples were prepared and analysed each in duplicate. The results quantified via CEAD are presented graphically showing mean values ± SD. The analytical parameters are displayed in table 3.5.

3.3.2. Analytic Trials for Dehydroascorbic Acid

As described in section 1.3.1.2, a common method to quantify dehydroascorbic acid (DHAA) is based on a reduction step and the two-fold determination of ascorbic acid (i.e. with and without preceding reduction). Due to the possibility to apply reducing potentials to the coulometric electrode array detector (up to -450 mV) it was supposed that reduction and following oxidation of total ascorbic acid could run simultaneously. Preliminary tests with DHAA standards (freshly prepared with oxalic acid and UHQ water, respectively) have been conducted with CEAD electrodes set at -450 to 600 mV in steps of 150 mV. The results are described in section 5.1.3.

HPLC parameters	
Instrument	HPLC-UV-CEAD system (refer to table 2.1)
Guard column	RP-18 Security Guard, 4 mm x 3 mm i. d., Phenomenex (Aschaffenburg, Germany)
Analytical column	Aqua RP-18, 150 mm x 4.6 mm i. d., particle size 3 μm, Phenomenex (Aschaffenburg, Germany)
Injection volume	5 μL
Mobile phase A	0.02 M NaH_2PO_4, set at pH 3.4 with phosphoric acid
Mobile phase B	Acetonitrile + 0.02 M NaH_2PO_4, pH 3.4 (2+1) (v+v)
Flow	0.7 mL/min
Elution type	Isocratic at 0 % B
Washing step	10 min with 100 % B
Re-equilibrating	30 min with 0 % B
UV wavelength	243 nm
UV-scan range	200-400 nm
Voltage coulometric cells	8 electrodes set at 400–700 mV: no. 1-5 at 400 mV, no. 6-8 at 500-700 mV in steps of 100 mV, maintained at 30 °C
Limit of quantification	14 pmol for CEAD and 56 pmol for UV detection

Table 3.5.: Quantification of ascorbic acid in NCA juice

However, the resulting chromatograms have shown that the reaction rate to reduce DHAA was distinctly lower than the flow rate of the HPLC system (at 0.7 mL/min the time of flight per channel accounts for a maximum of 1.5 sec). Thus, DHAA concentration was not determined in the context of both of the storage studies.

3.4. Antioxidative Capacity Assays

The antioxidative capacity of the fruit juices of the first storage study was determined using two different methods. While the TOSC assay is well-established at the institute, the VARP assay has been developed in the context of the first storage study (refer to section 4). The latter method has shown to provide reliable data and a sensitivity comparable to the TOSC assay. Therefore, the juices of the second storage study were analysed by using the VARP assay only.

3.4.1. TOSC Assay

The total oxidant scavenging capacity assay (TOSC assay) was applied in a modified and automated version [80, 45, 43, 44]. This assay is based upon the ethylene yielding reaction of α-keto-γ-methiolbutyric acid (KMBA) with one of three available reactive oxygen species (ROS). The time course of ethylene production was monitored during one hour by repeated headspace GC with a CombiPAL autosampler.

Peroxyl was chosen as ROS for analysis of the juice samples due to its lower reactivity in comparison to peroxynitrite and hydroxyl radicals. Peroxyl radicals are the most stable ones and thus, require the lowest concentration of antioxidants to be scavenged [43]. Therefore, peroxyl was considered to be the most sensitive ROS to detect even small changes in antioxidative capacity.

TOSC values were quantified by comparing the areas for (uninhibited) control and sample reaction: a sample without antioxidative capacity has a TOSC value of 0 %, a complete suppression of ethylene formation corresponds to a TOSC value of 100 % and prooxidants obtain negative TOSC values.

Samples of the juices were analysed each for two different dilutions which were prepared and measured each in duplicate. The results are presented graphically showing calculated TOSC values for each of the two dilutions. The analytical parameters are displayed in table 3.6 on page 31.

3.4.2. VARP Assay

The voltammographic analysis of the reducing potential (VARP) assay was developed to quantify the antioxidative capacity by means of a coulometric electrode array detector. Details of the method are described in chapter 4.

3.5. Oxygen Analysis

The oxygen measurements were conducted by the laboratory of the packaging company SIG combibloc. The headspace volume was calculated as mean value of three packages. For the determination of the oxygen content in the headspace, a CheckMate II gas analyser with electrochemical oxygen sensor was used.

The oxygen concentration in the juice was analysed by an optical sensor measuring the luminescence of dissolved oxygen. This analysis was conducted in triplicate. The results are presented graphically showing mean values $\pm$ SD.

Autosampler parameters	
Instrument	CombiPAL autosampler (refer to table 2.5)
Incubation temperature	37 °C
Incubation time	60 min
Sample agitating	Every 55 sec for 5 sec
Parallel analysed samples	6
Sampling times	0 to 60 min, every 12 min
Sample amount	100 μL from sample headspace
GC-system	
Instrument	GC-system (refer to table 2.5)
Column	Chrompack PoraPLOT Q column, 27.5 m x 0.53 mm x 20 μm, Varian (Darmstadt, Germany)
Carrier gas	Nitrogen
Flow rate	15 mL/min
Split	Off
Oven temperature	80 °C
Injector temperature	100 °C
Detector temperature	220 °C

Table 3.6.: Analytical parameters of the TOSC assay

3.6. Sensory Analysis

The evaluation of the juice color and taste was conducted by the laboratory of the packaging company SIG combibloc. Within regular intervals a trained sensory panel of at least four persons evaluated the stored juices. Prior to test, juice samples stored at LT and HT as well as refrigerated stored controls were equally tempered.

At first, the panel conducted a triangle test according to DIN ISO 4120 from April 2005 [39] to check the differentiability between the three samples. Afterwards, an unspecific evaluation scheme with a scale from 1 to 5 (refer to figure 3.1) was used to rate the juice color and taste in accordance with DIN 10 952 from September 1983 [38]. A juice failed to fulfil the demands of quality when the mean evaluation was grade four or higher. The results are presented graphically showing mean values of the evaluation scores.

Grade (Points)	Description	Demands on Quality according to DIN 10952 Part 2
0	no	fulfilled
1	weak	
2	noticeable	acceptable
3	distinct	
4	strong	not fulfilled
5	very strong	

Figure 3.1.: Applied sensory evaluation scheme for the color and taste changes

3.7. CIE Lab System Analysis

Beside the evaluation of the juice color by a sensory panel, the filtered juices (FA and RG juice) were also analysed by the laboratory of the packaging company SIG combibloc using a spectral photometer. According to the CIE Lab color space system the luminance and color values were determined and the Delta E value was calculated. The results are presented graphically.

Delta E describes the euclidean color distance between the stored juice and the fresh filled juice at the beginning of the storage period. It is defined as:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

3.8. Statistical Analysis

Changes in parameters over time (at identical storage conditions) were statistically tested using linear regression analysis. The Pearson correlation coefficient

R, its square (the coefficient of determination R^2) and its corresponding significance level were calculated using Excel (Microsoft Inc., Redmond, Wa, USA) and SPSS 14.0 (SPSS Inc., Chicago, Il, USA).

There was a significant linear correlation between parameter and storage time in case of $R^2 \geq 0.8$ and $p < 0.05$. Linear correlation reached high significance if $p < 0.01$.

In case of no linear correlation ($R^2 < 0.8$) a parameter was analysed statistically in view of a significant difference compared to its initial value using two-tailed Student's t-test for paired samples (Excel, Microsoft Inc., Redmond, Wa, USA).

The difference was significant from the time point when $p < 0.05$ for the rest of the storage period. High significance was reached in case of $p < 0.01$.

For the second storage study data were evaluated regarding the question whether the packaging or the storage temperature had a significant influence on a parameter. Thus, corresponding data of samples taken at comparable time points (stored either in different packages or at different storage temperatures) were statistically tested using two-tailed Student's t-test for independent samples (Excel, Microsoft Inc., Redmond, Wa, USA). A storage condition had significant influence if $p < 0.05$. High significance was reached in case of $p < 0.01$.

4. Development of the VARP Assay

4.1. Basic Concept

The voltammographic analysis of the reducing potential (VARP) assay quantifies the antioxidative capacity by means of a coulometric electrode array detector (CEAD). Without prior HPLC-separation the sample flows through serial cells with ascending potentials (see figure 4.1, orange juice sample, diluted 1:10). The contained substances which are oxidised at low potentials (0 to 700 mV) may react as possible antioxidants.

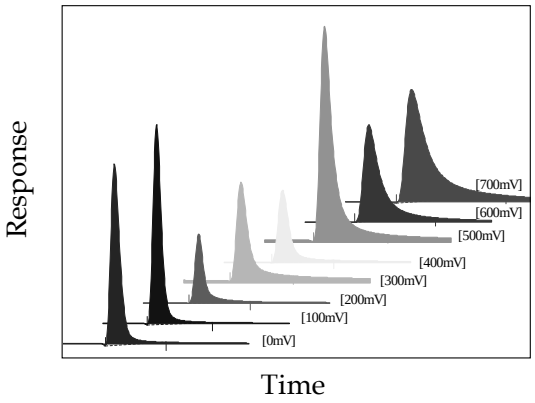


Figure 4.1.: VARP assay - peak response in ascending channels

Thus, the resulting voltammogram (current against applied potential) provides an indication of the antioxidative capacity of the sample (see figure 4.2). It shows the ability of the sample to reduce other molecules, e.g. oxidised juice components or cellular compounds suffering from oxidative stress.

This concept is supported by previous publications which showed the correlation between antioxidative capacity (determined by various assays like

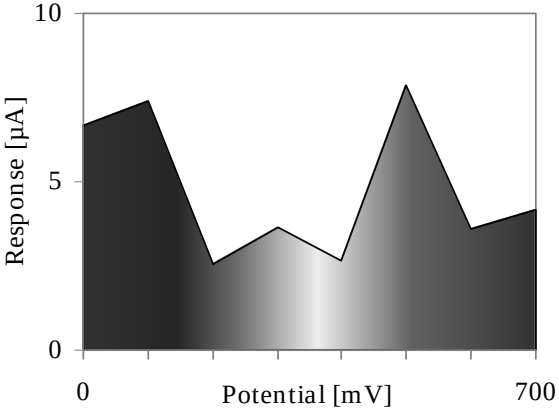


Figure 4.2.: VARP assay - formation of a voltammogram

ORAC[1], FRAP[2] or DPPH[3]) and the electrochemical data obtained from HPLC-CEAD analysis for standard compounds as well as for various food samples [1, 2, 25].

In detail, Guo *et al.* (1997) used HPLC analysis with CEAD for determination of antioxidants in various fruit and vegetable extracts. Then the CEAD data were compared with the results obtained from ORAC assay for these extracts.

The ORAC results correlated linearly with the total peak area and also with the total peak height (i.e. summed area of all peaks either from the dominant channels or from all channels). [25]

In conclusion, former research used CEAD data which were derived from HPLC chromatograms by summing up all peaks (area or height units) from specific or all CEAD channels. These data were used to describe antioxidative characteristics of a sample.

[1] oxygen radical absorbance capacity assay [14]
[2] ferric reducing/antioxidant power assay [9]
[3] 1,1-diphenyl-2-picrylhydrazyl assay [11, 66]

However, the VARP assay uses the CEAD without prior HPLC separation which drastically accelerates the analysis. Furthermore, not the summed up peak areas but the area under the resulting voltammogram is used to describe the antioxidative characteristics of a sample.

4.2. Analytical Process

Due to susceptibility of the CEAD to blockage, samples are filtered through a 0.2 μm syringe PET filter and are analysed diluted. The choice of dilution depends on the amount of antioxidants in the sample and shall prevent an overload of the electrodes. Dilutions are prepared with mobile phase A to minimise electrochemical effects other than caused by sample compounds. At isocratic conditions up to 40 serial injections are possible before a washing step and subsequent clean cell procedure (i.e. 1 min application of oxidative (900 mV) followed by reducing (-350 mV) potentials) becomes necessary. The analytical parameters are displayed in table 4.1.

HPLC parameters	
Instrument	VARP-system (refer to table 2.6)
Injection volume	5 μL
Number of serial injections	up to 40
Time between injections	5 min
Mobile phase A	0.02 M NaH_2PO_4, set at pH 3.4 with phosphoric acid
Mobile phase B	Acetonitrile + 0.02 M NaH_2PO_4, pH 3.4 (2+1) (v+v)
Flow	0.3 mL/min
Elution type	isocratic at 0 % B
Clean cell procedure	1 min at 900 mV and 1 min at -350 mV
Washing step	10 min with 100 % B
Re-equilibrating	40 min with 0 % B
Voltage coulometric cells	8 electrodes set at 0–700 mV in steps of 100 mV, maintained at 30 °C

Table 4.1.: Analytical parameters of the VARP assay

4.3. Data Evaluation

The current of each CEAD channel is plotted against the applied potential to derive a voltammogram. However, deduction of a single value from each voltammogram is necessary to improve the data presentation. It was suggested that the area under the voltammogram complies with the ability of the sample to reduce other molecules and thus, corresponds to its antioxidative capacity.

In the course of method development it was supposed that compounds which were oxidised in the first channels (i.e. at very low potentials) are stronger antioxidants than such ingredients that are oxidised in the latter channels. Thus, it was considered to be appropriate to sum up the weighted partial areas under the voltammogram according to the following formula:

$$WPA = \sum_{i=1}^{7} \frac{(x_{i+1} - x_i) * \frac{y_{i+1}+y_i}{2}}{x_{i+1}}$$

with WPA=weighted sum of partial areas [mV*μA], x_i=potential applied to channel no. i [mV], y_i=current (i.e. peak height) of channel no. i [μA].

However, even small variations in CEAD performance cause compounds to be oxidised in posterior channels. Thus, it was considered to be more appropriate to sum up the unweighted partial areas under the voltammogram (UPA). In fact, tests with standard antioxidants (e.g. catechin, ascorbic acid or the water soluble vitamin E analogon trolox) revealed that UPA values have lower standard deviations than corresponding WPA data. In addition, UPA plotted against the antioxidant concentration covers a major linear range than WPA plotted against concentration (see figure 4.3).

Thus, for all VARP calculations the unweighted area under the voltammogram is calculated by adding its partial areas using the following formula:

$$UPA = \sum_{i=1}^{7} \frac{(x_{i+1} - x_i) * \frac{y_{i+1}+y_i}{2}}{100}$$

with UPA=unweighted sum of partial areas [mV*μA], x_i=potential applied to channel no. i [mV], y_i=current (i.e. peak height) of channel no. i [μA].

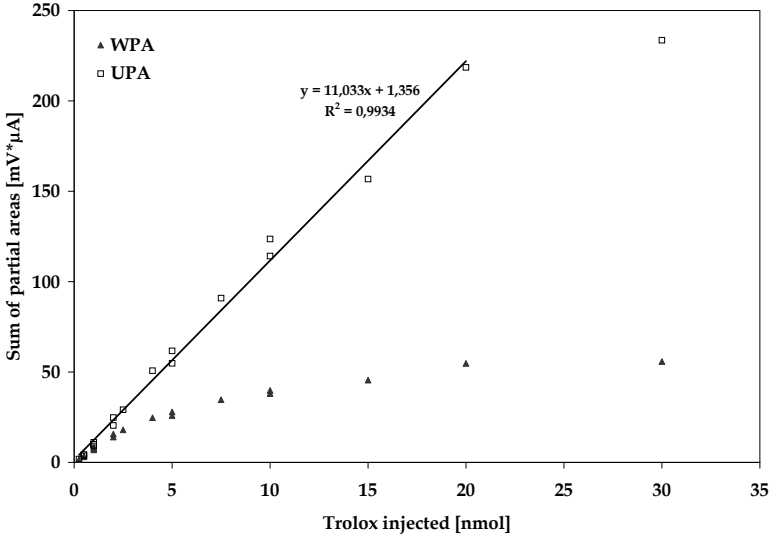


Figure 4.3.: Linear range of VARP calibration curves for WPA and UPA using trolox standard at various injection levels (5 to 20 μL).

4.4. Standard Antioxidants as Reference

The performance of each electrode of a CEAD depends on various parameters (e.g. pH, buffer concentration, temperature, pressure) which have to be maintained to get comparable voltammograms. However, even small differences, which cannot be completely eliminated, lead to different results.

In addition, CEAD response decreases within its lifetime due to deposit on the graphite surface of electrodes and abrasion of the electrodes especially at higher voltages. Thus, the area under the VARP voltammogram depends on the CEAD performance and so does its UPA value. To improve its robustness the VARP assay is calibrated using an external standard antioxidant. Catechin and trolox were tested as calibration standards. While the first one results in voltammograms comparable to typical juice samples (see figure 4.4) trolox is already completely oxidised at low potentials unless higher concentrations

cause an overload of the first channels (see figure 4.5).

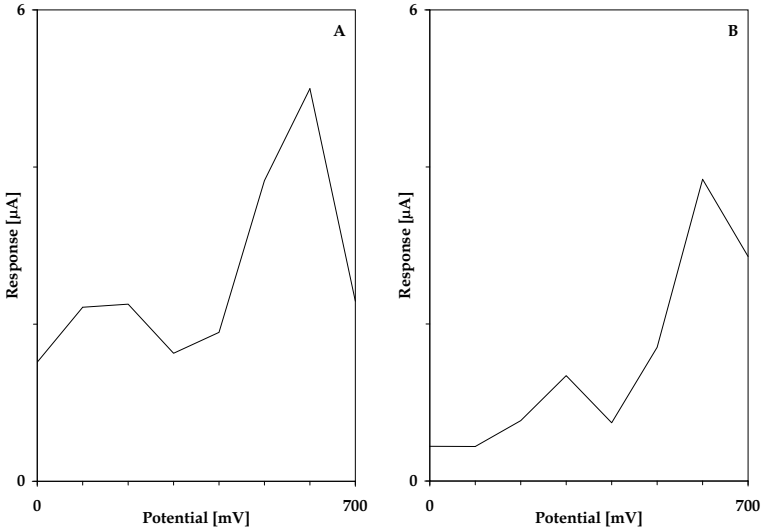


Figure 4.4.: Voltammograms of (A) catechin standard, 179 μM and (B) RG juice sample, 1:10 diluted (5 μL injected).

The linear range for trolox was verified to be from 0.5 to 20 nmol injection amount, for catechin it was from 0.36 to 5.37 nmol injection amount (injection volume ranges from 5 to 20 μL).

4.5. Generating VARP Data

Trolox and catechin were used as external standards and calibration curves were plotted on the basis of UPA. However, as it is the most prevalent standard antioxidant used for quantifying antioxidative capacity (e.g. for trolox equivalent antioxidative capacity method (TEAC) [50]) only trolox data were chosen for presentation of VARP results of the two storage studies.

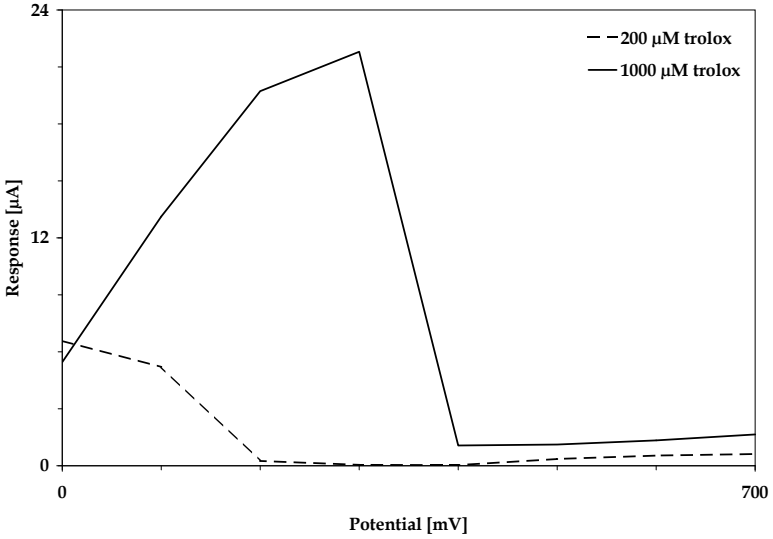


Figure 4.5.: Voltammograms of trolox standards at different concentrations (5 μL injected).

Dilutions of each sample were prepared and measured each in duplicate. The results are presented graphically showing mean values ± SD.

During the second storage study VARP data obtained from temporary divergent test series were analysed in detail. Comparison revealed that not only the absolute UPA results of standard compounds and juice samples (stored frozen until analysis) differed slightly from time to time, but also the VARP value calculated as trolox or catechin equivalents.

Thus, the VARP results depend on the CEAD performance and absolute VARP values of two temporary divergent measurements cannot be compared. However, to compare the antioxidative capacity of the juices stored during the first and the second study VARP ratios were calculated. Details are described in section 6.4.5.1 on page 123.

4.6. Comparison with Other Assays

In the context of the development of the assay the VARP results for seven different fruit juices were compared with TOSC (refer to section 3.4.1) and TEAC [50] values of the same samples. Their results yielded similar rankings of the analysed juices (see figure 4.6 and table 4.2) [10].

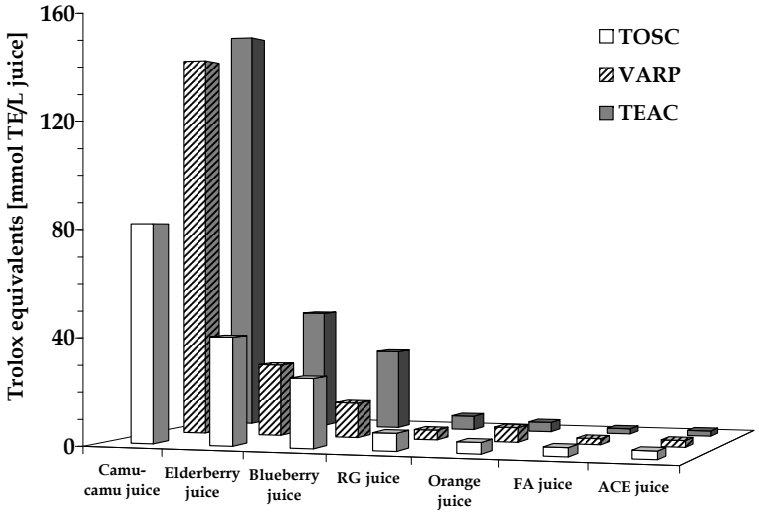


Figure 4.6.: Comparison of different fruit juices in terms of antioxidative capacity determined by VARP, TOSC and TEAC assay.

The TE ratios were calculated for VARP, TOSC and TEAC values of each juice in proportion to the respective TE value of ACE juice (see table 4.3). The differences between the three values of each juice reflect the differences between the three techniques. As all methods base on different reaction mechanisms this finding is plausible.

	TOSC	VARP	TEAC
	[mmol TE/L juice]		
Camu-camu juice	82.2	146.4	159.4
Elderberry juice	40.2	27.4	45.9
Blueberry juice	25.7	13.4	30.8
RG juice	6.6	3.8	5.4
Orange juice	4.2	5.5	3.7
FA juice	3.3	2.3	2.0
ACE juice	3.0	2.5	2.0

Table 4.2.: Antioxidative capacity of different fruit juices determined by VARP, TOSC and TEAC assay.

	TOSC	VARP	TEAC
	[TE ratio in proportion to ACE juice]		
Camu-camu juice	27	59	80
Elderberry juice	13	11	23
Blueberry juice	8.6	5.4	15
RG juice	2.2	1.5	2.7
Orange juice	1.4	2.2	1.9
FA juice	1.1	0.9	1.0
ACE juice	1.0	1.0	1.0

Table 4.3.: TE ratios calculated for VARP, TOSC and TEAC values of each juice in proportion to the respective TE value of ACE juice.

5. The First Storage Study

Influences of Storage Time and Temperature

The first study analysed the influences of oxidative processes on three different commercial juices (refer to section 2.1.1 on page 16) over a storage period of one year (in accordance with the usual period of shelf-life) at room temperature.

The results show the detailed changes during deterioration of the juices (partially dependent from oxygen influence). This storage study should give information to test the following hypothesis: As antioxidants are highly reactive (especially in presence of oxygen) they first undergo a degradation reaction and thus, influence the antioxidative capacity of the juice. If a minor antioxidative capacity was associated with a worse sensory evaluation, this could be a useful additional parameter to evaluate juice quality. The accurate determination of antioxidants during the storage period should give a hint which compounds were responsible for a possible decrease of the antioxidative capacity.

The alterations of the juices were analysed in terms of their antioxidative capacity and its influencing antioxidants like ascorbic acid and polyphenols as well as their HMF content and their sensory analysis of color and taste. The oxygen amount was determined by the headspace volume and its oxygen content as well as the oxygen concentration in the juice.

In opposite to the other tests the headspace volume, oxygen and sensory evaluations were also carried out for HT samples. For clarification whether a parameter was influenced by storage temperature all parameters tested were determined for two HT samples of each juice (i.e. short-term storage over 11 weeks and long-term storage over 46 weeks).

5.1. Storage of Apple Juices

5.1.1. Oxygen and Headspace Analysis

Immediately after filling of the two apple juices the headspace in the packaging accounted for 40 mL. The volume decreased fast due to the condensation of the water vapour that was blown into the packaging after filling directly before the packaging was sealed. After the first three weeks, the volume remained in the range of 20 mL in the NCA juice at LT storage (see figure 5.1). The headspace volume of the FA juice varied highly, but obtained a comparable mean value of 18 mL (see appendix figure C.1 on page 153). Headspace volume did not correlate linearly with time, neither for NCA nor FA juice and neither at LT nor HT storage.

After three weeks the headspace atmosphere contained almost no more oxygen. This was considered to be due to the degassing of the juice directly before filling and subsequent dissolving of the headspace oxygen in the juice. The figures C.2 and C.3 on page 154 show the results for the HT storage which are similar to the LT samples.

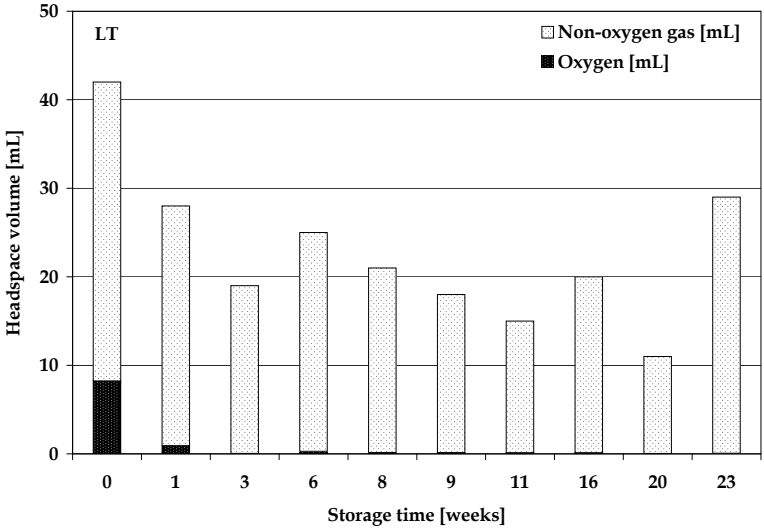


Figure 5.1.: Headspace atmosphere of NCA juice packaging: Influence of storage time during LT storage.

Figure 5.2 shows the fast decrease of the oxygen concentration in the NCA juice within the first three weeks of storage. Subsequently, the oxygen content leveled off at 0.25 ppm for both storage temperatures. Though oxygen also migrated from headspace into juice, its high concentration of antioxidants (especially ascorbic acid) caused fast consumption of the oxygen.

In contrast, the oxygen concentration in the FA juice stored at LT reduplicated during the first week of storage. Probably the oxygen from the headspace atmosphere dissolved faster in juice than it reacted with juice compounds. This was considered to be due to the lower concentration of antioxidants in the FA juice.

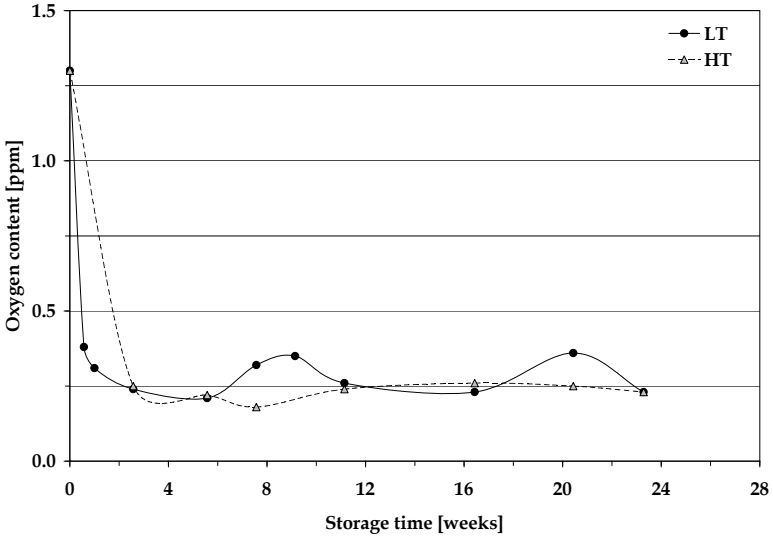


Figure 5.2.: Oxygen content in NCA juice: Influence of storage time and temperature.

Subsequently, the oxygen concentration diminished fast down to about 0.25 ppm which was comparable to the NCA juice (see figure 5.3). After three weeks of LT storage the juice contained three times as much oxygen compared to the HT sample indicating a higher reaction rate at higher storage temperatures. However, the following data points show similar oxygen levels at both storage temperatures.

5.1.2. Polyphenolic Compounds

The main polyphenol of the apple juices was chlorogenic acid. Figure 5.4 shows its stability in the NCA juice during LT storage. There was no linear correlation observed between chlorogenic acid concentration and storage time. However, in comparison to the initial value the loss reached statistical significance ($p < 0.05$) after 38 weeks of storage.

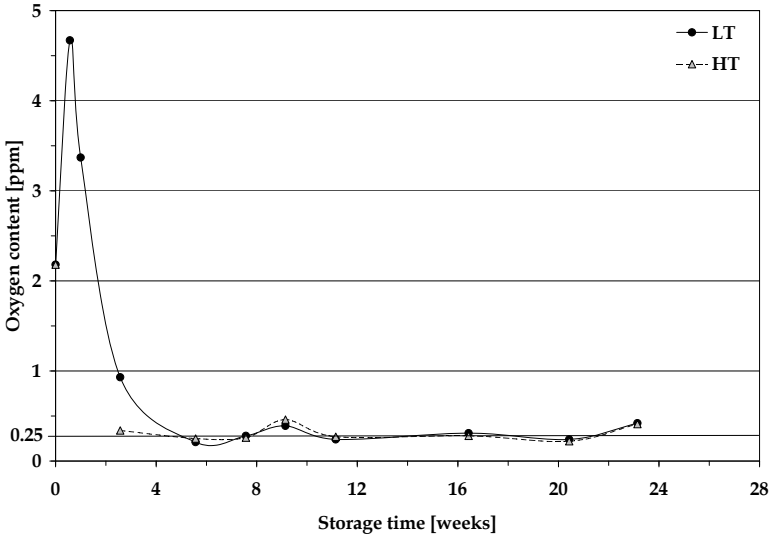


Figure 5.3.: Oxygen in FA juice: Influence of storage time and temperature.

The two HT samples contained significantly less chlorogenic acid than their LT correspondents (after 12 and 44 weeks of LT storage). However, the short-term HT sample was within the abnormally high standard deviation of the LT sample of week eight. Thus, this example was considered not providing a safe indication of the temperature influence on the degradation. A comparison between the degradation of the different polyphenols in NCA juice is shown in table 5.1.

Chlorogenic acid showed similar stability in the FA juice during LT storage (refer to figure C.4 on page 155). Its initial value was slightly lower than in the NCA juice. There was no linear autocorrelation and the change did not reach statistical significance in comparison to the initial value.

In opposite to the NCA juice, the chlorogenic acid concentration of the two HT samples was significantly lower ($p < 0.01$) than of the respective LT samples (after 8, 12 and 44 weeks of LT storage) indicating a temperature

dependent degradation of this polyphenol.

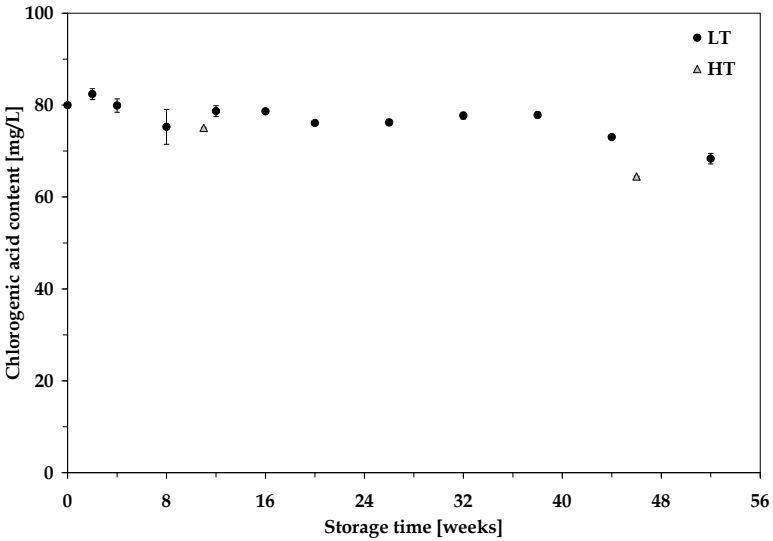


Figure 5.4.: Chlorogenic acid in NCA juice: Influence of storage time and temperature during one year of storage.

Another typical phenol in the apple juices was phloridzin. Figure 5.5 shows its stability in the NCA juice during LT storage. Phloridzin content was not linearly correlated with storage time. No distinct alteration occured within the first 38 weeks of storage, but then its concentration diminished significantly ($p < 0.05$) during the last 14 weeks of storage. It was considered that there were other compounds that fulfilled a protective function for the phloridzin during the first nine months of storage.

There was no significant difference between the short-term HT sample and its LT correspondents (8 and 12 weeks, respectively), but the long-term HT sample showed a highly significantly lower phloridzin level ($p < 0.01$) than the LT sample after 44 weeks. This underlined the hypothesis that other protective compounds inhibited the phloridzin degradation over a certain period of storage before a temperature-dependent loss occured.

Figure C.5 on page 155 shows a similar trend for the FA juice. However, it contained about twice as much phloridzin as the NCA juice. When long-term HT and LT storage samples (46 and 44 weeks, respectively) were compared in terms of their phloridzin content the difference was significant ($p < 0.05$).

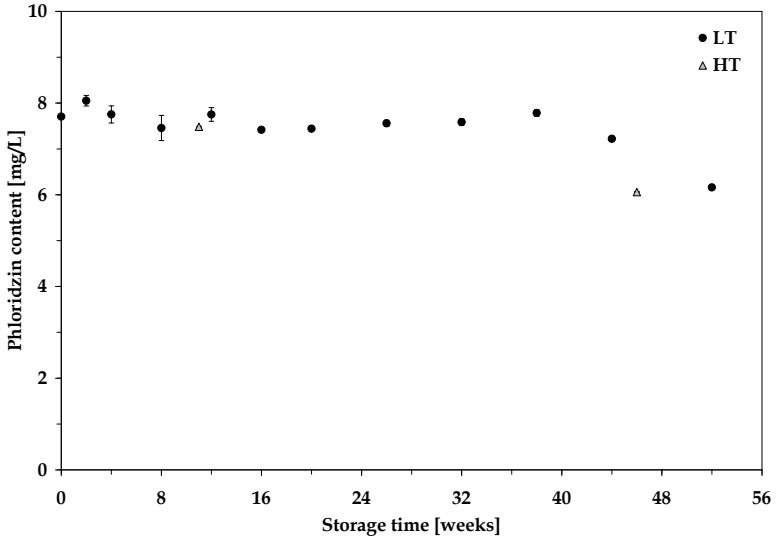


Figure 5.5.: Phloridzin in NCA juice: Influence of storage time and temperature during one year of storage.

Figure 5.6 indicates the characteristics of flavan-3-ols in the NCA juice during storage. Catechin and epicatechin showed similar stability, but no significant linear correlation with storage time was observed.

However, after 32 weeks there was significant loss of epicatechin in comparison to its initial concentration. For catechin the degradation reached significance after one year of storage.

In accordance with the phloridzin characteristics the short-term HT sample did not indicate a temperature-dependent degradation of the flavan-3-ols. In contrast, the long-term HT sample contained decisively less flavan-3-ols (high significance level with $p < 0.01$) providing an indication of a temperature dependent deterioration. There were no flavan-3-ols detectable in the FA juice.

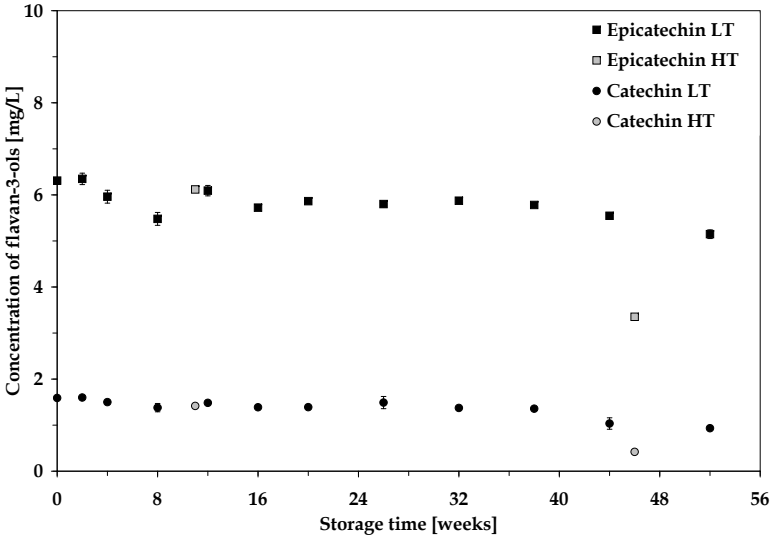


Figure 5.6.: Flavan-3-ols in NCA juice: Influence of storage time and temperature during one year of storage.

Storage time [weeks]	temp.	Chlorogenic acid [%]	Degradation of Phloridzin [%]	Epicatechin [%]	Catechin [%]
12	LT	1.6	nod	3.5	6.7
38	LT	2.7	nod	8.4	14.6
44	LT	8.7	6.3	12.1	34.9
52	LT	14.6	20.1	18.4	41.3
11	HT	6.2	2.9	3.0	10.9
46	HT	19.4	21.4	46.9	73.6

nod: no degradation

Table 5.1.: Degradation of polyphenols in NCA juice at different dates of storage

5.1.3. Ascorbic Acid in NCA Juice

The NCA juice was the only juice of the first storage study containing a determinable amount of ascorbic acid. Figure 5.7 demonstrates the changes of the ascorbic acid concentration during storage. For LT storage, this parameter correlated linearly with storage time ($R^2 = 0.824$, $p < 0.01$).

The two HT samples contained less ascorbic acid than the corresponding LT samples (high significance with $p < 0.01$). This temperature-dependent decomposition of ascorbic acid was already detected by various authors primarily during the storage of orange juices. [31, 35, 36, 40, 47, 48, 57, 65, 71]

After 20 weeks the juice contained less ascorbic acid than the given value the producer declared. At week 38 the concentration reached 70 % of the initial value and remained stable during the second half of the year.

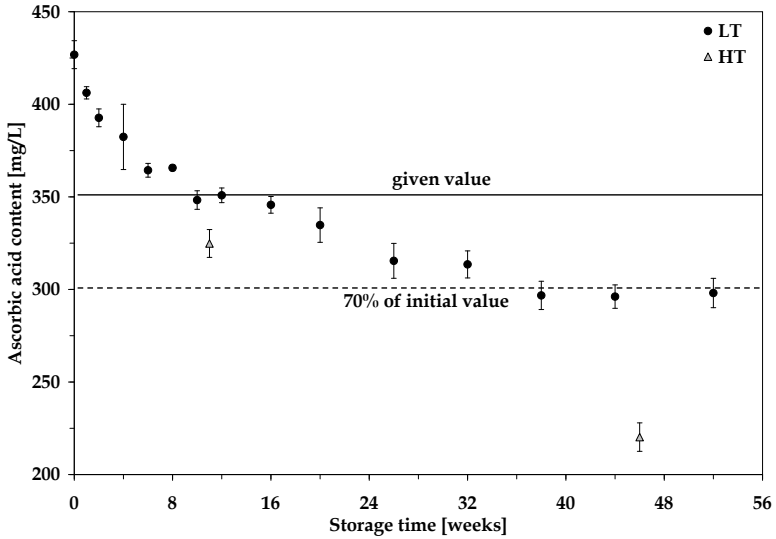


Figure 5.7.: Ascorbic acid in NCA juice: Influence of storage time and temperature during one year of storage.

Preliminary tests to determine the concentration of dehydroascorbic acid in the juice were conducted with DHAA standards (please refer to section 3.3.2

on page 28). However, the resulting chromatograms have shown that the reaction rate to reduce DHAA was distinctly lower than the flow rate of the HPLC system (at 0.7 mL/min the time of flight per channel accounts for a maximum of 1.5 sec). This finding was plausible as time periods of 5 to 15 min have been published by other authors to complete the reduction of DHAA to ascorbic acid [30, 46, 61]. Thus, DHAA concentration was not determined in the context of both of the storage studies.

5.1.4. HMF

In figure 5.8 graphs A and B show the increasing amount of HMF in both apple juices. Comparison of both graphs demonstrate similarities and differences between the two juices. For both juices formation of HMF correlated linearly with storage time ($R^2 > 0.95$, $p < 0.01$).

However, the low amount of HMF in the NCA juice remained stable during the first eight weeks. After this lag phase a constant formation rate led to a still low HMF level at week 52 (also compare table 5.2). A similar lag phase was observed for the sensory color evaluation of the NCA juice (refer to figure 5.11). As an intermediate of the maillard reaction that builds browning pigments, the formation of HMF is associated with the color changes of the juice [8].

The HMF concentration of the FA juice started on a more than 20 times higher level than in NCA juice. Without any lag phase the HMF content rose within one year of LT storage. For both juices the data of the HT samples pointed out the temperature-dependency of the HMF formation.

Storage time [weeks]	temp.	HMF content [mg/L] in NCA juice	FA juice
0	LT	0.11 ±0.01	2.40 ±0.01
12	LT	0.23 ±0.01	4.17 ±0.02
52	LT	1.79 ±0.02	9.86 ±0.08
11	HT	1.08 ±0.00	10.97 ±0.01
46	HT	23.45 ±0.05	61.48 ±0.94

Table 5.2.: HMF contents in both apple juices at different points of storage

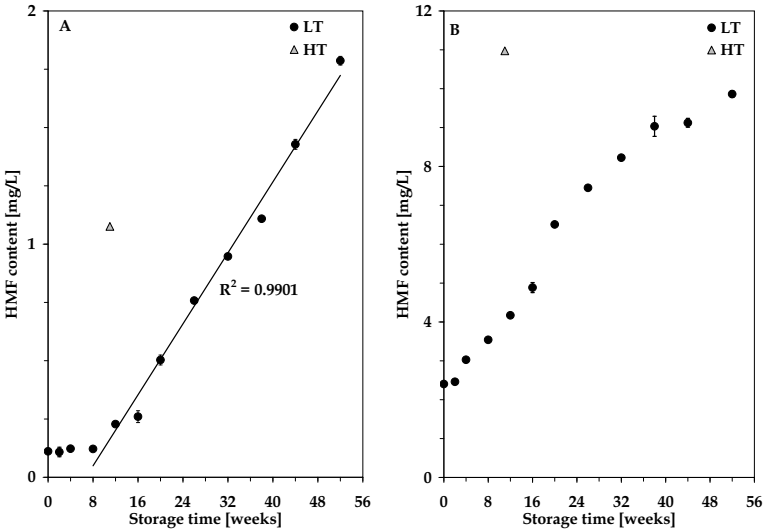


Figure 5.8.: HMF in NCA juice (A) and in FA juice (B): Influence of storage time and temperature during one year of storage.

5.1.5. Antioxidative Capacity

Two different methods were used to measure the antioxidative capacity of the juices of the first storage study. This was due to the fact that the VARP assay has just been developed during the first storage study. Furthermore, it was of interest to compare the results of the two assays.

5.1.5.1. TOSC Assay

Due to the fact that the TOSC assay provided only one value for each time point and dilution a Student's t-test could not be performed. However, regression analysis was carried out for LT samples. Furthermore, HT data were evaluated without prove of statistical significance.

Figure 5.9 shows the antioxidative capacity of NCA juice quantified via TOSC assay. The 1:100 diluted juice obtained a TOSC value which corre-

sponded to about 60 % of the TOSC value of the 1:50 dilution. This finding indicated a non-linear relationship between dilution and TOSC value which was in accordance with already published observations [43].

The TOSC values of the more diluted samples varied highly around the initial value. There was no linear correlation with time during one year of storage. It was concluded that the samples were too diluted to detect minor changes in case they occurred during the storage.

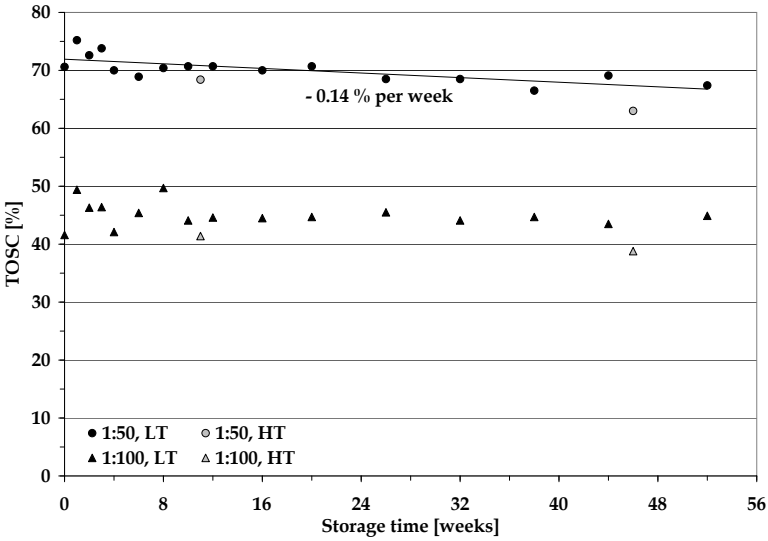


Figure 5.9.: TOSC data of NCA juice: Influence of storage time and temperature on the antioxidative capacity (TOSC against peroxyl radical) during one year of storage.

The TOSC values of the 1:50 diluted NCA juice indicated a slight decrease with time but failed to fulfil the criteria set for linear correlation (i.e. $R^2 = 0.54 < 0.8$, $p < 0.01$). However, for comparison with VARP data the slope and intercept of the regression line was calculated. The juice lost on average 0.14 % of its initial antioxidative capacity per week.

There were very slight differences between the short-term HT sample and its corresponding LT samples. The distance between the long-term HT sam-

ple and the corresponding LT sample increased but a temperature dependent effect remained unclear.

As figure C.6 in Appendix shows for the FA juice no apparent change of TOSC values occurred during storage and no temperature dependency was indicated in view of the two HT samples. In comparison to the NCA juice both dilutions of the FA juice provided similar TOSC values as the twice as much diluted NCA juice. Thus, the antioxidative capacity of NCA juice (determined by TOSC assay) was twice as high as for the FA juice.

5.1.5.2. VARP Assay

In comparison to the TOSC results of the apple juices their VARP data showed similar characteristics (see figure 5.10).

In accordance with the TOSC results the VARP data of the NCA juice indicated a slight decrease with time but failed to fulfil the criteria set for linear correlation (i.e. $R^2 = 0.57 < 0.8$, $p < 0.01$). However, regression equation was calculated and showed a higher average loss of about 0.24 % of its initial antioxidative capacity per week (compare to TOSC figure 5.9). Thus, the VARP assay reacted more sensitively to changes in the antioxidant composition of the NCA juice.

The VARP data of the FA juice did not correlate with time but ranged closely to its mean value within the complete storage period. Compared to each other the NCA juice had a 2.75-fold antioxidative capacity than the FA juice when determined by VARP assay. This provided another indication to the higher sensitivity of the VARP assay as the differences between the juices were smaller when analysed via TOSC assay (2-fold vs. 2.75-fold antioxidative capacity).

5.1.6. Sensory Deterioration

5.1.6.1. Changes in Juice Color

As shown in figure 5.11 the color evaluations of the two apple juices stored at LT exhibited similar trends, but the NCA juice revealed a faster deterioration. However, the sensory panel evaluated both juices as “acceptable” at the end of the storage time after 42 weeks. In detail, the FA juice received the grade “noticeable color change” while the alteration of the NCA juice was rated as “distinct”. The applied sensory scheme including the demands on quality

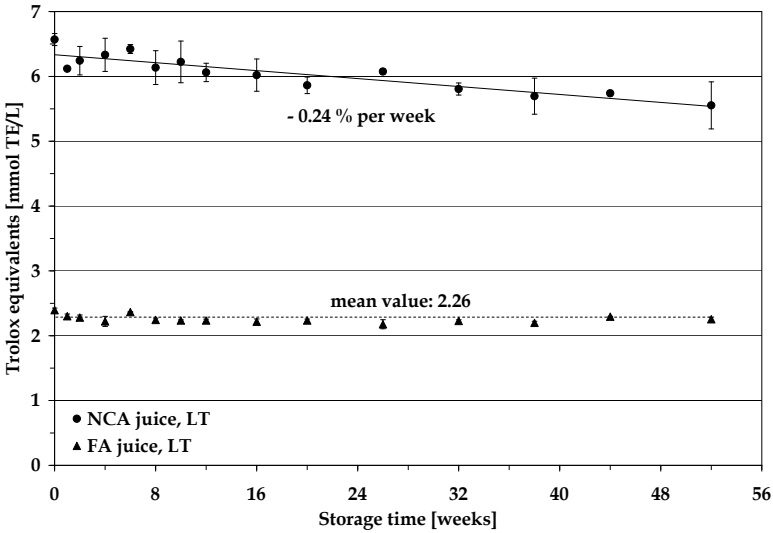


Figure 5.10.: VARP data of NCA and FA juice: Influence of storage time on the antioxidative capacity (measured as trolox equivalents) during one year of storage.

according to DIN 10952 Part 2 is presented in section 3.6.

Color evaluation of the NCA juice barely missed the criteria set for linear correlation with time ($R^2 = 0.73$, $p < 0.01$ for LT storage and $R^2 = 0.72$, $p < 0.01$ for HT storage). However, the FA juice color was correlated linearly with time at each storage temperature ($R^2 = 0.87$, $p < 0.01$ for LT storage and $R^2 = 0.89$, $p < 0.01$ for HT storage).

The color rating of the apple juices stored at HT proceeded almost in parallel course (see figure 5.12). However, the NCA juice color was judged 0.5 to 1.0 point higher than the FA juice color. For the NCA juice this resulted in a distinct color change already after eleven weeks of storage. Unacceptable color alterations were determined for both NCA and FA juice at week 42.

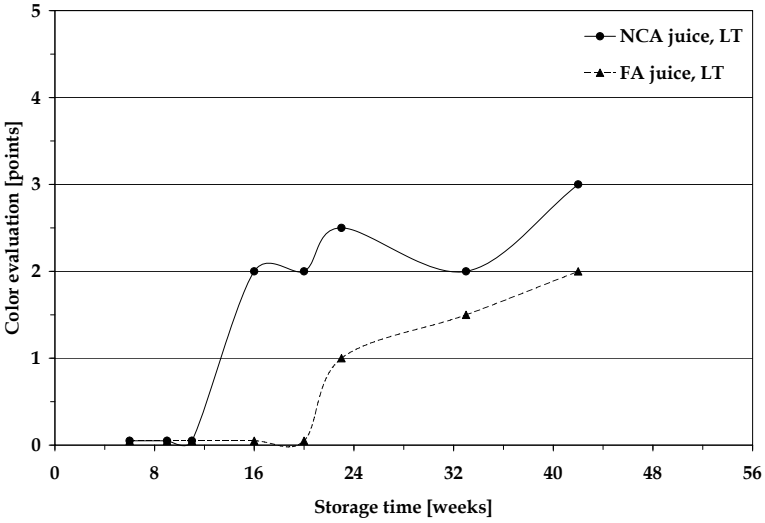


Figure 5.11.: Color evaluation of NCA and FA juice: Influence of storage time during 42 weeks of LT storage.

5.1.6.2. Changes in Taste

The sensory panel evaluated the taste of the apple juices stored at LT as illustrated in figure 5.13. Since week 16 the NCA juice was constantly rated one point higher than the FA juice. In detail the NCA juice taste alteration was estimated as "distinct" while the FA juice received the grade "noticeable taste change". However, both fulfilled the demands on quality after 42 weeks of LT storage.

Taste evaluation of the NCA juice stored at LT correlated linearly with time ($R^2 = 0.87$, $p < 0.01$) while for HT storage no significant linear correlation with time was observed.

The taste of the FA juice stored at LT showed no significant linear correlation with time. For HT storage taste evaluation barely missed the criteria set

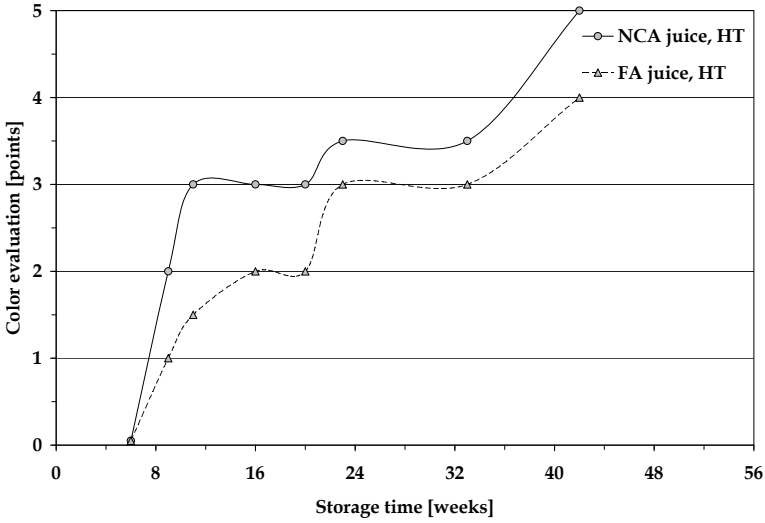


Figure 5.12.: Color evaluation of NCA and FA juice: Influence of storage time during 42 weeks of HT storage.

for linear correlation with time ($R^2 = 0.75$, $p < 0.01$).

The HT storage yielded similarly rising trends for both apple juices (see figure 5.14 on page 62) but they are rated consequently higher than the juices stored at LT apart from the first test at week six. The course of the taste evaluation of the NCA juice showed an abnormally high value at week nine. However, except for the last evaluation after 42 weeks the taste of the HT stored NCA juice was rated below grade four and thus, met the requirements of quality. In opposite, the FA juice fulfilled these demands even at week 42 when its taste alteration was evaluated as “distinct” but “acceptable”.

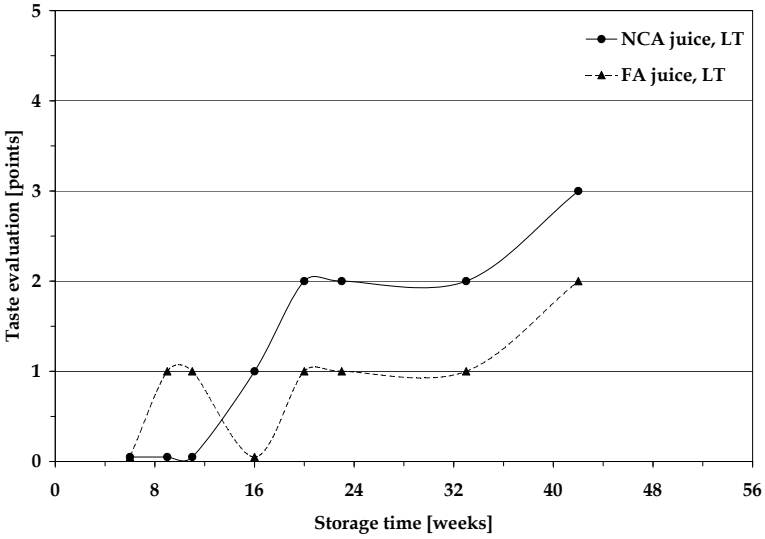


Figure 5.13.: Taste evaluation of NCA and FA juice: Influence of storage time during 42 weeks of LT storage.

5.2. Storage of Red Grape Juice

5.2.1. Oxygen and Headspace Analysis

Due to delivery time for the RG juice packages the laboratory that conducted the oxygen analysis could not run the first analysis until one week after filling. Furthermore, headspace volume and its oxygen concentration of the RG juice were only analysed during week one to six because at week six no oxygen was found in headspace at both storage temperatures (see figure 5.15). Determination of the oxygen concentration of the RG juice was also stopped after week six.

The headspace volume decreased slightly during the first six weeks of LT and HT storage and was in the same range for both storage temperatures. However, it did not correlate linearly with time, neither at LT nor HT storage.

The overall mean value of this parameter (combined LT and HT samples) was compared to the mean values of the apple juices (data taken from week

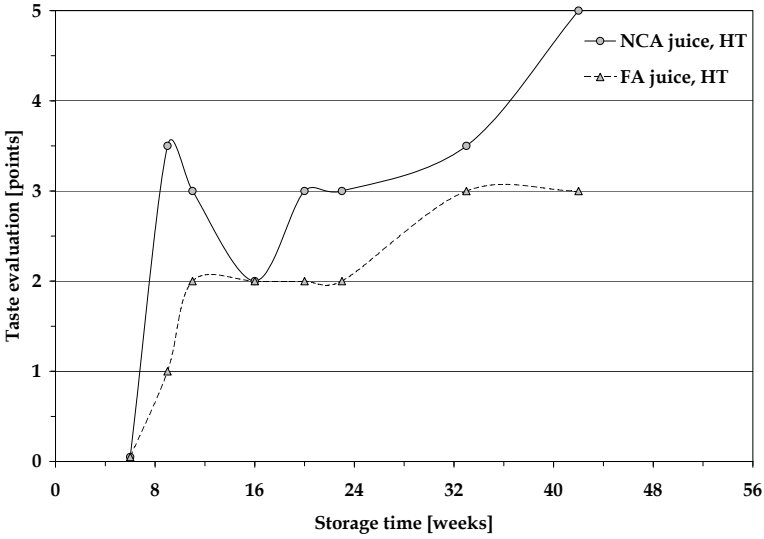


Figure 5.14.: Taste evaluation of NCA and FA juice: Influence of storage time during 42 weeks of HT storage.

one to six, refer to figure 5.1). Due to the smaller packaging volume (1.0 L for RG juice instead of 1.5 L for the apple juices) the relative headspace volume was calculated. It was the same for both apple juices (16 mL/L juice) and slightly lower for the RG juice (14 mL/L juice).

As figure 5.16 indicates the oxygen content of the RG juice leveled off at 0.2 ppm after three weeks of LT and HT storage, respectively. Thus, it was in the same range as for both apple juices (compare to figure 5.2).

5.2.2. Anthocyanins

The anthocyanin pattern of the RG juice is presented in table 5.3. At the time when the analysis was executed the only present standard compound was cyanidin-3-glucoside. Therefore, the anthocyanins were quantified as cyanidin-

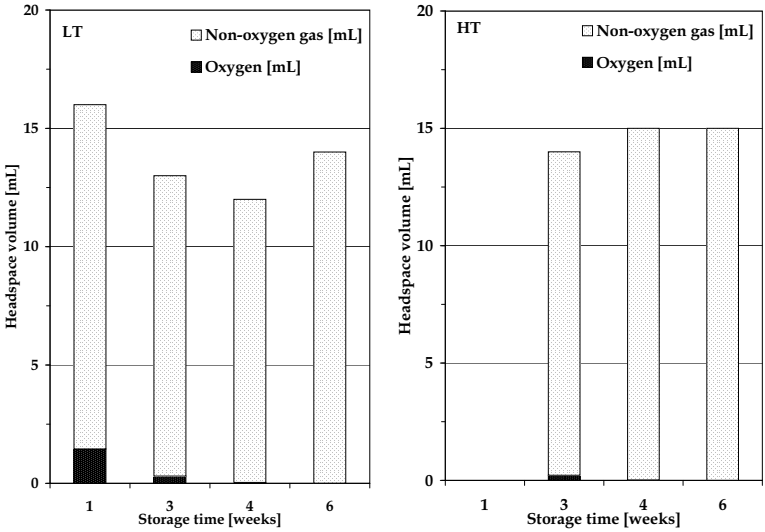


Figure 5.15.: Headspace atmosphere of RG juice packaging: Influence of storage time and temperature.

3-glucoside equivalents. Two anthocyanins were identified by LC-MS but not quantified as they were not separated clearly by HPLC-UV-Vis.

As shown in figure 5.17 and 5.18 the anthocyanins were subject to degradation. It is known from literature that the anthocyanins polymerize forming more stable pigments which still color the juice even though no more monomers are detectable. [12, 77, 76]

All anthocyanins diminished continuously during the period of storage. Whereas the main anthocyanins malvidin-3-glucoside and peonidin-3-glucoside obtained high degradation rates, while the lower concentrated anthocyanins decreased only slightly. At week 32 traces of malvidin-3-glucoside and cyanidin-3-glucoside were detected but they were below LOQ. The cyanidin-3-glucoside data at week 20 (see figure 5.18) was interpreted as an outlier as it was unlikely that it increased up to the three-fold value of week 16. When this outlier was excluded from statistical analysis, the degradation of all anthocyanins correlated linearly with time ($R^2 > 0.8$, $p < 0.01$).

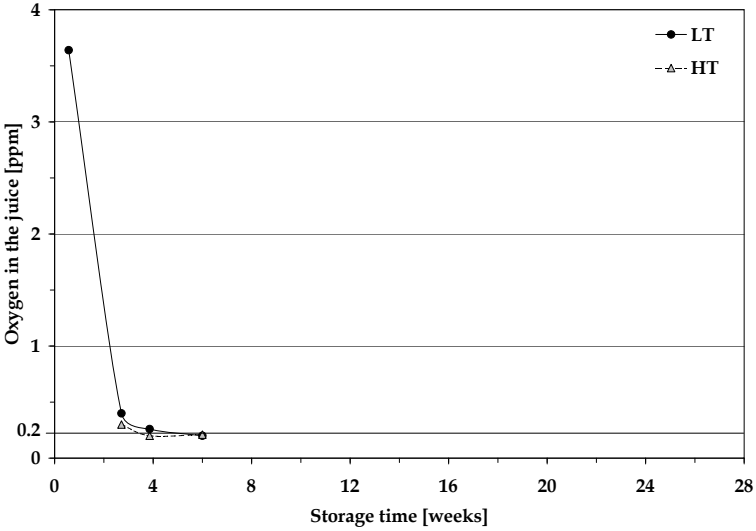


Figure 5.16.: Oxygen content in RG juice: Influence of storage time and temperature.

Anthocyanin	Proportion
Malvidin-3-glucoside	major
Peonidin-3-glucoside	major
Cyanidin-3-glucoside	minor
Petunidin-3-glucoside	minor
Delphinidin-3-glucoside	minor
Malvidin-3-(6”-acetoyl)-glucoside	traces, not quantified
Malvidin-3-(6”-coumaroyl)-glucoside	traces, not quantified

Table 5.3.: Anthocyanin pattern of the RG juice.

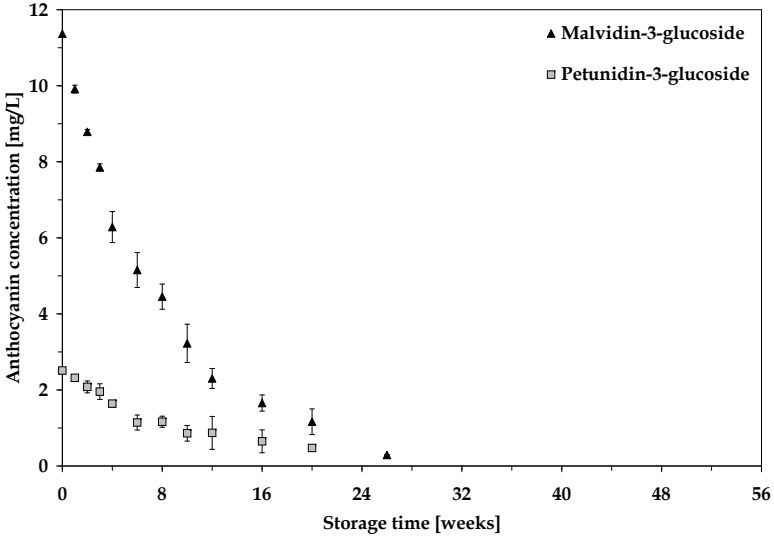


Figure 5.17.: Anthocyanins in RG juice, part I: Influence of storage time during one year of storage [quantified as cyanidin-3-glucoside].

To point out the temperature dependency of the degradation of the anthocyanins table 5.4 shows a comparison between the measured data of the short-term HT sample and the calculated data of the juice stored at LT (mean value of the samples at week ten and twelve). The higher HT/LT ratio shows that the cyanidin-3-glucoside was significantly more stable at HT storage than both of the main anthocyanins. This finding was consistent with the faster degradation of malvidin-3-glucoside and peonidin-3-glucoside when stored at LT.

5.2.3. Further Polyphenols

The main colorless polyphenol in RG juice was gallic acid. As figure 5.19 shows no degradation occurred during one year of LT storage. The parameter did not correlate with time and there was no significant difference between any data point and the initial value. However, the differences between the two HT

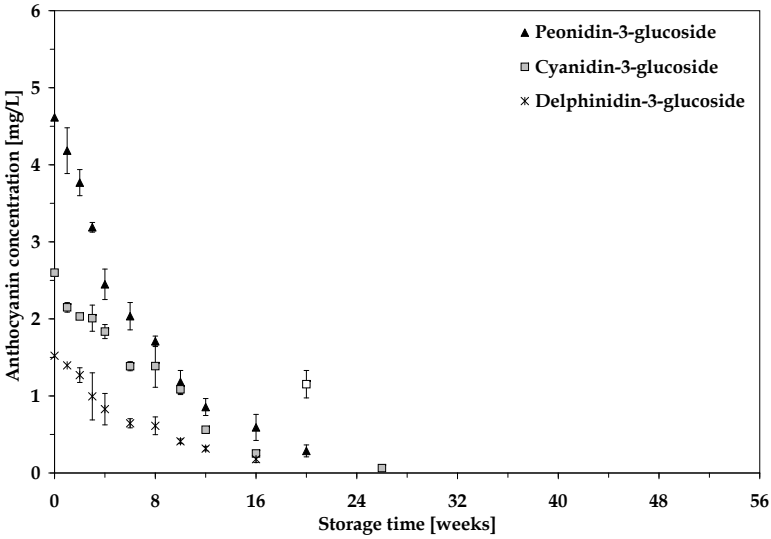


Figure 5.18.: Anthocyanins in RG juice, part II: Influence of storage time during one year of storage [quantified as cyanidin-3-glucoside].

	After eleven weeks of storage		
	Calculated data [AU]	Measured data [AU]	Ratio
Storage temperature	LT	HT	HT/LT
Malvidin-3-glucoside	8.5	2.6	0.31
Peonidin-3-glucoside	3.2	0.7	0.22
Cyanidin-3-glucoside	2.8	1.7	0.61

Table 5.4.: Influence of storage temperature on anthocyanins content (measured as UV peak area) after eleven weeks of storage

samples and their LT correspondents (after 8, 12 and 44 weeks, respectively) obtained high significance ($p < 0.01$) and indicated a temperature-dependent storage alteration.

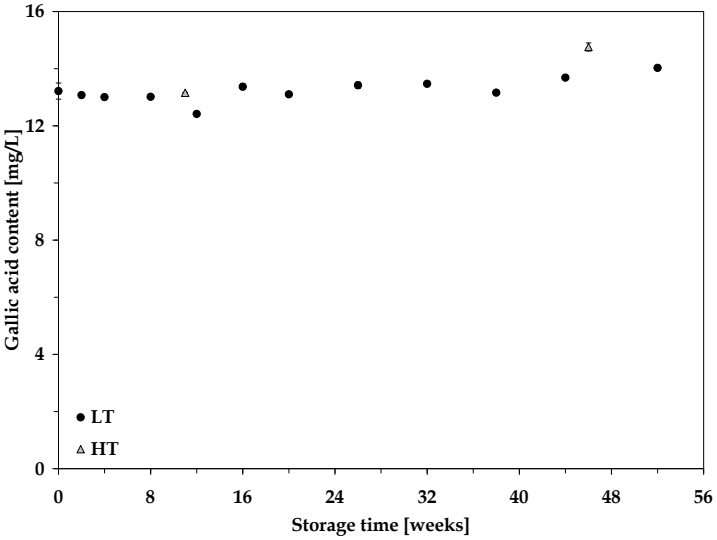


Figure 5.19.: Gallic acid in RG juice: Influence of storage time and temperature during one year of storage.

Figure 5.20 presents the changes of the flavan-3-ols concentrations in the RG juice. A similar trend was observed for catechin and epicatechin, respectively. The degradation correlated linear with time ($R^2 = 0.865$, $p < 0.01$ for catechin and $R^2 = 0.812$, $p < 0.01$ for epicatechin).

Furthermore, the HT samples showed a highly significant temperature-dependent degradation of the flavan-3-ols ($p < 0.01$). Though the corresponding LT samples contained twice as much catechin than epicatechin, their concentrations aligned in both of the HT samples.

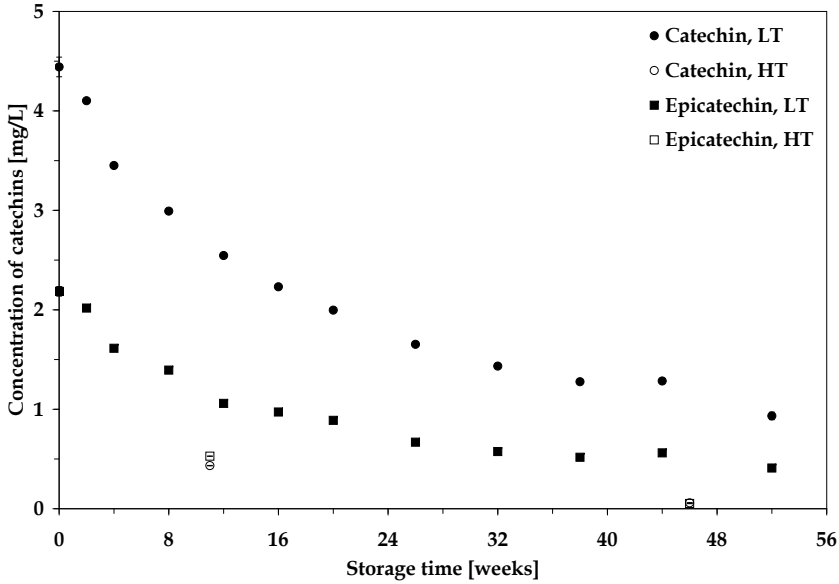


Figure 5.20.: Flavan-3-ols in RG juice: Influence of storage time and temperature during one year of storage.

5.2.4. Antioxidative Capacity

5.2.4.1. TOSC Assay

The antioxidative capacity of RG juice quantified by the TOSC assay is shown in figure 5.21. The TOSC data of the RG juice were in the same range as the NCA juice (refer to figure 5.9) at identical dilutions. The 1:100 diluted juice obtained a TOSC value which corresponded to about 70 % of the TOSC value of the 1:50 dilution. This result was in accordance with the non-linear relationship between dilution and TOSC value found for the apple juices (refer to figure 5.1.5.1).

The TOSC values of both dilutions indicated a decrease with time but failed to fulfil the criteria for linear correlation (i.e. $R^2 = 0.72$, $p < 0.01$ for 1:50 dilution and $R^2 = 0.59$, $p < 0.01$ for 1:100 dilution, respectively). However, for comparison with VARP data regression analysis was performed. The RG juice lost on average 0.14 % and 0.29 %, respectively, of its initial antioxidative capacity per week. Thus, the 1:100 diluted samples yielded a degradation rate twice as high as the one of the 1:50 dilution.

The HT samples showed slightly lower TOSC values especially at the 1:50 dilution, which provided an indication of the temperature dependency of the degradation.

5.2.4.2. VARP Assay

Figure 5.22 shows the antioxidative capacity of the RG juice determined via VARP assay. The data indicated a slight decrease with time but failed to fulfil the criteria for linear correlation (i.e. $R^2 = 0.33$, $p < 0.01$). However, regression equation showed that the RG juice lost about 0.15 % of its initial antioxidative capacity per week.

In accordance with the TOSC results of the HT samples also the VARP assay indicated a noticeable temperature-dependency of this parameter.

In comparison to the TOSC results of the RG juice similar results were obtained using the VARP assay. The VARP degradation rate was in the same range as the TOSC rate of the 1:50 dilution but about half of the TOSC rate of the 1:100 dilution.

Thus, the VARP assay reacted less sensitively than the TOSC assay to changes in the antioxidant composition of the RG juice. Furthermore, the RG juice exhibited a distinctly lower VARP value than the NCA juice whereas their TOSC values were in the same range. This was considered to be due to the different reaction mechanisms that proceeded in both of the assays.

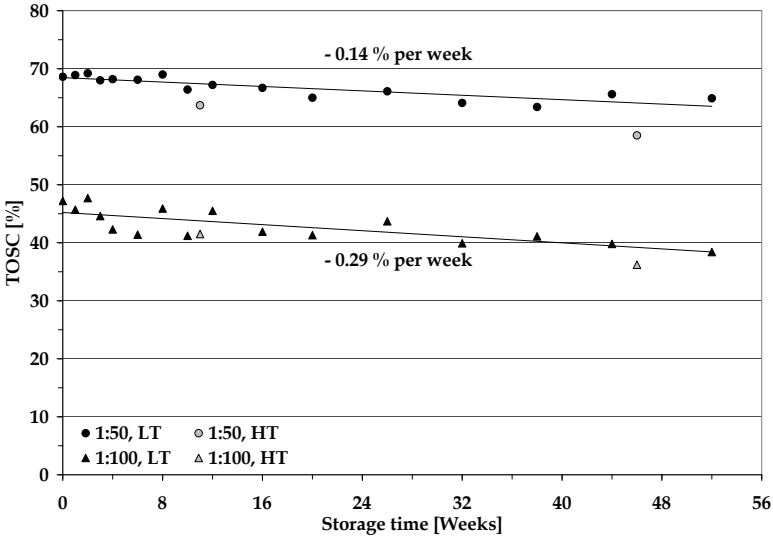


Figure 5.21.: TOSC value of RG juice: Influence of storage time and temperature on the antioxidative capacity (TOSC against peroxyl radical) during one year of storage.

5.2.5. Sensory Deterioration

5.2.5.1. Changes in Juice Color

As illustrated in figure 5.23 the color evaluation of the RG juice stored at LT and HT exhibited similar trends, but the HT samples revealed faster deterioration. The juice stored at LT showed slow but continuous deterioration up to a "noticeable" but "acceptable color change" at week 37 and 45. Its evaluation correlated linearly with time ($R^2 = 0.88$, $p < 0.01$).

From week 15 to 27 the color evaluation of the HT samples was inconsistent. However, it was obvious that the storage temperature influenced the alterations though they showed no significant linear correlation with time. After 24 weeks the juice failed to fulfil the demands on quality. The applied sensory scheme was explained in section 3.6.

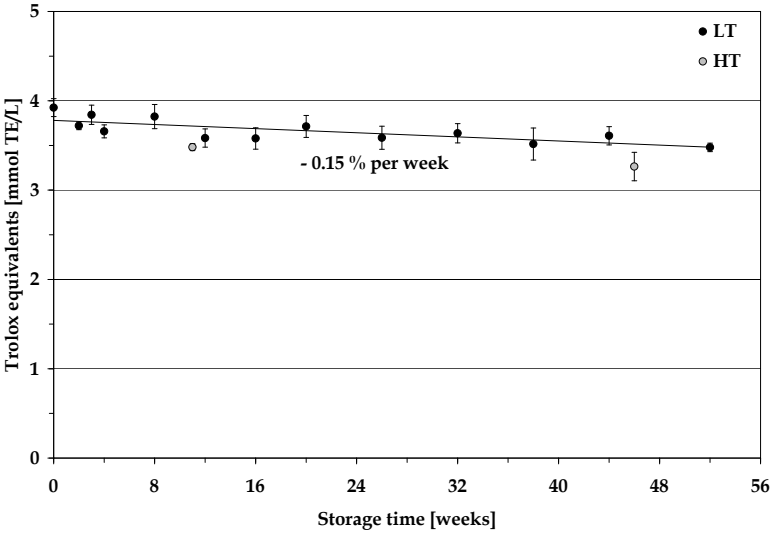


Figure 5.22.: VARP value of RG juice: Influence of storage time and temperature on the antioxidative capacity (measured as trolox equivalents) during one year of storage.

5.2.5.2. Changes in Taste

Figure 5.24 illustrates the taste changes of the RG juice during LT and HT storage. At the end of the test series the sensory panel rated the taste changes stored at LT as "noticeable but acceptable". The taste evaluation correlated linearly with time ($R^2 = 0.88$, $p < 0.01$).

Comparing the LT and HT samples the juice stored at HT yielded a stronger taste deterioration and thus, reached the limit of acceptance at week 24. However, taste changes showed no significant linear correlation with time.

5.3. Conclusions of the First Storage Study

The results of the first storage study showed that the sensory quality of the juices fulfilled the requirements over an LT storage period of one year. Though

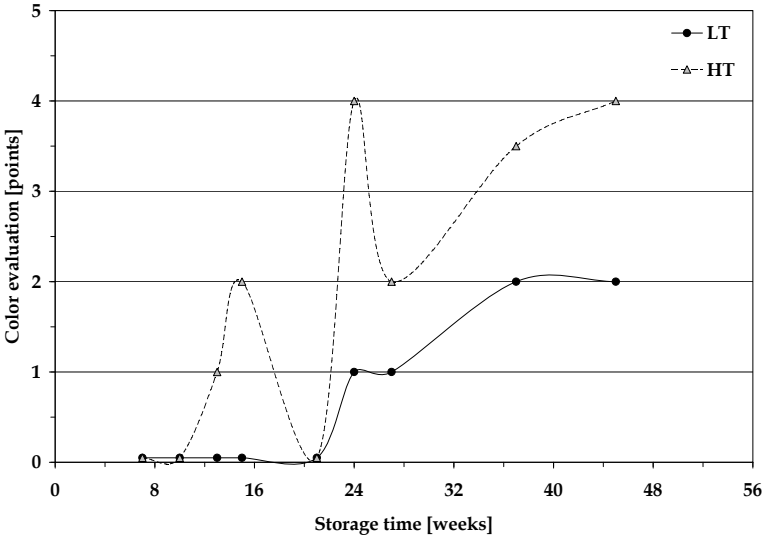


Figure 5.23.: Color evaluation of RG juice: Influence of storage time and temperature during one year of storage.

there were several compounds that altered within this time, only small effects on the antioxidative capacity were monitored. This was considered to be due to the fact that this parameter depended on numerous juice compounds.

For the NCA juice antioxidants like flavan-3-ols and ascorbic acid diminished during storage but maillard reaction formed HMF which possessed also antioxidative characteristics [8]. Furthermore, the anthocyanins of the RG juice diminished due to polymerization to pigments which still reacted as antioxidants. [43, 13, 23, 29]

The changes of the juice characteristics were too small to determine whether there was a correlation between the juice quality and the behaviour of its antioxidants. Thus, two possibilities for further research were conceivable:

1. This storage study could have been prolongated until the storage time showed more obvious effects.

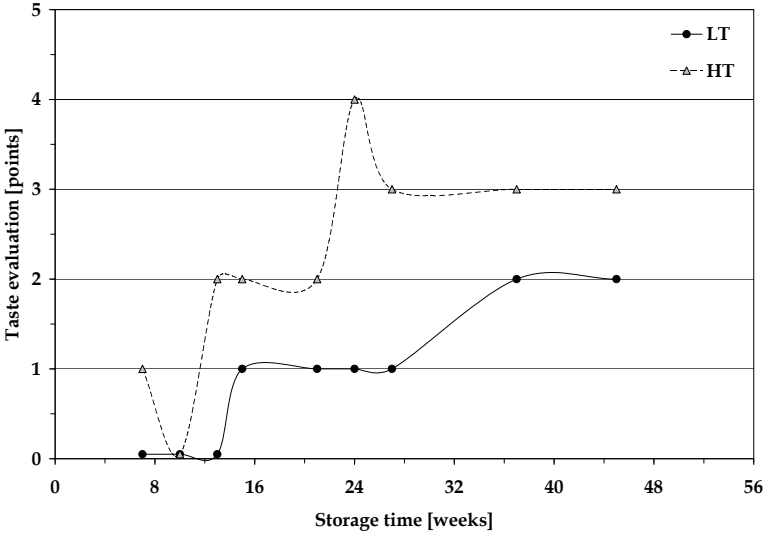


Figure 5.24.: Taste evaluation of RG juice: Influence of storage time and temperature during one year of storage.

2. A new storage study under charged conditions could force the alterations to appear faster.

The first possibility did not fit in the commercial shelf-life period of fruit juices filled in laminated carton packages. Therefore, a second storage study was designed which considered the higher reaction rate of the temperature-dependent compounds and furthermore, took into account the oxygen permeability of the packaging.

6. The Second Storage Study

Influences of Storage Time, Temperature and Oxygen Permeability of the Packaging

To observe the influences of time, temperature and oxygen on the juice quality during storage three commercial juices (refer to section 2.1.2 on page 16) were monitored over a storage period of one year.

In addition to the usual aluminium laminated composite packaging (ALC) as illustrated in figure 1.1 on page 4, the juice was also filled in aluminium-free polyamide composite packages (PAC) that permitted a higher oxygen permeability. As a consequence of the results of the preceding storage study more HT samples were analysed for antioxidative capacity and specific compounds. Thus, it should be determined whether changes were influenced by the storage temperature.

The aim of this study design was to force the juices to deteriorate faster so that possible correlations between antioxidative compounds and sensory quality of the juice could be determined. Actually, the reaction rates of the compounds might not only increase by the presence of more oxygen and at a higher storage temperature, but also other kind of reactions could appear. Therefore, the study was additionally conducted with the commercial juices filled in usual ALC packages and stored at room temperature.

The following parameters were monitored similarly to the first study: antioxidative capacity, its influencing antioxidants like ascorbic acid and polyphenols, HMF formation, the headspace volume, its oxygen content, the oxygen concentration in the juice and the sensory evaluation of color and taste. Furthermore, the weight of the filled package was surveyed and the color of the filtered juices (FA and RG juice) was determined via CIE Lab system.

6.1. Oxygen and Water Vapour Permeation

The oxygen permeability of the packages was observed in a separate test. The permeability range of five samples was determined each for ALC and PAC packaging. RG juice and both apple juices were filled at two different companies and thus, not using identical filling parameters. Therefore, oxygen permeation was determined for RG juice and apple juice package (see table 6.1). While ALC packaging showed slight differences between RG and apple juices the PAC packaging obtained an at least 20-fold higher value for all juices. Furthermore, data were calculated for 1.0 L packages in ppm/month (i.e. $mg\,O_2/L\,juice$ per month). The results were consistent with the data published by Ostermann and Lorenz (1988). They showed that within one year the oxygen permeability of a 1.0 L aluminium-laminated packaging ac-

counted for 2.2 to 7.5 mL (i.e. 0.18 to 0.63 $mL\,O_2/month$). [52]

Packaging	Oxygen permeability	
	$[mL\,O_2/month]$	$[ppm/month]$
ALC, apple juices	0.25 - 0.65	max. 0.9
ALC, RG juice	0.6 - 0.75	max. 1.0
PAC	> 16	> 21

Table 6.1.: Oxygen permeability of the packaging.

Furthermore, PAC packaging was influenced by evaporation. Therefore, the package weight was observed. Figure 6.1 shows the weight reduction for the FA juice during storage at different temperatures and in different packages. NCA and RG juice yielded similar results (data not shown).

ALC was not permeable for water vapour and thus, neither at HT nor at LT storage a significant weight loss was detected (LT storage data not shown). In contrast, the PAC package lost weight during storage and the weight correlated linearly with time at each storage temperature ($R^2 = 0.93$, $p < 0.01$ for LT storage, $R^2 = 0.99$, $p < 0.01$ for HT storage). Thus, from week seven the difference in evaporation was highly significant between both packages ($p < 0.01$).

Furthermore, for the PAC package the level of evaporation depended highly significantly on the storage temperature ($p < 0.01$ from week seven). During one year of LT storage each the packages of the three juices lost between 1.2 and 2.1 % of the initial weight, at HT storage the loss raised up to 4.6, 5.1 and 6.0 %, respectively. Thus, a minor enrichment of juice compounds resulted within the storage period, but it was not considered in the following calculations.

6.2. Storage of Naturally Cloudy Apple Juice

6.2.1. Oxygen and Headspace Analysis

In opposite to the first storage study all juices were filled in 1.0 L packages. Therefore, headspace volume of the NCA juice was lower than during the first storage study (about 11 mL at the beginning of the storage period, see figure 6.2).

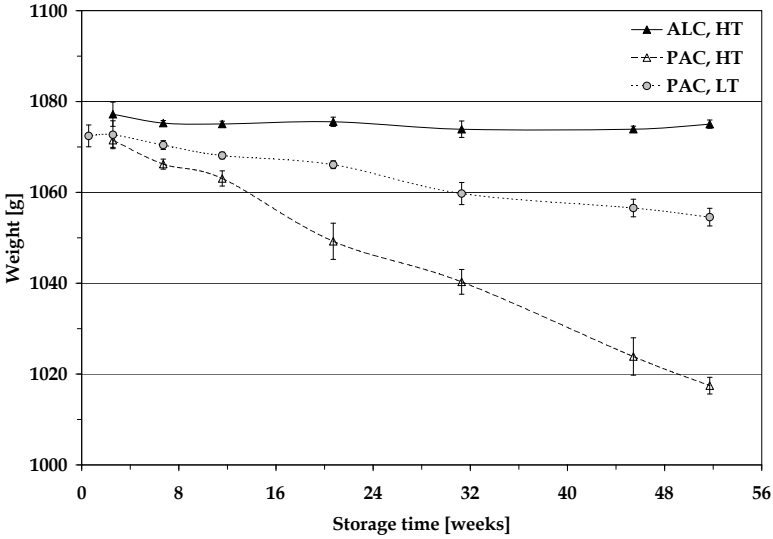


Figure 6.1.: Weight of filled packages of filtered apple juice: Influence of packaging, storage time and temperature during one year of storage.

Prior to filling both apple juices were degassed. Thus, the air from the headspace start solving in the juice directly after filling. Hence, at LT storage the volume decreased fast and after seven weeks there was no more headspace in the ALC package. The data point at week 45 was interpreted as an outlier.

The headspace diminished also in the PAC package, but the data varied highly during the first twelve weeks. However, the volume reached the base line at week 31.

Headspace volume of the HT stored ALC package decreased down to a level between 3 and 4 mL. At two time points (week seven and twelve) even 0 mL were measured. It was questionable whether these data could be interpreted as outliers or how the volume could have increased afterwards. A possible explanation for residual headspace volume was that due to the higher storage temperature less gas was dissolved in the juice and thus, remained in headspace. At week seven and twelve the test was potentially conducted after

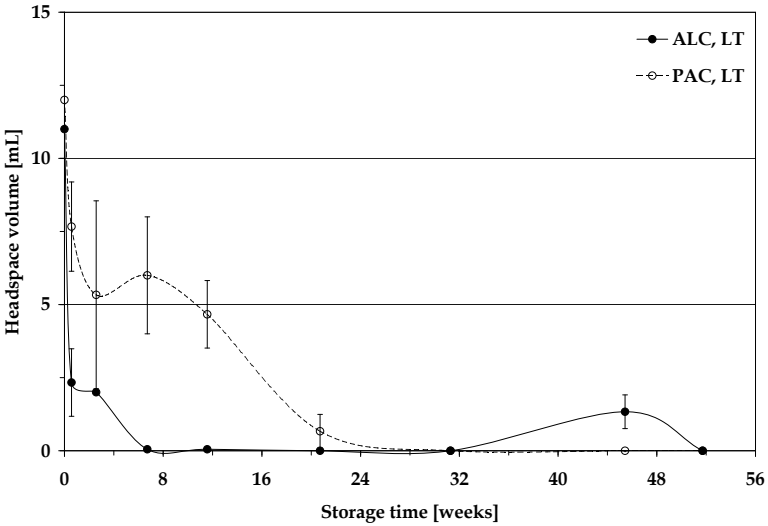


Figure 6.2.: Headspace volume in packages of NCA juice: Influence of storage time during LT storage.

the juice had cooled down and thus, the gas was already dissolved in the juice.

In contrast, headspace volume of the HT stored PAC package showed a similar decrease to 0 mL as for the LT storage (see figure 6.3). Unlike the ALC packaging, the higher gas permeability of the PAC package could have caused this different trend.

Irrespective of its volume, packaging and storage temperature the headspace atmosphere of the NCA juice only contained about 17 % of oxygen directly after the filling. In the packages with residual headspace only traces of oxygen (below 1 %) were detected after the first week (data not shown).

In opposite to the oxygen rate in the headspace its concentration in the juice varied with packaging and storage temperature. Figure 6.4 shows the oxygen content of the NCA juice during one year of storage. The LT data of the ALC

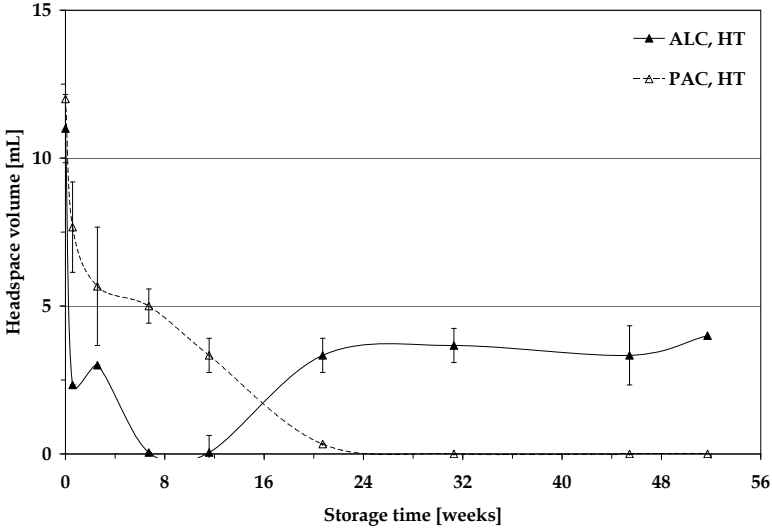


Figure 6.3.: Headspace volume in packages of NCA juice: Influence of storage time during HT storage.

packaging were not presented as they revealed no significant difference to the corresponding HT data.

Under all storage conditions observed the oxygen concentration diminished from 1.6 ppm down to 0.3 ppm within three weeks of storage. Thereafter, a slight reduction appeared in the ALC package until nearly no oxygen was left in the juice. This status remained until the end of the storage period at both temperatures.

Until week 31 the oxygen concentration of the NCA juice in the PAC packaging was not significantly effected by the storage temperature. Furthermore, the oxygen permeation through the PAC material was in the same range as the oxygen consumption by oxidative reactions in the juice. Thus, the oxygen content remained stable until week 31.

Subsequently, the LT data increased up to 3 ppm, while HT storage led to a slight rise of the oxygen content (significant at week 52, $p < 0.05$). It was

likely that after week 31 less oxidative reactions occurred in the juice stored at LT due to the lower storage temperature. Thus, the gas that permeated into the juice did not react.

In contrast, the higher storage temperature caused further oxidative processes and the diffusing oxygen reacted immediately. Thus, the oxygen concentration remained on a steady level at HT storage.

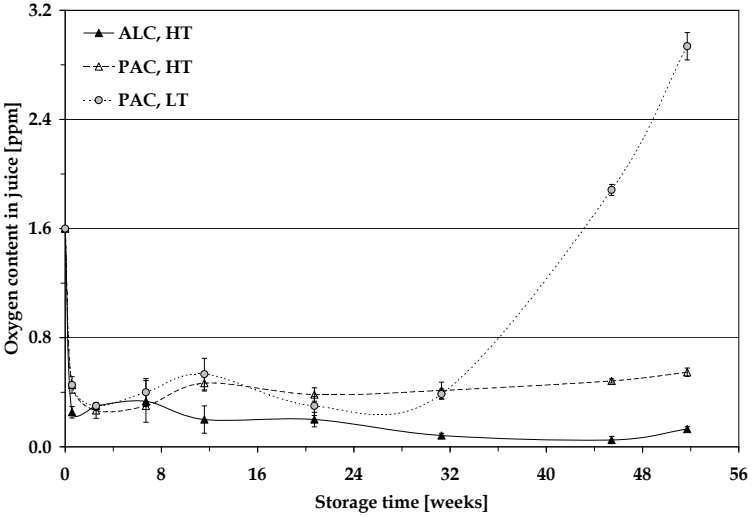


Figure 6.4.: Oxygen in NCA juice: Influence of packaging, storage time and temperature during one year of storage.

6.2.2. Polyphenolic Compounds

Figure 6.5 shows a representative part of a chromatogram of NCA juice sampled at the beginning of the storage period. The UV signal at 280 nm is presented in the upper part of the figure, the lower part shows CEAD channel 2 at 100 mV. While HMF provided only a UV signal, the other known peaks were detected by UV-DAD and CEAD. Phloridzin was oxidised at higher potentials (ch. 4 to 8, ≥ 300 mV) and thus, no peak was observed in ch. 1 to 3.

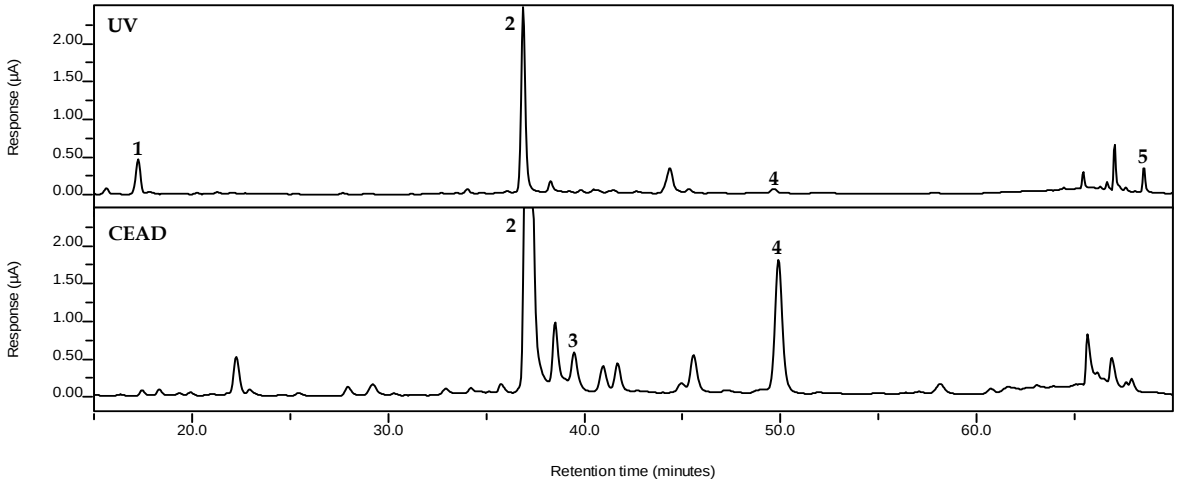


Figure 6.5.: A representative chromatogram of NCA juice sampled at the beginning of the storage period. Upper part: UV signal at 280 nm, lower part: CEAD channel 2 at 100 mV; Peak identification: HMF (1), chlorogenic acid (2), catechin (3), epicatechin (4), phloridzin (5).

In accordance with the first storage study the main polyphenol of the apple juices was chlorogenic acid. The NCA juice of the second study contained twice as much chlorogenic acid as the former apple juices. Figure 6.6 illustrates its trend in both packages during one year of LT and HT storage, respectively. The data of the ALC packaging stored at HT were significantly lower ($p < 0.05$) than at LT storage except at week 13. For the LT and HT stored ALC packaging there was no linear correlation observed between chlorogenic acid concentration and storage time.

While the juice in the ALC package lost no significant amount of chlorogenic acid in comparison to its initial concentration, this compound declined noticeably in the PAC package. For the LT storage this parameter correlated linearly with time ($R^2 = 0.943$, $p < 0.01$). However, the HT data of the PAC package were inconsistent: there was an abnormally high value at week 13 and at week 20 the chlorogenic acid concentration was below the value at week 31.

Potentially, the HT sample of week 13 was mixed up with the corresponding one stored at LT. Furthermore, the HT samples at week 20 and 31 could have been interchanged. However, no B specimens were taken from the HT stored juices and thus, the results could not be verified. From these inconsistent data it was concluded that there were insufficient data to perform statistical analysis but only a trend was evaluated.

Also the HT samples in PAC packaging showed a distinct decrease in chlorogenic acid concentration. Degradation rate was affected by storage temperature. In comparison with the ALC packaging samples, a stronger influence of storage temperature was apparent for the PAC package.

In conclusion, the data showed that packaging and storage temperature significantly influenced the degradation of chlorogenic acid in NCA juice.

The NCA apple juice contained another typical polyphenol in a similar concentration compared to the apple juices of the first storage study: phloridzin. Figure 6.7 illustrates its changes during the period of storage in different types of packaging and at different storage temperatures.

The similarity to the alterations of the chlorogenic acid were obvious. The juice in the ALC package lost no significant amount of phloridzin during one year of LT or HT storage and the parameter did not correlate linearly with time. However, from week 11 the difference between LT and HT samples became significant ($p < 0.05$).

There were significant changes of phloridzin concentration during storage in the PAC package at both temperatures. For the LT storage this parameter

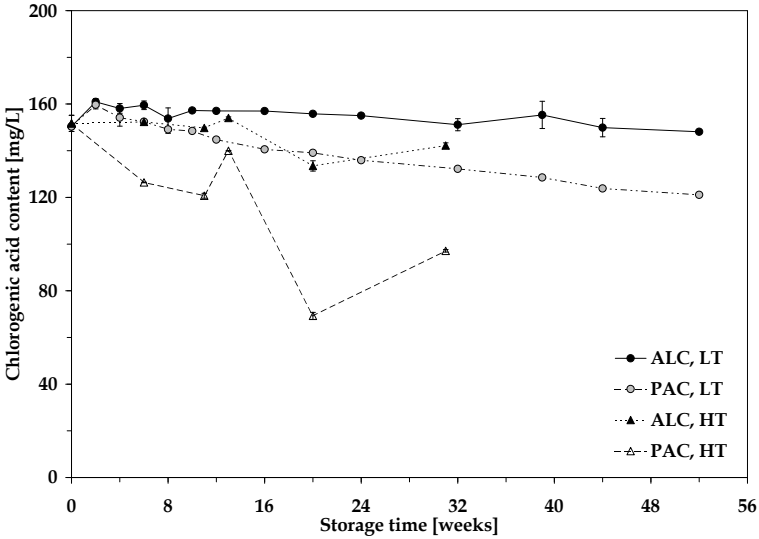


Figure 6.6.: Chlorogenic acid in NCA juice: Influence of packaging, storage time and temperature during one year of storage.

correlated linearly with time ($R^2 = 0.870$, $p < 0.01$). Due to a potential mix-up of samples in PAC packaging as described for chlorogenic acid the HT data were inconsistent.

However, the phloridzin concentration decreased distinctly for HT samples in PAC packaging. The storage temperature had a highly significant influence on the degradation rate ($p < 0.01$). In comparison with the ALC samples, the phloridzin in PAC package was more affected by storage temperature.

In conclusion, the data showed that packaging and storage temperature significantly influenced the degradation of phloridzin in NCA juice.

The flavan-3-ols of the NCA juice underwent a distinct alteration during one year of storage. Figure 6.8 illustrates the change of the catechin concentration in NCA juice. At LT storage in ALC packaging the parameter correlated linearly with time ($R^2 = 0.889$, $p < 0.01$). However, at HT storage in ALC package it barely missed the criteria set for linear correlation ($R^2 = 0.750$,

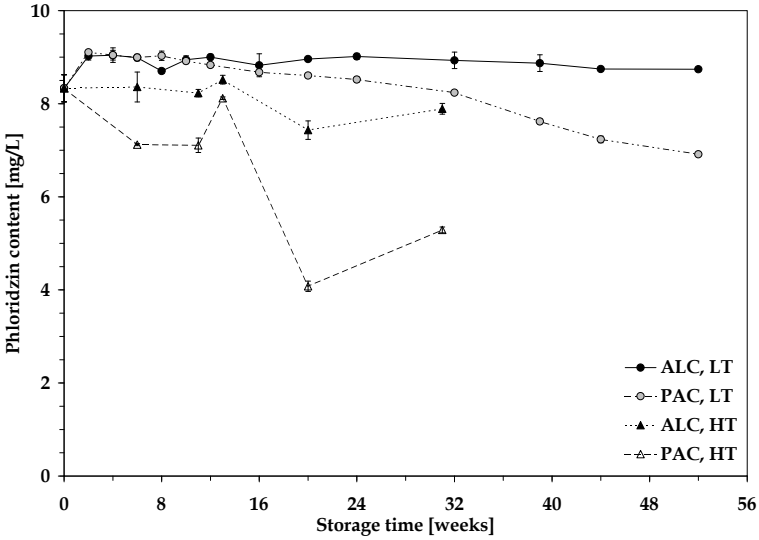


Figure 6.7.: Phloridzin in NCA juice: Influence of packaging, storage time and temperature during one year of storage.

$p < 0.01$). The difference between LT and HT storage was not continuously significant for ALC packaging.

The loss of catechin in PAC package did not correlate linearly with time neither at LT nor at HT storage (only two data points and thus, no statistical significance). In the PAC packaging the degradation rate was significantly higher than in ALC package at bot storage temperatures ($p < 0.05$).

In conclusion, the degradation of catechin in NCA juice strongly depended on the packaging as well as on the storage temperature.

As figure 6.9 illustrates, the epicatechin content in the NCA juice showed similar degradation characteristics as catechin. In ALC packaging at LT storage epicatechin content correlated linearly with time ($R^2 = 0.918$, $p < 0.01$). However, it barely missed the criteria set for linear correlation ($R^2 = 0.760$, $p < 0.01$) at HT storage in ALC package. Furthermore, storage temperature

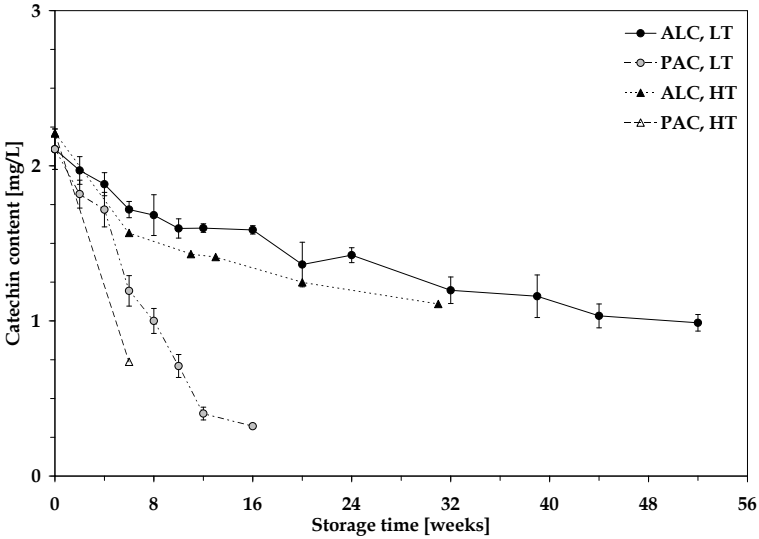


Figure 6.8.: Catechin in NCA juice: Influence of packaging, storage time and temperature during one year of storage.

had no continuously significant effect on epicatechin content for NCA juice in ALC packaging.

Due to its six times higher initial concentration epicatechin could be quantified in the PAC package until week 24 for LT storage and until week 20 for HT storage. Degradation did not correlate linearly with time. Furthermore, after eleven weeks the difference between LT and HT stored samples in PAC packaging minimised.

In conclusion, the reduction of epicatechin in NCA juice was mainly influenced by packaging and slightly by storage temperature.

At the beginning of the storage period this NCA juice contained about twice as much epicatechin and distinctly more catechin than the NCA juice of the first study (compare to section 5.1.2). However, in the first study flavan-3-ols only reduced slightly even during HT storage but the flavan-3-ols of this NCA juice were very unstable. This could be due to a different combination of the

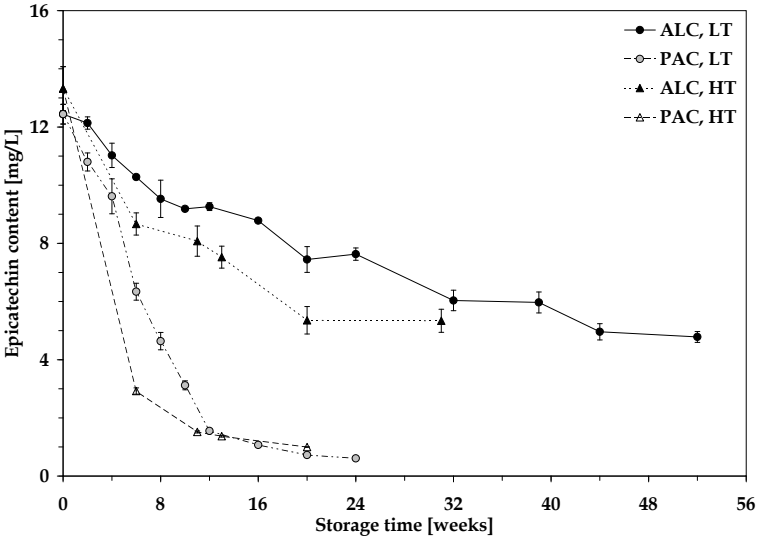


Figure 6.9.: Epicatechin in NCA juice: Influence of packaging, storage time and temperature during one year of storage.

found antioxidants. Thus, the flavan-3-ols in the NCA juice of the first study were more stable because there were other compounds with lower oxidation potentials that degraded first.

6.2.3. Ascorbic Acid

The NCA juice was the only one in the study with a noticeable ascorbic acid concentration. Since the juice was only filled for this storage study the producer did not declare the intended ascorbic acid content. Figure 6.10 shows the ascorbic acid degradation during the storage period for different packaging and storage temperatures.

Starting with a concentration of about 220 mg/L the ascorbic acid reduced considerably. At LT storage in ALC packaging the degradation did not correlate linearly with time. In the PAC package stored at LT its degradation

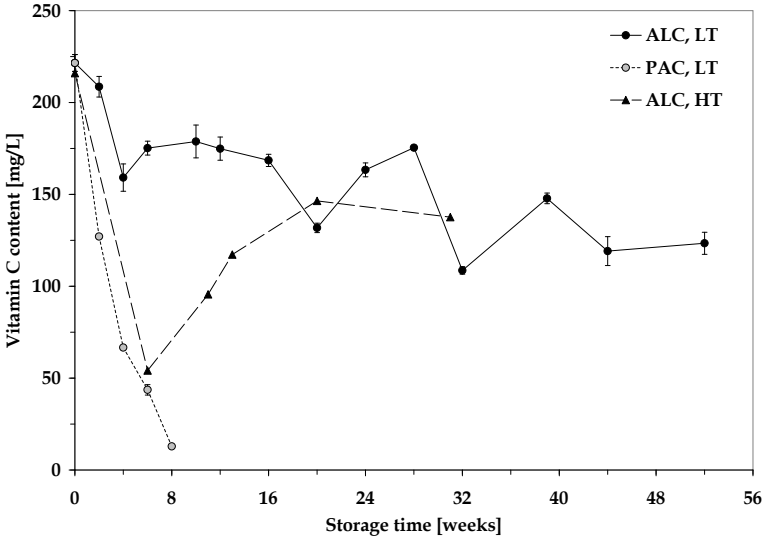


Figure 6.10.: Ascorbic acid in NCA juice: Influence of packaging, storage time and temperature during one year of storage.

correlated linearly with time ($R^2 = 0.921$, $p < 0.01$) and resulted in a total loss after ten weeks.

When the first HT samples were taken after six weeks there was no ascorbic acid found in the PAC packed juice. The data obtained for the HT storage in ALC packaging were inconsistent: it was very unlikely that after the drastic loss determined in week six the ascorbic acid amount would have increased again up to a level comparable with the corresponding LT storage data. However, only one specimen was taken at HT storage and thus, the data could not be verified.

Even smallest differences in manufacturing of the packaging could have caused a higher oxygen permeability and thus, a higher reduction rate. Potentially, this was the reason for the abnormal trend of the juice stored at HT in the ALC packages. Furthermore, it was a possible explanation for the major variation of the ascorbic acid content in the ALC packaging at LT storage when compared to the corresponding results of the first study.

6.2.4. HMF

The figure 6.11 illustrates the increment of HMF in NCA juice under different storage conditions. In ALC packaging the HMF content correlated linearly with storage time at both temperatures ($R^2 = 0.990$, $p < 0.01$ for LT storage and $R^2 = 0.927$, $p < 0.01$ for HT storage).

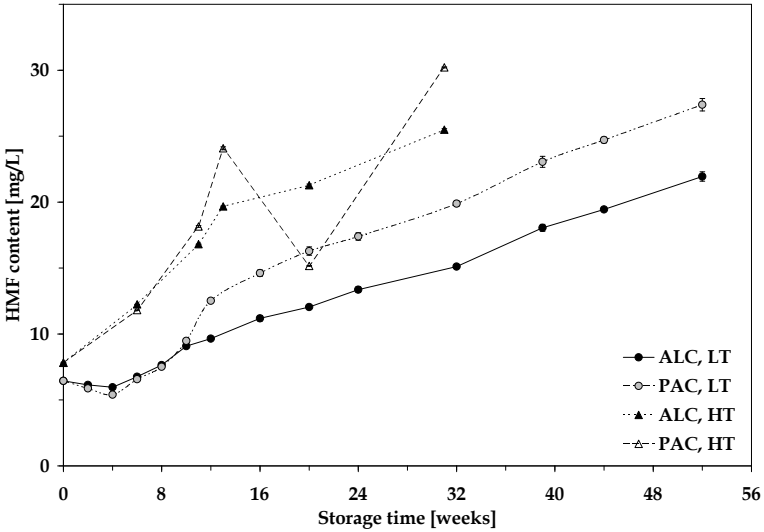


Figure 6.11.: HMF in NCA juice: Influence of packaging, storage time and temperature during one year of storage.

Linear correlation with time was also observed for HMF concentration in PAC packaging for LT storage ($R^2 = 0.968$, $p < 0.01$) and for HT storage when the abnormal low value at week 20 was excluded from statistical analysis ($R^2 = 0.887$, $p < 0.01$).

For both packages higher HMF concentrations were obtained at HT storage than at LT storage (high significance, $p < 0.01$). From week twelve, differences between the LT data of the juice in ALC and PAC package reached statistical significance ($p < 0.01$) with higher HMF levels in the PAC package. When the

aforementioned outlier at week 20 was excluded from statistical analysis the same conclusion could be drawn for the HT storage.

6.2.5. Antioxidative Capacity

For the second storage study the antioxidative capacity of the juices was determined using VARP assay. Figure 6.12 shows the changes of the antioxidative capacity of NCA juice during one year of LT storage in different packages. For storage in ALC packaging the VARP data did not correlate linearly with time. Instead, within the first 24 weeks of storage the VARP value was significantly higher than its initial value ($p < 0.01$). Afterwards, it remained stable on the initial level.

In contrast, storage in PAC packaging caused a linear degradation with time ($R^2 = 0.925$, $p < 0.01$). NCA juice lost 0.62 % of its initial antioxidative capacity per week and after one year of storage the VARP value has decreased by 32 %. In conclusion, packaging had a highly significant influence on this parameter for LT stored NCA juice ($p < 0.01$).

As figure 6.13 shows the VARP assay provided inconsistent results for the HT storage of NCA juice. For both packages the data did not correlate linearly with time. After six weeks the juice stored in ALC packages lost already 12 % of its initial VARP value but then remained stable until week 31.

Storage in PAC packages caused consequently lower VARP values though the trend did not ran consistently. However, the HT samples in PAC packaging showed a distinct decrease in antioxidative capacity. After 31 weeks VARP value has diminished by 34 % of the initial value. The abnormal values of week 13 and 20 corresponded to the characteristics observed for the chlorogenic acid and phloridzin content (refer to section 6.2.2). This further substantiated the possibility of mixed up samples.

When data of week 13 and 20 were excluded from analysis packaging had a highly significant influence on antioxidative capacity for HT stored NCA juice ($p < 0.01$).

The test series of LT and HT stored samples were conducted with temporary divergence. Due to the fact that the VARP results depended on the performance of the coularray detector the absolute VARP values of the LT and HT stored samples could not be compared (refer also to section 4.5).

On basis of the average loss rate of 0.62 % per week a theoretical decrease of 19 % of the initial value was calculated for NCA juice in PAC package stored

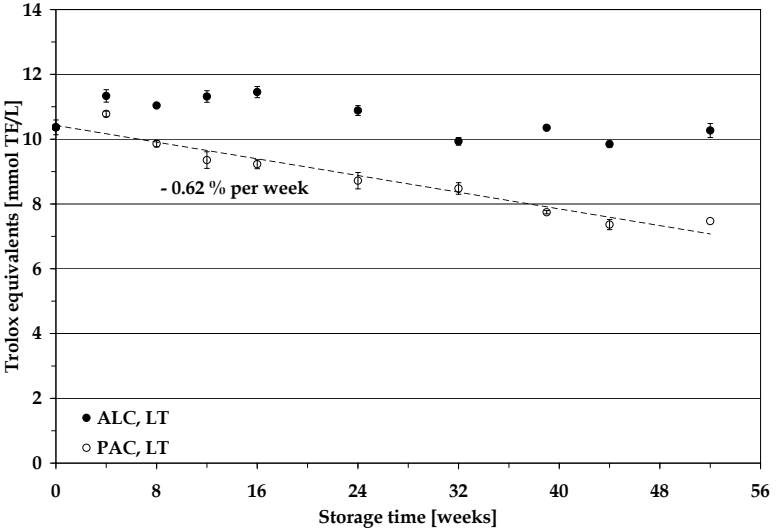


Figure 6.12.: VARP data of NCA juice: Influence of storage time and packaging on the antioxidative capacity (measured as trolox equivalents) during one year of LT storage.

at LT for 31 weeks. In comparison with 34 % reduction at HT storage there was a distinct influence of storage temperature on the antioxidative capacity of NCA juice stored in PAC packaging.

6.2.6. Sensory Deterioration

6.2.6.1. Changes in Juice Color

The graphs A and B in figure 6.14 show the color evaluation of the NCA juice under different storage conditions. Regardless of the packaging or storage temperature, the color rating increased with storage time. In ALC packaging color changes barely missed the criteria set for linear correlation ($R^2 = 0.748$, $p < 0.01$ at LT storage and $R^2 = 0.792$, $p < 0.01$ at HT storage). Furthermore, the color evaluation for PAC packages did not correlate linearly with time.

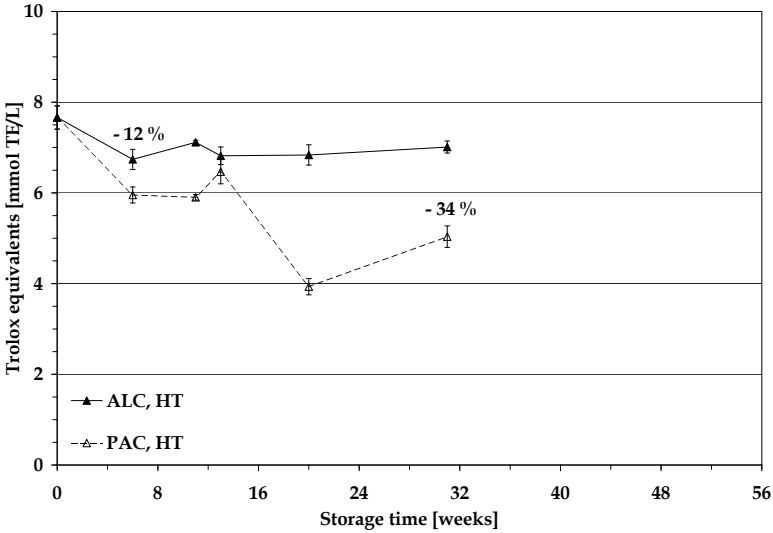


Figure 6.13.: VARP data of NCA juice: Influence of storage time and packaging on the antioxidative capacity (measured as trolox equivalents) during one year of HT storage.

Comparison of both diagrams revealed that color rating depended highly on the packaging. However, storage temperature exerted minor influence on color evaluation for the PAC packaging (see graph B of figure 6.14). The juice failed to fulfil the demands on quality after seven weeks of HT storage and 13 weeks of LT storage, respectively.

As shown in part A of figure 6.14 the juice quality stored in ALC packages depended on the storage temperature. The sensory panel evaluated the color of the LT stored juice as “acceptable” until week 44. At HT storage the juice deteriorated faster and thus, its color did not comply with the recommended quality standard from week 21.

A comparison of these results to the first storage study is shown in figure 6.15. Both of the NCA juices in an ALC packaging exhibited similar

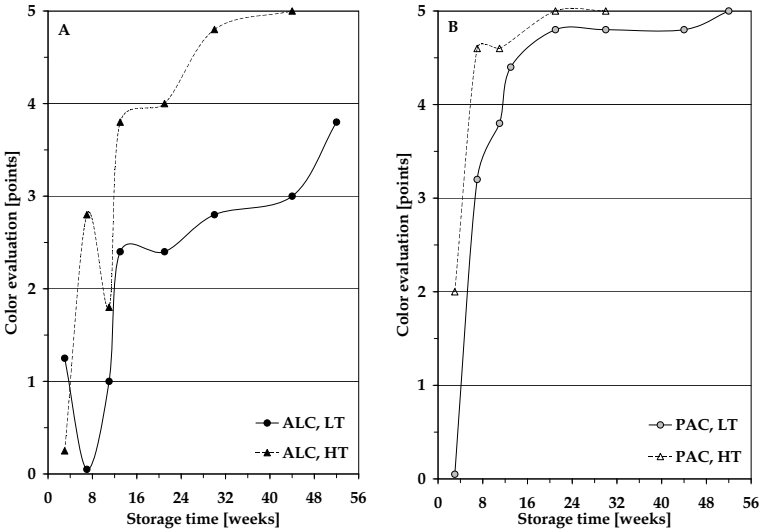


Figure 6.14.: Color evaluation of NCA juice in ALC (A) and PAC (B) packages: Influence of storage time and temperature during one year of storage.

characteristics at both storage temperatures. After 42 and 44 weeks of LT storage, respectively, the sensory panel evaluated the color alterations of both juices as "acceptable".

During HT storage color of the NCA juice of the second storage study was rated predominantly higher than the juice of the first study. Thus, it failed to fulfil the demands on quality after 21 weeks whereas the juice of the first study was rated acceptable until week 33.

6.2.6.2. Changes in Taste

The sensory panel rated the taste of the NCA juice stored in ALC packages as illustrated in part A of figure 6.16. At HT storage the taste evaluation correlated linearly with time ($R^2 = 0.891$, $p < 0.01$). However, for LT storage

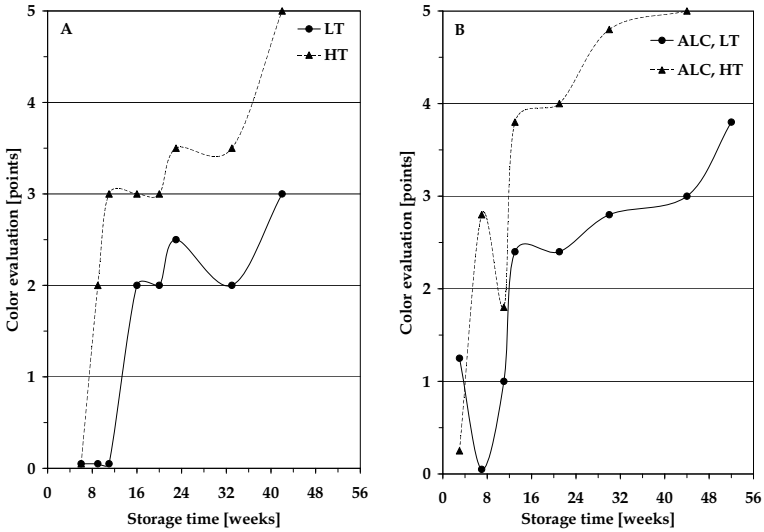


Figure 6.15.: Color evaluation of NCA juices in ALC packages during the first (A) and the second (B) storage study: Influence of storage time and temperature.

this parameter barely missed the criteria set for linear correlation ($R^2 = 0.789$, $p < 0.01$).

During one year of LT storage its taste complied with the quality standards. Whereas the HT stored juice received an unacceptable score at the end of the storage period. After week 13 the difference between the taste evaluations at LT and HT storage amounted more than one grade and subsequently, rose almost in parallel.

The taste changes of the NCA juice appeared faster when stored in PAC packaging. Part B of figure 6.16 illustrates the deterioration observed for LT and HT storage. For LT storage the taste changes correlated linearly wit time ($R^2 = 0.889$, $p < 0.01$) whereas no linear correlation was observed for HT storage. However, the courses of the taste evaluations for both temperatures increased in parallel but HT stored juice was rated at least one grade higher

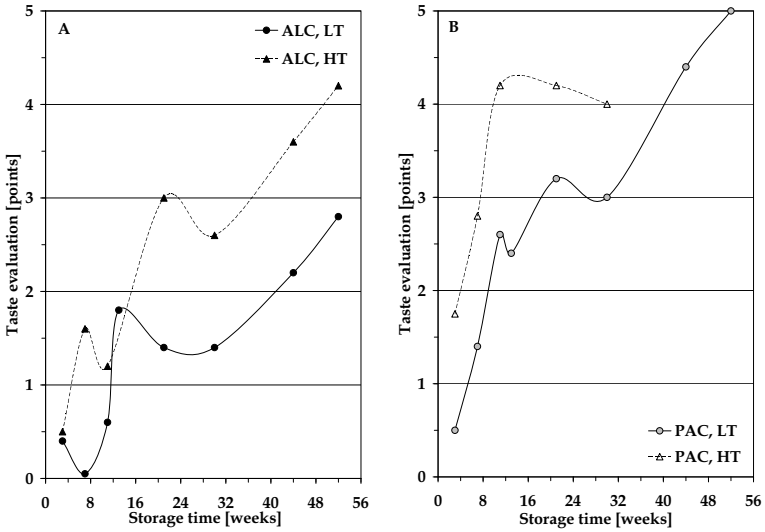


Figure 6.16.: Taste evaluation of NCA juice in ALC (A) and PAC (B) packages: Influence of storage time and temperature during one year of storage.

than LT stored juice. After eleven weeks the HT stored juice failed to fulfil the demands on taste quality whereas the LT stored juice did not comply with these claims anymore after 44 weeks.

Comparison between the taste evaluation of the NCA juice in the first and second storage study is illustrated in figure 6.17. During the LT storage the taste of the first study NCA juice reached a grade of three at week 42 whereas in the second study the taste change was rated below three points after one year of storage.

Only smaller differences appeared between the two juices at HT storage. However, comparison of the taste evaluation at week 42 and 44, respectively, showed that the NCA juice of the first study was rated 1.4 points higher than in the second study (5.0 in contrast to 3.6 points).

In conclusion, NCA juice color deteriorated faster in the first study than in the second one.

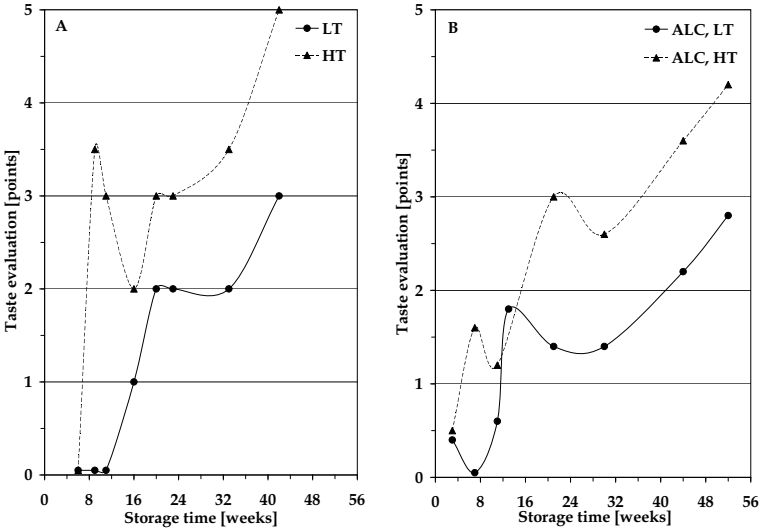


Figure 6.17.: Taste evaluation of NCA juices in ALC packages during the first (A) and the second (B) storage study: Influence of storage time and temperature.

6.3. Storage of Filtered Apple Juice

6.3.1. Oxygen and Headspace Analysis

Due to degassing prior to filling of the apple juices the headspace volume of the FA juice decreased rapidly (refer to section 6.2.1). From 8 mL containing 17.5 % of oxygen the headspace volume diminished completely within the first week of storage (data not shown).

However, the oxygen concentration in the FA juice varied highly during storage. As illustrated in figure 6.18 its value depended from packaging, storage time and temperature.

Due to low oxygen permeability of the ALC package (refer to section 6.1) the oxygen concentration of the juice in this packaging decreased fast and independently from the storage temperature (LT storage data are not shown). After only seven weeks it reached a low level of about 0.2 ppm and remained stable until the end of storage.

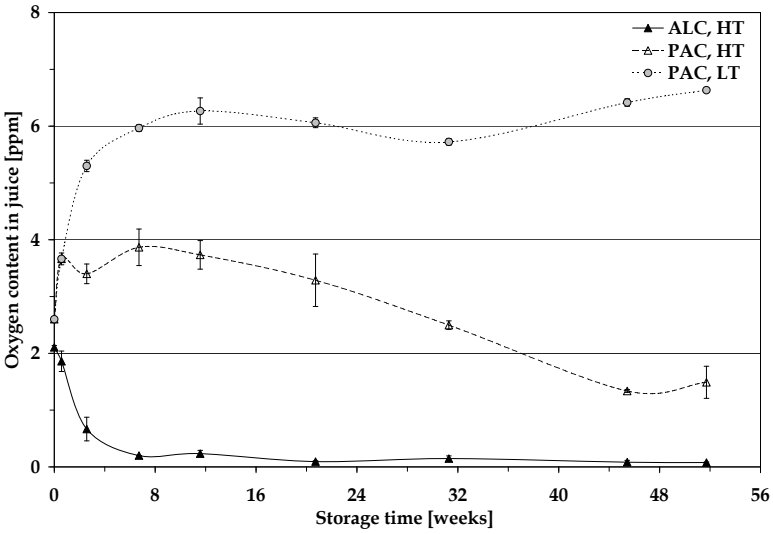


Figure 6.18.: Oxygen in FA juice: Influence of packaging, storage time and temperature during one year of storage.

However, the high permeability of the PAC package caused an increase of the oxygen concentration depending on the storage temperature. The oxygen level increased during the first seven weeks at HT and then diminished until week 45. The parameter barely missed the criteria set for linear correlation ($R^2 = 0.790$, $p < 0.01$).

Its trend pointed out that the higher temperature accelerated the oxidative reactions and thus, oxygen consumption exceeded oxygen permeation through the packaging material. The last data point indicated that the decline diminished probably due to a deceleration of oxidative processes.

In PAC packages stored at LT the oxygen content of the juice did not correlate linearly with time. However, it increased significantly until week twelve ($p < 0.05$). Afterwards, the data ranged around 6 ppm until the end of storage. Apparently, the lower storage temperature decelerated oxidative reactions and thus, oxygen consumption.

To clarify whether the increase slowed down because the juice was saturated with oxygen a saturation test was conducted: a juice sample was stirred continuously and oxygen concentration was determined after 24 hours. A maximum of 8 ppm was reached which was in accordance with the calculated saturation limit of oxygen in water (8.8 ppm at 20 °C and 1013 mbar) when taking into account that dissolved juice compounds lowered this theoretical value. [72]

Thus, the results obtained from the saturation test exceeded the value determined during storage by 33 %. As saturation was not reached the oxygen that permeated through the packaging material was consumed by oxidative processes and therefore, did not increase the oxygen content of the juice.

6.3.2. Polyphenolic Compounds

Figure 6.19 shows a representative chromatogram of FA juice sampled at the beginning of the storage period. The UV signal at 280 nm is presented in the upper part of the figure, the lower part shows CEAD channel 2 at 100 mV. HMF (peak 1) was only detected by UV-DAD, chlorogenic acid and phloridzin were observed via UV-DAD and CEAD. Phloridzin was oxidised at higher potentials (ch. 4 to 8, ≥ 300 mV) and thus, no peak was observed in ch. 1 to 3.

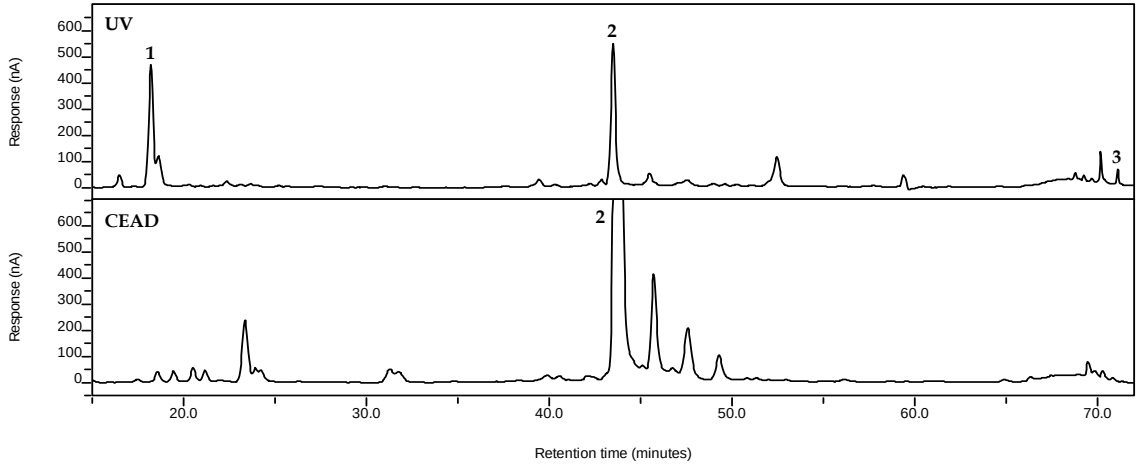


Figure 6.19.: A representative chromatogram of FA juice sampled at the beginning of the storage period. Upper part: UV signal at 280 nm, lower part: CEAD channel 2 at 100 mV; Peak identification: HMF (1), chlorogenic acid (2), phloridzin (3).

In accordance with the FA juice of the first storage study the polyphenol pattern of the FA juice of the second study also contained chlorogenic acid and phloridzin.

Figure 6.20 illustrates the changes of the chlorogenic acid content in FA juice depending on packaging, storage time and temperature. On closer examination the FA juice resembled the NCA juice regarding its characteristics of the main polyphenolic compounds. In comparison to the NCA juice of this study, FA juice contained only one third of its chlorogenic acid concentration.

In ALC packaging the LT stored juice did not correlate linearly with time and furthermore, there was no significant decrease during one year of storage. However, for LT storage degradation in PAC package was significant and correlated linearly with time ($R^2 = 0.941$, $p < 0.01$).

The data obtained for HT storage were inconsistent for both ALC and PAC packages. Except for week 13 the chlorogenic acid concentration was significantly lower than in the corresponding LT samples. However, the data determined for week 13 indicated that HT samples might have been interchanged with the corresponding LT samples. However, a mix-up of these samples could explain only some of the unusual results but did not suit all (see further results in figure 6.21 and section 6.3.3). From these inconsistent data it was concluded that the HT results of week 13 remained out of consideration.

In conclusion, the data showed that packaging and storage temperature significantly influenced the degradation of chlorogenic acid in FA juice.

Figure 6.21 illustrates the changes of the phloridzin concentration in FA juice under different storage conditions. The FA juice contained less than 20 % of the initial phloridzin concentration of the NCA juice.

The phloridzin content did not correlate with storage time regardless of packaging or storage temperature. Additionally, there was no significant difference from its initial concentration observed during one year of storage.

As discussed in the previous paragraph for chlorogenic acid there were abnormal data for the HT samples of week 13. When these results were excluded the HT stored samples contained distinctly less phloridzin than the LT samples in both ALC and PAC packaging. However, statistical significance ($p < 0.05$) was reached for all data except for the last HT time point at week 31 ($p = 0.056$ for ALC and $p = 0.054$ for PAC).

The differences between ALC and PAC packaging at each storage temperature were less distinct and did not reach consistent significance.

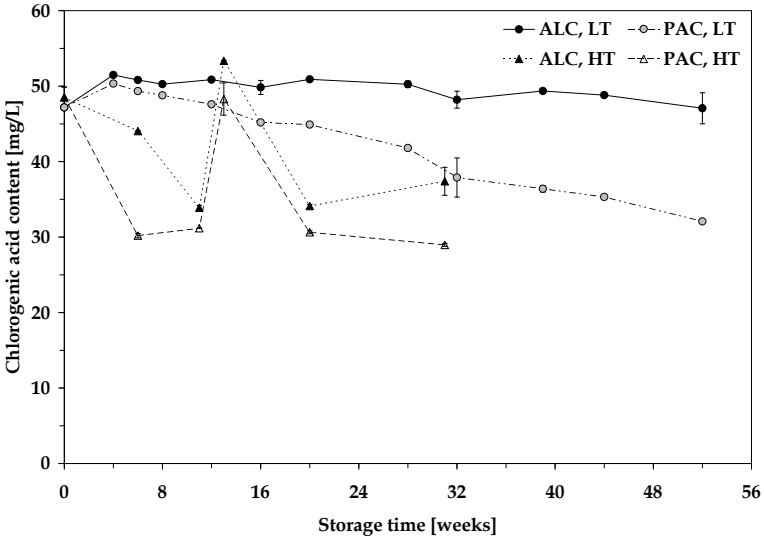


Figure 6.20.: Chlorogenic acid in FA juice: Influence of packaging, storage time and temperature during one year of storage.

In conclusion, due to the lower initial concentration and the relatively small changes during storage it could not be concluded that storage temperature or packaging had a significant influence on the phloridzin content in FA juice.

6.3.3. HMF

Figure 6.22 shows the rising of the HMF content of the FA juice under different storage conditions. Based on an initial value of 8.0 mg/L the HMF concentration increased depending on the storage temperature.

HMF formation correlated linearly with time at both storage temperatures and in both packages. The respective highly significant coefficients of determination ($p < 0.01$) are listed in table 6.2.

At LT storage the difference in HMF content between ALC and PAC packaging reached statistical significance from week 16 ($p < 0.05$). Subsequently,

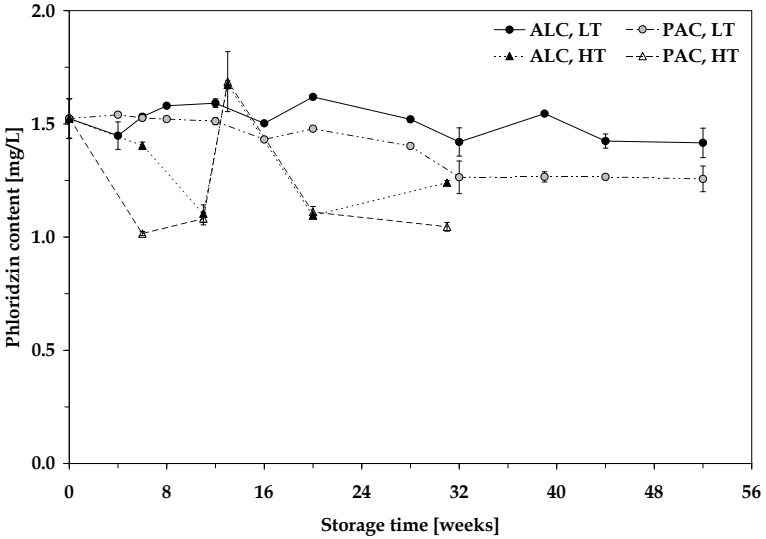


Figure 6.21.: Phloridzin in FA juice: Influence of packaging, storage time and temperature during one year of storage.

	R^2 value	
Packaging	at LT storage	at HT storage
ALC	0.992	0.841
PAC	0.986	0.821

Table 6.2.: Coefficients of determination for HMF formation in FA juice.

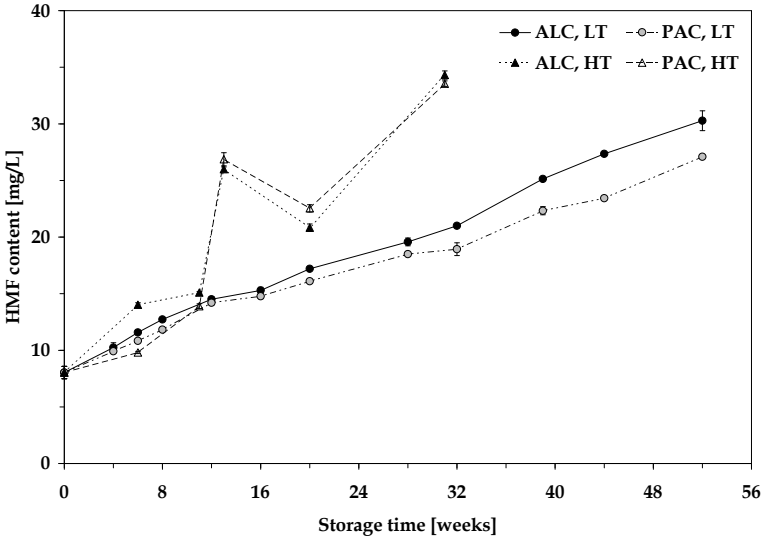


Figure 6.22.: HMF content of FA juice: Influence of packaging, storage time and temperature during one year of storage.

storage in PAC package decelerated HMF formation and led to a 10 % lower HMF end concentration at week 52.

HMF is an intermediate of the maillard reaction. It was concluded that in presence of oxygen not only the formation of HMF was accelerated [60] but also the increase of brown pigments as final products of the maillard reaction [24, 7]. Thus, the higher oxygen concentration accelerated more strongly the formation of melanoidins than the HMF synthesis. That led to a slowed increase of HMF in comparison to the juice in ALC packaging. The delta E results presented in section 6.3.5.1 supported this explanation.

At HT storage the HMF concentration increased faster than at LT storage. The differences between HT stored samples and their LT stored correspondents were significant in both packages ($p < 0.05$) except for week eleven. However, the HMF content developed inconsistently in the two packages. The samples of week six and eleven showed the same characteristics as the LT stored juice:

a significantly lower HMF concentration in the juice filled in PAC package. However, at week 20 the HMF content in ALC package was significantly lower. The differences at week 13 and 31 did not reach statistical significance. Furthermore, both samples at week 13 yielded abnormal high values. As discussed in section 6.3.2 the HT data of week 13 showed inconsistent results also for further parameters. However, the HMF measurements contradicted a potential mix-up with the corresponding LT stored samples as this would have caused lower HMF values than determined.

In conclusion, the data showed that storage temperature strongly influenced the HMF concentration in FA juice. Furthermore, packaging caused a slightly but significantly different formation rate with a predominantly stronger increase for the ALC packaging.

6.3.4. Antioxidative Capacity

The VARP results of FA juice during one year of LT storage in different packaging are illustrated in figure 6.23. Neither in ALC nor in PAC packages the parameter correlated linearly with time.

Starting with a lower initial value LT storage in ALC packaging caused the VARP data to remain stable in the range of 4.0 mmol TE/L for one year. Antioxidative capacity of the FA juice in PAC package decreased slightly and was significantly lower than the initial VARP value after 16 weeks of LT storage. However, in the second half of the storage period it remained stable in the range of 3.6 mmol TE/L and thus, 10 % below the ALC results.

As illustrated in figure 6.24 the VARP assay yielded inconsistent data for the HT storage in both packages. Due to the temporary divergent measurements the HT samples yielded distinctly lower VARP values than the LT samples (refer to section 4.5) and thus, could not be compared with each other. In both packages the VARP value did not correlate linearly with time.

When the outlier at week 13 was excluded from analysis (as discussed in section 6.3.2) the antioxidative capacity was significantly lower than the initial VARP value at all other data points. Between both packaging no significant differences appeared except at week 6 when the PAC package yielded a highly significantly lower VARP value ($p < 0.01$). After 31 weeks the loss averaged out at 20 % for both packages.

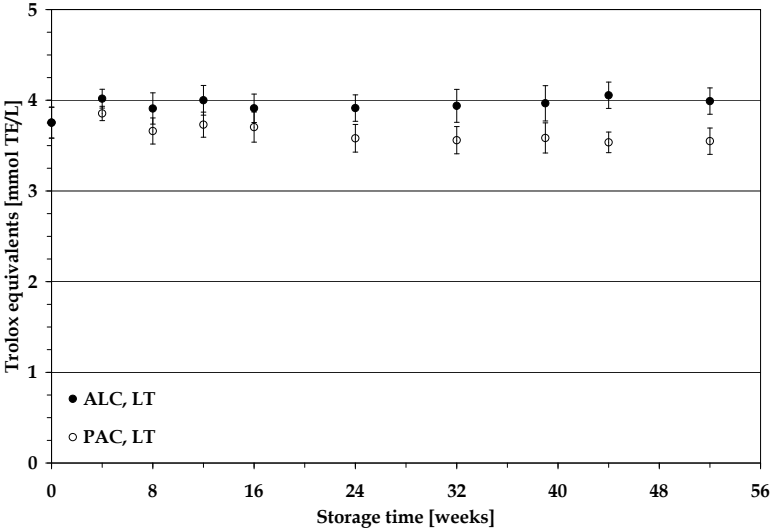


Figure 6.23.: VARP data of FA juice: Influence of storage time and packaging on the antioxidative capacity (measured as trolox equivalents) during one year of LT storage.

In conclusion, the data showed that packaging had a slight but significant influence on the antioxidative capacity of FA juice during LT storage. However, at HT storage the VARP value was not significantly influenced by the packaging.

6.3.5. Sensory Deterioration

6.3.5.1. Changes in Juice Color

The graphs A and B in figure 6.25 illustrate the increasing color evaluation of the FA juice during storage under different conditions. While the parameter barely missed the criteria set for linear correlation when stored at LT in ALC packaging ($R^2 = 0.733$, $p < 0.01$), it correlated linearly for HT storage ($R^2 = 0.915$, $p < 0.01$). Furthermore, storage in PAC package caused linear correlation between color rating and storage time at both temperatures

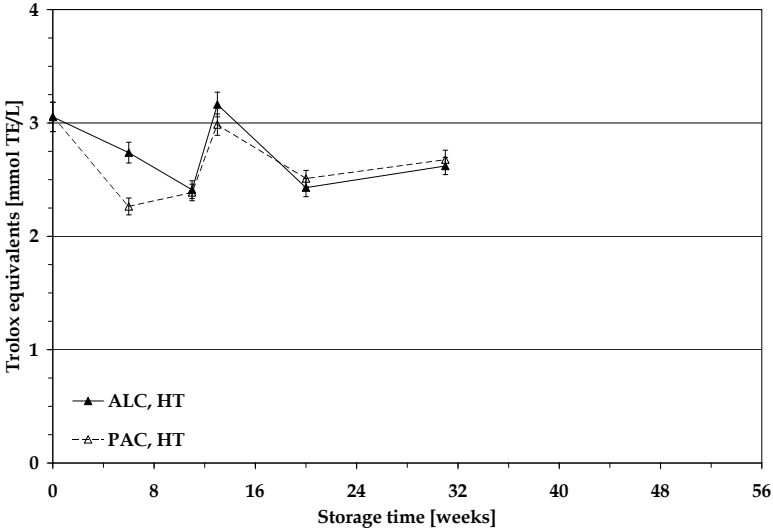


Figure 6.24.: VARP data of FA juice: Influence of storage time and packaging on the antioxidative capacity (measured as trolox equivalents) during one year of HT storage.

($R^2 = 0.841$, $p < 0.01$ for LT and $R^2 = 0.882$, $p < 0.01$ for HT storage).

For both LT and HT storage juices filled in ALC packages fulfilled the demands on quality during the complete period of storage. However, the HT stored juice was rated consequently higher (at least 0.5 points) than the LT stored samples. These results corresponded to the ones obtained for FA juice of the first storage study (refer to section 5.1.6.1).

Part B of figure 6.25 illustrates the results of the FA juice in PAC packages. During the first 21 weeks only minor differences appeared between the two storage temperatures. Subsequently, the sensory panel evaluated the color of the HT stored juice distinctly higher than the LT stored juice. While the LT samples fulfilled the demands on quality until the end of storage the HT samples were rated unacceptable at week 44 and 45. At week 52 the HT stored

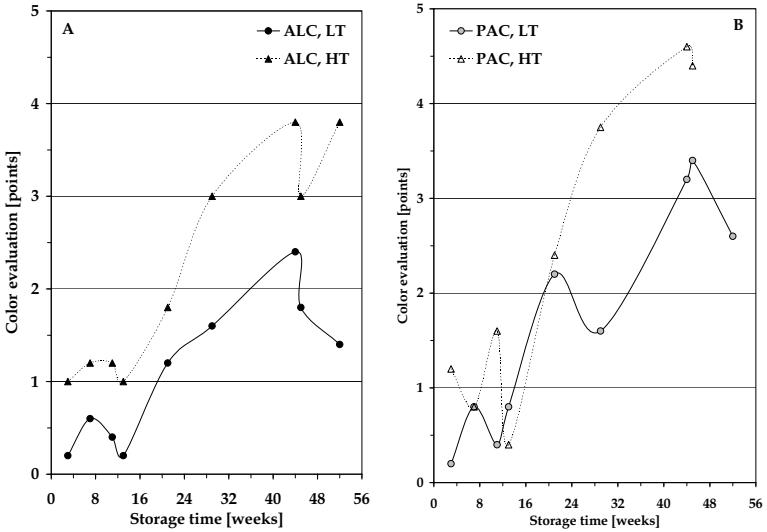


Figure 6.25.: Color evaluation of FA juice in ALC (A) and PAC (B) packages: Influence of storage time and temperature during one year of storage.

juice was not evaluated.

In spite of the high oxygen permeability of the PAC packaging the color quality of the FA juice stored at LT fulfilled the requirements during one year of storage. Even at HT storage the juice color was acceptable within the first half of the year. In comparison to the strong alterations of both the NCA and RG juice when stored in PAC packaging (refer to sections 6.2.6.1 and 6.4.6.1) this was a rather unexpected outcome.

Juice color was further evaluated by calculation of delta E values determined via CIE Lab system analysis (see figure 6.26). While the sensory panel attested the juice only minor color differences between both packages, the delta E values showed a distinctly stronger increase for the juice filled in PAC packages.

For the LT stored juice in ALC package the delta E value remained stable during one year of storage. However, HT stored samples yielded an increasing delta E value which correlated linearly with time ($R^2 = 0.972$, $p < 0.01$).

LT storage in PAC packaging caused a linear increase of the delta E value with time ($R^2 = 0.826$, $p < 0.01$) while this parameter barely missed the criteria set for linear correlation for HT stored samples ($R^2 = 0.791$, $p < 0.01$).

Due to formation of brown pigments by maillard reaction the delta E value increased. However, delta E results and sensory color evaluation did not obtain identical outcome. Thus, sensory analysis revealed that a more intense color was not directly associated with a worse juice quality.

Furthermore, the figure also gave information about the influence of storage temperature and oxygen on the extent of the maillard reaction in the juice.

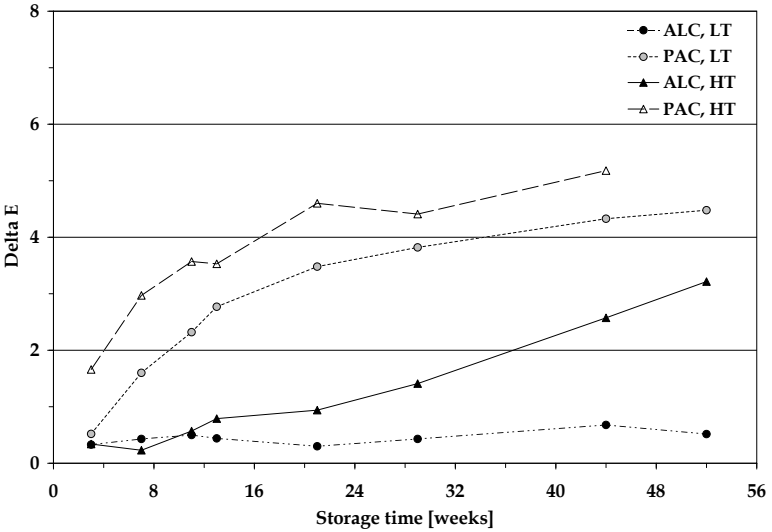


Figure 6.26.: Delta E measurement of FA juice: Influence of packaging, storage time and temperature during one year of storage.

6.3.5.2. Changes in Taste

The sensory panel rated the taste of the FA juice as illustrated in the graphs A and B of figure 6.27. Under all storage conditions observed the taste rating increased with time.

In ALC package this parameter correlated linearly with time only during HT storage ($R^2 = 0.829$, $p < 0.01$) but not during LT storage. In contrast, taste rating barely missed the criteria set for linear correlation when FA juice was stored in PAC packaging ($R^2 = 0.764$, $p < 0.01$ at LT and $R^2 = 0.729$, $p < 0.01$ at HT storage).

In ALC package the taste was only slightly influenced by storage temperature and the juice fulfilled the demands on quality during the complete period of storage at both temperatures. These results were similar to the data of the first storage study though the ratings between the two storage temperatures differed less than in the first study.

Part B of figure 6.27 shows the taste evaluation of the FA juice stored in PAC packages. The influence of oxygen on the taste evaluation was noticeable especially at HT storage. However, the taste of the FA juice stored at LT fulfilled the demands on quality during one year of storage. Furthermore, also the taste of HT stored juice was rated "acceptable" during the observation period except at the last time point after 45 weeks.

Taste evaluation together with color rating revealed that storage stability under usual conditions turned out satisfactory for FA juice filled in PAC packaging. Furthermore, all additional parameters determined during storage of FA juice did not contradict this finding.

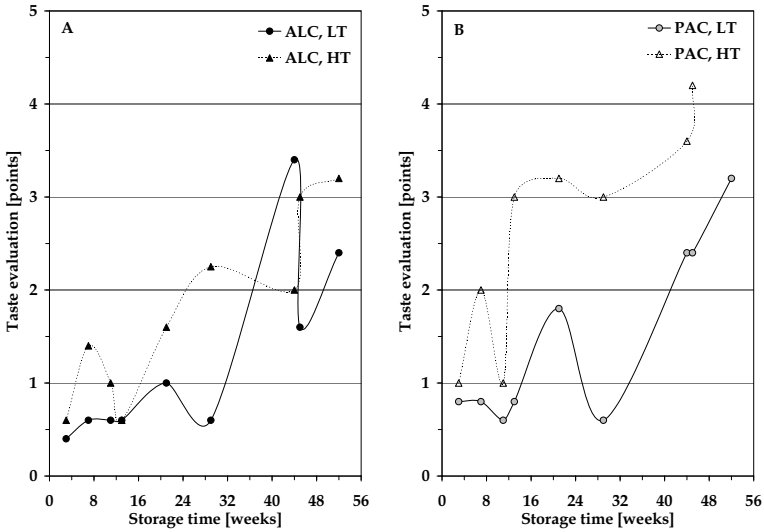


Figure 6.27.: Taste evaluation of FA juice in ALC (A) and PAC (B) packages: Influence of storage time and temperature during one year of storage.

6.4. Storage of Red Grape Juice

6.4.1. Oxygen and Headspace Analysis

Figure 6.28 shows the headspace volume of RG juice during one year of LT storage in ALC and PAC packaging. In opposite to the apple juices RG juice was not degassed prior to filling and thus, headspace volume did not reduce rapidly.

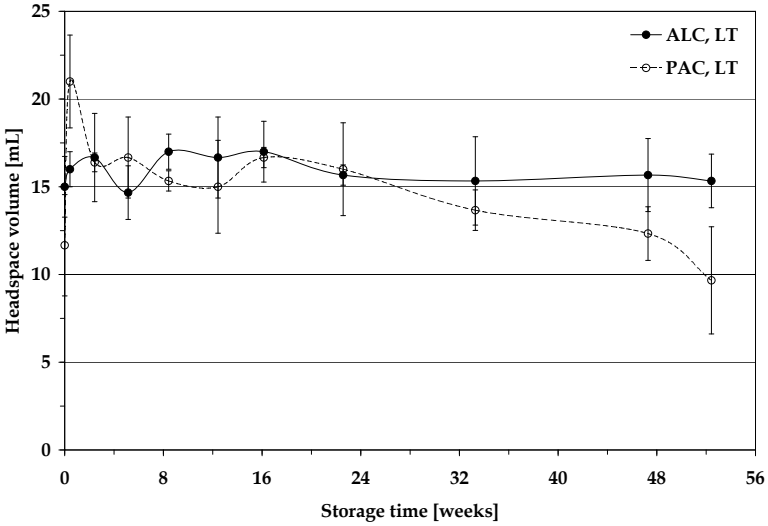


Figure 6.28.: Headspace volume in packages of RG juice: Influence of storage time during LT storage.

In the ALC packaging headspace volume remained stable at about 16 mL during one year of LT storage. Compared to the results of the first storage study (refer to section 5.2.1) the volume was in the same range.

Though the headspace volume of LT stored RG juice in PAC packages varied highly during storage the differences did not reach statistical significance. Furthermore, its overall mean volume was 15 mL and thus, in the same range

as for the ALC package.

Headspace volume of the HT stored samples in ALC packaging showed analogue characteristics as for LT storage (see figure 6.29). Due to high variation of the data the differences were not significant neither within the storage period, nor in comparison with the LT stored samples in ALC packaging.

However, headspace volume of RG juice decreased significantly during HT storage in PAC packaging. Furthermore, comparison with regard to packaging and storage temperature revealed that headspace volume of HT stored samples in PAC package was significantly lower from week 16 ($p < 0.05$).

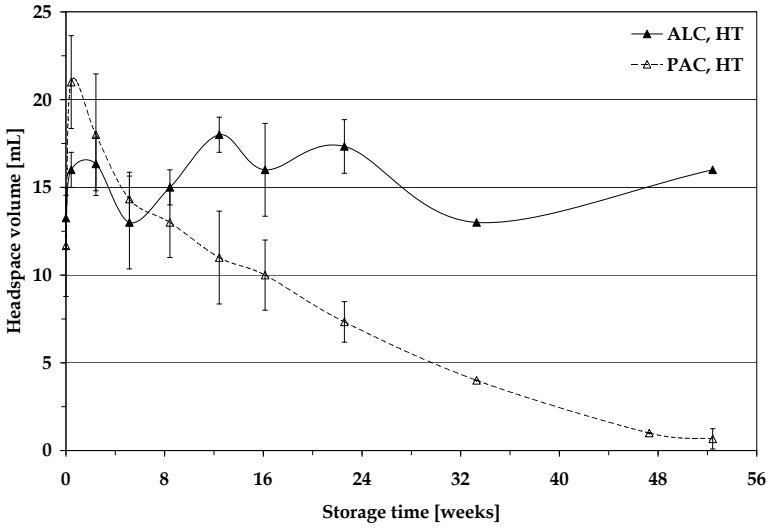


Figure 6.29.: Headspace volume in packages of RG juice: Influence of packaging and storage time during HT storage.

In conclusion, headspace volume remained stable under all storage conditions except for the HT stored juice in PAC packaging. Under latter conditions headspace decreased down to 1 mL after 47 weeks of storage.

Immediately after filling the oxygen content of the headspace in the ALC packaging diminished rapidly from 16.8 % down to zero within two weeks (data not shown). However, due to its higher oxygen permeability the PAC package obtained a higher oxygen percentage in headspace. Figure 6.30 shows the oxygen rate in headspace during one year of storage starting three days after filling.

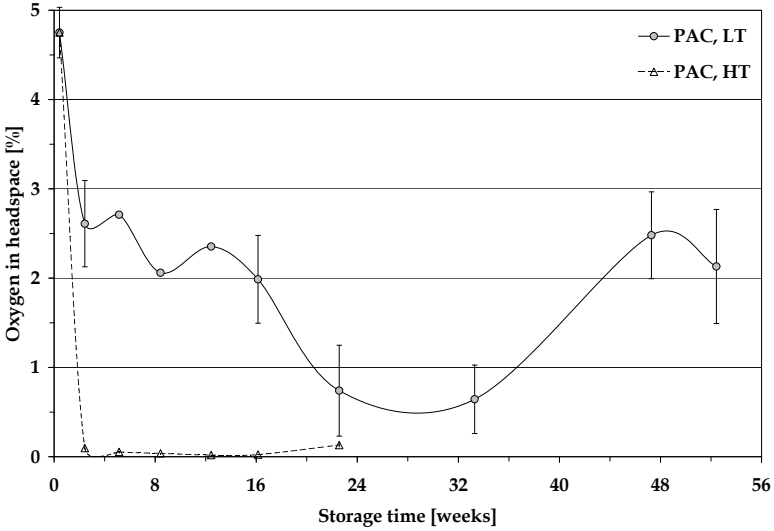


Figure 6.30.: Oxygen in headspace of RG juice in PAC packages: Influence of storage time and temperature during one year of storage starting with week 1.

Within two weeks of HT storage the oxygen percentage diminished down to almost zero. Due to the higher reaction rate at HT storage oxygen permeated directly into the juice.

Also during LT storage the oxygen rate decreased but a noticeable amount remained indicating a lower reaction rate of oxidative processes.

Figure 6.31 illustrates the trend of the oxygen content in the RG juice under different storage conditions. For the juice stored in ALC packages low

oxygen concentrations were obtained irrespectively of the storage temperature (LT data not shown). Furthermore, HT storage in PAC packages caused a comparably low oxygen level due to high consumption by oxidative reactions.

However, during LT storage oxygen permeation (> 21 ppm/month, refer to table 6.1) and consumption was in a similar range which resulted in oxygen levels that varied highly around 2.5 ppm.

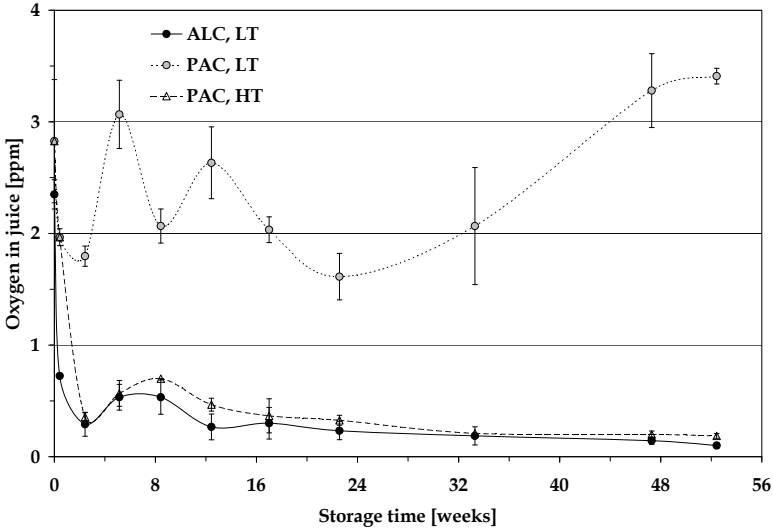


Figure 6.31.: Oxygen in RG juice: Influence of packaging, storage time and temperature during one year of storage.

6.4.2. Anthocyanins

The anthocyanin pattern of the RG juice is presented in table 6.3. In comparison to the results of the first storage study (refer to table 5.3) the RG juice of this study contained similar anthocyanins but in lower concentrations. However, for the second study the anthocyanins were quantified using malvidin-3-glucoside as standard compound. Therefore, explicit comparison

of absolute concentrations was not reasonable.

Anthocyanin	Proportion
Malvidin-3-glucoside	major
Petunidin-3-glucoside	minor
Malvidin-3-(6"-coumaroyl)-glucoside	minor
Delphinidin-3-glucoside	minor
Peonidin-3-glucoside	minor

Table 6.3.: Anthocyanin pattern of the RG juice.

The alteration of malvidin-3-glucoside during LT storage in different packaging is illustrated in figure 6.32. For both packages malvidin-3-glucoside content correlated linearly with time ($R^2 = 0.983$, $p < 0.01$ for ALC package, $R^2 = 0.985$, $p < 0.01$ for PAC package).

Concentrations of further anthocyanins of LT stored RG juice in ALC packaging are shown in figure 6.33. Due to the use of another HPLC system the malvidin-3-(6"-coumaroyl)-glucoside concentration could be quantified. In opposite to the first study cyanidin-3-glucoside and malvidin-3-(6"-acetoyl)-glucoside were not detected (refer to section 5.2.2). The degradation of delphinidin-3-glucoside correlated linearly with time ($R^2 = 0.982$, $p < 0.01$).

In conclusion, all anthocyanins of RG juice decreased fast and thus, after ten weeks of LT storage in ALC package none of them could be quantified. An even faster loss occurred for the LT stored juice in PAC package. At HT storage no anthocyanin was detected in the first sample taken after five weeks.

6.4.3. Further Polyphenols

Figure 6.34 shows a representative part of a chromatogram of RG juice sampled at the beginning of the storage period. The UV signal at 280 nm is presented in the upper part of the figure, the lower part shows CEAD channel 6 at 500 mV. While HMF (peak 1) provided only a UV signal, the other known peaks were detected by UV-DAD and CEAD. Due to higher sensitivity of the CEAD its peak height exceeded that of the UV signal. Thus, the scale was adjusted separately for each signal.

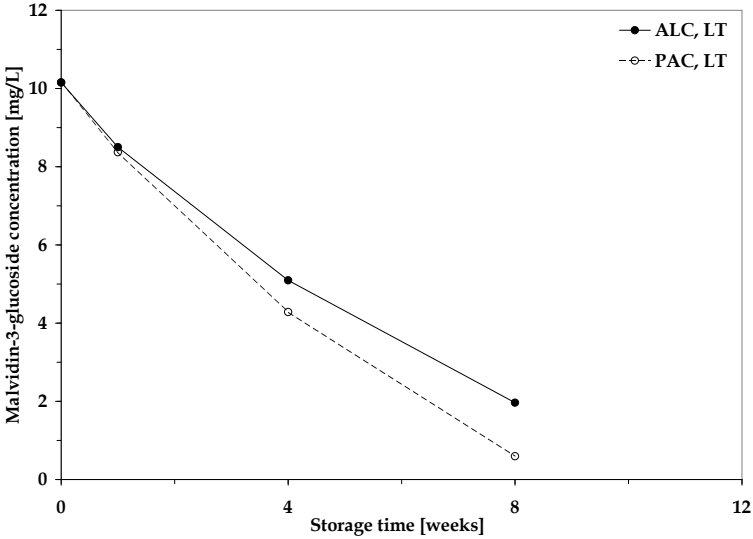


Figure 6.32.: Malvidin-3-glucoside in RG juice: Influence of storage time and packaging during one year of LT storage.

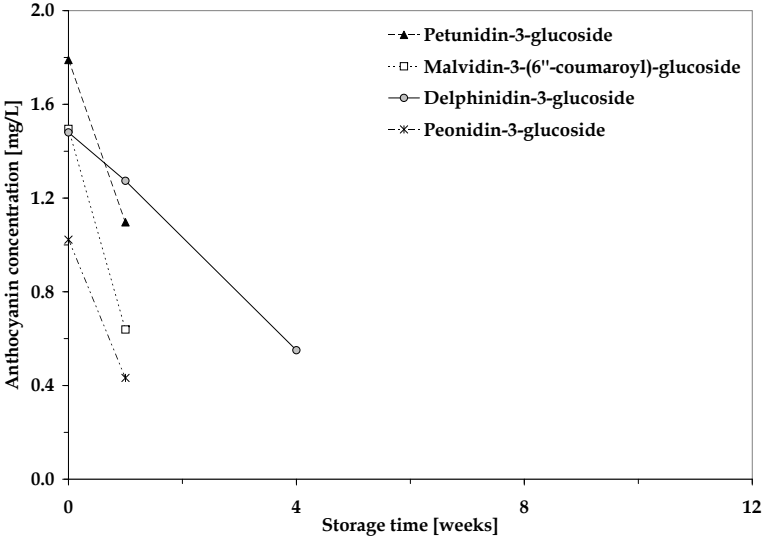


Figure 6.33.: Further anthocyanins of RG juice in ALC packages: Influence of storage time during LT storage [quantified as malvidin-3-glucoside].

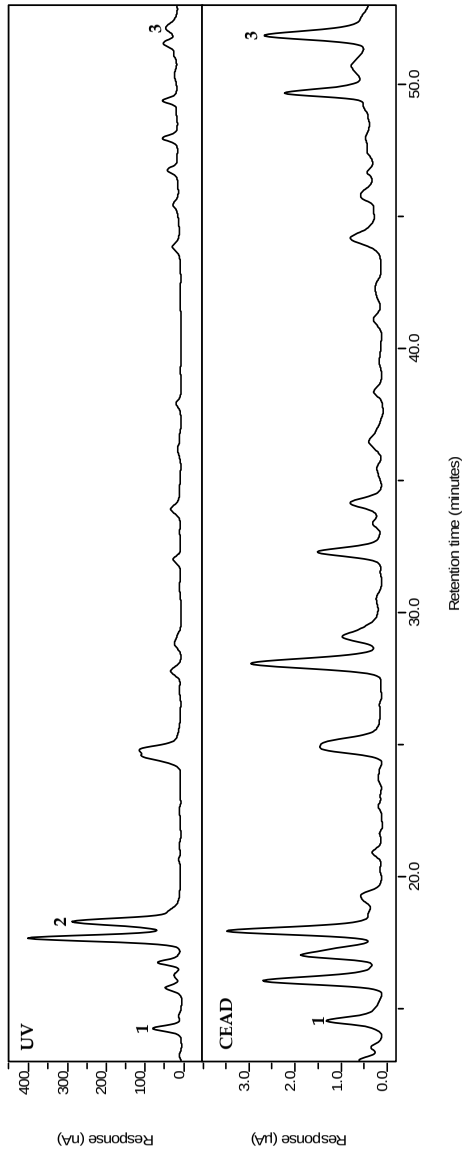


Figure 6.34.: A representative chromatogram of RG juice sampled at the beginning of the storage period. Upper part: UV signal at 280 nm, lower part: CEAD channel 6 at 500 mV; Peak identification: Gallic acid (1), HMF (2), catechin (3).

As one of the colorless polyphenols RG juice contained gallic acid. However, this juice had less than 25 % of the gallic acid concentration found in the RG juice of the first storage study.

Figure 6.35 shows its stability during storage under different conditions. Gallic acid concentration did not correlate linearly with time regardless of the storage conditions. Furthermore, this parameter ranged closely to its initial value and thus, no significant loss occurred during one year of storage.

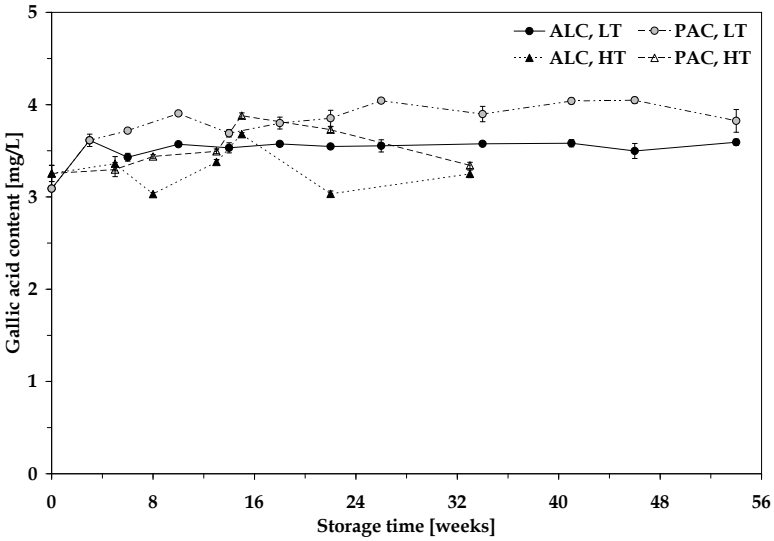


Figure 6.35.: Gallic acid in RG juice: Influence of packaging, storage time and temperature during one year of storage.

In conclusion, gallic acid concentration of RG juice was not significantly influenced during one year of storage under all conditions tested.

While the RG juice of the first storage study contained considerable amounts of catechin and epicatechin, only catechin was detected in this RG juice. Figure 6.36 illustrates the alterations during storage under different conditions.

In ALC packaging the catechin degradation missed the criteria set for linear correlation ($R^2 = 0.764$, $p < 0.01$ for LT storage and $R^2 = 0.752$, $p < 0.01$ for

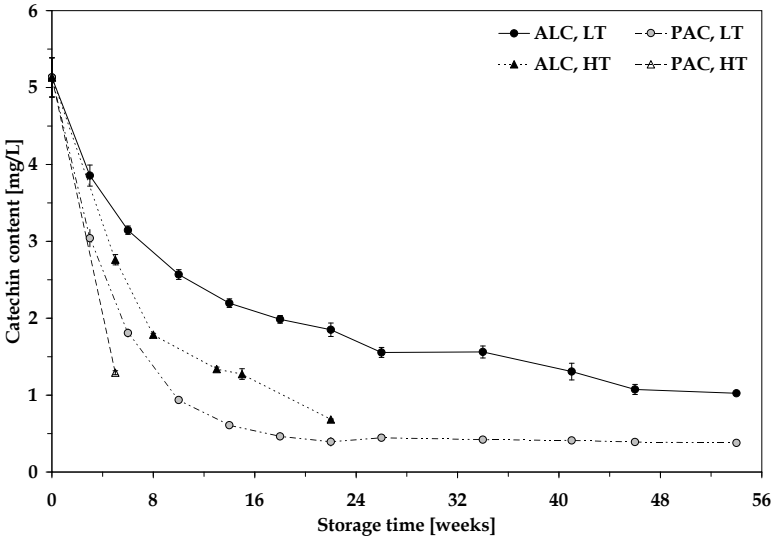


Figure 6.36.: Catechin in RG juice: Influence of packaging, storage time and temperature during one year of storage.

HT storage). Furthermore, for LT storage in PAC package no linear correlation with time was observed for this parameter. HT storage of RG juice in PAC packaging caused complete degradation of catechin within eight weeks and thus, calculation of linear correlation was not appropriate.

During LT storage in both packages the catechin content decreased fast within the first 18 weeks and then remained on a low but stable level until the end of storage. A similar but less pronounced characteristic was observed for catechin and epicatechin degradation in RG juice during the first study (refer to figure 5.20). Due to low oxygen permeability of the ALC packaging it was reasonable to conclude that oxidative processes diminished when oxygen level in the juice was low and thus, catechin degradation decelerated (refer to section 6.4.1).

Filled in PAC packaging RG juice was exposed to high oxygen permeation. However, figures 6.30 and 6.31 showed that during LT storage considerable amounts of oxygen remained in headspace and in juice indicating low reaction

rates for oxidative processes. Potentially, in the beginning of storage catechin degradation was enhanced by other compounds of the juice. Later this reaction slowed down though oxygen was present in the juice.

In conclusion, comparison of the catechin degradation showed that the influence of storage temperature was highly significant in both packaging ($p < 0.01$) causing complete loss during HT storage. Furthermore, the difference between both packaging at each storage temperature reached high significance for this parameter.

6.4.4. HMF

In opposite to the RG juice of the first study this one contained HMF. Its initial content of about 5 mg/L was within the normal range for juices made from concentrate (refer to section 6.2.4).

Figure 6.37 shows its increase during storage under different conditions. HMF content in RG juice stored at LT only rose slowly and correlated linearly with time ($R^2 = 0.992$, $p < 0.01$ for ALC and $R^2 = 0.984$, $p < 0.01$ for PAC packaging). During one year of LT storage HMF concentration did not exceed the limit value of 20 mg/L.

Within the first 26 weeks there was no significant difference between ALC and PAC package, respectively. Subsequently, storage in PAC packaging caused significantly slower HMF formation ($p < 0.01$) which was in accordance with the corresponding findings for the FA juice (see 6.3.3).

The temperature dependency of the HMF formation led to significantly higher concentrations during HT storage ($p < 0.01$). Analogue to LT storage the parameter correlated linearly with time when RG juice was stored at HT ($R^2 = 0.953$, $p < 0.01$ for ALC and $R^2 = 0.992$, $p < 0.01$ for PAC packaging).

Significant differences between the storage in ALC and PAC packages appeared already after five weeks. Finally, HMF concentration exceeded the limit value after 15 weeks of HT storage in both ALC and PAC packages.

6.4.5. Antioxidative Capacity

Figure 6.38 shows the antioxidative capacity of the RG juice (quantified via VARP assay) during LT storage in different packages.

During storage in ALC package no linear correlation with time was observed for this parameter. Furthermore, the VARP value remained stable and showed no significant decrease until week 46 ($p < 0.05$).

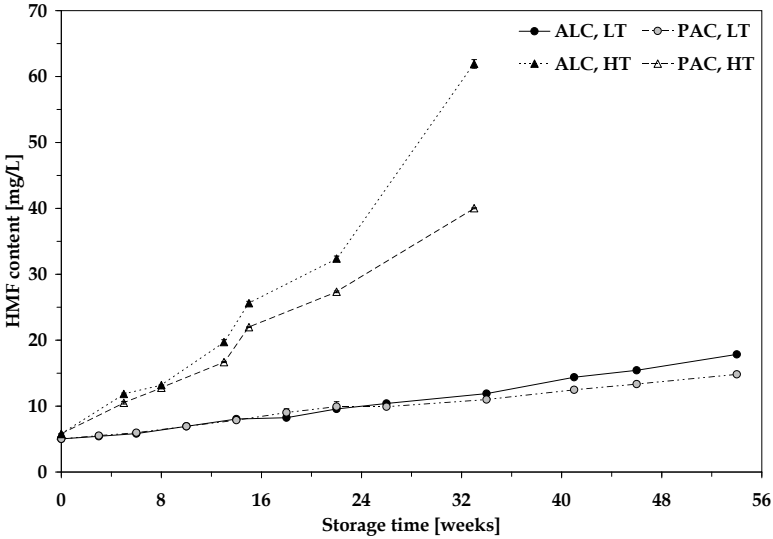


Figure 6.37.: HMF in RG juice: Influence of packaging, storage time and temperature during one year of storage.

However, antioxidative capacity decreased significantly for LT stored samples in PAC packaging. The VARP value correlated linearly with time ($R^2 = 0.880$, $p < 0.01$) and the loss amounted on average 0.58 % per week. Within one year of LT storage RG juice filled in PAC packaging lost 30 % of its initial antioxidative capacity.

Figure 6.39 illustrates the VARP changes for HT stored samples. The VARP value decreased significantly during storage in ALC packaging ($p < 0.05$) but reduction did not correlate linearly with time.

For PAC packaging the RG juice obtained inconsistent VARP results. The parameter did not correlate linearly with time. Furthermore, after highly significant reduction within the first five weeks the VARP value remained stable until week 13 and subsequently increased up to its initial level.

After 33 weeks there was a significant ($p < 0.05$) but minor loss of about 4 % of the initial VARP value. This finding showed that a higher storage

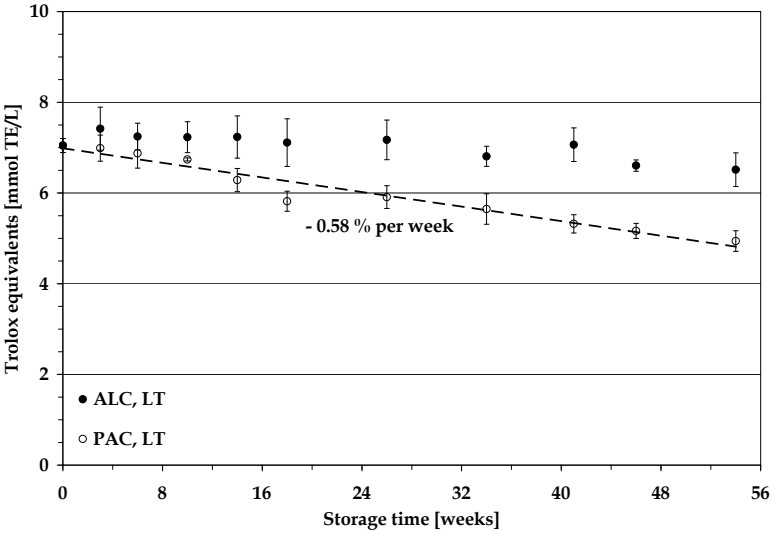


Figure 6.38.: VARP data of RG juice: Influence of storage time and packaging on the antioxidative capacity (measured as trolox equivalents) during one year of LT storage.

temperature did not necessarily lead to a stronger reduction of antioxidative capacity. Potentially, the accelerated HMF formation (refer to section 6.4.4) prevented the VARP value from stronger decline.

6.4.5.1. Comparison of the VARP Data

As mentioned in 4 the absolute VARP value depended on the performance of the coulometric array detector. Table 6.4 shows the absolute initial VARP values obtained for the juices of both storage studies. The ranking of the three juices was the same within each study. However, no further classification based on absolute results was reasonable due to the variation observed between temporary divergent measurements.

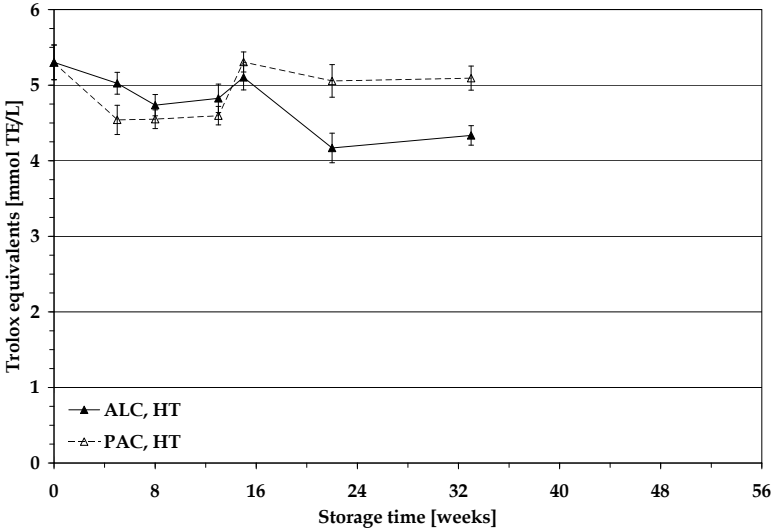


Figure 6.39.: VARP data of RG juice: Influence of storage time and packaging on the antioxidative capacity (measured as trolox equivalents) during one year of HT storage.

	NCA juice	RG juice	FA juice
First storage study	6.57	3.92	2.39
Second storage study	10.36	7.05	3.75

Table 6.4.: Comparison between the absolute initial VARP values [mmol TE/L] of the two storage studies

To compare the different juices among both studies the VARP ratio was calculated in relation to the VARP value of one of the juices (see table 6.5). Even though the two NCA juices contained ascorbic acid from different sources the ratios related to the FA juice were very similar.

In comparison to the RG juice of the first study the RG juice of the second storage study obtained a lower ratio. This might be explained by the fact that the second juice contained distinctly less antioxidative compounds (no epicatechin and less anthocyanins and gallic acid).

	NCA juice	RG juice	FA juice
First storage study	2.76	1.88	1.00
Second storage study	2.74	1.64	1.00

Table 6.5.: Ratios of the initial VARP data calculated in relation to the VARP value of FA juice of each study.

6.4.6. Sensory Deterioration

6.4.6.1. Changes in Juice Color

Part A of figure 6.40 illustrates the color evaluation of the RG juice under different storage conditions. During LT storage in ALC package the juice fulfilled the demands on quality over a storage period of one year but evaluation was not correlated linearly with time.

Furthermore, the color rating of the HT stored juice in ALC packaging correlated linearly with time ($R^2 = 0.891$, $p < 0.01$) reaching a higher score than for LT storage after twelve weeks. From week 15 the difference between LT and HT stored samples amounted at least 1.0 points and after 46 weeks the HT stored juice failed to comply with the recommended standards.

The color alterations of the juice stored in PAC packages (see part B of figure 6.40) appeared very fast and almost irrespectively of the storage temperature. Thus, the juice color did not comply with the quality requirements after twelve (at HT storage) and fifteen weeks (at LT storage), respectively. There was no linear correlation observed neither for LT nor for HT stored samples.

Juice color was also evaluated by calculation of delta E values determined via CIE Lab system analysis. Figure 6.41 shows its rising during storage under

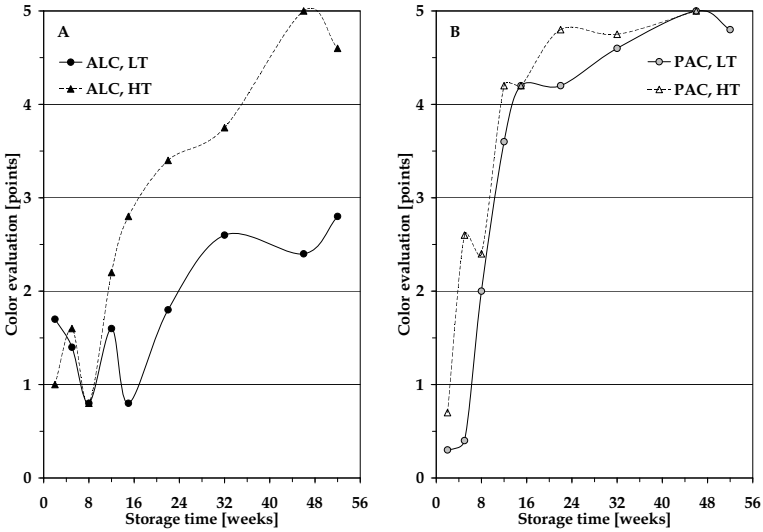


Figure 6.40.: Color evaluation of RG juice in ALC (A) and PAC (B) packages: Influence of storage time and temperature during one year of storage.

different conditions. The influences of packaging and storage temperature were obvious.

For storage in ALC packaging delta E values correlated linearly with time ($R^2 = 0.983$, $p < 0.01$ for LT and $R^2 = 0.844$, $p < 0.01$ for HT stored samples). Furthermore, LT storage in PAC package also showed linear correlation with time ($R^2 = 0.860$, $p < 0.01$). However, linear correlation was not observed for HT storage in PAC packaging.

While the sensory panel saw mostly no differences between the color of LT and HT stored juice in PAC packaging, the CIE Lab system analysis revealed significantly higher delta E values for HT stored samples. Furthermore, LT storage in PAC packages yielded delta E data comparable to HT stored samples in ALC package.

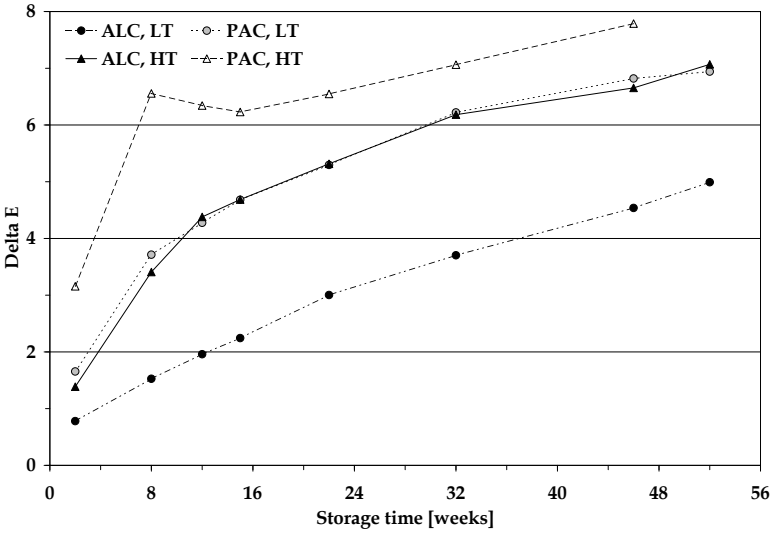


Figure 6.41.: Delta E measurement of RG juice: Influence of packaging, storage time and temperature during one year of storage.

In conclusion, storage temperature and oxygen permeation strongly influenced the optical characteristics of the RG juice, likely due to accelerated maillard reaction.

6.4.6.2. Changes in Taste

The graphs A and B in figure 6.42 illustrate the taste evaluation of the RG juice during storage under different conditions. In ALC packaging the LT stored juice complied with the demands on taste quality within one year of storage but taste changes did not correlate linearly with time.

However, linear correlation with time was observed for taste rating of HT stored samples in ALC packaging ($R^2 = 0.902$, $p < 0.01$). Until week 15 there was no clear differentiation between LT and HT stored samples in ALC packaging. Afterwards, the higher storage temperature caused faster deterioration of taste and thus, the RG juice failed to fulfil the recommended quality stan-

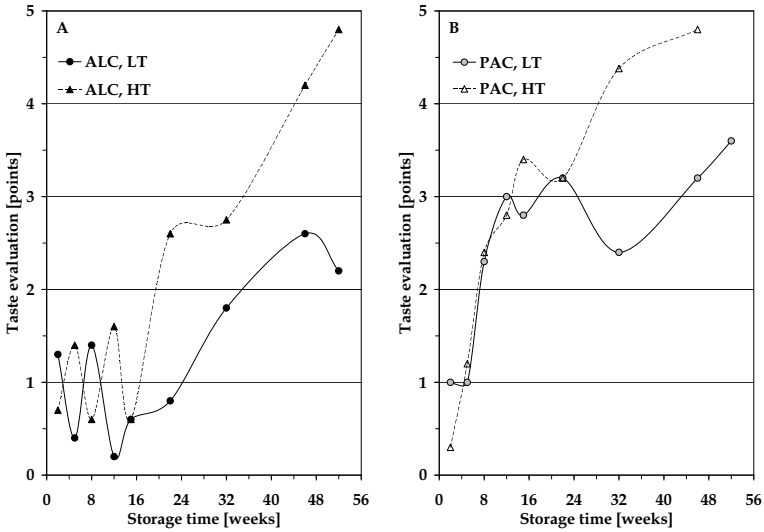


Figure 6.42.: Taste evaluation of RG juice in ALC (A) and PAC (B) packages: Influence of storage time and temperature during one year of storage.

dards after 46 weeks.

As shown in graph B of figure 6.42 the taste of the RG juice stored in PAC packages deteriorated faster than in ALC packaging, especially in the first 15 weeks of storage. Within this period the taste was rated equally regardless of storage temperature.

Subsequently, the taste of the LT stored juice in PAC package was judged "acceptable" until the end of storage whereas the corresponding HT stored samples reached the limit score of four after 32 weeks.

For LT storage in PAC packaging taste rating did not correlate linearly with time. However, for HT stored samples this parameter showed linear correlation with time ($R^2 = 0.814$, $p < 0.01$).

In conclusion, due to higher oxygen permeability PAC packaging caused faster deterioration of taste especially during the first 15 weeks. The influence

of storage temperature became apparent in both ALC and PAC packages during the second half of the storage period.

6.5. Conclusions of the Second Storage Study

The results of the second study observed the influences of storage time and temperature as well as oxygen permeability of the packaging on the juice quality of NCA, FA and RG juice during one year of storage.

Numerous parameters were monitored for detailed description of these juices: antioxidative capacity, its influencing antioxidants like ascorbic acid and polyphenols, HMF formation, the headspace volume, its oxygen content, the oxygen concentration in the juice and the sensory evaluation of color and taste. Furthermore, the weight of the filled package was surveyed and the color of the filtered juices (FA and RG juice) was additionally determined via CIE Lab system.

The overall influence of storage conditions on analysed parameters during one year of storage were illustrated in tabular form (see tables 6.6, 6.8, 6.10):

1. Column: The parameter observed,
2. Column: The main trend, i.e. the overall change of a parameter during one year of LT storage in ALC packaging,
3. Column: The influence of a higher storage temperature on the main trend,
4. Column: The influence of LT storage in PAC packaging (i.e. the higher oxygen permeability) on the main trend,
5. Column: Supporting or contrary influence of HT storage in PAC packaging on HT storage in ALC packaging,
6. Column: Supporting or contrary influence of HT storage in PAC packaging on LT storage in PAC packaging.

However, in case a parameter remained stable during storage in ALC package an alteration under different conditions is described using arrows as in the "main trend" column.

Furthermore, it was the aim of this study to clarify whether the juice quality was associated with its antioxidative status. Therefore, VARP data and the respective color and taste evaluations were correlated.

However, juice samples were not taken simultaneously at both laboratories. Therefore, it was necessary to correlate data of slightly divergent time points.

For example, the sensory evaluation of week 21 was related to the VARP results of week 24. The highest difference accounted for four weeks and was obtained for LT stored RG juice (VARP value at week 26 corresponded to delta E and sensory results of week 22).

The Pearson correlation coefficients (R) were calculated between color and taste measurements and the corresponding VARP data. There was a strong significant correlation in case of $|R| \geq 0.6$ and $p < 0.05$. Correlation was rated very strong for $|R| \geq 0.8$ and $p < 0.05$.

NCA juice

Table 6.6 illustrates which characteristics of the NCA juice changed within the storage period. For ALC packaging the higher storage temperature had a significant influence on most parameters. It either supported the main trend observed for LT storage or caused degradation when the main trend was stable. However, the weight and VARP value was not influenced neither by LT nor by HT storage.

Storage in PAC packaging influenced the juice quality in many respects. Already during LT storage oxygen permeation increased the reduction rate obtained for LT storage in ALC packaging. Furthermore, it even caused degradation of antioxidants which were stable in ALC package.

As a sum parameter VARP value was affected by the increased loss of antioxidants and thus, diminished during LT storage in PAC packaging. HT storage mostly caused an even faster deterioration. In conclusion, storage in PAC packaging was not appropriate for NCA juice under ordinary temperature condition (i.e. room temperature).

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
			Influence of		
Parameter	Main trend	Storage temp.		PAC packaging	
		(HT, ALC)	(LT, PAC)	(HT, PAC)	(HT, PAC)
		⇓	⇓	⇓	⇓
	(LT, ALC)	(LT, ALC)	(LT, ALC)	(HT, ALC)	(LT, PAC)
Weight	=	0	↓	↓	+
Headspace volume	↓	-	-	-	0
Oxygen in juice	↓	0	-	0	-
Chlorogenic acid	=	↓	↓	+	+
Phloridzin	=	↓	↓	+	+
Catechin	↓	+	+	+	+
Epicatechin	↓	+	+	+	0
Ascorbic acid	↓	+/-	+	+	+
HMF	↑	+	+	+	+
VARP	=	0	↓	↓	+
Color, sensory	↑	+	+	+	0
Taste, sensory	↑	+	+	+	+
= stable		0 no influence			
↑ rise		+ supporting influence			
↓ reduction		- contrary influence			
		+/- equivocal influence			

Table 6.6.: Overall influence of storage conditions on NCA juice parameters during one year of storage.

R values for NCA juice are presented in table 6.7. They revealed strong negative correlations between VARP data and sensory evaluations of the NCA juice under all storage conditions tested. For storage in PAC packaging very strong correlation was observed. The finding indicated that antioxidative capacity was associated with the sensory color and taste evaluation of NCA juice.

Storage conditions		Correlation between VARP data and	
Temp.	Packaging	Color, sensory	Taste, sensory
LT	ALC	-0.657	-0.593
HT	ALC	-0.831	-0.718
LT	PAC	-0.841	-0.980
HT	PAC	-0.874	-0.846

Table 6.7.: Pearson correlation coefficients for NCA juice.

FA juice

The overall alterations during storage of FA juice are presented in table 6.8. FA juice contained less antioxidants of the NCA juice which were already susceptible to degradation during storage under ordinary conditions (i.e. ascorbic acid and flavan-3-ols). Therefore, it was less affected by higher storage temperature or oxygen permeation.

The VARP value of the LT stored juice in PAC packaging was lower than the respective data for ALC package. However, unlike NCA juice its trend remained stable during LT storage and no continuous decline was observed in PAC packaging. Furthermore, comparison of VARP data from HT storage revealed no significant influence of packaging.

Together with the lack of oxygen influence on the sensory evaluation of the LT stored juice, this was a rather unexpected outcome of the storage study. It indicated that storage stability was sufficient for FA juice filled in PAC packaging.

This finding was remarkable as sales figures showed that apple juice (i.e. mainly filtered apple juice) was the most consumed fruit juice in Germany (12 L of 40 L per capita consumption [75]). The PAC packaging was cheaper to produce due to the absence of an aluminium layer. Thus, a considerable cost advantage could be obtained by filling FA juice in PAC packaging.

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
			Influence of		
Parameter	Main trend	Storage temp.		PAC packaging	
		(HT, ALC)	(LT, PAC)	(HT, PAC)	(HT, PAC)
		⇓	⇓	⇓	⇓
	(LT, ALC)	(LT, ALC)	(LT, ALC)	(HT, ALC)	(LT, PAC)
Weight	=	0	↓	↓	+
Headspace volume	↓	0	0	0	0
Oxygen in juice	↓	0	-	-	-
Chlorogenic acid	=	↓	↓	+	+
Phloridzin	=	↓	↓	+/-	+
HMF	↑	+	-	0	-
VARP	=	n. d.	↓	0	n. d.
Color, sensory	↑	+	0	+	+
Color, Delta E	=	↑	↑	+	+
Taste, sensory	↑	0	0	+	+
= stable		0 no influence			
↑ rise		+ supporting influence			
↓ reduction		- contrary influence			
n. d. not determined		+/- equivocal influence			

Table 6.8.: Overall influence of storage conditions on FA juice parameters during one year of storage.

Table 6.9 gives possible correlations between VARP results and the alteration of color and taste evaluation during storage of FA juice. Due to the fact that in many respects this juice was less affected by storage, strong correlations were more unlikely to occur than for both other juices.

Indeed, for LT storage in ALC packaging the small increase of the VARP value during storage (refer to figure 6.23 on page 105) even caused a positive R value which was considered not to be reliable. Furthermore, R values calculated for HT storage showed no strong correlation. The only (very) strong correlations were observed for LT stored juice in PAC packaging.

Storage conditions		Correlation between VARP data and		
Temp.	Packaging	Color, delta E	Color, sensory	Taste, sensory
LT	ALC	0.599	0.466	0.584
HT	ALC	-0.279	-0.442	-0.619
LT	PAC	-0.923	-0.886	-0.666
HT	PAC	-0.395	-0.157	0.036

Table 6.9.: Pearson correlation coefficients for FA juice.

RG juice

Table 6.10 shows the overall changes of the RG juice within the storage period. Regarding storage in ALC packaging temperature had a significant influence on single compounds (e.g. anthocyanins, catechin, HMF) as well as on sum parameter like sensory evaluation, VARP and delta E values. In general, gallic acid was the only compound which was not affected neither by storage temperature nor by higher oxygen permeation.

In accordance with the oxygen influence on NCA juice, the PAC packaging caused a sharper decline of antioxidants (catechin, anthocyanins, VARP value) in LT stored RG juice. It also led to stronger deterioration of color and taste.

HT storage accelerated the alteration of most parameters though it was not always possible to clearly determine the effect (e.g. due to inconsistent VARP data or too fast occurrence of complete loss of anthocyanins). Thus, under usual temperature condition storage in PAC packaging could not be recommended for RG juice.

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
			Influence of		
Parameter	Main trend	Storage temp.		PAC packaging	
		(HT, ALC)	(LT, PAC)	(HT, PAC)	(HT, PAC)
		⇓	⇓	⇓	⇓
	(LT, ALC)	(LT, ALC)	(LT, ALC)	(HT, ALC)	(LT, PAC)
Weight	=	0	↓	↓	+
Headspace volume	=	0	0	↓	↓
Oxygen in headspace	↓	0	-	0	-
Oxygen in juice	↓	0	-	0	-
Anthocyanins	↓	+	+	n. d.	+
Gallic acid	=	0	0	0	0
Catechin	↓	+	+	+	+
HMF	↑	+	-	-	+
VARP	=	↓	↓	+/-	+/-
Color, sensory	↑	+	+	+	0
Color, Delta E	↑	+	+	+	+
Taste, sensory	↑	+	+	+	+
= stable		0 no influence			
↑ rise		+ supporting influence			
↓ reduction		- contrary influence			
n. d. not determined		+/- equivocal influence			

Table 6.10.: Overall influence of storage conditions on RG juice parameters during one year of storage.

R data calculated for RG juice are presented in table 6.11. The results showed mainly strong and very strong negative correlations between the VARP data and the sensory evaluations of the RG juice. The low values calculated for HT stored juice in PAC packaging were considered to result from the corresponding inconsistent VARP data (refer to figure 6.39 on page 124). However, the further seen correlations indicated that antioxidative capacity was associated with rating of taste and color (determined via sensory analysis and delta E value) of RG juice.

Storage conditions		Correlation between VARP data and		
Temp.	Packaging	Color, delta E	Color, sensory	Taste, sensory
LT	ALC	-0.967	-0.814	-0.804
HT	ALC	-0.778	-0.684	-0.848
LT	PAC	-0.931	-0.830	-0.732
HT	PAC	-0.443	0.037	0.125

Table 6.11.: Pearson correlation coefficients for RG juice.

7. Overall Conclusion and Outlook

The Storage Studies

The above presented storage studies provided extensive knowledge about the stability of commercial grape and apple juices during storage under different conditions. Deterioration of each juice was observed periodically during storage over a usual period of shelf-life. Sensory quality as well as antioxidative capacity and concentrations of single juice compounds (especially antioxidants) were determined to clarify whether the antioxidative status of a juice could provide useful information concerning its storage stability.

Due to their high reactivity it was assumed that antioxidants would undergo degradation soon during storage and thus, would lower the antioxidative capacity of the juice. Indeed, the results of the first study showed that several compounds altered during storage. However, only minor effects on the antioxidative capacity were observed. Furthermore, the sensory deterioration of the juices occurred slowly.

Thus, it was concluded that under ordinary conditions the storage stability of the juices avoided to determine whether antioxidative characteristics were correlated with juice quality. Therefore, a second study was conducted taking into account a higher storage temperature and packaging with considerable oxygen permeability.

For RG and NCA juice the oxygen permeation was the dominant factor for sensory evaluation and thus, caused fast deterioration. The most oxygen-sensitive compounds were anthocyanins and catechin in RG juice and ascorbic acid as well as flavan-3-ols in NCA juice.

Due to the fact that FA juice contained neither flavan-3-ols nor ascorbic acid its low antioxidative capacity was less affected by storage temperature and oxygen permeation. Findings indicated that FA juice filled in PAC packaging might yield adequate storage stability under ordinary conditions. The use of this packaging could reduce the production costs since the aluminium foil accounts for a considerable share.

The VARP Assay

Furthermore, a new method was developed for fast determination of antioxidative capacity. The voltammographic analysis of the reducing potential (VARP) assay was based on the coulometric electrode array detector (CEAD). This method used the CEAD without prior HPLC separation and calculated the

area under the resulting voltammogram to describe the antioxidative capacity of a sample.

Though external standards (trolox and catechin) were applied to calibrate the analysis, the VARP results depended significantly on the CEAD performance. Thus, absolute VARP values of temporary divergent measurements could not be compared directly. However, the calculation of ratios between contemporaneously analysed juices provided further information.

Strong correlations were revealed between VARP data and sensory evaluations of NCA and RG juice under different storage conditions. For juices with considerable amounts of antioxidants this finding indicated that antioxidative capacity was associated with sensory color and taste evaluation.

In conclusion, the VARP assay was a fast, simple and sensitive method to provide useful information concerning the antioxidative status of juice samples. Though it did not supersede the sensory analysis of juice samples during a storage study it mostly supported the sensory results. Variations and abnormal trends observed for color and taste ratings point out that further methods like the VARP assay are needed to improve the evaluation of juice quality.

Furthermore, variations in sensory results might be minimised by implementation of a linear instead of an integer scale. First experiences by the laboratory of the packaging company SIG combibloc showed that a linear scale reduced the variations within a sensory panel as well as between temporary divergent evaluations of a reference sample.

Outlook

The second study showed which influences caused a significant decrease of the antioxidative capacity of a juice during storage. However, this decrease did not proceed as fast as expected. Though the juices with high antioxidative capacity (i.e. NCA and RG juice) demonstrated rapid and complete loss of some antioxidants the VARP value did not diminish by a similar extent.

Thus, it could be of interest to find out under which storage conditions a complete reduction of the VARP value would occur. Furthermore, the changes of the juice compounds which influence the VARP value should be observed in detail. Therefore, for the next storage study model juices should be designed which consist of a known amount of different antioxidants together with usual juice compounds (e.g. sugar and fruit acids). Alterations of these samples

might further elucidate the relation between sensory deterioration and antioxidative capacity.

Concerning the VARP assay, the set-up of a data base including numerous different fruit juices has just been started. First results indicate that comparable VARP values are obtained for juices of one kind from different producers. In case this trend will be approved by further tests the VARP assay might be used for verification of fruit contents for single fruit nectars.

In addition, further research could clarify the comparability of VARP to other methods to determine the antioxidative capacity (beyond TOSC and TEAC assay). Furthermore, the VARP assay could be modified to test antioxidative capacity in matrices other than juice (e.g. plant extracts, human plasma).

Appendix

Appendix A.

Abbreviations

ABAP	2,2'-azobis(2-methylpropionamidine) dichloride
ACE juice	mixed fruit juice, containing vitamin C, E and provitamin A (i.e. β-carotene)
ALC	aluminium laminated composite
AU	UV absorption units
CEAD	coulometric electrode array detection
ch.	Channel
CIE	International Commission on Illumination
Cya-3-gluc	Cyanidin-3-glucoside
DAD	diode array detector
Del-3-gluc	Delphinidin-3-glucoside
Delta E	euclidean color distance in the CIE Lab color space
DHAA	dehydroascorbic acid
DIN	German Institute for Standardization
DPHH	1,1-diphenyl-2-picrylhydrazyl assay
DTPA	diethylenetriaminepentaacetic acid
e.g.	exempli gratia (for example)
ESI	electrospray interface
FA juice	filtered apple juice

FRAP	ferric reducing/antioxidant power assay
GC	gas chromatography
HMF	hydroxymethylfurfural
HPLC	high-performance liquid chromatography
HT	high temperature storage
i. d.	inside diameter
i.e.	id est (that is)
IEL	Department of Nutrition and Food Sciences
ISO	International Organization for Standardization
KMBA	α-keto-γ-methiolbutyric acid
Lab	coordinates of the Hunter L, a, b color space
LC	liquid chromatography
LDPE	low density polyethylene
LOQ	limit of quantification
LT	low temperature storage
Mal-3-gluc	Malvidin-3-glucoside
MS	mass spectrometry
NCA juice	naturally cloudy apple juice
n. d.	not determined
nod	no degradation
ORAC	oxygen radical absorbance capacity assay
p	probability of error
PAC	polyamide composite, aluminium free

Peo-3-gluc	Peonidin-3-glucoside
PET	polyester
Pet-3-gluc	Petunidin-3-glucoside
PPO	polyphenol oxidase
R	Pearson correlation coefficient
R^2	coefficient of determination
RG juice	red grape juice
ROS	reactive oxygen species
RP	reversed phase
SD	standard deviation
TE	trolox equivalents
TEAC	trolox equivalent antioxidant capacity
TFA	tri-fluor acetic acid
TOSC	total oxidant scavenging capacity
trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxyl acid
UHQ	ultra high quality
UPA	unweighted partial areas under the voltammogram
UV	ultraviolet
v	volume
VARP	voltammographic analysis of the reducing potential
Vis	visible light
WPA	weighted partial areas under the voltammogram

Appendix B.

Optimised HPLC Gradient Elution Programs

Time [min]	Rate B [%]	Description
0	0	
2	0	injection + start file
7	0	
19	17	
35	22	
37	33	
52	33	
54	90	washing step
62	90	
64	0	re-equilibrating
67	0	stop file
109	0	

Table B.1.: Gradient elution program for quantification of polyphenols and HMF by HPLC-UV-CEAD for juices of the first storage study

Time [min]	Rate B [%]	Description
0	1	
2	1	injection + start file
11	4	
19	9	
25	9	
32	16	
36	17	
43	17	
45	18	
49	18	
55	20	
57	20	
59	31	
61	32	
63	46	
64	47	
68	47	
70	90	washing step
78	90	
80	1	re-equilibrating
83	1	stop file
120	1	

Table B.2.: Gradient elution program for quantification of polyphenols and HMF by HPLC-UV-CEAD for NCA juice of the second storage study

Time [min]	Rate B [%]	Description
0	1	
2	1	injection + start file
6	1	
16	6	
20	7	
28	7	
39	14	
44	16	
52	16	
61	19	
62	19	
68	57	
73	57	
75	90	washing step
82	90	
84	1	re-equilibrating
87	1	stop file
120	1	

Table B.3.: Gradient elution program for quantification of polyphenols and HMF by HPLC-UV-CEAD for FA juice of the second storage study

Time [min]	Rate B [%]	Description
0	1	
2	1	injection + start file
20	5	
23	5	
24	6	
28	7	
38	9	
41	13	
48	15	
51	15	
54	16	
69	22	
76	38	
79	42	
82	42	
83	90	washing step
90	90	
92	1	re-equilibrating
94	1	stop file
130	1	

Table B.4.: Gradient elution program for quantification of polyphenols and HMF by HPLC-UV-CEAD for RG juice of the second storage study

Appendix C.
Additional Results of the First Storage Study

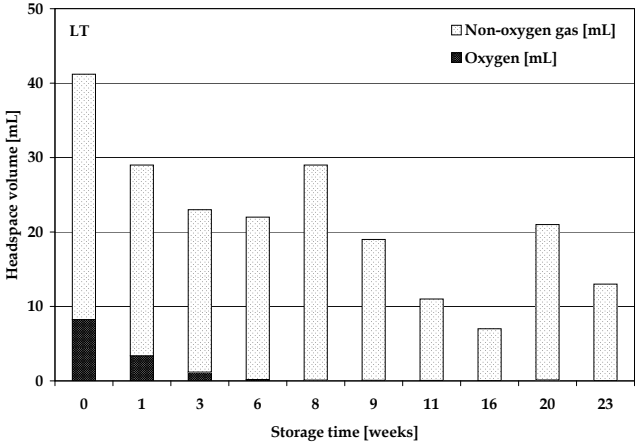


Figure C.1.: Headspace atmosphere of FA juice packaging: Influence of storage time during one year of LT storage.

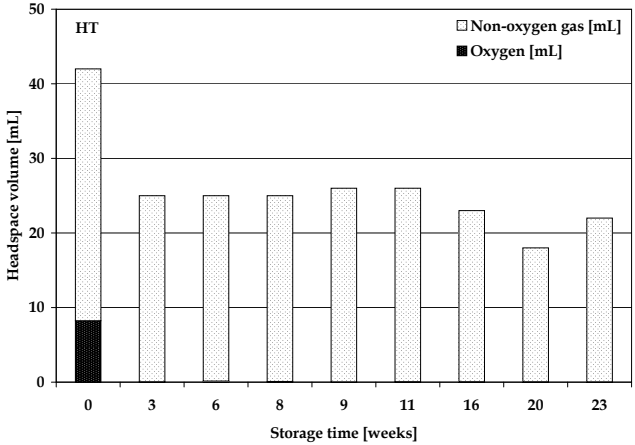


Figure C.2.: Headspace atmosphere of NCA juice packaging: Influence of storage time during one year of HT storage.

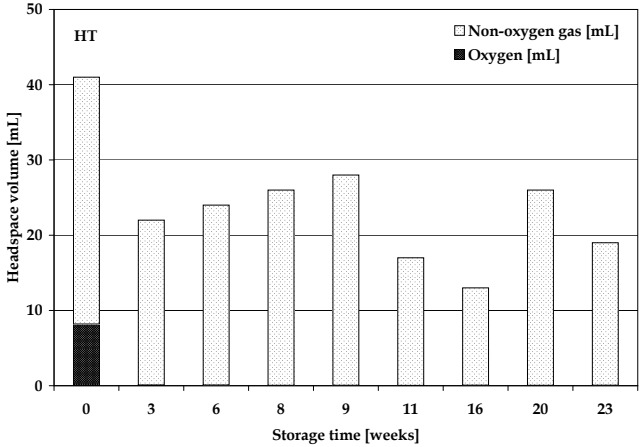


Figure C.3.: Headspace atmosphere of FA juice packaging: Influence of storage time during one year of HT storage.

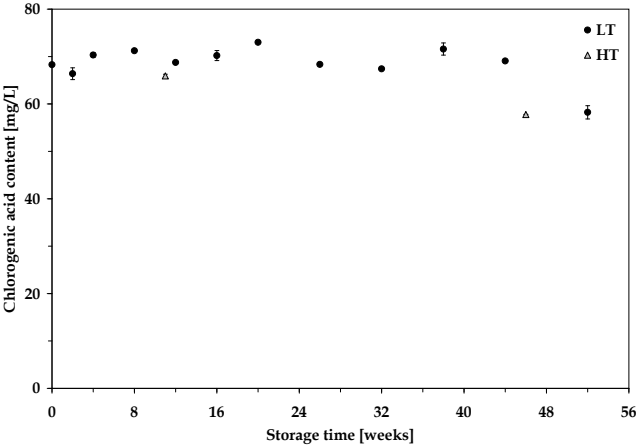


Figure C.4.: Chlorogenic acid in FA juice: Influence of storage time and temperature during one year of storage.

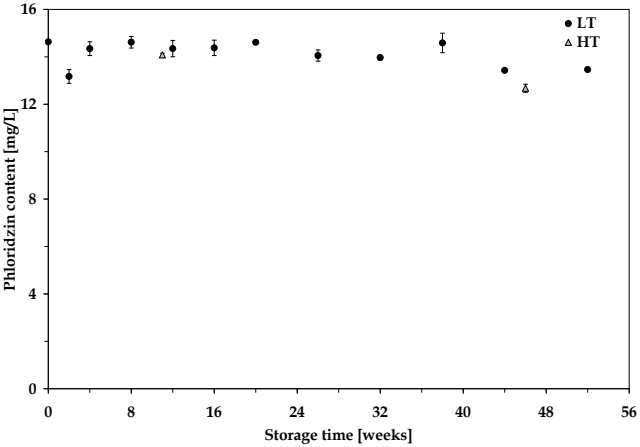


Figure C.5.: Phloridzin in FA juice: Influence of storage time and temperature during one year of storage.

Storage time [weeks]	Storage temperature	Degradation of Phloridzin [%]
0	LT	nod
38	LT	nod
44	LT	8.2
52	LT	8.0
11	HT	3.8
46	HT	13.4

nod: no degradation

Table C.1.: Phloridzin in FA juice at different points of storage

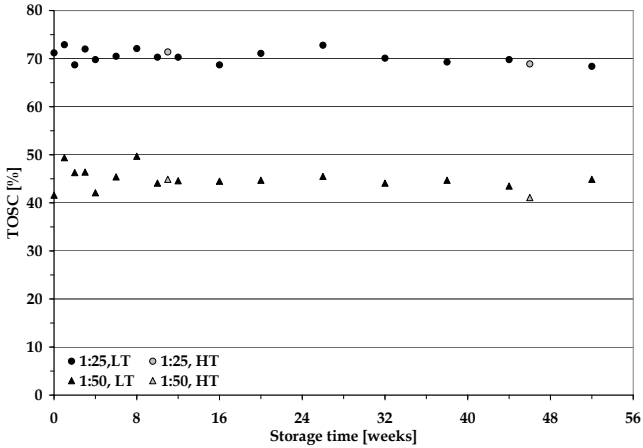


Figure C.6.: TOSC data of FA juice: Influence of storage time and temperature on the antioxidative capacity (TOSC against peroxyl radical) in one year of storage.

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