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Analytical and sensory characterization of black chokeberry (Aronia melanocarpa) juice

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Abstract

Black chokeberries (*Aronia melanocarpa*) have recently gained popularity for their health-promoting effects, which are linked to their exceptionally high concentrations of phenolic phytochemicals (proanthocyanidins, anthocyanins, phenolic acids and flavonoids).

However, very little research has been conducted regarding the flavor of aronia berries and products thereof. Therefore in this thesis a combination of instrumental and sensory analyses was used to characterize black chokeberry juice.

The volatile substances of Austrian aronia juices produced from the aronia cultivar “Nero” were analyzed with chromatographic methods and gas chromatography-olfactometry. This allowed the identification and semi-quantification of the substances relevant for their aroma. By means of multivariate analysis several juice samples were evaluated according to their similarities and dissimilarities. Some of those samples were further examined via sensory analysis in order to procure a description of their sensory properties and aroma profiles. With the aid of these different, yet complementary analyses a first-ever characterization of aronia juices from the cultivar “Nero” could be achieved.

Kurzfassung

Die schwarze Apfelbeere (*Aronia melanocarpa*) erfreut sich in letzter Zeit großer Beliebtheit aufgrund ihrer gesundheitsfördernden Wirkung, welche sich durch ihren hohen Gehalt an phenolischen sekundären Pflanzenstoffen (Proanthocyanidine, Anthocyane, Phenolsäuren und Flavonoide) begründen lässt.

Allerdings wurde das Aroma von Aroniabeeren aus sensorischer Sicht noch nicht ausreichend erforscht. Daher wurde in dieser Arbeit eine Kombination aus instrumentellen und humansensorischen Analysen verwendet, um eine Basischarakterisierung von Aroniasäften zu erhalten.

Die flüchtigen Substanzen von österreichischem Aroniasaft, der ausschließlich aus der Sorte „Nero“ hergestellt wurde, wurden chromatographisch und mittels GC-Olfaktometrie („Schnüffelanalyse“) untersucht. Dies erlaubte die Verbindungen, die für dessen Aroma ausschlaggebend sind, zu identifizieren und zu semi-quantifizieren. Mittels multivariater Datenanalyse wurden die verschiedenen Aroniasäfte bezüglich ihrer Gemeinsamkeiten beziehungsweise Unterschiede evaluiert. Des Weiteren wurde mit sensorischen Analysen eine Beschreibung der sensorischen Eigenschaften und Aromaprofile von einigen der Aroniasäfte erstellt. Mit Hilfe dieser unterschiedlichen, sich jedoch ergänzenden Analysenmethoden konnte eine allererste Charakterisierung von Aroniasäften der Sorte „Nero“ erzielt werden.

Contents

1 Introduction

Black chokeberries (*Aronia melanocarpa*) are called "superberries", as they distinguish themselves from other berries by their high content of phenolic phytochemicals (such as anthocyanins, flavonoids, proanthocyanidins and phenolic acids), which show great antioxidant potential.[1–3] Various clinical studies indicate that consumption of aronia has positive effects on diverse health problems, from hypertension[4] and high cholesterol[5,6] to diabetes[7], and it even displays anti-mutagenic[8,9] and anti-tumor activity[10–12].

This is why aronia berries and their juice have gained popularity in recent years in Austria; they have already become established in health-food shops and even some supermarkets' organic food sections. Areas under cultivation have also increased rapidly in Styria, where most of its produce is processed to juice.

In spite of many studies about black chokeberries' health-promoting effects, very little research has been conducted on the flavor profile of chokeberries. Therefore the aim of this thesis is to fill the knowledge gap that exists regarding the sensory character of aronia juice using sensory as well as analytical methods. This is supplemented with information on origin, appearance, composition and health-enhancing effects of black chokeberries. The theoretical background of the analytical and sensory methods employed is discussed, as well as aroma and how it is formed in fruits.

The juice samples used in this work were processed Austrian chokeberry juices made by different farmers from cultivar "Nero". The instrumental methods used were HS-SPME/GC-MS and HS-SMPE/comprehensive GCxGC-MS. The data obtained from the chromatographic measurements was evaluated with principal component analysis to sort the individual juices according to their similarities and dissimilarities. Additionally, the aroma-active components in aronia juice were analyzed with HS-SPME/GC-olfactometry and the results were linked to substances already identified with GC-MS. Furthermore, a trained sensory panel evaluated selected aronia juices using descriptive analysis and quantitative descriptive analysis methods. Finally, the data from all the aforementioned methods was used in conjunction in order to characterize the sensory properties of aronia juices.

2 General aspects

2.1 Black chokeberry - *Aronia melanocarpa*

Black chokeberry (*Aronia melanocarpa*) is a plant in the rose family (*Rosaceae*) and is native to the Eastern half of North America, from the Great Lakes region to the Appalachian Mountains.[13] It was introduced to Europe in the 18th century, where it was first cultivated in the Soviet Union and later brought to the west via Scandinavia.[14]

Aronia melanocarpa is a deciduous, perennial shrub of up to two meters height. In May to June the plant's small, white to pale pink flowers form clusters of 10 -30 in the shape of corymbs.[15] Due to their late bloom they are able to avoid most sudden spring frosts. These flowers develop into black-violet to black pomes in August and September. The development from flowers to fully ripe black chokeberries is depicted in Figure 1 to Figure 4. The pomes are about 6 - 16 mm in diameter and usually weigh between 0.32 g and 1.1 g, depending on variety.[16] They contain one to eight small, elongated, light-red to brownish seeds in a core that resembles an apple core.[14] Harvest is usually carried out mechanically using currant or grape harvesters.[17] Once the plants are mature, which takes about five years, 5-10 t/ha yield can be obtained.[18]

Figure 1: Aronia flowers in the shape of a corymb[19]

Figure 2: Green, unripe aronia fruits[20]

Figure 3: Different ripening stages of aronia berries

Figure 4: Ripe, black-violet aronia berries[21]

The common name “chokeberry” is thought to derive from the astringent, mouth-drying sensation when consuming the berries. [13]

Several cultivars of *Aronia melanocarpa* have been bred, such as “Nero”, “Viking”, “Aron”, “Hugin”, “Fertödi”, “Galicjanka” [16] and more. In Austria the cultivar “Nero” is grown, which is a shorter growing variety that was selected in the Czech Republic for commercial aronia berry production.[16,22]

2.1.1 Aronia cultivation in Austria

Although aronia has been cultivated in Europe for decades, cultivation of aronia in Austria did not start until 2001 with a mere six farmers in south-eastern Styria.[23,24] Therefore a few years ago the fruits were still mostly unknown. This changed rapidly however, and in 2015, roughly 70 agricultural holdings in Austria cultivated black chokeberries on approximately 250 hectares.[24] This number increased to 350 hectares in 2016 and thus aronia had climbed to the fifth most important type of fruit in Austria. The areas under aronia cultivation are expected to increase further.[25]

In 2015 the brand “Aronia Austria” was established by Austrian farmers as a quality seal. The products sold under this label are made solely from berries grown in Austria. Moreover, the manufacturers guarantee that further processing has to occur in Austria, with short transport routes and minimal time delay (processing within eight hours of harvest). Only fully ripe aronia berries are to be used.[23]

Most of these products are sold at farmers' markets or through farm-gate sales[24], however some of them are sold at Austrian health-food shops and even a few supermarkets.

2.2 Composition of *Aronia melanocarpa*

2.2.1 General composition

Aronia berries contain about 84 % water, 13.7-15.1 % carbohydrates, only 0.60-0.81 % proteins, 0.09-0.17 % fat and 0.37-0.49 % ash.[26] The carbohydrate portion includes 0.3-2 % phenolic phytochemicals, which will be discussed later in Chapter 2.2.3. [14]The amount of organic acids lies between 1-1.5 % of fresh berry weight, with L-malic acid being the most abundant organic acid, followed by quinic acid and succinic acid. Citric acid, iso-citric acid and shikimic acid were also found in lower concentrations.[14]

2.2.1.1 Vitamins and minerals

Aronia juice is rich in vitamins and minerals, especially vitamin K, C, E, A and β-carotene as well as potassium and iron. Literature values are listed in Table 1 and Table 2. [14,26]

Table 1: Vitamin content of aronia berries and aronia juice

Vitamin	µg/100 g fresh berries[26]	µg/100 mL aronia juice[14]
B1	50	18
B2	60	20
Niacin	340	300
B6	55	28
Pantothenic acid	220	279
Folic acid	n/a	3
A	n/a	77
C	20000	13700
E	n/a	1420
K	n/a	24

Table 2: Mineral and trace element content of aronia berries and aronia juice

Element	mg/100 g fresh berries[26]	mg/100 mL aronia juice[14]
Na	2.6	0.5
Mg	16.2	14
K	218	285
Ca	32.2	15
Mn	0.175	0.7
Zn	0.147	0.13
Fe	0.93	0.4
Cu	0.044	0.05

2.2.2 Amygdalin

Amygdalin is a cyanogenic glycoside and is present in black chokeberries at concentrations of about 20 mg/100 g fresh berries.[27] While amygdalin is present as glycoside in the intact plant, this substance can potentially be harmful. Enzymatic hydrolysis releases benzaldehyde and volatile, toxic hydrogen cyanide. The largest portion of amygdalin is present in the seeds, which is common for plants in the Rosaceae family (bitter almonds and the seeds of apricots, plums or apples are well known examples)[28].

When aronia pomace was sieved, the fraction with a size of up to 0.8 mm, which consisted of only shredded skins and fruit flesh, contained 7.7 mg amygdalin per 100 g dried berry weight. In contrast, the fraction with a size of 1.25 mm to 2 mm, containing the seeds, showed 185.7 mg/100 g dry weight. Mixed fractions showed amygdalin levels in-between these two values.[29] No data on the amygdalin level in commercially processed aronia juice has been released so far.

However, the consumption of aronia juice is harmless to health.[30] Hydrogen cyanide has a boiling point of 25.9°C.[31] Therefore, most of the formed HCN will evaporate before the juice is bottled, especially since the juice is pasteurized in the majority of cases. Likewise eating fresh berries is also non-hazardous to health. Since the fresh berries are very astringent, it is unlikely that large amounts of aronia berries will be consumed at once. Furthermore it is doubtful that a great number of the small seeds will be crushed while chewing.[30]

2.2.3 Phenolic phytochemicals in *Aronia melanocarpa*

Phenolic phytochemicals are ubiquitous secondary plant products. This is an extremely heterogeneous group of substances, however their common feature is the structural motive of phenol. In plants they fulfill various important tasks, such as protection against free radicals, pigmentation to attract pollinator animals, repelling vermin, defense against pathogens and more.[32]

In comparison to other fruits, aronia berries distinguish themselves through their exceptionally high content of phenolic compounds. Quantification of the average phenolic content in berries is difficult, however, and scientific results fluctuate greatly.[2] Naturally these values can vary depending on the cultivar, harvest date, location, maturation, climate and other factors. Furthermore, the analysis of phenolic compounds may be problematic, as they can be prone to interferences, which may lead to underestimation or overestimation.[33]

2.2.3.1 Proanthocyanidins

An abundant class of phenolic compounds in aronia berries are procyanidins, which are a subgroup of proanthocyanidins/condensed tannins. Proanthocyanidins are condensed flavan-3-ols, and if built from (-)-epicatechin and (+)-catechin (see Figure 5 and Figure 6) monomers only, they are called procyanidins. Oligomeric procyanidins are built from 2-10 monomers, polymeric procyanidins are made from >10 units.[34]

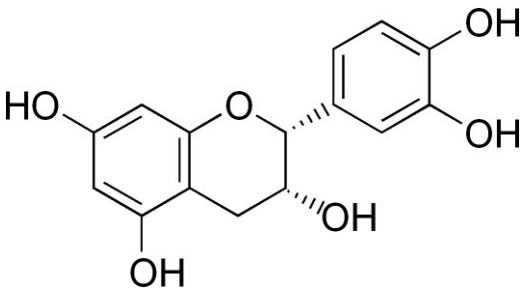

Figure 5: (-)-epicatechin[35]

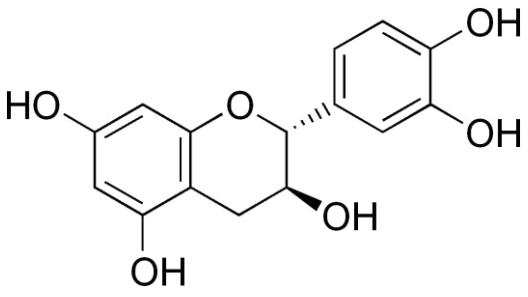

Figure 6: (+)-catechin[35]

Various studies show that the proanthocyanidins in aronia are comprised of only procyanidins. They are B-type and built from mainly (-)-epicatechin monomers.[36,37] The type refers to the location of the carbon-carbon bond between the monomer-units. B-type

proanthocyanidins have their bond between position 4 of the extending and position 8 (or less commonly 6) of the terminal monomer (examples shown in Figure 7 and Figure 8).[38]

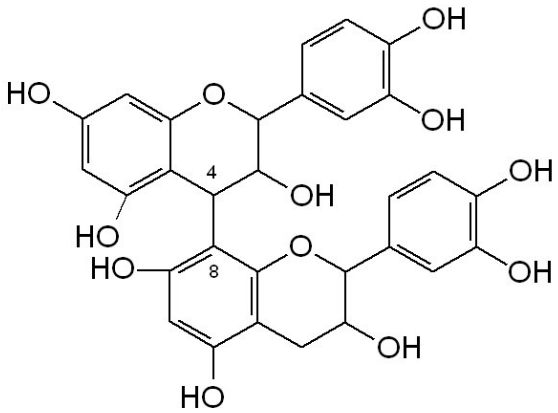

Figure 7: Dimeric 4→8 B type proanthocyanidin[39]

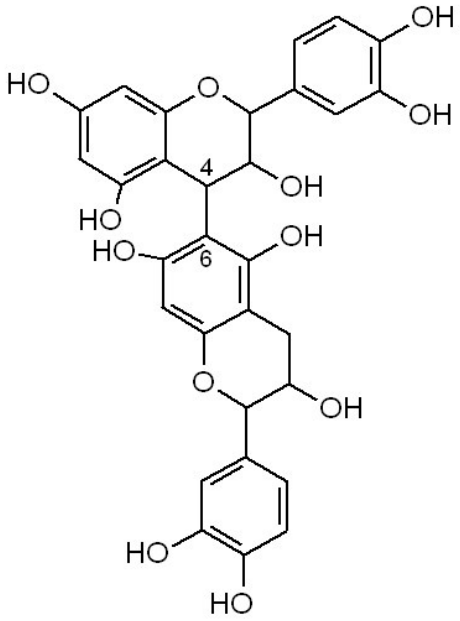

Figure 8: Dimeric 4→6 B type proanthocyanidin[39]

In chokeberries the concentrations of total procyanidins has been determined as 664 mg/100 g to 845 mg/100 g fresh berry weight. (Wu 2004; Wilkes 2014) It was determined that 82 % of total proanthocyanidins were polymeric procyanidins (>10 units).[37]

Proanthocyanidins are molecules which can precipitate proteins. Upon consumption they interact with the proteins present in saliva and therefore leave an astringent tactile sensation when consumed. This is likely the reason why aronia berries and their products have such a high astringency.[40]

2.2.3.2 Anthocyanins

Anthocyanins are plant pigments and are responsible for the almost black appearance of the aronia berries. Aronia contain primarily cyanidin glycosides: The most abundant are cyanidin-3-galactoside and cyanidin-3-arabinoside, in smaller concentrations cyanidin-3-xyloside and cyanidin-3-glucoside are also present.[36,37,41,42] In cultivar "Nero", total anthocyanins were estimated as 549 mg/100 g fresh berry weight. [43] The red color of the

aronia fruit juice is dominant - so much that even after a dilution of 1:100 decoloration was not observed.

2.2.3.3 Other phenolic phytochemicals

Hydroxycinnamic acids are present in moderate quantities in aronia. Their main representatives are chlorogenic acid and its isomer neochlorogenic acid. In cultivar “Nero”, chlorogenic acid and neochlorogenic acid were determined as 53 mg and 72 mg/100 g fresh berry weight, respectively.[43]

Total flavonols were estimated in concentrations of 35 mg/100 g fresh fruit[41] and >71 mg/100 g fresh fruit[44]. Depending on the literature, from 3 [36] to 6 different flavonols[44] were identified.

Accordingly, aronia is of interest mainly for two reasons, both related to its high polyphenolic content:

a. Due to their high concentration of anthocyanins they are an interesting source for the production of natural color additives in the food industry.[42]
b. Aronia berries and their products show high antioxidant capacity thanks to their rich phenolic phytochemicals, which are related to their health enhancing character. These benefits will be discussed in greater detail in Chapter 2.3.

2.3 Health benefits of *Aronia melanocarpa*

Aronia has long been renowned in folk medicine. The Native American Potawatomi tribe steeped aronia to make an infusion, which was a cold remedy. Other Northeast tribes were also aware of chokeberries as food and medicine.[13,45] In Russia *Aronia melanocarpa* is a medicinal plant and has been used in the time of the ex-Soviet Union to treat various diseases and conditions, among them hypertension, scarlet fever and somatic damage caused by radiation exposure.[46]

In recent years the potential of black chokeberries has been recognized and research into their health-promoting effects has increased. For many of these positive properties phenolic phytochemicals are deemed responsible. However, not just the abundance but also the specific composition and distribution pattern of phenolic phytochemicals might play an important part in the health-beneficial and disease-preventive attributes of *Aronia melanocarpa*. Below is a summary of some of the potential health-promoting activities:

2.3.1 Antioxidant potential

Oxidative stress is the excessive formation of free radicals or reactive oxygen species in the body, which can cause toxic effects and damage cells. This is thought to play a role in the development of various diseases including but not limited to cancer[47], arteriosclerosis[48] and neurodegenerative diseases[49].

Phenolic phytochemicals show great antioxidant potential. Black chokeberries contain high overall concentrations of different phenolic phytochemicals (see chapter 2.2.3). Several established *in vitro* assays, like oxygen radical absorbance capacity (ORAC)[37,1] or Trolox equivalence antioxidant capacity (TEAC)[36,42,3] have been used to examine the antioxidant capacitiy of aronia berries and aronia products. In a review written by Kulling and Rawel [2] the results of several publications have been summarized: Aronia has the highest antioxidant capacity measured as ORAC compared to seventeen other fruits, among them elderberry, lingonberry, blackberry and black currant.

2.3.2 Positive effect on cardio-vascular diseases

Since radical oxygen species and oxidative stress can contribute to the pathogenesis of cardiovascular diseases, the fact that chokeberries excel in radical scavenging and also display extraordinary antioxidant activity makes them an interesting field of research. They are thought to contribute to protect against cardiovascular disease risk factors, such as hypertension, diabetes mellitus and high cholesterol, and positively influence existing medical conditions. This is supported by many *in vitro*[3,50,51] and *in vivo* studies[4–7].

2.3.3 Anti-mutagenic and cytostatic and chemopreventive activity

Mutagens damage genetic material and this can cause mutations, which may lead to the development of cancer. In a study by Gąsiorowsk et al. (1997)[8] anthocyanins extracted from aronia demonstrated anti-mutagenic properties against the polycyclic aromatic hydrocarbons (PAH) benzo(a)pyrene and 2-aminoflorene. PAHs are ubiquitous carcinogenic and mutagenic substances that form for example during incomplete combustion and are therefore present in e.g. smoked meat.[8] In animal studies on rats aronia nectar showed an inhibitive effect on the endogenous formation of carcinogenic N-nitrosamines.[9] Research also indicates anti-proliferative and protective properties against cancer, especially colon cancer in vitro[12] and in animal studies[10,11] For example an anthocyanin-rich extract from aronia suppressed the proliferation of human HT-29 colon cancer cells and also demonstrated chemopreventive activity.[12]

2.3.4 Gastro-protective effect

In a study conducted by Matsumoto et al.[52] aronia extract was administered to rats which suffered from ethanol-induced gastric hemorrhagic lesions. Doses of 2 g/kg body weight black chokeberry extract suppressed the mucosal damage to less than 30% in comparison to the damage observed in the control group. It appears that the antiulcerative activity is linked to cyanidin derivatives.[52]

2.4 Overview of flavor, aroma and taste

Foods consist of a complex mixture of many different chemical substances. All human senses are involved in the perception of the physiochemical properties of food. What is referred to as flavor describes the overall oral sensory stimulus when food is consumed. This sensation is comprised of the synergy of odor, taste and texture (mouthfeeling). This interaction is schematically represented in Figure 9. Flavor plays vital part in quality and consumer acceptance of fresh and processed fruits and vegetables.[53,54]

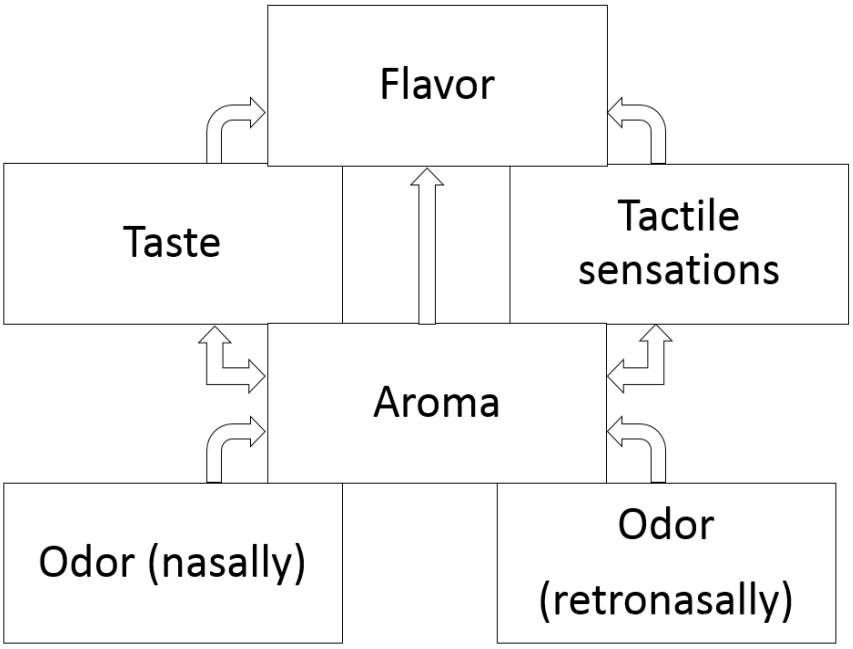

Figure 9: Schematic diagram of the interaction of odor, taste and texture to the perception of flavor

2.4.1 Taste

Taste is the sensory stimulus of usually nonvolatile substances detected by the taste buds on the tongue. The five commonly recognized taste perceptions are sweet, sour, salty, bitter and umami.

In fruits the most important taste stimulus is sweetness, which is caused by sugars like fructose, glucose or sucrose. But also polyols like sorbitol or xylitol can be found in some fruits. A moderately sour taste is also important; excessive sourness, however, is associated with unripe fruits. Responsible for sour perception are mostly organic acids, such as citric acid, malic acid and tartaric acid. Bitterness on the other hand is not associated with many fruits, however there are exceptions like grapefruits. In general the perception of

bitterness can be evoked by numerous and diverse substances, such as phenols, salts, amino acids, peptides, alkaloids as well as gylcosides, thiocarbamates and nitrogenous compounds.[55]

2.4.2 Tactile sensations

Flavor also includes the texture and mouthfeel of food, which plays an important role in consumer preference and acceptance. Texture reception is based on the interaction of the peripheral nervous system's sensory and motor nerves with the central nervous system. Simply put it is the qualities of food items that can be felt with the tongue, oral cavity, and teeth. The oral sensory system can be grouped in four major sensory functions: [56]

- Discriminative touch (recognition of shape, size and structure of food)
- Proprioception (sense of relative position and movement)
- Nociception (pain perception due to oral or nasal tissue damage)
- Temperature sense (warm and cold)

Especially important for the flavor of black chokeberries are their astringent properties. This tactile stimulus is detected by mechanoreceptors, therefore related to touch perception. It is described as "drying out the mouth", leaving a rough and almost "furred" sensation in the oral cavity. On a molecular level, the astringent compound precipitates or aggregates proteins in the saliva. It is suspected that the reduced lubrication, caused by the precipitation of saliva proteins, induces the rough and dry sensation upon consumption of the astringent.[57]

2.4.3 Aroma

Aroma encompasses the sensory stimulus caused by aroma substances, which can be perceived nasally and retronasally. "Nasally" refers to the odor impression received by the olfactory receptors when volatile compounds are inhaled through the nose. "Retronasally" stands for the indirect reception, when volatiles are released while chewing and transported through the throat to the olfactory bulb.[53] Since the food in the mouth is heated to body temperature, compounds with a lower volatility are released as well. Additionally the contact with enzymes and microflora in saliva/oral cavity can alter the aroma.

The characteristic odor of an individual fruit usually is a complex blend of dozens, up to hundreds of organic, volatile substances. They are present in low concentration (10-15 mg/kg) compared to other constituents.[53] These compounds are called aroma substances/aroma-active compounds and show diverse reactivities and polarities.

In order for substances to contribute to aroma, they must be volatile and perceivable by humans. Furthermore they have to be present above a certain threshold.

The so called “odor detection threshold” is the lowest concentration in a certain medium at which a human can detect a certain substance by its smell. At this level a human can perceive something, however the stimulus cannot be identified. The “odor recognition threshold” on the other hand refers to the concentration at which qualitative perception is possible. At the “difference threshold” the intensity of perception increases further with increasing concentration and differences between samples can be detected. When all receptors are saturated, an increase in physical concentration cannot increase the stimulus any more. This point is the “terminal threshold”.[58] These thresholds vary depending on the substance and the medium. For this reason highly odorous compounds can be perceived in low concentrations and vice versa. Thus, some substances might significantly influence the aroma of a certain food item, even though their concentration is only in the ng/kg range.[54] Naturally these thresholds can also differ from person to person, due to different genetic or environmental influencing factors.

2.4.4 Odor Activity Values

To estimate the relevance of an individual substance to the overall odor impression of a sample, the odor activity value (OAV) can be calculated using the formula shown in Equation 1.

$$OAV = \frac{c_x}{OT_x}$$

Equation 1: Odor Activity Value

OAV	Odor Activity Value
c_x	Concentration of substance X in the sample
OT_x	Odor Threshold of substance X

It is the ratio of the concentration to the odor threshold of the substance X (in a similar or identical matrix). To make a contribution to the odor, a substance has to be present at a concentration higher than its odor threshold. Substances with an OAV lower than 1 are expected to have an almost negligible influence on the sensory attributes, if any at all.[59]

However, even if a compound is present at concentration levels above its odor threshold, there are several factors that can influence the perception, such as antagonistic, synergistic and matrix effects. [59]

In most cases it is not a single compound alone that is responsible for the sensory identity of a sample. Most of the time the combination and interaction of a variety of substances at appropriate concentrations creates an individual aroma. For example, of the 850 volatile compounds found in coffee, the coffee aroma is composed of the interaction of about 40 odor-active substances (none of which smell like coffee).[53]

2.4.5 Flavor formation in fruits

Flavor compounds that are formed in the intact fruit during growth, maturation and ripening are referred to as primary flavor compounds. In most fruits however, these primary flavor compounds are mostly formed in the course of the ripening stage when metabolism stops and catabolism begins. Catabolic and anabolic pathways and numerous interactions between them yield many different volatile compounds. Secondary flavor compounds on the other hand are not present in the intact fruit and are only formed during or after damaging cell walls. This includes but is not limited to the processing of fruits through mechanical means and heat treatment. The reactions involved are diverse, comprising for example autoxidation or enzymatically catalyzed reactions. Consequently, the flavor composition in fruits and fruit products varies depending on the genetic composition of the plant, but also on ripeness and processing.[60]

An overview of the metabolic pathways of volatile substances found in fruits, as well as their non-volatile precursors is given in Figure 10.[60]

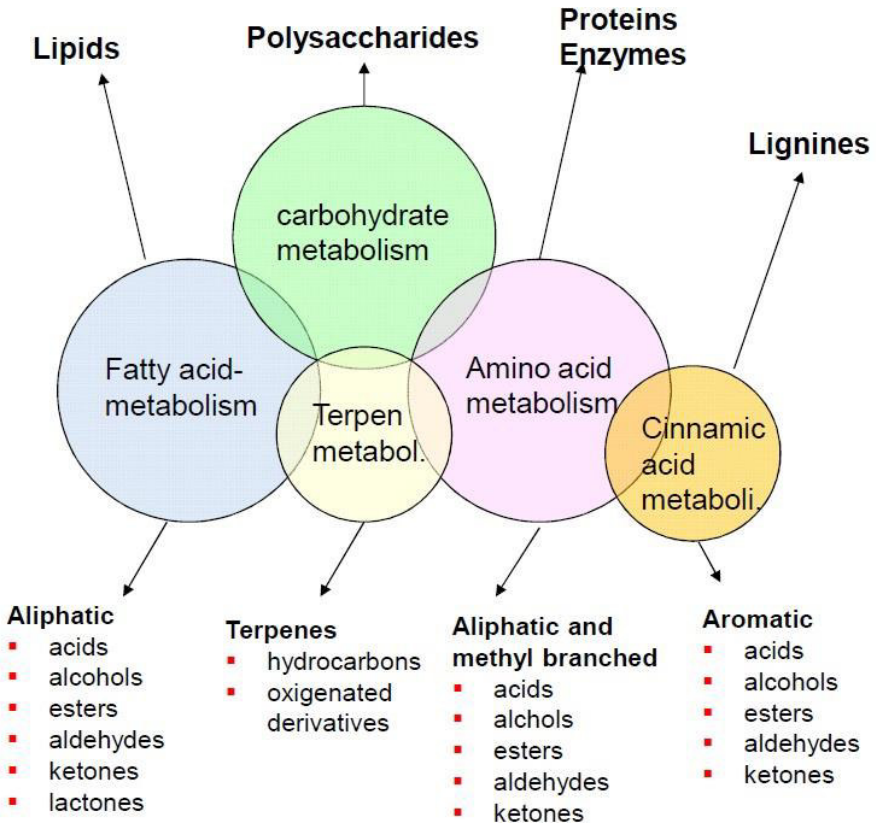

Figure 10: Overview of the metabolic pathways of volatile compounds and their non-volatile precursors[60]

2.4.5.1 Fatty acid metabolism

As precursors, fatty acids can form many different volatile substances, such as saturated and unsaturated acids, alcohols, esters, ketones, aldehydes and lactones. The two pathways involved are β-oxidation and the lipoxgenase (LOX) pathway.

β-oxidation takes place in the intact fruit and is an important pathway for the formation of various odor-active compounds, but the specific processes are not well understood.[61] Degradation of unbranched fatty acids may lead to saturated and unsaturated metabolites, like acids, alcohols, aldehydes, esters, lactones and methylketones.

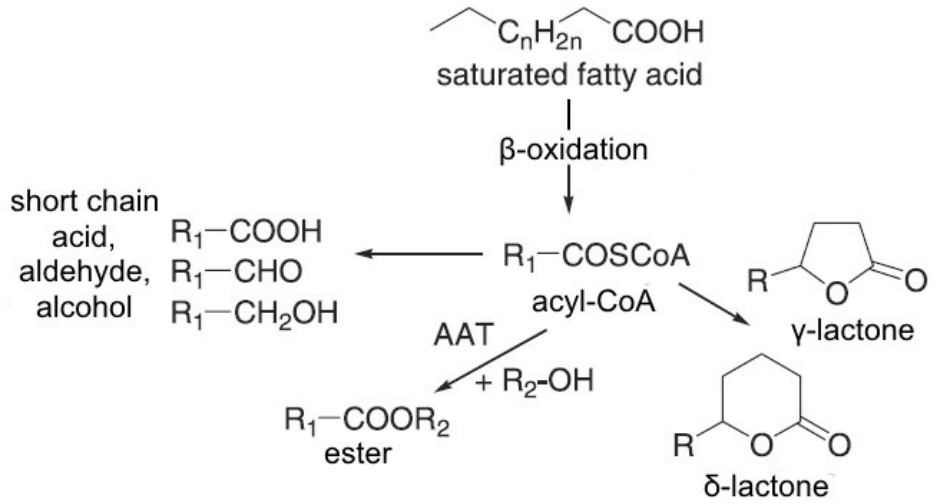

Figure 11: Formation of acids, aldehydes, alcohols, esters and lactones by β-oxidation (from [61])

The LOX pathway is believed to occur mainly upon damaging the cell walls of fruits, since oxygen from the surrounding air can enter the system. However, increased permeability of the membrane in the course of fruit ripening might also lead to activation of the LOX pathway. The LOX pathway leads to the formation of C6 and C9 aldehydes, both saturated and unsaturated. Depending on the enzyme systems present in the fruit, the initial product can further be altered and other volatile aroma substances can be formed. An example for the LOX pathway is shown in Figure 12, in which the formation of *(Z)*-3-hexenal, *(Z)*-3-hexen-1-ol, *(E)*-2-hexenal and *(E)*-2-hexen-1-ol from linolenic acid is illustrated.[62]

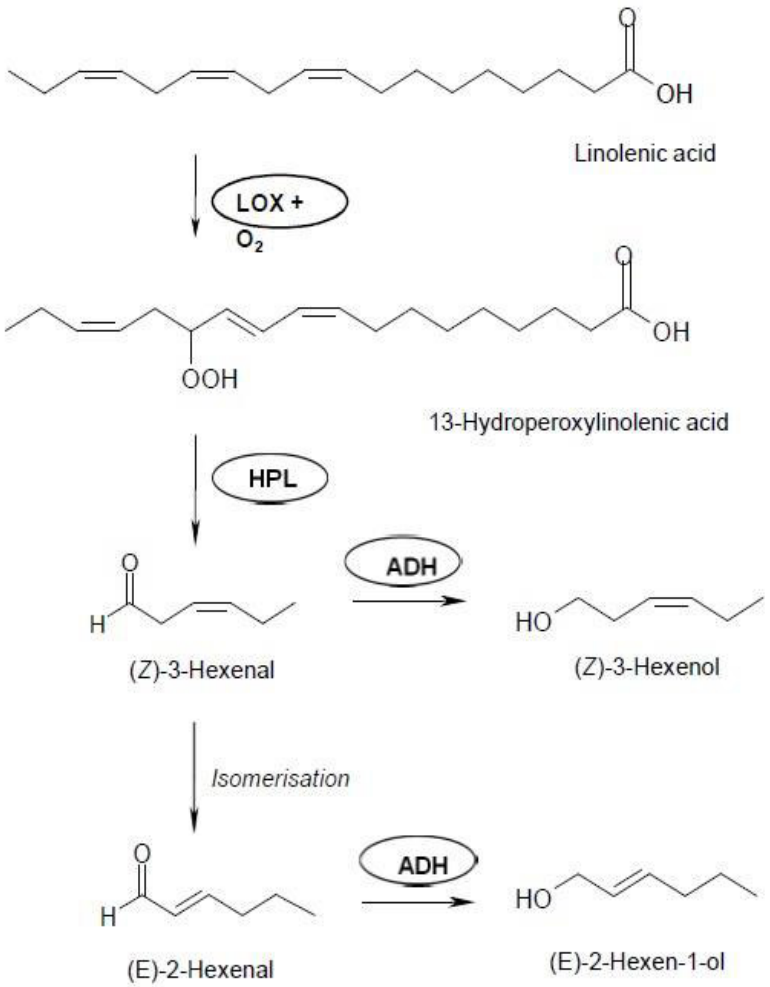

Figure 12: Formation of (Z)-3-hexenal, (Z)-3-hexen-1-ol, (E)-2-hexenal and (E)-2-hexen-1-ol from linolenic acid through the LOX pathway (LOX: lipoxygenase, HPL: hydroperoxide lyase; ADH: alcohol dehydrogenase). [62]

2.4.5.2 Amino acid metabolism

Free amino acids are the non-volatile precursors for aroma substances. Enzymatic de-animation of the amino acid, followed by decarboxylation forms an aldehyde. This aldehyde, which has lost one C-atom compared to the amino acid, can form other aroma compounds in follow-up reactions. Some aldehydes and their amino acid precursors are listed in Table 3 and the amino acid pathway is demonstrated using the example of leucine in Figure 13. [62]

Table 3: Aldehydes formed via amino acid metabolism from their amino acid precursors

Amino acid precursor	Aldehyde
Valine	2-Methylpropanal
Isoleucine	2-Methylbutanal
Leucine	3-Methylbutanal
Phenylalanine	Phenylacetaldehyde
Methionine	Methional

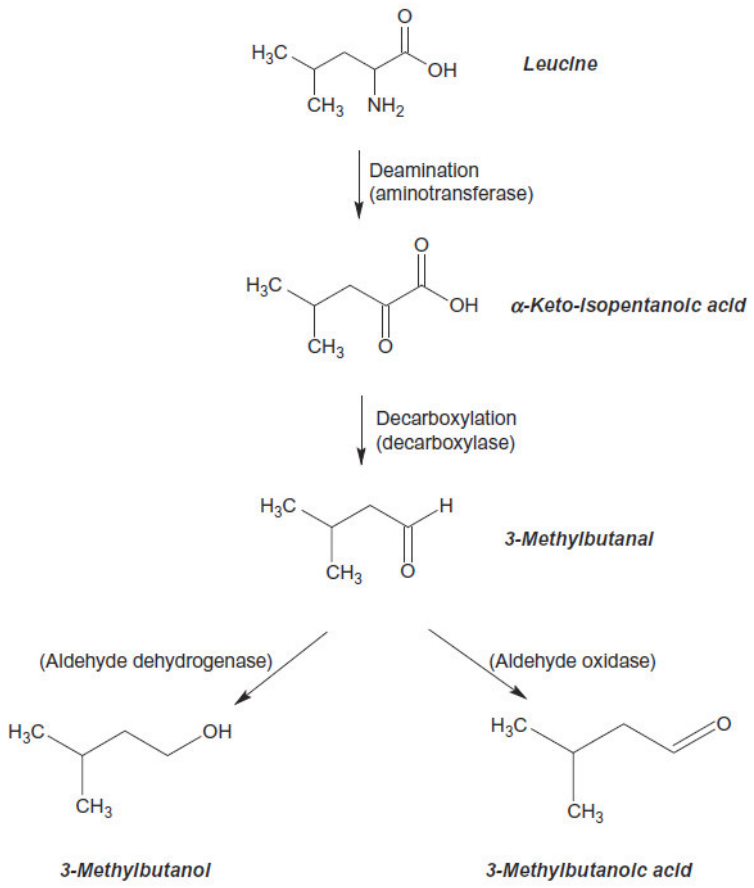

Figure 13: Amino acid pathway illustrated using the example of leucine [62]

2.4.5.3 Carbohydrate and terpene metabolism

Terpenes are built from isoprene subunits (five C-atoms). The condensation of two isoprene units yields monoterpene, consequently consisting of 10 C-atoms. Sesquiterpenes consist of three isoprene units, therefore 15 C-atoms. Cyclization and subsequent rearrangement forms a multitude of terpenes. Many terpenes contribute to the aroma of fruits. Important monoterpenes are limonene, camphene, α-thujene, γ-terpinene, α-pinene, β-pinene, myrcene and ocimene; noteworthy oxygenated monoterpenes are linalool, menthol and geraniol; examples for sesquiterpenes are β-farnesene, humulene and farnesol to name a few.[62]

Norisoprenoids, commonly named irregular terpenes, are oxidative degradation products of both acyclic and cyclic carotenoids. The degradation takes place during senescence of the fruit and/or after damaging the cells. Examples for norisoprenoids are β-damascenone, β-ionone, 6-methyl-5-hepten-3-one and vitispirane. [62]

2.5 Fruit juice

Fruit juice is a regulated product and the specifications that have to be met are defined by the Austrian fruit juice regulation[63]. This has since been amended on some points by the directives BGBl. II Nr. 441/2010[64] and BGBl. II Nr. 206/2013[65] of the European Parliament.

The regulation prescribes that juice is made from the palatable parts of ripe fruits and that the juice should retain the authentic taste and aroma characteristics of this fruit. One or more kinds of fruits may be used. Aroma, pulp and fruit cells may be added to fruit juice of the same kind. However, no acid or sugar may be added.

Not from concentrate (NFC) fruit juice is 100% juice, no concentration steps or addition of water is allowed before bottling. When fruit juice concentrate is rehydrated with water, the product is called “fruit juice from concentrate”. In contrast, fruit nectar contains pulp, and the addition of sugar up to 20% of the end-product’s total weight, as well as addition of water is permitted. [63]

In the course of this work only NFC fruit juice of *Aronia melanocarpa* has been used.

3 Methods

3.1 Head Space – Solid Phase Micro Extraction (HS-SPME)

Solid Phase Micro Extraction is used in sample preparation to extract and concentrate volatile analytes from a sample. A 1-2 cm long fused silica fiber, which is the stationary phase, is exposed to the sample. The analytes ab- or adsorbs onto the stationary phase. The extraction is done by placing the sample into a vial and sealing it with a septum cap. The cap is then pierced with a needle and the fiber is inserted into the vial.

In Head Space – Solid Phase Micro Extraction (HS-SPME) the fiber is not directly in contact with the sample, only with the gas phase above it. On the other hand, if the fiber is directly dipped into the liquid sample, this method is referred to as DI-SPME (direct immersion).

There are different types and thicknesses of fiber coatings and various combinations thereof are available commercially. The right choice for a given analytical task is crucial and recommendations are usually given by the manufacturer.[66] A non-polar fiber coating like e.g. PDMS (Polydimethylsiloxane) is used to investigate non-polar analytes. Conversely, for polar analytes polar fiber coatings like Carboxen/divinylbenzene (CAR/DVB) are used. This is based on the principle “like dissolves like”. For odor and flavor compounds a mixed type of coating is recommended, namely DVB/CAR/PDMS, which combines non-polar (PDMS) and polar (DVB/Carboxen) materials. That is why it is suited for a wide range of analytes (C3-C20) and analyte polarities.[66,67]

HS-SPME is well suited for the analysis of odorants, because only volatile compounds will ab- or adsorb to the fiber. To extract analytes with a lower volatility, the vial can be heated. Extraction can further be improved through modification of the matrix, such as the addition of salts or adjustment of pH. The amount of analyte extracted by the fiber is proportional to the amount of analyte in the gas phase above the sample. After extraction, the fiber is retracted into its protective covering and removed from the vial. Subsequently the analytes are desorbed, which is often done by heating the fiber in the injection port of the separating instrument.

The advantages of HS-SPME are its easy handling, possibility for automation and fast, solventless extraction. It is also a selective, sensitive and rather inexpensive method. HS-

SPME is commonly coupled to GC, GC-MS and HPLC. [68,69] This is why SPME is a valuable sample preparation technique particularly in the analysis of volatile and semi-volatile organic compounds for biological, environmental and food chemistry.[70,71]

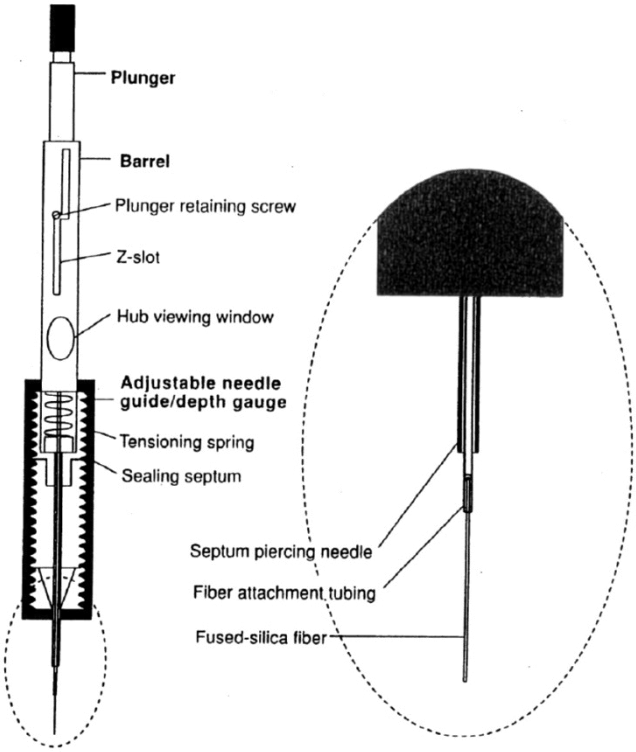

Figure 14: Schematic of an SPME fiber [69]

3.2 GC-MS-(EI)

3.2.1 Gas Chromatography (GC)

Gas chromatography is a routinely employed chromatography technique used to separate volatile and vaporizable compounds. The compounds are transported through the chromatographic column by the mobile phase or carrier gas, which is an inert gas such as nitrogen, helium or rarely hydrogen.

The stationary phase is located inside a fused silica or sometimes metal tube, also called a chromatographic column. Many different types of columns are available in varying lengths, film thicknesses and diameters. The most common stationary phases are polymers with a high molecular weight (such as polysiloxanes or polyethylene glycols) which coat the inside wall of a capillary column. These capillary columns are especially useful in the analysis of trace amounts of organic substances, as they possess excellent separation capabilities. Less frequently used column types are packed columns, which are filled tightly with packing material. They are shorter and thicker in comparison to capillary columns. Since they have higher capacities, larger amounts of sample can be injected into packed columns, but they display poorer separation efficiency.[72,73]

The analyte compounds are separated rough their interactions with the stationary phase. This process is based on continuous absorption into and extraction out of the stationary phase. The distribution between the mobile and the stationary phase is determined by (i) the solubility of a substance in the stationary phase, (ii) the substance's boiling point and (iii) the temperature of the column. Compounds that are more strongly retained in the column will elute later with a higher retention time and vice versa. The higher the boiling point of a substance, the smaller the portion in the mobile phase. Since the mobile phase facilitates the transport of the compound through the column, a low boiling point results in a long retention time. When the temperature of the column is increased, analyte molecules have higher kinetic energy and therefore their statistical distribution in the mobile gas phase is also increased, which leads to a faster elution and better separation. This is why a temperature program is often used, during which the temperature is increased at a defined rate over time. To ensure precise temperature control the column is located in an oven.[74,73]

3.2.2 Flame Ionization Detector (FID)

One possible detector that can be used with GC analysis is a flame ionization detector (FID). Oxidizable eluents from the column are burned in the FID in a H_2/O_2 flame. Meanwhile the ions formed are detected. However, as all substances are combusted, no further analysis is possible.

3.2.3 Mass Spectrometry – Electron Ionization

GC-MS analysis is one of the standard techniques to analyze volatile compounds in e.g. foodstuffs. The hyphenated technique stands for a GC instrument coupled to a mass spectrometer.

After the passage through the GC column the analyte compounds have to be identified. One of the possible methods (among others) of detection is using a mass spectrometer.

A mass spectrometer consists of a vacuum system, an ion source to create ions, a mass analyzer that sorts the ions by their mass-to-charge ratio and lastly an ion detector to record the ions formed. [73]

While gas chromatography works at atmospheric pressure, the pressure has to be reduced for ionization and mass separation (to around 10^{-4} Pa). Therefore an interface between the gas chromatograph and the mass spectrometer has to be installed. To remove gas molecules and introduce sample molecules into the mass spectrometer, various interfaces have been developed, for example the Jet orifice interface or the direct split interface.

A commonly employed “hard” ionization technique is Electron Ionization, short EI. The stream of gaseous sample molecules is bombarded with electrons created from a heated wire filament. The electrons are attracted to an electrode with an appropriate potential and are accelerated to a standardized energy of 70 eV. Upon collision the energy of an electron is transmitted to the molecule. When the ionization energy of the molecule is lower than the energy of the electron, the sample molecule will lose an electron. This process creates a cation with the mass of the molecule and a charge of +1. However, in the majority of cases more ion fragments than just the molecular ion are formed due to the high energy (70 eV) supplied by the electron. These other fragments with a smaller mass form because weak bonds are broken. Depending on the temperature and the energy of

the bombarding electrons, these fragments are characteristic and also reproducible, given that the same conditions are provided. The comparison of the obtained mass spectrum to other mass spectra in libraries or of an authentic reference substance is one way molecules can be identified. In aroma analyses this is often used in combination with the retention index, which is explained in chapter 3.5.1, to identify an unknown substance.[73,75,76]

The different masses of the ions that were formed are then utilized to sort them by their mass/charge ratio. Several mass analyzers have been developed, such as the magnetic sector, time of flight or quadruple mass spectrometer. The latter is often used in GC-MS instruments, because it has high sensitivity and scans rapidly.[77]

In quadrupole mass analyzers, the ion beam passes through the center of four parallel rods. An alternating current is applied to two opposing rods, and simultaneously a direct current is applied to the others.[78] In this manner the trajectory of ions in the center of the rods can be influenced. Only ions with a specific m/z ratio will have the right trajectory and arrive at the detector, all the other ions will be ejected.[79]

3.3 GC-O

Gas Chromatography-Olfactometry (GC-O) is a technique that uses a human assessor to determine not only the presence of an odor-active compound but also the odor impression and quality at the given concentration in the sample, the duration of this odor, and its intensity.[80]

The human nose is used like a sensitive detector after the individual components of a sample have been separated by the GC. Often the eluate gas stream is split, so that one portion is sniffed by the assessor and the other is carried to a conventional detector, such as a flame ionization detector (FID). The assessor sniffs the eluate through a specially designed sniffing port, which is usually a glass cone. The sniffing port is connected to the GC by an adjustable transport line of about 30-60 cm length. To ensure that the volatile substances won't condense, this line has to be heated. Since the gas eluting is very dry, it is humidified to 50-75% relative humidity to ensure the assessor's nose does not dry out and cause discomfort.[81] A schematic of a GC-O is shown in Figure 15.

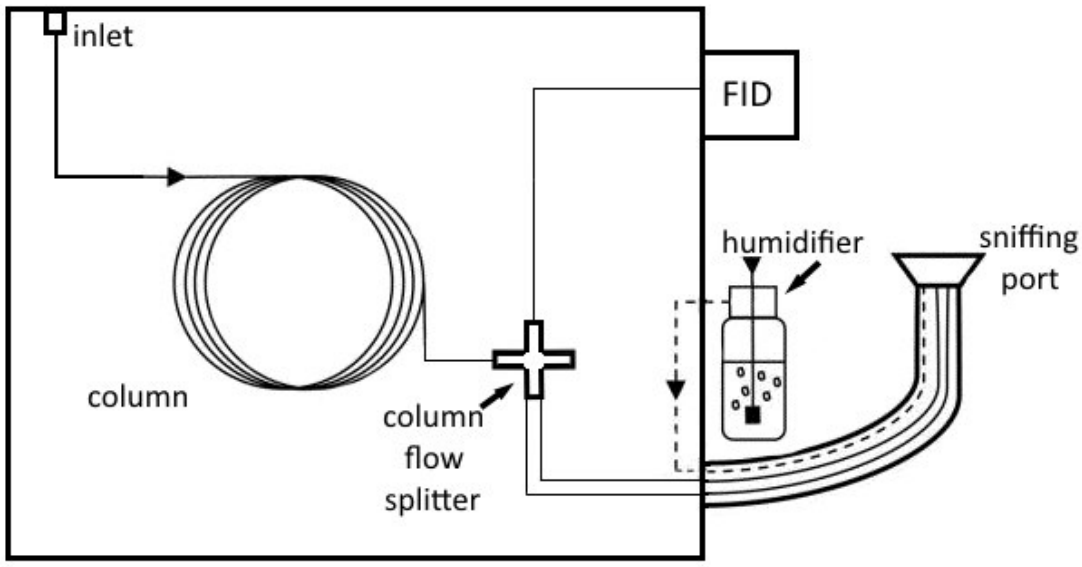

Figure 15: Schematic of GC-Olfactometer

With GC-O analysis three categories of GC-O methods have been conceived: detection frequency, dilution, and direct intensity.[81]

The detection frequency (DF) method is applied to determine the impact of odorants in a sample. For this method a number of n assessors (usually 6-12 people) sniff a certain

sample with the same concentration using GC-O. While the classic DF methods use untrained people to sniff the effluents, in the course of this work trained panelists evaluated the sample. When an assessor detects an odor, they press a button to record that they (i) have an olfactory impression and (ii) can additionally specify the odor impression (so e.g. floral, moldy) with voice recording. All recordings are then combined to create an aromagram. Each assessor's results represent 1/n NIF (Nasal Impact Frequency) in the final aromagram. If a compound was perceived by all participants, it has a NIF of 100%.[82] It is assumed that the higher the NIF is, the more important this odor is to the sample.

3.4 Comprehensive two-dimensional gas chromatography (comp. GCxGC)

In comp. GCxGC (comprehensive two-dimensional gas chromatography) two columns are connected sequentially with a modulator (a transfer device). The modulator is the heart of comprehensive GCxGC. This device collects small fractions of effluents from the first column, focuses them, and then injects them onto the second column in a continuous way. This means that the entire sample undergoes two separations on two GC columns in a single analytical run and no information from the first separation is lost during the second one. Therefore the enormous advantage of comp. two-dimensional GC systems is the amplified separation capacity. Especially for complex mixtures or substances which tend to co-elute this is the method of choice.[83]

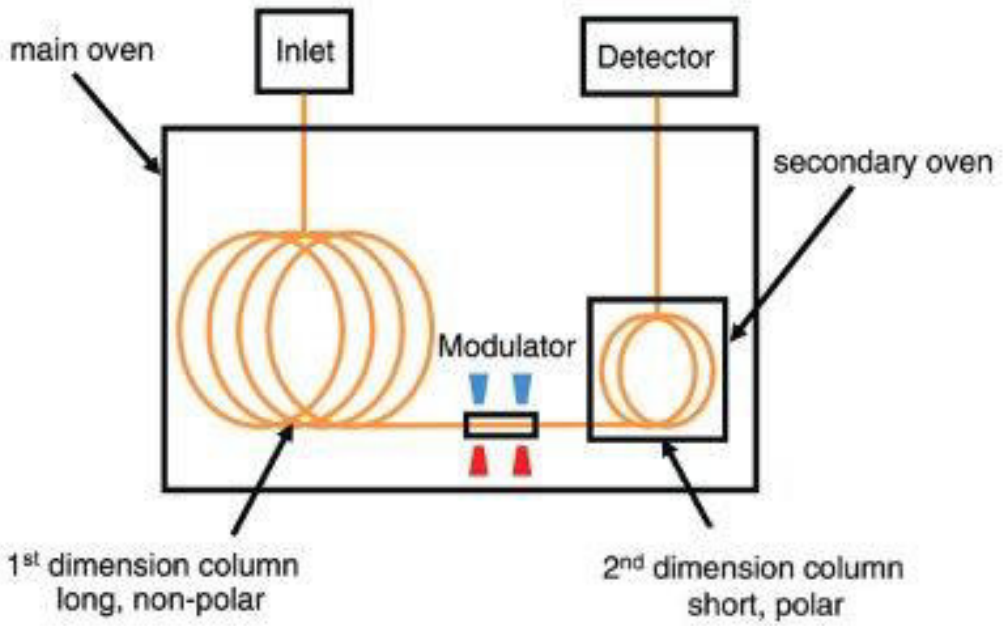

Figure 16: Schematic of a GCxGC system from [84]

A two dimensional chromatogram is created using special software such as *ChromSquare* by *Chromaleont*.[85] On the x-axis the separation of compounds by the first column is displayed, as it would be in a "normal" chromatogram. On the y-axis the retention time of the second dimension is plotted. Peaks are visualized as spots and the intensity is indicated through colors like in a topographical map (see also Figure 17).[86,87]

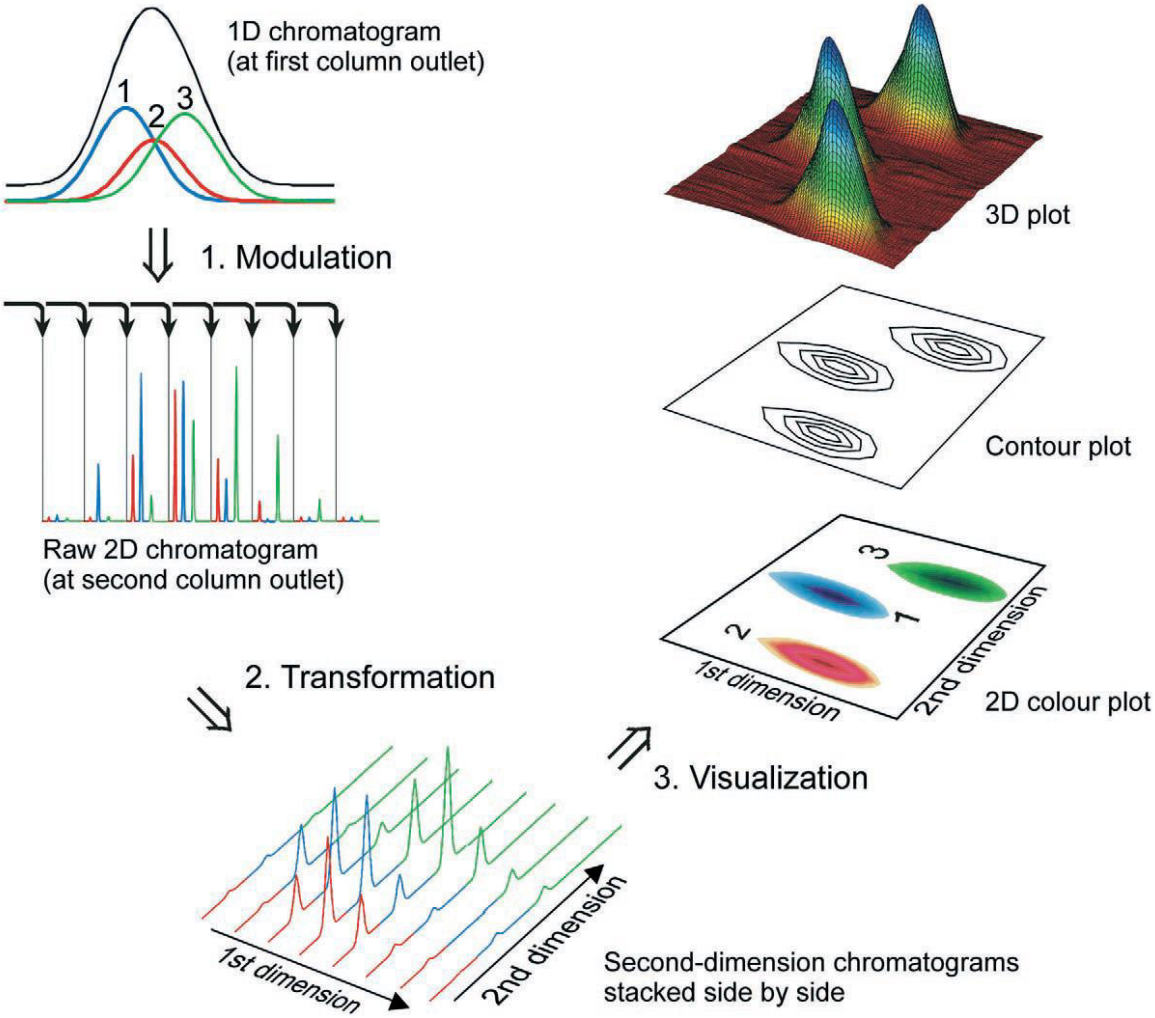

Figure 17: Relation of 1- and 2-dimensonal chromatogram from [86]

3.5 Identification of compounds

The task of identifying aroma-active compounds can be extremely challenging. Not just the large number of substances, but also their at times low concentrations make identification difficult.

3.5.1 Retention index

Retention indices (RIs) are a tool to help with the identification of substances.

While the retention time (RT) of a compound changes depending on the individual chromatographic system that is used in a laboratory, the RI is independent of the chromatographic system. However, a necessary prerequisite is that the polarities of columns match. The RI is calculated by referring and then normalizing the RT of a compound x to the RT of standards for which the *n*-alkanes (C_5-C_{20}) are used. The RI can then be compared to a reference sample's RI or database RIs. However, to compare the agreement of RIs, the experiment's and referenced databases' column types (polarity) have to match.

The individual substance's RI is calculated using the RT's of the adjacent *n*-alkanes (the one eluting before and the one after) according to Equation 2: [88]:

$$RI = 100 * \left(z + \frac{T_x - T_z}{T_{z+1} - T_z}\right)$$

Equation 2: Calculation of the Retention Index [88]

RI	Retention Index
T_x	RT of the compound x
T_z, T_{z+1}	RTs of the *n*-alkanes eluting adjacently to compound x
z, z+1	Number of C-atoms of respective *n*-alkane z or z+1

3.6 Quantification of compounds

For the (semi-)quantification of compounds with HS-SMPE/GC-MS one method is the internal standard approach. A known amount of internal standard (a substance that is not present in the sample) is added to the sample. It is assumed that the volatile internal standard will be trapped on the fiber proportional to its concentration, and the same is assumed for the unknown substances in the sample. Therefore the ratio of internal standard to the compound in question is used to calculate the concentration of the individual substances contained in the sample.

Of course, as the word “semi” in parenthesis at the beginning illustrates, this method has its limitations. Since volatile compounds have diverse polarities and reactivities some might adhere better to the SPME fiber than the internal standard or vice versa. Therefore the concentrations can only be given relative to the IS.

3.7 Sensory analysis

In sensory evaluation human assessors utilize all their senses (visual, auditory, olfactory, gustatory and tactile sense) to examine and evaluate products, such as agricultural products and other foodstuffs, but occasionally also non-food items. Sensory evaluation is a scientifically acknowledged method and can be divided into analytical and hedonic methods.

In the analytical approach the properties of a product are evaluated with (quantitative)-descriptive or discriminative methods. For this purpose specially trained panelists are employed. These skilled panelists have to meet high demands and have to be trained and reevaluated regularly according to ISO 8586:2012. For descriptive tests and specific analytical questions, the panelists have to undergo even further training. This is done to e.g. normalize the panel's vocabulary to obtain consistent descriptors. Since panelists are highly qualified, a smaller number of assessors is sufficient compared to hedonic tests. The test location (sensory laboratory) also has to meet standardized requirements regarding many factors, such as no noise and odor nuisance, special furniture and equipment, plus constant temperature and humidity (noted in DIN 10962). All this is necessary to ensure that the results obtained are objective and validated, comparable to chemical or physical measurements. It can be used to characterize a product, assess its quality and shelf life, as well as monitor production, product development and process changes.[58,89]

The hedonic method uses untrained consumers as assessors for acceptance and preference tests. It is an instrument in market research, product development and studies exploring consumer behavior and dietary habits. The assessors should be part of the target group, if required, but otherwise do not have to fulfill any special requirements. Results of such investigations are subjective, and to obtain statistical significance a large number of participants is required (n=60-400). While hedonic sensory analysis can take place in sensory labs, this is not imperative. For example, so called home use tests can be conducted in the participant's own home.[58,89]

In this work the following analytical sensory evaluations were utilized and will therefore be described in more detail:

3.7.1 Odor Threshold Determination

While the odor detection (and also recognition) thresholds for many substances have already been published, this is not the case for every substance's threshold.

Several methods exist to determine the detection threshold; one such method is the three-alternative forced-choice (3-AFC) test. In the 3-AFC test a series of three samples is given to the panelists simultaneously. Generally, the panelists have to select one among those three samples which they deem as an outlier in regard to a certain, specified sensory stimulus.

In the case of a threshold determination one sample contains the compound in question, while the others are blanks with no odor. Therefore the stimulus is compared to level "zero", and determination of the presence of the stimulus alone is enough. Increasing concentrations are provided from sample series to series for the substance in question. The panelists are also provided with information about the added substance. The test reveals at which concentration step the presence of the substance is perceivable. With these results the odor or taste thresholds can be calculated using the geometric mean:

1. For panelist i:

$$ES_i = \sqrt{C_E + C_{E-1}}$$

Equation 3: Detection threshold for panelist i

2. For the entire panel:

$$\overline{Threshold} = \sqrt[n]{\prod_{i=1}^{n} ES_i}$$

Equation 4: Detection threshold for the entire panel

C_E	Concentration of the first correct answer [g/L]
C_{E-1}	Concentration of the sample prior to the correct answer [g/L]
ES_i	Individual threshold of panelist i
n	Number of panelists

3.7.2 Descriptive Analysis

In descriptive analysis the perception of the product is characterized through attributes selected freely by the panelist. At first the panelists have to be familiarized with the product and have to expand their vocabulary to accurately express their perception using all senses. For this product-specific training is required. One such training method is to dip cellulose strips in standard-solutions of compounds, which are thought to influence the aroma of the investigated product. These strips are then sniffed by the panel to accustom them to the typical aroma and train the description of these odors. Descriptive analysis yields a qualitative description of characteristic attributes for the product in question. It has its application in characterization of products and monitoring of the influence of changes in recipes or raw materials. Furthermore it can provide the basis to set up other sensory methods, such as conventional profiling.[90]

3.7.3 Profiling

In profiling, the descriptive attributes are not only gathered, but their intensity is also quantified. This method is utilized to compare different products to each other, to monitor product quality compared to standards, and also as a tool in product development and optimization.[90]

The attributes that are going to be quantified have to be selected through suitable sensory analysis, like a descriptive analysis, first. The intensity of an attribute can be measured in different ways, for example by having participants rate it on an intensity scale (which can be either discrete or continuous) ranging from “not intense” to “extremely intense”. 15 characteristics is the maximum amount of attributes that are to be assessed at once. Not only must the panel be able to accurately identify the attributes, but also rate them in a consistent manner. At least 6 panelists are required for profiling and training is complex

and time-consuming.[90] Well known profiling methods include for example Quantitative Descriptive Analysis (QDA®) and the Spectrum™ Method.

Humans are good at judging relative sensory differences, and QDA® is a method that takes advantage of this ability. Trained panelists evaluate the samples in separate booths without a reference sample. Therefore the results of QDA® can give relative difference among the samples, which can be diagnosed with one-way Analysis of Variance (ANOVA) methods. The results can also be used to examine the panel performance based on interaction of panelist and product, or to verify whether project goals were met. Means of attributes in the same category are often presented in "spider-web" graphs.[91]

4 Samples

4.1 Juices

In this work the following aronia fruit juices from 100% aronia berries were investigated (see Table 4). The samples are referred to by the numbers listed below.

Table 4: Juice samples investigated in this work

Juice	Producer
111	Ragger
1000	Retter
1297	Weiß
1537	Karner
1634	Köck
1635	Eisvogel
1746	Reinhart
1747	Steinkleibl
1832	Gangl
1922	Wallner
1923	Eberhart
1924	Kober
1925	Gölles
1926	Liebminger
1949	Konrad
2000	Christandl
2098	Liebmann
2138	Trummer
2150	Unger

Figure 18: Austrian aronia juice samples

The pH measurement was the first analysis conducted, and at this point only three aronia juice samples were available.

For the characterization of aronia juice with HS-SPME/GC-MS and subsequent principal component analysis (PCA) 15 aronia juices were analyzed. These juices were produced by Austrian agriculturists from cultivar “Nero” and assessed at the Styrian state evaluation 2016 (Steirische Landesbewertung 2016) in the category “aronia juice”.

From these juices 4 were chosen for descriptive analysis (1746, 1747, 1925 and 1949). These 4 juices had scored 18 points or better at the Styrian state evaluation 2016, which is comparable to a gold medal in this fruit juice category. The results of descriptive analysis were used to select the attributes for QDA®.

In QDA® juices 1000, 1634, 1747 and 2000 were evaluated. Of these four only 1747 was an awarded juice sample; the other samples were bought from health food stores or supermarkets for the analysis, since awarded juices were not available at this point. These

four juices plus juice 111, which was manufactured with a steam juicer, were analyzed with HS-SPME/GC-MS and subsequently evaluated in PCA (marked with * in Table 5.)

Juice 2000 was used in GC-O analysis, since it represented the characteristic and complex flavor of aronia without any defects. The pH values of juices 1635, 2000 and 2150 were measured. The latter two juices were also examined in comprehensive GCxGC-MS analysis.

The variation of juice samples is due to limited availability of larger quantities of juice from a single manufacturer. A listing of juices and the corresponding methods that were used for their analysis can be seen in Table 5.

Open bottles of aronia juice were storeddark and cool at 6°C in a cold-storage room for a few days. If analysis was to be conducted later than a few days after opening of a bottle, juice samples were frozen and stored at -23°C and only thawed if needed.

Table 5: Juices and the corresponding methods that were used for their analysis

Juice	Producer	GC-MS	GC-O	Comp. GCxGC	Descript. Analysis	1st Profiling	2nd Profiling	pH
111	Ragger[a]	*						
1000	Retter	*				✓		
1297	Weiß	✓						
1537	Karner	✓						
1634	Köck	✓*				✓		
1635	Eisvogel							✓
1746	Reinhart	✓			✓			
1747	Steinkleibl	✓*			✓	✓	✓	
1832	Gangl	✓						
1922	Wallner	✓						
1923	Eberhart	✓						
1924	Kober	✓						
1925	Gölles	✓			✓			
1926	Liebminger	✓						
1949	Konrad	✓			✓			
2000	Christandl	✓*	✓	✓		✓	✓	✓
2098	Liebmann	✓						
2138	Trummer	✓						
2150	Unger			✓				✓

[a] Juice 111 was produced with steam juicing.

4.2 Plant parts

Since chokeberries are commonly harvested mechanically, it is possible that the raw material used in the juice production is not completely clear of small plant parts, such as pedicels, twigs or leaves. To investigate their influence, as well as the influence of crushed aronia seeds on the juice, they were analyzed with HS-SPME/GC-MS. These plant parts were taken from a chokeberry shrub grown in Graz, Styria in July 2016.

Figure 19: Separated aronia pedicels

Figure 20: Separated aronia seeds

5 Experimental

In the following chapters the experimental conditions and equipment used in this work are described.

5.1 HS-SPME/GC-MS

HS-SPME/GC-MS analysis was conducted for the investigation of odorants present in aronia juice samples and aronia plant parts. The following parameters were used in the HS-SPME/GC-MS analysis:

Before usage, all headspace vials were washed and rinsed with acetone, then placed in a compartment dryer at 200°C for at least an hour and finally cooled off for the respective measurement. The blanks used in the analysis were simply empty HS vials only equipped with a glass-coated stirring bar. To calculate the RIs a mixture of *n*-alkanes from C_5 to C_{26} in methanol were measured. Their concentration in the HS vial was 100 ng absolute, with the exception of C_6 at 150 ng and C_{20} at 200 ng.

To verify the general condition of the GC-system and the sensitivity of the column, 10 µL of a special mixture containing 1-dodecanol, acenaphtene, acenaphtylene, 1,8-cineol, p-cymene, menthol, methyldecanoate, α-pinene and β-pinene in methanol was also added to every GC-MS sequence. The concentration of this SPME-mix was 1 mg/L in methanol, which results in 10 ng absolute in a headspace vial for the measurement.

The optimal sample preparation for aronia juice was established as follows: For improved extraction of volatile compounds and sensitivity, about 50 mg NaCl were weighed into a 20 mL headspace vial with a glass-coated magnetic stirrer. Then 200 µL of aronia juice (well shaken) and 10 µL internal standard were added for the quantification of the compounds. The vial was sealed immediately afterwards. 2-octanol was used as internal standard. The concentration of the stock solution was 1 g/L and this was diluted to 10 mg/L, which resulted in a final concentration of 0.5 mg internal standard/kg sample. All juice samples were measured in a randomized order. For all samples 4 replicates were measured.

The DVB/CAR/PDMS fiber was exposed to the gas phase above the sample for 20 minutes while the vial was heated to a temperature of 60°C.

The HS/SPME-GC-MS equipment and conditions are listed in Table 6 and Table 7.

Table 6: Equipment used in HS/SPME-GC-MS analysis

SPME Fiber	Supelco 50/30 µm; DVB/Carboxen/PDMS on a 2 cm StableFlex fiber (Supleco, Bellefonte, PA, USA)
Autosampler	CTC Combi PAL sampler (CTC Analytics, Switzerland)
GC	Agilent Technologies 7890A (Agilent Technologies, CA, USA) (Serial No. CN10925072)
Column	HP-5MS UI Length: 30 m Diameter (mm) 0.250 Film (µm) 1.00 (Serial No. US9482512H) Part.No 19091S-233UI
Detector	Agilent Technologies 5975C VL MSD with Triple-Axis Detector

Table 7: Conditions for GC-MS analysis

Carrier gas	He
Injection mode	Splitless
Flow	19,95 mL/min
Solvent Delay	4 min
Injection Temperature	270°C
Temperature program	
Start	-10°C for 1 min
Gradient	12°C/min
End	280°C for 3 min
Detector	
Detector temperature	280°C
Mass range	35-300 amu
Voltage EI	70 eV
Voltage photomultiplier	1906 V

5.2 GC-O

To identify the odor-active compounds in aronia juices, analyses with GC-olfactometry were conducted. Aronia juice 2000 from producer “Christandl” was studied.

For the investigation 1 mL aronia juice and 500 mg NaCl were placed in a 20 mL Headspace vial, which was then placed in a heating block at 60°C. The SPME fiber was exposed to the gas phase for 20 minutes for equilibration. Insertion and exposure of the fiber and subsequent injection were done manually in contrast to GC-MS analysis. Five assessors sniffed the samples and their time and odor impressions were recorded with *Gerstel*. The samples were sniffed 10 times.

Table 8: Equipment used in GC-O analysis

GC-FID	Hewlett Packard 5890 Series II
Column	DB-5 Length (m) 30 Diameter (mm) 0.320 Film (µm) 0.25 Part. No. 123-5032
Olfactory Detection Port	Gerstel ODP (Gerstel GmbH & Co. KG)
Detector	FID

Table 9: Conditions for GC-O analysis

Column pre-pressure	1 bar (constant pressure)
Carrier gas	He
Injection mode	Splitless
Split ratio (ODP:FID)	1:1
Temperature	
Injection temperature	270°C
Detector temperature	300°C
Transport line	345°C
Temperature program	
Start	35°C (1 min hold)
Gradient	10°C/min
End	280°C (1 min hold)

5.3 Comprehensive GCxGC-MS

Analysis with comp. GCxGC-MS was conducted to make use of the amplified separation capacity, which is especially useful for complex mixtures or substances that tend to co-elute.

Aronia juices “Christandl” and “Unger” were analyzed in double determination using comprehensive GCxGC-MS. 200 µL juice sample and 100 mg NaCl were placed in a HS vial with a glass-coated magnetic stirrer.

The conditions and equipment used in comprehensive GCxGC-MS are listed in Table 10 and Table 11.

Table 10: Equipment used in comprehensive GCxGC-MS analysis

GC	Shimadzu GC-2010 Plus (Shimadzu Europa GmbH)
Autosampler	Shimadzu AOC 5000
Modulator	ZOEX ZX-1 (Zoex Corp., Huston, TX, USA)
First column	ZB5-MS capillary column (Phenomonex Inc., USA) Length (m) 30 Diameter (mm) 0.25 Film (µm) 0.25
Second column	BPX-50 (SGE GmbH, Germany) Length (m) 2.5 Diameter (mm) 0.15 Film (µm) 0.15
Detector	Shimadzu GCMS-QP2010 Ultra (Shimadzu Europa GmbH)

Table 11: Conditions for comprehensive GCxGC-MS analysis

Carrier gas	He
Injection mode	Splitless
Column flow	1.45 mL/min
Solvent Delay	4.9 min
Injection Temperature	270°C
Modulation frequency	5 s
Temperature program	
Start	35°C (1 min hold)
Gradient	3°C/min to 210°C and 20°C/min to 280°C
End	280°C (3 min hold)
Detector	
Detector voltage	Relative to tuning result
Mass range	35-300 m/z
Acquisition mode	Scan

5.4 Sensory Analysis

The sensory analysis was conducted with an expert panel of up to 15 trained panelists, fulfilling the requirements for the selected methods according to DIN 10961.

The test setting was a sensory laboratory, which is equipped according to DIN 10962. A sample questionnaire is attached in the Appendix for all tests that were performed. The order of samples was also randomized and the samples were coded with random three or four digit numbers to ensure unbiased results.

5.4.1 Threshold determination

The odor threshold values of *(E)*-3-penten-2-one vary strongly in literature, ranging from 0.015 mg/kg to 1.20 mg/kg in water as matrix [92]. In order to clear this discrepancy and to assess the importance of *(E)*-3-penten-2-one to the aroma of aronia juices with its odor activity value, a threshold evaluation of *(E)*-3-penten-2-one was performed.

6 dilutions, guided by the values of the literature, were made in the following, increasing concentrations, (see Table 12). With these, a 3-alternative forced-choice test was performed as described in Chapter 3.7.1.

The intermediate dilutions of *(E)*-3-penten-2-one were prepared in ethanol. From these, 150 µL were added into 1.5 L water as the final dilution for the evaluation. To eliminate errors of nonconformance, 150 µL of pure ethanol was added to 1.5 L water, which served as the blanks. The dilutions and blanks were offered in plastic cups and sealed with a plastic lid. The threshold evaluation was performed twice on two consecutive weeks through smelling of the offered samples.

Table 12: Concentrations for threshold evaluation of (E)-3-Penten-2-one

Dilution	Concentrations in threshold analysis [mg/L]
1	0.0001
2	0.001
3	0.01
4	0.1
5	1
6	10

5.4.2 Description of standard solutions

Since most people are unacquainted with aronia juice, it was important to train the panel in regard to the characteristic flavor of aronia. In order to do this, cellulose strips were dipped into standard solutions of reference substances which were deemed important to the aroma of aronia. The substances were chosen based on the GC-MS analysis and are listed in Table 13. All standard solutions were made with ethanol (96%), concentrations are also found in Table 13. 12 panel members took part in the evaluations.

Table 13: Reference substances and their concentration used in sensory training

Substance	Concentration [%]
p-cymene	1
***(E)*-2-Hexenal**	1
***(E)*-2-hexen-1-ol**	1
***(E)*-3-Penten-2-on**	2
***(Z)*-3-Hexenol**	1
1-Penten-3-ol	1
2-Ethylhexanol	1
2-Methylbutyric acid	1
2-Methylhexanoic acid	1
Benzyl alcohol	2
Eugenol	1
Guaiacol	1
Hexanal	1
Hexanoic acid	1
Hexanol	1
Methyl butanoate	1
Phenylacetaldehyde	1
2-Phenylethanol	5
α-Terpineol	1
β-Damascenone	1
β-Ionone	1
γ-Terpinene	1

5.4.3 Descriptive analysis

To obtain characteristic attributes that describe aronia juices, the juice samples 1746, 1747, 1925 and 1949 were evaluated with descriptive analysis. All juice samples were served in wine glasses in randomized order, coded with a random number and had room temperature when they were served. The test sheets used can be found in the Appendix. 14 panelists took part in the analysis.

5.4.4 Profiling/QDA®

The following characteristic attributes, which were taken from descriptive analysis, were selected for the profiling of aronia juices 1000, 1634, 1747 and 2000:

- Sweetness
- Bitterness
- Astringency
- Green/grassy notes
- Fruitiness
- Acidity[b]

The evaluation of aronia juices was performed twice by 8 panelists each, of whom five took part in both assessments. However, acidity was only quantified in the second assessment. The juice samples were coded with a random number and were served in wine glasses at room temperature in a randomized order.

For the first assessment, a discrete scale was chosen that consisted of five options, symbolizing intensities from “not intense” to “extremely intense” for every attribute. The panelists were asked to rate the intensity by choosing the appropriate option. “Not intense” was mapped to a value of 0, while “extremely intense” was given a value of 10. The three options in-between amounted to intensities of 2.5, 5 and 7.5 respectively, so they were comparable to the slightly different test sheet used in the second assessment.

[b] The attribute “acidity” was only evaluated in the second assessment for juices 1747 and 2000.

For the second assessment the following week, a continuous scale of 10 cm length was used, where one end represented that the attribute was “not intense” and the other that it was “extremely intense”. The panelists rated the intensity of an attribute with a mark on the line. The length was then measured and thereby converted into an intensity ranging from 0 (not intense) to 10 (extremely intense), corresponding to 0 cm to 10 cm respectively.

5.5 pH measurement

The pH meter used for the determination of the pH of selected aronia juice samples was a Thermo Scientific Orion 3-star TM benchtop pH meter.

6 Results and Discussion

6.1 pH measurement

The pH measurement was among the first measurements taken, and only a limited number of juice samples were available.

The results of pH measurements of 3 Austrian aronia juices are presented in Table 14:

Table 14: Results of pH measurement

Sample	Temperature [°C]	pH
Unger 2150	22.1	3.933
Christandl 2000	21.7	3.720
Eisvogel 1635	22.3	3.944

This is in good agreement with the results found by other researchers. Ara[14] reported pH ranging from 3.3 to 3.9 in aronia juice and Tanaka and Tanaka[26] measured pH values from 3.36 to 3.79 in fresh berries. Since this is a natural product, these values can vary depending on climate, cultivar, water supply, ripeness and many other factors.

6.2 HS-SPME/GC-MS

Substances that were identified with HS-SPME/GC-MS analysis are presented in Table 15. The compounds were identified through their mass spectra by computer-assisted comparison to MS databases and through comparison of their respective retention indices with indices found in previous publications. For the calculation of the RI's Equation 2 was used.

Table 15: Substances identified in aronia juices with HS-SPME/GC-MS analysis with their RT, RI compared to literature RI and odor

Substance	RT [min]	exp.RI (HP-5)	RI Lit	Odor Lit
Ethyl acetate	7.310	617	613 [i]	Ethereal, fruity, sweet, weedy, green [g]
Acetoin (3-hydroxybutan-2-one)	9.180	717	711 [i]	Sweet, buttery, creamy, dairy, milky, fatty [g]
***(E)*-3-Penten-2-one**	9.604	741	735 [i]	Sharp and acetone-like and fruity, phenolic and fishy with a chemical glue nuance [g]
Hexanal	10.64	800	800 [i]	Green, grass, fatty, fresh, sweet [e]
Furfural	11.22	836	830 [i]	Woody, almond, sweet, fruity, flowery [i]
2-Methylbutyric acid	11.36	843	839 [f]	Cheesy, like butyric acid, sweaty feet, sweat, vomit, fermented [e]
***(Z)*-3-Hexen-1-ol**	11.57	857	858 [f]	Green, nuts [e]
***(E)*-2-Hexen-1-ol**	11.07	866	862 [i]	Green, nuts, stagnant water [e]
1-Hexanol	11.73	868	865 [i]	Green, flower [e]
γ-Butyrolactone	12.51	917	915 [i]	Sweet, aromatic, caramel [i]
n-Hexanoic acid	13.31	968	981 [h]	Cheesy, pungent, sweaty, acidic, buttery, fermented [i]
Benzaldehyde	13.34	973	960 [f]	Almond, burnt sugar [f]
***(E)*-3-Hexenoic acid**	13.44	979		Cheesy, green, dairy-like with a waxy fruity nuance [g]
***(E)*-2-Hexenoic acid**	13.88	1002		Powerful, fruity, sweet, warm, herbal [g]
2-Ethylhexanol	14.16	1029	1032 [f]	Green, fresh, floral, mint, alcoholic [e]

Benzyl Alcohol	14.35	1044	1039 [f]	Sweet, medicinal, chemical, pungent, solvent, fruity, plastic [e]
Eucalyptol	14.43	1049	1039 [i]	Camphor, minty, sweet, liquorices, mentholic, pine [i]
δ-lactone-5-hydroxy-2-pentenoic acid	14.99	1090	n/a	n/a
γ-Terpinene	14.73	1071	1074 [f]	Gasoline, thyme, solvent [e]
***trans*-Furan linalool oxide**	15.13	1100	1089 [i]	Sweet, floral, creamy, leafy, earthy, green [i]
Nonanal	15.22	1107	1104 [i]	Waxy, aldehydic, rose, fresh, orris, orange peel, fatty, peely [g]
2-Phenylethanol	15.50	1129	1121 [h]	Rose, floral, sweet, honey, green [e]
Benzoic acid	15.89	1160	1159 [h]	Urine [f]
Ethyl benzoate	16.22	1184	1170 [i]	Chamomile flower, celery, fruity, musty, tea[f]
Terpinen-4-ol	16.41	1199	1179 [i]	Cooling, woody, earthy, clove spicy with a citrus undernote [g]
Decanal	16.53	1209	1209 [f]	Sweet, aldehydic, waxy, orange peel, citrus, floral [g]
3-Phenylpropan-1-ol (Hydrocinnamylalcohol)	16.98	1247	1252 [i]	Fruity, spicy, cinnamon, floral, anise [i]
4-Ethylguaiacol	17.57	1296	1282 [h]	Clove, phenolic, flowery [i]
Eugenol	18.49	1377	1364 [f]	Clove, honey [e]
β-Damascenone	18.85	1409	1393 [i]	Apple, rose, honey [f]

RT *Retention time (RT) in minutes of the individual compounds obtained with one dimensional GC-MS analysis*
exp. RI *Calculated Retention Index (RI) on a HP-5 column*
RI Lit *Retention Index found in the literature*
Odor Lit *Odor descriptions found in the literature*
e *6.5.2 Descriptions of standard solutions*
f *http://www.flavornet.org/flavornet.html*
g *http://www.thegoodscentscompany.com/*
h *http://webbook.nist.gov/chemistry/*
i *http://www.pherobase.com/*

The semi-quantification of the substances was obtained through their ratio to the internal standard (IS) 2-Octanol, as described in Chapter 3.6. This results in the following formula (see Equation 5) to calculate the concentration of a substance X. The resulting average concentrations relative to the IS for every juice are shown in Table 16 and Table 17. For all

juice samples the GC-MS analysis was performed 4 times, and the relative standard deviation for the quantified substances was mostly below 10%. The exceptions were acids such as benzoic acid, which is due to the column type used and regions of co-elution.

$$c(subsance\ X) = \frac{area(subsance\ X) * c(IS)}{area(IS)}$$

Equation 5: Calculation of concentration of substance X

Still, the quantification performed in this way of the individual substances has its limitations. Additionally to the reasons explained in Chapter 3.6, some substances showed co-elution or incomplete separation in one dimensional GC-MS. Therefore the concentrations of volatile substances are to be taken as a point of reference to estimate whether, and if so how much, they contribute to the aroma of aronia juices. To identify substances which might have been missed with 1-dimensional GC-MS, analysis with comprehensive GCxGC-MS was also performed (see Chapter 6.3 Comprehensive GCxGC-MS).

Table 16: Average concentrations in juices 1297, 1537, 1634, 1746, 1747, 1832, 1922 obtained through HS-SPME/GC-MS analysis (n=4);

Substance	**Concentration relative to IS [µg/L]**						
	Aronia juice sample						
	1297	1537	1634	1746	1747	1832	1922
***(E)*-3-Penten-2-one**	1134	833	494	1036	833	1068	735
Hexanal	nd	3.95	nd	nd	1.50	nd	Nd
2-Methylbutyric acid	22.6	14.9	34.3	22.6	16.1	17.1	18.3
***(Z)*-3-Hexen-1-ol**	113	310	111	117	305	449	203
***(E)*-2-Hexen-1-ol**	240	nd	128	199	208	494	52.6
1-Hexanol	nd	nd	242	nd	nd	nd	Nd
γ-Butyrolactone	17.6	11.7	7.36	20.0	11.5	nd	15.8
Benzaldehyde	185	59.0	24.3	107	148	113	111
n-Hexanoic acid	49.3	41.4	87.2	50.4	56.5	59.5	68.4
2-Ethylhexanol	nd	2.85	18.1	nd	nd	nd	7.08
Benzyl Alcohol	303	68.5	166	230	259	82.8	203
δ-lactone-5-hydroxy-2-pentenoic acid	1226	322	156	839	488	445	415
γ-Terpinene	10.5	nd	nd	13.9	10.4	nd	Nd
Nonanal	7.75	9.12	12.4	15.5	9.56	10.1	13.2
2-Phenylethanol	79.5	7.29	53.7	76.6	105	9.70	52.3
Ethyl benzoate	32.1	4.58	10.2	19.6	7.96	nd	6.76

4-Terpineol	14.5	nd	nd	14.0	13.0	nd	Nd
Eugenol	8.26	nd	nd	3.90	4.15	nd	5.38
Acetovanillone	11.1	7.58	nd	9.49	9.65	13.0	Nd
β-Damascenone	13.4	14.5	6.20	8.85	3.82	17.3	5.62

nd not detectable

Table 17: Average concentrations in juices 1923, 1924, 1925, 1926, 1949, 2000, 2089, 2138 HS-SPME/GC-MS analysis obtained through HS-SPME/GC-MS analysis (n=4)

Substance	**Concentration relative to IS [µg/L]**							
	Aronia juice sample							
	1923	1924	1925	1926	1949	2000	2098	2138
***(E)*-3-Penten-2-one**	827	891	861	1243	684	925	1050	998
Hexanal	nd	nd	nd	nd	5.15	nd	nd	5.28
2-Methylbutyric acid	32.0	30.6	26.2	38.1	11.3	27.0	19.9	18.1
***(Z)*-3-Hexen-1-ol**	257	106	158	33.8	382	147	117	498
***(E)*-2-Hexen-1-ol**	nd	176	70.4	34.8	151	268	243	127
1-Hexanol	nd	296	243	143	518	370	275	382
γ-Butyrolactone	15.0	22.0	19.1	8.74	nd	13.6	16.5	Nd
Benzaldehyde	34.2	75.3	71.9	572	172	75.4	220	120
n-Hexanoic acid	77.6	68.8	125	32.8	83.6	45.2	70.2	85.6
2-Ethylhexanol	nd	nd	nd	3.86	nd	6.54	nd	3.08
Benzyl Alcohol	111	317	395	517	179	315	311	74.2
δ-lactone-5-hydroxy-2-pentenoic acid	548	1246	670	678	527	666	1055	453
γ-Terpinene	nd	16.8	18.7	nd	nd	nd	14.6	Nd
Nonanal	5.49	17.0	nd	14.0	10.0	14.5	9.05	11.2
2-phenylethanol	28.5	105	112	66.3	21.0	91.1	81.5	17.4
Ethyl benzoate	14.3	54.2	26.8	24.3	23.7	32.9	28.3	Nd
4-Terpineol	5.75	16.2	20.7	nd	nd	nd	15.0	Nd
Eugenol	2.05	7.92	10.98	14.22	nd	6.68	8.76	6.57
Acetovanillone		10.5	8.28	nd	nd	nd	nd	Nd
β-Damascenone	4.30	7.70	11.1	7.66	8.80	13.7	14.1	11.5

nd not detectable

Notable is the lack of so called “fruit esters” in black chokeberry juices. These esters usually contribute significantly to the aroma of many fruits, such as in strawberries, for which over 100 esters have been found.[62] However, in the aronia juices examined, numerous alcohols, ketones, aldehydes and acids, as well as terpenes and sesquiterpenes are present. The

most abundant compound is *(E)*-3-penten-2-one, which corresponds well to the literature.[93]

Aronia juice also contains lactones, such as δ-lactone-5-hydroxy-2-pentenoic acid, and small amounts of γ-butyrolactone. γ-Butyrolactone is known as for its intoxicating effects and use in knockout drops, but it can also be found in low concentrations in wines. Intermolecular esterification of the hydroxycarboxylic acid 4-hydroxybutanoic acid yields γ-butyrolactone. In the same manner the intermolecular esterification of 5-hydroxy-2-pentenoic acid could form 5,6-Dihydro-2H-pyran-2-one.

However, 5,6-Dihydro-2H-pyran-2-one was only tentatively identified through its mass spectrum. The conformance with the library mass spectra was around 70%. Still 5,6-Dihydro-2H-pyran-2-one was also identified with comprehensive GCxGC-MS analysis. The biochemical origins of this substance could not be identified. The idea was that δ-lactone-5-hydroxy-2-pentenoic acid might be introduced to the juice by contamination with ligneous or other parts of chokeberry bushes, such as twigs, leaves or crushed seeds. However, the investigation did not reveal δ-lactone-5-hydroxy-2-pentenoic acid in neither the leaves, nor twigs, nor seeds. The results are presented in Chapter 6.2.2.

Not every substance could be detected in each juice sample with one-dimensional GC-MS, as can be seen in Table 16 and Table 17. This does not imply that the compound was absent in a certain juice sample though. In fact, analysis of chokeberry juice samples with two-dimensional gas chromatography (see Chapter 6.3) suggests that the present volatile substances are always the same, which is not the case for the concentrations of the individual compounds. The differences in concentration can be due to circumstances such as microclimate, ripeness, or storage before pressing, as well as other influencing factors. Unfortunately no data could be collected about these factors. Consequently no connections could be made about the influence of location, climate, ripeness and storage on the volatile substances found in chokeberry juice.

The sensory evaluation of selected juices (aronia juices 1000, 1634, 1746, 1747, 1925, 1949 and 2000) reinforces the assumption that varying concentrations of volatile substances play an important role in the aroma. These juices were made from the same cultivar and pressing method, but were from different producers, and showed varying characteristics

in sensory analysis (see Chapter 6.5). Furthermore, a calculation of the odor activity values was done with the concentrations and the results are presented in Chapter 6.6.1.

6.2.1 Principal Component Analysis

To characterize and group the GC-MS data according to their similarities, an evaluation with Principal Component Analysis (PCA) was performed.

The data obtained through GC-MS analysis was assessed with the software MASstat® by Analyt MTC. This program uses the statistical method of PCA to visualize the similarities and dissimilarities of samples. This is done through a graph where samples with similar composition are closer together. Samples that are located far apart from each other have different composition features. The following masses, which are known as common contaminants in GC analysis (such as siloxanes from the SPME fiber and fiber glue), were excluded from the evaluation with MASstat®: 73, 133, 147, 151, 207, 221, 267, and 281.

The results of the evaluation with MASstat® are shown in Figure 21.

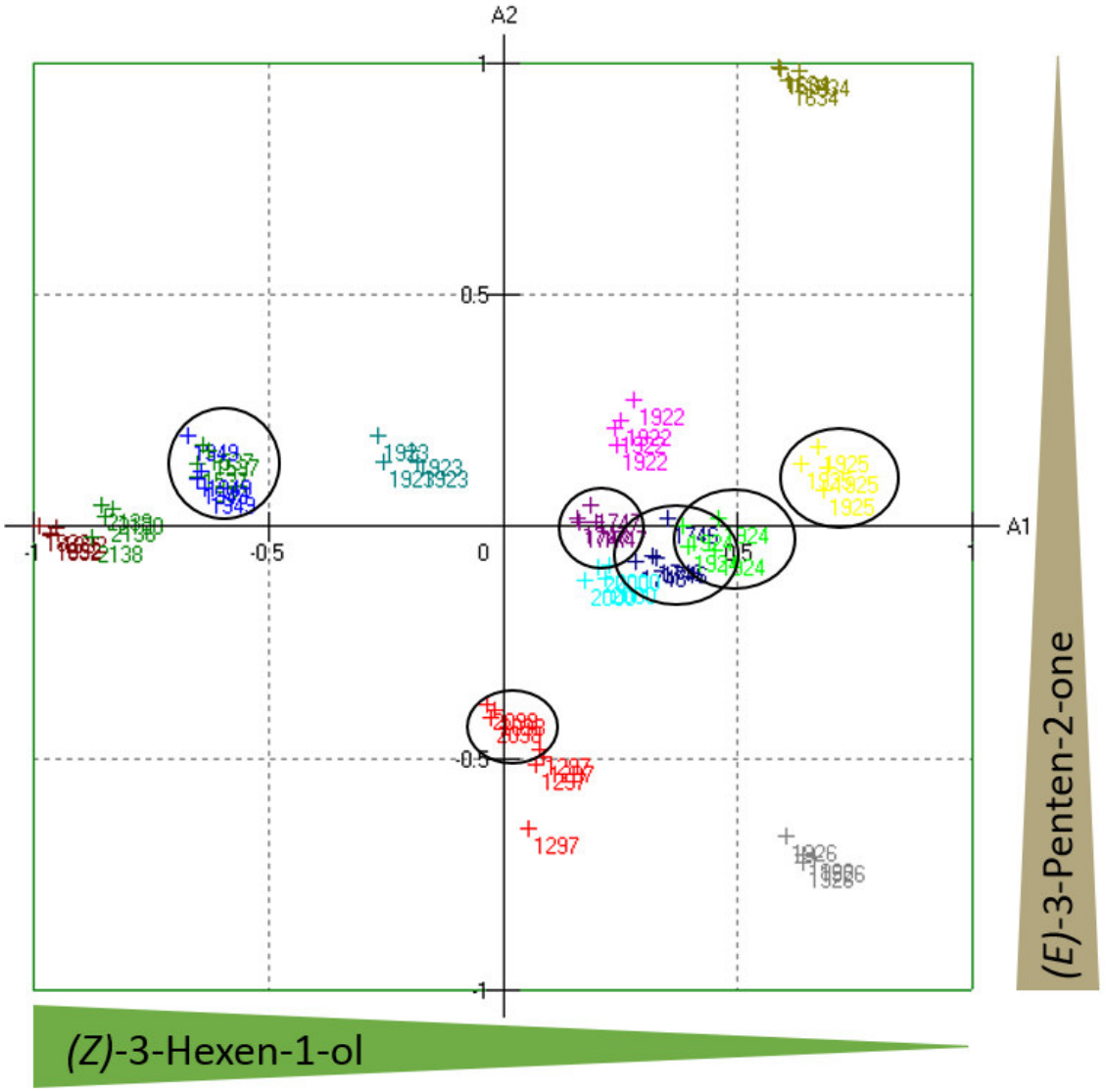

Figure 21: MASstat® evaluation of 15 aronia juices
(encircled black: 18 or more points at Styrian state evaluation 2016)

Most of the aronia juices with 18 or more points at the Styrian state evaluation 2016, which is equal to a gold medal in the aronia juice category (marked with a black circle in Figure 21), are located within the cluster right of the center of the graph: Juices 1746 (19 points), 1747 (20 points), 1924 (18 points), 1925 (18 points). The only exceptions are juice 1949 (to the left middle) and 2089 (½ down from the center), with 18 points each. Juice 2000 was not awarded 18 points, but is also located in the right-side cluster. In a preliminary evaluation it was considered to be a characteristic aronia juice without defects and used in GC-Olfactometry and QDA®. The closeness to the awarded juices only emphasizes this fact.

(E)-3-penten-2-one and *(Z)*-hexen-3-ol are both contained in aronia juice at quite high concentrations (ranging from 735 to 1134 µg/L *(E)*-3-penten-2-one and from 33.8 to 498 µg/L for *(Z)*-hexen-3-ol). When the juices were sorted in ascending order of their *(E)*-3-penten-2-one and *(Z)*-hexen-3-ol concentrations respectively, noticeable correlation was observed with results obtained from PCA analysis based on the GC-MS chromatograms: The higher the *(E)*-3-penten-2-one concentration, the lower they are plotted on the y-axis value. This is visualized in Figure 22, in which the chromatograms of five exemplary juices are overlaid. The peaks are the respective *(E)*-3-penten-2-one peaks. Similarly, the higher the *(Z)*-hexen-3-ol concentration, the lower they are plotted on the x-axis.

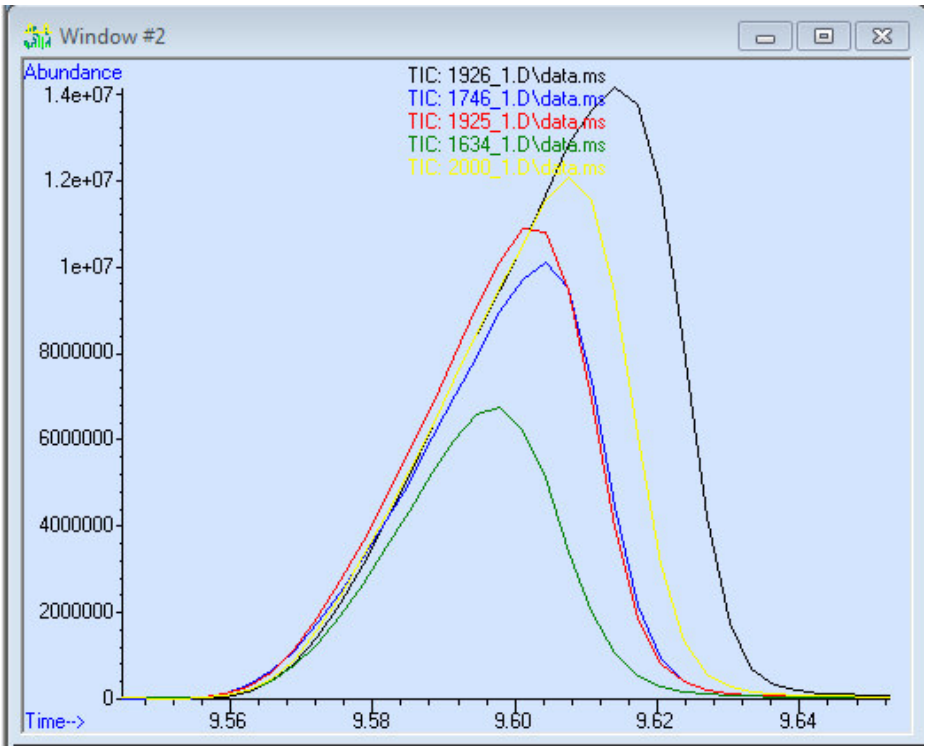

Figure 22: Increasing concentration of (E)-3-penten-2-one in 5 exemplary aronia juices

Juices 1000, 1634, 1747 and 2000 were also measured with GC-MS in quadruple determination and the results were evaluated with MASstat® (see Figure 23). The juices are labeled with other numbers in this graph, the number they are referred to in this work is written next to them in the same color. The distribution pattern in the MASstat® graph also depicts juice 2000 (blue) and juice 1747 (brown green) close together in the left center, while the other juices are at a greater distance from them. While juice 1000 (green) is located in the bottom right corner, juice 1634 (purple) is located in the upper right corner. Next to juice 1634 another juice depicted with red color can be found (juice 111). 111 is an Austrian aronia juice, however, it was produced with a steam juicer instead of a belt press.

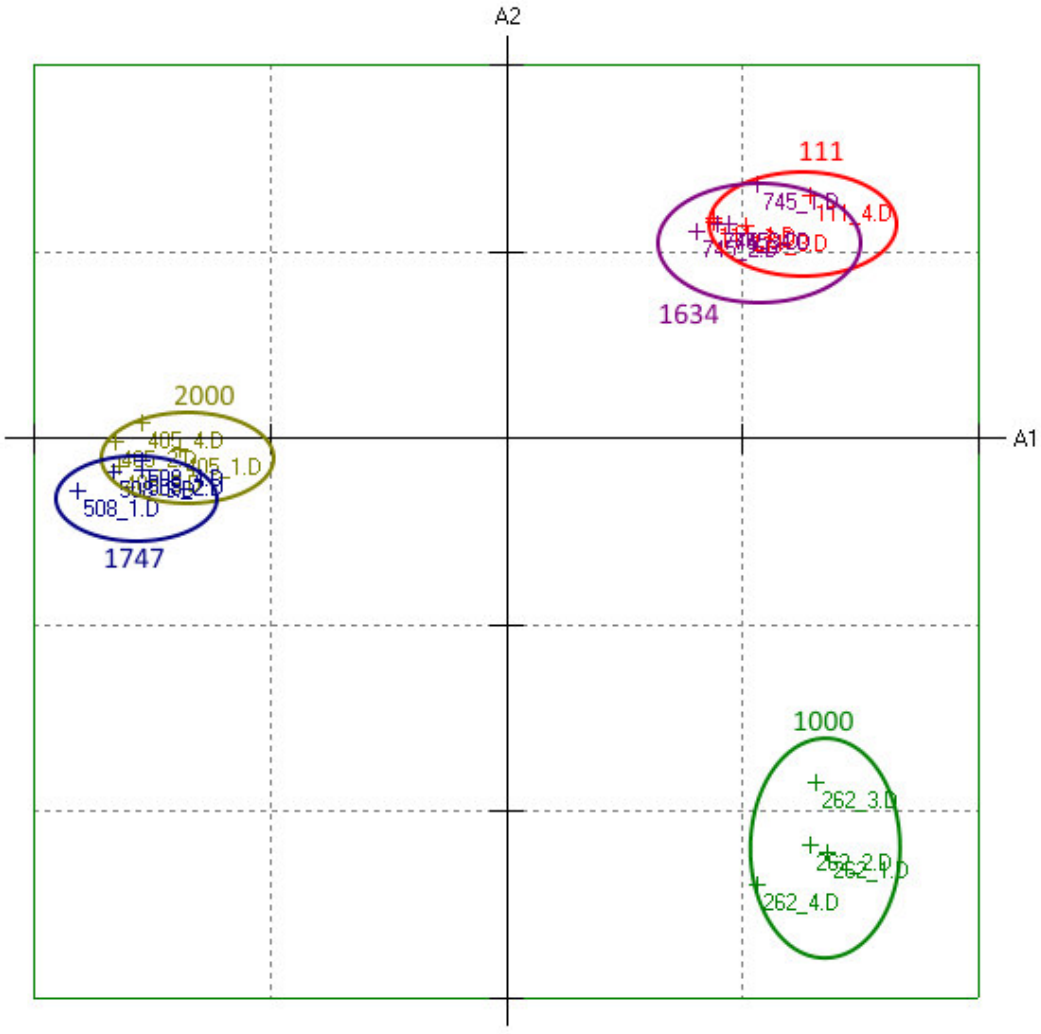

Figure 23: MASstat® evaluation of 5 aronia juices

6.2.2 Aronia seeds, leaves and pedicels

The following individual plant parts of aronia shrubs were also investigated with HS-SPME/GC-MS analysis with quadruple determination:

- Leaves
- Pedicels and twigs
- Seeds

This was done to estimate the influence of plant parts on the processed aronia juice samples. It is possible that leaves or small twigs might not be fully sorted out before juice production. The pedicels directly attached to the aronia pomes are even more difficult to remove. Seeds on the other hand might contribute to the aronia juice aroma, depending on the amount of crushed kernels.

Furthermore the question of the origin of δ-lactone-5-hydroxy-2-pentenoic acid should be resolved. One possible explanation is that it was introduced to the juice by contamination by ligneous parts or by the aforementioned plant matter.

Figure 24: Mechanical harvest of aronia berries mixed with various unwanted plant parts[94]

The results of the HS-SPME/GC-MS analysis of the plant parts are listed in Table 18.

Table 18: Substances identified in aronia leaves with HS-SPME/GC-MS analysis with their RT and % peak area (n=4)

Aronia leaves		
Substance	exp. RI (HP-5)	Peak area [%]
2-Pentanol	698	1.35
***(E)*-3-Penten-2-one**	739	1.33
***(Z)*-2-Penten-1-ol**	770	0.66
Welches Isomer? 2-Methyl-2-pentenal	797	1.01
Hexenal	799	0.96
4-Hydroxy-2-pentanone	822	0.74
***(E)* 2-Hexenal**	857	16.7
***(Z)*-3-Hexen-1-ol**	860	19.3
1-Hexanol	869	2.72
Welches Isomer? 2,4-Hexadienal	913	1.80
Benzaldehyde	976	44.3
Benzyl alcohol	1044	6.95
Phenylethyl alcohol	1128	0.46
Eugenol	1376	1.78

exp. RI Calculated Retention Index (RI) on a HP-5 column

Table 19: Substances identified in aronia pedicels and twigs with HS-SPME/GC-MS analysis with their RT and % peak area (n=4)

Aronia pedicels and twigs		
Substance	exp. RI (HP-5)	Peak area [%]
Benzaldehyde	981	98.3
Benzyl alcohol	1044	1.64

exp. RI Calculated Retention Index (RI) on a HP-5 column

Table 20: Substances identified in crushed aronia seeds with HS-SPME/GC-MS analysis with their RT and % peak area (n=4)

Aronia seeds (crushed)		
Substance	exp. RI (HP-5)	Peak area [%]
Benzaldehyde	979	89.0
Benzyl alcohol	1045	11.0

exp. RI Calculated Retention Index (RI) on a HP-5 column

The results of the GC-MS analysis of the plant parts show that for seeds, pedicels and twigs the main constituent is benzaldehyde. In twigs and pedicels benzaldehyde amounts to 98.3 % of the volatiles; benzyl alcohol, which is an oxidation product of benzaldehyde (see

Figure 37), accounts for 1.64 %. The volatiles found in seeds are about 89 % benzaldehyde and 11 % benzyl alcohol. In the leaves, benzaldehyde makes up 44 % of the head space volatiles, and its oxidation product benzyl alcohol around 7 %. The high amount of benzaldehyde is hardly surprising. Many plants in the Rosaceae family (and especially their seeds) contain the cyanogenic compound amygdalin.[28] Upon tissue disruption the enzymatic hydrolysis leads to the formation of benzaldehyde and hydrogen cyanide. Benzaldehyde and its role in the investigated juice samples is further discussed in Chapter 6.6.2

The second and third most abundant compounds in leaves are *(Z)*-3-hexen-1-ol (leaf alcohol) and *(E)*-2-hexenal, which are both formed from linolenic acid through the LOX pathway of fatty acid metabolism after the cell walls were damaged and oxygen was introduced to the system. [62]

However, none of the plant parts analyzed contains 5-hydroxy-2-pentenoic acid or the cyclized δ-lactone-5-hydroxy-2-pentenoic acid. Therefore the origins of this compound remain unclear.

6.3 Comprehensive GCxGC-MS

Aronia juices 2000 and 2150 were analyzed with comprehensive GCxGC-MS to reveal substances which might not have been visible in 1-dimensional GC analysis.

Since the resulting chromatogram contains a considerable amount of information, it has been split into two parts: from beginning to 20 minutes RT (Figure 25 and Figure 26) and from 20 minutes RT until the end (Figure 27 and Figure 28). The probable substances are numbered consecutively and presented in Table 21.

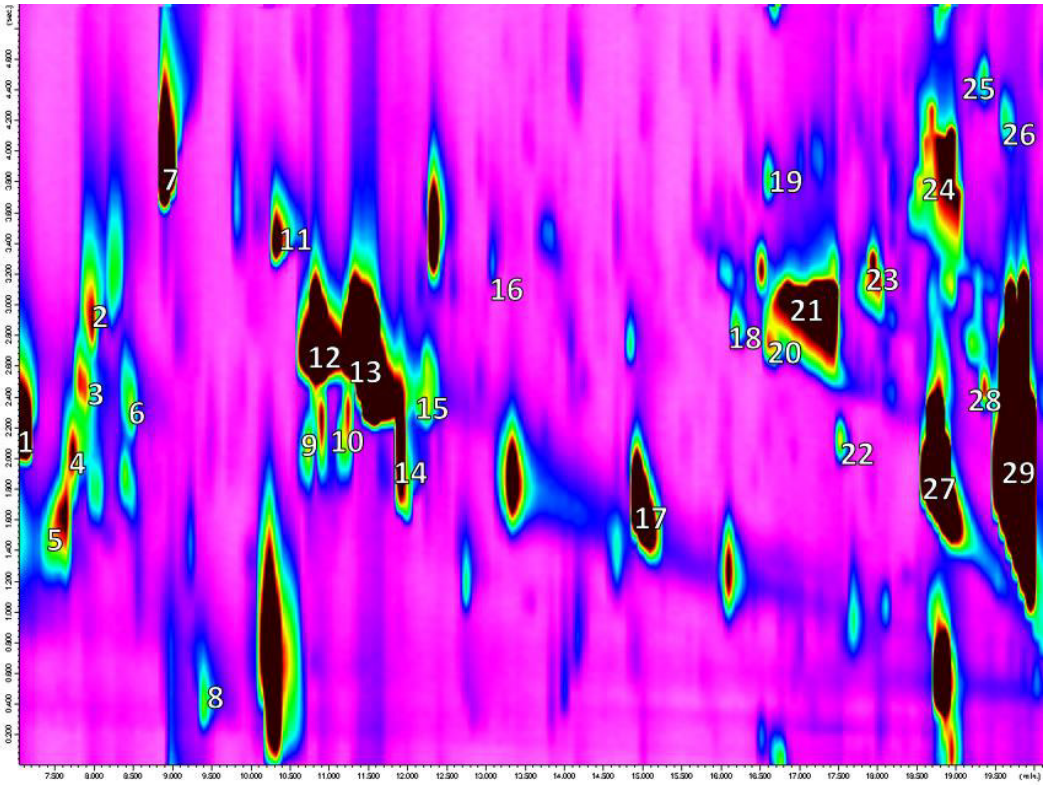

Figure 25:comprehensive GCxGC-chromatogram of juice 2000, from beginning to RT 20 minutes, created with ChromSquare ®

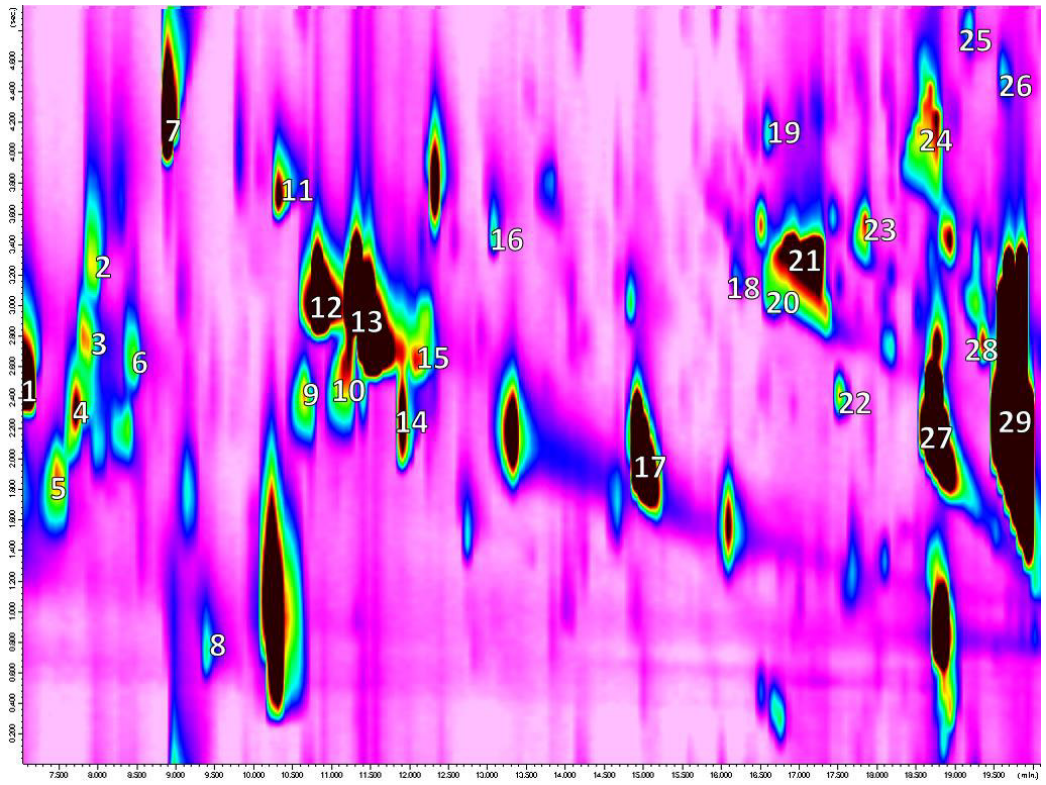

Figure 26: comprehensive GCxGC-chromatogram of juice 2150, from beginning to RT 20 minutes, created with ChromSquare ®

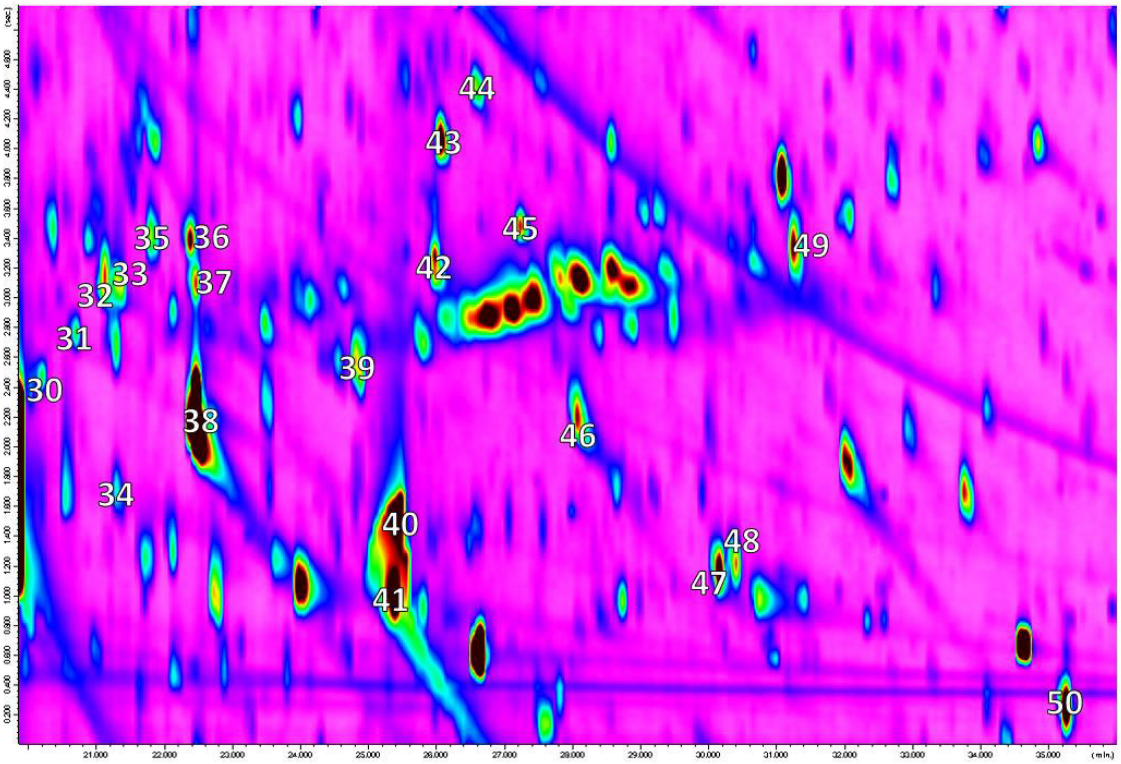

Figure 27: comprehensive GCxGC-chromatogram of juice 2000, from RT 20 minutes until end, created with ChromSquare ®

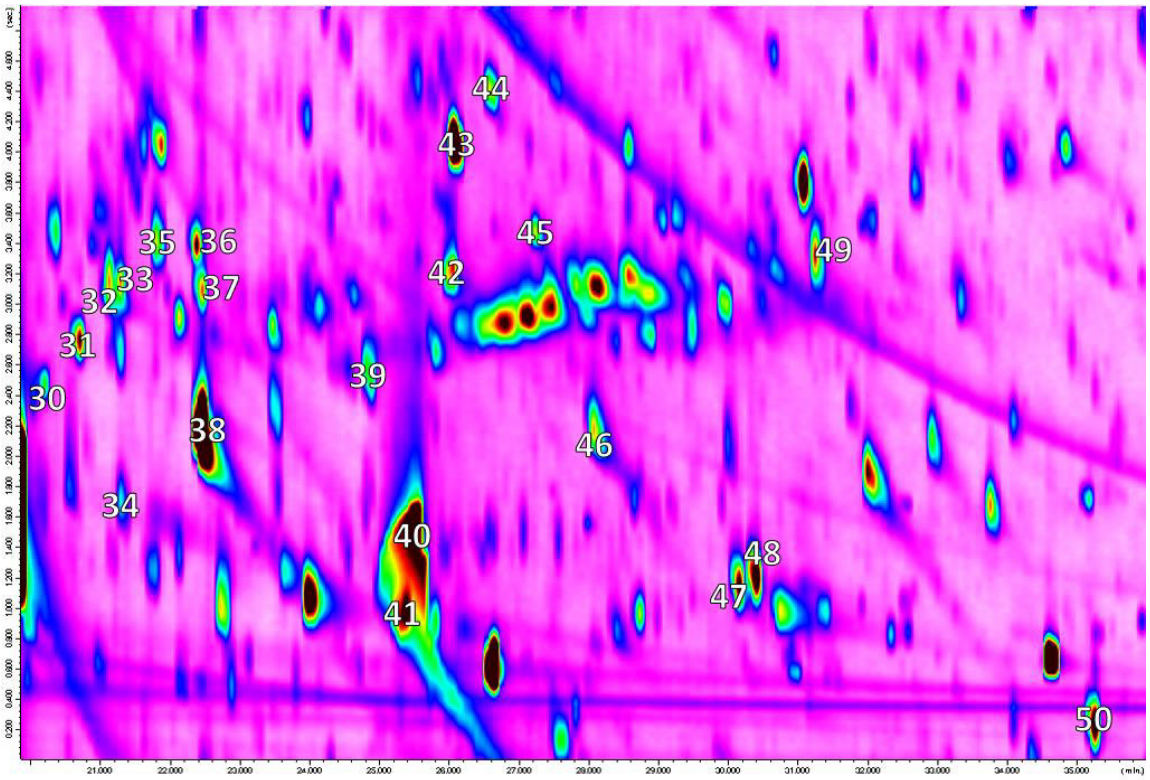

Figure 28: comprehensive GCxGC-chromatogram of juice 2150, from RT 20 minutes until end, created with ChromSquare ®

Table 21: Substances identified with comprehensive GCxGC-MS analysis

Number	Substance
1	*(E)*-3-Penten-2-one
2	2,3-Butandiol
3	Acetylacetone
4	*(Z)*-2-Penten-1-ol
5	2-Methylpropanoic acid
6	Hexanal
7	4-Hydroxy-2-pentanone
8	Furfural
9	2-Methylbutanoic acid
10	3-Methylbutanoic acid
11	*(E)*-2-Hexenal
12	*(Z)*-Hexen-3-ol
13	*(E)*-2-Hexen-1-ol
14	Butyrolactone
15	1-Hexanol
16	Heptanal
17	Benzaldehyde
18	1-Heptanol
19	6-Methyl-5-hepten-2-one
20	1-Octen-3-ol
21	Hexanoic acid
22	β-Myrcene
23	*(E)*-3-Hexenoic acid
24	2-Hexenoic acid
25	Sorbic acid
26	Hexyl-2-methyl-2-propenoate
27	Benzyl alcohol
28	Limonene
29	δ-lactone-5-hydroxy-2-pentenoic acid
30	β-Ocimene
31	γ-Terpinene
32	*cis*-Linalool oxide (furanoid)
33	Heptanoic acid
34	Guaiacol
35	trans-Linalool oxide (furanoid)
36	Nonanal
37	Linalool
38	2-Phenylethanol
39	1-Phenyl-1,2-propanedione
40	Benzoic acid
41	Ethyl benzoate
42	Octanoic acid
43	Terpinen-4-ol

44	L-α-Terpineol
45	Decanal
46	3-Phenylpropanol
47	4-Ethylguaiacol
48	2,3,6-Trimethylphenol
49	Acetovanillone
50	β-Damascenone

It is evident that there are many substances eluting in the same time frame, such as heptanoic acid and guaiacol (33 and 34 in Figure 27 and Figure 28) or nonanal, linalool and 2-phenylethanol (36, 37 and 38). This might explain why some substances cannot be identified with conventional GC-MS analysis, such as 1-octen-3-ol, which is covered by hexanoic acid (20 and 21 in Figure 25 and Figure 26). Moreover the sensitivity of comp. GCxGC is much higher than that of one-dimensional GC.

Two different juice samples were investigated with comprehensive GCxGC-MS, yet concerning the presence of substances, they were similar. This is not at all surprising, since the juices were produced from the same cultivar. However there are minute variations in the concentration of substances, based on the size of blobs relative to one another. Juice sample 2000 contains less 1-hexanol, γ-terpinene, and terpinen-4-ol, but more 1-octen-3-ol, 3-hexenoic acid, 2-hexenoic acid, hexanoic acid than juice 2150. The ratios of each of these seven compounds have been estimated based on their peak areas and are listed in Table 22:

Table 22: Comparison of substance ratios of juice 2000 to juice 2150

Substance	Ratio of substance in juice 2000 : 2150
1-Hexanol	0.66 : 1
γ-Terpinene	0.59 : 1
Terpinen-4-ol	0.64 : 1
1-Octen-3-ol	3.13 : 1
3-Hexenoic acid	1.57 : 1
2-Hexenoic acid	1.99 : 1
Hexanoic acid	1.53 : 1

6.4 GC-O

To identify the odor-active compounds in aronia juices, analyses with GC-olfactometry were conducted. The method of choice was based on detection frequency. However, contrary to classic detection frequency analysis, five trained panelists sniffed the samples. Their odor impressions and the retention time at which the odor was detected, were recorded. The individual results were combined and resulted in an aromagram (n=10), with peaks for each odor impression recorded. The height of an individual peak corresponds to the number of assessors who detected an odor impression at the specific retention time. The recorded odor impression was used in combination with the calculated RI to identify odor-active substances. Since RI are system-independent (under the condition that the columns are of the same polarity) a comparison with the substances identified through previous GC-MS experiments could be made.

Only if the RI of the substance in question was compatible with the RI calculated (on both instruments) and the odor impression recorded fit together with the substance's odor in the literature, it was considered a possible match. For some substances, retention indices and odor impressions were further verified through comparison with authentic reference substances (β-damascenone, methyl benzoate, ethyl benzoate, *(E)*-3-penten-2-one, *(E)*-2-Hexenal, 1-Octen-3-ol, nonanal, decanal, 2-phenylethanol, 3-methylbutanoic acid).

The odor threshold of a potentially odor active substance had to be considered as well. If the substance's concentration was far below the odor threshold, it was unlikely to cause the odor impression found with GC-O analysis. Therefore a calculation of the odor activity values was done and the results are presented in Chapter 6.6.1.

The detection frequency and the GC-FID chromatogram are split into two parts, from beginning to RI 1050 in Figure 29 and from there until the end of recorded odor impressions in Figure 30. The individual peaks are numbered and the likely odor-active substances are listed below in Table 23.

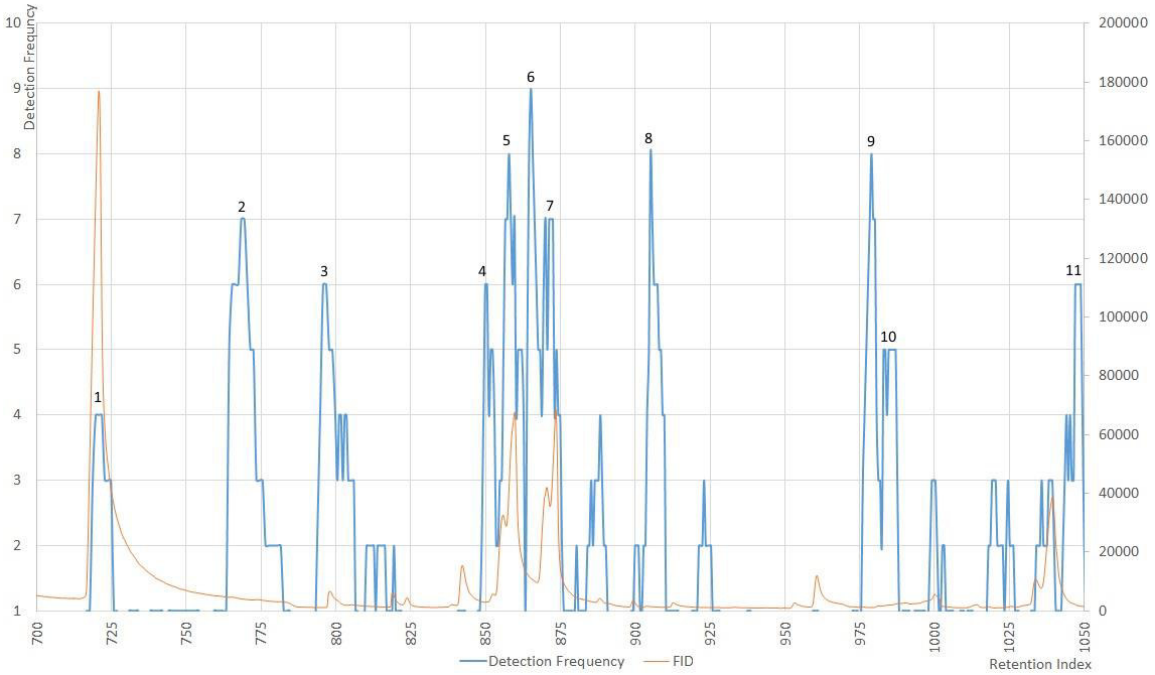

Figure 29: FID chromatogram and GC-olfactogram of juice 2000, n=10

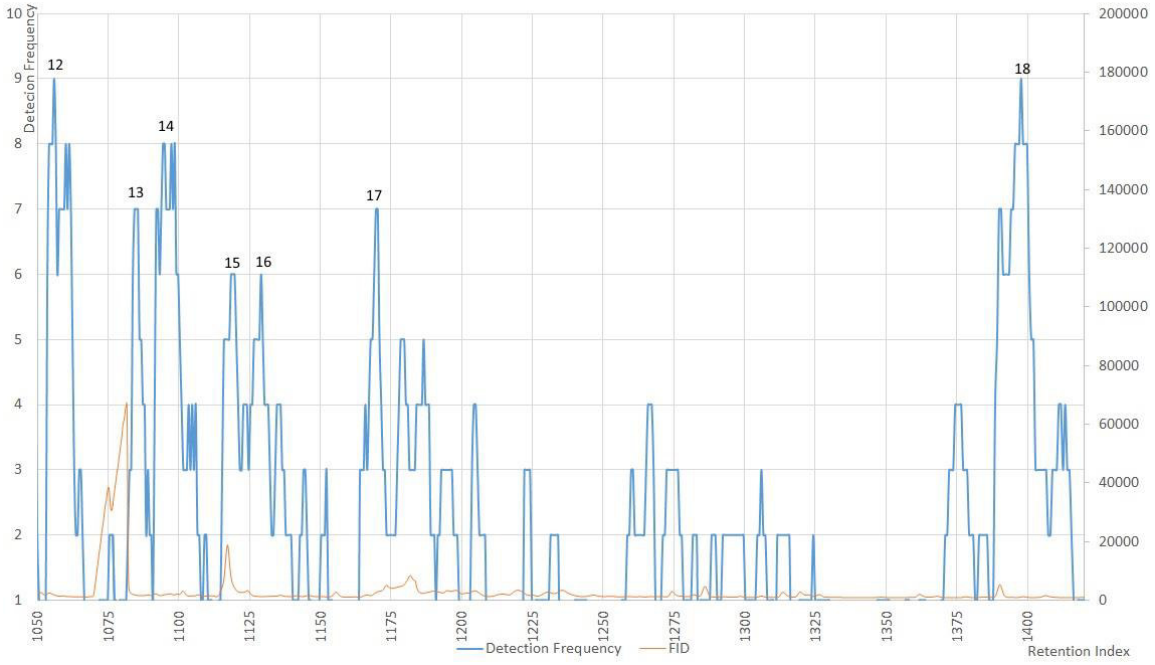

Figure 30: FID chromatogram and GC-olfactogram of juice 2000, part 2; n=10

In Table 23 the odor impressions detected most often are presented, together with a likely substance which matches in odor description and RI found in the literature.

Table 23: Odor-active compounds detected with GC-O

Peak	exp. RI (HP-5)	Odor Impression	Possible Substance	Lit. RI (HP-5)
1	722	Weak, burnt, musty	*(E)*-3-Penten-2-one	735 [i]
2	769	Warm plastic, glue, solvent	*(Z)*-2-Penten-1-ol	767 [i]
3	797	Green, grassy, fresh, pungent, floral	Hexanal	800 [i]
4	850	Fruity, red berries, sweet, green apple	Ethyl-2-methylbutanoate/ Ethyl-3-methylbutanoate	846 [i] 854 [f]
5	857	Freshly cut grass, green, grass, leafy, green apple	*(E)*-2-Hexenal/ *(Z)*-3-Hexen-1-ol	854 [i] /857 [i]
6	865	Roasted, earthy, meat soup	Not identified	n/a
7	871	Sweaty feet, cheesy	3-Methylbutanoic acid	877 [f]
8	905	Potato, cooked potato	Methional	909 [f]
9	979	Mushrooms, forest soil	1-Octen-3-ol	982 [f]
10	986	Burnt motor, solvent, pungent	β-Myrcene/ 6-Methyl-5-heptene-2-one	991 [i]/ 985[i]
11	1048	Tagetes (marigold), waxy, sweet, honey-like, goat willow, floral, dry	β-Ocimene	1043 [i]
12	1056	Unpleasant, musty, carton, burnt, sweet, green, floral, smoky	Not identified	n/a
13	1084	Roasted, popcorn-like, dry, sweet, stifling	Benzyl alcohol	1091 [i]
14	1097	Medicinal, woody, sweet, becomes green, green peas, vegetable	Guaiacol/ Nonanal	1089/1104
15	1119	Floral, sweet, rose, fresh	Linalool	1112 [i]
16	1129	Floral, honey, waxy, rose	2-Phenylethanol	1121 [h]
17	1170	Herbal tea, diffuse, burnt, sweet, green	Ethyl benzoate	1170 [i]
18	1396	Fruity, apple, cooked apple, floral, rose, sweet	β-Damascenone	1393 [i]

exp. RI *Retention Indices calculated from experimental retention times on a HP-5 column*
Lit. RI *Retention Indices found in the literature for HP-5 column*
f *http://www.flavornet.org/flavornet.html*
g *http://www.thegoodscentscompany.com/*
h *http://webbook.nist.gov/chemistry/*
i *http://www.pherobase.com/*

From RI 1048 (= RT 7.95 min) to about RI 1100 (= RT 8.98 min), which corresponds to about a minute in time in the GC-O experiment, numerous different odor impressions are recorded. It is likely that not only many odor-active substances elute within this time frame, but also quite possible that some of them overlap or at least elute closely to one another. Depending on the breathing cycle, it is conceivable that some substances might be missed during a single run or that only one of two were detected. This is one of the reasons why the sample was sniffed multiple times. The comment on peak 14 for example is noteworthy, in which one assessor mentioned the odor impression switching from medicinal to green. In this case it could point to guaiacol (which is described in the literature as smoke, sweet, medicine (fn)) and nonanal (fat, citrus, green) eluting closely.

Kraujalyte et al.[93] found ethyl-2-methylbutanoate and ethyl-3-methylbutanoate as compounds responsible for a fruity, berry odor perceived in GC-O analysis around RIs 851 and 854 respectively. Since both compounds have low odor thresholds (6 ng/L for ethyl-2-methylbutanoate and 10 ng/L for ethyl-3-methylbutanoate[92] they could be responsible for the detected odor. Unfortunately, this is difficult to verify using instrumental analysis methods.

Table 24: Possible aroma substances in aronia juice and their perceived odor compared to the odor and odor thresholds (OT) found in the literature

Peak	Substance	Odor	Odor Descriptions	OT [µg/L]
1/2	*(E)*-3-Penten-2-one	Weak, burnt, musty/ Warm plastic, glue, solvent	Sharp and acetone-like and fruity, phenolic and fishy with a chemical glue nuance [g]	88
3	Hexanal	Green, grassy, fresh, pungent, floral	Green, grass, fatty, fresh, sweet [g]	4.5 [L]
4	Ethyl-2-methylbutanoate / Ethyl-3-methylbutanoate	Fruity, red berries, sweet, green apple	Sweet, Fruity, Strawberry, Blackberry, Green apple [i]/ Cashew, Fruity, Anise, Sweet, Apple, Blackcurrant [i]	0.006[92] / 0.01 [92]
5	*(E)*-2-Hexenal/ *(Z)*-3-Hexen-1-ol	Freshly cut grass, green, grass, leafy, green apple	Green, freshly cut grass, grassy [e]	17 [L] 70 [L]
7	3-Methylbutanoic acid	Sweaty feet, cheesy	Sweaty, cheesy, rancid [i]	120 [L]
8	Methional	Potato, cooked potato	Backed potato, grassy [i]	0.04 [92]
9	1-Octen-3-ol	Mushrooms, forest soil	Mushroom [f]	1 [L]
10	β-Myrcene/6-Methyl-5-heptene-2-one	Burnt motor, solvent, pungent	Metallic, musty, Geranium, sweet, fruity, ethereal, soapy, lemon, spicy, woody[i]/ mushroom, earthy, vinyl, rubbery, woody, blackcurrant, boiled fruit[i]	13 [92]
11	β-Ocimene	Tagetes (marigold), waxy, sweet, honey-like, goat willow, floral, dry	Warm, floral, herb, flower, sweet [g]	34 [92]
13	Benzyl alcohol	Roasted, popcorn-like, dry, sweet, stifling	Sweet, medicinal, chemical, pungent, solvent, fruity, plastic [e]	100 [92]
14	Guaiacol/ Nonanal	Medicinal, woody, sweet, becomes green, green peas, vegetable	Medicine, smoke, ham [e]/ Waxy, aldehydic, rose, fresh, orris, orange peel, fatty, peely [g]	0.75 [92] 1.1 [92]
15	Linalool	Floral, sweet, rose, fresh	Muscat, sweet, green, floral, lemon, parsley, lavender, fruity [i]	0.22 [92]

16	2-Phenylethanol	Floral, honey, waxy, rose	Rose, floral, sweet, honey, green [e]	140 [92]
17	Ethyl benzoate	Herbal tea, diffuse, burnt, sweet, green	chamomile, flower, celery, fruit [f]	53 [92]
18	β-Damascenone	Fruity, apple, cooked apple, floral, rose, sweet	Apple, rose, honey [f]	0.002 [L]

fn *http://www.flavornet.org/flavornet.html*
gsc *http://www.thegoodscentscompany.com/*
L *http://www.leffingwell.com/odorthre.htm*
e *6.5.2 Descriptions of standard solutions*
h *http://webbook.nist.gov/chemistry/*
i *http://www.pherobase.com/*
OT *Odor threshold*

In Table 24 only tentatively identified substances are listed with their respective odor thresholds. Odor impressions to which no corresponding substance could be attributed, were omitted from this table and are presented in Table 25.

Table 25: RI and odor impressions of not identified substances

Peak	exp. RI (HP-5)	Odor
6	865	Roasted, earthy, meat soup
12	1056	Unpleasant, musty, carton, burnt, sweet, green, floral, smoky

Many odorants are aroma substances derived from the fatty acid metabolism, such as *(E)*-2-hexenal, *(Z)*-3-hexen-1-ol. They are likely responsible for the green attributes of black chokeberry juices. β-Damascenone has a pleasant floral and apple-like odor, and due to its low odor threshold this norisoprenoid also plays an important part for aronia juices. Degradation products of amino acids were also among the odorants, namely methional and 3-methyl butanoic acid. Ethyl-2-methylbutanoate, ethyl-2-methylbutanoate and methional were not found during instrumental analysis and only tentatively identified based on their odor, retention index and comparison to the literature.[93].

(E)-3-Penten-2-one was detected with GC-O analysis, however it was also described as weak, therefore the contribution to the overall aroma of aronia juices might not be very impactful.

Unfortunately some odorants could not be identified unambiguously and a few odor impressions could not be matched to an odor-active substance.

The odor activity values for substances deemed important for the aronia juice's aroma were calculated and the results are presented in Chapter 6.6.1.

6.5 Sensory analysis

6.5.1 Threshold determination

(E)-3-penten-2-one is a substance present in considerable amounts (94 µg/L to 1243 µg/L relative to IS) in the investigated aronia juices. To assess the importance of this compound to the aroma and calculate its odor activity value, the odor threshold of *(E)*-3-penten-2-one was determined.

(E)-3-penten-2-one was diluted and the minimal concentration at which a distinction from a blank was possible, was assessed. For the calculation, Equation 1 was used. Compared to the odor thresholds of *(E)*-3-penten-2-one found in the literature, which are 1.5 µg/L and 1200 µg/L [92] the result lies in between these two, but closer to the former.

Table 26: Determined odor threshold of (E)-3-penten-2-one

Substance	Odor threshold [µg/L]
***(E)*-3-penten-2-one**	88

δ-lactone-5-hydroxy-2-pentenoic acid was found in the examined black chokeberry juices in concentrations of up to 1246 µg/L. However no odor threshold of this substance could be found in the literature. Therefore a threshold determination was planned just like for *(E)*-3-penten-2-one, but preliminary tests revealed that odor threshold of δ-lactone-5-hydroxy-2-pentenoic acid lies above 100 mg/L; pure δ-lactone-5-hydroxy-2-pentenoic acid has a faint, slightly sweet odor. Even so, this result was not evaluated through threshold determination and only serves as a guideline.

6.5.2 Descriptions of standard solutions

The descriptors chosen by the sensory panel are compared to the descriptors found in the literature in Table 27, while the concentrations of the standard solutions are listed in Table 13.

Table 27: Comparison of panel results to literature

Substance	Odor description panel	Odor description literature
p-cymene	Green, slightly fruity, grassy, floral, petrol	Harsh chemical, woody and terpene-like with an oxidized citrus lemon note [g]
***(E)*-2-Hexenal**	Green, fruity, sweet, fresh green apple, grassy, foliage	Sharp, penetrating fresh leafy green, clean, fruity with herbal and spicy herbal nuances [g]
***(E)*-2-Hexen-1-ol**	Green, nuts, stagnant water	Green, leaf, walnut [f]
***(E)*-3-penten-2-one**	Cocoa-notes, sweet, pungent, green, fruity, fishy, berry, medicinal	Sharp and acetone-like and fruity, phenolic and fishy with a chemical glue nuance [g]
***(Z)*-3-Hexenol**	Green, freshly cut grass, grassy	Fresh, green, cut grass, foliage, vegetable, herbal, oily [g]
1-Hexanol	Green, flower	Resin, flower, green [f]
1-Penten-3-ol	Fruity, pungent, horseradish, sweet, green, burnt	Pungent, horseradish-like, green vegetable and tropical fruity nuances [g]
2-Ethylhexanol	Green, fresh, floral, mint, alcoholic	Rose, green [f]
2-Methylbutyric acid	Cheesy, like butyric acid, sweaty feet, sweat, vomit, fermented	Acidic, fruity, dirty, cheesy with a fermented nuance [g]
2-Methylhexanoic acid	Pungent, acidic, fatty, sweat	Acidic, oily, fatty, lard and chicken fat with roasted and roasted savory nuances [g]
Benzyl alcohol	Sweet, medicinal, chemical, pungent, solvent, fruity, plastic	Sweet, floral, fruity with chemical nuances [g]
Eugenol	Clove, sweet, woody	Sweet, spicy, clove like, woody, with phenolic savory ham and bacon notes and cinnamon and allspice nuances [g]
Guaiacol	Medicinal, smoke, ham	Smoke, sweet, medicine [f]
Hexanal	Green, grass, fatty, fresh, sweet	Grass, tallow, fat [f]

Hexanoic acid	Cheesy, pungent, sweaty, acidic, buttery, fermented	Cheesy, fruity, phenolic, fatty, goaty [g]
Methyl butanoate	Medicinal, fruity, phenolic, solvent, sweet	Ether, fruit, sweet [f]
Phenylacetaldehyde	Floral, sweet, honey, grass	Hawthorne, honey, sweet [f]
2-Phenylethanol	Rose, floral, sweet, honey, green	Floral, rose, dried rose, flower, rose water [g]
α-Terpineol	Citrus, woody, sweet, floral, fresh, musty, rose, flowers	Oil, anise, mint [f] pine, terpene, lilac, citrus, woody, floral [g]
β-Damascenone	Floral, sweet, clove, woody, walnut	Woody, sweet, fruity, earthy with green floral nuances [g]
β-Ionone	Violet, sweet, dusty, green, floral	Seaweed, violet, flower, raspberry [g]
γ-Terpinene	Gasoline, thyme, solvent	Gasoline, turpentine [f]

f *http://www.flavornet.org/flavornet.html*
g *http://www.thegoodscentscompany.com/*
h *http://webbook.nist.gov/chemistry/*
i *http://www.pherobase.com/*

The odor descriptions found in the literature match well with the descriptions provided by the panel. However, some additional descriptors, which were given by the panelists, could be to better link odor impressions to a corresponding substance in other analytical procedures.

6.5.3 Descriptive analysis

The 4 Austrian aronia juices, which were awarded a gold medal, were characterized with the following attributes in descriptive analysis (see Table 28).

Table 28: Results of descriptive analysis of 4 Austrian aronia juices

Sample	Odor Description	Taste	Overall sensory impression
Juice 1746	Black currant, sweet, red berries, fruity, earthy, green	Astringent, sweet, bitter	Typical aronia taste
Juice 1747	Slightly green, sweet, shrubbery/stalks, vegetables	Little sweetness, little fruit, tart, highly acidic, green	Not enough fruit character, disharmonious,
Juice 1925	Slightly fermented, beetroot, fruity, red berries, cough syrup	Bitter, high astringency, barely sweet, little acid	Very bitter, little fruit, red wine, intense
Juice 1949	Green, barely fruity, woody, shrubbery/stalks, musty, stale, plastic, solvent	Sweet, little fruit, bitter, green, tart	Astringent, barely fruity

The results of this descriptive analysis gave a basic characterization of aronia juices, and from these attributes common ones were selected for the following quantitative descriptive analysis. Despite sharing attributes like astringency, sweet, bitter and fruity, every juice has its own distinctive characteristics: Juice 1746 is overall a typical aronia juice, with strong fruity and black currant notes, while also being somewhat green and earthy. Juice 1747 on the other hand appears disharmonious and is not overly fruity, but green and reminds of stalks and vegetables. Juice 1925 is very astringent and bitter, and exhibits fruity notes reminiscent of red berries, such as black currant and red grapes, and fermented notes like in red wine. Juice 1949 is distinctly green (stalks and shrubbery), with musty, stale, plastic and woody notes.

The results of this descriptive analysis were correlated to the results of HS-SPME/GC-MS analysis with the help of PCA analysis (see Figure 31).

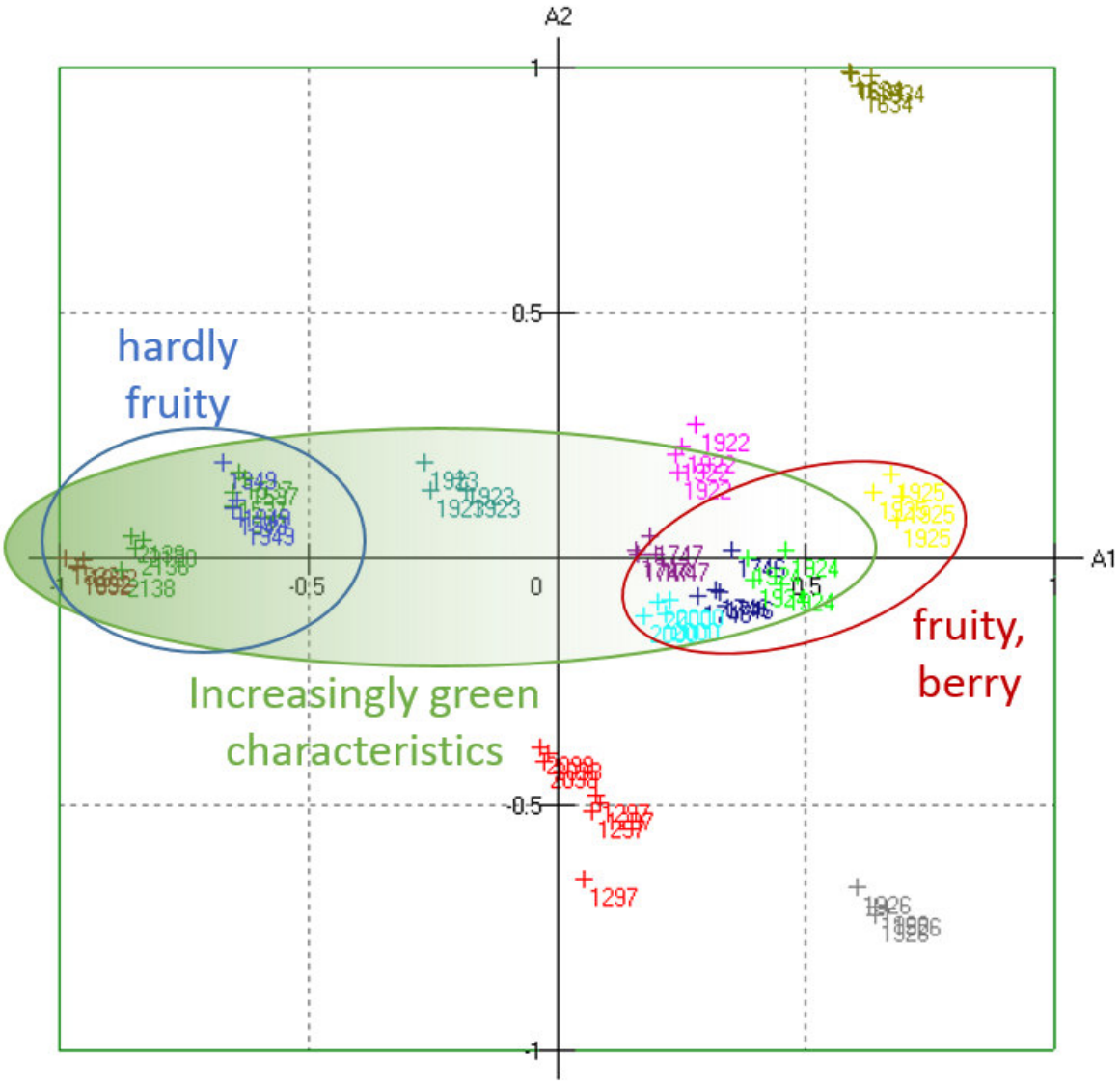

Figure 31: MASstat® evaluation of 15 aronia juices correlated to attributes found with descriptive analysis

The juices located in the cluster on the right of the center of the graph were not only awarded gold medals in the Styrian State evaluation for aronia juices, but also showed fruity and berry attributes in the descriptive analysis. Chokeberry juice samples on the left side have more prominent green attributes and the fruitiness decreases. The increased "green" perception corresponds well to the increasing *(Z)*-3-hexen-1-ol (leaf alcohol) concentration on the left side of the MASstat® graph.

6.5.4 QDA ®

The results of the first QDA ® are presented in Table 29 and Figure 32. The juices distinguish themselves through their sweetness, fruitiness, astringency and bitterness. Juice 1000 is the most bitter and astringent, but least sweet juice out of these four. In the descriptive analysis, juice 1000 was further described as sour (reminiscent of malolactic fermentation), and disharmonic, musty and stale. Juice 1634 on the other hand is the least astringent and bitter juice. It was characterized as green, woody, earthy and not very fruity. Juice 2000 is the sweetest and the second most astringent. Its bitterness is also quite pronounced, and it has "cooked" notes. Juice 1747 is musty, woody, and fruity, but not very sweet. It gives a slightly green, bitter and somewhat unripe impression.

Table 29: Conventional profiling, descriptive analysis of 4 aronia juices, first assessment

Sample	Sensory Evaluation
1000	Bitter, fruity, sour (like malolactic fermentation), musty, stale, astringent, disharmonic, grassy
1634	Green, stalks, not that fruity, woody, musty, earthy, sour, bitter, "cooked" notes
1747	Fruity, musty, woody, slightly green, slightly sour, tart, slightly bitter, unripe, fresh
2000	Fruity, berry, green, bitter, musty, woody, sweet, astringent, tart, "cooked" notes

The results of the quantitative evaluation of the attributes "Bitterness", "Fruitiness", "Green", "Astringency" and "Sweetness" are in good agreement with the descriptive analysis. All juices have a unique profile and differ in almost all attributes. The smallest variation was found for the green attributes, the largest for astringency, bitterness and sweetness.

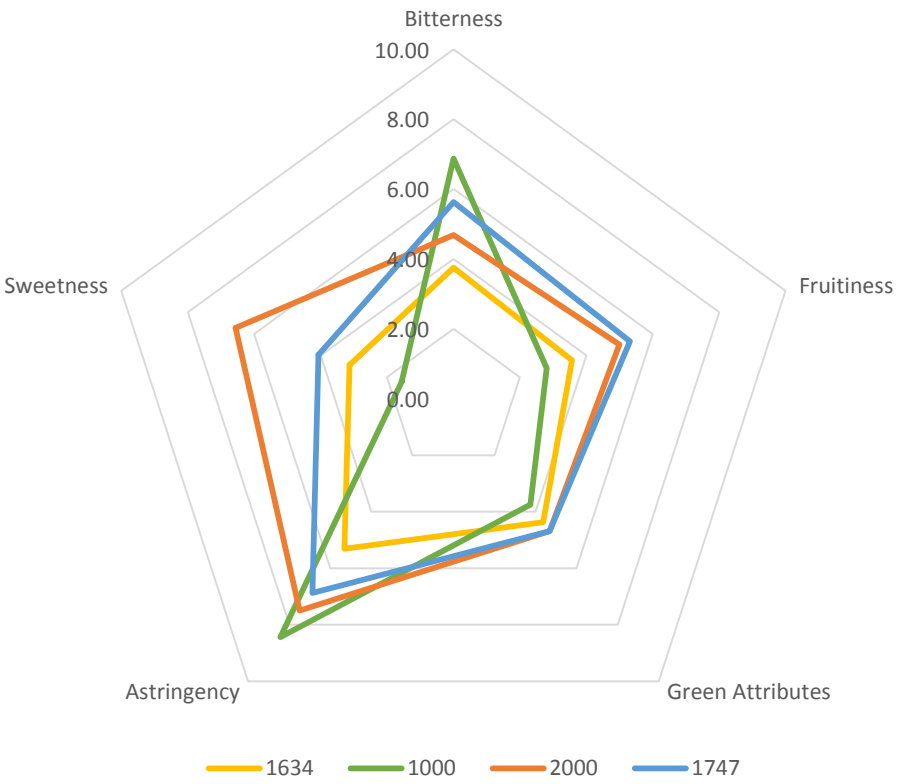

Figure 32: Results of the conventional profiling of 4 aronia juices (first assessment)

Moreover, sweetness and bitterness mutually suppress each other.[95] This might play a role in the perception of juices 1000, 1634, 1747 and 2000. Chokeberry juice 1000 is the bitterest and the least sweet one among the evaluated juices. It might be the case that the low sweetness enhances the bitterness or the bitterness suppresses the sweet taste.

In the second assessment the juices 1747 and 2000 were evaluated a second time, however the attribute “acidity” was added. The results are presented in Figure 33 and Table 30.

Table 30: Conventional profiling, descriptive analysis of 2 aronia juices, second assessment

Sample	Odor Impression	Overall impression
1747	Red berry, fruity, green, earthy, tart, musty, apple, bitter almond	Fruity, potent astringency, bitter, less sweet (than 2000), apple
2000	Red berry, fruity, green, woody, earthy, beetroot, musty	Potent astringency, very sweet, slightly bitter, slightly acidic, fruity

The first three attributes of the odor impressions of juice samples 1747 and 2000 are identical: red berries, fruity and green. This is not surprising, since their profiles in both assessments are similar, and also in the MASstat® graphs (Figure 21) these juices are always quite close to each other. However juice 1747 shows some apple and also bitter almond notes, while 2000 reminds of red beets.

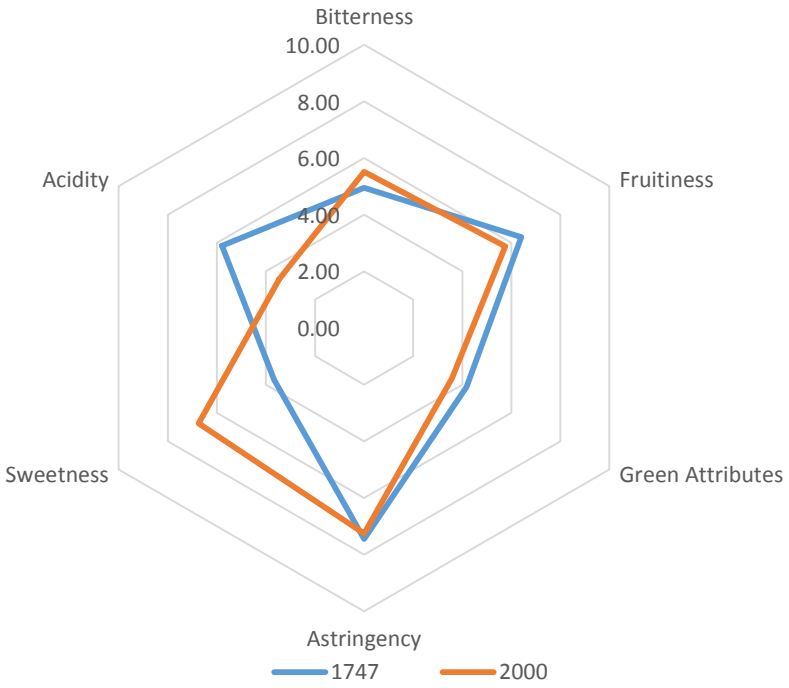

Figure 33: Results of the conventional profiling of 2 aronia juices (second assessment)

What distinguishes the two juices 1747 and 2000 is evident in the overall impression as well as the spider web plot: Juice 1747 is perceived as less sweet and more acidic than 2000.

While both juices are described as potently astringent, juice 2000's astringency was observed as quite long lasting.

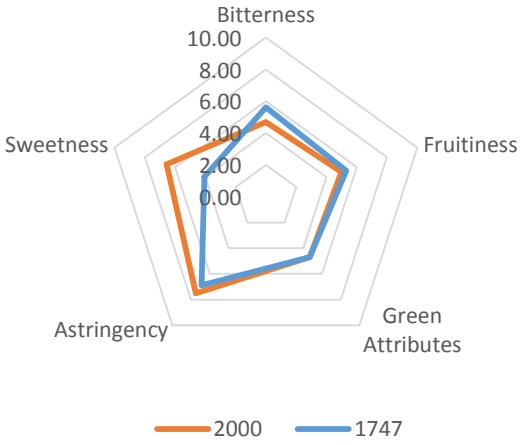

Figure 34:Profile of 2 aronia juices (first assessment)

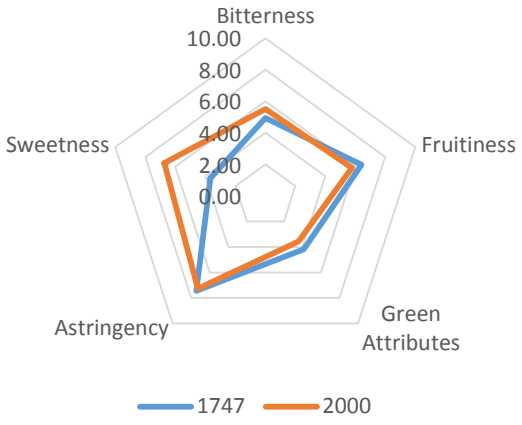

Figure 35: Profile of 2 aronia juices (second assessment without attribute "acidity")

A comparison of the first and the second assessment for only juice 1747 and 2000 can be seen in Figure 34 and Figure 35, however, the attribute "acidity" has been removed from the second assessment to allow for a better comparison. In both the first and second assessment the evident difference is the sweetness of the juices.

The results of juice 1747 and 2000 have also been evaluated with statistical analysis as well. The results of the 2-way ANOVA are visualized with the software PanelCheck[c] in the Figure 36 below.

[c] http://www.panelcheck.com/ , April 19th 2017

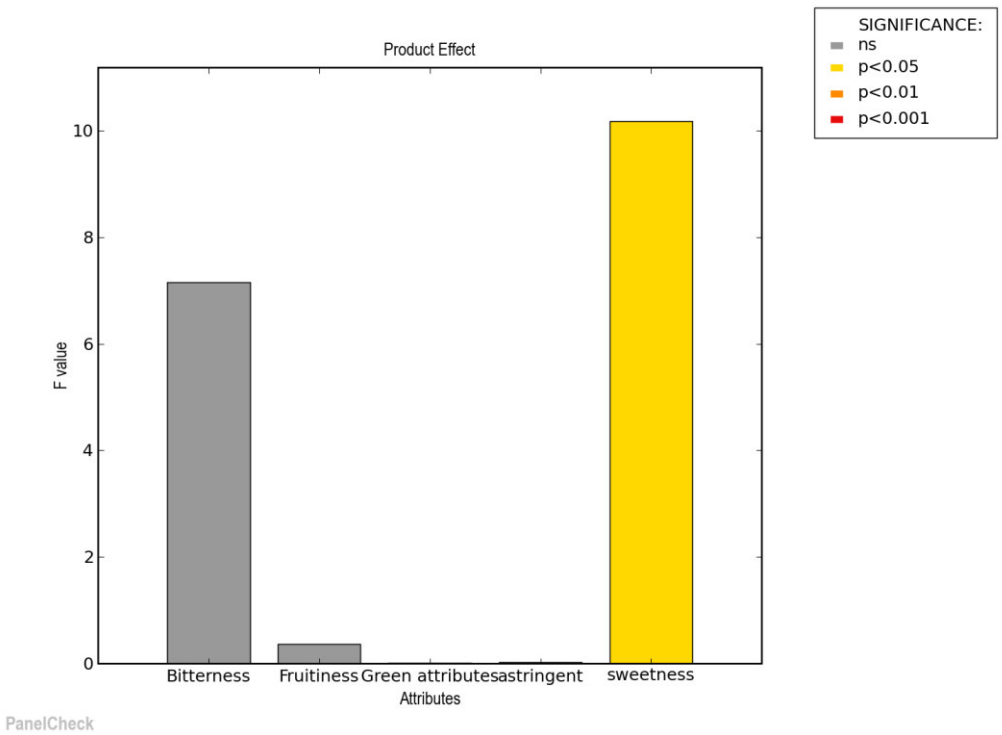

Figure 36: 2-way ANOVA for two replicates of QDA, product effect

In a 2-way analysis of variance (ANOVA) for both quantitative sensory assessments of samples 1747 and 2000, the aronia juices differed with 95% significance in the attribute “sweetness”. For the other attributes the differences were not significant.

6.6 Correlation of results

The aim of this chapter is to correlate the individual results of the different analytical techniques and draw a bigger picture of the investigated aronia juices.

6.6.1 Odor Activity Values

The activity value is an indication of the importance of the substance for the aroma. An activity value below 1 indicates that the substance is present at concentrations lower than its threshold and is therefore unlikely to contribute to the aroma.

With the concentrations relative to the IS obtained through GC-MS analysis and the odor thresholds for the individual substances found in the literature, the odor activity values (OAV) can be calculated using Equation 1. The odor activity values for substances of interest are listed in Table 31 and Table 32.

Table 31: Odor activity values of substances of interest, part one

Substance	**Odor activity value**						
	Aronia juice sample						
	1297	1537	1634	1746	1747	1832	1922
β-damascenone	6675	7256	3102	4423	1911	8644	2811
Nonanal	24	29	39	49	30	31	41
***(E)*-3-Penten-2-one**	13	9	6	12	9	12	8
Methyl benzoate	9	n/a	n/a	n/a	n/a	n/a	n/a
Benzyl Alcohol	3	<1	2	2	3	<1	2
***(E)*-2-Hexen-1-ol**	2	<1	1	2	2	5	1
***(Z)*-3-Hexen-1-ol**	2	4	2	2	4	6	3
Eugenol	1	n/a	n/a	1	1	n/a	<1
Phenylethyl Alcohol	1	<1	1	1	2	<1	1
Ethyl benzoate	<1	<1	<1	<1	<1	n/a	<1
Benzaldehyde	<1	<1	<1	<1	<1	<1	<1
Hexanal	n/a	<1	n/a	<1	n/a	n/a	n/a

n/a not available

Table 32: Odor activity values of substances of interest, part two

Substance	**Odor activity value**							
	Aronia juice sample							
	1923	1924	1925	1926	1949	2000	2089	2138
β-damascenone	2148	3849	5570	3830	4402	6860	7050	5751
Nonanal	17	53	n/a	44	31	45	28	35

***(E)*-3-Penten-2-one**	9	10	10	14	8	11	12	11
Methyl benzoate	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Benzyl Alcohol	1	3	4	5	2	3	3	<1
***(E)*-2-Hexen-1-ol**	<1	2	1	<1	2	3	2	1
***(Z)*-3-Hexen-1-ol**	4	2	2	<1	5	2	2	7
Eugenol	<1	1	2	2	n/a	1	1	1
Phenylethyl Alcohol	<1	2	2	1	<1	2	1	<1
Ethyl benzoate	<1	1	<1	<1	<1	1	<1	n/a
Benzaldehyde	<1	<1	<1	2	<1	<1	<1	<1
Hexanal	n/a	n/a	n/a	n/a	1	n/a	n/a	1

n/a not available

Even though there are slight variations in all chokeberry juice samples, the substance with the highest activity value is β-damascenone. B-damascenone has a pleasant, sweet odor, reminiscent of apples, roses and honey. The high activity value is due to the low odor threshold of β-damascenone, as well as the relatively high concentrations ranging from 4.30 µg/L to 17.3 µg/L. β-damascenone is present in many other fruits as well, such as raspberries, blackberries, apricots, grapes, kiwis, mangoes and many more. [96]

Nonanal had the second highest odor activity for all juices. This is in agreement with the results from GC-O analysis, where nonanal could be detected.

Concentrations of *(E)*-3-penten-2-one in the aronia juice investigated lie between 494 µg/L at the bottom of the list and 1243 µg/L as the maximum. But even for the lowest concentration this is above the odor threshold and therefore likely to play a role in aronia's aroma. *(E)*-3-penten-2-one has also been detected with GC-O analysis of juice sample 2000.

(Z)-3-Hexen-1-ol is present in all aronia juices above its odor threshold, with the exception of juice 1926. The highest activity value of 7 was found for juice 2138. This is in good agreement with the results of the GC-O analysis of juice 2000, where *(Z)*-3-Hexen-1-ol has also been detected.

The activity values for other substances vary depending on the juice sample. This variation is likely to play an important role in the different perception of the individual juices.

6.6.2 Comparison to the literature

6.6.2.1 Benzaldehyde

Little research has been conducted on the flavor profile of chokeberries and chokeberry juices so far. Ara[14] reported that freshly pressed chokeberry juice's aroma is dominated by benzaldehyde and prussic acid, which are formed upon enzymatic hydrolysis of the cyanogenic glycoside amygdalin. Other sources also state that aronia smells like bitter almonds, attributing this to benzaldehyde. [2]

However, while benzaldehyde is contained in all examined aronia juices, its odor activity value lies below 1 for the majority of juice samples. The only exceptions are juice 1926 and juice 1000, which have an odor activity value for benzaldehyde of 2 and 1.6 respectively. For all other juices, the concentrations are still lower than the odor threshold benzaldehyde, which lies around 350 µg/L. These findings were reinforced by GC-O analysis, in which benzaldehyde could not be perceived either. Consequently, in the processed aronia juices examined, benzaldehyde only makes a negligible contribution to the aroma, if at all.

Nonetheless, the varying concentrations of benzaldehyde could be resulting from amygdalin contained in aronia berries. The small kernels in the aronia pomes contain the highest amount of amygdalin, as is the case for many fruits of the rose family. Therefore a higher concentration of benzaldehyde could indicate a larger amount of crushed seeds during production. This is in agreement with the results of HS-SPME/GC-MS analysis of the aronia seeds, in which benzaldehyde dominated (see Table 20).

The benzaldehyde then gives rise to other substances such as benzoic acid and benzyl alcohol, which are also present in the examined juice samples. Benzoic acid and benzyl alcohol are oxidation products of benzaldehyde, and the reaction is shown in Figure 37.

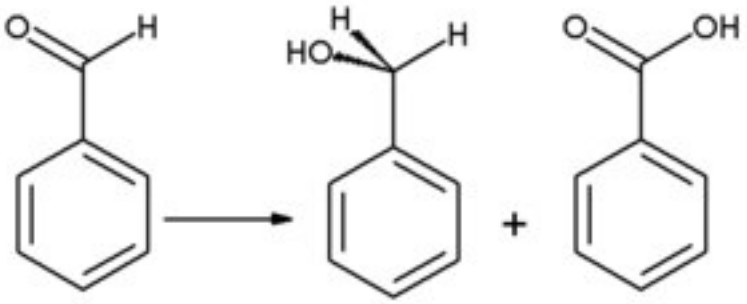

Figure 37: Oxidation of benzaldehyde to benzyl alcohol and benzoic acid[97]

6.6.2.2 Comparison of GC-O results

The only scientific article describing headspace analysis of aroma-active compounds in aronia was done by Kraujalyte et al. in 2013[93]. This publication also includes a GC-O analysis of freshly prepared aronia juices (from cultivars "Aron" and "Aronia var. cleata"). Kraujalyte et al. revealed 22 odor impressions with detection frequency analysis and was able to identify 15 of them. A comparison of all 22 odor impressions with their retention times to the aroma-active compounds found in the course of this work is given in Table 33.

Table 33: GC-O analysis comparison to literature

RI[93]	**Odor description[93]**	**Compound[93]**	**exp. RI (HP-5)**	**Odor description**	**Compound**
681	Pungent	1-Penten-3-ol	n/a	n/a	n/a
706	Fruits, berry	Ethyl propanoate	n/a	n/a	n/a
n/a	n/a	n/a	722	Weak, burnt, , musty	*(E)*-3-Penten-2-one
768	Fruity, berry	Not identified	769	Warm plastic, glue, solvent	*(Z)*-2-Pentene-1-ol
783	Fruity	Not identified	n/a	n/a	n/a
801	Sour fruits, apple	Ethyl butanoate	802	Green, grassy, fresh, pungent, floral	Hexanal

851	Fruity	Ethyl-2-methyl butanoate	850	Fruity, sweet, berry	Ethyl-2-methyl butanoate/ Ethyl-3-methyl butanoate
854	Fruity, berry	Ethyl-3-methyl butanoate			
n/a	n/a	n/a	857	Freshly cut grass, green	*(E)*-2-Hexenal/ *(Z)*-3-Hexen-1-ol
n/a	n/a	n/a	865	Roasted, earthy, meat soup	Not identified
n/a	n/a	n/a	870	Sweaty feet, cheesy	3-Methylbutanoic acid
901	Rancid, stinky	Heptanal	n/a	n/a	n/a
906	Sea food	Not identified	905	Potato, cooked potato	Methional
981	Mushroom	1-Octen-3-ol	979	Mushroom, forest soil	1-Octen-3ol
986	Mushroom, fungi	6-Methyl-5-heptene-2-one	986	Burnt motor, solvent, pungent	β-Myrcene/ 6-Methyl-5-heptene-2-one
1002	Fruity, berry	Ethyl hexanoate	n/a	n/a	n/a
1033	Mint	1,8-Cineole	n/a	n/a	n/a
n/a	n/a	n/a	1049	Tagetes (marigold), waxy, sweet, honey-like, goat willow, floral, dry	β-Ocimene
1056	fungi, moldy, stinky	Not identified	1056	Unpleasant, musty, carton	Not identified
n/a	n/a	n/a	1084	Roasted, popcorn-like, dry, sweet, stifling	Benzyl alcohol
1097	Pelargonium, green	Nonanal	1097	Medicinal, woody, sweet, becomes green, green peas, vegetable	Guaiacol/ Nonanal
n/a	n/a	n/a	1118	Floral, sweet	Linalool
1145	Stinky, strong sweet	Not identified	n/a	n/a	n/a
1169	Fruity, bonbon	Ethyl benzoate	1170	Diffuse, herbal tea, woody	Ethyl benzoate
1185	Green	Not identified	n/a	n/a	n/a
1191	Dill	3,9-Epoxy-p-meth-1-ene	n/a	n/a	n/a
1209	Fruity	Not identified	n/a	n/a	n/a
1259	Caraway, dill like	Carvone	n/a	n/a	n/a

1395	Fruity, berry, sweet	Ethyl decanoate	1396	Fruity, floral, apple, rose	β-Damascenone

Matching substances are highlighted in gray. When the odor description and the calculated retention index matched, but a different compound was attributed, the row was marked in light gray. Kraujalyte et al. identified ethyl decanoate responsible for a “fruity, berry and sweet” odor with a retention index of 1395. Nevertheless, ethyl decanoate was not found in the examined aronia juices with either one- or two-dimensional gas chromatography. Instead, β-damascenone could be identified with matching RI, odor description and reference compound. A “sea food”-like odor was perceived with an RI of 906. Whether or not the compound responsible for this odor is the same as the methional identified in this thesis is questionable.

Of the 15 substances identified by Kraujalyte et al. with GC-O analysis, seven were esters, four were terpenes, two were aldehydes and two were alcohols. In contrast to this, the processed aronia juices examined in this work contained merely three odor-active esters, of which only one was also identified with gas chromatography. This could be due to different treatments of the fruit juices (freshly prepared versus processed); the fruit’s esters are often hydrolyzed by the hydrolase enzymes during the homogenization and processing, and therefore fruit aroma flattens.[53]

Whereas Kraujalyte et al. observed 10 compounds with a fruity character, in the analyzed juice only two odor impressions were recorded as “fruity”, namely β-damascenone and ethyl-2-methyl butanoate and/or ethyl-3-methyl butanoate. β-damascenone has by far the highest odor activity value, between 1911 and 8644, of all substances observed in the aronia juice samples.

Furthermore green odor descriptors increased in comparison to the fresh juice. Compounds deemed responsible for these green notes are *(E)*-2-hexenal, *(Z)*-3-hexen-1-ol and hexanal. All these compounds are associated with the LOX pathway of fatty acid metabolism, which is believed to occur mainly upon damaging the cell walls of fruits and the introduction of oxygen to the system. Therefore the processing step influences the aroma-active compounds present. The fact that the cultivars differ might also have an impact on the different odor-active compounds found.

6.6.3 Further PCA analysis including sensory data

6.6.3.1 PCA analysis of OAV and sensory data

In order to correlate the odor activity values with the sensory data of the juices, principal component analysis was performed using the software XLSTAT by Addinsoft.

The chart obtained is shown below in Figure 38.

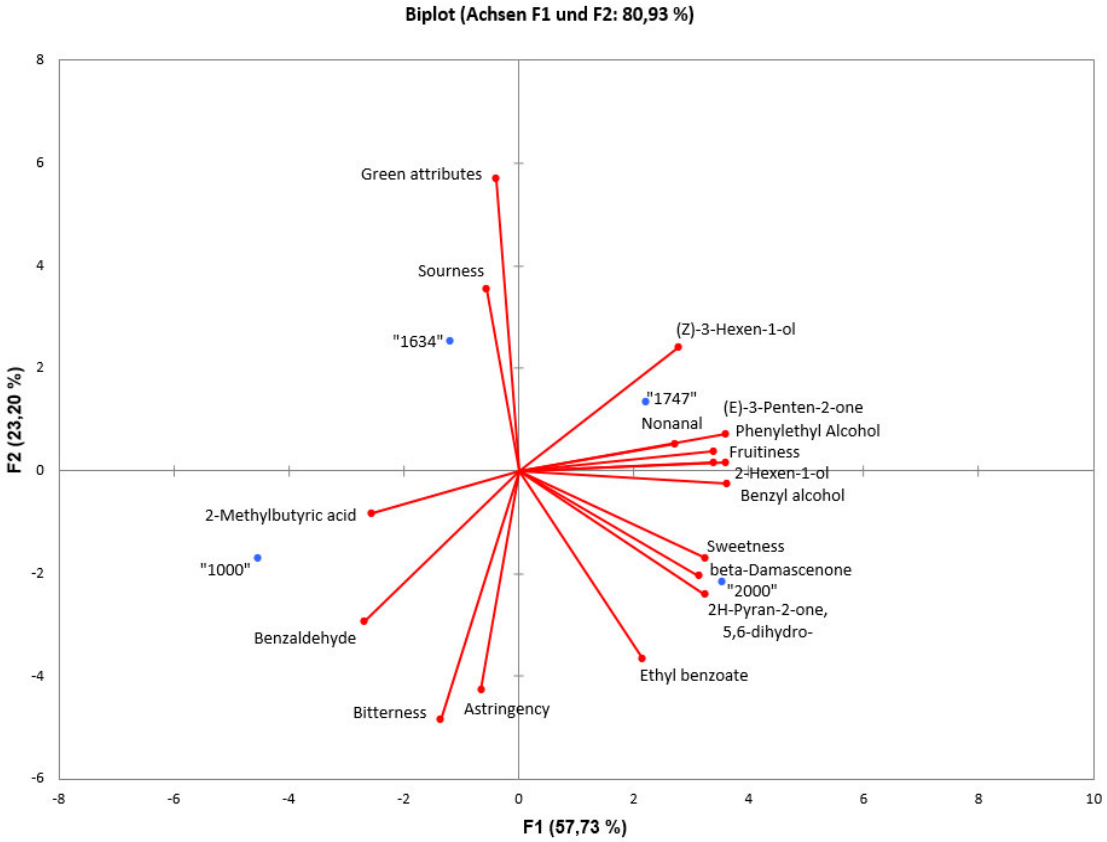

Figure 38: XLSTAT evaluation of OAV and sensory data of aronia juice samples 1000, 1634, 1747 and 2000

All juice samples are well separated, speaking for the differences in the perception of their sensory attributes as well as their aroma based on the activity values of odor-active compounds.

Aronia juice sample 1000 is located in the bottom left of the representation, moderately close to the attributes bitterness and astringency as well as to 2-methylbutyric acid and benzaldehyde. Since the seeds of aronia berries are the most likely source for elevated levels of benzaldehyde, this could indicate higher pressure applied during production of juice 1000 (compared to the other NFC aronia juices). This might also explain the higher

astringency and bitterness of juice 1000, because the skin of aronia berries is rich in anthocyanins and other polyphenols, and the higher pressure could lead to an increased extraction of bitter and astringent substances (see also Chapter 6.6.4). It is noteworthy that even though 2-methyl butyric acid's OAV in the juice samples is well below zero, it still impacts the PCA analysis.

Juice 2000 in the right bottom corner is in close proximity to the attribute sweetness and to β-damascenone, δ-lactone-5-hydroxy-2-pentenoic acid, and ethyl benzoate. Not only is juice 2000 the sweetest juice, but these results offer compelling evidence for the importance of β-damascenone in its relation to the sweetness of the aronia juices.

The QDA® analysis of juice samples 2000 and 1747 for six basic aronia juice attributes (see Chapter 6.5.4) depicts both juices as similar in all attributes except sweetness and acidity. Likewise the PCA analysis of data obtained through GC-MS placed juices 1747 and 2000 relatively close to each other (Chapter 6.2.1). It is important to note that this is no longer the case when the OAV are taken into account. Juice 1747 is surrounded by fruitiness, nonanal, *(E)*-3-penten-2-one, phenylethyl alcohol, benzyl alcohol and the C6 alcohols 3-hexen-1-ol and 2-hexen-1-ol. *(E)*-3-penten-2-one, phenylethyl alcohol and benzyl alcohol are very different in their odor perceptions, ranging from sweet flowery to chemical and solvent-like. Nonanal, and especially 3-hexen-1-ol and 2-hexen-1-ol are associated with green, grassy and fatty notes. However, contrary to expectations, the attribute "green notes" is relatively far apart from these substances in the top left quarter of the graph. This is also the quadrant where juice sample 1634 and acidity are located. However, the results regarding sourness should be treated with caution, since acidity was not evaluated for juices 1634 and 1000. For this evaluation the values were based on mean data from the other two juice samples.

6.6.3.2 PCA analysis of concentrations and sensory data

A PCA analysis was performed to correlate the sensory data of the juices with the concentrations relative to the IS obtained through HS-SPME/GC-MS.

First of all the odor activity values are better suited to evaluate the importance of a substance to the aroma of samples as explained in Chapter 2.4.4. Still, the final results of both PCA evaluations concur well with each other. The overall distribution of attributes and juice samples are comparable, only flipped vertically.

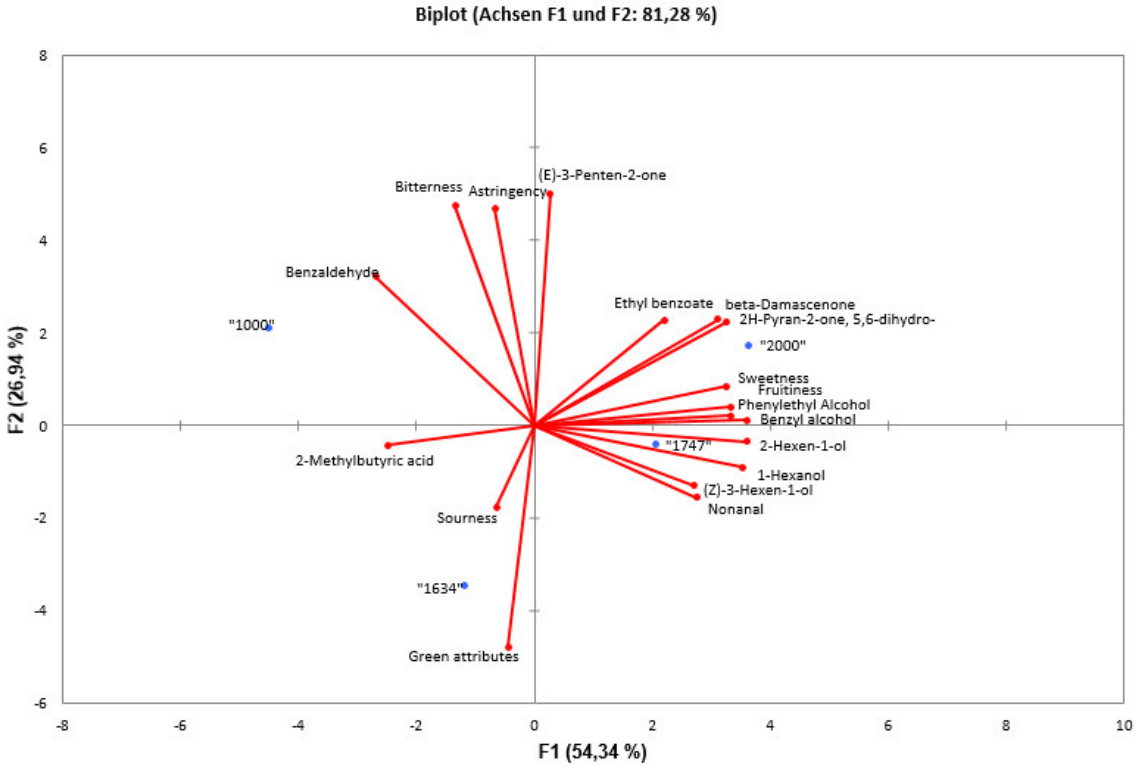

Figure 39: XLSTAT evaluation of concentrations of odor-active substances and sensory data of aronia juice samples 1000, 1634, 1747 and 2000

6.6.4 Astringency and Bitterness of aronia juices

Astringency is a distinctive and typical feature of black chokeberry juices. For some food items such as coffee, chocolate or beer a certain level of bitterness is accepted. Likewise some foods are moderately astringent, such as red wine. Nevertheless, both astringent and bitter characteristics are often associated negatively and generally have low acceptance. However consumers are more tolerant towards astringent and bitter properties when they expect health benefits from the food item. [57]

Early on it was established that a certain degree of astringency is not only characteristic, but also desirable in aronia juice, since it is a sign of high tannin content and therefore linked to the health-promoting aspects of aronia berries.

In general, numerous different groups of molecules can induce a bitter sensation, such as phenols, salts, amino acids, peptides, alkaloids as well as gylcosides, thiocarbamates and nitrogenous compounds.[55] The perception of bitterness of an individual compound can vary from person to person, this can be due to their genetic make-up, gender, age, diseases and so on. An astringent sensation is caused by water soluble phenols, acids and/or salts, which precipitate proteins present in saliva.[55] In black chokeberries the compounds at least co-responsible for the astringent sensation and for bitter taste are probably procyanidins (flavan-3-ol polymers made from (-)-epicatechin and (+)-catechin monomers). While the bitterness of these substances decreases with the degree of polymerization, the astringency on the other hand increases with the degree of polymerization.[57] In black chokeberries 82% of the proanthocyanidins are >10-mers[37]. The stereochemistry can greatly influence the astringency and bitterness as well. Despite both flavan-3-ol monomers exhibiting astringency and bitterness, the (-)-epicatechin monomer is more bitter and astringent than its diastereomer (+)-catechin.[98] Procanthocyanidins in aronia berries are predominantly built with the (-)-epicatechin monomer, and non-polymerized (-)-epicatechin was also found in concentrations of 12.7 mg/100g dried juice weight.[36]

Astringency and bitterness can intensify over several sips, but the full extent of these sensations take a few seconds to completely develop.[55]

Furthermore the matrix and other factors like temperature can influence and interact with the perceptions of bitterness and astringency. A higher pH was reported to decrease the

astringency of cranberry juice and vice versa. Likewise, a decrease in viscosity or a lowering in temperature were also able to reduce the observed astringency, but to a smaller extent.[99]

7 Conclusion

In the course of this work aronia juices from cultivar “Nero” by different producers were investigated.

By means of instrumental methods over 50 substances could be identified and 20 of them also quantified. Noteworthy is the lack of “fruit esters” in black chokeberry juices, which are usually important to the aroma of fruits and their products. However, the examined juices contained numerous alcohols, ketones, aldehydes and acids, as well as terpenes and sesquiterpenes. The calculation of odor activity values revealed that β-damascenone has by far the highest activity, followed nonanal, *(E)*-3-penten-2-one, methyl benzoate, benzyl alcohol, *(E)*-2-hexen-1-ol, *(Z)*-3-hexen-1-ol and eugenol. However the concentrations of individual volatile substances vary from juice to juice. This could be due to many influencing factors such as microclimate, ripeness, or storage before pressing. Unfortunately no data was available and therefore no conclusions about them could be drawn. It is likely that these factors and the variations in aroma substances play a large role in the different perceptions of the juices.

Sensory analysis demonstrated that the aronia juice samples differed from one another, even though they were made from the same cultivar. Furthermore the first-ever aroma profiles of aronia juices were created.

The results of the GC-MS measurement were evaluated with multivariate data analysis. With this the juice samples were sorted according to their similarities. A connection of the distribution on the resulting graph to the most abundant volatile substances was also detected. Further PCA evaluation allowed to correlate the chemical composition of the aronia juice’s volatile fraction to its sensory properties. For example higher concentrations of β-damascenone could be linked to sweet attributes with the aid of PCA analysis.

With GC-olfactometry odor stimuli were associated with odorants present in aronia juices. This revealed that aldehydes, alcohols, norisoprenoids, monoterpenes and phenolic compounds are relevant for the aroma of black chokeberry juices. Some substances could only be identified with comprehensive GCxGC-MS.

This work provides a basic characterization of the aroma of NFC aronia juices, and can serve as a base for further research of the aroma and flavor of chokeberries and their products.

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Abbreviations

ANOVA	Analysis of Variance
CAR	Carboxen
FID	Flame ionization detector
DC	Direct current
DF	Detection Frequency
DVB	Divinylbenzene
GC	Gas Chromatography
Comprehensive GCxGC	Comprehensive Gas Chromatography x Gas Chromatography
GC-MS	Gas Chromatography – Mass Spectrometry
GC-O	Gas Chromatography – Olfactometry
HS-SPME	Head Space – Solid Phase Micro Extraction
IS	Internal standard
LOX	Lipoxygenase
MS	Mass Spectrometry
m/z	Mass-to-charge (ratio)
NFC	Not from concentrate
OAV	Odor activity value
ODP	Olfactory detection port
ORAC	Oxygen radical absorbance capacity
PAH	Polycyclic aromatic hydrocarbon
PCA	Primary Component Analysis
PDMS	Polydimethylsiloxane
QDA®	Quantitative Descriptive Analysis
RI	Retention index
SPME	Solid Phase Micro Extraction
TEAC	Trolox equivalent antioxidant capacity
TIC	Total ion chromatogram

Appendix

Geruchsbeschreibung

Name:	
Prüfer Nr.:	

Prüfanleitung: 1. Auf dem Prüfplatz befinden sich Riechstreifen in Zellophanhüllen. Die Prüfproben sind in der angegebenen Reihenfolge zu prüfen.
2. Der Geruch der Proben ist so genau wie möglich zu beschreiben.

Nr.	Verbindung	Geruchsbeschreibung (Literatur)	Kommentare
1	**Methylbutanoat**	ether, fruit, sweet	
2	**Benzylalkohol**	sweet, flower; Sweet, floral, fruity with chemical nuances	
3	**1-Penten-3-ol**	butter, pungent; Pungent, horseradish-like, green vegetable and tropical fruity nuances	
4	**α- Terpineol**	oil, anise, mint; pine terpene lilac citrus woody floral	
5	**3-Penten-2-on**	fruity acetone phenolic fishy; Sharp and acetone-like and fruity, phenolic and fishy with a chemical glue nuance	
6	**β-Damascenone**	apple, rose, honey; Woody, sweet, fruity, earthy with green floral nuances	

Bestimmung des Schwellenwerts von *(E)*-3-Penten-2-on

Name	
Prüfer-Nr.	

Prüfanleitung: Es sind 6 Triangeltests mit je drei Proben zu prüfen. Innerhalb eines Triangeltests sind 2 Proben sind identisch und eine abweichend. Die abweichende Probe wurde mit 3-Penten-2-on versetzt – die Konzentration steigt von Triangeltest zu Triangeltest.
Die Probenmatrix ist Wasser.

***(E)*-3-Penten-2-on**: grün, Aceton-artig, fruchtig, fischig, Kakaonote

Prüffrage: Welches ist die abweichende Probe?

Probennr.	***Nummer der abweichenden Probe***	***Worin besteht der Unterschied?***	***Auswertung*** *Richtig/falsch*	
761 111 993				
984 752 158				
102 485 788				
463 367 576				
252 899 172				

Deskriptive Beurteilung

Name:	
Prüfer Nr.:	

Prüfanleitung: 1. Auf dem Prüfplatz befinden sich 4 Proben Aronia-Saft von unterschiedlichen Produzenten.
2. Zuerst der Geruch und dann der Geschmack der Proben sind so genau wie möglich zu beschreiben.

No.	Geruchsbeschreibung	Geschmacksbeschreibung	Gesamtbewertung
746			

Deskriptive Beurteilung

Name:	
Prüfer Nr.:	

Prüfanleitung:

1. Auf dem Prüfplatz befinden sich 4 Proben Aroniasaft von unterschiedlichen Produzenten.
2. Zuerst sind der Geruch und dann der Geschmack der Proben so genau wie möglich zu beschreiben. Des Weiteren sollen fünf ausgewählte Attribute der Säfte auf einer fünfteiligen Skala nach ihrer Intensität beurteilt werden.

Nr. 508

Deskriptive Beurteilung:

Bewertung von unterschiedlichen Attributen:

Bitterkeit:

☐	☐	☐	☐	☐
sehr bitter				nicht bitter

Fruchtigkeit/Rotbeerigkeit:

☐	☐	☐	☐	☐
sehr fruchtig				nicht fruchtig

Grüne Noten/grasig:

☐	☐	☐	☐	☐
sehr grün				nicht grün

Astringenz:

☐	☐	☐	☐	☐
sehr astringierend				nicht astringierend

Süße:

☐	☐	☐	☐	☐
sehr süß				nicht süß

Sensorische Beurteilung von Aroniasaft

Prüfperson	
Prüfernr.	

1. Auf dem Prüfplatz stehen zwei Gläser mit unterschiedlichen Aroniasäften. Versuche bitte, den Geruch und Geschmack der Proben so gut wie möglich zu beschreiben.

2. Versuche bitte, die angeführten sensorischen Eigenschaften in ihrer Intensität zu beurteilen.

Probennr.	Geruch	Geschmack- Gesamteindruck
617		

nicht ausgeprägt — stark ausgeprägt

Süße

Säure

Bitterkeit

Astringenz

Grün/grasig

Fruchtigkeit

Poster contribution

Lebensmittelchemikertage 2016; vom 8. Bis 10. Juni 2016 in St. Pölten [100]

Aronia melanocarpa – die steirische „Superbeere“

Eine Aroma-Basischarakterisierung von Saft der schwarzen Apfelbeere

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Die schwarze Apfelbeere (Aronia melanocarpa) erreichte in den letzten Jahren vor allem aufgrund der gesundheitsfördernden Wirkung einen hohen Bekanntheitsgrad. Aronia-Produkte sind in so gut wie jedem Reformhaus zu finden. Die gesundheitsfördernde Wirkung von Aronia melanocarpa ist vor allem begründet auf den außerordentlich hohen Gehalten an Polyphenolen mit stark antioxidativer Wirkung - das sind im Fall der schwarzen Apfelbeere Verbindungen aus den Substanzklassen der Anthocyane, Flavonole, Proanthocyanidine und Phenolsäuren [1]. Über die hohe Konzentration der Polyphenole hinaus ist die schwarze Apfelbeere reich an Mineralstoffen und Spurenelementen (v.a. Kalium und Zink) sowie an einigen Vitaminen [2]. Klinische Studien zeigten, dass der Konsum der schwarzen Apfelbeere oder daraus hergestellter Extrakte positiven Einfluss auf die Biomarker verschiedenster Erkrankungen hat, so zum Beispiel hinsichtlich kardiovaskulärer Erkrankungen, oxidativem Stress speziell bei Personen aus Hochrisikogruppen, aber auch positiven Einfluss auf Cholesterolgehalte, Triglyceridwerte, Bluthochdruck etc. [2]. Es gibt auch Studien, die einen entzündungshemmenden Effekt, anti-tumor Aktivität sowie positiven Einfluss auf die Gesundheit der Augen beschreiben [1]. Der Zusammenhang zwischen (Brust-) Krebs und der stark antioxidativen Wirkung von Aronia melanocarpa wird von B. Olas, 2014 [3] übersichtlich zusammengefasst.

Im Gegensatz zu den gesundheitsfördernden Eigenschaften von Aronia Produkten ist über das Aroma der schwarzen Apfelbeere in der Literatur vergleichsweise wenig beschrieben. Es gibt nur einige wenige Studien, in denen die flüchtigen Verbindungen aus Aronia Beeren untersucht wurden [4-6]. Eine sensorische Charakterisierung der schwarzen Apfelbeere bzw. daraus hergestellter Produkte unter Verwendung von sensorischen Methoden ist nach unserem Wissensstand nicht publiziert.

Die Aronia-Anbauflächen haben in den letzten Jahren in der Steiermark sehr stark zugenommen, da die schwarze Apfelbeere (die „steirische Superbeere“) aus verschiedenen Blickwinkeln aus betrachtet eine hervorragende Ergänzung zu den aktuell kultivierten Obstsorten darstellt – ein Großteil der Beeren wird zur Produktion von Aronia-Saft verwendet. Neben der gesundheitsfördernder Wirkung entscheiden Geruch und Geschmack darüber, ob das Produkt wiederholt gekauft wird. Um das zu garantieren liegt jedoch – wie schon zuvor erwähnt – kein ausreichendes Wissen über das Aroma von Aronia Produkten vor.

Es ist Ziel dieser Studie, die beschriebene „Wissenslücke“ in Hinblick auf die sensorischen Eigenschaften und die flüchtigen Verbindungen von Aronia-Saft zu schließen. Um dieses Ziel zu erreichen, werden einerseits sensorische Methoden (vor allem deskriptive Methoden) unter Einsatz eines produktspezifisch geschulten Panels eingesetzt sowie andererseits instrumentelle Techniken der Aromastoffforschung (i.e. gaschromatographische Methoden unter Einsatz verschiedener Detektoren, Gaschromatographie-Olfakometrie zur Identifizierung der geruchsaktiven Verbindungen). Multivariate statistische Verfahren werden eingesetzt, um die Ergebnisse, die mit diesen beiden, sich in ihrem Informationsgehalt ergänzenden Techniken erzielt werden, zu korrelieren. Die Ergebnisse dieser Untersuchungen sollen als Basis für weiterführende Studien dienen, bei denen der Einfluss von unterschiedlichen (Press-) Technologien, aber auch von Faktoren wie Standort oder Ernte- bzw. Reifezeitpunkt der Beeren auf die sensorischen Eigenschaften von Aronia-Saft im Mittelpunkt steht.

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[2] S.E. Kulling, H.M. Rawel (2008) *Planta Med.* 74 (2008) 1625-1634.

[3] B. Olas. in V. Breedy (Ed.) Cancer: Oxidative Stress and Dietary Antioxidants, Elsevier (2014) pp.151-157.

[4] E. Leitner, A.W. Strigl, I. Mayer, A. Schaffer, W. Pfannhauser. In Flavour Perception Aroma Evaluation: Proceedings of the 5th Wartburg Aroma Symposium (Kruse, H. P., Rothe, M., Eds); Eigenverlag Universitat Potsdam. (1997).

[5] V.Kraujalyte, E. Leitner, P. R. Venskutonis. *J. Agric. Food Chem*. 61 (2013) 4728-4736.

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Aronia melanocarpa – die steirische „Superbeere“
Eine Aroma-Basischarakterisierung des Safts der schwarzen Apfelbeere

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Einleitung

Die schwarze Apfelbeere (*Aronia melanocarpa*) wird als „Superbeere“ bezeichnet, da sie sich durch ihren hohen Gehalt an Polyphenolen mit großem antioxidativem Potential (Anthocyane, Flavonole, Proanthocyanidine und Phenolsäuren) auszeichnet. Etliche klinisch Studien zeigen, dass der Verzehr von Aronia positive Wirkung bei verschiedensten Beschwerden aufweist, von kardiovaskulären Erkrankungen über Bluthochdruck, bis hin zu Anti-Tumor Aktivität, etc.

So erfreut sich die Aroniabeere großer Beliebtheit; sie hat bereits die Reformhäuser und sogar die Bio-Sektionen einiger Supermärkte erobert. Auch die Anbauflächen für Aronia haben in den letzten Jahren in der Steiermark stark zugenommen, wobei von diesen Beeren ein Großteil zu Saft verarbeitet wird.

Während über die gesundheitsfördernde Wirkung der schwarzen Apfelbeere bereits viele Studien existieren, gibt es nur wenige Studien über deren Aroma. Ziel dieser Studie war es daher, mit Hilfe sensorischer als auch analytischer Methoden die „Wissenslücke“ in Hinblick auf den sensorischen Charakter des Aroniasafts und dessen flüchtiger Verbindungen zu füllen.

Material und Methoden

Für die Charakterisierung des Aroniasafts wurden 15 Direktsäfte der Aroniasorte „Nero“ von unterschiedlichen steirischen Produzenten herangezogen. Folgende, sich ergänzende Methoden wurden eingesetzt, um die sensorischen Eigenschaften des Aroniasafts zu beurteilen.

- Alle Saftproben wurden hinsichtlich ihrer flüchtigen Substanzen mit Headspace-SPME GC-MS analysiert.
- Von diesen 15 Säften wurden vier, die bei der steirischen Landesbewertung mit Gold ausgezeichnet wurden, von einem fachspezifisch geschulten Personal deskriptiv beurteilt.
- Darüber hinaus wurden die geruchsaktiven Komponenten eines sensorisch einwandfreien Safts, der ein für Aronia typisches Aroma aufweist, mittels GC-O analysiert.
- Die gewonnen Informationen wurden mittels multivariater Datenanalyse in Zusammenhang gebracht.

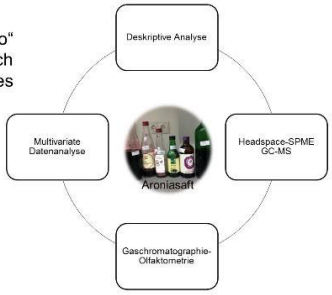

Abb. 1: Kombination von der sensorischen und instrumentellen Methoden zur Basischarakterisierung von verschiedenen Aroniasäften

Ergebnisse

Die Ergebnisse dieser Studie zeigen, dass sich die verschiedenen Aroniasäfte trotz Verwendung einheitlicher Sorte deutlich voneinander unterscheiden. Der Vergleich der GC-MS Daten zeigt, dass meisten bei der steirischen Landesbewertung mit Gold ausgezeichneten Säfte durch eine ausgeprägte Fruchtig- und Beerigkeit mit ausgewogen grünen Noten charakterisiert werden.
Je weiter links ein Saft in der PCA (Abb. 3) liegt, desto höher ist der Gehalt an (Z)-3-Hexen-1-ol. Die grünen Noten nehmen auf der linken Seite stark zu und die Fruchtigkeit tritt in den Hintergrund.

GC-MS

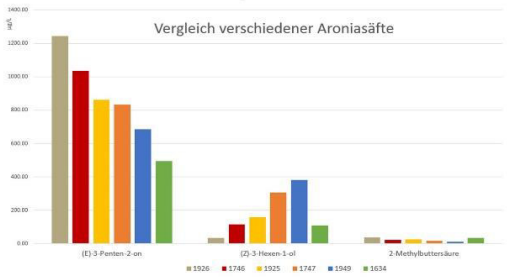

Abb. 2: Auftragung der berechneten Konzentrationen an (E)-3-Penten-2-on und (Z)-3-Hexen-1-ol von einigen Saftproben

Multivariate Datenanalyse

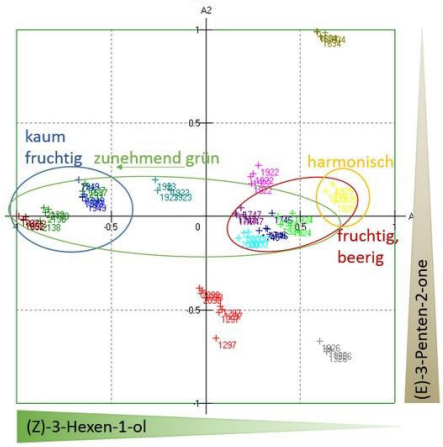

Abb. 3: Hauptkomponentenanalyse (PCA-Plot) der GC-MS Daten aller 15 Aroniasäfte und Korrelation mit den Ergebnissen der deskriptiven, sensorischen Bewertung.

Danksagung Besonderer Dank geht an die Mitglieder des Sensorikpanels des ACFC für die sensorische Beurteilung der Proben und an DI Georg Thünauer (Landwirtschaftskammer Steiermark) und Aronia Austria für die Bereitstellung der Aroniasäfte.