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Stability of Selected Nutrients in Home-Made Fruit Juices under Different Ways of Processing

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Zusammenfassung

Das Ziel dieser Masterarbeit war die Stabilität von ausgewählten Nährstoffen in selbstgemachten Fruchtsäften zu analysieren. Die Herstellung der Fruchtsäfte erfolgte mit einer Schneckenpresse mit einer anti-Schaum Vorrichtung und einem Standmixer unter verschiedenen Atmosphären: normale Luft (Referenz), Vakuum, CO_2 und N_2O. Für die Herstellung der Säfte wurden Erdbeere, Orange, Heidelbeere, Kiwi, Roter Paprika, Apfel, Rote Weintraube und Matcha Pulver verwendet und anschließend wurden die Säfte in verschlossenen Glasflaschen 24 Stunden lang bei 3 °C im Kühlschrank gelagert.

Die folgenden Nährstoffe wurden untersucht: Vitamin C (gesamter Ascorbinsäuregehalt), Anthocyane, gesamter Phenolgehalt und zusätzlich unterschiedliche Catechine im Matcha Pulver.

Der **Vitamin C**-Gehalt der verschiedenen Fruchtsäfte hatte eine gute Stabilität über eine Lagerungsdauer von 24 Stunden. Mit Ausnahme vom Kiwisaft hatte die Applikation von Vakuum keinen Einfluss auf die Stabilität von Vitamin C. Der Vitamin C-Gehalt des Kiwisafts wurde durch das Vakuum besser stabilisiert und ist somit das einzige positive Ergebnis für die Applikation von Vakuum. Die Applikation der Gase CO_2 und N_2O führte zu einer sehr guten Stabilisierung des Vitamin C-Gehaltes aller Fruchtsäfte, denn es wurden nur vernachlässigbare oder keine Verluste detektiert. Ähnliche Resultate lieferte die Schneckenpresse mit der anti-Schaum Vorrichtung. Deren Fruchtsäfte wiesen auch eine sehr gute Stabilität des Vitamin C-Gehaltes auf.

Ein großer Verlust des **Anthocyan**-Gehaltes wurde für den Standmixer mit normaler Luft festgestellt. Die Applikation von Vakuum führte zu einer viel besseren Stabilität der Anthocyane, da nur kleine Verluste detektiert wurden. Weiters führte die Applikation von CO_2 und N_2O zu einer etwas besseren Stabilität der Anthocyane als das Vakuum. Auch der Traubensaft der Schneckenpresse mit der anti-Schaum Vorrichtung zeigte eine sehr gute Stabilität der Anthocyane. Allerdings war der Anthocyan-Gehalt geringer als bei den Traubensäften der anderen Herstellungsverfahren, da die Schneckenpresse die anthocyan-reichen Traubenschalen während des Pressens vom Saft trennt.

Der **gesamt Phenolgehalt** der Fruchtsäfte zeigte generell eine geringere Stabilität als der Vitamin C Gehalt. Die Applikation von Vakuum zeigte keine positiven Effekte für die Stabilität

des gesamt Phenolgehalts der Fruchtsäfte. Im Gegensatz dazu erhöhten die Gase CO_2 und N_2O die Stabilität des gesamt Phenolgehalts. Wieder lieferte die Schneckenpresse mit der anti-Schaum Vorrichtung ähnliche Resultate in Bezug auf erhöhte Stabilität des gesamt Phenolgehalts. Eine spezieller Fall ist der Matcha Saft, dessen gesamt Phenolgehalt komplett stabil blieb. Der Grund dafür könnte sein, dass Matcha bereits ein prozessiertes Lebensmittel ist.

Catechin und Epicatechin hatten eine sehr gute Stabilität im Matcha Saft, wohingegen große Verluste des Epigallocatechin-gallates entdeckt wurden. Die Applikation von Vakuum, CO_2 und N_2O zeigte keinen Einfluss auf die Stabilität der unterschiedlichen Catechine. Das Mixen des Matcha Pulvers führte zu höheren Catechin-Gehalten der Säfte als das Mischen per Hand.

Zusammenfassend lässt sich sagen, dass nicht viele positive Effekte für die Applikation von Vakuum in Bezug auf Nährstoffstabilität der Fruchtsäfte gefunden wurden. Die einzig positiven Effekte waren die erhöhte Stabilität des Vitamin C Gehaltes im Kiwisaft und besonders des Anthocyan-Gehaltes des Traubensaftes. Die Anwendung von CO_2 und N_2O lieferte immer bessere Ergebnisse als die Applikation von Vakuum. Der Grund dafür könnte die Leistung der Vakuumpumpe sein, da sie nur ein Vakuum von -0,7 bar erzeugen kann. Das bedeutet 0,3 bar normale Luft befinden sich noch immer im Behälter des Mixers. Weiters wurden sehr gute Ergebnisse in Bezug auf die Nährstoffstabilität mit der Schneckenpresse mit anti-Schaum Vorrichtung erhalten. Der einzige Nachteil der Schneckenpresse war der geringe Anthocyan-Gehalt des Traubensaftes, aufgrund der Trennung der anthocyan-reichen Traubenschalen vom Saft.

Abstract

The aim of this master thesis was to analyse the stability of selected nutrients in self-made fruit juices after processing with a masticator with an antifoaming device and with a blender under different atmosphere conditions: normal air (reference), vacuum, CO_2 and N_2O. Therefore, strawberry, orange, blueberry, kiwi, red pepper, apple, red grape and matcha powder were used for the processing and the juices were stored in capped glass bottles for 24 hours at 3 °C in the refrigerator.

The following nutrients were investigated: vitamin C (total ascorbic acid content), anthocyanins, total phenolic content and in addition different catechins of the matcha powder.

The **vitamin C** of the different fruit juices showed good stability over a storage time of 24 hours. The application of vacuum did not influence the stability of vitamin C, except in the kiwi juice, which showed the only positive trend for vacuum application in regards to vitamin C stability. The application of CO_2 and N_2O led to a very good stabilisation of the vitamin C content of all juices, only negligible losses or no losses were detected. Similar results were obtained for the juices from the masticator with the antifoaming device, which showed very good stability for the vitamin C content.

A huge loss of the **anthocyanin** content of the grape juice from the blender with normal air was detected. The application of vacuum led to a much better stability of the anthocyanins, as only minor losses of the anthocyanin content were detected. Nevertheless, the application of CO_2 and N_2O increased the stability of the anthocyanins a little bit better than the application of vacuum. Also, the grape juice from the masticator with the antifoaming device showed a very good stability of the anthocyanin content. However, the anthocyanin content was very low in comparison to the blending procedures, due to the separation of the grape skins from the juice, which contain the most anthocyanins.

The **total phenolic content** of the fruit juices showed less stability over a storage time of 24 hours compared to the ascorbic acid content. Vacuum application did not show any positive effect on the stability of the total phenolic content. In contrast, the application of CO_2 and N_2O increased the stability of the total phenolic content. Again, similar results with regards to increased stability of the total phenolic content were obtained for the juices from the

masticator with the antifoaming device. A special mention is the total phenolic content of the matcha juice, which was totally stable, maybe due to matcha being an already processed food product.

Catechin and epicatechin had very good stability in the matcha juice over a storage time of 24 hours, whereas rather large decreases of the epigallocatechin-gallate were detected. The application of vacuum, CO_2 and N_2O did not affect the stability of the different catechins. Blending of the matcha powder led to higher catechin contents of the juices than manual mixing.

To **conclude**, the application of vacuum did not show many positive trends in terms of nutrient stability for the various fruit juices. The only discovered positive effects were the increased stability of the vitamin C content of kiwi juice and especially of the anthocyanin content of grape juice. The application of CO_2 and N_2O always led to better results than the application of vacuum. Reason for this might be the power of the vacuum pump, which is only capable to produce a vacuum of -0.7 bar, hence 0.3 bar of normal air are still present in the jar of the blender. Also very good results in regards to nutrient stability were obtained for the masticator with the antifoaming device. The only disadvantage of the masticator was the overall low anthocyanin content of the grape juice, because the anthocyanin rich grape skins are removed by the masticator during the processing of the juice.

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CO_2	carbon dioxide
DHAA	dehydroascorbic acid
DKGA	2,3-diketo-L-gulonic acid
EGCG	epigallocatechin-gallate
HCA	hydroxycinnamic acid
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
mAU	milli absorbance unit
N_2O	nitrous oxide/laughing gas
RP-HPLC	reversed phase high performance liquid chromatography
Rpm	rounds per minute
TAC	total anthocyanin content
TCEP	Tris(2-carboxyethyl)phosphin
TPC	total phenolic content

Introduction

Vitamin C

Structure and terminology

Vitamin C is a generic term used for all compounds which exhibit qualitatively the same biological activity as ascorbic acid. L-ascorbic acid is the natural occurring compound with vitamin C activity and is classified as a water soluble-vitamin. L-ascorbic acid is systematically named L-threo-2-hexenono-1,4-lactone (Ball, 1994). There are three diastereomers of the L-ascorbic acid: D-ascorbic acid, L- and D-isoascorbic acid. None of this three enantiomers show vitamin C activity. There exist two oxidation products of L-ascorbic acid that belong to a whole reversible redox system: L-semidehydroascorbic acid and L-dehydroascorbic acid (DHAA). Both of those oxidation products show the same biological vitamin C activity (Velisek, 2014).

The chemical structure of the isomers of ascorbic acid and DHAA can be seen in figure 1.

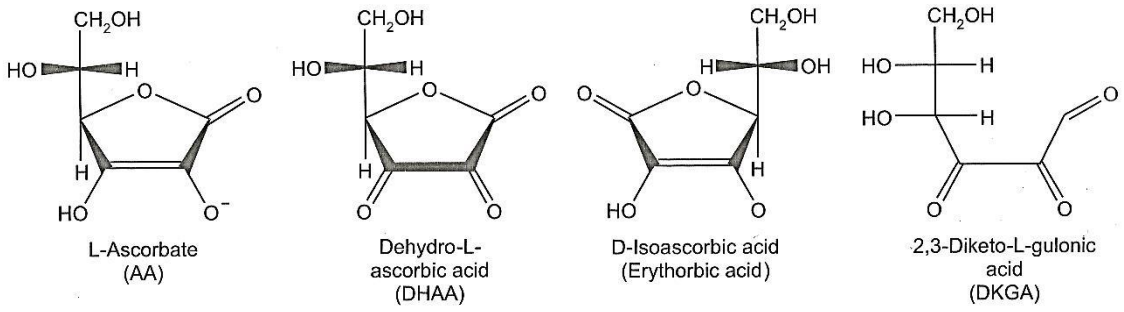


Figure 1: Chemical structure of L-ascorbic acid, one of its enantiomers and of the two oxidation products (adapted from Coultate, 2016).

Physical and chemical properties

Ascorbic acid is considered as a diprotic acid, because both enolic hydroxyl groups of ascorbic acid can dissociate. It belongs to one of the least stable vitamins, because it is sensitive to heat, light and oxygen. The stability of the vitamin C is also influenced by the pH of the food. Vitamin C has the greatest stability between a pH of 4 and 6 (Naidu, 2003).

Vitamin C is a reducing agent because it can donate two electrons. All known physiological and biochemical actions are based on vitamin C being an electron donor. It prevents the oxidation of other compounds by donating its electrons and is thus called an antioxidant. Vitamin C is oxidized during the process, losing its electrons sequentially. Semidehydroascorbic acid, a free radical, is formed after loss of one electron, but this ascorbyl radical is relatively stable compared to other free radicals. Dehydroascorbic acid is formed when two electrons are lost (Padayatty et al., 2003).

Many enzymes catalyse the oxidation of ascorbic acid to DHAA, especially in mechanically damaged plant tissues, where mainly ascorbic acid oxidase catalyses this reaction in presence of atmospheric oxygen. Also peroxidases and other enzymes are associated with the loss of vitamin activity in some plant tissues. Ascorbic acid also reacts with oxidised phenols, the so called quinones, which form brown polymeric pigments and therefore are the cause of the browning of fruits and vegetables. These quinones are reduced back to the parent phenols by ascorbic acid and an equal amount of DHAA is formed. Oxidation of ascorbic acid by oxygen is the most important reaction and mainly the cause for vitamin C losses in foods during processing (Velisek, 2014).

At least three separate enzyme pathways are able to reduce the ascorbyl radical and the DHAA back to ascorbic acid. Also reducing compounds like glutathione are able to reduce these oxidation products back to ascorbic acid. Humans are only capable of a partial reduction, thus not all of the oxidised ascorbic acid is recovered. Some of the DHAA is hydrolysed and lost (Padayatty et al., 2003).

DHAA is less stable than ascorbic acid and can undergo a ring-opening of the lactone ring, which produces 2,3-diketo-L-gulonic acid (DKGA). DKGA has no vitamin activity and this reaction is irreversible and strongly influenced by the pH. The higher the pH, the faster this formation of DKGA occurs (Coultate, 2016). The chemical structure of DKGA can be seen in figure 1.

Synthesis and metabolism

All photoautotrophic plants are capable of synthesising ascorbic acid. They provide their energy needs by photosynthesis and ascorbic acid helps to protect this important photosynthetic machinery of the chloroplasts from oxidation (Velisek, 2014; Coultate, 2016).

The synthesis of ascorbic acid could have appeared 590 – 500 million years ago in the evolution of fishes. The synthesis of ascorbic acid occurs in the kidneys of fishes, amphibians and reptiles and in the liver of mammals. Animals synthesise ascorbic acid from glucose via the glucuronic acid pathway.

A number of species, such as teleost fishes, passeriform birds, some bats, guinea pigs, and primates, including humans, have lost the ability to synthesise ascorbic acid. This ability was lost due to a deficiency of the enzyme L-gulonolactone oxidase, which oxidises L-gulonolactone to L-ascorbic acid. The gene for this enzyme is still present in all species, but it is highly mutated and thus non-functional. This enzyme deficiency causes the dietary need of ascorbic acid and renders, an otherwise normal metabolite, a vitamin (Linster & Van Schaftingen, 2007).

Ascorbic acid is mainly metabolised to DHAA by oxidation. The DHAA is then hydrolysed to 2,3-diketo-L-gulonic acid, which is then either converted to CO_2 and 5-carbon fragments by decarboxylation, or converted to oxalic acid and 4-carbon fragments by oxidation. Humans can also use ascorbic acid for the formation of L-ascorbic acid 2-sulfate (Combs, 2008).

Ascorbic acid is easily absorbed by active transports in the intestines but not stored in the body, due to being a water soluble compound. It has a half-life of about 10-20 days and a daily turnover of 1 mg/kg bodyweight. The absorption of ascorbic acid declines with high levels of intake of about 500 mg/day. When extremely high doses are consumed it is excreted unchanged through the urine (Naidu, 2003).

Physiological functions

Vitamin C has various known metabolic functions, such as an enzyme cofactor and as a protective agent. Furthermore it is also involved in reactions with transition metal ions in the form of the ascorbyl radical. The redox properties of vitamin C are involved in each of these functions.

The basis of most, if not all, essential biological functions of vitamin C is the reaction with free radicals, because of its antioxidant properties. This ability to reduce the toxic, reactive oxygen species, as well as organic and nitrogen oxy radicals, is extremely important in aerobic cells, since they must defend against the toxicity of the element, on which they depend to produce energy via the respiratory chain (Combs, 2008).

Ascorbic acid also serves as a biochemical redox system, because it can lose its electrons easily and is thus involved in many electron transport reactions and also functions as a co-factor for monooxygenase and hydrolase enzymes. Therefore vitamin C is involved in the following important metabolic functions: synthesis of collagen, tyrosine metabolism, synthesis of noradrenalin and desaturation of fatty acids (Combs, 2008; Naidu, 2003).

The synthesis of collagen proteins is one of the best characterised functions of vitamin C. It acts as a co-factor for the enzymes prolyl 4-hydroxylase, prolyl 3-hydroxylase and lysyl hydroxylase. For this reason, ascorbic acid is important for the hydroxylation of specific prolyl and lysyl residues and is therefore involved in the folding of the triple helical structure and formation of molecular cross-links of collagen.

Another important metabolic role of vitamin C is its involvement in the catecholamine biosynthesis. It is an electron donor for dopamine β-monooxygenase, which forms the neurotransmitter noradrenaline out of dopamine. Furthermore, the biosynthesis of carnitine, which is required to transport fatty acids into the mitochondria to produce energy for the cell, involves vitamin C as a cofactor. Vitamin C is also involved in the tyrosine metabolism, where it is needed for the oxidative degradation of tyrosine.

Other important functions of vitamin C include the enhancement of the bioavailability of iron, enhancement of the immune system, lowering the risk of atherosclerosis, prevention of diabetic complications and reduction or delay of tumour formation for some type of cancers (Combs, 2008).

Scurvy

Scurvy is the best known ascorbic acid deficiency disease (Coultate, 2016). This deficiency manifests when the vitamin C of the total body pool becomes less than 300 mg. Considering normal vitamin C levels of 1,500 mg for the total body, it takes human adults around 45 – 80 days of no vitamin C consumption to contract from scurvy (Combs, 2008).

Due to the defects in the connective tissue formation, symptoms include poor wound healing, bleeding of the gum and internal organs, joints become painful and in advanced stages bones become extremely painful and can fracture. Also changes in the behaviour, in personality and listlessness can be observed, due to reduced production of noradrenaline

(Coultate, 2016). Because of the impaired formation of carnitine, fatigue and lassitude can manifest in patients (Combs, 2008).

With scurvy being a disease caused by malnutrition it is very rare encountered in industrialised countries. Thus it is encountered mostly in malnourished people, which include people with malabsorption, poor and elderly people with an inadequate diet, alcoholics and other drug addicts (Shils & Shike ,2006).

Scurvy in children is called Moeller-Barlow disease and usually manifests in non-breastfed infants at an age of 6 months. This deficiency of vitamin C can result in widening of the bone-cartilage boundaries, especially of the rib cage, severe joint pain, fever and anaemia (Combs, 2008).

Nutrition and stability

A consumption of 10 mg of vitamin C each day is enough to prevent scurvy. Today, many national agencies have set recommendations for daily vitamin C intake and these range from 40 to 95 mg/day. The amount of consumed vitamin C can go up to 500 – 1,000 mg/day or even more, if people suffer from respiratory diseases, cancer or during convalescence. 2,000 mg/day is the maximum intake, which is tolerated by most adults without suffering hypervitaminosis. Symptoms for vitamin C hypervitaminosis are higher excretion of oxalic acid increasing the risk of stones in the urinary tract, possible iron overload, increased uptake of toxic metals and interference with some medications, like antidepressants and aspirin (Velisek, 2014).

Rich sources of vitamin C are green vegetables and fresh fruits, especially citrus fruits and black currants. Also potatoes, which contain only moderate amounts of vitamin C, can be an important source because they can be eaten in large quantities (Ball, 1994). Thus it has to be taken into account that foods that are eaten in large quantities can be an important source of vitamin C, although they contain only modest amounts. In contrast, parsley contains high amounts of vitamin C, but is most of the time only used as a garnish and therefore the overall amount of vitamin C provided by the parsley is nutritionally insignificant (Coultate, 2016). Some examples of vitamin C containing foods are listed in table 1.

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

	Vitamin C content [mg/100 g]	Reference
Rose-hips	1,000	Ball, 1994
Black currants	200	Ball, 1994
Sweet red peppers	140	Ball, 1994
Parsley	150	Ball, 1994
Broccoli	110	Ball, 1994
Oranges	30 – 60	Velisek, 2014
Strawberries	40 – 70	Velisek, 2014
Kiwi	70 - 163	Velisek, 2014
Spinach	35 – 84	Velisek, 2014
Brussel sprouts	100 – 103	Velisek, 2014
Potato	10 – 30	Combs, 2008
Kale	120 – 180	Combs, 2008
Beans	10 – 30	Combs, 2008
Liver	10 – 40	Combs, 2008
Kidney	10 – 40	Combs, 2008

In general the vitamin C content of crops is strongly influenced by many pre-harvest factors, such as climatic conditions and cultural practices (Weston & Barth, 1997). Further important factors that influence the vitamin C content of fruits and vegetables are the maturity at harvest, the harvesting method and the post-harvest handling (Kader, 1988). The degradation of vitamin C can occur rapidly after harvest, depending on the commodity, temperature, pH and other factors. Vitamin C has a better stability in fruits than in vegetables, because of their lower pH (Velisek, 2014).

Significant losses of vitamin C can result from storage of foods, cooking procedures and industrial processes. Leaching and oxidation are the cause for the most significant losses. (Velisek, 2014).

Washing, blanching and cooking of fruits and vegetables lead to losses of vitamin C due to leaching, but the losses during washing are generally lower than during blanching and

cooking. Those losses depend also on the pH, water quantity, temperature, surface area that is in contact with water and presence of metals and oxygen. In general losses are higher in green leafy vegetables than in root vegetables, due to their larger surface area (Velisek, 2014).

Rapid heating of fruits and vegetables or pasteurization of fruit juices prevent vitamin loss during storage, because the ascorbic acid oxidase is completely inactivated at 65 °C and thus unable to oxidise vitamin C (Bender, 1979). To lower the oxidation of ascorbic acid during processing, vacuum deaeration and treatment with inert gas can be carried out. The headspace in cans and bottles should be minimised in order to minimise the present oxygen and maximise the vitamin stability. Glass containers are favourable, because plastic bottles and cardboard cartons are permeable to oxygen, leading to larger losses of vitamin C. Also copper and iron food contact material should be avoided, since ions of those metals are able to oxidise vitamin C (Ball, 1994).

Industrial application

Due to the properties of vitamin C as a vitamin, antioxidant and chelating agent, it is widely used as a food additive. Especially in canned foods and fermentation processes. Vitamin C is added to fruit juices and other fruit products to prevent oxidation during storage or processing. Therefore it can stop the enzymatic browning reactions or undesirable changes in flavour. Citric acid is often used in combination with vitamin C to enhance the stability thereof. Addition of ascorbic acid can also prevent the formation of colloidal turbidity in beer. Together with nitrites it can enhance and speed up the production of meat products and is thus of functional and economic importance. The smoking time of the meat can also be shortened and formation of nitrosamines, which are carcinogenic, can be inhibited. Addition of vitamin C also improves the baking properties of flour and reduces the production time of the bread (Velisek, 2014).

Phenolic compounds

Structure and terminology

Polyphenols are one of the most numerous and ubiquitous secondary plant metabolites and thus an important and integral part of the human diet. They are important for the growth, pigmentation and reproduction of the plant and provide the plants with resistance to pathogens and predators. Furthermore they increase the astringent taste of the food. More than 8000 different phenolic structures are already known (Bravo, 1998).

Phenolic compounds consist of an aromatic ring, which is substituted with one or more hydroxyl groups and they can range from simple phenolic molecules to highly polymerised compounds. They are often conjugated with one or more sugar residues. Those conjugated sugar residues can be mono-, di- and even oligosaccharides. Common are also linkages to other phenols and associations with organic acids. (Bravo, 1998).

Polyphenols can be divided into several classes, due to their different quantity of phenol rings and bound structural elements. The main groups of polyphenols are:

phenolic acids, flavonoids, tannins, stilbenes and lignans (Ignat et al., 2011).

Phenolic acids

Phenolic acids can be divided into two main groups: derivatives of the benzoic acid and of the cinnamic acid. Both acids have an aromatic ring as a back bone, which is linked either to a one carbon structure or to a three carbon structure (Tsao, 2010). The backbone of hydroxybenzoic and hydroxycinnamic acids can be seen in figure 2.

Common examples for hydroxybenzoic acids are gallic acid and protocatechuic acid. Only red fruits and tea contain a high amount of hydroxybenzoic acids, apart from that they are currently not considered to be of great nutritional interest. In general, hydroxycinnamic acids are much more common in edible plants. Caffeic acid is the most abundant one, especially in fruits. 75 – 100% of the total hydroxycinnamic acids content of fruits can be caffeic acid. Ferulic acid is highly present in cereals and grains and may represent up to 90% of total polyphenols (D'Archivio et al., 2007).

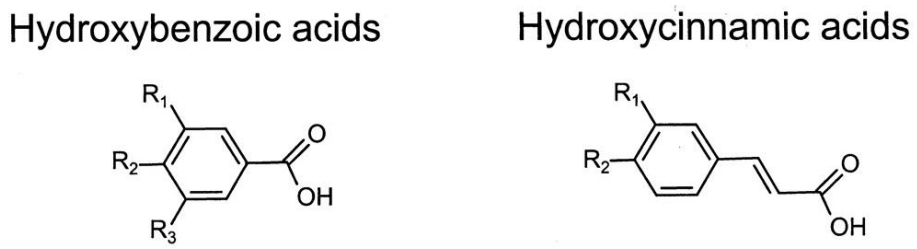


Figure 2: Chemical structure of the hydroxybenzoic and hydroxycinnamic acid backbones (Ignat et al., 2011).

Flavonoids

Flavonoids are structurally based on two aromatic rings linked together by a three carbon chain, which may form a closed pyran ring with one of the aromatic rings. This basic structure can be seen in figure 3. Based on the oxidation state of the central pyran ring, flavonoids can be separated into 6 subclasses: flavonols, flavones, flavanones, isoflavones, anthocyanidins and flavanols. Furthermore, flavonoids represent the largest group of polyphenols with more than 4,000 identified members. This is due to the occurrence of many substitution patterns, in which primary substituents can be further substituted. These substitutions can lead to highly complex structures (D'Archivio et al., 2007).

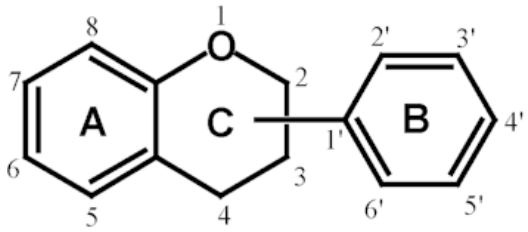


Figure 3: Chemical structure of flavonoid backbone (Tsao, 2010).

Tannins

Tannins are the third important group of polyphenols and are high molecular compounds, which can be divided into condensed and hydrolysable tannins (Ignat et al., 2011).

Condensed tannins are also called proanthocyanidins and are a group of polyhydroxy-flavan-3-ol oligomers and polymers, which are linked to other flavanol subunits by carbon-carbon bonds (Schofield et al., 2001). These condensed tannins are much more common in fruits

than the hydrolysable ones. They are distributed throughout the flesh of fruits, but are usually primarily located in the skin or the peel (Shahidi & Naczk, 1995). The model structure of a proanthocyanidin can be seen in figure 4.

Hydrolysable tannins are formed when many gallic acid molecules are esterified to a core polyol. The galloyl groups may be further esterified or oxidatively crosslinked in order to yield more complex hydrolysable tannins (Ignat et al., 2011). Based on the phenolic compounds yielded upon hydrolysis of the hydrolysable tannins, they can be further divided into gallo- and ellagitannins. Ellagitannins consist of many esterified hexahydroxydiphenic acid molecules instead of gallic acid molecules (Shahidi & Naczk, 1995).

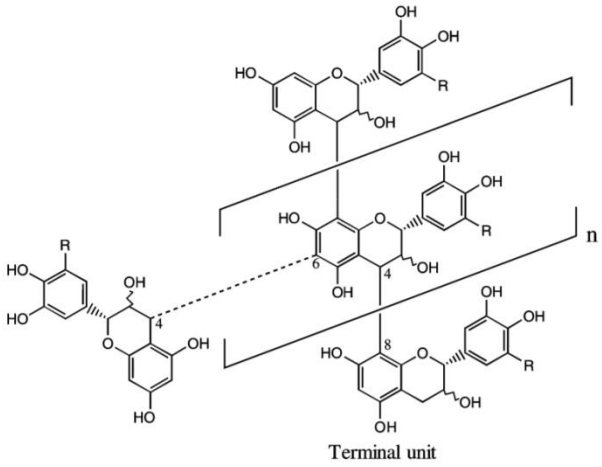


Figure 4: Model structure of a proanthocyanidin. The dotted line represents an alternative interflavan bond. The terminal unit is at the bottom of the multi-unit structure (Schofield et al., 2001).

Stilbenes

Over 30 stilbenes and stilbene glycosides occur in nature. The structure is based on two aromatic rings, which are connected by a two carbon unit. The number and position of hydroxyl groups can differ and they can also be substituted with sugars, methyl, methoxy and other residues (Soleas et al., 1997). The most common stilbene is resveratrol and exists in *Z* and *E* form. The chemical structure of resveratrol can be seen in figure 5. Overall, stilbenes are only present in low quantities in the human diet (Ignat et al., 2011).

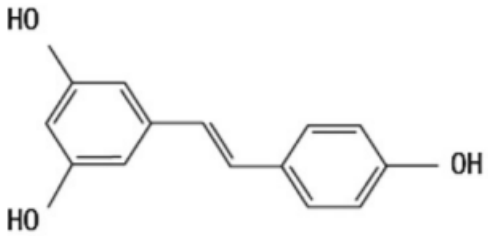


Figure 5: Chemical structure of *E*-resveratrol (Ignat et al., 2011).

Lignans

Lignans are formed of two phenylpropane units, which are linked together by oxidative dimerization. The major form of lignans in nature is the free form, whereas their glycosides are only a minor form. The main dietary source for lignans is flaxseeds (D'Archivio et al., 2007). The chemical structure of a lignan, secoisolariciresinol, can be seen in figure 6.

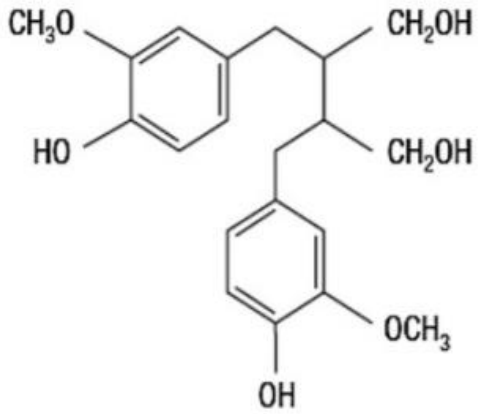


Figure 6: Chemical structure of secoisolariciresinol (Ignat et al., 2011).

Biosynthesis

Phenylalanine is the most common precursor of most phenolic compounds. The enzymes phenylalanine ammonialyase, cinnamate 4-hydroxylase and hydroxycinnamate CoA ligase are important enzymes which transform phenylalanine to p-coumaric acid. These three steps are necessary for the biosynthesis of all phenolic compounds (Macheix & Fleuriet, 1990).

Hydroxycinnamic acids (HCA), like caffeic and ferulic acid, are formed from p-coumaric acid through hydroxylation and methylation reactions. Other derivates like chlorogenic acid are

formed by transesterification of HCA-CoA esters with hydroxyacids (Macheix & Fleuriet, 1990).

Benzoic acids are either derived directly from the shikimate pathway, which is the main route for gallic acid, or they can be produced by the degradation of cinnamic acids. The latter works similar as the β-oxidation of fatty acids (Macheix & Fleuriet, 1990).

Acetate and phenylalanine are the fundamental building blocks of the carbon skeleton for all flavonoids. At first, acetyl-CoA-carboxylase executes the conversion of acetyl-CoA into malonyl-CoA. This first step is followed by the formation of the 15 carbon skeleton, which is formed from a molecule 4-coumaryl-CoA and three molecules malonyl-CoA by the chalcone synthase. This formed intermediate is termed naringenin chalcone and is further converted into flavanone by a chalcone flavanone isomerase. This flavanone is the building block for all different flavonoids, which result from hydroxylation, oxidation, methylation and glycosylation reactions of the different rings (Macheix & Fleuriet, 1990).

In contrast, the precursors for all stilbenes are synthesised from a molecule p-coumaryl-CoA or cinnamoyl-CoA and three molecules malonyl-CoA by the stilbene synthase. (Jeandet et al., 2010).

The biosynthesis of proanthocyanidins branches off from the flavonoid pathway and the resulting catechin and epicatechin derivatives are oxidized to quinones and further polymerised. Nevertheless, it is still unknown if this polymerisation occurs enzymatically or non-enzymatically (Vogt, 2010).

The biosynthesis of lignans involves the oxidative polymerisation of p-coumaryl, coniferyl and sinapyl alcohols by peroxidases. The final stage of lignan biosynthesis is the polymerisation and accumulation in the cell wall (Macheix & Fleuriet, 1990).

Metabolism and health benefits

Many factors, such as the degree of glycosylation or acylation, basic structure, size of the molecule, solubility and degree of polymerization influence the absorption and metabolism of polyphenols. Non-extractable polyphenols, such as condensed tannins, can be recovered in faeces to a large degree, verifying their resistance to digestion and absorption (Bravo, 1998). However, simple phenolic acids and non-glycosylated phenolic compounds can be absorbed via the small intestinal mucosa (King et al., 1996). The glycosylated phenolic

compounds are passed into the large intestine and are hydrolysed by the caecal microflora. These aglycones are absorbed through the epithelial cells of the gut. After absorption they are methylated and/or conjugated with glucuronic acid or sulphate in the liver, which is the main organ involved in the metabolism. Also other organs, such as the kidneys and intestinal mucosa, may be involved in the metabolism of phenolic compounds. Conjugated or methylated derivatives are either secreted in the urine or in the bile, where the polyphenols can enter an enterohepatic cycle. This means that they are deconjugated or metabolised to simple phenolic acids by the colon microflora and are afterwards reabsorbed. To conclude, evidence of the absorption and metabolism of polyphenols in the gut exists, but not much is known about the efficiency of the absorption and the permanence of the polyphenols (Bravo, 1998).

Polyphenols are considered to be strong antioxidants, because they can donate an electron or hydrogen atom and thus neutralise free radicals. Hence polyphenols reduce the rate of oxidation by inhibiting the generation of free radicals. Furthermore polyphenols are also metal chelators and thus prevent the oxidation caused by hydroxyl radicals. They are also able to stop the lipid peroxidation chain reactions by scavenging of the radicals. Many chronic diseases including cancer, cardiovascular disease, chronic inflammation and many degenerative diseases are linked to oxidative stress, caused by reactive oxygen and nitrogen species. Therefore polyphenols can be related to lower the risk of these diseases (Tsao, 2010).

In addition they can act as synergistic antioxidants by reducing α-tocopheroxyl radical to regenerate α-tocopherol, an essential vitamin and antioxidant (Zhou et al., 2005).

Furthermore, polyphenols are also able to increase the endogenous antioxidant enzymes in cardiomyocytes and they also inhibit the expression of xanthine oxidase. These properties led to a protection against oxidative cell injury and cardiac cell apoptosis (Du et al., 2007).

Sources and stability

Polyphenols are present in a wide range of foods from plant origin. Major sources of polyphenols in the human diet are fruits, vegetables and beverages, such as red wine and green tea. The TPC of different fruits and vegetables can differ greatly. Even the TPC of fruits or vegetables from the same cultivar can have a wide variation. Reasons for these

differences may be the complexity of these compounds and the different methods for analysis (Balasundram et al., 2006). The TPC content of various foods can be seen in table 2.

Table 2: Total phenolic content of some selected fruits and vegetables. Values are given in mg gallic acid equivalent/100 g edible fresh fruit.

	TPC [mg/100 g]	Reference
Apple	294	Sun et al., 2002
Cranberry	527	Sun et al., 2002
Red Grape	201	Sun et al., 2002
Strawberry	160	Sun et al., 2002
Blackberry	417 – 555	Sellappan et al., 2002
Blueberry	270 – 930	Sellappan et al., 2002
Broccoli	102	Chu et al., 2002
Cabbage	55	Chu et al., 2002
Carrot	56	Chu et al., 2002
Spinach	91	Chu et al., 2002

The polyphenol content is influenced by many factors, such as the environment, germination, ripeness, processing and storage. Light is also a very important factor, because it influences the formation of flavone and flavonol glycosides greatly. Leaves in the outer part of the plant have much higher concentrations than those in the subterranean parts (Bravo, 1998).

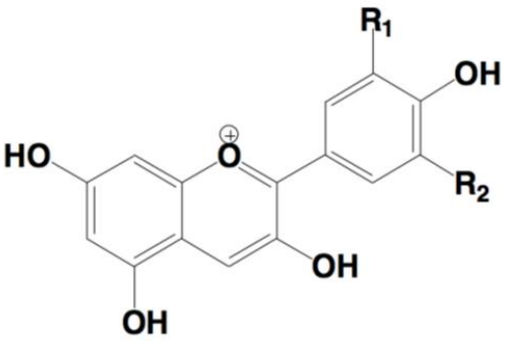
The enzyme polyphenoloxidase is the main reason for the spoilage of food. This enzyme causes an enzymatic discolouration reaction by oxidising polyphenols to highly active quinones, which can polymerise and thus contribute to the browning of foods. These quinones may also react with proteins and anthocyanins leading to changes in the physical, chemical and nutritional characteristics. However, these oxidative changes are important for the sensory properties of black tea. There are also non-enzymatic discolouration reactions, which lead to a bluish-grey colour. The presence of metal ions and alkaline conditions enhance these non-enzymatic discolouration reactions. Polyphenoloxidases can be inactivated by heat, whereas 90 °C are necessary to totally inactivate the enzymes. Another method is the lowering of the pH, because most polyphenoloxidases have an optimum pH between 4 and 7 (Shahidi & Naczk, 1995).

Anthocyanins

Structure and terminology

Anthocyanins are pigments that cause the red, violet and blue colour of flowers, fruits and some vegetables. Anthocyanins are glycosylated forms of anthocyanidins, the aglycone component. The anthocyanidins themselves are flavonoids and thus are assigned to the polyphenols. The basic structure of anthocyanidins is called the flavylium cation and can be seen in figure 7 (Coultate, 2016). All of the different anthocyanidins are substituted with hydroxyl groups at various positions and even methoxyl groups may occur.

Anthocyanins are always glycosylated in position C-3 with one of the five monosaccharides: D-glucose, L-rhamnose, D-galactose, D-xylose and L-arabinose. Also a glycosylation with glucose at position C-5 is possible. Further and exceptional cases of glycosylation with mono-, di-, and trisaccharides can occur at positions C-7, C-3', C-5' and C-4'. Those bound sugars are also often acylated with phenolic acids, such as p-coumar-, caffeic-, ferulic- and sinapic acid (Velisek, 2014).



Anthocyanidin	R^1	R^2
pelargonidin	-H	-H
cyanidin	-OH	-H
peonidin	$-OCH_3$	-H
delphinidin	-OH	-OH
petunidin	$-OCH_3$	-OH
malvidin	$-OCH_3$	$-OCH_3$

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

17 different anthocyanidins exist in nature, whereof 6 are the most occurring ones in food. These six important anthocyanidins are: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. 300 different anthocyanins have already been identified in nature (Velisek, 2014).

Colouring

The colour of the anthocyanins depends strongly on the pH. Under very acidic conditions, which means a pH of 1.0 and lower, anthocyanins exist solely as red coloured flavylium salts. The red colour starts to fade when the pH increases, because the colourless carbinol pseudo base is formed. Anthocyanins are completely colourless at a pH of 4.0 to 4.5. Further increase of the pH leads to the formation of a neutral quinoid base with a purplish-red colour. A blue coloured quinoid base is formed at a pH of 7 and after another increase in the pH, a formation of yellow chalcones occur. Those blue quinoid and colourless carbinol bases can be transformed back to the red flavylium cation by decreasing the pH to 1.0 (Velisek, 2014).

The colour of the anthocyanins is also affected by the type and number of substituents of the anthocyanidin. A higher amount of hydroxyl groups lead to a blue colour, whereas a higher amount of methoxyl groups lead to a red colour (Velisek, 2014).

Anthocyanins can interact with other colourless flavonoids and this interaction leads to a better stabilisation of the blue quinoidal form and to an enhancement of the colour intensity. In general the pH and the interaction with other substances have a much greater influence on the colour than the substituents of the anthocyanidins (Coultate, 2016).

Biochemistry and health benefits

In general, not much research has been done to explore the biological properties of anthocyanins (Velisek, 2014).

Studies confirm that the gastrointestinal tract of rats is able to rapidly absorb anthocyanins. It seems that the small intestine tissue is much more capable of absorbing anthocyanins than the reported bioavailability based on plasma and urine concentrations (He et al., 2009). This indicates that anthocyanins are indeed absorbed efficiently but are not transported by circulation, but rather transported across the apical membrane (Steinert et al., 2008).

Endothelial dysfunction has an important role in the development of cardiovascular diseases. Anthocyanins improve the function of endothelial cells by protecting them against oxidative stressors (Youdim et al., 2000).

Freeze-dried extracts of strawberries and black raspberries displayed some anticancer properties. Those extracts were able to inhibit the transformation of cells by unknown

mechanisms, which may involve the detoxification or the DNA binding of the carcinogen and the repair of the DNA (Xue et al., 2001).

A high consumption of anthocyanins leads to significantly lower insulin resistance. Therefore anthocyanins may reduce the type 2 diabetes risk (Jennings et al., 2013).

Sources and stability

Anthocyanins are located in the call vacuoles of the plants and the main sources are foods of the grapevine family Vitaceae and of the rose family Rosaceae. Many fruits belong to those plant families, including grapes, cherries, strawberries, blackberries, apples and plums. There are also a lot of anthocyanin containing fruits, which do not belong to those two plant families. Examples are black- and redcurrants, blueberries, cranberries and olives.

The number of present anthocyanins can differ between various fruits and vegetables. Strawberries and blackberries contain only a few different anthocyanins, whereas red grapes and blueberries can have more different anthocyanin pigments (Velisek, 2014).

The total anthocyanin content can vary a lot between individual fruits and different cultivars of the same fruit. Light, temperature, agronomic and composition factors can influence the anthocyanin content to a large degree. Low temperatures promote the formation of anthocyanins in apples, whereas warm weather conditions promote the formation of anthocyanins in blackberries. The intensity and quality of light is the predominant factor for the anthocyanin content. Olives ripened in the dark contain ten times less anthocyanins than those ripened under normal conditions (Shahidi & Naczk, 1995).

The total anthocyanin content of some foods can be seen in table 3.

Table 3: Anthocyanin content of some selected fruits and vegetables. Values are given as mg anthocyanins/100 g edible fresh fruit.

	Anthocyanin content [mg/100 g]	Reference
Cranberry	45 – 100	Shahidi & Naczk, 1995
Cherry sour	45	Shahidi & Naczk, 1995
Grape muscadine	40 – 403	Shahidi & Naczk, 1995
Strawberry	45 – 75	Shahidi & Naczk, 1995
Apple	10 – 20	Velisek, 2014
Black olives	500	Velisek, 2014
Purple potato	15 – 50	Velisek, 2014

The stability of anthocyanins is usually low, as their stability is affected by certain enzymes, pH, temperature, radiation and presence of oxygen. In contrast to other molecules, anthocyanins show increased stability under high temperatures. One reason for this phenomenon can be the condensation of monomers that leads to formation of more stable oligomers. Anthocyanins can be oxidised to colourless or brown products by atmospheric oxygen or through other compounds, which are preferably oxidised like ascorbic acid. Enzymes, like glucosidases and polyphenol oxidases, may cause the loss of anthocyanins. Glucosidases can hydrolyse the glycosidic bond, which leads to a formation of a less stable anthocyanidin. Polyphenol oxidases are involved in the enzymatic browning reaction of fruits and vegetables. Also the type of bound sugar and presence of acyl groups can influence the stability (Velisek, 2014).

Anthocyanins can also form complexes with metal ions, which can lead to unusual colouring of canned fruits. For example the pink colour of canned pears is due to the formation of iron or tin complexes. Sulphur dioxide causes irreversible bleaching of anthocyanins at high concentrations, whereas at low concentrations colourless compounds are formed (Coultate, 2016).

Industrial applications

Anthocyanins already have been used for more than 100 years as food colourings. Due to the growing interest in natural substances, they are now used for a variety of foods as the red colour of choice (Velisek, 2014; Coultate, 2016).

There are limitations in the potential sources, caused by the availability of the plant materials and the economic conditions of their production. Most anthocyanins are derived from black grapes. Further rich sources of anthocyanins for production are elderberries, blackberries, chokeberries, black carrots and also red cabbage (Velisek, 2014).

Nonetheless anthocyanins as food colourings have a disadvantage as the highest intensity of the colour is in acidic solutions with a pH < 3.5 (Velisek, 2014).

Tea Catechins

Structure and terminology

Flavanols or flavan-3-ols are a subgroup of the flavonoids and are often called catechins. In contrast to other flavonoids, 4 possible diastereomers exist due to the structure of the flavan-3-ol backbone. The trans-configuration is called catechin, whereas the cis-configuration is called epicatechin. Both of those can have two isomers (figure 8). Proanthocyanidins, also called condensed tannins, are polymers formed from catechin monomers (Tsao, 2010). Each of the 4 isomers can be esterified with gallic acid to yield the corresponding gallate. An example, epigallocatechin-gallate (EGCG), can be seen in figure 8.

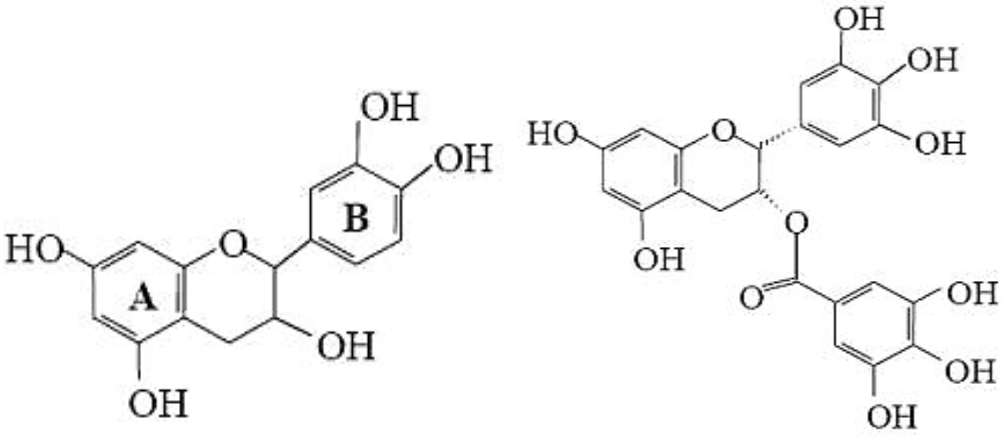


Figure 8: Chemical structure of the flavan-3-ol backbone on the left side and an example, EGCG, on the right side (Singh et al., 2011).

Tea

Tea is one of the most popular beverages in the world. Tea is made from leaves of the plant *Camellia sinensis*. Black and green tea, the two most commercial tea products, are produced by different methods of manufacture. Green tea is mostly consumed in the Far East, whereas fermented black tea is more popular in other countries. Both teas have differences in the chemical composition due to the different methods of manufacture. The polyphenols of green tea remain relatively intact during the production, because the polyphenol oxidase is inactivated during the process. This is usually achieved by heat treatments of the tea

leaves, such as pan-firing or steaming. In contrast, black tea production uses this enzymatic oxidation of the catechins to form theaflavins and thearubigins, which are polymerised flavonoids and important for the colour and taste of the black tea (Astill et al., 2001). The main occurring polyphenols in tea are flavanols, which can constitute up to 35% of the dry weight. Their composition in tea may vary due to the variety of the tea, geographical origin, environmental and agronomic conditions. Epigallocatechin-gallate (EGCG) is the major catechin found in green tea (Shahidi & Naczk, 1995).

Biochemistry and health benefits

Tea polyphenols are indeed bioavailable, as high concentrations of the catechins were found in human plasma after green tea consumption. Furthermore, epicatechin and epigallocatechin were discovered in urine after green tea consumption. There are further indications that catechins are also absorbed through the oral mucosa (Yang & Landau, 2000).
The most important property of tea polyphenols is the antioxidant activity. They are able to scavenge reactive oxygen and nitrogen species. Thus they are able to reduce the damage to lipid membranes, proteins and nucleic acids *in vitro*. They can also bind metal ions and block them from catalysing peroxidative reactions (Yang & Landau, 2000). Therefore, tea consumption may reduce the risks of cardiovascular disease, some forms of cancer and promotion of other physiological functions. (McKay & Blumberg, 2002).
Tea catechins seem to have many beneficial effects on the vascular function, such as the inhibition of oxidation, vascular inflammation, atherogenesis and thrombogenesis. Catechines can also directly act on immune und vascular cells in various ways in order to regulate their functions (Babu et al., 2008).
Most of the cancer chemopreventive properties of green tea are mediated by EGCG. EGCG can target specific cell signalling pathways and thus has the ability to regulate cellular proliferation and apoptosis. This activation of cell death signals and induction of apoptosis have been shown to be selective for cancer cells (Singh et al., 2011).

Other sources and stability

Catechins are found in many types of fruits, although red wine, chocolate and green tea are by far the richest sources. The main flavanols of most fruits are catechin and epicatechin, whereas gallocatechin, epigallocatechin, and epigallocatechin gallate are found in grapes and in seeds of leguminous plants and especially in tea. Unlike other flavonoids, flavanols are not glycosylated in foods (Manach et al., 2004). The flavanol content of some fruits can be seen in table 4. The content of flavanols is influenced by the same conditions, which are discussed in the stability of the polyphenols. Furthermore the flavanol content can have a wide variation between cultivars (Macheix & Fleuriet, 1990).

Table 4: Flavanol content of some selected fruits. Values are given as mg catechins/100 g edible fresh fruit.

	Flavanol content [mg/100 g]	Reference
Apricot	10 – 25	Manach et al., 2004
Cherry	5 – 22	Manach et al., 2004
Grape	3 – 17.5	Manach et al., 2004
Peach	5 – 14	Manach et al., 2004
Blackberry	12.6	Macheix & Fleuriet, 1990
Apple	7.9	Macheix & Fleuriet, 1990
Plum	6.1	Macheix & Fleuriet, 1990
Strawberry	4.2	Macheix & Fleuriet, 1990

Catechins are together with chlorogenic acid the natural substrates for the polyphenol oxidases and thus heavily involved in the browning reactions of foods (Macheix & Fleuriet, 1990). These browning reactions are already discussed in the stability of the polyphenols.

Materials and Methods

Materials

Instruments

All instruments which were used for sample preparation and sample analysis are listed in table 5.

Table 5: List of used instruments and their manufacturing company.

Instrument	Manufacturer
Precision Balance, Entris 2201I-1S	Sartorius Lab Instruments GmbH & Co, Göttingen, Germany
Analytical Balance, AG245	Mettler Toledo GmbH, Vienna, Austria
High Performance Liquid Chromatograph, Series 1100	Hewlett Packard, California, USA
Vortex-Genie 2	Scientific Industries, Inc., New York USA
pH 3110	Carl Roth GmbH & Co., Karlsruhe, Germany
Spectrophotometer U-1100	Hitachi Ltd. Corporation, Tokyo, Japan
Centrifuge 5804R	Eppendorf AG, Hamburg, Germany
Centrifuge Hermle Z323	Hermle Labortechnik GmbH, Wehingen, Germany

Chemicals

All used analytical standards are listed in table 6. Chemicals used for sample preparation and analysis are listed in table 7.

Table 6: List of used analytical standards and their manufacturing company.

Analytical Standards	Manufacturer
L-ascorbic acid	Sigma Aldrich, St. Louis, USA
Gallic acid	Sigma Aldrich, St. Louis, USA
Caffeine	Sigma Aldrich, St. Louis, USA
Epicatechin	Sigma Aldrich, St. Louis, USA
Epigallocatechin-gallate	Sigma Aldrich, St. Louis, USA
Catechin	Sigma Aldrich, St. Louis, USA

Table 7: List of used chemicals and their manufacturing company.

Chemicals	Manufacturer
0.1 M Hydrochloric acid	Carl Roth GmbH & Co., Karlsruhe, Germany
Acetonitrile	ChemLab analytical, Zedelgem, Belgium
Formic acid	Merck KGaA, Darmstadt, Germany
Folin & Ciocalteu phenol reagent	Sigma Aldrich, St. Louis, USA
Methanol	ChemLab analytical, Zedelgem, Belgium
meta-Phosphoric acid	Sigma Aldrich, St. Louis, USA
di-Sodiumhydrogenphosphate	Merck KGaA, Darmstadt, Germany
Sodiumdihydrogenphosphate	Merck KGaA, Darmstadt, Germany
Sodiumcarbonat	Merck KGaA, Darmstadt, Germany
Tris(2-carboxyethyl)phosphin (TCEP)	Sigma Aldrich, St. Louis, USA

Raw materials

All fruits and vegetables, which were used for the production of the self-made juices, were purchased at local supermarkets. The used fruits and vegetables, their variety and their origin are listed in table 8.

Table 8: List of the fruits and vegetables used for the juice production.

Fruit/Vegetable	Variety	Origin
Strawberries	Da komm' ich her! – no further information	Austria
Oranges	Lane Late	South Africa
Red Grapes	Crimson	Italy
Red Peppers	"Spar Paprika" – no further information	Austria
Matcha	"Fuku" Matcha	Japan
Tomatoes	"Rispentomaten" – no further information	Austria
Apples	Golden Delicious	Austria
Kiwis	Hayward	Italy
Blueberries	"Powerblueberry" – no further information	Austria

Methods

High Performance Liquid Chromatography (HPLC)

HPLC is an analytical method used to separate, identify and quantify individual substances of a mixture. A pressurized liquid is passed through a separation column and separation is achieved by partitioning of the sample substances between a mobile and a stationary phase. For normal-phase HPLC measurements a nonpolar mobile and a polar stationary phase are used, whereas for reversed-phase HPLC measurements a polar mobile and a nonpolar stationary phase are used. Due to different interactions of the analyte with the mobile and stationary phase, the analyte is retained in different degrees, which results in specific retention times for the individual substances. The composition of the mobile-, stationary phase and the temperature play a major role in the separation process. After the separation, the substances can be detected by using various different detectors, such as UV/VIS-, fluorescence-, refraction index- or photodiode array detectors.

HPLC was used to determine the total ascorbic acid content, anthocyanin content and the catechin content of the juice samples.

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

Folin-Ciocalteu Assay

The Folin-Ciocalteu assay is a simple method to measure the total phenolic content (TPC) of different foods. It is a development of the Folin-Denis assay, which was used for determination of tyrosine in proteins (Folin & Ciocalteu, 1927). The Folin-Ciocalteu assay has been used for many years now to determine the polyphenol content of foods and food products and is based on a reduction-oxidation mechanism. Folin-Ciocalteu reagent consists of heteropoly-, phosphomolybdic- and phosphotungstic acids. Phenolic hydroxyl groups of polyphenols reduce the phosphomolybdic- and phosphotungstic acids to molybdenum blue and tungsten blue (Agbor et al., 2014). This Folin-Ciocalteu assay was further improved with a molybdotungstophosphoric heteropolyanion reagent that reduces polyphenols more specifically, resulting in a blue coloured product with an absorption maximum of 765 nm

(Singleton & Rossi, 1965). By using the Folin-Ciocalteu assay, it is possible to detect a wide range of polyphenols, for example: flavonols, flavones, anthocyanins and phenolic acids (Agbor et al., 2014). However, the Folin-Ciocalteu assay has some limitations caused by interfering compounds that may be present in high abundance in various foods, especially in fruits and vegetables. Ascorbic acid, dehydroascorbic acid and reducing sugars have the highest impact on lowering the accuracy of the assay (Rangel et al., 2013).

Philips Juicers

Philips Masticating Juicer + Antifoaming Device

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

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

Functional principle: The P. Avance Masticating Juicer consists 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. The raw juice from the masticator is transferred into a spinning drum of the antifoaming device, in which the air-water mixture spins at an acceleration of around 900 m/s^2. Due to the centrifugal force, the heavier, foam free, liquid phase separates in a matter of about 30 seconds from the lighter, foamy, liquid phase. The foam free liquid phase exits the drum over a Blocking Wall "Wehr" after a certain liquid level is reached, while the foam is kept inside.

Table 9: Abbreviations and descriptions of the construction scheme 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

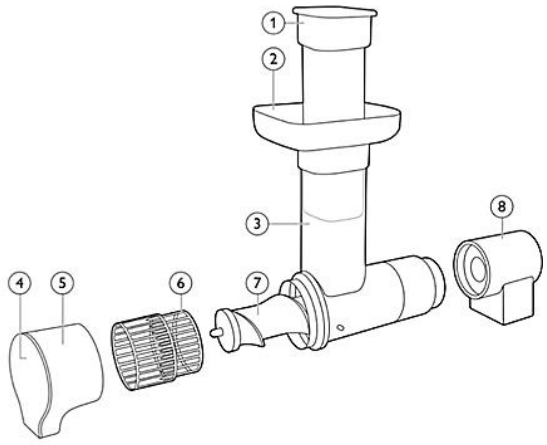


Figure 9: Construction scheme of the Philips Masticating Juicer (Philips instruction manual, 2015).

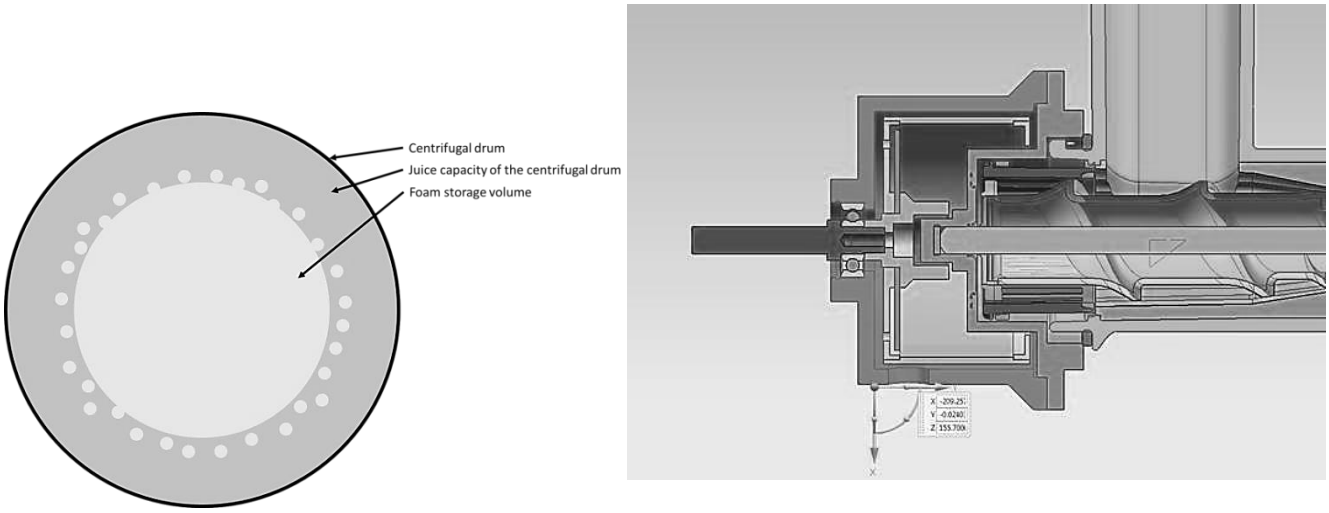


Figure 10: Functional principle of the antifoaming device on the left side, construction scheme of the antifoaming device in conjunction with the masticating juicer on the right side (Philips Foamless-Masticator Prototype-description, 2017).

Vidia Vacuum Blender

Complete description: Vidia Vacuum Blender BL-001R/BL-001S, "Ozen"

Technical data: not known

Functional principle: The Vidia Vacuum Blender consists of a glass jar with six knifes (hexablade) at the bottom, which cut and smash the fruits and vegetables at high speed. There is no separation of solid particles from the juice. The integrated vacuum pump can be used to apply a vacuum of -0.7 mbar prior to the blending of the fruits and vegetables. This blender was also used without vacuum, which is referred to as "Blender Reference".

Atmosphere exchange: The lid of this blender was modified with a valve for the atmosphere exchange. Therefore it was possible after degassing to fill the glass jar with the desired gases (CO_2 or N_2O). In order to completely exchange the atmosphere of the glass jar, it was necessary to redo the atmosphere exchange twice before blending. Otherwise there would still be 0.3 mbar of air in the glass jar.

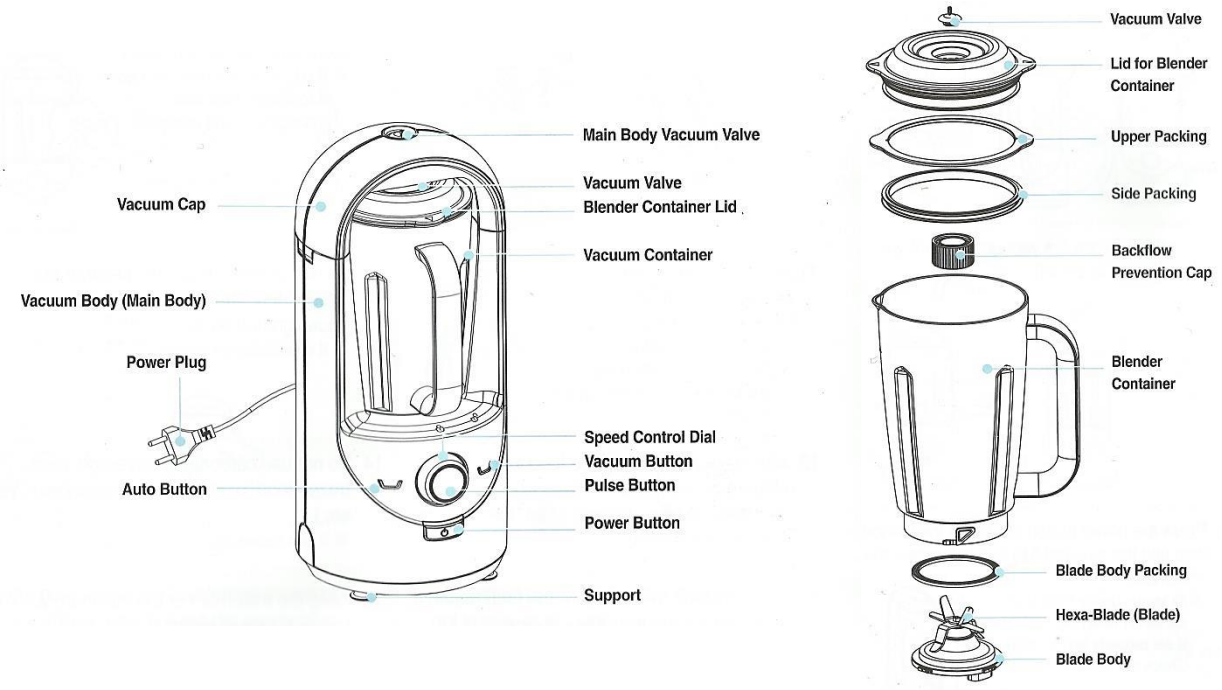


Figure 11: Construction scheme of the Vidia Vacuum Blender (Vidia user manual, 2016).

Sample preparation

In advance to the juicing procedure and the weighting in, the fruits and vegetables from the local supermarkets were washed with water, the leaves, peduncles and non-edible peels were removed and finally they were cut into small pieces. The apples and matcha were mixed with water, so that it was possible to get a juice with a nice texture. It was not possible to use the masticator with the antifoaming device for the matcha. Therefore the matcha was mixed with water manually, which is referred to as “Blank” in the results. The masticator with the antifoaming device was always used without water addition. The amount of fruits or vegetables and added water can be seen in the table 10.

Table 10: List of the amount of fruits and additional water used for the juicing procedure.

Fruit/Vegetable	Quantity
Strawberries	400 g
Oranges	400 g
Kiwis	400 g
Apples + water	250 g + 250 ml
Red Grapes	400 g
Matcha + water	3 g + 300 ml
Blueberries	400 g
Red Peppers	400 g

The juices were stored in capped glass bottles for 24 hours at 3 °C in the fridge. Sampling was done after 0 and 24 hours of storage. To gain a representative mean value the measurements were carried out in triplicates. The table 11 lists the analysed nutrients for the several fruits and vegetables.

Table 11: List of the investigated nutrients of the individual fruits and vegetables.

Nutrients	Fruit/Vegetable
Total ascorbic acid content	Strawberries Oranges Kiwis Blueberries Red Peppers
TPC	Apples Blueberries Matcha
Anthocyanin content	Red Grapes
Catechin content	Matcha

Analysis of the total ascorbic acid content

Sample preparation

The juice samples were diluted with 1 % meta-phosphoric acid in order to stabilize the ascorbic acid. Different dilutions were chosen for the various fruits and vegetables, so that the ascorbic acid concentration is in a nice detection range. The dilutions for the various juices are listed in table 12.

Table 12: Dilutions used for the analysis of the individual juices.

Juice	Dilution
Strawberry juice	1:10
Orange juice	1:10
Kiwi juice	1:20
Blueberry juice	1:2
Red Pepper juice	1:20

After the dilution step the samples were mixed and kept in the freezer at -20 °C until the analysis. To be able to analyse the total ascorbic acid content, the samples were centrifuged for 10 minutes at 14,000 rpm and the present dehydroascorbic acid was reduced to ascorbic acid with a reducing agent. Therefore the reducing agent TCEP, dissolved in 1 % meta-phosphoric acid, was added to the supernatant. The final concentration of TCEP in the sample was 5 mM and the incubation time 45 minutes.

HPLC measurement

The table 13 depicts the parameters of the HPLC measurement. One of the standard chromatograms can be observed in figure 12. The retention time of the L-ascorbic acid standard was 1.71 minutes.

Table 13: HPLC parameters for the analysis of the total ascorbic acid content.

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

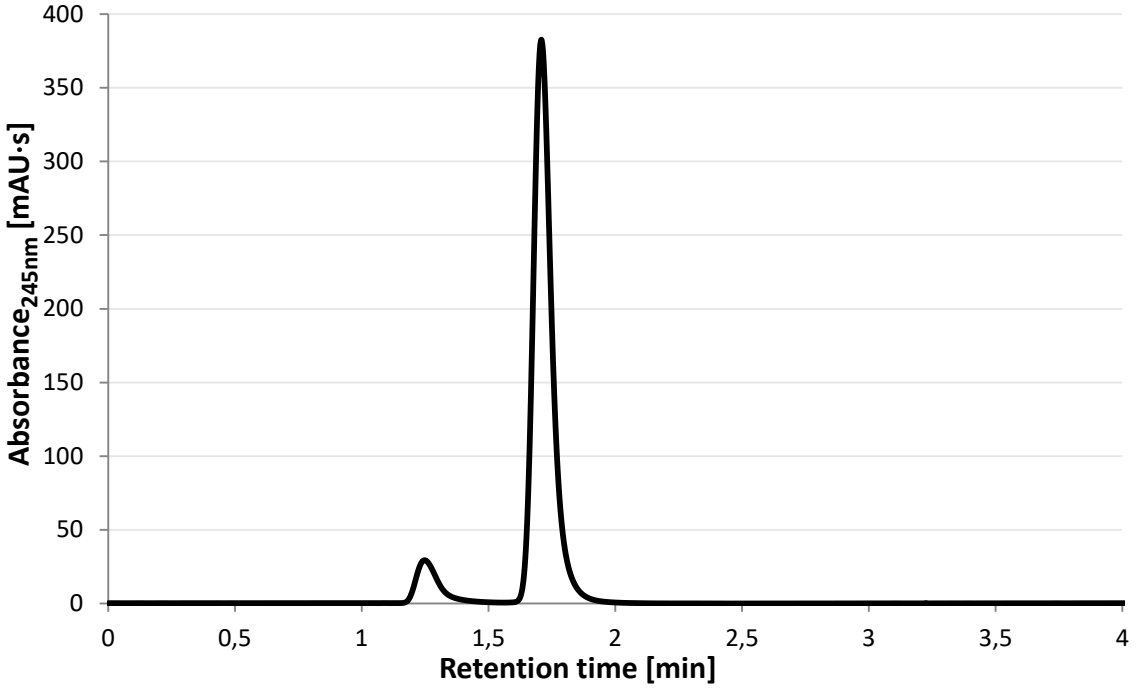


Figure 12: Chromatogram of a L-ascorbic acid standard with a concentration of 65 µg/ml. The absorption was measured at 245 nm. The first peak is from the meta-phosphoric acid, the second peak from the L-ascorbic acid**.**

Analysis of the anthocyanin content

Sample preparation

In order to stabilize the present anthocyanins of the juice samples, these were diluted 1:2 with 0.1 M HCl. The samples were stored in the freezer at -20 °C until the analysis. Centrifugation of the samples for 10 minutes at 14,000 rpm was done and the supernatants were used for the HPLC measurement.

HPLC measurement

The table 14 depicts the parameters of the HPLC measurement. No analytical standards were available, thus the results only show the detected peak areas. The retention times of the peaks were compared with the results from Brar et al., 2008.

Table 14: HPLC parameters for the analysis of the anthocyanin content.

Column	Agilent LiChrospher® 100 RP-18e (5µm)		
Column temperature	30 °C		
Detection	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		

Analysis of the total phenolic content

The measurement of the total phenolic content was based on the procedure by Singleton and co-workers (1999). Therefore, the juice samples were centrifuged for 10 minutes at 14,000 rpm. 5 µl of the supernatant were mixed with 600 µl deionized water in 1.5 ml plastic cuvettes. Then 50 µl of Folin & Ciocalteu´s phenol reagent were added, incubated for 1 minute, followed by an addition of 150 µl of 20 % Na_2CO_3. After 8 minutes of incubation time another 150 µl of 20 % Na_2CO_3 were added and finally the samples were incubated for 2 hours in the dark until the absorption was measured with a spectrophotometer at a wavelength of 765 nm. For the blank, 5 µl of deionized water were used. The blueberry samples were diluted 1:2 with deionized water, so that the adsorption was in calibration range.

Gallic acid was used as a standard substance, thus the results of the TPC measurements were expressed as gallic acid equivalent in micrograms/millilitre.

A spectrophotometer U-1100 was used for the measurements and data evaluation.

Figure 13: Calibration for the Folin & Ciocalteu assay. Gallic acid was used as standard substance.

Analysis of the catechins

Sample preparation

The measurement of the catechins was based on the procedure by Friedman and co-workers (2006). Samples of the matcha juice were stored in the freezer at -20 °C until the analysis. The samples were centrifuged for 10 minutes at 14,000 rpm and the supernatants were used for the HPLC measurement.

HPLC measurement

The table 15 depicts the parameters of the HPLC measurement. A chromatogram for the standards can be observed in figure 14.

The retention times of the standards were:

Caffeine: 5.07 min

Catechin: 5.56 min

Epicatechin: 8.00 min

EGCG: 9.11 min

Table 15: HPLC parameters for the analysis of the catechin content.

Column	pre-column: Phenomenex® AJ0-9297 EVO C18 Phenomenex, Kinetex, 5 µm EVO C18 100 Å		
Column temperature	23 °C		
Detection	UV/VIS detector, 280 nm		
Flow rate	0.6 ml/min		
Mobile phase	A: Acetonitrile B: 20 mM KH_2PO_4		
Gradient	time [min]	A %	B %
	2	7	93
	60	25	75
Injection volume	1.5 µl		
Stop time	12 min		
Post time	4 min		

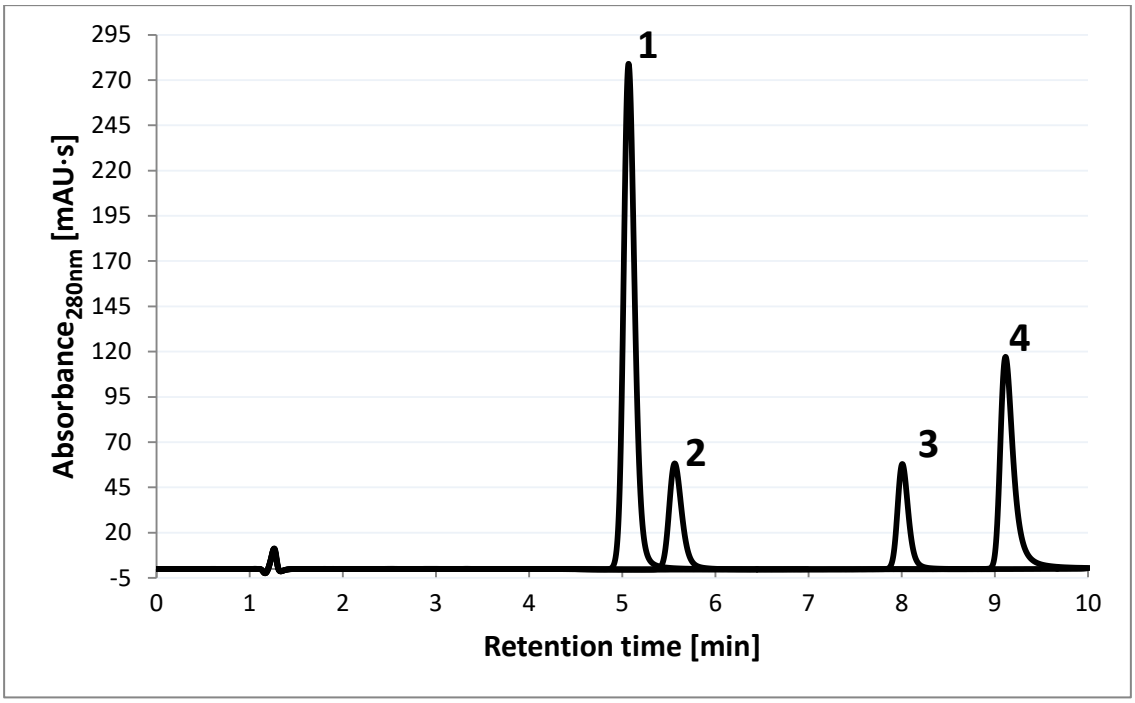


Figure 14: Calibration curve of the different catechin standards. Absorption was measured at 280 nm. The concentrations of the individual standards were 100 µg/ml. 1: caffeine; 2: catechin; 3: epicatechin; 4: EGCG

Results and discussion

Vitamin C

The preparation and analysis of the fruits was carried out as described in the chapter "Materials and Methods". Mean values and standard deviations were calculated for all obtained data and represented in tabular and graphic form. Standard calibration, measured peak areas and a sample chromatogram are listed in the appendix.

Orange juice

Overall, the total ascorbic acid content of orange juice was stable over a storage time of 24 hours, regardless of the used juicer or applied atmosphere (table 16, figure 15). The juice from the blender with vacuum application had the highest loss of ascorbic acid, with a loss of almost 9 % of the initial content. The juice from the blender with normal air showed a loss of 5 % of the initial ascorbic acid content. Nevertheless, these are very minor losses of ascorbic acid. Negligible or no losses of ascorbic acid were obtained in experiments using the masticator with the antifoaming device and the blender with the CO_2 and N_2O atmospheres. The highest concentration of ascorbic acid was detected in experiments using the blender with normal air. However this difference in the initial ascorbic acid concentration is caused by the variation in the nutrient content and freshness of the fruits. This variation in the nutrient content and freshness of the fruits may be the reason, why the blender with normal air showed a better stability of the ascorbic acid content than the blender with vacuum application. The measured ascorbic acid concentrations are similar to the ones mentioned in literature, which state a vitamin C concentration of 50 mg/100 g and 30 – 60 mg/100 g (Combs, 2008; Velisek, 2014).

Table 16: Stability of the vitamin C content in orange juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Orange – Vitamin C [mg/100 ml]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	69.9 ± 0.5	74.1 ± 0.8	53.5 ± 2.6	52.5 ± 1.9	53.4 ± 0.3
24	70.4 ± 0.9	70.1 ± 2.3	48.7 ± 2.9	52.9 ± 3.5	52.4 ± 2.5

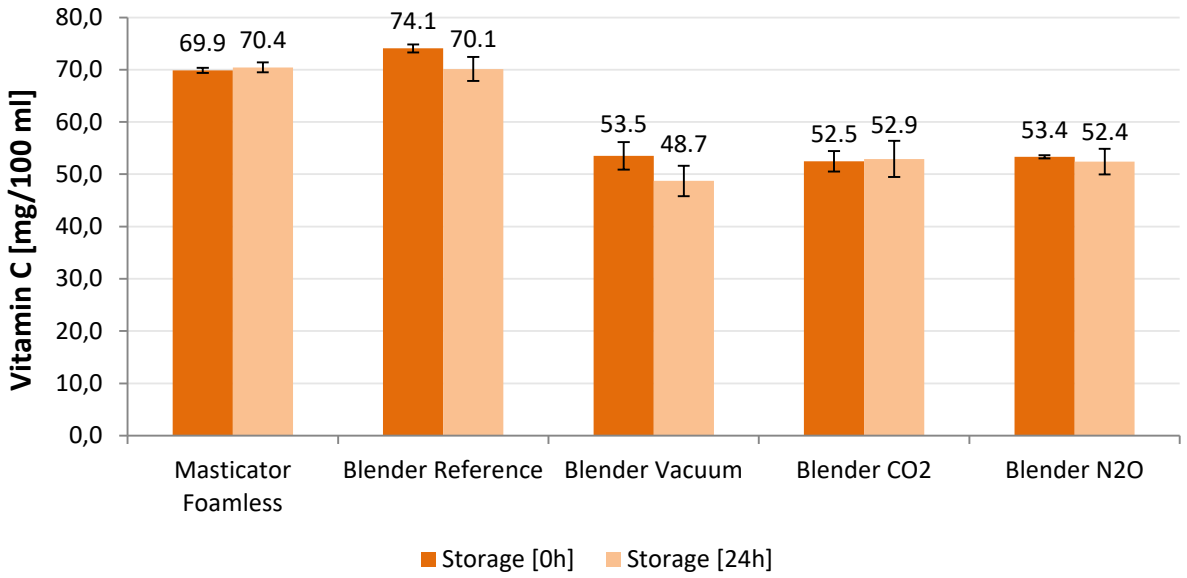


Figure 15: Stability of the vitamin C content of orange juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Strawberry juice

The ascorbic acid content of the strawberry juices was stable over a storage time of 24 hours (table 17, figure 16). The highest loss of ascorbic acid was detected in experiments using the blender with normal air, with a loss of almost 10 % of the initial ascorbic acid content. Strawberry juices from the masticator with the antifoaming device and blender with applied vacuum and inert gases showed negligible or no losses of ascorbic acid. The highest concentration of ascorbic acid was obtained in experiments using the masticator with the antifoaming device. However, this difference in the initial ascorbic acid concentration is caused by the variation in the nutrient content and freshness of the fruits. Stated vitamin C contents for strawberries in the literature are 40 – 90 mg/100 g strawberries and 40 – 70

mg/100 g strawberries (Combs, 2008; Velisek, 2014). Thus the measured vitamin C contents fit well to the mentioned ones of the literature.

Table 17: Stability of the vitamin C content in strawberry juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Strawberry – Vitamin C [mg/100 ml]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	59.5 ± 2.7	46.5 ± 3.3	44.9 ± 0.5	42.3 ± 1.3	46.7 ± 2.7
24	57.8 ± 0.8	41.9 ± 0.9	43.3 ± 0.9	41.9 ± 1.5	46.8 ± 1.2

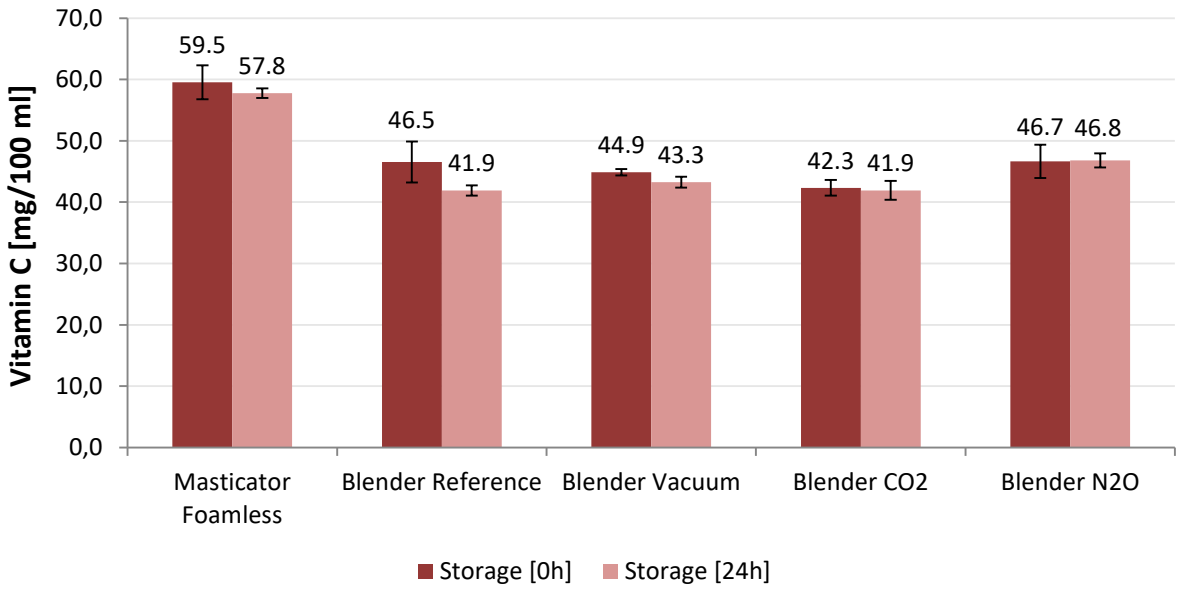


Figure 16 Stability of the vitamin C content of strawberry juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Blueberry Juice

Almost no loss of ascorbic acid in blueberry juice was detected over a storage time of 24 hours (table 18, figure 17). Ascorbic acid was very stable in all blueberry juices, regardless of the used juicer or applied atmosphere. Again, there are differences in the initial ascorbic acid concentration of the juices. The lowest ascorbic acid content was obtained in experiments

using the blender with applied vacuum and the highest ascorbic acid content was obtained in experiments using the blender with applied CO_2, but those differences are caused by the variation in the nutrient content and freshness of the fruits. The literature states ascorbic acid concentrations of 13 mg/100 g and 9.8 mg/100 g for blueberries, which fit well to the obtained ascorbic acid concentrations (Combs, 2008; Shivembe, 2017).

Table 18: Stability of the vitamin C content in blueberry juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Blueberry – Vitamin C [mg/100 ml]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	10.4 ± 0.6	10.8 ± 0.6	9.3 ± 0.4	12.1 ± 0.2	10.2 ± 0.7
24	10.9 ± 0.1	11.0 ± 0.2	8.9 ± 0.1	11.9 ± 0.2	10.3 ± 0.2

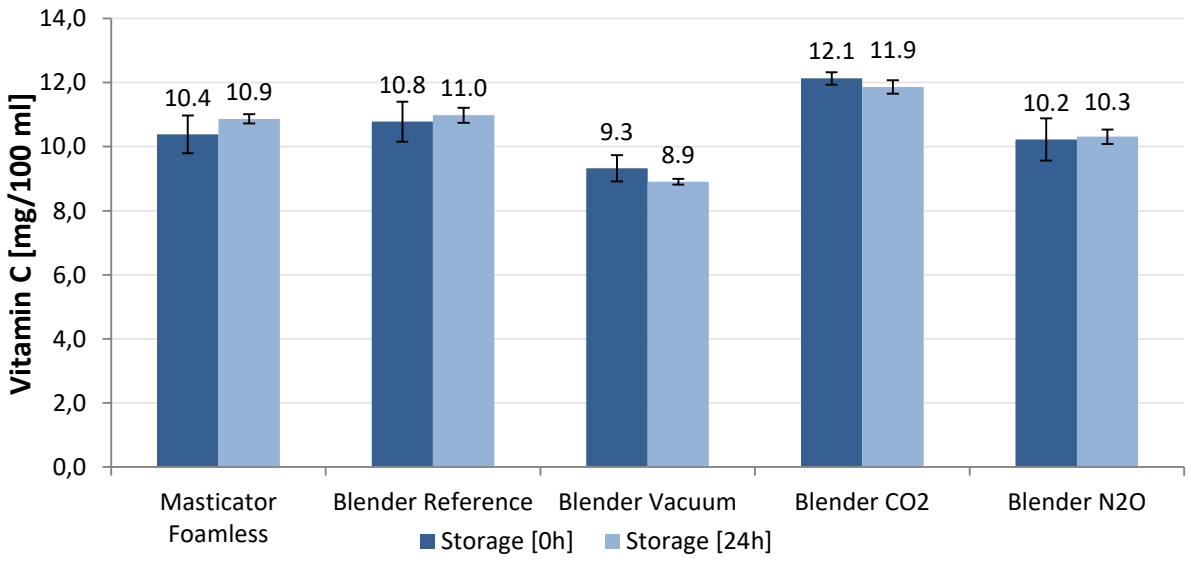


Figure 17: Stability of the vitamin C content of blueberry juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Kiwi juice

Overall, the ascorbic acid content of the kiwi juices was quite stable over a storage time of 24 hours (table 19, figure 18). An exception is the kiwi juice from the blender with normal air, which had a rather large loss of 19 % of the initial ascorbic acid content. Blending with vacuum application had a positive effect on the stability of the ascorbic acid, since there was only a loss of 8 % of the initial ascorbic acid content detected. The juices from the masticator with the antifoaming device and from the blender with the applied inert gases had only negligible or almost no losses of the initial ascorbic acid content. The kiwi juice from the blender with applied N_2O had noticeable lower concentration of ascorbic acid than the other kiwi juices, but these different ascorbic acid contents result from the variation in the nutrient content and freshness of the fruits. The obtained values fit nice to the ones mentioned in the literature, which state an ascorbic acid content of 98 mg/100 g and 70 – 163 mg/100 g for kiwis (Combs, 2008; Velisek, 2014).

Table 19: Stability of the vitamin C content in kiwi juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Kiwi – Vitamin C [mg/100 ml]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	82.3 ± 5.1	81.0 ± 8.1	87.1 ± 7.5	77.2 ± 4.2	66.3 ± 4.6
24	80.7 ± 2.3	65.5 ± 4.5	80.0 ± 1.9	74.8 ± 5.2	66.3 ± 2.2

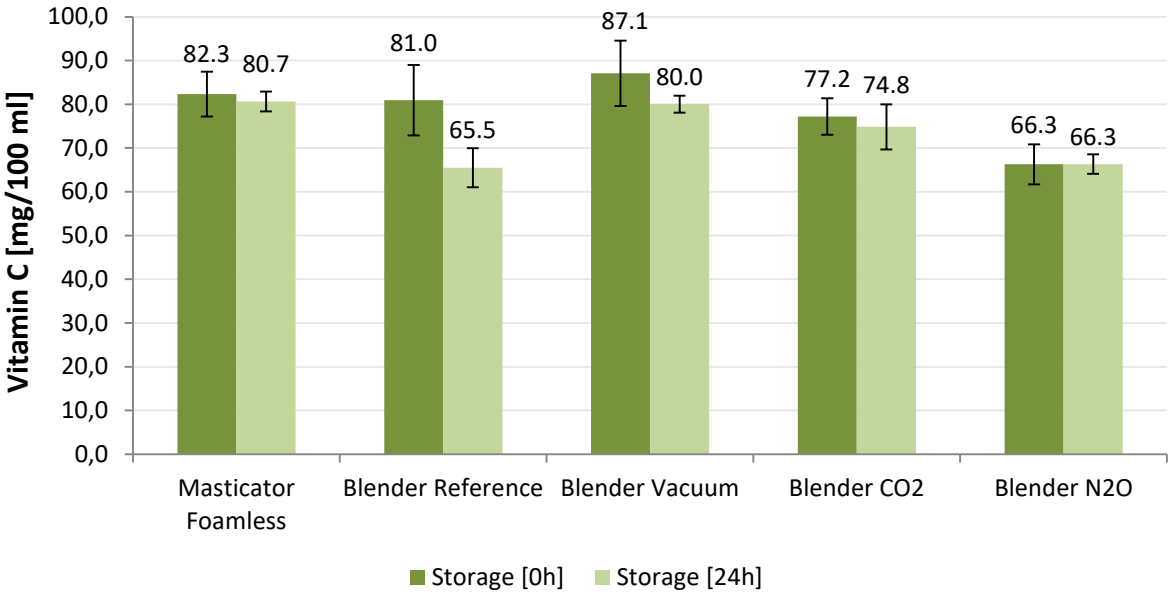


Figure 18: Stability of the vitamin C content of kiwi juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Red pepper juice

The ascorbic acid content of red pepper juice showed either very good stability or an increase thereof over a storage time of 24 hours (table 20, figure 19). Large increases of the ascorbic acid content were obtained in experiments using the blender with normal air and applied CO_2, which had the highest increase of almost 29 % of the initial ascorbic acid content. This increase may be explained by solid particles of the red pepper, which were not broken up during the blending process. These solid particles lysed during storage, thus their ascorbic acid became available for the measurement. Again, the differences in the initial ascorbic acid contents of the red pepper juices can be explained by the variation in the nutrient content and freshness of the peppers. Literature states an ascorbic acid content of 190 mg/100 g and 62 – 300 mg/100 g for peppers (Combs, 2008; Velisek, 2014). Therefore the obtained data fits to the one, mentioned in the literature.

Table 20: Stability of the vitamin C content in red pepper juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Red Pepper – Vitamin C [mg/100 ml]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	139 ± 10	120 ± 9	133 ± 3	155 ± 2	121 ± 3
24	148 ± 5	144 ± 11	135 ± 7	200 ± 4	126 ± 1

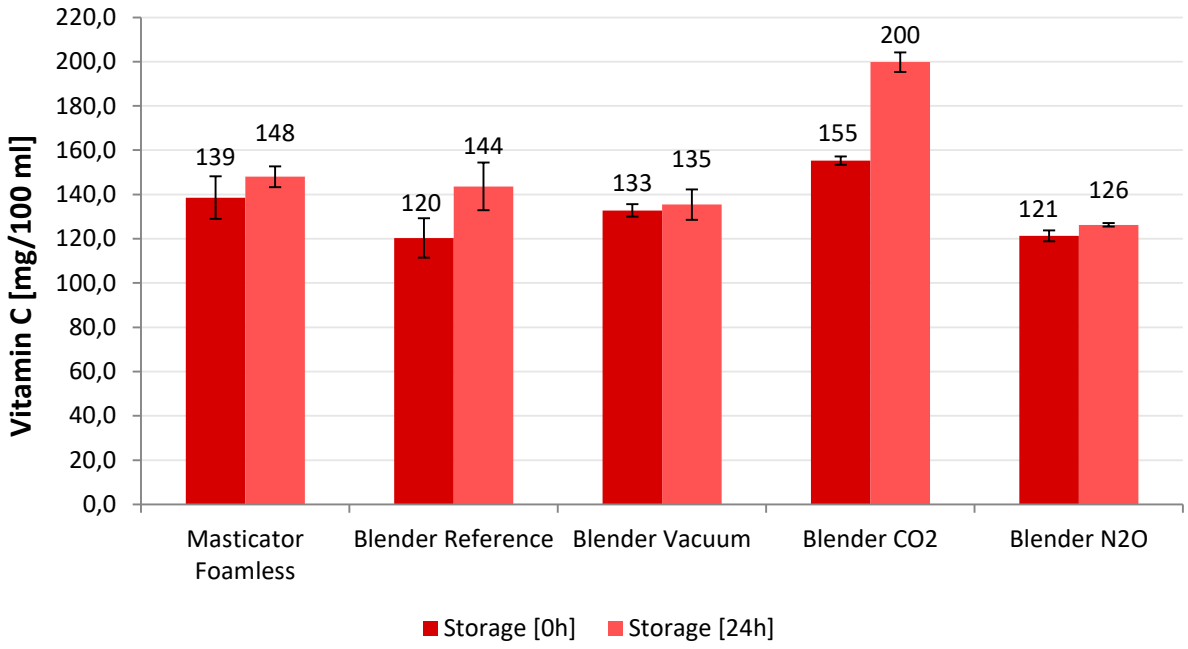


Figure 19: Stability of the vitamin C content of red pepper juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Conclusion Vitamin C

Overall, the ascorbic acid content of the different fruit juices showed good stability over a storage time of 24 hours, regardless of the used juicing procedure. Especially blueberry and red pepper juice had extremely good stability.

The application of vacuum did not lead to an increased stability of the ascorbic acid content in orange, blueberry and red pepper juice. Positive effects of the vacuum application were discovered for the strawberry and kiwi juice. In strawberry juice vacuum application led to slightly better stability of the ascorbic acid content than normal blending, but in general the losses were fairly low. As a result the increased stability of the vacuum application was neither really important nor significant. Therefore, a positive trend was obtained only for kiwi juice with vacuum blending. The loss of ascorbic acid from normal blending was 19 %, which was a considerable amount and also the largest loss of ascorbic acid, which was detected in this study. In vacuum only 8 % were lost. Hence, the application of vacuum led to a better stability of the ascorbic acid in the kiwi juice.

The application of the inert gases CO_2 and N_2O led to negligible or no losses of the ascorbic acid in any of the juices, although decreases were detected for normal blending or vacuum blending. Therefore, the application of these gases before the blending process enhanced the ascorbic acid stability much better than blending with vacuum conditions.

The reason why the applied gases enhanced the stability greater than vacuum blending might be the power of the vacuum pump, which is only able to apply a vacuum of -0.7 bar, hence 0.3 bar of the normal air are still left in the jar of the blender. The remaining oxygen of the 0.3 bar of the normal air (0.06 bar O_2) was able to dissolve in the juice during the blending process and then degrade the ascorbic acid of the stored juice. As mentioned in the chapter “Material and Methods”, it was possible to exchange the whole atmosphere with CO_2 or N_2O. As very low levels of oxygen were present in the blender jar, the stability of the juices was superior. These reasons indicate that a vacuum pump with more power should lead to an increased stability of the ascorbic acid content.

The juices from the masticator with the antifoaming device showed also an extremely good stability of the ascorbic acid content, comparable to the juices from the blender with CO_2 and N_2O atmosphere. Thus, it can be concluded that the antifoaming device is very effective in removing the oxygen from the juice, resulting in the nice stability of the ascorbic acid content.

Anthocyanins

The preparation and analysis of the fruits was carried out as described in the chapter "Materials and Methods". Mean values and standard deviations were calculated for all obtained data and represented in tabular and graphic form. Measured peak areas and a sample chromatogram are listed in the appendix.

Red grape juice

The main detected anthocyanins in this experiment were cyanidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside and peonidin-3-O-(6"-O-coumaroyl)-glucoside. The sum of the peak areas of those 4 major anthocyanins was stated as the TAC of the grape juice. The TAC of the grape juices were quite stable for over a storage time of 24 hours, except the juice from the blender with normal air showed a huge decrease of 63 % of the initial TAC (figure 21, table 20). The other juices showed much lower decreases of the initial TAC, ranging from 11 % to negligible amounts. Thus it can be assumed, that the stability of the TAC in red grape juice is strongly influenced by the oxygen, which dissolves in the juice during the blending process. Because of this, the application of vacuum or inert gases before the juicing procedure increased the stability of the TAC to a large degree, whereas the applied inert gases were a little bit more effective in stabilising the TAC than the vacuum. The reason for this might be the power of the vacuum pump, which is also discussed in the chapter "Conclusion Vitamin C". The difference in the initial TAC of the juices is explained by the variation in the nutrient content and freshness of the grapes. Important to note is that the low TAC from the juice of the masticator is also influenced by the separation of the grape skins from the juice, because most anthocyanins are present in the grape skin and are not fully extracted during the juicing procedure.

Table 21: Stability of the anthocyanin content of red grape juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Red Grape – TAC [mAU·s]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	300 ± 3	604 ± 6	968 ± 32	575 ± 61	434 ± 25
24	277 ± 31	221 ± 12	853 ± 12	624 ± 34	394 ± 28

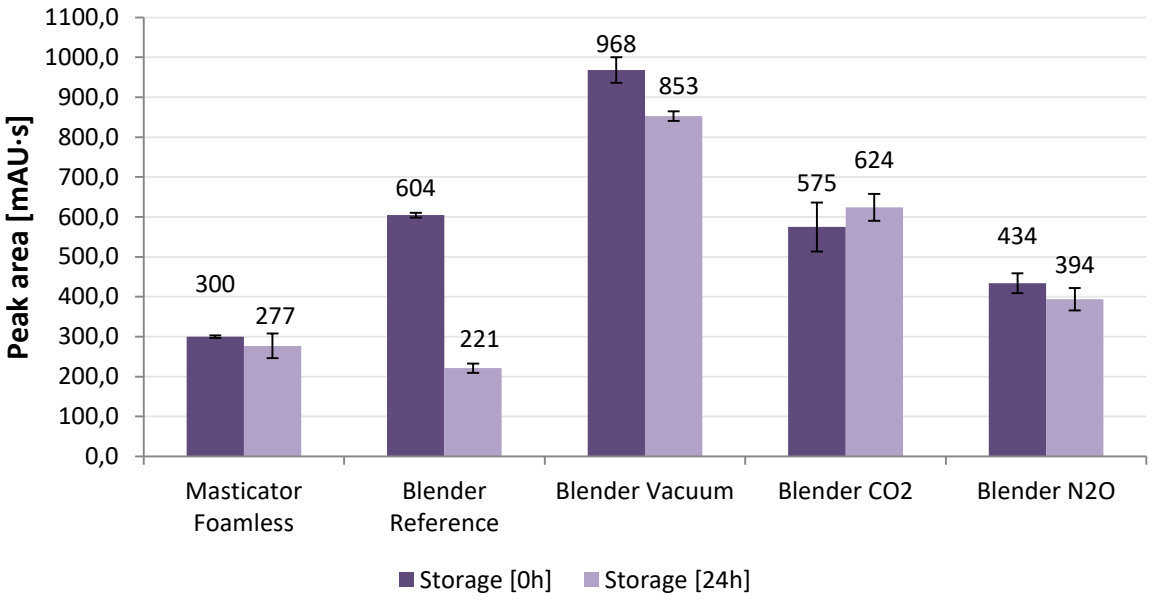


Figure 20: Stability of the total anthocyanin content of red grape juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Total phenolic content

The preparation and analysis of the fruits was carried out as described in the chapter "Materials and Methods". Mean values and standard deviations were calculated for all obtained data and represented in tabular and graphic form. Standard calibration and measured absorptions of the samples are listed in the appendix.

Blueberry juice

The TPC of blueberry juice decreased significantly in almost all juices over a storage time of 24 hours (table 22, figure 21). The juice from the blender with N_2O atmosphere showed the best stability for the TPC, with a loss of almost 9 % of the initial TPC. The largest decreases of the TPC were obtained for the blender with natural air and applied vacuum, which showed losses of 27 % and 24 % of the initial TPC. Lower losses of 17 % and 19 % of the initial TPC were detected for the masticator with the antifoaming device and the blender with CO_2 atmosphere. The highest TPC were detected for the juice from the blender with CO_2 atmosphere and the lowest TPC for the juice from the masticator with the antifoaming device, but those differences of the initial TPC were caused by the variation in the nutrient content and freshness of the blueberries. Literature state a TPC of 264 – 528 mg/100 g (Dragović-Uzelac et al., 2010). The obtained TPC are lower than the ones mentioned in literature, because the TPC strongly depends on the variety of the blueberry, cultivation and harvest time.

Table 22: Stability of the TPC of blueberry juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Blueberry – TPC [mg/100 ml]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	154 ± 11	206 ± 12	157 ± 3	206 ± 3	165 ± 4
24	128 ± 3	151 ± 6	118 ± 4	170 ± 7	150 ± 2

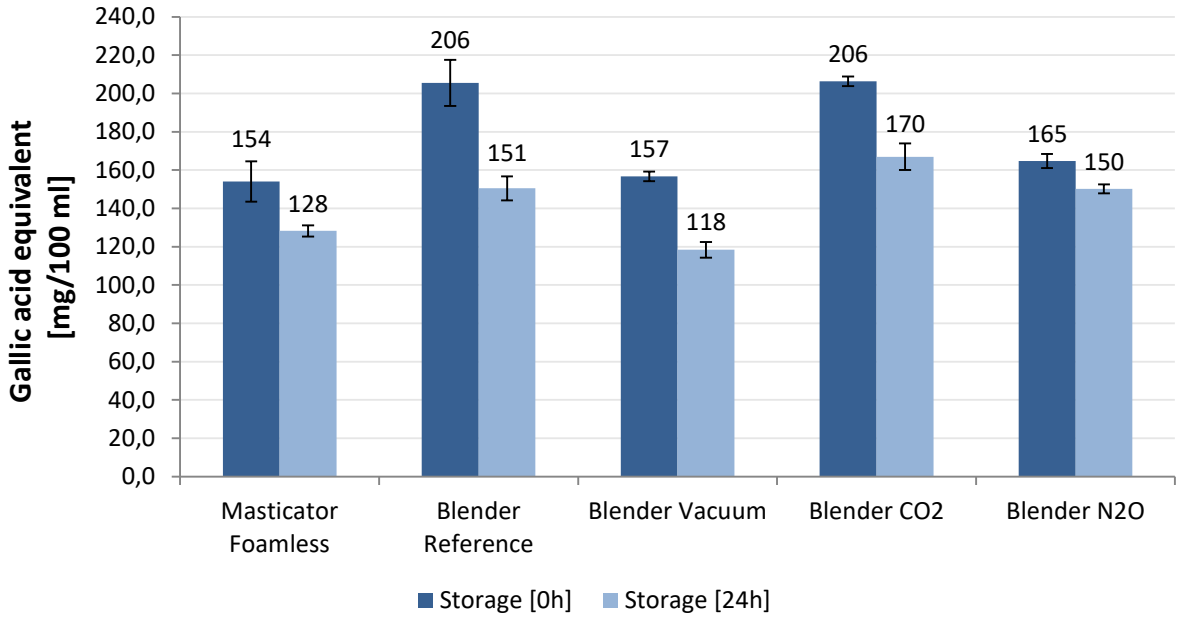


Figure 21: Stability of the TPC of blueberry juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Apple juice

Decreases of the TPC content over a storage time of 24 hours were detected for the juices from the blender with normal air and applied vacuum, which showed a decrease of 18 % and 23 % of the initial TPC (table 23, figure 22). The juice from the masticator with the antifoaming device showed a minor loss of 5 % of the initial TPC. Very good stability of the TPC was obtained for the juices from the blender with the applied inert gases CO_2 and NO_2. It is important to note that the TPC of the juice from the masticator with the antifoaming device is extremely high in comparison to the other juices, because the other juices were diluted 1:2 with water, as mentioned in the "sample preparation" of the chapter "Material and Methods". The general differences in the initial TPC are explained by the variation in the nutrient content and freshness of the apples. This may also be the reason why the juice from the blender with applied vacuum showed a larger decrease of the TPC in comparison to the juice from the blender with normal air. The apples must have had a lower quality, because the initial TPC of this juice is noticeable lower than the others and this overall lower quality of the apples could have also influenced the stability of their nutrients. Literature state a TPC of 68 mg/100 g and 27 mg/100 g for golden delicious (Fu et al., 2011 & Matthes et al., 2009). These mentioned TPC are much lower than the obtained values, considering the apples were

diluted 1:2 with water. Thus apples, even from the same variety, can differ a lot in the nutrient content.

Table 23: Stability of the TPC of apple juice over a storage time of 24 hours. Comparison of the different juicing procedures. Mean values and standard deviations were obtained from three separate experiments.

Apple – TPC [mg/100 ml]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	164 ± 0	77.2 ± 1.9	56.9 ± 1.5	76.7 ± 1.9	69.0 ± 2.0
24	157 ± 4	63.3 ± 2.4	44.0 ± 2.6	75.9 ± 0.5	67.9 ± 4.3

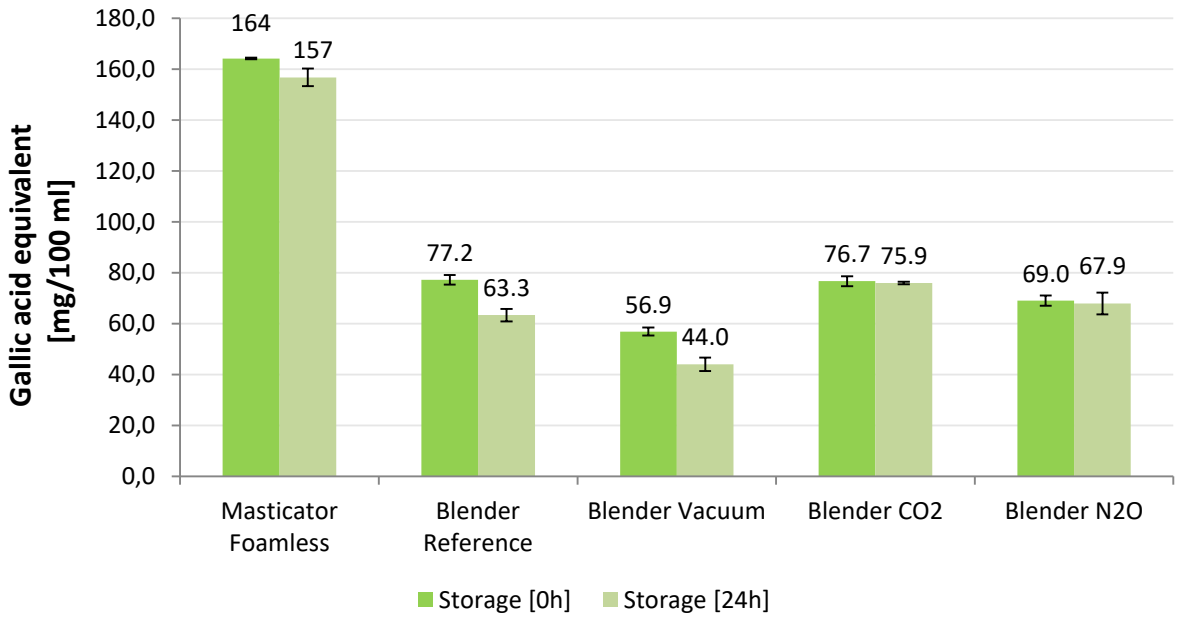


Figure 22: Stability of the TPC of apple juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Matcha juice

The TPC of the different matcha juices were extremely stable over a storage time of 24 hours (table 24, figure 23). Only negligible or no losses of the TPC were detected. Matcha is in comparison to the other used fruits an already processed food product, which is a very homogenous, packaged powder and therefore no difference in the nutrient content should

be detected. Nevertheless, different initial TPC were obtained for the juices. The blank, which was matcha mixed manually, yielded the lowest TPC. Thus it can be said, that blending enhances the dissolution of the matcha powder and therefore the TPC of the juice. Similar TPC were detected for nearly all of the matcha juices, which were produced by blending. An exception is the juice from the blender with CO_2 atmosphere that had a much higher TPC in comparison to the other juices. It is possible that the applied CO_2 lowered the pH of the juice and thus stabilised the polyphenols during the blending process, leading to a higher TPC. Literature state TPC of 65.8 – 106 for green teas (Khokhar & Magnusdotti, 2002). Therefore it can be concluded that this "Fuku Matcha" has a rather low TPC compared to other green teas.

Table 24: Stability of the TPC in matcha juice over a storage time of 24 hours. Comparison of the different mixing procedures. Mean values and standard deviations were obtained from three separate experiments.

Matcha – TPC [mg/100 ml]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	56.8 ± 0.3	67.1 ± 0.9	66.2 ± 0.3	82.8 ± 1.7	61.5 ± 1.0
24	56.6 ± 0.7	70.2 ± 0.6	65.6 ± 1.1	81.0 ± 1.8	60.3 ± 0.5

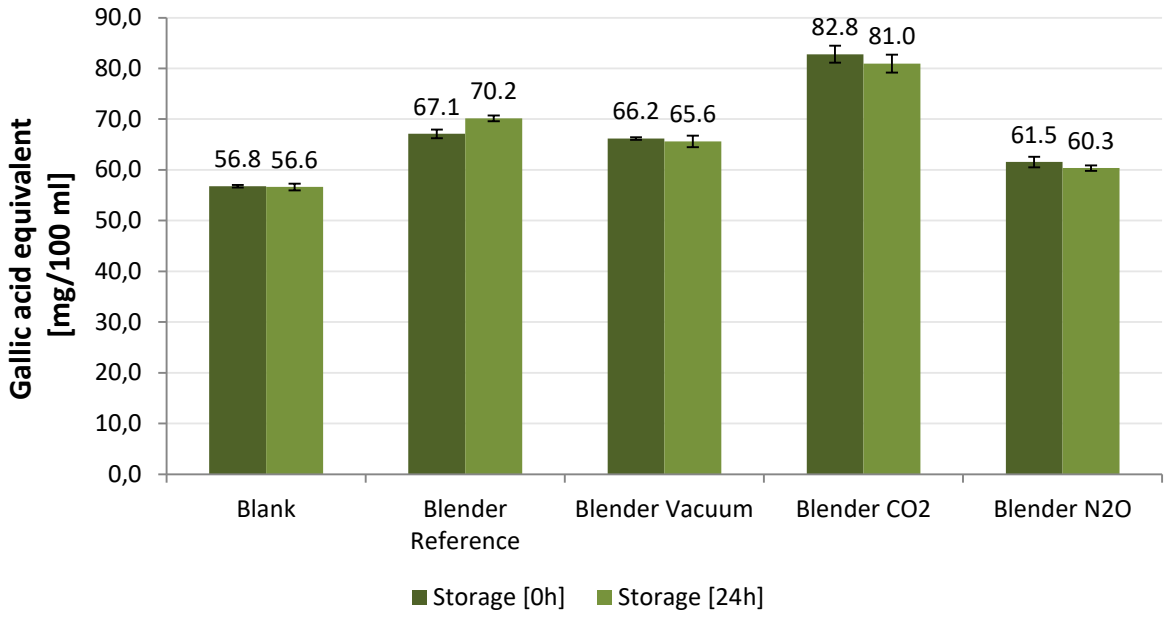


Figure 23: Stability of the TPC of matcha juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different mixing procedures.

Conclusion TPC

Overall, the TPC of the fruit juices showed less stability over a storage time of 24 hours compared to the ascorbic acid content. Especially the blueberry juices showed a significant decrease of the TPC. The TPC of the matcha juices were completely stable, due to matcha being an already processed food product in comparison to the fruits.

Vacuum application did not increase the stability of the TPC of the fruit juices. The difference in the decrease of the TPC of blueberry juice between normal blending and vacuum blending was only 3 %, a very minor amount. The decrease of the TPC of apple juice was even worse for vacuum blending than normal blending. As discussed in the results of the apple juice, the low quality of the apples could have influenced the TPC and the stability thereof.

The application of CO_2 and N_2O led to an increased stability of the TPC in comparison to normal blending and vacuum blending. Particularly the TPC of the apple juice showed very good stability, when the gases were applied before the juicing procedure. CO_2 and N_2O also enhanced the stability of the TPC of the blueberry juice in comparison to normal or vacuum blending, but noticeable losses of 19 % and 9 % were still detected. Therefore it can be said, that the TPC of blueberry juice is very prone to degradation by oxygen.

The reason why vacuum blending did not show positive results might be the power of the vacuum pump, which is also discussed in the chapter “Conclusion Vitamin C”.

The juices from the masticator with antifoaming device showed a good stability of the TPC, especially the TPC of the apple juice had a very good stability. Nonetheless there was still a noticeable degradation of the TPC of blueberry juice detected, but this degradation was clearly lower than the degradation of the juices from normal and vacuum blending. Overall, the performance of the masticator with the antifoaming device is comparable to blending with CO_2 and N_2O atmosphere in regards to the stabilisation of the TPC. This similar performance was also observed for the ascorbic acid content of the juices.

The matcha juice showed very interesting results, as there was no loss of the TPC detectable. Blending of the matcha powder led to higher TPC than manual mixing and the application of CO_2 before the juicing procedure also increased the TPC of the juice. As mentioned in the results of the matcha juice, CO_2 could have lowered the pH of the matcha juice and therefore stabilised the present polyphenols. Because of the lower pH, the hydroxyl groups of the polyphenols would have been protonated and thus better protected versus oxidations.

Tea Catechins

The preparation and analysis of the matcha was carried out as described in the chapter "Materials and Methods". Mean values and standard deviations were calculated for all obtained data and represented in tabular and graphic form. Standard calibration, measured peak areas and a sample chromatogram are listed in the appendix.

Caffeine

Very similar caffeine contents were detected for all matcha juices, except for the blank (table 25, figure 24). The reason for this might be that manual mixing was not as effective as machine blending of the matcha powder. The published caffeine contents for different green tea extracts range from 0.3 to 26.8 mg/g. (Friedman et al., 2006; Khokhar & Magnusdotti, 2002). Therefore it can be concluded that this used "Fuku matcha" has a rather high caffeine content in comparison to most green teas.

Table 25: Stability of caffeine in matcha juice over a storage time of 24 hours. Comparison of the different mixing procedures. Mean values and standard deviations were obtained from three separate experiments.

Matcha – Caffeine [mg/g]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	18.4 ± 0.2	19.1 ± 0.2	19.7 ± 0.1	20.3 ± 0.1	20.0 ± 0.2
24	18.3 ± 0.4	19.8 ± 0.1	19.6 ± 0.1	20.1 ± 0.1	19.7 ± 0.1

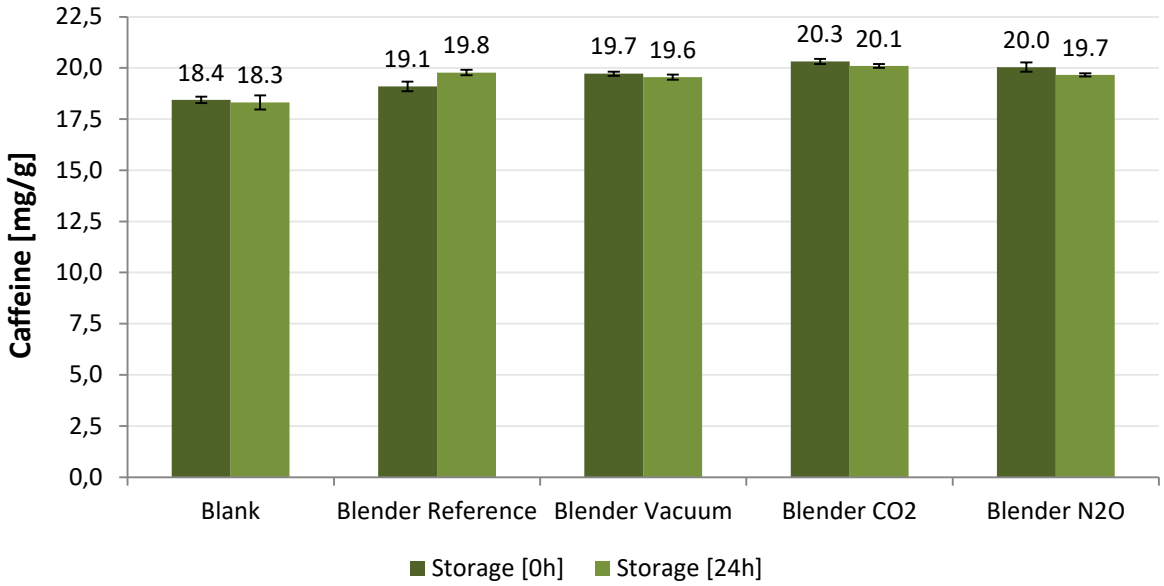


Figure 24: Stability of the caffeine in matcha juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Catechin

There was a big difference in the catechin content of the matcha juices detected, but catechin was very stable in all juices over a storage time of 24 hours (table 26, figure 25). The juices of the blank and the blender with CO_2 atmosphere contained only trace amounts of catechin, which were not quantifiable. Nearly identical amounts of catechin were obtained for the juices from normal and vacuum blending, whereas the juice from the blender with N_2O atmosphere contained the highest amount of catechin, which was 70 % more than in the juices from normal and vacuum blending. So it can be assumed that either a variation in the catechin content of the matcha powder from the same package exists, or that the applied inert gases influenced the catechin content of the juices.

Table 26: Stability of catechin in matcha juice over a storage time of 24 hours. Comparison of the different mixing procedures. Mean values and standard deviations were obtained from three separate experiments.

Matcha – Catechin [mg/g]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	-	2.1 ± 0.1	2.1 ± 0.2	-	3.6 ± 0.0
24	-	2.3 ± 0.2	2.1 ± 0.2	-	3.7 ± 0.1

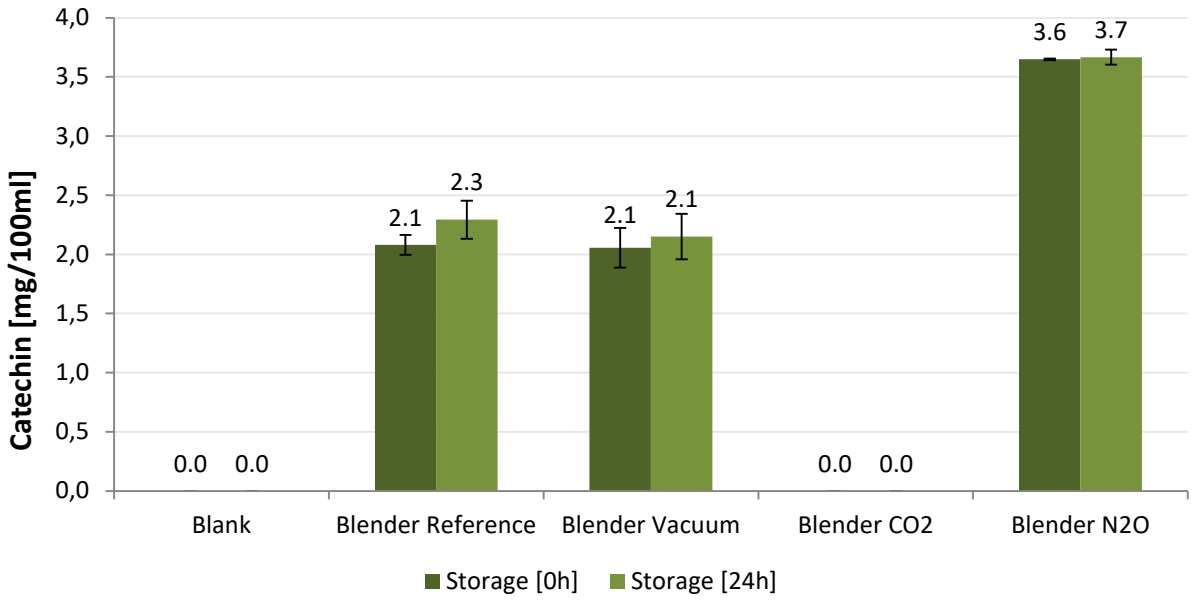


Figure 25: Stability of the catechin in matcha juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Epicatechin

The epicatechin in all juices was very stable for a storage time of 24 hours (table 27, figure 26). The blank contained the lowest amount of epicatechin and the reason for this might be that the blank was mixed manually, which is also discussed in the results of the caffeine content. Similar epicatechin contents were obtained for all juices from the different blending procedures.

Table 27: Stability of epicatechin in matcha juice over a storage time of 24 hours. Comparison of the different mixing procedures. Mean values and standard deviations were obtained from three separate experiments.

Matcha – Epicatechin [mg/g]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	6.5 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.9 ± 0.2	7.5 ± 0.1
24	6.5 ± 0.1	7.4 ± 0.1	7.4 ± 0.1	7.7 ± 0.2	7.3 ± 0.1

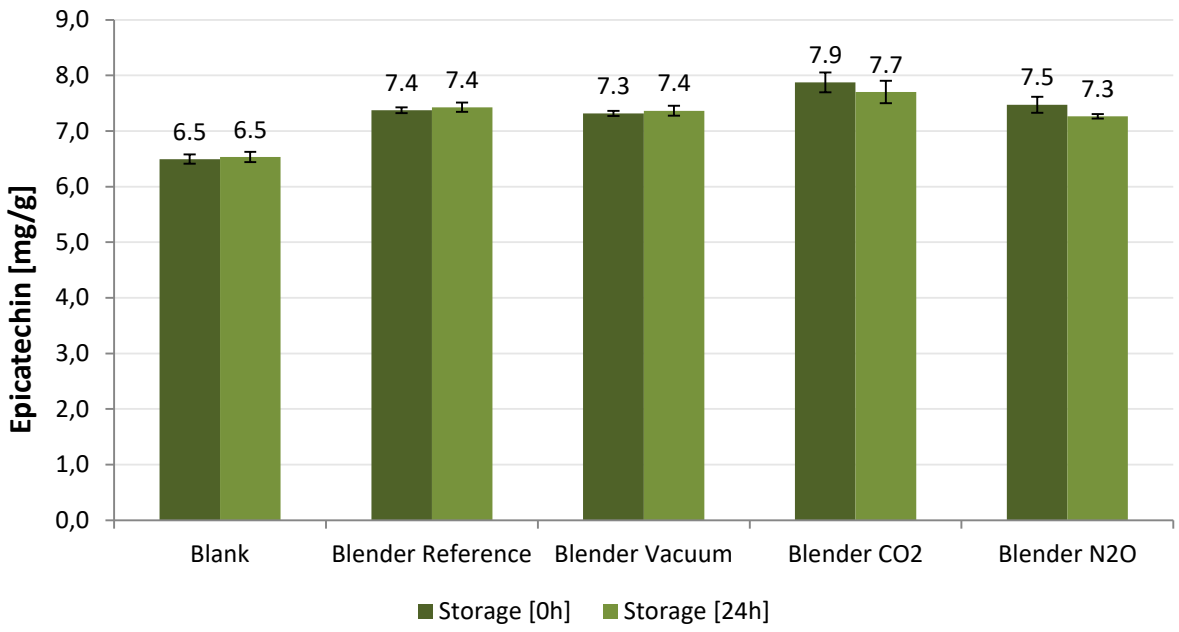


Figure 26: Stability of the epicatechin in matcha juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

EGCG

The EGCG content is the only one of the measured catechins, which decreased over a storage time of 24 hours (table 28, figure 27). Only the juice from normal blending showed a good stability of the EGCG content, but this juice contained the lowest initial amount of EGCG. The highest losses of the EGCG content were detected for the juices from the blank and the blender with N_2O atmosphere. Both juices showed a decrease of roughly 25 % of their initial EGCG content. Losses of 19 % and 10 % of the EGCG content were obtained for the blender with applied vacuum and CO_2 atmosphere. The juice from the blender with CO_2 atmosphere contained a huge amount of EGCG compared to the other juices, almost 100 % more than the juice from normal blending. Therefore it can be concluded, that CO_2 had a stabilising effect on the EGCG content during the blending process. The reason for this could be that the applied CO_2 lowered the pH of the juice and thus stabilised the polyphenols during the blending process. Due to the lower pH, the hydroxyl groups of the EGCG would have been protonated and thus better protected versus oxidation.

Table 28: Stability of EGCG in matcha juice over a storage time of 24 hours. Comparison of the different mixing procedures. Mean values and standard deviations were obtained from three separate experiments.

Matcha – EGCG [mg/g]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	10.8 ± 0.5	10.1 ± 0.8	12.1 ± 0.8	20.0 ± 0.9	12.7 ± 1.0
24	8.1 ± 0.1	9.8 ± 0.5	9.9 ± 0.8	18.0 ± 0.1	9.4 ± 0.9

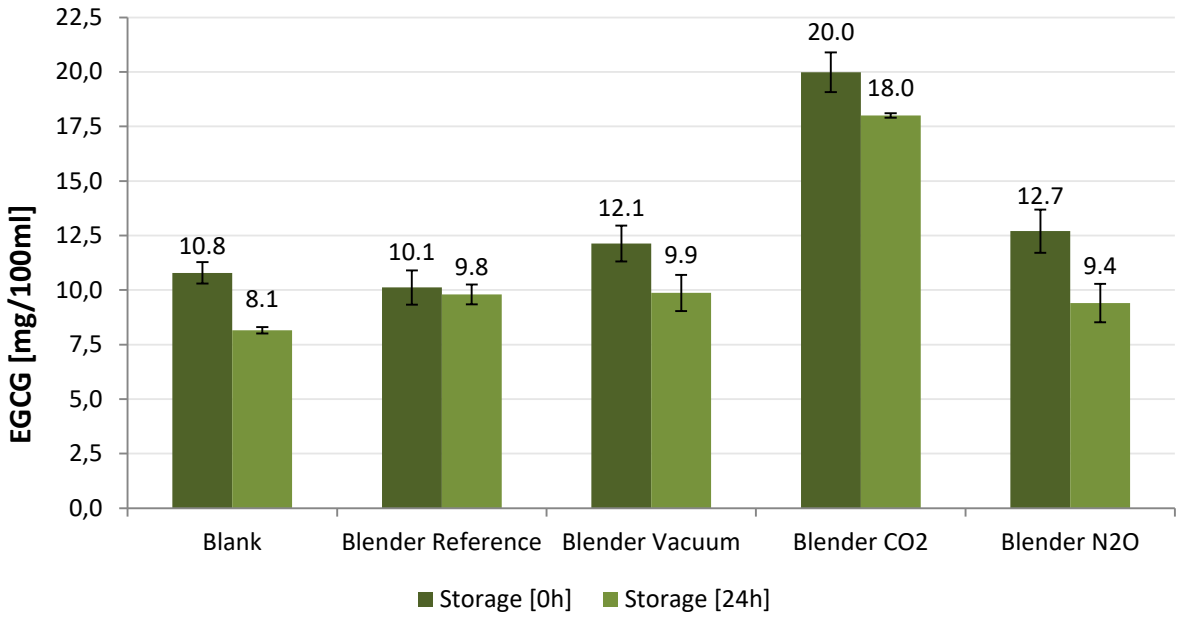


Figure 27: Stability of the EGCG in matcha juice over a storage time of 24 hours. The juices were stored in capped glass bottles at 3 °C. Comparison of the five different juicing procedures.

Conclusion Catechines

Caffeine, catechin and epicatechin had very good stability for a storage time of 24 hours, whereas rather large decreases of the EGCG were detected. The application of vacuum or inert gases did not affect the stability of the different catechins.

However the gases CO_2 and N_2O did affect the content of EGCG and catechin of the juices. The application of N_2O led to an increase of 70 % of the catechin content compared to normal blending, whereas the application of CO_2 led to only trace amounts of catechin. Also the EGCG content of the juice was affected by the applied CO_2, which resulted in an 100 %

increase thereof compared to normal blending. The reason for this may be the influence on the pH, which is discussed in the results of the EGCG.

The matcha juice, which was mixed manually (blank), contained lower amounts of the measured catechins in comparison to the other juices. Thus it can be concluded that blending is more effective than manual mixing.

Literature ranked the amounts of different catechins in green teas: EGCG > epicatechin > catechin (Khokhar & Magnusdotti, 2002). This was also detected in these catechin measurements, where EGCG was the most abundant, followed by epicatechin and of catechin were only very small amounts detected.

Comparison of TPC and HPLC measurement

The results of the TPC and the HPLC measurement correlate with each other, because the blank showed the lowest and the application of CO_2 the highest polyphenol content in both determination methods. However, there was no loss of the TPC content, although significant losses of the EGCG were detected in the HPLC measurement. The reason for this might be the formation of polymerised catechins, such as theaflavins or bisflavanols. Tea enzymes can catalyse the oxidation of EGCG and other catechins to theaflavins when oxygen is present (Shahidi & Naczk, 1995). These theaflavins have the same antioxidant capacity and also the conversion of catechins to theaflavins does not result in loss of their free radical scavenging activity (Leung et al., 2001). Therefore no losses of the TPC of the matcha juices were detected.

Appendix

Data of the vitamin C measurements

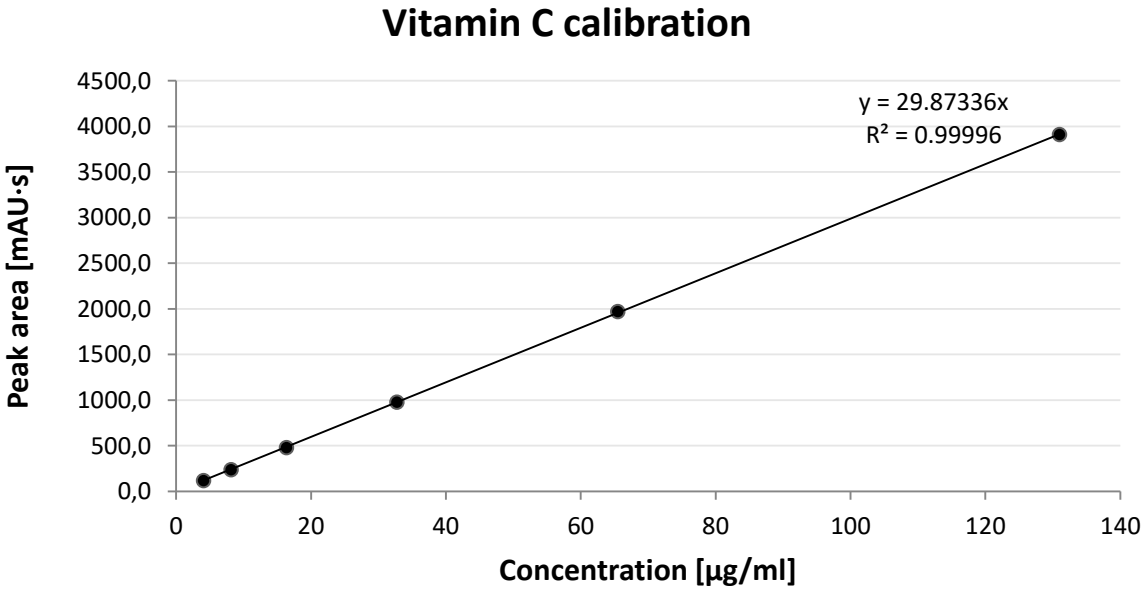


Figure 28: Standard calibration curve of L-ascorbic acid. Calibration equation was used to calculate the total ascorbic acid content of the different juices.

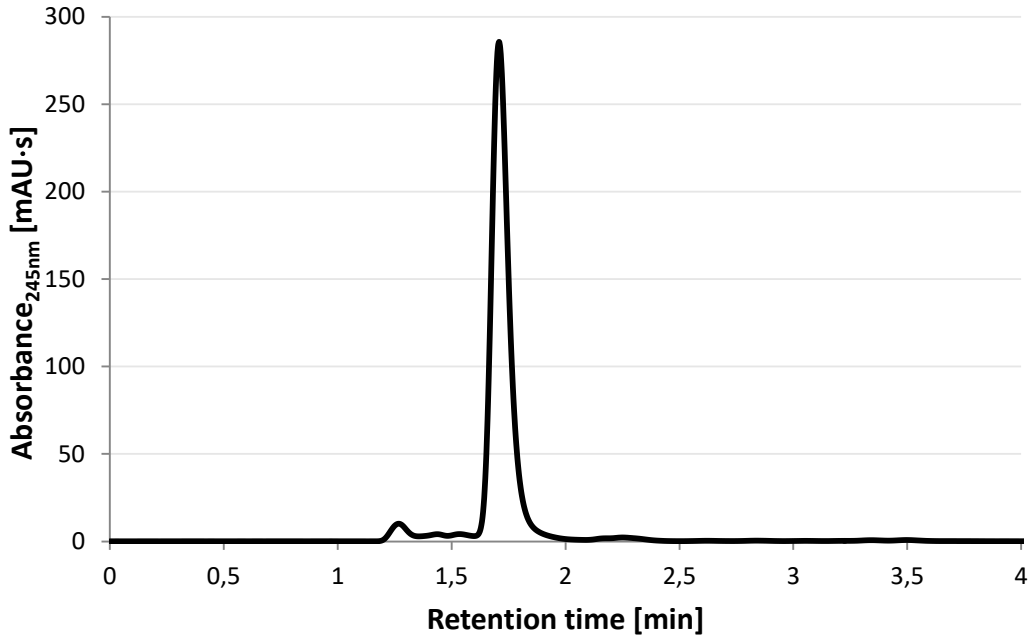


Figure 29: Sample chromatogram of the total ascorbic acid measurement from orange juice.

Table 29: Peak areas obtained from the HPLC measurement of the total ascorbic acid content in the various juices. Measurement was done in triplicates.

Orange juice – peak areas [mAU·s]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	1556.0	1650.3	1221.5	1152.3	1191.1
	1577.0	1679.4	1132.6	1149.5	1191.8
	1563.9	1648.9	1242.9	1226.5	1203.1
24	1582.3	1529.3	1107.0	1243.3	1225.7
	1596.5	1555.9	1020.3	1215.5	1116.0
	1555.0	1629.3	1148.1	1097.5	1180.2
Strawberry juice – peak areas [mAU·s]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	1154.5	877.0	923.3	892.0	890.4
	1266.0	1012.8	917.0	845.9	960.9
	1217.5	953.1	902.7	849.2	999.4
24	1307.8	932.1	969.3	978.7	1027.4
	1274.2	960.4	989.1	919.0	1040.9
	1300.5	923.9	949.3	919.1	1077.6
Blueberry juice – peak areas [mAU·s]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	1188.2	1166.9	1097.4	1384.1	1171.9
	1088.4	1287.6	1021.0	1343.9	1201.3
	1213.5	1167.7	1015.4	1348.0	1061.2
24	1225.8	1200.6	1004.6	1304.7	1171.0
	1227.6	1237.3	985.5	1352.0	1168.1
	1198.7	1251.9	1002.5	1330.2	1125.4
Kiwi juice – peak areas [mAU·s]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	986.1	1011.1	1064.0	811.0	762.0
	905.1	853.1	897.1	891.6	781.4
	875.3	856.4	965.7	892.7	684.4
24	918.0	743.7	916.5	891.2	751.1
	874.3	679.5	899.4	847.1	715.3
	918.3	778.3	873.5	776.4	762.9
Red pepper juice – peak areas [mAU·s]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	1427.9	1359.6	1493.5	1758.5	1376.9
	1611.5	1242.9	1453.3	1717.3	1326.7
	1617.9	1442.6	1516.1	1743.3	1371.2
24	1705.7	1660.8	1552.4	2203.3	1417.8
	1667.2	1695.3	1570.8	2294.3	1404.1
	1601.8	1470.6	1428.9	2216.5	1421.2

Data of the anthocyanin measurements

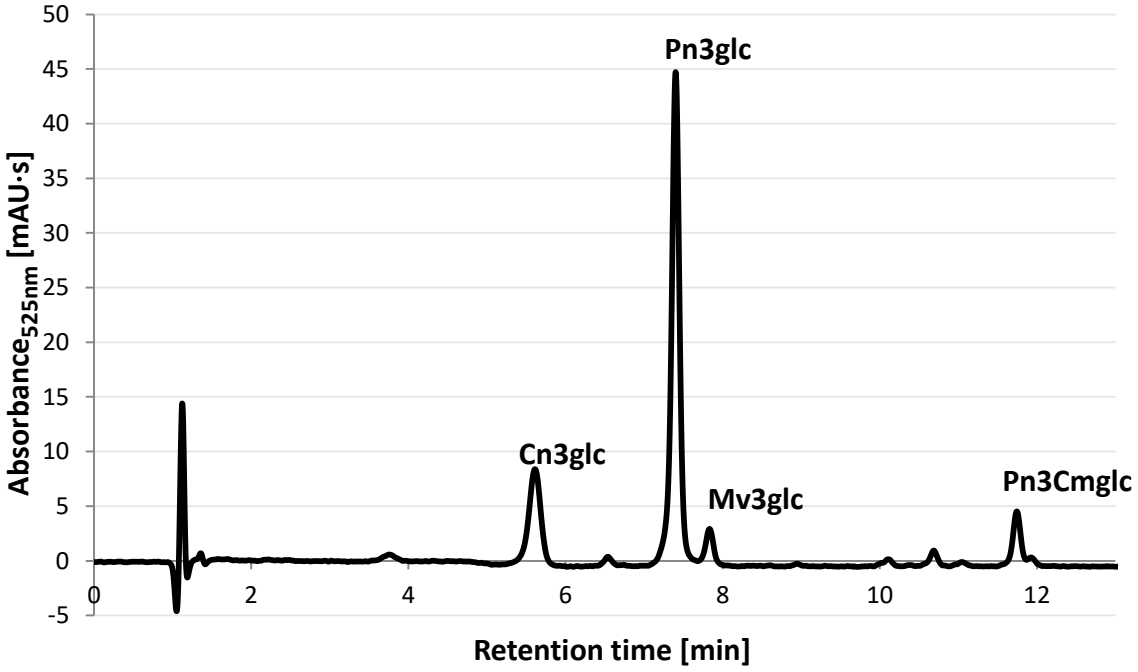


Figure 30: Sample chromatogram of the anthocyanin measurement from red grape juice. Cn3glc: cyanidin-3-O-glucoside; Pn3glc: peonidin-3-O-glucoside; Mv3glc: malvidin-3-O-glucoside; Pn3Cmglc: peonidin-3-O-(6″-O-coumaroyl)-glucoside (Annotation of peaks from Brar et al., 2008)

Table 30: Peak areas obtained from the HPLC measurement of the anthocyanin content in red grape juice. Measurement was done in triplicates.

Red grape juice – peak areas [mAU·s]					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	151.0	303.5	491.6	309.0	204.5
	150.7	304.4	494.8	300.8	229.4
	148.0	298.6	465.5	252.3	216.9
24	120.9	116.8	428.2	293.3	191.6
	150.2	105.3	419.8	316.4	213.0
	144.1	109.7	431.2	326.2	186.4

Data of the TPC measurement

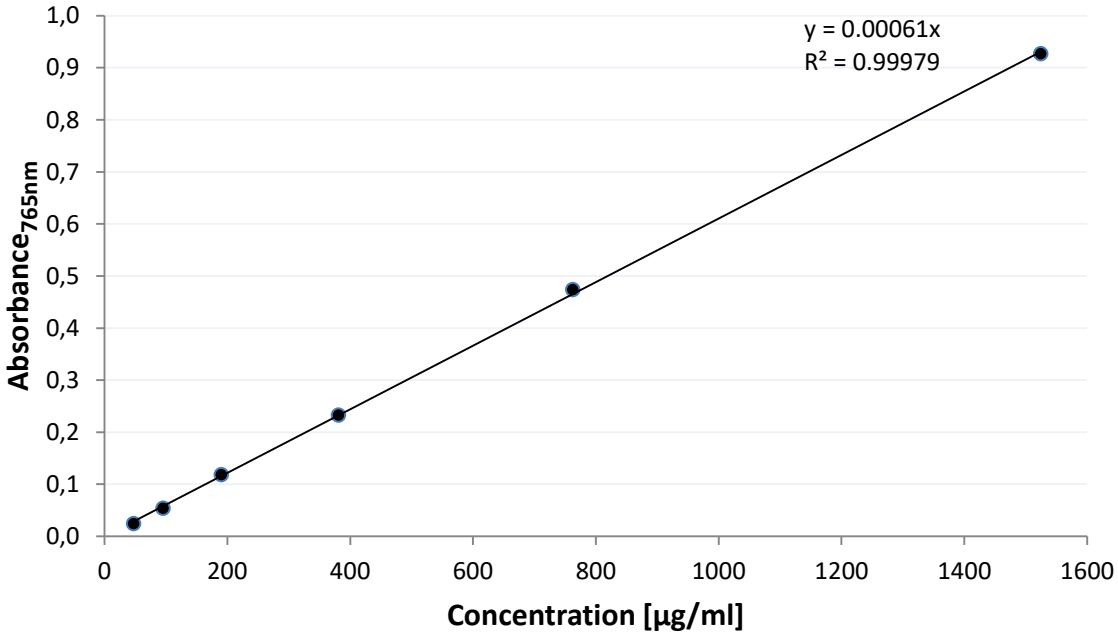


Figure 31: Standard calibration curve of gallic acid. Calibration equation was used to calculate the TPC of the different juices.

Table 31: Absorption obtained from the photometric measurement of the TPC of the various juices. Three separate measurements were done and each measurement was performed as triplicates.

Blueberry juice – absorbance					
Storage [h]	**Masticator F.**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0/1	0.488	0.640	0.481	0.622	0.501
	0.472	0.635	0.484	0.617	0.495
	0.465	0.628	0.489	0.623	0.494
0/2	0.434	0.653	0.473	0.633	0.502
	0.440	0.646	0.479	0.625	0.491
	0.433	0.679	0.457	0.636	0.492
0/3	0.491	0.581	0.480	0.632	0.521
	0.504	0.590	0.477	0.640	0.514
	0.503	0.589	0.482	0.635	0.511
24/1	0.404	0.432	0.357	0.538	0.454
	0.370	0.444	0.363	0.532	0.476
	0.376	0.440	0.364	0.522	0.465
24/2	0.378	0.460	0.375	0.502	0.456
	0.397	0.466	0.378	0.481	0.453
	0.393	0.459	0.368	0.482	0.466

24/3	0.398	0.473	0.352	0.516	0.455
	0.404	0.474	0.353	0.505	0.448
	0.401	0.482	0.341	0.504	0.450

Apple juice – absorbance

Storage [h]	Masticator F.	Blender R.	Blender V.	Blender CO_2	Blender N_2O
0/1	0.529	0.232	0.179	0.220	0.191
	0.501	0.236	0.180	0.224	0.188
	0.472	0.244	0.186	0.222	0.204
0/2	0.519	0.248	0.180	0.292	0.212
	0.489	0.248	0.174	0.227	0.223
	0.500	0.247	0.178	0.227	0.221
0/3	0.510	0.234	0.167	0.229	0.213
	0.499	0.228	0.161	0.229	0.222
	0.487	0.203	0.156	0.235	0.220
24/1	0.459	0.189	0.113	0.216	0.169
	0.528	0.208	0.115	0.233	0.167
	0.529	0.207	0.112	0.234	0.181
24/2	0.447	0.206	0.137	0.220	0.207
	0.491	0.207	0.142	0.237	0.234
	0.441	0.200	0.147	0.246	0.230
24/3	0.453	0.165	0.143	0.241	0.220
	0.481	0.188	0.136	0.232	0.231
	0.472	0.168	0.163	0.233	0.225

Matcha juice – absorbance

Storage [h]	Blank	Blender R.	Blender V.	Blender CO_2	Blender N_2O
0/1	0.357	0.408	0.404	0.519	0.380
	0.347	0.392	0.408	0.514	0.387
	0.340	0.412	0.404	0.518	0.373
0/2	0.346	0.429	0.403	0.494	0.374
	0.353	0.405	0.401	0.500	0.368
	0.340	0.410	0.405	0.501	0.363
0/3	0.350	0.410	0.404	0.498	0.377
	0.339	0.416	0.406	0.511	0.382
	0.345	0.402	0.397	0.491	0.375
24/1	0.331	0.430	0.403	0.516	0.369
	0.345	0.422	0.416	0.461	0.372
	0.347	0.431	0.398	0.483	0.367
24/2	0.346	0.421	0.393	0.518	0.362
	0.350	0.422	0.393	0.474	0.380
	0.344	0.431	0.391	0.527	0.370
24/3	0.346	0.433	0.407	0.485	0.360
	0.348	0.431	0.395	0.505	0.359
	0.352	0.431	0.406	0.476	0.374

Data of the catechin measurement

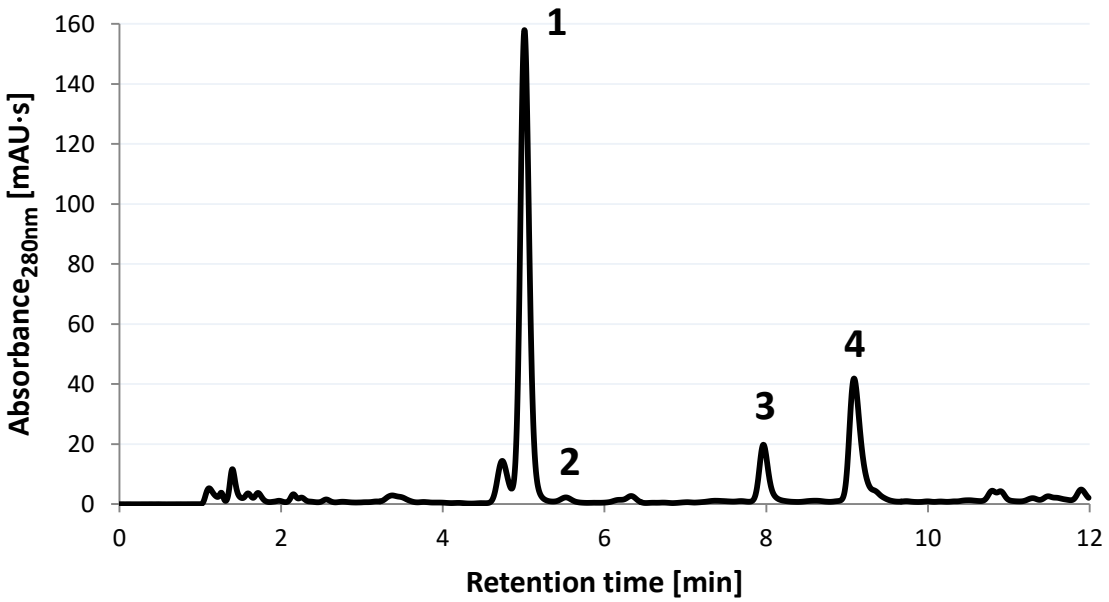


Figure 32: Sample chromatogram of the catechin measurement from matcha juice. 1: caffeine; 2: catechin; 3: epicatechin; 4: EGCG

Caffeine

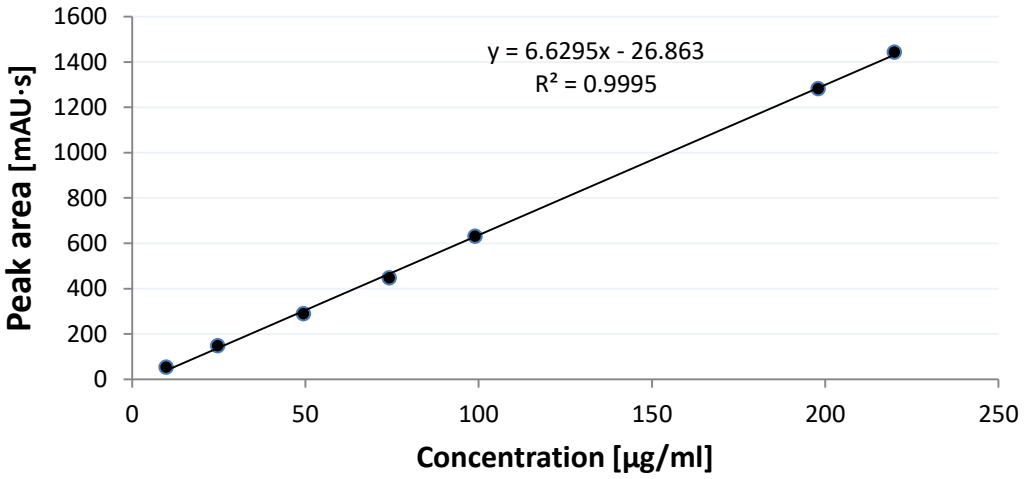


Figure 33: Calibration curve of caffeine. Calibration equation was used to calculate the caffeine content of the different juices.

Table 32: Peak areas obtained from the HPLC measurement of the caffeine content in matcha juice. Measurement was done in triplicates.

Matcha juice – peak areas of caffeine [mAU·s]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	1183.7	1247.9	1285.5	1325.0	1308.0
	1198.7	1249.1	1272.3	1325.4	1312.7
	1203.4	1221.0	1283.0	1310.5	1284.8
24	1214.2	1288.8	1271.9	1311.5	1282.1
	1173.2	1289.5	1275.5	1304.8	1273.0
	1174.6	1274.4	1260.5	1299.3	1273.5

Catechin

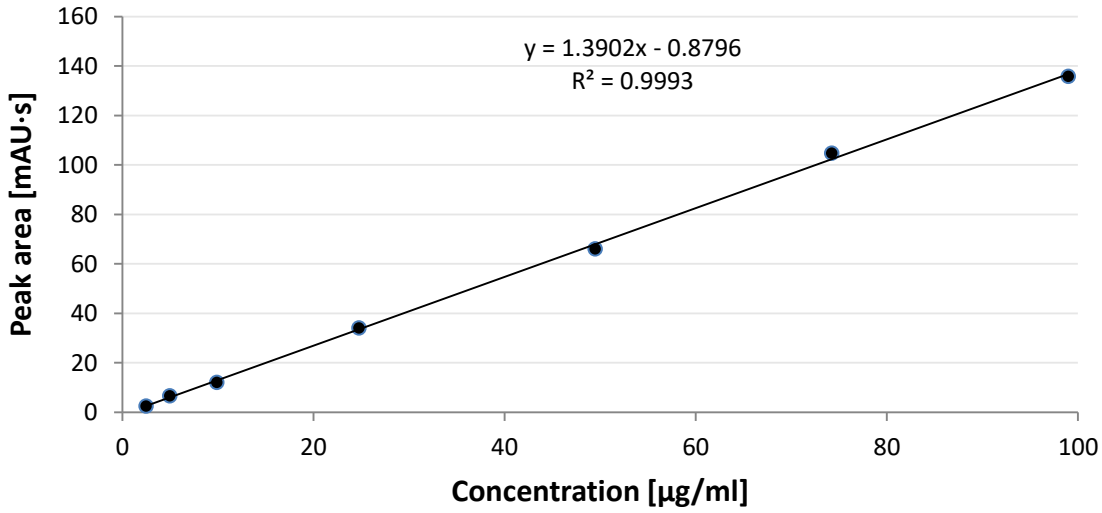


Figure 33: Calibration curve of catechin. Calibration equation was used to calculate the catechin content of the different juices.

Table 33: Peak areas obtained from the HPLC measurement of the catechin content of matcha juice. Measurement was done in triplicates.

Matcha juice – peak areas of catechin [mAU·s]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	-	21.0	18.0	-	23.9
	-	20.2	21.0	-	35.9
	-	19.3	20.8	-	35.8

24	-	22.1	19.3	-	35.3
	-	24.0	20.3	-	36.5
	-	20.8	23.0	-	36.3

Epicatechin

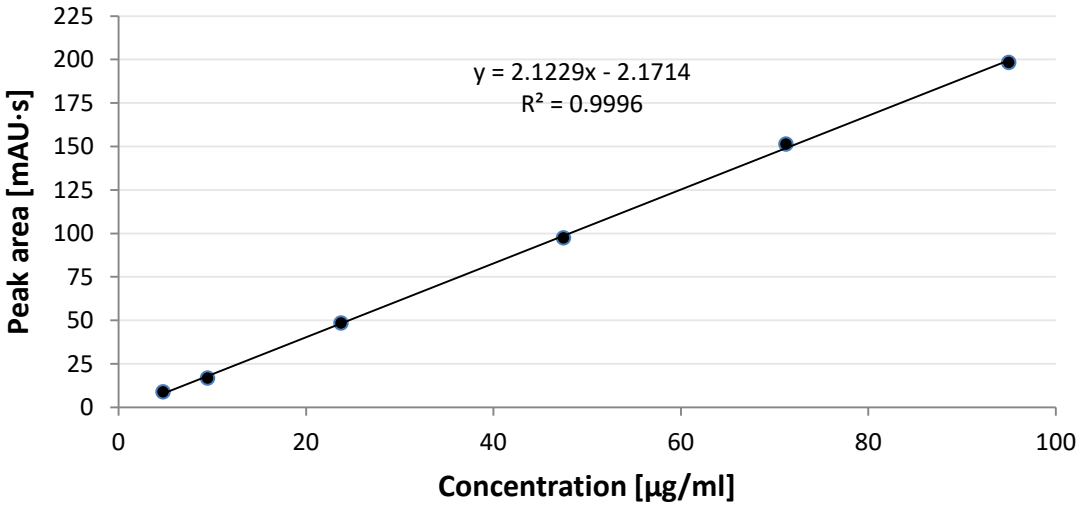


Figure 34: Calibration curve of epicatechin. Calibration equation was used to calculate the epicatechin content of the different juices.

Table 34: Peak areas obtained from the HPLC measurement of the epicatechin content in matcha juice. Measurement was done in triplicates.

Matcha juice – peak areas of epicatechin [mAU·s]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	135.4	153.6	152.8	163.6	158.9
	134.1	155.6	152.4	162.1	157.5
	137.6	153.8	154.3	169.3	153.0
24	138.8	157.5	153.6	164.6	153.0
	135.1	154.5	156.3	162.9	151.9
	135.7	154.5	152.6	156.5	151.3

EGCG

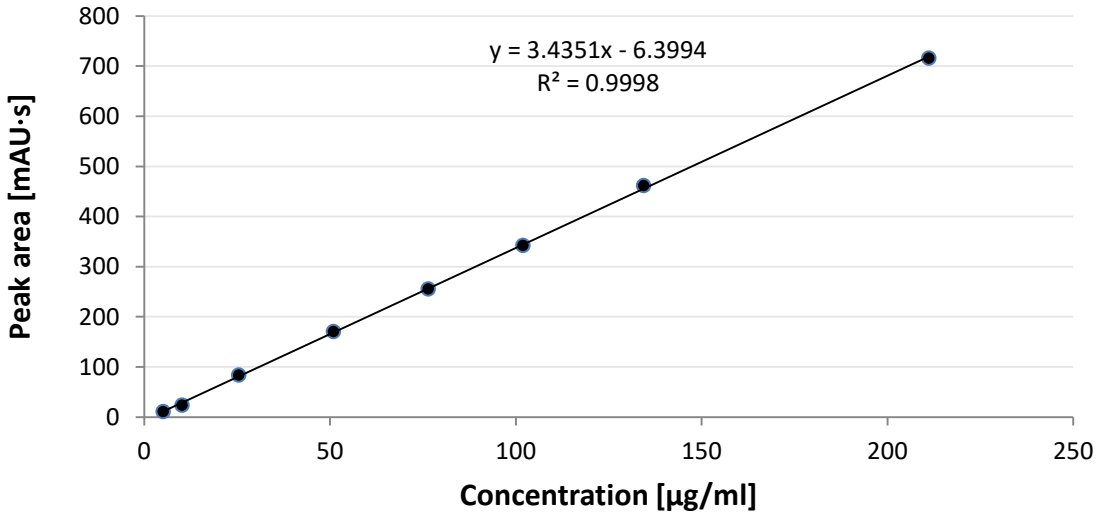


Figure 35: Calibration curve of EGCG. Calibration equation was used to calculate the EGCG content of the different juices.

Table 35: Peak areas obtained from the HPLC measurement of the EGCG content of matcha juice. Measurement was done in triplicates.

Matcha juice – peak areas of EGCG [mAU·s]					
Storage [h]	**Blank**	**Blender R.**	**Blender V.**	**Blender CO_2**	**Blender N_2O**
0	345.9	367.6	441.4	692.3	468.3
	379.3	342.1	385.9	703.2	416.2
	366.9	313.5	403.7	644.6	404.8
24	381.7	328.5	345.7	609.1	350.9
	277.1	346.8	352.2	610.8	303.6
	270.0	315.2	300.0	615.9	294.5

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