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Stability of Selected Nutrients in Home-Made Fruit Juices under Oxygen-Free Storage Conditions

Comparison of different juice extractors

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Zusammenfassung

Das Ziel dieser Masterarbeit war, den Einfluss von Sauerstoff auf die Stabilität von ausgewählten Nährstoffen in selbstgepressten Fruchtsäften zu analysieren. Dazu wurden die Säfte zuerst unter Sauerstoffatmosphäre für 24 Stunden bei 4 °C im Kühlschrank gelagert. Anschließend wurden die Säfte für den gleichen Zeitraum bei 4 °C unter Stickstoffatmosphäre in Exsikkatoren gelagert, um die Stabilität der Nährstoffe unter sauerstofffreien Lagerbedingungen zu untersuchen. Es wurden Säfte von verschiedenen Obst- und Gemüsesorten (Orange, Erdbeere, Karotte und rote Traube), sowie drei verschiedene Entsafter-Modelle (Zentrifuge, Standmixer, Schneckenpresse) verglichen. Folgende Nährstoffe und Parameter wurden untersucht: Vitamin C, 5-Methyltetrahydrofolat, Vitamin B_6, Gesamt-Phenolgehalt, Anthocyane und Hesperidin. Zusätzlich wurde eine Keimzahlbestimmung durchgeführt, um die Koloniezahlen von Hefen, Schimmel und Bakterien zu untersuchen.

Zusammenfassend lässt sich sagen, dass Vitamin C und Hesperidin über 24 Stunden unter Sauerstoffatmosphäre nicht stabil waren, wobei die Stabilität durch Sauerstoffausschluss erhöht wurde. Alle anderen gemessenen Parameter zeigten kaum einen Konzentrationsabfall über 24 Stunden.

Es wurde kein optimaler Entsafter für alle Obst- und Gemüsesorten und Nährwerte gefunden, bei allen Modellen gibt es Vor- und Nachteile.

Die höchsten **Vitamin C**-Konzentrationen in Orangen- und Erdbeersaft waren 50,2 und 50,0 mg/100 ml und wurden in Säften der Schneckenpresse bzw. der Zentrifuge gefunden. In den Säften des Standmixers sank der Vitamin C -Gehalt unter Sauerstofflagerung um 70 % bzw. 80 %. Auch in den Säften der anderen Entsafter kam es zu einem Abbau der Konzentration während der Lagerung unter Sauerstoff, wobei die Lagerung unter Stickstoff zu einer stabilen Vitamin C-Konzentration führte.

Der Gehalt an **5-Methyltetrahydrofolat** war sowohl in den Orangen- als auch in den Erdbeersäften unter Sauerstoffbedingungen über 24 Stunden stabil. Der höchste Gehalt in Orangesaft wurde mit dem Standmixer erzielt (25,4 mg/100 ml) und der höchste Gehalt in Erdbeersaft mit der Zentrifuge (771 mg/100 ml).

Die Konzentration von **Vitamin B_6** in Karottensaft war sowohl unter Sauerstoff, als auch

unter Stickstoffatmosphäre stabil. Der höchste Gehalt wurde in Säften der Zentrifuge nachgewiesen (177 mg/100 ml).

Der **Gesamt-Phenolgehalt** in Erdbeer-, Karotte- und Traubensaft war unter beiden Lagerbedingungen stabil. Die höchste Konzentration in Erdbeersaft war 366 mg/100 ml, in Karottensaft 32,2 mg/100 ml und in Traubensaft 45,0 mg/100 ml und wurde jeweils in Säften des Standmixers gefunden.

Der Gehalt an **Anthocyanen** in Erdbeer- und Traubensaft war unter Sauerstoff- und Stickstoffbedingungen stabil. Bei Erdbeersaft erzielten alle drei Entsafter sehr ähnliche Werte, während der Anthocyangehalt in Traubensäften des Standmixers doppelt so hoch wie in den Säften der Zentrifuge und der Schneckenpresse war.

Unter Sauerstofflagerung sank die **Hesperidin**-Konzentration in Orangensäften bei allen Entsaftern. Die höchste gemessene Konzentration betrug 108 mg/100 ml und wurde in Säften des Standmixers nachgewiesen, wobei bei diesem über 24 Stunden 60 % der ursprünglichen Konzentration abgebaut wurden. Die Lagerung unter Stickstoff führte zu einer stabilen Konzentration in allen Orangensäften.

Die durchschnittliche **Lagertemperatur** im Kühlschrank wurde mit 3,9 ± 4,7 °C errechnet. Die Bestimmung der **Keimzahlen** ergab eine hohe bakterielle Belastung der Karottensäfte aller Entsafter. Die höchsten Werte lagen bei über 300.000 koloniebildenden Einheiten (CFU)/ml. Die meisten **Bakterienkolonien** in Orangen-, Erdbeer- und Weintraubensaft wurden in Säften der Zentrifuge gefunden, wobei der höchste Wert bei 24.000 CFU/ml lag. Die höchsten Werte bezüglich **Hefekolonien** wurden in Karottensäften der Schneckenpresse gefunden (151.000 CFU/ml). Bei allen anderen Früchten zeigten wieder die Säfte der Zentrifuge die höchsten Kontaminationen. **Schimmelpilze** wurden nur in den Weintraubensäften der Schneckenpresse nachgewiesen (14.000 CFU pro ml). Die Lagerung unter Stickstoffatmosphäre führte sowohl bei Bakterien als auch bei Hefen zu einem verminderten Wachstum.

Abstract

Aim of this master thesis was to analyse the influence of oxygen on the stability of selected nutrients in home-made fruit juices. Therefore, the juices were either stored for 24 hours at 4 °C under oxygen atmosphere or under nitrogen atmosphere in desiccators. Juices from different fruits and vegetables (orange, strawberry, carrot and red grape) and three different juice extractors (centrifuge, blender, and masticating juicer) were compared.

The following nutrients and parameters were analysed: vitamin C, 5-methyltetrahydrofolate, vitamin B_6, total phenolic content, anthocyanins, and hesperidin. In addition, the colony forming units of yeasts, moulds, and bacteria were determined.

There was no optimal juicer for all fruits and vegetables, as every device has its advantages and disadvantages.

All in all it can be said that vitamin C and hesperidin were not stable over 24 hours when stored under oxygen atmosphere, whereas the stability was enhanced by oxygen exclusion. The other parameters measured were stable for 24 hours.

The highest **vitamin C** concentrations in orange- and strawberry juice were 50.2 and 50.0 mg/100 ml and were found in juices from the masticating juicer and the centrifuge. The vitamin C content in juices from the blender was reduced by 70 % and 80 % during the storage under oxygen. When stored under oxygen, a degradation of the vitamin C content was detected in juices from all three extractors, while storing under nitrogen led to an enhanced stability.

The content of **5-methyltetrahydrofolate** in orange and strawberry juice was stable over 24 hours when exposed to oxygen. The highest content in orange juice was achieved by using the blender (25.4 mg/100 ml) and the highest content in strawberry juice was achieved by using the centrifuge (771 mg/100 ml).

The **vitamin B_6** content in carrot juice was stable under oxygen and nitrogen atmosphere. The highest content was detected in juices from the centrifuge (177 mg/100 ml).

The **total phenolic content** in strawberry, carrot and grape juice was stable under both storage conditions. The highest concentration in strawberry juice was 366 mg/100 ml, in carrot juice 32.3 mg/100 ml and in grape juice 45.0 mg/100 ml. They were all detected in juices processed with the help of the blender.

The content of **anthocyanins** in strawberry and grape juice was stable under oxygen and nitrogen storage conditions. The obtained values of the three juicers were similar for strawberry juice, while the anthocyanin content in grape juices from the blender was twice as high as in juices from the centrifuge and the masticating juicer.

The **hesperidin** concentration decreased in orange juices from all three extractors when stored under oxygen. The highest detected concentration was 108 mg/100 ml, and was found in juices from the blender, whereas 60 % of the initial content was degraded over 24 hours. Storing under nitrogen atmosphere enhanced the stability during the storage period. The average **storage temperature** in domestic fridges was assumed to be 3.9 ± 4.7 °C.

Carrot juices from all three juice extractors showed high contaminations of **bacteria** with values greater than 300,000 colony forming units (CFU)/ml. Juices from the centrifuge showed the highest bacterial growth in orange, strawberry and grape juice, with values up to 24,000 CFU/ml. The highest number of **yeasts** was found in carrot juices from the masticating juicer (151,000 CFU/ml), whereas for all other fruits the highest contamination was, again, found in juices from the centrifuge. **Moulds** were only detected in significant amounts in grape juices from the masticating juicer (14,000 CFU/ml). Storing under nitrogen led to a reduced growth of both, bacteria and yeasts, in all juices.

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IV. List of abbreviations

5-Me-THF	5-methyltetrahydrofolate
CFU	colony forming units
DHAA	dehydroascorbic acid
DHF	7,8 dihyrofolate
DNA	deoxyribonucleic acid
DTT	dithiothreitol
DXP	deoxyxylose 5-phosphate
HMDHP	hydroxymethyldihydropterin
HPLC	high performance liquid chromatography
LU	luminescence unit
Mal-3-glc	malvidin-3-glucoside
mAU	milli absorbance unit
MeOH	methanol
PC	plate count
PL	pyridoxal
Pl-3-glc	pelonidin-3-glucoside
Pl-3-malonyl-glc	pelonidin-3-malonyl-glucoside
PLP	pyridoxal-5-phosphate
PM	pyridoxamine
PMP	pyridoxamine-5-phopshate
PN	pyridoxine
Pt-3-glc	petunidin-3-glucoside
Pt-3-O-(6-p-c)-glc	petunidin-3-O-(6-p-coumaroyl)-glucoside
RP-HPLC	reversed-phase high performance liquid chromatography
SAX	strong anion exchange

SPE	solid-phase extraction
THF	5,6,7,8 tetrahydrofolate
TPC	total phenolic content
TRIS	tris(hydroxymethyl)-aminomethan
YGC	yeast extract glucose amphenicol

1. Introduction

1.1 Vitamin C

Chemical structure

Vitamin C is a general term used for all compounds that qualitatively obtain the biological effects of ascorbic acid. The natural occurring compound exhibiting vitamin C activity is called L-ascorbic acid and belongs to the group of water-soluble vitamins. The systematic name of L-ascorbic acid is L-threo-2-hexenono-1,4-lactone (Ball, 1994). There are four possible enantiomers: L- and D-ascorbic acid as well as L- and D-isoascorbic acid (Velisek, 2014). The structures of L-ascorbic acid and its oxidation product dehydroascorbic acid (DHAA) are depicted in Figure 1.

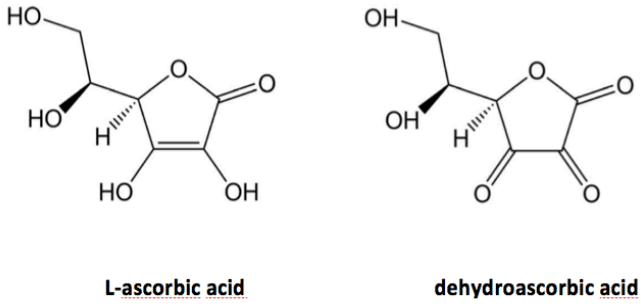


Figure 1. Chemical structure of L-ascorbic acid and dehydroascorbic acid (Adapted from Carr & Vissers, 2013)

Physical properties

Ascorbic acid is sensitive to light, high temperatures and oxygen; therefore, it is one of the least stable vitamins. Also, the pH value of food influences the stability, whereas vitamin C exhibits the greatest stability between pH 4 and 6 (Naidu, 2003).

As vitamin C is an electron donor, it can act as a reducing agent by donating its two electrons, and therefore, it prevents other compounds from oxidation. During this reaction, vitamin C itself gets oxidized and thus is called an antioxidant. The electron donation occurs sequentially (Velisek, 2014). The loss of one electron results in the formation of the relatively

stable and unreactive ascorbyl radical. By losing the second electron, dehydroascorbic acid (DHAA) and hydrogen peroxide are formed.

DHAA possesses the same biological vitamin activity as ascorbic acid and its stability strongly depends on the surrounding temperature and pH value (Coultate, 2016). In humans, DHAA can be partially reduced back to ascorbic acid by enzymes or biological reducing compounds (e.g. glutathione). Irreversible cleavage of the lactone ring leads to the formation of 2,3-diketogulonic acid (Padayatty *et al.*, 2003).

Vitamin C synthesis and metabolism

All phototrophic plants are able to synthesize ascorbic acid (Velisek, 2014), and also most mammalian species are able to produce ascorbic acid from D-glucose or D-galactose via the glucuronic acid pathways in the liver. Exceptions are humans, non-human primates, some fruit-eating bats and guinea pigs. Due to a mutation, the latter lack the gene for the enzyme gulonolactone oxidase, which synthesizes ascorbic acid from the precursor 2-keto-1-gulonolactone (Padayatty *et al.*, 2003). Therefore, humans have to ingest sufficient amounts of ascorbic acid with food to avoid vitamin C deficiency diseases. Presently, the recommended dietary intake for adults is defined as 95 – 110 mg ascorbic acid/day for adults (Deutsche Gesellschaft für Ernährung e.V., 2017).

Ascorbic acid is mainly metabolized to dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid, which is then excreted through urine (Naidu, 2003). In case extensive amounts of vitamin C are consumed, it remains unchanged and is excreted in the urine. Nevertheless, high doses of ascorbic acid can cause diarrhoea and gastrointestinal disturbances (Anderson *et al.*, 1997).

Physiological functions

In the human body, ascorbic acid is mainly involved in hydroxylation reactions. It acts as co-factor for monooxygenase and hydroxylase enzymes, which are, for example, involved in the synthesis of collagen, neurotransmitters and carnitine (Naidu, 2003).

Collagen is part of the skin, teeth and blood vessels and contributes one third of the total proteins in the human body. During collagen synthesis the vitamin C-dependent enzyme prolyl-4-hydroxylase is required for proper formation and cross-linking of collagen fibres (Velisek, 2014). Failures in connective tissue formation often result in scurvy, which is one of

the best-investigated ascorbic acid deficiency diseases. Typical symptoms of scurvy are gum bleeding, internal bleeding, poor wound healing and in advanced stages bone pain, bone fractures and also behavioural disturbances. The latter conditions can probably be linked to a reduced activity of the vitamin C-dependent enzyme dopamine hydroxylase. This enzyme is responsible for the conversion of the neurotransmitter dopamine to noradrenaline (Coultate, 2016).

The biosynthesis of carnitine, which is needed for the transport of activated fatty acids into mitochondria for energy production, also includes two vitamin C-dependent hydroxylation steps. *In vitro* studies showed that the activity of the involved enzymes is stimulated in the presence of ascorbic acid. Consequently, a lack of ascorbic acid could decrease the synthesis of carnitine and the oxidation rate of fatty acids in muscles and other tissues, resulting in exhaustion and weariness (Englard & Seifter, 1986).

Other biological functions of vitamin C include the absorption of inorganic iron, enhancement of the immune system and reduction of the risk of arteriosclerosis and possibly some forms of cancer (Harris, 1996).

Vitamin C - sources and stability

More than 90 % of the daily vitamin C requirements are covered by fruits and vegetables (Ball, 1994). Rose hips, blackcurrants, citrus fruits and broccoli are particularly rich in vitamin C (Coultate, 2016). Due to their high consumption, potatoes are also an important source of ascorbic acid, although they only contain a moderate amount of vitamin C (Ball, 1994). Further examples are listed in Table 1.

Table 1. Ascorbic acid content of some selected fruits and vegetables. Values are given in milligram ascorbic acid/100 gram edible fresh fruit

	Ascorbic acid content	Reference
Carrots	5 – 10	Velisek, 2014
Oranges	30 – 60	Velisek, 2014
Potatoes	30	Coultate, 2016
Red grapes	3.2	California Table Grape Commission Vitamin C in grapes, 1999
Rose hips	1000	Coultate, 2016
Strawberries	60	Coultate, 2016

Nevertheless, the content of vitamin C strongly depends on the species and cultivars as well as on climatic conditions and cultural practice (Weston & Barth, 1997). Critical factors also include the maturity at the harvest time, the method of harvesting and post-harvest handling (Kader, 1988).

Loss of vitamin C is favoured during long periods of storage, higher temperature treatments, industrial processing, chilling and cooking procedures. Also the presence of oxygen and heavy metal ions, like Cu_2^+ and Fe_3^+, as well as alkaline conditions enhance the oxidation of ascorbic acid (Parviainen & Nyyssonen, 1992). In general, the stability of ascorbic acid is higher in fruits than in vegetables, as the pH is usually lower in fruits (Velisek, 2014).

In plants, the copper dependent enzyme ascorbate oxidase was identified to be the major reason for enzymatic degradation of ascorbic acid (Saari *et al.*, 1995). Ascorbate oxidase is mainly present in fast-growing regions of plants, where it is either bound to the cell walls or present as soluble protein in the cytosol. Stress caused by chemical exposure or pathogenic infestation increases the ascorbate oxidase level in plants (Loewus & Loewus, 1987). The ascorbate oxidase is completely inactivated at 65 °C; therefore, rapid heating of fruits and vegetables, as well as pasteurization of fruit juices prevents the loss of vitamin C during storage (Bender, 1979).

Industrial applications of ascorbic acid

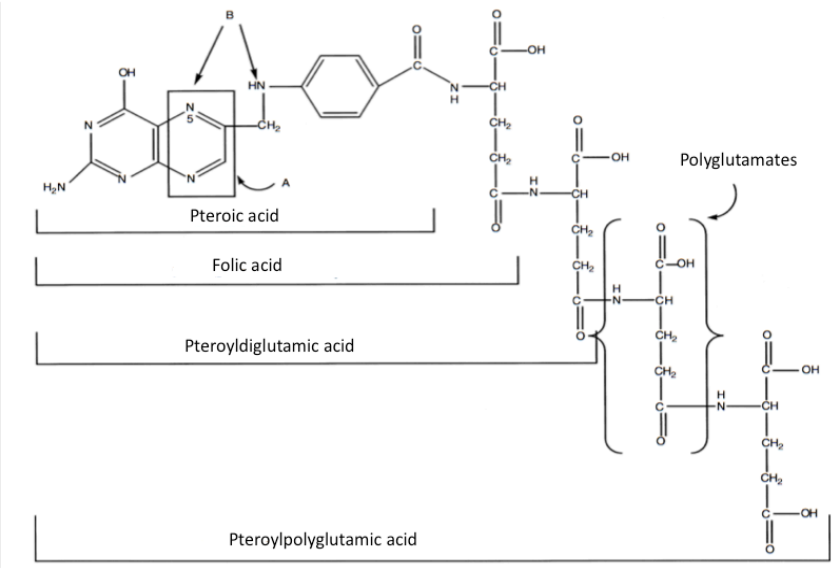
In the food industry, ascorbic acid is used for a wide range of purposes. For example, it is added to fruit beverages to preserve the aroma of fruits and the colouring by preventing enzymatic browning. Furthermore, if drinks are required to contain a certain amount of vitamin C, the losses that occur during processing and storage can be compensated. Also, the nutritive value of fruit juices, like apple or grape that naturally contain little or no vitamin C, can be improved by adding ascorbic acid. Other applications in the food industry include wine and beer making, where ascorbic acid promotes turbidity (Ball, 1994) and bread making, where it improves dough handling and the texture of bread (Elkassabany *et al*., 1980).

1.2 Folates

Chemical structure

Folates are a group of water-soluble B-vitamins that are structurally related to folic acid (pteroylmonoglutamic acid) and exhibit the same biological function (Jastrebova, 2003). Folic acid consists of a bicyclic pterin-ring, which is linked to *p*-aminobenzoic acid and one molecule of L-glutamic acid (Ball, 1994).

In food and feed, folic acid is naturally present in reduced forms as 7,8-dihydrofolate (DHF) or 5,6,7,8-tetrahydrofolate (THF), whereas only the latter one acts as co-factor for many enzyme reactions. THF can be substituted with one-carbon adducts at the nitrogen-atom on positions 5, 10 or both. Folates predominantly exist as polyglutamates, carrying 3 to 8 L-glutamic acid residues. The main occurring polyglutamyl folate derivates are 5-methyltetrahydrofolate (5-me-THF) and 10-formyltetrahydrofolate (Velisek, 2014). Figure 2 depicts the general structure of folates.



A – Pterin ring oxidation – reduction; B – one carbon fragment attachment; n – number of glutamates

Figure 2. Structural relationships of folates (Modified from Eitenmiller *et al.*, 2008)

Physical properties

Most folates are sensitive to heat, UV-radiation, aerobic conditions and low pH and their degradation is enhanced when the pH value drops below 3.5 (Frommherz *et al.*, 2014). When exposed to oxygen, THFs can be partially oxidized to DHFs or fully oxidized to folic acid

(Combs, 2008). All natural occurring folates show native fluorescence. The maximum fluorescence is obtained with an excitation wavelength between 300 and 320 nm. The fluorescence emission shows a maximum between 360 nm and 425 nm (Ball, 1994).

Folate synthesis and metabolism

In plants, folates are present in plastids, cytosol, mitochondria and vacuoles. The starting point for the synthesis of the pterin ring is the conversion of guanosine-5´-triphosphate to 6-hydroxymethyldihydropterin (HMDHP) in the cytosol. *p*-Aminobenzoic acid is synthesized in the plastids from chorismate, a product obtained from the Shikimate pathway. HMDHP and *p*-aminobenzoic acid are assembled in the mitochondria and a glutamic acid moiety is added. A polyglutamyl tail is enzymatically linked to the glutamate residue of the folates (Hanson *et al.*, 2011).

In spite of many microorganisms and plants, animals and humans lack the possibility to synthesize folates on their own, but they are able to reduce folic acid and DHF to THF, and also to add or hydrolyse glutamic acid moieties. Nevertheless, mammals have to take up sufficient amounts of folates with their diet. In literature the recommended daily intake varies from 0.065 mg for infants to 0.6 mg for adults (Velisek, 2014).

Prior to folate absorption, food-folates, which are predominantly present as reduced polyglutamyl folates, are cleaved by the enzyme folate conjugase to mono- or diglutamate forms (Ball, 1994). Conjugases are highly present in the proximal small intestine, but also in liver, kidney and plasma. There are three mechanisms involved in the absorption of folates valid for most species: 1.) Na^+-coupled folate-transporters that carry the folates actively across the jejunum, 2.) Folate receptors that bind folates with high affinity in the intestine, 3.) Passive absorption via diffusion (Combs, 2008).

Physiological functions

THF acts as a co-enzyme in single-carbon metabolism. It is essential for the synthesis of the DNA-bases adenine, guanine and thymine, and thus required for the *de novo* synthesis of DNA, DNA replication and repair, and cell division (Combs, 2008).

Furthermore, folates are involved in the metabolism of amino acids, especially serine, histidine, glycine, and methionine (Ball, 1994; Cook, 2001). Methionine participates in the synthesis of polyamines and proteins and it is the precursor of the important methyl donor

S-adenosylmethionine (Combs, 2008).

The folate function is strongly influenced by the length of the polyglutamyl tail, whereas enzymes usually prefer polyglutamyl folates, while folate transporters favour monoglutamyl folates. Furthermore, polyglutamylation also enhances the stability of folates against oxidative degradation (Hanson *et al.* 2011).

Folate deficiency

Insufficient folate in-take can result in several severe disorders and diseases, which basically can be divided into three groups: hypomethylation, DNA biosynthesis failure, and homocysteinaemia. Adequate methylation is important for many reactions including the stabilisation of myelin proteins in the layer around nerve axons (Blancquaert *et al.*, 2010). Therefore, inadequate folate levels are also linked to neurodegenerative disorders like Alzheimer´s disease (Seshardi *et al.*, 2002).

Folate deficiency can also lead to a reduced synthesis of deoxythymidine monophosphate. As a consequence, deoxyuridine triphosphate may be incorporated in the DNA instead, resulting in possible point mutations and instabilities causing DNA-strand ruptures or even chromosomal breaks (Blancquaert *et al.*, 2010). High levels of circulating homocysteine (homocysteinaemia) are caused by insufficient conversion of homocysteine to methionine, which is controlled by the folate-dependent enzymes methionine synthase and methylene-THF reductase (Combs, 2008).

Other severe disorders include megaloblastic anaemia or abnormal embryogenesis. Therefore, the demand of folates is particularly high during pregnancy to avoid neural tube defects like anencephaly and spina bifida caused by impaired cell division in the bone marrow and the gastrointestinal tract (Ball, 1994; Geisel, 2003).

Folate - sources and stability

Folates are widely distributed in foods, including green leafy vegetables, beetroots, cheese, and whole-grain cereals. They are usually bound to proteins or storage polysaccharides, like starch and glycogen (Ball, 1994).

Table 2 summarizes the folate content of some selected foods.

Table 2. Folate content of selected foods. Values are given as microgram folate/100 gram edible food

	Folate content	Reference
Beetroots	70 – 90	Jastrebova *et al.*, 2003
Cabbage	15 – 45	Combs, 2008
Oranges	25	Combs, 2008
Peanuts	110	Ball, 1994
Strawberries	644	Stralsö *et al.*, 2003
Yeast extract	1010	Ball, 1994

The main reasons for folate reduction in foods are leakage and photo oxidative degradation. For example, cooking in boiling water results in losses of 84 % of the total folates in cauliflower (Combs, 2008). During the pasteurization of milk and spray-drying processes of cereals or porridge, also high folate losses are detected (Witthöft *et al.*, 1999). 5-Me-THF and DHF are the most sensitive folate forms, while folic acid and 5-formyl-THF are comparatively stable compounds (Blancquaert, 2010). Therefore, synthetic folic acid is often used for fortification in wheat flour with added amounts of 140 – 220 µg/100 g (Crider *et al.*, 2011). Also the addition of ascorbic acid is a common method to prevent folates from oxidation and to enhance their storage stability (Witthöft *et al.*, 1999).

1.3 Vitamin B_6

Chemical structures

Vitamin B_6 is a general term for six interconvertible, water-soluble derivatives of 3-hydroxy-2-methylpyridine, namely pyridoxine or pyridoxol, pyridoxal, pyridoxamine, and their 5´-phosphate esters. Pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM) either possess alcohol, aldehyde or amine groups at the position C-4 of the pyridine-ring (Ball, 1994). All six forms are active vitamins, but pyridoxal-5-phosphate (PLP) and pyridoxamine-5-phosphate (PMP) also act as co-enzymes in more than 100 enzymatic reactions (Wrenger *et al.*, 2005). The structures of the vitamin B_6 compounds and their 5´-phosphate esters are depicted in Figure 3.

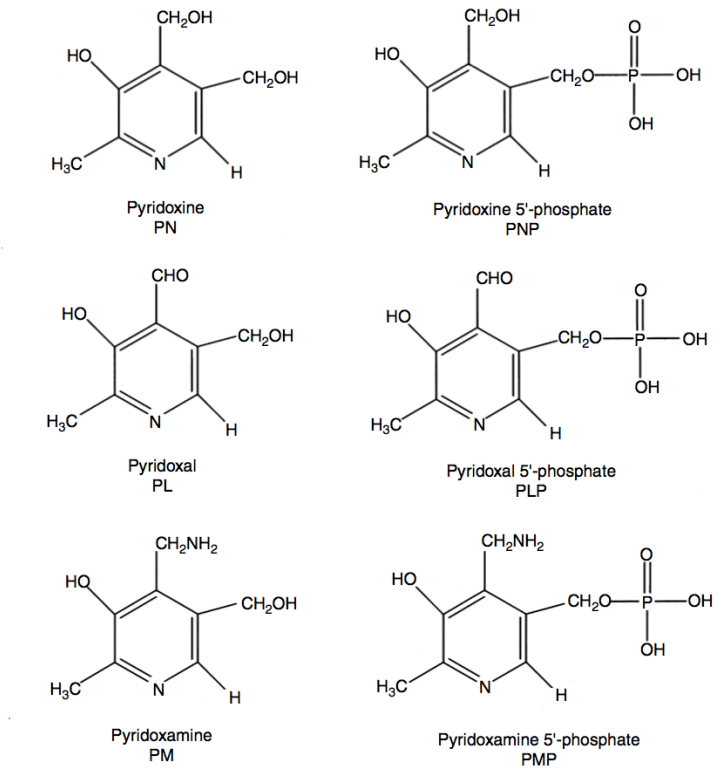


Figure 3. Structures of pyridoxine, pyridoxal and pyridoxamine and their 5´-phosphate esters (Eitermiller *et al.*, 2008)

Foods of plant origin predominantly contain PN and PL (Velisek, 2014), where the vitamers are mostly bound to β-glycosides and oligo glucosides (Kall, 2003). In contrast, PL and PM are the main compounds in foods of animal origin, which may be bound to proteins like imines in form of Schiff bases (Velisek, 2014).

Physical properties

The B_6 vitamers show the highest stability under acid conditions and their stability is decreasing with increasing pH, or when exposed to UV-radiation and high temperatures (Velisek, 2014). PN, PL and PM show natural pH-dependent fluorescence, with PM as the most fluorescent compound. The 5´-phosphate esters display only weak fluorescence. The maximal excitation wavelength is between 285 nm and 300 nm and the wavelength of maximal fluorescence is between 350 nm and 410 nm (Ball, 1994).

Vitamin B_6 synthesis and metabolism

Nowadays, three different pathways for the biosynthesis of vitamin B_6 are known: two *de novo* pathways and one *salvage* pathway (Mooney *et al.*, 2009).

Eubacteria, including *Escherichia coli*, use 4-phosphohydroxy-L-threonine and deoxyxylose-5-phosphate (DXP) to synthesize PNP; therefore, this way is called DPX-dependent. Fungi, plants and archaea have a DPX-independent pathway, where PLP is synthesized *de novo* from the sugar precursors ribose-5-phosphate or ribulose-5-phosphate together with glyceraldehyde 3´-phosphate or dihydroxyacetone phosphate and glutamine.

In addition to the two *de novo* pathways, most organisms also have a *salvage* pathway that converts the different B_6 vitamers to PLP (Hellmann & Mooney, 2010). The enzyme pyridoxine/pyridoxamine 5'-phosphate oxidase is involved in the synthesis of PLP from PN and PM, while the enzyme pyridoxal kinase is needed for the conversion of PL to PLP.

Like most mammals, humans lack the enzymes for the *de novo* synthesis of vitamin B_6, but they have a salvage pathway for interconversion (Roje, 2007). Nevertheless, mammals have to ingest the sufficient amount of B_6 vitamins via their diet. The recommended daily allowance value ranges from 1.6 mg to 1.8 mg for adults (Ball, 1994).

Absorption of vitamin B_6 mainly takes place in the jejunum and ileum via passive diffusion, after dephosphorylation of the 5´-phosphate esters by the membrane-bound enzyme alkaline phosphatase (Combs, 2008). Dephosphorylation is important to enable the vitamers to cross membranes in the intestine.

The vitamers are then transported to the liver (Ball, 1994), phosphorylated by the enzyme pyridoxal kinase, and a flavin-dependent oxidase converts PNP and PMP to PLP (Merrill & Henderson, 1987). One part of the PLP is directly utilized in the liver, while the other part is released into the blood plasma and forms a tight complex with albumin as protection against

hydrolysis. PLP may also occur tightly bound to haemoglobin in erythrocytes (Merrill & Henderson, 1987). Prior to tissue-entry the PLP-albumin-linkage is hydrolysed; the free PL is absorbed and re-phosphorylated to retain the vitamin in the cells (Ball, 1994; Combs, 2008). The highest quantities of the vitamers are stored in the skeletal muscles, bound to glycogen phosphorylase, or in the liver. The main degradation product of B_6-vitamers is pyridoxic acid, which results from oxidation processes and is excreted in the urine (Ink & Henderson, 1984).

Physiological functions

PLP - and to a lesser extent also PMP - functions as an important co-enzyme in many metabolic pathways (Ball, 1994). PLP is involved in the biosynthesis of amino acids, but also in their catabolism by operating as co-enzyme for transaminases. It is involved in the transsulfuration of methionine to cysteine and the conversion of tryptophan to niacin (vitamin B_3). Other important PLP-dependent steps are racemization, decarboxylation and α,β-eliminations (Mooney *et al.*, 2009). In gluconeogenesis, PLP is needed to transfer a phosphate-group to glycogen to yield glucose-1-phosphate. Other PLP-dependent enzymatic steps are involved in the biosynthesis of the neurotransmitters adrenalin, noradrenalin and serotonin (Combs, 2008) and in the biosynthesis of haemoglobin and chlorophyll (Mooney *et al.*, 2009). The synthesis of polyunsaturated fatty acids is also catalysed by PLP-dependent enzyme reactions. Furthermore, vitamin B_6 supports the immune competence, whereas this mechanism has not been fully elucidated yet (Combs, 2008).

Vitamin B_6 deficiency

Vitamin B_6 is present in many different kinds of foods; therefore, inadequate supplies are not very common nowadays and when detected, they are connected with other severe nutrient deficiencies (Ball, 1994). Signs of vitamin B_6 deficiency have been reported in developing countries. Typical symptoms include exhaustion, insomnia, anaemia, inflammations on lips and tongue, but also nervous disorders, like peripheral neuropathies and weakness of the skeleton muscles (Eitenmiller *et al.*, 2008).

Nevertheless, deficiency symptoms can be treated quickly by administration of vitamin B_6 (Combs, 2008).

Vitamin B_6 – sources and stability

As mentioned above, vitamin B_6 is present in a wide range of unprocessed foods. Good sources include meats, vegetables like potatoes, whole-grain and nuts (Ball, 1994). In contrast, milk products and fruits contain comparatively low amounts of vitamin B_6. Pyridoxine is mostly present in plant-derived foods, while foods from animal sources predominantly contain pyridoxal and pyridoxamine (Combs, 2008). Table 3 summarizes the total vitamin B_6 content of some selected foods.

Table 3. Total vitamin B_6 content of selected foods. Values are given as milligram total vitamin B_6/100 gram edible food

	Total vitamin B_6 content	Reference
Beef	0.33	Combs, 2008
Carrots	0.17	Eitenmiller *et al.*, 2008
Liver, ox	0.83	Ball, 1994
Oranges	0.06	Combs, 2008
Peanuts	0.40	Combs, 2008
Strawberries	0.06	Combs, 2008

Vitamin B_6 shows the highest stability in foods under acid conditions, with pyridoxine as the most stable vitamer (Combs, 2008). Therefore, heating and dehydrating steps during food processing result in greater vitamin losses in animal derived foods than in vegetables and fruits. Especially dehydration processes induce high vitamin degradation up to 40 – 70 % (Ball, 1994). For food fortification, usually pyridoxine hydrochloride is used, as it is especially stable (Eitenmiller *et al.*, 2008).

1.4 Phenolic compounds

Chemical structure

With more than 8,000 known compounds, polyphenols constitute one of the major groups of secondary metabolites in plants (Williams *et al.*, 2004; Tsao, 2010). Polyphenols are involved in processes like plant growth, plant morphology (especially pigmentation), reproduction and protection against UV-radiation. They also provide resistance against pathogens and herbivores (Bravo, 1998). In addition, polyphenols are important for the astringent and bitter taste and colouring of fruits (Cheynier, 2005).

Polyphenols obtain phenolic structures with numerous hydroxyl groups linked to aromatic rings in order to form rather simple monomers and oligomers or complex polymers (Gharras, 2009). Due to their wide distribution and high diversity among the plant kingdom, different categorization strategies for polyphenols evolved. Therefore, they may be classified by their chemical structure, source of origin or biological function (Tsao, 2010). According to Manach and co-workers, polyphenols are categorized in the following groups: phenolic acids, flavonoids, stilbenes and lignans (Manach *et al.*, 2004).

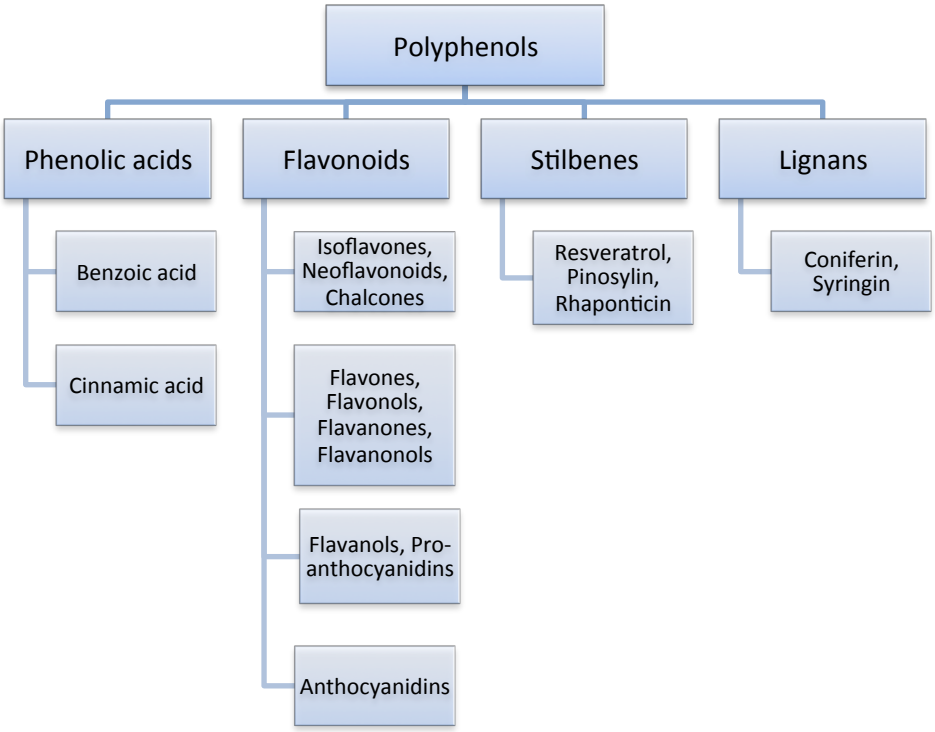


Figure 4. Categorization of polyphenols based on their chemical structure

Phenolic acids

Phenolic acids can be separated in derivatives from cinnamic and benzoic acids with their backbone based on a phenolic ring linked to a C1 or C3 structure. Typical phenolic acid structures are depicted in Figure 5. In general, hydroxybenzoic acids are more common in foods than hydroxycinnamic acids, with caffeic acid being the most abundant, especially in fruits (Gharras, 2009). The predominant hydroxycinnamic acid in foods is ferulic acid, which is highly present in cereals grains (Bourne & Rice-Evans, 1998).

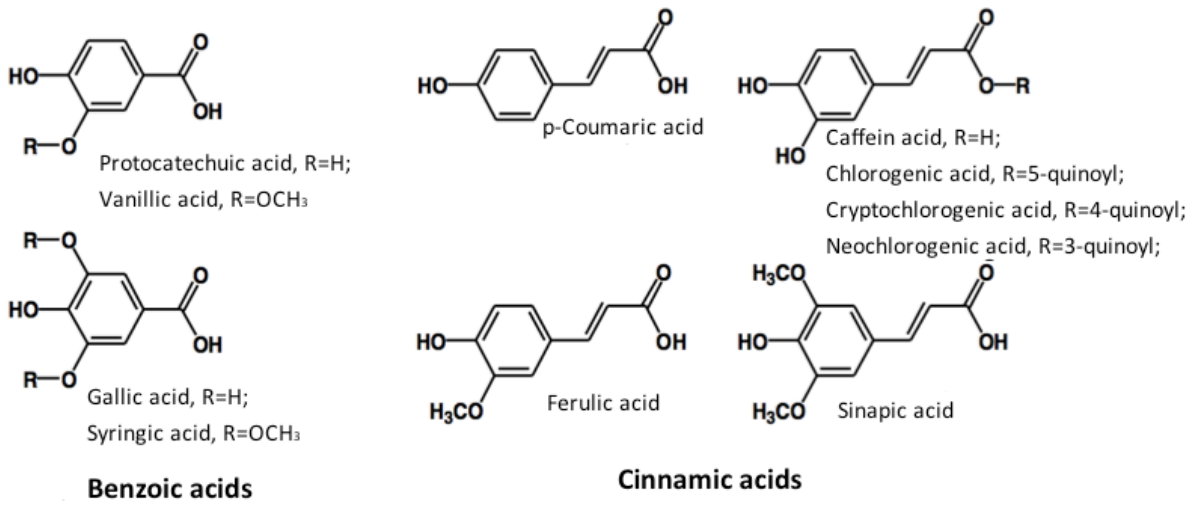


Figure 5. Typical phenolic acids present in foods; left: benzoic acids; right: cinnamic acids (Tsao, 2010)

Flavonoids

Flavonoids represent the prevalent group of polyphenols with more than 4,000 identified members (Cheynier, 2005). Structurally, flavonoids are based on two phenolic C6 units (Rings A and B) connected by a C3 unit. Flavonoids show different hydroxylation patterns and vary in the structure of the heterocyclic chromane ring (Ring C). In some flavonoids, the B ring is attached to the C-2 atom of ring C, while in some it is linked to the C-atoms at positions 3 or 4. The basic flavonoid backbone is depicted in Figure 6.

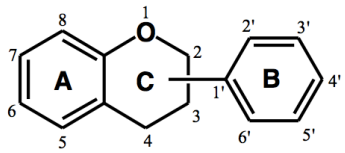


Figure 6. Basic backbone of the flavonoid compounds (Tsao, 2010)

Based on these structural differences, they can be separated further into sub-groups. In foods, flavonoids are usually present as glycosides, whereas their biological and antioxidant activity strongly depends on their glycosylation patterns (Tsao, 2010).

Stilbenes

The structure of stilbenes is based on two phenolic rings, linked by a C2 unit. The most common stilbene is resveratrol, which is highly abundant in red grapes. Resveratrol occurs either as *Z* or *E* isomer and is usually present as glycoside (Crozier *et al.*, 2006). In general, stilbenes are only found in very low quantities in human nutrition (Manach *et al.*, 2004).

Lignans

Lignans contain two condensed phenylpropane units, which are linked by the central C-atom at position 8 in the side chains (Umezawa, 2003). In the human diet, they are only present in significant amounts in flaxseed and flaxseed oil (Adlercreutz & Mazur, 1997). Lignans have oestrogen agonist and oestrogen antagonist properties and are, therefore, recognized as phytoestrogens (Scalbert & Williamson, 2000).

Biosynthesis of polyphenols and metabolism

Similar to other phenolic compounds like aromatic amino acids, phenolic acids (cinnamic acid and benzoic acid) are synthesized from phosphoenolpyruvat via the Shikimate pathway. In contrast, the biosynthesis of complex flavonoids takes place in the cytoplasm and requires intermediates from the primary metabolism of mitochondria and plastids (Tsao, 2010). Nevertheless, flavonoid biosynthesis is also linked to the Shikimate pathway, as the amino acid phenylalanine acts as precursor for the aromatic ring B and also the chromane ring C. Phenylalanine is converted to cinnamate, which leads to the C6-C3 structure. Ring A originates from three malonyl-CoA units, which are added via sequential decarboxylation reactions to initiate the biosynthesis of flavonoids (Knaggs, 2001). The final condensation of 4-coumaroyl-CoA and three malonyl-CoA units results in naringenin chalcone. Isomerization by the chalcone flavone isomerase yields a flavone intermediate, which is the branching point of all flavonoid classes, stilbenes, and lignans (Tsao, 2010; Humphreys & Chapple, 2002).

In case of stilbenes, the condensation reaction of p-coumaroyl-CoA or cinnamoyl-CoA with

three units of malonyl-CoA, catalysed by the enzyme stilbene synthase, leads to the precursor of all stilbenes-structures (Jeandet *et al.*, 2010).

Glucosyltransferases catalyse the addition of glucosyl-residues like glucose, arabinose, or xylose to the phenolic structures (Tsao, 2010; Scalbert & Williamson, 2000).

Metabolism of some phenolic compounds takes place in the gastrointestinal tract, were phenolic acids and non-glycosylated phenolic compounds are directly absorbed via the small intestinal mucosa (King *et al.*, 1996). Glycosylated forms pass into the large intestine, where they are hydrolysed by a bacterial β-glycosidase. After absorption of the aglycones through the epithelial cells in the gut, three main conjugation steps follow: methylation, sulfation and/or glucuronidation. The main metabolism of polyphenols takes place in the liver, but also kidneys and the intestinal mucosa may be involved (Bravo, 1998). The exact metabolic fate of the conjugates in the liver is still unknown, but a complex set of carriers and enzymes is expected to control the uptake and release of phenolic metabolites in the hepatocytes (Manach *et al.*, 2004). Conjugated and methylated compounds are either secreted in the urine or hydrolysed into simple phenolic acids by bacteria and are fully metabolized. Alternatively, they can be deconjugated, enter the enterohepatic cycle and are reabsorbed in the colon (Bravo, 1998).

Physiological functions

Due to their high abundance in fruits and vegetables, polyphenols play an important role in the human diet and polyphenol-rich nutrition is thought to have numerous positive effects on human health (Tsao, 2010). Because of their highly conjugated structure and hydroxylation patterns, polyphenols are considered to obtain strong antioxidant properties. Polyphenols act as radical scavengers by providing hydrogen atoms or electrons and thus may lead to the neutralization of free radicals (Pietta, 2000). Furthermore, it is known that polyphenols act as metal chelators. Consequently, they are expected to reduce oxidation caused by hydroxyl radicals (Bravo, 1998). Another important purpose is the potential inhibition of low density lipoprotein oxidation (Fuhrman *et al.*, 1995).

Moreover, degenerative diseases like Morbus Alzheimer, certain types of cancer, chronic inflammation and cardiovascular diseases are linked to oxidative stress caused by reactive oxygen or nitrogen species. Therefore, high dietary intake of polyphenols is thought to lower the risk of these chronic diseases (Tsao, 2010).

Nevertheless, the degree of absorption and metabolism strongly influences the antioxidant properties of polyphenols (Bravo, 1998), and due to their low concentration in plasma, some scientists claim that polyphenols may not act as direct antioxidants *in vivo*. However, the effects of polyphenols on signalling pathways are taken into account to trigger regulation mechanisms in cells (Williams *et al.*, 2004).

Polyphenols – sources and stability

Polyphenols are present in a wide range of fruits, vegetables and beverages. Rich sources for polyphenols are for example strawberries, orange juice, onions and cereals like barley and sorghum. Coffee, tea and red wine also contain high amounts of polyphenols, while peanuts and apple juice contain relatively low amounts. Table 4 summarizes the total phenolic content (TPC) of some selected foods.

Table 4. Total phenolic content of selected foods. Values are given as milligram total phenolic content/100 gram edible food or as milligram total phenolic content/100 millilitre

	TPC	Reference
Carrot	19 – 342	Leja *et al.*, 2013
Grape	50	Bravo, 1998
Onion	90	Vinson *et al.*, 1998
Orange juice	750	Rousseff *et al.*, 1987
Strawberry	38 – 1441	Bravo, 1998; Panico *et al.,* 2009

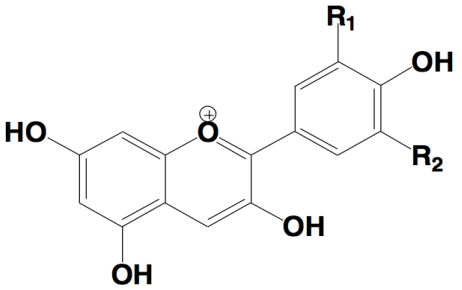
It has to be mentioned that the polyphenolic content fluctuates strongly between different cultivars. Genetic factors, germination and cultivation conditions, like access to sunlight, but also ripeness, storage circumstances, and processing have a great impact on the total phenolic content of foods (Mazza, 1995).

Degradation of polyphenols in foods occurs throughout processes like cooking or boiling in water (Lima *et al.*, 2009). Oxidation of polyphenols takes place during storage periods. In case of cacao and black tea, oxidative changes and polymerization lead to the typical sensory properties of the products, while enzymatic and non-enzymatic browning reactions in fruits and vegetables lead to undesired taste and colouring (Shahidi & Naczek, 1995; Bravo, 1998).

1.5 Anthocyanins

Chemical structure

Anthocyanins are water-soluble pigments that are responsible for the orange, red, purple and violet colouring of fruits, vegetables and other plants (Velisek, 2014). Chemically, they are assigned to the polyphenol-group of flavonoids (Gharras, 2009). The structure of anthocyanins is based on the 2-phenylbenzopyrylium-cation (Dimitrovska *et al.*, 2011). Anthocyanins are the glycosylated forms of anthocyanidins and can be substituted with hydroxyl or methoxyl groups at different positions. At position C3, all anthocyanins contain sugar monosaccharides, namely D-glucose, L-rhamnose, D-galactose, D-xylose and L-arabinose. Disaccharides, like rutinose or sambubiose, and trisaccharides can be attached at different positions, such as C7, C3´, C5´ and C4 (Velisek, 2014). The general structure of the anthocyanidin backbone is shown in Figure 7. Due to the high variety in the hydroxylation, methoxylation and glycosylation pattern, more than 500 anthocyanins have already been identified so far (Tsao, 2010).



Anthocyanidin	R_1	R_2
Cyanidin	-OH	-H
Delphinidin	-OH	-OH
Pelargonidin	-H	-H
Malvidin	$-OCH_3$	$-OCH_3$
Peonidin	$-OCH_3$	-H
Petunidin	-OH	$-OCH_3$

Figure 7. General structure of the anthocyanidin backbone and residues of the most common anthocyanins (Tsao, 2010)

Anthocyanidins are highly unstable, while their stability against UV-radiation, oxygen and pH is strongly increased through glycosylation. Furthermore, the esterification with phenolic and organic acids and complex-formation with other flavonoids also enhances their stability (Manach *et al.*, 2004).

17 different anthocyanidins have been identified in nature (Velisek, 2014), whereas the great majority of anthocyanins is structurally based on cyanidin, delphinidin, pelargonidin, malvidin, peonidin, and petunidin (Tsao, 2010).

Colouring

Anthocyanins obtain different colouring depending on the surrounding pH value. Under acid conditions, the red coloured flavylium cation is predominant, while the colour changes from colourless over purple to blue, when the pH is increased. Under strong alkaline conditions, a yellow coloured chalcone is formed (Velisek, 2014; Tsao, 2010).

Nevertheless, the colour of anthocyanins is also determined by the degree and type of substituents of the aromatic rings (Tsao, 2010). A high degree of hydroxylation leads to a blue dye, while a higher number of methoxyl groups leads to a red tint of the plants. Also other pigments like chlorophyll, carotenoids, and anthoxanthins contribute to the resulting colour (Velisek, 2014).

Anthocyanins - sources and stability

In the human diet, anthocyanins are most abundant in fruits like cherries, strawberries, red and blackcurrants and grapes (Scalbert & Williamson, 2000). However, they can be also found in cereals and vegetables, including aubergines, onions, cabbage, and beverages like red wine (Manach *et al.*, 2004).

Anthocyanins are present in the vacuoles of cells (Velisek, 2014) and are usually located in the skin of fruits and vegetables. Exceptions are cherries and strawberries, where anthocyanins can also be found in the flesh (Manach *et al.*, 2004).

The number of anthocyanins differs according to several fruits and vegetables. Blueberries and maize contain up to ten different anthocyanins, while strawberries and blackberries only contain a few (Velisek, 2014).

Table 5 summarizes the anthocyanin content of some selected foods.

Table 5. Anthocyanin content of selected foods. Values are given as milligram of anthocyanins/100 gram edible food or as milligram anthocyanins/100 millilitre

	Anthocyanin content	Reference
Apple	10 – 20	Velisek, 2014
Black olive	500	Velisek, 2014
Grape	59 – 151	Janik *et al.*, 2007
Pear	5 – 10	Velisek, 2014
Red wine	20 – 35	Manach *et al.*, 2004
Strawberry	13	Riedl & Murkovic, 2011

The most abundant anthocyanins in strawberries are pelargonidin (2 mg/100 g fresh fruit) and cyanidin (0.12 mg/100 g fresh fruit).

Red grapes contain mainly malvidin and peonidin (1 mg/100 g fresh fruit), followed by cyanidin (0.38 mg/100 g fresh fruit) (Belitz *et al.*, 2008).

In general, cyanidin is the most abundant anthocyanin in foods. Nevertheless, the anthocyanin content in food demonstrates high variations within species, and the values increase with progressed ripeness of the food (Manach *et al.*, 2004).

Temperature and pH of the surrounding, and also the presence of oxygen, influence the degradation of anthocyanins. In contrast to other molecules, anthocyanins show increased stability under high temperatures. One possible reason therefore is the condensation of monomers, which then form more stable oligomeric pigments. Enzymatic degradation of anthocyanins is mainly caused by glycosidases and polyphenol oxidases, which are involved in enzymatic browning reactions (Velisek, 2014).

Applications in the food industry

Anthocyanins are used as additives (E163) by the industry to improve the colour of foods. As anthocyanins are only stable under acid conditions, they can be added to foods like jams, red wine and soft drinks (Jackman *et al.*, 1987). Grape skin and sediments are mainly used for food colouring, but also extracts of elderberries and blackberries are commonly added. As no toxicity or mutagenicity of anthocyanins could be attested, there are no limits concerning concentration in most countries (Velisek, 2014).

1.6 Hesperidin

Chemical structure

Hesperidin belongs to the group of flavanones, which is a sub-group of flavonoids. Like other flavanones, hesperidin is a derivative from (2*S*)-5,7-dihydroxyflavanone, carrying an additional hydroxyl group at position 3´ of ring C and a methoxyl group at position 4´of ring C (Velisek, 2014). Hesperidin (2-S-hesperetin-7-rutinoside) is the glycosylated form of hesperetin and is mainly found in oranges (Di Mauro *et al.*, 1999; Magwaza *et al.*, 2015). The chemical structure of hesperidin is depicted in Figure 8.

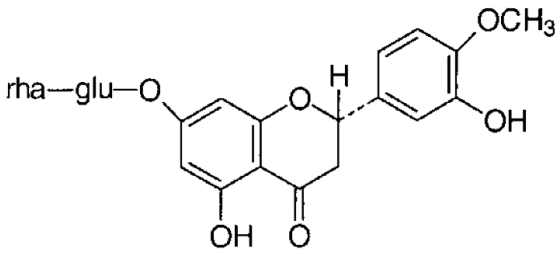


Figure 8. Chemical structure of hesperidin (Roohbaksh et al., 2014)

With more than 90 % of the total flavanone concentration, hesperidin contributes the major flavanone glycoside in oranges (Manach *et al.*, 2005). In contrast to other flavanone glycosides, like naringin or neohesperidin that usually contribute a bitter taste to fruits, hesperidin is tasteless. Furthermore, flavanones are light yellow or colourless and are therefore not significant as plant pigments (Velisek, 2014).

Physiological effects

Like all polyphenols, flavanones are expected to obtain certain protective effects against oxidative stress. Furthermore, a reduction in plasma and hepatic cholesterol caused by hesperidin has been shown in animal studies. These results support the conclusion that hesperidin may be beneficial for preventing cardiovascular diseases. Administration of high amounts of hesperidin also leads to the protection of animals against chemically induced cancers of skin and intestine. However, the exact mechanisms remain unknown (Magwaza *et al.*, 2015).

Hesperidin – sources and stability

Flavonoids are highly abundant in foods, but flavanones are almost exclusively present in citrus fruits, including oranges, lemons and mandarins and some herbs like mint (Manach *et al.*, 2003). It is highly abundant in orange blossoms, unripe fruits and in the rind tissue of ripe oranges (Hendrickson & Kesterson, 1964), whereas the hesperidin concentration is decreasing during the ripening process of the fruits (El Nawawi, 1995). Variations in the hesperidin concentration can be further caused by different cultivation and storage conditions. Also postharvest treatments contribute to differences in the concentration profile (Abad-Garcìa *et al.*, 2012).

Hesperidin is hardly soluble in water, and therefore it is expected to be one reason for the turbidity of orange juice by forming white crystals (Kimball, 1991). Crystallization usually occurs during storage of processed orange juices, and the extent is influenced by factors like extraction pressure, pasteurization conditions, and holding time. Because of this and the previous stated reasons, the hesperidin content of orange juices shows a high quantitative variability, according to literature. Concentrations between 10 – 100 mg/100 ml can be found in literature (Velisek, 2014; Gil-Izquierdo *et al.*, 2001).

1.7 Yeasts, moulds and bacteria

Fruit juices are an ideal environment for the growth of numerous microorganisms, as they contain carbohydrates, amino acids, mineral salts, and vitamins (Ashurst, 2016). The presence of microorganisms in juices is strongly influenced by the quality of the raw materials. The skin layer usually protects fruits against spoilage and pathogens, but during handling, cutting, and juice processing, the flesh is exposed to environmental impacts that favour the growth of pathogenic microorganism (Raybaudi-Massilia *et al.*, 2009).
Fruits and vegetables contain large amounts of organic acids and sugars and obtain a pH between 2.0 and 4.5. Thus, spoilage is mainly caused by fungi (yeasts and moulds), and in some cases acid tolerant bacteria (Dewanti-Hariyadi, 2013).

Yeasts include more than 8,000 species, while only ten species are commonly responsible for spoilage of foods (Wareing & Davenport, 2005). Yeasts are tolerant against acid conditions, and they can grow in aerobe and anaerobe surroundings. Their typical growth temperature is around 25 °C, but some yeasts also persist temperatures of 0 – 10 °C or 70 °C. The presence of yeasts results in the formation of vinegary, ethanolic, buttery, and acetoin-like tastes (Ashurst, 2016). *Saccharomyces* spp., *Zygosaccharomcyes* spp., *Candida* spp., and *Pichia* spp. are commonly found in fruit and vegetable juices (Raybaudi-Massilia *et al.*, 2009).

Moulds grow aerobically and also belong to the class of fungi. Therefore, moulds typically form layers on surfaces. Some fungi are able to produce mycotoxins as secondary metabolites. The most abundant mycotoxin in fruit juices is patulin (Wareing & Davenport, 2005). Common moulds are *Aspergillus* sp., *Penicillium* sp., or *Cladosporium* (Aneja *et al.*, 2014).

Bacteria are usually found to a lesser extent in fruit juices, as they prefer a pH from 6.5 to 7.5. Typical representatives are *Acetobacter*, *Lactobacillus*, *Saccharobacter* and *Zymobacter*. Bacterial growth leads to the formation of a sour, vinegary or cheesy taste in the juices. Also gas production and increased cloudiness can result (Juvonen *et al.*, 2011).

2. Materials and Methods

2.1 Materials

2.1.1 Instruments

All instruments used for sample preparation and sample analysis are listed in Table 6.

Table 6. List of used instruments and manufacturing companies

Instrument	Manufacturer
Analysis level AG135 & AG245	Mettler Toledo GmbH, Vienna, Austria
Data logger testo 175T2	Testo GmbH, Vienna, Austria
Centrifuge 5804 R	Eppendorf AG, Hamburg, Germany
High Performance Liquid Chromatograph, Series 1100	Hewlett Packard, California, USA
Vortex	Scientific Industries, New York, USA
DU® 800 Photometer	Beckman Coulter™ Inc, Vienna, Austria
ThermoStat plus	Eppendorf AG, Hamburg, Germany
LiChrolut vacuum chamber	Merck, Darmstadt, Germany
LiChrolut SAX SPE columns	Merck Darmstadt, Germany

2.1.2 Chemicals

All used standard reagents are listed in Table 7. Other chemicals and solvents for the HPLC analysis are listed in Table 8.

Table 7. List of used standard chemicals and manufacturing companies

Standard substance	Manufacturer
5-Methyltetrahydrofolic acid disodium salt	Sigma Aldrich Chemie GmbH, Vienna, Austria
Gallic acid	Sigma Aldrich Chemie GmbH, Vienna, Austria
Hesperidin	Sigma Aldrich Chemie GmbH, Vienna, Austria
L-Ascorbic acid	Sigma Aldrich Chemie GmbH, Vienna, Austria
Pyridoxal hydrochloride	Sigma Aldrich Chemie GmbH, Vienna, Austria
Pyridoxamine dihydrochloride	Sigma Aldrich Chemie GmbH, Vienna, Austria
Pyridoxine hydrochloride	Sigma Aldrich Chemie GmbH, Vienna, Austria

Table 8. List of used chemicals and HPLC solvents and the manufacturing companies

Chemicals	Manufacturer
0.1 M Hydrochloric acid	Roth, Karlsruhe, Germany
2-mercaptoethanol	Sigma Aldrich Chemie GmbH, Vienna, Austria
Acetic acid	ChemLab analytical, Zedelgem, Belgium
Acetonitrile	ChemLab analytical, Zedelgem, Belgium
Ammonium sulphate	Fluka, Vienna, Austria
di-Sodiumhydrogenphosphate	Merck, Darmstadt, Germany
Dithiothreitol	Formedium, Norfolk, UK
Folin & Ciocalteu´s phenol reagent	Sigma Aldrich Chemie GmbH, Vienna, Austria
Formic acid	J.T. Baker, Pennsylvania, USA
Hog kidney acetone powder	Sigma Aldrich Chemie GmbH, Vienna, Austria
L-cysteine hydrochlorid	Fluka, Vienna, Austria
Methanol	ChemLab analytical, Zedelgem, Belgium
ortho-Phosphoric acid 50 %	Fluka, Vienna, Austria
Peptone water	Biokar diagnostics, Allone, France
Plate count agar	Biokar diagnostics, Allone, France
Potassium acetate	Roth, Karlsruhe, Germany
Sodiumacetate	Roth, Karlsruhe, Germany
Sodiumcarbonat	Merck, Darmstadt, Germany
Sodiumdihydrogenphophate	Merck, Darmstadt, Germany
Sulfuric acid	J.T. Baker, Pennsylvania, USA
Tris(hydroxymethyl)-aminomethan	Merck, Darmstadt, Germany
Ringer solution	Merck, Darmstadt, Germany
YGC agar	Merck, Darmstadt, Germany

2.1.3 Raw materials

All raw materials for the home-made juices were purchased at local supermarkets. The used fruits and vegetables, the variety and countries of origin are listed in Table 9.

Table 9. List of used fruits and vegetables from local supermarkets used for the juice production

Fruit/vegetable	Variety	Country of origin
Oranges	Spanish Navelia	Spain
Red Grapes	Crimson	Italy
Strawberries	Da komm´ ich her (no further information)	Austria
Carrots	Da komm´ ich her (sugar carrots)	Austria

2.2 Methods

High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was used for the determination of vitamin C, 5-me-THF, vitamin B_6, anthocyanins, and hesperidin in fruit juice samples. Separation is achieved by different partitions of the sample substances between a mobile and a stationary phase, which is packed in a separation column. In normal-phase HPLC, the stationary phase is polar and the mobile phase is non-polar, while in reversed-phase HPLC (RP-HPLC), a non-polar stationary phase like octadecyl-modified silica and a polar mobile phase are used. Other types of HPLC include for example size-exclusion or affinity chromatography. Depending on the interactions with the stationary and mobile phase, the analytes are retained in different degrees, resulting in a specific retention time for every substance. After elution, the substances can be verified by using different detector types.

The HPLC-instrument by Hewlett Packard, Series 1100 (consisting of a vacuum-degasser, a quaternary pump, an auto sampler, a UV/VIS detector and a fluorescence detector) was used. The software ChemStation for LC 3D© 1990 – 2003 by Agilent Technologies was used for data evaluation.

Solid-phase extraction (SPE)

Complex sample matrices can cause numerous problems during chromatographic analysis. These problems include plugging of columns, resolution issues, baseline interferences, and quantification errors (Arsenault, 2003). Therefore, solid-phase extraction (SPE) is a common method for sample preparation. The main aims are to remove undesired compounds and sample matrix, to exchange solvents and to concentrate the analytes (Simpson, 2000).

The principle of SPE is to trap the analytes by a proper sorbent, when they are passed through a plastic or glass cartridge (Nollet, 2000). The whole process can be separated into the following main parts: sorbent conditioning, sample loading, sorbent washing and elution

of the analyte with a selective organic solvent. The main steps are depicted in Figure 9 (Simpson, 2000).

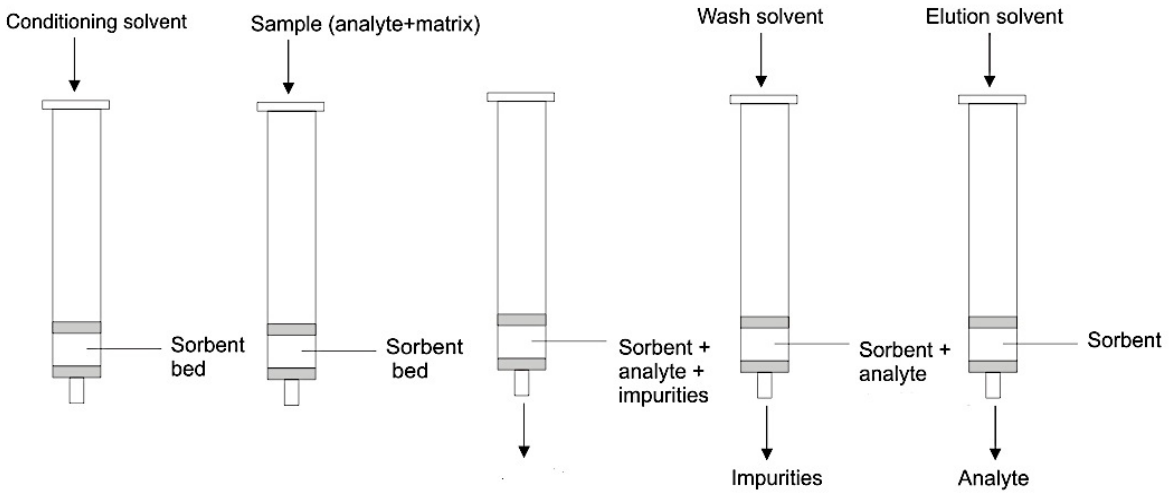


Figure 9. Steps of the solid-phase extraction procedure (Modified from Żwir-Ferenc and Bizuik, 2006)

The most common retention mechanisms include polar, non-polar and ionic interactions (Żwir-Ferenc & Bizuik, 2006). For polar interactions, silica or polyamide sorbents are typically used. For non-polar interactions, octadecyl-modified silica and polystyrene vinyl benzene copolymers are recommended (Nollet, 2000). In ion-exchange SPE, silica sorbents with aliphatic sulfonic acid groups are used to isolate cationic compounds, and silica sorbents with aliphatic quaternary amine groups are used to isolate anionic compounds. During the experiments, strong anion-exchange (SAX) sorbets based on trimethylaminopropyl silica were used for the purification of folates. The use of SAX-cartridges for folate purification is highly recommended in literature (Nilsson *et al*., 2004, Philips *et al*., 2005, Vahteristo *et al*., 1997; Jastrebova *et al*., 2003; Hamacek *et al*., 2014).

Folin-Ciocalteu assay

The total phenolic content (TPC) can be measured by using the Folin-Ciocalteu reagent assay. The Folin-Ciocalteu assay is a modification of the Folin-Denis assay, which was originally used to detect tyrosine and tryptophan in protein hydrolysates (Vermerris & Nicholson, 2008). The Folin-Ciocalteu reagent contains a mixture of sodium molybdate and

sodium tungstate, and is based on a reduction-oxidation mechanism. Under basic conditions, the molybdotungstate reagent is reduced by the phenolate anion of phenolic compounds, while the phenols are oxidized. To achieve a pH value around 10, sodium carbonate is added to the samples (Jadhav *et al*. 2012). The product of this redox-reaction is a blue coloured complex with an absorption maximum of 765 nm. For quantification, the absorption can be measured by using a visible-light spectrophotometer (Everette *et al*., 2010).

By using the Folin-Ciocalteu assay, soluble phenols, for example anthocyanins, as well as complex phenols, like condensed tannins, can be detected (Vermerris & Nicholson, 2008). Nevertheless, there are some limitations caused by interfering compounds, namely sugars, organic acids, enediols like ascorbic acid, reductones and aromatic amines (Prior *et al.*, 2005).

Philips Juicers

Juices from carrots, strawberries, red grapes and oranges were produced by using the Philips juice-makers, which are depicted in Figure 10.

Figure 10. Pictures of the juicers used during the experiment. From left to right: Philips Avance Collection, Philips ProBlender 6 and Philips Masticating Juicer (Philips instruction manuals, 2014 – 2015)

a.) Philips Avance Collection

Complete description: Philips HR1870/00 Avance Quick Clean 2.5 l

Technical data: power: 700 W, voltage: 220 – 240 V, frequency: 50/60 Hz

Juicing-principle: The P. Avance Collection obtains a filter sieve with small, sharp knives. The knives chop the fruits and vegetables in small pieces and then high-speed spinning separates solid and liquid particles. The pulp remains in a repertoire while the juice passes through a small opening.

Table 10. Abbreviations and descriptions of the construction schema of the Philips Avance Collection

A	Driving shaft
B	Locking arm
C	Control knob
D	Cord storage facility
E	Overload protection button
F	Cover of pusher
G	Pusher with pre-clean function
H	Feeding tube
I	Lid
J	Sieve
K	Direct serve spout accessory
L	Juice collector with detachable spout (R)
M	Pulp window
N	Pulp container

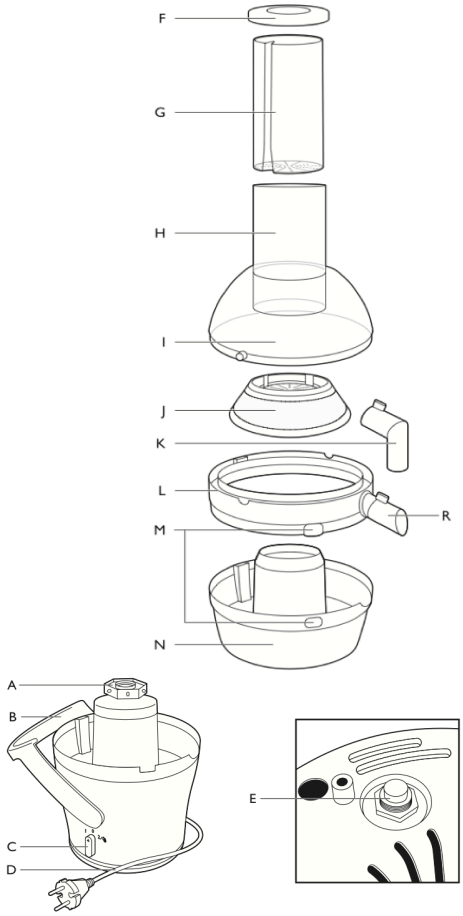


Figure 11. Construction scheme of the Philips Avance Collection (Philips instruction manual, 2014)

b.) Philips ProBlender 6

Complete description: Philips ProBlender 6 HR2096/00, 2l

Technical data: power: 800 W, voltage: 220 – 240 V, frequency: 50/60 Hz

Juicing principle: The P. ProBlender consists of a glass vessel with four knifes at the bottom. The fruits and vegetables are smashed and no separation between solid and liquid particles takes place.

Table 11. Abbreviations and descriptions of the construction schema of the Philips ProBlender 6

1	Measuring cup
2	Spatula
3	Lid
4	Blender jar
5	Blender unit
6	Collar
7	Rotary knob and present buttings

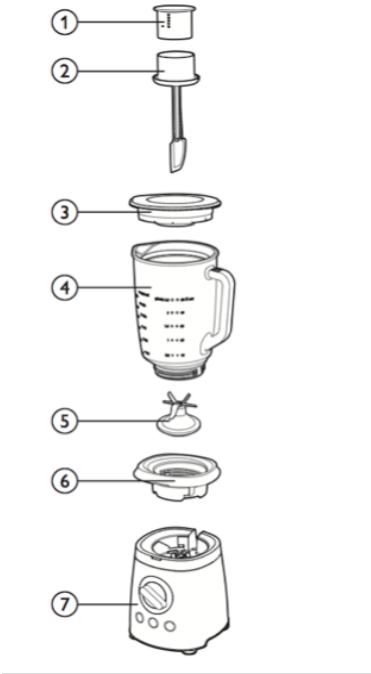


Figure 12. Construction scheme of the Philips ProBlender 6 (Philips instruction manual, 2014)

Philips Masticating Juicer

Complete description: Philips Avance Masticating Juicer HR 1897, 1 l

Technical data: power: 200 W, voltage: 220 – 240 V, frequency: 50 – 60 Hz

Juicing principle: The P. Avance Masticating Juicer consist of a screw-press to crush and smash the fruits and vegetables at very low speed. Solid particles are separated from the juice by passing through a sieve.

Table 12. Abbreviations and descriptions of the construction schema of the Philips Avance Collection

1	Pusher
2	Berry tray
3	Feeding tube
4	Drip stop
5	Spout
6	Black two-part filter for clean juice
7	Juice screw
8	Pulp outlet

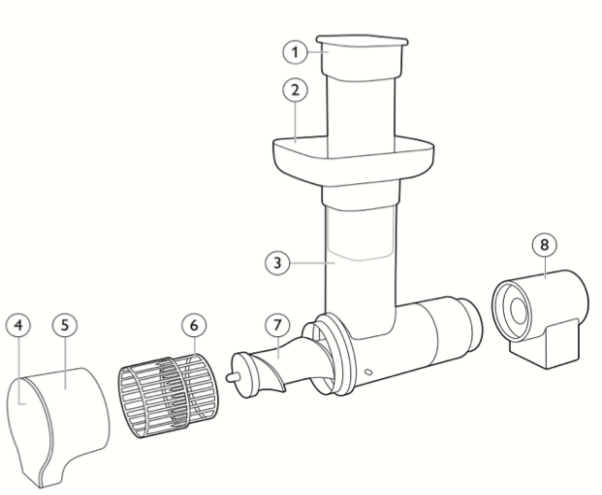


Figure 13. Construction scheme of the Philips Masticating Juicer (Philips instruction manual, 2015)

Sample preparation

Before the pressing procedure, 0.5 kg raw material from local supermarkets were weighted in and rinsed with water; leaves and peduncles were removed, oranges were peeled. The fruits and vegetables were cut into small pieces, were homogenised or pressed.

Sampling was done after 0, 2, 5, 8 and 24 hours. During the sampling, the juices were stored in glass bottles at 4 °C in the refrigerator - on time under oxygen atmosphere and the other time under nitrogen atmosphere in desiccators. All experiments were carried out in triplicates to achieve a representative mean-value. Table 13 summarizes the chemical parameters, which were analysed for the several fruits and vegetables.

Table 13. Summary of all chemical parameters analysed under oxygen atmosphere and nitrogen atmosphere

Raw material	**Chemical parameter**
Carrots	Total phenolic content
	Vitamin B_6
	Total microbial counts
Orange	5-Methyltetrahydrofolate
	Hesperidin
	Vitamin C
	Total microbial counts
Red Grapes	Anthocyanins
	Total phenolic content
	Total microbial counts
Strawberry	Anthocyanins
	5-Methyltetrahydrofolate
	Total phenolic content
	Vitamin C
	Total microbial counts

2.2.1 Analysis of total vitamin C content

Sample preparation

In order to stabilize vitamin C, the fruit juice samples were first diluted 1:10 with 1 % ortho-phosphoric acid, and then mixed and kept in the freezer at -18 °C until the analysis.

For the analysis of the total vitamin C content, dehydroascorbic acid was reduced to ascorbic acid. For this purpose, the samples were centrifuged for 10 minutes at 14,000 rpm, mixed with DTT-TRIS-solution to obtain a pH value between 5 and 7, and kept in the dark for 10 minutes. The final concentration of DTT in the sample solution was 5.5 mM.

50 % ortho-phosphoric acid was added to reach a pH between 2.5 and 3, and to stop the reduction reaction.

Then, the samples were analysed using isocratic RP-HPLC. The exact sample preparation is summarized in Table 14.

The L-ascorbic acid standard was solved in 1 % ortho-phosphoric acid, and concentrations of 3.9 µg – 125/ml were prepared.

Table 14. Method for the reduction of dehydroascorbic acid to ascorbic acid

Strawberry juice	- 46.29 mg DTT were dissolved in TRIS-solution (0.4 mol/l) - 550 µl sample were mixed with 125 µl DTT-TRIS-solution (pH 6.8) - 5 µl 50 % ortho-phosphoric acid were added (pH 2.7)
Orange juice	- 106 mg DTT were dissolved in TRIS-solution (0.4 mol/l) - 575 µl sample were mixed with 50 µl DTT-TRIS-solution (pH 6) - 1.5 µl 50 % ortho-phosphoric acid were added (pH 2.65)

HPLC measurement

The parameters of the HPLC measurements are summarized in Table 15. One example of the resulting standard chromatograms is depicted in Figure 14. The retention time of the L-ascorbic acid standard was 1.78 minutes.

Table 15. HPLC parameters for the analysis of the total vitamin C content in strawberry juice and orange juice

Column	pre-column: phenomenex® AJ0-9297 EVO C18 phenomenex Kinetex, 5 µm EVO C18 100 Å
Column temperature	23 °C
Detection	UV/UV-vis Detector, 245 nm
Flow rate	0.5 ml/min
Mobile phase	50 mM phosphate buffer, pH 2.7
Injection volume	3 µl
Stop time	10 min

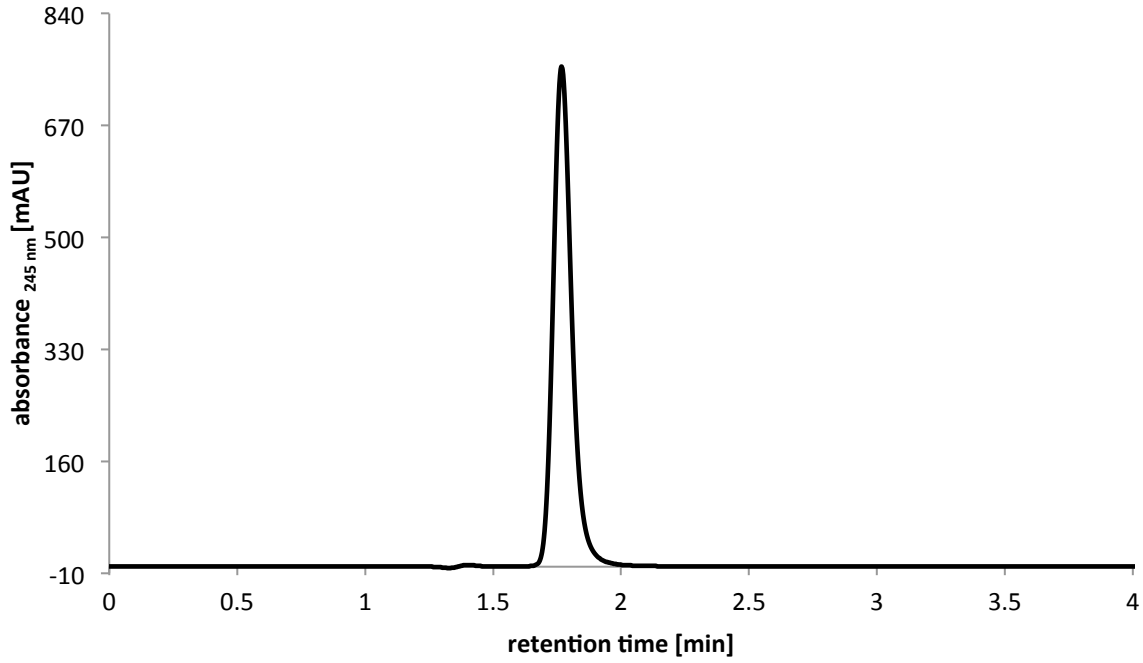


Figure 14. Chromatogram of the L-ascorbic acid standard with a concentration of 125 µg/ml. The absorption was measured at 245 nm

2.2.2 Analysis of 5-methytetrahydrofolate (5-me-THF)

The analytical procedure was based on the method of Jastrebova, Witthöft *et al.* (2003).

Enzyme isolation

The conjugase enzyme was isolated by homogenising 10 mg of lyophilized hog kidney acetone powder in 100 ml L-cysteine hydrochloride solution (pH 4.6, 10 mg/ml). Then the suspension was incubated for 4 hours at 37 °C in a heating block, followed by a centrifugation step at 14,000 rpm for 30 minutes.

In order to isolate the enzyme according to Gregory, Sartain & Day (1984), the resulting supernatant was mixed with 75 % ammonium sulphate solution in a ratio of 1:1 for protein precipitation. After centrifugation, the pellet was re-dissolved in 100 ml 50 mM potassium acetate buffer with a pH of 4.5 containing 10 mM 2-mercaptoethanol. The resulting conjugase solution was stored in 2 ml aliquots at -20 °C until further analysis.

Sample preparation

250 ± 5 mg samples were exactly weighted in and homogenized in 830 µl 0.1 M phosphate buffer, pH 6.0 containing 0.1 % (v/v) 2-mercaptoethanol and 2 % (w/v) L-ascorbic acid. After mixing and flushing with nitrogen, the samples were extracted at 100 °C for 10 minutes and shaken twice in the meanwhile. Then, the samples were put on ice immediately and the pH was adjusted to 4.9 by using 96 % acetic acid.

125 µl hog kidney conjugase were added, and the extracts were incubated for 3 hours at 37 °C and 350 rpm in a thermo cycler.

For enzyme inactivation, the extracts were heated for 5 minutes at 100 °C, rapidly cooled on ice and centrifuged at 14,000 rpm for 15 minutes at 4 °C. The resulting supernatants were transferred into 2 ml flasks, and the pellets were re-dissolved in 830 µl 0.1 M phosphate buffer, pH 6.0 containing 0.1 % (v/v) 2-mercaptoethanol. The samples were flushed with nitrogen and centrifuged at 14,000 rpm for 15 minutes at 4 °C.

The obtained supernatants were transferred to the same flasks as before, and the volume was filled up to 2 ml by using 0.1 M phosphate buffer, pH 6.0 containing 0.1 % (v/v) 2-mercaptoethanol. After flushing with nitrogen and tight capping, the samples were stored at -20 °C.

Sample clean-up

Prior to HPLC analysis, a solid-phase extraction with strong-anion exchange cartridges (500 mg, 3 ml, Agilent Technologies, USA) was used to purify the sample extracts. A LiChrolut vacuum chamber from Merck, Darmstadt, Germany was used to work under reduced pressure. The columns were pre-conditioned with 2 x 2 ml methanol and 2 x 2 ml deionized water.

2 ml sample extracts were applied and in order to remove interfering compounds, the cartridges were washed with deionized water (2 x 2 ml). To elute the retained folates, 2 ml 0.1 M sodium acetate buffer containing 0.1 % (v/v) 2-mercaptoethanol, 10 % (w/v) sodium chloride and 1 % (w/v) L-ascorbic acid was used. The first 560 µl eluate were discarded, and the remaining eluate was collected, flushed with nitrogen and stored at -20 °C until analysis.

HPLC measurement

The parameters for the HPLC measurements are summarized in Table 16. One example of the resulting standard chromatograms is depicted in Figure 15. The retention time of the 5-methyltetrahydrofolic acid disodium salt standard was 2.96 minutes.

Table 16. HPLC parameters for the analysis of the 5-me-THF content in strawberry juice and orange juice

Column	pre-column: phenomenex® AJ0-9297 EVO C18 phenomenex Kinetex, 5 µm EVO C18 100 Å		
Column temperature	23 °C		
Detection	Fluorescence detector	λ_{ex} : 290 nm λ_{em} : 360 nm	
Flow rate	0.6 ml/min		
Mobile phase	A: 30 mM potassium phosphate buffer pH 2.3 B: acetonitrile		
Gradient	time [min]	% A	% B
	0	94	6
	4	94	6
	9	20	100
Injection volume	5 µl		
Stop time	10 min		
Post time	5 min		

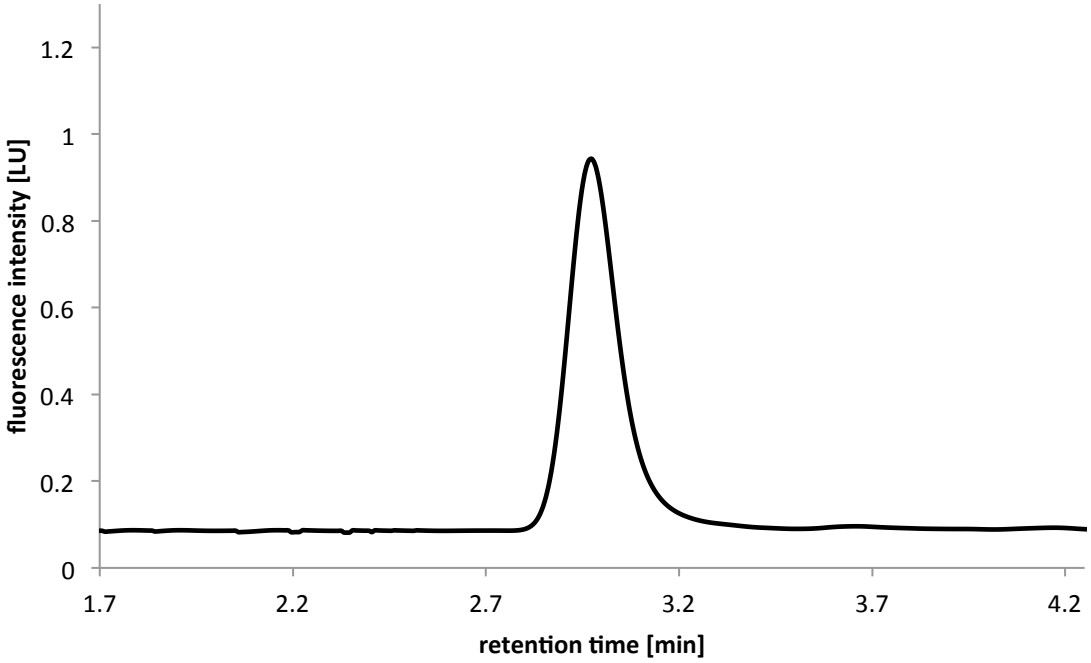


Figure 15. Chromatogram of the 5-me-THF standard with a concentration of 10 µg/ml. The fluorescence was measured at 360 nm

Reproducibility and recovery of the SPE method

To analyse the reproducibility, a 2 µg/ml standard, a strawberry juice sample from P. Avance Collection and the same sample with 50 % of the concentration added as standard were purified with SPE and analysed with HPLC. The standard was analysed three times, the sample five times and the sample with added standards three times.

The recovery of the SPE method was determined by using the method of standard addition. Therefore, 200 %, 250 % and 400 % of the analysed sample concentration were added as standard, and a calibration curve was generated. The recovery was calculated by using the following formula:

$$Recovery = \frac{k\ standard\ calibration}{k\ standard\ addition}$$

Formula 1. Used formula to calculate the recovery after the solid – phase extraction

2.2.3 Analysis of vitamin B_6

Sample preparation

The sample preparation and the further chromatographic analysis were performed based on the method by Brubacher, Müller-Mulot & Southgate (1985); 250 µl carrot juice were homogenised in 250 µl 0.1 N H_2SO_4, heated and shaken on a heating block for 30 minutes at 99 °C. After cooling the samples, 500 µl of deionized water were added, and the samples were centrifuged for 10 minutes at 14,000 rpm. The supernatant was then analysed by using RP-HPLC.

For the standards, 10 mg of pyridoxine hydrochloride, pyridoxal hydrochloride and pyridoxamine dihydrochloride were dissolved in deionized water and further diluted with water to obtain concentrations between 0.00508 and 0.325 µg/ml in the final solution.

HPLC measurement

The parameters for the HPLC measurements are summarized in Table 17. One example of the resulting standard chromatograms is depicted in Figure 16. The retention time of pyridoxamine, pyridoxal, and pyridoxine were 2.76 minutes, 6.08 minutes and 9.38 minutes, respectively.

Table 17. HPLC parameters for the analysis of the vitamin B_6 content in carrot juice

Column	pre-column: phenomenex® AJ0-9297 EVO C18 YMC-Triart C18 150x2.1 mm S-3 µm, 12 nm			
Column temperature	25° C			
Detection	Fluorescence detector	λ_{ex}: 285 nm λ_{em}: 400 nm		
Flow rate	0.2 ml/min			
Mobile phase	A: methanol B: deionized water C: 40 mM sulphuric acid			
Gradient	time [min]	% A	% B	% C
	10	0	0	100
	10.01	0	100	0
	12	20	80	0
Injection volume	5 µl			
Stop time	25 min			

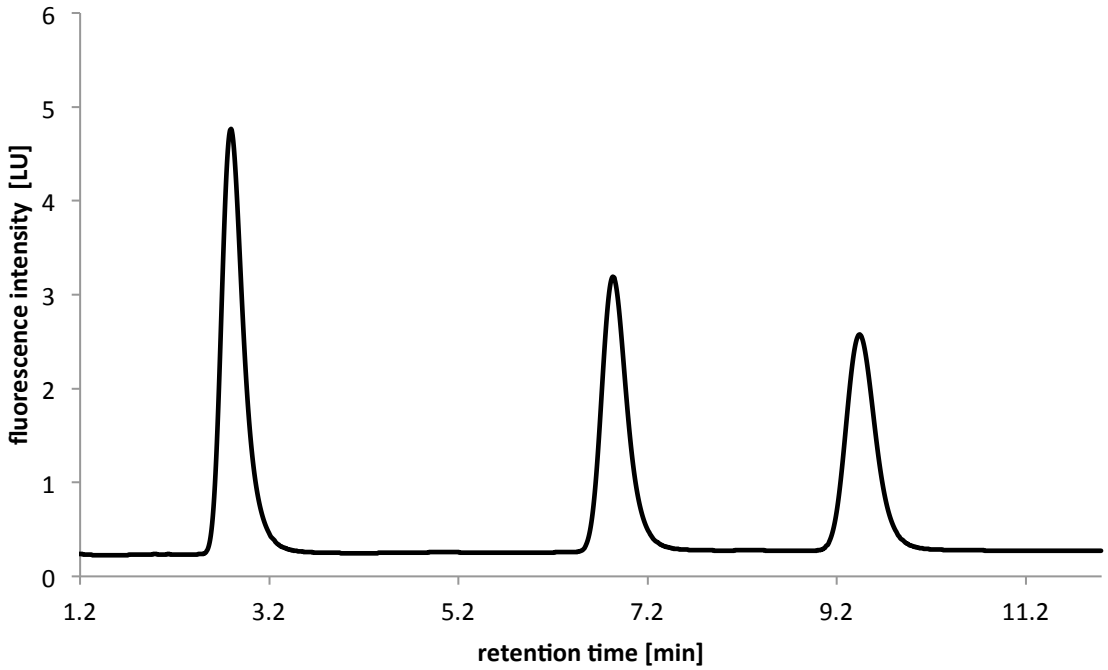


Figure 16. Chromatogram of the standards pyridoxine, pyridoxal and pyridoxamine with a concentration of 0.32 µg/ml. The fluorescence was measured at 400 nm

2.2.4 Analysis of the total phenolic content

The total phenolic content (TPC) of the fruit juice samples was measured based on the procedure by Singelton *et al.* (1999). Therefore, the samples were centrifuged for 10 minutes at 14,000 rpm, and 10 µl of the supernatants were mixed with 600 µl deionized water and 50 µl Folin & Ciocalteu´s phenol reagent in 1 cm plastic cuvettes. After waiting for one minute, 150 µL $NaCO_3$ (20 %) were added, and after eight more minutes another 150 µl $NaCO_3$ were added. The solutions were then incubated for 2 hours in the dark, and the absorption was measured at 765 nm by using a photometer. For the blank, 10 µl deionized water were used instead of a sample.

Gallic acid was used as standard substance, and the TPC of the samples was expressed as gallic acid equivalent in micrograms/millilitre.

During the experiment, a Beckman Coulter DU® 800 photometer and the associated software DU® 800 Spectrophotometer were used for data evaluation.

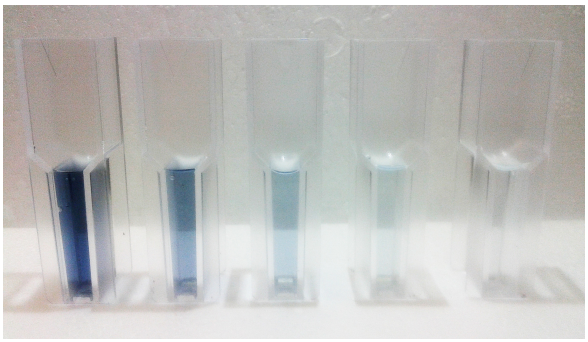


Figure 17. Gallic acid calibration curve for the Folin-Ciocalteu assay to analyse the total phenolic content of fruit juices

2.2.5 Analysis of anthocyanins

Sample preparation

0.1 M hydrochloric acid (HCl) was used to stabilize present anthocyanins. Therefore, 750 µl of the juice samples were diluted with 750 µl 0.1 M HCl, mixed and kept in the freezer at -18 °C until further analysis.

Shortly before the RP-HPLC measurement, the samples were centrifuged for 10 minutes at 14,000 rpm, and the supernatants were transferred to HPLC-vials. The vials were flushed with nitrogen, and the samples were analysed.

HPLC measurement

The parameters for the HPLC measurements are summarized in Table 18. The anthocyanins in strawberry juice were identified by comparing the retention times of the separated compounds with the results from Riedl & Murkovic, 2011. The retention times from the grape juices were compared with the results from Dimitrovska *et al.*, 2011.

Table 18. HPLC parameters for the analysis of anthocyanins in strawberry juice and red grape juice

Column	Agilent LiChrosphere® RP C18, 125x4.5 µm		
Column temperature	30 °C		
Detection	UV/UV-vis Detector, 525 nm		
Flow rate	1 ml/min		
Mobile phase	A: H_2O: formic acid: acetonitrile (87:10:3)		
	B: H_2O: formic acid: acetonitrile (40:10:50)		
Gradient	time [min]	% A	% B
	3	90	10
	18	0	100
Injection volume	5 µl		
Stop time	18 min		
Post time	3 min		

2.2.6 Analysis of hesperidin

Sample preparation

The sample preparation was accomplished based on a paper by Gorinstein *et al.*, 2006 with slight modifications. Therefore, 3.6 g of orange juice were extracted with the help of 4 ml deionized water. The suspensions were mixed and centrifuged for 10 minutes at 4,500 rpm. The supernatants were then transferred into new sample vials, and the pellets were extracted two more times with 3 ml deionized water, respectively. Between the extraction steps, the samples were again centrifuged, and the supernatants were collected to obtain a total volume of 10 ml extraction solution, which was stored at -18 °C until further analysis. Before the RP-HPLC analysis, the samples were diluted 1:10 with methanol (MeOH). For the hesperidin-standard, 10 mg hesperidin were solved in 70 % MeOH-solution and diluted with 80 % MeOH-solution to obtain hesperidin concentrations between 3.9 and 125 µg/ml.

HPLC measurement

The parameters for the HPLC measurements are summarized in Table 19. One example of the resulting standard chromatograms is depicted in Figure 18. The retention time of the hesperidin standard was 9.18 minutes.

Table 19. HPLC parameters for the analysis of hesperidin in orange juice

Column	pre-column: phenomenex® AJ0-9297 EVO C18 phenomenex® Kinetex, 5 µm EVO C18 100 Å		
Column temperature	40 °C		
Detection	UV/UV-vis Detector, 285 nm		
Flow rate	0.6 ml/min		
Mobile phase	A: 2% acetic acid in water B: acetonitrile		
Gradient	time [min]	% A	% B
	2	90	10
	10	30	70
	20	10	90
Injection volume	5 µl		
Stop time	20 min		
Post time	5 min		

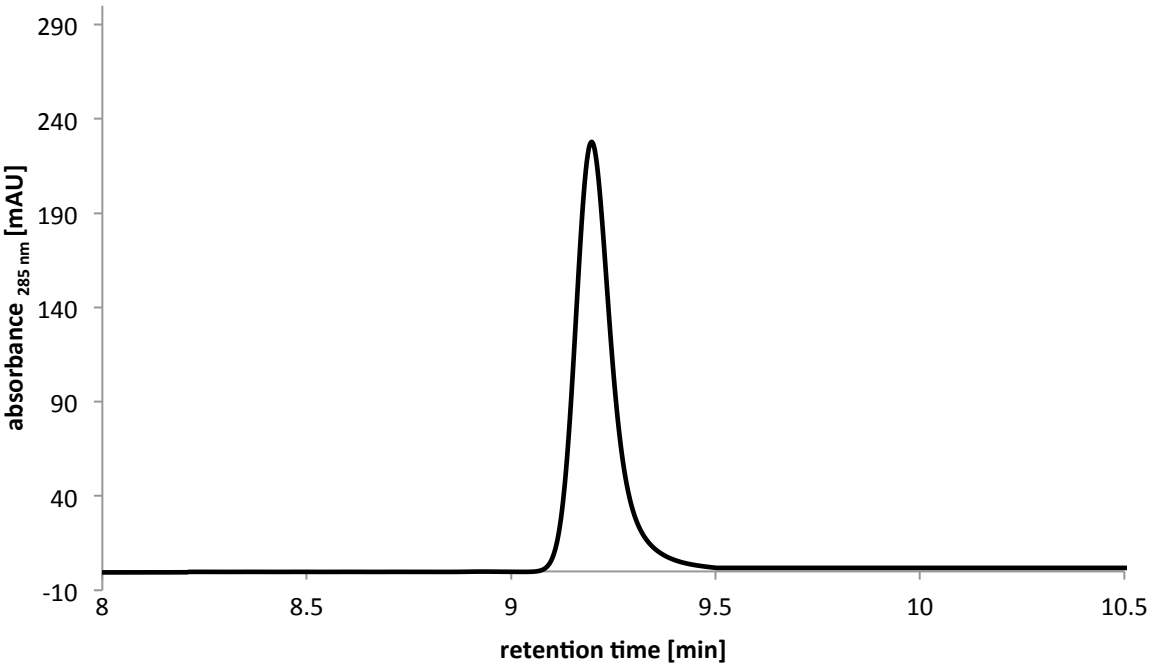


Figure 18. Chromatogram of the hesperidin standard with a concentration of 125 µg/ml. The absorption was measured at 285 nm

2.2.7. Determination of the total microbial counts

To determine the growth of yeasts and moulds in the fruit juice samples, YGC (Yeast extract glucose chloramphenicol) agar was used, and for monitoring the total bacterial growth, PC (plate count) agar was taken. For media preparation, 32 g YGC agar powder and 16.4 g PC agar powder were weighted in and diluted with 800 ml deionized water, respectively. After subsequent stirring, both media were put in boiling water for 30 minutes to solve the agar. Afterwards, the media were autoclaved for 20 minutes at 121 °C.

Peptone water was used for sample homogenization. Therefore, 51 g buffered peptone powder were diluted with two litres deionized water and autoclaved for 20 minutes at 121 °C.

500 ml Ringer solution were prepared by dissolving one Ringer solution tablet in deionized water.

The samples were homogenized and diluted 1:10 with peptone water. After subsequent dilution with Ringer solution (1:10), 0.1 ml homogenate were applied to petri dishes in order to obtain a total dilution factor of 1:1,000. Approximately 20 ml of each agar were poured in each petri dish. After solidification of the agar, the plates with YGC agar were incubated at 25 °C for four days, and the PC agar plates were incubated at 30 °C for three days.

2.2.8 Temperature profile analysis

The surrounding temperature has a great impact on the quality and stability of fruit juices and other foods. Low temperatures decrease the growth rate of microorganisms and therefore reduce the risk of microbial contaminations and prolong the storability of foods. The average temperature of domestic fridges shows high fluctuations between different households in Austria. Optimal fridge temperatures are recommended to be 1 – 5 °C (Hölzl & Aldrian, 2011)

Thus, one additional part of this master´s thesis was to compile a temperature profile analysis in order to gain an overview of the typical storage conditions in local fridges.

Therefore, a testo 175T2 data logger was used to record the temperature of different fridges over a period of seven days. Fridges from private persons, the laboratory and members of the laboratory staff from the Food Chemistry group (Technical University of Graz) were analysed. To ensure equal conditions, the testo 175T2 data logger was placed in the rear section of the middle shelf in every fridge. Measuring points were recorded every third second.

The testo Comfort Software Basic 5.0 was used to record the surrounding temperatures, and the mean value and standard deviation were calculated.

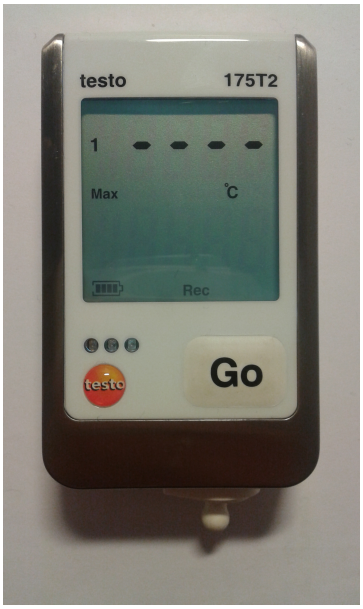


Figure 19. Picture of the testo 175T2 data logger used for the temperature profile analysis

3. Results and discussion

3.1 Vitamin C

The fruits were washed or pealed, prepared as described in the chapter “Materials and Methods”, and analysed by using HPLC. Mean values and standard deviations were calculated for all obtained data. The results as well as the concentration differences between oxygen and nitrogen storage atmosphere are represented in tabular and graphic form. Standard calibration, measured peak areas and a sample chromatogram are listed in the appendix. The vitamin C content was measured in orange juice and strawberry juice.

Orange juice

Under oxygen storage conditions, L-ascorbic acid displayed a low stability over 24 hours in orange juices from all three juice-makers. The highest degradation was found in the P. ProBlender, where about 70 % of the initial vitamin C content was degraded. The P. Avance Collection and the P. Masticating Juicer obtained similar degradation characteristics, with a loss of about 25 % of the initial concentration. The great loss of vitamin C with the ProBlender machine is caused, as during the high-speed mixing a lot of oxygen is added to the juice, which favours the oxygen induced degradation of vitamin C. In contrast, the spinning and chewing methods of the P. Avance Collection and the P. Masticating Juicer lead to less oxygen input in the juices (Table 20, Figure 20). Storing under nitrogen conditions led to a great enhancement of the L-ascorbic acid stability. When protected from oxygen, the vitamin C concentration remained almost constant over 24 hours for all three juice-makers. When comparing the vitamin C concentrations of the juices under oxygen and nitrogen atmosphere, the concentrations are considerably higher under nitrogen storage conditions. In addition, the obtained vitamin C contents in the first experiment are at the lower end of literature references, as the average vitamin C concentration of orange juice is indicated to be 30 – 60 mg/ 100 ml (Velisek, 2014). One reason therefore is the strong dependence of the vitamin C content on both, harvest time and ripeness of the fruits. The typical harvest

season for oranges is from October till March. The first experiment (storing under oxygen atmosphere) was performed at the end of April, while the second experiment was completed at the end of October.

Therefore it can be assumed that the different harvest times are plausible explanations for the concentration variances among the orange juices.

Table 20. Stability of the vitamin C content in orange juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

Vitamin C [mg/100 g]						
time [h]	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
	O_2	N_2	O_2	N_2	O_2	N_2
0	34.3 ± 2.6	44.5 ± 8.0	29.5 ± 6.6	37.6 ± 1.8	34.8 ± 2.3	48.6 ± 9.7
2	36.5 ± 2.8	44.0 ± 6.9	18.2 ± 2.7	37.7 ± 4.2	30.8 ± 3.6	50.2 ± 10.1
5	37.6 ± 6.4	43.8 ± 5.0	21.6 ± 5.1	36.6 ± 1.7	29.8 ± 3.8	46.8 ± 9.7
8	34.3 ± 7.1	44.2 ± 5.7	13.8 ± 5.1	36.9 ± 2.6	25.9 ± 2.8	47.2 ± 8.3
24	27.2 ± 2.6	43.7 ± 2.7	8.7 ± 3.8	37.1 ± 3.9	26.1 ± 4.2	48.1 ± 10.7

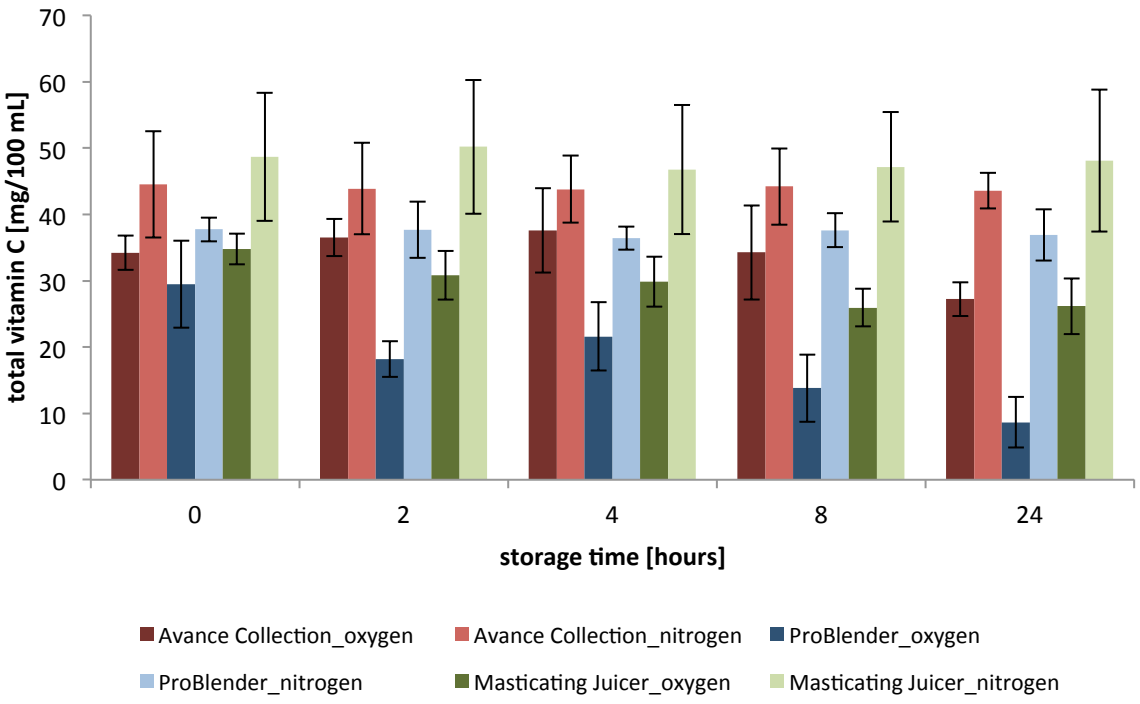


Figure 20. Stability of the vitamin C content in orange juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Strawberry juice

The vitamin C content in strawberry juice strongly decreased over a storage period of 24 hours under oxygen atmosphere. After 24 hours, the highest remaining vitamin C concentration was detected in juices from the P. Masticating juicer, with a loss of about 39 %. The degradation rate in the juices from the P. Avance Collection and the P. ProBlender were considerably higher, with a loss of 64 % and 80 % of the initial concentration, respectively. The high degradation rate of the P. ProBlender can again be explained by the high oxygen in-put during the juice making process. By storing the juices under oxygen-free conditions, the stability of vitamin C was greatly enhanced. Under nitrogen atmosphere, the concentration was moderately stable over 24 hours. There was a slight loss of vitamin C in the juices from the P. ProBlender, even when stored under nitrogen conditions. Possible explanations can be that the exposure to UV-light or temperatures over 4 °C during sample withdrawal could have induced degradation processes.

The maximum vitamin C concentrations could be obtained in juices from the P. Avance Collection (50 mg/100 ml), while juices produced by the P. ProBlender had the lowest content (39.1 mg/100 ml, see Table 21, Figure 21). The vitamin C concentrations measured in this experiment are lower than references from the literature, with values of 60 mg/ 100 ml (Velisek, 2014). This can be explained by general variations in the nutrient content, resulting from different harvest times and cultivation conditions. Furthermore, also the treatment of the strawberries before processing (removing of damaged areas, stems and leaves) exposed the fruits to oxygen and could have led to vitamin C degradation.

Table 21. Stability of the vitamin C content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

Vitamin C [mg/ 100 g]						
time	**P. Avance Collection**		**P. ProBlender 6**		**P. Masticating Juicer**	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	49.6 ± 6.1	49.5 ± 9.3	39.1 ± 2.9	40.6 ± 6.2	46.1 ± 1.1	46.3 ± 5.9
2	40.7 ± 4.2	49.6 ± 5.4	11.4 ± 3.1	40.5 ± 2.0	45.1 ± 1.2	46.4 ± 4.9
5	29.0 ± 2.8	50.0 ± 5.7	9.0 ± 2.8	40.2 ± 1.8	35.4 ± 3.6	46.0 ± 2.5
8	19.4 ± 2.1	48.5 ± 7.2	7.5 ± 2.1	40.0 ± 2.8	31.3 ± 2.6	45.2 ± 0.9
24	17.7 ± 2.4	49.2 ± 7.1	7.6 ± 2.7	40.2 ± 1.4	28.3 ± 2.4	45.9 ± 1.2

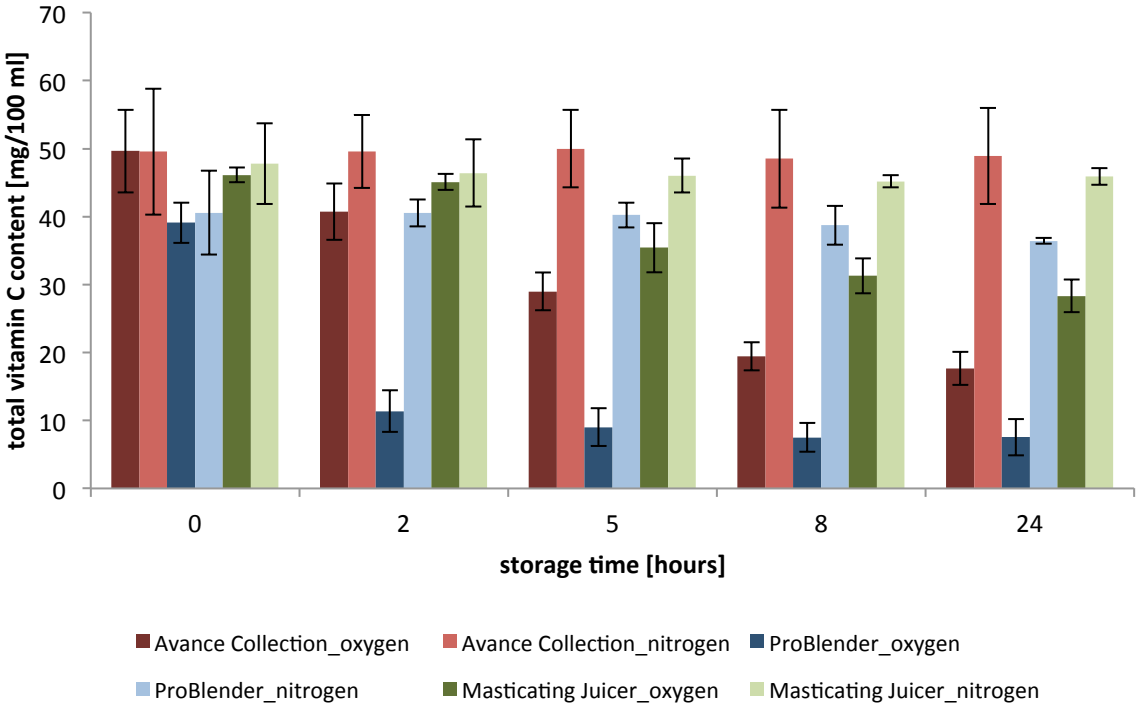


Figure 21. Stability of the vitamin C content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Comparison of orange and strawberry juice

In both, orange and strawberry juice, a degradation of the vitamin C concentration could be detected over 24 hours under oxygen conditions. The greatest losses occurred in juices of the P. ProBlender. The highest concentration of vitamin C in orange juice could be obtained from the P. Masticating Juicer, and the highest L-ascorbic acids content in strawberry juice from the P. Avance Collection. As vitamin C is very sensitive, the exclusion of oxygen during storage times led to great improvements in the vitamin C stability in both, orange and strawberry juices.

The highest average vitamin C concentration in orange juice was 50.2 mg/100 ml, and the highest measured content in strawberry juice was 50.0 mg/100 ml. At this point is has to be mentioned again that there are many factors which influence the nutritional composition of fruits, including the variety, cultivation, harvest time, and storage conditions. During the experiments, also noteworthy deviations in the vitamin C content in the juices from the same experiment were obtained, resulting in high standard deviations.

3.2 5-Methyltetrahydrofolate

The fruits were washed or pealed and prepared as described in the chapter “Materials and Methods”. Mean values and standard deviations were calculated for all obtained data. The results as well as the concentration differences between oxygen and nitrogen storage atmosphere are represented in tabular and graphic form. Furthermore, the reproducibility and the recovery of the SPE method were analysed. Standard calibration, measured peak areas, sample quantity and a sample chromatogram can be found in the appendix. The 5-me-THF content was measured in orange juice and strawberry juice.

Reproducibility and recovery

The calculated reproducibilities were **99.2 %** for the 5-me-THF standard, **101 %** for the sample and **99.3 %** for sample plus 50 % of the concentration added as standard. These results indicate that the used SPE method is highly reproducible and reliable. The concentrations, mean values, and standard deviations are listed in Table 22 and represented in graphic form in Figure 22.

The calculated recovery of 5-me-THF after the SPE is **78 %**. Despite the high reproducibility of the SPE method a noteworthy amount of folate is lost during the purification process. One possible reason is a lack of time for the folate to sufficiently bind to the column sorbent, due to the applied vacuum. Another explanation is that the used solvent for folate elution was too weak to rupture the sorbent-folate linkage, and parts of the analyte remained on the column. Furthermore, the applied vacuum could not be adjusted precisely, which also impeded the sample clean up. Nevertheless, at this point, it has to be mentioned that some losses may always occur during sample purification. The obtained standard addition curve and the calibration curve are depicted in Figure 23. The calculated concentration of the analysed sample was **1.1 µg/ml** folate and the peak area was **19 LU·s**. The sample concentration obtained from the HPLC analysis was **0.91 µg/ml** and the peak area was **19.4 LU·s**.

Even though there are small deviations in concentrations and peak areas, the results of the first HPLC analysis and the calculations from the standard addition are suitable.

All in all, it can be said that although the used SPE method exhibits slight losses of the analyte, the obtained results show a high reproducibility.

Table 22. Reproducibility analysis of the SPE method to purify 5-me-THF previous to the HPLC analysis

	Concentration in µg/ml					Mean value ± standard deviation	Reproducibility in %
Standard	2.11	2.12	2.05			2.09 ± 0.04	99.2 %
Sample	0.90	0.92	0.89	0.92	0.92	0.91 ± 0.01	101 %
Sample + 50 % standard	1.81	1.86	1.82			1.83 ± 0.1	99.3 %

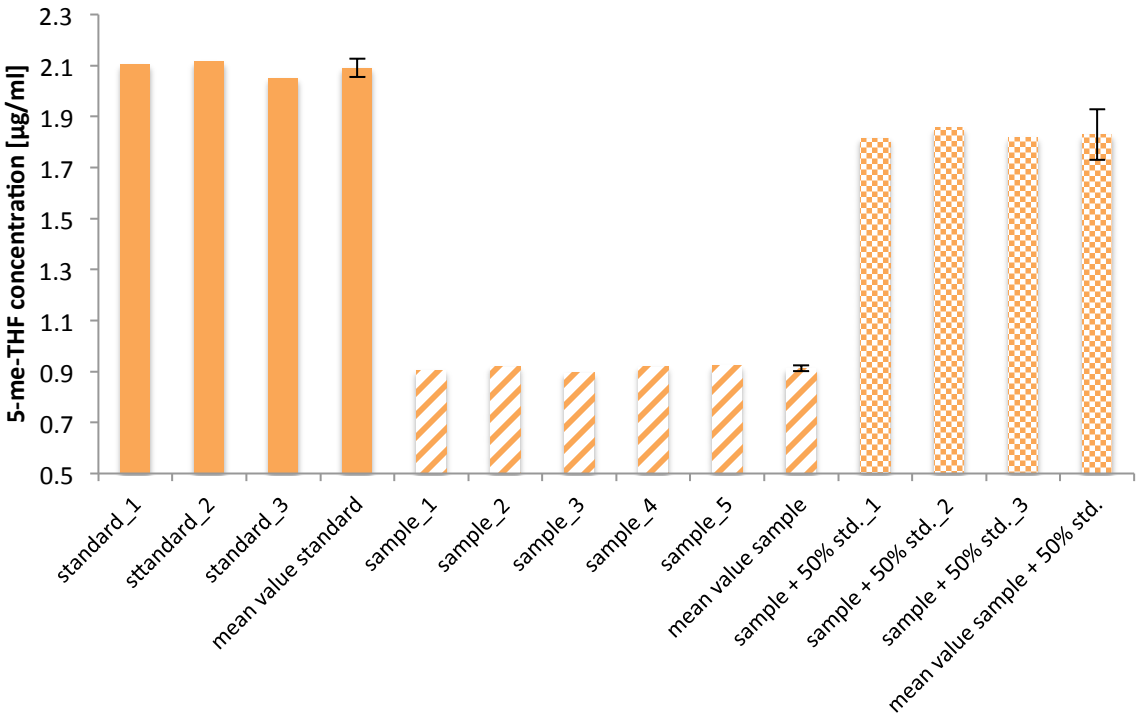


Figure 22. Reproducibility analysis of the SPE method to purify 5-me-THF previous to the HPLC analysis. Analysis of the 2 µg/ml standard, a strawberry juice sample from P. Avance Collection and the sample plus 50 % of the concentration added as standard

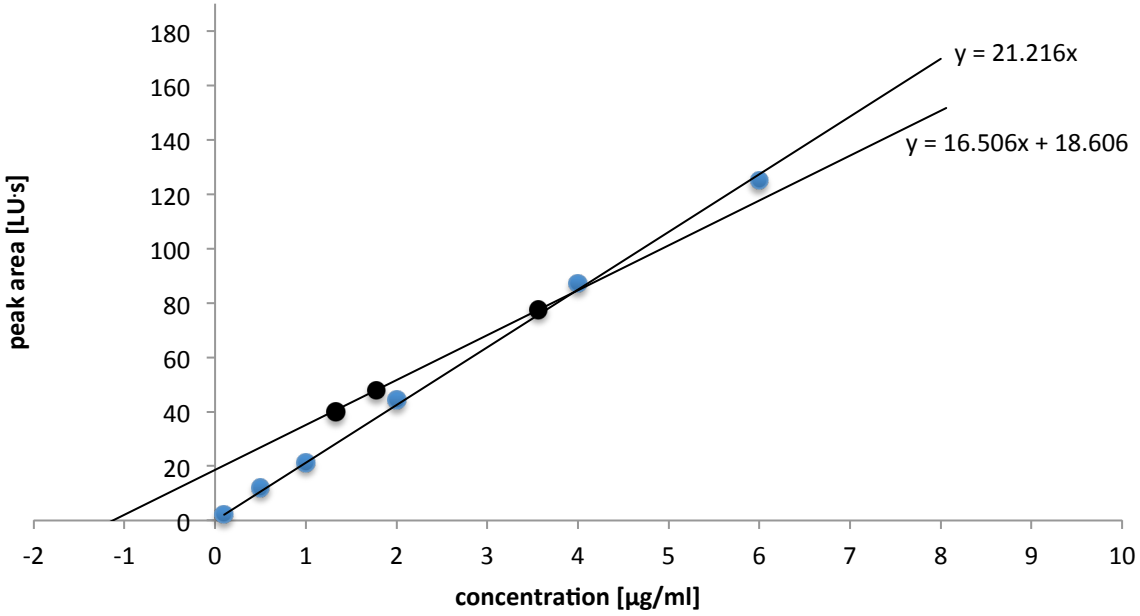


Figure 23. Calibration curve of the 5-me-THF standard (blue) and standard addition curve of the strawberry juice sample from P. Avance Collection (black) to obtain the recovery after the SPE. Calibration equations: y = 21.216 x (for the standard addition) and y = 16.506 x + 18.606 (for the standard calibration curve)

Table 23. Values of the peak areas of the 5-me-THF standard for the calibration curve and the strawberry juice sample for the standard addition

5-Me-THF standard		Strawberry juice sample		
Concentration [µg/m]	**Peak area [LU·s]**	**Concentration [µg/ml]**	**Peak area [LU·s]**	**Standard addition in %**
6	125	1.34	40.0	200 %
4	87.2	1.78	48.0	250 %
2	44.3	3.56	77.6	400 %
1	21.2			
0.5	12.0			
0.1	2.18			

Orange juice

The 5-me-THF content in orange juice was stable over 24 hours under oxygen conditions. The highest concentration was obtained from juices from the P. Masticating Juicer, and the lowest concentration from the P. ProBlender. Under nitrogen atmosphere, the

concentration was also moderately constant over 24 hours in juices from all three juice-makers.

Therefore, it can be assumed that 5-me-THF is not as sensitive to oxygen as for example vitamin C.

For the P. Avance Collection and the P. ProBlender, the folate contents measured in the nitrogen experiments were higher than in the first experiment under oxygen atmosphere. The concentrations in juices from the P. Masticating Juicer were almost equal under both conditions. Furthermore, the P. ProBlender obtained the lowest concentration under oxygen storage, but the highest concentration under nitrogen atmosphere. Thus, it is hard to say which juicer is most favourable to extract the highest content of 5-me-THF from orange juice (Table 24, Figure 24).

The measured folate contents were in general lower than the literature references, which are about 25 µg/100 g (Combs, 2008). Only the juices from the P. ProBlender under nitrogen storage yielded a concentration of 24 – 25 µg/100 g.

The concentration differences between the two experiments and also to the literature references can be explained by the different harvest times and ripeness of the oranges. Experiment 1 (oxygen storage) was performed in April, while the second experiment (nitrogen storage) was conducted in October, within the typical harvest time of oranges. Nevertheless, it appears that there is even a variation in the nutrient content in fruits of the same variety, which have been purchased at the same time in local supermarkets.

Table 24. Stability of the 5-me-THF content in orange juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

5-Me-THF [µg/100 g]						
time	**P. Avance Collection**		**P. ProBlender 6**		**P. Masticating Juicer**	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	15.1 ± 2.2	18.5 ± 3.1	14.5 ± 0.1	24.3 ± 2.1	17.5 ± 5.0	17.4 ± 4.0
2	16.6 ± 3.5	19.5 ± 1.5	14.2 ± 0.7	24.6 ± 1.5	16.2 ± 3.5	17.8 ± 4.7
5	14.7 ± 2.6	18.6 ± 2.2	13.6 ± 5.6	24.4 ± 2.6	16.7 ± 2.6	17.2 ± 4.4
8	15.4 ± 2.2	18.5 ± 3.2	14.1 ± 4.6	25.4 ± 1.6	17.2 ± 2.9	16.9 ± 3.7
24	16.1 ± 1.5	19.0 ± 0.5	14.5 ± 5.9	25.3 ± 1.1	16.5 ± 1.9	17.4 ± 3.7

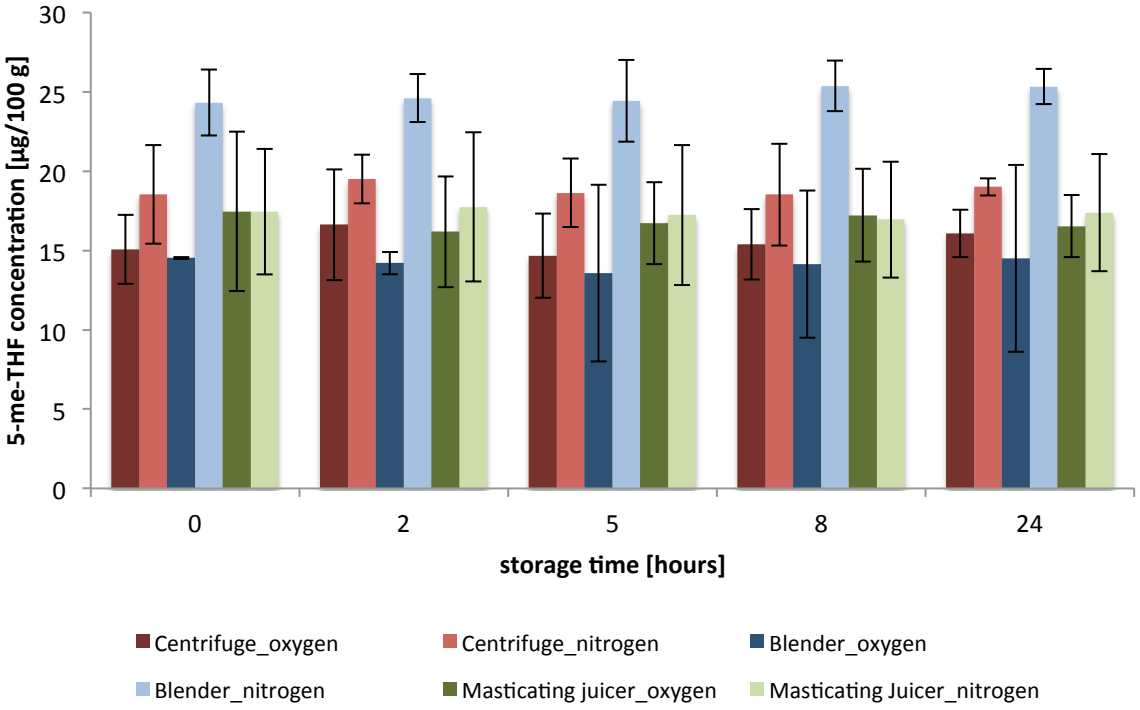


Figure 24. Stability of the 5-me-THF content in orange juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Strawberry juice

5-Me-THF in strawberry juice was stable over 24 hours under oxygen atmosphere. The highest content was detected in strawberry juices of the P. Avance Collection, and the lowest was found in juices from the P. Masticating Juicer. Under nitrogen storage conditions, the content of folate was also stable over 24 hours. The highest concentration was measured in juices gained from the P. ProBlender. The concentration in the juices from the P. Avance Collection and the P. Masticating Juicer were relatively equal under nitrogen atmosphere.

By comparing the data of the two experiments it is impossible to tell which juicer leads to the best results in terms of folate content. However, the concentration in the juices produced by the P. ProBlender showed the highest similarity between the two trails (Table 25, Figure 25).

Furthermore, high variation in the folate content could be detected between the juices within one experiment, which leads to high standard deviations.

Literature references for the 5-me-THF content in strawberry juice are about 644 µg/ 100 g (Straslö *et al.*, 2008). The highest detected concentration was 770 µg/100 g, and the lowest concentration was 490 µg/100 g. The first experiment was performed in the middle of May, which can be an explanation for the higher folate contents than in literature. The second experiment was conducted at the end of August, after the typical harvest time of strawberries. Thus, some fruits may have been unripe which could be a reason for the comparatively low folate contents in the juices from the P. Avance Collection and the P. Masticating Juicer.

Table 25. Stability of the 5-me-THF content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

5-Me-THF [µg/ 100 g]						
time	**P. Avance Collection**		**P. ProBlender 6**		**P. Masticating Juicer**	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	764 ± 25	490 ± 58	686 ± 115	660 ± 74	589 ± 33	498 ± 53
2	724 ± 26	508 ± 80	637 ± 26	626 ± 45	707 ± 84	502 ± 6
5	723 ± 5	492 ± 65	619 ± 27	615 ± 49	707 ± 59	528 ± 44
8	743 ± 8	512 ± 82	621 ± 30	682 ± 69	696 ± 43	505 ± 60
24	771 ± 29	552 ± 94	598 ± 43	650 ± 38	670 ± 58	516 ± 61

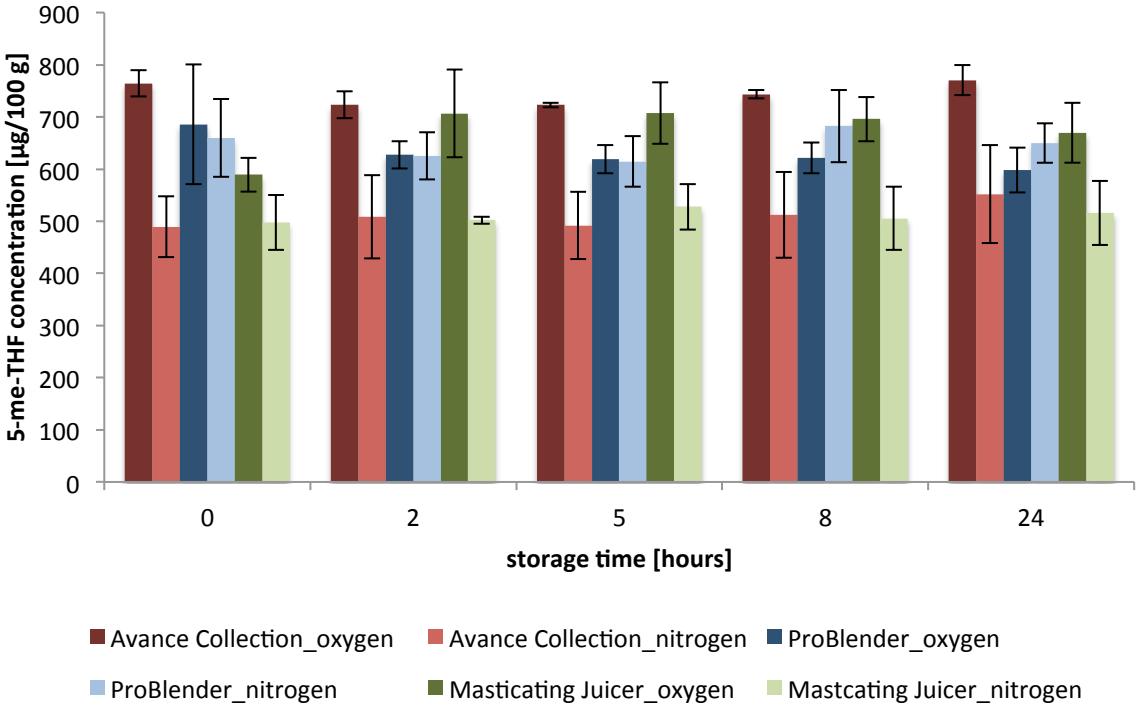


Figure 25. Stability of the 5-me-THF content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Comparison of orange and strawberry juice

The concentration of 5-me-THF was stable under oxygen and nitrogen conditions in both, orange and strawberry juice, from all three juice-makers. Therefore it can be concluded that 5-me-THF is not degraded over 24 hours when exposed to oxygen. Concentration deviations under oxygen and nitrogen storage conditions were obtained in both juices. They may result from different harvest times and maturity of the fruits.

The highest folate content in orange juice could be obtained with the P. ProBlender and the highest concentration in strawberry juices with the P. Avance Collection. The concentrations show small deviations from literature references, but all in all, the values match.

In conclusion it can be said that none of the juicers can be explicitly highlighted to obtain the highest folate concentration, neither in orange juice nor in strawberry juice. Nevertheless, by comparing the results of orange and strawberry juices, it was observed that the folate content in strawberry juice is at least 20 times higher than in orange juice. Thus, strawberry juice is definitely the better source of folates than orange juice.

3.3 Vitamin B_6

The fruits were washed or pealed, prepared as described in the chapter "Materials and Methods", and analysed using HPLC. Mean values and standard deviations were calculated for all obtained data. The results as well as the concentration differences between oxygen and nitrogen storage atmosphere are represented in tabular and graphic form. Standard calibration, measured peak areas, and a sample chromatogram can be found in the appendix. The vitamin B_6 content was measured in carrot juice.

Carrot juice

The vitamin B_6 content in carrot juice was stable over 24 hours under oxygen storage conditions in juices from all three juice-makers. Therefore, it can be assumed that vitamin B_6 is not degraded within 24 hours when exposed to oxygen. Under oxygen storage, the highest content was obtained from the P. ProBlender, and the lowest concentration from the P. Avance Collection. However, the calculated concentrations of the juices stored under oxygen conditions are comparatively similar within all three juicers. Concentration deviations can be seen in the juices from the P. Avance Collection, and the vitamin B_6 concentration in juices from the P. ProBlender increases over 24 hours. These variations can be explained by small measurement inaccuracies. As expected, the vitamin B_6 content in the juices stored under nitrogen atmosphere was also stable. The highest concentration was obtained from the P. Avance Collection, and the lowest concentration in juices produced from P. Masticating Juicer. Nevertheless, the concentration differences within the juices of the several juice-makers are, again, relatively low (Table 26, Figure 26). There are no significant concentration differences between the juicers under both storage conditions. Thus it is not possible to identify which juicer is the most favourable to extract high amounts of vitamin B_6 from carrots. The obtained vitamin B_6 contents of the first experiment (storage under oxygen atmosphere) were lower than the concentrations of the second experiment (storage under nitrogen atmosphere). This can be explained by the fact that the first trail was performed in March using early carrot varieties and the second trail was performed in May using late carrot varieties. The cultivation conditions and the access to sunlight strongly influence the

nutritional content of fruits and vegetables.

According to literature, the vitamin B_6 content of carrots is 170 µg/100 g (Eitenmiller, 2008). The concentrations gained from the late variety carrots are highly compatible with the literature references, while the vitamin content in juices from early variety carrots was slightly lower.

Table 26. Stability of the vitamin B_6 content in carrot juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

Vitamin B_6 [µg/100 ml]						
time	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	137 ± 8	177 ± 19	143 ± 9	175 ± 11	146 ± 2	163 ± 27
2	156 ± 18	177 ± 20	146 ± 7	177 ± 10	146 ± 10	163 ± 16
5	137 ± 27	176 ± 13	144 ± 10	175 ± 6	1512 ± 22	163 ± 13
8	153 ± 17	176 ± 15	156 ± 7	175 ± 18	153 ± 19	163 ± 33
24	141 ± 30	175 ± 16	158 ± 5	174 ± 6	157 ± 7	163 ± 19

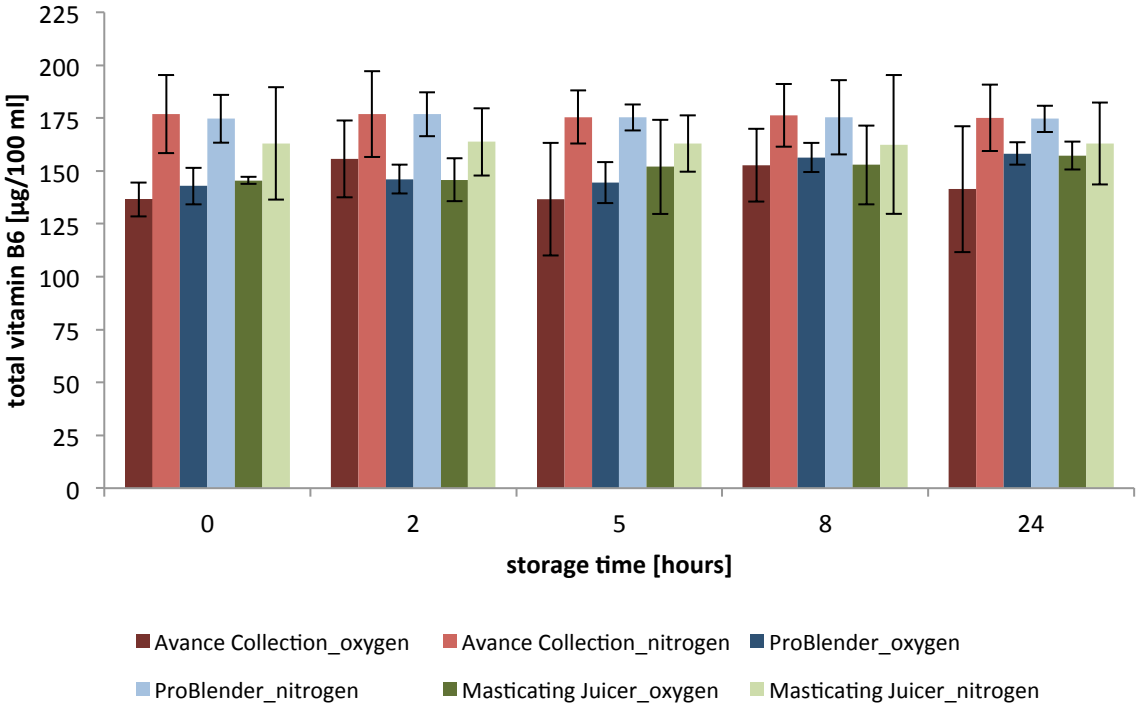


Figure 26. Stability of the vitamin B_6 content in carrot juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

3.4 Total phenolic content

The fruits were washed or pealed, prepared as described in the chapter "Materials and Methods", and analysed with the photometer. Mean values and standard deviations were calculated for all obtained data. The results as well as the concentration differences between oxygen and nitrogen storage atmosphere are represented, absorptions are in the appendix. The total phenolic content was measured in strawberry, carrot and grape juice.

Strawberry juice

The total phenolic content in strawberry juice was stable over 24 hours when stored under oxygen atmosphere. No significant degradation could be detected in the juices produced by the several machines. Consequently, most phenolic compounds are stable under oxygen storage conditions. The highest initial TPC was found in juices from the P. ProBlender, and the lowest content in juices from the P. Masticating Juicer. As expected, the TPC was also stable when the juices were stored under nitrogen conditions. The highest concentrations were again obtained from the P. ProBlender, and the lowest TPC was measured in juices from the P. Avance Collection (Table 27, Figure 27).

There is a tendency that the P. ProBlender leads to the highest TPC, whereas the calculated contents from the three juices are quite similar within the several storage conditions. The obtained TPC from the second experiment are higher than the contents from the first experiment. One reason is that the first trail was performed in April, before the typical strawberry harvest time, and the second trail was performed at the end of May, directly within the harvest time. Furthermore, the production of polyphenols in the skin of fruits strongly depends on the exposure to sunlight. More sunlight leads to increased production of polyphenols to protect the fruits against UV-radiation (Bravo, 1998). Depending on the variety, the land of origin and the harvest time, different values for the TPC in strawberry juices can be found in literature. References range from 38 – 1441 mg/100 g (Bravo, 1998; Panico *et al.,* 2009). During the experiments, values of 219 – 366 mg/100 ml strawberry juice were obtained.

Table 27. Stability of the total phenolic content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

TPC [mg/100 ml]						
time	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	234 ± 0	293 ± 3	241 ± 15	356 ± 63	228 ± 4	330 ± 25
2	223 ± 14	312 ± 48	244 ± 33	362 ± 49	246 ± 9	330 ± 19
5	233 ± 15	303 ± 42	219 ± 13	353 ± 60	237 ± 13	339 ± 4
8	219 ± 12	308 ± 31	246 ± 13	366 ± 54	247 ± 22	320 ± 6
24	223 ± 15	300 ± 41	237 ± 16	351 ± 61	252 ± 8	327 ± 17

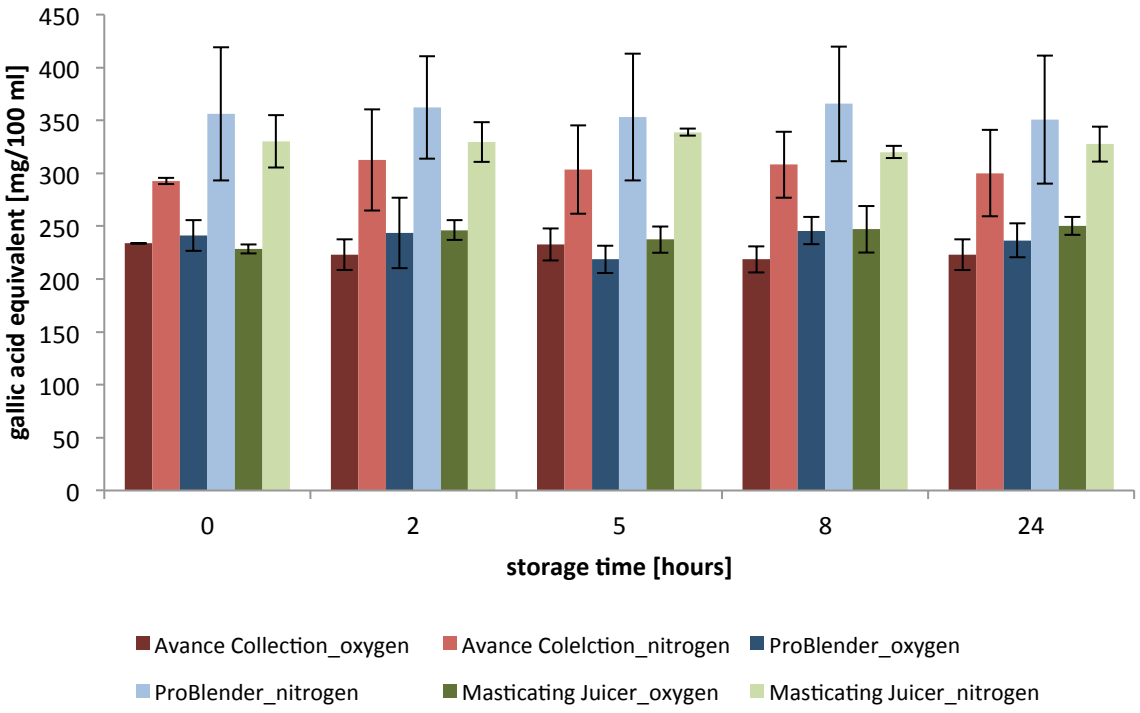


Figure 27. Stability of the total phenolic content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Carrot juice

The total phenolic content in carrot juice was comparatively stable over 24 hours, when exposed to oxygen during the storage time. Only a slight degradation could be observed in all three machines. The highest TPC was reached when the P. ProBlender was used. The

lowest initial TPC was observed in juices produced by the P. Masticating Juicer, whereas the concentration differences between all three juice-makers are rather small. During the storage under oxygen, 9 % of the initial TPC was lost in the carrot juices from the P. ProBlender, while 5 % were lost in juices from the P. Avance Collection. The P. Masticating Juicer had the lowest degradation rate with 2 % of the initial TPC. The observed degradation characteristics of the P. ProBlender and the P. Masticating Juicer fit the previous results, as during the slow chewing process of the P. Masticating Juicer less oxygen is added than during the high-speed mixing of the P. ProBlender.

Obviously, the TPC was also stable when the juices were stored under nitrogen conditions. The highest concentration was, again, obtained from the P. ProBlender and the lowest from P. Masticating juicer (Table 28, Figure 28).

As detected before, there are concentration differences between the two experiments (storing under oxygen and under nitrogen conditions), and the TPCs of the second trail are slightly higher. Nevertheless, the concentration variations are quite low, which indicates that the TPC in carrots is considerably stable in early and late varieties.

Reference values of the TPC in carrots range from 19 – 342 mg/100 g (Leja *et al.*, 2013). The resulting TPC in the carrot juices from all three machines vary between 18.7 – 29.4 mg/100 ml. Thus, the measured contents are at the lower end of the range, whereas the literature values refer to carrots and not to carrot juice, and during the juice processing, losses in the TPC and other nutrients may definitely occur.

Table 28. Stability of the total phenolic content in carrot juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

TPC [mg/100 ml]						
time	**P. Avance Collection**		**P. ProBlender 6**		**P. Masticating Juicer**	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	19.7 ± 0.5	28.1 ± 1.8	20.4 ± 1.9	29.7 ± 2.5	19.4 ± 1.5	20.9 ± 2.6
2	18.9 ± 0.1	29.4 ± 0.6	20.3 ± 0.1	32.2 ± 3.6	18.9 ± 0.3	22.1 ± 1.2
5	19.5 ± 1.1	27.0 ± 5.1	18.6 ± 1.4	25.6 ± 1.3	18.7 ± 1.1	20.9 ± 2.2
8	18.8 ± 0.5	28.0 ± 2.9	20.1 ± 0.2	23.9 ± 3.8	19.1 ± 1.2	21.7 ± 2.6
24	18.8 ± 0.3	27.1 ± 50	18.6 ± 1.6	27.6 ± 6.5	19.0 ± 14	21.1 ± 0.7

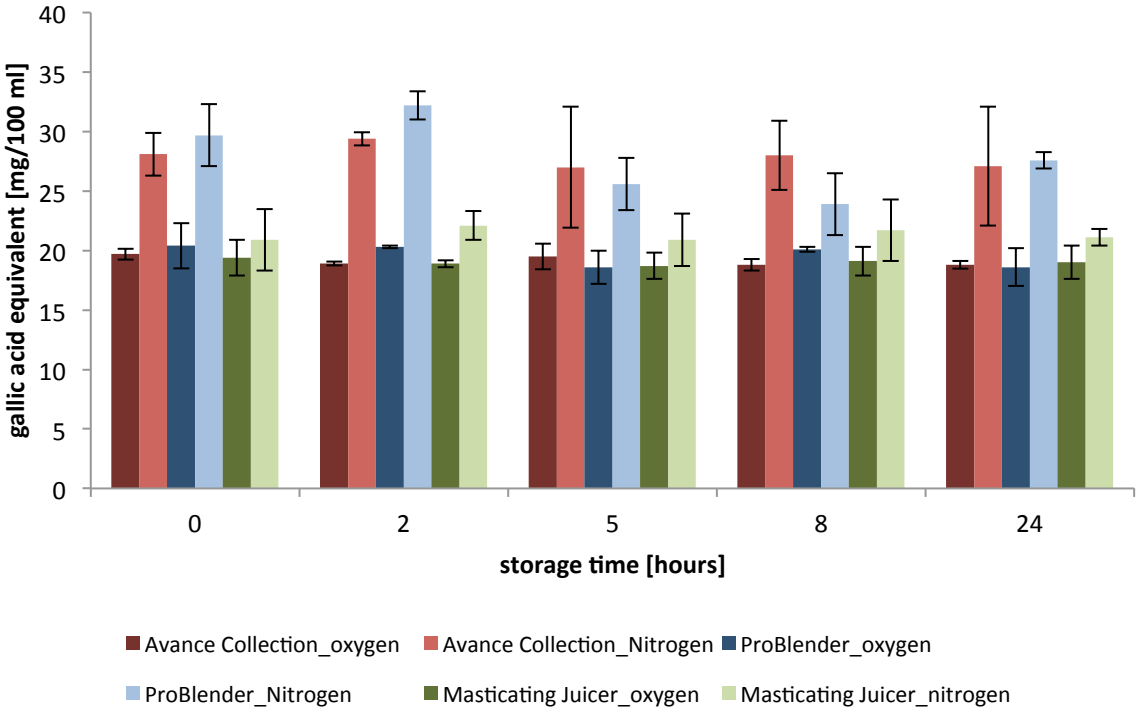


Figure 28. Stability of the total phenolic content in carrot juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Grape juice

Similar to the results of strawberry and carrot juice, the TPC in grape juice was also stable over 24 hours under oxygen atmosphere. The highest concentration was found in juices from the P. ProBlender, and the lowest in juices from the P. Masticating Juicer.

Also storing under nitrogen atmosphere resulted in a stable TPC profile. The highest TPC in the nitrogen storage experiment was, again, obtained from the P. ProBlender, and the lowest from the P. Masticating Juicer. Thus, it can be said that the blender uses the most efficient technique to extract high amounts of polyphenols from grapes. The mashing technique of the P. Masticating juicer may not sufficiently break up the cell walls of the fruits to release polyphenolic compounds (Table 29, Figure 29).

The first experiment (storage under oxygen atmosphere) was performed in April and the second experiment (storage under nitrogen) at the end of September. The typical harvest time of grapes starts in autumn; this may explain the concentration differences between the two trails.

The literature reference of the TPC in grapes is roughly 50 mg/100 g (Bravo, 1998). The

values measured in the experiment range from 19 – 45 mg/100 ml. So, the TPC of the grape juices obtained from this experiments match the numbers found in literature.

Table 29. Stability of the total phenolic content in grape juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

TPC [mg/100 ml]						
time	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	26.4 ± 2.1	27.0 ± 3.0	37.7 ± 1.3	42.0 ± 5.1	19.0 ± 1.5	24.7 ± 0.3
2	24.0 ± 1.4	30.6 ± 3.8	37.3 ± 1.1	45.0 ± 3.6	19.1 ± 1.4	23.6 ± 2.3
5	22.6 ± 0.3	29.1 ± 2.8	37.3 ± 1.0	41.0 ± 8.7	21.4 ± 1.7	21.6 ± 1.8
8	21.5 ± 0.9	30.1 ± 4.3	36.4 ± 1.0	39.1 ± 4.9	19.9 ± 1.5	23.4 ± 2.6
24	21.6 ± 2.5	27.1 ± 2.3	37.9 ± 1.7	37.4 ± 5.3	19.0 ± 1.1	20.7 ± 2.5

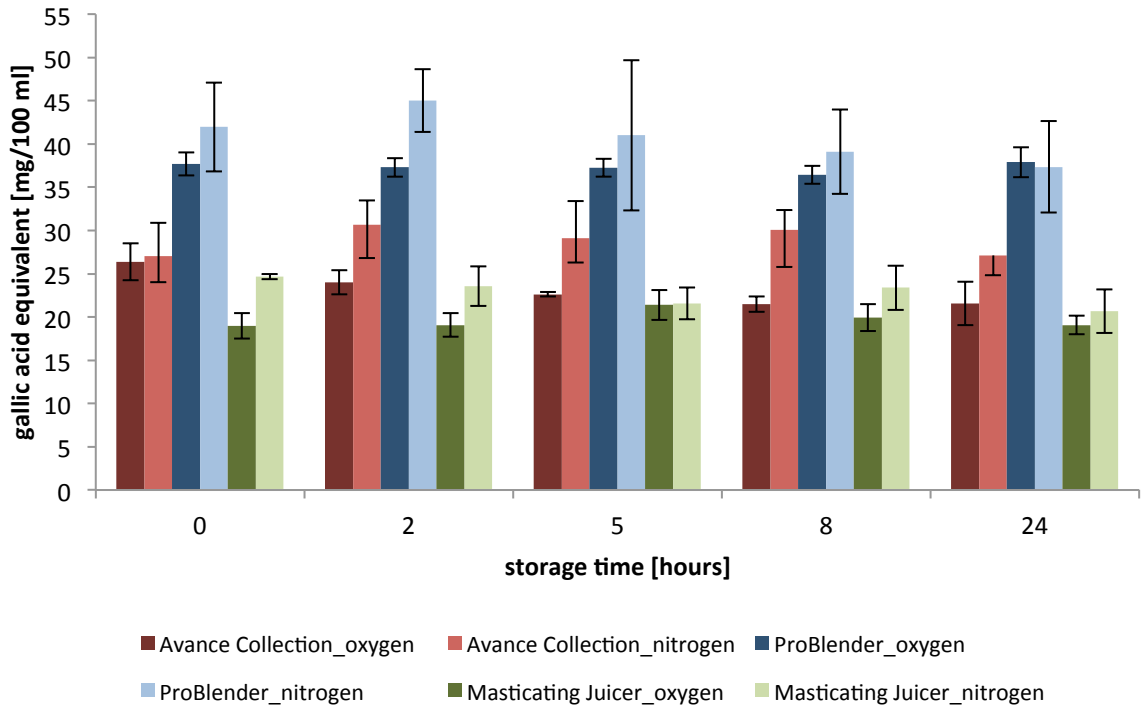


Figure 29. Stability of the total phenolic content in grape juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Comparison of strawberry, carrot and grape juice

The measured total phenolic contents were stable under oxygen and nitrogen conditions in strawberry and grape juices produced by all three machines. Only in carrot juice, the TPC was slightly decreasing under oxygen storage conditions. This can be explained by the different pH of the raw materials. Strawberries and grapes obtain a pH between 3 – 3.9 and 2.8 – 3.8, respectively. In contrast, the pH value of carrots ranges between 5.9 and 6.4 (Bridges & Mattice, 1939). Polyphenols are more stable under acid conditions (Jackman *et al.*, 1987); thus, strawberries and grapes provide more favourable conditions for polyphenols. Nevertheless, these losses could be avoided by storing the juices under nitrogen.

By comparing the obtained results, there is a slight tendency that the P. ProBlender extracts the highest amounts of polyphenols. This sounds plausible, as during the chopping and smashing process, the cell walls are broken up and the phenols are released. In contrast, the P. Masticating Juicer usually attained the lowest amounts. Therefore, one possible reason could be that during the chewing process, the cell walls are not broken up efficiently. Furthermore, strawberry juice is the best source of polyphenols, while carrot juice contains the lowest TPC. Whereas the harvest time again strongly influences the nutritional composition of fruits and vegetables.

3.5 Anthocyanins

The fruits were washed or pealed, prepared as described in the chapter "Materials and Methods", and analysed using HPLC. Mean values and standard deviations were calculated for all obtained data. The results as well as the concentration differences between oxygen and nitrogen storage atmosphere are represented in tabular and graphic form, sample chromatograms can be found in the appendix. Anthocyanins were measured in strawberry and grape juice.

Strawberry juice

Referring to literature, pelargonidin is the most abundant anthocyanin in strawberries with a concentration of 2 mg/100 g (Berlitz *et al.*, 2008). In this experiment, pelargonidin-3-glucoside (Pl-3-glc) and pelargonidin-3-malonyl-glucoside (Pl-3-malonyl-glc) were identified to be to predominant anthocyanin forms. The content of Pl-3-glc was about three times higher than the concentration of Pl-3-malonyl-glc. No further anthocyanins were detected in the strawberry juice samples.

When exposed to oxygen, the content of Pl-3-glc and Pl-3-malonyl-glc in strawberry juice was stable over a storage time of 24 hours. This could be observed in juices from all three machines.

Juices from the P. Avance Collection contained the largest amounts of Pl-3-glc, and the juices from the P. Masticating Juicer contained the lowest amount. In contrast, the content of Pl-3-malonyl-glc was fairly equal in juices from all three machines. Storing under nitrogen conditions also resulted in a stable concentration profile. In the second experiment, the highest Pl-3-glc was again obtained with the P. Avance Collection, while the least anthocyanins were present in juices from P. Masticating Juicer.

The concentration of Pl-3-malonyl-glc was quite the same in all juices (Table 30, Figure 30, Figure 31, Figure 32).

There were again concentration differences between the two trails, which can be explained by the different seasons when the experiments were conducted and the varying ripeness of the fruits.

Table 30. Stability of anthocyanin content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements. Pl-3-glc = Pelargonidin-3-glucoside, Pl-3-malonyl-glc = pelargonidin-3-malonyl-glucoside;

Pl-3-glc [mAU·s]						
time [h]	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
	O_2	N_2	O_2	N_2	O_2	N_2
0	915 ± 171	966 ± 116	823 ± 63	906 ± 299	755 ± 136	855 ± 84
2	850 ± 159	947 ± 36	872 ± 56	888 ± 258	825 ± 154	866 ± 84
5	858 ± 186	947 ± 74	838 ± 48	867 ± 254	759 ± 125	853 ± 68
8	891 ± 133	927 ± 98	845 ± 38	876 ± 291	778 ± 127	856 ± 95
24	854 ± 158	941 ± 89	838 ± 72	868 ± 279	796 ± 146	864 ± 88
Pl-3-malonyl-glc [mAU·s]						
time [h]	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
	O_2	N_2	O_2	N_2	O_2	N_2
0	270 ± 36	313 ± 34	265 ± 49	335 ± 44	269 ± 49	303 ± 39
2	264 ± 50	306 ± 1	279 ± 48	294 ± 44	270 ± 42	325 ± 58
5	264 ± 34	304 ± 20	263 ± 40	297 ± 49	264 ± 34	316 ± 42
8	267 ± 40	296 ± 24	256 ± 29	314 ± 34	266 ± 45	319 ± 52
24	266 ± 37	301 ± 19	262 ± 44	303 ± 57	268 ± 43	3201 ± 41
Total Anthocyanins [mAU·s]						
time [h]	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
	O_2	N_2	O_2	N_2	O_2	N_2
0	1185 ± 210	1279 ± 50	1088 ± 109	1242 ± 172	1023 ± 172	1159 ± 134
2	1114 ± 200	1253 ± 44	1150 ± 104	1224 ± 208	1095 ± 208	1191 ± 124
5	1122 ± 233	1251 ± 93	1100 ± 87	1164 ± 167	1023 ± 167	1169 ± 101
8	1159 ± 162	1223 ± 122	1101 ± 68	1191 ± 177	1044 ± 177	1175 ± 138
24	1120 ± 110	1242 ± 108	1101 ± 113	1171 ± 186	1064 ± 186	1184 ± 130

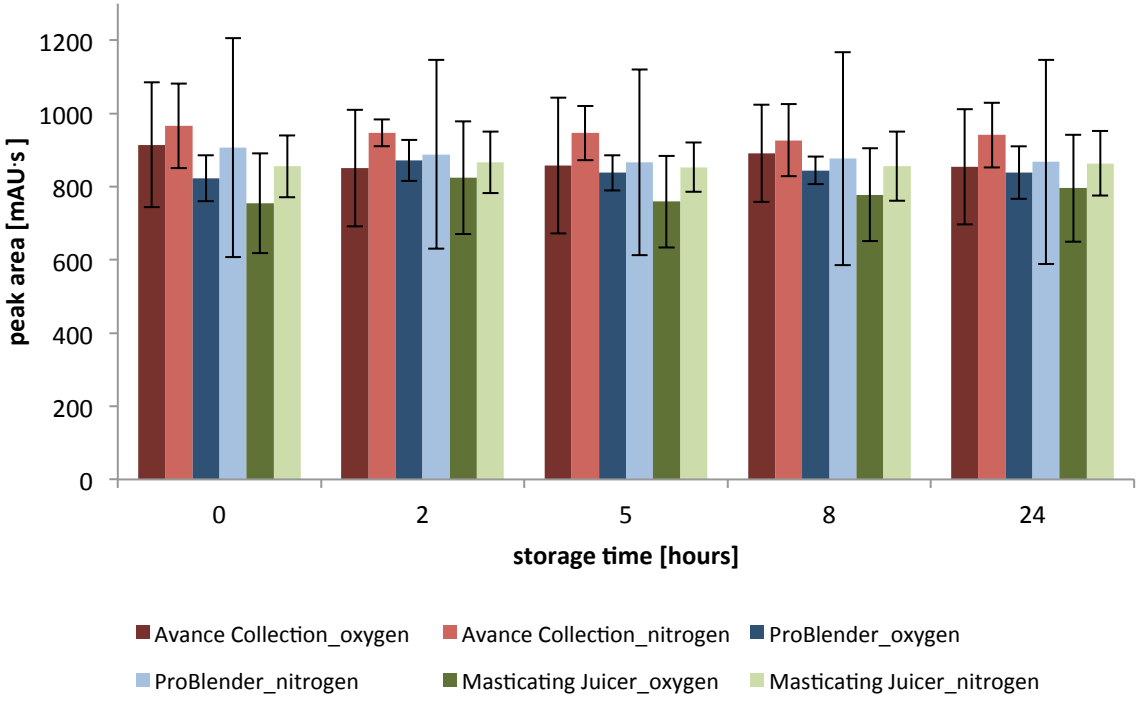


Figure 30. Stability of the pelargonidin-3-glucoside content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

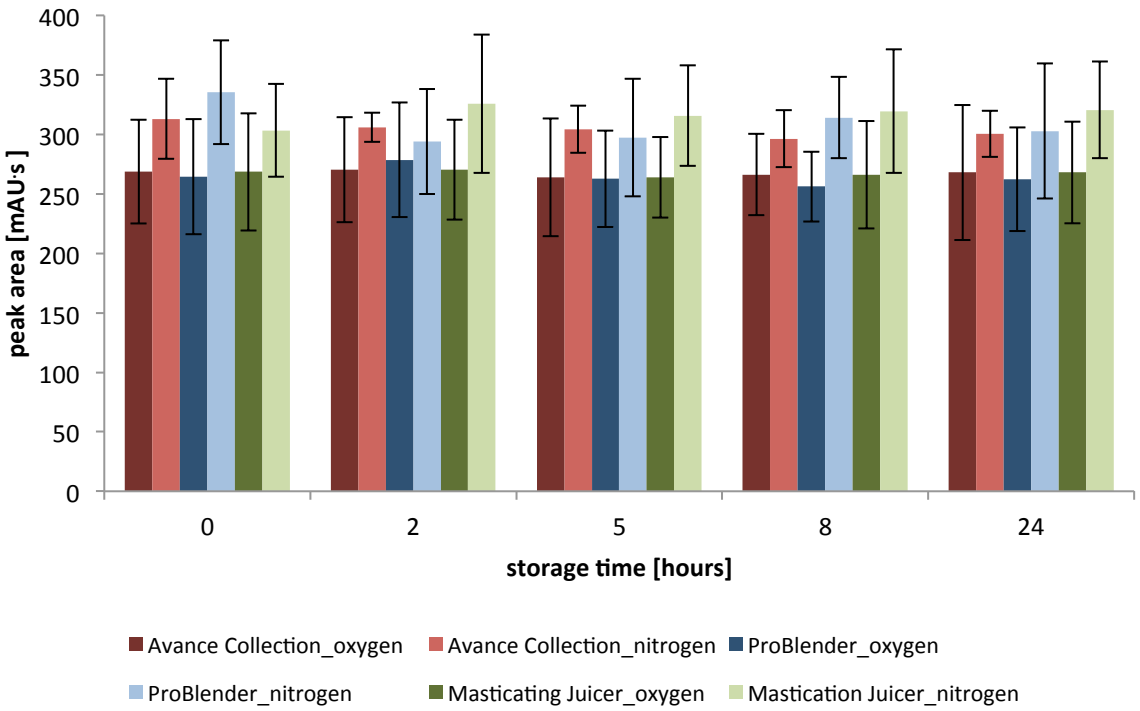


Figure 31. Stability of the pelargonidin-3-malonyl-glucoside content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

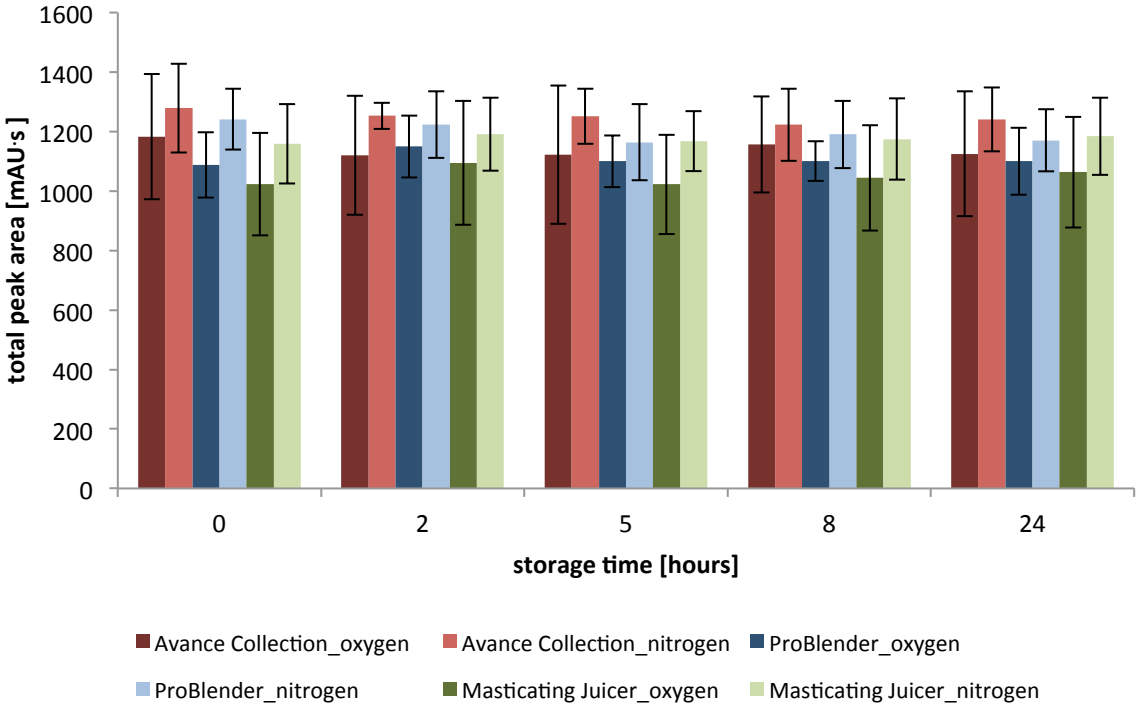


Figure 32. Stability of the total anthocyanin content in strawberry juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Grape juice

According to literature, the main anthocyanidins in grapes are malvidin and peonidin, which are present in a concentration of about 1 mg/100 g. Cyanidin is also one of the main anthocyanins and usually has a concentration of about 0.38 mg/100 g (Berlitz *et al.*, 2008). In this experiment, the main detected anthocyanins are petunidin-3-glucoside (Pt-3-glc), malvidin-3-glucoside (Mal-3-glc) and petunidin-3-O-(6-p-coumaroyl)-glucoside (Pt-3-O-(6-p-c)-glc). The obtained concentrations of Mal-3-glc and Pt-3-O-(6-p-c)-glc are about ten times higher than the content of Pt-3-glc. When the juices were stored under oxygen atmosphere, the concentration of all three identified compounds decreased over 24 hours. It was observed that about 54 % of the initial Pt-3-glc amount was degraded in juices from the P. Masticating Juicer. The lowest degradation rate was found for Pt-3-O-(6-p-c)-glc in juices from the P. Avance Collection, where 84 % of the initial concentration remained after 24 hours. The largest amounts of anthocyanins could be detected in juices from the P. ProBlender, while the lowest amounts of Pt-3-glc and Pt-3-O-(6-p-c)-glc were found in juices from P. Avance Collection.

In the juices for the second experiment (storage under nitrogen conditions), only Mal-3-glc was detected in noteworthy amounts in the grape juice, and the exclusion of oxygen during the storage period enhanced the stability of Mal-3-glc. The highest Mal-3-glc content was again found in juices from the P. ProBlender (Table 31, Figure 33, Figure 34, Figure 35 and Figure 36). These results indicate that the composition and present concentration of anthocyanins in grapes is variable and strongly influenced by previously mentioned factors.

Table 31. Stability of the anthocyanin content in grape juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements. Pt-3-glc = Petunidin-3-glucoside, Mal-3-glc = Malvidin-3-glucoside, Pt-3-O-(6-p-coumaroyl)-glc = Petunidin-3-O-(6-p-coumaroyl)-glucoside;

Pt-3-glc [mAU·s]						
time [h]	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
	O_2	N_2	O_2	N_2	O_2	N_2
0	22.1 ± 8.8	-	56.8 ± 27.8	-	29.6 ± 14.7	-
2	21.5 ± 9.7	-	43.9 ± 11.3	-	27.6 ± 12.5	-
5	17.5 ± 9.0	-	39.5 ± 12.5	-	20.1 ± 5.0	-
8	17.5 ± 9.4	-	43.4 ± 5.5	-	20.8 ± 9.8	-

24	11.8 ± 5.7	-	36.6 ± 1.5	-	13.9 ± 7.7	-
Mal-3-glc [mAU·s]						
time [h]	**P. Avance Collection**		**P. ProBlender 6**		**P. Masticating Juicer**	
	O_2	N_2	O_2	N_2	O_2	N_2
0	217 ± 62	609 ± 47	316 ± 69	623 ± 116	227 ± 42	259 ± 36
2	208 ± 47	615 ± 72	290 ± 36	627 ± 116	221 ± 46	258 ± 33
5	172 ± 36	563 ± 73	241 ± 18	617 ± 103	203 ± 48	255 ± 28
8	164 ± 33	557 ± 56	227 ± 9	615 ± 67	184 ± 36	261 ± 31
24	128 ± 34	583 ± 33	178 ± 15	639 ± 78	133 ± 15	258 ± 27
Pt-3-O-(6-p-c)-glc [mAU·s]						
time [h]	**P. Avance Collection**		**P. ProBlender 6**		**P. Masticating Juicer**	
	O_2	**N_2**	**O_2**	**N_2**	**O_2**	**N_2**
0	230 ± 86	-	469 ± 259	-	242 ± 62	-
2	237 ± 99	-	346 ± 194	-	238 ± 58	-
5	202 ± 87	-	317 ± 146	-	217 ± 42	-
8	204 ± 94	-	301 ± 146	-	211 ± 57	-
24	195 ± 96	-	262 ± 119	-	166 ± 51	-
Total anthocyanins [mAU·s]						
time [h]	**P. Avance Collection**		**P. ProBlender 6**		**P. Masticating Juicer**	
	O_2	**N_2**	**O_2**	**N_2**	**O_2**	**N_2**
0	469 ± 67	609 ± 47	842 ± 202	623 ± 116	499 ± 36	259 ± 36
2	467 ± 49	615 ± 72	681 ± 135	627 ± 116	487 ± 37	258 ± 33
5	391 ± 44	563 ± 73	597 ± 83	617 ± 103	440 ± 35	255 ± 28
8	386 ± 34	557 ± 56	572 ± 80	615 ± 67	416 ± 25	261 ± 31
24	335 ± 30	583 ± 33	477 ± 61	639 ± 78	312 ± 24	258 ± 27

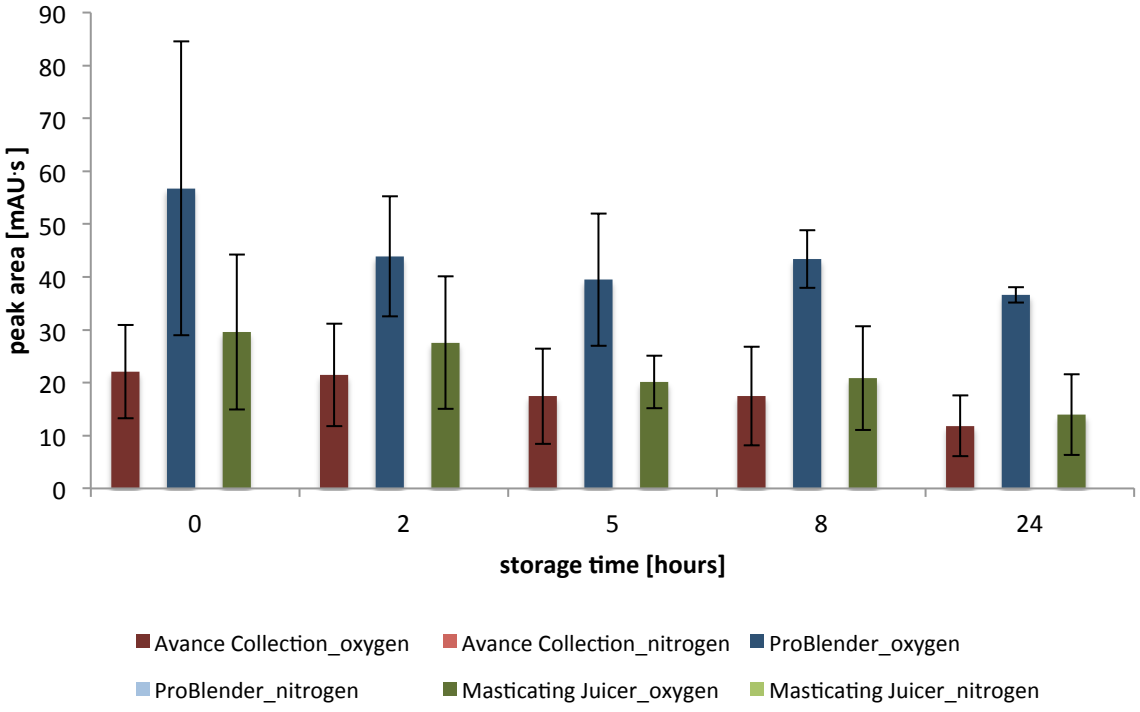


Figure 33. Stability of the petunidin-3-glucoside content in grape juice over 24 hours. Comparison of all three juicers under oxygen storage conditions at 4 °C

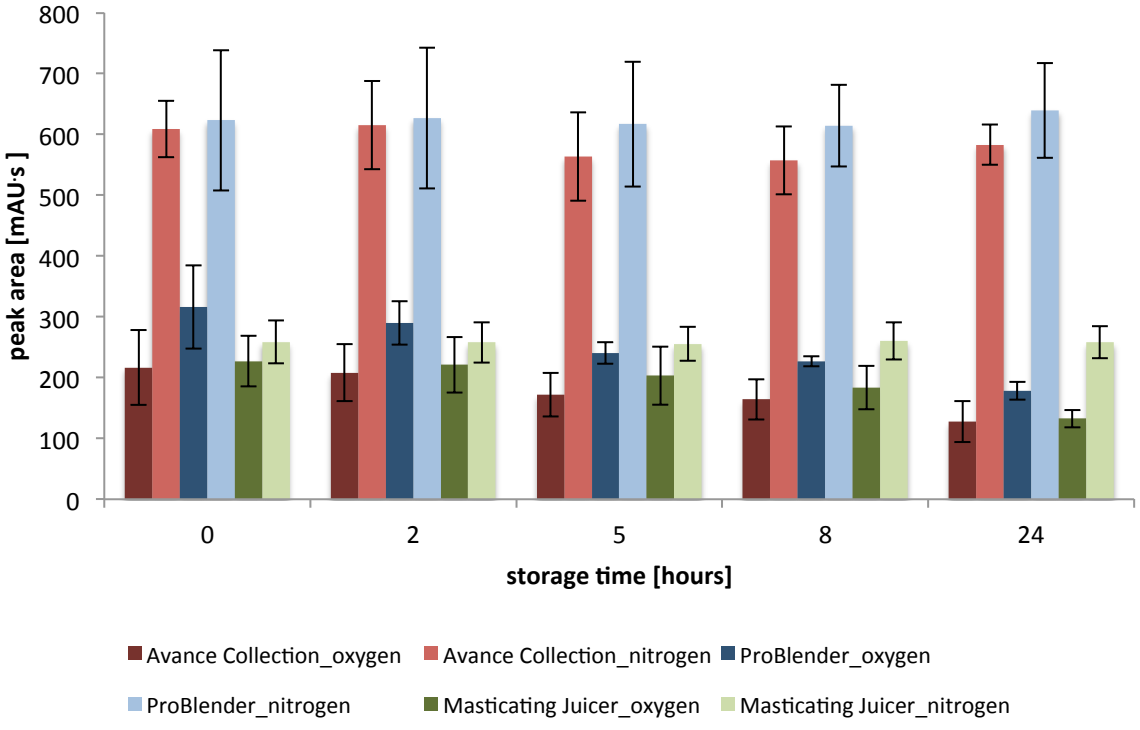


Figure 34. Stability of the malvidin-3-glucoside content in grape juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

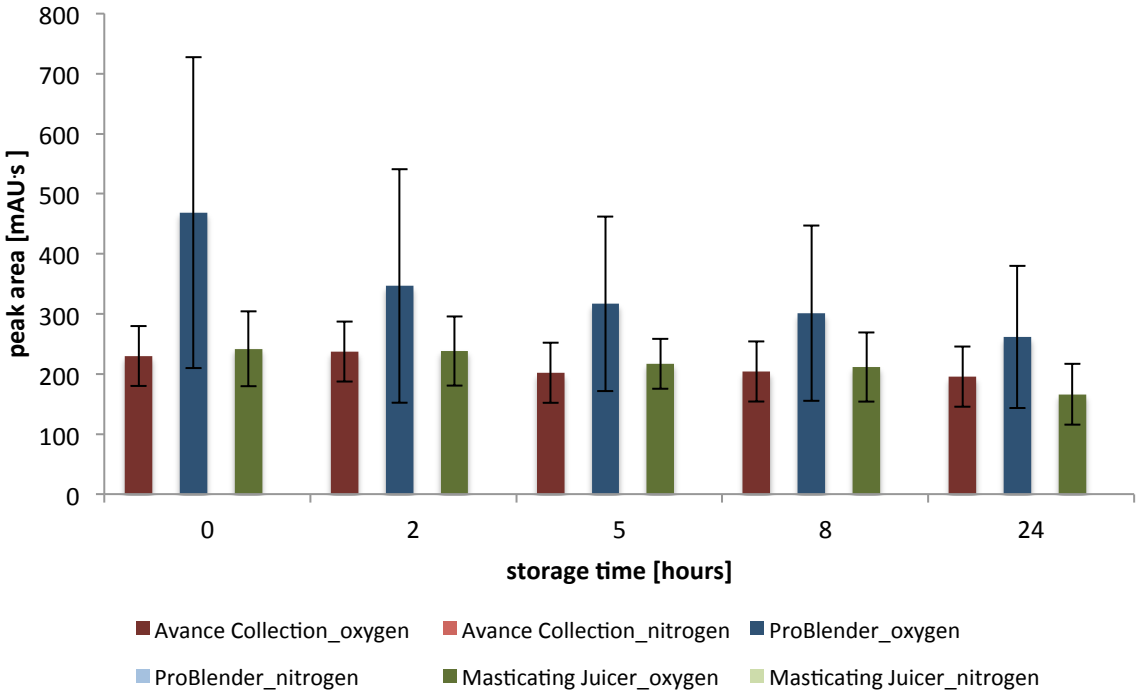


Figure 35. Stability of the petunidin-3-O-(6-p-coumaroyl)-glucoside content in grape juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

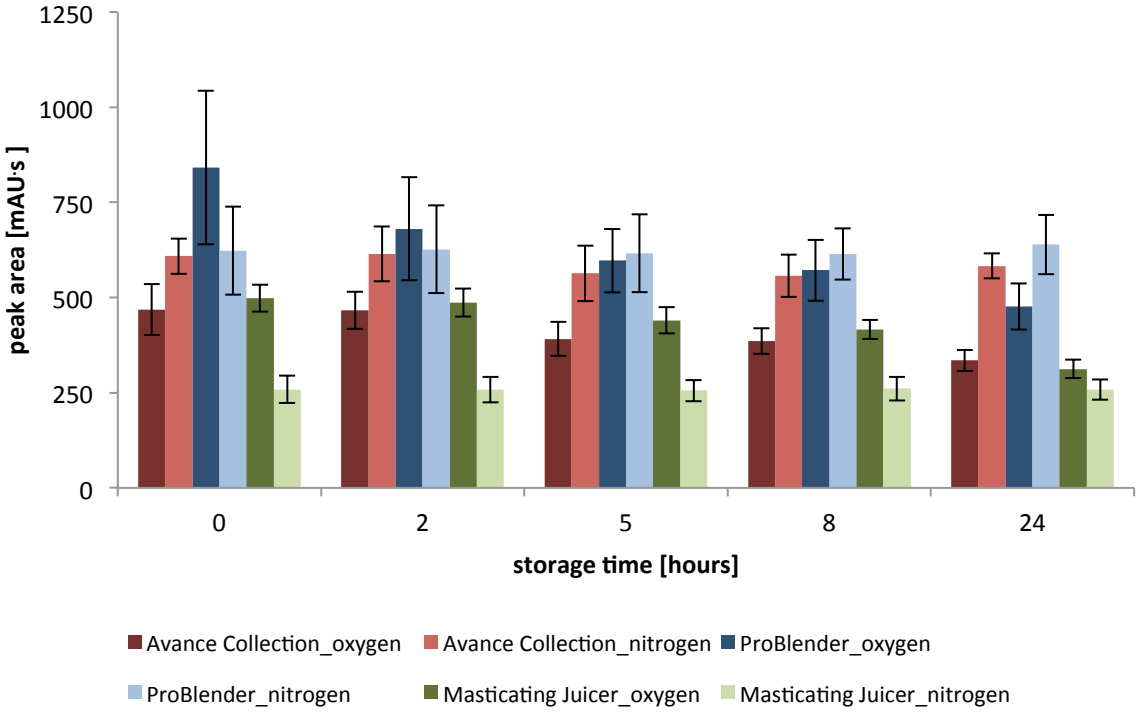


Figure 36. Stability of the total anthocyanin content in grape juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

Comparison of strawberry and grape juice

The anthocyanins in strawberry juice were stable over 24 hours when exposed to oxygen, while in grape juice a reduction in the content was detected. The stability of anthocyanins strongly depends on influencing factors like UV-radiation or pH changes, which may explain the reduced concentration (Velisek, 2014).

In general, storage under nitrogen conditions has a positive effect on some anthocyanins, whereas other compounds are stable over 24 hours even when stored under oxygen atmosphere.

Juices from the P. Avance Collection showed the highest content of anthocyanins in strawberry juice, while the lowest amounts were found in juices from P. Masticating Juicer. The P. ProBlender is clearly the most suitable machine to extract anthocyanins from grapes, while the lowest amounts were found in juices from the P. Masticating Juicer. These results also fit to those of the TPC analysis.

Furthermore, as indicated by the results from the TPC analysis, the concentration of anthocyanins in strawberry juice is generally higher than the concentration in grape juice. Consequently, strawberry juice is a richer source of anthocyanins than grape juice.

3.6 Hesperidin

The fruits were washed or pealed, prepared as described in the chapter “Materials and Methods”, and analysed using HPLC. Mean values and standard deviations were calculated for all obtained data. The results as well as the concentration differences between oxygen and nitrogen storage atmosphere are represented in tabular and graphic form. The hesperidin content was measured in orange juice.

Orange juice

The hesperidin concentration in orange juice decreased over a period of 24 hours when the juices were stored under oxygen conditions. Thus, it can be assumed that exposure to oxygen leads to degradation of hesperidin. The greatest loss was detected in juices produced by the P. ProBlender, where about 60 % of the initial hesperidin content was degraded. In juices from the P. Avance Collection and the P. Masticating Juicer, about 40 % and 47 % of the initial hesperidin concentration were lost after 24 hours. The highest concentration was obtained from the P. ProBlender and the lowest concentration from the P. Masticating Juicer, whereas the initial concentration of hesperidin is very similar in the juices from the P. Avance Collection and the P. ProBlender.

By storing the orange juices under nitrogen atmosphere, the stability of hesperidin could be enhanced, and no degradation over 24 hours occurred. In the second experiment, the highest concentration was also obtained from the P. ProBlender, and the lowest concentration was again found in juices from the P. Masticating Juicer (Table 32, Figure 37). Thus, the slow chewing technique of the P. Masticating Juicer is not as efficient in extracting hesperidin from oranges as the other two juicing methods.

The degradation characteristics of hesperidin in juices from the P. ProBlender are similar to those found for vitamin C. Hesperidin is sensitive to oxygen, and during the high-speed mixing of the blender, high amounts of oxygen are added to the juices, leading to unfavourable conditions. During the spinning and chewing processes of the P. Avance Collection and the P. Masticating Juicer, less oxygen in-put occurs.

The hesperidin concentrations of orange juice found in literature ranged from 10 – 100

mg/100 ml (Velisek, 2014; Gil-Izquierdo *et al.*, 2001). The concentrations obtained from the experiment range from 53 – 108 mg/100 ml, which are consistent with literature references. Variations in the detected concentrations can result from different harvest times and ripeness of the oranges.

Table 32. Stability of the hesperidin content in orange juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C. Mean values and standard deviations were obtained from three separate measurements

Hesperidin [mg/100 g]						
time	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
[h]	O_2	N_2	O_2	N_2	O_2	N_2
0	103 ± 7	101 ± 19	108 ± 14	85 ± 8	85 ± 4	53 ± 7
2	71 ± 4	100 ± 19	65 ± 11	85 ± 10	68 ± 7	50 ± 6
5	51 ± 13	100 ± 20	60 ± 8	84 ± 13	64 ± 8	49 ± 5
8	65 ± 11	101 ± 18	56 ± 6	84 ± 13	57 ± 6	50 ± 4
24	61 ± 5	101 ± 18	44 ± 1	86 ± 11	45 ± 4	51 ± 4

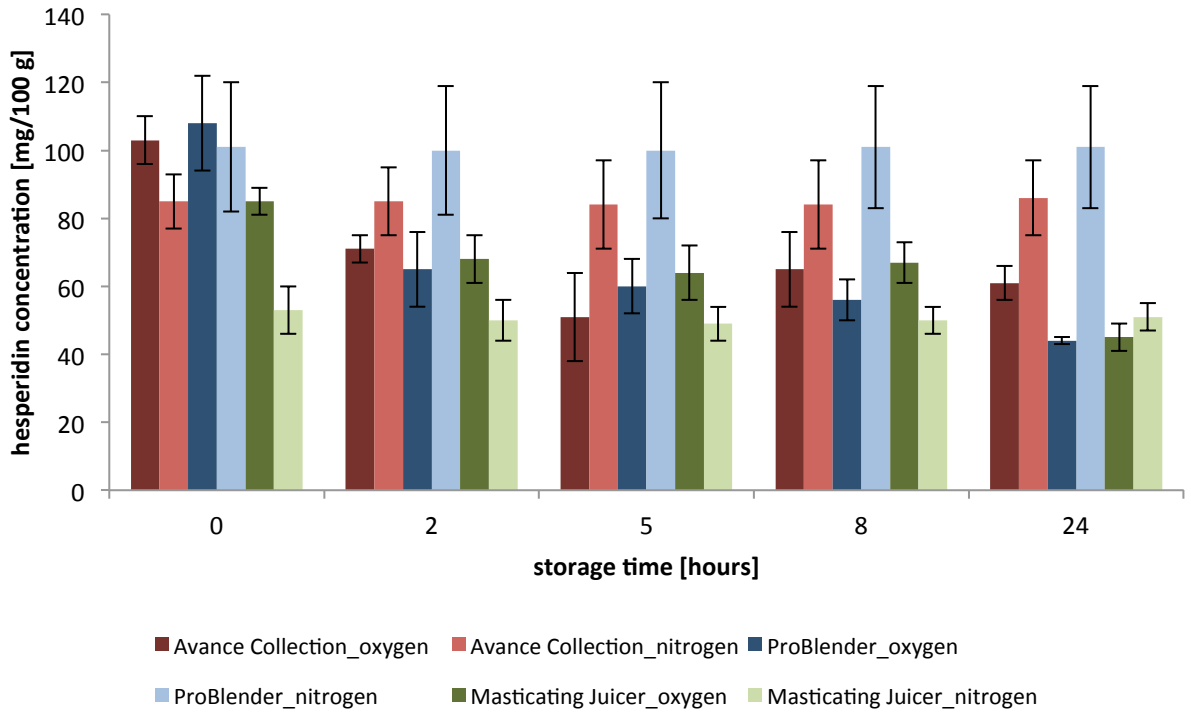


Figure 37. Stability of the hesperidin content in orange juice over 24 hours. Comparison of all three juicers under oxygen and nitrogen storage conditions at 4 °C

3.7 Temperature profile analysis

To analyse the average storage temperatures of domestic fridges, temperature profile analysis of seven different fridges were complied. A testo data logger was used to generate the temperature profiles. The calculated average temperatures including standard deviations are shown in Table 33 and depicted in Figure 38. The temporal temperature curves over 7 days are shown in Figure 39.

The average fridge temperature of all tested fridges was determined with **3.9 ± 4.7 °C**. The lowest calculated average temperature was -4.8 °C (fridge 6), and the highest fridge temperature was 8.4 °C (fridge 1). In general, high deviations between the different device types, but also high temperature fluctuations of the several fridges within the test period were observed. Especially the profile from fridge 2 shows a substantial temperature oscillation, which ranges from -2.5 °C to 6.9 °C. This can also be observed in the high standard deviation of fridge 2. In contrast, fridges 5 and 7 obtain the most uniform temperature profile, whereas fridge 5 was used to store the fruit juice samples during the experiments. The cooling characteristics of fridges depend on the age and conditions of the instruments, but also external factors like out-door temperatures and season. Furthermore, other electrical devices including kitchen stoves that radiate and transfer heat influence the fridge temperatures.

In literature, the optimal fridge temperature for many foods ranges between 1 and 5 °C (Hölzl & Aldrian, 2011). Thus, the average temperature of all devices obtained from this experiment matches the recommendations. Nevertheless, high standard deviations and temperature fluctuation need to be kept in mind.

Table 33. Calculated average fridge temperatures and standard deviations of different fridges over a period of 7 days

Average fridge temperatures over 7 days in °C	
Fridge 1	8.4 ± 1.9
Fridge 2	-4.8 ± 2.5
Fridge 3	5.0 ± 0.7
Fridge 4	6.2 ± 1.1
Fridge 5	4.8 ± 0.3
Fridge 6	0.1 ± 1.7
Fridge 7	7.9 ± 0.3
Average	3.9 ± 0.8

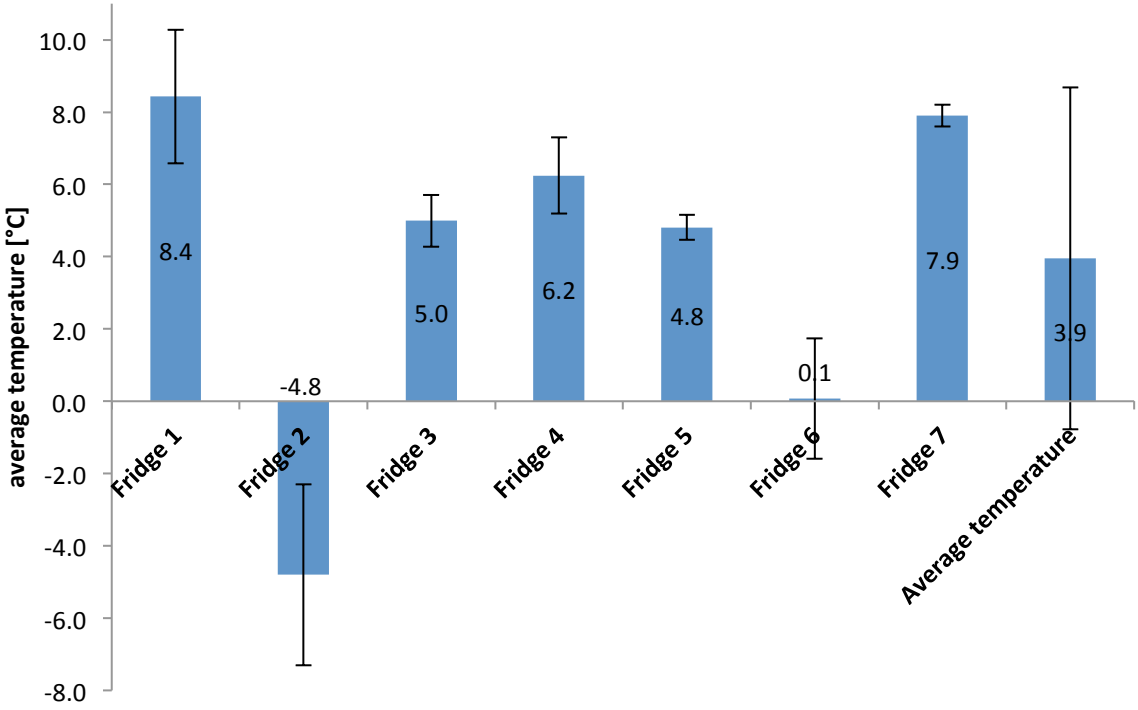


Figure 38. Average fridge temperatures over a period of 7 days. Mean values and standard deviations are depicted in the diagram

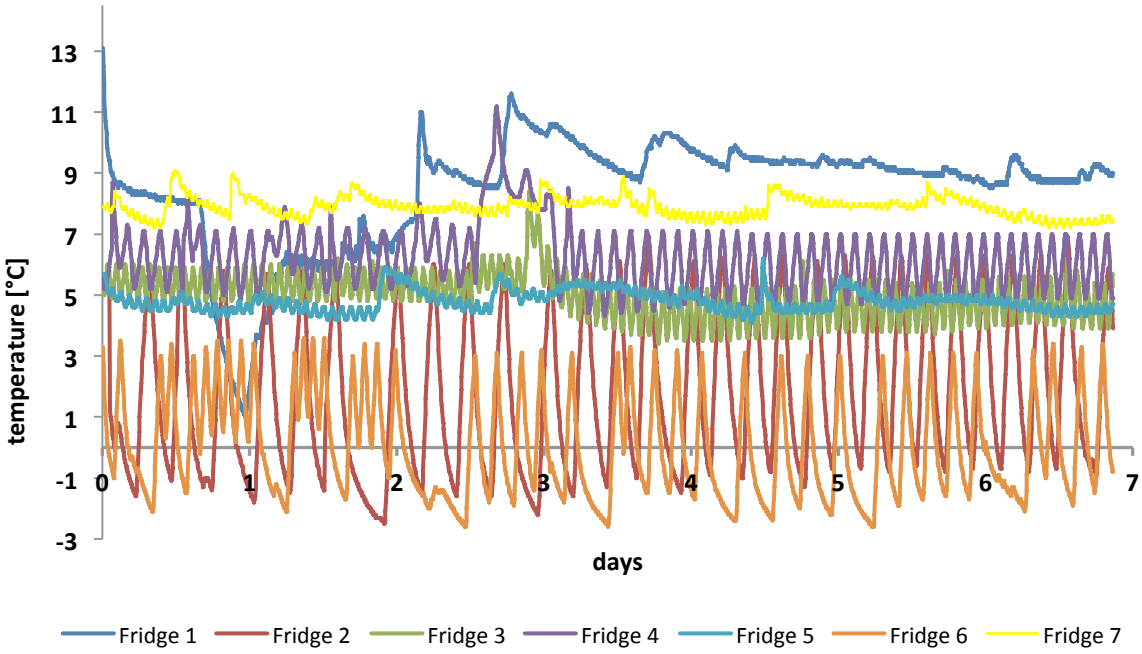


Figure 39. Temperature profile analysis of different fridges over a period of 7 days

3.8 Determination of the total microbial counts

The colony forming units (CFU/ml) of yeasts, moulds and bacteria where determined in all fruit and vegetable juices. The samples were analysed directly after squeezing and after a storage period of 24 hours under oxygen and under nitrogen conditions. The obtained CFU values are listed in Table 34. Significant CFU values of moulds were only found in grape juices. Consequently, the results are not depicted graphically. The results of bacteria and moulds are shown in Figure 40 – Figure 45.

In **orange juice,** the highest amounts of bacteria were found in juices from the P. Avance Collection. The lowest initial number of bacteria was obtained from the P. ProBlender, and the lowest number of yeasts from the Masticating Juicer. Moulds were only found in juices from the P. Avance Collection after a storage time of 24 hours under oxygen conditions. The highest amounts of bacteria and yeasts in **strawberry juices** were obtained from the P. Avance Collection. The lowest number of bacteria was found in juices from the P. ProBlender and the lowest number of yeasts in juices from the P. Masticating Juicer. Moulds were not detected in any of the analysed fruit juices.

The results of bacteria in **carrot juice** were greater than 300,000 CFU/ml for the juices of all three machines. The results also showed high contaminations with yeasts, whereas the largest amounts were found in juices from the P. Masticating Juicer. The lowest number of yeasts was found in juices from the P. ProBlender. Otherwise, moulds were only found in juices from the P. ProBlender.

The highest values of bacteria and yeasts in **grape juice** were both obtained from the P. Avance Collection. The lowest number of bacteria and yeasts were detected from the P. Masticating Juicer, and also moulds were only found in juices from this machine.

All in all, it can be said that the highest contamination of both, bacteria and yeasts, were found in carrots juices, whereas orange and strawberry juices showed comparatively low contaminations. Possible explanations may be that due to their high physiological pH value of 5.9 to 6.4 (Bridges & Mattice, 1939), carrots provide a more favourable environment for the growth of microorganisms. Oranges and strawberries obtain an acid pH, and thus could impede microbiological growth.

By comparing all results, the P. Avance Collection showed the highest bacterial contamination of orange, strawberry and grape juices. The largest numbers of yeasts in orange, strawberry and grape juice were also found in juice from the P. Avance Collection. One possible reason could be that due to the complex construction of this extractor, the several features, especially the sieve, were more difficult to clean by hand than the features of the other juicers.

The results of bacteria and yeasts in juices from the P. ProBlender and the P. Masticating Juicer obtained comparatively low numbers. Nevertheless, the highest contaminations of yeasts and moulds were found in juices from the P. Masticating Juicer (carrot and grape juice). By comparing the results from this experiment with the CFU values of a previous conducted trail (results are listed in Table 43 in the appendix), it can be seen that the obtained contaminations are higher in this work. It is noteworthy that the P. Avance Collection and the P. ProBlender were frequently in use over two years and the devices were only rinsed with pure water and washed by hand. Thus, it can be said that due to the low washing temperatures and the frequent use, an accumulation of microorganisms is a logic outcome. Consequently, the use of a dishwasher is highly recommended. Storing under nitrogen led to a reduced growth of both bacteria and yeasts in all juices, thus this storage condition it can be definitely considered as a possibility to prolong the storage time of fruit juices.

Table 34. Comparison of the colony forming units (CFU) of bacteria, yeasts and moulds in orange, strawberry, carrot and grape juice. Values are given in CFU/ml

Orange juice				
Juicer	**Storage time [h]**	**CFU/ml**		
		Bacteria	**Yeasts**	**Moulds**
Avance Collection	0	13,000	15,000	1,000
	24 (oxygen)	16,000	18,000	
	24 (nitrogen)	5,000	12,000	
ProBlender	0	1,000	5,000	
	24 (oxygen)	5,000	4,000	
	24 (nitrogen)	3,000	3,000	
Masticating Juicer	0	5,000	2,000	
	24 (oxygen)	1,000	<1,000	
	24 (nitrogen)	<1,000	<1,000	

Strawberry juice				
Juicer	Storage time [h]	CFU/ml		
		Bacteria	Yeasts	Moulds
Avance Collection	0	21,000	17,000	
	24 (oxygen)	24,000	22,000	
	24 (nitrogen)	6,000	13,000	
ProBlender	0	2,000	2,000	
	24 (oxygen)	3,000	2,000	
	24 (nitrogen)	2,000	1,000	
Masticating Juicer	0	4,000	1,000	
	24 (oxygen)	3,000	<1,000	
	24 (nitrogen)	<1,000	<1,000	
Carrot juice				
Juicer	Storage time [h]	CFU/ml		
		Bacteria	Yeasts	Moulds
Avance Collection	0	300000	98,000	
	24 (oxygen)	>300,000	151,000	
	24 (nitrogen)	>300,000	142,000	
ProBlender	0	>300,000	29,000	1000
	24 (oxygen)	>300,000	57,000	
	24 (nitrogen)	>300,000	17,000	5,000
Masticating Juicer	0	>300,000	150,000	
	24 (oxygen)	>300,000	150,000	
	24 (nitrogen)	>300,000	150,000	
Grape juice				
Juicer	Storage time [h]	CFU/ml		
		Bacteria	Yeasts	Moulds
Avance Collection	0	19,000	15,000	3,000
	24 (oxygen)	21,000	21,000	
	24 (nitrogen)	13,000	8,000	1,000
ProBlender	0	5,000	<1.000	
	24 (oxygen)	18,000	15,000	
	24 (nitrogen)	13,000	5,000	
Masticating Juicer	0	<1,000	1,000	4,000
	24 (oxygen)	1,000		14,000
	24 (nitrogen)	1,000	1,000	12,000

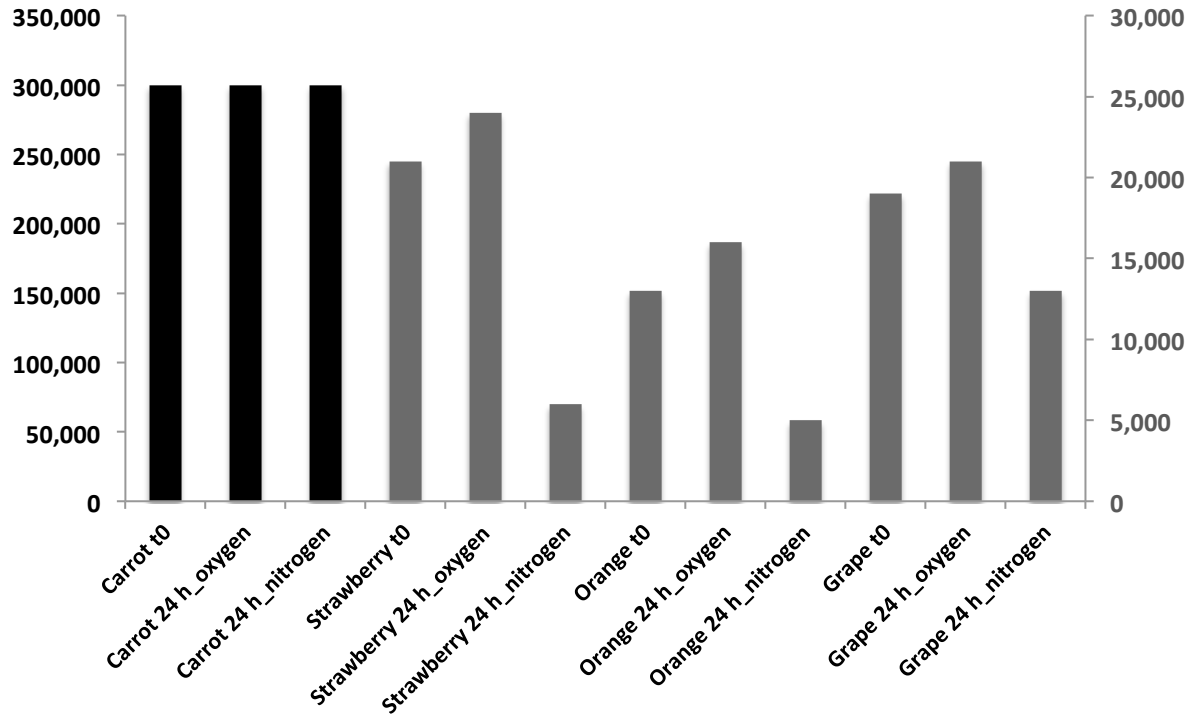


Figure 40. Colony forming units of bacteria in juices from P. Avance Collection. Comparison between oxygen and nitrogen storage conditions

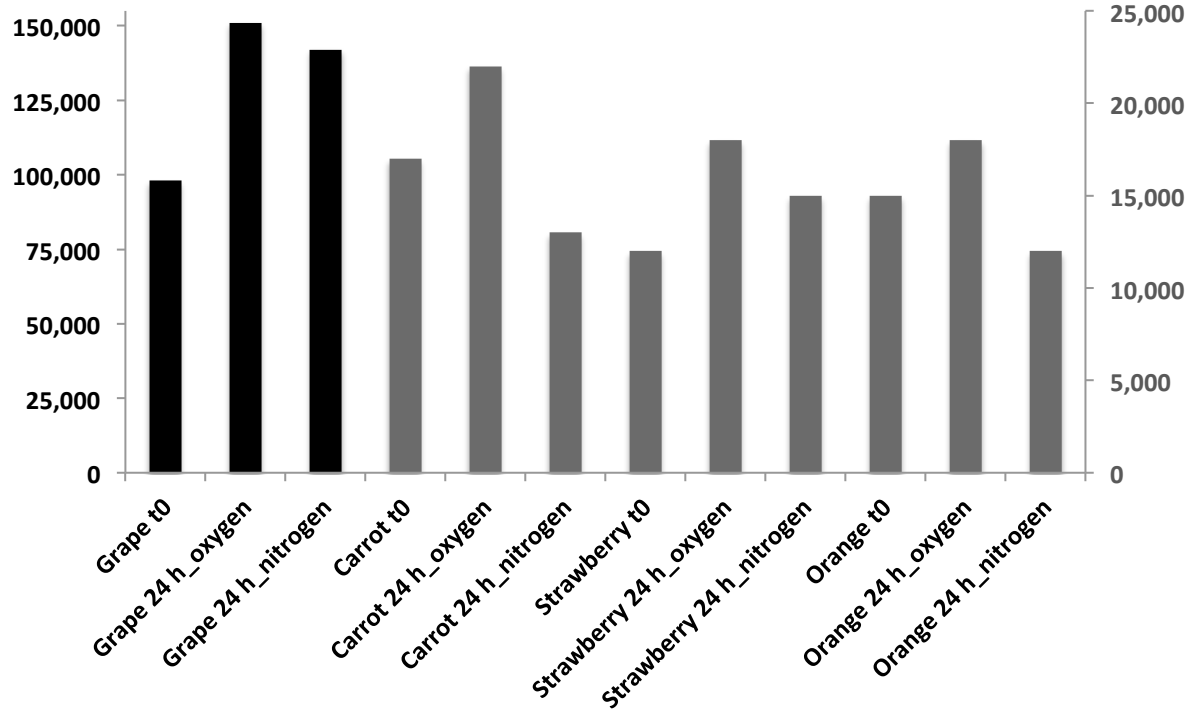


Figure 41. Colony forming units of yeasts in juices from P. Avance Collection. Comparison between oxygen and nitrogen storage conditions

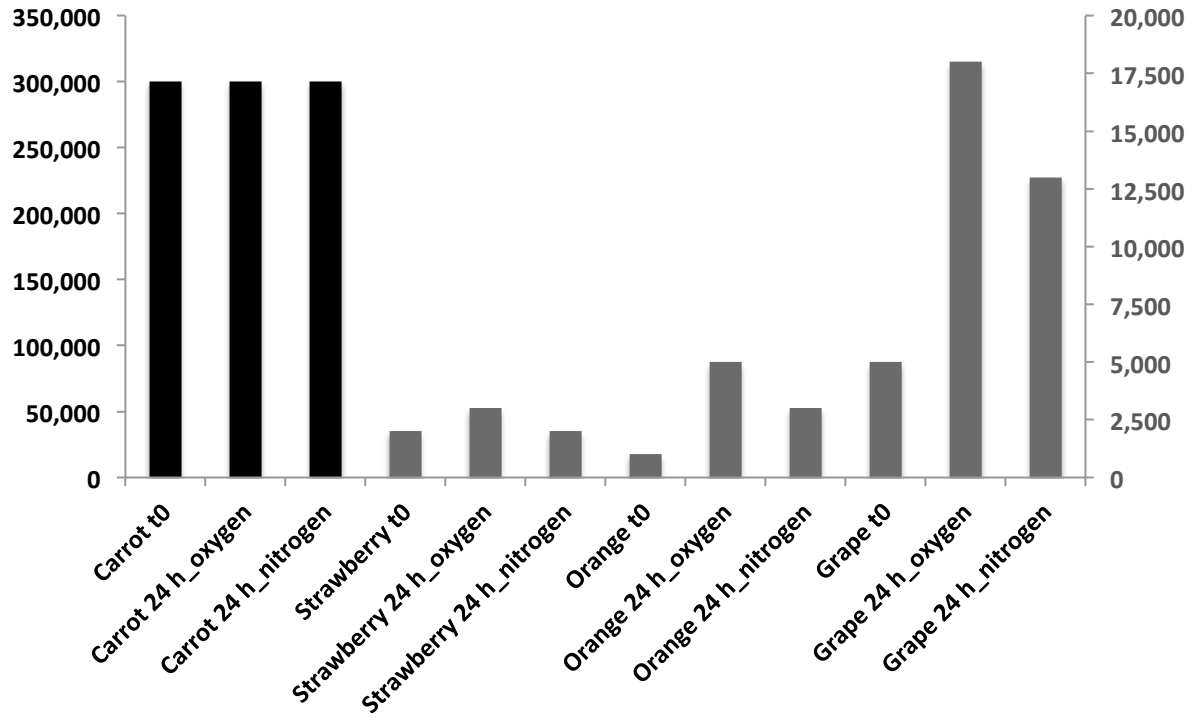


Figure 42. Colony forming units of bacteria in juices from P. ProBlender. Comparison between oxygen and nitrogen storage conditions

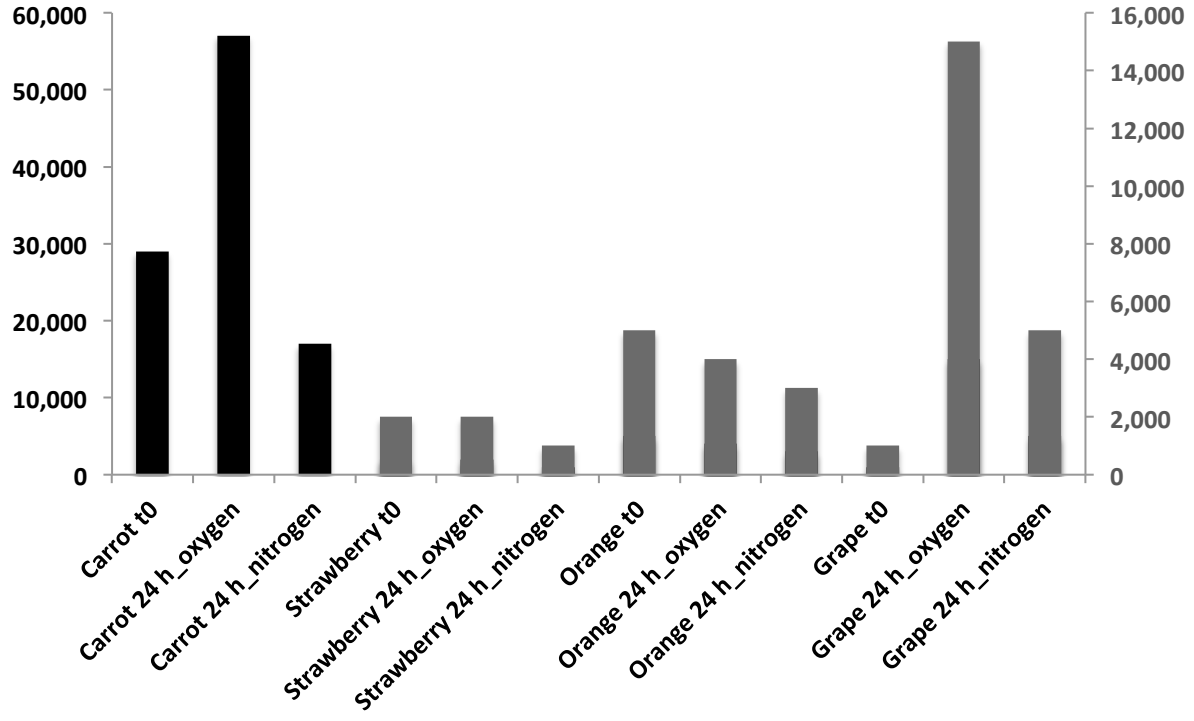


Figure 43. Colony forming units of yeasts in juices from P. ProBlender. Comparison between oxygen and nitrogen storage conditions

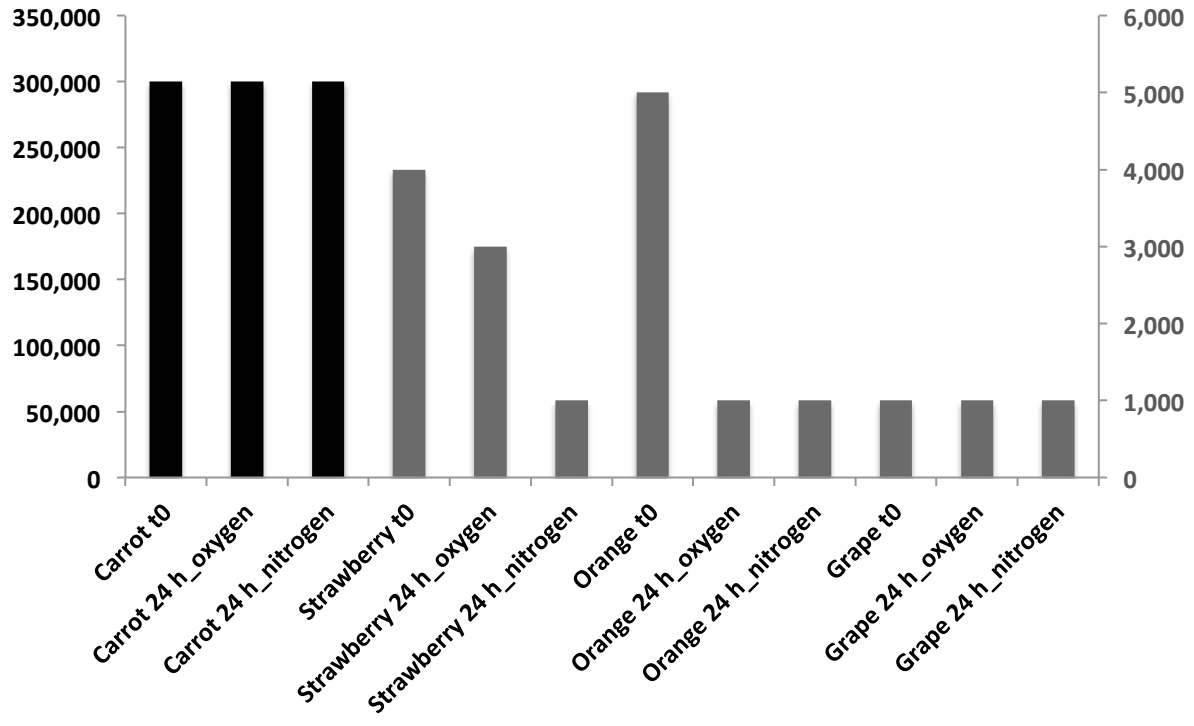


Figure 44. Colony forming units of bacteria in juices from P. Masticating Juicer. Comparison between oxygen and nitrogen storage conditions

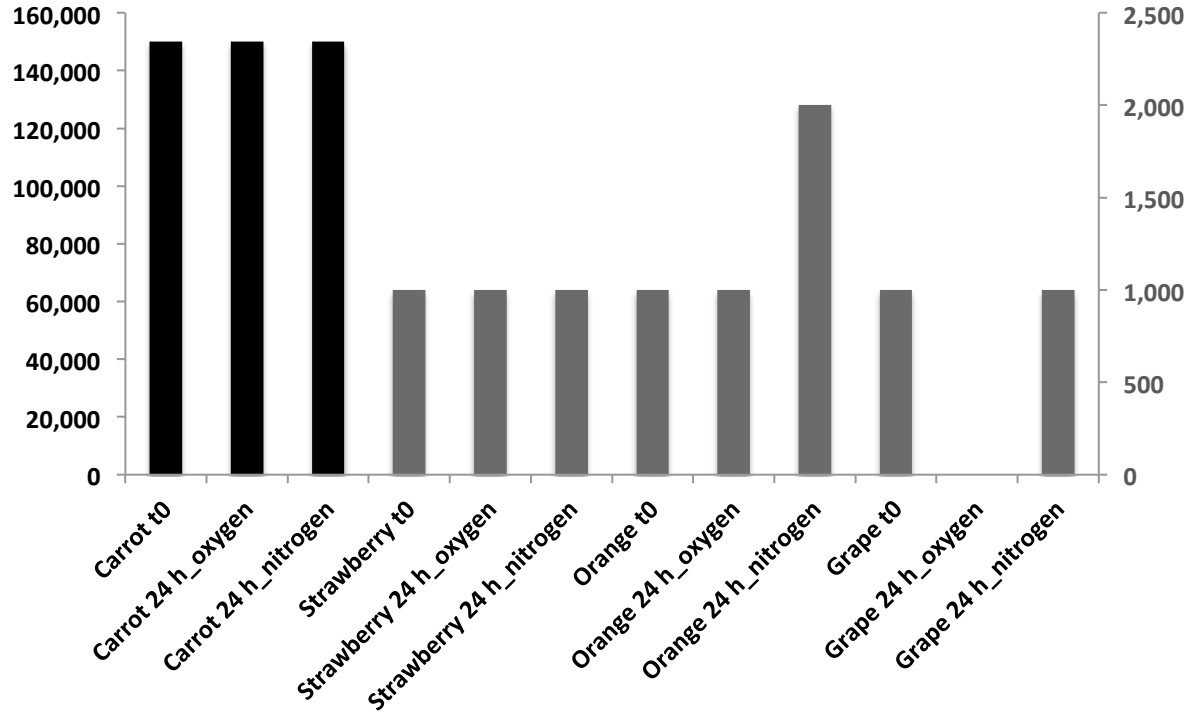


Figure 45. Colony forming units of yeasts in juices from P. Masticating Juicer. Comparison between oxygen and nitrogen storage conditions

V. Appendix

Data of the vitamin C measurements

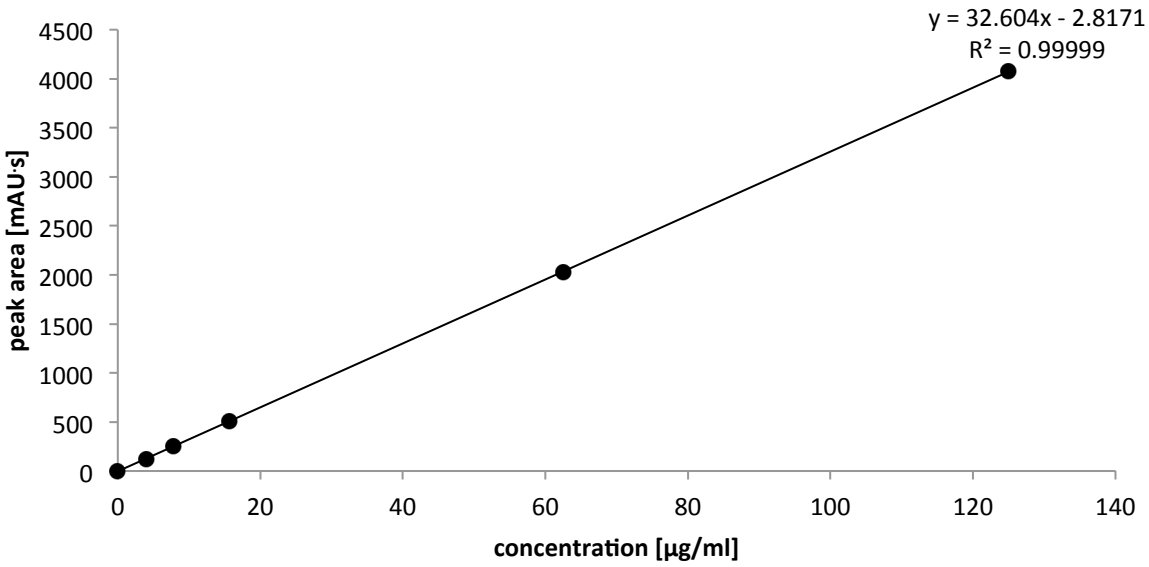


Figure 46. Standard calibration curve of L-ascorbic acid. Calibration equation was used to calculate the total vitamin C content in orange and strawberry juice. Calibration equation: y = 32.604x – 2.8171

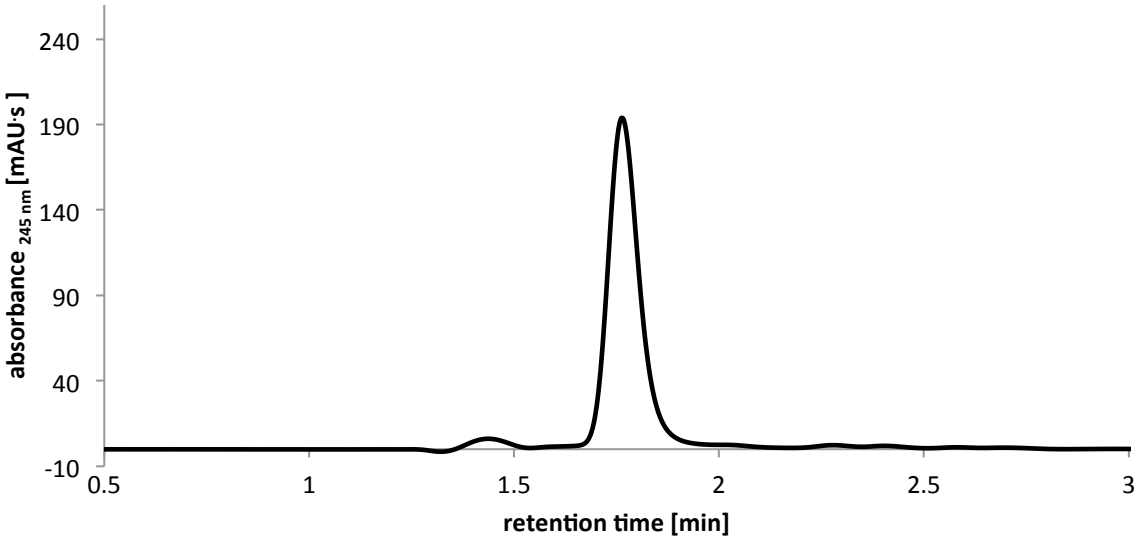


Figure 47. Sample chromatogram of the L-ascorbic acid measurement in strawberry juice from P. ProBlender

Table 35. Peak areas obtained from the analysis of vitamin C in orange and strawberry juice

Peak areas [mAU*s] of the vitamin C measurement under oxygen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	1201.9	1154.0	1083.8	1084.3	893.8	657.9	912.1	1149.6	1049.4
2	990.1	1169.7	1102.7	763.2	716.6	453.2	837.7	987.8	928.9
5	886.2	1078.3	1193.4	639.4	518.8	469.9	747.5	962.5	958.5
8	905.8	1287.6	870.1	600.9	344.5	292.1	754.2	873.4	692.9
24	812.4	894.5	728.6	402.6	200.9	174.5	829.9	884.5	625.1
Peak areas [mAU*s] of the vitamin C measurement under nitrogen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	1303.8	1598.0	1077.1	1135.0	1177.9	1061.0	1464.4	1613.7	1270.0
2	1323.7	1523.7	1075.6	1272.7	1097.3	999.7	1425.6	1661.1	1297.5
5	1308.1	1524.2	1032.3	1175.4	1133.7	963.9	1408.7	1658.7	1211.0
8	1297.3	1556.3	1005.1	1147.1	1152.2	996.6	1447.1	1616.6	1251.2
24	1277.5	1553.0	1078.0	1161.7	1109.6	1045.2	1425.3	1636.5	1238.5
Peak areas [mAU*s] of the vitamin C measurement under oxygen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	1370.2	1377.3	1686.8	1190.4	1066.5	1238.3	1336.6	1389.7	1394.0
2	1157.9	1125.9	1353.8	443.3	306.0	270.2	1321.1	1383.6	1323.4
5	945.4	864.7	780.7	364.4	209.3	236.9	1092.1	934.1	1138.7
8	649.2	555.9	534.1	296.5	174.6	203.6	1001.8	944.8	850.3
24	609.3	497.2	474.7	315.2	159.8	204.7	925.8	814.1	793.5
Peak areas [mAU*s] of the vitamin C measurement under nitrogen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	1792.0	1288.7	1346.1	1364.2	1254.8	1006.2	1387.2	1250.0	1502.0
2	1658.2	1407.4	1363.0	1259.5	1221.3	1141.7	1377.3	1237.2	1530.5
5	1685.0	1394.7	1386.7	1147.0	1256.0	1193.4	1287.4	1393.8	1432.1
8	1624.2	1383.2	1323.4	1184.2	1242.3	1150.5	1372.3	1319.6	1345.5
24	1642.9	1374.0	1381.2	1153.1	1261.5	1140.0	1382.1	1392.4	1325.5

Data of the 5-me-THF measurements

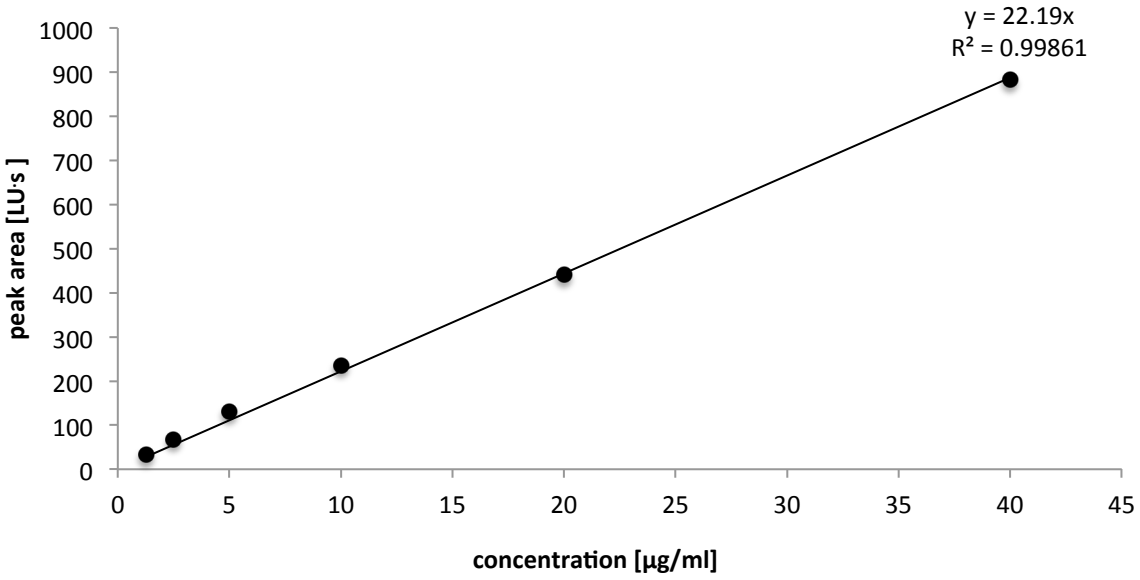


Figure 48. Standard calibration curve of 5-me-THF. Calibration equation was used to calculate the folate content in orange and strawberry juice. Calibration equation: y = 22.19x

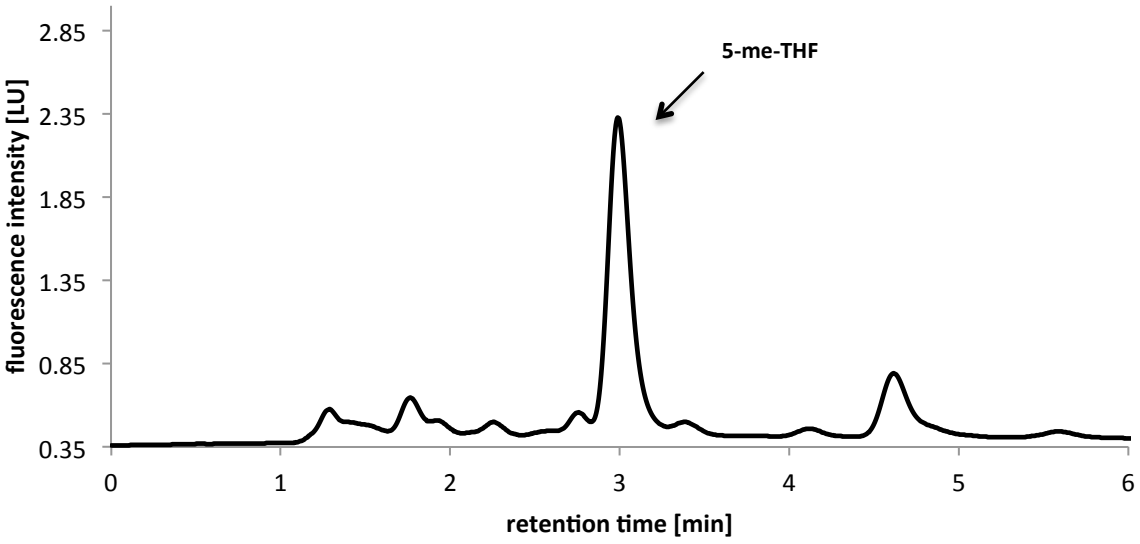


Figure 49. Sample chromatogram of the 5-me-THF measurement in strawberry juice from P. Avance Collection

Table 36. Sample weigh-in of orange and strawberry juice samples for solid-phase extraction

Sample weigh-in [mg] for the 5-me-THF measurement under oxygen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	257.5	256.5	252.3	252.5	253.4	249.0	252.5	250.9	252.9
2	257.2	254.5	254.7	252.6	246.4	255.4	250.9	248.9	253.8
5	250.5	251.0	248.4	246.6	237.8	255.7	250.6	250.4	248.9
8	254.3	249.7	252.7	247.9	254.7	249.8	250.0	250.9	254.7
24	249.7	253.8	248.8	247.5	253.5	253.5	247.9	255.0	255.6
Sample weigh-in [mg] for the 5-me-THF measurement under nitrogen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	255.9	252.6	246.5	251.0	248.4	253.4	251.9	249.9	254.7
2	255.5	252.8	252.4	256.6	257.4	256.4	249.4	254.2	250.5
5	252.3	257.1	246.8	246.4	250.5	245.7	254.9	248.3	249.6
8	248.5	248.2	250.4	245.8	246.4	251.4	253.5	252.9	249.7
24	249.3	255.6	246.7	246.7	256.8	252.5	251.3	251.8	250.2
Sample weigh-in [mg] for the 5-me-THF measurement under oxygen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	252.2	251.7	249.2	250.9	248.8	253.2	252.5	250.2	251.4
2	249.1	250.2	251.3	253.1	251.3	251.1	251.8	249.0	253.6
5	251.7	248.5	249.1	256.2	251.4	251.8	251.0	251.9	247.4
8	252.5	250.4	249.8	253.3	255.1	254.6	252.0	254.7	248.6
24	250.8	250.9	253.8	247.7	249.5	250.3	253.2	250.0	252.2
Sample weigh-in [mg] for the 5-me-THF measurement under nitrogen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	252.8	248.1	251.0	250.8	253.8	252.4	254.8	249.4	248.9
2	250.1	250.2	251.0	251.3	252.3	254.5	255.4	249.5	251.2
5	255.5	248.8	248.9	252.2	247.3	254.7	253.6	246.8	251.4
8	248.9	248.7	254.8	254.6	250.9	250.9	254.1	247.8	254.9
24	247.9	253.3	255.1	249.7	255.7	247.9	252.2	248.9	248.5

Table 37. Peak areas obtained from the analysis of 5-me-THF in orange and strawberry juice

Peak areas [LU˙s] of the 5-me-THF measurement under oxygen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	4.5	5.5	5.5	5.0	5.1	4.9	5.3	7.3	4.7
2	4.5	6.1	6.2	5.0	4.9	4.7	5.2	6.4	4.6
5	4.3	4.9	5.7	6.0	4.7	4.1	4.9	6.3	5.4
8	4.6	5.2	5.8	6.2	4.5	3.8	5.1	6.6	5.4
24	5.8	5.3	3.0	6.7	4.3	3.8	5.2	6.2	5.2
Peak areas [LU˙s] of the 5-me-THF measurement under nitrogen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	6.5	6.7	5.0	8.2	7.0	7.7	7.0	5.3	5.0
2	6.9	6.1	6.1	8.2	7.4	8.0	7.2	5.6	4.7
5	5.5	6.8	6.0	7.3	7.1	8.3	7.1	5.4	4.6
8	6.8	6.1	5.1	7.3	7.9	8.3	6.8	5.3	4.8
24	6.2	6.1	6.2	8.0	8.2	7.6	6.9	5.3	5.0
Peak areas [LU˙s] of the 5-me-THF measurement under oxygen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	19.4	19.8	20.6	17.8	17.9	18.3	16.0	18.0	17.3
2	18.0	19.1	19.3	18.9	17.5	17.9	17.8	21.6	21.8
5	18.8	18.6	18.8	17.7	17.2	18.7	19.2	22.3	19.9
8	19.2	19.2	19.6	17.2	17.7	18.8	18.7	20.5	20.7
24	19.6	21.0	19.6	17.0	18.6	16.3	17.5	20.0	20.5

Peak areas [LU*s] of the 5-me-THF measurement under nitrogen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	10.8	16.2	15.9	18.1	17.4	21.4	12.9	14.4	15.8
2	9.8	17.6	17.2	19.0	16.9	18.0	14.8	14.3	14.9
5	10.3	16.4	16.9	18.9	17.7	16.1	15.5	13.9	16.7
8	10.1	15.9	18.9	18.2	18.6	22.0	14.1	13.4	16.5
24	10.3	18.0	19.7	18.7	17.8	20.0	14.6	13.7	16.7

Data of the vitamin B_6 measurements

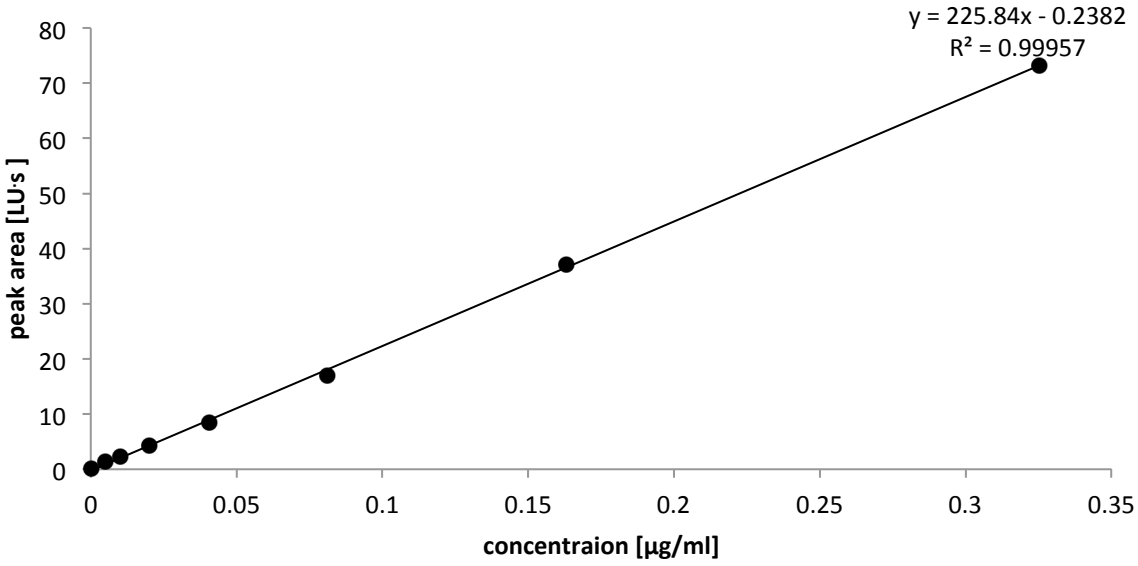


Figure 50. Standard calibration curve of pyridoxamine. Calibration equation was used to calculate the vitamin B_6 content in carrot juice. Calibration equation: y = 225.84x – 0.2382

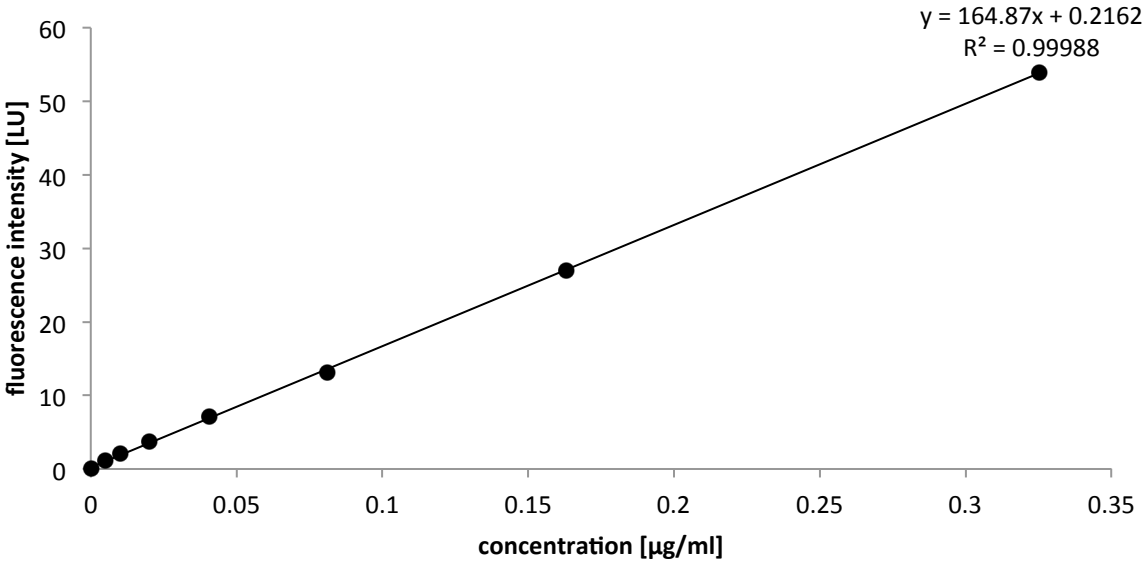


Figure 51. Standard calibration curve of pyridoxal. Calibration equation was used to calculate the vitamin B_6 content in carrot juice. Calibration equation: y = 164.87x + 0.2162

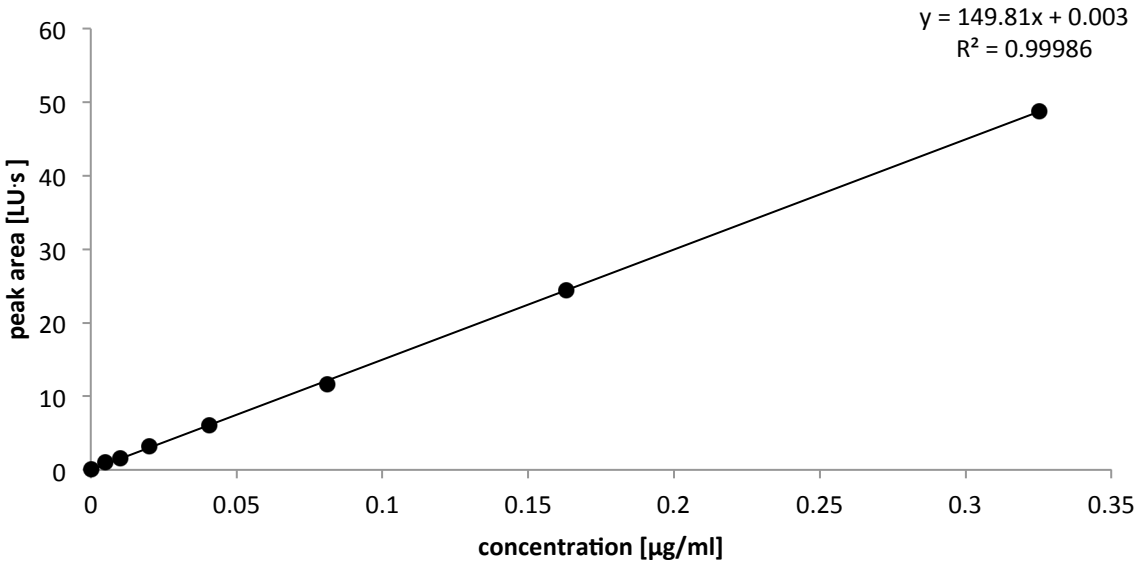


Figure 52. Standard calibration curve of pyridoxine. Calibration equation was used to calculate the vitamin B_6 content in carrot juice. Calibration equation: y = 149.81 + 0.003

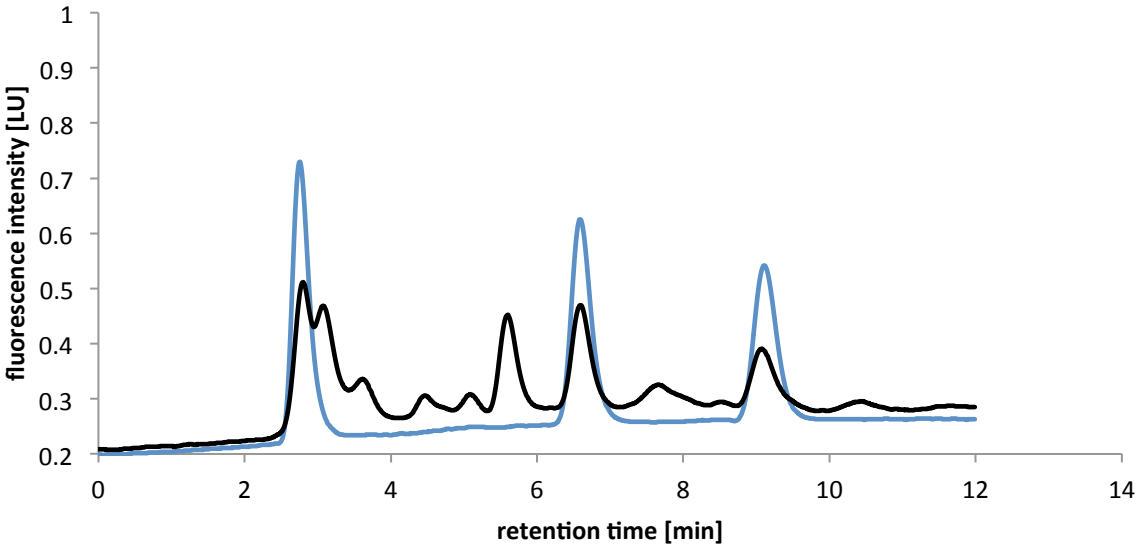


Figure 53. Sample chromatogram of the vitamin B_6 measurement in carrot juice from P. ProBlender. Standard calibration curve is depicted in blue, the sample chromatogram in black. Peak order from left to right: pyridoxamine, pyridoxal, and pyridoxine;

Table 38. Peak areas obtained from the analysis of vitamin B_6 in carrot juice

Peak areas [LU·s] of the vitamin B_6 measurement under oxygen storage conditions in carrot juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	PM	PL	PN	PM	PL	PN	PM	PL	PN
0/1	7.1	1.7	0	3.4	3.5	2.5	4.4	2.9	0
0/2	7.1	2.2	0	3.4	3.6	1.9	4.3	2.9	0
0/3	7.9	1.7	0	3.6	3.7	1.7	3.8	3.2	0

2/1	8.5	2.2	0	3.7	3.6	2.2	4.4	3.3	0
2/2	7.4	1.6	0	3.6	3.9	2.1	4.3	2.8	0
2/3	9.4	2.4	0	3.5	3.9	1.6	3.9	2.8	0.5
5/1	7.0	1.7	0	3.4	3.5	2.4	4.6	3.2	0.3
5/2	9.0	2.3	0	3.7	3.8	2.1	3.8	2.5	0
5/3	6.1	1.8	0	3.5	3.9	1.4	5.0	3.4	0
8/1	8.3	2.0	0	3.7	3.9	2.5	4.6	3.1	0
8/2	7.3	1.8	0	3.1	3.7	2.5	4.0	2.6	0
8/3	9.2	2.6	0	3.3	3.8	2.1	5.0	3.4	0
24/1	9.5	3.1	0	3.0	3.2	2.2	4.6	3.2	0
24/2	6.4	3.1	0	2.7	3.6	2.6	4.3	3.1	0
24/3	6.8	3.4	0	3.2	4.2	2.3	4.9	3.2	0

Peak areas [LU·s] of the vitamin B_6 measurement under nitrogen storage conditions in carrot juice

Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	PM	PL	PN	PM	PL	PN	PM	PL	PN
0/1	8.5	2.0	0	7.5	2.3	0	8.4	1.9	0
0/2	7.9	1.0	0	7.2	1.8	0	6.4	1.5	0
0/3	8.0	1.2	0	6.3	2.3	0	6.7	1.2	0
2/1	8.7	1.9	0	6.6	2.9	0	8.0	1.7	0
2/2	7.6	1.6	0	7.5	1.4	0	6.2	2.0	0
2/3	7.4	1.2	0	6.7	2.5	0	7.0	1.2	0
5/1	8.5	1.6	0	6.8	2.6	0	8.2	1.4	0
5/2	7.6	1.7	0	7.4	1.7	0	6.5	1.6	0
5/3	7.5	1.3	0	6.5	2.4	0	6.5	1.8	0
8/1	8.9	1.5	0	7.0	2.8	0	9.0	1.7	0
8/2	7.3	1.5	0	7.1	1.3	0	5.9	1.4	0
8/3	7.4	1.7	0	6.6	2.6	0	6.5	1.5	0
24/1	8.4	1.7	0	7.0	1.9	0	8.6	1.4	0
24/2	7.2	1.3	0	7.8	1.5	0	6.3	1.7	0
24/3	7.4	2.0	0	6.6	2.7	0	6.3	1.7	0

Data of the total phenolic content measurements

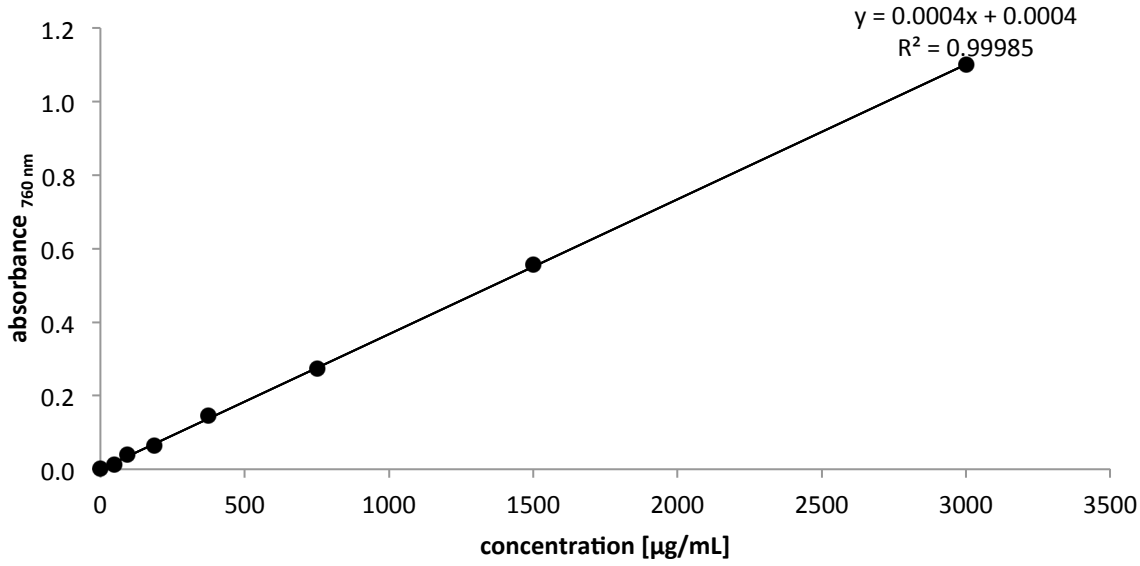


Figure 54. Standard calibration curve of gallic acid. Calibration equation was used to calculate the total phenolic content in strawberry, carrot and grape juice, Calibration equation: y = 0.0004x + 0.0004

Table 39. Absorption obtained from the TPC measurements in strawberry, carrot and grape juice

Absorbance of the TPC measurement under oxygen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	0.9373	0.9352	0.9347	1.031	0.9328	0.9291	0.9236	0.8927	0.9233
	0.9374	0.9360	0.9346	1.035	0.9330	0.9291	0.9236	0.8927	0.9235
	0.9371	0.9363	0.9345	1.030	0.9330	0.9291	0.9236	0.8927	0.9233
2	0.9248	0.8257	0.9245	0.9992	0.8308	1.093	0.9733	0.9547	1.0259
	0.9248	0.8260	0.9257	0.9991	0.8305	1.093	0.9730	0.9548	1.0258
	0.9255	0.8261	0.9282	0.9992	0.8304	1.093	0.9729	0.9549	1.0259
5	0.9926	0.9273	0.8726	0.8731	0.9297	0.8256	0.9621	0.9913	0.8939
	0.9925	0.9267	0.8725	0.8705	0.9296	0.8256	0.9609	0.9914	0.8942
	0.9925	0.9270	0.8727	0.8702	0.9296	0.8255	0.9608	0.9916	0.8941
8	0.8276	0.9256	0.8726	1.026	0.9268	0.9971	1.0222	0.9522	0.9254
	0.8254	0.9260	0.8725	1.0257	0.9268	0.9973	1.0222	0.9522	0.9227
	0.8271	0.9261	0.8725	1.026	0.9268	0.9975	1.0223	0.9522	0.9227
24	0.8253	0.9258	0.9264	1.025	0.9295	0.9828	0.9923	0.9713	1.0371
	0.8256	0.9255	0.9260	1.025	0.9296	0.8921	0.9923	0.9715	1.0373
	0.8252	0.9255	0.9258	1.0257	0.9293	0.8928	0.9923	0.9715	1.0369
Absorbance of the TPC measurement under nitrogen storage conditions in strawberry juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	1.1855	1.1661	1.640	1.7158	1.2819	1.2771	1.3147	1.2268	1.4243
	1.1852	1.1659	1.1636	1.7166	1.2816	1.2769	1.3144	2.2262	1.4239
	1.1850	1.1656	1.1638	1.7158	1.2810	1.2763	1.3141	1.2252	1.4226
2	1.4715	1.1473	1.1330	1.6741	1.3493	1.3266	1.2467	1.3144	1.3982
	1.4706	1.1471	1.1325	1.6741	1.3490	1.3262	1.2461	1.3147	1.3974
	1.4701	1.1467	1.1323	1.6741	1.3490	1.3259	1.2456	1.3134	1.3970

	1.4066	1.1283	1.1082	1.6837	1.3329	1.2248	1.3392	1.3619	1.3677
5	1.4062	1.1281	1.1080	1.6830	1.3326	1.2243	1.3385	1.3615	1.3662
	1.4062	1.1278	1.1078	1.6820	1.3322	1.2238	1.3426	1.3612	1.3648
	1.3748	1.1891	1.1364	1.7014	1.4152	1.2742	1.2547	1.2867	1.3014
8	1.3744	1.1889	1.1362	1.7007	1.4148	1.2736	1.2556	1.2867	1.3008
	1.1741	1.1889	1.1360	1.7007	1.4148	1.2733	1.2550	1.2864	1.3005
	1.3893	1.1086	1.1036	1.6888	1.3452	1.1967	1.2407	1.3202	1.3713
24	1.3889	1.1086	1.1036	1.6588	1.3452	1.1964	1.2407	1.3196	1.3713
	13889	1.1084	1.1034	1.6588	1.3452	1.1940	1.2404	1.3199	1.3713

Absorbance of the TPC measurement under oxygen storage conditions in carrot juice

Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	0.2236	0.2242	0.2153	0.2093	0.2280	0.2505	0.2339	0.2002	0.2182
0	0.2229	0.2239	0.2146	0.2091	0.2277	0.2503	0.2336	0.1999	0.2182
	0.2226	0.2234	0.2143	0.2089	0.2275	0.2500	0.2335	0.1996	0.2179
	0.2103	0.2118	0.2136	0.2285	0.2262	0.2282	0.2147	0.2086	0.2150
2	0.2100	0.2112	0.2133	0.2300	0.2258	0.2278	0.2155	0.2083	0.2151
	0.2102	0.2113	0.2134	0.2282	0.2256	0.2278	0.2143	0.2080	0.2147
	0.2100	0.2330	0.2159	0.1929	0.2239	0.2091	0.2119	0.1978	0.2207
5	0.2099	0.2327	0.2149	0.1922	0.2236	0.2088	0.2119	0.1967	0.2205
	0.2100	0.2329	0.2145	0.1918	0.2234	0.2086	0.2117	0.1966	0.2203
	0.2170	0.2075	0.2099	0.2261	0.2273	0.2238	0.2182	0.1995	0.2244
8	0.2176	0.2073	0.2096	0.2257	0.2276	0.2235	0.2182	0.1995	0.2244
	0.2177	0.2074	0.2094	0.2256	0.2270	0.2233	0.2181	0.1993	0.2241
	0.2130	0.2072	0.2134	0.1936	0.2278	0.2052	0.2170	0.1985	0.2257
24	0.2128	0.2068	0.2134	0.1933	0.2284	0.2051	0.2167	0.1957	0.2255
	0.2126	0.2066	0.2128	0.1930	0.2270	0.2052	0.2165	0.1954	0.2256

Absorbance of the TPC measurement under nitrogen storage conditions in carrot juice

Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	0.3016	0.3001	0.3365	0.3625	0.3228	0.3085	0.2435	0.2567	0.2022
0	0.3033	0.3016	0.3375	0.3624	0.3225	0.3082	0.2434	0.2567	0.2022
	0.3039	0.3023	0.3376	0.3622	0.3224	0.3081	0.2432	0.2564	0.2021
	0.3331	0.3249	0.3210	0.3133	0.3792	0.3850	0.2620	0.2368	0.2445
2	0.3344	0.3262	0.3222	0.3133	0.3791	0.3848	0.2620	0.2367	0.2445
	0.3349	0.3266	0.3235	0.3132	0.3788	0.3849	0.2618	0.2365	0.2442
	0.3660	0.2749	0.2639	0.2892	0.2701	0.2983	0.2517	0.2073	0.2443
5	0.3657	0.2744	0.2635	0.2887	0.2700	0.2983	0.2516	0.2072	0.2442
	0.3654	0.2739	0.2631	0.2888	0.2700	0.2981	0.2515	0.2071	0.2442
	0.3700	0.3088	0.3580	0.2213	0.3023	0.2787	0.2302	0.2748	0.2223
8	0.3697	0.3086	0.3576	0.2210	0.3021	0.2787	0.2313	0.2748	0.2223
	0.3694	0.3082	0.3575	0.2205	0.3017	0.2786	0.2209	0.2747	0.2222
	0.4372	0.3361	0.4263	0.2847	0.3890	0.2528	0.2301	0.2343	0.2449
24	0.4370	0.3359	0.4261	0.2845	0.3889	0.2528	0.2300	0.2344	0.2449
	0.4369	0.3356	0.3259	0.2844	0.3889	0.2527	0.2300	0.2342	0.2448

Absorbance of the TPC measurement under oxygen storage conditions in grape juice

Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	0.267	0.304	0.306	0.409	0.410	0.432	0.2021	0.2000	0.2292
0	0.263	0.304	0.306	0.406	0.409	0.434	0.2020	0.2000	0.2293
	0.264	0.306	0.305	0.406	0.406	0.432	0.2019	0.2001	0.2293
	0.250	0.280	0.267	0.424	0.396	0.411	0.2023	0.2034	0.2287
2	0.249	0.280	0.267	0.423	0.402	0.413	0.2021	0.2035	0.2286
	0.250	0.280	0.267	0.424	0.402	0.411	0.2019	0.2034	0.2286
	0.248	0.248	0.253	0.399	0.421	0.417	0.2183	0.2362	0.2564
5	0.249	0.249	0.254	0.398	0.420	0.416	0.2182	0.2361	0.2561
	0.249	0.248	0.254	0.398	0.418	0.416	0.2180	0.2360	0.2560
	0.227	0.242	0.244	0.415	0.392	0.404	0.2014	0.2330	0.2270
8	0.226	0.241	0.245	0.416	0.397	0.399	0.2012	0.2331	0.2275
	0.227	0.242	0.246	0.414	0.396	0.397	0.2012	0.2334	0.2276

	0.220	0.227	0.270	0.417	0.444	0.407	0.2081	0.2012	0.2241
24	0.218	0.227	0.270	0.403	0.436	0.408	0.2079	0.2012	0.2240
	0.218	0.228	0.270	0.403	0.441	0.406	0.2076	0.2010	0.2243

Absorbance of the TPC measurement under nitrogen storage conditions in grape juice

Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	0.2677	0.3034	0.3338	0.5288	0.4511	0.4186	0.2787	0.2763	0.2717
0	0.2677	0.3032	0.3337	0.5287	0.4509	0.4184	0.2787	0.2763	0.2716
	0.2676	0.3031	0.3335	0.5284	0.4506	0.4183	0.2787	0.2763	0.2718
	0.2929	0.3600	0.3709	0.4768	0.5454	0.4763	0.2848	0.2690	0.2360
2	0.2929	0.3599	0.3709	0.4766	0.5461	0.4763	0.2848	0.2690	0.2359
	0.2928	0.3598	0.3707	0.4765	0.5461	0.4761	0.2848	0.2693	0.2358
	0.3018	0.3600	0.3123	0.5164	0.5051	0.3452	0.2554	0.2184	0.2509
5	0.3016	0.3599	0.3121	0.5163	0.5050	0.3451	0.2554	0.2184	0.2507
	0.3014	0.3598	0.3120	0.5153	0.5048	0.3451	0.2553	0.2183	0.2506
	0.2996	0.3887	0.3175	0.4950	0.4151	0.3933	0.2469	0.2941	0.2433
8	0.2993	0.3888	0.3172	0.4949	0.4149	0.3932	0.2468	0.2940	0.2434
	0.2992	0.3887	0.3171	0.4948	0.4148	0.3931	0.2467	0.2940	0.2434
	0.2846	0.3319	0.2921	0.4324	0.4625	0.3504	02.414	0.2534	0.2001
24	0.2844	0.3318	0.2921	0.4323	0.4625	0.3505	0.2415	0.2533	0.2000
	0.2844	0.3319	0.2920	0.4323	0.4624	0.3503	0.2412	0.2533	0.2000

Data of the anthocyanin measurements

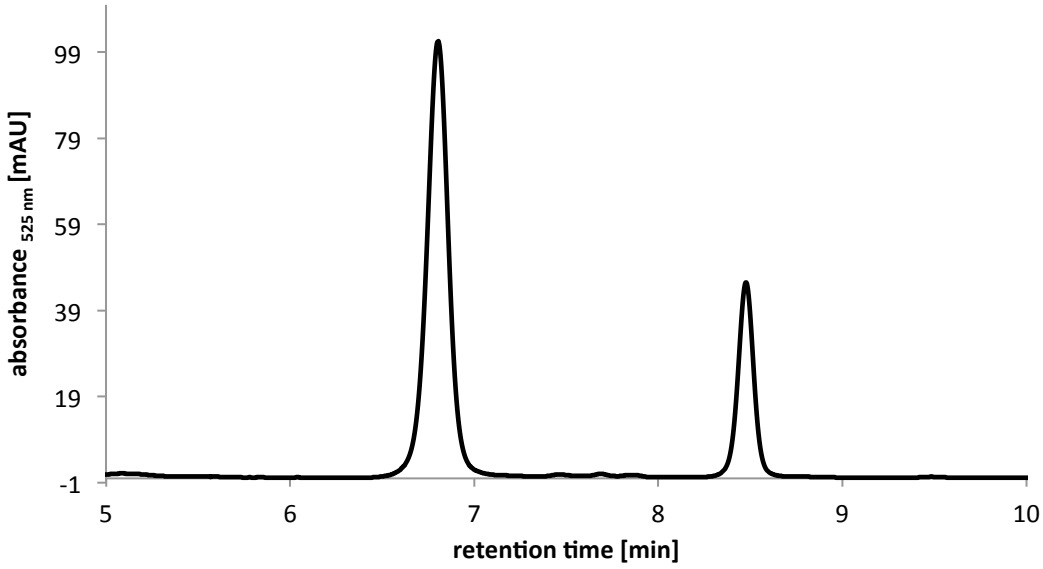


Figure 55. Sample chromatogram of the anthocyanin measurement in strawberry juice from P. Avance Collection. Left peak: pelargonidin-3-glucoside, right peak: pelargonidin-3-malonyl-glucoside

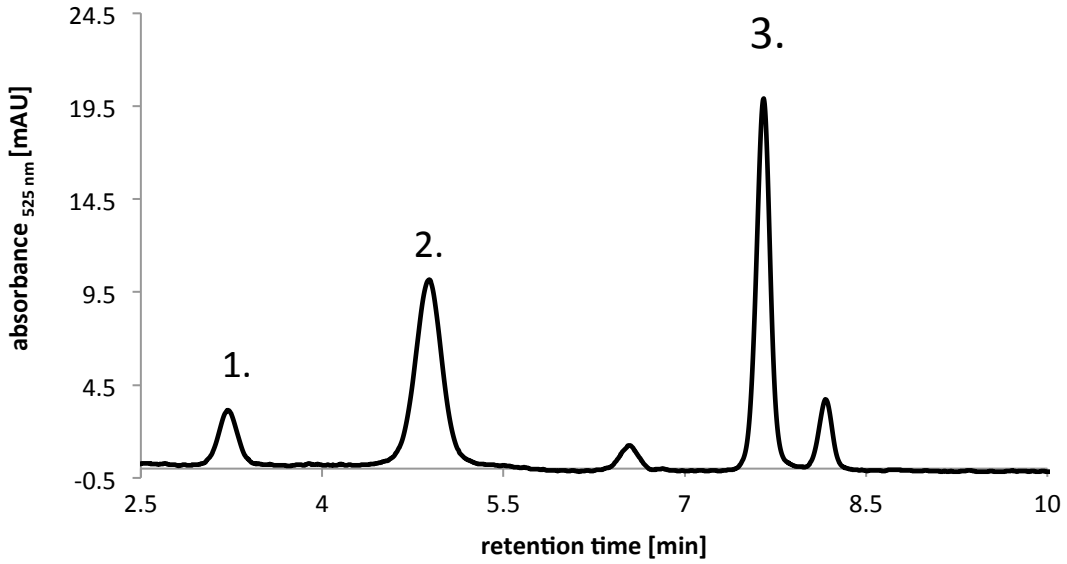


Figure 56. Sample chromatogram of the anthocyanin measurement in grape juice from P. Avance Collection. Peak 1: petunidin-3-glucoside, peak 2: malvidin-3-glucoside, peak 3: petunidin-3-O-(6-p-coumaroyl)-glucoside

Table 40. Peak areas obtained from the analysis of anthocyanins in strawberry juice

Peak areas [mAU*s] of the anthocyanin measurement under oxygen storage conditions in strawberry juice						
Time [h]	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
	Pl-3-glc	Pl-3-malonyl-glc	Pl-3-glc	Pl-3-malonyl-glc	Pl-3-glc	Pl-3-malonyl-glc
0	725.9	228.8	751.4	209.9	945.5	322.6
	1057.6	297.6	849.6	302.2	778.4	225.8
	960.0	283.9	868.1	281.7	842.3	257.2
2	677.0	207.6	810.7	227.1	922.3	312.1
	990.0	303.2	921.4	322.6	769.3	228.0
	884.0	281.2	882.3	286.3	905.6	270.5
5	656.5	234.0	783.0	216.8	891.8	297.7
	1022.2	301.2	852.0	293.2	774.7	230.1
	893.8	257.0	873.4	277.8	891.5	263.9
8	746.5	240.8	800.5	222.2	923.3	311.0
	1007.1	313.4	868.3	274.1	747.7	220.7
	919.5	248.1	864.9	272.1	895.9	266.7
24	718.7	239.0	767.3	212.2	937.0	311.5
	1027.7	307.8	835.4	284.1	766.2	226.0
	816.6	252.0	911.6	290.8	887.7	266.7
Peak areas [mAU*s] of the anthocyanin measurement under nitrogen storage conditions in strawberry juice						
Time [h]	P. Avance Collection		P. ProBlender 6		P. Masticating Juicer	
	Pl-3-glc	Pl-3-malonyl-glc	Pl-3-glc	Pl-3-malonyl-glc	Pl-3-glc	Pl-3-malonyl-glc
0	957.4	314.5	906.2	298.8	785.4	292.1
	1086.6	346.1	1001.6	383.6	873.4	346.7
	854.8	278.8	1056.2	323.9	605.8	271.2
2	962.1	320.0	888.2	257.6	774.5	280.6
	973.6	301.0	1024.6	343.2	998.0	391.2
	905.4	297.1	1009.4	281.2	701.6	305.2
5	973.1	320.1	866.6	254.0	701.9	302.3
	1004.3	310.6	1018.9	351.2	902.5	363.2
	862.8	282.2	1003.9	287.0	672.4	281.9
8	947.3	309.0	876.4	290.8	746.7	289.2
	1013.1	311.7	1040.3	353.4	917.5	379.4
	820.0	268.6	983.9	298.1	669.8	289.7
24	944.2	307.1	867.7	279.0	759.1	301.7
	1028.4	315.9	987.5	367.8	956.8	367.3
	850.5	278.7	978.2	262.0	671.1	292.9

Table 41. Peak areas obtained from the analysis of anthocyanins in grape juice

Peak areas [mAU*s] of the anthocyanin measurement under oxygen storage conditions in grape juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	Pt-3-glc	Mal-3-glc	Pt-3-O-(6-p-c)-glc	Pt-3-glc	Mal-3-glc	Pt-3-O-(6-p-c)-glc	Pt-3-glc	Mal-3-glc	Pt-3-O-(6-p-c)-glc
0	18.2	281.5	126.3	65.6	358.9	170.1	46.3	193.4	156.5
	15.9	209.4	67.5	79.1	353.2	383.3	23.5	214.0	98.8
	32.2	159.2	151.3	25.6	237.2	149.6	18.9	273.9	107.5
2	16.1	254.4	118.8	50.6	301.5	127.1	41.7	184.9	151.9
	15.6	209.1	69.2	50.3	319.1	284.8	23.1	206.0	97.7
	32.7	160.5	168.1	30.8	250.0	107.7	17.9	272.3	107.6
5	12.3	208.7	101.2	53.5	256.3	132.3	23.4	162.9	132.1

	12.2	169.9	57.6	35.3	244.1	240.7	22.6	190.7	92.3
	27.9	136.8	144.2	20.6	221.4	102.5	14.4	255.6	101.3
8	10.0	192.2	94.5	48.7	231.7	117.7	30.5	153.5	138.6
	11.0	172.5	59.4	43.7	232.4	234.2	21.1	174.7	86.1
	26.7	127.8	152.3	37.8	217.4	99.9	10.9	223.4	92.3
24	6.9	150.2	91.2	38.1	163.2	99.2	21.7	121.2	112.2
	7.7	144.7	53.4	36.4	179.7	199.2	13.7	127.3	66.8
	20.9	89.0	148.4	35.3	192.3	94.1	6.4	149.1	70.0

Peak areas [mAU*s] of the anthocyanin measurement under nitrogen storage conditions in grape juice

Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
	Pt-3-glc	Mal-3-glc	Pt-3-O-(6-p-c)-glc	Pt-3-glc	Mal-3-glc	Pt-3-O-(6-p-c)-glc	Pt-3-glc	Mal-3-glc	Pt-3-O-(6-p-c)-glc
0	0	592.4 572.4 661.3	0	0	752.2 529.1 588.4	0	0	287.3 270.0 218.9	0
2	0	574.0 572.8 698.7	0	0	741.6 510.4 628.4	0	0	289.1 262.1 222.9	0
5	0	500.3 547.0 642.9	0	0	722.5 517.4 610.9	0	0	262.9 278.7 224.7	0
8	0	508.2 545.6 618.0	0	0	686.4 553.2 604.0	0	0	289.7 263.7 228.2	0
24	0	594.3 546.0 608.9	0	0	709.9 555.7 652.6	0	0	280.0 266.2 228.7	0

Data of the hesperidin measurements

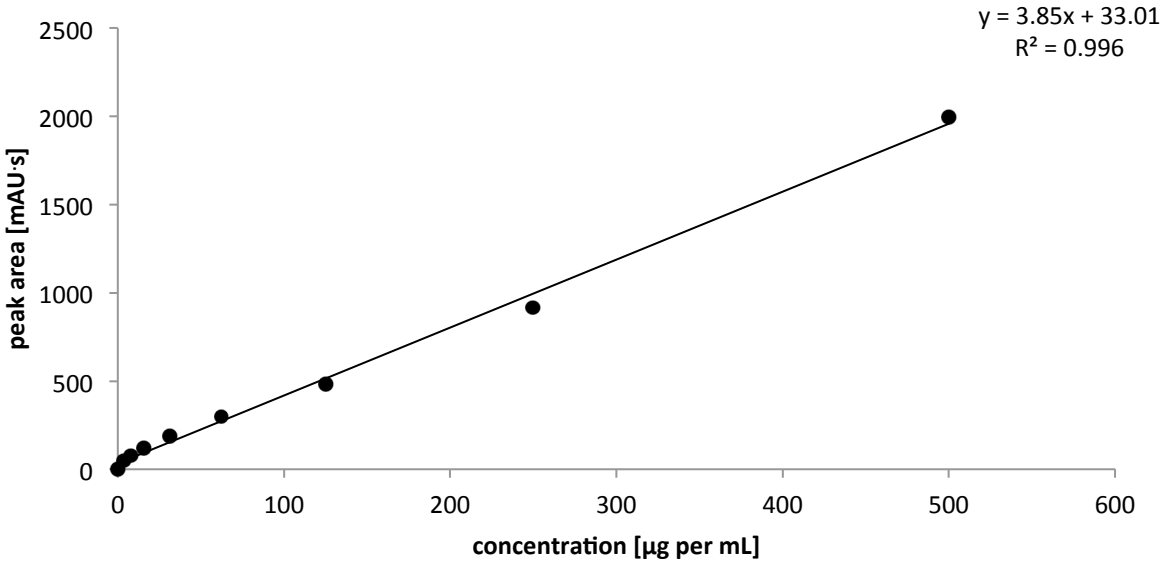


Figure 57. Standard calibration curve of hesperidin. Calibration equation was used to calculate the hesperidin content in orange juice. Calibration equation: y = 3.85x + 33.01

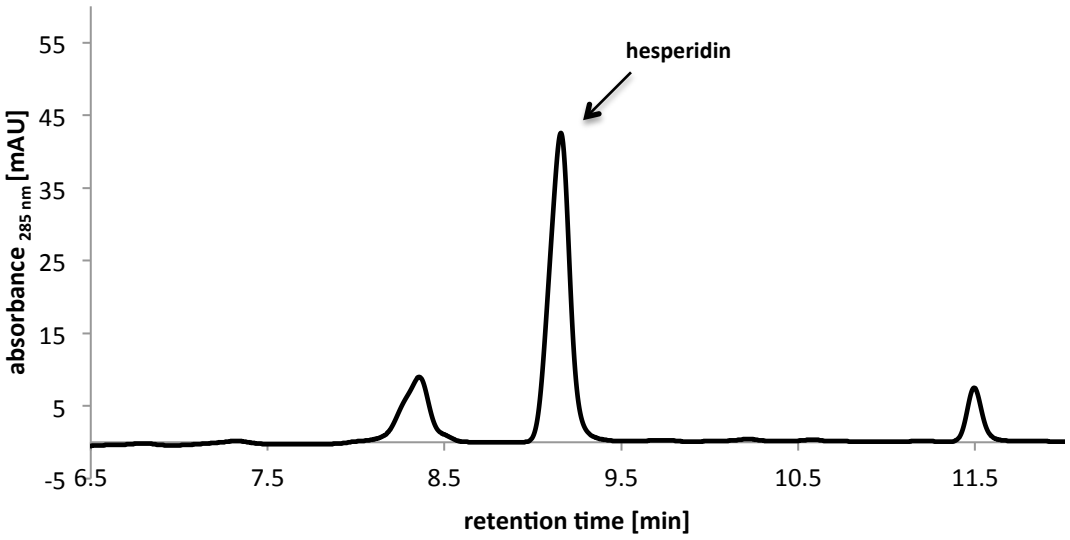


Figure 58. Sample chromatogram of the hesperidin measurement in orange juice from P. Masticating Juicer

Table 42. Peak areas obtained from the analysis of hesperidin in orange juice

Peak areas [mAU*s] of the hesperidin measurement under oxygen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	439.1	368.9	424.7	457.8	423.9	397.2	335.2	306.2	317.0
2	293.8	221.3	215.7	268.3	297.4	298.8	283.9	254.7	230.8
5	250.2	189.0	233.0	161.8	193.8	265.6	264.6	242.5	206.0
8	233.0	192.3	194.8	281.6	211.5	303.2	236.9	202.0	196.4
24	158.7	164.5	156.1	240.4	274.7	230.9	176.8	146.1	170.3
Peak areas [mAU*s] of the hesperidin measurement under nitrogen storage conditions in orange juice									
Time [h]	P. Avance Collection			P. ProBlender 6			P. Masticating Juicer		
0	298.1	309.4	354.6	300.0	410.3	440.9	214.7	165.1	204.3
2	297.3	297.7	367.1	292.6	405.1	437.0	196.8	160.5	199.9
5	281.4	300.2	374.1	291.6	410.8	440.2	188.5	161.8	196.3
8	280.4	299.8	376.9	303.1	412.1	438.3	194.2	164.2	193.8
24	293.3	306.0	372.0	302.8	418.4	433.4	198.1	168.4	195.6

Data of the determination of the total microbial counts

This experiment was part of the master thesis of Daniela Krieg and conducted by Sandra Holzer at the company Biomerx in Pasching, Austria in 2015.

Table 43. Determination of the total microbial counts in fruit juices conducted in 2015

Orange Juice				
Juicer	**Storage time [h]**	**CFU/ml**		
		Bacteria	**Yeasts**	**Moulds**
Avance Collection	0	<1000	<1000	<1000
	24	<1000	4000	<1000
ProBlender	0	23000	7000	<1000
	24	31000	6000	<1000
Avance Juicer	0	2000	8000	<1000
	24	10000	9000	<1000
Strawberry Juice				
Juicer	**Storage time [h]**	**CFU/ml**		
		Bacteria	**Yeasts**	**Moulds**
Avance Collection	0	<1000	2000	<1000
	24	1000	<1000	<1000
ProBlender	0	9000	2000	<1000
	24	2000	<1000	<1000
Avance Juicer	0	1000	3000	<1000
	24	3000	<1000	<1000
Grape Juice				
Juicer	**Storage time [h]**	**CFU/ml**		
		Bacteria	**Yeasts**	**Moulds**
Avance Collection	0	7000	<1000	<1000
	24	2000	<1000	<1000
ProBlender	0	61000	3000	<1000
	24	76000	6000	<1000
Avance Juicer	0	7000	3000	<1000
	24	2000	5000	<1000

VI. Literature

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